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TITLE OF THESIS... STUDIES ... ON ... SOIL ... AND ...

... FRESHWATER ..... CYTOPHAGAS ...

.....

UNIVERSITY.. OF ... ALBERTA, ... EDMONTON .....

DEGREE FOR WHICH THESIS WAS PRESENTED..... Ph. D .....

YEAR THIS DEGREE GRANTED..... 1973 .....

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THE UNIVERSITY OF ALBERTA

STUDIES ON SOIL AND FRESHWATER CYTOPHAGAS

by

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A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF SOIL SCIENCE

EDMONTON, ALBERTA
Spring, 1973

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Studies on Soil and Freshwater Cytophagas," submitted by Penelope Janet Christensen, B.Sc., Dip. Ag. (Soil Science), M.Sc., in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Fred A. Coota
Supervisor

Date April 19, 1973.

ABSTRACT

The author has undertaken a detailed review of the history and definition of the genus *Cytophaga*, and has examined the relationships of the cytophagas with the *Flavobacteria* and the Flexibacters. This is followed by an examination of several different aspects of this group, including a review of a taxonomic analysis of a large group of spreading and non-spreading cytophagas, some of which resembled *C.johnsonae*. Improved media and techniques are recommended for the isolation and enumeration of cytophagas and for encouraging the spreading growth habit. In a study of cytophagas from the N. W. T. it was found that this group is widely distributed in arctic lakes, that most were mesophilic, and that flexing and silkiness were better diagnostic criteria than spreading.

Growth studies on *C.johnsonae* 405 have indicated a generation time of about one hour, an optimum pH of 8.9, and that the longest cells occur during the first half of the logarithmic phase. The literature on the ultrastructure of this group has been reviewed in detail. Electron microscopic work with *C.johnsonae* 405 has confirmed the existence of the separable outer membrane and slime layers in this species, and interesting 'vesicles' containing up to five pairs of cells have been discovered.

New approaches have been made to the precise description of two morphological characters, colour and spreading. The author

has introduced the Munsell system of colour notation which is widely applicable in bacteriology. A growth code has been developed to describe the various spreading growth forms produced by cytophagas, and the definition of and conditions leading to spreading have been investigated.

The degradation of polysaccharides and proteins by the cytophagas and their allies, and their lytic and antibiotic properties have been reviewed. An extensive series of morphological and metabolic tests on 84 cultures has resulted in the creation of one new high GC% myxobacter genus, *Lysobacter*, and six new species (*L.antibioticus*, *L.enzymogenes*, *L. gummosus*, *Flexibacter canadensis*, *Cytophaga brunescens* and *C.compacta*). The genus *Cytophaga* is redefined and considered better placed in the Flexibacterales, and *C.johnsonae* is redefined as a denitrifier. The definition of *Flexibacter* is also amended, and several former *Flexibacter* and *Flavobacterium* species have been reassigned.

ACKNOWLEDGEMENTS

The author acknowledges with gratitude the invaluable assistance of Dr. F. D. Cook, the supervisor of this thesis, whose wise and friendly counsel has always been a source of great encouragement.

She is indebted to Drs. D. C. Gillespie, E. J. Ordal, R. Y. Stanier, D. White and R. S. Wolfe for kindly donating bacterial cultures, and thanks go to Drs. R. R. Colwell and R. A. Lewin as well as colleagues in Soil Science, Plant Pathology and Microbiology for helpful advice. The author wishes to thank Dorothea Donass, Linda Webster and Ann Webster for cheerful and efficient technical assistance and Pearl Williams for neat and thoughtful typing of the manuscript. She would like to extend special thanks to her husband, Gus, for his steadfast support, and to her mother, Mrs. G. D. Gardner, for her encouragement throughout her studies.

Acknowledgement is gratefully given to the National Research Council of Canada for a Graduate Research Assistantship and two Postgraduate Scholarships, and to the University of Alberta for a Graduate Research Assistantship and a Dissertation Fellowship.

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INTRODUCTION

Vegetative cells of the Order Myxobacterales can be distinguished from those of rod-shaped eubacteria by their characteristic gliding, non-flagellar movement, by their remarkable flexibility, by their low refractility, by their copious production of a tenacious slime, and by the capacity of many to aggregate and to form fruiting bodies.

For several decades following Thaxter's reports at the turn of the century, the Myxobacterales were conceived solely as microbes with a complex developmental cycle involving a unicellular vegetative phase and a communal fruiting phase that is formed by a process of aggregation of cells and their conversion to microcysts. This process, already known in the Myxomycetes, had not been described previously among the bacteria. Subsequently, the polysaccharide-decomposing soil cytophagas were interpreted as nonfruiting "imperfect myxobacteria," which exist entirely in the vegetative state and never form microcysts. The genus *Sporocytophaga* was considered to be a group transitional between the cytophagas and the fruiting myxobacteria on the basis that *Sporocytophaga* produces spherical cells which are similar in appearance and possibly in mode of formation to the microcysts of the fruiting *Myxococcus* and *Chondrococcus*. However, the *Sporocytophaga* microcysts lie scattered at random among the vegetative cells, instead of being grouped into organized structures like the fruiting bodies of the higher myxobacters.

More recently, support has been placed behind Soriano's suggestion of removing *Cytophaga* and possibly *Sporocytophaga* to an Order Flexibacteriales which would also include other flexing and gliding organisms such as *Flexibacter* and *Beggiatoa*. In addition, the systematic relationship between the cytophagas and the fruiting Myxobacteria has been challenged by the discovery that the DNA base composition of *Cytophaga* and *Sporocytophaga* is in the range of 32 to 40 per cent G + C, in contrast to the 66 to 70 per cent G + C found in the fruiters.

Much of the current confusion as to the taxonomical position of the genus *Cytophaga* is due to the paucity of data included in the early descriptions of species, together with the scarcity of extant type cultures with which to do further work, or with which new isolates may be compared. In this thesis attempts have been made to formulate media for the isolation, enumeration and routine culture of freshwater and terrestrial *Cytophaga* and allied species, and to describe and further define some special features of this group, for example their spreading habit on solid media, their colour, and the silkiness produced in liquid media. A considerable part of the thesis is devoted to a detailed taxonomic study of several *Cytophaga* and *Flavobacterium* type cultures of non-marine origin together with a selection of more recent unidentified soil and freshwater isolates which may be cytophagas.

Note: The system adopted in this thesis is that only generic and specific names have been italicized.

CHAPTER I

AN HISTORICAL APPROACH TO THE CYTOPHAGAS

I.1 The Opsimorphs (Myxomycetes, Acrasiales and Myxobacteria)

The cytophagas are at present classified (375) as primitive members of the Order Myxobacterales (the slime bacteria), the higher members of which show a strong superficial resemblance to those organisms known as slime moulds, the Myxomycetes and the Acrasieae. The Myxomycetes or slime moulds, which are not really very slimy, the Acrasieae or cellular slime moulds, which are not moulds, and the Myxobacteria or slime bacteria, whose identity as bacteria has been questioned (74), have at different times during the past 300 years been considered as Gasteromycetes, Hyphomycetes, Protozoa Cyanophytes, insect eggs (396), Eubacteria, Myxobacteria, Actinomycetes (53), Spirochaetes (54) and even as symbioses between moulds and Eubacteria (439). The three groups have in common a distinct separation of the vegetative from the morphogenetic phase. The gelatinous vegetative phase (myxomycetous plasmodium , acrasian pseudoplasmodium, and myxobacterial swarm or pseudoplasmodium) is of constantly changing shape and is the feeding and growing stage, whereas the culminating fruiting phase has a fixed and definite anatomy, often of delicate beauty. Since the increase in mass and development are sharply demarcated in time,Cohen (74) coined the termed "opsimorphic," from the Greek 'late form', to describe these organisms.

I.2 The Myxobacteria

Most of the errors in assigning myxobacteria to the fungi were made by cryptogamic botanists prior to the general understanding of bacteria. Thus, Link (248) who first described a myxobacterium considered *Polyangium* with its brilliantly coloured fruiting bodies as a Gasteromycete, and Berkeley and Curtis (32) described *Chondromyces* as a Hyphomycete. Schroeter (343) was probably the first to recognize the bacterial nature of these organisms (190). However, the first extensive study was that of Thaxter in his classic papers (393, 394, 395, 396) containing detailed descriptions and beautiful drawings of the fruiting myxobacteria, which he included in the Schizomycetes, thus laying the foundation for the current classification as Order VIII of the bacteria.

I.3 The Lower Myxobacteria (*Cytophaga* and *Sporocytophaga*)*

Later on, organisms were discovered which resembled the fruiting myxobacteria in their vegetative state, but which could not be found to fruit, and there was some confusion amongst the early workers as to their correct systematic position. Merker, in 1911 (274), described an organism which he called *Micrococcus cytophagus* which was probably a variety of *Sporocytophaga myxococcoides*. This work has been little recognized and Hutchinson and Clayton are

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*Table I should be consulted throughout this section.

Table 1

The History and Current Status of Cytophaga Species

Original Description and Author
Micrococcus cytophagus Merker 1911
Spirochaeta cytophaga Hutchinson and Clayton 1919
Cytophaga hutchinsonii Winogradsky 1929 (incorrectly assumed synonymous with *Spirochaeta cytophaga*)
Cytophaga aurantiaca Winogradsky 1929
Cytophaga lutea Winogradsky 1929
Cytophaga rubra Winogradsky 1929
Cytophaga tenuissima Winogradsky 1929
Cytophaga anularis Stapp and Bortels 1934
Cytophaga crocea Stapp and Bortels 1934
Cytophaga flavicula Stapp and Bortels 1934
Cytophaga globulosa Stapp and Bortels 1934
Cytophaga sylvestris Stapp and Bortels 1934
Cytophaga winogradskii Verona 1934
Cytophaga ellipsospora Imsenecki and Solntseva 1936
Cytophaga hutchinsonii Imsenecki and Solntseva 1936
Myxococcus hutchinsonii Imsenecki 1940
Cytophaga krzemieniewskii (sic) Stanier 1940
Cytophaga diffluens Stanier 1940

Subsequent description, if any	Current Status
	Sporocytophaga myxococcoides Stanier
(i) *Mycococcus cytophagus* Bokor 1930	*Mycococcus cytophagus* not accepted
(ii) *Cytophaga myxococcoides* Krzemieniewska 1933	*Sporocytophaga myxococcoides* Stanier
Restricted to amicrocystogenous forms (Krzemieniewska 1933)	as original description, type species
	as original description
	prob. var. of *C.hutchinsonii* Winogradsky
	as original description
	as original description
	synonymous with *C.hutchinsonii* Winogradsky
	synonymous with *C.hutchinsonii* Winogradsky
	synonymous with *C.hutchinsonii* Winogradsky
Sporocytophaga globulosa Stanier 1940	*Sporocytophaga globulosa* Stanier
	synonymous with *C.hutchinsonii* Winogradsky
	as original description
Sporocytophaga ellipsospora Stanier 1942	*Sporocytophaga ellipsospora* Stanier
Sporocytophaga myxococcoides Stanier 1940	*Sporocytophaga myxococcoides* Stanier
	Sporocytophaga myxococcoides Stanier
corrected to *krzemieniewskae* (Stanier 1941)	as original description
see Lewin's emendation 1969	as original description

Table 1 - continued
The History and Current Status of Cytophaga Species

Original Description and Author
Cytophaga albogilva Fuller and Norman 1943
Cytophaga deprimata Fuller and Norman 1943
Sporocytophaga congregata Fuller and Norman 1943
Bacillus columnaris Davis 1922
Promyxobacterium flavum Imsenecki and Solntseva 1945
Promyxobacterium lanceolatum Imsenecki and Solntseva 1945
Cytophaga sensitiva Humm 1946
Cytophaga johnsonae Stanier 1947
C.johnsonae var. *denitrificans* Stanier 1947
Promyxobacterium johnsonii (sic) Krasil'nikov 1949
P.johnsonii (sic) var. *denitrificans* Krasil'nikov 1949
Promyxobacterium krzemieniewskae var. *defluens* (sic) Krasil'nikov 1949
Promyxobacterium sensitivum Krasil'nikov 1949
Sporocytophaga ochracea Ueda, Ishikawa, Itami and Asai 1952
Cytophaga haloflava Kadota 1953
C.haloflava var. *non-reductans* Kadota 1954
Cytophaga rosea Kadota 1954
Cytophaga fermentans Bachmann 1955
C.fermentans var. *agarovorans* Veldkamp 1961
Cytophaga salmonicolor Veldkamp 1961
C.salmonicolor var. *agarovorans* Veldkamp 1961
Cytophaga succinicans Anderson and Ordal 1961

Subsequent description, if any	Current Status
	as original description
	as original description
	as original description
(i) *Chondrococcus columnaris* Ordal and Rucker 1944	poss. syn. *Cytophaga columnaris* Garnjobst 1945
(ii) *Cytophaga columnaris* Garnjobst 1945	*Cytophaga columnaris* Garnjobst
	? *Cytophaga flava*
	? *Cytophaga lanceolata*
	as original description
Often mis-spelled *johnsonii*	as original description
Often mis-spelled *johnsonii*	as original description
	Cytophaga johnsonae Stanier
	C.johnsonae var. *denitrificans* Stanier
	? *Cytophaga krzemieniewskae*
	Cytophaga sensitiva Humm
	as original description
	as original description
	as original description
	as original description
	as original description
	as original description
	as original description
	as original description
	as original description

Table 1 - continued

The History and Current Status of Cytophaga Species

Original Description and Author
Bacterium anitratum Schaub and Hauber 1948
Moraxella lwoffi Audureau 1940
Sphaerocytophaga filiformis Gräf 1961
Sphaerocytophaga fusiformis Gräf 1961
Sporocytophaga cauliformis Gräf 1962
Sphaeromyxa xanthochlora Bauer 1962
Cytophaga marinoflava Colwell et al 1966
Cytophaga psychrophila Borg 1948 (not validly published)
Cytophaga diffluens Stanier emend. Lewin 1969
C.diffluens Stanier emend. Lewin var. *aprica* 1969
C.diffluens Stanier emend. Lewin var. *carnea* 1969
Cytophaga latercula Lewin 1969
Cytophaga lytica Lewin 1969

Subsequent description, if any	Current Status
Cytophaga anitrata Lautrop 1961	Latest proposal: *Lingelsheimia*
Also in genera *Achromobacter*, *Acinetobacter*, *Diplococcus*, *Herellea*, *Mima* and *Moraxella*	*anitrata* Seeliger, Schubert and Schlieber 1966
Cytophaga lwoffi Lautrop 1961	*Moraxella* Audureau
	Cytophaga sp.
	Cytophaga sp.
	Sporocytophaga sp.
	Cytophaga sp.
	as original description
Cytophaga psychrophila Pacha 1968	*Cytophaga psychrophila* Pacha
	emendation is a suggestion only
	as original description
	as original description
	as original description
	as original description

generally acknowledged to have described the first cytophaga. In 1919, Hutchinson and Clayton isolated from soil an aerobic cellulose decomposer characterized by its peculiar morphology and by the fact that it could use only cellulose as an energy source (174). Two morphological types always occurred; a long, thin, flexible, pointed rod and a large coccus which they termed the "sporoid." The rod form predominated in young cultures, while in older ones it was replaced by the coccus. The two could not be separated and transitional forms occurred, therefore Hutchinson and Clayton concluded that they were stages in the developmental cycle of a single species. The organism was assigned to the genus *Spirochaeta* of the Order Spirochaetales because of its flexibility and because the authors considered that its peculiar developmental cycle excluded it from the true bacteria, but they noted that it exhibited a number of features atypical of this genus. The specific epithet *cytophaga* (Greek = cell destroyer) was given to this organism. Winogradsky (436) worked with several, albeit impure, isolates physiologically similar to *Spirochaeta cytophaga*, some of which did not form sporoids. He placed the whole group in the Order Actinomycetales (53) giving them the generic name *Cytophaga*, rightly concluding that the morphology did not justify their inclusion in the genus *Spirochaeta* (370). On the basis of pigmentation and cell size five species were differentiated; *C.hutchinsonii* the type species identical with *Spirochaeta cytophaga*, *C.aurantiaca*, *C.lutea*, *C.rubra* and *C.tenuissima*. Bokor (36) claimed to have isolated a pure culture of *Spirochaeta cytophaga* on which he made morphological studies. As a result of

these, he considered its cycle of development to be similar to that of the Actinomycetes, with which he placed it as the new species *Mycococcus cytophaga*. It seems from his illustrations however that he was dealing with a mixed culture of an *Actinomyces* and *Spirochaeta cytophaga* (now *C.hutchinsonii*) (370) or possibly *C.aurantiaca* (42).

A critical examination of previous work on the cytophagas was carried out by H. Krzemieniewska (230, 231) who was able to grow the organisms on cellophane, thereby facilitating microscopic observations in situ. She showed that although they were very similar in vegetative morphology and pigmentation, Hutchinson and Clayton's *Spirochaeta cytophaga* and Winogradsky's *Cytophaga hutchinsonii* were not identical, as the coccus stage occurred only in *S.cytophaga*. Mme. Krzemieniewska was the first to recognize that the transition of rod to coccus and its subsequent germination to form the rod again showed a remarkable similarity to the development of the myxobacteria of the genus *Myxococcus*. She drew attention to this fact (23) but was hesitant to transfer all of these organisms to the Order Myxobacterales because a developmental cycle was only known in *S.cytophaga*, and not in the other five *Cytophaga* species. Krzemieniewska called the sporoids "microcysts" thus emphasizing their difference from the endospores of the Eubacteriales, *Spirochaeta cytophaga* she renamed *Cytophaga myxococcoides* (231).

Further representatives of the genus, *C.anularis*, *C.crocea*, *C.flavicula*, *C.globulosa* and *C.sylvestris*, were described by Stapp and Bortels in 1934 (378). These differed in pigmentation and

temperature optima from the previously known species, and one of them, *C.globulosa* had a developmental cycle similar to that of *C.myxococcoides*. Stanier (370) considered that possibly some of these forms were identical with previously described species. An important contribution made by Stapp and Bortels was the discovery of motility in cytophagas, confirming Hutchinson and Clayton's initial observations of rotatory and flexing movements in hanging drops. Winogradsky had been unable to confirm this or to demonstrate flagella, but Stapp and Bortels observed flexing movements and slow creeping movement in the direction of the long axis of the cell quite different from the flagellar motility of the Eubacteriales. Soon after the publication of Stapp and Bortels' work Verona (412) proposed another species *C.winogradskii* which was different in pigmentation and cell dimensions from those already described. Verona's description is not sufficient, however, to warrant comparison with currently recognised species. In Russia Imsenecki and Solntseva (180) reported finding *C.hutchinsonii*, but it was later established that this was not the same organism as *C.hutchinsonii* Winogradsky, and Imsenecki and Solntseva's organism is now recognised as *Sporocytophaga myxococcoides*. A later work by Imsenecki (178) described *Myxococcus hutchinsonii* which is also now thought to be synonymous with *Sporocytophaga myxococcoides* (373).

Thus up until 1940 the genus contained only the "classical" cellulose-decomposing soil cytophagas, which have a high degree of nutritional specialization (370, 422). Although it has since been

discovered that some simpler carbohydrates can be metabolized in addition to cellulose (110, 372, 373), they remain a sharply delimited group from the nutritional standpoint. A considerable number of non-fruiting myxobacteria with much broader nutritional requirements are now know. The first to be described were the marine, agar-digesting cytophagas which can use peptides and a wide range of carbohydrates as energy sources, are not inhibited by heat-sterilized sugars, and require growth factors for their development (370). Two obligately halophilic species were initially differentiated by Stanier, *C.krzemieniewskii* (the spelling of which was later (371) corrected to *C.krzemieniewskae*), a pale pink form which also produces a diffusible dark brown pigment, and *C.diffluens* which is salmon-coloured and has smaller cells (370, 371). Both species always occur in a variety of forms; straight, arcuate, U-shaped, S-shaped and even sometimes looped around into a full circle. The ends of these flexible cells may be slightly pointed especially in *C.diffluens*, and star-shaped aggregates are often formed in liquid media. Stanier concluded (370) from his studies on the motility and morphology of various cytophagas and myxococci, that the Order Myxobacterales should be characterized on the basis of the structure of the vegetative cell and its manner of locomotion, which he considered sufficiently distinctive to exclude forms belonging to any other Order of bacteria, rather than on the production of fruiting bodies and cysts. The cytophagas would then be included, as Krzemieniewsk a had tentatively suggested a decade before (230),

as the simplest representatives of the Order, in a new Family Cytophagaceae. Essentially the same ideas on the systematic position of the cytophagas were expressed by Imsenecki and Solntseva (180, 181). Stanier then proposed that the microcyst-forming cytophagas could be most conveniently placed in the family Myxococcaceae under a new genus *Sporocytophaga*. The type species proposed was *Sporocytophaga myxococcoides* which was synonymous with *Spirochaeta cytophaga* Hutchinson and Clayton and *Cytophaga myxococcoides* Krzemieniewska, but not synonymous with *Cytophaga hutchinsonii* Winogradsky. Although not actually stated by Stanier, his work infers that *C.globulosa* Stapp and Bortels of which he was aware, now be renamed *Sporocytophaga globulosa*.

Our knowledge of the physiological abilities of the lower myxobacteria was broadened by Fuller and Norman (119, 120) who isolated from soil three new flexing organisms which could grow on a wide variety of carbohydrates in the laboratory, including starch and pectin but not cellulose. These were *Cytophaga albogilva* a pale yellow form, *C.deprimata* a yellow-pigmented type with larger cells and the capacity to etch agar, and *Sporocytophaga congregata* which was also yellow.

In 1945 Imsenecki and Solntseva proposed that a new Family Promyxobacteriaceae be set up for the simpler members of the Myxobacterales (182). This Family was to include the previously described spindle-shaped species of the genera *Cytophaga* and *Sporocytophaga*, together with a new genus *Promyxobacterium* which was erected to

encompass the species which had cells with rounded ends. It had been observed by several authors (182, 373) that when grown on certain nutritionally richer media the colonies and round-ended (cylindrical) cells of some cytophagas were indistinguishable from eubacteria, notably of the genera *Flavobacterium*, *Bacterium* and *Achromobacter* (29). Imsenecki and Solntseva considered that their new genus *Promyxobacterium* would provide a much needed bridge to the Eubacteriales (373). These authors described two soil species, *P. flavum* a yellow cylindrical form, and *P.lanceolatus* a greyish cream form having thicker cells which are described, however, as having "slightly tapered ends." This would seem to make nonsense of their new genus! At least two more species *P.johnsonii* (sic) and *P.krzemieniewskae* var. *defluens* have since been described (279). The former is now considered to be identical with *C.johnsonae* Stanier (375), and *P.krzemieniewskae* var. *defluens* may correspond with *C.krzemieniewskae* Stanier. Although still in use in the U.S.S.R. (420), the genus *Promyxobacterium* is now in demise in the western world (375) because several workers have remarked upon the fact that living cells of soil and marine cytophagas alike are only very slightly spindle-shaped and have rounded ends for the most part (246, 374). Fixed and stained preparations, however, often show spindle-shaped artefacts (174, 373, 436). No suggestions have been made to date that *Promyxobacterium flavum* or *P.lanceolatum* are synonymous with any described *Cytophaga* species, and hence they may become *C.flava* and *C.lanceolata* respectively.

Mention should be made at this point of the organism causing an important dermal disease of numerous freshwater fishes (7, 91, 111, 192, 280, 281, 297, 298, 333, 407, 421) currently recognized as a fruiting myxobacterium of the genus *Chondrococcus* in Bergey's Manual (375). The causal agent of "columnaris disease" is a rod-shaped bacterium capable of columnar (almost trichomatous), aflagellar, progressive movement on surfaces, and was described by Davis (90) as *Bacillus columnaris*. A subsequent, more detailed characterization (121) assigned this organism to the genus *Cytophaga*, and hence it became the first member of this group known to be an animal parasite. However, Garnjobst was evidently unaware of the concurrent work of Ordal and Rucker (293) who claimed discovery of fruiting body formation in strains which appeared similar to those studied by Garnjobst, and hence reclassified the organism as *Chondrococcus columnaris* (Davis) Ordal and Rucker, by which name it is recognized in Bergey's Manual (375). More recently, doubts have been expressed about this taxonomic position. Johnson and Ordal (194, 195) report that DNA homology is lacking between *Chondrococcus columnaris* and fruiting myxobacteria and between it and the cytophagas. Examination of an authentic culture (275) has failed to demonstrate fruiting bodies comparable with those of *Chondrococcus coralloides* or other higher myxobacteria, and GC ratios of 29.8 to 35.9 (Tables 2 and 3) are within the range for *Cytophaga* and quite different from all reported for authentic strains of fruiting myxobacteria (275). There have also been reports (118, 333), that cultures of *Chondrococcus columnaris* lose their ability to fruit after

laboratory culture. Therefore there is reasonable evidence that *Chondrococcus columnaris* should be classified with the lower myxobacteria. If it is to be transferred to *Cytophaga* a nomenclatural problem arises since it is not absolutely certain that this is the same organism that was described as *Cytophaga columnaris* by Garnjobst (121). The latest text by Stanier et al. (376) prefers *Cytophaga columnaris*, and Jeffers and Holt (188) are of the same opinion.

Table 2

GC ratios of *Cytophaga* and *Sporocytophaga*

Species	Base ratios reported (%G+C)
Cytophaga aurantiaca	42 (253)
C.diffluens	34.7, 7 strains 40.3-42.3 (254)
C.diffluens var. *aprica*	3 strains 35.2-37.2 (254)
C.diffluens var. *carnea*	37.2 (254)
C.fermentans	39 (253)
C.hutchinsonii	39 (253)
C.johnsonae	33 (253)
C.johnsonae var. *denitrificans*	35 (253)
C.latercula	34.2 (254)
C.lytica	5 strains 33.2-34.2 (254)
C.marinoflava	37 (80)
Cytophaga spp.	5 strains 33-42 (100), 31.7, 33.6, 34.2 (275), 37.1 (272)
Sporocytophaga myxococcoides	36, 36 (253)
Sporocytophaga sp.	41 (272)

Table 3

GC ratios of fruiting myxobacteria

Species	Base ratios reported (%G+C)
Archangium gephyra	67.8, 68.3 (272)
Archangium sp.	68 (100)
Chondrococcus columnaris	29.8, 30.3, 35.9 (275)
C.coralloides	67.6, 68.1 (272)
Chondromyces apiculatus	69.3 (272), 70 (253)
C.brunneus	68.7 (272)
C.crocatus	69.6, 69.7 (272)
C.medius	68.5, 68.7 (272)
Myxococcus fulvus	67.4 (272), 69, 71 (253)
M.virescens	67.6 (272), 68, 69, 69, 70 (253)
M.xanthus	67.1 (272), 68, 70 (253)
Myxococcus spp.	69, 69 (253)
Polyangium fuscum	68.3, 68.5 (272)
Polyangium	67 (100)
Sorangium cellulosum	69, 69 (253)
Sorangium sp.	67 (100)

Investigations on marine agar-digesting bacteria (173) revealed the existence of a light orange flexing organism which produced no water soluble pigment. It was obligately halophilic, extremely fastidious in its nutritional requirements and proved extremely difficult to culture. Hence it was given the name *Cytophaga sensitiva*, although it is also known as *Promyxobacterium sensitivum* (57, 229).

A few years before the existence of non-fruiting myxobacteria was generally recognized, Johnson (191) published a brief

account of certain myxobacteria which attack chitin. Some of these appear to have been typical *Myxococci*, but others failed to form fruiting bodies while possessing similar vegetative morphological characteristics. Miss Johnson's descriptions were not very complete, but Stanier (374) isolated and described similar organisms which he named in her honour *Cytophaga johnsonae*. This bright yellow species and its denitrifying variety, which was the first facultatively anaerobic *Cytophaga* described, were capable of using various sugars and more complex carbohydrates including starch, inulin and chitin, and therefore the nutritional range of the cytophagas was again extended.

In 1952 a new species of *Sporocytophaga*, *S.ochracea* was reported by Ueda et al (403) but it has not yet been found outside Japan. Another two contributions from Japan introduced new marine cellulose-utilizing species *C.haloflava* (198), *C.haloflava* var. *non-reductans* and *C.rosea* (199). Both species were obligately halophilic but neither could degrade agar, in contrast to the previously described marine species (*C.diffluens*, *C.krzemieniewskae* and *C.sensitiva*). *C.haloflava* was a yellow form causing deterioration of stored fishing nets, while the pink *C.rosea* was isolated from sea water. The first facultatively anaerobic marine cytophaga described was *Cytophaga fermentans* (14), a bright yellow obligately halophilic form which could ferment or oxidize simple sugars and starch, but not cellulose, agar or chitin. However, a variety which could decompose agar *C.fermentans* var. *agarovorans* was found, as

well as a new salmon-coloured, marine, facultative anaerobe *C.salmonicolor* with a similar agar-decomposing variety (408). The same year a facultatively anaerobic freshwater form was isolated (12), which was named *C.succinicans* because the predominant end product of its fermentation was succinic acid.

A further complication of the generic history was perpetrated at this time by the transference on the basis of cellular morphology and gliding motility of *Bacterium anitratum* Schaub and Hauber (336) and *Moraxella lwoffi* Audureau to the genus *Cytophaga* (239). However the validity of *Cytophaga anitrata* and *Cytophaga lwoffi* was later questioned by the same author (240), because their form of motility was more closely allied with that of certain eubacteria, some of which were flagellate and the proposal was therefore withdrawn (240). The species *Moraxella lwoffi* is conserved, and various suggestions have been made at different times to place *B.anitratum* in the genera *Achromobacter, Acinetobacter, Diplococcus, Herellea, Mima* and *Moraxella*. The latest proposal is the erection of a new genus *Lingelsheimia* with *L.anitrata* (Schaub and Hauber 1948) Seeliger, Schubert and Schlieber 1966 (344) as the type species.

Gräf and Bauer have described a series of so-called new genera and species of myxobacteria. These are *Sphaerocytophaga filiformis* and *S.fusiformis* (133), *Sporocytophaga cauliformis* (134), and *Sphaeromyxa xanthochlora* (21). The genera *Sphaerocytophaga* and *Sphaeromyxa* are erected on the formation of "sphaeroids," claimed to be part of the life cycle and to undergo germination. The

photographs indicate that these "spheroids" are clearly sphaeroplasts, and no evidence for germination is presented. Thus these organisms are obviously cytophagas which, like most myxobacteria, will form sphaeroplasts when old or under adverse conditions. Poindexter has examined Gräf's cultures of *"Sporocytophaga" cauliformis*, which are claimed to go through a life cycle involving a stalked form similar to *Caulobacter*. She has suggested that the "stalk" was no more than the slender, tapered end of the cell which persisted for some time after cell division (313). Dworkin's comment on this matter is apt: "The creation of new genera and species on the basis of sphaeroplast formation and faulty morphological observations only confuses an already difficult taxonomic situation." (100).

A marine bacterium which had previously been known as an "unidentified *Flavobacterium*" NCMB 397 (369), and was the host strain for two interesting bacteriophages was subjected to taxonomic scrutiny by Colwell's group (80). The organism was facultatively halophilic and aflagellate, and although creeping or flexuous movements could not be demonstrated, the results of a computer analysis indicated that it was best placed with the genus *Cytophaga*. They recommended that it be named *Cytophaga marinoflava*.

The etiological agent of bacterial cold-water disease of salmon was originally isolated by Borg (37) and identified as a non-fruiting myxobacterium with a low optimal growth temperature and no growth above 25°C. In contrast to the first fish pathogen suspected of being a *Cytophaga (Chondrococcus columnaris* Ordal and

Rucker 1944, but probably a *Cytophaga)*, fruiting has never been observed and the name *Cytophaga psychrophila* was proposed (37, 38). The description was somewhat incomplete and it was not included in Bergey's Manual (375). Recently this organism has been reinvestigated (38, 296), and a more detailed description is now available. Several tests have not revealed fruiting bodies, thus this is the second documented fish pathogen in the genus. It is not known how closely *Cytophaga psychrophila* resembles *Chondrococcus (Cytophaga) columnaris*. An interesting comparative study by Lewin and Lounsbery (246) has indicated that a strain of *C.psychrophila* isolated by Ordal, and one of *C.aurantiaca* isolated by Dworkin seem to be identical, although Lewin and Lounsbery were unable to show cellulolysis by the latter species.

Several new marine *Cytophaga* species have been proposed by Lewin (245) in a detailed and thoughtful evaluation of various types of gliding microbes, which unfortunately contains a few small errors in the historical presentation. The data on the isolates was subjected to two independent computer analyses, and Lewin has used Fager's recurrent group analysis (109) to help arrange the strains into sensible taxa. Colwell's Adansonian analysis (77) agrees well with Lewin's arrangement of the *Cytophaga* species (245). One of the known marine types *C.diffluens* Stanier is an orange-pigmented organism which digests cellulose (filter paper), agar and alginate but not starch. Lewin was unable to observe degradation of cellulose (cigarette paper) by his cytophagas, but they all liquefied carboxy methyl cellulose (CMC). He has emended Stanier's description

(although the emendation is not formally presented), to include carboxy methyl cellulose liquefaction instead of cellulose degradation, and starch degradation. Lewin then proposes two new colour varieties of *C.diffluens* Stanier emend Lewin; viz. var. *aprica* which tolerates lauryl sulphate, and var. *carnea* which does not digest starch. New species include the red, short-celled *C.latercula* which digests CMC, agar and alginate but not starch, and the yellow *C.lytica* which degrades all four carbohydrates. It appears that Lewin originally wished to designate these organisms *Agarophaga diffluens* (for *C.diffluens* Stanier emend Lewin), *A.aprica* (for *C.diffluens* var. *aprica*), *A.carnea* (for *C.diffluens* var. *carnea*), *Flexibacter laterculum* (for *C.latercula*) and *A.lytica* (for *C.lytica*) but later decided on a more conservative approach (87).

1.4 Definition of the Genus *Cytophaga*

The genus *Cytophaga* Winogradsky was defined by Skerman (355) using the information in Bergey's Manual (42) as follows:

> "DIFFERENTIATING CHARACTERS: Unicellular, nonphotosynthetic, flexible, rod-shaped organisms less than 2μ wide and 10μ or less in length; arranged singly; motile by a gliding motion on solid surfaces; not flagellated; Gram negative; aerobic; heterotrophic; no microcysts or resting cells are formed. Not known to deposit iron.
> Type species: *Cytophaga hutchinsonii* Winogradsky.
> NOTES: Eleven species are listed, three of which are obligate halophiles. They are fusiform or round-ended

rods varying from 0.3 to 1.0 wide and, on the average, 2 to 10μ long. Two species extend to 20μ. In old cultures degenerate coccoid forms, not microcysts, are formed.

Nine of the eleven species have been cultured on various media, including filter paper silica gels, glucose-mineral salts media, starch agar, peptone agar, and sea water-peptone agar.

In the more restrictive media, growth is in the form of a barely visible film, like etching on the surface. On richer media, raised, moist colonies may be produced. Colonies are usually pigmented yellow, orange, pink, olive green, or gray.

Agar is hydrolysed by marine species particularly.

Four species examined utilize, but do not produce acid from a wide range of carbohydrates. Heated carbohydrates are often toxic.

Of six species tested, four use NH_3 and NO_3 for nitrogen. All six use peptone and yeast extract.

Cellulose is hydrolyzed by 7/8 species. Ability to hydrolyze precipitated cellulose in agar plates is dependant upon agar sufficiently soft to permit migration of the cells through the medium.

Gelatin is liquefied by 4/5 species.

Information on other biochemical tests is too fragmentary to record.

Aerobic; optimal temperature 22-30°C.

Found in rotting vegetable matter in soil and water, both fresh and marine."

In addition, the characterization of the Order Myxobacterales (375) refers to the low refractility of the vegetative cells, "which multiply by binary transverse fission to produce a thin, flat, rapidly extending colony. Actively motile cells at the periphery

of the colony commonly occur as groups of two or three to several hundred individuals in the form of tongue-like extensions or isolated islands whose presence is virtually diagnostic of the order. The moving cells may pave the substrate with a thin layer of slime on which they rest." Since that time two freshwater fish pathogens, a freshwater facultative anaerobe and eleven marine forms, including a facultative halophile and facultatively anaerobic species have been added. There have been no new soil forms described since *C.johnsonae* in 1947 (374).

In a paper presented in 1968 and published in 1969 (275) Mitchell, Hendrie and Shewan have attempted a redefinition of this genus, taking into account results by techniques such as DNA base ratios and problems posed by the morphologically atypical strains. Their definition, presented below recognizes the occurrence of facultatively anaerobic species, and describes the cell boundary as having a slime layer on the outside and often lacking a rigid cell wall. They note the autolysis of older cells and add three biochemical characteristics (oxidase +, phosphatase + and ribonuclease +). They state that they are non-lytic to viable cells and that most strains are resistant to polymyxin B, (although no data exists in the literature for these two statements, as far as the author is aware). The DNA base ratio range is also given.

Mitchell, Hendrie and Shewan's definition

"The genus *Cytophaga* comprises Gram negative rods, varying from short and regular to long, curved and filamentous; slime layer present; nonflagellate but many lack a rigid cell

wall and show gliding motility on surfaces. No resting cells or fruiting bodies formed. The cells are pigmented in the mass from pale to deep yellow, orange or salmon pink to red. Growth on agar varies from flat and spreading to discrete convex colonies depending on medium composition and species. Colonies change from semi-opaque to transparent on aging, apparently associated with the lysis of the majority of the cells: oxidase, phosphatase and ribonuclease positive : many species characterized by ability to attack polysaccharides : non-lytic to viable eubacterial cells. DNA base ratio in range 29-45% GC : most strains resistant to polymyxin B."

Mitchell et al. recognize that their revised definition will not fit easily into any existing hierarchical scheme of classification of the Myxobacterales. In addition it may be found that it is unnecessarily wide, overlapping with proposed genera such as *Flexibacter*. The alternative would be to restrict the genus *Cytophaga* to organisms fitting the classical concept and erect new genera for other forms.

Lewin (245) has also proposed a more precise delimitation of the genus *Cytophaga* and his interest in the relationship between all forms of gliding microbes is apparent. He separates cytophagas from other gliding forms, notably the Beggiatoales (colourless trichome-forming 'bacteria' resembly Cyanophytes), as non-photosynthetic types having carotenoid pigments, unbranched, unsheathed and not helical. He extends the maximum length from 20μ (355) to 50μ , and restricts the maximum width from 1.0μ (355) to 0.7μ.

Lewin's definition

"(Additional characters, not strictly part of the definition, are added in parentheses.)

Non-photosynthetic (normally with yellow, orange or red carotenoid pigments); non-flagellate but capable of gliding (and, if sufficiently long, of flexing) on solid substrata; short or elongate rods or filaments (usually 5 to 50μ long, 0.3 to 0.7μ wide, with rounded or tapered ends); unbranched, unsheathed, not helical; not forming distinct fruiting bodies (though the cells may aggregate in pustular assemblages); not forming either endospores or microcysts (though inflated, more or less spherical cells are commonly formed in some cultures); Gram negative (generally with a lower refractility than most eubacteria); obligately or facultatively aerobic; obligately heterotrophic; usually capable of digesting (or depolymerizing) several insoluble or macromolecular colloidal polysaccharides such as cellulose (or carboxymethyl cellulose), chitin, agar, alginates, etc."

Lewin also observes that the ability of certain strains of *Cytophaga* to dissolve and digest cellulose is known to be a labile feature, which tends to be 'lost' in the course of cultivation when the organism is regularly maintained on more readily assimilable organic nutrients. He suggests that probably the same is true for the faculty of certain strains to digest other polysaccharides such as agar.

Tables 4 and 5. Explanatory Notes

GENERAL	+ = positive, - = negative, ± = both positive and negative reported, -? = probably negative, w = weak, s = slow, blank = no report.
HABITAT	F = freshwater, M = marine mud or sand, P = marine plant, S = soil, W = sea water, X = freshwater fish.
GC RATIO	Molar % guanine + cytosine in DNA
ROUND OR POINTED ENDS	R = rounded, P = pointed
COLOUR	C = cream, G = olive green, O = orange, P = pink, R = red, S = salmon pink, W = white, Y = yellow
WSP	Water soluble pigment
NaCl REQUIREMENT	+ = required for growth, ± = grows extremely slowly without NaCl, - = not required
AGAR	+ = softening or pitting, G = gelase fields, - = no action
D.O.P. ALANINE	Growth on (+ or -) and degradation of dihydroxyphenyl alanine : C = clearing, D = black or grey halo
TYROSINE	Growth (+ or -) and degradation of tyrosine (5 g/l) : C = clearing (dissolution of crystals), R = red or pink halo
GROWTH FACTORS	T = thiamine
LITMUS MILK	Growth (+ or -), C = clotting, R = reduction of dye, A = acidification, P = proteolysis (redigestion of curd).

Tables 4 and 5. Explanatory Notes - continued

ANTIBIOTICS

R = resistant, S = sensitive to the following discs: Actinomycin D 100 µg; Aureomycin 30 µg; Bacitracin 10 units; Chloromycetin 25 or 30 µg, numbers 5.0 - 6.7 = minimal inhib. conc'n. in µg/ml; Dihydrostreptomycin 10 µg; Erythromycin 2 or 15 µg; Kanamycin 30 µg; Neomycin 30 µg; Novobiocin 30 µg; Penicillin 10 units, numbers 6 - 9 = neg. log 10 of highest conc'n. permitting growth, numbers 15-29 = minimal inhib. conc'n. in units/ml; Polymyxin B 300 units; Pteridin = 0/129 Vibriostat; Pteridin discs conc'n. not reported; S.L.S. (sodium lauryl sulphate) .01%; Sulfadiazine 1 mg; Terramycin 30 µg; Tetracycline 30 µg.

Table 4

Known characteristics of soil and freshwater cytophagas

	Habitat	G C Ratio	Morphology: Width μ	Morphology: Length μ
Cellulolytic species				
C.hutchinsonii	S	39	.3-.5	2-10
*C.anularis**	S		.2-.3	2.5-5
C.crocea *	S			
*C.flavicula**	S			
*C.lutea**	S		.4	6-8
*C.sylvestris**	S		.2-.3	3-7
C.aurantiaca	S	42	1.0	6-8
C.rubra	S		.4-.7	3.0-11
C.tenuissima	S		very slender	
C.winogradskii	?S		.3-.5	6.6-8.3
Amylolytic species				
C.albogilva	S		.3-.5	4.5-7.5
C.deprimata	S		.3-.5	5.5-10
C.johnsonae	S	33-35	.2-.4	1.5-15
C.johnsonae var. *denitrificans*	S		.2-.4	1.5-15
C.succinicans	F		.6	5
Pathogenic species				
C.psychrophila	X		.75	1.5-7.5
C.columnaris	X	29.8-35.9	.5-.7	4.0-8.0

*These five species are now considered synonymous with *C.hutchinsonii*.

	Broth		Colony	Physiology							
				NaCl				O_2			
Round or pointed ends	Star-shaped aggregates	Silky	Pellicle	Colour	WSP	Requires 0.5%	Minimum %	Optimum %	Maximum %	Grows aerobically	Grows anaerobically
		+		Y		-				+	I+
	I+			Y						+	
				Y						+	
				Pale Y						+	
				Y		-				+	
	+			Y						+	
P		+		Y-O		-				+	I+
		+		P		-				+	
				G		-				+	I+
R				Y						+	
P				C		-				+	-?
P				O-Y		-				+	-?
R		+	I+	Y		-				+	-
R		+	I+	Y		-				+	+
R				W-YO		-				+	+
				Y	-	-	0		.8-1	+	-
				Y						+	

Table 4 - continued

Known characteristics of soil and freshwater cytophagas

	Physiology - continued						
	Temperature			pH			
	Minimum	Optimum	Maximum	Minimum	Optimum	Maximum	
Cellulolytic species							
C.hutchinsonii	15	30,20	<37, 30				
C.anularis	10	30	32				
C.crocea		20-28					
C.flavicula		20-24	26				
C.lutea			< 37				
C.sylvestris			40-41				
C.aurantiaca		20	30				
C.rubra	15	30	37, 30				
C.tenuissima			< 37				
C.winogradskii							
Amylolytic species							
C.albogilva		22-30	37				
C.deprimata		25-30					
C.johnsonae		25-30	< 37				
C.johnsonae var. *denitrificans*		25-30					
C.succinicans	2	25	37		7-7.5		
Pathogenic species							
C.psychrophila	4	20	23				
C.columnaris					7.3		

Sugars																		
Monosaccharides									Disaccharides					Trisaccharides				
Hexoses						Pentoses												
Fructose	Galactose	Glucose	Mannose	Sorbose		Arabinose	Rhamnose	Ribose	Xylose	Cellobiose	Lactose	Maltose	Sucrose	Trehalose		Melezitose	Raffinose	
-	-	+	-			-			-	+								
	+	+											+					
-	-	+	+			-			+	+								
	+	+									+	+	+					
		+									+	+	+					
	+	+	+			+			+	+	+	+	+				+	
	+	+	+			+			+	-	-	+	+w				-	
-	+	+	+	-		±			-	±	+	+	±	±		-	-	
		-								-								
		+																

Table 4 - continued

Known characteristics of soil and freshwater cytophagas

	Alcohols							
	Dulcitol	Ethanol	Erythritol	Glycerol	Inositol	Mannitol	Sorbitol	
Cellulolytic species								
C.hutchinsonii						-		
C.anularis								
C.crocea								
C.flavicula								
C.lutea								
C.sylvestris								
C.aurantiaca								
C.rubra						-		
C.tenuissima								
C.winogradskii								
Amylolytic species								
C.albogilva								
C.deprimata								
C.johnsonae	-					-		
C.johnsonae var. *denitrificans*	-					-	-	
C.succinicans	-		-	-	-	-	-	
Pathogenic species								
C.psychrophila								
C.columnaris								

Polysaccharides													Organic acids				
Cellulose	Hemicellulose	Cellulose dextrins	Dextrin	CMC	Agar	Alginate	Chitin	Gum arabic	Inulin	Pectin	Starch	Acetate	α Keto glutarate	Aspartate	Citrate	Fumarate
+					-						-			+		
+												+				
+																
+																
+											-					
+												+				
+				+							±+	+				
+					G									+		
+																
+																
±w	+		+					+		+	+					
±w	+	+			G					+	+					
-					G		+		+	+	+	-		+	-	±+
-					G		+w		+w		+	-			-	+
-									-		+					
-											-					
-					-		-				-				-	

Table 4 - continued

Known characteristics of soil and freshwater cytophagas

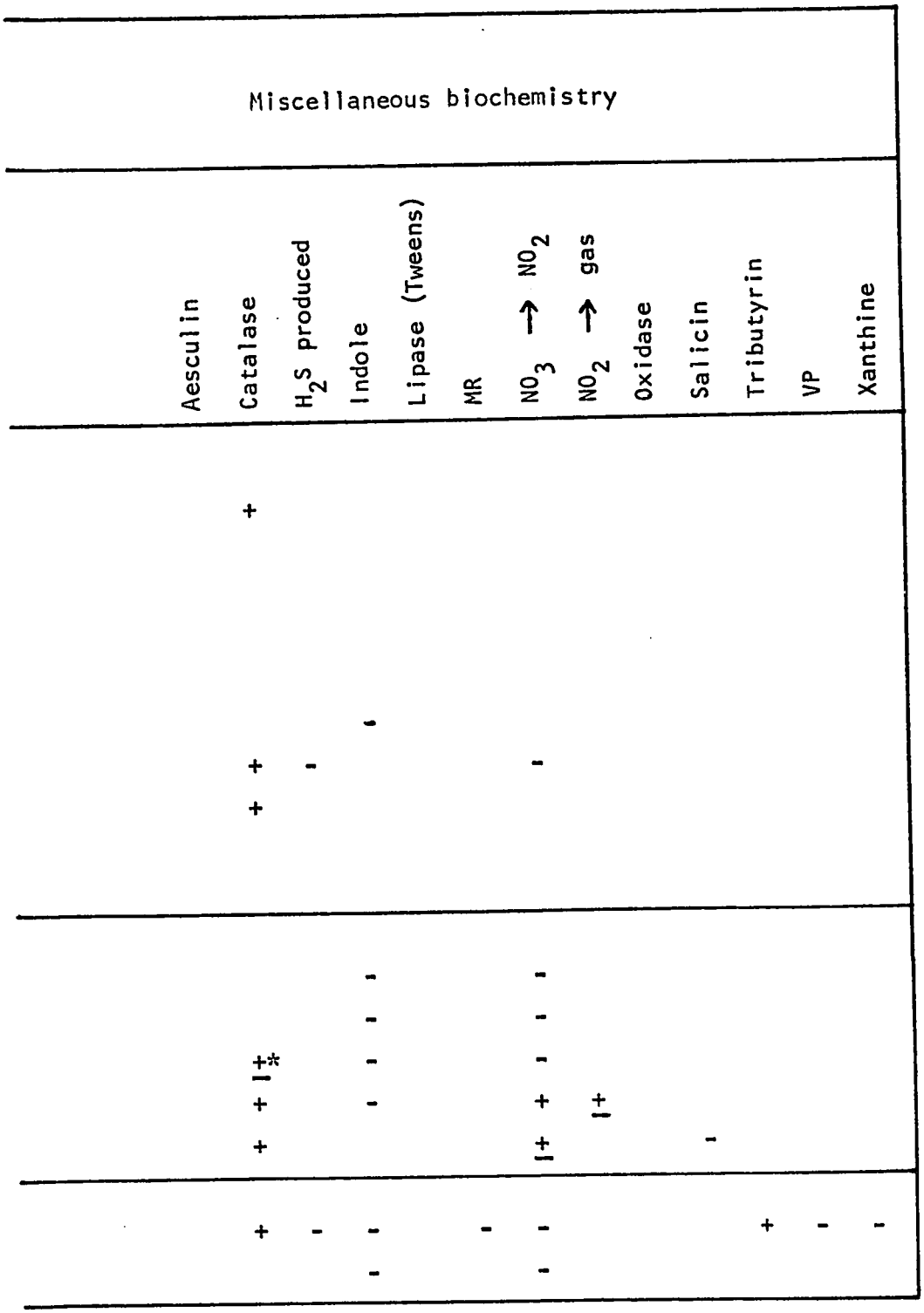
	Organic acids continued							
	Glutamate	Lactate	Malate	Malonate	Pyruvate	Succinate	Tartrate	
Cellulolytic species								
C.hutchinsonii								
C.anularis								
C.crocea								
C.flavicula								
C.lutea								
C.sylvestris								
C.aurantiaca	+							
C.rubra								
C.tenuissima								
C.winogradskii								
Amylolytic species								
C.albogilva								
C.deprimata								
C.johnsonae		-	+/-	-		+/-	-	
C.johnsonae var. *denitrificans*		-	-	-		+	-	
C.succinicans		-	-					
Pathogenic species								
C.psychrophila								
C.columnaris								

Amino acids										Nitrogen sources						
Alanine	Arginine	Asparagine	D. O. P. Alanine	Glutamine	Glycine	Histidine	Lysine	Ornithine	Tyrosine	NH_4NO_3	NH_4	NO_3	NO_2	Urea	Casamino acids	Peptone
		+									+	+				+
+		+									+			+		
											+	+				
											+	+				
-		+									+	+		+		
			+c						+c			+			+	
		+									+	+				+
											+	+				+
+		+			-					+						+
+		+			-						+	+				+
											+	+				+
											+	+				+
								-	-							

Table 4 - continued

Known characteristics of soil and freshwater cytophagas

	Nitrogen sources continued				Proteolysis				
	Tryptone	Yeast extract	Nutrient Broth	Growth factors required	Albumen	Gelatin	Litmus milk	Milk lysed	
Celluiolytic species									
C.hutchinsonii		+		-					
C.anularis									
C.crocea									
C.flavicula									
C.lutea									
C.sylvestris						+	CA		
C.aurantiaca	+					+	+R		
C.rubra		+							
C.tenuissima									
C.winogradskii									
Amylolytic species									
C.albogilva						+	-		
C.deprimata		+				+	-		
C.johnsonae						±s		+	
C.johnsonae var. *denitrificans*						+		+	
C.succinicans						+s			
Pathogenic species									
C.psychrophila	+				+	+	-		
C.columnaris	+					+			



*Reported as + (1947) and - (1957) by Stanier

Table 4 - continued

Known characteristics of soil and freshwater cytophagas

	Antibiotics etc.						
	Actinomycin D	Bacitracin	Chloromycetin	Dihydrostreptomycin	Erythromycin	Neomycin	Penicillin
Cellulolytic species							
C.hutchinsonii			R25 6.0				25
C.anularis							
C.crocea							
C.flavicula							
C.lutea			R25 5.0				15
C.sylvestris							15
C.aurantiaca	S		S25 5.5				6
C.rubra			R25 5.0				18
C.tenuissima			S25 5.5				15
C.winogradskii							
Amylolytic species							
C.albogilva			R25 6.0				20
C.deprimata			R25 6.0				20
C.johnsonae			R25 5.5				26
C.johnsonae var. *denitrificans*							
C.succinicans			S25				
Pathogenic species							
C.psychrophila		S	S30		S	S2	S S10
C.columnaris							

Polymyxin B	Sulfadiazine	S.L.S.	Terramycin	Tetracycline	References
					42, 54, 373, 423, 424.
					378.
					378.
					378.
					42, 423, 424.
					378.
		R			42, 54, 103, 246, 423, 424.
					42, 54, 423, 424.
					42, 423, 424.
					412
					42, 119, 423, 424.
					42, 119, 423, 424.
					42, 202, 375.
					42, 375, 423, 424.
					12, 423.
S30	R30	R	S	S	296.
					42.

Table 5

Known characteristics of marine cytophagas

			Morphology	
	Habitat	G C Ratio	Width μ	Length μ
Agarolytic species				
C.diffluens Stanier emend. Lewin*	WM	40-43	.5-1.5	4-40
C.diffluens var. *aprica*	M	35.5-37	.5-1.0	15-50
C.diffluens var. *carnea*	M	37.5	.5-1.0	> 30
C.fermentans var. *agarovorans*	M		.3-.5	2-30
C.krzemieniewskae	W		.5-1.5	5-20
C.latercula	W	34.5	.5-1.0	5
C.lytica	WM	33.5-34.5	.5-1.0	10-20
C.salmonicolor var. *agarovorans*	M		.3-.5	2-30
C.sensitiva	P		.8-1.0	7-20
Non-agarolytic species				
C.fermentans	M	39	.5-.7	5->30
C.haloflava	W		.3-.45	2.5-10
C.haloflava var. *non-reductans*	W		.3-.45	2.5-10
C.marinoflava	W	37	1.0	2.2
C.rosea	W		.3-.4	2-8
C.salmonicolor	M		.3-.5	2-30

C.diffluens Stanier is cellulolytic but does not use starch

		Broth		Colony		Physiology								
						NaCl				O_2		Temperature		
Round or pointed ends	Star-shaped aggregates	Silky	Pellicle	Colour	WSP	Requires 0.5%	Minimum %	Optimum %	Maximum %	Grows aerobically	Grows anaerobically	Minimum	Optimum	Maximum
P	+		+	P-O	-	+	1.5		5.0	+	-?	15	22-30	30-40
				O	-	+	1.25		5.0	+	-?			30-35
				O	-	+	1.25		5.0	+	-?			30
R				C-Y		+	1		3	+	+		28-37	<37
PR	+	+		P	+	+	1.5		5	+	-?	15	22-25	30
				R		+	2.5		2.5	+	-			30
				Y		+	1.25		5	+	-			40
R	+			S		+	1		3	+	+		28-37	
R				O		+				+				
R				Y		+	2.5	3	3	+	+		30	<37
R				Y		+	1	3	5	+		15	20-25	30
R				Y		+	1	3	5	+		15	20-25	30
P				Y		+				+	+			30
R				P		+	>1	3	<5	+		20	25	30
R	+			S		+	1		3	+	+		28-37	<37

Table 5 - continued

Known characteristics of marine cytophagas

	Physiology continued			Sugars					
	pH			Monosaccharides					
				Hexoses					
	Minimum	Optimum	Maximum	Fructose	Galactose	Glucose	Mannose	Sorbose	
Agarolytic species									
C.diffluens Stanier emend Lewin					+	+			
C.diffluens var. *aprica*					+	+			
C.diffluens var. *carnea*					-	-			
C.fermentans var. *agarovorans*				+	+	+	+	-	
C.krzemieniewskae					+	+			
C.latercula					+	+			
C.lytica					+	+			
C.salmonicolor var. *agarovorans*				+	+	+	+	-	
C.sensitiva									
Non-agarolytic species									
C.fermentans		6.7-8.3		+	-	+	+	-	
C.haloflava		7.8		-		+w	-		
C.haloflava var. *non-reductans*		7.8		-		+w	-		
C.marinoflava					+	+			
C.rosea	7	7.2	9	-	-	+	-		
C.salmonicolor				+	+	+	+	-	

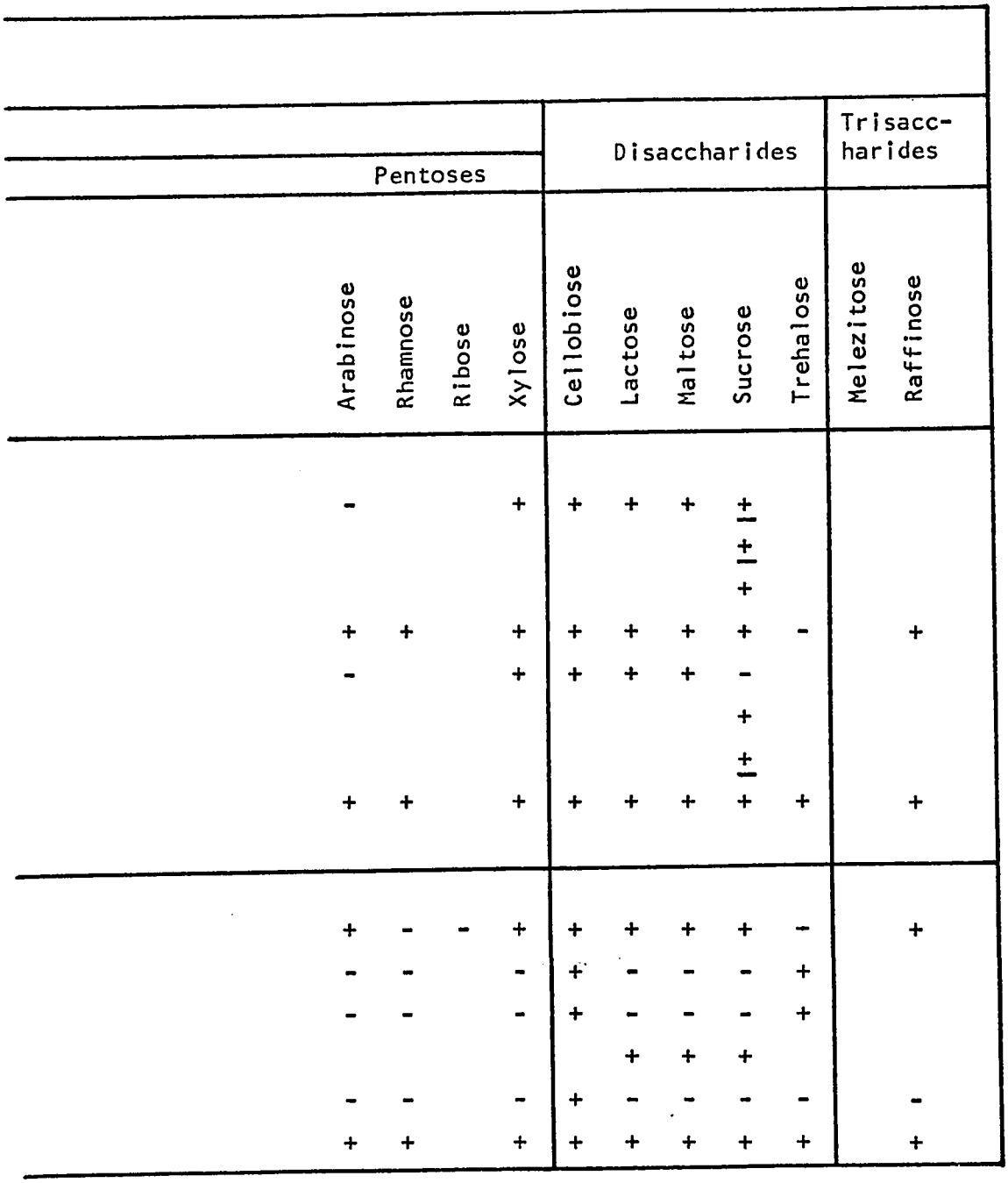


Table 5 - continued

Known characteristics of marine cytophagas

	Alcohols							
	Dulcitol	Ethanol	Erythritol	Glycerol	Inositol	Mannitol	Sorbitol	
Agarolytic species								
C.diffluens Stanier emend. Lewin				-				
C.diffluens var. *aprica*				+				
C.diffluens var. *carnea*				-				
C.fermentans var. *agarovorans*						+	+	
C.krzemieniewskae								
C.latercula				-				
C.lytica				+				
C.salmonicolor var. *agarovorans*						-	-	
C.sensitiva								
Non-agarolytic species								
C.fermentans	-	-		-		+		
C.haloflava			-	-	-	-		
C.haloflava var. *non-reductans*			-	-	-	-		
C.marinoflava						-		
C.rosea			-	-	-	-		
C.salmonicolor						-	-	

Polysaccharides													Organic acids				
Cellulose	Hemicellulose	Cellulose dextrins	Dextrin	CMC	Agar	Alginate	Chitin	Gum arabic	Inulin	Pectin	Starch	Acetate	α Keto glutarate	Aspartate	Citrate	Fumarate
-				+	+	+	-				+	+				
-				+	+	+	-				+	+				
-				+	+	+	-				-	-				
					+		-		+		+					
+					+	+	-				+					
-				+	+	+	-				-	-				
-				+	+	+	-				+	+				
					+		-		+		+					
					+											
-					G	-	-				+	-	-	-	-	-
+					-				-		-					
+					-				-		-					
-?					-										-	
+			-		-				-		-					
					-		-		+		+					

Table 5 - continued

Known characteristics of marine cytophagas

	Organic acids continued							
	Glutamate	Lactate	Malate	Malonate	Pyruvate	Succinate	Tartrate	
Agarolytic species								
C.diffluens Stanier emend. Lewin	+	-						
C.diffluens var. *aprica*	+	-						
C.diffluens var. *carnea*	+	-						
C.fermentans var. *agarovorans*								
C.krzemieniewskae								
C.latercula	+	-						
C.lytica	+	±						
C.salmonicolor var. *agarovorans*								
C.sensitiva								
Non-agarolytic species								
C.fermentans	-	-	-		-	-		
C.haloflava								
C.haloflava var. *non-reductans*								
C.marinoflava								
C.rosea								
C.salmonicolor								

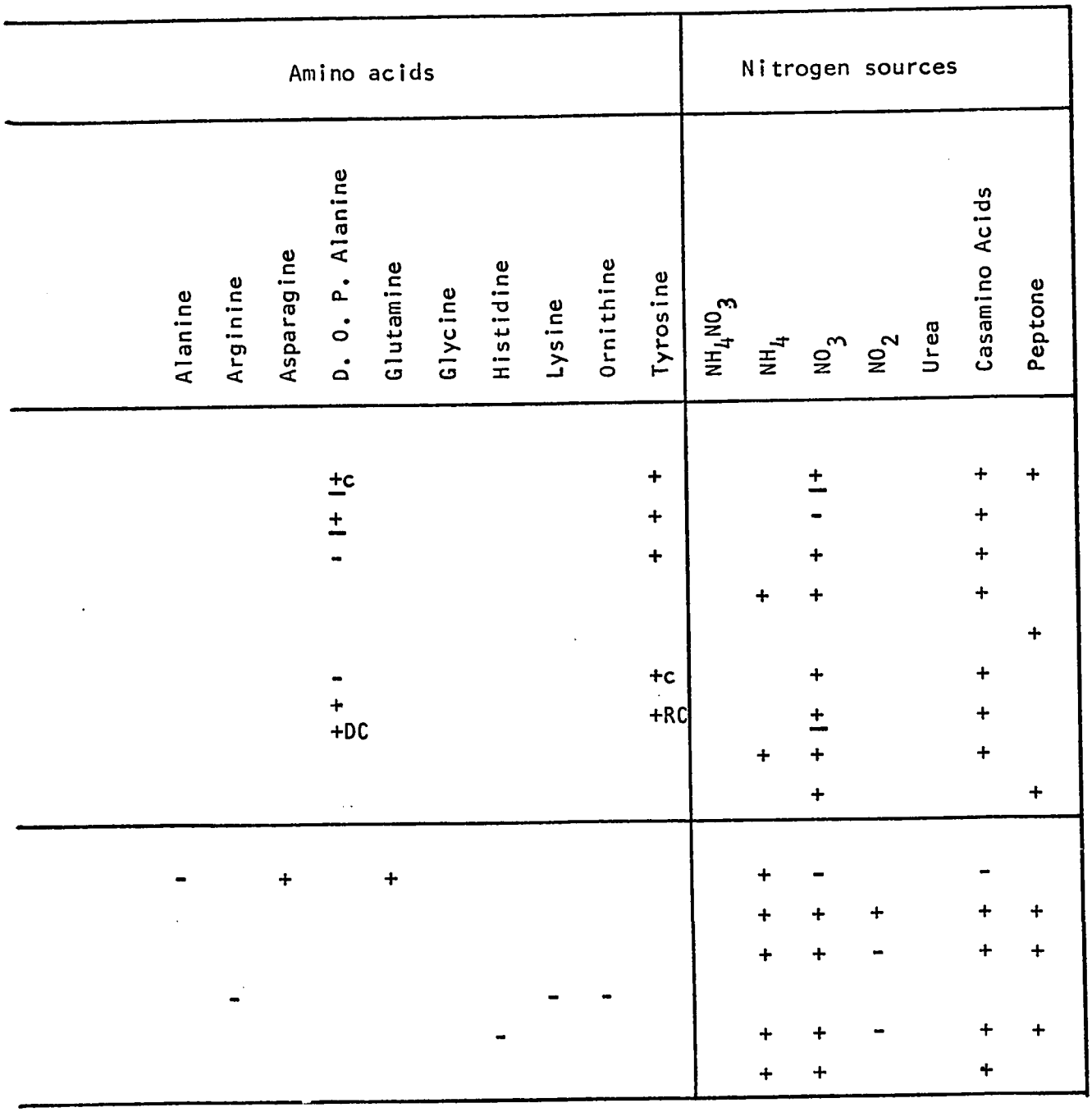

Amino acids
Nitrogen sources
Alanine
Arginine
Asparagine
D. O. P. Alanine
Glutamine
Glycine
Histidine
Lysine
Ornithine
Tyrosine
NH_4NO_3
NH_4
NO_3
NO_2
Urea
Casamino Acids
Peptone
+c
+DC
+RC

Table 5 - continued

Known characteristics of marine cytophagas

	Nitrogen sources continued				Proteolysis				
	Tryptone	Yeast extract	Nutrient Broth	Growth factors required	Albumen	Gelatin	Litmus milk	Milk lysed	
Agarolytic species									
C.diffluens Stanier emend. Lewin	+	+		-		+	CR+PA		
C.diffluens var. *aprica*	+			-		+	RA CRA		
C.diffluens var. *carnea*	+			-		+	-		
C.fermentans var. *agarovorans*		+	+	-		+s			
C.krzemieniewskae		+				+			
C.latercula	+			-		+	CRA		
C.lytica	+			-		+	CR CPR		
C.salmonicolor var. *agarovorans*		+	+	-		+s			
C.sensitiva				+		-	-	-	
Non-agarolytic species									
C.fermentans				T					
C.haloflava						-		-	
C.haloflava var. *non-reductans*						-		-	
C.marinoflava						+	-		
C.rosea						-		-	
C.salmonicolor		+	+	-		+s			

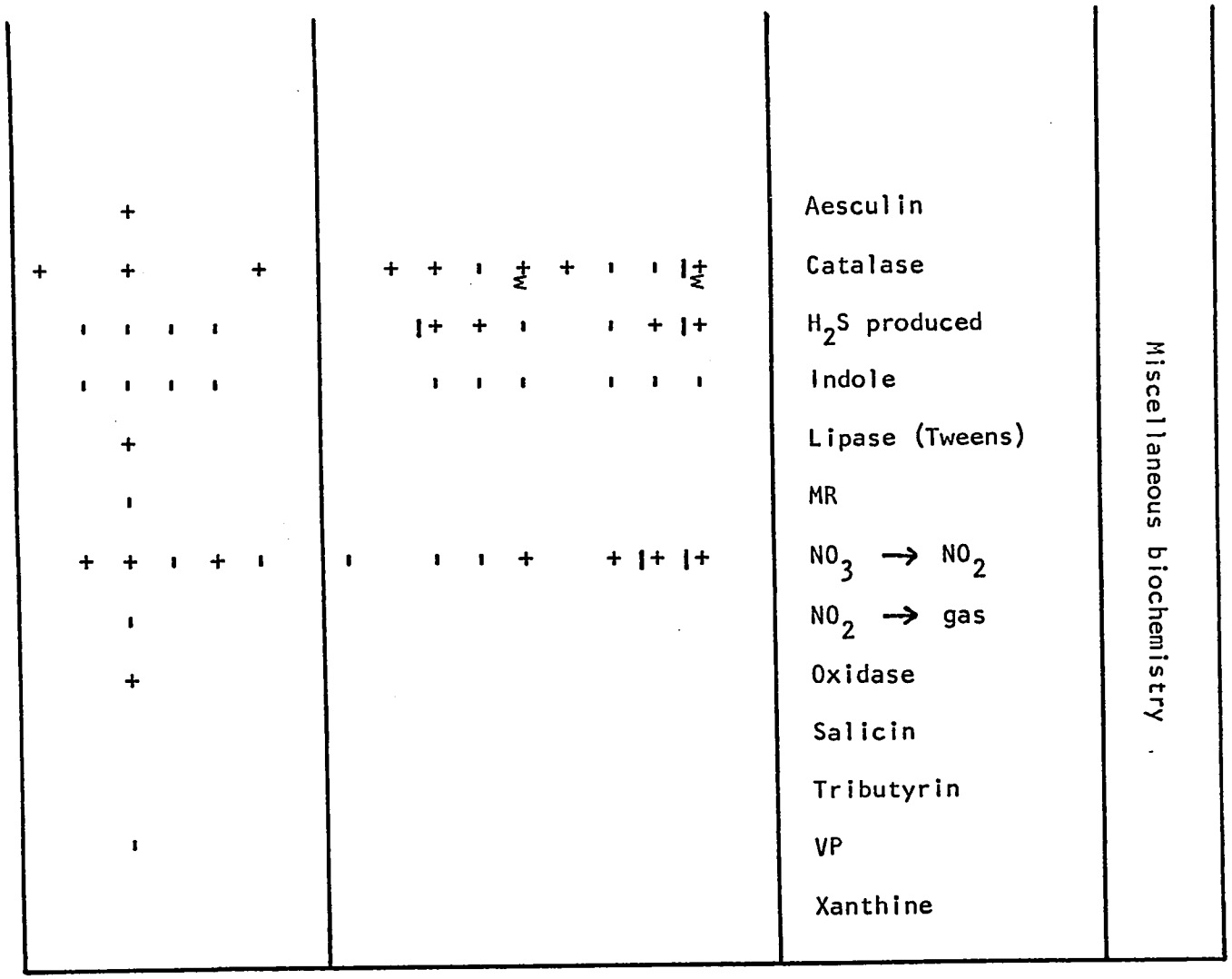

Aesculin
Catalase
H_2S produced
Indole
Lipase (Tweens)
MR
$NO_3 \rightarrow NO_2$
$NO_2 \rightarrow$ gas
Oxidase
Salicin
Tributyrin
VP
Xanthine
Miscellaneous biochemistry

Table 5 - continued

Known characteristics of marine cytophagas

	Antibiotics, etc.					
	Aureomycin	Chloromycetin	Dihydrostreptomycin	Erythromycin	Kanamycin	Novobiocin
Agarolytic species						
C.diffluens Stanier emend. Lewin						
C.diffluens var. *aprica*						
C.diffluens var. *carnea*						
C.fermentans var. *agarovorans*		R25 6.3				
C.krzemieniewskae						
C.latercula						
C.lytica						
C.salmonicolor var. *agarovorans*						
C.sensitiva						
Non-agarolytic species						
C.fermentans		R25 6.0				
C.haloflava						
C.haloflava var. *non-reductans*						
C.marinoflava	R	S30	R	S15	R	S
C.rosea						
C.salmonicolor		R25 6.7				

Penicillin	Polymyxin B	Pteridin (Vibriostat)	Pteridin discs	S.L.S.	Terramycin	Tetracycline	References
				S			42, 246, 373.
				R			246.
				S			246.
25							408, 423, 424.
							42, 373.
7				S			246.
6				S			246.
							408.
							42, 173.
29 6-7				S			14, 423, 424.
6-7				R			198.
				S			199.
R10	R	S	R		S	S	80.
							199.
27							408, 423, 424.

Table 6

A Key to the Cytophagas

Soil and freshwater forms. Not obligately halophilic.

Cellulose + Starch -. Not pathogenic.

Yellow..... *C.hutchinsonii*
(including the synonymous *C.anularis*, *C.crocea*, *C.flavicula*, *C.lutea*, *C.sylvestris*)

Orange yellow..... *C.aurantiaca*

Pink..... *C.rubra*

Olive green..... *C.tenuissima*

Cellulose -. Starch +. Not pathogenic.

Strict aerobes. Do not denitrify.

Cream..... *C.albogilva*

Orange yellow..... *C.deprimata*

Yellow..... *C.johnsonae*

Facultative anaerobes

Do not ferment carbohydrates. Denitrify.
Xylose +, Lactose -..... *C.johnsonae* var. *denitrificans*

Ferment carbohydrates. Some strains denitrify.
Xylose - . Lactose + ... *C.succinicans*

Cellulose -. Starch -. Pathogenic to fish.

Glucose oxidized. Causes columnaris disease..... *C.columnaris*

Glucose not oxidized. Causes bacterial cold water disease..... *C.psychrophila*

Marine forms. Obligately Halophilic.

Starch +.

Ferment carbohydrates. Agar + or -.

Agar -.

Yellow. Trehalose -. Mannitol + ... *C.fermentans*

Salmon pink. Trehalose +. Mannitol. ..... *C.salmonicolor*

Agar +.

Yellow. Trehalose -.
Mannitol +. ..... *C.fermentans* var. *agarovorans*

Salmon pink. Trehalose +.
Mannitol -. ..... *C.salmonicolor* var. *agarovorans*

Table 6 (continued)

A Key to the Cytophagas

Do not ferment carbohydrates. Agar +.

Cellulose +. ..... *C.krzemieniewskae*

Cellulose -.

Resistant to .01% SLS, Orange ..... *C.diffluens* Stanier emend. Lewin var. *aprica*

Sensitive to .01% SLS, Pink-Orange. Glycerol-. ..*C.diffluens* Stanier emend. Lewin

Yellow. Glycerol -. ..... *C.lytica*

Starch -.

Cellulose -. Agar +.

Orange. Denitrify. Galactose -. H_2S *C.diffluens* Stanier emend. Lewin var. *carnea*

Red. Do not denitrify. Galactose +. H_2S +. .... *C.latercula*

Cellulose +. Agar + or -.

Denitrify.

Agar +. Xylose +. Lactose +. Maltose -. Pink-Orange ..... *C.diffluens* Stanier

Agar -. Xylose -. Lactose -. Maltose -.

Pink. Trehalose -. ..... *C.rosea*

Yellow. Trehalose +. ..... *C.haloflava*

Do not denitrify. Yellow. Trehalose +. Agar -. ..... *C.haloflava* var. *non-reductans*

1.5 Species Differentiation of Cytophagas

Information about most *Cytophaga* species is not as complete as could be hoped, especially for those described earlier in the literature (Tables 4 and 5). A species key has been constructed (Table 6), although it is realized that because of the paucity of data available, this is unsatisfactory in certain respects. In 1948 Tchan, Pochon and Prévot suggested that the genus be divided into two sub-genera, those able to decompose only polysaccharides, and a polyphagous group, of unspecified yet broader nutritional capabilities (392). Several groups are now well established (275) mainly on the basis of habitat and degradation of the three ecologically significant polymers for which the most information is available, cellulose, starch and agar.

(i) Cellulolytic species from soil

This group includes the type species of the genus *C.hutchinsonii* and five probably indistinguishable variants of it (Table 6). Relatively few strains have been successfully isolated and maintained in pure culture, and none have been added in the last 40 years. Differences between the species *C.hutchinsonii*, *C.rubra*, *C.aurantiaca* and *C.tenuissima* are restricted to colour of the cell mass and cell size, their physiology and cultural properties being very similar. *C.winogradskii* seems to belong in this group, although there is too little information in the only description of it (412) to warrant formal inclusion (Table 6, 375). Starch

is not attacked, differentiating this group from two described species *C.albogilva* and *C.deprimata* which reputedly had weak cellulolytic activity when first isolated (120). There is no growth on ordinary nutrient media and when cellulose agar plates are used for culture the agar concentration must be kept to a minimum (about 1%). An interesting feature of the group is their apparent failure to utilize soluble carbohydrates, subsequently found to be due to the toxicity of heat sterilized solutions and the sensitivity of some strains to high concentrations of soluble carbohydrates (348).

(ii) Soil and freshwater amylolytic species

The major species in this non-cellulolytic group is *C.johnsonae* characterized by utilization of chitin. Two species, *C.albogilva* and *C.deprimata*, were described by Fuller and Norman (120) but authentic cultures are not available. The differences between them are minor (colour and slight difference in cell size range), and it is impossible to tell to what extent they overlap with *C.johnsonae* since they were not originally examined for chitin degradation (275). A facultative anaerobe was added to this group in 1961 (12), *C.succinicans* ferments carbohydrates, has weak chitinoclastic ability and has different sugar reactions from *C.johnsonae* (Tables 4 and 6).

(iii) Fish pathogens

Two very similar organisms which degrade neither cellulose nor starch are included here, *C.psychrophila* and the organism

presently known as *Chondrococcus columnaris* but which most probably is a *Cytophaga* (page 17). *C.columnaris* causes lesions of exterior and interior fish tissues and oxidizes glucose, whereas *C.psychrophila* causes dermal lesions, does not utilize glucose and has a lower optimum temperature (11, Tables 4 and 6).

(iv) Obligately halophilic marine forms

Two actively agarolytic *Cytophaga* spp., *C.krzemieniewskae* and *C.diffluens*, were described by Stanier (370). They both attack cellulose and alginate but differ by the production of a brown soluble pigment and attack of starch by the former. In spite of the lack of the type cultures of these species their original descriptions are probably adequate to allow their future re-identification (275). It is more doubtful whether the description of the agarolytic *C.sensitiva* (173) is sufficient in the absence of an authentic culture (375). The first non-agarolytic marine forms to be isolated, *C.haloflava* and *C.rosea* (198, 199) are often overlooked yet their descriptions are reasonably detailed. The two species differ in colour and growth in trehalose. A rather confusing redefinition of *C.diffluens* Stanier has recently been suggested by Lewin (245 and page 23) in which carboxy methyl cellulose (CMC) degradation is substituted for cellulose digestion, and ability to attack starch is added. Both *C.diffluens* Stanier and *C.diffluens* Stanier emend. Lewin have been included in the key (Table 6). Two varieties which do not utilize CMC are added, one of them does not use starch either. Lewin has also described two new species, *C.lytica* and *C.latercula* which are

very similar to his emended *C.diffluens* (Tables 5 and 6).

C.marinoflava (80), a halophile which will grow very slowly on non-saline media, can grow anaerobically if nitrate is provided, and digests neither agar nor cellulose has not been included in the key.

(v) Human pathogens

There are indications that a fifth group of cytophagas occur as pathogens in homoiotherms, since Gräf (133) isolated what he thought were two species of a new genus *Sphaerocytophaga*. These have since been identified as *Cytophaga* spp. (page 21), but nevertheless, the fact that they were found in the human oral cavity and seem to cause Vincent's angina (345) lends them considerable interest. In addition the etiological agent of meningitis of the newborn, *Flavobacterium meningosepticum*, is suspected of more properly belonging to the genus *Cytophaga* (275).

1.6 Taxonomy of *Sporocytophaga*

Separation of *Sporocytophaga* from *Cytophaga* rests on the demonstration of microcysts, and this is a labile feature (97, 180). The only well recognized species is the cellulolytic *S.myxococcoides*, which in culture appears otherwise identical with the classical cellulolytic *Cytophaga* group (*C.hutchinsonii* etc.). The microcysts contrast sharply in shape with the vegetative cells and are abundantly formed on filter paper media (164). The true fruiting myxobacteria share a number of features with both *Cytophaga* and

Sporocytophaga and Stanier (373) proposed classifying *Sporocytophaga* with the myxococci rather than with *Cytophaga*, and this separation has continued (375). The evidence of DNA base ratios has now provided strong reason for revising this position and placing *Sporocytophaga* in the Cytophagaceae (Tables 2 and 3). In a taxonomical analysis of 40 strains of 14 species belonging to all five families of the Myxobacterales (271) six groups of fruiters were recognized at 94%S and a clear distinction between fruiting and non-fruiting myxobacters (56%S) was again confirmed. In addition studies of the cell wall composition of *C.hutchinsonii* and the two forms of *S.myxococcoides* (410, 411) have shown that all are essentially similar. It has even been suggested that the microcyst stage of *C.myxococcoides* is merely a multilayered structure of the same composition as the vegetative cells (275). The present consensus of opinion (97, 275, 309, 316, 368) seems to favour the inclusion of *Sporocytophaga* in the same family as *Cytophaga*, and not in the Myxococcaceae as in Bergey's Manual (375). Lewin's proposal that all the cytophagas be included together with the other gliding bacteria in the Cytophagaceae, Order Flexibacterales, still leaves *Sporocytophaga* in a doubtful position. It is the only microcystogenous genus and has obvious links with the fruiting myxobacteria in the separate Order Myxobacterales.

An interesting postscript to the discussion of the systematics of the Myxobacterales is the observation (33) of the tendency of most groups of living creatures to evolve from a

condition adapted to an aquatic existence to a terrestrial form. This is especially noticeable in the reproductive and distributive structures and is clearly discernible in the Myxobacterales. The morphologically unspecialized cytophagas are marine and freshwater organisms and are also found abundantly in sloughs and soils, especially wet ones (70). Sporocytophagas likewise are soil and water organisms, but the "higher" fruiting myxobacters are regularly found only in soil and dryer habitats such as dung, rotting wood and tree trunks. There is no report of an aquatic, marine, fruiting myxobacter (310, 379), although myxococci have been isolated from semi-marine beach sand (50). Occasionally fruiting myxobacteria are found in freshwater, but in such small numbers (51) that it is considered that their resistant forms have been washed there fortuitously (310). The myxobacteria are well adapted to a terrestrial existence (33, 132) by their creeping type of motility which requires a mechanical support. The highest evolutionary development in this group can be seen where the microcysts are grouped into fruiting bodies, often of very elaborate design. In the 'transitional' genus *Sporocytophaga* the microcysts, although very resistant to drying and inanition, are formed and distributed singly. It is possible, therefore, that they may be the ancestors of the fruiting myxobacteria.

CHAPTER II

THE RELATIONSHIPS OF THE CYTOPHAGAS WITH THE FLAVOBACTERIA AND THE FLEXIBACTERIA

II.1 Introduction

The genus *Cytophaga* commenced as a somewhat natural taxon and became much less finite as strains which shared one or more attributes with the classical group were added, and as cytophagal properties were studied in more detail. The *Flavobacteria* share with the cytophagas several characteristics; they are pigmented (often yellow), aerobic, rod-shaped, nonmotile and Gram negative. *Flavobacterium*, however, cannot be defended as a natural taxon as it has traditionally been a collecting heap for bacteria with the above characteristics which could not readily be assigned to other genera. The resulting generic heterogeneity is due partially to unintentional exclusion of some strains from *Cytophaga* (427). The cytophagas also seem to blend taxonomically with other gliding organisms. Physiology apart, there is little to distinguish the members of the genus *Cytophaga* from certain unicellular, rod-shaped blue-green algae (375). In recent years many new, colourless, gliding organisms have been discovered, the new genus *Flexibacter* (364, 365, 366, 367) being an example, and their relationships with both the photosynthetic Cyanophytes and the cytophagas will have to be clarified. By long tradition colourless organisms such as the

Beggiatoaceae, which show very close morphological relationships to specific genera of blue-green algae, have been treated taxonomically as "bacteria" (377). Thus not only are the relationships of cytophagas with the fruiting myxobacteria in question, but two other boundaries have to be established, differentiating *Cytophaga* from *Flavobacterium* on the one hand and from the many different forms of flexing bacteria on the other.

II.2 The Flavobacteria

The genus *Flavobacterium*, which is now placed in the Family Achromobacteraceae of the Order Eubacteriales has existed since 1923 (30), when it was accompanied by a description allowing virtually any yellow-pigmented rod to be ascribed to it, regardless of the extent to which other characteristics might suggest a relationship to better defined but normally unpigmented groups. Pigmentation and an aerobic nature continued to distinguish *Flavobacterium* in successive editions of Bergey's Manual even though the original rationale to separate chromogenic bacteria into a common hierarchy was no longer used. The concept of *Flavobacterium* was not changed with each new edition of Bergey's Manual except to exclude polar flagellates (29) and Gram positive forms (42). It is hardly surprising that of the 133 species of *Flavobacterium* described (57, 161, 441) 44 have been subsequently assigned to quite diverse genera. Twelve polar flagellates have been reassigned to *Pseudomonas*, eight Gram positives to *Brevibacterium*, seven other forms to *Agarbacterium*, five to *Halobacterium*, two to

Bacterium, two to *Nocardia*, and one each to *Bacillus*, *Chromobacterium*, *Corynebacterium*, *Escherichia*, *Micrococcus*, *Salmonella*, *Vibrio* and *Zettnowia*.

Since the last edition of Bergey's Manual the genus *Empedobacter* has been proposed (46) to encompass nonmotile rods producing a yellow water insoluble pigment. It has been suggested that 19 of the 89 presently recognized *Flavobacteria* be transferred to *Empedobacter* (46, 47, 48, 200, 315, 402), although these moves have been questioned (164). In addition proposals have been made to reassign one species of *Flavobacterium* to *Beneckea* (66) and one more to *Pseudomonas* (58).

The type species of the genus is *Flavobacterium aquatile* (Frankland and Frankland 1889) Bergey et al. 1923 which was originally described as *Bacillus aquatilis* (117). There is no authentic culture extant, and Taylor's 1941 reisolate F36 from the same deep chalk wells in Kent that were studied by the Franklands does not conform exactly to the original description. Strain Taylor F36 is said to be motile and peritrichate (30), whereas the Franklands described *B.aquatilis* as nonmotile and showing slow oscillatory movement. In 1939 *F.aquatile* was deprived of its flagella to conform with the original description (29). The problem is made more complex by the fact that this supposed reisolate of the type species has since proved to be a *Cytophaga* (82, 115, 426). No formal proposal of the generic name change from *F.aquatile* to *Cytophaga aquatilis* has yet been made, but there is however another,

complicating, formal proposal to reassign the species as *Empedobacter aquatile* (48). The organism has a DNA base ratio within the *Cytophaga* range and its growth characteristics easily allow its inclusion in this genus (Table 7). Three other *Flavobacteria* which have *Cytophaga*-like base ratios and characteristics (Table 7), have been cited as probable cytophagas (275) but again, formal proposals have not yet been made. These species are *F.heparinum, F.meningosepticum* and *F.pectinovorum* (also proposed as *Empedobacter pectinovorum* (200)), which would become *C.heparina, C.meningoseptica* and *C.pectinovora* respectively. One organism originally described as a *Flavobacterium* sp., NCMB 397 (369), has since been reassigned as *Cytophaga psychrophila* (80).

Several other species of *Flavobacterium* have been challenged but not formally assigned elsewhere. *F.acidificum*, for example, may be a strain of *Erwinia herbicola* (275). *F.flavescens, F.suaveolans, F.arborescens* and *F.esteroaromaticum* have been found to be Gram positive (275), and hence cannot remain in the revised genus (42). The latter two species have been proposed as *Empedobacter arborescens* (315) and *E.esteroaromaticum* (402) respectively. The taxonomic affinities of four others whose current position as *Flavobacteria* has been questioned viz: *F.aurantiacum* (398), *F.buchneri, F.capsulatum* and *F.odoratum* (275) have yet to be determined.

Table 7

Characteristics of selected *Flavobacterium* species

Species	Cultures available * = type culture	Habitat	% GC
F.aquatile	Taylor F 36* (ATCC 11947, NCIB 8694) ATCC 8375 (NCIB 8535) ATCC 9758	Freshwater high in $CaCO_3$	32-34
F.heparinum	ATCC 13125* (NCIB 9290)	Soil	42.2
F.meningosepticum	ATCC 13253* (NCTC 10016) ATCC 13254 ATCC 13255 ATCC 19248	Infant throat and spinal fluid	36.4 36.4 38.3
F.pectinovorum	NCIB 9059* (ATCC 19366)	Soil	32.7 32.9

* Cb. = coccobacilli, Colours BY = brownish yellow, DY = dark yellow, GW = grey-white, LY = light yellow, O = orange, Y = yellow.

Morphology									Physiology			
									NaCl Growth in		O_2	
Length μ	Width μ	Shape*	Rounded ends	Capsule	Gram	Swarming	Motility	Colour*	1%	3%	Aerobic	Anaerobic
1.0-40	.5-.7	Cb. to rods			±		±	Y, BY or O	±	±	+	±
1	.3-.4				-		-	LY to DY			+	+
		Rods	+	±	-	-	-	GW to Y			+	-
3	.5	Rods	+		-	-	-	Pale Y	+	-	+	-

Table 7 - continued

Characteristics of selected *Flavobacterium* species

	Physiology continued						Sugars				
							Monosaccharides				
	Temperature 0°C			pH			Hexoses				
	Minimum	Optimum	Maximum	Minimum	Optimum	Maximum	Fructose	Galactose	Glucose	Mannose	
F.aquatile	10		30-37	6.5	7.2-7.4	7.8	+	+	±	+	
F.heparinum	>0	24	<37		6.5-7.0				+		
F.meningosepticum	25	37	42				+	-	+	+	
F.pectinovorum		25-30	<37						+		

	Pentoses			Di- and Tri-saccharides						Alcohols					Poly-saccharides		
	Arabinose	Rhamnose	Xylose	Cellobiose	Lactose	Maltose	Sucrose	Trehalose	Raffinose	Adonitol	Dulcitol	Ethanol	Glycerol	Mannitol	Dextrin	Heparin	Inulin
	±		±	+	+	±	+		+		-	-	±	-	-		
																+	
	-	-	-		+	+	-	+	-	-	-			+			
	±		±		+	+	+						±				+

Table 7 - continued

Characteristics of selected *Flavobacterium* species

	Polysaccharides continued		N sources			Proteolysis			Miscellaneous			
	Pectin	Starch	NH_4	Urea	Peptone	Gelatin liq.	Litmus milk*	Haemolysis	Arginine → NH_4	Catalase	Citrate	Cysteine → H_2S
F.aquatile		-		-	+	I+	+ R P	I+	-	+	-	+
F.heparinum			+									
F.meningosepticum		-		-		+	+ P	-		+	+	
F.pectinovorum	+	+	+	poor, - -	-	+	+ C A	-	-	+ weak	-	-

* + = growth, A = acidification, C = clotting, P = proteolysis (redigestion of curd), R = reduction of dye.

biochemistry										References
Indole	MB reduced	MR	$NO_3 \rightarrow NO_2$	Oxidase	Prototrophic	Salicin	Tributyrin	TSI	VP	
±+	-	-	-		-	-	±+		-	42, 96.
										275, 305.
+		-	-	++		-		H_2S on paper above. Alk.	-	213.
-	-	-	+			±+	+		-	96.

Differentiation of *Flavobacterium* from *Cytophaga* relies at present on the demonstration of swarming on solid media or flexing and gliding movement by the latter genus (162). However it is now recognized that swarming need not be restricted to *Cytophaga* (100, 162). The apparent association of spreading growth with the group of organisms designated Pleiston A by Floodgate and Hayes (113) led those authors to regard this group as consisting of *Cytophaga* strains. Subsequent examination of some of them has shown their GC ratios to be about 61% (93), whilst the group which Floodgate and Hayes considered to be *Flavobacterium* (Pleiston G) can now be identified as *Cytophaga*. Other differentiating characteristics will have to be found, and this depends on more specific definitions of both genera concerned. Pragmatic authors advocate that every effort be made to assign an unknown nonmotile, Gram negative, yellow-pigmented, aerobic rod to a well-defined genus before relegating it to *Flavobacterium*.

At the present time then, there is no adequate means of defining the genus *Flavobacterium*. For convenience, two subgroups may be considered (164).

(a) Nonmotile rods, not identifiable as *Cytophaga*, Gram negative, oxidative or not attacking sugars. Pleiston A of Floodgate and Hayes (113) may belong here although some strains appear to be Gram variable and they also appear highly sensitive to penicillin.

(b) Gram negative rods, motile by usually few peritrichous

flagella, oxidative in sugars. The relationship of these strains to genera such as *Agrobacterium* or *Rhizobium* needs investigation, especially since one species of yellow-pigmented *Agrobacterium* is known *(A.gypsophilae)* (164).

Hayes would restrict *Flavobacterium* to nonmotile forms and considered that the few peritrichous forms were sufficiently different from the nonmotile nonswarming ones to merit a separate genus. Brisou (315) has also separated flagellated flavobacteria from nonmotile forms but he would reserve the genus for motile species and place the nonmotile ones in a new genus *Empedobacter*. Hendrie, Mitchell and Shewan (275) are of the opinion however, that most of the strains bearing this name which they have examined could be assigned to better defined genera. Thus further work will be required before this nomenclature can be accepted. Neither Hayes nor Brisou have reported nucleotide base ratio analyses for their cultures: were these values known confusion still might not be removed. As an illustration of this, while typical *Cytophagae* have characteristically low GC mole values there is at least one seemingly typical *Cytophaga* sp. NCIB 9497 (279) which has a higher value, 73.2% GC (275). The few reported DNA base ratios of *Flavobacteria* not identified as *Cytophaga* (93, 257, 275) seem to be only of these species of questionable taxonomic affinities and show a wide range (34-71% GC, Table 8) obviously indicative of a heterogeneous group of organisms.

Table 8

DNA base ratios of some Flavobacteria

Species	Strain	GC %	Source of data
F.acidificum		48-50	257
F.arborescens		66-68	257
F.buchneri	A-1	42.2	275
F.capsulatum	NCIB 9890	63	275
F.esteroaromaticum		68-70	257
F.flavescens		66-68	257
F.odoratum		34-36	257
F.suaveolans	ATCC 958	64.5	82
F.suaveolans		66-68	257
F.vitarumen	ATCC 10234	63.3	82
Flavobacterium sp	NCIB 9491	51.2	275
Flavobacterium sp	NCIB 9942	64.4	275
Flavobacterium sp	NCIB 9776	66.9	275
Chitin digester	NCIB 8501	71	275
Unnamed	NCMB 296	57.3	275
Unnamed (Pleiston A)	NCMB 244	62.9	93
Unnamed (Pleiston A)	NCMB 259	63.7	93

It may well be that on closer examination of the remaining *Flavobacterium* species, that there is no necessity for this "regrettable genus" (374) after all.

11.3 The Flexibacteria

Unbranched filamentous microbes capable of slow gliding movements on solid substrata are common in nature, but have been

generally neglected by microbiologists, in part because most routine culture media contain concentrations of organic nutrients inhibitory to their growth There are certainly hundreds of distinct species, nevertheless their taxonomy presents problems that are only just being understood. Three groups have been generally recognized (368):

(i) Those which are clearly blue-green are assigned without question to the Cyanophyta; so are those such as *Phormidium persicinum* which have a pink or lilac colour due to an excess of phycoerythrin over chlorophyll. All such pigmented forms are presumed to be capable of liberating oxygen by photosynthesis, although this has only been established in a few cases. In this respect they resemble the eucaryotic algae and differ from the photosynthetic bacteria. It is noteworthy that there are quite a few apochlorotic gliding organisms which bear a strong morphological resemblance to certain genera of Cyanophytes.

(ii) Those filamentous forms which lack pigmentation but whose cells contain recognizable globules of sulphur (though the chemical nature of these globules is more usually presumed than established), are mostly assigned to the genera *Beggiatoa* and *Thioplaca*. Some authors have included them on physiological grounds with the heterogeneous "sulphur bacteria" in the Beggiatoaceae, while others have preferred to classify them on morphological criteria among the Oscillatoriaceae (Cyanophyta).

(iii) Those shorter apochlorotic forms with flexuous cells of which some at least secrete extracellular polysaccharases are usually

recognized as members of the genus *Cytophaga* or of other genera within the Myxobacterales.

In addition there remains an assemblage of heterogeneous forms for most of which the taxonomic position has either been ignored altogether, or selected more by subjective than by objective criteria. Members of this assemblage have been variously assigned to one of the foregoing groups, or to new orders and classes, with a tendency to disregard earlier publications on the subject. This state of disorganization may be due to the scattered nature of the literature, species having been described from almost every conceivable habitat by many types of biologists, and also to the lack of pure culture studies to determine physiological and biochemical facts. The situation is now improving, thanks largely to the pioneering efforts of Pringsheim (317), who has been concerned with the phylogenetic relationships between bacteria and blue-green algae. Recently an organism with properties akin to flexibacteria, blue-green algae and to the green sulfur bacteria has been isolated from hot springs (311). This organism is morphologically similar to the apochlorophyllous flexibacteria, its gliding movement resembles that of flexibacteria and blue-green algae, and yet it contains two bacteriochlorophylls found previously in the green sulphur bacteria. Such organisms will be of value in establishing the relationships between the photosynthetic bacteria and gliding filamentous prokaryotes.

Buchanan (55) set off the sulphur-containing filamentous forms, together with certain others, with a new ordinal name, the

Beggiatoales. This order would comprise the three trichome-forming Families; the Beggiatoaceae(genera *Beggiatoa, Thiospirillopsis, Thioplaca* and *Thiothrix*) whose cells contain sulphur granules when growing in the presence of hydrogen sulphide, the Vitreoscillaceae (genera *Vitreoscilla, Bactoscilla* and *Microscilla*) which do not contain sulphur granules, and the Leucotrichaceae (genus *Leucothrix*) whose nonmotile trichomes may have exterior sulphur particles, as well as the single-celled family Achromatiaceae (genus *Achromatium*).

A taxonomic milestone was reached when Soriano proposed that the Myxobacterales be restricted to forms which possess fruiting bodies (364, 365, 366, 367). The non-fruiting species (*Cytophaga* and *Sporocytophaga*) together with the Beggiatoaceae he classified in the new Order Flexibacterales which comprised the Families Cytophagaceae, Flexibacteraceae and Beggiatoaceae. Soriano (366) separated the flexible-celled organisms of the Orders Flexibacterales and Myxobacterales in the Subclass Flexibacteria from the rigid-celled Subclass Eubacteria, as was originally advocated by Breed, Murray and Hitchens (40). Tchan, Pochon and Prévot (392) concurred with the creation of the new genus *Flexibacter* but they preferred to group the three genera *Cytophaga, Sporocytophaga* and *Flexibacter* in a "lower" suborder Asporangiales of the Myxobacterales, because they considered them strongly related to the higher myxobacteria. Soriano's scheme has been thoughtfully amended (368) and reamended (245) until in the most recent proposal the majority of Buchanan's Order Beggiatoales is now included together with *Cytophaga,*

Sporocytophaga, *Saprospira* (Gross 1910) emend. Lewin 1962 (formerly misplaced in the Spirochaetales), *Herpetosiphon* Holt and Lewin 1968, *Flexibacter* Soriano 1945, and *Simonsiella* Simons 1922 (formerly included in the Caryophanales) in the Order Flexibacterales. The classification suggested (245, 368) is as follows:

	Main Characters
Division: Schizophyta (Procaryota)	No nuclear membrane, etc.
Class: Cyanophyta (Schizophyceae)	Photosynthetic, using chlorophyll *a* and evolving oxygen.
Class: Bacteria (Schizomycetes)	Generally not photosynthetic; if so, do not evolve oxygen.
Subclass: Eubacteria	Non-motile, or motile with flagella.
Subclass: Flexibacteria	Motile by gliding; none flagellated.
Order: Myxobacterales (excluding *Cytophaga*)	Fruiting bodies.
Order: Flexibacterales	No fruiting bodies.
Family: Beggiatoaceae	Cylindrical; with sulphur granules.
Family: Leucotrichaceae	Tapering
Family: Simonsiellaceae	Flattened.
Family: Vitreoscillaceae (excluding *Microscilla*)	Filaments constricted at nodes.
Family: Cytophagaceae (or Flexibacteraceae)	Cylindrical; with carotenoids.
Genus: *Saprospira*	Helical.
Genus: *Flexithrix*	Branched.
Genus: *Herpetosiphon*	Sheathed.
Genus: *Sporocytophaga*	Microcysts formed.
Genus: *Cytophaga*	Mostly <20μ, polysaccharolytic.
Genus: *Flexibacter*	Lengths various: mostly from freshwater or mud (see Table 9).
Genus: *Microscilla*	Mostly >20μ, marine

Note: Lewin (245) includes *'Sphaerocytophaga'* in the Family Cytophagaceae but this seems now to be an unnecessary genus (see discussion page 21).

These ideas were discussed but not accepted by Stanier (375), although he notes that if 'cytophagas' are white and albuminous, "They may belong to Soriano's genus *Flexibacter*." More recently Soriano and Lewin's treatment of the systematics of gliding microbes has been accepted by several authors (77, 303, 304, 349, 350). Prévot and Fredette also group *Flexibacter*, *Cytophaga* and *Sporocytophaga* together in the Cytophagaceae but keep this family in the Myxobacterales (316). In a numerical analysis of Lewin's strains Colwell (77) has reported that the organisms of the genus *Flexibacter* as well as some of those identified as *Cytophaga* and *Microscilla* form a broad group with high phenotypic similarity, which she felt could not be subdivided on the basis of the existing data.

It is apparent that the four genera *Saprospira*, *Flexithrix*, *Herpetosiphon* and *Sporocytophaga* are fairly easily distinguished by their morphology. There remains the *Cytophaga + Flexibacter + Microscilla* complex. Stanier (370) defined *Cytophaga* as cellulolytic and Soriano originally differentiated *Flexibacter* from *Cytophaga* by the lack of cellulolytic ability (Table 9). Since that time a variety of *Cytophaga* species of increasingly diverse morphological and physiological characteristics have been described. The limits of this genus have become inconveniently diffuse, and new definitions are required for both *Flexibacter* and *Cytophaga* as a consequence.

Table 9. Explanatory notes

SPECIES	Type species is *F.flexilis*. *F.aurantiacus* is composed of one strain each of *Cytophaga aurantiaca* and *C.psychrophila*. *F.litoralis* Lewin 1969 is synonymous with *F.litorale* (sic) Lewin 1963 which was not validly published. *F.ruber* Lewin 1969 is synonymous with *F.rubrum* (sic) Lewin 1963 which was not validly published. *F.marinum* was also described by Lewin in 1963, it is now known as *Microscilla marina*.
HABITAT	F = freshwater, Ff = freshwater fish, Fs = stagnant freshwater, H = hotspring, M = marine, Mc = marine copepod, S = soil.
SHAPE	F = filaments, R = rods.
COLOUR	O = orange, P = pink, R = red, W = white, Y = yellow, YG = yellow gilt, * = dark water soluble pigment.
SINGLE AMINO ACIDS	Those tested (separately) were alanine, arginine, asparagine, aspartic acid, glutamic acid, glutamine, glycine, histidine, isoleucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan and valine.
D.O.P. ALANINE	Growth (+ or -) and degradation of dihydroxyphenyl alanine; C = clearing, D = black or grey halo.
GROWTH FACTORS REQUIRED	# = necessity depends on the temperature of growth.

Table 9. Explanatory notes continued

LITMUS MILK	+ = growth, A = acidification, C = clotting, P = proteolysis (redigestion of curd), R = reduction of dye.
TYROSINE	Growth (+ or -) and degradation of tyrosine: B = black or grey halo, C = clearing, R = red or pink halo.
ACTINOMYCIN D	* = more sensitive than *E.coli* B but not as sensitive as *Myxococcus xanthus* FB or *B.subtilis* 168.
S.L.S. .01%	R = resistant, S = sensitive.

Table 9

Characteristics of *Flexibacter* species

Species and references	Habitat	% GC	Morphology: Cells: Width μ	Morphology: Cells: Length μ
F. albuminosus[1]	Fs		.3-.4	4-10
F.aurantiacus[2]	Ff, S	31.5-32	.5-1.0	5-20
F.aurantiacus var. *copepodarum*[2]	Mc	33	.5-1.0	<5
F.aurantiacus var. *excathedrus*[2]	F	34.5	.5-1.0	<10
F.aureus[1]	Fs		.3-.4	3-5
F.elegans[1,2]	Fs, H	47.5	.4-1.0	20-50
F.flexilis (type species)[1,2]	F, Fs, H	40.5-43	.5-1.0	10-50
F.flexilis var. *iolanthae*[2]	?	41.3	.5-1.0	10-30
F.flexilis var. *pelliculosus*[2]	F	39.5	.5-1.0	10-30
F.giganteus[1,2]	F, Fs	32	.7-1.0	100
F.litoralis[2,4]	M, Fs	31	.5-1.0	180
F.roseolus[2]	H	34-5-38	.5-1.0	50
F.ruber[2]	H	37	.5-1.0	50
F.sancti[2]	S,?	46-47	.5-1.0	5-50
Flexibacter sp. FS-1[3]	S	48.7	.7	10-500

[1] Soriano 1945 a, b, c, 1947 (364, 365, 366, 367).

[2] Lewin and Lounsbery 1969 (246).

[3] Simon and White 1971 (350).

[4] Fox and Lewin 1963 (116).

							Physiology										
				Colony			NaCl			O_2		Temp. °C		
	Shape	Rounded ends	Slime layer	Flexing	Colour	Opacity	Fringe	Requires 0.5%	Minimum %	Maximum %	Aerobic	Anaerobic	Minimum	Optimum	Maximum
	R			±	W*	+	±								
	RF			+	YO			-	0	2.5	+	-		30	
	R			+	Y			+	2.5	2.5	+	-		30	
	R			+	Y			-	0	0	+	-		40	
	R			±	YG,W,O		±								
	RF	+		++	O,Y		?+	-	0	2.5	+	-		40	
	RF	+	+	+	O		++	-	0	0	+	-		40	
	F			+	O			-	0	0	+	-		35	
	F			+	O			-	0	0	+	-		35	
	F			++	P,OR		++	-	0	0	+	-		35	
	F			+	R, P			+	1.25	5	+	-		30	
	F			+	R			-	0	5	+	-		40	
	F			+	R			-	0	0	+	-		40	
	RF			+	Y			-	0	0	+	-		35	
	F	R	+	++	O	-	+	-			+	-	<25	30-35	38

Table 9 - continued

Characteristics of *Flexibacter* species

	Physiology continued	Sugars					
	pH						
	Opt. pH	Fructose	Galactose	Glucose	Mannose	Ribose	Sucrose
F.albuminosus							
F.aurantiacus			±	±			±
F.aurantiacus var. *copepodarum*			-	-			-
F.aurantiacus var. *excathedrus*			-	-			-
F.aureus							
F.elegans			-	-			-
F.flexilis (type species)			±	±			+
F.flexilis var. *iolanthae*			-	-			-
F.flexilis var. *pelliculosus*			+	+			+
F.giganteus			±	+			-
F.litoralis			-	-			-
F.roseolus			-	-			-
F.ruber			+	+			+
F.sancti			+	+			+
Flexibacter sp. FS-1	6.8-7.2	-	+	+	+	-	+

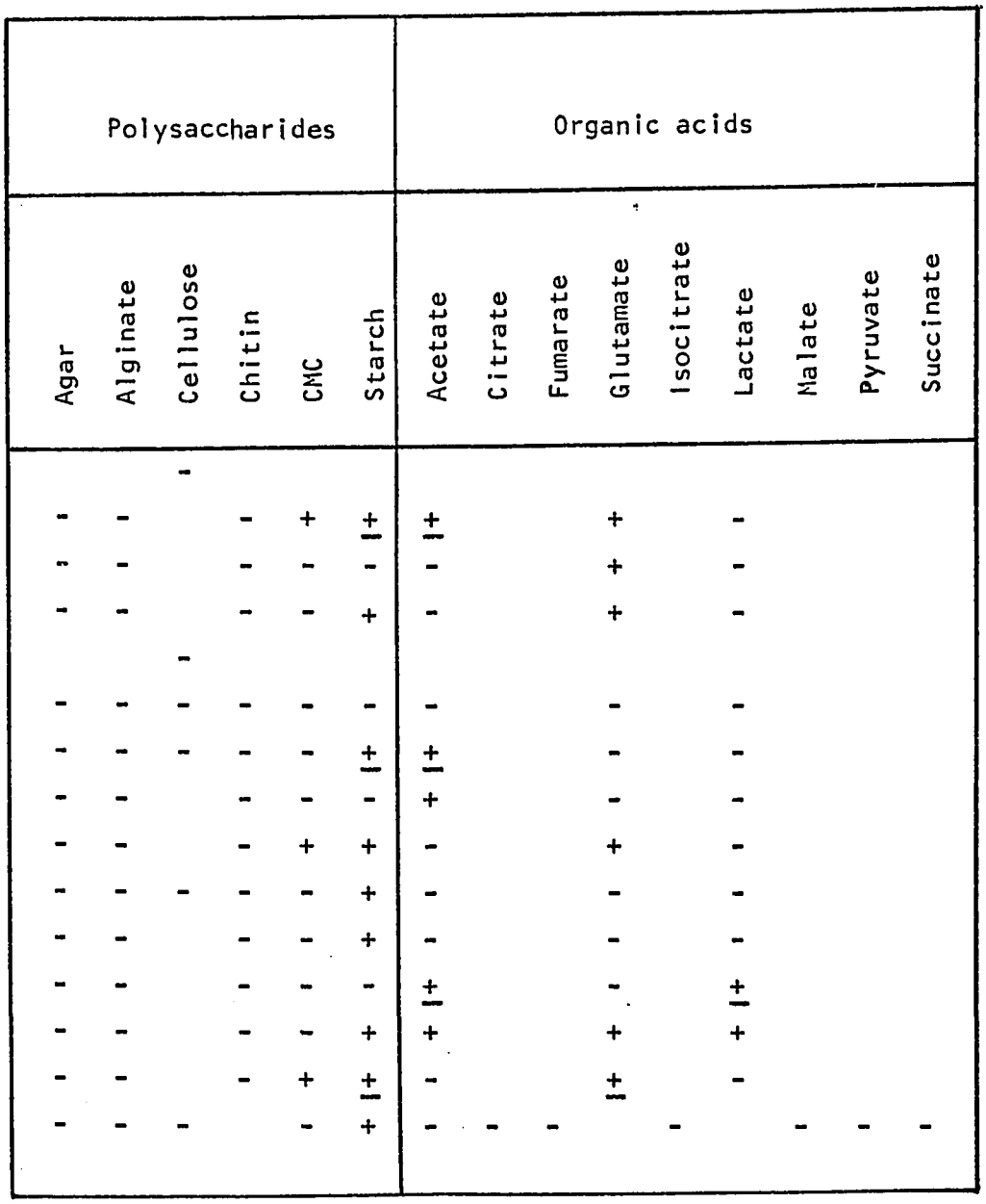

Polysaccharides
Organic acids
Agar
Alginate
Cellulose
Chitin
CMC
Starch
Acetate
Citrate
Fumarate
Glutamate
Isocitrate
Lactate
Malate
Pyruvate
Succinate

Table 9 - continued

Characteristics of *Flexibacter* species

	Nitrogen sources							
	NO_3	NH_4	Casamino acids	Peptone	Tryptone	Single amino acids	Bovine albumin	Catalase
F.albuminosus				++				
F.aurantiacus	+		+		+			+
F.aurantiacus var.*copepodarum*	-		+		+			-
F.aurantiacus var. *excathedrus*	-		+		+			-
F.aureus				+				
F.elegans	-		+	+	+			-
F.flexilis (type species)	-		+	±	+			-
F.flexilis var. *iolanthae*	-				+			-
F.flexilis var. *pelliculosus*	+		+		+			-
F.giganteus	-		+	+	+			-
F.litoralis	-		-		+			-
F.roseolus	-		+		+			-
F.ruber	+		+		+			-
F.sancti	+		+		+			-
Flexibacter sp. FS-1	+	+	+			+	+	-

Miscellaneous biochemistry										Antibiotics etc.		
D.O.P. alanine	Gelatin	Glycerol	Growth factors required	H_2S produced	Indole	Litmus milk	$NO_3 \rightarrow NO_2$	Tributyrin	Tyrosine	Actinomycin D	Penicillin	S.L.S. .01%
+C	+	-	-	-	-	+ +R	-		+C		6	R
-	+	-	-	-	-	+ CA	-		+C		6	S
+DC	+	-	-	-	-	+ CPR	-		+BC		7	S
+	+	-	-	-	-	+	-		+C		8	S
+	+	-	+	+	-	+ R	-		+		6-8	S
+	+	-	-	-	-	+	-		+		6	S
+DC	+	-	-	+	-	+ R	+		+R		6	S
-	+	-	+	±	-	+ +CP	-		±		8-9	S
-	+	-	+	-	-	+C	-		+RC		6	S
-	+	-	+	-	-	+	-		-		9	S
-	+	-	+	-	-	+	+		±		8	S
+ +DC	+	±	-	±	-	+ +R +AR	±		+C		6-7	S
	+	-	±	-	-		-	+		S		

Lewin (245) has redefined *Cytophaga* (page 28) as "usually capable of digesting (or depolymerizing) several insoluble or macromolecular colloidal polysaccharides such as cellulose (or carboxy methyl cellulose), chitin, agar, alginates etc." and he states that *Flexibacter* and *Microscilla* "have generally more limited extracellular polysaccharase activity" (Table 9). He specifies inability to attack cellulose (although curiously CMC is allowed), agar and alginate, but starch utilization is permitted. Both genera are proteolytic and may form long filaments, and thus the only means of differentiating them is on the range of polysaccharides degraded. *Microscilla* (Pringsheim 1951) emend. Lewin 1969 is very similar to *Flexibacter*, with long filaments 20 to 100μ or longer which may, however, form slender trichomes without perceptible septation. Lewin (245) has redefined these two genera as follows:

Flexibacter and *Microscilla*. Characters in common.

> "Flexible but not helical rods or filaments, usually 1μ or less in width; crosswalls not apparent (at magnifications of about x1000); not branched or sheathed; without flagella; capable of gliding on solid substrata; Gram negative; without photosynthetic pigments or intracellular sulphur granules; unable to attack cellulose or agar; reproduction by simple fragmentation; not forming fruiting bodies, spores or microcysts."

Note: None of the 12 strains of *Flexibacter*, seven of *Microscilla* and five of *Cytophaga* that Lewin tested were able to digest chitin. No marine Cytophaga has yet been found to degrade chitin (Table 5,

page 48), and all Lewin's *Cytophaga* strains were marine. Some soil Cytophagas do have this capacity however (Table 4) and would otherwise be indistinguishable from flexibacters as neither will digest cellulose, CMC, agar or alginate. It is therefore felt that 'inability to attack chitin' should be added to the definition of *Flexibacter* and *Microscilla* in order to differentiate them from certain *Cytophaga* species.

Flexibacter characters.

> "Filaments generally 5 to 50μ long; colour (seen only in packed masses) pink, red, orange or yellow; liquefying gelatin but not alginate. Habitats: mostly along freshwater banks, hot springs etc. (and some marine)".

Microscilla characters.

> "Filaments usually 20 to 100μ or longer; colour (seen only in packed masses) yellow or orange; some species liquefy alginate and gelatin. Habitat: marine shores".

The 1957 Bergey's Manual lists two species from freshwater as well. It is evident that the extreme length of the cells and the formation of trichomes, as well as its limited polysaccharase production, will differentiate *Microscilla* from *Cytophaga* and *Flexibacter* It appears that the boundary between *Cytophaga* and *Flexibacter* is not yet clearly resolved, too few characters distinguish them at present. If a non-cellulolytic variant of a *Cytophaga* is encountered, or if a laboratory test for a polysaccharase is equivocable, then this strain will be assigned to *Flexibacter*. This has already happened;

page 48), and all Lewin's *Cytophaga* strains were marine. Some soil Cytophagas do have this capacity however (Table 4) and would otherwise be indistinguishable from flexibacters as neither will digest cellulose, CMC, agar or alginate. It is therefore felt that 'inability to attack chitin' should be added to the definition of *Flexibacter* and *Microscilla* in order to differentiate them from certain *Cytophaga* species.

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Microscilla characters.

> "Filaments usually 20 to 100μ or longer; colour (seen only in packed masses) yellow or orange; some species liquefy alginate and gelatin. Habitat: marine shores".

The 1957 Bergey's Manual lists two species from freshwater as well. It is evident that the extreme length of the cells and the formation of trichomes, as well as its limited polysaccharase production, will differentiate *Microscilla* from *Cytophaga* and *Flexibacter* It appears that the boundary between *Cytophaga* and *Flexibacter* is not yet clearly resolved, too few characters distinguish them at present. If a non-cellulolytic variant of a *Cytophaga* is encountered, or if a laboratory test for a polysaccharase is equivocable, then this strain will be assigned to *Flexibacter*. This has already happened;

strains of *C.aurantiaca* and *C.psychrophila* would not degrade cigarette paper in Lewin's laboratory (246), and as other characteristics were also similar, he has combined the two into a new species *F.aurantiacus*. No other Cytophagas have yet been transferred to this genus. Ten species of *Flexibacter* and four varieties have been described (116, 245, 364, 365, 366, 367) and at least one other has been assigned to this genus without being given a specific epithet (349, 350). One of the species, *F.marinum*, has been reassigned as *Microscilla marina*.

A necessary, but not sufficient, condition for substantial genetic homology between organisms is an overall similarity in DNA base composition (257). The GC values of several flexibacteria were analysed and discussed by Mandel and Lewin (254). The % G + C for 90 cultures of flexibacteria (*Cytophaga, Flexibacter, Flexithrix, Herpetosiphon, Microscilla* and *Saprospira*) ranged from 31 to 53. The 21 strains of *Flexibacter* had values of 30 to 47% (Table 9), the lower two-thirds of this range coinciding with the 31 to 42% reported for *Cytophaga*.(253). The gliding bacteria may be regarded as apochlorotic relatives of the filamentous Cyanophyta, notably the Oscillatoriales (154, 317). Representatives of the latter Order have GC values ranging from 39 to 51% (104) which overlap the range for the filamentous flexibacteria.

CHAPTER III

A TAXONOMIC ANALYSIS OF A GROUP OF CYTOPHAGAS

III.1 Introduction

Results from a computerized analysis performed by Quadling, Cook and Colwell in the early sixties, but only published in abstract form (320), were subjected to scrutiny. They picked representative yellow-orange colonies from streptomycin (30 µg/ml) Difco Plate Count agar, each from a different soil sample. They were tested for creeping motility on Cook's Cytophaga agar (71; see Table 16 page 105) and were subjected to tests to determine attributes previously found to be characteristic of cold-tolerant arctic *Cytophaga* strains (319). Coded results were subjected to numerical analysis, following the methods of Sneath (357) and of Colwell and Liston (81).

III.2 Description of the analysis and its results

Seventy three tests (Table 10) were performed on 68 *Cytophaga*-like organisms from Canadian and Scottish agricultural soils and scored on a simple plus or minus system. A Q analysis resulted in the identification of 13 clusters and of two main groups linked at 61% S (Figure 1). The organisms 15D and 18H represented the central concept of Group I, which also included 11 other organisms within the fairly tightly knit 75% S boundary. The larger Group II comprised 33 organisms within a 70% S core, with the organisms 425, 439, 440 and 499 being the most closely linked. A subgroup of arctic isolates near

Group II, a small *(? Alcaligenes)* subgroup of Group I, and a "Myxobacter' subgroup which possibly forms an intergrade between Groups I and II and the fruiting myxobacteria were also identified (79).

All members of Groups I and II had 23 characteristics in common (Table 11), and a further 12 characteristics were shared by a similar % of isolates in each group (Table 12). The members of Group I were 100% positive for another 15 characteristics for which Group II had a variable response (Table 13); similarly there were 6 characteristics which all members of Group II shared but where the Group I response was varied (Table 14). The 13 organisms comprising Group I represented a more compact group, with a 75% S limit and 45 characteristics for which all of its members were positive, whereas the larger Group II had a wider limit of 70% and only 29 wholly positive charcteristics. The nine main differences between the two groups are shown in Table 15.

Table 10

Tests Used for the Taxonomic Analysis

Cell Morphology
- Rods long
- Rods short
- Rods of variable size
- Rods irregular
- Rods slender
- Rods curved
- Chains present
- Blebs present
- Cells flagellated and motile
- Jerking movement
- Good contrast
- Gram negative

Colony Morphology
- Scanty growth
- Visible insoluble pigment
- Other pigments
- Creeping motility on agar

Growth in Liquid Medium
- Even turbidity when shaken
- Ring in broth culture
- Pellicle in broth culture

Physiology
- Growth at pH 5.5
- Growth at pH 8.0
- Growth at 0°C good
- Growth at 30°C good
- Growth at 35°C good
- Survive 1 hr at 50°C
- Survive 1 hr at 45°C
- Survive 1 hr at 42°C
- Survive 1 hr at 35°C
- Growth in 0% NaCl
- Growth in 3% NaCl

Biochemistry of Carbohydrates
- Prototrophic (glucose + salts)
- Starch hydrolysed
- H&L glucose → acid*
- H&L lactose → acid
- H&L sucrose → acid
- H&L maltose → acid
- H&L mannitol → acid
- H&L glycerol → acid
- H&L oxidative
- B&H glucose → acid*
- B&H lactose → acid
- B&H sucrose → acid
- B&H maltose → acid
- B&H mannitol → acid
- B&H glycerol → acid

* H&L - Hugh and Leifson (172)
B&H - Board and Holding (35)

Table 10 - continued

Tests Used for the Taxonomic Analysis

Miscellaneous Biochemistry	Antibiotic and Antibacterial Sensitivities
Gelatin liquified	S.L.S. .01%*
Hydrolysis of skim milk	Streptomycin 100μ tubes
Catalase produced	Streptomycin 10μ discs
$NO_3 \longrightarrow NO_2$	Penicillin 100μ tubes
$NO_3 \longrightarrow$ gas	Penicillin 10μ tubes
Anaerobic growth with NO_3	Penicillin 10μ discs
Phosphatase at pH 6.2	Chloromycetin 30μ discs
Phosphatase at pH 8.3	Polymyxin B 30μ discs
Ornithine $\longrightarrow$ acid	Tetracycline 30μ discs
Hydrolysis of Tween 40	Novobiocin 30μ discs
Hydrolysis of Tween 60	Erythromycin 30μ discs
Hydrolysis of Tween 80	Aureomycin 30μ discs
Cysteine $\rightarrow$ H_2S	Kanamycin 30μ discs
Thiosulphate $\longrightarrow$ H_2S	Terramycin 30μ discs

* S.L.S. = Sodium lauryl sulphate

Figure 1. Groupings obtained from the taxonomic analysis

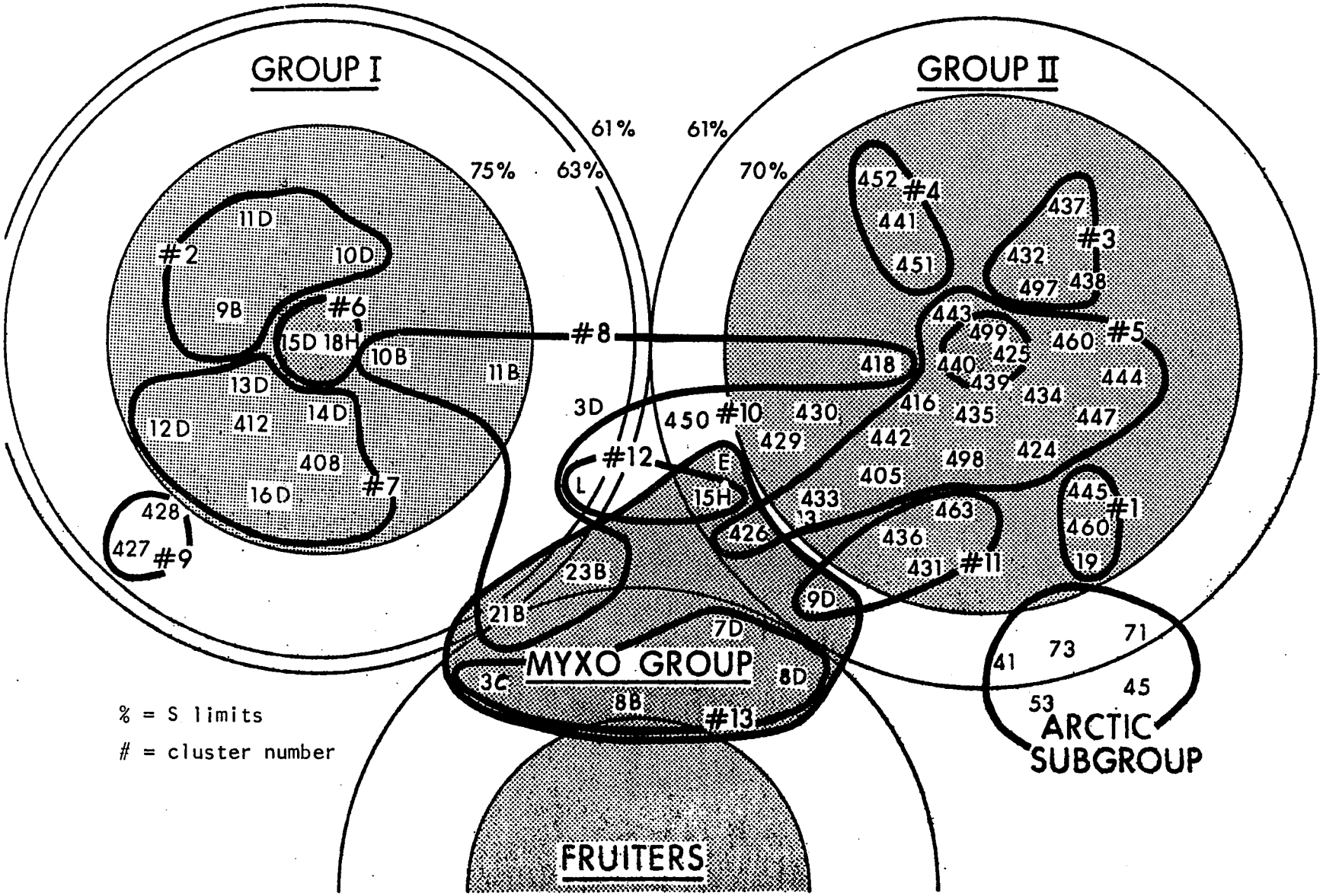


Table 11

Characters shared by all organisms of Groups I & II

Morphology	Rods not irregular
	Not in chains
	Cells not flagellate/motile
	Gram negative
	Not scanty growth
Physiology	Growth at pH 8.0
	Growth at 30°C
	Survive 1 hr 35°C
	Survive 1 hr 42°C
	Growth in 0% NaCl
Biochemistry	Not prototrophic
	Hydrolyse starch
	No acid from lactose (H & L)
	No acid from mannitol (H & L)
	No acid from glycerol (H & L)
	Not oxidative (H & L)
	Hydrolyse skim milk
	Phosphatase at pH 6.2
	Phosphatase at pH 8.3
	No acid from ornithine
Antibiotics	Resistant to Penicillin 10 μ discs
	Resistant to Erythromycin 30 μ discs
	Resistant to Kanamycin 30 μ discs

Table 12

Characters shared by a similar %
(within 10%) of organisms in Groups I & II

Expressed as % of each group positive for each character, in order of decreasing positivity.

Characters	Group I	Group II
Gelatin liquified	100	96.6
Visible insoluble pigment	100	96.55
Other pigments	100	96.52
Resistant to Streptomycin 10μ discs	100	96.5
Resistant to Polymyxin B 30μ discs	100	93.1
Catalase produced	100	93
No blebs present	100	92.86
Growth at pH 5.5	84.6	93.1
Resistant to Penicillin 10μ tubes	66.67	68.00
Growth at 0°C good	46.2	48.3
$NO_3 \rightarrow NO_2$	23	31
Growth in 3.0% NaCl	15.38	10.34

Table 13

Characters 100% positive for
Group I and variable for Group II

Characters	% positive Group II
Hydrolyse Tween 40	89.66
No gas from nitrate	86.21
No acid from lactose (B & H)	82.76
Survive 1 hr at 45°C	75.86
Sensitive to Novobiocin 30μ discs	68.97
Sensitive to S.L.S. .01%	48.28
Resistant to Streptomycin 100μ tubes	48.28
No acid from sucrose (H & L)	25.81
Rods not long	25
No acid from glucose open (B & H)	12.13
Cysteine $\rightarrow H_2S$	10.34
No acid from glucose (H & L)	3.13
No acid from maltose (H & L)	3.13
Ring in broth culture	0
Pellicle in broth culture	0

Table 14

Characters 100% positive for
Group II and variable for Group I

Characters	% positive Group I
No growth at 35°C	79.3
Acid from maltose (B & H)	61.54
Do not survive 1 hr at 50°C	38.46
Acid from mannitol (B & H)	23.08
Rods curved	15.38
Poor contrast	7.69

Table 15

Main differences between the two Groups of cytophagas

Character	% Group I Positive	% Group II Positive
Ring in broth culture	100	0
Pellicle in broth culture	100	0
Acid from glucose (H & L)	0	96.87
Acid from maltose (H & L)	0	96.87
Good contrast	92.31	0
Cysteine $\rightarrow$ H_2S	100	10.34
Acid from glucose, open (B & H)	0	87.87
Rods curved	15.38	100
Creeping motility on agar	7.69	93.10

III.3 Discussion

Strain Cook 405 is a chitinoclastic denitrifier and has been identified as *C.johnsonae* var. *denitrificans*. It is included in Group II in this analysis on overall similarity. Chitin degradation was not included in this series of tests because of difficulties in finding a suitable medium,and this is a pity. Decomposition of cellulose was not achieved by the few isolates tested (83), and about one quarter of the organisms of both Groups reduced nitrate. On this basis it was assumed that Group I and Group II both fell within the species *C.johnsonae*.

Recent work (see Chapter VIII) has established that none of the organisms 15D and 18H (Group I), 405 (Group II), 9D and 3C (Myxobacter subgroup) degrade cellulose or alginate. All of these except 3C decompose starch and polypectate, and only 405 and 3C utilize chitin. Thus if *C.johnsonae* is to be defined mainly on its ability to attack chitin, it would appear that Group II could be equated with *C.johnsonae* and that Group I would be a closely related but distinct species.

One consequence of the application of numerical taxonomy is the ease with which small differences in rank may be recognized. The two groups described here have their highest linkage at 61% S and are mainly bounded by cones of 75% S (Group I) and 70% S (Group II). The question of assigning to them specific or varietal names rests on the degree of similarity necessary for species status. Sokal

and Sneath (362) wisely did not suggest absolute S limits for species but preferred to designate various 'phenons'. In this case the core of Group I would be a 75-phenon and that of Group II would be a 70-phenon, and the two groups together would constitute a 61-phenon. It is probably preferable to leave the assignation of names to these phenons until more work has been done on soil and freshwater cytophagas.

CHAPTER IV

THE ISOLATION AND ENUMERATION OF CYTOPHAGAS

IV.1 Myxobacterial media

Myxobacters have been widely cultivated on milk and its derivatives since 1913 when Kofler (223) used a skim milk - nutrient agar medium formulated by Hastings (160). Various milk hydrolysates have also been employed (99, 263, 282, 294). Ordal and Rucker (293) reported success with a tryptone agar, subsequently augmented with yeast extract, sodium acetate and beef extract (9). A simple 0.2% tryptone - 1% agar medium (Cook's Cytophaga Agar) has been used as a test for colonial spreading (78, 432). Peptonised milk was introduced as a myxobacter medium by Jeffers (187), and this was later supplemented with tryptone, yeast extract, sodium acetate and beef extract (67). In this laboratory a variety of cytophagas and fruiting myxobacteria have been maintained for some years on a skim milk - yeast extract agar or broth, or on autoclaved yeast cell agar (83, 356). Suppression of contaminants during isolation of myxobacters has been reported with actidione (52), neomycin and cycloheximide (67), and penicillin G, chloramphenicol or bile salts (424). The object of the present study was to find media which supported the growth of large numbers of cytophagas from natural sources, and solid media which encouraged the spreading habit of these myxobacters and facilitated their isolation into pure culture.

IV.2. Materials and Methods

Examination of Media: The media used comprised some previously successful ones, together with several original modifications (Table 16). All except one had a protein base consisting of tryptone or a degraded milk or dead yeast cells, or a combination of these. Yeast extract and/or beef extract supplements were included in most of the formulae, and sodium acetate in many of them. Experience in this laboratory has shown that cytophagas often grow better, and certainly demonstrate spreading better (374), on nutritionally more dilute media , hence many of the new formulations reflect these observations.

Various water and mud samples from known *Cytophaga* habitats were employed to evaluate the media. Inocula of 0.1 ml of suitable dilutions were spread with bent glass rods on the dried surfaces of agar plates, using five replicates of two dilutions of each sample. All plates were incubated at room temperature (25°C) for five days. The non-fruiting myxobacteria of typical colonial morphology were estimated quantitatively, and various qualitative characteristics of the colonies were assessed.

Media were evaluated on their effectiveness in:

(i) supporting the growth of large numbers of cytophagas;
(ii) producing colonies which were easy to identify and isolate; and
(iii) allowing expression of the spreading growth habit.

To verify the nature of the yellow colonies counted as cytophagas, all such colonies from several different plates were checked for typical microscopic morphology.

Table 16

Composition of media

Name	Constituents % w/v									
	Tryptone	Pep. milk	Skim milk	Bakers' yeast	Yeast extract	Beef extract	Sodium acetate	Glucose	Mineral salts	Agar
Cook's Cytophaga (432)	.2									1.0
T.Y.A.	.2				.25		.02			1.5
Weak T.Y.A.	.1				.02		.002			1.5
Cytophaga Agar*	.2				.05	.02	.02			1.5
Plate Count (Difco)	.5				.25			.1		1.5
1/10 Plate Count	.05				.025			.01		1.5
Plate Count Acetate	.5				.25		.02	.1		1.5
Peptonised Milk (67)	.005	.05			.005	.002	.002			1.5
Pep. Milk I	.005	.05			.01		.002			1.5
Pep. Milk II	.1	.1			.02		.02			1.5
Pep. Milk III	.01	.05			.005	.002	.002			1.5
Pep. Milk IV	.005	.05				.01	.002			1.5
P.M.Y.A. I		.2			.25		.02			1.5
P.M.Y.A. II		.1			.02		.002			1.5
P.M.Y.A. II'		.15			.1		.02			1.5
Skim Milk (356)			.5		.05					1.5
Skim Acetate			.5		.05		.02			1.5
Yeast Agar (356)				.5						1.5
Yeast Tryptone Agar	.1			.25						1.5
Y.E.A. Min.					.1		.1		**	1.5

* Modified from Anderson and Ordal (12).

** K_2HPO_4 = 0.8%, KH_2PO_4 = .02%, $MgSO_4$ = .02%.

Tween 20 experiments: The dispersing agent Tween 20 was evaluated for its effectiveness in increasing the counts of myxobacters (12, 126). Using the three best enumeration media (Table 17), and Plate Count as a comparative standard, water and mud samples were tested as follows:

(i) Tween in dilution blank: Dilution series for the samples were made up with different concentrations, (0, 0.001, 0.01, 0.1, 1, 10, 100 and 1000µ litre/litre) of separately autoclaved Tween 20 in the inoculating dilution water blank. Five replicate plates of two suitable dilutions were incubated at 25°C for five days.

(ii) Tween in medium: One plain dilution series for each sample was plated in quintuplicate on seven sets of media containing 0, 0.01, 0.1, 1, 10, 100 and 1000µ litre/litre of Tween 20 respectively.

Sodium lauryl sulphate (S.L.S.) experiments: This selective bacteriocide was chosen after a large number of possible agents were exhaustively screened (83, 320). Experiments were carried out to ascertain whether S.L.S. could be a useful taxonomic aid and/or a differential counting tool. Preparatory studies were carried out to determine the concentration of S.L.S. in agar medium which would effectively inhibit growth of organisms that were considered morphologically as cytophagas. Fourteen cytophagas and four non-cytophagas were inoculated from liquid culture on to fresh Plate Count plates containing 0, 0.005, 0.01, 0.05, 0.1, 0.5 and 1.0% S.L.S. respectively. A further 26 cytophagas and seven other bacteria were tested at 0, 0.05 and 0.1% S.L.S. levels.

The three best isolation media (Table 18) were used to test 116 pure cultures on triplicate plates with, and without 0.1% S.L.S. incorporated into the agar medium prior to autoclaving. The organisms comprised 66 cytophagas, 15 *Flavobacterium* type cultures and 26 other common soil and water organisms (Tables 23 and 24). After five days at 25°C growth was scored as luxuriant, fair, trace or negative, and proteolysis (clearing) of the skim acetate medium was noted.

To test the usefulness of S.L.S. in enumeration of cytophagas three dilution plates, from soil, creek mud and creek water respectively, which contained 20-50 colonies of various organisms were replicated with velvet on to three types of fresh media. Duplicate plates of plain media were replicated first, and then further duplicate plates with 0.1% S.L.S incorporated were inoculated. These were incubated at 25°C for five days and growth of cytophagas and non-cytophagas was recorded.

IV.3. Results and discussion

Media: Many different samples were tested with the 20 listed media (Table 16), and although no one medium always gave the highest numbers of cytophagas or the best quality of growth, for each category there were certain media which were consistently better than the others. To simplify matters we have included in the tabular presentation only those three or four media which consistently performed better for enumeration of cytophagas (Table 17), for ease of recognition and isolation (Table 18) and for expression of the spreading habit (Table 19).

Table 17

Recommended media for enumeration. Examples of data.

Medium	Whitemud Creek: Upper creek sample — Cytophagas x 10^4/ml: Range	Av.	% cytophagas	Whitemud Creek: Creek mouth sample — Cytophagas x 10^4/ml: Range	Av.	% cytophagas
P.M.Y.A. II	4-8	7.0	25	18-28	21.4	25
T.Y.A.	3-8	5.6	17	10-29	18.8	24
Skim Acetate	2-9	4.6	12	15-28	21.4	21

Table 18

Recommended media for isolation. Example of data.

Medium	Average numbers x 10^4/ml	Quality of cytophaga growth			Characteristics of medium
		Colour	Size	Compactness of colonies	
		Range = V. Pale to V. Bright	Range = Pinpoint to large	Range = Discrete to spreading	
Pep. Milk II	14.0	Bright	Large	Discrete	Clear, colourless
Skim Acetate	15.5	V.Bright	Variable	Spreading	Cloudy, able to be lysed
P.M.Y.A. II	10.25	Bright	Medium	Discrete	Slightly cloudy, colourless
Plate Count	11.3	Bright	Large	Spreading	Clear but yellowish

Table 19

Recommended media for spreading. Example of data.

Medium	Average numbers x 10^3/ml	% of cytophagas which spread	Largest diameter (mm)	Distinguishability from medium
	White Mud Creek - Upper sample			
Pep. Milk I	50	60	70	very good
P.M.Y.A. II	70	85	25	very good
Yeast Agar	40	33	7	good
	White Mud Creek - Mouth sample			
Pep. Milk I	21	10	70	fair
P.M.Y.A. II	21	5	38	good
Yeast Agar	15	7	68	good

The most generally useful media were Skim Acetate and PMYA II, and these two were also superior in the later enumeration experiments with Tween. Skim milk and peptonised milk are thus confirmed as excellent nutritional sources for cytophagas, with yeast extract and sodium acetate being stimulatory for this group.

Tween: The most effective concentration of Tween 20 in the inoculating dilution water was 0.1µ litre/litre (Figure 2), but this did vary slightly with the sample and medium. Counts of cytophagas were increased two to three and one half times over the control dilutions. However, at concentrations between 1 and 1000µ litre/litre there was an increasingly pronounced inhibitory effect. The incorporation of Tween 20 into the agar media did not increase counts of cytophagas significantly or consistently but an inhibition of numbers of organisms was again apparent at 100 and 1000µ litre/litre (Figure 3). If Tween is indeed having a detergent action breaking up clumps of cells in the sample, then it is obvious that it would be more effective in a liquid sample being shaken than in a solid agar medium. In the particular sample used for Figure 2, a slight increase in numbers is apparent at 0.001µ litre/litre, then a drop at 0.01µ litre/litre before the 0.1µ litre/litre peak is reached. This was often noted in various samples, and it can only be speculated that Tween 20 is having two different effects, or an effect at two levels. The depression of cytophaga numbers by high concentrations of Tween is shown by both methods, presumably due to cell membrane rupture by the surfactant.

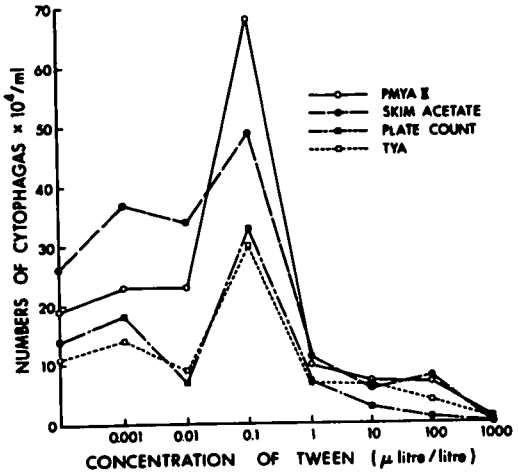


Figure 2. The influence of Tween 20 in the inoculating dilution blank on numbers of cytophagas from a small roadside slough near Gainford, Alberta.

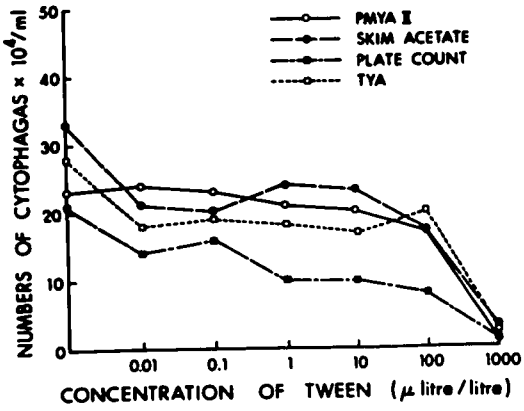


Figure 3. The influence of Tween 20 in the plating medium on numbers of cytophagas from a small roadside slough near Gainford, Alberta.

S.L.S.: In the preparatory studies to determine the effective concentration, 0.05% S.L.S. completely inhibited 77.5% of the cytophagas but only 36% of the non-cytophagas, while 0.1% S.L.S. inhibited 92.5% of the cytophagas compared to 45% of the non-cytophagas (Tables 20 and 21). The decision was made to use 0.1% sodium lauryl sulphate as a screening agent for cytophaga cultures. Some of the cytophaga-like organisms tested in the preliminary experiments did in fact grow at this concentration (Table 20), but because of other work proceeding concurrently (83) these were ignored. It is now considered that the organisms 3C, 17B, 21B and 23B do not fit the main concept of the *Cytophaga* group as we recognize it, but are more closely related to the fruiting myxobacteria (see Chapter III).

In the experiments with pure cultures 97% of the cytophagas, as well as 80% of the *Flavobacteria*, and 85% of the other organisms tested were sensitive to 0.1% S.L.S. (Tables 22, 23 and 24). However, 91% of the cytophagas were proteolytic on Skim Acetate and sensitive to S.L.S., compared to 53% of the *Flavobacteria* and 50% of the other cultures. Of the lattermost group of organisms there were none that were not easily differentiated morphologically from cytophagas. Hence the S.L.S. susceptibility test should be done on Skim Acetate so that the proteolytic reaction can also be observed.

The replicate plating of soil and water samples with and without S.L.S. resulted in the inhibition of 75-98% of the organisms by the S.L.S. in samples containing about 15% cytophagas. As it was shown above that S.L.S. susceptibility is not restricted to the cytophagas, it is thus not surprising that the attempt to utilize it as a differential counting tool was not successful.

Table 20

Growth of cytophagas on Difco Plate
Count with various concentrations of S.L.S.

Organisms Our laboratory numbers	Concentration of S.L.S. %						
	0	0.005	0.01	0.05	0.1	0.5	1.0
15 D	+	-	-	-	-	-	-
E, L, 8D	+	+	-	-	-	-	-
Group a*	+			-	-	-	-
9D	+	+	trace	-	-	-	-
13, 19, 405#, 11B, 3D, 14D, 18H	+	+	+	-	-	-	-
7B	+	+	+	trace	-	-	-
3C	+	+	+	+	-	-	-
13B, 12I	+			+	-		
8I, 10I	+			+	trace		
17B	+			+poor	+poor		
21B, 23B	+			+	+		

\+ = good growth
\- = no growth
(blank) = not tested

* Group a 416, 418, 4040, 4041, 4042, 4043, 4044, 4045, 4046, 4047, 4048, 4049, 8B, 9B, 10B, 12B, 22B, 2D, 4D.

\# *C.johnsonae*

Table 21

Growth of non-cytophagas on Difco Plate Count with various concentrations of S.L.S.

Non-cytophagas	Concentration of S.L.S. % 0	0.005	0.01	0.05	0.1	0.5	1.0
Micrococcus denitrificans CDA 449	+	-	-	-	-	-	-
Arthrobacter CDA 852	+			-	-		
Arthrobacter aurescens CDA 579 (ATCC 13344)	+			-	-		
Flavobacterium aquatile ATCC 11947	+			-	-		
Agrobacterium radiobacter CDA 418	+	+	+	+	trace	-	-
Serratia marcescens	+				+	+	
Aerobacter aerogenes	+				+	+	
Escherichia coli	+				+	+	
Rumen organism R_2	+				+	+	
Agrobacterium radiobacter CDA 590	+	+	+	+	+	-	-
Pseudomonas stutzeri NCIB 9040	+	+	+	+	+	+	-

\+ = good growth

\- = no growth

(blank) = not tested

CDA = Canadian Department of Agriculture, Ottawa, Ontario

Table 22

Growth of Cytophagas on
three media with and without 0.1% S.L.S.

Organisms Our laboratory numbers	Plate Count Plain	Plate Count +S.L.S.	Pep. Milk II Plain	Pep. Milk II +S.L.S.	Skim Acetate Plain	Skim Acetate +S.L.S.	Proteolysis
Extremely Sensitive							
Group 1*	+++	-	+++	-	+++	-	✓
5, 4048, 2D	+++	-	+++	-	+++	-	0
Very Sensitive							
13B	+++	-	+++	+	+++	-	✓
Group 2*	+++	-	+++	-	+++	+	✓
3D, 4D	+++	-	+++	-	+++	+	0
F-2-25	+++	+	+++	+	+++	-	✓
405#, B-2-25	+++	+	+++	-	+++	+	✓
Group 3*	+++	-	+++	+	+++	+	✓
Group 4*	+++	+	+++	+	+++	+	✓
Sensitive							
PC5	++	-	+++	-	+++	+	✓
PC10	++	-	++	-	++	-	✓
PC7	+	-	+++	-	+++	+	✓
PC8	+++	-	+++	++	+++	+	✓
PC1	++	-	+++	+	+++	+	✓
4049	+++	-	+++	+	++	+	0
Weakly Sensitive							
PC4, H_2O-1A	+++	++	+++	+	+++	+	✓
PC2, E-1-15	+++	+	+++	+	+++	++	✓
D-2-25	+++	++	+++	+	+++	++	✓
PC12	+	+	+++	+	+++	+	✓

* Group 1 495, 4045, 22B, 3C, 10I, 12I, PC9, PC15, PC16, D-1-25, G-2-15, G-2-25, G3B, Gill-1-25, H-1-25, H_2O-1-15, H_2O-4-25, I-1-15, K24, K54, G-1-25.

Group 2 18H, 8I, B-1-15, B-3-15, B-4-15, E-1-25, FBB, Gill-2-25, H-2-25.

Group 3 15D, A25, F-1-15, G-3C-25, Gill-1-15, Kidney 2A.

Group 4 9D, PC11, PC14, A15, B-1-25, C-15, F-3-15, G-1-25, Kidney 2.

C.johnsonae

+++ luxuriant, ++ fair, + trace, - negative.
✓ lysis, 0 no lysis.

Table 23

Growth of *Flavobacteria* type cultures
on three media with and without 0.1% S.L.S.

Organisms NCIB Nos.	Plate Count		Pep. Milk II		Skim Acetate		
	Plain	+S.L.S.	Plain	+S.L.S.	Plain	+S.L.S.	Proteolysis
Extremely Sensitive							
Group I*	+++	-	+++	-	+++	-	✓
Group II*	+++	-	+++	-	+++	-	0
Very Sensitive							
F. resinovorum 8767 a#	+++	+	+++	-	+++	-	0
F. suaveolans 8992	+++	-	+++	+	+++	+	✓
Group III*	+++	+	+++	+	+++	+	✓
Not Sensitive							
F. proteus 8771	+++	++	++	++	++	++	0
F. rhenanum 9157 a#	+++	+++	++	+	++	++	0
F. rhenanum 9157 b#	+++	+++	+++	+++	+++	+++	0

*Group I — *F. arborescens* 8185, *F. esteroaromaticum* 8186, *F. flavescens* 8187, *F. lucecoloratum* 9324a #, *F. lucecoloratum* 9324b #

Group II — *F. devorans* 8195, *F. aurantiacum* 8204, *F. resinovorum* 8767b #

Group III — *F. aquatile* 8535, *F. pectinovorum* 9059

Two different organisms were isolated from these newly acquired cultures.

+++ luxuriant growth, ++ fair growth, + trace, - negative growth
✓ lysis, 0 no lysis.

Table 24

Growth of other bacteria and actinomycetes on three media with and without 0.1% S.L.S.

Organisms	Plate Count		Pep. Milk II		Skim Acetate		
Our laboratory numbers	Plain	+S.L.S.	Plain	+S.L.S.	Plain	+S.L.S.	Proteolysis
Extremely Sensitive							
Group A*	+++	-	+++	-	+++	-	✓
Group B*	+++	-	+++	-	+++	-	0
Very Sensitive							
Group C*	+++	-	+++	-	+++	+	✓
B.polymyxa 4615	+++	-	+++	+	+++	+	✓
B.alvei 4619	+++	+	+++	+	+++	-	?
Group D*	+++	+	+++	+	+++	+	✓
B.megatherium	+++	+	+++	+	+++	+	0
Weakly Sensitive							
Agrobacterium tumefaciens A21	+++	+	+++	+	+++	++	0
Rhizobium meliloti RM9	+++	-	+++	+	+++	+++	0
Alcaligenes faecalis 4455	+++	++	+++	+	+++	++	0
Rhizobium meliloti 110	+++	+	+++	+	+++	+++	0
Micrococcus dentrificans 623	+++	+++	+++	+	+++	+	0
Not Sensitive							
Group E*	+++	+++	+++	+++	+++	+++	✓
Group F*	+++	+++	+++	+++	+++	+++	0

* Group A *Bacillus brevis* 4612, *B. subtilis* 4611, *B. pumilis* 4614, *B. licheniformis* 4617, B. *circulans* 4618.

Group B *B. cereus* 4616, *Pseudomonas stutzeri* M305, Actinomycete 4398, Actinomycete 4460, Actinomycete 4467.

Group C "White", *Chromobacterium*.

Group D 'sporeformer', *Arthrobacter globiformis* 4165.

Group E *Serratia marcescens* 5127, *Escherichia coli*.

Group F *Agrobacterium radiobacter* 515, *Pseudomonas aeruginosa*.

+++ luxuriant growth, ++ fair growth, + trace, - negative growth
✓ lysis, 0 no lysis.

CHAPTER V

A STUDY OF SOME ARCTIC CYTOPHAGAS

V.1 Introduction

There have been a few studies establishing the existence of myxobacteria (52) and of cytophagas (273, 319) in the Canadian arctic, but so far little detailed information about them has been accumulated. In this investigation a series of cytophagas from five lakes in the North West Territories were isolated and described in some detail as part of a more general study of the microflora of these lakes.

V.2 Materials and methods

The samples were collected by R. L. Hare and W. G. M. Gattinger of the Inland Waters Branch during the summer of 1971 and are outlined in Table 25. Although the samples were taken aseptically, their subsequent handling did not allow quantitative estimations to be made. Cook's Cytophaga Agar, T.Y.A. and Skim Milk Agar plates (71, see Table 16, page 105) were inoculated with dilutions of each sample and incubated at room temperature (about 25°C) for 11 days or at 10°C for 18 days. Colonies which could be cytophagas were picked and purified for further work. Tests, which were carried out at the isolation temperature of each organism (10° or 25°C) were as follows:

Table 25

Arctic Samples

Sample	Lab. #	Location in N. W. T.	Zone	Lake	pH	Conductivity (mhos/cm)
4765 water	10	106N 9 8-616900-7507900 Decp Lake, 45 miles NE of	Subarctic	Limnetic*	7.2	135
4765 mud	5	Arctic Red River				
4794 water	8	106M 6 8-484400-7482950 15 miles W	Tundra	Littoral*	6.7	25
4794 mud	7	of Ft. McPherson				
4796 water	9	106M 7 8-502350-7461200. In flood plain of Peel River, 15 miles S of Ft.	Coniferous Forest	Littoral	8.8	210
4796 mud	6	McPherson				
4810 water	1	106M 8 8-526000-7475250 15 miles E	Coniferous Forest	Littoral	7.5	90
4810 mud	2	of Ft. McPherson				
4824 water	3	106K 13 8-551500-740980 20 miles W	Coniferous Forest	Littoral	7.5	Not done
4824 mud	4	of Martin House				

*Limnetic = no light to bottom of lake

*Littoral = light to bottom of lake

(i) Cook's Cytophaga Agar streak description and microscopic examination.

The amount of growth, occurrence of spreading, colour, length, width, shape and motility of each organism were described at three to four days (25°C) or five to six days (10°C).

(ii) Skim Milk broth description and microscopic examination.

Proteolysis (indicated by the clearing of the solution), and silkiness of the liquid culture were noted at two, three and six days. Microscopic observations of length, width, shape, flexing and motility were made at 10-14 hours and two days (25°C), or 24-42 hours and five days (10°C).

(iii) Skim Milk plate description and tests.

Growth at 5° and 30°C, and proteolysis were noted at six days. At four to five days Gram stains were made, and the Munsell colour (277) determined in the following manner. The total surface growth was scraped up with a loop and well mixed on the agar surface, then a blob was transferred to a flat white porcelain plate. It was quickly shaped into a mound 1-1.5mm high with a flat surface of at least four mm^2. Comparison with standard Munsell colour chips was done immediately to forestall colour changes which occur on drying. The criteria for defining a culture as a *Cytophaga* were the following. Organisms were Gram negative, nonmotile rods which in Skim Milk broth

were (a) Very thin to thin in width ($<0.5\mu$)

(b) Medium to long in length ($>5\mu$)

(c) Flexuous

(d) Silky when culture gently shaken

(e) Proteolytic

and on Skim Milk Agar

(f) Produce a pigment within the range of 7.5 to 10 YR 6 to 7/8 to 12

and (g) Show spreading growth on Cook's Cytophaga Agar.

Up to three characteristics of the group (a) to (g) may not be shown, with the exception of combinations of

(i) Non-flexuous and non-spreading

(ii) Short and not thin

or (iii) Not thin and non-flexuous.

V.3 Results

The results of the individual tests on the isolates are presented in Tables 26 and 27. The first figure of the colony number indicates the laboratory sample number, and the second is specific. A summary of the types found in each sample (Table 28) shows considerable variation between the samples. The large number of cytophagas isolated from sample #1 (4810 water) reflects the more intensive investigation of that sample as well as the preponderance of yellow-orange colonies found on the plates.

Table 26

Characteristics of the arctic isolates on Cook's Cytophaga agar*

Colony Number	Isolation Temp.	Growth	Spreading	Colour	Length			Width	Motility	Shape
					Short	Medium	Long			
1-1	25	±	-	Cream	+	+	-	V.thin	-	Rod
1-3	25	±	-	OY	+	-	-	Thin	-	Rods in groups
1-10	25	++	+	OY	-	+	+	Thin	-	Rod
1-11	25	++	+	OY	+	+	+	Thin	-	Rod
1-12	10	+	+	OY	+	+	+	Thin	-	Rod
1-13	25	++	+	OY	+	+	-	Thin	-	Rod
1-14	25	++	+	OY	-	+	+	Thin	-	Rod
1-15	10	+	+	OY	-	+	+	Thin	-	Rod
1-16	25	++	+	OY	+	-	-	Thin	-	Rod
1-17	10	+	+	OY	+	+	+	Thin	-	Rod
1-18	10	+	-	OY	+	+	-	Thin	-	Rod
1-19	25	++	+	OY	+	+	-	Thin	-	Rod
1-20	25	++	+	OY	+	-	-	Thin	-	Rod
1-21	25	++	+	OY	+	+	+	Thin	-	Rod
1-22	25	++	+	OY	+	+	-	Thin	-	Rod
1-23	25	++	+	OY	-	+	+	Thin	-	Rod
1-24	25	++	+	OY	+	+	-	Thin	-	Rod
1-25	25	++	+	OY	-	+	+	Thin	-	Rod
1-26	25	++	+	OY	+	-	-	Thin	-	Rod
1-27	10	+	+	OY	+	-	-	Thin	-	Rod
1-35	10	++	+	OY	+	-	-	Thin	-	Rod
2-1	25	+	-	OY	-	+	+	Thin	-	Rod
2-2	25	±	-	Cream	-	+	-	Thin	-	Rod
2-3	25	±	-	Cream	-	+	-	Thin	-	Rod

*'Growth' column: ++ = good, + = fair, ± = slight, - = negative.
All other quantitative columns: + = positive, - = negative.
Cb. = coccobacillus, OY = orange-yellow.

Table 26 - continued

Characteristics of the arctic isolates on Cook's Cytophaga agar

Colony Number	Isolation Temp.	Growth	Spreading	Colour	Length			Width	Motility	Shape
					Short	Medium	Long			
2-4	25	±	-	Yellow	-	+	-	Thin	-	Rod
2-5	25	+	-	Cream	+	+	+	Thin	-	Rod
2-6	25	±	-	Yellow	+	+	+	Thin	-	Rod
2-7	25	+	-	OY	+	+	-	Thin	-	Rod
2-9	25	++	+	OY	+	-	-	Medium	-	Rod
2-10	25	+	+	OY	+	+	-	Thin	-	Rod
2-14	25	±	-	OY	+	-	-	Thin	-	Rod
3-1	25	+	-	OY	-	+	+	Thin	-	Rod
3-2	25	++	-	OY	+	+	-	Thin	-	Rod
3-3	25	±	-	White	-	+	+	Thin	-	Rod
3-4	25	++	+	OY	+	-	-	Thin	-	Rod
3-11	25	++	+	OY	+	-	-	Thin	-	Rod
3-12	25	++	+	OY	+	+	-	V.Thin	-	Rod
3-14	25	++	+	OY	+	-	-	Thin	-	Rod
3-19	25	++	+	OY	+	-	-	Thin	-	Rod
3-22	10	++	+	OY	+	-	-	Thin	-	Cb.*
3-23	10	++	+	OY	+	-	-	Thin	-	Cb.
5-6	10	++	-	OY	-	-	+	Thin	-	Rods, many circles
6-1	25	+	+	Cream	-	+	-	Thin	-	Rod
6-2	25	+	-	OY	-	+	+	Thin	-	Rod
6-3	25	+	-	OY	+	+	-	Thin	-	Rod
6-4	25	+	-	OY	+	+	+	Thin	-	Rod
6-9	10	+	-	OY	+	+	-	Medium	-	Rod
7-1	25	±	-	Cream	+	+	+	Thin	-	Rod
7-4	10	++	±	OY	-	+	-	Thin	-	Rod
9-2	25	+	-	OY	+	+	+	Thin	-	Rod
9-4	25	+	-	OY	-	+	+	Thin	-	Rod
10-6	10	++	+	OY	+	-	-	Thin	-	Rod

Table 27

Characteristics of the arctic isolates on skim milk media*

Colony number	Skim Milk Broth									Skim Milk Agar				
	Length										Growth at			
	Short	Medium	Long	Width	Flexing	Silkiness	Lysis	Motility	Shape	Lysis	5°	30°C	Gram	Munsell colour
1-1	-	+	+	Thin	+	+	+	-	Rod	+	-	++	-	10 YR 6/12
1-3	+	+	-	Thin	+	+	+	-	Rod	+	-	++	-	10 YR 6/12
1-10	-	+	+	Thin	+	+	+	-	Rod	+	++	++	-	10 YR 6/12
1-11	-	+	-	Thin	+	+	+	-	Rod	+	+	++	-	7.5 YR 7/10
1-12	+	+	-	Thin	+	+	+	-	Rod	+	±	++	-	7.5 YR 7/10
1-13	-	+	+	Thin	+	+	+	-	Rod	+	+	++	-	7.5 YR 6/10
1-14	-	+	+	Thin	+	+	+	-	Rod	+	+	++	-	7.5 YR 6/10
1-15	+	+	-	Thin	+	+	+	-	Rod	+	±	++	?-	7.5 YR 7/8
1-16	-	+	+	Thin	+	+	+	-	Rod	+	+	++	-	7.5 YR 6/10
1-17	-	+	-	Thin	+	+	+	-	Rod	+	±	++	-	7.5 YR 7/10
1-18	+	+	+	Thin	+	+	+	-	Rod	+	+	+	-	10 YR 7/10
1-19	-	+	-	Thin	+	+	+	-	Rod	+	+	++	-	10 YR 7/8
1-20	-	+	-	Thin	+	+	+	-	Rod	+	+	++	-	7.5 YR 6/8
1-21	-	+	+	Med.	+	+	+	-	Rod	+	+	++	-	7.5 YR 6/10
1-22	-	+	-	Thin	+	+	+	-	Rod	+	+	++	-	7.5 YR 6/8
1-23	-	+	-	Thin	+	+	+	-	Rod	+	+	++	-	7.5 YR 6/8
1-24	-	+	-	Thin	+	+	+	-	Rod	+	+	++	-	7.5 YR 6/10
1-25	-	+	-	Thin	+	+	+	-	Rod	+	+	++	-	7.5 YR 6/10
1-26	-	+	-	Thin	+	+	+	-	Rod	+	+	++	-	10 YR 7/10
1-27	-	+	-	Thin	+	+	+	-	Rod	+	+	++	-	7.5 YR 6/8
1-35	+	+	-	Thin	+	+	+	-	Rod	+	+	++	-	7.5 YR 6/10
2-1	+	+	+	Thin	+	+	+	-	Rod	+	±	++	-	7.5 YR 6/12

*"Growth at 5° and 30°C" column: ++ = good, + = fair, ± = slight, - = negative. All other quantitative columns: + = positive, - = negative. "Lysis of skim milk" = partial or complete clearing.

Table 27 - continued

Characteristics of the arctic isolates on skim milk media

Colony number	Skim Milk Broth									Skim Milk Agar				
	Length			Width	Flexing	Silkiness	Lysis	Motility	Shape	Lysis	Growth at 5°	Growth at 30°C	Gram	Munsell colour
	Short	Medium	Long											
2-2	+	+	+	Thin	+	+	+	-	Rod	+	±	++	-	10 YR 6/12
2-3	+	+	+	Thin	+	+	+	-	Rod	+	-	++	-	10 YR 6/12
2-4	+	+	-	Thin	+	+	+	-	Rod	+	-	++	-	10 YR 6/12
2-5	+	+	-	Thin	+	+	+	-	Rod	-	±	++	-	2.5 Y 7/10
2-6	+	+	+	Thin	+	+	+	-	Rod	+	++	++	-	10 YR 6/12
2-7	+	+	+	Thin	+	+	+	-	Rod	+	++	++	-	10 YR 6/12
2-9	-	+	-	Thin	+	+	+	-	Rod	+	++	++	-	7.5 YR 6/8
2-10	-	+	-	Thin	+	+	+	-	Rod	+	+	++	-	7.5 YR 6/8
2-14	+	+	-	Thin	+	-	+	-	Rods some chains	+	-	++	-	7.5 YR 6/10
3-1	-	+	+	Thin	+	+	+	-	Rod	+	+	++	-	7.5 YR 6/12
3-2	+	+	-	Thin	+	+	+	-	Rod	+	+	++	-	10 YR 6/10
3-3	+	+	-	V.Thin	+	+	+	-	Rod	+	-	++	-	10 YR 6/12
3-4	-	+	-	Thin	+	+	+	-	Rod	+	+	++	?-	7.5 YR 6/8
3-11	-	+	+	Thin	+	+	+	-	Rod	+	+	++	-	7.5 YR 6/8
3-12	-	+	+	Thin	+	+	+	-	Rod	+	+	++	-	7.5 YR 6/10
3-14	-	+	-	Thin	+	+	+	-	Rod	+	+	++	-	7.5 YR 6/10
3-19	+	+	-	Thin	+	+	+	-	Rod	+	+	++	-	7.5 YR 6/8
3-22	+	-	-	Thin	-	+	+	-	Rods, some chains	+	++	++	?-	7.5 YR 6/10
3-23	+	-	-	Thin	-	+	+	-	Rods, some chains	+	+	++	?-	7.5 YR 6/10
5-6	-	+	+	V.Thin	+	+	+	-	Rod	+	++	-	-	10 YR 7/10

Table 27 - continued

Characteristics of the arctic isolates on skim milk media

Colony number	Skim Milk Broth									Skim Milk Agar				
	Length			Width	Flexing	Silkiness	Lysis	Motility	Shape	Lysis	Growth at 5°	Growth at 30°C	Gram	Munsell colours
	Short	Medium	Long											
6-1	-	+	-	Thin	+	+	+	-	Rod	+	-	++	-	7.5 YR 6/10
6-2	-	+	+	Thin	+	+	+	-	Rod	+	++	±	?-	10 YR 7/10
6-3	+	+	+	Thin	+	+	+	-	Rod	+	++	±	?+	10 YR 7/10
6-4	+	-	+	Thin	+	+	+	-	Rod	+	++	±	-	10 YR 7/10
6-9	-	+	+	Thin	+	+	+	-	Rod	+	++	-	?-	10 YR 6/12
7-1	-	+	+	Thin	+	+	+	-	Rod	+	++	++	-	10 YR 7/12
7-4	-	+	+	Thin	+	+	+	-	Rod	+	+	++	-	10 YR 6/10
9-2	+	+	-	Thin	+	+	+	-	Rod	+	++	++	?-	10 YR 6/12
9-4	+	+	-	Thin	+	+	+	-	Rods, many chains	+	+	+	-	10 YR 6/12
10-6	+	+	-	Thin	+	+	+	-	Rod	+	-	++		

Table 28

Cytophagas found in arctic samples

Sample	Total	Number of Cytophagas Grow at 5°C	Grow at 30°C	Spreading	Proteolytic
4765 water (10)	1	0	1	1	1
mud (5)	1	1	0	0	1
4795 water (8)	0	0	0	0	0
mud (7)	2	2	2	1	2
4796 water (9)	2	2	2	2	2
mud (6)	5	4	1	1	5
4810 water (1)	21	16	21	18	21
mud (2)	10	4	10	2	10
4824 water (3)	10	9	10	10	10
mud (4)	0	0	0	0	0

A summary of the characteristics of the 52 arctic cytophagas appears as Table 29.

Several groups of cytophagas were recognized and are outlined below. All were very thin to thin rods of medium to long maximum length, were flexuous, silky, proteolytic and of orange-yellow colour (7.5 to 10 YR 6 to 7/8 to 12) on Skim Milk media, and grew very well on both Cook's Cytophaga Agar and Skim Milk Agar unless otherwise stated.

Table 29

Summary of the
Characteristics of the Arctic Cytophagas

Characteristic	Resulting Group	Cytophagas Number	%
Cook's Cytophaga agar			
Growth	++	25	48
	+	18	35
	±	9	17
	-	0	0
Spreading	+	29	56
	±	1	2
	-	22	42
Note: Of the 44 (85%) that grow on this medium 30 (65%) show some spreading.			
Motility			
Cook's Cytophaga agar	-	52	100
Skim Milk broth	-	52	100
Morphology			
Cook's Cytophaga agar	Bacilli	50	96
	Coccobacilli	2	4
Skim Milk broth	Bacilli	52	100
Maximum Length			
Cook's Cytophaga agar	Short	15	29
	Medium	17	33
	Long	20	38
Skim Milk Broth	Short	2	4
	Medium	28	54
	Long	22	42

Table 29 - continued

Characteristic	Resulting Group		Cytophagas Number	%
Width				
Cook's Cytophaga agar	Very thin		2	4
	Thin		48	92
	Medium		2	4
	Fat		0	0
Skim Milk broth	Very thin		2	4
	Thin		49	94
	Medium		1	2
	Fat		0	0
Colour on Cook's Cytophaga agar	Orange Yellow		43	83
	Cream		6	12
	Yellow		2	4
	White		1	2
Munsell Colours on Skim Milk agar	Orange Yellow	Total	50	96
	7.5 YR	6/8	9	17
		6/10	13	25
		6/12	2	4
		7/8	1	2
		7/10	3	6
	10 YR	6/10	2	4
		6/12	12	23
		7/8	1	2
		7/10	6	12
		7/12	1	2
	Yellow	Total	1	2
	2.5 Y	7/10	1	2
	Untested		1	2

Table 29 - continued

Characteristic	Resulting Groups	Cytophagas Number	%
Flexing in Skim Milk broth	+	50	96
	-	2	4
Silkiness in Skim Milk broth	+	51	98
	-	1	2
Proteolysis (Clearing)			
Skim Milk broth	+	52	100
Skim Milk agar	+	51	98
	-	1	2
Gram reaction	G- and ?G-	50	96
	?G+	1	2
	Untested	1	2
Temperature relations			
Growth at 5°C	++	12	23
	+	26	50
	±	6	12
	-	8	15
Growth at 30°C	++	45	87
	+	2	4
	±	3	6
	-	2	4
Growth Range			
'Strict psychrophiles'	(no growth at 30°C)	2	4
'Psychrophiles'	(better growth at 5°C)	3	6
'Tolerant'	(similar growth at 5° and 30°C)	7	13
'Mesophiles'	(better growth at 30°C)	32	61
'Strict Mesophiles'	(no growth at 5°C)	8	15

Spreading cytophagas

(1) Moderate growth on Cook's Cytophaga and Skim Milk. Mesophilic Organisms 1-12, 1-15, 1-17 and 1-27.

(2) Mesophilic Organisms 1-11, 1-13, 1-14, 1-16, 1-19, 1-20, 1-21, 1-22, 1-23, 1-24, 1-25, 1-26, 1-35, 2-10, 3-4, 3-11, 3-12, 3-14, 3-19 and 7-4.

(3) Tolerant Organisms 1-10 and 2-9.

(4) Coccobacilli on Cook's Cytophaga, and short rods on Skim Milk. Tolerant. Organisms 3-22 and 3-23.

(5) Cream coloured, moderate growth on Cook's Cytophaga. Strict mesophile. Organism 6-1.

Non-spreading cytophagas

(6) Cream coloured, poor growth on Cook's Cytophaga. Strict mesophiles. Organisms 1-1, 2-2 and 2-3.

(7) Cream coloured, moderate growth on Cook's Cytophaga. Colour 2.5 Y 7/10 on Skim Milk. Not lytic on agar. Strict mesophile. Organism 2-5.

(8) Cream coloured, poor growth on Cook's Cytophaga. Tolerant. Organism 7-1.

(9) Orange-cream coloured, moderate growth on Cook's Cytophaga as short rods. Colour 10 YR 5/8 on Skim Milk. Strongly proteolytic. Strict mesophile. Organism 4-4.

(10) Yellow coloured, poor growth on Cook's Cytophaga. Strict mesophile. Organism 2-4.

(11) Yellow coloured, poor growth on Cook's Cytophaga. Tolerant. Organism 2-6.

(12) Poor growth on Cook's Cytophaga as short rods. Strict mesophile. Organism 1-3.

(13) Poor growth on Cook's Cytophaga as short rods. Not silky. Strict mesophile. Organism 2-14.

(14) Moderate growth on Cook's Cytophaga. Mesophiles. Organisms 2-1, 3-1 and 3-2.

(15) Moderate growth on Cook's Cytophaga and Skim Milk. Tolerant. Organisms 1-18, 2-7, 9-2 and 9-4.

(16) Moderate growth on Cook's Cytophaga. Strict psychrophiles. Organisms 5-6, 6-2, 6-3, 6-4 and 6-9.

(17) White coloured, poor growth on Cook's Cytophaga. Strict mesophile. Organism 3-3.

V.4 Discussion

The three media used for isolation, namely Cook's Cytophaga Agar, T.Y.A. and Skim Milk should screen the samples exhaustively for cytophagas (71). Of course if more and different media were used it is inevitable that other organisms could be found. The *Cytophaga* group of organisms (some of which may belong to *Flexibacter*) were well represented, being recovered from all five lakes examined although not from every sample. The group was especially numerous in 4765 and 4810 water samples.

Spreading on Cook's Cytophaga Agar was shown by only 56% of the 52 cytophagas found in this study. In addition there were a few other organisms which exhibited creeping motility on this medium,

notably motile bacteria and organisms producing copious mucilaginous material. Hence spreading on this medium should not be considered as an infallible criterion for the identification of cytophagas. It is still an excellent confirmatory characteristic nevertheless.

The use of liquid Skim Milk broth, as opposed to the agar media tested, gave consistently superior estimates of three important criteria for cytophagas, maximum length, thinness of cells and proteolysis (clearing) of milk. Thus it is suggested that one estimation of these characteristics from Skim Milk broth would be sufficient. The use of this liquid medium also permits the observation of flexing by the cells and of silkiness of the growth medium on gentle tapping or shaking of the container. Silkiness, which has been noted before (83, 374) but never proposed as a diagnostic criterion of this genus (245, 275, 375) was found to correlate rather well with other characteristics such as flexing and thinness of the cells. It has the advantage of being a very easy characteristic to determine. It has to be noted, however, that silkiness is also achieved by other long-celled or chain-forming organisms, by the production of large amounts of polysaccharide, and sometimes by single motile or non-motile rods (unpublished results).

The cytophagas isolated from these sites showed a good conformity of colouring, as measured by the Munsell system. Ninety six percent were deep orange-yellow falling within the notation 7.5 to 10 YR 6 to 7/8 to 12 with one organism slightly yellower.

The only 'strict psychrophiles' (grew at 5°C but not at 30°C) found in the study were cytophagas, however the vast majority

of the cytophagas were mesophilic, and only 23% grew really well at 5°C. Their activities must therefore be limited at normal lake temperatures.

The grouping of morphologically similar cytophagas was done mainly to facilitate the selection of organisms to be kept in stock culture for further study. It showed, interestingly enough, that the 30 spreading organisms belonged to only five types, whereas the 22 non-spreaders were more varied, forming 13 types.

Although it is difficult to compare the results of Chapters III, IV and V because of differences in the objectives and techniques used, some comments follow. In all three studies the large majority of the cytophagas were yellow-orange in colour, with less than 5% of pink, brown or other colours observed. Both spreading and non-spreading cytophagas were encountered in all three studies. The taxonomic investigation (Chapter III) resulted in two groups being recognized, the first were shorter-celled organisms of which only seven percent spread on Cook's Cytophaga Agar. Group II were longer-celled and 93% of these organisms were spreaders. In the enumeration and isolation study (Chapter IV) from five to 85% of the cytophagas encountered on any one plate were spreaders. The arctic organisms (Chapter V) were mostly medium or long in length and only 56% of these exhibited spreading. It is therefore concluded that these populations are very similar.

VI.2 ELECTRON MICROSCOPY

VI.2.1 Literature review

The ultrastructure of the fruiting members of the Myxobacterales is well documented. There are published accounts of several species including *Archangium gephyra* and *A.violaceum* (234,342), *Archangium* sp. (440), *Chondrococcus coralloides* (342), *Chondromyces apiculatus* (2, 4, 234, 236, 342), *C.crocatus* (1, 3, 264), *Myxococcus fulvus* (168), *M.rubescens* (234, 236, 342), *M.virescens* (168, 234, 342), *M.xanthus* (17, 168, 217, 234, 260, 261, 340, 341, 342, 413, 414, 415, 416, 417), *Polyangium fuscum* (234, 236), *Sorangium cellulosum* (183), *Sorangium* spp. (179, 234, 342) and *Stigmatella aurantiaca* (326, 327, 418). The surface structure of *Sporocytophaga myxococcoides* has been investigated (258, 410, 411) as well as the internal anatomy (166, 167). The ultrastructure of four species of *Cytophaga* has been described, namely of *Cytophaga (Chondrococcus) columnaris* (237, 299, 303, 304), *C.hutchinsonii* (258, 410, 411), *C.johnsonae* (114, 115, 420) and *C.marinoflava* (405). Follett and Webley's limited electron microscope studies on *Flavobacterium aquatile* NCIB 8694 (115) demonstrated a 'remarkable' morphological similarity to *Cytophaga johnsonae*, but this appears not so remarkable in view of the similarity in DNA base composition (93) and general cultural characteristics (275, see page 66). The ultrastructure of two *Flexibacter* species were described by Hageage (149) but no illustrations were provided, and *Flexibacter* FS-1 has been studied by Simon's group (314, 349, 350).

The vegetative cells of the myxobacteria are Gram negative rods which are of three size ranges (100). They may be thick rods with blunt ends (eg. 1.2 x 2.5μ) exemplified by *Sorangium;* thin, delicate rods (eg. 0.5 x 5-10μ) such as found in the genera *Cytophaga* and *Sporocytophaga,* or they may be of an intermediate size (eg. 0.75 x 5.0μ) exemplified by *Myxococcus*. The cells of *Flexibacter* appear to be less than 1μ wide and may form extremely long, often septate filaments (eg. 0.7 x 400μ). By contrast the *Cytophaga*-like strains presently known as *Flavobacteria* can appear as regular rods or even coccobacilli (275).

The low optical refractility, flexibility (at least for the longer cells, and this may be a function of their length), and gliding motility of the vegetative cells of myxobacteria had previously led to the acceptance of the assumption that these cells either lacked a rigid cell wall (375), or that they had no cell wall at all (179, 232, 404), even though the microcysts supposedly possessed a refractile, deeply-staining wall. The sensitivity of gliding bacteria to actinomycin D suggested to Dworkin (102, 103) that their surfaces were different from other Gram negative bacteria. In the last 15 years. electron micrographs of sufficient resolution to permit an examination of this question have revealed clearly that these micro-organisms possess a cell wall essentially indistinguishable from that of other Gram negative bacteria. Studies on the surface layers of the vegetative cells of *Cytophaga (Chondrococcus) columnaris* (304) have indicated that a third, electron-dense layer exists outside the unit plasma membrane (Figure 7).

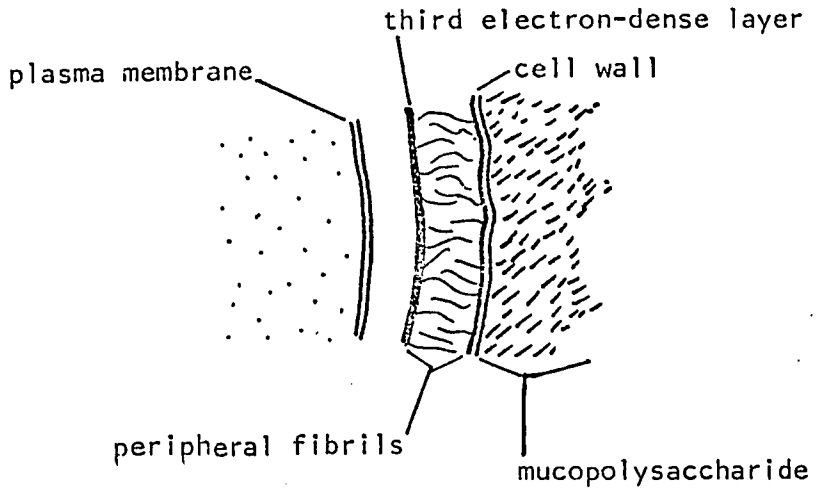


Figure 7. Diagrammatic transverse section of the surface layers of *C.columnaris* (after Pate and Ordal 1967 b).

Between this new layer and the tripartite wall there is a layer of peripheral fibres which, it is suggested, may play a role in gliding motility. On the very outside lies a slimy substance, probably an acid mucopolysaccharide. This interesting third wall layer has also been described in *Myxococcus xanthus* (414), *Stigmatella aurantiaca* (327), myxobacter N-5 (385), in the microcyst but not the vegetative cells of *Sporocytophaga myxococcoides* (166, 167) and in *C.johnsonae* (115) but not in *C.marinoflava* (405). The "extra" layer is not unique to the Myxobacterales; it has also been described for two species of *Flexibacter* (149), *E.coli* and *Spirillum serpens* (382), *Vitreoscilla, Simonsiella, Beggiatoa, Acetobacter* and *Nitrosomonas* species (278). It is the "solid membrane" in *Veillonella* (34), and the "dense layer" of *Asticacaulis* (300). This electron-dense layer takes part in septum formation when this form of division occurs in

Gram negative bacteria (382). The cell wall of these gliding organisms is thus seen to be of similar morphology to several other flexing and non-flexing Gram negative bacteria belonging to five different Orders. It may be that the difference in flexibility resides in a difference in macromolecular arrangement not detectable by methods used so far.

Copious amounts of an extracellular slime are produced during active growth in liquid by *Sporocytophaga myxococcoides* and *Cytophaga hutchinsonii* (258). This was found to consist of a densely interwoven network of filamentous material, possibly anionic heteropolysaccharide, originating on the cell surface and extending far into the medium without any discernible boundary. Three years before this observation, Follett and Webley had noted the loose, crenated, flexible outer membrane (cell wall) of *C.johnsonae* which is external to the 'amorphous' or 'peripheral fibril' layer. This layer may be very thick and slimy, with slime extruding through postulated pores in the outer membrane, forming a slime layer comparable to the extracellular capsules of other bacteria (115). The origin of the slime in various fruiting myxobacteria was also found to be within the cell wall (342).

As early as 1958 it was reported the *M.xanthus* cell walls had the same chemical components as those of eubacteria (196, 260). The wall of *C.columnaris* was found to consist mainly of galactosamine glycan (193). Similarly the major cell wall polymer of *S.myxococcoides* and *C.hutchinsonii* has been found to be a mucopeptide (410, 411, 434, 435) with muramic acid, glucosamine, 2,6-diaminopimelic acid,

glutamic acid and alanine occurring as the minor constituents in the molar ratio 1:1:1:1:2. Other typical macromolecules which are prominent accessory cell wall materials in eubacteria, such as teichoic acids, proteins and polysaccharides were not found in these *Cytophaga* and *Sporocytophaga* walls.

Vegetative cells of *Myxococcus xanthus* (413, 415), *Stigmatella aurantiaca* (327), *Chondromyces apiculatus* (4), *C.crocatus* (3), *Sporocytophaga myxococcoides* (167), *Cytophaga (Chondrococcus) columnaris* (303), *Cytophaga marinoflava* (405) and *Flexibacter* species (149, 350) possess peripherally located granular cytoplasm and fibrillar material, mostly axially situated, which are typical of bacterial cells. Mesosomes occur in many Gram negative organisms (413) but in contrast to Gram positive species they do not seem to occur regularly near the division plane (112). Various types have been recognized in *M.fulvus, M.virescens M.xanthus, C.apiculatus, C.crocatus, S.myxococcoides, C.columnaris* and *C.marinoflava*. Other intracytoplasmic membrane systems have also been observed especially in dividing cells and in cells in which viruses were being formed. Ribosomal particles or materials are reported in *M.xanthus, S.aurantiaca, S.myxococcoides, C.columnaris* and in two species of *Flexibacter*. Storage materials, probably metaphosphate granules (153, 419) have been described in *M.xanthus, S.aurantiaca* and *S.myxococcoides*, and possible polysaccharide granules in *S.aurantiaca*.

Curious rod-shaped particles, at first described as 'rhapidosomes' when they were observed in *Saprospira grandis* (85, 244)

have been found in lysates from several genera of myxobacteria. Rhapidosomes resemble bacteriophage tails, for instance of the coliphage T4 (276), but are often found alone, with no phage heads, coats or other phage-like appendages. Reichenbach (324) came to the conclusion that the structures that he found in *Archangium violaceum* were defective phage particles. So far seven phages have been demonstrated in *Cytophaga (Chondrococcus) columnaris* (8, 215, 216, 237, 299, 301, 302), and phages or rhapidosomes have been described for *Archangium violaceum* (324), *Chondrococcus coralloides* (299), *Myxococcus xanthus* (59, 61, 416), *?Sorangium* 495 (299), *Sporocytophaga myxococcoides* (137, 167, 299), *S.cauliformis* and *Cytophaga* species (137) and *Cytophaga marinoflava* (69, 405, 406) as well as in the flexibacterium *Saprospira grandis* (85, 244, 328).

Rhapidosomes are channelled rods often with a contracted sheath and protruding core. Their size has been variously reported as averaging 170 Å long for *S.myxococcoides* (167), 920 x 240 Å for *A.violaceum* (322), 1720 x 228 Å for *S.grandis* (328) and 5-15,000 x 300 Å for *C.columnaris* (299). Myxobacterial phages have been reported as having typical polyhedral heads and a tail encased in a contractile sheath and terminating in a tail plate. The sizes reported are for myxophage MX-1 of *M.xanthus*, head 750 Å diameter, tail 1000 Å long; and for myxophage C2 of *C.columnaris*, head 600 Å diameter, tail 1000 x 200 Å. These double-stranded DNA phages are species-specific and their size, morphology, base composition (55.5% GC for *M.xanthus* myxophage MX-1), and life cycle are grossly similar to those of other such bacterial viruses for example the coliphage T-2 (44).

VI.2.2 Materials and methods

The organism used was *Cytophaga johnsonae* var. *denitrificans* strain Cook 405, which was grown for varying periods of time in 0.2% tryptone broth (see Cook's Cytophaga Agar, Table 16, page 105) on a New Brunswick G-2 rotator at 100 rpm or on Skim Milk agar plates at room temperature (about 25°C).

The basic procedure for the preparation of thin sections entailed centrifuging a one-or-two-day old culture broth for 10 minutes at 7,000 rpm, washing in 0.1 M phosphate buffer (pH 7.2) and recentrifuging. A small amount of the slimy pellet was placed in the base of a glass vial, forming a layer 1-1.5 mm thick on the bottom, and fixed in 3% v/v glutaraldehyde in 0.1M phosphate buffer (pH 7.2) for two hours at room temperature (334). Washing in buffer for 3 to 48 hours (3 changes) was followed by fixing in osmium tetroxide (1% w/v in 0.1M PO_4 buffer, pH 7.2) for three hours at room temperature. After further washing with three changes of buffer in 2 to 24 hours, the material was dehydrated in an alcohol series; 70% ethyl alcohol (10 minutes), 85% (10 minutes), 98.5% (10 minutes) and 98.5% (20 minutes). Propylene oxide was then added for 10 minutes, and fresh propylene oxide for a further 20 minutes, to which an equal volume of a 70:30 Epon 812 mixture (251) was subsequently added and mixed thoroughly. The vial was left overnight with the cork removed to facilitate the penetration of the Epon and the volatilization of the propylene oxide. This mixture was then substituted with a pure 70:30 Epon 812 mixture and heated at 60°C for 18-24 hours.

Sectioning was done on a Porter-Blum Mark II ultra-microtome with glass knives, using 10% acetone to float the sections. These were then supported on #200 copper grids with Formvar films. Staining of sections was done with 3% w/v uranyl acetate (5-10 minutes) and/or 0.2% w/v lead citrate in 1/10 N NaOH (5-10 minutes) (331). All of the electron micrographs were made with a Philips EM 200 electron microscope. This basic procedure was variously modified during the course of this largely methodological study. Notably the length of fixation time in osmium tetroxide was increased to experimental times up to 24 hours, and uranyl acetate staining of the pellet was introduced immediately before the dehydration with ethanol. Problems were also encountered with penetration and hardening of the Epon blocks, and to help remedy this smaller pieces of the fixed material were used in the final embedding, and also the blocks were rehardened for a further period of up to 24 hours.

For negative staining broth and plate cultures of various ages were used. Concentration of broth cultures by centrifugation to about 1/10 volume was found to be necessary to get an adequate number of bacterial cells on a grid. The grids were dried, washed in 0.1M phosphate buffer (pH 7.2) and stained for varying lengths of time with acid, neutral, or basic 1% phosphotungstic acid (PTA) (43) or with 3% w/v uranyl acetate (170). The stain was washed off with distilled water and the grid dried.

Both broth and plate cultures of various ages were used for shadowed preparations, shadowing with uranium oxide was done unilaterally and on a revolving turntable at a low angle (39).

VI.2.3. Results

(i) Methodological

Penetration of the Epon resin was better when only a very small amount of cell material was used. The initial blocks made with glutaraldehyde and 3 hours OsO_4 fixation (Methods 1 and 2, see page 146) gave sections with poor contrast, and so further blocks were given 6 (Method 3), 9, 12 (Method 4), or 24 hours in OsO_4 respectively. Of these it seems that the 6 hour blocks are the best. Uranyl acetate staining of the pellet just prior to dehydration was an improvement on the unstained material of course, but a longer period of staining would give slightly better results. Experiments with uranyl acetate and lead citrate staining of the mounted sections indicated that about five minutes exposure to each is best for this particular material (Methods 2, 3 and 4). As can readily be seen from the photomicrographs, there are still problems with definition of cellular structure. It may be that properties of the dense slime layer surrounding these bacteria and/or reactions of the fixatives and stains with cellular components account for some of these problems. Some lead carbonate spots are also present on some sections, but the final technique has eliminated their production.

In the negative staining studies some difficulty was experienced with clumping of cells from the air-drying of liquid broth concentrates, and from the slime layers around cells from plate cultures. Nevertheless, some single separated cells were present on all grids. Staining times necessary for the various

mixtures were:

Uranyl acetate	3 mins	(Method 9)
Acid PTA	2 mins	(Method 7)
Neutral PTA	2-3 mins	(Methods 5 and 6)
Basic PTA	20-40 mins	(Method 8)

As could be expected, the four stains had different reactions with the cells and hence different types of information were obtained.

Clumping of cells was also a problem in the shadowing experiment. The unidirectional shadowing (Method 11) gave very little information but the later rotating shadowing (Method 12) was more profitable.

(ii) Observations on *C. johnsonae*

The longitudinal (Figures 8a, 8e) and transverse (Figure 8c) sections generally show features similar to cells of other cytophagas and fruiting myxobacteria. In transverse section the cells are often slightly ovoid rather than round and their diameter is about 0.3 to 0.4 μ. This species can have quite long, thin cells but the longest seen in this study was just over 4 μ. The technique has not been refined sufficiently to allow much in the way of high resolution work, however some observations follow. The cells seem to be bounded by both outer and inner membranes, the outer being about 0.007 to 0.02 μ wide and the inner one about 0.011 to 0.015 μ wide. The peripheral cytoplasmic region surrounds the inner nucleoid area, and one to several dense bodies (possibly storage products) were found to occur towards the central region of the cell (Figure 8a). Nothing that could be interpreted as a third inner layer of the cell wall has yet been seen.

Key to Fixing and Staining Methods for Figures 8 - 14.

Thin sections

Method 1. Fixed in glutaraldehyde, then in O_sO_4 for 3 hours, pellet stained with uranyl acetate, sections stained with lead citrate. Figures 9c, 10c.

Method 2. Fixed in glutaraldehyde, then in O_sO_4 for 3 hours, pellet stained with uranyl acetate, sections stained with uranyl acetate and lead citrate. Figures 8b, f, 9b, d, e, f, g, h, 10 a, 11 a.

Method 3. As above except O_sO_4 for 6 hours. Figures 8a, c, d, 9a, 10b.

Method 4. As above except O_sO_4 for 12 hours. Figures 8e, 9i, 10 d.

Whole cells

Method 5. Negatively stained with neutral phospho-tungstic acid (PTA) for 3 mins. Figures 11b, c.

Method 6. Negatively stained with neutral PTA for 2 mins. Figures 10e, 12a.

Method 7. Negatively stained with acid PTA for 2 mins. Figures 11e, 12b, c.

Method 8. Negatively stained with basic PTA for 22 mins. Figure 11d.

Method 9. Negatively stained with uranyl acetate for 3 mins. Figure 13b.

Method 10. Negatively stained with uranyl acetate for 4 mins. Figure 13a.

Method 11. Shadowed unilaterally with uranium oxide. Figure 12d.

Method 12. Rotationally-shadowed with uranium oxide. Figures 14a, b.

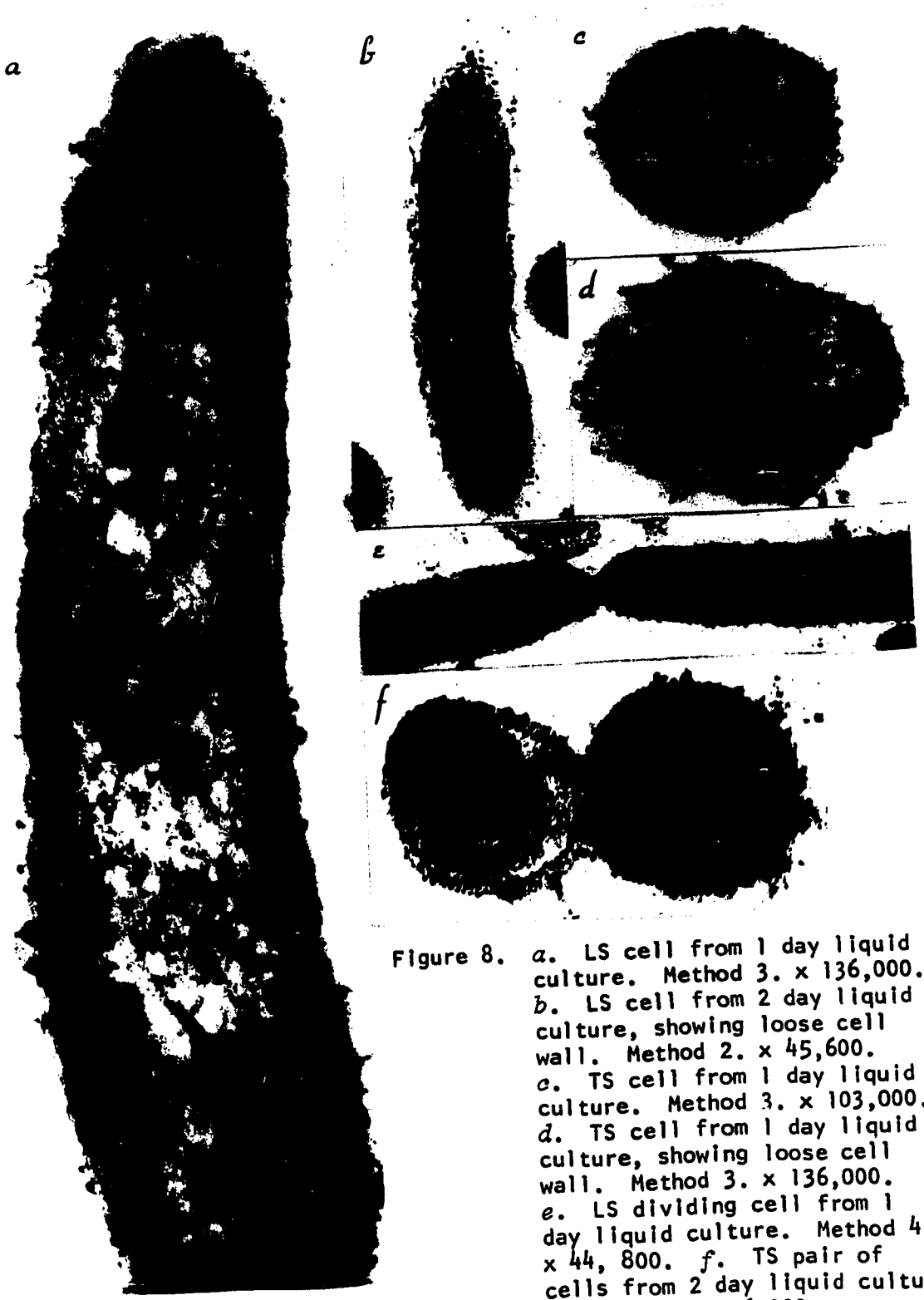


Figure 8. *a*. LS cell from 1 day liquid culture. Method 3. x 136,000. *b*. LS cell from 2 day liquid culture, showing loose cell wall. Method 2. x 45,600. *c*. TS cell from 1 day liquid culture. Method 3. x 103,000. *d*. TS cell from 1 day liquid culture, showing loose cell wall. Method 3. x 136,000. *e*. LS dividing cell from 1 day liquid culture. Method 4. x 44, 800. *f*. TS pair of cells from 2 day liquid culture. Method 2. x 136,000.

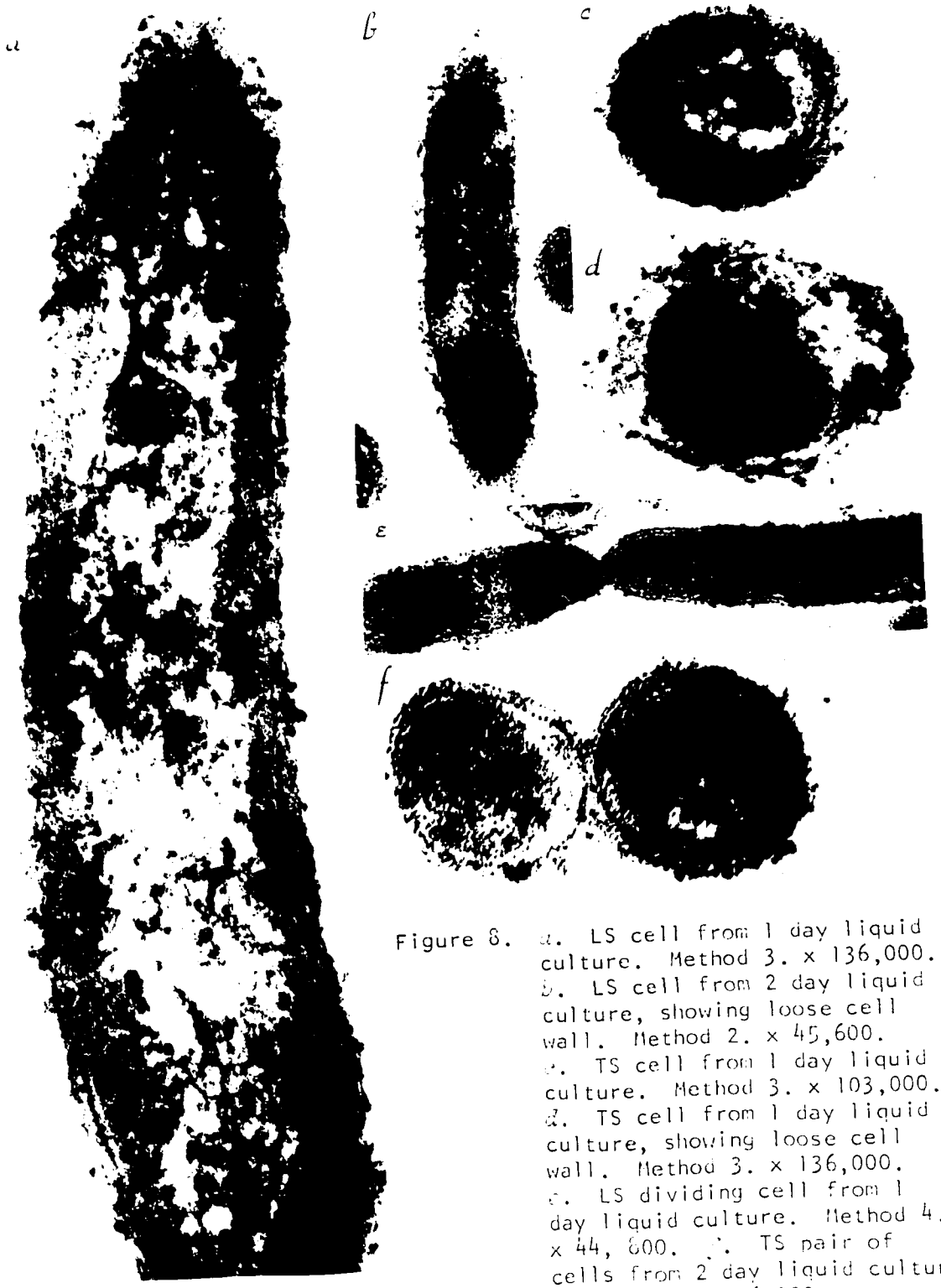


Figure 8. a. LS cell from 1 day liquid culture. Method 3. x 136,000. b. LS cell from 2 day liquid culture, showing loose cell wall. Method 2. x 45,600. c. TS cell from 1 day liquid culture. Method 3. x 103,000. d. TS cell from 1 day liquid culture, showing loose cell wall. Method 3. x 136,000. e. LS dividing cell from 1 day liquid culture. Method 4. x 44, 800. f. TS pair of cells from 2 day liquid culture. Method 2. x 136,000.

The most interesting discovery was the presence of various membrane-bound aggregations in all of the preparations examined. The photomicrographs showed a mixture of two types of transverse sections, one in which the outer and inner membrances were in contact (Figures 8a, 8c), and the other in which the membranes had separated and the inner part of the cell remained closer to one side, leaving an outer cavity at the other side (Figures 8b, 8d). Some cells were found with expanded cavities or "vesicles" attached (Figure 9a). Pairs of cells occurred with, seemingly, their outer membranes (cell walls) fused and inner (plasma) membranes intact (Figure 8f), and sometimes pairs were found within a large 'vesicle' (Figure 9b). Triplets were found without (Figure 9c) and with 'vesicles' (Figure 9d). The most common tetrad was square (Figure 9e), but flattened configurations occurred (Figure 9f), and fours were also found in 'vesicles' (Figure 9g). A 'vesicle' containing five cells and one empty cell, with a couplet perhaps about to join up with the group (Figure 9h), and a seven-celled 'vesicle' with a couplet in close proximity (Figure 10a) were also seen. Groups of eight (Figure 9i) and 10 cells (Figure 10b) within a 'vesicle' were the largest such groupings observed. It was noted that in most cases the cells were arranged in pairs within the 'vesicle (Figures 9g, 9i), and that the cells were almost invariably at the edge of the 'vesicle' with at least some of their inner membrane closely adpressed to the outer ('vesicle') membrane. The continuity of this outer membrane and its separation from the inner one can be seen especially well in Figures 8f, 9e and 9h.

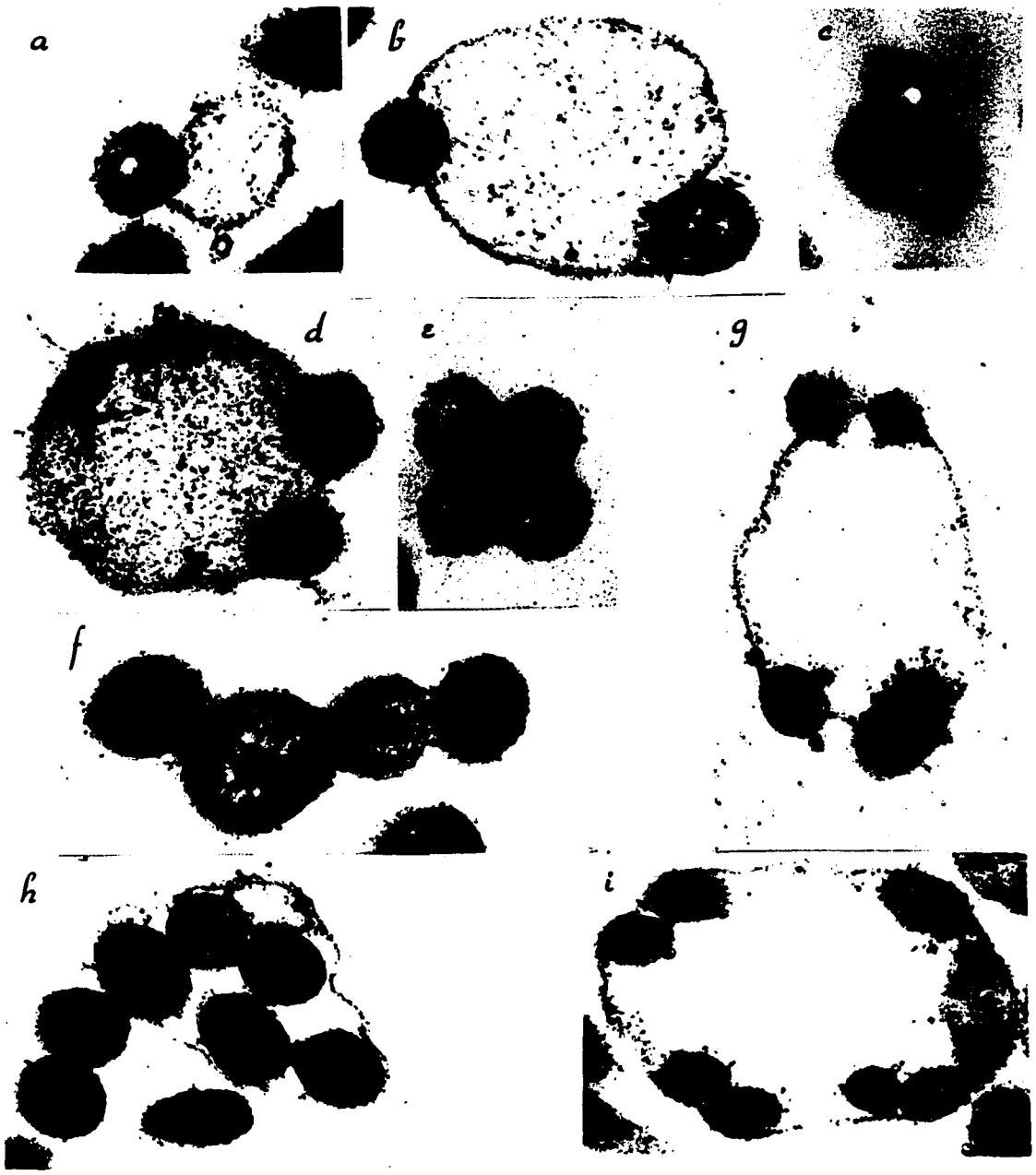


Figure 9. Transverse sections, 9*a* and *i* from 1 day, 9*b*-*h* from 2 day liquid cultures. *a*. Cell with expanded vesicle. Method 4. x 45,600. *b*. Two cells within vesicle. Method 2. x 45,600. *c*. Triplet. Method 1. x 45,600. *d*. Triplet within vesicle. Method 2. x 45,600. *e*. Quartet. Method 2. x 45,600. *f*. 'Flattened quartet'. Method 2. x 45,600. *g*. Vesicle containing 2 pairs of cells. Method 2. x 45,600. *h*. Vesicle containing 6 cells (1 empty) with another pair perhaps about to join. Method 2. x 45,600. *i*. 4 pairs of cells within a vesicle. Method 4. x 37,800.

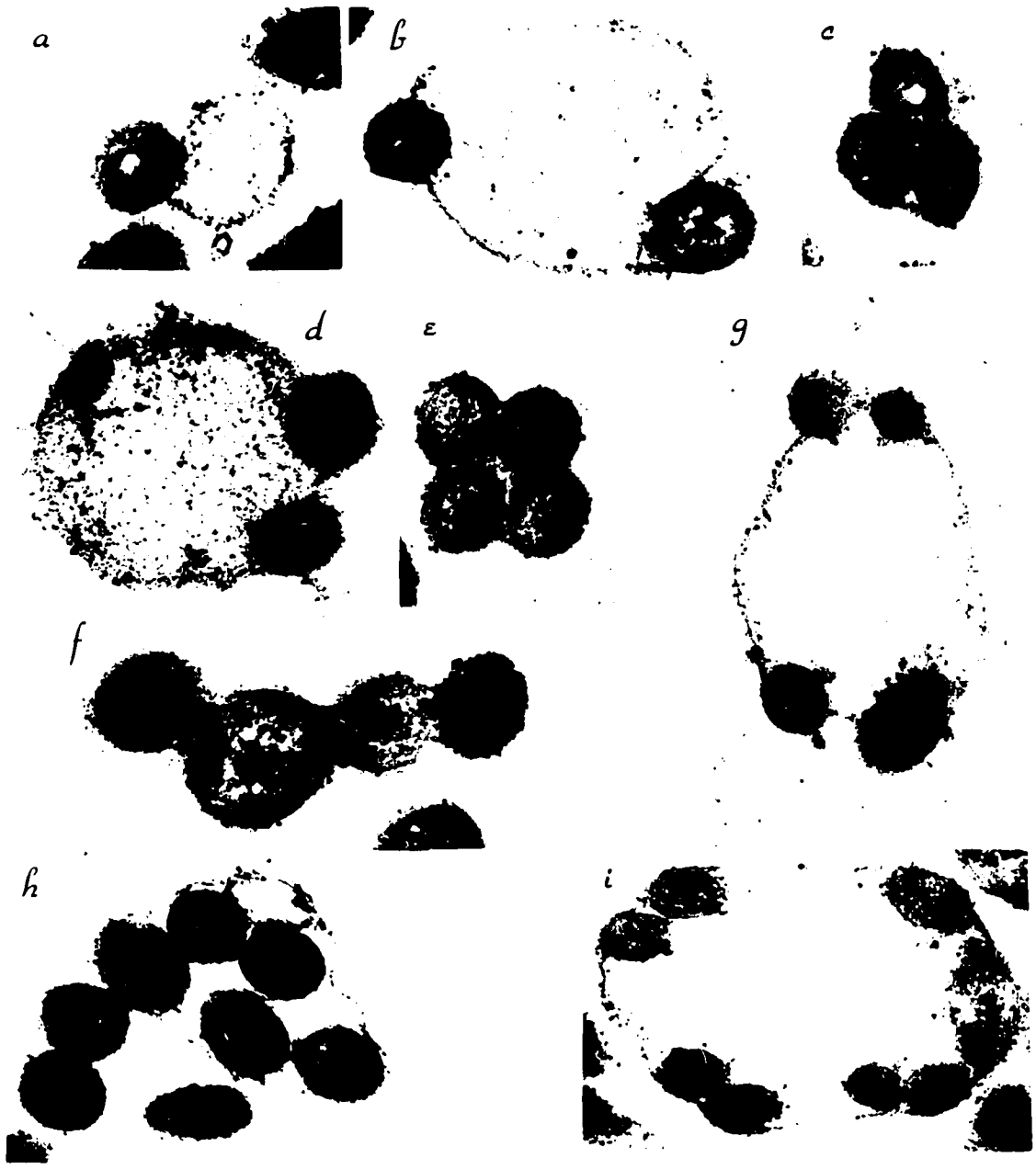


Figure 9. Transverse sections, 9*a* and *i* from 1 day, 9*b*-*h* from 2 day liquid cultures. *a*. Cell with expanded vesicle. Method 4. x 45,600. *b*. Two cells within vesicle. Method 2. x 45,600. *c*. Triplet. Method 1. x 45,600. *d*. Triplet within vesicle. Method 2. x 45,600. *e*. Quartet. Method 2. x 45,600. *f*. 'Flattened quartet'. Method 2. x 45,600. *g*. Vesicle containing 2 pairs of cells. Method 2. x 45,600. *h*. Vesicle containing 6 cells (1 empty) with another pair perhaps about to join. Method 2. x 45,600. *i*. 4 pairs of cells within a vesicle. Method 4. x 37,800.

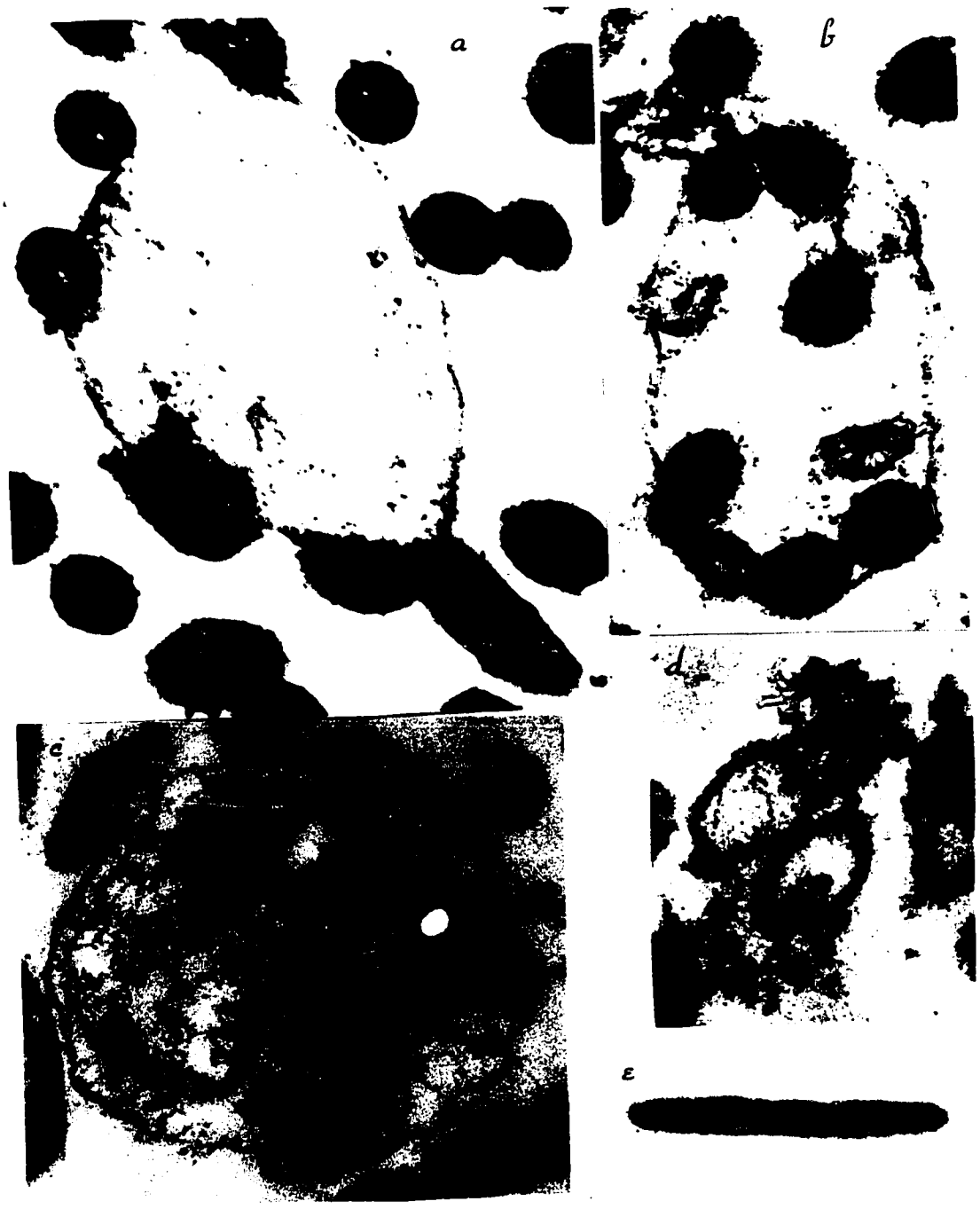


Figure 10. *a*. TS 7-celled vesicle with a pair nearby, from 2 day liquid culture. Method 2. x 44,800. *b*. TS 10-celled vesicle from 1 day liquid culture. Method 3. x 45,600. *c*. TS vesicle containing 'expanded cells', from 2 day liquid culture. Method 1. x 45,600. *d*. TS 'Budded sacs' from 1 day liquid culture. Method 4. x 45,600. *e*. Neg. stained cell from 2 day plate, showing dense spots. Method 6. x 26,700.

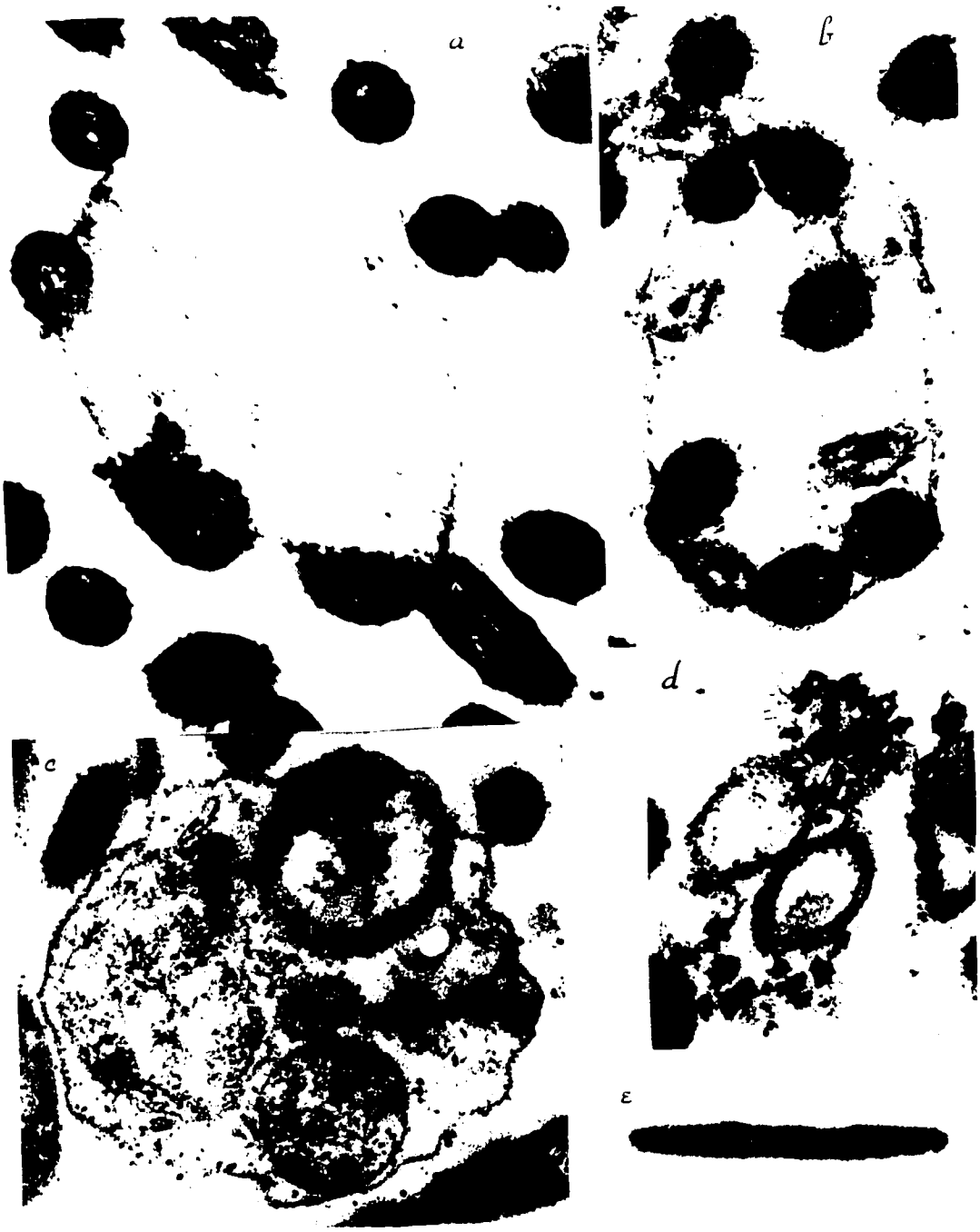


Figure 10. *a*. TS 7-celled vesicle with a pair nearby, from 2 day liquid culture. Method 2. x 44,800. *b*. TS 10-celled vesicle from 1 day liquid culture. Method 3. x 45,600. *c*. TS vesicle containing 'expanded cells', from 2 day liquid culture. Method 1. x 45,600. *d*. TS 'Budded sacs' from 1 day liquid culture. Method 4. x 45,600. *e*. Neg. stained cell from 2 day plate, showing dense spots. Method 6. x 26,700.

The thin sections also show other structures some of which may well be artefacts. These include 'vesicles' containing objects resembling 'expanded' cells (Figure 10c), and various miscellaneous 'vesicles', prolongations from outer membranes, and apparently budded sacs (Figures 10a, 10d).

Negative staining revealed further characteristics, but it is not yet known if any of these were artefacts caused by the method of staining. Plate cultures stained with neutral pH phosphotungstic acid revealed dense spots in the cells (Figure 10e), slime layers around the cell (Figure 11b), and curious small extrusions or filaments around the cell edge (Figure 11c). Cells grown in liquid culture and similarly stained, showed many more dark spots in the cells, many of which seemed to be attached to the inner surface of the membrane, and longer extrusions, resembling thick flagella were seen all around the cells (Figure 12a). When basic PTA was used a slightly different impression was obtained, the internal components being emphasized and the 'extrusions' not apparent (Figure 11d). Using acidified PTA some more observations on the surface structure were made. A 14 hour liquid-cultured cell showed a less dense outer layer and a surrounding aura of material, probably slime (Figure 11e). At 24 hours this layer of material was again seen (Figure 12b), but the 68 hour cells were remarkable for the presence of short stubby projections (Figure 12c), about 0.066 μ long and 0.03 μ wide occurring at regular intervals (about 0. 11 μ) all around the bacterial cell.

Figure 11. *a*. TS 'budded sacs' from 2 day liquid culture. Method 2. x 45,600. *b*. Neg. stained cells from 3 day plate, showing slime layer. Method 5. x 58,800. *c*. Neg. stained cells from 3 day plate, showing extrusions. Method 5. x 45,600. *d*. Neg. stained cell from 24 hr. liquid culture, showing dense areas. Method 8. x 26,700. *e*. Neg. stained cell from 14 hr. liquid culture, showing slime layer. Method 7. x 26,000.

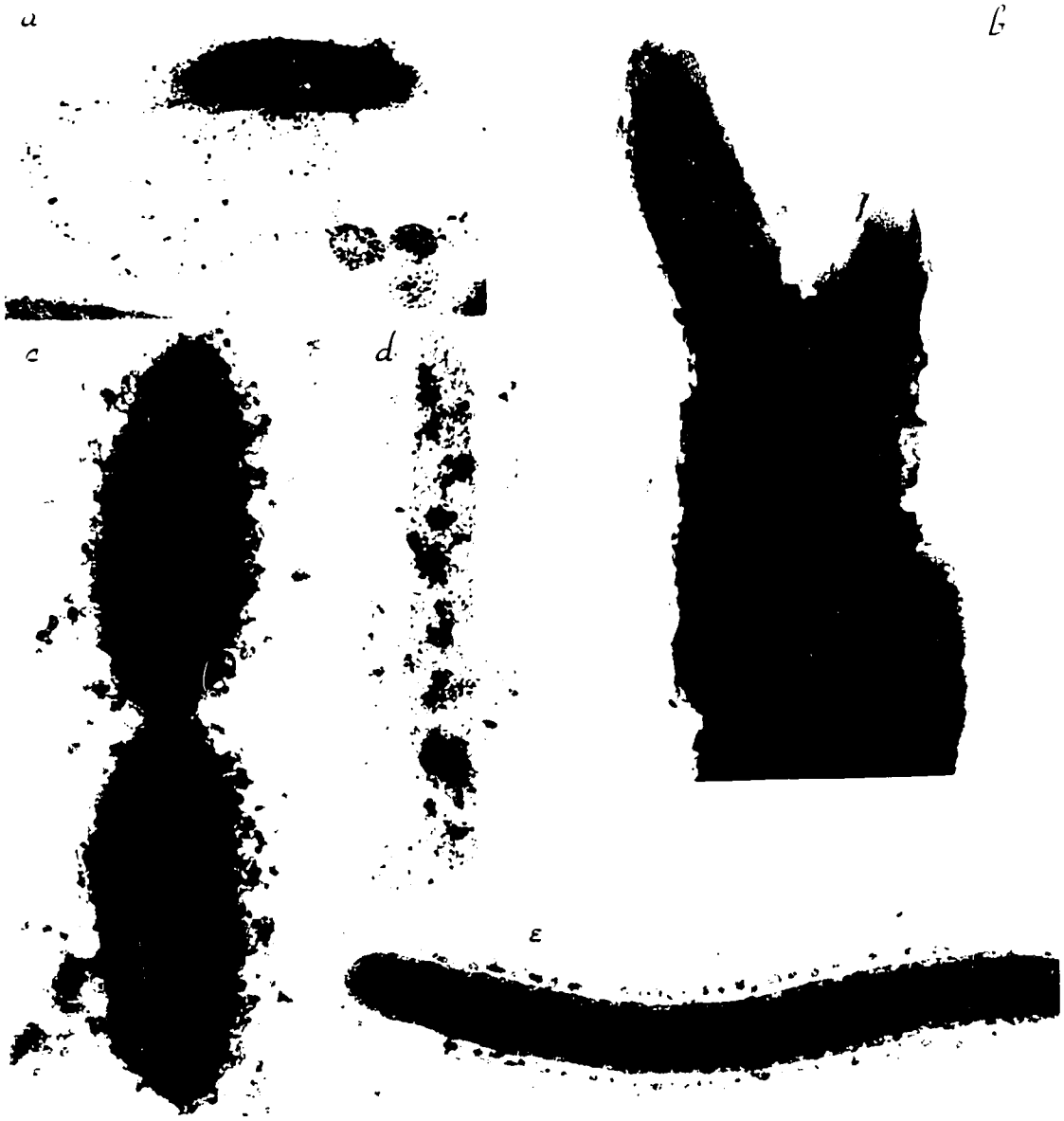


Figure 11. *a*. TS 'budded sacs' from 2 day liquid culture. Method 2. x 45,600. *b*. Neg. stained cells from 3 day plate, showing slime layer. Method 5. x 58,800. *c*. Neg. stained cells from 3 day plate, showing extrusions. Method 5. x 45,600. *d*. Neg. stained cell from 24 hr. liquid culture, showing dense areas. Method 8. x 26,700. *e*. Neg. stained cell from 14 hr. liquid culture, showing slime layer. Method 7. x 26,000.

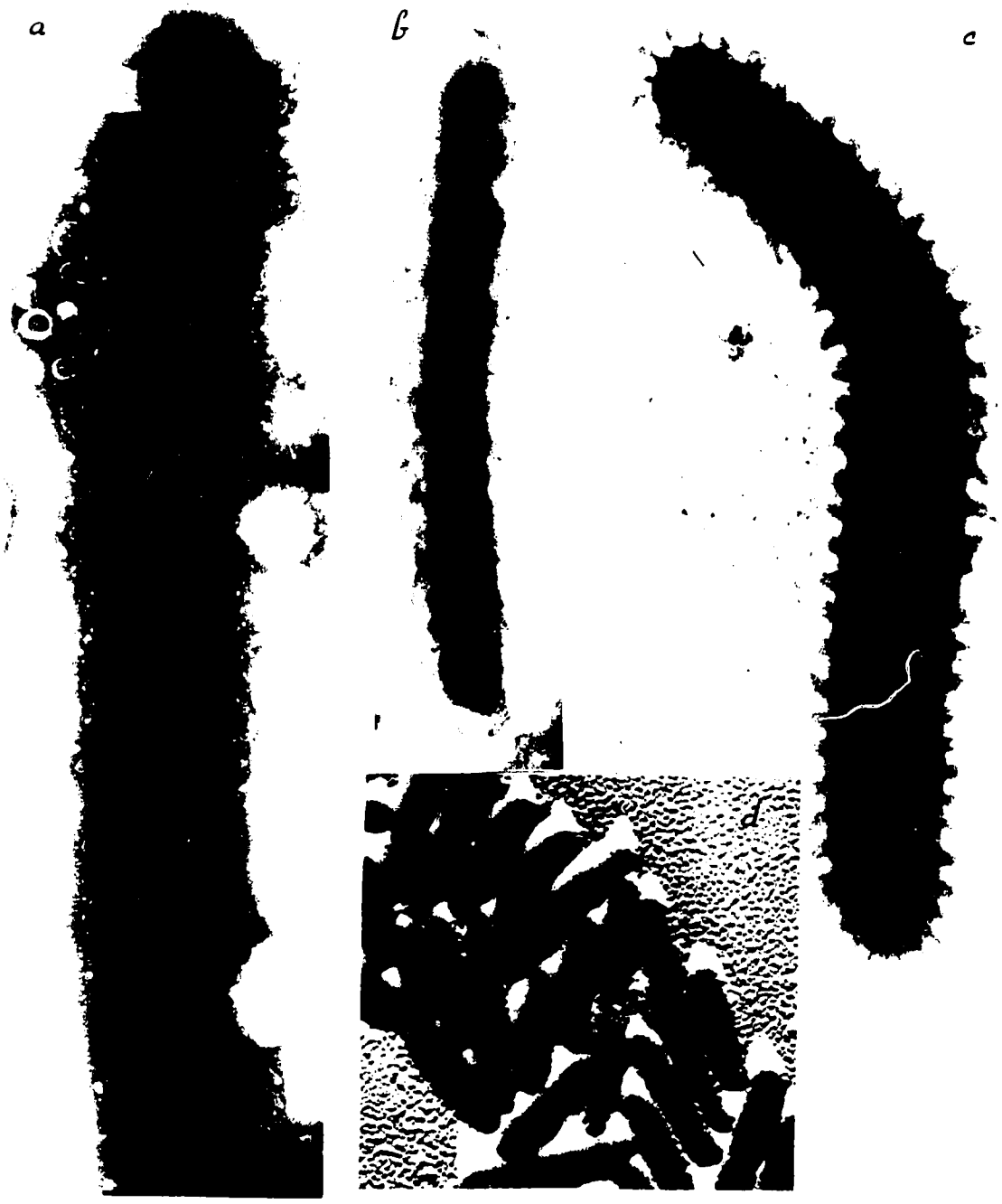


Figure 12. *a*. Neg. stained cell with extrusions, from 27 hr. liquid culture. Method 6. x 37,800. *b*. Neg. stained cell showing slime layer, from 24 hr. liquid culture. Method 7. x 37,800. *c*. Neg. stained cell with stubby projections, from 68 hr liquid culture. Method 7. x 45,600. *d*. Shadowed cells from 3 day plate. Method 11. x 16,100.

Figure 12. *a*. Neg. stained cell with extrusions, from 27 hr. liquid culture. Method 6. x 37,800. *b*. Neg. stained cell showing slime layer, from 24 hr. liquid culture. Method 7. x 37,800. *c*. Neg. stained cell with stubby projections, from 68 hr liquid culture. Method 7. x 45,600. *d*. Shadowed cells from 3 day plate. Method 11. x 16,100.

The cells exhibited the same overall dimensions as before, the longest measured being 18.8 μ x 0.34 μ. Negative staining with uranyl acetate was tried on a few liquid-cultured specimens. Where cells were separated from one another the stain was taken up fairly evenly, with the production of one or more electron-dense centres in each cell, and an indication of a slime layer surrounding it (Figure 13a). In a younger population of cells which were closely packed in small packets across the grid, (presumably a drying phenomenon) the stain was most easily retained where two bacteria were in contact, and the membrane was only distinguishable here (Figure 13b). Electron-dense bodies were again observed.

The unidirectional shadowing with uranium oxide (Figure 12d) did not give much information about the *Cytophaga* cells, this was therefore abandoned in favour of rotational shadowing. Further, the preliminary trials with plate cultures indicated that separation of the individual cells on the grids was impossible with the cells embedded in slime threads but single cells could be studied from liquid culture. These first efforts showed an abundance of fairly short rods, between 1 and 3.5μ long and about 0.25μ wide, in a three-day plate culture.

The liquid-cultured 19 hour cells prepared by rotating the grids while shadowing are featured in Figures 14a and 14b. The general appearance of the cells indicates that one or two dense bodies exist in each cell, and in many cells the rounded ends are also more electron-dense than the rest of the cell. The cells are longer (up to 5 μ long) than those from the plate culture, and some curious spheres occur at the juxtapositions of two ends of cells in some cases (Figure 14a).

Figure 13. *a*. Neg. stained cell showing dense spots and slime, from 24 hr. liquid culture. Method 10. x 45,600. *b*. Neg. stained cells showing dense spots and membranes, from 14 hr. liquid culture. Method 9. x 45,600.

Figure 13. *a*. Neg. stained cell showing dense spots and slime, from 24 hr. liquid culture. Method 10. x 45,600. *b*. Neg. stained cells showing dense spots and membranes, from 14 hr. liquid culture. Method 9. x 45,600.

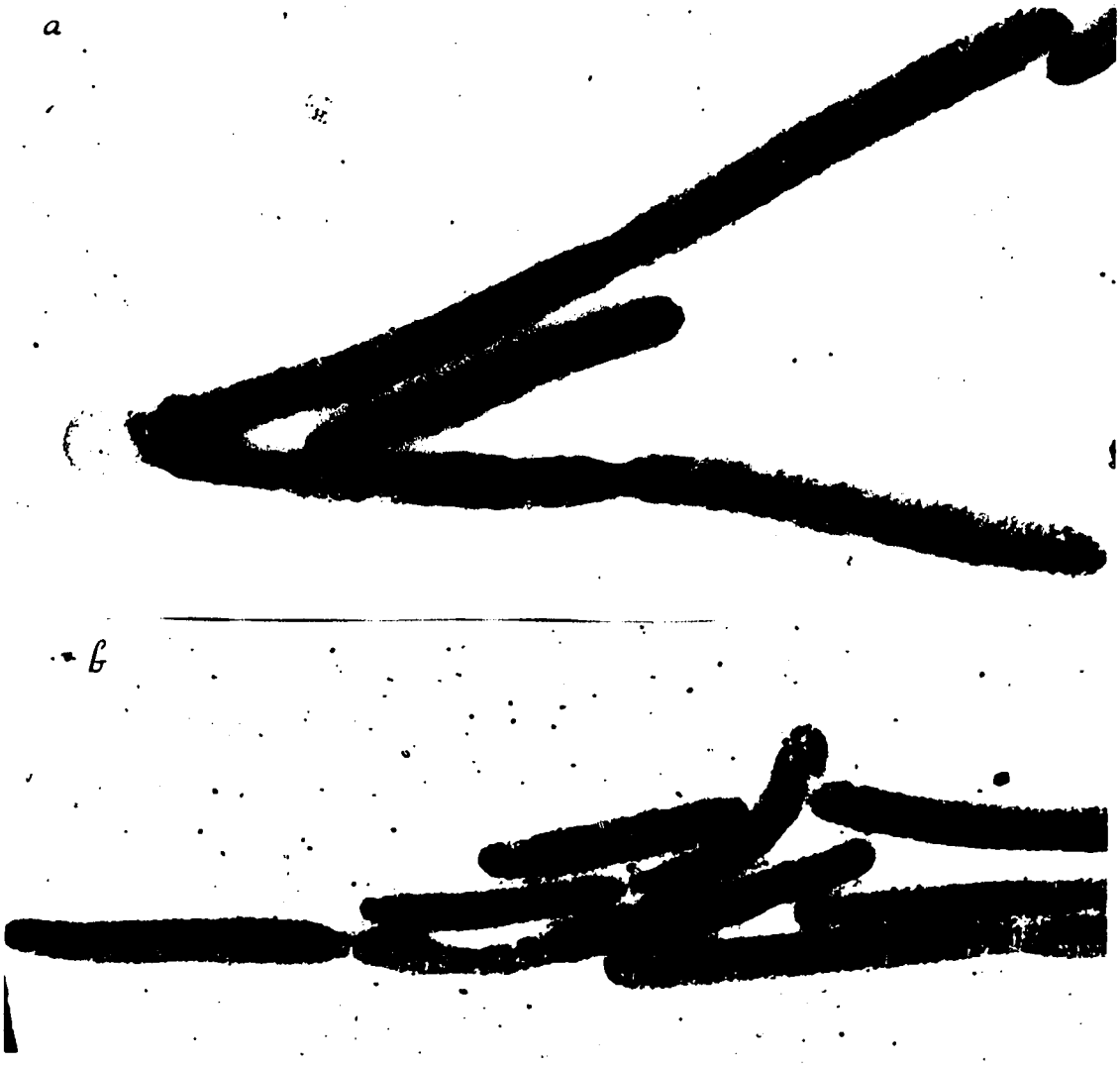


Figure 14. Shadowed cells from 19 hr. liquid culture. Method 12. *a*. Cells with sphere at end. x 20,300. *b*. Cell with dense spots centrally and terminally located. x 16,100.

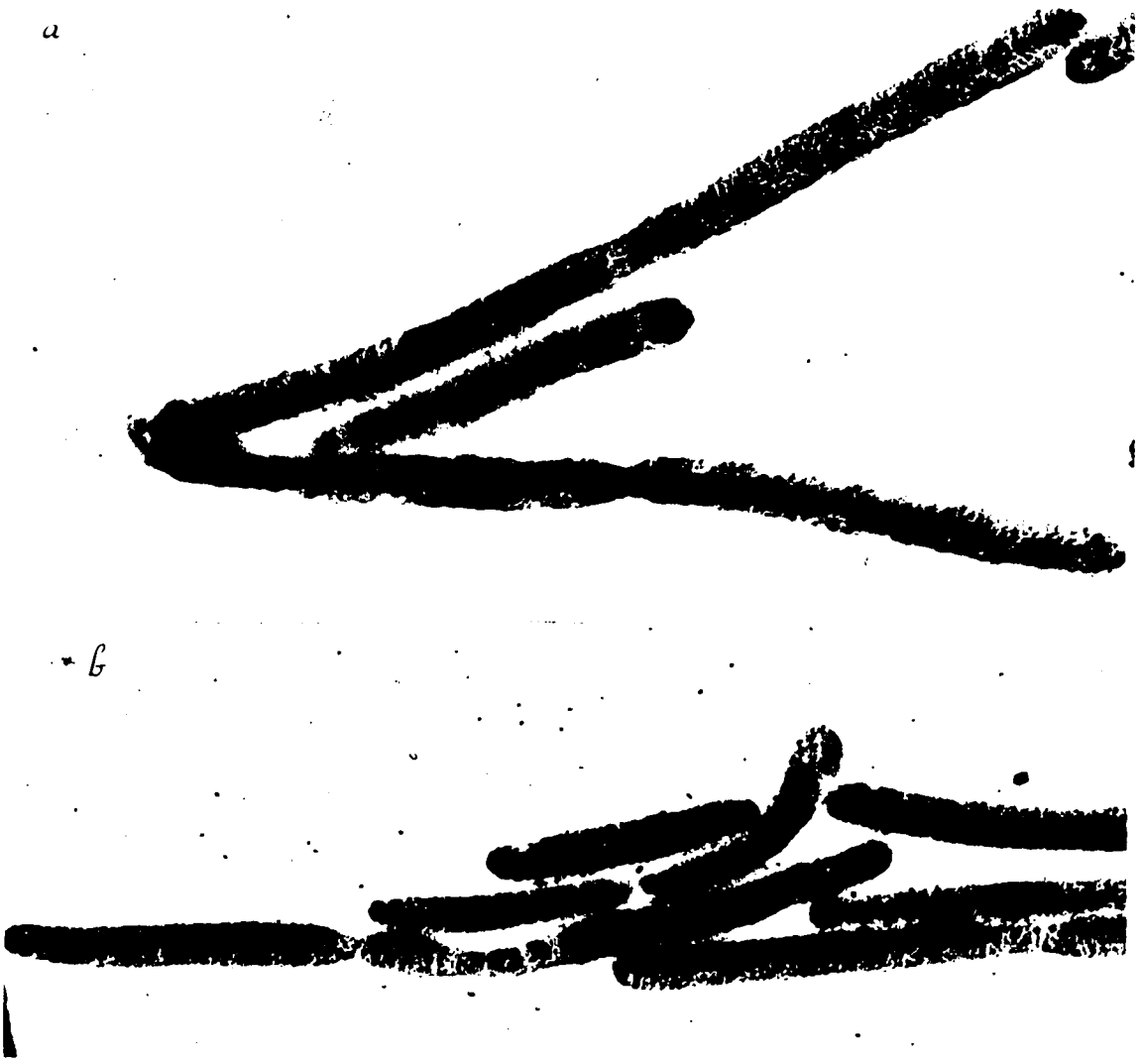


Figure 14. Shadowed cells from 19 hr. liquid culture. Method 12. *a*. Cells with sphere at end. x 20,300. *b*. Cell with dense spots centrally and terminally located. x 16,100.

VI.2.4. Discussion

The observations on the thin sections and whole cells indicate that *C.johnsonae* has a similar morphology and structure to that of the fruiting myxobacters and other *Cytophaga* species investigated. This is especially true in the case of the separable outer membrane, which bears close resemblance to that observed in *Cytophaga (Chondrococcus) columnaris* (303, 304) and was also seen in *C.johnsonae* by Follett and Webley (115). The dense inner bodies too have been frequently observed in fruiting genera and also in *Cytophaga marinoflava* (405) and *C.johnsonae* (115). These have been interpreted as nuclei in *Sorangium* (179) although this has been repudiated (236), as non-nuclear "unstructured inclusions" in *Chondromyces apiculatus*, *Myxococcus rubescens* and *Polyangium fuscum* (236), and most recently as deposits of polyphosphates in *Myxococcus xanthus* (419), *Sporocytophaga myxococcoides* (167) and *Stigmatella aurantiaca* (327).

The most interesting and important finding seems to be that of 'vesicle' formation, a hypothetical summary of which is given in Figure 15. Cells seem to pair up and then join in the formation of 'vesicles'. These are composed of fused outer membranes, and contain the cells each surrounded by a single (inner) membrane. Each of these membranes seems to be a typical 'unit membrane' of two electron-opaque fine lines separated by an electron-transparent area. The cells were nearly always distinctly paired and very few odd numbers of cells were encountered in the vesicles. It is suggested therefore that cells join this association in pairs, and that triplets

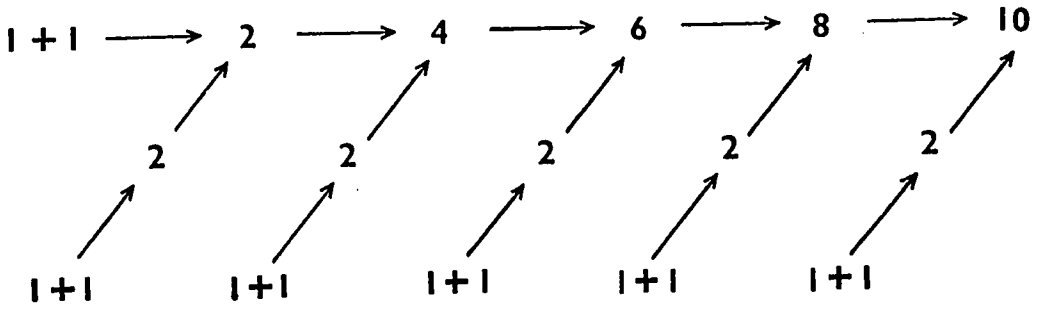
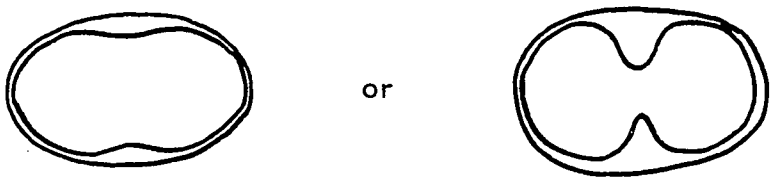


Figure 15. Hypothesis of 'vesicle' formation.

or other odd-numbered groups are artefacts of sectioning, for it is impossible to cut through every cell in such a three-dimensional object every time if they are randomly oriented. It is to be noted that Hendrie et al. (164) suggested that the pairing of cells in older cultures is very characteristic of the genus *Cytophaga*. It could also be argued that instead of the pairing hypothesized for these cells, they are in fact dividing into two, four, etc. with an incomplete division of the membranes. However no intermediate stages such as



have ever been seen, and this species usually replicates by fission of the shortest axis not the longest one, as is usual with most bacteria. Ten is the largest number of cells seen so far in one 'vesicle'. The subsequent history of these associations is indeed food for speculation, as the genus *Cytophaga* has been classified largely on its apparent inability to form fruiting structures or microcysts. It is intended that further work will be initiated in this area.

Explanations of the various other structures seen in the sections are more difficult. The 'vesicles' containing what appear to be 'expanded' cells could possibly be older cells which have taken on the role of slime production. Such cells have been suggested for *Myxococcus xanthus* (340). The 'vesicles' devoid of contents could well be sections through cell-containing 'vesicles' which by chance do not pass through any cells. The miscellaneous sacs and apparently budded membranous spherical bodies could be breakdown products upon lysis or severe change in the cells caused by the fixation, embedding and staining.

The photomicrographs of the negatively stained cells agree extremely well with those of *C.johnsonae* and *F.aquatile* (115), and of *C.hutchinsonii* and *S.myxococcoides* (258). This is especially noteworthy since Follett and Webley used ammonium molybdate, Martin's group used sodium phosphotungstate, and acid, neutral or basic phosphotungstic acid or uranyl acetate were used in this study. A slime layer could be readily detected in most preparations and the surface appeared crinkled in some. Strands and sacs, perhaps containing

slime, were evident along the cell edge, sometimes in a regular fashion and at others very irregularly. Similar extrusions have been seen in *Myxococcus xanthus* (115, 304, 340), *S.myxococcoides* and *C.hutchinsonii* (258), as well as in *C.johnsonae* (115) and *Flexibacter* FS-1 (349, 350). Up to eleven mesosome-like membranous objects were seen in neutral PTA-stained cells (Figure 12a). The electron-dense areas seen with neutral PTA and with uranyl acetate were presumed to be storage polyphosphates.

The general image presented by the shadowed cells was of a highly crenated surface with irregular depressions of varying length and height. Follett and Webley also found this kind of surface in *C.johnsonae* and correlated it with the deep and irregular undulations of the outer membrane in their thin sections. However few thin sections in the present study showed cells with a wavy outer membrane, most were fairly regular. The electron-dense areas were presumed to be polyphosphate granules again, but the curious spheres at the ends of two juxtaposed cells are unexplained.

The slime or extracellular polysaccharide mentioned earlier was very evident in the cells of this species. With the Myxobacterales, the fruiting members of which have long been known to aggregate, and with the possibility now of a co-operative alliance between *Cytophaga* cells it is interesting to comment on the work of Kalckar (201) on 'cell sociology'. He has pointed out the involvement of polysaccharides in the 'social patterns' of cells, for example when mutations result in changes in extracellular polysaccharides then co-operative

ventures of these cells such as colony morphology are changed as well as such individual properties as immunological response and phage receptor sites. In higher organisms certain surface polysaccharides seem to be important for the existence of recognition sites on erythrocytes. In a study of the attachment areas of cells in the epidermis of developing newts (212), it was shown that acid mucopolysaccharides were heavily concentrated in the desmosomes. In view of the characters controlled by surface polysaccharides in general and the adhesive properties attributed to acid mucopolysaccharides (specifically demonstrated in *Myxococcus xanthus*, *M.fulvus* and *C.columnaris* (304) in particular, the possible significance of such extracellular substances in the myxobacteria and cytophagas becomes apparent.

CHAPTER VII

NEW APPROACHES TO THE MORPHOLOGY OF CYTOPHAGAS

VII.1 COLOUR: INTRODUCTION OF THE MUNSELL SYSTEM TO BACTERIOLOGY

VII.1.1. Colour as a taxonomic criterion

For years colour has been one of the first characteristics noted about a micro-organism, mainly because it is conspicuous, relatively constant and easy to record. Many names of taxa in current use spring to mind as being based upon a colour relationship for instance *Chromobacterium violaceum*, Achromobacteraceae, *Chlorochromatium* and *Rhodomicrobium*. Myxobacteria are invariably pigmented and many of the genera, notably *Myxococcus*, *Polyangium*, *Angiococcus* and *Cytophaga*, are classified in Bergey's Manual (42) largely on the basis of their pigmentation. This ease of observation has however led to problems where other characters have not been studied or given due weight. For example the genus *Flavobacterium* was created in 1923 (30) not because the bacteria which were included had been studied and found to be a natural group, but rather because colour was very noticeable (427). It is obviously fallacious to assume that pigmentation describes a biological relationship and that differentiation may be based upon hue alone.

Cowan (86) considers colour among his 'top ten' important taxonomic characteristics. He notes however, that many cultural characteristics notably colour are subjective and are also influenced

by temperature, duration of incubation and the nutritional qualities of the medium. The colours of myxangia for example are usually intensified on richer media (187). In a study of a large number of flexibacteria isolates Lewin and Lounsbery (246) concluded that colour was a relatively constant character, and in the course of laboratory subculturing for several years, they did not observe any case of a major change in the general colour of any strain. Cohen (74) is more conservative but concedes that colour may be taxonomically valuable in some instances as in spore colour of the Myxomycetes, and can be used with discretion.

Since superficially similar colony colour may be due to the presence of different compounds, it is essential to know the type of pigment present as well as its colour in any definitive study. Pigmentation has proven taxonomic value when the chemistry is known for example in the work on photosynthetic bacteria by Jensen (189) and on Gram positive carotenoid-producers (428). Chemical characterization may even show species or generic specificity in certain cases for instance in *Xanthomonas* (380). However, many colours and individual pigments are widely distributed among bacteria, the ability to produce yellow or orange pigments for example is very common, and carotenoids alone have been described from 12 different families (164).

The non-diffusible pigments of myxobacteria are red, orange, yellow, cream or sometimes green. Jahn (186) suggested that these were carotenoids, and subsequent analyses have confirmed this in *Myxococcus xanthus* (60, 62, 63, 145, 146), *M.fulvus* (145, 146, 325), *M.virescens* (145, 146, 147), *Polyangium* sp. (219), *Sorangium*

cellulosum (329), *S.compositum* (221), *Stigmatella aurantiaca* (218, 220), *Cytophaga aurantiaca, C.latercula, C.psychrophila* and also in the flexibacterium *F.roseolus* (246). Diffusible dark brown pigments, probably melanin-like (385), are also known for example in *Archangium violaceum* (323), *Cytophaga krzemieniewskae* (375), *C.succinicans* (385), and in numerous unidentified strains (83). Woods in 1948 (437) and later Greene and Leadbetter (146, 147) reported that the nature of the pigmentation in *Myxococcus* was a function of whether the cells were grown in the light or dark. This photo-induction of carotenoids is probably a generalized phenomenon (20). The carotenoids of *Myxococcus xanthus* have been shown to serve a photo-protective function (60, 62, 63) as originally described by Stanier's group for the Athiorhodaceae (354).

Even though precise characterization of pigments is now being undertaken, it is nevertheless inevitable that routine descriptions of bacterial colonies will include their colour, and this would be better designated in a more objective manner than is used at present. After all, there are a limited number of names that can be applied to colours, and these are subjective and apt to change with the times. Hence the introduction of a qualitative descriptive system to bacteriology is long overdue.

Such a system was developed by Munsell in 1915 (277) and has found uses in such widely diverse fields as textiles, soil profiles, plant nutrient deficiencies and packaged meat (73). The Munsell numerical code is simple and has the added advantage that

difficulties in translation into other languages such as occur with the nuances of word meanings will never occur. Visual comparison of the sample is made against Munsell colour chips (glossy or matte finish) under standard illumination. The Munsell system of colour notation identifies colour in terms of three attributes, hue, value and chroma in that order. The notation for a typical cytophaga for example, 7.5 YR 7/10 specifies the hue 7.5 Yellow Red which is one of the intermediate steps between Red and Yellow Red, the value 7 on the scale of lightness and darkness where 0 is Black and 10 is White, and 10 on the level of chroma, where the chroma or intensity of the colour increases from 0 (neutral) to 16 or 18 depending on the hue. Figure 16 illustrates a range of hues of constant value and chroma, and Figure 17 will serve as an example of the variation in value and chroma for the hue 7.5 YR.

VII.1.2 Method

A selection of different strains of cytophagas, flexibacteria and flavobacteria were grown on a variety of agar media (see Chapter VIII), and incubated at 25°C for various lengths of time. Several repeat plates were done at different times to assess the reproducibility of the results. Standard mounds of bacterial cells for comparison with the reference colours were made as follows. The total surface growth of the bacterial colony was scraped up with a loop and mixed well on the agar surface. A blob was then transferred to a flat white porcelain plate (the edge of a spot-plate is ideal), and quickly shaped into a mound 1-1.5 mm high with a flat

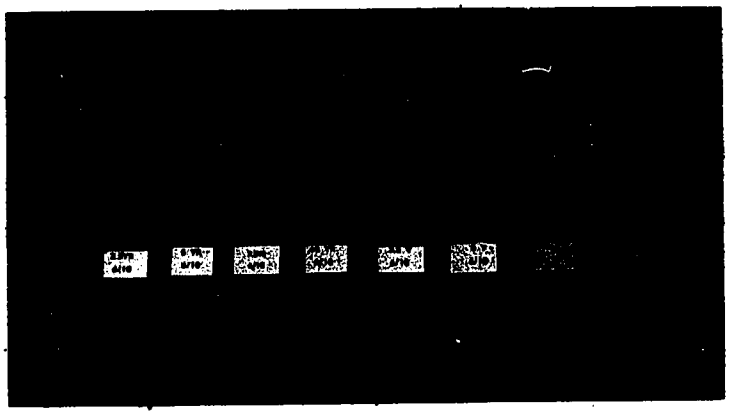


Figure 16. Munsell colour chips of value 6 and chroma 10 through the hue range 2.5 YR to 10Y. Note: the exact colours do not reproduce at all well photographically, and this is meant as a general example only.

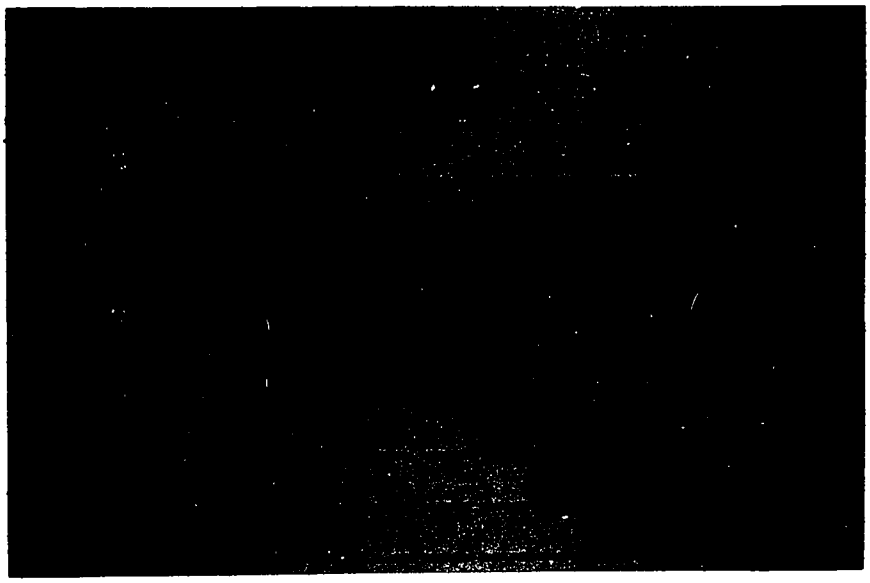


Figure 17. The 7.5 YR hue page from the Munsell Book of Colour (277). The value (lightness or darkness) is seen to range from 2 to 9, and the chroma (intensity) from 2 to 16.

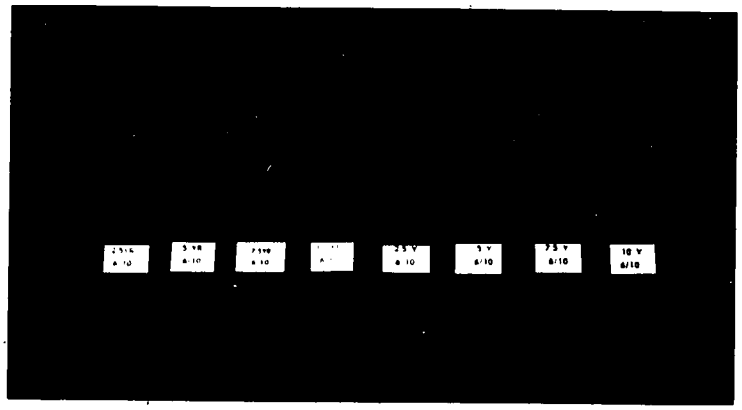


Figure 16. Munsell colour chips of value 6 and chroma 10 through the hue range 2.5 YR to 10Y. Note: the exact colours do not reproduce at all well photographically, and this is meant as a general example only.

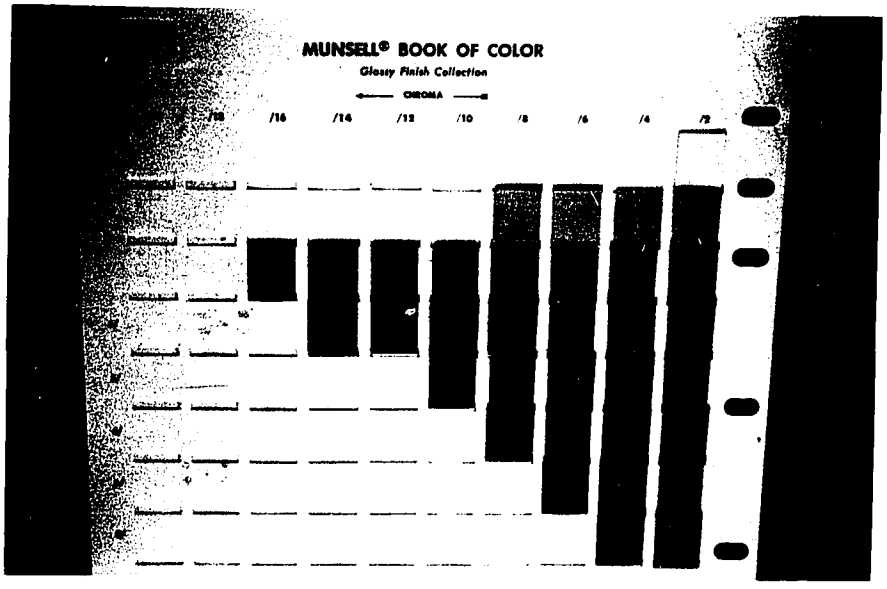


Figure 17. The 7.5 YR hue page from the Munsell Book of Colour (277). The value (lightness or darkness) is seen to range from 2 to 9, and the chroma (intensity) from 2 to 16.

surface of at least four square millimetres. Comparison with glossy finish Munsell colour chips (277) under standard illumination was done immediately to forestall colour changes which occur on drying.

VII.1.3 Results and discussion

A quite acceptable slight variation in colour was shown on the replicate plates produced at different times and read at different ages. A selection of examples from Skim Acetate agar and Cook's Cytophaga agar (71) appears in Tables 30 and 31 respectively. A wider range was expected in the organisms 2, D, 6 and 4541 which constitute a new *Cytophaga* species, *C.brunescens* (see Chapter VIII) and characteristically produce a brown to black water soluble pigment at different ages on different media.

The colours produced by the organisms on six media differing in nutritional complexity (Table 32) showed more variation of course. The average range of colour was 2.5 or 5 units of hue, one unit of value and about four of chroma. The organism *Flexibacter* FS-1 will serve as an example of an average variation, it shows the following range 5 to 7.5 YR 5 to 6/8 to 12. Some organisms had a quite narrow range, for instance *Flavobacterium resinovorum* NCIB 8767 with a range of 7.5 YR 5 to 6/10 to 12 on these six media. *Cytophaga johnsonae* ATCC 17061, however, was observed to have a very wide variation in colour depending on the nutritional quality of the supporting medium, its range was 2.5 YR to 2.5 Y 4 to 7/10.

Table 30

Munsell colours on Skim Acetate Agar

Organism (see Table 41 Chapter VIII)	Colours at 6 to 8 days All from different plates			
Yellow				
NCIB 8186	5Y 7/10	5 Y 7/10	7.5 Y 7.5/8	7.5 Y 8/10
NCIB 8187	5Y 6/10	5 Y 7/6	5 Y 7/10	5 Y 8/10
NCIB 8535	5Y 7/8	5 Y 7/10	5 Y 7/10	7.5 Y 8/10
Yellow-orange				
405	7.5 YR 6/10	10 YR 6/8	10 YR 6/10	10 YR 7/10
Bryant	5 YR 5/10	5 YR 5.5/10	5 YR 5/12	5 YR 5/12
A15	5 YR 4/9	5 YR 5/10	5 YR 5/10	5 YR 6/12
B-2-25	7.5 YR 6/8	7.5 YR 6/10	7.5 YR 6/10	7.5 YR 6/10
E-1-25	7.5 YR 6/8	7.5 YR 6/10	7.5 YR 6/10	7.5 YR 6/10
H_2O-1A	7.5 YR 6/10	7.5 YR 6/10	7.5 YR 6/10	10 YR 6/10
3	5 YR 5/10	5 YR 6/10	7.5 YR 6/10	7.5 YR 6/12
4433	5 YR 5.5/10	7.5 YR 6/10	7.5 YR 6/10	7.5 YR 7/8
4539	7.5 YR 6/10	7.5 YR 7/8	10 YR 7/8	10 YR 7/10
4707	7.5 YR 6/10	7.5 YR 6/10	7.5 YR 7/9	7.5 YR 7/10
NCIB 9059	2.5 YR 4/12	5 YR 5/10	5 YR 5/10	5 YR 5/12
15D	5 YR 5.5/10	7.5 YR 6/10	7.5 YR 6/10	7.5 YR 6/10
18H	5 YR 5/12	7.5 YR 6/10	7.5 YR 6/10	7.5 YR 6/12
13B	7.5 YR 6/6	10 YR 6/6	10 YR 6/6	10 YR 7/6
4553 (ii)	7.5 YR 5.5/6	7.5 YR 6/6	10 YR 6/6	10 YR 6/8
Brown				
2	10 YR 3/4	10 YR 4/6	10 YR 4/6	10 YR 5/8
D	10 YR 4/6	10 YR 4/6	10 YR 4/6	10 YR 5/8
6	10 YR 3/6	2.5 Y 4.5/6	5 Y 2/2	5 Y 3/4
4541	7.5 YR 4/8	7.5 YR 5/8	10 YR 5/8	10 YR 5/10
White and Cream				
9D	10 YR 7/2	10 YR 8/2	2.5 Y 7/2	2.5 Y 8/2
495	2.5 Y 6.5/6	2.5Y 8/6	5 Y 7/4	5 Y 7/6
4553 (i)	2.5 Y 7/4	2.5 Y 7/4	5 Y 8/4	5 Y 8/4

Table 31

Munsell colours on Cook's Cytophaga Agar

Organism (see Table 41 Chapter VIII)	Colours at 5 to 8 days All from different plates		
Yellow			
NCIB 8186	5 Y 7/5	7.5 Y 7.5/4	7.5 Y 8/8
NCIB 8187	5 Y 7/6	5 Y 7.5/6	7.5 Y 7/8
NCIB 8535	5 Y 6.5/6	5 Y 7/6	7.5 Y 7/8
4553 (i)	5 Y 7/4	5 Y 7/4	2.5 Y 6.5/4
Yellow and yellow-orange			
405	7.5 YR 6/8	7.5 YR 6/10	10 YR 7/10
Bryant	7.5 YR 6/9	7.5 YR 6/10	7.5 YR 7/10
A15	5 YR 5/10	7.5 YR 5/10	7.5 YR 6/10
B-2-25	7.5 YR 6/10	7.5 YR 6/10	10 YR 6/10
E-1-25	7.5 YR 6/10	7.5 YR 6/10	7.5 YR 6/12
H_2O-1A	7.5 YR 6/11	7.5 YR 6/12	2.5 Y 7/10
3	5 YR 5/10	5 YR 5/12	10 YR 6/12
4433	7.5 YR 6/10	10 YR 6/10	2.5 Y 7/10
4539	7.5 YR 6/10	9 YR 6/8	10 YR 6/10
4707	10 YR 6/10	10 YR 7/10	2.5 Y 7/10
NCIB 9059	5 YR 5/10	7.5 YR 6/10	10 YR 7/10
15D	5 YR 5/12	5 YR 6/10	7.5 YR 6/12
18H	6 YR 6/12	7.5 YR 6/10	7.5 YR 6/12
13B	7.5 YR 6/6	7.5 YR 6/8	10 YR 7/6
4553 (ii)	10 YR 6/6	10 YR 6/8	2.5 Y 6/5
Brown			
2	2.5 Y 4/6	2.5 Y 4/6	5 Y 4/6
D	2,5 Y 4/6	2.5 Y 4/6	2.5 Y 5/6
6	2.5 Y 4/6	5 Y 2/1	5 Y 3/4
4541	10 YR 5/8	2.5 Y 4/6	2.5 Y 5/6
White and cream			
9D	10 YR 7/3	2.5 Y 8/2	2.5 Y 8/2
495	5 YR 8/4	2.5 Y 6/6	2.5 Y 7/4

Table 32. Munsell colours on six different media*

Organism (see Table 41 Chapter VIII)	Salts + Glu. + YE	Cook's Cytophaga	Skim Acetate	Casein	Yeast	Plate count
Yellow						
NCIB 8188	7.5 Y 8/8	7.5 Y 7.5/4	6 Y 8/10	5 Y 6/8	5 Y 7/8	5 Y 7/8
NCIB 9324	9 Y 8/6	5 Y 7/5	7.5 Y 8.5/2	5 Y 6.5/8	6 Y 7/8	2.5 Y 6.5/6
Yellow-orange						
ATCC 17061	7.5 YR 6/10	1.5 Y 7/10	5 YR 5/10	2.5 YR 4/10	2.5 Y 4.5/10	2.5 YR 4/10
405		10 YR 7/10	10 YR 6/8	10 YR 6/8	10 YR 6/8	7.5 YR 5/8
NCIB 9059		10 YR 7/10	2.5 YR 4/12	5 YR 5/12	2.5 YR 4/10	5 YR 5/10
FS-1	7.5 YR 5/8	7.5 YR 6/10	5 YR 5/12	7.5 YR 5/10	7.5 YR 5/10	5 YR 5/10
NCIB 10782	7.5 YR 5/10	2.5 Y 8/10	10 YR 6/10	10 YR 6/12	10 YR 6/12	7.5 YR 6/12
NCIB 8767	7.5 YR 6/10	7.5 YR 6/10	7.5 YR 6/10	7.5 YR 6/12	7.5 YR 6/10	7.5 YR 5/10
NCIB 8992	2.5 Y 7/8	2.5 Y 7/6	7.5 YR 6/12	10 YR 6/10	2.5 Y 7/10	10 YR 6/8
NCIB 9290	10 YR 7/6	2.5 Y 7/4	10 YR 7/4	10 YR 6.5/6	10 YR 7/6	10 YR 6/4
Orange-red						
NCIB 8185	10 R 5/12	2.5 YR 6/10	5 YR 6/12	2.5 YR 5/12	5 YR 6/10	5 YR 5.5/10
RL8	5 YR 5/10	4 YR 5/10	2.5 YR 6/12	5 YR 5/12	2.5 YR 5/10	2.5 YR 5/12
Brown						
2		2.5 Y 4/6	10 YR 4/6	2.5 Y 4/6	2.5 Y 4/6	2.5 Y 3/4
Cream						
AL-1	5 Y 7/4	5 Y 8/4	5 Y 7.5/4	2.5 Y 5/4	5 Y 7/4	5 Y 6/6
495		2.5 Y 6/6	2.5 Y 6.5/6	2.5 Y 6/6	2.5 Y 7/6	2.5 Y 5/4

*Salts + Glucose + Yeast extract read at 11 days; Cook's Cytophaga, Skim Acetate and Plate Count read at 6 days; Casein read at 8 days; Yeast read at 10 days.

Although there were no outstanding general colour shifts with an increase in nutrient level, there was a tendency for the organisms to become less yellow and more red, and for them to produce a darker pigmentation. In other words the hue sometimes shifted from say 7.5 Y to 5 Y or from a Y hue to a YR hue, and the value often dropped by one or two units. The chroma showed no general tendency to change with nutrient concentration.

In the taxonomic study of a large number of cytophagas, *Flavobacteria* and similar organisms (Chapter VIII) colours were observed on Skim Acetate Agar in the various groups as follows.

Spreading yellow and orange cytophagas and *Flexibacter* FS-1

19 strains

Range: 5-10 YR 4-7/8-12 and 2.5 Y 6-7/810

This is comprised of the following individual colours,

5 YR 4/9, 5/10, 5/12, 5.5/10, 6/10 and 6/12

7.5 YR 5/10, 6/8, 6/10, 6/12, 7/8, 7/9 and 7/10

10 YR 6/8, 6/10, 6/12, 7/8 and 7/10

2.5 Y 6/10 and 7/8

Non-spreading yellow-orange cytophagas

3 strains

Range: 2.5-7.5 YR 5-6/10-12

Individual colours: 2.5 YR 6/12

5 YR 5/12 and 5.5/10

7.5 YR 6/10 and 6/12

Whitish spreading cytophaga *(Flexibacter canadensis)*

1 strain

Range: 10 YR 7-8/2 and 2.5 Y 7-8/2

Individual colours: 10 YR 7/2 and 8/2
2.5 Y 7/2 and 8/2

Brown spreading cytophagas *(C.brunescens)*

4 strains

Range: 7.5 - 10 YR 3-5/4-10 and 2.5-5 Y 2-4.5/2-6

Individual colours: 7.5 YR 4/8 and 5/8
10 YR 3/4, 3/6, 4/6, 5/8 and 5/10
2.5 Y 4.5/6
5 Y 2/2 and 3/4

"495 group"of cytophagas *(Lusobacter enzumogenes)*

34 strains having two colour variants

Range: 6-10 YR 5.5-7/5-8 and 2.5-7.5 Y 6-8/2-6

Individual colours: 6 YR 7/4
7.5 YR 5.5/6, 6/6 and 6/7
8.5 YR 6/6
9 YR 6/6
10 YR 6/6, 6/7, 6/8, 6.5/5 and 7/6
2.5 Y 6.5/6, 7/4, 7/5, 7/6, 7.5/4, 7.5/6, 8/4 and 8/6
3 Y 7/4
4 Y 7/4, 8/4 and 8/6
5 Y 7/4, 7/6, 7.5/4, 7.5/5, 8/2, 8/4, 8/6 and 8.5/4
7.5 Y 8/2 and 8/4

Flavobacteria

16 strains

Range: 10 R 5/8, 2.5-10 YR 4-7/4-12 and 2.5-7.5 Y 6-8.5/2-10

Individual colours: 10 R 5/8
2.5 YR 4/10 and 4/12
5 YR 5/10, 5/12 and 6/12
7.5 YR 6/10 and 6/12
10 YR 7/4
2.5 Y 7/6, 7/8 and 7/10
5 Y 6/10, 7/6, 7/8, 7/10, 8/8 and 8/10
6 Y 8/10
7.5 Y 7.5/8, 8/10 and 8.5/2

In the study of arctic cytophagas (Chapter V) the following colours were observed on Skim Milk Agar in the two groups.

Spreading arctic cytophagas

29 strains

Range: 7.5-10 YR 6-7/8-12

Individual colours: 7.5 YR 6/8, 6/10, 7/8 and 7/10
10 YR 6/10, 6/12, 7/8 and 7/10

Non-spreading arctic cytophagas

22 strains

Range: 7.5-10 YR 6-7/10-12 and 2.5 Y 7/10

Individual colours: 7.5 YR 6/10 and 6/12
10 YR 6/10, 6/12, 7/10 and 7/12
2.5 Y 7/10

The general colour range for the typical yellow-orange spreading cytophagas (5-10 YR 4-7/8-12 and 2.5 Y 6-7/8-10) and non-spreading cytophagas (2.5 - 10 YR 5-7/10-12 and 2.5 Y 7/10) are very similar. The spreading forms are generally yellower, darker and of more intense colouration, that is they have a narrower hue range

especially in the redder hues, and narrower value and chroma ranges in the lighter and more intense regions respectively. It is to be emphasized that the spreading mode of growth should not affect these results directly because of the standard method of preparing bacterial mounds for colour observations. Nevertheless there could be indirect effects if, say, the spreading organisms produced more slime material which diluted the colour for instance. After examining about 120 strains of these organisms including *C.johnsonae*, *C.johnsonae* var. *denitrificans*, *C.hutchinsonii* and *C.succinicans* it is proposed that the colour range

2.5-10 YR 4-7/8-12 and 2.5 Y 6-7/8-10

is characteristic of most soil and freshwater cytophagas. There are other species with different colours however, and examples studied here are the organisms producing a brown water-soluble pigment *C.brunescens*, the whitish strain 9D *Flexibacter canadensis*, and the cream "495 group" *L.enzymogenes* (Chapter VIII). The very wide range of colours exhibited by the *Flexibacteria* species from pure red, through all the yellow-reds and most of the yellow hues as well, only emphasizes the heterogeneous nature of this genus as presently constituted.

VII.2. SPREADING: TOWARDS A DEFINITION

VII.2.1 The swarming phenomenon

The typical spreading growth of the myxobacteria, including the cytophagas, takes the form of a thin film extending rapidly over the surface of the agar from the site of inoculation. Some strains will cover the surface of a 9cm petri plate in a few days from a central inoculum. Delayed spreading occurs with some cultures, outgrowths suddenly appearing after a week or more of incubation (164). The very thin layer of cells, which is easily detected in reflected light, has been called a swarm (186), a "moiré" (367) and also a pseudoplasmodium (393). The latter term is an undesirable one since it suggests a connection with the myxomycetes, and more particularly with the Acrasieae, to which the myxobacteria are not even remotely related (373).

Stanier (370) introduced the following method for observing the development of myxobacterial swarms. A thin layer of agar is spread on a sterile cover slip, allowed to solidify and subsequently inoculated with a mass of young cells from the tip of a needle. The coverslip is then mounted over a moist chamber, incubated at a suitable temperature, and examined microscopically at intervals. Within one or two hours the first changes along the periphery of the artificial colony generally become evident. At many points groups of cells begin to move out; when the columns become longer they branch, fuse and interlace with one another so that the edge consists entirely of tongue-like extensions and isolated islands of actively

motile cells. The extension of a cytophaga colony is thus not a passive matter of cell growth and division as in the eubacteria, but rather the result of the active gliding motility of non-flagellated cells that move in large masses across the agar surface. The moving cells may pave the substrate with a layer of slime ahead of the cell mass (unpublished results; 375), however Stanier has also suggested that inability to swarm in certain *Cytophaga* strains is probably due to the copious synthesis of a microbial gum (373).

The rate of movement varies considerably and the factors controlling this are unknown. Groups of cells moving vigorously may suddenly slow up and stop whilst others will as suddenly start creeping across the agar. The fastest rate measured has been that of *C.krzemieniewskae* at 15 μ/min, although this species averages five to six μ per minute. As well as the generally forward gliding movement, actively motile cells show rapid flexing movements ("Krümmungsbewegungen"). These have also been referred to as "twitching" or "jerking" (240) and are probably faster than the flexing or bending described for many other flexibacteria. Rapid flexing movements are sometimes caused by collision with say a neighbouring non-motile cell, but mostly seem to be spontaneous. Since only a small minority of cells in a moving mass exhibit them they are evidently not causally related to the forward movement. They occur in young, healthy cells under normal conditions and so it is unlikely that they are pathological in nature as was suggested by Stapp and Bortels (378).

There is an apparent co-ordination between the different cells in a swarm. The slime is the only physical connection observed between the cells, although membranous vesicles have now also been described (Chapter VI). The direction and speed of movement of a few cells at the apex of a moving column control the activities of a large number of following cells. Single, isolated cells are very rarely motile; the unit of effective movement seems to consist of 20 to 30 cells (370). The obvious advantage of this type of movement is that the cells are not confined to a small area where the food supply is rapidly used up. This may be especially important to a group of bacteria which depend largely on macromolecules insoluble in aqueous solutions and which seem to need close physical contact in order that these materials may be enzymologically solubilized (436).

Although swarming is so marked in some *Cytophaga* strains as to constitute a cultural nuisance, it cannot be demonstrated at all in others thought to belong to this genus from other evidence. Some fruiters and cytophagas spread through the agar gel rather than on the surface (164), and this is generally true of many other species of these organisms at agar concentrations of one per cent or less (408). Some cellulolytic species, for example *C.hutchinsonii* and *C.rubra* have been shown not to swarm when grown on salts - glucose agar (373), and certain agarolytic and cellulolytic forms produce colonial variants that never show swarming motility (14, 373, 408). These exceptions must be borne in mind when considering studies that have excluded organisms which failed to swarm on a certain medium, for instance that of Lewin and Lounsbery (246) who used a screening

medium composed of 0.02% tryptone, 1% agar and 0.001% actidione.

Conversely, both Hayes (162) and Dworkin (100) have stated that swarming need not be restricted to the myxobacteria and cytophagas. This does not only apply to certain alleged *Flavobacteria*, *F.aquatile* and *F.heparinum* for example (275), which are most probably mis-classified cytophagas (see pages 66 and 243) but also to various members of the Cyanophyceae, Beggiatoaceae and Vitreoscillaceae, and to other species such as *Proteus vulgaris*, *P.mirabilis*, *Lingelsheimia anitrata* and aflagellate variants of *Pseudomonas fluorescens*. One cannot even reliably distinguish between the genera *Cytophaga* and *Flavobacterium* on the basis of spreading, as the following example will demonstrate. Following an Adansonian study of yellow pigmented rods Floodgate and Hayes (113) assigned one group to *Cytophaga* on the basis of swarming movement, and a second non-swarming group to *Flavobacterium*. Further investigation indicated that the non-swarming strains were more probably related to *Cytophaga* (93), the likely identity of the other group still remains in doubt (275).

The composition of the medium is critical for demonstration of the phenomenon of swarming. The first requirement is a solid surface upon which to glide (370), in soft agar (1% or less) the cells tend to penetrate into the agar and surface spreading is greatly retarded (409). It is generally agreed that swarming is best observed on freshly prepared, moist media containing small concentrations of nutrients. Dworkin in fact has suggested using a non-nutrient medium (101), but Hayes (162) demonstrated that if the peptone concentration

was decreased sufficiently (0.01%) then growth was inhibited and swarming could not be observed.

In 1947 Stanier's work with the chitinovorous cytophagas showed that swarming could be partially controlled by varying the agar concentration. He found that 1.5 to 2% agar resulted in the best expression of the spreading habit; Hayes (162) also used this range of concentration, but Hendrie (164) found that 0.8% agar was best for their isolates. Stanier (374) has noted that a 1% agar gel facilitates the separation and purification of cytophagas as these can spread down and through the agar away from contaminating bacteria which, even if flagellated, cannot move through a 1% agar gel unless possessing the ability to liquefy agar.

At a peptone concentration of 1% or over the majority of the cytophagas tested (162, 374) form a compact, raised colony with an entire edge such as is typical of many eubacteria. As the peptone concentration is dropped below 1% swarming becomes more and more evident. Both Stanier (374) and Hayes (162) observed that many isolates produced 'intermediate' colonies which had raised centres and flattened peripheries but did not exhibit true swarming. At peptone levels of about 0.25% completely flat, rapidly spreading, almost invisible swarms are produced. At the lowest peptone concentrations used by Hayes swarming was less in evidence, the colonies being minute because of the extreme lack of available nutrients. Upon microscopic examination of cells from colonies grown on high nutrient and low nutrient 1.5% agar media, both Hayes and Stanier observed

that gliding and flexing were manifested only by the latter cells. Thus a gradation in motility corresponding to the colony form is suggested, confirming that gliding motility is characterized macroscopically by spreading growth of the colony.

Colony structure in these organisms is therefore not fixed, but can be changed at will by varying the nutrient concentration. Some unusual procedures have been successful in causing certain organisms to exhibit a spreading form of growth, for example, prolonged incubation at 15°C was useful in the case of *Flavobacterium aquatile* NCIB 8694, and *F.heparinum* NCIB 9290 was grown on a medium containing yeast RNA as the major nutrient (275). Both of these strains appear to belong to *Cytophaga* but form discrete colonies under most cultural conditions. In the work of Schmidt, Gaydos and Jeffries (339), two non-swarming colonies of *Proteus mirabilis* grown next to one another sometimes resulted in one or both colonies developing a typical swarm. The authors suggested that the failure to swarm derives from the inability to produce some metabolite. Such a substance, when supplied by the adjacent colony, may induce swarming.

VII.2.2 Introduction to the present investigation

The fruiting myxobacteria, probably because they were first described by botanists, have traditionally been classified on their morphology and colour (42), although the recent detailed work by McCurdy (265, 266, 267, 268, 269) has now put their classification on a sounder basis by including physiological characteristics as well. In contrast the cytophagas were first described by bacteriologists

and their classification reflects this; in Bergey's Manual (42) they are differentiated on the basis of habitat, polysaccharides degraded and colour.

Although they do not form interesting fruiting structures of defined shape, they nevertheless show very characteristic spreading patterns on different media. Three major colony forms have been recognized, but all kinds of variations within and between these types are immediately obvious. The present investigation has attempted to define this morphology in codified form. Using this code, the author then tried to resolve the problem of distinguishing cytophagas from non-cytophagas by the possession of spreading ability in the following ways. In order to be able to recognize those cytophagas which do not spread on Cook's Cytophaga Agar, which is the medium recommended to demonstrate spreading behaviour (78), the proportions of the constituents were varied in an attempt to find a better formulation. The influence of the concentration of agar and of tryptone on various morphological parameters of spreading colonies was examined in some detail. Also, tests were carried out on various *Cytophaga*-like strains and on certain *Flavobacteria* to discover whether they were able to swarm on these nutritionally varied media. An experiment was undertaken to examine the difference in spreading achieved on routine agar plates with dry surfaces, and on fresh, moist plates of identical nutrient and agar composition. Conditions leading to the production of subsurface growth, that is spreading through the agar rather than on its surface, were studied as well. A discussion leading towards a definition of the term 'spreading' concludes the investigation.

VII.2.3. Materials and methods

The following organisms, grouped according to their customary behaviour on Cook's Cytophaga Agar, were used for all of the experiments.

"Spreaders":	*Cytophaga johnsonae* var. *denitrificans* 405, 4539, 4433, 4707, BRYANT, A15, B-2-25, E-1-25, H_2O-1A, 3, 9D, 2, D, 6, 4541 and *Flavobacterium pectinovorum* NCIB 9059.
"Intermediates":	(wide mucilaginous growth, sometimes with a very narrow spreading edge): 13B, 495.
"Non-spreaders":	18H, 15D, *F. esteroaromaticum* NCIB 8186, *F.flavescens* NCIB 8187 and *F.aquatile* NCIB 8535.

Further organisms were also used in the dry v. moist experiment, as indicated in Table 37. Isolation, characteristics and other taxonomic information about these organisms can be found in Chapter VIII.

Twenty-four different variations of the formula of Cook's Cytophaga Agar (0.2% tryptone in 1.0% agar) were used for this series of experiments, with the concentration of tryptone ranging from 0.05 to 2%, and that of the agar ranging from 0.75 to 2% (Table 33). While preparing the spreading code, nine modifications of Skim Acetate Agar (routinely 0.5% skim milk powder, 0.05% yeast extract, 0.02% sodium acetate and 1.5% agar) were also used, with the skim milk at levels of 0.1%, 0.5% and 1.0%, and the agar at 0.75%, 1.0% and 1.5%. Active cultures were streaked in one straight line down the centre

of the dried surface of the plates and incubated at 25°C for six days. On the second and sixth days the growth codes were assessed on Cook's Cytophaga Agar; measurements on Skim Acetate plates were made at four days.

VII.2.4 Results and discussion

Development of growth code

The growth of a cytophaga 'streak colony' can be divided into three areas, the central portion around the initial streak which is usually the thickest, the intermediate portions on each side which are usually thinner and wider, and the outermost, very thin, flat, spreading edge or "fringe" as it is called here. These colonies are often difficult to photograph successfully, both because of their shiny appearance and because reflected light is needed to observe the fringe. A code to describe the many variations of the spreading form was therefore developed through a number of stages, the final form is basically a four-digit code with a small, variable number of suffixes. The code for *C.johnsonae* var. *denitrificans* 405 on routine Skim Acetate Agar after two days growth at 25°C,

III C 53 β w

will serve as an example (Figure 18). The first digit, a Roman numeral, describes the width of the fringe, in this case an average one for a cytophaga, measuring between 0.5 and 2.0 mm. The second digit, an upper case letter of the alphabet, describes the total width of growth, here it is between eight and 12mm.

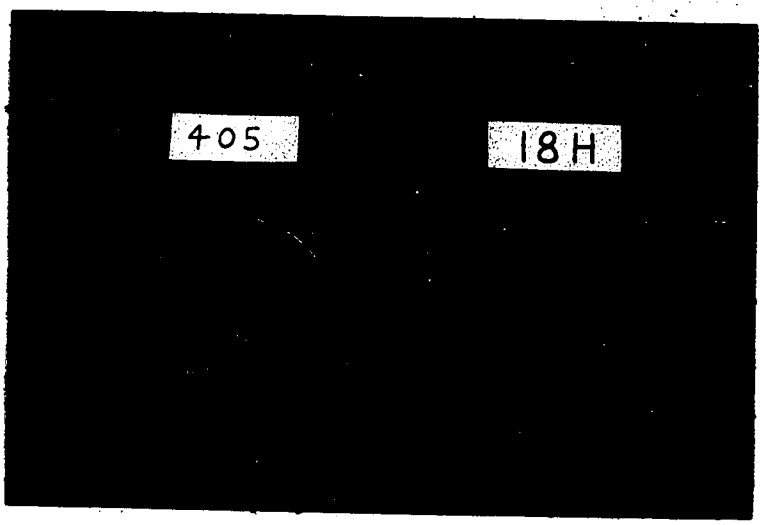


Figure 18. *C.johnsonae* var. *denitrificans* strain Cook 405, and 18H on Skim Acetate Agar after two days at 25°C.

Growth codes: 405 = I I I C 53 β w
18H = I A 1

Figure 19. *E.coli*, *S.marcescens* and *B.subtilis* grown on Difco Plate Count Agar (upper row) and on Cook's Cytophaga Agar (lower row) at 25°C for three days. The growth codes are all I A 1

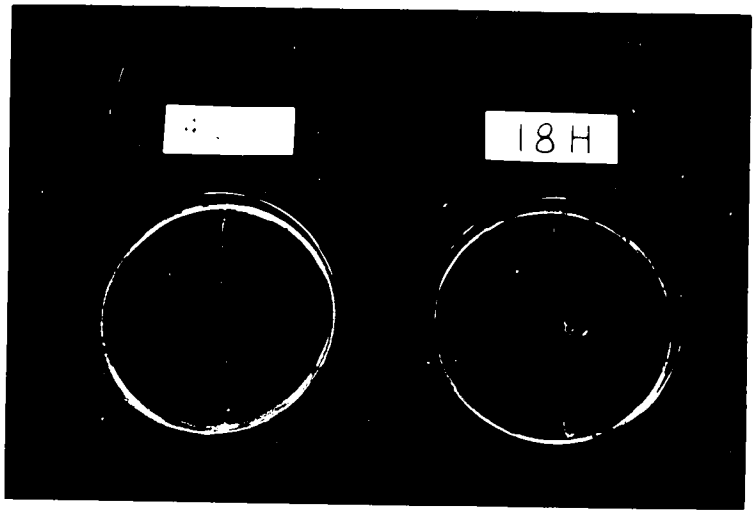


Figure 18. *C. falsenia* var. *denitrificans* strain Cook 405, and 18H on Skim Acetate Agar after two days at 25°C.

Growth codes: 405 = I I I C 53 B w
18H = I A I

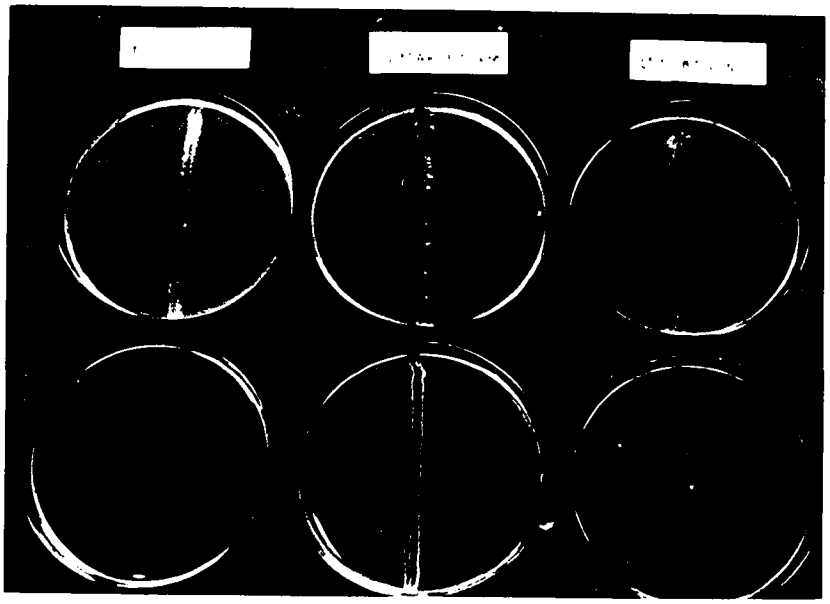


Figure 19. *A. odi*, *C. [illegible]* and *A. [illegible]* grown on Difco Plate Count Agar (upper row) and on Cook's Cytophaga Agar (lower row) at 25°C for three days. The growth codes are all I A I

The third digit is an Arabic number indicating the growth form which in the example is thin and transparent, with a plain central zone (Code 5) and an intermediate zone composed entirely of large 'fingers' from the central zone (Code 3). The fourth digit, a lower case Greek letter, describes the comparative width of the central zone and the growth on one side of it, in this case these two areas are of approximately the same width. Suffixes are lower case letters indicating special features which tend to occur irregularly along the length of growth, and which have not been dealt with in the preceding part of the code. In the example above the edge of the growth was a wavy line, not a more or less straight one.

An example of a nonspreading organism 18H on the same medium and under the same conditions,

I A 1

will show (Figure 18), that the code indicates no fringe (Code 1), a width up to five mm (Code A) and one simple, thick, opaque zone of growth (Code 1). No further digits were required for this simple growth form.

Figure 19 indicates that several common bacteria also have simple I A 1 growth codes. The organisms *E.coli*, *S.marcescens* and *B.subtilis* were grown for three days on Difco Plate Count Agar, and on Cook's Cytophaga Agar at 25°C. The growth code is explained in detail in the following pages.

Growth code for surface growth

1st digit. Width of fringe in mm.

I = None
II = Minute <0.5
III = Average 0.5 - 2
IV = Wide >2

2nd digit. Width of total growth in mm.

A = <5	F = >24 - 30
B = >5 - 8	G = >30 - 37
C = >8 - 12	H = >37 - 47
D = >12 - 18	J = >47 - 77
E = >18 - 24	K = >77

These ranges were determined after several hundred diameters were plotted graphically and natural groupings determined. The diameter of the plastic petri plates used was 85 mm.

3rd digit. Growth form

Thick, opaque growth

1 zone only, no fringe = 1

2 zones

Central wider than intermediate zone, no fringe = 2

Central and intermediate zones about same width.

Total width of growth narrow (<E), regular edge, mostly without a fringe = 3

Total width of growth wide (>C), irregular edge, with or without a fringe, complex texture or colouration = 4

Thin, slight, transparent or translucent growth, with fringe

Central zone plain = 5
Central zone banded in colour = 6
Central zone banded in texture = 7
No separate central zone, but a gradation occurs from the centre to where the fringe begins. Sometimes an intermediate area occurs in part, of sufficient size to be described below = 8

Types 5, 6, 7 and 8 are subdivided on the form of their intermediate zone, as follows, creating two-number digits eg. 58, 81.

Intermediate zone

0 = very shiny, rough, medium thickness, plain
1 = rather dull, rough, very thin, plain
2 = shiny, smooth, plain
3 = consisting entirely of large fingers from central zone
4 = consisting of large blobs, fairly thick
5 = fairly shiny, very rough, many medium-sized blobs, fairly thick
6 = shiny, consists of wide bands of smooth and rough, medium thickness
7 = consists of wide bands of smooth and rough, very thin
8 = rather dull, rough with many small warts, very thin and transparent

4th digit. Relative width of central zone

(not used when 3rd digit is 1 or 8)

α = central zone narrower than intermediate + fringe on one side
β = central zone is roughly the same width as intermediate + fringe on one side
γ = central zone wider than intermediate + fringe on one side

Suffixes

One or more may be added, a double letter eg. cc indicates a very strong characteristic. No suffix indicates a regular growth form. Listed in code in decreasing order of their importance in the morphology.

b = bands occur parallel to the growth front
c = crenate to clubby edge
e = fingers from intermediate zone extend into fringe
f = fingers from central zone extend into the intermediate zone
g = small separate growths (colonial variants) occur on, or extending from the main growth area
l = fine lines occur parallel to the growth front

o = many blobs throughout intermediate zone

p = 'puffs' occur along edge of growth, presumably due to groups of more rapidly growing cells

s = slime layer present ahead of growth front (ss = two slime layers)

w = wavy edge, not more or less straight

Growth code for subsurface growth

The code is prefixed "SUB" and consists of only three other digits. No spreading edge or fringe is discernible.

1st digit. Total width

A to K as above.

2nd digit. Growth form

This consists of two figures

(a) Central zone

Submerged (dull)	= 1
On surface (shiny)	= 2

(b) Intermediate zone edge

No intermediate zone	= 0
Regular	= 1
Wavy	= 2
Extremely irregular	= 3

3rd digit. Relative width of central zone

α, β, γ, as above

Suffixes may be added as for surface growth.

An example is the organism *C.johnsonae* var. *denitrificans* 405 on 0.05% tryptone and .75% agar, after 6 days growth

SUB E 23 β pp

This indicates that the growth was subsurface, 18 to 24 mm wide, the central zone had surface growth and the intermediate zone had a very irregular edge, the central and one intermediate zone were of roughly equal width, and a large number of 'puffs' occurred. Examples of the use of the growth code for selected organisms are presented in Tables 33 and 38, and complete lists for twenty three organisms on the twenty four media at both two and six days appear as Appendices I and II respectively.

Obviously the growth form and hence the corresponding growth code will vary with the stage of growth. Figure 20 shows the spreading organism 3-19 on Cook's Cytophaga Agar. The growth code at two days was III C 53 β w, but by six days the total growth had widened and the growth form had changed, giving the code III H 82 α f w. Similarly Figure 21 indicates the changes which occurred to the intermediate organism 3C between two and 14 days on Skim Acetate Agar. At two days there existed a simple growth form I A 1, but a small fringe and considerable complexity developed later, giving the growth code II E 52 α b p.

The growth medium also affects the growth form, and this can be seen with strain 405 *(C.johnsonae* var. *denitrificans)* after two days at 25°C on three different agar media (Figure 22). The growth codes were as follows, on Plate Count I A 1, on Skim Acetate III C 53 β w, and on Cook's Cytophaga Agar I B 53 γ e. Hence a considerable variation in width, fringe production and other features occurs, and as with other bacteriological techniques, the test conditions have to be reported along with the growth code.

Table 33

Examples of growth codes on Cook's Cytophaga Agar at two days

		Agar % 0.75	1.0	1.5	2.0
405	0.05	SUB C 12 α	IV J 51α	III E 51 α	III F 88
'Spreader'	0.1	III E 51 α pf	IV G 51 α p	III G 51 α o	III G 88
Tryptone %	0.2	III C 51 β f	IV J 58 α	III F 58 α f	III F 58 α f
	0.5	III D 55 α fp	IV H 58 α	III G 58 α fe	III E 58 α f
	1.0	III E 55 α f	III E 85 f	III E 50 β f	III E 50γ ffe
	2.0	II E 85 fpe	III H 55 β f	III G 55 α fe	II F 50 γ ffe
495	0.05	II B 82 γ	II B 82 γ f	I A 1	I A 1
'Intermediate'	0.1	II C 82 γ f	II B 82 γ f	I A 1	I A 1
	0.2	II C 82 γ f	II B 82 γ f	I A 1	I A 1
	0.5	II C 82 γ c	II B 82 γ f	I A 1	I A 1
	1.0	I B 1 e	I B 1	I A 1	I A1
	2.0	III C 82 γ f	I B 1	I A 1	I A1
18H	0.05	I B 1 s	I B 2 β s	I A 1	I A 1
'Non-spreader'	0.1	I B 1 s	I B 2 β ss	I A 1	I A 1
	0.2	I B 1 s	I B 2 β s	I A 1	I A 1
	0.5	I B 1 s	I B 1 ss	I A 1	I A 1
	1.0	I C 1 ss	I B 1 s	I A 1	I A 1
	2.0	I C 1 s	I B 1 s	I B 1	I A 1
8535	0.05	I A 1	I A 1	I A 1	I A 1
'Non-spreading' *Flavobacterium*	0.1	I A 1	I A 1	I A 1	I A 1
	0.2	I A 1	I A 1	I A 1	I A 1
	0.5	I A 1	I A 1	I A 1	I A 1
	1.0	I B 1	I A 2 β	I A 1	I A 1
	2.0	I B 2 α	I A 1	I A 1	I A 1

Variation of growth forms and codes with time

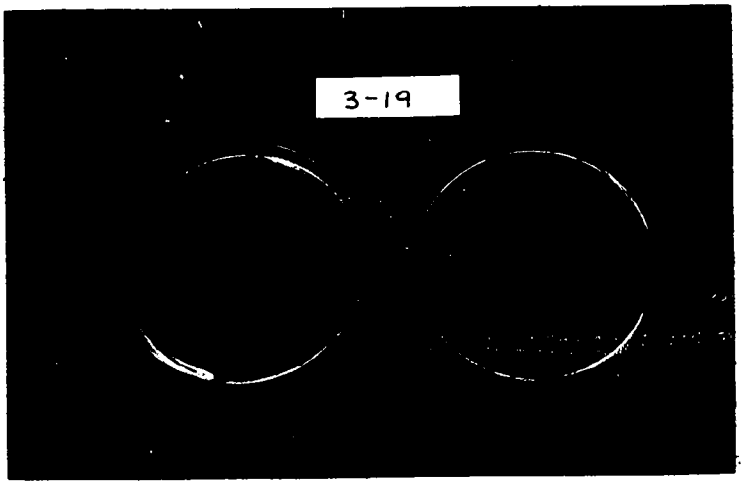


Figure 20. Growth codes for strain 3-19 on Cook's Cytophaga Agar. Two days (left) = III C 53 β w, six days (right) = III H 82 α f w.

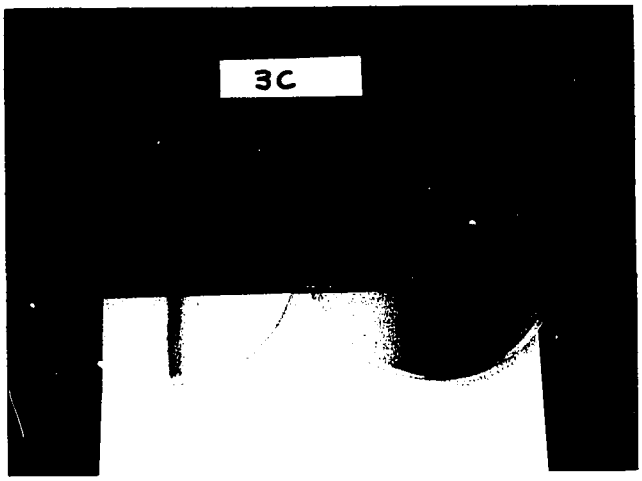


Figure 21. Growth codes for strain 3C on Skim Acetate Agar. Two days (left) = I A 1, 14 days (right) = II E 52 α b p.

Variation of growth forms and codes with time

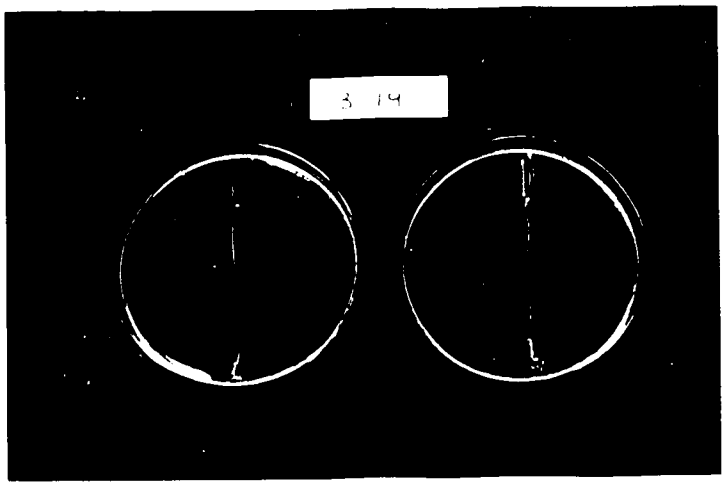


Figure 20. Growth codes for strain 3-19 on Cook's Cytophaga Agar. Two days (left) = III C 53 β w, six days (right) = III H 82 α f w.

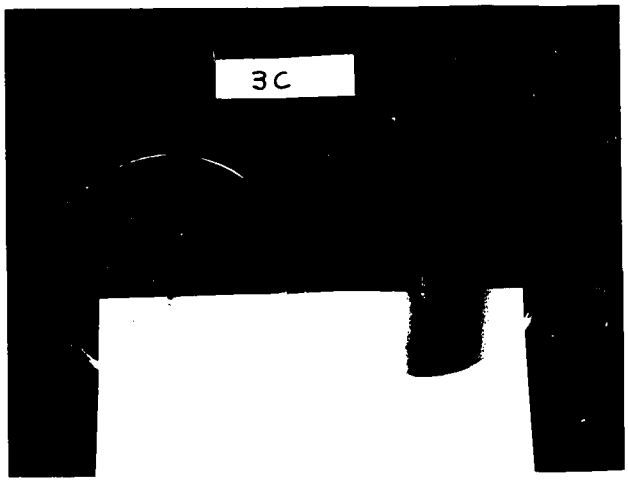


Figure 21. Growth codes for strain 3C on Skim Acetate Agar. Two days (left) = I A 1, 14 days (right) = II E 52 α b p.

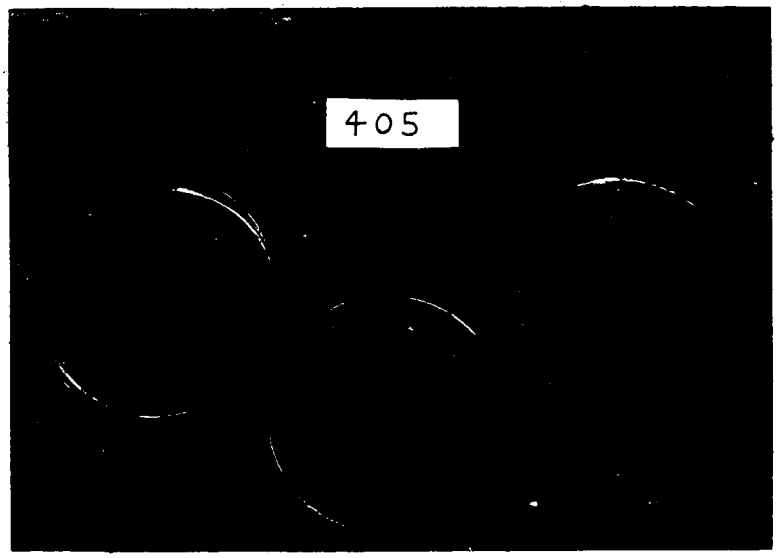


Figure 22. Variation in growth code on different media. *C.johnsonae* var. *denitrificans* 405 after two days at 25°C. Growth codes on Plate Count (left I A 1), Skim Acetate (centre) III C 53 β w, and Cook's Cytophaga Agar (right) I B 53 γ e.

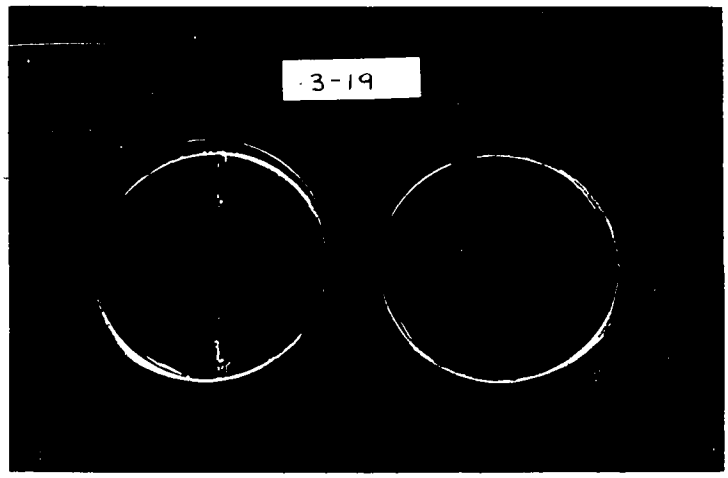


Figure 23. Growth codes on fresh and dry Cook's Cytophaga Agar after six days at 25°C. Strain 3-19, code on dry plate (left) I C 51 β w p, on fresh plate (right) III G 51 α w.

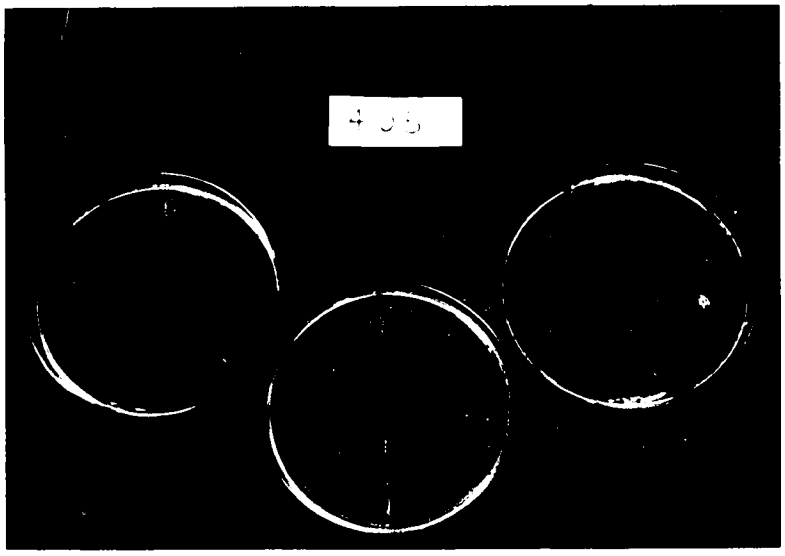


Figure 22. Variation in growth code on different media. *C. johnsoniae* var. *denitrificans* 405 after two days at 25°C. Growth codes on Plate Count (left I A 1), Skim Acetate (centre) III C 53 β w, and Cook's Cytophaga Agar (right) I B 53 γ e.

Figure 23. Growth codes on fresh and dry Cook's Cytophaga Agar after six days at 25°C. Strain 3-19, code on dry plate (left) I C 51 a w p, on fresh plate (right) III G 51 a w.

Characteristics of spreading organisms

Perhaps the most characteristic and unique feature of a spreading cytophaga is the fringe. This was observed in all of the 'spreading' and 'intermediate' organisms on Skim Acetate Agar at four days, and in all of them on Cook's Cytophaga Agar except H_2O-1A at two days, and 9059 at two and six days. No fringe was observed on any medium in any of the 'nonspreaders' Tables 34, 35 and 36). A fringe was not usually produced when the growth was subsurface, thus these instances have been ignored for this part of the discussion. There was no general correlation between the percentage of agar and the expression of the spreading habit, although there was a tendency for a wider fringe to be produced at a lower agar concentration within the range of 0.75 to 2%. Other authors have found that 0.8% (164) or 1.5 to 2.0% (162, 374) agar resulted in the best swarming. In this study the tryptone seemed to be the major factor controlling the production and width of the fringe. The optimum concentration (resulting in the widest fringe) was 0.05% or sometimes 0.1% tryptone, although good fringes were also seen at 0.2% as well. The observations of Stanier (374) and Hayes (162) that more than 1% tryptone usually results in compact eubacterial-like colonies, that 0.25-1.0% gives 'intermediate' colonies, and that below 0.25% results in the best swarming have been corroborated here.

Table 34

Occurrence of a fringe at 2 days on Cook's Cytophaga Agar*

Agar Concentration%	Tryptone Concentration (%) → 0.05		0.1		0.2		0.5		1.0		2.0	
0.75	(405)*	(13B)	405	13B	405		405		405		405	
	4539	(3)	4539	(3)		(3)						495
	(4433)	495	4433	495	4433	495	4433	495				
	4707	9D	4707	9D		9D		9D		9D		
		(2)		2		2		2	Bryant	2	Bryant	
	(A15)	(D)	A15	D		D		D		D		
	B-2-25	(6)	B-2-25	6	B-2-25	6		6				
	(E-1-25)	4541	E-1-25	4541	E-1-25	4541	E-1-25	4541		4541		
	H_2O-1A		H_2O-1A									
1.0 ↓	405	(13B)	405	13B	405		405		405		405	
	4539	(3)	4539	(3)		(3)						
	4433	495	4433	495	4433	495		495				
	4707	9D	4707	9D		9D		9D		9D		9D
	Bryant	2	Bryant	2	Bryant	2	Bryant	2	Bryant	2	Bryant	
	A15	D	A15	D		D		D		D		
	B-2-25	6	B-2-25	6	B-2-25	6	B-2-25	6				
	E-1-25	4541	E-1-25	4541	E-1-25	4541	E-1-25	4541		4541		
	H_2O-1A		H_2O-1A									

Table 34 - continued

Occurrence of a fringe at 2 days on Cook's Cytophaga Agar*

Tryptone concentration (%) ⟶

Agar Concentration %

	0.05		0.1		0.2		0.5		1.0	2.0
1.5	405	13B	405	13B	405	13B	405		405	405
	4539	3	4539	3		3		3		
	4433		4433		4433					
	4707	9D	4707	9D	4707	9D		9D		
						2	Bryant			
		D		D		D		D		
	B-2-25		B-2-25	6	B-2-25	6	B-2-25	6		
	E-1-25	4541	E-1-25	4541	E-1-25	4541	E-1-25		E-1-25	
2.0 ↓	405	13B	405	13B	405	13B	405		405	405
	4539	3	4539	3		3		3		
	4433		4433		4433					
	4707	9D	4707	9D	4707	9D	4707	9D	9D	9D
										Bryant
		D		D		D				
	B-2-25		B-2-25	6	B-2-25	6	B-2-25			
	E-1-25	4541	E-1-25	4541	E-1-25		E-1-25		E-1-25	

* Subsurface growth is indicated by parentheses, no fringe can be observed.
No fringe or subsurface growth were observed in the organisms 18H, 15D, 8186, 8187, 8535 and 9059.

Table 35

Occurrence of a fringe at 6 days on Cook's Cytophaga Agar*

Agar concentration (%) ↓	Tryptone concentration (%) → 0.05	0.1	0.2	0.5	1	2
0.75	(405)* (13B)	(405) 13B	(405) 13B	405	405	
	4539 (3)	(3)	(3)	3		
	(4433) (495)	(4433) (495)	4433 495	4433	4433	
	4707 (9D)	4707 (9D)	4707 9D	4707 9D	9D	
	?(Bryant) 2	?Bryant 2	?Bryant 2	Bryant 2	2	
	(A15) D	A15 D	A 15 D	A15 D	A15	
	B-2-25 (6)	B-2-25 6	B-2-25 6	B-2-25	B-2-25	
	(E-1-25) 4541	E-1-25 4541	E-1-25 4541	E-1-25 4541		
1.0	405 (13B)	405 13B	405 13B	405		
	4539 (3)	4539 (3)	(3)	3		
	4433 (495)	4433 (495)	4433 495		4433	
	4707 9D	4707 9D	4707 9D	4707 9D	9D	
	(Bryant) 2	Bryant 2	Bryant 2	2		
	A15 D	A15 D	A15 D	A15 D		
	B-2-25 6	B-2-25 6	B-2-25 6	B-2-25 6		
	E-1-25 4541	E-1-25 4541	4541	4541	4541	

Table 35 - continued

Occurrence of a fringe at 6 days on Cook's Cytophaga Agar*

Agar concentration (%)	Tryptone concentration (%) → 0.05	0.1	0.2	0.5	1	2
1.5	(405) (13B) (3)	405 (13B) 3	405 13B	13B		
	4433 495	4433 495	495 9D	495		
	(Bryant) (2) (D)	Bryant 2 D	Bryant 2 D	2 D		
	6 E-1-25 4541	B-2-25 6 E-1-25 4541	4541	4541		
2.0 ↓	405 (13B) 3	405 13B 3	405 13B 3	13B		
	4433 495	495	495	495		
	4707 9D	4707 9D	9D	9D		
	Bryant 2 D	2 D	2 D	2 D		
	B-2-25 6	B-2-25 6	B-2-25	B-2-25		
	E-1-25 4541	E-1-25 4541	4541	4541		

* Subsurface growth is indicated by parentheses.

No fringe or subsurface growth were observed in the organisms H_2O-1A, 18H, 15D, 8186, 8187, 8535 and 9059.

Table 36

Occurrence of a fringe at 4 days on Skim Acetate Agar*

Agar concentration (%)	Skim Milk concentration (%) → 0.1		0.5		1.0	
0.75	(405)*	(13B)			405	
	(4539)	(3)	4539	(3)	4539	
	(4433)	(495)	4433		4433	495
	(4707)	(9D)	4707	9D	4707	9D
	(Bryant)	2	Bryant	2	Bryant	2
	(A15)	D	A15	D	A15	D
	(B-2-25)	6	B-2-25		B-2-25	
	(E-1-25)	(4541)	E-1-25	(4541	E-1-25	4541
	(H_2O-1A)	9059	H_2O-1A	9059	H_2O-1A	
1.0	(405)	13B	405		405	
	4539	3	4539	3		3
	4433		4433		4433	495
	4707	9D	4707		4707	
		2		2		2
	(A15)	D	A15	D		D
	(B-2-25)	6	B-2-25		B-2-25	6
	(E-1-25)	4541	E-1-25	4541	E-1-25	4541
	H_2O-1A	9059	H_2O-1A	9059	H_2O-1A	
1.5	405	13B			405	
	4539	3	4539	3		3
	4433	495	4433		4433	
	4707	9D	4707	9D		
		2		2		2
	A15	D	A15	D		D
	B-2-25	6	B-2-25	6	B-2-25	
	E-1-25	4541	E-1-25	4541	E-1-25	4541
	H_2O-1A	9059	H_2O-1A	9059	H_2O-1A	

*Subsurface growth is indicated by parentheses.
No fringe or subsurface growth were observed in the organisms 18H, 15D, 8186, 8187 and 8535.

The rapid spreading of cytophaga colonies often results in a very wide growth on a streak plate. The influence of the concentration of agar and of tryptone upon the width of growth was investigated (Appendices I and II). With an increase in the percentage of agar the width of all the organisms tested generally decreased, the widest growth occurring at 0.75 and/or 1.0% agar. Increasing the tryptone concentration had different effects on the different groups of organisms. There was a tendency for the width of the 'spreaders' to decrease, whilst that of the 'intermediates' stayed about the same, whereas the lateral growth of the 'non-spreaders' increased. The largest diameter in the 'spreading' group was always shown on 0.05 to 0.2% tryptone. It is apparent that the use of a low tryptone concentration, even below the 0.2% in current use in Cook's Cytophaga Agar, would enhance the lateral growth of spreading types whilst restricting the non-spreaders.

As the tryptone concentration was increased the organisms showed a deeper colouration, and where a brown water soluble pigment was produced it too increased with the tryptone level (2, D, 6 and 4541), or was only produced at the higher concentrations for example 0.5% and over (13B), 1% and over (495) or 2% and over (8186, 8187, 8535). The agar concentration had little influence on the colours observed.

The experiment on fresh and dry plates of Cook's Cytophaga Agar showed firstly that large differences do occur between the morphology on agar of different moisture contents and different physical properties (Figure 23 and Table 37).

Table 37

Growth codes on fresh and
dry Cook's Cytophaga Agar plates after 5 days at 25°C

Organism	'Dry' 3-week-old	'Fresh' 1-hour-old
"Spreaders"		
ATCC 17061	SUB E 23 α bf	III K 53 α f
405	III D 55 α ppf	III J 55 α f
Alfalfa	IB 50 α c	I D 80 β fw
Bryant	II A 3 γ f	II B 5 γ f
A 15	I A 1 s	I E 50 β bes
B-2-25	I H 58 α w	I J 54 α
E-1-25	I H 58 α w	I J 54 α
H_2O-1A	I C 52 γ b	I E 50 α bcess
1-10	I H 83 α ew	III J 83 α ew
3	III C 51 β ppf	III D 51 β ppf
3-19	I C 51 β w p	III G 51 α w
3-22	II C 50 β wf	II D 50 β wf
4433	III H 50 α fw	I J 50 α ffw
4539	I F 50 α b	I G 50 α be
4707	II D 56 α ffee	II F 56 α ffe
NCIB 9059	I B 3 γ	I B 3 γ
FS-1	IV H 81 α s	IV J 81 α s
Stanier 6	no growth	no growth
10782 (C.H.)	no growth	no growth
2	III H 55 α fe	III H 55 α fe
D	III G 55 α fe	III H 55 α fe
6	III C 55 β	III C 5 γ f
4541	III F 55 α bf	III F 55 β bf
PC 15	IV B 3 γ o	no growth
9-11	II C 51 β ef	II C 51 β ef
9D	III E 54 α f	III J 54 α

Table 37 - continued

Growth codes on fresh and

dry Cook's Cytophaga Agar plates after 5 days at 25°C

Organism	'Dry' 3-week-old	'Fresh' 1-hour-old
495	I B 52 β e	II C 52 γ f
AL-1	I B 1 s	plate covered
21123	II B 3 β	III K 72 α pp
18LY	I C 51 β c	II C 51 β w
18LW	I C 3 β sw	III D 8 ppw
4553 (i)	II C 3 γ s	I B 2 β w
4553 (ii)	III D 51 β	III D 51 β
4554	II B γ s	plate covered
4555 (i)	I B 4 γ cs	I B 1 eog
4555 (ii)	I C 1 ws	plate covered
4556 (i)	I C 3 γ s	plate covered
4556 (ii)	I A 3 γ c	I B 3 γ c
4557 (i)	I B 1 sw	I C 4 β pw
4557 (ii)	I A 1	I A 1
4558 (i)	I B 1 s	plate covered
4558 (ii)	I B 1	I A 1
4559 (i)	I C 1 s	II C 4 β w
4559 (ii)	II B 51 γ c	II B 3 γ c
4560 (i)	I B 32 γ sw	plate covered
4560 (ii)	I B 1 s	plate covered
4560 (iii)	I B 3 γ c	I B 3 γ c
4561 (i)	III C 8 fw	IV A 8 fw
4561 (ii)	II B 1 s	plate covered
4562 (i)	II C 1 sf	III C 3 γ pw
4562 (ii)	II C 51 β s	III D 52 β wfs
4563 (i)	I B 1 pws	III D 52 α psf
4563 (ii)	I C 3 γ ws	plate covered

Table 37 - continued

Growth codes on fresh and

dry Cook's Cytophaga Agar plates after 5 days at 25°C

Organism	'Dry' 3-week-old	'Fresh' 1-hour-old
4564 (i)	II C 3 β ws	plate covered
4564 (ii)	II B 1 s	plate covered
4565 (i)	I B 1 ws	plate covered
4565 (ii)	I B 1 ws	plate covered
13B	II C 52 β c	I K 52 α cc
3C	III C 3 β w	III F 4 β p
"Non-spreaders"		
7-1	I A 1	I A 1
15D	I B 3 γ	I A 3 γ
18H	I B 3 β cs	I H 82 pp s
NCIB 8186	I A 1	I A 1
NCIB 8187	I A 1	I A 1
NCIB 8188	I A 1	I A 1
NCIB 8195	I A 1	I A 1
NCIB 8535	I A 1	I A 1
NCIB 8767	I B 1 c	I D 3 α w
NCIB 8771	I A 1	I A 1
NCIB 8992	I A 1	I A 1
NCIB 9157	I A 1	I A 1
NCIB 9290	I A 1	I F 53 α cfw
NCIB 9324	I A 1	I A 1
RL 8	I A 1	I A 1
NCIB 8185	I A 1	I A 1
NCIB 8204	I A 1	I A 1
5-9	I B 1 w	I B 1 w
14	I A 1	I B 1 c
402	I C 1	I C 1

Out of the 80 tested, seven organisms produced a fringe on the fresh plates but not on the older ones, and a further four strains had a wider fringe on the moist plates. Conversely three organisms had a fringe on the old but not the fresh plates. Over half of the cultures, mostly the 'spreaders', increased their width on fresh plates and 15% achieved a greater complexity of growth, whilst two organisms decreased in complexity. Only two of the 14 *Flavobacteria* showed a difference in growth form on the two types of plates. All of the 26 'spreading' cytophagas plus 28 out of 33 'intermediates' and five out of seven 'non-spreading' cytophagas reacted differently to the moist and dry media.

Towards a definition of 'spreading'.

The definition of 'spreading' in terms of an instantly recognizable, unique and constant feature of cytophagas is problematical. From the foregoing work it appears that the possession of a fringe is the best characteristic with which to work. It is not always present on all media at all stages of growth and thus some searching may have to be done in order to establish its presence. The total width of growth and the complexity of the morphology are also good yardsticks, and these will now be examined in relation to the possession of a fringe. The maximum fringe width and maximum width of growth relate quite well in the spreading organisms (Table 38 and Figure 24), but the relationship obviously means less in the narrower 'intermediate' organisms.

Table 38

Maximum fringe and width on Cook's Cytophaga Agar*

Organism	Maximum fringe		Maximum width		Relationship+	
	2 days	6 days	2 days	6 days	2 days	6 days
Spreaders						
405	IV	III	J	K	+	+
4539	IV	II	H	J	+	+
4433	IV	III	J	K	+	+
4707	IV	IV	H	K	+	±
Bryant	III	III	F	H	±	-
A15	II	IV	D	G	±	+
B-2-25	III	IV	J	K	±	+
E-1-25	III	III	J	K	±	+
H_2O-1A	III	I	F	G	+	n.a.
3^2	III	III	E	J	+	+
9D	III	IV	H	J	±	+
2	III	III	G	K	±	+
D	IV	III	G	J	+	+
6	III	III	C	D	+	+
4541	IV	III	D	H	±	+
Intermediates						
13B	III	III	B	D	-	-
495	III	IV	C	D	+	-
Nonspreaders						
18H	I	I	B	D	n.a.	n.a.
15D	I	I	B	C	n.a.	n.a.
Flavobacteria						
8186	I	I	B	C	n.a.	n.a.
8187	I	I	A	B	n.a.	n.a.
8535	I	I	B	B	n.a.	n.a.
9059	I	I	D	D	n.a.	n.a.

* Subsurface growth has been ignored for the purposes of this table.

+ Relationship of the increase in fringe width (I to IV) with increasing total width (A to K), for examples see Figure 24.

n.a. = not applicable because no fringes occurred.

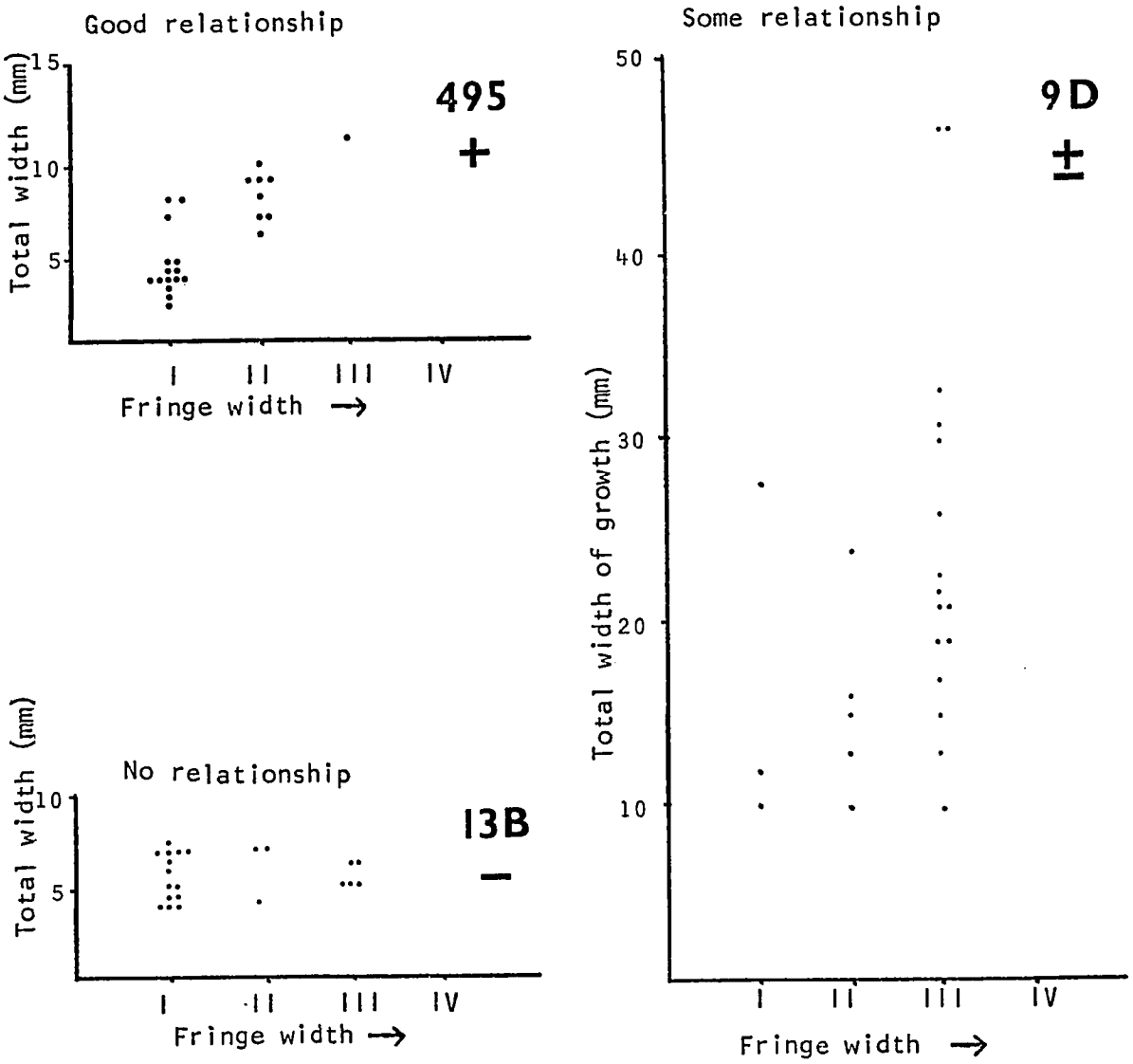


Figure 24. Examples of fringe/total width relationships. Each point refers to one plate (Table 32) after 2 days growth.

In the 23 organisms that were tested on 24 different tryptone/agar media it was found that the production of a fringe (Code II, III or IV) related rather well to the development of a fairly complex growth form (Code 50 to 88). In the 552 tests read at two days only three of those having a fringe had a simple growth form (Code 1 to 4), and less than eight percent of those showing no fringe had a complex development. At six days there were no fringed simple forms, but just over 15% had complex morphologies which lacked a fringe. It is thought that the production of a complex growth form is characteristic of most cytophagas, but that sometimes a fringe may be 'overgrown' by the slimy, thicker mass of cells following it. This idea is borne out firstly by the fact that the percentage of tests showing a complex growth form but no fringe doubled between two and six days growth. In all 69 plates showed a disappearance by six days of a fringe which was present on that plate at two days. However, there were also 44 plates in which a fringe developed in the same time period. Secondly, this hypothesis may explain the position of the so-called 'intermediate' organisms which are all very mucoid and may or may not show a fringe, it being overlain by excess slime. Although it is now known that the production of a fringe varies with the medium,the concentration of agar and tryptone, with moisture level and with time, the factors governing the appearance of this unique characteristic are not yet fully understood.

A standard medium for fringe production?

Complications arise when it is desired to recommend a standard medium for testing the presence or absence of fringes as an indication of spreading growth. Firstly, among the wide range of organisms studied here many different patterns of fringe production on the various media were observed. Secondly, subsurface spreading through the agar, the dimensions of which obviously cannot be compared directly with those of surface growth, is encouraged at low agar and low tryptone concentrations (Table 39). Subsurface growth was found to occur in one or more strains at all agar concentrations tested and from 0.05 to 0.2% tryptone, which are precisely the same proportions at which fringes are best developed in most organisms. It is clear that no one medium can be ideal for expressing spreading in all organisms in which it occurs. Therefore it is suggested that a range of media be tested, namely from 0.75 to 2% agar at concentrations of tryptone ranging from 0.05 to 0.2% viz:

		Tryptone %		
		0.05	0.1	0.2
Agar %	0.75	SUB	SUB	F
	1.0	SUB	SUB	F
	1.5	SUB	F	
	2.0	F		

SUB = subsurface growth

F = fringe

Table 39

Occurrence of subsurface growth

Agar % ↓	Cook's Cytophaga Agar Tryptone % → 2 days						Cook's Cytophaga Agar Tryptone % → 6 days						Skim Milk Agar Skim Milk % →			
	0.05	0.1	0.2	0.5	1.0	2.0	0.05	0.1	0.2	0.5	1.0	2.0	0.1	0.5	1.0	
0.75	405 3 4433 2 A15 D E-1-25 13B 6	3	3				405 4433 E-1-25 13B 3 495 9D 6	405 4433 3 495 9D	405 3				405 A15 3 4539 13B 4541 4433 3 4707 495 Bryant 9D B-2-25 E-1-25 H_2O-1A 4541			
1.0	13B 3	3	3				Bryant 3 13B 495	3 495	3				405 A15 B-2-25 E-1-25			
1.5							405 2 13B 13B D 3									
2.0							13B									

Indicated in the above figure are the most likely places for subsurface growth (SUB) and fringes (F) to occur. To obviate the use of 12 different media for each organism required to be tested, it is further suggested that the central two media be tried first, that is 0.1% tryptone and 1.0% agar, and 0.1% tryptone and 1.5% agar. With the organisms tested here 17 out of 17 at 2 days and 16 out of 16 at 6 days produced a fringe on one or both of these media. However if subsurface growth is encountered on both media, or only compact colonies are produced, then further media can be made with higher or lower concentrations of the constituents respectively. It is suggested that colonies be observed at least twice, at two and six days.

VII.2.5 Conclusion

Spreading of cytophagas has been defined primarily as the production of an extremely thin layer of advancing cells at the growth front (the fringe), with also the attainment of great width and complexity of the colony. The production of a fringe may be masked under certain conditions such as when large amounts of slime are present. Media and conditions are described with which to enhance spreading growth, notably fringe production. The two non-spreading organisms 18H and 15D which have previously been regarded as fairly closely related to *C.johnsonae* var. *denitrificans* (see Chapter III page 101), have not yet been observed to produce a fringe, and have a fairly narrow and simple growth form. It would be interesting now to grow

these two organisms on Pep. Milk I medium which has also been shown to enhance spreading of cytophagas (see Chapter IV, page 110; 71). Their systematic position as cytophagas is reviewed further in Chapter VIII. The three non-spreading *Flavobacterium* strains, *F.esteroaromaticum* NCIB 8186, *F.flavescens* NCIB 8187 and *F.aquatile* NCIB 8535 are also very different from typical *Cytophaga* species, whilst *F.pectinovorum* NCIB 9059 was shown to be able to produce a fringe and wide, complex growth, which adds further evidence to the suggestion that this is really a *Cytophaga* species (275).

A codified description, from which the colonial growth can be visualized, has been developed. It describes the width of the fringe, total width of growth, morphological form, comparative width of the central and outer zones, and certain special features of individual colonies. The difficulty in obtaining good photographs of spreading colonies and especially of the fringe area can be compensated in part or wholly by the use of such a code, which is also simpler and cheaper than keeping a photographic record of every plate. As with a photograph, the code allows detailed comparisons to be made within the present data and with future experimental material, and it could also be fed into a computer as a photograph could not.

CHAPTER VIII

TAXONOMIC STUDIES ON CYTOPHAGAS AND THEIR ALLIES

VIII.1. Important characteristics of the myxobacteria

Some of the prominent characteristics of the myxobacteria, namely the DNA base composition, growth rate, vegetative cell morphology, ultrastructure, colour and swarming morphology, have already been discussed. Three other important properties will be treated in this section, they are the degradation of carbohydrates and polysaccharides, proteolysis and the predatory capabilities of this intriguing group.

The use of both soluble and insoluble macromolecules has long been recognized as a general characteristic of the myxobacteria (100, 373). Substrates utilized include various proteins, DNA, RNA, cell walls, lipids, cellulose, agar, chitin, starch, alginate, inulin, pectin, keratin, laminarin, heparin, porphyran and glycogen. Such molecules are too large to enter the cell hence the enzyme systems are extracellular, usually being bound to the cell wall or to the slime polysaccharide exuded by the cells (371). There has been little quantitative work done until recently, and more is known qualitatively about the cytophagas than about the fruiting myxobacteria. The fruiters are usually capable of hydrolysing starch, glycogen, casein, gelatin, RNA, DNA and various sugars, and cellulose, inulin and aesculin can be utilized by some species, but probably none are capable of attacking agar (100, 262, 267, 268, 269, 285).

The cytophagas are able to degrade a wide range of carbohydrates (381) and polysaccharides as well as numerous proteinaceous materials, and the list is longer for many species mainly because more work has been done on these organisms than on the higher myxobacteria. For example, Napier (279) has reported that culture filtrates of a *Cytophaga* sp. (NCIB 9497) had lipase, protease, elastase, laminarinase, keratinase and chitinase activities and were able to lyse mould mycelium. Classical cellulose deomposers like *Sporocytophaga myxococcoides* and *Cytophaga hutchinsonii* and the marine species *C.haloflava* and *C.rosea* show a highly distinctive nutritional specialization, as they are usually unable to use any source of carbon except carbohydrates, mostly cellulose, cellobiose and glucose (372, 373). This means that they must compete very efficiently with other more versatile cellulose decomposers. In this connection it may be mentioned that these cytophagas prefer direct contact with the cellulose fibre, and due to their ability to creep on solid surfaces they rapidly invest the fibres,which are then decomposed in a very short time. Though rather restricted in their choice of energy source, the "classic" cellulose degraders are able to use inorganic as well as a number of organic nitrogen sources (Table 4, pages 31-42).

A number of nutritionally versatile cytophagas have been described that are also capable of attacking cellulose. The marine *C.diffluens*, and some soil species (eg. *C.albogilva*, *C.deprimata*) belong here. It is interesting to note that the cellulolytic ability of these organisms is only very feeble in comparison with that of the

'specialists' mentioned above. Fuller and Norman (119, 120) reported that the cellulolytic ability of *C.albogilva* and *C.deprimata* was weak on first isolation and was soon lost when they were cultured on simpler carbohydrates.

The agarolytic (Table 5, pages 43-54) and chitinoclastic *(C.johnsonae)* cytophagas are very versatile organisms that attack a wide variety of simple carbohydrates and nitrogenous compounds. The hydrolytic enzymes from a *Cytophaga* sp.(NCMB 1327) which catalyse the degradation of agarose and related polysaccharides, notably the algal galactan sulphate porphyran, have recently been described (98, 400, 401). The regular occurrence of agar decomposers in the marine environment indicates their important role in the natural decomposition of seaweed polysaccharides. However, agarolytic bacteria are not confined to sea and shore; there are several soil forms and some angiosperm pathogens, but so far no nonmarine agarolytic cytophagas. The only chitin-degrading cytophaga hitherto named, *C.johnsonae*, has been found in soils in many parts of the world (409), which indicates the ubiquitous distribution of a very competitive population of such organisms. Various enzymes produced by *C.johnsonae* are also being studied, notably those which catalyze the breakdown of chitin (390). Unlike other chitinolytic bacteria some strains of *C.johnsonae* do not liberate an extracellular chitinase, but need close contact with the chitin particles in order to hydrolyse them. Other strains liberate an extracellular chitinase and a chitobiase. Other studies have shown that the endo-polygalacturonate lyase of *C.johnsonae* has a different

mode of action from that of the polygalacturonate lyases of *B.polymyxa*, *Erwinia caratovora* and *Xanthomonas campestris* (389), and that glucanases produced by *C.johnsonae* have facilitated examination of the cell wall structure of yeasts (15, 16). It is reasonable to expect that chitinoclastic marine cytophagas will be found, as chitin forms the bulk of the exoskeletons of the Foraminifera and Crustacea as well as occurring in other forms, for example in the nematocysts of the Hydrozoa, and these residues must ultimately be recycled.

The amylolytic non-cellulolytic organisms *C.succinicans*, *C.fermentans* and *C.salmonicolor* are also capable of using a variety of sugars as carbon sources, but little else. A number of other unnamed strains have been isolated, for instance by Stanier (374), which decompose starch but not agar, chitin or cellulose. The fish pathogens *C.columnaris* and *C.psychrophila* so far as is known do not degrade polysaccharides. It has to be emphasized that many of the above statements are based on somewhat incomplete data (see Tables 4 and 5, pages 31-54).

Of the four *Flavobacterium* species of especial interest here (Table 7, pages 67-72), *F.aquatile*, *F.meningosepticum* and *F.pectinovorum* will use a variety of sugars but not alcohols. *F.aquatile* will not degrade dextrin or starch, *F.meningosepticum* does not use starch, but *F.pectinorovum* will utilize inulin, pectin and starch. Little has been published about *F.heparinum* apart from its ability to degrade heparin (249).

Most of the information on the 15 *Flexibacter* strains described in Table 9 (pages 83-88) is derived from the work of Lewin (245, 246). He found that up to four sugars could be degraded by any one species, but six species are not yet known to use any sugars. Glycerol is used only by *F.sancti*, and up to three organic acids may be used by several strains, while six species are not known to use any organic acids. The genus *Flexibacter* is separated from *Cytophaga* on its inability to degrade a wide range of polysaccharides, notably cellulose. Of the 15 known species and varieties isolated from various marine and non-marine habitats, none are known to decompose agar, alginate, cellulose or chitin, although in the author's opinion the chitin test employed by Lewin was not satisfactory. He used a suspension of chitin particles and incubated the tests for "one or two weeks", whilst experience with *C.johnsonae* and related strains has shown that a partially hydrolysed chitin suspension and incubation for at least one month are necessary (see Method 20, page 249). About one half of the *Flexibacter* species and varieties attack starch, and three out of 13 tested liquefy CMC. This raises the question of the interrelation of CMC and cellulose degradation. According to King and Vessal (214) the enzymatic degradation of these two polymers is not identical, but nevertheless the series of enzymes involved with each substrate are part of the same 'cellulase complex'. It thus seems inappropriate to the author to include CMC-decomposers in *Flexibacter* if cellulose decomposition, however measured, is the major (or indeed only) generic differentiation from *Cytophaga*. The three organisms in question are *F.aurantiacus*, *F.flexilis* var.

pelliculosus and *F.sancti* and all three also degrade starch. *Flexibacter aurantiacus* is composed of one strain of *Cytophaga psychrophila*, and one strain of *C.aurantiaca* which Lewin and Lounsbery found would not decompose cigarette paper (246). The medium used to determine cellulolytic ability contained several other simpler carbon sources including glucose, so their result is not entirely convincing. It has even been suggested that *C.aurantiaca* is merely a variety of *C.hutchinsonii* (375) and the author feels that this strain should have acted as a control on Lewin and Lounsbery's method, rather than accepting such a result and erecting a new species. It is also possible, of course, that this particular strain of *C.aurantiaca* has been cultivated on non-cellulosic material and had lost its ability to degrade cellulose, but this is hardly reason enough to transfer the whole species to a new genus. The author feels that it would be better to leave *C.psychrophila* in the genus *Cytophaga* for the time being until more work has been done on it, and on *C.columnaris* the other fish pathogen, to determine their polysaccharase potential. Similarly on the basis of polysaccharide degradation, *F.sancti* and *F.flexilis* var. *pelliculosus* would be better transferred to Cytophaga, even though this would split the latter species and necessitate a name change. The separation of the genera *Cytophaga* and *Flexibacter* will continue to be difficult until further characteristics are found to differentiate them, or until it is decided that they cannot be differentiated and that they should therefore constitute only one genus.

All species of higher and lower myxobacteria tested are proteolytic, and many are markedly so. Peptone (22), casein (294) and gelatin (321) are commonly utilized, and haemolysis and softening of coagulated egg and serum media have been reported in *Chondrococcus*, *Myxococcus*, *Sorangium* (203) and *Cytophaga* (273). Ribonuclease and deoxyribonuclease activity are practically always detectable (144, 275), and Norén reported that RNA or DNA could serve as the sole carbon and nitrogen source for *Myxococcus virescens* (285). Various potentially useful instances of proteolytic activity have been described, for example the solubilization of a structurally bound enzyme from fish muscle was achieved using a *Cytophaga* species (252), and an important protease has been described from myxobacter *(?Sorangium)* 495 (128, 431). The degradation of the highly insoluble protein keratin, which is the main component of vertebrate epidermal structures (hair, nails, feathers, etc.) is known to be accomplished by dermatophytic fungi (49) and by certain soil actinomycetes (238). Several *Cytophaga* strains, for example 10D, 15D, 16D, 161, 442 and others (259) and NCIB 9497 (279) have been found to produce keratinases. Strains 10D, 15D and 16D which were put in Group I, and 442 which was placed in Group II of Quadling, Cook and Colwell's taxonomic analysis (Chapter III) may be related to *C.johnsonae*. The isolates tested by Martin and So (259) used autoclaved feathers or wool as their sole source of carbon and nitrogen, but they did not solubilize the unmodified substrates. Strain NCIB 9497 had keratinase activity against unmodified hair and stratum corneum.

The extracellular proteases of *Cytophaga* 16D are present naturally as a complex with acidic polysaccharides of the slime layer (72). This slime-enzyme complex is hypothesized as responsible for attaching cells to an insoluble substrate as well as ensuring that degradative enzymes are kept in close contact with the substrate (72). This observation would also explain the rather poor solubilization of autoclaved feathers by both the culture filtrate and the isolated enzyme when compared with the rapid rate of digestion of the feathers by the bacterial cultures. Another postulated role for the enzyme-polysaccharide conjugate is the stabilization of the enzyme. Duckworth and Turvey (98) found that separation of a *Cytophaga* agarase from its complex with slime polysaccharide resulted in marked instability of the enzyme. Calcium ions and soluble proteins were found to be important in the stabilization of the isolated protease from 16D against denaturation by pH and heat, and this may indicate that the slime polysaccharides have a similar role in the natural environment.

Although the fish pathogens *Cytophaga (Chondrococcus) columnaris* and *Cytophaga psychrophila* do not seem to degrade polysaccharides they are actively proteolytic. Perhaps this activity plays an important part in their pathogenicity by breaking down tissues at sites of infection.

Studies on the *Flavobacterium* species of interest here, *F.aquatile, F.meningosepticum* and *F.pectinovorum* have shown all three to degrade gelatin and litmus milk, but haemolysis is not usually shown. No information on the proteolytic ability of *F.heparinum* is

available to the author's knowledge (Table 7, pages 67-72).

All the described species of *Flexibacter* are proteolytic, using gelatin, peptone, tryptone and casamino acids, and one strain is reported as decomposing albumin (Table 9, pages 83-88). Lewin and Lounsbery reported that a number of strains of flexibacteria required certain amino acids in the nutrient medium (246). The concentrations of free amino acids found in natural waters (45, 92) would not suffice for appreciable growth however, unless the organisms were able to accumulate them effectively. One must therefore conclude that the majority of the strains requiring specific amino acids for growth obtain these compounds by the action of extracellular enzymes on proteins in their environment.

Lytic agents have been isolated from a wide variety of micro-organisms including *B.subtilis* (332), *Flavobacterium* sp. (205), *Staphylococcus aureus* (337), *Streptomyces* spp. (124, 335) and the fungus *Charalopsis* sp. (155). These enzymes attack only Gram positive bacteria, and very few organisms are known to exhibit lytic properties in mixed cultures or to use living cells as their nutrient substrate. Activity of this kind has been found in various myxobacteria and a voluminous literature exists on the antibiotic, bactericidal and bacteriolytic products of these micro-organisms (290, 379). It is well known that many of the higher myxobacteria are active and specialized destroyers of other forms of microbial life (373). When grown with yeasts or bacteria as a food source, species which fruit poorly or not at all on dung agar will fruit normally and abundantly (356). Their frequent

occurrence on dung in nature is due largely to the exceptionally high bacterial content of this material (373).

In general, myxobacteria feed better on Gram negative than on Gram positive bacteria (13, 23, 24, 290, 352, 386), although much variation exists in the preferences of individual species and indeed strains. Species of fruiting myxobacteria are known to attack many Gram negative, Gram positive and acid-fast bacteria, yeasts and other fungi including plant and animal pathogens, green and blue-green algae and even human viruses and nematodes (Table 40). Considerably less work has been done with cytophagas but they are known to attack Gram negative and Gram positive bacteria, actinomycetes, yeasts and other fungi including dermatophytes and phytopathogens, green and blue-green algae and nematodes (Table 41) and some cytophagas are human pathogens (135, 136 and page 60). Various 'Sorangiaceous' and other unidentified non-fruiting myxobacters have also been shown to attack Gram negative, Gram positive and acid-fast bacteria, actinomycetes,fungi including yeasts and plant and animal pathogens, green and blue-green algae and nematodes (Table 42). In the literature mostly lysis of live or dead cells has been reported, but in many instances it is difficult to ascertain whether lytic or antibiotic activity was encountered by the authors. For simplicity, in the following tables no attempt has been made to differentiate between lysis of living or dead cells, lysis of cell wall preparations, antibiotic action or the various combinations of these that have been observed.

Table 40

Micro-organisms attacked by fruiting myxobacteria*

ANGIOCOCCUS sp. (204).

G-	*Aerobacter aerogenes*		*S. typhosa*
	A.cloacae		*Shigella dysenteriae*
	Escherichia coli	G+	*Bacillus cereus*
	Proteus morganii		*Sarcina lutea*
	P.vulgaris		*Staphylococcus albus*
	Pseudomonas aeruginosa		*S.aureus*
	Salmonella schottmuelleri		*Streptococcus faecalis*

ARCHANGIUM GEPHYRA and *A.PRIMIGENIUM* (204).

G-	*A.aerogenes*	G+	*B.cereus*
	A.cloacae		*B.subtilis*
	E.coli		*S.albus*
	P.morganii		*S.aureus*
	P.vulgaris		*S.faecalis*
	S.schottmuelleri	Yeasts	*Candida albicans*
	S.typhosa		*Saccharomyces cerevisiae*
	S.dysenteriae		

ARCHANGIUM VIOLACEUM (175, 176, 177).

G+ *Micrococcus lysodeikticus* Yeast *S.cerevisiae*

CHONDROCOCCUS BLASTICUS (23, 24).

G- non-sporeforming rods, mostly white and yellow, common soil forms.

CHONDROCOCCUS EXIGUUS (352, 353).

G- *E.coli* *Pseudomonas fluorescens*

* Note: Owing to the confused state of nomenclature in the fruiting myxobacteria, names have been left as designated by the author hence some may overlap. Where possible the names of other species conform with the seventh edition of Bergey's Manual (42).

Table 40 - continued

Micro-organisms attacked by fruiting myxobacteria.

CHONDROCOCCUS CORALLOIDES (204, 283, 284, 286).

G-	*A.aerogenes*	G+	*B.cereus*
	A.cloacae		*B.subtilis*
	Aerobacter sp.		*S.albus*
	E.coli		*S.aureus*
	P.morganii		*S.faecalis*
	P.vulgaris	Acid-fast	*Mycobacterium phlei*
	P.aeruginosa		*M.smegmatis*
	S.schottmuelleri	Yeast	*C.albicans*
	S.typhosa		*S.cerevisiae*
	S.marcescens		
	S.dysenteriae		

CHONDROCOCCUS LACTICUS (290).

G-	*E.coli*		*B.megatherium*
	Flavobacterium sp.		*B.subtilis*
	P.fluorescens		*Micrococcus* sp.
	S.marcescens		*S.lutea*
G+	*A.aerogenes*		

CHONDROCOCCUS MEGALOSPORUS (204).

G-	*A.aerogenes*	G+	*B.cereus*
	A.cloacae		*B.subtilis*
	E.coli		*S.albus*
	P.morganii		*S.aureus*
	P.vulgaris		*S.faecalis*
	P.aeruginosa	Acid-fast	*M.phlei*
	S.schottmuelleri		*M.smegmatis*
	S.typhosa	Yeast	*C.albicans*
	S.dysenteriae		*S.cerevisiae*

Table 40 - continued

Micro-organisms attacked by fruiting myxobacteria

CHONDROMYCES APICULATUS (233).

G-	*Alcaligenes* sp.		*S.marcescens*
	E.coli	G+	*S.albus*
	P.vulgaris		*S.aureus*
	S.schottmuelleri	Acid-fast.	*M.diphtheriae*
	S.typhosa		

CHONDROMYCES CROCATUS (204, 290, 312).

G-	*A.aerogenes*	G+	*B.cereus*
	A.cloacae		*B.megatherium*
	E.coli		*B.subtilis*
	Flavobacterium sp.		*Micrococcus* sp.
	P.morganii		*S.lutea*
	P.vulgaris		*S.aureus*
	P.flucrescens		*S.faecalis*
	S.marcescens		

MYXOCOCCUS FULVUS (68, 165, 204, 222, 256, 283, 284, 290, 292, 335, 352, 353, 384).

G-	*A.aerogenes*	G+	*Nocardia corallina*
	A.cloacae		*S.lutea*
	E.coli		*S.aureus*
	Flavobacterium sp.		*S.faecalis*
	P.morganii	Acid-fast.	*M.phlei*
	P.vulgaris		*M.smegmatis*
	P.aeruginosa	Yeasts	*Candida* sp.
	P.fluorescens		*C.albicans*
	S.marcescens		*C.pulcherrima*
	S.schottmuelleri		*S.cerevisice*
	S.typhosa		*Saccharomyces gracilis*
	S.dysenteriae		*Torula aurantiaca*

Table 40 - continued

Micro-organisms attacked by fruiting myxobacteria

G+	*B.cereus*	BG Alga.	*Nostoc muscorum*
	B.megatherium	G. Algae.	*Chlamydomonas eugametos*
	B.subtilis		*Scenedesmus Naegelii*
	Micrococcus sp.		*Stichococcus bacillaris*
	M.lysodeikticus		*S.mirabilis*
MYXOCOCCUS LACTIIS (290).			
G-	*A.aerogenes*	G+	*B.megatherium*
	E.coli		*B.subtilis*
	Flavobacterium sp.		*Micrococcus* sp.
	P.fluorescens		*S.lutea*
	S.marcescens		
MYXOCOCCUS OVALISPORUS (204).			
G-	*A.aerogenes*	G+	*B.cereus*
	A.cloacae		*B.subtilis*
	E.coli		*S.lutea*
	P.morganii		*S.aureus*
	P.vulgaris		*S.faecalis*
	P.aeruginosa	Acid-fast.	*M.phlei*
	S.schottmuelleri		*M.smegmatis*
	S.typhosa	Yeasts	*C.albicans*
	S.dysenteriae		*S.cerevisiae*
MYXOCOCCUS STIPITATUS (204, 290, 292).			
G-	*A.aerogenes*	G+	*B.subtilis*
	A.cloacae		*Micrococcus* sp.
	E.coli		*S.lutea*
	Flavobacterium sp.		*S.aureus*
	P.morganii		*S.faecalis*
	P.vulgaris	Acid-fast.	*M.phlei*

Table 40 - continued

Micro-organisms attacked by fruiting myxobacteria

G-	*P.aeruginosa*	Acid-fast	*M.smegmatis*
	P.fluorescens	Yeasts	*C.albicans*
	S.schottmuelleri		*Mycoderma valide*
	S.typhosa		*S.cerevisiae*
	S.marcescens		*S.gracilis*
	S.dysenteriae		*Schizosaccharomyces pombe*
G+	*B.cereus*		*Torulopsis utilis*
	B.megatherium		*Zygosaccharomyces* sp.

MYXOCOCCUS VIRESCENS (156, 157, 158, 159, 204, 206, 207, 208, 222, 283, 284, 286, 287, 288, 290, 294, 295, 352, 353)

G-	*Aerobacter* sp.		*N.corallina*
	A.aerogenes		*S arcina* sp.
	A.cloacae		*S.agilis*
	Agrobacterium tumefaciens		*S.lutea*
	E.coli		*S.albus*
	Flavobacterium sp.		*S.aureus*
	P.morganii		*S.faecalis*
	P.vulgaris	Acid-fast.	*M.phlei*
	P.fluorescens		*M.smegmatis*
	S.schottmuelleri	Yeasts	*C.albicans*
	S.typhosa		*S.cerevisiae*
	S.marcescens		*S.gracilis*
	S.dysenteriae		*T.aurantiaca*
G+	*B.cereus*		
	B.megatherium	G.Algae	*C.eugametos*
	B.mesentericus		*Hormidium flaccidum*
	B.mycoides		*S.Naegelii*
	B.subtilis		*S.bacillaris*
	Flavobacterium sp.		*S.mirabilis*
	Micrococcus sp.	Viruses	Influenza A
	M.lysodeikticus		Influenza B
			Mumps

Table 40 - continued

Micro-organisms attacked by fruiting myxobacteria

MYXOCOCCUS XANTHUS (26, 27, 65, 289, 384, 387).			
G-	*A.aerogenes*		*B.subtilis*
	Azotobacter beijerinckii		*M.lysodeikticus*
	A.chroococcum	Yeast	*Pullularia pullulans*
	A.vinelandii	BG Alga	*N.muscorum*
G+	*B.megatherium*		
MYXOCOCCUS SPP. (190, 235, 359, 360, 384, 438).			
G-	*Escherichia freundii*		*Ustilago zeae*
	Pseudomonas mildenbergii		*U.avenae*
	P.putida		*U.levis*
G+	*Micrococcus lysodeikticus*	BG Algae	*Lyngbya* sp.
Yeast	*S.cerevisiae*		*N.muscorum*
Fungi	*Saprolegnia* sp.		*Synechococcus cedrorum*
	Sorosporium reilianum		Five others
PODANGIUM ERECTUM (292).			
G-	*A.aerogenes*		*S.albus*
	E.coli		*S.aureus*
	P.vulgaris	Yeasts	*M.valide*
G+	*B.luteus*		*S.gracilis*
	B.megatherium		*T.aurantiaca*
	B.mesentericus	G.Algae.	*C.eugametos*
	S arcina sp.		*S.bacillaris*
	S.agilis		*S.mirabilis*
POLYANGIUM PARASITICUM (122).			
	Aquatic green algae		
POLYANGIUM VIOLACEUM (235).			
Fungus	*Saprolegnia* sp.		

Table 41

Micro-organisms attacked by cytophagas

SPOROCYTOPHAGA CAULIFORMIS (141).

G-	*Achromobacter* sp.		*S.typhi-murium*
	A.aerogenes	G+	*B.anthracis*
	E.coli		*B.anthracoides*
	E.freundii		*B.cereus*
	Klebsiella ozaenae		*B.mesentericus*
	K.pneumoniae		*B.mycoides*
	Salmonella bareilly		*B.panis-viscosi*
	S.braenderup		*B.sphaericus*
	S.enteritidis		*Clostridium perfringens*
	S.muenster		*S.lutea*
	S.paratyphi		*S.albus*
	S.typhi		*S.aureus*

CYTOPHAGA JOHNSONAE (335).

G-	*E.coli*	Yeast	*C.pulcherrima*
	P.aeruginosa		

CYTOPHAGA NCIB 9497 (279).

Fungi	*Aspergillus fumigatus*		*Rhizopus nigricans*
	Cephalosporium sp.		*Trichophyton rubrum*
	Microsporium canis		*T.sulphureum*
	Penicillium chrysogenum		

CYTOPHAGA 15D (209, 210).

Nematodes	*Aphelenchus avenae*		*Panagrellus* sp.
	Caenorhabditis briggsae		*Rhabditis oxycerca*
	Heterodera trifolii		

Table 41 - continued

Micro-organisms attacked by cytophagas

CYTOPHAGA N-5 (383, 385).

G-	*A.aerogenes*		*Lyngbya* sp.
	Alcaligenes providence		*Nostoc* sp.
	Citrobacter sp.		*Oscillatoria* sp.
	E.coli		*Phormidium* sp.
	K.pneumoniae		*Plectonema* sp.
	P.mirabilis	G.Algae	*Chlamydomonas* sp.
	S.typhosa		*Chlorosarcinopsis* sp.
	S.marcescens		*Dictyochloris* sp.
G+	*B.subtilis*		*Radiosphaera* sp.
	Gaffkya tetragena		*Spirogyra* sp.
	M.lysodeikticus		*Spongiococcum* sp.
	S.aureus		*Tetracystis* sp.
BG Algae	*Anaebaena* sp.		*Trichosarcina* sp.
	Calothrix sp.		*Zygnema* sp.

CYTOPHAGA FP-1 (346, 347).

G-	*A.aerogenes*	*Nostoc* sp.
	E.coli	*Oscillatoria prolifera*
	P.fluorescens	*Plectonema boryanum*
	S.typhimurium	*Spirulina platensis*
BG Algae	*Anacystis nidulans*	*S.tenuis*
	Coccochloris penyocystis	*Synechococcus cedorum*

PROMYXOBACTERIUM FLAVUM (182).

Fungi	*Botrytis* sp.
	Fusarium lini

Table 42

Micro-organisms attacked by unidentified non-fruiting myxobacteria

MYXOBACTER 3C (? *SORANGIUM*) (307, 308, 384).

G-	*A.aerogenes*		*S.sphericus*
	Agrobacterium radiobacter		*B.subtilis*
	A.tumefaciens		*Corynebacterium insidiosum*
	Alcaligenes faecalis		*C.sepedonicum*
	Arthrobacter globiformis		*Micrococcus denitrificans*
	Erwinia amylovora		*S.aureus*
	E.carotovora	Acid-fast.	*M.phlei*
	E.coli		*M.tuberculosis*
	Pseudomonas atrofaciens	Actinos.	*Streptomyces candidus*
	P.coronataciens		*S.griseus*
	P.fluorescens		*S.lavendulae*
	P.phaseolicola		*S.scabies*
	P.pisi		*Streptomyces* spp. (7)
	P.stutzeri	Yeasts	*Candida krusei*
	Rhizobium leguminosarum		*C.utilis*
	R.lupini		*Candida* sp.
	R.meliloti		*Endomycopsis vernalis*
	R.phaseoli		*Rhodotorula mucilaginosa*
	R.trifolii		*S.cerevisiae*
	Xanthomonas campestris		*S.fragilis*
	X.phaseoli var. *fuscans*		*S. logos*
G+	*Bacillus alvei*		*S.pombe*
	B.cereus		*Z.acidifaciens*
	B.coagulans		*Z.ravennatis*
	B.laterosporus		*Z.rugosus*
	B.pulvifaciens		

Table 42 - continued
Micro-organisms attacked by unidentified non-fruiting myxobacteria

Fungi	*Absidia* sp.	*Leptographium* sp.
	Acrospeira levis	*Menispora ciliata*
	Alternaria sp.	*Monilia sitophila*
	Aspergillus niger	*Mortieralla* sp.
	Bisporomyces chlamydosporus	*Mucor ramannianus*
	Botrytis cinerea	*Myrothecium* sp.
	Byssochlamys nivea	*Nigrospora* sp.
	Cephalosporium sp.	*Oidiodendron* sp.
	Chaetomium sp.	*Paecilomyces marquandii*
	Chrysosporium pannorum	*Papularia sphaerosperma*
	Circinella sp.	*Penicillium sacculum*
	Cladosporium herbarum	*Periconia macrospinosa*
	Corynespora cassiicola	*Pullularia pullulans*
	Coryneum umbonatum	*Rhinocladiella* sp.
	Curvularia lunata	*Rhizopus nigricans*
	Cylindrocarpon radicola	*Scolecobasidium constrictum*
	Cylindrocladium scoparium	*Stachybotrys atra*
	Emericellopsis minima	*S.aurantia*
	Fusarium culmorum	Mortierella *Stilbum* sp.
	F.oxysporum	*Stysanus*
	F.solani	*Trichoderma veride*
	Gliocladium roseum	*Trichothecium roseum*
	Gonytrichum macrocladum	*Verticillium albo-atrum*
	Helminthosporium sativum	*Zygorhynchus* sp.
	Humicola sp.	

NON-FRUITERS (384).

15/25 killed BG Alga *N.muscorum* and B Alga *Tetracystis intermedium*

6/25 killed *N.muscorum* only

4/25 killed neither

Table 42 - continued

Micro-organisms attacked by unidentified non-fruiting myxobacteria

MYXOBACTERS CP-1 to CP-4 (88).

BG Algae. *Anabaena ambigua*
A.catenula
A.cylindrica
A.flos-aquae
A.oscillariodes
Anabaenopsis circularis
Cylindrospermum sp.
Lyngbya sp.
Microcystis sp.
Nostoc calcicola
N.ellipsosporum
N.muscorum
Phormidium sp.
P.foveolarum
P.luridum
P.uncinatum
Plectonema spp.
P.boryanum
P.calothricoides
P.notatum

MYXOBACTER 495 (127, 128, 209, 210, 307, 308, 399, 433).

G- *A.radiobacter*
A.tumefaciens
Alcaligenes faecalis
Arthrobacter atrocyaneus
A.citreus
A.duodecadis
A.globiformis
A.nicotaniae
A.oxydans
A.pascens
A.ramosus
A.simplex
A.tumescens
E.coli
A.atrofaciens
P.stutzeri
Rhizobium meliloti
Xanthomonas phaseoli

G+ *B.subtilis*
C.insidiosum
C.michiganense
C.sepedonicum
M.lysodeikticus
N.corallina
N.globerula
N.opaca
Sarcina sp.
S.aureus

Actinos various

Fungi *Fusarium oxysporum*
Trichoderma sp.

Nematodes *Aphelenchus avenae*
Caenorhabditis briggsae
Heterodera trifolii
Panagrellus sp.
Rhabditis oxycerca

Table 42 - continued

Micro-organisms attacked by unidentified non-fruiting myxobacteria

MYXOBACTER AL-1 (106, 107, 108, 384).

G-	*Arthrobacter* spp. (6)	G+	*Micrococcus aureus*
	A.crystallopoietes		*M.lysodeikticus*
	Rhodospirillum rubrum		*S.lutea*
	Spirillum itersonii	G.Alga	*T.intermedium*
	S.serpens	BG.Alga	*N.muscorum*

MYXOBACTER 18L (? *Sorangium*) (209, 210).

Nematodes	*A.avenae*	*Panagrellus* sp.
	C.briggsae	*R.oxycerca*
	H.trifolii	

MYXOBACTER SPP. (224, 225, 363)

Phytopathogenic bacteria

Fungi *Verticillium dahliae*

Pinoy (312) was the first to propose that a symbiotic or parasitic relationship existed between myxobacteria and true bacteria, and Beebe (23, 24) considered myxobacters as obligate eubacterial parasites, which is not the case. In 1946 Oxford and Singh (295) proposed that myxobacteria had a dual action on their prey, first an antibiosis and then lysis. This assumption that antibiotics might play a role in the initial phase of lysis was supported by Norén (283, 284) and later by Kühlwein (233). It has since been shown however (26, 222, 256, 290), that antibiotic activity is not necessarily associated with lytic activity. Although the mechanism of lysis of living bacteria by myxobacteria is still not completely clear (386), Kletter and Henis (222) have attempted to define the process in *Myxococcus fulvus* and *M.virescens*. The first stages in the lysis of attacked cells were the adsorption of the myxobacters on to the bacterial cells and then their aggregation by means of slime. Adhesion to the culture flasks then took place followed by lysis and the release of bacterial proteins. The myxobacters were in close contact with their prey and their lytic enzymes remained bound to the cells until after lysis was completed, when they were detected in the culture filtrates.

The enzyme systems of some fruiting myxobacteria have been the subjects of detailed biochemical work. These include the cell wall-lytic enzyme system of *Myxococcus xanthus* (387, 388), the muramidase and protease of *Chondrococcus coralloides* (151, 152) and the bacteriolytic enzyme of *Archangium violaceum* (176, 177). Haska and

co-workers have studied various bacteriolytic and proteolytic enzymes from *M.virescens* (156, 157, 158, 159). Two non-fruiting "Sorangiaceous' myxobacters are responsible for the two best known bacteriolytic enzymes from this group. The first is Ensign and Wolfe's organism (? *Cytophaga*) AL-1 which produces a cell wall-lytic enzyme (106, 107, 108, 171, 184, 185), and the second is Cook's isolate (? *Sorangium*) 495 (128) which elaborates both α- and β-proteases (5, 89, 197, 399, 429, 430, 431, 432, 433).

Oxford (294) first demonstrated that a culture of myxobacter *(M.virescens)* had antibiotic activity. A variety of fruiting members of this Order, including *M.fulvus, M.lacteus, M.stipitatus, M.virescens, Chondromyces blasticus* and *C.crocatus* produce antibiotics that are effective against Gram positive bacteria, but inactive against Gram negative ones (290). Nevertheless these same Gram negative bacteria are lysed rapidly, which demonstrates that no correlation exists between the antibacterial activity and the bacteriolytic capacity of these myxobacters. The potent wide-spectrum antibiotic 'Myxin' is produced by the non-lytic, non-fruiting but high GC 'Sorangiaceous' myxobacter strain Cook 3C (84, 129, 242). This purified antibiotic is active against Gram positive, Gram negative and acid-fast bacteria, actinomycetes, yeasts and many other fungi including phytopathogens and dermatophytes (Table 42).

The anti-viral agent produced by *M.virescens* (206, 207, 208) may be of importance but there seems to have been no follow-up on these remarkable observations (100, 345). Bacteriocins have been detected in two species, *Sporocytophaga myxococcoides* (391) and *Cytophaga (Chondrococcus) columnaris* (10).

The unusual properties of the myxobacteria, and especially of the cytophagas, play a major role in the biochemical ecology of the soil and of natural waters. Cytophagas can be detected in most wet habitats that have been examined, and both aerobic and facultatively anaerobic species are known. Most significant is their decomposition of the structural elements and storage products of eukaryotic organisms, molecules which are often relatively resistant to breakdown by other micro-organisms. Their role in the humification of plant and animal remains in soil is now recognized (226, 227), and work is even in progress on the effects of herbicides on the activities of this group (241). The myxobacteria constitute a major group of slime-producing bacteria, and they therefore have importance in the stabilization of soil aggregates (425). Many other plant and animal wastes are recycled through the proteolytic activites of these bacteria. It has also been suggested that certain varieties of a *Sporocytophaga* species can act as indicators of the trophic levels of lake water (138, 139, 140). The discovery of the lytic and antibiotic activities of the myxobacteria has given further insights into their unique role in the ecological balance.

VIII.2. Selection of isolates for the present study

The scarcity of named *Cytophaga* species available from culture collections is a hindrance to any taxonomic study of this group. The position is worse for the soil and freshwater species than for the marine ones (Table 43). In addition the soil and freshwater forms were generally discovered earlier and many of the descriptions are really not adequate to allow comparison with present-day isolates (182,

378, 412). In this study an effort was made to acquire at least one named culture of each soil and freshwater *Cytophaga* reported to date (Table 1, pages 5-10). Nevertheless, representative cultures of only four of the 16 species and varieties so far described were able to be studied: two strains of *C.hutchinsonii*, two of *C.johnsonae*, and one each of *C.johnsonae* var. *denitrificans* and *C.succinicans* (Table 44). Three other strains were available but were not tested for the following reasons; the first culture of *C.aurantiaca* NCIB 8628 sent failed to survive lyophilization, however a subsequent slant culture is now growing well; the slant culture of *C.psychrophila* 144a received from Dr. E. J. Ordal failed to grow on any of the recommended or routine media; and the strain of *C.columnaris* has not yet been received. Type cultures for *C.fermentans* and *C.hutchinsonii* were suggested by Sneath and Skerman (358) and Hendrie et al. (163), but none of these are still available from institutional collections. The replacement for NCIB 9469, the suggested working type of *C.hutchinsonii* (163) is NCIB 10782 which is represented here. Efforts to obtain other authentic cultures from over 400 Type Culture Collections and also from various authors were unsuccessful.

Table 43

Extant Cultures of Currently Recognised Cytophagas

Species	Cultures Available	Isolated and/or Deposited By
Freshwater and Soil Species		
C.albogilva	None	
C.aurantiaca	NCIB 8628 (ATCC 12208)	Bortels 1954
C.deprimata	None	
C.hutchinsonii	NCIB 10782 (formerly "C.H.")+	Palleroni 1970
	Sneath D 465	Sneath
	Stanier and Doudoroff strain "6"	Kihara
C.anularis	None	
C.crocea	None	
C.flavicula	None	
C.lutea	None	
C.sylvestris	None	
C.johnsonae	ATCC 17061	van Niel
	ATCC 21123	Kyowa Ferm. Ind. 1968
	NCIB 10150	Webley 1967
" var. *denitrificans*	Cook 405	Cook 1961
C.rubra	None	
C.succinicans	Ordal RL8	Anderson 1961
C.tenuissima	None	
Freshwater Fish Pathogens		
C.columnaris	NCMB 1038	Ordal
C.psychrophila	Ordal 144a	Borg 1948

Table 43 (continued)

Extant Cultures of Currently Recognised Cytophagas

Species	Cultures Available	Isolated and/or Deposited By
Marine Species		
C.diffluens Stanier	None	
C.diffluens Stanier emend. Lewin	ATCC 23125, 23140, 23144, 23149 and 23155	Lewin 1969
" var. *aprica*	ATCC. 23126* and 23132	Lewin 1969
" var. *carnea*	ATCC 23142*	Lewin 1969
C.fermentans	ATCC 19072*	Veldkamp 1961
" var. *agarovorans*	None	
C.haloflava	None	
" var, *non-reductans*	None	
C.krzemieniewskae	None	
C.latercula	ATCC 23177*	Lewin 1969
C.lytica	ATCC 23157, 23169, 23174, 23176, and 23178*	Lewin 1969
C.marinoflava	NCMB 397 (ATCC 19326)*	Spencer 1960
C.rosea	None	
C.salmonicolor	ATCC 19041	Veldkamp 1961
" var. *agarovorans*	ATCC 19043	Veldkamp 1961
C.sensitiva	None	
Others		
Strain C	NCIB 9336	Gibson 1961
Strain H	NCIB 9337	Gibson 1961
? *Flavobacterium*	NCMB 292 (ATCC 19854)	MacLeod 1957
Cytophaga spp.	ATCC 21119, 21120, 21121, 21122, 21238	Kwoya Ferm. Ind. 1968
Flavobacterium heparinum (probably a *Cytophaga*)	ATCC 13125 (NCIB 9290)	Korn 1956

* = type strain + = suggested working type

Table 44

Codes, names and sources of strains*

Code	Name	Habitat	Isolation Location	Isolated by
Spreaders				
ATCC 17061	*Cytophaga johnsonae*	? Soil	? USA	C.B. van Niel
405	*C.johnsonae* var. *denitrificans*	Soil	Ottawa, Ontario	F.D. Cook
Alfalfa		Alfalfa roots	Edmonton, Alberta	F.D. Cook
Bryant		Soil	Alberta	F.D. Cook
A15		Diseased fish	Lake, Manitoba	D.C. Gillespie
B-2-25		Diseased fish	Lake, Manitoba	D.C. Gillespie
E-1-25		Diseased fish	Lake, Manitoba	D.C. Gillespie
H_2O-1A		Diseased fish	Lake, Manitoba	D.C. Gillespie
1-10		Lake water	Ft.McPherson, NWT	P.J. Christensen
3			Alberta	F.D. Cook
3-19		Lake water	Martin House, NWT	P.J. Christensen
3-22		Lake water	Martin House, NWT	P.J. Christensen
4433		Moose dung	Alberta	F.D. Cook
4539		Moose dung	Elk Island Pk.,Alta.	F.D. Cook
4707		Soil	Alberta	F.D. Cook
NCIB 9059	*Flavobacterium pectinovorum*	Soil	S.E. England	M.J. Dorey
FS-1	*Flexibacter* sp.	Soil	Samoa	G.D. Simon and D. White

*A photographic record of each strain is available as Appendix IV (author's copy).

Table 44 - continued

Codes, names and sources of strains

Code	Name	Habitat	Isolation Location	Isolated by
Stanier 6	*C.hutchinsonii*	? Soil	? USA	R. Y. Stanier
NCIB 10782 (C.H.)	*C.hutchinsonii*	? Soil	? USA	N. Palleroni
2		Lake water	Martin House, NWT	F.D. Cook
D		Lake water	Martin House, NWT	F.D. Cook
6		Lake water	Martin House, NWT	F.D. Cook
4541		Slough water	Alberta	F.D. Cook
PC 15		River water	Whitemud Ck., Alta.	P.J. Christensen
9-11		Lake water	Ft.McPherson, NWT	P.J. Christensen
9D		Soil	Ottawa, Ontario	F.D. Cook
Intermediates				
495	*? Sorangium*	Soil	Ottawa, Ontario	F.D. Cook
AL-1 i, ii, iii*		Soil	? Illinois, USA	R.S. Wolfe
ATCC 21123	*Cytophaga johnsonae*	? Soil	? Japan	Kwoya Ferm. Ind.
18LY, W*	*? Sorangium*	Soil	Ottawa, Ontario	F.D. Cook
4553 i, ii*		Soil	Carmangay, Alberta	F.D. Cook
4554		Soil	Angus Ridge, Alta.	F.D. Cook

Table 44 - continued

Codes, names and sources of strains

Code	Name	Habitat	Isolation Location	Isolated by
4555 i, ii*		Soil	Malmo, Alberta	F.D. Cook
4556 i, ii*		Soil	Vegreville, Alberta	F.D. Cook
4557 i, ii*		Soil	Breton, Alberta	F.D. Cook
4558 i, ii*		Soil	Peace River, Alta.	F.D. Cook
4559 i, ii*		Soil	Lac la Biche, Alta.	F.D. Cook
4560 i, ii, iii*		Soil	Lloydminster, Alta.	F.D. Cook
4561 i, ii, iii*		Soil	Olds, Alberta	F.D. Cook
4562 i, ii*		Soil	Vermilion, Alberta	F.D. Cook
4563 i, ii*		Soil	Alberta	F.D. Cook
4564 i, ii*		Soil	Alberta	F.D. Cook
4565 i, ii*		Soil	Alberta	F.D. Cook
13B		Soil	Ottawa, Ontario	F.D. Cook
3C	*? Sorangium*	Soil	Ottawa, Ontario	F.D. Cook
Nonspreaders				
7-1		Lake mud	Ft. McPherson, NWT	P.J. Christensen
15D		Soil	Ottawa, Ontario	F.D. Cook
18H		Soil	Ottawa, Ontario	F.D. Cook
NCIB 8186	*Flav. esteroaromaticum*	? Organic material		C.P. Hegarty

Table 44 - continued

Codes, names and sources of strains

Code	Name	Habitat	Isolation Location	Isolated by
NCIB 8187	*Flav. flavescens*			C. Darling
NCIB 8188a, b†	*Flav. suaveolans*	Creamery waste		M. Levine
NCIB 8195	*Flav. devorans*	? Freshwater		N. Porges
NCIB 8535	*Flav. aquatile*	Well water	Kent, England	C.D. Kelly
NCIB 8767	*Flav. resinovorum*	Soil	? France	P. Daste
NCIB 8771	*Flav. proteus*	Ale yeast	Brooklyn, USA	Schaefer Brewing Corp.
NCIB 8992	*Flav. suaveolans*	Butter		M. Grimes
NCIB 9157 a, b†	*Flav. rhenanum*	? Freshwater	? Czechoslovakia	M. Kocur
NCIB 9290	*Flav. heparinum*	Soil	? Maryland, USA	E.K. Korn
NCIB 9324	*Flav. lucecoloratum*			G.D. Wilkin
RL 8	*C.succinicans*	Freshwater fish	Washington, USA	R.L. Anderson
NCIB 8185	*Flav. arborescens*	? Freshwater		A. Stewart
NCIB 8204	*Flav. aurantiacum*	? Freshwater		
5-9		Lake water	Arctic Red R., NWT	P.J. Christensen
14		River water	N. Sask. R., Alta.	D. Donass
402		Soil	Ottawa, Ontario	F.D. Cook

*At least two cultural variants, a dirty-white mucoid one and a yellowish non-mucoid one, have been isolated from these strains by the author.

†Two colonial variants were isolated by the author from cultures received directly from the NCIB.

Four species of *Flavobacteria* have been implicated as possible cytophagas, *F.aquatile*, *F.heparinum*, *F.meningosepticum* *F.pectinovorum*, and one strain of each except *F.meningosepticum* were included in this study so that they could be compared with present *Cytophaga* species and if necessary be transferred to this genus. Representatives of ten other *Flavobacterium* species were used for comparative purposes (Table 44). One freshwater strain of *Flexibacter*, namely FS-1 recently described by Simon and White (349) was also included. Seven other *Flexibacter* species and varieties have been described from freshwater and five of these are now available.

Representative isolates from the arctic study (Chapter V) the yellow spreaders 1-10, 3-19 and 3-22, the yellow non-spreader 7-1, the pink spreader 9-11 and the pink non-spreader 5-9 were tested as well as strains used by Colwell, Cook and Quadling in their taxonomic analysis (see Chapter III), namely the white iridescent spreader 9D, the pink mucoid 'intermediate' 3C which produces the wide-spectrum antibiotic 'Myxin" (see page 234), and the yellow non-spreading 18H and 15D. The lattermost organism, 15D, has been shown to produce a lytic nematocidal enzyme (209, 210, 128). A further 34 interesting unidentified cytophaga-like strains were studied and these comprised several yellow, brown and pink spreaders, one pink and one white non-spreader, a cream 'intermediate' 13B, and 16 cream 'intermediates' known as '495 types', most of which had at least two colonial variants which were tested separately (Table 44). Included in this '495 group' was firstly, strain Cook 495 which has previously been regarded as a

Sorangium species and which produces a commercially important protease and is also nematocidal (see page 234 and Table 42). Also included is strain Cook 18L, also previously identified as a *Sorangium* (128). which has proteolytic and nematolytic activity (Table 44). Ensign and Wolfe's organism AL-1 (see page 234 and Table 44) is another member of the '495 group' and it produces an interesting cell wall-lytic enzyme (see page 234). A further 13 proteolytic Canadian soil isolates, numbers 4553 to 4565 inclusive also seem to be closely allied with this group. In all 65 organisms and 19 cultural variants of these were included in the study, and most of them have also been utilized for the colour and spreading studies (Chapter VII).

VIII.3. Methods

Cell morphology

1. Cell length and width

These were determined from living preparations examined at 1000x from one day and three day liquid cultures grown at 25°C in Skim Acetate broth (Table 16, page 105) except for the *Flavobacteria* NCIB 8185, 8195, 8204, 8767, 8771 and 9157 which were grown in Penassay broth (Difco Antibiotic Medium 3).

2. Cell shape, motility, arrangement and Gram stain

These were determined from one-and eight-day-old Skim Milk broth cultures and from wet mounts from two-day-old Skim Acetate plates, except in the case of certain *Flavobacteria* (see Method 1) which grew better in Penassay broth and on Difco Plate Count Agar (94).

Colony Morphology

3. Form, elevation, and optical, surface and edge characteristics

Descriptions (361) were made from single colonies grown on two day old Cook's Cytophaga (Table 16, page 105) Plate Count and Skim Acetate plates for five days at 25°C.

4. Colour

The Munsell notation for colour (see Chapter VII.1) was determined as follows. Standard mounds of bacterial cells were made by scraping up the total surface growth of a bacterial streak or giant colony, then mixing well with the loop on the agar surface. A blob was then transferred to a flat, white, porcelain plate (the edge of a spot plate is ideal), and quickly shaped into a mound about one and one half millimetres high with a flat surface of at least four square millimetres. The mounds were compared immediately with glossy finish Munsell colour chips (277) under standard illumination, to forestall colour changes which occur on drying.

5. Water soluble (diffusible) pigment

The presence or absence of a brown water soluble pigment was determined on Cook's Cytophaga, Skim Acetate and Plate Count Agar for all organisms, and on numerous other agar media for many of them.

6. Spreading ability, subsurface growth and slime layers

The ability to swarm on an agar surface, and to spread within it, as well as the production of layers of slime ahead of the growth front were determined on many different agar media.

Growth in liquid media

7. Turbidity, viscosity and flocculence

These observations were made from cultures grown in 10 ml of Skim Acetate broth in a 50 ml flask shaken at 100 rpm for 18 hours at room temperature (about 25°C). The *Flavobacteria* mentioned under Method 1 were grown in Penassay broth.

8. Flexing, silkiness, pellicle and ring formation

Microscopical observation of flexing movement, and determination of the silkiness of a culture when gently shaken, as well as the formation of rings and pellicles were made every day for 12 days and then on the 16th and 20th day from cultures grown in unshaken Skim Milk broth tubes (Table 16 page 105), and also from the one day shake flask cultures (Method 7).

Physiology

9. Salinity tolerance

The dried surfaces of four Skim Acetate plates made up with 0%, 1%, 2% and 3% NaCl respectively were inoculated in that order with a central streak of the test organism. Incubation was at 25°C for two weeks with readings of the growth, spreading and lysis being taken at seven and 14 days.

10. Anaerobicity

Skim Acetate plates were inoculated with a single, central streak of the test organism and incubated for one week at room temperature (about 25°C) in three different oxygen regimes. The first

group were incubated aerobically, the second in a candle jar (about 10% O_2) and the third were in a Gas-Pak anaerobic jar under hydrogen. At three and seven days estimates of the amount of growth and lysis were made.

11. Temperature limits for growth

Replicate Skim Acetate streak plates were incubated at temperatures ranging from 0° to 50°C for six days when the type and width of growth were recorded. The 0°C plates were incubated for 10 days, and those at 35°C or more were wrapped in polythene bags to prevent excess moisture loss.

12. pH range for growth

Batches of Skim Acetate Agar were adjusted with HCl or NaOH to different pH levels before autoclaving and rechecked afterwards. When the surfaces had dried the plates were inoculated with spots of different test organisms, four per plate. Agar acidified to pH 3.0 and 4.0 did not gel, and hence tests were carried out on pH 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0. Growth was estimated on a comparative basis after 5 days at 25°C.

Carbohydrates

13. Prototrophic growth

Salts-glucose agar plates were made with Hutchinson and Clayton's salt solution (174) (0.25% $NaNO_3$, 0.1% K_2HPO_4, 0.03% $MgSO_4$, 0.01% $CaCl_2$, 0.01% NaCl, 0.001% $FeCl_3$, pH 7.2-7.3) to which was added 0.1% w/v filter sterilized glucose and 1.5% agar. A duplicate set of

plates were made with the addition of 0.05% yeast extract. Streak inoculations were made and incubation was at 25°C, with readings at four and nine days.

14. O.F. test

Poured tubes of Board and Holding's medium (35) (0.5%, filter sterilized glucose, 0.5% agar, 0.05% $NH_4H_2PO_4$, 0.05% K_2HPO_4, 0.05% yeast extract, pH 7.2) were inoculated by stabbing with a thin wire. One set was sealed with about one centimetre of paraffin wax, and both were incubated at 25°C for 15 days. The colours of the test and control tubes were noted at one, four, 11 and 15 days.

15. Sugars and alcohols

Five sets of aerobic tubes of Board and Holding's medium (Method 14) were made, substituting 0.5% solutions of cellobiose, sucrose, lactose, glycerol and mannitol respectively for the glucose. After incubation at 25°C the colours of the test and control tubes were noted at one, four, 11 and 17 days.

Polysaccharides

16. Alginate, carboxymethyl cellulose and pectate

Tubes of Hutchinson and Clayton's salt solution (Method 13) with 0.05% yeast extract and 3% sodium alginate (246, 355), 3% sodium carboxymethyl cellulose Fisher grade 7 HSP (105, 246), or 3% sodium polypectate (Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.) (355) were inoculated with the test cultures and incubated at 25°C.

Estimates of liquefaction were made at intervals up to one month in the test and control tubes after cooling them at 10°C for 20 minutes, when the sloppy gel in the control tubes had attained a firm consistency. Liquefaction was taken as evidence of alginase, carboxymethyl cellulase and polypectase activity respectively.

17. Cellulose

Strips of Whatman #1 filter paper 1 x 10 cm (255, 373) were placed in Skim Acetate broth tubes which were then inoculated and incubated at 25°C. The control organism, *C.hutchinsonii*, took about 12 days to break the paper strip at the air/water interface. Observations on the test organisms were made at intervals up to 30 days.

18. Agar + yeast extract tubes

Tubes of 1.5% Bacto-agar containing 0.05% yeast extract only were inoculated and left at 25°C. Growth of the cultures and any softening of the agar were noted at intervals for 40 days.

19. Gelase field

The appearance of 'gelase fields' around the colonies after flooding a plate with iodine is regarded by some workers as a reliable test for agar decomposition (142, 371, 408). Skim Acetate plates were used in the present study.

20. Chitin

Chitin agar was made with 40 mls of an hydrolysed chitin solution and 10 grams of agar per litre of water (143, 247, 330). This produces a slightly milky gel within which digestion of the

chitin can be seen as a clear zone around the organism. A duplicate set of plates with 0.05% yeast extract added was also used. Inoculated plates were kept in a damp chamber at 25°C for 40 days, and the growth and clearing were noted at intervals.

21. Salts-yeast extract-starch plates

Two percent Fisher 'soluble' starch, 0.05% yeast extract and 1.0% agar were added to Hutchinson and Clayton's salt solution (Method 13) (361). Streak-inoculated plates were incubated for four days at 25°C when growth and hydrolysis (iodine reaction) (246) were noted.

22. Nutrient Broth-starch tubes

Two percent Fisher 'soluble' starch was added to Bacto Nutrient Broth solution (361). After incubation at 25°C growth, pH and hydrolysis (iodine spot test) were recorded at five, eight, 16 and 25 days.

23. Potato Infusion Agar

This standard medium for estimating the colour of micro-organisms (361) was made up as follows: mashed potato 20%, glucose 0.5%, agar 1.5%, $CaCO_3$ 0.3%, $(NH_4)_2SO_4$ 0.1%. After eight days at 25°C the Munsell colour (Method 4) and the hydrolysis of starch (iodine test) were noted for the test organisms.

Proteolysis

24. Gelatin liquefaction

Tubes of 12% gelatin (361) were stab-inoculated and then

incubated at 25°C for 24 days. At various intervals the tubes were cooled at 10°C for one hour to set unaltered gelatin, and then the depth of liquefaction measured.

25. Haemolysis

Blood Agar plates (361) were inoculated with the test cultures in a manner to produce single colonies and then incubated at 25°C. Growth and haemolysis were noted at two and 12 days.

26. Lysis of Milk

The number of days taken to clear Skim Milk broth (Table 16, page 105), and the presence or absence of a lytic zone around the culture when grown on Skim Acetate Agar, after incubation at 25°C was recorded.

27. Casein broth

Duplicate tubes containing only 0.5% Fisher casein were inoculated and incubated for one month at 25°C. Growth and ammonia production (Nessler's reagent) were recorded at 14 and 28 days.

28. Casein plates

Plates of 0.5% Fisher casein in 1.5% agar were streaked with the test cultures and incubated for one week at 25°C. The type and amount of growth, and clearing of the casein were noted at three and seven days.

29. Salts-casitone broth

A medium containing 2.0% Bacto-casitone, 0.2% $MgSO_4$, 0.1% K_2HPO_4 and 0.06% KH_2PO_4 adjusted to pH 7.6 was used. Observations

on growth, ammonia production (Nessler's reagent) and pH were made at seven and 14 days in duplicate tubes.

30. Salts-casamino acids broth

A solution of 1.0% Difco vitamin-free casamino acids, 0.1% K_2HPO_4, 0.05% KNO_3, 0.02% $MgSO_4$, 0.01% NaCl and 0.001% $FeCl_3$ adjusted to pH 7.0 to 7.1 was employed. Two tubes were inoculated with each organism, and growth and ammonia production (Nessler's reagent) were recorded at three and seven days.

31. Tryptone agar

Cultures were streaked on Cook's Cytophaga agar (Table 16, page 105) and incubated at 25°C for 4 days, when the quality of growth and spreading were observed.

32. Ammonification in Penassay broth

Duplicate open tubes of Penassay broth (Difco Antibiotic Medium 3) were inoculated and incubated at 25°C for 11 days. Growth, production of ammonia (Nessler's reagent) and pH (bromthymol blue) were recorded at four and 11 days.

Nitrogen sources

33. Ammonia

Board and Holding's medium (Method 14) without the yeast extract was used and the colour of the medium was noted at intervals up to 17 days.

34. Urea

The medium (131) was made up of two parts. Solution A

consisted of 1g K_2HPO_4, 0.2g $MgSO_4$, 0.1g $CaCl_2$, 0.1g NaCl, 0.01g $FeCl_3$ and 15g agar in 900 mls distilled water, adjusted to pH 6.8-7.0 and autoclaved. Solution B contained 20g urea, 5g glucose and 10 ml of a 2% bromthymol blue solution in 90 mls distilled water, and was filter sterilized before adding it to the cooled solution A prior to pouring plates. Growth and pH changes from streak inoculations were recorded at six, 12 and 20 days after incubation at 25°C.

35. Amino acids

Hutchinson and Clayton's salts medium (Method 13) minus the $NaNO_3$ was supplemented with either 0.1% monosodium glutamate or 0.1% sodium asparaginate, and gelled with 1.5% agar. A filter sterilized glucose solution was added to the cooled, autoclaved medium to a final concentration of 0.1%. Growth of the spot inocula , four per plate was noted at four and nine days.

Miscellaneous biochemistry

36. H_2S from cysteine

Tubes containing 0.01% cysteine hydrochloride (361), sterilized by filtration, in Skim Milk broth were inoculated with the test organisms. Dried strips of filter paper impregnated with a 5% lead acetate solution were folded over the tip of the tube, which was capped as usual. Blackening of the paper strip, owing to the formation of lead sulphide, indicated H_2S production (246).

37. Catalase production

Various plates were flooded with 10% hydrogen peroxide (361).

Copious evolution of bubbles was evidence for catalase activity and hence presumptive aerobicity.

38. Oxidase production

A solution of 0.1 gm tetramethyl-para-phenylenediamine dihydrochloride in 10 mls distilled water (228) was allowed to stand for 15 minutes and then drops were placed on a piece of Whatman #1 filter paper. Test colonies from various agar plates were smeared on the reagent-saturated paper. If oxidase was present a dark purple colour developed rapidly (18).

39. Denitrification in open tubes

Duplicate open tubes of Penassay broth (Difco Antibiotic Medium 3) containing 0.1% KNO_3 (6) were inoculated and incubated at 25°C for 11 days. Spot plate tests for ammonia production (Nessler's reagent), pH (bromthymol blue), nitrate and nitrite (acidified diphenylamine) and nitrite (acidified Trommsdorf's reagent) and a hot wire test for nitrogen gas bubbles were carried out at four and 11 days. A similar series of tests was done with tubes containing 0.1% KNO_2, and these were all compared with the Penassay tubes used to estimate growth and ammonification (Method 32).

40. Denitrification in anaerobic system

Duplicate tubes of Penassay broth containing 0.1% KNO_3 were inoculated with the test cultures and incubated anaerobically in Gas-Pak jars under hydrogen. Tests for growth, ammonia production, pH, nitrate and nitrite were carried out as above (Method 39), and the results were compared with those from the open tube tests (Methods 32 and 39).

41. Phosphatase production

Twenty mls of a filter sterilized 0.5% phenolphthalein diphosphoric acid solution were added to 1 litre of routine Skim Acetate agar just before pouring plates, to give a final concentration of 0.01% (19). Cultures were spotted four per plate and after four days incubation at 25°C the plates were exposed to ammonia vapour. Colonies producing sufficient phosphatase to liberate free phenolphthalein became bright pink, others were unchanged.

42. Formation of indole

A small amount of Kovac's reagent (361) was added to 14 and 28 day-old casein broth cultures (Method 27), and to seven and 14 day-old casitone broth cultures (Method 29). After mixing and standing for up to half-an-hour a red colour at the interface indicated the formation of indole from tryptophan.

43. Stimulation by yeast extract

The results from two sets of tests were compared:

salts + glucose ± yeast extract (Method 13)
chitin ± yeast extract (Method 20).

Antibiotic and antibacterial sensitivities

44. Sodium lauryl sulphate (S.L.S.)

Streak inoculations were made on the three groups of Skim Acetate Agar plates containing 0%, 0.01% and 0.1% S.L.S. respectively (71) and incubated at 25°C. Growth with S.L.S. was compared with that on the control plate at two and five days.

45. Chloramphenicol, dihydrostreptomycin, penicillin and polymyxin B

A sufficient amount of an overnight Skim Acetate broth culture of a test organism was spread with a bent glass rod on the surface of a Skim Acetate plate to form confluent growth. After the surface of the plate had dried BBL discs of four antibiotics of the following concentrations were placed firmly on the agar; chloramphenicol 30 µg, dihydrostreptomycin 10 µg, penicillin G 10 units and polymyxin B 300 units. The diameters of growth inhibition were noted after two days at 25°C.

46. Actinomycin D

Four sets of discs were made containing 0.1, 1.0, 10 and 40 µg/disc of actinomycin D respectively. These were placed on the dried surfaces of Skim Acetate plates previously inoculated with the test bacteria as in Method 45. The results were read at two and three days after incubation at 25°C.

47. Nitrite

Growth in the Penassay + NO_2 denitrification tubes (Method 39) was compared with that in the Penassay tubes (Method 32) to see if nitrite inhibition had taken place.

Antimicrobial lytic action

48. Bacteria

A selection of five bacterial species, *E.coli*, *Pseudomonas aeruginosa* 9027, *Arthrobacter* sp. 4165, *Serratia marcescens* and *Bacillus subtilis*, was used to screen the lytic activity of the

test cultures against bacteria. Tests were made in a manner similar to antibiotic disc sensitivity tests, using one drop of an overnight Skim Acetate shaken culture of the 'predator' on a lawn of the 'prey' organism. The *Flavobacteria* mentioned under Method 1 were grown in Penassay broth. Two sets of Plate Count plates were used for each 'prey' bacterium, the lawns being allowed to grow for one hour and for two days respectively before inoculation with the potentially lytic test organisms. After one, three and eleven days at 25°C growth of the 'predators' and lysis of the 'prey' were determined.

49. Fungi, actinomycetes and an alga

The lysis of autoclaved yeast cells was examined on Yeast Agar plates (Table 16, page 105). The test cultures were streaked down the centre of a plate and after four days at 25°C the growth of the cultures and their lytic action were noted.

The lytic spectrum of the test cultures was estimated on the fungi *Penicillium notatum*, *Rhizopus* sp. and *Sclerotinia sclerotiorum*, two actinomycetes, 4432 which produces a brown water-soluble pigment, and 4441 which does not, and on the green alga *Chlorella* sp. These organisms were grown on Plate Count agar and were tested in the same manner as the bacteria (Method 48). However, these 'prey' organisms were allowed to grow for one and for four days respectively (two weeks for *Chlorella*) before drops of the potential 'predator' broth cultures were added.

VIII.4. Results and Discussion

Discussion of the tables of results

Tables 45-53 inclusive summarize the results of the morphological and biochemical tests. Detailed information is available but was considered too voluminous for inclusion here. Comments on each of the tables follow.

Table 45. Cultural characteristics

The majority of the organisms in the study were of course Gram negative, with the exception of four *Flavobacteria* which were consistently Gram positive, and three *Flavobacteria*, 7-1 and 402 which gave variable reactions. Motility of the flagellar type was observed only in some *Flavobacteria* and also in the purported *Cytophaga succinicans* RL8. The 'spreading' organisms were generally very long, thin and flexing, the exceptions being the medium-length but very thin *C.hutchinsonii* strains, and the fatter, shorter, nonflexing pinks' PC15 and 9-11. The 'intermediate' group were thin, medium length rods except for 495, A1-1 (i), 4565 (ii) and 13B which attained greater lengths. Most of these demonstrated flexing. The 'nonspreaders' were very mixed in these respects. As expected all of the spreading and intermediate organisms except *C.hutchinsonii*, PC15 and 9-11 were silky and flexuous in liquid medium but most of the nonspreaders were silky too, and a few were also flexuous. Extracellular slime or gum produced by the intermediates, most of the 'pinks' (PC15, 9-11, 14), 402 and *F.devorans* caused a noticeable rise in viscosity in liquid media.

Table 45

Cultural characteristics*

Organism	Gram 1	Gram 2	Gram 3	Motility	Length μ	Width μ	Silky	Flexing	Viscosity	Flocculence	Ring or pellicle	WSP	Sub-surface	Slime layers	Fringe
Spreaders															
ATCC 17061	-	-	-	-	2-36	0.2	+	+	-	-	R	-	+	-	+
405	-	-	-	-	3-20	0.4	+	+	-	-	-	±	+	-	+
Alfalfa	-	-	-	-	3-25	0.2	+	+	-	+	-	-	+	-	+
Bryant	-	-	-	-	3-105	0.2	+	+	-	-	P	-	+	+	+
A15	-	-	-	-	4-20	0.4	+	+	-	-	PR	-	+	+	+
B-2-25	-	-	-	-	3-20	0.4	+	+	-	-	-	-	+	+	+
E-1-25	-	-	-	-	3-15	0.5	+	+	-	-	-	-	+	+	+
H_2O-1A	-	-	-	-	3-25	0.4	+	+	-	-	-	-	+	+	+
1-10	-	-	-	-	2-20	0.2	+	+	-	+	R	-	+	-	+
3	-	-	-	-	3-25	0.3	+	+	-	-	P	-	+	-	+
3-19	-	-	-	-	1-9	0.3	+	+	-	-	-	-	+	-	+
3-22	-	-	-	-	1-15	0.3	+	+	-	-	P	-	+	-	+
4433	-	-	-	-	3-12	0.4	+	+	-	-	-	-	+	-	+
4539	-	-	-	-	3-20	0.5	+	+	-	-	P	-	+	-	+
4707	-	-	-	-	3-20	0.4	+	+	-	-	-	-	+	-	+
NCIB 9059	-	-	-	-	1-25	0.3	+	+	-	-	R	-	+	-	+
FS-1	-	-	-	-	10-400	0.4	+	+	-	-	-	-	-	-	+
Stanier 6	-	-	-	-	1-7	0.1	-	+	-	-	R	-	-	-	+
NCIB 10782	-	-	-	-	1-6	0.1	-	+	-	-	R	-	-	-	+

*Gram. 1 = 2 day plate, 2 = 1 day broth, 3 = 8 day broth cultures, ± = both observed.
Ring or pellicle. R = ring, P = pellicle seen at least once.
WSP. Brown water soluble pigment always seen (+), occasionally seen (±), or never seen (-).
Other columns. + = seen at least once, - = never seen.

Table 45 - continued

Cultural characteristics

Organism	Gram 1	Gram 2	Gram 3	Motility	Length μ	Width μ	Silky	Flexing	Viscosity	Flocculence	Ring or pellicle	WSP	Sub-surface	Slime layers	Fringe
2	-	-	-	-	2-20	0.3	+	+	-	-	PR	+	+	-	+
D	-	-	-	-	2-11	0.3	+	+	-	-	PR	+	+	-	+
6	-	-	-	-	2-15	0.2-0.5	+	+	-	-	PR	+	+	-	+
4541	-	-	-	-	1-21	0.3-0.5	+	+	-	-	R	+	+	-	+
PC 15	-	-	-	-	2-4	0.6	+	-	+	-	-	-	-	-	+
9-11	-	-	-	-	1-5	0.7	-	-	+	-	-	-	+	-	+
9D	-	-	-	-	3-60	0.3	+	+	-	-	-	-	+	-	+
Intermediates															
495	-	-	-	-	2-30	0.5	+	+	+	-	PR	±	+	-	+
AL-1 (i)	-	-		-	1-20	0.3	+	+	+	-	-	±	-	-	+
(ii)	-	-		-	1-7	0.4	+	+	+	-	-	±	-	-	+
(iii)	-	-		-	1-8	0.4	+	+	+	-	-	±	-	-	+
ATCC 21123	-	-	-	-	1-9	0.4	+	+	+	-	-	±	+	-	+
18L Y	-	-	-	-	1-6	0.4	+	+	-	-	R	±	+	-	+
18L W	-	-	-	-	1-6	0.4	+	+	+	-	R	±	+	-	+
4553 (i)	-	-	-	-	1-4	0.4-0.5	+	+	+	+	PR	±	-	-	+
4553 (ii)	-	-	-	-	1.5-3	0.4	+	+	+	+	R	±	-	-	+
4554	-	-	-	-	2-5	0.5	+	+	+	-	P	±	+	-	+
4555 (i)	-	-	-	-	1-3	0.5	+	-	+	-	P	±	-	+	-
4555 (ii)	-	-	-	-	2-6	0.4	+	+	+	-	R	±	-	+	+
4556 (i)	-	-	-	-	1-5	0.4	+	+	-	-	R	±	+	-	+
4556 (ii)	-	-	-	-	1.5-4	0.4	+	-	+	-	PR	±	-	-	-
4557 (i)	-	-	-	-	1-5	0.3-0.5	+	+	+	-	R	±	-	-	+
4557 (ii)	-	-	-	-	2-5	0.5	+	+	+	+	R	±	-	-	+

Table 45 - continued

Cultural characteristics

Organism	Gram 1	Gram 2	Gram 3	Motility	Length μ	Width μ	Silky	Flexing	Viscosity	Flocculence	Ring or pellicle	WSP	Sub-surface	Slime layers	Fringe
4558 (i)	-	-	-	-	2-6	0.4	+	+	+	-	P	I+	-	+	+
4558 (ii)	-	-	-	-	1-4	0.4	+	-	-	-	R	I+	-	-	+
4559 (i)	-	-	-	-	1-6	0.4	+	+	-	-	P	I+	-	+	+
4559 (ii)	-	-	-	-	1-5	0.4	+	+	+	-	R	I+	-	-	+
4560 (i)	-	-	-	-	1-7	0.4	+	+	+	-	R	I+	+	-	+
4560 (ii)	-	-	-	-	1-7	0.4	+	+	+	-	PR	I+	+	-	+
4560 (iii)	-	-	-	-	1-5	0.4	+?	+	+	+	P	I+	-	-	+
4561 (i)	-	-	-	-	1-4	0.4	+	+	+	-	R	I+	-	+	+
4561 (ii)	-	-	-	-	1-4	0.4	+	+	+	-	R	I+	-	-	+
4561 (iii)	-	-	-	-	1-8	0.3	+	+	+	-	R	I+	-	-	
4562 (i)	-	-	-	-	1-7	0.4	+	+	+	-	R	I+	-	+	+
4562 (ii)	-	-	-	-	1-7	0.4	+	+	+	-	P	I+	-	+	+
4563 (i)	-	-	-	-	1-5	0.4	+	+	+	-	P	I+	-	-	+
4563 (ii)	-	-	-	-	1-5	0.5	+	+	+	-	P	I+	-	-	+
4564 (i)	-	-	-	-	1-6	0.3	+	+	+	-	R	I+	-	+	+
4564 (ii)	-	-	-	-	1-3	0.3	+	-	+	-	R	I+	-	-	+
4565 (i)	-	-	-	-	1-4	0.4	+	+	+	-	R	I+	+	-	+
4565 (ii)	-	-	-	-	1-11	0.4	+	+	+	-	R	I+	+	-	+
13B	-	-	-	-	3-13	0.5	+	+	+	-	R	I+	+	+	+
3C	-	-	-	-	1-2.5	0.4	+	+	+	-	-	+	+	-	+

Table 45 - continued

Cultural characteristics

Organism	Gram 1	Gram 2	Gram 3	Motility	Length μ	Width μ	Silky	Flexing	Viscosity	Flocculence	Ring or pellicle	WSP	Sub-surface	Slime layers	Fringe
Nonspreaders															
7-1	-	±	-	-	1-30	0.3	+	+	-	-	-	-	+	-	-
15D	-	-	-	-	1-60	0.6	+	+	-	+	PR	-	+	+	-
18H	-	-	-	-	3-75	0.7	+	+	-	+	R	-	+	+	+
NCIB 8186	+	+	+	-	1-2.5	0.3	-	-	-	-	-	-	+	-	-
NCIB 8187	+	+	+	+	0.6-2.4	0.3	+?	-	-	-	-	-	+	-	-
NCIB 8188 a	+	±	+	+	0.5-1	0.2	-	-	-	-	-	-	+	-	-
NCIB 8188 b	+	±	+	+	0.5-1	0.2	-	-	-	-	-	-	+	-	-
NCIB 8195	-	-	-	+	1-9	0.8-1.0	+	+	+	-	P	-		-	-
NCIB 8535	+	+	+	+	1-3	0.3	+	-	-	-	-	-	+	-	-
NCIB 8767	-	-	-	-	1-15	0.4	+	+	-	-	P	-	+	-	+
NCIB 8771	-	-	-	-	1-40	0.5	+	+	-	-	P	-	-	-	+
NCIB 8992	-	-	-	-	1-2	0.3	+	-	-	-	-	-	-	-	-
NCIB 9157 a	-	-	-	+	2-3	0.5	+	-	-	-	-	-	+	-	-
NCIB 9157 b	-	-	-	+	2-9	0.5	-	-	-	-	R	-	+	-	-
NCIB 9290	-	-	-	-	1-9	0.3	+	+	-	-	-	-	-	-	+
NCIB 9324	-	-	±	+	1-2	0.3	+	-	-	+	R	±	+	-	-
RL 8	-	-	-	+	2-4	0.6	+	-	-	-	-	-	-	-	-
NCIB 8185	-	-	+	+	0.5-1.5	0.2	+	-	-	+	R	-	+	-	-
NCIB 8204	-	-	-	-	1-2	0.5	-	-	-	-	R	-	-	+	-
14	-	-	-	-	3-6	0.4	-	-	+	-	-	-	+	-	-
402	±	-	-	-	0.5-2	0.4	+	-	+	-	PR		-	-	-

Brown water soluble pigments were observed consistently in only five organisms, 2, D, 6, 4541 and 3C, but were also produced by all of the intermediates and two others especially in older cultures.

Table 46. Physiology.

Most of the bacteria studied were typical mesophiles having optima between 20° and 30°C, however A 15, 3-22 and *F.resinovorum* had lower ranges. The intermediate group showed a general tendency for higher maximum and optimum temperatures, as did a number of the *Flavobacteria*. The four 'browns' (2, D, 6 and 4541) and three of the *Flavobacteria* had higher temperature optima of 35-40°C. The pH range of most of these organisms was relatively wide with a general preference for alkalinity. Two organisms, 9D and 5-9, grew best only around neutrality, and six (3-19, 3-22, PC 15, 3C, 7-1 and 14) were acidophilic.

None of the organisms grew on Skim Acetate Agar in the absence of oxygen, but many of the spreaders and nonspreaders preferred a lower oxygen tension than is present in the atmosphere. All except one (21123) of the spreaders and intermediates were at least partially inhibited by NaCl concentrations less than that found in seawater (roughly 2.5%). However a number of the nonspreaders grew quite well with 3% NaCl.

Table 46

Physiology

Organism	Temperature °C		pH		O_2		NaCl%	
	Range	Opt.	Range	Opt.	Opt. growth	Opt. lysis*	Partial Inhibition	Complete Inhibition
Spreaders								
ATCC 17061	10-35	30	7-10	7-10	low O_2	low O_2	2	3
405	8-35	18	5-10	6-10	low O_2	low O_2	1	2
Alfalfa	0-30	20-25	5-10	6-10	low O_2	O_2	1-2	2
Bryant	18-40	20-30	5-10	6-10	low O_2	low O_2	1	2
A 15	0-25	8-20	6-10	6-10	low O_2	low O_2	1	2
B-2-25	0-30	18	5-10	6-10	low O_2	low O_2	1	3
E-1-25	0-30	20	5-10	6-9	low O_2	low O_2	1	2
H_2O-1A	0-25	20	6-10	6-10	low O_2	low O_2	1	3
1-10	0-30	20	5-10	5-10	O_2	O_2	2	>3
3	0-35	30	5-10	5-10	low O_2	low O_2	1	2
3-19	5-30	25	5-10	5	low O_2	O_2	2	3
3-22	0-30	10	5-10	6	low O_2	low O_2	1-2	2
4433	0-30	18	5-10	5-10	low O_2	low O_2	1	3
4539	0-30	18-20	5-10	5-10	low O_2	low O_2	1	3
4707	0-30	18-25	5-10	5-10	low O_2	low O_2	1	2
NCIB 9059	0-30	18-20	5-10	5-10	low O_2	low O_2	1	3
FS-1	15-35	25	7-10	7-10	low O_2	low O_2	< 1	1
Stanier 6	20-35	25-35			O_2	-	1	2
NCIB 10782	20-35	20-30			O_2	-	1	2
2	15-40	40	5-10	5-10	O_2	O_2	1	2
D	15-35	30-35	5-10	5-10	low O_2	O_2	1	2
6	20-40	40	6-10	6-10	O_2	low O_2	<1	1
4541	15-40	40	5-10	7-10	O_2	O_2	1	2
PC 15	10-30	25	5-10	5-8	low O_2	O_2	?2	3
9-11	10-30	20	6-10	6-9			1	2
9D	10-40	18-30	5-10	6-8	low O_2	low O_2	1	2

*Denotes clearing of milk.

Table 46 - continued

Physiology

Organism	Temperature °C		pH		O_2		NaCl%	
	Range	Opt.	Range	Opt.	Opt. growth	Opt. lysis	Partial Inhibition	Complete Inhibition
Intermediates								
495	10-35	30	5-10	5-10	O_2	low O_2	1	3
A1-1 (i)	5-35	30	7-10	7-10	low O_2	O_2	2	3
(ii)	5-35	30	6-10	6-10	O_2	low O_2	1	3
(iii)	5-35	30	6-10	6-10	O_2	O_2	1	3
ATCC 21123	10-35	30-35	5-10	5-10	O_2	O_2	3	>3
18 L Y	10-40	30	6-10	6-10	O_2	O_2	2	>3
18 L W	10-40	30	5-10	5-10	O_2	O_2	2	>3
4553 (i)	10-35	30	5-10	5-10	O_2	O_2	2	>3
4553 (ii)	10-35	25	6-10	6-10	O_2	O_2	2	>3
4554	10-40	35	5-10	5-10	O_2	low O_2	2	>3
4555 (i)	10-40	25	5-10	5-10	low O_2	O_2	2	3
4555 (ii)	10-35	25	5-10	5-10	low O_2	O_2	1	3
4556 (i)	5-35	30-35	5-10	5-10	O_2	O_2	1	3
4556 (ii)	10-35	30	6-10	6-10	O_2	O_2	2	3
4557 (i)	5-35	30	5-10	5-10	O_2	O_2	1	3
4557 (ii)	10-35	30	6-10	7-10	O_2	low O_2	1	3
4558 (i)	5-40	30	6-10	6-10	O_2	O_2	1	3
4558 (ii)	10-40	35	7-10	7-10	O_2	low O_2	1	3
4559 (i)	10-40	35	6-10	6-10	O_2	O_2	1	3
4559 (ii)	10-40	25-35	6-10	7-10	O_2	O_2	1	3
4560 (i)	10-40	30-35	6-10	6-10	O_2	O_2	2	>3
4560 (ii)	10-40	20	7-10	7-10	O_2	low O_2	1	>3
4560 (iii)	10-40	25-35	7-10	7-10	low O_2	low O_2	1	>3
4561 (i)	10-40	25	6-10	6-10	O_2	O_2	1	3
4561 (ii)	10-35	35	7-10	7-10	low O_2	O_2	1	3
4562 (i)	10-40	20-30	7-10	7-10	O_2	O_2	1	3
4562 (ii)	10-40	25	6-10	6-10	O_2	O_2	1	3
4563 (i)	10-40	30	6-10	6-10	O_2	O_2	1	3

Table 46 - continued

Physiology

Organism	Temperature °C		pH		O_2		NaCl%	
	Range	Opt.	Range	Opt.	Opt. growth	Opt. lysis	Partial Inhibition	Complete Inhibition
4563 (ii)	10-35	30	6-10	6-10	0_2	0_2	1	3
4564 (i)	10-40	25	6-10	6-10	0_2	0_2	1	3
4564 (ii)	10-40	25	6-10	6-10	?low 0_2	low 0_2	1	3
4565 (i)	10-40	10-35	7-10	7-10	0_2	0_2	1	>3
4565 (ii)	10-40	25-30	7-10	7-10	0_2	0_2	2	>3
13B	8-35	30	5-10	5-10	0_2	0_2	1	3
3C	10-40	25	5-10	5	low 0_2	0_2	2	3
Nonspreaders								
7-1	0-40	30	5-10	6	0_2	0_2	>3	»3
15D	8-35	25-30	5-10	6-9	0_2	low 0_2	1	3
18H	8-35	25	5-10	5-9	low 0_2	low 0_2	1	3
NCIB 8186	15-40	25-40	5-10	5-10	0_2	0_2	3	>3
NCIB 8187	15-40	30-35	5-10	5-10	low 0_2	0_2	3	>3
NCIB 8188 a	10-40	35	7-10	7-10	low 0_2	0_2	2	>3
NCIB 8188 b	10-40	20-40	6-10	6-10	low 0_2	0_2	2	>3
NCIB 8195	10-40	35-40	7-10	7-10	low 0_2	-	>3	»3
NCIB 8535	15-40	25-35	6-10	6-10	low 0_2	low 0_2	3	>3
NCIB 8767	5-25	15-20	6-10	6-10	0_2	0_2	1-2	2
NCIB 8771	15-35	25-35	6-10	6-10	low 0_2	-	1	2
NCIB 8992	10-35	30	7-10	7-10	low 0_2	0_2	2	3
NCIB 9157 a	10-35	10-25	6-10	6-10	low 0_2	-	1	3
NCIB 9157 b	10-35	35	6-10	7-10	low 0_2	-	1-2	2
NCIB 9290	10-35	20-35	7-10	7-10	low 0_2	-	3	>3
NCIB 9324	10-35	30-35	7-10	7-10	? 0_2	0_2	>3	»3
RL 8	10-35	15-30	6-10	7-10	? 0_2	-	>3	»3
NCIB 8185	10-40	35	6-10	6-10	low 0_2	0_2	>3	»3
NCIB 8204	10-40	20-35	5-10	5-10	low 0_2	-	>3	»3
5-9	10-40	30	6-10	6-8	low 0_2	-	1-2	2
14	10-35	20-30	6-10	6	low 0_2	-	<1	1
402	10-40	20	6-10	6-10	0_2	0_2	2	3

Table 47. Use of carbohydrates.

All of the tested organisms with the exception of PC 15 and 9-11 could use glucose aerobically, although a few showed no growth in Board and Holding's medium, and some showed no pH change probably due to the use of the acids as soon as they were formed (173). A few spreaders and *Flavobacteria* were also capable of fermenting glucose. Cellobiose was utilized by about nine tenths of the cultures, with the notable exception of the 'browns', most of the 'pinks' (PC 15, 9-11 and 14), 15D and 18H and *F.aurantiacum*. The cellobiose-negative organisms were also unable to use the other sugars tested. About three fourths of the isolates could use sucrose and lactose, the ability to use one being generally parallelled by the ability to use the other. The use of the alcohols glycerol and mannitol was mostly confined to the *Flavobacteria*.

Table 48. Use of polysaccharides.

Cellulose in the form of filter paper was only degraded by the two strains of *C.hutchinsonii*, but many more cultures liquefied carboxy methyl cellulose, including many of the spreaders, all of the intermediates and a few of the nonspreaders. Starch was used by all of the spreading organisms except *C.hutchinsonii* and by some of the nonspreaders, but none of the intermediates nor RL8 and 402 could use it. Chitin was decomposed by most of the spreaders, all of the intermediates and by 15D, 402 and *F.resinovorum*.

Table 47

Use of carbohydrates

Organism	Ability to use glucose as C source with NO_3	NO_3 +YE	NH_4	NH_4 +YE	Urea	O.F.	Sugars* Cb.	Suc.	Lac.	Alcohols* Glyc.	Mann.
Spreaders											
ATCC 17061	-	+	+	+	+	O, slow F	+	-	+	-	-
405	-	+	+	+	-	O	+	+	+	-	-
Alfalfa	+	+	+	+	+	O	+	+	+	?+slow	+
Bryant	+	+	-	+	-	OF	+	+	+	?+slow	-
A 15	+	+	+	+	-	O	+	-	-	-	-
B-2-25	+	+	+	+	-	OF	+	+	+	+	-
E-1-25	+	+	+	+	-	OF	+	+	+	+	-
H_2O-1A	+	+	+	+	-	O	+	-	-	-	-
1-10	+	+	+	+	+	O	+	+	+	-	+
3	+	+	+	+	-	O	+	+	+	-	-
3-19	-	+	+	+	+	O	+	+	?+slow	-	-
3-22	-	+	+	+	+	O	+	+	+	-	-
4433	+	+	+	+	-	O	+	-	+	-	-
4539	+	+	+	+	-	O	+	+	+	-	-
4707	+	+	+	+	-	O	+	+	+	?+slow	-
NCIB 9059	+	+	+	+	+	O	+	+	+	-	-
FS-1	+	+	+	+	+	O	+	+	+	-	-
Stanier 6			+	+	-	O	+	-	?	-	+
NCIB 10782	-	-	+	+	+	O	+	-	-	-	-
2	+	+	+	+	-	O	-	-	-	-	-
D	+	+	+	+	-	O	-	-	-	-	-
6	-	+	+slow	?+	-	No growth	-	-	-	-	-
4541	+	+	+	+	-	O	-	-	-	-	-
PC 15	-	+	-	?+	-	No action	-	-	-	-	-
9-11	-	+	-	?+	-	No action	-	-	-	-	-
9D	-	+	+	+	-	OF	?+slow	-	+	+	-

*Cb. =cellobiose, Suc. = sucrose, Lac. = lactose, Glyc. = glycerol, Mann. = mannitol.

Table 47 - continued

Use of carbohydrates

Organism	Ability to use glucose as C source with: NO_3	NO_3 +YE	NH_4	NH_4 +YE	Urea	O.F.	Sugars: Cb.	Suc.	Lac.	Alcohols: Glyc.	Mann.
Intermediates											
495	+	+	+	+	-	0	+	+	+	-	-
AL-1 (i)	-	+	-	?+	-	No action	?+ slow	-	?+ slow	-	-
(ii)	+	+	-	?+	-	No action	?+ slow	-	+	-	-
(iii)	-	+	-	?+	-	No action	?+ slow	-	?+ slow	-	-
ATCC 21123	+	+	+	+	+	0	+	+	+	-	-
18L Y	+	+	+	+	+	0	+	+	+	-	+
18L W	+	+	+	+	+	0	+	+	+	-	-
4553 (i)	+	+	+	+	-	0	+	+	+	-	-
4553 (ii)	+	+	+	+	-	0	+	+	+	-	-
4554	+	+	+	+	-	0	+	+	+	-	-
4555 (i)	+	+	+	+	-	0	+	+	+	-	-
4555 (ii)	+	+	+	+	-	0	+	+	+	-	-
4556 (i)	+	+	+	+	-	0	+	+	+	-	-
4556 (ii)	+	+	+	+	-	0	+	+	+	-	-
4557 (i)	+	+	+	+	-	0	+	+	+	-	-
4557 (ii)	+	+	+	+	-	0	+	+	+	-	-
4558 (i)	+	+	+	+	-	0	+	+	+	-	-
4558 (ii)	+	+	+	+	-	0	+	+	+	-	-
4559 (i)	+	+	+	+	-	0	+	+	+	-	-
4559 (ii)	+	+	+	+	-	0	+	+	+	-	-
4560 (i)	+	+	+	+	-	0	+	+	+	-	-
4560 (ii)	+	+	+	+	-	0	+	+	+	-	-
4560 (iii)	+	+	+	+	-	0	+	+	+	-	-
4561 (i)	+	+	+	+	-	0	+	+	+	-	-
4561 (ii)	+	+	+	+	-	0	+	+	+	-	-
4561 (iii)	+	+	+	+	-	0	+	+	+	-	-

Table 47 - continued

Use of carbohydrates

Organism	Ability to use glucose as C source with: NO_3	NO_3 +YE	NO_4	NO_4 +YE	Urea	O.F.	Sugars: Cb.	Suc.	Lac.	Alcohols: Glyc.	Mann.
4562 (i)	+	+	+	+	-	O	+	+	+	-	-
4562 (ii)	+	+	+	+	-	O	+	+	+	-	-
4563 (i)	+	+	+	+	-	O	+	+	+	-	-
4563 (ii)	+	+	+	+	-	O	+	+	+	-	-
4564 (i)	+	+	+	+	-	O	+	+	+	-	-
4564 (ii)	+	+	+	+	-	O	+	+	+	-	-
4565 (i)	+	+	+	+	-	O	+	+	+	-	-
4565 (ii)	+	+	+	+	-	O	+	+	+	-	-
13B	-	+	+	+	+	O	+	+	+	-	-
3C	+	+	+	+	+	O	+	-	-	-	-
Nonspreaders											
7-1	?-	?-	+	+	+	O	+	+	-	+	+
15D	-	+	+	?+	-	No action	-	?+	-	-	-
18H	-	+	+slow	?+	-	No action	-	slow -	-	-	-
NCIB 8186	-	+	+	+	+	O	+	+	+	+	-
NCIB 8187	-	+	+	+	+	O	+	+	+	+	-
NCIB 8188 a	-	+	+	+	+	O	+	+	+	+	-
NCIB 8188 b	-	+	+	+	+	O	+	+	+	+	-
NCIB 8195	-	+	+	+	+	O	+	+	+	-	-
NCIB 8535	-	+	+	+	+	O	+	+	+	+	-
NCIB 8767	-	+	+	+	+	O,slowF	+	-	-	-	-
NCIB 8771	-	+	+	+	+	OF	?+slow	-	-	+	+
NCIB 8992	-	+	+ slow	+	-	OF	+	-	-	-	-

Table 47 - continued

Use of carboyhydrates

Organism	Ability to use glucose as C source with						Sugars			Alcohols	
	NO_3	NO_3 +YE	NO_4	NO_4 +YE	Urea	O.F.	Cb.	Suc.	Lac.	Glyc.	Mann.
NCIB 9157 a	-	+	+	+	+	OF	+	+	+	+	+
NCIB 9157 b	-	+	+	+	+	OF	+	+	+	+	+
NCIB 9290	-	-	+	+	+	O,slow F	+	+	+	?+ slow	+
NCIB 9324	-	+	+ slow	+	+	O,slow F	+	+	+	+	+
RL 8	-	+	+ slow	+	+	O	+	+	+	?+ slow	?+ slow
NCIB 8185	-	+	+	+	+	OF	+	+	+	+	+
NCIB 8204	-	+	+ slow	?+	+	No action	-	+	-	?+ slow	+
5-9	+	+	+	+	+	O	+	+	+	-	-
14	+	+	+	+	+	O	-	-	-	-	-
402	+	+	+	+	+	O	?+ slow	?+ slow	+	-	-

Table 48

Use of polysaccharides

Organism	Cellulose CMC	Cellulose Paper	Starch SYS	Starch S-NB	Starch Potato	Chitin	Agar Tubes	Agar Gelase	Algi-nate	Pec-tate
Spreaders										
ATCC 17061	+	-	+	+	?+	+	-	+	+	-
405	-	-	+	+	?	+	-	+	+	-
Alfalfa	+	-	+	+	+	+	-	+	+	-
Bryant	+	-	+	+	+	+	-	+	+	-
A 15	-	-	+	+	+	+	-	+	-	-
B-2-25	+	-	+	+	+	+	-	+	?	-
E-1-25	+	-	+	+	+	+	-	+	?	-
$H_2$0-1A	-	-	+	+	+	+	-	+	-	-
1-10	+	-	+	+	+	+	-	+	+	-
3	+	-	+	+	+	+	-	+	+	-
3-19	+	-	+	+	?+	-	-	+	+	-
3-22	+	-	+	+	?+	-	-	+	+	-
4433	+	-	+	+	+	+	-	+	-	-
4539	-	-	+	+	+	+	-	+	?	-
4707	-	-	+	+	?+	+	-	+	?	-
NCIB 9059	+	-	+	+	+	+	-	+	+	-
FS-1	+	-	+	+	-	+	-	+	+	-
Stanier 6	+	+	-	-	-	-	-	+	-	-
NCIB 10782	+	+	-	-	-	-	-	+	-	-
2	-	-	+	+	+	+	-	+	-	-
D	-	-	+	+	+	+	-	+	-	-
6	-	-	+	+	+	+	-	+	-	-
4541	-	-	+	+	+	+	-	+	-	-
PC 15	-	-	+	+	+	+	-	+	-	-
9-11	-	-	+	+	?+	-	-	+	-	-
9D	-	-	+	+	+	-	-	+	+	-

Table 48 - continued

Use of polysaccharides

	Cellulose		Starch			Chitin	Agar		Algi-	Pec-
	CMC	Paper	SYS	S-NB	Potato		Tubes	Gelase	nate	tate
Intermediates										
495	+	-	-	?+	-	+	-	+	-	+
AL-1 (i)	+	-	-	-	-	+	-	+	?	+
(ii)	+	-	-	-	-	+	-	+	?	+
(iii)	+	-	-	-	-	+	-	+	-	+
ATCC 21123	+	-	-	-	-	+	-	+	+	+
18L Y	+	-	-	?-	-	+	-	+	-	-
18L W	+	-	-	-	-	+	-	+	-	-
4553 (i)	+	-	-	-	-	+	-	+	-	+
4553 (ii)	+	-	-	-	-	+	-	+	-	+
4554	+	-	-	-	-	+	-	+	-	+
4555 (i)	+	-	-	-	-	+	-	+	-	+
4555 (ii)	+	-	-	?-	-	+	-	+	+	+
4556 (i)	+	-	-	-	-	+	-	+	+	+
4556 (ii)	+	-	-	-	-	+	-	+	+	+
4557 (i)	+	-	-	-	-	+	-	+	-	+
4557 (ii)	+	-	-	-	-	+	-	+	-	+
4558 (i)	+	-	-	-	-	+	-	+	-	+
4558 (ii)	+	-	-	-	-	+	-	+	-	+
4559 (i)	+	-	-	-	-	+	-	+	-	+
4559 (ii)	+	-	-	-	-	+	-	+	+	+
4560 (i)	+	-	-	-	-	+	-	+	-	+
4560 (ii)	+	-	-	-	-	+	-	+	?	+
4560 (iii)	+	-	-	-	-	+	-	+	?	-
4561 (i)	+	-	-	-	-	+	-	+	-	+
4561 (ii)	+	-	-	-	-	+	-	+	-	+
4561 (iii)	+	-	-	-	-	+	-	+		
4562 (i)	+	-	-	-	-	+	-	+	?	+
4562 (ii)	+	-	-	-	-	+	-	+	?	+
4563 (i)	+	-	-	-	-	+	-	+	?	+

Table 48 - continued

Use of polysaccharides

Organism	Cellulose CMC	Cellulose Paper	Starch SYS	Starch S-NB	Starch Potato	Chitin	Agar Tubes	Agar Gelase	Algi-nate	Pec-tate
4563 (ii)	+	-	-	-	-	+	-	+	-	-
4564 (i)	+	-	-	-	-	+	-	+	?	+
4564 (ii)	+	-	-	-	-	+	-	+	-	+
4565 (i)	+	-	-	-	-	+	-	+	-	+
4565 (ii)	+	-	-	-	-	+	-	+	-	+
13B	+	-	-	-	+	+	-	+	+	-
3C	+	-	-	-	-	+	-	+	-	-
Nonspreaders										
7-1	-	-	+	+	-	-	-	+	-	-
15D	-	-	+	+	+	+	-	+	+	-
18H	-	-	+	+	+	-	-	+	+	-
NCIB 8186	-	-	+	+	+	-	-	+	+	-
NCIB 8187	-	-	+	+	+	-	-	+	-	-
NCIB 8188 a	-	-	+	+	+	-	-	+	-	-
NCIB 8188 b	-	-	+	+	+	-	-	+	-	-
NCIB 8195	-	-	-	+	+?	-	-	-	?	-
NCIB 8535	-	-	+	+	+	-	-	+	?	-
NCIB 8767	-	-	+	+	+	+	-	+	?	-
NCIB 8771	+	-	-	-	-	-	-	+	-	-
NCIB 8992	-	-	+ poor	+	+	-	-	+	?	-
NCIB 9157 a	+	-	-	-	-	-	-	+	?	-
NCIB 9157 b	+	-	-	-	-	-	-	+	-	-
NCIB 9290	-	-	-	-	-	-	-	+	?	-
NCIB 9324	-	-	-	+	-	-	-	+	-	-
RL 8	-	-	-	-	-	-	-	+	?	-
NCIB 8185	+	-	-	-	-	-	-	+	-	-
NCIB 8204	-	-	-	?+	?+	-	-	+	?	-
5-9	+	-	+	+	?+	-	-	+	?	-
14	+	-	+	+	?+	-	-	+	-	-
402	+	-	-	-	-	+	-	+	-	-

As expected, none of these non-marine isolates softened agar, but all but one displayed a gelase field with the iodine test on agar plates. This reaction does not therefore correlate with agarolytic activity and the most likely explanation is that the organisms excrete some compound which interferes with the iodine reaction. Problems were encountered with interpretation of the pectate degradation test, hence the large number of doubtful results. Liquefaction was definitely shown by nearly half of the spreaders including *F.pectinovorum*, and by a few of the other cultures, but it did not seem to be correlated with any particular groups. Degradation of alginate was limited to the members of the 495 group (the intermediates minus 13B and 3C), with the exception of the two varieties of 18L.

Table 49. Proteolysis.

All of the organisms tested were proteolytic except *C.hutchinsonii*. Most of them were strongly active on a wide variety of protein media except for the browns (2, D, 6 and 4541), the 'pinks' (PC 15, 9-11, 5-9 and 14), most *Flavobacteria* and RL8 which had more limited abilities. Milk was lysed (cleared) especially rapidly by the 'browns', *Flexibacter* FS-1, 9D, many of the intermediates, 15D and 18H. The intermediate group was also strongly haemolytic. The only two media which supported the growth of all proteolytic organisms were tryptone (Cook's Cytophaga Agar) and casitone + N-free salts.

Table 49

Proteolysis (proteins used as both C and N source)

Organism	Gelatin	Milk	Tryptone	Penassay Growth	Penassay → NH_3	Penassay Blood	Casein Growth	Casein → NH_3	Casamino Casitone Growth	Casamino Casitone → NH_3	Growth	→ NH_3
Spreaders												
ATCC 17061	+	4	+	+	+	-	+	+	+	+	+	+
405	+	3	+	+	+	+	+	+	+	+	+	+
Alfalfa	+	2	+	+	-	-	+	+	+	-	+	+
Bryant	+	5	+	+	-	-	+	+	-	-	+	+
A 15	+ slow	3	+	+	+	+	+	+	+	+	+	+
B-2-25	+	7	+	+	+	±	+	+	+	+	+	+
E-1-25	+	3	+	+	+	+	+	+	+	+	+	+
H_2O-1A	+	2	+	+	-	+	+	+	+	+	+	+
1-10	+	2	+	+	+	+	+	+	+	-	+	+
3	+	2	+	+	+	+	+	+	+	+	+	+
3-19	?	2	+	+	?+	-	+	+	+	+	+	+
3-22	?	4	+	+	+	-	+	+	+	+	+	+
4433	+	3	+	+	+	+	+	+	+	+	+	+
4539	+	2	+	+	+	+	+	+	+	+	+	+
4707	+	2	+	+	+	+	+	+	+	+	+	+
NCIB 9059	+	6	+	+	+	+	+	+	+	+	+	+
FS-1	+	1	+			-	+	+			+	-
Stanier 6	-	-	-	-	-	-	-	-	-	-	-	-
NCIB 10782	-	-	-	-	-	-	-	-	-	-	-	-
2	+	1	+	+	-	-	+	-	+	-	+	+
D	+	1	+	+	-	-	+	-	+	-	+	+
6	+	1	+	+	-	-	+	-	+	-	+	+
4541	+	1	+	+	-	-	+	-	+	-	+	+
PC 15	+	1	+	+	-	-	+	-	+	-	+	+
9-11	?	3	+	+	-	-	-	-	+	-	+	+
9D	+	2	+	+	+	-	+	+	+	+	+	+

Table 49 - continued

Proteolysis (proteins used as both C and N source)

Organism	Gelatin	Milk	Tryptone	Penassay Growth	Penassay → NH_3	Blood	Casein Growth	Casein → NH_3	Casamino Growth	Casamino → NH_3	Casitone Growth	Casitone → NH_3
Intermediates												
495	+	1	+	+	+	++	+	+	+	+	+	+
A1-1 (i)	+	2	+	+	-	++	+	+	+	+	+	-
(ii)	+	2	+	+	-	+	+	+	+	+	+	-
(iii)	+	2	+	+	-	++	+	+	+	+	+	-
ATCC 21123	+	1	+	+	+	++	+	+	+	+	+	+
18L Y	+	2	+	+	+	++	+	+	+	+	+	+
18L W	+	1	+	+	+	++	+	+	+	+	+	+
4553 (i)	+	2	+	+	+	++	+	+	+	+	+	+
4553 (ii)	+	1	+	+	+	+	+	+	+	+	+	+
4554	+	2	+	+	+	++	+	+	+	+	+	+
4555 (i)	+	2	+	+	+	++	+	+	+	+	+	+
4555 (ii)	+	3	+	+	+	++	+	+	+	+	+	+
4556 (i)	+	1	+	+	+	++	+	+	+	+	+	+
4556 (ii)	+	3	+	+	+	+	+	+	+	+	+	+
4557 (i)	+	1	+	+	+	++	+	+	+	+	+	+
4557 (ii)	+	2-3	+	+	+	-.	+	+	+	+	+	+
4558 (i)	+	3	+	+	+	++	+	+	+	+	+	+
4558 (ii)	+	4	+	+	+	+	+	+	+	+	+	+
4559 (i)	+	3	+	+	+	++	+	+	+	+	+	+
4559 (ii)	+	2	+	+	+	+	+	+	+	+	+	+
4560 (i)	+	1	+	+	+	++	+	+	+	+	+	+
4560 (ii)	+	1	+	+	+	++	+	+	+	+	+	+
4560 (iii)	+	4	+	+	+	+	+	+	+	+	+	+
4561 (i)	+	2	+	+	+	++	+	+	+	+	+	+
4561 (ii)	+	3	+	+	+	++	+	+	+	+	+	+
4561 (iii)	+		+	+	+		+	+	+	+	+	+
4562 (i)	+	3	+	+	+	++	+	+	+	+	+	+

Table 49 - continued

Proteolysis (proteins used as both C and N source)

Organism	Gelatin	Milk	Tryptone	Penassay Growth	Penassay → NH_3	Penassay Blood	Casein Growth	Casein → NH_3	Casamino Growth	Casamino → NH_3	Casitone Growth	Casitone → NH_3
4562 (ii)	+	2	+	+	+	+	+	+	+	+	+	+
4563 (i)	+	2	+	+	+	++	+	+	+	+	+	+
4563 (ii)	+	1	+	+	+	++	+	+	+	+	+	+
4564 (i)	+	2	+	+	+	++	+	+	+	+	+	+
4564 (ii)	+	3	+	+	+	++	+	+	+	+	+	+
4565 (i)	+	2	+	+	+	++	+	+	+	+	+	+
4565 (ii)	+	1	+	+	+	++	+	+	+	+	+	+
13B	+	2	+	+	+	+	+	+	+	+	+	+
3C	?	1	+	+	-	?	+	+	+	-	+	+
Nonspreaders												
7-1	?	4	+	+	-	-	+	+	+	+	+	+
15D	+	1	+	+	+	+	+	+	+	+	+	+
18H	+	1	+	+	+	+	+	+	+	+	+	+
NCIB 8186	+	7	+	+	+	-	+	+	+	+	+	+
NCIB 8187	+	7	+	+	+	+	+	+	+	+	+	+
NCIB 8188 a	+ slow	20	+	+	-	-	+	+	+	+	+	-
NCIB 8188 b	+ slow	8-20	+	+	-	-	+	+	+	+	+	-
NCIB 8195	-	-	+	+	+	+	-	+	+	+	+	+
NCIB 8535	+	7	+	+	+	+	+	+	+	+	+	+
NCIB 8767	+ slow	9-12	+	+	+	-	+	+	+	+	+	-
NCIB 8771	?	-	+	+	+	-	-	-	+	-	+	+
NCIB 8992	+	>16	+	+	+	-	+	+	+ slow	+ slow	+	-
NCIB 9157 a	-	-	+	+	+	-	-	+	+	+	+	-

Table 49 - continued

Proteolysis (proteins used as both C and N source)

Organism	Gelatin	Milk	Tryptone	Penassay Growth	Penassay → NH_3	Penassay Blood	Casein Growth	Casein → NH_3	Casamino Growth	Casamino → NH_3	Casitone Growth	Casitone → NH_3
NCIB 9157 b	-	-	+	+	+	+	-	?+	+	+	+	+
NCIB 9290	-	-	+	+	-	+	-	+	+ slow	+ slow	+	-
NCIB 9324	+	13-16	+	+	-	-	+	+	+ slow	+ slow	+	-
RL 8	-	-	+	-	-	-	-	?+	+	-	+	-
NCIB 8185	?	3	+	+	+	-	+	+	+	-	+	+
NCIB 8204	-	-	+	+	-	-	-	-	-	-	+	-
5-9	?	-	+	+	-	-	+ weak	+	+	+	+	+
14	?	-	+	-	-	-	-	+	+	-	+	+
402	?	+	+	+	+	+	+	+	+	-	+	+

Table 50. Nitrogen sources and necessity of vitamins.

All of the isolates except PC 15 and 9-11 could use glucose (Table 47) therefore this was used as a carbon source for the first group of N source experiments. Ammonia was able to be used by all organisms that assimilated glucose readily, but NO_3 was utilized by fewer of them, namely many of the spreaders, nearly all of the intermediates but none of the *Flavobacteria*. Urea was used by only one third of the spreaders, five of the intermediates but by all except three of the nonspreaders.

Generally speaking all of the organisms, except *C.hutchinsonii* of course, could obtain both their carbon and nitrogen from the single amino acids and single proteins tested. The non-spreaders were a little more exacting, and this ties in with their generally more pronounced need for vitamins. The ability to synthesize their own growth factors was characteristic of the intermediates, although a few other organisms from both groups were also self-sufficient.

Table 51. Miscellaneous biochemical tests.

H_2S was produced by nearly all of the spreading and non-spreading organisms, but by only two of the intermediate group. Most of the organisms in the study produced catalase, the exceptions were ten nonspreaders and five spreaders, including *C.hutchinsonii* which may have been handicapped by the conditions of the experiment as it is recorded as positive in Bergey's Manual. Oxidase was elaborated by all of the isolates except a few *Flavobacteria*.

Table 50

Nitrogen sources and necessity of vitamins

Organism	Glucose=C source			No other C source				Yeast extract needed	
	NO_3	NH_4	Urea	Glut.	Asp.	Tryp.	Gel.[a]	Salts + glucose	Chitin#
Spreaders									
ATCC 17061	-	+	+	+	+	+	+	+	-
405	-	+	-	+	+	+	+	+	±
Alfalfa	+	+	+	+	+	+	+	-	-
Bryant	+	+	-	+	+	+	+	-	-
A15	+	+	-	+	+	+	+slow	-	±
B-2-25	+	+	-	+	+	+	+	-	±
E-1-25	+	+	-	+	+	+	+	-	±
H_2O-1A	+	+	-	+	+	+	+	-	-
1-10	+	+	+	+	+	+	+	-	-
3	+	+	?-	+	+	+	+	-	±
3-19	-	+	+	+	+	+	?-	+	+
3-22	-	+	+	+	+	+	?-	+	+
4433	+	+	-	+	+	+	+	-	+
4539	+	+	-	+	+	+	+	-	±
4707	+	+	-	+	+	+	+	-	±
NCIB 9059	+	+	+	+	+	+	+	-	±
FS-1	+	+	+	+	+	+	+	-	+
Stanier 6	-	+	-	-	-	-	-	no growth	
NCIB 10782	-	+	?+	-	-	-	-	no growth	
2	+	+	-	+	+	+	+	-	-
D	+	+	-	+	+	+	+	-	+
6	* ?-	+slow	-	+	+	+	+	?	+
4541	+	+	-	+	+	+	+	-	-
PC 15	† -	?-	-	-	+	+	+	?	-
9-11	† -	?-	-	+	+	+	?-	?	+
9D	-	+	-	+	+	+	+	+	+

* = use of gluscose is slow, † = glucose probably not used, #chitin + no growth without Y.E., ± = stimulated by Y.E., - = good growth without Y.E.,

a. glut. = glutamate, asp. = asparaginate, tryp. = tryptone.

Table 50 - continued

Nitrogen sources and necessity of vitamins

Organism	Glucose=C source			No other C source				Yeast extract needed	
	NO_3	NH_4	Urea	Glut.	Asp.	Tryp.	Gel.	Salts + glucose	Chitin
Intermediates									
495	-	+	-	+	+	+	+	-	-
AL-1 (i)*	-	?-	-	+	+	+	+		-
(ii)*	+	?-	-	+	+	+	+	-	-
(iii)*	-	?-	-	+	+	+	+		-
ATCC 21123	+	+	+	+	+	+	+	-	-
18L Y	+	+	+	+	+	+	+	-	-
18L W	+	+	+	+	+	+	+	-	-
4553 (i)	+	+	-	+	+	+	+	-	-
4553 (ii)	+	+	-	+	+	+	+	-	-
4554	+	+	-	+	+	+	+	-	-
4555 (i)	+	+	-	+	+	+	+	-	-
4555 (ii)	+	+	-	+	+	+	+	-	-
4556 (i)	+	+	-	+	+	+	+	-	-
4556 (ii)	+	+	-	+	+	+	+	-	-
4557 (i)	+	+	-	+	+	+	+	-	-
4557 (ii)	+	+	-	+	+	+	+	-	-
4558 (i)	+	+	-	+	+	+	+	-	-
4558 (ii)	+	+	-	+	+	+	+	-	-
4559 (i)	+	+	-	+	+	+	+	-	-
4559 (ii)	+	+	-	+	+	+	+	-	-
4560 (i)	+	+	-	+	+	+	+	-	-
4560 (ii)	+	+	-	+	+	+	+	-	-
4560 (iii)	+	+	-	+	+	+	+	-	-
4561 (i)	+	+	-	+	+	+	+	-	-
4561 (ii)	+	+	-	+	+	+	+	-	-
4561 (iii)	+	+	-	+	+	+	+	-	-
4562 (i)	+	+	-	+	+	+	+	-	-
4562 (ii)	+	+	-	+	+	+	+	-	-
4563 (i)	+	+	-	+	+	+	+	-	-

Table 50 - continued

Nitrogen sources and necessity of vitamins

Organism	Glucose=C source			No other C source				Yeast extract needed	
	NO_3	NH_4	Urea	Glut.	Asp.	Tryp.	Gel.	Salts + glucose	Chitin
4563 (ii)	+	+	-	+	+	+	+	-	-
4564 (i)	+	+	-	+	+	+	+	-	-
4564 (ii)	+	+	-	+	+	+	+	-	-
4565 (i)	+	+	-	+	+	+	+	-	-
4565 (ii)	+	+	-	+	+	+	+	-	-
13B	-	+	+	+	+	+	+	+	-
3C	+	+	+	+	+	+	?-	-	-
Nonspreaders									
7-1	?-	+	+	-	-	+	?-		+
15D	-	+	-	+	+	+	+	+	+
18H	+	+slow	-	+	+	+	+	-	+
NCIB 8186	-	+	+	+	+	+	+	+	+
NCIB 8187	-	+	+	+	+	+	+	+	+
NCIB 8188 a	-	+	+	+	-	+	+slow	+	-
NCIB 8188 b	-	+	+	+	-	+	+slow	+	-
NCIB 8195	-	+	+	+	+	+	-	+	+
NCIB 8535	-	+	+	+	+	+	+	+	+
NCIB 8767	-	+	+	+	+	+	+slow	+	+
NCIB 8771	-	+	+	+	+	+	? -	+	+
NCIB 8992	-	+slow	-	+	+	+	+	+	+
NCIB 9157 a	-	+	+	+	+	+	-	+	+
NCIB 9157 b	-	+	+	+	+	+	-	+	+
NCIB 9290	-	+	+	-	+	+	-		+
NCIB 9324	-	+slow	+	+	+	+	+	+	+
RL 8	-	+slow	+	+	+	+	-	+	+
NCIB 8185	-	+	+	+	+	+	?-	+	+
NCIB 8204	-	+slow	+	+	+	+	-	+	+
5-9	+	+	+	+	+	+	?-	-	-
14	+	+	+	+	+	+	?-	-	-
402	+	+	+	+	+	+	?-	-	-

Table 51

Miscellaneous biochemical tests

Organism	H_2S	Catalase*	Oxidase*	Denitrification			Phosphatase	Indole	
				$NO_3 \rightarrow NO_2$	$NO_2 \rightarrow$ gas	Anaerobic		Casitone	Casein
Spreaders									
ATCC 17061	+	5/6	6/6	-	+	-	+	-	-
405	+	5/7	6/6	+	+	+	+	-	-
Alfalfa	+	4/7	5/7	+	-	+	+	-	-
Bryant	+	1/7	6/6	-	-	-	+	-	-
A15	+	0/9	6/6	-	-	-	+	-	-
B-2-25	+	6/7	6/6	+	-	+	+	-	-
E-1-25	+	5/7	6/6	+	-	+	+	-	-
H_2O-1A	+	2/7	6/6	-	-	-	+	-	-
1-10	+	3/7	6/7	-	-	+	+	-	-
3	+	5/7	6/6	+	+	+	+	-	-
3-19	+	1/8	4/7	+	+	+	+	-	-
3-22	+	2/8	6/7	+	-	+	+	-	-
4433	+	5/7	6/6	+	-	+	+	-	-
4539	+	4/7	6/6	+	-	+	+	-	-
4707	+	5/7	6/6	+	-	+	+	-	-
NCIB 9059	+	5/7	6/6	+	-	+	+	-	-
FS-1	-	3/7	6/6	no growth			+	-	-
Stanier 6	-	1/6	2/2	no growth			no growth	-	-
NCIB 10782	-	0/7	3/3	no growth			slight+	-	-
2	+	5/7	6/6	-	-	-	+	-	-
D	+	5/7	6/6	-	-	-	+	-	-
6	+	5/7	6/6	-	-	-	+	-	-
4541	+	5/7	6/6	-	-	-	+	-	-

*Number of positive tests/plates tested.

Table 51 - continued

Miscellaneous biochemical tests

Organism	H_2S	Catalase	Oxidase	Denitrification			Indole		
				$NO_3 \rightarrow NO_2$	$NO_2 \rightarrow$ gas	Anaerobic	Phosphatase	Casitone	Casein
PC 15	+	3/9	4/8	-	-	+	+	-	-
9-11	+	3/7	5/6	-	-	+	+	-	-
9D	+	5/7	6/6	+	+	+	+	-	-
Intermediates									
495	+	5/7	6/6	-	-	-	+	-	-
AL-1 (i)	-	7/7	6/6	-	-	-	+	-	-
(ii)	-	4/7	6/6	-	-	-	+	-	-
(iii)	-	5/7	6/6	-	-	-	+	-	-
ATCC 21123	+	5/7	6/7	+	-	+	+	-	-
18L Y	-	3/7	7/7	-	-	+	+	-	-
18L W	-	5/7	6/7	-	-	+	+	-	-
4553 (i)	-	6/6	4/4	+	+	-	+	-	-
4553 (ii)	-	5/6	4/4	+	+	-	+	-	-
4554	-	6/6	4/4	+	+	-	+	-	-
4555 (i)	-	6/6	4/4	+	?+	-	+	-	-
4555 (ii)	-	6/6	4/4	+	?+	-	+	-	-
4556 (i)	-	6/6	4/4	+	?+	-	+	-	-
4556 (ii)	-	6/6	4/4	+	+	-	+	-	-
4557 (i)	-	6/6	4/4	+	+	-	+	-	-
4557 (ii)	-	4/6	4/4	+	+	-	+	-	-
4558 (i)	-	6/6	4/4	+	+	-	+	-	-
4558 (ii)	-	3/6	4/4	+	+	-	+	-	-
4559 (i)	-	6/6	4/4	+	+	-	+	-	-
4559 (ii)	-	6/6	4/4	+	+	-	+	-	-
4560 (i)	-	6/6	4/4	+	+		+	-	-
4560 (ii)	-	6/6	4/4	+	?+	-	+	-	-
4560 (iii)	-	4/6	4/4	+	?+	-	+	-	-

Table 51 - continued

Miscellaneous biochemical tests

Organisms	H_2S	Catalase	Oxidase	Denitrification			Phosphatase	Indole	
				$NO_3 \rightarrow NO_2$	$NO_2 \rightarrow$ gas	Anaerobic		Casitone	Casein
4561 (i)	-	6/6	4/4	+	+	-	+	-	-
4561 (ii)	-	5/6	4/4	+	+	-	+	-	-
4561 (iii)	-						+	-	-
4562 (i)	-	6/6	4/4	+	+	+	+	-	-
4562 (ii)	-	5/6	4/4	+	+	+	+	-	-
4563 (i)	-	6/6	4/4	+	?+	+	+	-	-
4563 (ii)	-	4/5	4/4	+	?+	+	+	-	-
4564 (i)	-	5/5	4/4	+	?+	+	+	-	-
4564 (ii)	-	5/5	4/4	+	?+	-	+	-	-
4565 (i)	-	5/5	4/4	+	?+	-	+	-	-
4565 (ii)	-	5/5	4/4	+	?+	-	+	-	-
13B	-	4/7	7/7	-	-	-	+	-	-
3C	-	5/7	7/7	+	-	+	+	-	-
Nonspreaders									
7-1	+	0/9	6/6	-	-	+	+	-	-
15D	+	5/7	6/6	-	+	-	+	+	+
18H	+	5/7	6/6	-	+	-	+	+	+
NCIB 8186	+	0/9	2/6	-	-	-	-	-	-
NCIB 8187	+	0/9	2/6	-	-	-	+	-	-
NCIB 8188 a	+	0/6	2/6	-	-	-	±	-	-
NCIB 8188 b	+	0/6	2/6	-	-	-	±	-	-
NCIB 8195	-	5/6	6/6	-	-	-	+	-	-
NCIB 8535	+	0/9	2/6	-	-	-	+	-	-
NCIB 8767	+	1/6	6/6	-	-	-	+	-	-
NCIB 8771	+	4/5	0/5	+	-	+	±	-	-

Table 51 - continued

Miscellaneous biochemical tests

Organism	H_2S	Catalase	Oxidase	Denitrification			Phosphatase	Indole	
				$NO_3 \rightarrow NO_2$	$NO_2 \rightarrow$ gas	Anaerobic		Casitone	Casein
NCIB 8992	+	5/6	6/6	-	-	-	+	-	+
NCIB 9157 a	+	3/5	2/5	+	-	+	+	-	-
NCIB 9157 b	+	6/6	0/5	+	-	+	+	-	-
NCIB 9290	+	6/6	6/6	-	-	+	+	-	-
NCIB 9324	+	5/6	3/6	-	-	-	+	-	-
RL 8	+	4/5	5/5	+	-	-	-	-	-
NCIB 8185	+	5/6	3/6	-	-	-	-	-	-
NCIB 8204	+	6/6	0/5	-	-	-	-	-	-
5-9	+	1/7	6/7	-	-	-	+	-	+
14	-	1/7	4/5	no growth			+	-	-
402	-	0/7	6/7	-	-	-	+	-	-

The complete denitrification process, $NO_3 \rightarrow NO_2 \rightarrow$ gas (probably N_2O as no bubbles were released), was carried out by the spreaders *C.johnsonae* var. *denitrificans* 405, 3, 3-19 and 9D, and by most of the intermediate group, but none of the nonspreaders had this ability. Thirteen organisms representing all of the three groups were capable of reducing nitrate to nitrite only, and there were three organisms (*C.johnsonae* 17061, 15D and 18H) which did not possess a nitrate reductase but which were able to denitrify NO_2 to an unidentified gaseous product. In the cases of 15D and 18H this end product has previously been identified as N_2O (83), a highly soluble gas which is not released as bubbles when a hot loop is immersed in the culture medium. It is very significant that *C.johnsonae* 17061 was able to utilize NO_2 but not NO_3, because this has an important bearing on the taxonomy of this species (see page 314). It is interesting to note that the isolates 405, 3-19, 3-22, 9D, 8771, 9157 and RL8 were not able to use NO_3 as an N source (Table 50) but were able to denitrify it.

Nitrate could be used as an alternate electron acceptor by 29 of the 84 isolates and hence these organisms, which were from all groups but were mostly spreaders, are facultatively anaerobic when NO_3 is present. Only seven of these 29 were fermentative on Board and Holding's medium (Table 47). There were also six organisms which were fermentative but did not use NO_3 anaerobically. It should also be noted that ten organisms (405, 3-19, 3-22, PC 15, 9-11, 9D, 7-1, 8771, 9157 and 9290) were not able to use NO_3 as an N source (Table 50) but were able to use it as an alternate electron acceptor.

The phosphatase test was positive for all of the spreading and intermediate organisms, (except for one strain of *C.hutchinsonii* which did not grow well in phosphatase medium), and for all of the nonspreaders except for three *Flavobacteria* and RL 8. Indole was only produced by four organisms, by 15D and 18H on both media employed, and by one strain only of *F.suaveolans* 8992 and 5-9 in casein broth alone.

Table 52. Antibiotic and antibacterial sensitivities.

All except a very few of the organisms used in this study had their growth reduced by the presence of 0.01% or slightly more of sodium lauryl sulphate, and were completely inhibited by 0.1% S.L.S. The spreaders were slightly more sensitive than the other two groups, and the pink organisms PC 15, 9-11 and 14 were especially sensitive, being inhibited completely at the 0.01% level. Six of the intermediates, *F.rhenanum* and 402 were more resistant than the rest, and *F.proteus* was not affected by even the strongest concentration of S.L.S. used.

Warke and Dhala (423) reported that three out of 12 species of *Cytophaga* were sensitive to 25 µg of chloramphenicol, and in this study the reaction to 30 µg discs of chloramphenicol was also mixed, half of the isolates being susceptible and one third resistant. The spreaders were mostly sensitive, and more of the intermediates were resistant. The results with 10 µg discs of dihydrostreptomycin were also varied, the only consistent effect was the resistance of the intermediate group.

Table 52

Antibiotic and antibacterial sensitivities*

Organism	S.L.S. % Growth reduced	S.L.S. % Growth inhibited	Chloramphenicol	Dihydrostrep.	Penicillin	Polymyxin B K-B	Polymyxin B PC	Actinomycin D	NO_2
Spreaders									
ATCC 17061	.01	.1	S	I	R	R	I	S	-
405	.01	.1	R	I	R	R	R	S	-
Alfalfa	.01	.1	S	I	R	R	R	S	-
Bryant	.01	.1	I	I	R	R	R	S	+
A 15	.01	.1	I	S	R	R	S	S	+
B-2-25	.01	.1	S	R	R	R	S	-	+
E-1-25	.01	.1	S	R	R	R	S	S	+
H_2O-1A	.01	.1	I	S	R	R	S	S	+
1-10	>.01	.1	S	R	R	R	R	S	-
3	.01	.1	I	S	I	I	S	S	-
3-19	.01	>.1	S	R	I	R	S		-
3-22	>.01	.1	R	R	I	R	S		-
4433	.01	.1	S	R	R	R	S	S	+
4539	.01	.1	S	I	R	R	I	S	+
4707	.01	.1	S	I	R	R	R	S	+
NCIB 9059	.01	>.1	I	I	I	R	I	S	+
FS-1	.01	.1	I	R	R	I	S	S	

*Chloramphenicol 30 µg, Dihydrostreptomycin 10 µg, Penicillin 10 units. Polymyxin B 300 units (K-B). S = sensitive I = intermediate, R= resistant according to the Kirby-Bauer interpretative scheme (18).

Polymyxin B (PC). Interpretative scheme based on behaviour of control organisms *E.coli*, *P.aeruginosa*, *Arthrobacter* sp. *S.marcescens* and *B.subtilis*.

Actinomycin D = not sensitive (same or less than *E.coli*). S = sensitive (more than *E.coli*). HS = highly sensitive (same as *B.subtilis*). VHS = very highly sensitive (more than *B.subtilis*)

NO_2 + = inhibited by .1% KNO_2, - = not inhibited.

Table 52 - continued

Antibiotic and antibacterial sensitivies

Organism	S.L.S. % Growth reduced	S.L.S. % Growth inhibited	Chloram-phenicol	Dihydro-strep.	Penicillin	Polymyxin B K-B	Polymyxin B PC	Actinomycin D	NO_2
Stanier 6	.01	.1	S	I	I	R	S	VHS	
NCIB 10782			S	S	I	R	R	VHS	
2	.01	.1	S	S	S	I	S	S	+
D	.01	.1	S	S	S	I	S	S	+
6	.01	.1	S	S	I	I	S	S	+
4541	>.01	.1	S	I	I	I	S	S	+
PC 15	<.01	.01	S	I	I	R	I	S	+
9-11	<.01	.01	S	R	S	R	S		-
9D	>.01	.1	S	S	R	R	S	S	-
Intermediates									
495	.01	.1	S	R	R	I	S	S	+
AL-1 (i)	.01	.1	R	R	R	R	S	S	-
(ii)	.01	.1	R	R	R	I	S	S	-
(iii)	>.01	.1	R	R	R	R	S	S	-
ATCC 21123	.1	>.1	I	R	R	R	S	S	-
18L Y	>.01	.1	I	R	R				-
18L W	>.01	.1	S	R	R	R	S		-
4553 (i)	>.01	.1	R	R	R	R	S	S	-
4553 (ii)	>.01	.1	R	R	R	I	S	-	-
4554	>.01	.1	S	R	R	R	S	S	-
4555 (i)	>.01	.1	S	R	R	R	S	S	-
4555 (ii)	>.01	.1	S	R	R	R	S	S	-
4556 (i)	>.01	.1	R	R	R	R	S	-	-
4556 (ii)	.1	>.1	R	R	R	I	S	-	-
4557 (i)	>.01	.1	S	R	R	I	S	-	-
4557 (ii)	.1	>.1	R	R	R	I	S	-	-

Table 52 - continued

Antibiotic and antibacterial sensitivities

Organism	S.L.S % Growth reduced	S.L.S % Growth inhibited	Chloram-phenicol	Dihydro-strep.	Penicillin	Polymyxin B K-B	Polymyxin B PC	Actino-mycin D	NO_2
4558 (i)	>.01	.1	R	R	R	R	I	S	-
4558 (ii)	.01	.1	R	R	R	I	S	S	-
4559 (i)	>.01	.1	R	R	R	R	S	S	-
4559 (ii)	>.01	.1	R	R	R	R	S	S	-
4560 (i)	>.01	.1	S	R	R	I	S	-	-
4560 (ii)	>.01	.1	S	R	R	I	S	S	-
4560 (iii)	.1	>.1	R	R	R	I	S	S	-
4561 (i)	>.01	.1	R	I	R	R	I	S	-
4561 (ii)	.1	>.1	R	I	R	R	S	S	-
4561 (iii)			S	R	R	R	S		-
4562 (i)	>.01	.1	I	R	R	R	I	-	-
4562 (ii)	>.01	.1	I	R	R	R	S	S	-
4563 (i)	>.01	.1	S	R	R	R	I	-	-
4563 (ii)	.1	>.1	R	R	R	I	S	-	-
4564 (i)	>.01	.1	R	R	R	I	S	S	-
4564 (ii)	>.01	.1	R	R	R	R	S	S	-
4565 (i)	>.01	.1	S	R	R	I	S	-	-
4565 (ii)	>.01	.1	R	R	R	I	S	S	-
13B	.01	.1	S	R	R	I	S	S	+
3C	>.01	.1	R	R	R	I	S		-

Table 52 - continued

Antibiotic and antibacterial sensitivities

Organism	S.L.S. Growth reduced	S.L.S. Growth inhibited	Chloram-phenicol	Dihydro-strep.	Penicillin	Poly-myxin B K-B	Poly-myxin B PC	Actino-mycin D	NO_2
Nonspreaders									
7-1	.01	.1	S	I	S	R	S		-
15D	.01	.1	R	R	R	R	I	S	-
18H	>.01	.1	R	R	R	R	I	S	-
NCIB 8186	>.01	.1	R	S	I	R	I	S	-
NCIB 8187	>.01	.1	I	S	I	R	I	S	-
NCIB 8188 a	>.01	.1	I	S	I	R	I	S	-
NCIB 8188 b	>.01	.1	I	S	I	R	S	S	-
NCIB 8195	.01	.1	S	R	I	R	R	S	-
NCIB 8535	>.01	.1	R	S	I	R	R	S	-
NCIB 8767	>.01	.1	S	S	R	I	S	S	-
NCIB 8771	>.1	>.1	S	I	R	I	S	S	-
NCIB 8992	.01	.1	S	I	R	I	S	S	-
NCIB 9157 a	.1	>.1	S	I	R	R	S	S	-
NCIB 9157 b	.1	>.1	S	I	R	R	S	S	-
NCIB 9290	.01	.1	R	R	R	I	S	S	-
NCIB 9324	>.01	.1	R	R	I	R	R	S	-
RL 8	>.01	.1	S	S	R	I	S	S	-
NCIB 8185	.01	.1	S	S	I	R	R	VHS	-
NCIB 8204	>.01	.1	S	S	S	R	I	VHS	-
5-9	.01	.1	R	R	I	I	S	HS	+
14	<.01	.01	S	R	R	R	I	S	-
402	.1	>.1	R	R	R	I	S	S	-

Only five organisms (2, D, 9-11, 7-1 and *F.aurantiacum*) were sensitive to 10 units of penicillin G, and most of each group including all of the intermediates were resistant to this antibiotic. This is in line with the results of Warke and Dhala (423) who showed that 12 *Cytophaga* species were resistant to 25 units/ml.

Mitchell, Hendrie and Shewan (275) have suggested (although no supporting data were presented) that the resistance of *Cytophaga* to polymyxin B is a useful diagnostic criterion, as most Gram negative bacteria including the fruiting myxobacteria are sensitive. The interpretation of polymyxin B inhibition zones is always difficult because of the small zone sizes due to the limited diffusion of such a large molecule. Using the Kirby-Bauer chart (column K-B in Table 52), although it is realized that this was not constructed for these organisms or these experimental conditions, 55 out of the 84 organisms were indeed resistant to 300 unit discs of polymyxin B, and the others were of intermediate status. Two difficulties were apparent, the first was that no differentiation between *Cytophaga* and *Flavobacterium* was apparent, and the second was that the "sensitive control" organisms *E.coli* and *P.aeruginosa* were resistant. Hence a new scheme of interpretation was drawn up (column PC) based on the behaviour of the control organisms. Under this scheme the position was roughly reversed with 49 organisms being classed as sensitive, 15 as intermediate and 10 as resistant. There was still no clear distinction between *Cytophaga* and *Flavobacterium* and other inconsistencies were frequently observed. Also Stewart and Brown (385) had noted that *C.johnsonae* 17061 was sensitive to 300 units of polymyxin B, whereas it was found here to be

one of the most resistant organisms tested. It is therefore felt that until convincing data appear in the literature the polymyxin B sensitivity test is not applicable as a diagnostic criterion for *Cytophaga*.

Actinomycin D inhibits the growth of many forms of Gram negative gliding bacteria (102, 103), and only 11 of the organisms tested in this study were found to be as resistant as the control *E.coli*. Most organisms (including the Gram positive as well as Gram negative *Flavobacteria*) were sensitive, and five (*C.hutchinsonii*, *F.arborescens*, *F.aurantiacum* and 5-9),were extremely sensitive. The growth of more than half of the spreaders was inhibited by 0.1% KNO_2, however all but three (495, 13B and 5-9) of the other 48 organisms were unaffected by this concentration of nitrite.

Table 53. Antimicrobial lytic action.

The Gram negative bacteria were the least affected by the lytic activities of the cytophagas, and they were not affected at all by nonspreaders. The Gram positive bacteria, actinomycetes, fungi and *Chlorella* were each attacked mainly by the intermediate organisms and by a few isolates of both the other groups.

The spreading cytophagas lysed every 'prey' organism between them except *S.marcescens*. The most frequently affected was the yeast however this may have been due to the fact that a different method was used for this organism and dead cells only were present.

Table 53

Antimicrobial lytic action*

Organism	Bacteria										Actinos				Fungi								Alga
	E.coli		*Ps.aer.*		*Arth.*		*S.mar.*		*B.sub.*		4432		4441		*Rhizo.*		*P.not.*		*Sclero.*		Yeast		*Chlorella*
Spreaders	1	2	1	2	1	2	1	2	1	2	3	4	3	4	3	4	3	4	3	4	5	6	
ATCC 17061									L				?				?		L	?	-	±	
405																	?				±	±	
Alfalfa											?		?						?		-	-	
Bryant													?								+	±	
A 15																	?				±	±	
B-2-25															?		?				±	±	
E-1-25															?		?				±	±	
H_2O-1A														?			?				-	±	
1-10									?		L		L						?		-	±	
3																	?				±	+w	
3-19														L			?		?		-	-	L
3-22														L			?		?		-	-	L
4433																	?				+	+	

*L = good lysis, ? = possible lytic activity. Bacteria. *E.coli*, *P.aeruginosa*, *Arthrobacter* sp., *S.marcescens*, *B.subtilis*. 'Predators' added 1 hr (column 1) or 2 days (column 2) after bacterial lawn seeded. Actinomycetes and fungi. Actinos 4432 and 4441, *Rhizopus* sp., *P.notatum*, *Sclerotinia sclerotiorum*. 'Predators' added 1 day (column 3) or 4 days (column 4) after lawn seeded. Alga. *Chlorella*. 'Predators' added to 2 week old algal lawns. Yeast (probably *S.cerevisiae*). Column 5 = 4 days, column 6 = 9 days, + = complete lysis, ± = partial lysis, - = no lytic action, w = weak.

Table 53 - continued

Antimicrobial lytic action

Organism	Bacteria										Actinos				Fungi								Alga
	E.coli		*Ps.aer.*		*Arth.*		*S.mar.*		*B.sub.*		4432		4441		*Rhizo.*		*P.not.*		*Sclero.*		Yeast		*Chlorella*
	1	2	1	2	1	2	1	2	1	2	3	4	3	4	3	4	3	4	3	4	5	6	
4539																	?				±	±	
4707														?			?				-	-	
NCIB 9059									L				?				?			?	±	±	
FS-1									?										L		-	±	
Stanier 6																					-	-	
NCIB 10782																					-	-	
2	L		L	L		L			L				L				L			L	±	±	L
D	L		L	L		L			L		?		L		L		L			L	±	±	L
6						?			L				L								±	±	L
4541	L		L			L			L				L		?		L				+	+	L
PC 15																			?		-	-	
9-11																					-	±	
9D													?								-	-	?
Intermediates																							
495			?		L	L			L	?	L		L		L		L				±	±	L
A1-1 (i)			?		L				L		L		L		L		L	?	L		+	+	L
(ii)	L				L				L		L		L	?	L		L	?	L	L	+	+	L
(iii)			?		L				L		L	?	L		L		L	?	L	L	+	+	L
ATCC 21123					L		L		L	L	L		L	L	L		L		?	L	+	+	L

Table 53 - continued

Antimicrobial lytic action

Organism	Bacteria										Actinos				Fungi								Alga
	E.coli		*Ps.aero.*		*Arth.*		*S.mar.*		*B.sub.*		4432		4441		*Rhizo.*		*P.not.*		*Sclero.*	Yeast			*Chlorella*
	1	2	1	2	1	2	1	2	1	2	3	4	3	4	3	4	3	4	3	4	5	6	
18L Y			?								L		L				L		?		±	+	
18L W			?		L		L		L		L		L	L			L	L	?	L	+	+	L
4553 (i)					L		L		L	L	L	L	L		L		L	?		?	+	+	L
4553 (ii)							L				?		L		L		?			L	+	+	L
4554					L		L		L		L		L	?	L		L	?		L	+	+	L
4555 (i)	L				L		L		L		L		L		L		L	?		L	+	+	L
4555 (ii)					L		?		L		L		L		L		L			L	+	+	L
4556 (i)	?		?		L		L		L		L	?	L		L		L			L	+	+	L
4556 (ii)	L						L						L		L		?			?	+	+	L
4557 (i)					L		L		L	L	L		L		L		L			L	+	+	L
4557 (ii)	L						L						L				L				±	±	L
4558 (i)					L		L		L		L		L		L		L	?		?	+	+	L
4558 (ii)							L						?		?		?				+	+	L
4559 (i)	L				L		?		L		L		L		L		L			?	+	+	L
4559 (ii)					L		L				?		L		L		L				+	+	?
4560 (i)					L		L		L		L	?	L		L		L	?		L	+	+	L
4560 (ii)					L		L		L		L	?	L	?	L		L	?			+	+	L
4560 (iii)			?				L						?				?				±	±	?
4561 (i)			?		L			L	L		L		L		L		L	L		L	+	+	?

Table 53 - continued

Antimicrobial lytic action

Organism	Bacteria										Actinos				Fungi								Alga
	E.coli		*Ps.aero.*		*Arth.*		*S.mar.*		*B.sub.*		4432		4441		*Rhizo.*		*P.not.*		*Selero.*		Yeast		*Chlorella*
	1	2	1	2	1	2	1	2	1	2	3	4	3	4	3	4	3	4	3	4	5	6	
4561 (ii)		L	?		L		?		L		L	L	L		L		L	?		L	+	+	
4561 (iii)																					+	+	
4562 (i)		L	?		L				L		L	?	L		L		L	?		L	+	+	
4562 (ii)			?				?	?	?		L	?	L				L	?		L	+	+	
4563 (i)					L		L		L		L	?	L		L		L	L		L	+	+	L
4563 (ii)			?		L		L		L		L		L		?		L			?	+	+	?
4564 (i)			?		L				L		L		L		L		L	?		L	+	+	
4564 (ii)			?		L				L		L	?	L		L		L	?		?	±	±	
4565 (i)					L		L		L		L		L	L	L		L	L	L	L	+	+	L
4565 (ii)					L		L		L		L	L	L	?	L		L	L	L	L	±	±	L
13B					L				L		L	?	L	?	L		L	L			+	+	L
3C		L			L		L		L	L	?		L				L		?		±	+	L
Nonspreaders																							
7-1																	?		?		-	-	L
15D											?		?	?			?				±	±w	
18H					L				L		?		L	?			L				±	±w	?
NCIB 8186													?				?				-	±w	
NCIB 8187													?				?				-	±w	
NCIB 8188 a											?		?				?		?		-	-	

Table 53 - continued

Antimicrobial lytic action

Organism	Bacteria										Actinos				Fungi								Alga
	E.coli.		*Ps.aero*		*Arth.*		*S.mar.*		*B.sub.*		4432		4441		*Rhizo.*		*P.not.*		*Sclero.*		Yeast		*Chlorella*
	1	2	1	2	1	2	1	2	1	2	3	4	3	4	3	4	3	4	3	4	5	6	
NCIB 8188 b											?		?				?		?		-	-	
NCIB 8195											?		?					?	?		-	-	?
NCIB 8535													?				?				-	+w	
NCIB 8767											L		?	?					L		-	-	
NCIB 8771							?				L		?								-	-	
NCIB 8992											?		?						?		-	-	
NCIB 9157 a									?		?		?						?		-	-	
NCIB 9157 b									?		?		?						L		-	-	
NCIB 9290											L		?								-	-	?
NCIB 9324											?		?	?					?		-	-	
RL 8											?			?							-	-	
NCIB 8185									?	?	L								?		±	±	
NCIB 8204											?		?							?	-	-	
5-9									?										?		-	-	
14																				?	-	-	
402					L				L	L	L		L	L	?	?	L		?	L	±	+	L

Lytic activity has been definitely ascribed to all of the spreaders except Alfalfa, 4707, *C.hutchinsonii*, PC 15 and 9D. The 'browns' (2, D, 6 and 4541) were the significantly active predators in the group, they lysed all organisms except *S.marcescens* and actino 4432. Strain 6 was the least active and D was lytic toward the most organisms.

It is immediately obvious from Table 53 that the organisms of the intermediate group are potent lytic agents. Although difficulty was experienced with the Gram negative bacteria, they were all extremely active against a large number of other organisms. Autoclaved yeast was the only organism attacked by all of the intermediates, and no intermediate lysed every one of the cultures, however nearly half of the group were lytic against all but the Gram negative bacteria.

Very few of the nonspreaders had definite lytic ability, and none attacked Gram negative bacteria, *S.marcescens* or *Rhizopus*. Only 18H and 402 had significant activity, and the other six predators (7-1, 8767, 8771, 9157b, 9290 and 8185) only attacked three organisms (actino 4432, *S.sclerotiorum* and *Chlorella*) between them.

As a postscript to this study it must be noted that in this test the pink organisms PC 15 and 5-9 were found to produce typical myxococcal fruiting bodies after growing on *Penicillium notatum* for about two weeks. These organisms had never previously been found to fruit, but they should now of course be reassigned to the higher myxobacteria and probably to the genus *Myxococcus*. This discovery raises two further questions, firstly whether other 'nonfruiting pinks' encountered from time to time in soils and waters are also *Myxococci*,

and secondly whether or not the method of producing fruiting bodies by growing the organism with a living fungal mycelium is generally applicable or even superior to the usual methods (dung pellets, starvation media etc.).

This consideration of the results has shown that the 'spreaders' are a fairly homogeneous group with several small subgroups (*C.hutchinsonii*, 'browns', 'pinks', 9D). The 'intermediates' form a more compact group, but the 'nonspreaders' are very heterogeneous both morphologically and metabolically. Table 54 lists the main differentiating characteristics of these three groups of organisms, and a complete profile for each organism can be found in Appendix III.

Taxonomic discussion

Based on the present state of knowledge of the genus (Chapter I) and on the work encompassed by this thesis, the following redefinition of *Cytophaga* is proposed.

Proposed redefinition of the genus *Cytophaga* Winogradsky

- Unicellular, Gram negative, flexible rods of low refractility, arranged singly, multiplying by binary transverse fission. Unbranched, unsheathed and not helical. Rods fusiform or round-ended, varying from short and regular to long, curved and filamentous; 0.2-1.5μ x 1.5-50μ.
- Aflagellate but motile by gliding, or by flexing if sufficiently long, on solid substrata. Slime layer present outside cell wall. Silky in liquid media when gently shaken. No microcysts or resting cells formed although degenerate coccoid forms may be present in old cultures.

Table 54

Differentiation of 'spreaders,' 'intermediates' and 'nonspreaders'

Property*	Organisms exhibiting the property		
	Spreaders	Intermediates	Nonspreaders
Fringe	All	Most	Few
W.s. pigment	Few	All	One only
Grow in 3% NaCl	None	One only	Many
Ferment glucose	Few	None	Few
Use alcohols	Few	One only	Many
Use starch	All except *C.hutchinsonii*	None	Many
Use alginate	None	Most	None
Haemolysis	Some, weak	Most, strong	Few, weak
NO_3 as N source	Most	Most	Few
Urea as N source	Some	Few	Most
Need vitamins	Many	One only	Most
H_2S produced	Most	Two only	Most
$NO_3 \rightarrow$ gas	Few	Most	None
Streptomycin Sens.	Some	None	Some
Good lytic activity	'Browns' only	All	Two only

*The characteristics referred to are these outlined in Tables 45-53.

Characteristic autolysis of older cells hence colonies become transparent on aging.

- Growth on agar plates may be in the form of a barely visible film or extend rapidly as a thin fringe from the central part of a colony especially in more restrictive media. On richer media more raised, compact colonies without fringes may be produced. Pigmented in the mass from pale to deep yellow, orange or salmon-pink to red (7.5-10 YR 6-7/8-12) ;olive-green and grey also reported. Carotenoid as far as is known.

- Nonphotosynthetic, do not deposit iron, nor do they produce sulphur granules in the presence of H_2S. Heterotrophic. Aerobic or facultatively anaerobic, some need NO_3 as an alternate electron acceptor when grown anaerobically. A few are obligate halophiles. Able to decompose one or more of the following polysaccharides: agar, alginate, cellulose, carboxy methyl cellulose, chitin. Most can use proteins as C and N source. Sensitive to Actinomycin D.

- Habitat: soil, freshwater and marine, two fish pathogens.

- DNA base ratio 29-45% G + C.

The members of the genus *Flexibacter* are further redefined (see page 215) as being unable to degrade any of the polysaccharides mentioned above (agar, alginate, cellulose, CMC and chitin), although starch hydrolysis is common and pectin may be utilized. Some other, non-exclusive differences between the two genera have been extracted from the literature (Tables 4 and 5 pages 31-54, Table 9 pages 83-88) and are presented in Table 55.

Table 55

Non-exclusive differences between *Flexibacter* and *Cytophaga*

Property	*Flexibacter*	*Cytophaga*
Cell length	3-500 μ	1.5-50 μ
Optimum temperature	Mostly 30-40°C	Mostly 20-30°C
Use glucose	Mostly -	Mostly +
Ferment sugars	-	±
If non-cellulolytic then use starch	±	+
NO_3 used as N source	Mostly -	Mostly +
Catalase	-	Mostly +

A new genus *Lysobacter* is being erected (see page 325) to encompass the 'Sorangiaceous non-fruiters' ('intermediate' group) studied here. A typical *Lysobacter* is a very mucoid, non-fruiting organism which sometimes produces a fringe and has a high GC ratio. It digests chitin, alginate and CMC, but not cellulose (filter paper) or starch, is highly proteolytic and strongly lytic against a variety of other micro-organisms. The genus is envisaged as a transition between *Cytophaga* and the fruiting Myxobacterales.

With these definitions of the genera *Cytophaga*, *Flexibacter* and *Lysobacter* in mind, following the currently held view of *Flavobacterium* as a Gram negative, peritrichate rod (42), the isolates studied here have been assigned to genera (Table 56). A taxonomic discussion of each of the isolates follows.

Table 56

Generic classification of isolates*

Organism	Gram neg.	Non-motile	Rods	V.thin-thin	Med.-long	Flexing	Silky	Fringe	Colour	Polymers	Act.D.	% GC	Genus
Spreaders													
ATCC 17061	+	+	+	+	+	+	+	+	+YR	+	+	33	*Cytophaga*
405	+	+	+	+	+	+	+	+	+	+	+	30-35	*Cytophaga*
Alfalfa	+	+	+	+	+	+	+	+	+	+	+		*Cytophaga*
Bryant	+	+	+	+	+	+	+	+	+R	+	+		*Cytophaga*
A 15	+	+	+	+	+	+	+	+	+D	+	+		*Cytophaga*
B-2-25	+	+	+	+	+	+	+	+	+	+	-		*Cytophaga*
E-1-25	+	+	+	+	+	+	+	+	+	+	+		*Cytophaga*
H_2O-1A	+	+	+	+	+	+	+	+	+	+	+		*Cytophaga*
1-10	+	+	+	+	+	+	+	+	+	+	+		*Cytophaga*
3	+	+	+	+	+	+	+	+	+	+	+		*Cytophaga*
3-19	+	+	+	+	+	+	+	+	+	+			*Cytophaga*
3-22	+	+	+	+	+	+	+	+	+	+			*Cytophaga*
4433	+	+	+	+	+	+	+	+	+	+	+		*Cytophaga*
4539	+	+	+	+	+	+	+	+	+	+	+		*Cytophaga*
4707	+	+	+	+	+	+	+	+	+	+	+		*Cytophaga*
NCIB 9059	+	+	+	+	+	+	+	+	+Y	+	+	32.9	*Cytophaga*
FS-1	+	+	+	+	+	+	+	+	+	+	+	48.7	*Cytophaga*

Conform (+) or do not conform (-) to the definition of *Cytophaga* given in the text (page 306).

V.thin-thin = <0.5 μ wide; Med.-long = >5μ long; Colour = within range 7.5-10YR 6-7/8-12 (+ or -), or if close then D = darker, l = less intense, R = redder, Y = yellower; Silky and Fringe + = observed at least once, - = never observed.
Polymers = degrade one or more of the polysaccharides cellulose, CMC, chitin or alginate.

Table 56 - continued

Generic classification of isolates

Organism	Gram neg.	Non-motile	Rods	V.thin-thin	Med.-long	Flexing	Silky	Fringe	Colour	Polymers	Act.D.	%GC	Genus
Stanier 6	+	+	+	+	+	+	-	+	+	+	+		*Cytophaga*
NCIB 10782	+	+	+	+	+	+	-	+	+	+	+	39	*Cytophaga*
2	+	+	+	+	+	+	+	+	DY	+	+		*Cytophaga*
D	+	+	+	+	+	+	+	+	DY	+	+		*Cytophaga*
6	+	+	+	+	+	+	+	+	DY	+	+		*Cytophaga*
4541	+	+	+	+	+	+	+	+	DY	+	+		*Cytophaga*
PC 15	+	+	+	-	-	-	+	+	-	+	+		*Myxococcus*
9-11	+	+	+	-	+	-	-	+	-	-			?
9D	+	+	+	+	+	+	+	+	-	-	+	37	*Flexibacter*
Intermediates													
495	+	+	+	+	+	+	+	+	-	+	+		*Lysobacter*
AL-1 (i)	+	+	+	+	+	+	+	+	-	+	+		*Lysobacter*
(ii)	+	+	+	+	+	+	+	+	-	+	+	69.2	*Lysobacter*
(iii)	+	+	+	+	+	+	+	+	-	+	+		*Lysobacter*
ATCC 21123	+	+	+	+	+	+	+	+	Y	+	+		*Lysobacter*
18 L Y	+	+	+	+	+	+	+	+	Y	+			*Lysobacter*
18L W	+	+	+	+	+	+	+	+	-	+			*Lysobacter*
4553 (i)	+	+	+	+	-	+	+	+	-	+	+		*Lysobacter*
4553 (ii)	+	+	+	+	-	+	+	+	Y	+	-		*Lysobacter*
4554	+	+	+	+	+	+	+	+	Y	+	+		*Lysobacter*
4555 (i)	+	+	+	+	-	-	+	-	-	+	+		*Lysobacter*
4555 (ii)	+	+	+	+	+	+	+	+	-	+	+		*Lysobacter*
4556 (i)	+	+	+	+	+	+	+	+	-	+	-		*Lysobacter*
4556 (ii)	+	+	+	+	-	-	+	-	-	+	-		*Lysobacter*
4557 (i)	+	+	+	+	+	+	+	+	-	+	-		*Lysobacter*
4557 (ii)	+	+	+	+	+	+	+	+	Y	+	-		*Lysobacter*

Table 56 - continued

Generic classificiation of isolates

Organism	Gram neg.	Non-motile	Rods	V.thin-thin	Med.-long	Flexing	Silky	Fringe	Colour	Polymers	Act.D.	%GC	Genus
4558 (i)	+	+	+	+	+	+	+	+	-	+	+		*Lysobacter*
4558 (ii)	+	+	+	+	-	-	+	+	+	+	+		*Lysobacter*
4559 (i)	+	+	+	+	+	+	+	+	-	+	+		*Lysobacter*
4559 (ii)	+	+	+	+	+	+	+	+	Y	+	+		*Lysobacter*
4560 (i)	+	+	+	+	+	+	+	+	Y	+	-		*Lysobacter*
4560 (ii)	+	+	+	+	+	+	+	+	-	+	+		*Lysobacter*
4560 (iii)	+	+	+	+	+	+	+	+	Y	+	+		*Lysobacter*
4561 (i)	+	+	+	+	-	+	+	+	-	+	+		*Lysobacter*
4561 (ii)	+	+	+	+	-	+	+	+	-	+	+		*Lysobacter*
4561 (iii)	+	+	+	+	+	+	+		Y	+			*Lysobacter*
4562 (i)	+	+	+	+	+	+	+	+	-	+	-		*Lysobacter*
4562 (ii)	+	+	+	+	+	+	+	+	Y	+	+		*Lysobacter*
4563 (i)	+	+	+	+	+	+	+	+	-	+	-		*Lysobacter*
4563 (ii)	+	+	+	+	+	+	+	+	-	+	-		*Lysobacter*
4564 (i)	+	+	+	+	+	+	+	+	-	+	+		*Lysobacter*
4564 (ii)	+	+	+	+	-	-	+	+	-	+	+		*Lysobacter*
4565 (i)	+	+	+	+	-	+	+	+	-	+	-		*Lysobacter*
4565 (ii)	+	+	+	+	+	+	+	+	Y	+	+		*Lysobacter*
13B	+	+	+	+	+	+	+	+	+	+	+		*Lysobacter*
3C	+	+	+	+	-	+	+	+	DI	+		62-69.2	*Lysobacter*

Table 56 - continued

Generic classification of isolates

Organism	Gram neg.	Non-motile	Rods	V.thin-thin	Med.-long	Flexing	Silky	Fringe	Colour	Polymers	Act.D.	%GC	Genus
Nonspreaders													
7-1	+	+	+	+	+	+	+	-	+	-			*? Flexibacter*
15D	+	+	+	-	+	+	+	-	+	+	+		*Cytophaga*
18H	+	+	+	-	+	+	+	+	+	-	+	36	*Cytophaga*
NCIB 8186	-	+	+	+	-	-	-	-	-	-	+		*?*
NCIB 8187	-	-	+	+	-	-	?+	-	-	-	+		*?*
NCIB 8188a	-	-	-	+	-	-	-	-	-	-	+		*?*
NCIB 8188 b	-	-	-	+	-	-	-	-	-	-	+		*?*
NCIB 8195	+	-	+	-	+	+	+	-	-	-	+		*?*
NCIB 8535	-	-	+	+	-	-	+	-	-	-	+		*?*
NCIB 8767	+	+	+	+	+	+	+	+	+	+	+		*Cytophaga*
NCIB 8771	+	+	+	+	+	+	+	+	+I	+	+		*?*
NCIB 8992	+	+	+	+	-	-	+	-	+	-	+		*?*
NCIB 9157 a	+	-	+	+	-	-	+	-	Y	+	+		*?*
NCIB 9157 b	+	-	-	+	+	-	-	-	Y	+	+		*?*
NCIB 9290	+	+	+	+	+	+	+	+	+	-	+	42.2	*Flexibacter*
NCIB 9324	±	-	+	+	-	-	+	-	-	-	+		*?*
RL8	+	-	+	-	-	-	+	-	-	-	+		*?*
NCIB 8185	-	-	+	+	-	-	+	-	-	+	+		*?*
NCIB 8204	+	+	-	+	-	-	-	-	-	-	+		*?*
5-9	+	+	+	-	+	+	+	-	-	+	+		*Myxococcus*
14	+	+	+	+	+	-	-	-	-	+	+		*? Vibrio*
402	±	+	+	+	-	-	+	-	-	+	+		*Lysobacter*

Cytophaga johnsonae

In the past confusion has existed over the definition of 'denitrification'. To some it has meant the reduction of nitrate to nitrite, and to others the reduction of nitrate to an unspecified gas. No consideration has been given to the fact that the second half of this process, that is the reduction of nitrite to a gas (usually nitrogen or nitrous oxide), may equally well be termed denitrification. It is now realized that many bacteria have the ability to reduce nitrate to nitrite, and that some also take the often toxic nitrite to a gaseous form. A few microbes are now known to reduce only nitrite and not nitrate, hence physiological symbioses in nature are possible. This study has shown that *C.johnsonae* ATCC 17061 is one of the latter organisms, possessing a nitrite but not a nitrate reductase. *C.johnsonae* var. *denitrificans* Cook 405 was confirmed as being capable of complete denitrification. Hence there is no alternative but to redefine *C.johnsonae* as being a denitrifier, with different strains possessing one or more of the different enzymes involved in this process. *C.johnsonae* var. *denitrificans* is then superfluous. The key characteristics of *C.johnsonae* as envisaged here are chitin degradation and denitrifying ability.

Strain ATCC 21123 has previously been classified as *C.johnsonae*, probably for want of anywhere else to put it as it is chitinoclastic and non-fruiting. It has been amply demonstrated in the present series of tests however, that this organism does not belong to this species. It is unlike ATCC 17061 and 405 in the

following respects; it is a cream, very mucoid and fairly compactly growing organism (Figure 25), with or without a small fringe (Figure 26), it prefers an aerobic atmosphere rather than one low in oxygen, it is only partially inhibited by 3% NaCl, does not degrade starch but does use alginate, shows excellent haemolysis and rapid lysis of milk, uses nitrate as an N source, does not need vitamins, is not affected by 0.01% S.L.S. and is very lytic to other micro-organisms. It is felt that it would be better placed in *Lysobacter enzymogenes* (see page 324).

The following isolates are being included in the species *C.johnsonae*. They all conform to the emended published descriptions (Table 4 pages 31-42 + denitrifying) as far as was determined here, except for the characteristics noted in parentheses.

ATCC 17061	(sucrose -, NO_3 not an N source)
405	(NO_3 not an N source)
Alfalfa	(mannitol +)
B-2-25	(pectin -)
E-1-25	(pectin -)
3	
4433	(sucrose -, pectin -)
4539	(pectin -)
4707	(pectin -)
NCIB 9059	formerly *Flavobacterium pectinovorum*

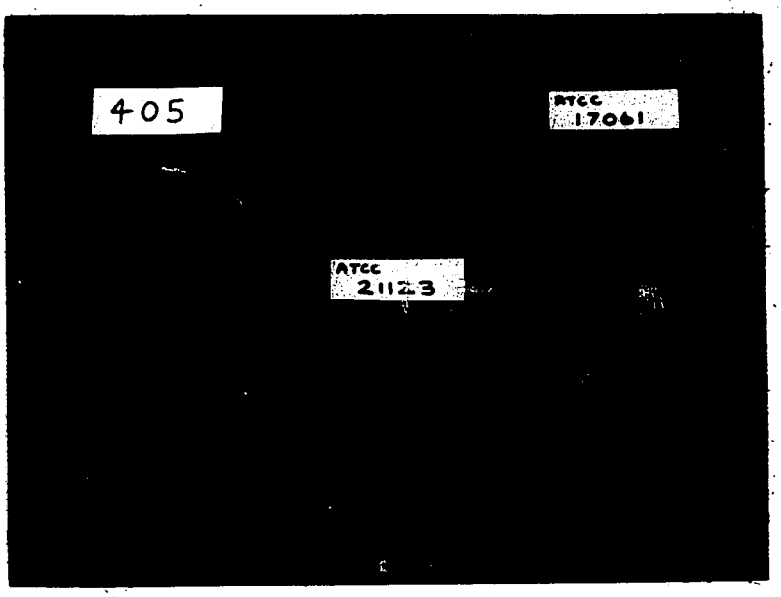


Figure 25. Comparison of growth form of 2 day *C.johnsonae* strains on Skim Acetate Agar:
405 (formerly var. *denitrificans*) code III C 53 βw, colour 10 YR 6/10
ATCC 17061 code III C 53 γ, colour 5 YR 5/10
ATCC 21123 (now *Lysobacter enzymogenes*)code IA1, colour 2.5 Y 7.5/6

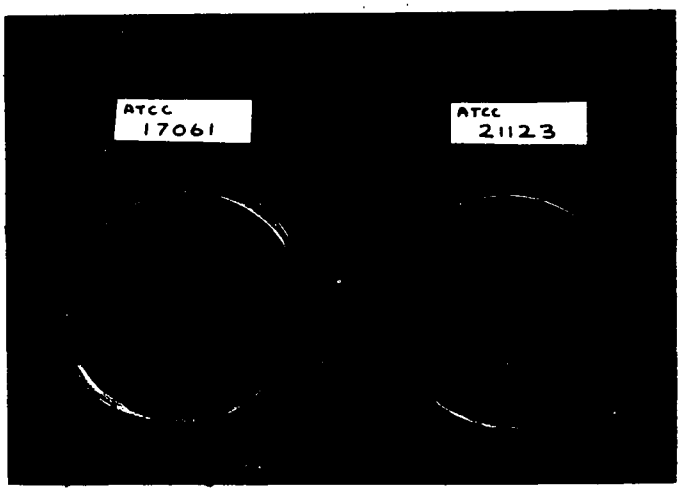


Figure 26. Comparison of growth form of 3 day *C.johnsonae* strains on Cook's Cytophaga Agar:
ATCC 17061 code III D 51 βfw, colour 1.5 Y 7/10,
ATCC 21123 (now *Lysobacter enzymogenes*) code IA1, colour 5 Y 7/6.

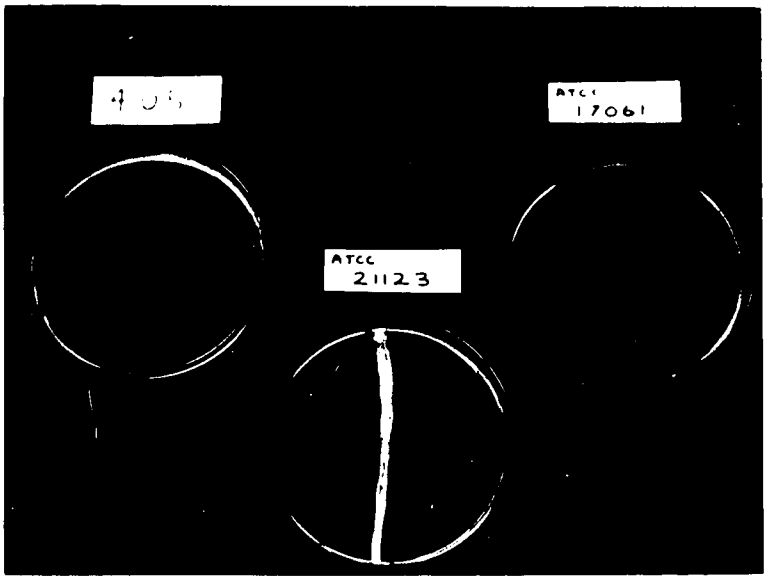


Figure 25. Comparison of growth form of 2 day *C.johnsonae* strains on Simm Acetate Agar:
405 (formerly var. *denitrificans*) code III C 53 Bw, colour 10 YR 6/10
ATCC 17061 code III C 53 Y, colour 5 YR 5/10
ATCC 21123 (now *Lysobacter enzymogenes*)code IA1, colour 2.5 Y 7.5/6

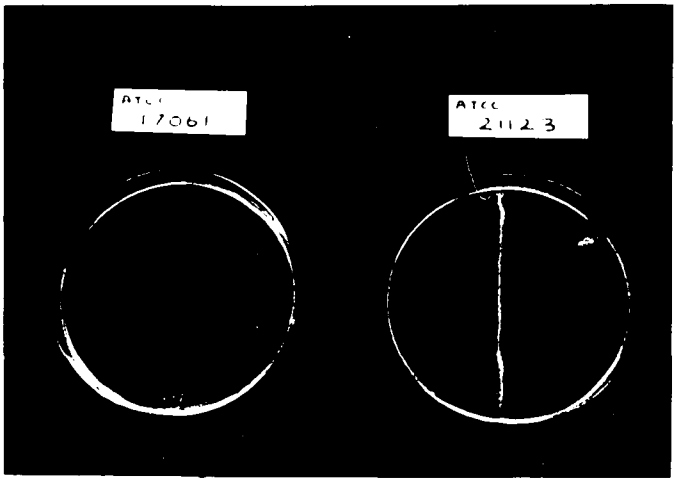


Figure 26. Comparison of growth form of 3 day *C.johnsonae* strains on Cook's Cytophaga Agar:
ATCC 17061 code III D 51 Bfw, colour 1.5 Y 7/10,
ATCC 21123 (now *Lysobacter enzymogenes*) code IA1, colour 5 Y 7/6.

The presence of the type species of *F.pectinovorum* in this list confirms the feelings of several workers that this organism would more properly be classified as a *Cytophaga*. The GC ratio of this isolate is 32.9, which is within the range for *Cytophaga* and its morphological similarity to other isolates of *C.johnsonae* can be seen in Figure 27.

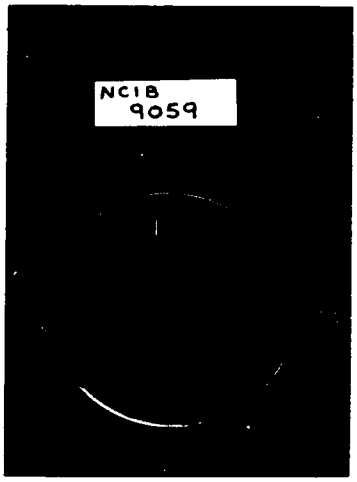


Figure 27. 3 day Skim Acetate culture of NCIB 9059 (formerly *Flavobacterium pectinovorum* and now proposed as synonymous with *C.johnsonae*). Code III E 53 α, colour 5 YR 5/10.

There are seven spreading, yellow-orange *Cytophagae* which do not belong in *C.johnsonae* as redefined here. The arctic isolates 3-19 and 3-22 do not degrade chitin, which is considered a key characteristic because of its historical association with *C.johnsonae*. In addition they do not use nitrate as a nitrogen source, they do not liquefy gelatin and are catalase negative. 3-22 also has a much lower optimum temperature (Table 46). There are five further isolates which do not denitrify even though otherwise similar to *C.johnsonae*. These, and their other atypical characteristics, are

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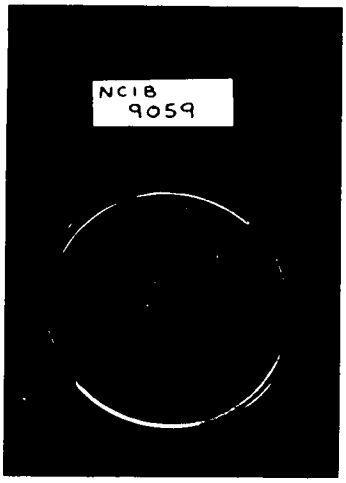

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FS-1	(formerly *Flexibacter* sp.)
1-10	(mannitol +)
Bryant	(max. 40°C, catalase -)
A15	(low opt. temp., lactose -, sucrose -, pectin -, catalase -)
H_2O-1A	(lactose -, sucrose -, pectin -, catalase -).

The isolate FS-1 was placed in the genus *Flexibacter* when it was initially found not to degrade complex polysaccharides (350). Chitin was not tested by Simon and White, and in the present study chitinolysis has been demonstrated, hence the change in genus. FS-1 did not grow in the nitrate medium employed here and if denitrification can be demonstrated in another medium then this isolate will belong in *C.johnsonae*. The GC ratio is high (48.7) for *Cytophaga*, however, and fits better into the *Flexibacter* range.

None of the above seven organisms seem to be identifiable as *C.succinicans*, and there is too little known about *C.albogilva* and *C.deprimata*, the only other non-cellulolytic nonmarine types described. for valid comparisons to be made. At present it is proposed not to create new species for them but to leave them as *Cytophaga* spp. until more is known about them.

Cytophaga hutchinsonii

The two isolates 'Stanier 6' and NCIB 10782, which were both derived from Dr. R. Y. Stanier's laboratory were very slightly different from the published descriptions of this species (Table 4 pages 31-42). Silkiness in liquid culture was not observed, isolate

Stanier 6 used mannitol, neither used nitrate as an N source, and they were both catalase negative. The isolates were identical in all respects in the present series of tests, with the exceptions of mannitol, the probable use of urea and production of phosphatase by 10782, and in their reactions to polymyxin B (Table 52). A certain amount of new information is presented about *C.hutchinsonii* and this is summarized as follows,

NaCl inhibition, partial 1%, complete 2%	
Sucrose -	Lactose -
Glycerol -	Mannitol ±
CMC +	Chitin -
Gelase +	Pectate -
Alginate -	H_2S -
Oxidase +	Indole -

Cannot use amino acids (glutamate or asparaginate) or proteins (gelatin, milk, tryptone, peptone, blood, casein casamino acids, casitone) as C and N sources.

Chloramphenicol, sensitive to 30 µg disc.

Dihydrostreptomycin, sensitive or intermediate to 10 µg discs.

Penicillin G, intermediate to 10 unit discs.

Actinomycin D, very highly sensitive (more than *B.subtilis*).

No lytic action on various micro-organisms.

Cytophaga brunescens n.sp.
(etym. M.L. becoming brown).

The four isolates constituting this new species are 2, D, 6 and 4541 (the 'browns'). They produce yellow colonies which almost

invariably turn darker and browner due to the release of a water soluble pigment which diffuses well in agar media (see Tables 30-32 pages 168-170, page 172, Figures 28 and 29). The surface layers of liquid cultures too become brown after a couple of days. The temperature range is higher than for any presently described *Cytophaga*, about 15° to 40°C, with an optimum between 35° and 40°C. The group does not use cellobiose, sucrose, lactose, glycerol, mannitol, cellulose, CMC, agar, pectate or alginate but good growth is obtained with glucose, starch, chitin or proteins. They are strongly proteolytic but no haemolysis is demonstrated. They are strict aerobes which do not ferment glucose, denitrify or use NO_3 as an alternate electron acceptor. In addition they are strongly lytic on Gram negative and Gram positive bacteria, actinomycetes, fungi and algae.

A marine *Cytophaga* which produces a brown water soluble pigment is already known, but *C.krzemieniewskae* is a pink organism which differs in various other respects too. It is an obligate halophile requiring at least 1.5% NaCl, while *C.brunescens* is strongly inhibited by this concentration. *C.krzemieniewskae* has a lower temperature range and degrades cellobiose, lactose, cellulose, agar and alginate but not chitin. It is also H_2S negative and reduces nitrate to nitrite. There can thus be no doubt that *C.brunescens* is a valid new species.

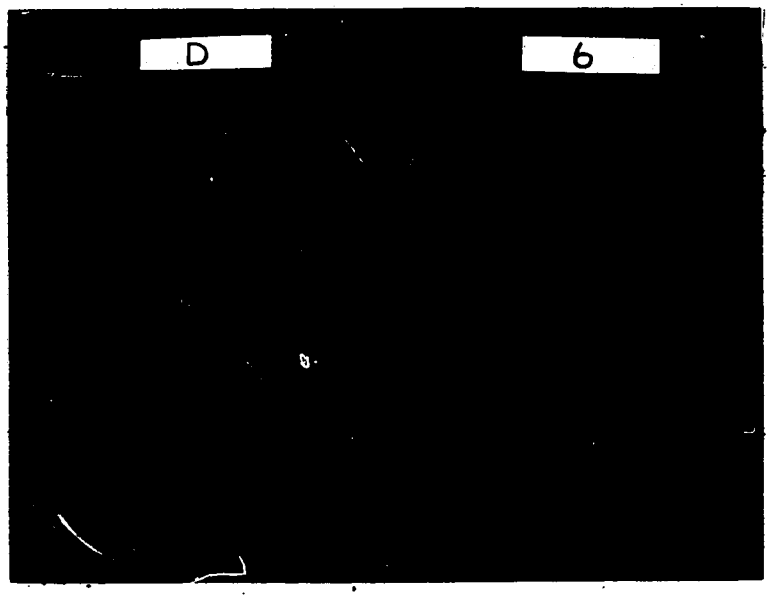

Figure 28. *C.brunescens*. 3 day cultures of D and 6 on Skim Acetate Agar (upper) and Cook's Cytophaga Agar (lower plates). Codes and colours:
D (SA) III B 73 γe 10 YR 4/6 , (CC) III C 53β 2.5Y 4/6.
6 (SA) II A 73 γe 2.5 Y 4.5/6 , (CC) III C 53β 5Y 3/4 .

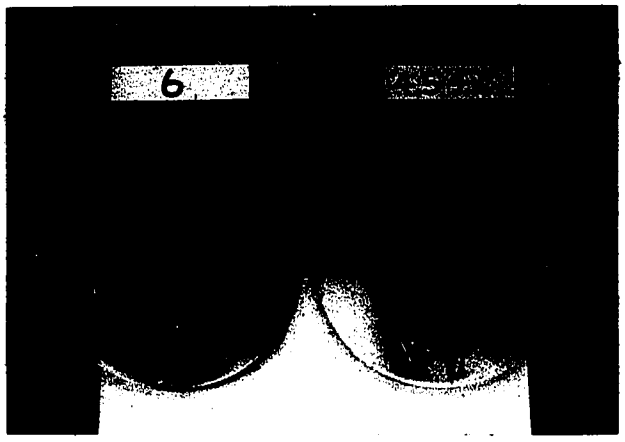

Figure 29. *C.brunescens*. 15 day cultures of 6 and 4541 on Skim Acetate Agar. Codes and colours:
6 II H 58 α b 2.5 Y 4/6, 4541 II G 58 αb 10 YR 4/6.

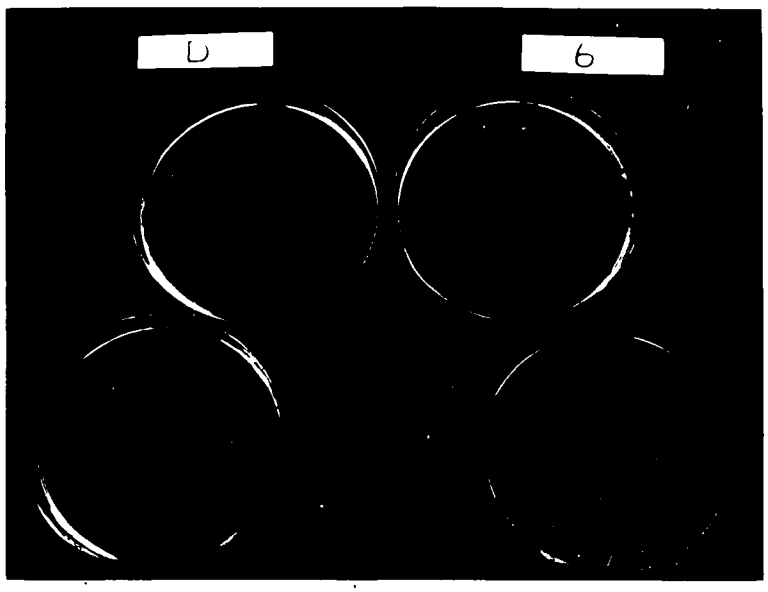

Figure 28. *C.bruneoccens*. 3 day cultures of D and 6 on Skim Acetate Agar (upper) and Cook's Cytophaga Agar (lower plates). Codes and colours:
D (SA) III B 73 ye 10 YR 4/6 , (CC) III C 538 2.5Y 4/6.
6 (SA) II A 73 ye 2.5 Y 4.5/6 , (CC) III C 538 5Y 3/4 .

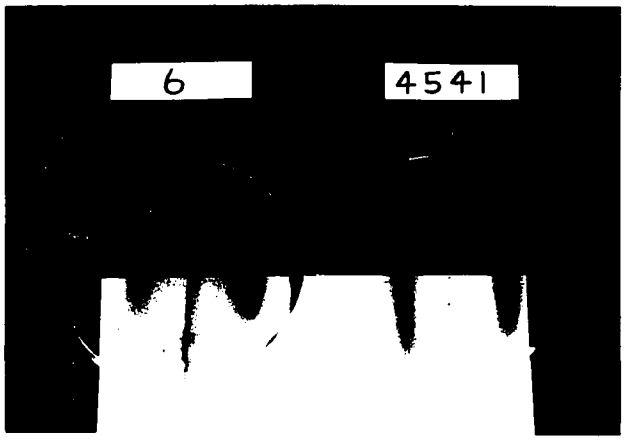

Figure 29. *C.bruneoccens*. 15 day cultures of 6 and 4541 on Skim Acetate Agar. Codes and colours:
6 II H 58 a b 2.5 Y 4/6, 4541 II G 58 ab 10 YR 4/6.

Spreading pink organisms

Isolate PC 15 has now been found to produce fruiting bodies typical of the genus *Myxococcus* (see page 301), probably *M.fulvus* or *M.cruentus*. As far as can be determined the other properties of this organism agree with the generic definition of *Myxococcus*.

The assignation of 9-11 is problematical. It has not yet produced fruiting bodies and does not degrade any polysaccharide except starch, thus it is excluded from the fruiting Myxobacterales and *Cytophaga*. It bears no resemblance to *Lysobacter*, and its similarity to *Flexibacter* is complicated by the fact that flexing has not yet been observed, although it does produce a fringe. There are four red and pink species of *Flexibacter*, *F.litoralis* is an obligate halophile and 9-11 seems to differ from *F.giganteus*, *F.roseolus* and *F.ruber* in several respects. Until further work is done with this organism and with other 'spreading pinks', it will be left unclassified.

Flexibacter canadensis n.sp.
(etym. M.L. native or resident in Canada).

The white, iridescent spreading organism 9D has very long, thin, gracefully flexing cells and does not degrade any polysaccharides other than starch and pectate. It has therefore been placed in the genus *Flexibacter*. Two creamy-white species, *F.albuminosus* and *F.aureus* are known, but both have short cells and are mucilaginous with a small fringe. 9D is also the first facultatively anaerobic *Flexibacter* species to be described. Isolate 9D was isolated from soil

on the Central Experimental Farm, Ottawa, Ontario (Table 44) and hence receives the specific epithet *canadensis*.

Flexibacter canadensis n.sp. is a remarkably good spreader, and its very long, thin, elegant cells (0.3 x 3-60 µ), exhibit a classic flexing motility. It is white and rather rhizoid or finger-like in its appearance (9D, Figure 30), and shows different iridescent colours on different media. Its colour range is 10 YR 7-8/2 and 2.5 Y 7-8/2 (page 172). Temperature range is 10-40°C, and growth is best between pH 6 and 8. Growth is better at a reduced oxygen level, and fermentation of sugars occurs. NO_3 is used as an alternate electron acceptor anaerobically and both nitrate and nitrite are reduced to a gas, probably N_2O. Uses glucose, cellobiose, lactose, glycerol, starch and pectate but not sucrose, mannitol, cellulose (filter paper), CMC, chitin, agar or alginate. Proteolytic, lyses milk, but not haemolytic. Proteins and single amino acids used as C and N sources. Uses NH_4 but not NO_3 or urea as N source. Yeast extract usually required as vitamin source in synthetic media. H_2S, catalase, oxidase and phosphatase positive, indole negative. Completely inhibited by 0.1% S.L.S., sensitive to 30 µg chloramphenicol, 10 µg dihydrostreptomycin and actinomycin D. Not affected by 10 units of penicillin G or 0.1% nitrite. No lytic ability on other micro-organisms. GC ratio of strain 9D is 37%.

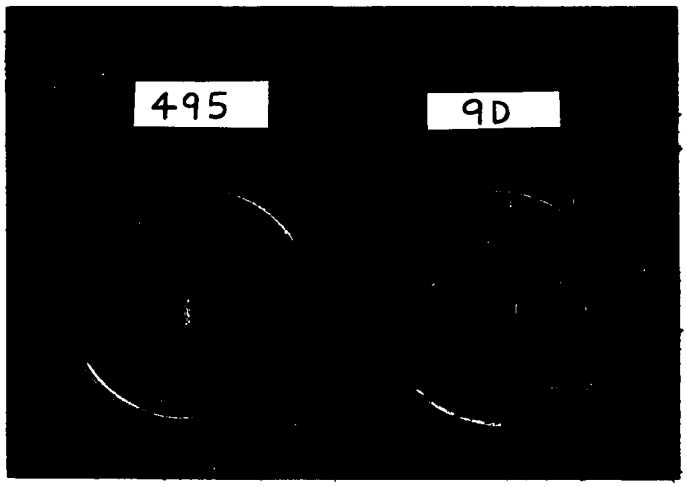

Figure 30. 3 day cultures on Cook's Cytophaga Agar. *Lysobacter enzymogenes* strain 495, code IA1 colour 2.5 Y 6/6. *Flexibacter canadensis* strain 9D, code II D 53 e colour 2.5 YR 8/2.

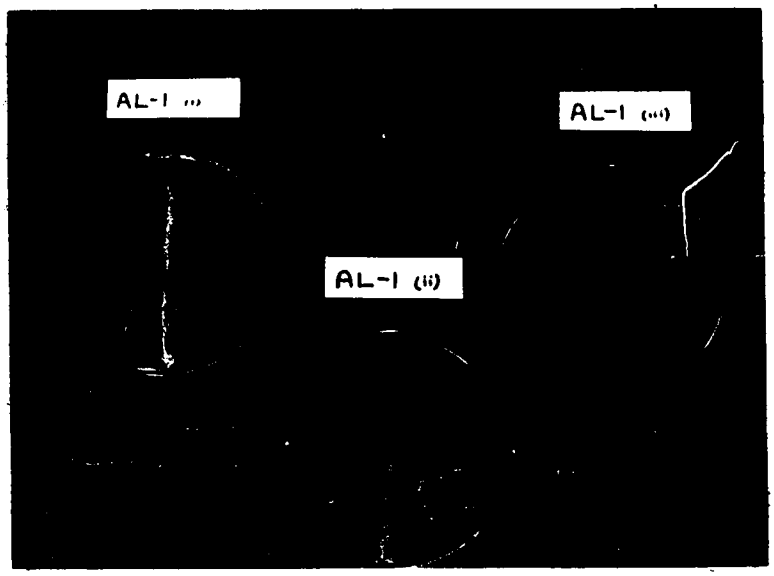

Figure 31. 3 cultural variants of strain A1-1 *Lysobacter enzymogenes* after 2 days growth on Skim Acetate Agar. Codes and colours:

(i)	IB1	5 Y 7.5/4
(ii)	IB1	5 Y 7.5/4
(iii)	II D 62 β	5 Y 8/4

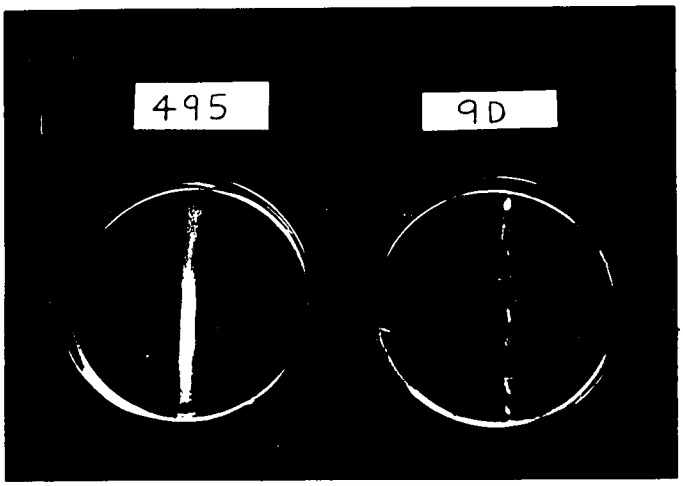

Figure 30. 3 day cultures on Cook's Cytophaga Agar. *Lysobacter enzymogenes* strain 495, code IA1 colour 2.5 Y 6/6. *Flexibacter canadensis* strain 9D, code II D 53 e colour 2.5 YR 8/2.

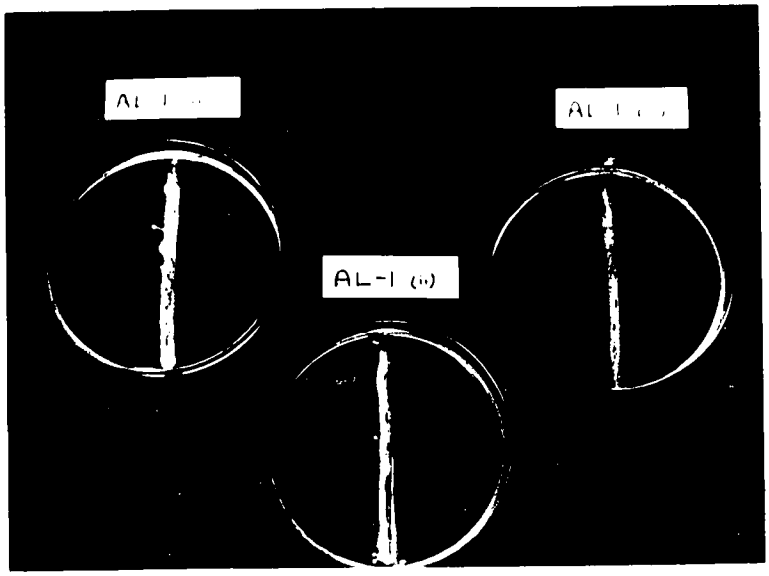

Figure 31. 3 cultural variants of strain Al-1 *Lysobacter enzymogenes* after 2 days growth on Skim Acetate Agar. Codes and colours:

(i) IB1 5 Y 7.5/4
(ii) IB1 5 Y 7.5/4
(iii) II D 62 β 5 Y 8/4

Lysobacter n.gen.
(etym. M.L. the lysing bacterium)

This new genus is designed to encompass the non-fruiting, 'Sorangiaceous', highly mucoid, brownish-cream, creamy-white, white or salmon-pink organisms which sometimes produce a fringe (the 'intermediates') and which have a high GC ratio circa 62-69%. They have fairly thin, medium length cells, 0.3-0.5 x 1-9μ, occasionally a length of up to 30 μ is attained, and flexing is generally observed. They have a relatively high resistance to salinity, although they are not of marine origin they are inhibited only by 3% or more NaCl. Their attack on glucose is oxidative, and most produce a brownish water soluble pigment in older cultures. The three species all use glucose, cellobiose, CMC and chitin, but not agar, many isolates use sucrose and lactose and one species uses starch and another alginate. Mannitol is attacked by only one strain, pectin by a few, but glycerol and cellulose (filter paper) are not decomposed. They are strongly proteolytic and haemolytic and growth factors are not usually required in synthetic media. Most use NO_3 and NH_4 but not urea as nitrogen sources, and most of the isolates of two species denitrify nitrate and many also reduce NO_2. Typically the genus is indole and H_2S negative and catalase, oxidase and phosphatase positive. Generally resistant to 0.01% SLS but most are completely inhibited by 0.1% S.L.S. Resistant to 10 μg dihydrostreptomycin and to 10 units of penicillin G. Most strains are sensitive to actinomycin D and not affected by 0.1% nitrite. The members of the genus *Lysobacter* characteristically lyse a variety of micro-organisms including bacteria,

actinomycetes, fungi and algae. Gram negative bacteria are less often attacked than Gram positive ones. The genus is a transitional one between *Cytophaga* and the fruiting myxobacters, and is placed in the Order Myxobacterales. The type species is *L.enzymogenes*.

The isolates included in *Lysobacter* are the '495 group' and 13B *(Lysobacter enzymogenes)*, 3C *(Lysobacter antibioticus)* and 402 *(Lysobacter gummosus)*. The previously mentioned creamy-white, mucoid *Flexibacter albuminosus* and *F.aureus* may also belong to *Lysobacter*, however neither their use of CMC, chitin and alginate nor their lytic abilities were tested by Soriano, and no extant cultures exist, thus it is impossible to assess them further. Their position in the genus *Flexibacter* is also tenuous since this genus is defined largely on its inability to attack polysaccharides. The fact that Gram negative bacteria are more resistant to attack by *Lysobacter* species than are Gram positive bacteria suggests that the composition of the Gram negative cell wall is the factor preventing autolysis of *Lysobacter* cells by thier own powerful lytic enzymes.

Lysobacter enzymogenes n.sp. Type species.
(etym. M.L. enzyme-producing).

Description as for genus with the following additions. Creamy-white often with a pale yellow-brown, nonspreading, less mucoid colonial variant (Figures 30-32). Colour range 6-10 YR 5.5-7/5-8 and 2.5-7.5 Y 6-8/2-6 (page 172). Most strains have been seen to produce a fringe. The cells are thin, medium length rods mostly 0.3-0.5 x 1-9 μ and flexing, occasionally longer.

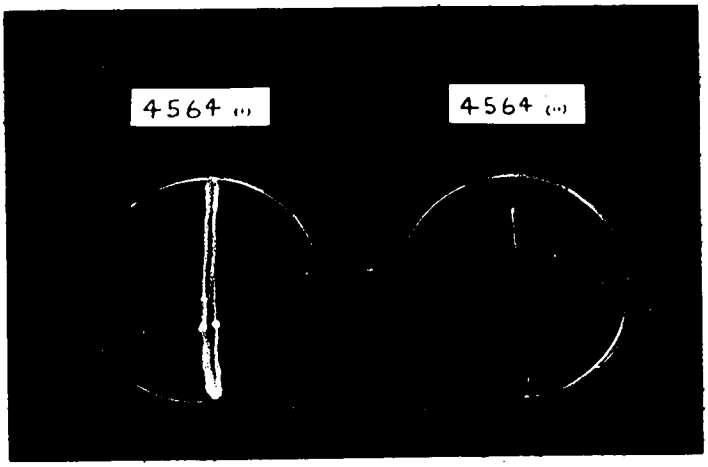

Figure 32. *Lysobacter enzymogenes* strain 4564, two cultural variants after 3 days growth on Cook's Cytophaga Agar. Codes and colours:
(i) IA1 5 Y 7.5/6
(ii) IB1 2.5 Y 6.5/6

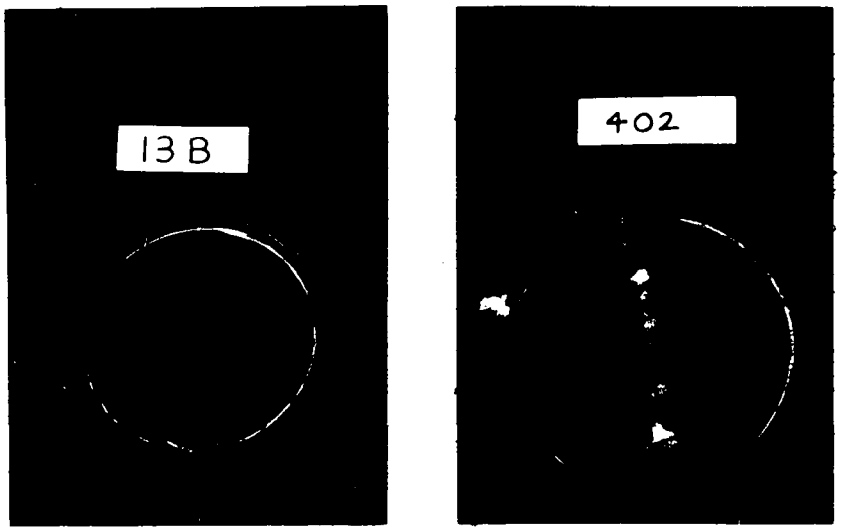

Figure 33. Cultures on Skim Acetate Agar.
Lysobacter enzymogenes var. *cookii* strain 13B (2 days), code IA1 colour 7.5 YR 6/6. *Lysobacter gummosus* strain 402 (6 days), code IE1 pp colour 5 Y 8/2.

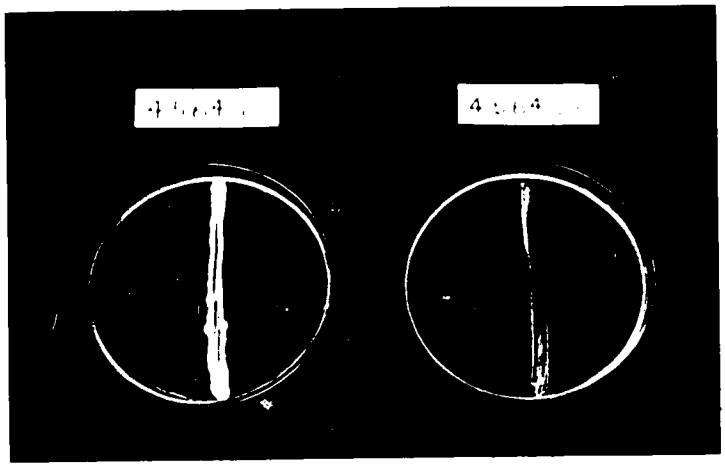

Figure 32. *Lysobacter enzymogenes* strain 4564, two cultural variants after 3 days growth on Cook's Cytophaga Agar. Codes and colours:
(i) IA1 5 Y 7.5/6
(ii) IB1 2.5 Y 6.5/6

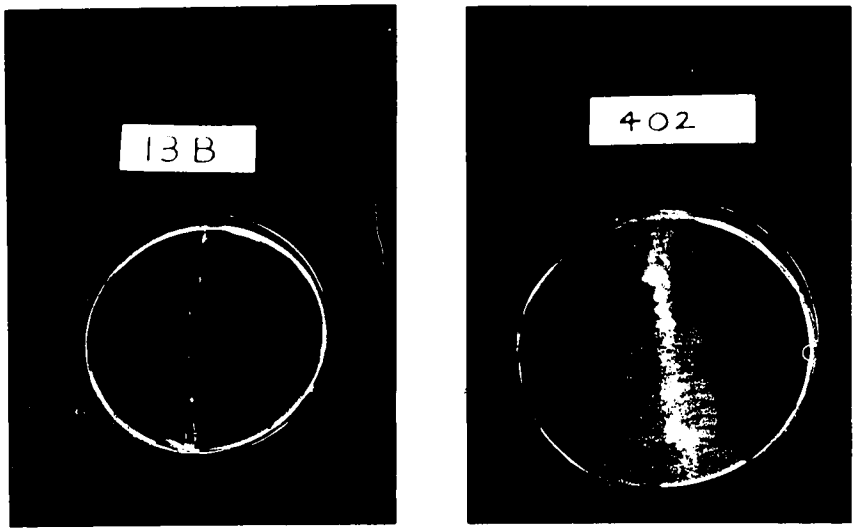

Figure 33. Cultures on Skim Acetate Agar.
Lysobacter enzymogenes var. *cookii* strain 13B (2 days), code IAi colour 7.5 YR 6/6. *Lysobacter gummosus* strain 402 (6 days), code IE1 pp colour 5 Y 8/2.

One strain (AL-1) grows but shows no pH change on Board and Holding's glucose medium and also does not use sucrose. One strain only (18LY) uses mannitol. All strains degrade CMC and chitin and most liquefy alginate. A few are pectolytic but none use starch. Most are strongly haemolytic, and will use NO_3 and NH_4 but not urea as N sources. Yeast extract is not required. H_2S is produced by two strains (495, 21123), and all produce catalase, oxidase and phosphatase but not indole. The isolates 4553 to 4565 inclusive denitrify NO_3 and NO_2 to $?N_2O$, 21123 reduces NO_3 only. Nitrate is used as an alternate electron acceptor anaerobically by five strains. Most are sensitive to Actinomycin D, and one strain (495) is sensitive to 0.1% nitrite. They attack Gram negative and Gram positive bacteria, actinomycetes, fungi, green and blue-green algae and nematodes (Table 42). GC ratio of AL-1 is 69.2.

The isolates belonging to this species are 495, AL-1, ATCC 21123 (formerly referred to as *C.johnsonae* see page 310), 18L and 4553 to 4565 inclusive, with their cultural variants. The lytic enzymes produced by strains 495 and AL-1 have been discussed on pages 224 and 238.

Lysobacter enzymogenes var. *cookii* n.var.

(etym. M.L. named for Dr. F. D. Cook, the microbiologist who first isolated the three species of this genus, and who recognized their lytic and antibiotic potential.)

Description as for *L.enzymogenes* with the following exceptions. Offwhite to deep brownish-cream, mucoid organism (Figure 33), with a colour range 7.5-10 YR 6-7/6-8 and 2.5 Y 6/6. Often produces a fringe

and produces much water soluble brownish pigment especially in older cultures. Cell dimensions 0.5 x 3-13 μ and flexing. Uses glucose, cellobiose, sucrose and lactose but not glycerol or mannitol. Hydrolyses potato starch and liquefies pectate but not alginate. Uses NH_4 and urea but not NO_3 as N sources, and growth factors are required in salts + glucose medium. Does not denitrify or use NO_3 as an anaerobic electron acceptor. Does not produce H_2S from cysteine. Sensitive to 30 μg discs of chloramphenicol, to actinomycin D and to 0.1% nitrite. Does not lyse Gram negative bacteria. A single isolate is known as yet, 13B. This variety is the member of the genus *Lysobacter* which seems closest to the genus *Cytophaga*.

Lysobacter antibioticus n.sp.
(etym. Gr. pref. anti = against; Gr. noun bius = life; M.L. adj. antibioticus = against life, antibiotic.)

Description as for genus with the following additions. Pink to salmon-pink, mucoid organism (Figure 21 page 191) with the colour range 7.5-10 YR 2.5-4/3-6 often with considerable brown water soluble pigment, and often with a fringe. Cells are 0.4 μ wide by 1-2.5 μ long and have been observed to flex. Uses cellobiose and glucose but not sucrose, lactose, glycerol or mannitol. Degrades CMC and chitin but not cellulose (filter paper), starch, agar, pectate or alginate. Can use NO_3, NH_4 and urea as a nitrogen source, and yeast extract is not required in synthetic media. The species is able to denitrify nitrate but not nitrite, and uses NO_3 as an alternate electron acceptor. Catalase, oxidase and phosphatase positive, indole and H_2S negative. Resistant to 0.01 % SLS but completely inhibited by

0.1%, resistant to 30 µg discs of chloramphenicol and to 0.1% nitrite. Shows lytic activity against Gram negative and Gram positive bacteria, actinomycetes, fungi and green and blue-green algae (Table 42). GC ratio of the only strain known, 3C, is 62-69.2%. This culture has been intensely studied because of its production of the potent wide-spectrum antibiotic 'Myxin' (see pages 233-234,238).

Lysobacter gummosus n.sp.

(etym. L.n.gummi = gum, L.suf. -osus = full of, abounding in. L.adj. gummosus = gummy, full of gum.)

Description as for genus with the following additions. White, intensely gummy organism (Figure 33) with the colour range 5 Y 7-8/2-4. No water soluble pigment or fringe produced. The thick, gelatinous colonies of this organism are extremely difficult to work with and almost rubbery in consistence. Cell dimensions 0.4 x 0.5-2 µ and flexing has not been observed, presumably it is inhibited in such a gummy matrix. The species uses glucose and lactose, and also cellobiose and sucrose rather slowly, but it does not use glycerol or mannitol. CMC and chitin are degraded but not cellulose (filter paper), starch, agar, pectate or alginate. NO_3, NH_4 and urea can all be used as a nitrogen source and growth factors are not required. Denitrification does not take place, neither is NO_3 used as an alternate electron acceptor anaerobically. Oxidase and phosphatase are produced but not H_2S, catalase or indole, (the gum could interfere with the catalase reaction however). Growth is merely reduced but not completely inhibited by 0.1% S.L.S., resistant to 30 µg discs of chloramphenicol and to 0.1% nitrite, but sensitive

to actinomycin D. Does not lyse Gram negative bacteria.

It is interesting to note that other algicidal nonfruiting myxobacters have been described by Daft and Stewart (88), Napier (279) and Shilo (347). These have not been examined by the author but their GC ratios are also high, being 68.9, 73.2 and 70 respectively, and it would not be surprising if these too were *Lysobacter* species.

Cytophaga compacta n.sp.

(etym. L. adj. compact, in the sense of nonspreading).

The isolates 15D and 18H comprise this first nonspreading *Cytophaga* species. Other nonspreaders have been assigned to this genus (14, 93, 373, 408), but no species have yet been erected to encompass any of these. A considerable amount is now known about the closely related isolates 15D and 18H, and they are known to be fairly closely allied to *C.johnsonae* (Chapter III). A fringe was seen in 18H once on PMYA II medium but has not been observed in 15D. Also 15D degrades chitin and pectate, and so far 18H is only known to degrade pectate. The GC ratio of 18H is 36, which is within the *Cytophaga* range. Taking all these characteristics into account it is felt that the best solution is to set up a new, nonspreading species of *Cytophaga* for them.

Description as for genus with the following exceptions. The cells are long, thick, flexible rods 0.6-0.7 x 1.75 μ. Autolysis of older cells not apparent, fringe and characteristic spreading is not typically observed. Yellow-orange colonies (Figure 34) which have a colour range 5-7.5 YR 5-6/10-12 (see Tables 30-31, pages 168-169).

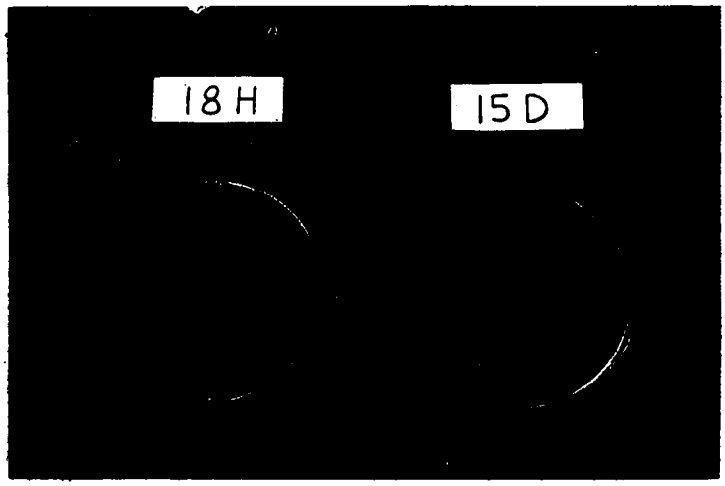

Figure 34. *Cytophaga compacta*. 2 day Skim Acetate cultures. Codes and colours:

18 H	IA1	5 YR 5/12
15 D	IA1	5 YR 5.5/10

Temperature range 8-35°C, optimum 25-30°C; pH range 5-10; partially inhibited by 1% NaCl, completely by 3% NaCl. Utilize glucose but no pH change is observed on Board and Holding's medium. Do not utilize cellobiose, sucrose, lactose, glycerol or mannitol, CMC or cellulose (filter paper), agar or alginate. Decompose starch, chitin (1/2 isolates) and pectate. Proteolytic, lyse milk rapidly and haemolytic; and proteins, glutamate and asparaginate can be used as C and N sources. NH_4 and NO_3 (1/2 isolates), but not urea used as N sources. Vitamins usually required. Nitrite but not nitrate is denitrified, and

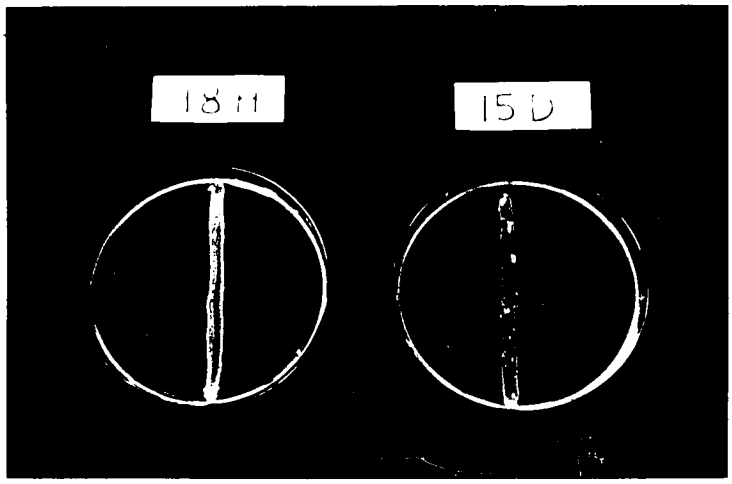

Figure 34. *Cytophaga compacta*. 2 day Skim Acetate cultures. Codes and colours:

18 H	IA1	5 YR 5/12
15 D	IA1	5 YR 5.5/10

Temperature range 8-35°C, optimum 25-30°C; pH range 5-10; partially inhibited by 1% NaCl, completely by 3% NaCl. Utilize glucose but no pH change is observed on Board and Holding's medium. Do not utilize cellobiose, sucrose, lactose, glycerol or mannitol, CMC or cellulose (filter paper), agar or alginate. Decompose starch, chitin (1/2 isolates) and pectate. Proteolytic, lyse milk rapidly and haemolytic; and proteins, glutamate and asparaginate can be used as C and N sources. NH_4 and NO_3 (1/2 isolates), but not urea used as N sources. Vitamins usually required. Nitrite but not nitrate is denitrified, and

NO_3 is not used as an alternate electron acceptor. *C.compacta* produces H_2S from cysteine, catalase, oxidase, phosphatase and indole. Sensitive to about 0.01% S.L.S. and completely inhibited by 0.1% S.L.S. Resistant to 30 µg chloramphenicol, 10 µg dihydrostreptomycin and 10 unit penicillin G discs and to 0.1% nitrite. Strain 18H is lytic to Gram positive bacteria, an actinomycete and fungi, and 15D is known to lyse nematodes (Table 41). The GC ratio of strain 18H is 36%.

Other yellow nonspreading organisms.

Isolate 7-1 is a flexing 0.3 x 1-30 µ rod which is silky in liquid culture but does not attack any of the prescribed polysaccharides. It is thus not a *Cytophaga*. It is unlike most of the yellow or orange species of *Flexibacter* in several respects, but it only differs in two characters (resistance to 3% NaCl and CMC degradation) from *F.sancti* (which should be classified as a *Cytophaga*, see page 220), and likewise in only two respects (resistance to 3% NaCl and use of glycerol) from *F.flexilis*. Until it can be compared with further isolates of *Flexibacter* species, it will remain without a specific epithet, and hence is a *Flexibacter* sp.

The *Flavobacteria*:*F.arborescens* NCIB 8185, *F.esteroaromaticum* NCIB 8186, *F.flavescens* NCIB 8187, *F.suaveolans* NCIB 8188, *F.aquatile* NCIB 8535 and *F.lucecoloratum* NCIB 9324 were found to be Gram positive and hence by definition must now be excluded from this genus. In the case of *F.aquatile* either the original identification of this strain was erroneous, or a contaminant has been introduced

in subculturing, because strain 8535 here bears no resemblance to *Cytophaga* and it has been argued most convincingly (see pages 65-66) that other strains of this species do indeed belong to *Cytophaga*.

Flavobacterium devorans NCIB 8195 and *F.rhenanum* NCIB 9157 are Gram negative and motile, hence they cannot be transferred to either *Cytophaga* or *Flexibacter*. The reddish organism sent as *Cytophaga succinicans* is likewise motile and its other characteristics (size, shape, nonspreading, no polysaccharides attacked, etc.) also indicate that the original *C.succinicans* had died and that this was a contaminant. This is a pity because it is one of the few type cultures of the genus available. Further efforts will be made to obtain an authentic specimen.

Flavobacterium resinovorum NCIB 8767 has been identified as a *Cytophaga* as a result of this study. A fringe has been observed once only but the dimensions (0.4 x 1-15 μ) and flexibility of the cell, as well as its silkiness and chitinolysis and other characteristics lead to this conclusion. In plate culture it resembles the isolates 15D and 18H (*Cytophaga compacta)* but it is very different from them metabolically. It seems to be more similar to the published description of *C.succinicans*, but its nomenclature will now have to wait until it can be compared with an isolate of this species. ATCC 12524 is the type species of *F.resinovorum*, so that this transference of NCIB 8767 to *Cytophaga* does not affect the status of this species.

F.suaveolans NCIB 8992, unlike strain NCIB 8188 is Gram negative. The species is described as Gram variable and motile (42) but strain 8992 was not observed to be motile here. Furthermore,

it is a short, non-flexing bacterium which does not spread or degrade polysaccharides. Hence it could not be reclassified as a *Cytophaga* or a *Flexibacter*.

F.proteus NCIB 8771 is a refractile,non-flexing,very short rod which however has been seen to produce a fringe once and does degrade CMC. It does not fit the definition of either *Cytophaga* or *Flexibacter*.

F.heparinum has been suggested as being a *Cytophaga* (see page 66 and Table 7 pages 67-72) and the type culture has been studied here (Figure 35).

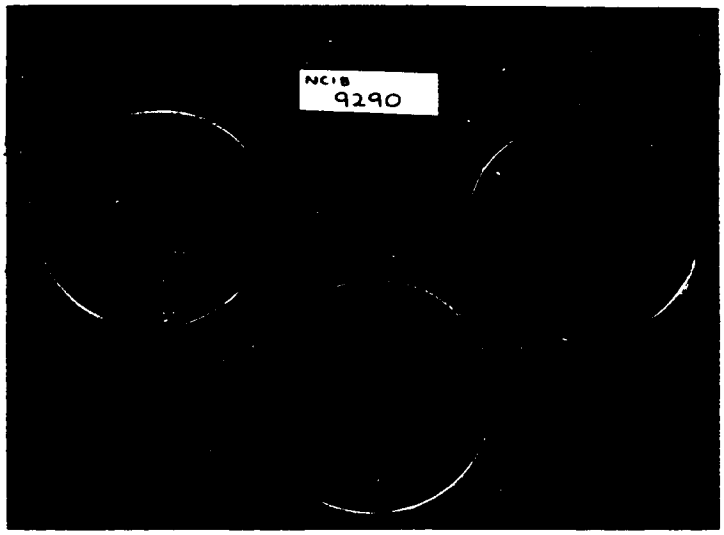

Figure 35. *Flexibacter heparinus* (formerly *Flavobacterium heparinum*) NCIB 9290. 2 day cultures. Codes and colours on Plate Count (left) IA1 10 YR 6/4, Skim Acetate (centre) IB1 10 YR 7/4, and Cook's Cytophaga Agar (right) IB1 2.5 Y 7/4.

it is a short, non-flexing bacterium which does not spread or degrade polysaccharides. Hence it could not be reclassified as a *Cytophaga* or a *Flexibacter*.

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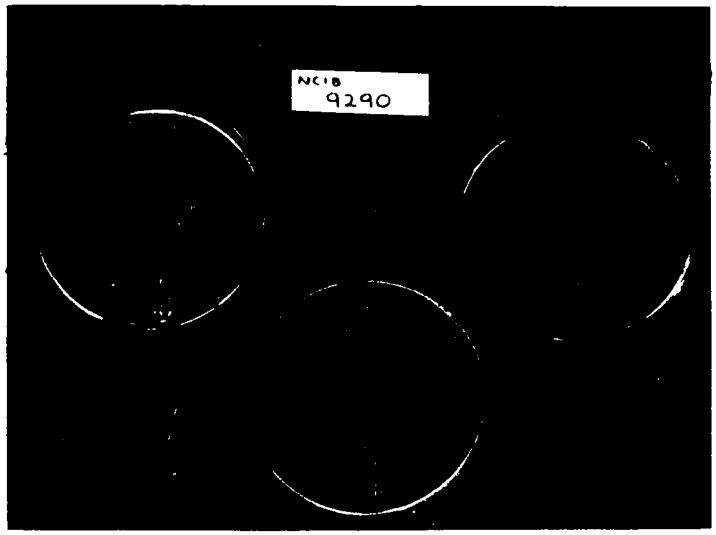

Figure 35. *Flexibacter heparinus* (formerly *Flavobacterium heparinum*) NCIB 9290. 2 day cultures. Codes and colours on Plate Count (left) IA1 10 YR 6/4, Skim Acetate (centre) IB1 10 YR 7/4, and Cook's Cytophaga Agar (right) IB1 2.5 Y 7/4.

It agrees with all the characteristics of the genus *Cytophaga* except that it degrades no polymers, not even starch. It is also highly resistant to salinity (Table 46) and does not use gelatin or lyse milk. It is hence, probably better grouped with *Flexibacter*. No fermentative or gelatin-negative *Flexibacter* species have yet been described (with the exception of the white, spreading *F.canadensis* described here). Otherwise the nearest species would be *F.flexilis*, but this will not tolerate even 1% NaCl and is catalase negative. As these are fairly basic characters in which NCIB 9290 differs, it is proposed that it be transferred to a new species *Flexibacter heparinus*. The GC ratio of 42.2% is in the centre of the range for this genus.

Flavobacterium aurantiacum NCIB 8204 has been questioned as a member of the genus *Flavobacterium* (see page 66) but its characteristics (Table 56) obviously do not allow it to be placed in *Cytophaga* or *Flexibacter* either.

Nonspreading pink organisms

The arctic isolate 5-9 has now been found to produce fruiting bodies of the *Myxococcus* type (see page 305), and hence it is not a *Cytophaga*. The taxonomy of strain 14 is more difficult and it probably does not belong to this group at all. At first slightly curved rods were observed, but in some media curly, almost circular cells were seen. It may be a *Vibrio* or related species.

VIII.5 Summary

Following the ideas of Soriano and Lewin (see page 79), it is felt that the low GC ratio genus *Cytophaga* should be transferred from the Order Myxobacterales to the new Order Flexibacterales, and the Family Cytophagaceae. This Family would then comprise the genera *Cytophaga, Flexibacter, Microscilla, Sporocytophaga, Herpetosiphon, Flexithrix* and *Saprospira*. The Myxobacterales would then be characterized by a high GC ratio and consist of the fruiting genera and the new nonfruiting genus *Lysobacter*.

Amongst the soil and freshwater *Cytophaga* species it is recommended that there are several which no longer warrant serious consideration because of the lack of extant cultures and of sufficient information concerning them. These species are *C.winogradskii, C.albogilva, C.deprimata, Promyxobacterium flavum (?Cytophaga flava),* and *P.lanceolatum (?C.lanceolata)* and probably also *C.rubra* and *C.tenuissima*. *C.anularis, C.crocea, C.flavicula, C.lutea* and *C.sylvestris* are considered to be synonymous with *C.hutchinsonii*. *C.johnsonae* and its variety *denitrificans* have been combined and the description of *C.johnsonae* amended to include denitrification (see page 311). Four soil and freshwater species therefore remain, *C.hutchinsonii, C.aurantiaca, C.johnsonae* and *C.succinicans,* to which have now been added *C.brunescens* (see page 315) and *C.compacta* (see page 327).

The fish pathogens *C.psychrophila* and *C.columnaris* will be left as they are until more work has been done on them, although there is reason to doubt this systematic position (see page 218).

The marine *Cytophaga* species have not been studied here, but the following comments can be offered. The emendation of *C.diffluens* Stanier by Lewin has created a confusing situation (see page 23) in this species which will have to be resolved. The lack of data on polysaccharide utilization together with the lack of an available culture of *C.sensitiva* means that this species should be abandoned, and the descriptions and lack of cultures of *C.fermentans* var. *agarovorans*, *C.krzemieniewskae* and *C.rosea* are also bound to cause problems. A similar situation with regard to denitrification as in *C.johnsonae* (page 311) may occur in *C.haloflava* and its variety *nonreductans*.

The new myxobacterial, nonfruiting genus *Lysobacter* has been proposed, together with three new species *L.enzymogenes* (the '495 group'), *L.enzymogenes* var. *cookii* (strain 13B), *L.gummosus* (strain 402) and *L.antibioticus* (strain 3C). It is felt that several other reported, unnamed strains may belong in this genus.

It is recommended that the genus *Flexibacter* be slightly reamended to exclude CMC and chitin degradation (see page 90). It is suggested that *F.aurantiaca* be disbanded; further work on the two varieties *copepodarum* and *excathedrus* may indicate them worthy of a new specific name(s). Three strains, *F.flexilis* var. *pelliculosus*, *F.sancti* and *Flexibacter* sp. Fs-1, should be reassigned

to *Cytophaga* (see page 215), and two species, *F.aureus* and *F.albuminosus* will have to be discarded as no cultures are available and their polysaccharase production is incompletely known (see page 322). In addition a new species *F.canadensis* has been described here (see page 318), and *Flavobacterium heparinum* is suggested as being more appropriately assigned as *Flexibacter heparinus* (see page 331).

As a result of this investigation some much-needed pruning has been achieved on the genus *Flavobacterium* (see pages 65-66, 329 - 332). *F.pectinovorum* has been identified as synonymous with *C.johnsonae* and *F.meningosepticum* and *F.resinovorum* NCIB 8767 are also thought to belong to *Cytophaga*. *F.heparinum* is reassigned as *Flexibacter heparinus*. In addition the following six strains have been rejected as *Flavobacteria; F.arborescens* NCIB 8185, *F.aquatile* NCIB 8535, *F.esteroaromaticum* NCIB 8186, *F.flavescens* NCIB 8187, *F.lucecoloratum* NCIB 9324 and *F.suaveolans* NCIB 8188. Three others are questioned, namely *F.aurantiacum* NCIB 8204, *F.proteus* NCIB 8771 and *F.suaveolans* NCIB 8992.

Buchanan (56) has made a suggestion that, "Names of bacterial taxa are not validly published unless they have been recognized or evaluated in the bacteriological literature since 1 January 1950." This would handily dispose of some of the poorly defined species, notably *C.winogradskii* Verona 1934, *P.flavum* Imsenecki and Solntseva 1945, *P.lanceolatum* Imsenecki and Solntseva 1945, *Flexibacter aureus* Soriano 1945 and *F.albuminosus*, were it not for the publication in 1961 of Jeffers and Holt's evaluation of the taxonomic status of members of the Myxobacterales (188). They evaluated *C.winogradskii*,

P. flavum and *P. lanceolatum* as validly published and legitimate species. However, it would seem to take care of the two *Flexibacter* species.

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APPENDIX I

Growth codes on Cook's Cytophaga Agar at two days

	Tryptone %	Agar % 0.75	1.0	1.5	2.0
405	0.05	SUB C 12 α	IV J 51 α	III E 51 α	III F 88
	0.1	III E 51 α pf	IV G 51 α p	III G 51 α o	II G 88
	0.2	III C 51 β f	IV J 58 α	III F 58 α f	III F 58 α f
	0.5	III D 55 α fp	IV H 58 α	III G 58 α fe	III E 58 α f
	1.0	III E 55 α f	III E 85 f	III E 50 β f	III E 50 γ ffe
	2.0	II E 85 fpe	III H 55 β f	III G 55 α fe	II F 50 γ ffe
4539	0.05	IV G 58 α	III H 58 α	III E 51 α	II C 51 α
	0.1	III F 58α	III G 58 α	II C 51 α	II C 50 α
	0.2	I F 82 γ e	I F 52 α e	I B 2 α c	I A 1 c
	0.5	I B 1	I A 2 α	I A 1	I A 1
	1.0	I B 1	I A 2 α	I A 1	I A 1
	2.0	I A 1 c	I A 1	I A 1	I A 1
4433	0.05	SUB E 22 α f	IV J 51 α	III F 51 α	III D 51 α
	0.1	III F 53 α	IV J 58 α	III E 51 α	III D 51 α
	0.2	III F 53 α	III D 51 α f	II D 50 α f	III D 51 α
	0.5	II F 53 α	I D 51 α f	I C 50 β f	I B 3 β e
	1.0	I F 52 α e	I E 52 α e	I A 1	I B 1 c
	2.0	I D 82	I C 52 β e	I B 2 β	I B 1
4707	0.05	III E 11 α f	IV H 58 α	III D 51 α	III D 51 α
	0.1	III E 52 α f	III F 53 α e	III D 58 α	III D 51 α
	0.2	I E 52 α fe	I G 83 γ fe	III D 51 α	III C 51 β p
	0.5	I B 1	I B 3 p	I A 1 p	II B 3 γ pe
	1.0	I B 2 α	I A 2	I A 1	I A 1
	2.0	I A 2 α c	I A 1	I A 1	I A 1

APPENDIX I - continued

Growth codes on Cook's Cytophaga Agar at two days

	Tryptone %	Agar % 0.75	1.0	1.5	2.0
Bryant	0.05	-	II B 2 β fss	I A 1	I A 1
	0.1	-	III C 52 β fs	I A 1	I A 1
	0.2	-	II B 52 γ fss	I A 1	I A 1
	0.5	I D 51 β s	III C 52 γ fs	II A 52 γ e	I A 55 β c
	1.0	III F 52 α fs	II E 53 α s	I A 1 c	I A 52 β c
	2.0	III D 52 β s	II D 53 β s	I B 52 α w	III C 82
A15	0.05	SUB D 11 β s	II D 51 β s	I A 1 s	I A 1
	0.1	II D 52 γ fs	II C 52 γ ess	I A 1 s	I A 51 γ
	0.2	I B 1 s	I A 1 ss	I A 1	I A 51 γ
	0.5	I C 3 β s	I B 2 γ ss	I A 1	I A 51 γ
	1.0	I B 3 β	I B 2 γ	I A 1	I A 1
	2.0	I A 1	I A 1	I A 1	I A 1
B-2-25					
	0.05	III G 52 α	III J 51 α	III G 51 α	III E 51 α
	0.1	III H 51 α o	III J 51 α o	III G 51 α	III E 51 α
	0.2	III G 54 α	III H 54 α	III F 51 α	III E 51 α
	0.5	I H 53 α e	II H 54 α	II E 58 α	II C 58 β
	1.0	I J 52 α	I G 50 α c	I B 55 γ f	I B 1 e
	2.0	I D 82 e	I C 3 c	I B 1	I B 1
E-1-25					
	0.05	SUB D 13 α	III J 58 α	III E 51 α	III D 51 α
	0.1	III F 52 α b	III J 58 α	III E 51 α	III D 51 α
	0.2	III H 58 α p	III F 55 α p	III C 58 α	III D 51 α
	0.5	III H 54 α	II F 55 α fp	II D 55 β	III C 55 β f
	1.0	I H 54 α	I F 55 α f	II B 82 γ f	II B 55 γ f
	2.0	I D 82 γ bc	I C 82 γ	I B 1	I B 1

APPENDIX I - continued

Growth codes on Cook's Cytophaga Agar at two days

	Agar %	0.75	1.0	1.5	2.0
H_2O-1A					
Tryptone %	0.05	III F 52 α bc	II E 52 α s	I A 1	I A 2 γ
	0.1	II E 52 β fes	II C 52 β fes	I A 2 γ	I A 2 β
	0.2	I B 3 β s	I A 1 s	I A 1	I A 2 β
	0.5	I B 3 β	I B 3 β	I A 1	I A 2 α
	1.0	IB 3 β	I B 1	I A 1	I A 2 α
	2.0	I B 1	I A 1	I A 1	I B 2 β
18H	0.05	I B 1 s	I B 2 β s	I A 1	I A 1
	0.1	I B 1 s	I B 2 β ss	I A 1	I A 1
	0.2	I B 1 s	I B 2 β s	I A 1	I A 1
	0.5	I B 1 s	I B 1 ss	I A 1	I A 1
	1.0	I C 1 ss	I B 1 s	I A 1	I A 1
	2.0	I C 1 s	I B 1s	I B 1	I A 1
15D	0.05	I A 1	I B 2 γ	I A 1	I A 1
	0.1	I A 2 γ	I A 2 γ	I A 1	I A 1
	0.2	I A 2 γ	I A 2 γ	I A 2 β	I A 1
	0.5	I B 1	I A 1	I A 2 β	I A 1
	1.0	I B 1	I B 1	I A 1	I A 1
	2.0	I B 1	I B 1	I B 1	I A 1
13B	0.05	SUB B 21 γ	SUB B 21 γ	III B 82 γ	III B 82 γ
	0.1	II B 2 γ	II B 2 β	III A 82 γ	III A 82 γ
	0.2	I B 2 β	I B 2 β	III A 82 γ	II A 82 γ
	0.5	I B 2 β	I B 2 α	I A 1	I A 2 β
	1.0	I B 1	I B 2 α	I A 1	I A 1
	2.0	I B 1	I A 1	I A 1	I A 1

APPENDIX I - continued

Growth codes on Cook's Cytophaga Agar at two days

	Tryptone %	Agar % 0.75	1.0	1.5	2.0
3	0.05	SUB B 11 β	SUB B 22 β f	III C 51 β f	II D 58 α e
	0.1	SUB C 11 β	SUB B 22 α f	III C 51 β ffp	III D 58 α f
	0.2	SUB B 22 β	SUB B 22 α f	III D 51 α f	III E 51 α bfw
	0.5	I D 50 β f	I D 55 α f	III D 50 β f	III D 58 α
	1.0	I B 1	I B 1	I A 1	I B 2 α c
	2.0	I B 3 γ	I B 1	I A 1	I B 2 β
495	0.05	II B 82 γ	II B 82 γ f	I A 1	I A 1
	0.1	II C 82 γ f	II B 82 γ f	I A 1	I A 1
	0.2	II C 82 γ f	II B 82 γ f	I A 1	I A 1
	0.5	II C 82 γ e	II B 82 γ f	I A 1	I A 1
	1.0	I B 1 e	I B 1	I A 1	I A 1
	2.0	III C 82 γ f	I B 1	I A 1	I A 1
9D	0.05	III F 53 α	III H 54 α	III E 54 α	III D 54 α
	0.1	III F 53 α	III H 54 α	III E 54 α	III D 54 α
	0.2	III F 53 α	III E 53 β	II D 55 β fe	II C 54 β fe
	0.5	III E 53 β e	III G 53 β	II D 50 γ fe	III C 82 e
	1.0	III D 53 γ e	III D 53 γ	I C 50 β fe	III C 82 ce
	2.0	I F 52 α e	II E 52 β e	I C 82 ce	II C 82 ce

APPENDIX I - continued

Growth codes on Cook's Cytophaga Agar at two days

	Tryptone %	Agar % 0.75	1.0	1.5	2.0
2	0.05	SUB E 22 α	III E 82 β	I B 51 β o	I B 51 γ
	0.1	III E 82 γ	III E 82 α f	I B 51 β o	I A 51 γ
	0.2	III F 82 β f	III E 58 α	II B 85 γ e	I A 2 γ
	0.5	III G 52 α f	III F 53 β	I A 52 γ e	I A 2 γ
	1.0	III F 52 α f	II E 53 β	I A 3 γ	I A 1
	2.0	I A 1	I B 1	I A 1	I A 1
D	0.05	SUB E 12 β	III D 52 α f	II B 51 β	II A 51 β
	0.1	III E 52 α	III E 52 α f	II B 51β	II A 85 β
	0.2	IV F 52 α b	III D 53 γ	II B 55 γ e	II B 55 β e
	0.5	III G 55 α f	III E 53 γ e	II B 55 γ e	I A 2 β
	1.0	III F 53 γ	II E 53 β e	II A 55 γ	I A 2 γ
	2.0	I B 1	I B 2 α	I A 1	I A 2 γ
6	0.05	SUB B 1	II A 50 β	I A 1	I A 1
	0.1	III C 50 β	II A 50 β	III A 50 β	II A 50 β
	0.2	III C 50 β	II A 50 β	II A 50 β	II A 50 β
	0.5	II A 50 β	II A 50	II A 50 β	I A 1
	1.0	No growth	No growth	I A 1	I A 1
	2.0	No growth	No growth	No growth	No growth
4541					
	0.05	III D 50 γ	III C 50 γ f	III B 8	II A 8
	0.1	III D 50 γ	III C 50 γ f	III B 8	III B 8
	0.2	IV D 50 γ f	IV D 53 β	III B 8	I A 1
	0.5	III E 50 β f	IV E 53 β	I A 1	I A 1
	1.0	III D 50 γ f	IV D 50 γ f	I A 1	I A 2 β
	2.0	I A 1	I A 1	I A 1	I A 1

APPENDIX I - continued

Growth codes on Cook's Cytophaga Agar at two days

	Tryptone %	Agar % 0.75	1.0	1.5	2.0
8186	0.05	I A 1	I A 1	I A 1	I A 1
	0.1	I A 1	I A 1	I A 1	I A 1
	0.2	I A 1	I A 1	I A 1	I A 1
	0.5	I B 2 α	I A 1	I A 1	I A 1
	1.0	I B 1	I A 1	I A 1	I A 1
	2.0	I B 1	I A 1	I A 1	I A 1
8187	0.05	I A 1	I A 1	I A 1	I A 1
	0.1	I A 1	I A 1	I A 1	I A 1
	0.2	I A 1	I A 1	I A 1	I A 1
	0.5	I A 1	I A 1	I A 1	I A 1
	1.0	I A 1	I A 1	I A 1	I A 1
	2.0	I A 1	I A 1	I A 1	I A 1
8535	0.05	I A 1	I A 1	I A 1	I A 1
	0.1	I A 1	I A 1	I A 1	I A 1
	0.2	I A 1	I A 1	I A 1	I A 1
	0.5	I A 1	I A 1	I A 1	I A 1
	1.0	I B 1	I A 2 β	I A 1	I A 1
	2.0	I B 2 α	I A 1	I A 1	I A 1
9059	0.05	I B 1	I A 1	Poor growth	Poor growth
	0.1	I A 1	I A 1	Poor growth	Poor growth
	0.2	I D 55 β c	I B 55 β c	Poor growth	Poor growth
	0.5	I D 55 α c	I C 55 γ c	I A 1	Poor growth
	1.0	I C 55 γ c	I C 55 γ c	I A 1	I A 1
	2.0	I D 3 β	I B 52 β	I A 1	I A 1

Appendix II

Growth codes on Cook's Cytophaga Agar at six days

		Agar % 0.75	1.0	1.5	2.0
405	0.05	SUB E 23βpp	III J 51αe	SUB H 12α	III J 88w
	0.1	SUB H 23αppf	III J 51αe	III J 51α	III J 88w
Tryptone %	0.2	SUB F 23αppf	III K 58α	II J 58αf	II J 58α
	0.5	III F 50βppf	III J 50αf	IH 50αb	IG 58αe
	1.0	II F 50γbc	IF 50 βbf	IF 50βfbp	IE 58αfcb
	2.0	IF 50βcb	IH 55βccbf	IH 55αffbc	ID 50β
4539	0.05	II J 58αbe	II J 51αb	IF 51αc	ID 51αbe
	0.1	I J 51αbe	II J 51αbe	ID 51αp	IC 52αcc
	0.2	IF 52αbc	IF 50αc	IB 50βcc	IB 50βc
	0.5	IB 3γc	IB 2β	IA 2	IA 2 β
	1.0	IA 1	IB 2βc	IA 1	IA 1
	2.0	IB 3γco	IB 3γco	IB 3gg	IB3γg
4433	0.05	SUB J 23αpp	III K 51α	III J 51α	III F 51α
	0.1	SUB J23αppf	III K 51α	II H 51α	I E 51αw
	0.2	III H 52αpf	III F 50αbffw	I D 52αw	I E 51αw
	0.5	II G 52αff	I E 50αffb	I C 52βw	I B 3βc
	1.0	II F 52αwc	II E 52αbc	I B 1	I C 1 c
	2.0	I D 52βc	I D 52βbc	I B 1	I B 1
4707	0.05	SUB J 11αb	III K 50α	I H 58αe	III H 51α
	0.1	IV J 52αb	IV J 50αffbw	I G 58αc	II H 58αw
	0.2	IV G 50αbw	II H 50αbcf	I F 50αbc	I D 52αc
	0.5	III D 52γp	III D 62αbwc	I A 1	I B 3γc
	1.0	I B 1 c	I A 1 c	I A 1	I A 1
	2.0	I B 3γpp	I C 3γgg	I A 3βg	I A 1 g
Bryant					
	0.05	SUB A 11β	SUB B 12βp	SUB A 11β	II A 55γf
Tryptone %	0.1	SUB A 11β	II E 58βpb	II B 55αf	II A 55γf
	0.2	SUB A 11β	III C 58βf	II B 55γfe	I A 1
	0.5	II E 57αbpcs	I D 55γbpss	I C 55γfp	I B 50βp
	1.0	I H 52αbbwos	I F 50αbpfc	I B 1 c	I B 52βw
	2.0	I D 52βbws	I D 52αbwf	I C 1 w	I C 1

Appendix II - continued

Growth codes on Cook's Cytophaga Agar at six days

	Agar %	0.75	1.0	1.5	2.0
A15	0.05	SUB D 22βss	IV G 8γfp	I A 1	I A 1 e
	0.1	IV G 52γcwss	IV F 8βfp	I A 1	I B 1 c
	0.2	IV D 8γss	III D 51γss	I A 1	I B 1 c
	0.5	III D 52βggps	III C 55γggs	I B 1	I B 1 c
	1.0	II B 3γggcs	I B 1 g s	I B 1	I B 1
	2.0	I A 1 g	I A 1 g	I B 1 g	I A 1 g
B-2-25					
	0.05	IV H 52α	III J 52αof	I J 51αe	III H 51α
	0.1	III K 52αo	III K 54α	III J 51α	III G 51α
	0.2	III J 52αfo	III J 54αf	I G 58α	II F 58α
	0.5	III K 54αf	III J 54αb	I F 55α	II D 58α
	1.0	II J 52αob	I H 50αoobcp	I C 3 β	I C 52β
	2.0	I D 82βccb	I D 3βc	I C 3 β	I C 1
E-1-25					
	0.05	SUB F 13αcc	III K 51α	III J 51α	III G 51αc
	0.1	III K 51αco	III K 58α	III H 58α	II G 51α
	0.2	III K 54α	I H 51αcf	I F 50αb	I F 51α1
	0.5	III J 54α	I G 50αcb	I E 50αb	I D 58αb
	1.0	I H 54αbc	I F 50αbc	I C 52γ	I C 50γ
	2.0	I D 50γcb	I C 52β	I C 1 w	I C 3γ
H_2O-1A					
	0.05	III G 52αbps	II E 82 eb	I A 1 c	I B 3γc
	0.1	II G 52αbes	II D 52βc	I B 1 c	I A 1
	0.2	II B 3 γ	II A 3 γ	I A 1	I A 1
	0.5	II B 3γp	I C 3 β	I B 1	I B 1
	1.0	I B 1 g	I B 1 g	I A 1	I B 1

Appendix II - continued

Growth codes on Cook's Cytophaga Agar at six days

	Agar %	0.75	1.0	1.5	2.0
18H	0.05	I B 3βp	I B 2βcs	I B 2	I A 2
	0.1	I C 3βc	I B 2βcs	I A 2	I A 2
	0.2	I B 2βcs	I B 2βcs	I A 2	I A 2
	0.5	I C Bαcs	I C 3βws	I B 3βc	I B 3αc
	1.0	I D 3αs	I C 3βs	I B 1	I B 1
	2.0	I D 3αs	I C 3αws	I C 1	I C 1
15D	0.05	I B c	I B 1	I A 3γc	I A 2β
	0.1	I B 1 c	I A 1 c	I A 2γ	I A 2β
	0.2	I B 1 c	I A 1 c	I A 2γ	I A 2β
	0.5	I B 1	I B 1	I B 2γc	I A 2α
	1.0	I C 1	I C 1	I A 1	I B 1 c
	2.0	I C 1	I C 1	I C 3γ	I B 1
13B	0.05	SUB D 12β	SUB C 21β	SUB c 21β	SUB C 11β
	0.1	II C 52β	II C 52β	SUB C 21β	III C 52β
	0.2	II C 52βf	II C 52β	III C 52β	III C 52β
	0.5	I D 3 γ	I C 3 γ	II C 52γf	II B 55γ
	1.0	I C 1	I B 1	I B 50 γ	I B 3 γ
	2.0	I C 1	I B 3 γ	I C 1	I B 3 α
3	0.05	SUB D 12β	SUB H 23αppf	SUB D 13αpp	II H 51αf
	0.1	SUB E 21αp	SUB H 23αppfo	III F 51αppf	III J 58αf
	0.2	SUB E 23βpb	SUB D 23βppbf	I H 58αfb	II H 58αfw
	0.5	II D 50αbc	II D 50αbfc	I E 50αfb	I D 50αc
	1.0	I C 1	I C 1 c	I B 3γc	I B 2αc
	2.0	I C 1 c	I C 1	I B 1 w	I B 3γp

Tryptone %

Appendix II - continued

Growth codes on Cook's Cytophaga Agar at six days

	Agar %	0.75	1.0	1.5	2.0
495	0.05	SUB D 21γ	SUB C 21γ	III B 52α	III B 8
	0.1	SUB D 22γ	SUB D 21γ	IV B 52α	III B 8
	0.2	III D 52γ p	III C 52γ p	II B 8	II B 8
	0.5	I C 52γ p	I D 52γ b	III B 8	II B 8
	1.0	I C 3 γ	I C 1	I B 1	I B 1
	2.0	I C 1	I C 1	I B 1	I B 1
9D	0.05	SUB J 23α f	II J 54α e	I H 54α	III J 54α
	0.1	SUB J 23α fo	II J 54α e	I J 54α	III J 54α
	0.2	III J 53α w	IV J 53α	II J 54α fe	II G 54α
	0.5	III H 53α p	IV J 54α f	I G 50α f	II F 50β w
	1.0	III G 53α e	II G 50α oe	I D 52β w	I E 52β bwe
	2.0	I H 52α e	I G 52α c	I D 52β ce	I D 50β eew
2	0.05	III J 51γw	III J 87 e	SUB D 11β	III C 51βf
	0.1	III J 87	III J 51βec	II D 55αf	III C 51βf
	0.2	III K 87	III J 55αce	II D 55βfe	II C 55βfe
	0.5	III J 55γ	III J 55αfc	II C 55βf	II B 55γf
	1.0	II H 50αb	I E 50βe	I A 1	I B 1
	2.0	I B 3γ	I B 1	I B 1	I A 1
D	0.05	III J 81	III H 81	SUB D 20	III C 51β
	0.1	III J 51α	III H 51β	II D 81α	II C 55αf
	0.2	III J 80	III G 55βef	II D 55αf	II C 55βf
	0.5	III J 80 b	III H 55αfb	II C 55βfe	II A 55γf
	1.0	I G 52βo	I E 50βc	I B 1 e	I A 3γ
	2.0	I B 3γc	I B 1	I B 1	I A 1

Tryptone %

Appendix II - continued

Growth codes on Cook's Cytophaga Agar at six days

	Agar %	0.75	1.0	1.5	2.0
6	0.05	SUB C 10	II B 57β	II B 55βf	II A 55γf
	0.1	III D 51βw	II B 51γ	II B 55βf	II B 55γf
	0.2	III D 51γw	II B 55γw	I B 55βf	I A 55γf
	0.5	I A 1	II A 5 γ	I A 1	I A 1
	1.0	No growth	No growth	I A 1	I A 1
	2.0	No growth	No growth	No growth	No growth
4541	0.05	III G 51γ	III F 66α	III D 52α	III B 51βf
	0.1	III H 51γ	III E 66α	III D 51αf	III D 51βf
	0.2	III H 58γ	III H 66αo	III D 58αf	III C 55γf
	0.5	III H 56αf	III G 66αo	III C 58β	II A 8
	1.0	I F 50αo	III F 55βb	I A 1	I A 1
	2.0	I B 1	I B 1	I A 1	I A 1
8186	0.05	I A 1	I A 1	I A 1	I A 1
Tryptone %	0.1	I A 1	I A 1	I A 1	I A 1
	0.2	I A 1	I A 1	I A 1	I A 1
	0.5	I B 1	I A 1	I B 1	I B 1
	1.0	I B 3 γ	I B 3 γ c	I B 1	I B 1 c
	2.0	I C 1	I B 1	I B 1	I B 1
8187	0.05	I A 1	I A 1	I A 1	I A 1
	0.1	I A 1	I A 1	I A 1	I A 1
	0.2	I A 1	I A 1	I A 1	I A 1
	0.5	I A 1	I B 1	I B 1	I A 1
	1.0	I B 3 β	I A 1	I B 1	I B 1
	2.0	I B 1	I B 3 γ	I B 1	I B 1

Appendix II - continued

Growth codes on Cook's Cytophaga Agar at six days

	Agar %	0.75	1.0	1.5	2.0
8535	0.05	I A 1	I A 1	I A 1	I A 1
	0.1	I B 1	I B 1	I A 1	I A 1
	0.2	I B 1	I A 1	I A 1	I A 1
	0.5	I B 3 γ	I B 1	I A 1	I A 1
	1.0	I B 3 γ	I B 3 γ	I A 1	I A 1
	2.0	I B 3 γ	I B 3 γ	I B 1	I B 1
9059	0.05	I B 3 γ	I B 2 γ	Poor growth	Poor growth.
	0.1	I A 3 γ	I B 2 γ	Poor growth	Poor growth
	0.2	I D 50βppb	I B 2 β	Poor growth	Poor growth
	0.5	I D 50αbp	I C 3βcb	I A 1	I A 1
	1.0	I C 50γcb	I C 3 β	I B 1	I B 1
	2.0	I D 3 γ	I C 1	I B 1	I B 1

APPENDIX III

PROFILES OF THE ORGANISMS USED IN THE TAXONOMIC STUDY (CHAPTER VIII)

PROFILE OF ORGANISM:- *CYTOPHAGA JOHNSONAE* ATCC 17061

CELLS

Length	2-36 μ	Shape	Rods	Arrangement	Single
Width	0.2 μ	Motility	-	Flexing +	Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Rough	Rough	Rough/Smooth
Edge	Erose	Lobate/Erose	Erose
Elevation	Effuse	Effuse	Effuse/Convex
Opt. Props.	Transparent	Translucent	Translucent
Colour	1.5 Y 7/10	5 YR 5/10	2.5 YR 4/10

GENERAL WSP - Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM

Turbidity	++	Flocculence	-	Silky	+
Viscous	-	Ring	+	Colour	YC

PHYSIOLOGY

TEMP°C	Min. 10	Opt. 30	Max. 35
PH	Min. 7	Opt. 7-10	Max. 10

O_2 Growth Anaerobic - Candle Jar + Air + Best Candle

NaCl Good growth up to 1% Partial inhibition 2% Total inhibition 3%

CHO

SUGARS OF Glucose O+Slow F Glucose + Sucrose - Lactose + Cb.+

ALCOHOLS Glycerol - Mannitol - STARCH SSY + NB + Potato ? +

CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +

POLYPECTATE + ALGINATE - CHITIN +

PROTEINS

Tryptone +	Gelatin liq. +	Skim milk lysis +	Haemolysis -
Casamino A use +	ammonif. +	Casitone use +	ammonif. +
Casein lysis +	ammonif. +	Penassay use +	ammonif. +

BIOCHEMISTRY

$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? + NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ +

Indole - Phosphatase + Catalase + Oxidase +

N SOURCES

NO_3 - NH_4 + Urea + Glutamate + Asparaginate + Tryptone +

Casitone + Gelatin + Casein + SM + acetate + Prototrophic -

Stim. by Y.E. + Needs Y.E. + glu - chitin

ANTIBIOTIC etc. SENSITIVITIES

Actinomycin D	S	NO_2 -	Polymyxin B R 1
Chloramphenicol	S	Dihydrostrep. 1	Penicillin G R
SLS growth reduced	.01	growth inhibited	.1

PREDATION

E. coli -	Ps.aeruginosa -	Arthrobacter -	S.marcescens -
B.subtilis +	Chlorella -	Actino 32 -	Actino 41 ?
Yeast ±	Rhizopus -	Penicillium ?	Sclerotinia +

PROFILE OF ORGANISM: *CYTOPHAGA JOHNSONAE* var. *DENITRIFICANS* 405 *(C.JOHNSONAE)*

CELLS
Length 3-20 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Rough	Rough	Rough
Edge	Undulate	Lobate	Lobate
Elevation	Effuse	Effuse	Effuse
Opt. Props.	Transparent	Transparent	Transparent
Colour	10 YR 7/10	10 YR 6/8	7.5 YR 5/8

WSP - Slime Layers - Spreading S
GENERAL WSP Casein only Subsurface +

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous - Ring - Colour OY

PHYSIOLOGY

TEMP°C	Min. 8	Opt. 18	Max. 35
PH	Min. 5	Opt. 6-10	Max. 10

O_2 Growth Anaerobic - Candle Jar + Air + Best Candle
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 2%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY + NB + Potato ?
CELLULOSE Filter Paper - CMC - AGAR Pits - Gelase +
POLYPECTATE ?+ ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis +
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? + NO_3 as e^- acceptor ++ Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
$NO_3 - NH_4$ + Urea - Glutamate + Asparaginate - Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic -
Stim. by Y.E.+glu-chitin Needs Y.E. +

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B RR
Chloramphenicol R Dihydrostrep. 1 Penicillin G R
SLS growth reduced .01 growth inhibited .1

PREDATION
E.coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis - Chlorella - Actino 32 - Actino 41 -
Yeast ± Rhizopus - Penicillium ? Sclerotinia -

PROFILE OF ORGANISM: ALFALFA *(CYTOPHAGA JOHNSONAE)*

CELLS
Length 3-25 μ Shape Rods Arrangement Single
Width 0.2 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Smooth	Rough	Smooth
Edge	Entire	Erose	Entire
Elevation	Raised	Raised	Raised
Opt. Props.	Translucent	Translucent	Opaque
Colour	7.5 YR 6/10 WSP -	7.5 YR 6/8 -	7.5 YR 5/6 -

GENERAL WSP - Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence ± Silky +
Viscous - Ring - Colour Dirty C

PHYSIOLOGY

TEMP°C	Min. 0	Opt. 20-25	Max. 30
PH	Min. 5	Opt. 6-10	Max. 10

O_2 Growth Anaerobic - Candle Jar + Air + Best Candle
NaCl Good growth up to 1% Partial inhibition<2% Total inhibition 2%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb.+
ALCOHOLS Glycerol ?+slow Mannitol + STARCH SSY + NB + Potato +
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE ?+ ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis -
Casamino A use + ammonif. - Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. -

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor ?+ Cystein$\rightarrow H_2S$ +
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3+ NH_4 + Urea + Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E.-glu+chitin Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES

Actinomycin D	S	NO_2 -	Polymyxin B R R
Chloramphenicol	(S)	Dihydrostrep. 1	Penicillin G R
SLS growth reduced	.01	growth inhibited	.1

PREDATION

E.coli -	Ps.aeruginosa -	Arthrobacter -	S.marcescens -
B.subtilis-	Chlorella -	Actino 32 ?	Actino 41 ?
Yeast -	Rhizopus -	Penicillium -	Sclerotinia ?

PROFILE OF ORGANISM: BRYANT *(CYTOPHAGA* sp.)

CELLS
Length 3-105 μ Shape Rods Arrangement Single
Width 0.2 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Rough	Rough	Rough
Edge	Undulate	Erose	Erose
Elevation	Effuse/Umbonate	Convex	Convex
Opt. Props.	Transparent	Translucent	Translucent
Colour	7.5 YR 6/9 WSP -	5 YR 5.5/10 -	4 YR 5/9 -

GENERAL WSP - Subsurface + Slime Layers + Spreading (R)

LIQUID MEDIUM
Turbidity + Flocculence - Silky +
Viscous - Ring Pellicle Colour Offwhite

PHYSIOLOGY
TEMP°C Min. 18 Opt. 20-30 Max. 40
PH Min. 5 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 2%

CHO
SUGARS OF Glucose F Glucose + Sucrose + Lactose + Cb.+
ALCOHOLS Glycerol ?+ Slow Mannitol - STARCH SSY + NB + Potato ?
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE ?+ ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis -
Casamino A use - ammonif. - Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. -

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone - Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. + glu-chitin Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 + Polymyxin B R R
Chloramphenicol 1 Dihydrostrep. 1 Penicillin G R
SLS growth reduced .01 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis - Chlorella - Actino 32 - Actino 41 ?
Yeast ± Rhizopus - Penicillium - Sclerotinia -

PROFILE OF ORGANISM: A 15 *(CYTOPHAGA* sp.)

CELLS
Length 4-20 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Rough	Rough	Rough
Edge	Undulate	Undulate	Undulate
Elevation	Effuse/Umbonate	Raised	Raised
Opt. Props.	Transparent	Translucent	Translucent
Colour	7.5 YR 6/10 WSP -	5 YR 5/10 -	7.5 YR 5/10 -

GENERAL WSP - Subsurface + Slime Layers + Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous - Ring + or Pellicle Colour OY

PHYSIOLOGY
TEMP°C Min. 0 Opt. 8-20 Max. 25
PH Min. 6 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 2%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose - Lactose - Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY + NB + Potato +
CELLULOSE Filter Paper - CMC - AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq.+ slow Skim milk lysis + Haemolysis +
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase ? Oxidase +

N SOURCES
NO_3 + NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone - Gelatin ± slow Casein + SM + acetate +. Prototrophic +
Stim. by Y.E. +glu-chitin Needs Y.E. -glu + chitin

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 + Polymyxin B R S
Chloramphenicol I Dihydrostrep. S Penicillin G R
SLS growth reduced .01 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis - Chlorella - Actino 32 - Actino 41 -
Yeast ± Rhizopus - Penicillium ? Sclerotinia -

PROFILE OF ORGANISM: B-2-25 *(CYTOPHAGA JOHNSONAE)*

CELLS
Length 3-20 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Rough	Rough	Rough
Edge	Lobate	Lobate	Undulate
Elevation	Effuse	Effuse	Raised
Opt. Props.	Transparent	Transparent	Translucent
Colour 10 YR	6/10 WSP -	7.5 YR 6/8 -	7.5 YR 6/10 -

GENERAL WSP - Subsurface + Slime Layers + Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous - Ring - Colour OY

PHYSIOLOGY
TEMP°C Min. 0 Opt. 18 Max. 30
PH Min. 5 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose F Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol + Mannitol - STARCH SSY + NB + Potato +
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE ?- ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis ±
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor ?+ Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
$NO_3 + NH_4$ + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E.+glu-chitin Needs Y.E. -glu + Chitin

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 + Polymyxin B RS
Chloramphenicol S Dihydrostrep. R Penicillin G R
SLS growth reduced .01 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis - Chlorella - Actino 32 - Actino 41 -
Yeast ± Rhizopus ? Penicillium ? Sclerotinia -

PROFILE OF ORGANISM: E-1-25 *(CYTOPHAGA JOHNSONAE)*

CELLS

Length	3-15 μ	Shape	Rods	Arrangement	Single
Width	0.5 μ	Motility	-	Flexing +	Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Rough	Rough	Rough
Edge	Lobate	Erose	Undulate
Elevation	Effuse	Effuse	Convex
Opt. Props.	Transparent	Transparent	Translucent
Colour	7.5 YR 6/10	7.5 YR 6/10 -	7.5 YR 5/10 -

GENERAL WSP - Subsurface + Slime Layers + Spreading S

LIQUID MEDIUM

Turbidity	++	Flocculence	-	Silky	+
Viscous	-	Ring	-	Colour	OY

PHYSIOLOGY

TEMP°C	Min. 0	Opt. 20	Max. 30
PH	Min. 5	Opt. 6-9	Max. 10

O_2 Growth Anaerobic - Candle Jar + Air + Best Candle

NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 2%

CHO

SUGARS OF Glucose F Glucose + Sucrose + Lactose + Cb. +

ALCOHOLS Glycerol + Mannitol - STARCH SSY + NB + Potato +

CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +

POLYPECTATE ?- ALGINATE - CHITIN +

PROTEINS

Tryptone +	Gelatin liq. +	Skim milk lysis +	Haemolysis +
Casamino A use +	ammonif. +	Casitone use +	ammonif. +
Casein lysis +	ammonif. +	Penassay use +	ammonif. +

BIOCHEMISTRY

$NO_3 \rightarrow NO_2$ +	$NO_2 \rightarrow$ gas or ? -	NO_3 as e^- acceptor ?+	Cystein $\rightarrow H_2S$ +
Indole -	Phosphatase +	Catalase +	Oxidase +

N SOURCES

$NO_3 + NH_4$ + Urea - Glutamate + Asparaginate + Tryptone +

Casitone + Gelatin + Casein + SM + acetate + Prototrophic +

Stim. by Y.E. + glu-chitin Needs Y.E. - glu + chitin

ANTIBIOTIC etc. SENSITIVITIES

Actinomycin D S	NO_2 +	Polymyxin B	R S
Chloramphenicol S	Dihydrostrep. R	Penicillin G	R
SLS growth reduced .01	growth inhibited .1		

PREDATION

E. coli -	Ps. aeruginosa -	Arthrobacter -	S. marcescens -
B. subtilis -	Chlorella -	Actino 32 -	Actino 41 -
Yeast ±	Rhizopus -	Penicillium ?	Sclerotinia -

PROFILE OF ORGANISM: H_2O - 1A *(CYTOPHAGA sp.)*

CELLS
Length 3-25 μ
Width 0.4 μ
Shape Rods
Motility -
Arrangement Single
Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Rough	Rough	Smooth/Rough
Edge	Erose	Lobate	Erose
Elevation	Effuse/Umbonate	Effuse	Raised
Opt. Props.	Transparent	Transparent	Translucent
Colour	7.5 YR 6/11	10 YR 6/10 -	7.5 YR 5/10 -

GENERAL WSP - Subsurface + Slime Layers + Spreading S

LIQUID MEDIUM
Turbidity ++
Viscous -
Flocculence -
Ring -
Silky +
Colour OY

PHYSIOLOGY
TEMP°C Min. 0 Opt. 20 Max. 25
PH Min. 6 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose - Lactose - Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY + NB + Potato +
CELLULOSE Filter Paper - CMC - AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis +
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. -

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone - Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. + glu-chitin Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S
NO_2 +
Polymyxin B R S
Chloramphenicol 1
Dihydrostrep. S
Penicillin G R
SLS growth reduced .01
growth inhibited .1

PREDATION
E. coli - Ps. aeruginosa - Arthrobacter - S. marcescens -
B. subtilis - Chlorella - Actino 32 - Actino 41 ?
Yeast ± Rhizopus - Penicillium ? Sclerotinia -

PROFILE OF ORGANISM: 1-10 (*CYTOPHAGA* sp.)

CELLS
Length 2-20 μ Shape Rods Arrangement Single
Width 0.2 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Smooth	Rough	Smooth
Edge	Undulate	Erose	Entire
Elevation	Flat	Effuse	Raised
Opt. Props.	Transparent	Translucent	Translucent
Colour	7.5 YR 6/12	7.5 YR 6/10	7.5 YR 5/10

WSP - Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence + Silky +
Viscous - Ring + Colour Dirty Y

PHYSIOLOGY

TEMP°C	Min. 0	Opt. 20	Max. 30
PH	Min. 5	Opt. 5-10	Max. 10

O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 1% Partial inhibition 2% Total inhibition > 3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol + STARCH SSY + NB + Potato +
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE + ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis +
Casamino A use + ammonif. - Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor + Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
$NO_3 + NH_4$ + Urea + Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. + Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B R R
Chloramphenicol (S) Dihydrostrep. R Penicillin G R
SLS growth reduced < .1 growth inhibited .1

PREDATION
E. coli - Ps. aeruginosa - Arthrobacter - S. marcescens -
B. subtilis ? Chlorella - Actino 32 + Actino 41 +
Yeast ± Rhizopus - Penicillium - Sclerotinia ?

PROFILE OF ORGANISM: 3 *(CYTOPHAGA JOHNSONAE)*

CELLS
Length 3-25 μ Shape Rods Arrangement Single
Width 0.3 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Rough	Rough	Smooth
Edge	Lobate	Erose	Entire
Elevation	Effuse	Effuse/Umbonate	Convex
Opt. Props.	Transparent	Translucent	Translucent
Colour	10 YR 6/12	5 YR 6/10-	5 YR 5/10 -

GENERAL WSP - Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous - Ring Pellicle Colour OY

PHYSIOLOGY
TEMP°C Min. 0 Opt. 30 Max. 35
PH Min. 5 Opt. 5-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air+ Best Candle
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 2%

CHO
SUGARS OF Glucose O Glucose + Sucrose + Lactose + Cb.+
ALCOHOLS Glycerol - Mannitol - STARCH SSY + NB + Potato +
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE ?+ ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis +
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? + NO_3 as e^- acceptor ++ Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea ?- Glutamate + Asparaginate + Tryptone +
Casitone - Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. - Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B I S
Chloramphenicol I Dihydrostrep. S Penicillin G I
SLS growth reduced .01 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis - Chlorella - Actino 32 - Actino 41 -
Yeast ± weak Rhizopus - Penicillium ? Sclerotinia -

PROFILE OF ORGANISM: 3-19 *(CYTOPHAGA sp.)*

CELLS
Length 1-9 μ Shape Rods Arrangement Single
Width 0.3 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Smooth	Rough	Rough
Edge	Lobate	Undulate	Undulate
Elevation	Effuse	Flat	Raised
Opt. Props.	Transparent	Translucent	Translucent
Colour	7.5 YR 5/10	7.5 YR 6/8	8 YR 5/8

WSP - GENERAL WSP- Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous - Ring Colour OY

PHYSIOLOGY

TEMP°C	Min. 5	Opt. 25	Max. 30
PH	Min. 5	Opt. 5	Max. 10

O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 1% Partial inhibition 2% Total inhibition 3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose ?+ slow Cb. +
ALCOHOLS Glycerol - Mannitol- STARCH SSY + NB + Potato ?+
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE + ALGINATE - CHITIN -

PROTEINS
Tryptone + Gelatin liq. ? Skim milk lysis + Haemolysis -
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. Penassay use + ammonif. ?+

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? + NO_3 as e^- acceptor ?+ Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase ? Oxidase +

N SOURCES
$NO_3 - NH_4$ + Urea + Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin ?- Casein + SM + acetate + Prototrophic -
Stim. by Y.E.+glu-chitin Needs Y.E. + glu n.g. chitin

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D NO_2^- Polymyxin B R S
Chloramphenicol (S) Dihydrostrep.(R) Penicillin G I
SLS growth reduced .01 growth inhibited > .1

PREDATION

E. coli -	Ps.aeruginosa -	Arthrobacter -	S.marcescens -
B.subtilis-	Chlorella +	Actino 32 -	Actino 41 +
Yeast -	Rhizopus -	Penicillium ?	Sclerotinia ?

PROFILE OF ORGANISM: 3-22 (*CYTOPHAGA* sp.)

CELLS
Length 1-15 μ Shape Rods Arrangement Single
Width 0.3 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Smooth	Smooth	Smooth
Edge	Undulate	Undulate	Undulate
Elevation	Effuse	Flat	Raised
Opt. Props.	Transparent	Translucent	Translucent
Colour	10 YR 6/10	10 YR 6/12	7.5 YR 5/10

GENERAL WSP - Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous - Ring Colour OY

PHYSIOLOGY

TEMP°C	Min. 0	Opt. 10	Max. 30
PH	Min. 5	Opt. 6	Max. 10

O_2 Growth Anaerobic - Candle Jar + Air + Best Candle
NaCl Good growth up to 1% Partial inhibition <2% Total inhibition 2%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb.+
ALCOHOLS Glycerol - Mannitol - STARCH SSY + NB ?+ Potato ?+
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE + ALGINATE - CHITIN -

PROTEINS
Tryptone + Gelatin liq. ? Skim milk lysis + Haemolysis -
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor ?+ Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase ? Oxidase +

N SOURCES
NO_3 - NH_4 + Urea + Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin ?- Casein + SM + acetate + Prototrophic -
Stim. by Y.E.+glu-chitin Needs Y.E. + glu n.g. chitin

ANTIBIOTIC etc. SENSITIVITIES

Actinomycin D	NO_2 -	Polymyxin B R S
Chloramphenicol R	Dihydrostrep. R	Penicillin G I
SLS growth reduced <.1	growth inhibited .1	

PREDATION

E. coli -	Ps.aeruginosa -	Arthrobacter -	S.marcescens -
B.subtilis -	Chlorella +	Actino 32 -	Actino 41 +
Yeast -	Rhizopus -	Penicillium ?	Sclerotinia ?

PROFILE OF ORGANISM: 4433 *(CYTOPHAGA JOHNSONAE)*

CELLS
Length 3-12 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Rough	Rough	Smooth
Edge	Lobate	Undulate	Undulate
Elevation	Effuse	Effuse	Umbonate
Opt. Props.	Transparent	Transparent	Translucent
Colour	10 YR 6/10	5 YR 5.5/10	7.5 YR 5/8

WSP - GENERAL WSP - Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous - Ring - Colour OY

PHYSIOLOGY
TEMP°C Min. 0 Opt. 18 Max. 30
PH Min. 5 Opt. 5-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose- Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY + NB + Potato +
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis +
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor ?+ Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea - Glutamate + Asparaginate - Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. + glu-chitin Needs Y.E. - glu + chitin

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 + Polymyxin B R S
Chloramphenicol S Dihydrostrep. R Penicillin G R
SLS growth reduced .01 growth inhibited .1

PREDATION
E.coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis - Chlorella - Actino 32 - Actino 41 -
Yeast + Rhizopus - Penicillium ? Sclerotinia -

PROFILE OF ORGANISM: 4539 *(CYTOPHAGA JOHNSONAE)*

CELLS

Length	3-20 μ	Shape	Rods	Arrangement	Single
Width	0.5 μ	Motility	-	Flexing +	Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Circular
Surface	Smooth	Rough	Smooth
Edge	Lobate	Erose	Entire
Elevation	Effuse	Effuse	Flat
Opt. Props.	Transparent	Transparent	Translucent
Colour	7.5 YR 6/10	WSP - 7.5 YR 7/8	- 9 YR 6/8 -

GENERAL WSP - Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM

Turbidity	++	Flocculence	-	Silky	+
Viscous	-	Ring	Pellicle	Colour	deep COY

PHYSIOLOGY

TEMP°C	Min. 0	Opt. 18-20		Max. 30
PH	Min. 5	Opt. 5-10		Max. 10
O_2	Growth Anaerobic -	Candle Jar +	Air +	Best Candle
NaCl	Good growth up to 0%	Partial inhibition 1%		Total inhibition 3%

CHO

SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb. +

ALCOHOLS Glycerol - Mannitol - STARCH SSY + NB + Potato +

CELLULOSE Filter Paper - CMC - AGAR Pits - Gelase +

POLYPECTATE ?- ALGINATE - CHITIN +

PROTEINS

Tryptone	+	Gelatin liq.	+	Skim milk lysis	+	Haemolysis	+
Casamino A use	+	ammonif.	+	Casitone use	+	ammonif.	+
Casein lysis	+	ammonif.	+	Penassay use	+	ammonif.	+

BIOCHEMISTRY

$NO_3 \rightarrow NO_2$	+	$NO_2 \rightarrow$ gas or ?	-	NO_3 as e^- acceptor	?+	Cystein $\rightarrow H_2S$	+
Indole	-	Phosphatase	+	Catalase	+	Oxidase	+

N SOURCES

NO_3 + NH_4	+	Urea	-	Glutamate	+	Asparaginate	+	Tryptone	+
Casitone	-	Gelatin	+	Casein	+	SM + acetate	+	Prototrophic	+
Stim. by Y.E.	-	Needs Y.E.	-						

ANTIBIOTIC etc. SENSITIVITIES

Actinomycin D	S	NO_2	+	Polymyxin B	R I
Chloramphenicol	S	Dihydrostrep.	I	Penicillin G	R
SLS growth reduced	.01	growth inhibited	.1		

PREDATION

E. coli	-	Ps.aeruginosa	-	Arthrobacter	-	S.marcescens	-
B.subtilis	-	Chlorella	-	Actino 32	-	Actino 41	-
Yeast	±	Rhizopus	-	Penicillium	?	Sclerotinia	-

PROFILE OF ORGANISM: 4707 *(CYTOPHAGA JOHNSONAE)*

CELLS

Length	3-20 μ	Shape	Rods	Arrangement	Single
Width	0.4 μ	Motility	-	Flexing +	Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Smooth	Rough	Smooth
Edge	Erose	Undulate	Undulate
Elevation	Effuse	Effuse	Convex
Opt. Props.	Transparent	Transparent	Translucent
Colour	10 YR 6/10	7.5 YR 7/9	7.5 YR 6/10

GENERAL WSP - Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM

Turbidity	++	Flocculence -	Silky +
Viscous	-	Ring -	Colour COY

PHYSIOLOGY

TEMP°C	Min. 0	Opt. 18-25	Max. 30
PH	Min. 5	Opt. 5-10	Max. 10

O_2 Growth Anaerobic - Candle Jar + Air + Best Candle

NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 2%

CHO

SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb.+

ALCOHOLS Glycerol ?+ slow Mannitol - STARCH SSY + NB + Potato ?+

CELLULOSE Filter Paper - CMC - AGAR Pits - Gelase +

POLYPECTATE ?- ALGINATE - CHITIN +

PROTEINS

Tryptone +	Gelatin liq. +	Skim milk lysis +	Haemolysis +
Casamino A use +	ammonif. +	Casitone use +	ammonif. +
Casein lysis +	ammonif. +	Penassay use +	ammonif. +

BIOCHEMISTRY

$NO_3 \rightarrow NO_2$ +	$NO_2 \rightarrow$ gas or ? -	NO_3 as e^- acceptor ?+	Cystein $\rightarrow H_2S$ +
Indole -	Phosphatase +	Catalase +	Oxidase +

N SOURCES

NO_3 + NH_4 +	Urea -	Glutamate +	Asparaginate +	Tryptone +	
Casitone -	Gelatin +	Casein +	SM +	acetate +	Prototrophic +
Stim. by Y.E. -	Needs Y.E. -				

ANTIBIOTIC etc. SENSITIVITIES

Actinomycin D	S	NO_2	+	Polymyxin B	R R
Chloramphenicol	S	Dihydrostrep.	I	Penicillin G	R
SLS growth reduced	.01	growth inhibited	.1		

PREDATION

E. coli -	Ps.aeruginosa -	Arthrobacter -	S.marcescens -
B.subtilis -	Chlorella -	Actino 32 -	Actino 41 ?
Yeast -	Rhizopus -	Penicillium ?	Sclerotinia -

PROFILE OF ORGANISM: *FLAVOBACTERIUM PECTINOVORUM* 9059 *(CYTOPHAGA JOHNSONAE)*

CELLS
Length 1-25 μ Shape Rods Arrangement Single
Width 0.3 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	≃Circular
Surface	Rough	Rough	Rough
Edge	Undulate	Erose	Erose
Elevation	Flat	Effuse	Convex
Opt. Props.	Transparent	Translucent	Translucent
Colour	10 YR 7/10	2.5 YR 4/12	5 YR 5/10

WSP - GENERAL WSP - Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous - Ring + Colour OY

PHYSIOLOGY

TEMP°C	Min. 0	Opt. 18-20	Max. 30
PH	Min. 5	Opt. 5-10	Max. 10

O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose O Glucose + Sucrose + Lactose + Cb.+
ALCOHOLS Glycerol - Mannitol - STARCH SSY + NB + Potato ?
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE ?+ ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis +
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor ?+ Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea + Glutamate + Asparaginate + Tryptone +
Casitone - Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. + glu - chitin Needs Y.E. - glu + chitin

ANTIBIOTIC etc. SENSITIVITIES

Actinomycin D	S	NO_2 +	Polymyxin B R I
Chloramphenicol	(S) R	Dihydrostrep. I	Penicillin G I
SLS growth reduced	.01	growth inhibited	>.1

PREDATION

E.coli -	Ps.aeruginosa -	Arthrobacter -	S.marcescens -
B.subtilis +	Chlorella -	Actino 32 -	Actino 41 ?
Yeast ±	Rhizopus -	Penicillium ?	Sclerotinia ?

PROFILE OF ORGANISM: *FLEXIBACTER* FS-1 *(CYTOPHAGA JOHNSONAE)*

CELLS
Length 10-400 μ Shape Rods Arrangement Single
Width 0.7 μ (0.4 μ) Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Rough	Rough	Rough
Edge	Erose	Erose	Erose
Elevation	Effuse	Effuse	Undulate
Opt. Props.	Transparent	Transparent	Translucent
Colour	7.5 YR 6/10	5 YR 5/12	5 YR 5/10

WSP -
GENERAL WSP - Subsurface - Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity + Flocculence - Silky +
Viscous - Ring Colour Offwhite

PHYSIOLOGY
TEMP°C Min. 15 Opt. 25 Max. 35
PH Min. 7 Opt. 7-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle
NaCl Good growth up to 0% Partial inhibition <1% Total inhibition 1%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb.+
ALCOHOLS Glycerol - Mannitol - STARCH SSY + NB + Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE + ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis no growth
Casamino A use ammonif. Casitone use + ammonif. -
Casein lysis + ammonif. + Penassay use ammonif.

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ $NO_2 \rightarrow$ gas or ? NO_3 as e^- acceptor Cystein $\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea + Glutamate + Asparaginate + Tryptone +
Casitone ?+ Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. ± glu + chitin Needs Y.E. - glu + chitin

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 Polymyxin B I S
Chloramphenicol (S) R Dihydrostrep. R Penicillin G R
SLS growth reduced .01 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis ? Chlorella - Actino 32 - Actino 41 -
Yeast ± Rhizopus - Penicillium - Sclerotinia +

PROFILE OF ORGANISM: *CYTOPHAGA HUTCHINSONII* STANIER 6

CELLS
Length 1-7 μ Shape Rods Arrangement Single
Width 0.1 μ Motility - Flexing + Gram -

COLONIES #1. SA. PC.
Form
Surface
Edge
Elevation
Opt. Props.
Colour
GENERAL WSP - WSP Subsurface - Slime Layers - Spreading R

LIQUID MEDIUM
Turbidity +
Viscous -
Flocculence granules from paper Silky -
Ring on paper only Colour C

PHYSIOLOGY
TEMP°C Min. 20 Opt. 25-35 Max. 35
PH Min. Opt. Max.
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 2%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose - Lactose ? Cb. +
ALCOHOLS Glycerol - Mannitol + STARCH SSY - NB - Potato -
CELLULOSE Filter Paper + CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN -

PROTEINS
Tryptone - Gelatin liq. - Skim milk lysis - Haemolysis no growth
Casamino A use ammonif. Casitone use ammonif.
Casein lysis ? ammonif. - Penassay use ammonif.

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ $NO_2 \rightarrow$ gas or ? NO_3 as e^- acceptor Cystein $\rightarrow H_2S$
Indole - Phosphatase no growth Catalase + Oxidase +

N SOURCES
NO_3 NH_4 + Urea Glutamate Asparaginate Tryptone
Casitone Gelatin Casein SM + acetate Prototrophic
Stim. by Y.E. n.g. chitin Needs Y.E. n.g. chitin

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D HS NO_2 Polymyxin B R S
Chloramphenicol S Dihydrostrep. I Penicillin G (S) R
SLS growth reduced .01 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis - Chlorella - Actino 32 - Actino 41 -
Yeast - Rhizopus - Penicillium - Sclerotinia -
ng

PROFILE OF ORGANISM: *CYTOPHAGA HUTCHINSONII* NCIB 10782 (C.H.)

CELLS
Length 1-6 μ Shape Rods Arrangement Single
Width 0.1 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form			Punctiform
Surface			Smooth
Edge			Entire
Elevation			Raised
Opt. Props.			Translucent
Colour			7.5 YR 6/12

GENERAL WSP - Subsurface - Slime Layers - Spreading R

LIQUID MEDIUM
Turbidity ++ Flocculence granules from paper Silky -
Viscous - Ring on paper only Colour YC

PHYSIOLOGY
TEMP°C Min. 20 Opt. 20-30 Max. 35
PH Min. Opt. Max.
O_2 Growth Anaerobic - Candle Jar + Air + Best Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 2%

CHO
SUGARS OF Glucose O Glucose + Sucrose - Lactose- Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper + CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN -

PROTEINS
Tryptone - Gelatin liq. - Skim milk lysis - Haemolysis no growth
Casamino A use ammonif. Casitone use ammonif.
Casein lysis ? ammonif. - Penassay use ammonif.

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ $NO_2 \rightarrow$ gas or ? NO_3 as e^- acceptor Cystein$\rightarrow H_2S$
Indole - Phosphatase ? Catalase Oxidase +

N SOURCES
NO_3 NH_4 + Urea ?+ Glutamate Asparaginate Tryptone
Casitone Gelatin Casein SM + acetate Prototrophic
Stim. by Y.E. ng. chitin Needs Y.E. ng. chitin

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D H S NO_2 Polymyxin B R R
Chloramphenicol S Dihydrostrep. S Penicillin G I
SLS growth reduced growth inhibited

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis - Chlorella - Actino 32 - Actino 41 -
Yeast - ng Rhizopus - Penicillium - Sclerotinia -

PROFILE OF ORGANISM: 2 *(CYTOPHAGA BRUNESCENS)*

CELLS
Length 2-20 μ Shape Rods Arrangement Single
Width 0.3 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Rough	Rough	Rough
Edge	Lobate	Lobate	Undulate
Elevation	Effuse	Effuse/Convex	Effuse/Umbonate
Opt. Props.	Transparent	Transparent	Translucent
Colour	2.5 Y 4/6 WSP ±	10 YR 4/6 +	2.5 Y 3/4 +

GENERAL WSP almost always Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous - Ring + Pellicle Colour Dirty OY

PHYSIOLOGY

TEMP°C	Min. 15	Opt. 40	Max. 40
PH	Min. 5	Opt. 5-10	Max. 10

O_2 Growth Anaerobic - Candle Jar + Air + Best Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 2%

CHO
SUGARS OF Glucose $O^{?Slow}_{+F}$ Glucose + Sucrose - Lactose - Cb. -
ALCOHOLS Glycerol - Mannitol - STARCH SSY + NB ?+ Potato +
CELLULOSE Filter Paper - CMC - AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis -
Casamino A use + ammonif. - Casitone use + ammonif. +
Casein lysis + ammonif. - Penassay use + ammonif. -

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ n.g. $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea - Glutamate - Asparaginate - Tryptone +
Casitone - Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. + glu - chitin Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES

Actinomycin D	S	NO_2 +	Polymyxin B I S
Chloramphenicol	S	Dihydrostrep. S	Penicillin G S
SLS growth reduced	.01	growth inhibited	.1

PREDATION

E. coli +	Ps.aeruginosa +	Arthrobacter +	S.marcescens -
B.subtilis +	Chlorella +	Actino 32 -	Actino 41 +
Yeast ±	Rhizopus -	Penicillium +	Sclerotinia +

PROFILE OF ORGANISM: D *(CYTOPHAGA BRUNESCENS)*

CELLS
Length 2-11 μ Shape Rods Arrangement Single
Width 0.3 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Rough	Rough	Rough
Edge	Lobate	Undulate	Undulate
Elevation	Effuse	Effuse/Raised	Effuse/Umbonate
Opt. Props.	Transparent	Transparent	Translucent
Colour	2.5 Y 4/6	10 YR 4/6 ±	2.5 Y 3/4 +

GENERAL WSP almost always Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous - Ring + or Pellicle Colour Brownish OY

PHYSIOLOGY
TEMP°C Min. 15 Opt. 30-35 Max. 35
PH Min. 5 Opt. 5-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 2%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose - Lactose - Cb -
ALCOHOLS Glycerol - Mannitol - STARCH SSY + NB ?+ Potato +
CELLULOSE Filter Paper - CMC - AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis -
Casamino A use + ammonif. - Casitone use + ammonif. +
Casein lysis + ammonif. - Penassay use + ammonif. -

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ ng. $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
$NO_3 + NH_4$ + Urea - Glutamate - Asparaginate - Tryptone +
Casitone - Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. ±glu ±chitin Needs Y.E. - glu + chitin

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 + Polymyxin B I S
Chloramphenicol S Dihydrostrep. S Penicillin G S
SLS growth reduced .01 growth inhibited .1

PREDATION
E.coli + Ps.aeruginosa + Arthrobacter + S.marcescens -
B.subtilis + Chlorella + Actino 32 ? Actino 41 +
Yeast ± Rhizopus + Penicillium + Sclerotinia +

PROFILE OF ORGANISM: 6 *(CYTOPHAGA BRUNESCENS)*

CELLS
Length 2-15 μ Shape Rods Arrangement Single
Width 0.2-0.5 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	
Surface	Rough	Rough	
Edge	Erose	Erose	
Elevation	Effuse	Effuse	
Opt. Props.	Transparent	Transparent	
Colour	5 Y 3/4	2.5 Y 4.5/6 +	

GENERAL WSP almost always + Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous - Ring + or Pellicle Colour COY

PHYSIOLOGY
TEMP°C Min. 20 Opt. 40 Max. 40
PH Min. 6 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition <1% Total inhibition 1%

CHO
SUGARS OF Glucose no growth Glucose + slow Sucrose - Lactose - Cb. -
ALCOHOLS Glycerol - Mannitol - STARCH SSY + NB ?+ Potato +
CELLULOSE Filter Paper - CMC - AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis no growth
Casamino A use + ammonif. - Casitone use + ammonif. +
Casein lysis + ammonif. - Penassay use + ammonif. -

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ ng. $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
$NO_3 - NH_4$ + slow Urea - Glutamate - Asparaginate - Tryptone +
Casitone - Gelatin + Casein + SM + acetate + Prototrophic
Stim. by Y.E. ± glu chitin Needs Y.E. + chitin

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 + Polymyxin B I S
Chloramphenicol S Dihydrostrep. S Penicillin G I
SLS growth reduced .01 growth inhibited .1

PREDATION
E. coli - Ps. aeruginosa - Arthrobacter ? S. marcescens -
B. subtilis + Chlorella + Actino 32 - Actino 41 +
Yeast ± Rhizopus - Penicillium - Sclerotinia -

PROFILE OF ORGANISM: 4541 *(CYTOPHAGA BRUNESCENS)*

CELLS

Length	1-21 μ	Shape	Rods	Arrangement	Single
Width	0.3-0.5 μ	Motility	-	Flexing +	Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Rough	Rough	Rough
Edge	Undulate	Erose	Erose
Elevation	Effuse/Convex	Effuse/Convex	Effuse/Convex
Opt. Props.	Transparent	Translucent	Translucent
Colour 10 YR	5/8 WSP-	7.5 YR 4/8 ±	10 YR 5/8 no wsp to 10 YR 4/6 + wsp

GENERAL WSP almost always Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM

Turbidity	++	Flocculence	-	Silky	+
Viscous	-	Ring	+	Colour	Dirty OY

PHYSIOLOGY

TEMP°C	Min. 15	Opt. 40		Max. 40
PH	Min. 5	Opt. 7-10		Max. 10

O_2 Growth Anaerobic - Candle Jar + Air + Best Air

NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 2%

CHO

SUGARS OF Glucose O Glucose + Sucrose - Lactose - Cb.-

ALCOHOLS Glycerol - Mannitol - STARCH SSY + NB ?+ Potato +

CELLULOSE Filter Paper - CMC - AGAR Pits - Gelase +

POLYPECTATE - ALGINATE - CHITIN +

PROTEINS

Tryptone	+	Gelatin liq.	+	Skim milk lysis	+	Haemolysis	-
Casamino A use	+	ammonif.	-	Casitone use	+	ammonif.	+
Casein lysis	+	ammonif.	-	Penassay use	+	ammonif.	-

BIOCHEMISTRY

$NO_3 \rightarrow NO_2$ ng $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein$\rightarrow H_2S$ +

Indole - Phosphatase + Catalase + Oxidase +

N SOURCES

NO_3 + NH_4 + Urea - Glutamate + Asparaginate + Tryptone +

Casitone + Gelatin + Casein + SM + acetate + Prototrophic +

Stim. by Y.E. - Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES

Actinomycin D	S	NO_2	+	Polymyxin B	I S
Chloramphenicol	S	Dihydrostrep.	I	Penicillin G	I
SLS growth reduced	<.1	growth inhibited	.1		

PREDATION

E.coli	+	Ps.aeruginosa	+	Arthrobacter	+	S.marcescens	-
B.subtilis	+	Chlorella	+	Actino 32	-	Actino 41	+
Yeast	+	Rhizopus	?	Penicillium	+	Sclerotinia	-

PROFILE OF ORGANISM: PC 15 *(MYXOCOCCUS)*

CELLS
Length 2-4 μ Shape Rods Arrangement Single
Width 0.6 μ Motility - Flexing - Gram -

COLONIES	#1.	SA.	PC.
Form	Circular	Circular	Circular
Surface	Smooth	Smooth	Smooth
Edge	Erose	Entire	Entire
Elevation	Raised	Raised	Convex
Opt. Props.	Transparent	Opaque	Opaque
Colour	2.5 YR 6.5/4	much redder than 2.5 YR 4/10	redder than 2.5 YR 4/10

GENERAL WSP - Subsurface - Slime Layers - Spreading (S)

LIQUID MEDIUM
Turbidity + Flocculence - Silky -
Viscous - Ring Colour v.pale pink

PHYSIOLOGY
TEMP°C Min. 10 Opt. 25 Max. 30
PH Min. 5 Opt. 5-8 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose no action Glucose ? Sucrose - Lactose - Cb.-
ALCOHOLS Glycerol - Mannitol - STARCH SSY + NB + Potato
CELLULOSE Filter Paper - CMC ?- AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis no growth
Casamino A use + ammonif. - Casitone use + ammonif. +
Casein lysis + ammonif. - Penassay use + ammonif. -

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor + Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase ? Oxidase +

N SOURCES
NO_3 - NH_4 ? Urea - Glutamate - Asparaginate - Tryptone -
Casitone + Gelatin + Casein + SM + acetate + Prototrophic
Stim. by Y.E. + Needs Y.E. - chitin

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 + Polymyxin B R I
Chloramphenicol S Dihydrostrep. I Penicillin G I
SLS growth reduced <.01 growth inhibited .01

PREDATION
E.coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis - Chlorella - Actino 32 - Actino 41 -
Yeast - Rhizopus - Penicillium - Sclerotinia ?

PROFILE OF ORGANISM: 9-11 (sp.?)

CELLS
Length 1-5 μ Shape Rods Arrangement Single
Width 0.7 μ Motility - Flexing - Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	≃Circular
Surface	Rough	Rough	Smooth
Edge	Erose	Erose	Entire
Elevation	Effuse	Effuse	Pulvinate
Opt. Props.	Transparent	Transparent	Opaque
Colour	Pinker than 2.5 YR 6/7 WSP -	Redder than 2.5 YR 4/8-	Redder than 2.5 YR 4/10-
GENERAL	WSP- Subsurface +	Slime Layers -	Spreading S

LIQUID MEDIUM
Turbidity + Flocculence - Silky-
Viscous - Ring Colour V.pale pink

PHYSIOLOGY
TEMP°C Min. 10 Opt. 20 Max. 30
PH Min. 6 Opt. 6-9 Max. 10
O_2 Growth Anaerobic Candle Jar Air Best
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 2%

CHO
SUGARS OF Glucose No action Glucose ?+ Sucrose - Lactose - Cb.-
ALCOHOLS Glycerol - Mannitol - STARCH SSY + NB + Potato ?+
CELLULOSE Filter Paper- CMC ?- AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN -

PROTEINS
Tryptone + Gelatin liq. ? Skim milk lysis + Haemolysis no growth
Casamino A use + ammonif. - Casitone use + ammonif. +
Casein lysis + ammonif. - Penassay use + ammonif. -

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor + Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 - NH_4 ? Urea - Glutamate - Asparaginate - Tryptone +
Casitone + Gelatin ?- Casein ?+ SM + acetate +
Prototrophic Stim. by Y.E. + Needs Y.E. + chitin

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D NO_2 - Polymyxin B R S
Chloramphenicol S Dihydrostrep. R Penicillin G S
SLS growth reduced <.01 growth inhibited .01

PREDATION
E. coli - Ps.aeruginosa- Arthrobacter - S.marcescens -
B.subtilis - Chlorella - Actino 32 - Actino 41 -
Yeast ± weak Rhizopus - Penicillium - Sclerotinia -

PROFILE OF ORGANISM: 9D *(FLEXIBACTER CANADENSIS)*

CELLS

Length	3-60 μ	Shape	Rods	Arrangement	Single
Width	0.3 μ	Motility	-	Flexing +	Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Rough	Rough	Smooth
Edge	Lobate	Lobate	Lobate
Elevation	Effuse	Effuse	Effuse/Raised
Opt. Props.	Transparent	Translucent	Translucent/Opaque
Colour	2.5 YR 8/2	10 YR 8/2	2.5 YR 6/3

GENERAL WSP - Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM

Turbidity	++	Flocculence	-	Silky	+
Viscous	-	Ring	-	Colour	White

PHYSIOLOGY

TEMP°C	Min. 10	Opt. 18-30	Max. 40	
PH	Min. 5	Opt. 6-8	Max. 10	

O_2 Growth Anaerobic - Candle Jar + Air + Best Candle

NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 2%

CHO

SUGARS OF Glucose F Glucose + Sucrose - Lactose + Cb. ?+ slow

ALCOHOLS Glycerol + Mannitol - STARCH SSY + NB + Potato ?

CELLULOSE Filter Paper - CMC - AGAR Pits - Gelase +

POLYPECTATE ?+ ALGINATE - CHITIN -

PROTEINS

Tryptone +	Gelatin liq. +	Skim milk lysis +	Haemolysis -
Casamino A use +	ammonif. +	Casitone use +	ammonif. +
Casein lysis +	ammonif. -	Penassay use +	ammonif. +

BIOCHEMISTRY

$NO_3 \rightarrow NO_2$ +	$NO_2 \rightarrow$ gas or ? +	NO_3 as e^- acceptor ++	Cystein $\rightarrow H_2S$ +
Indole -	Phosphatase +	Catalase +	Oxidase +

N SOURCES

$NO_3 - NH_4$ +	Urea -	Glutamate -	Asparaginate -	Tryptone +
Casitone +	Gelatin +	Casein +	SM + acetate +	Prototrophic -
Stim. by Y.E. + glu - chitin	Needs Y.E. +			

ANTIBIOTIC etc. SENSITIVITIES

Actinomycin D S	NO_2 -	Polymyxin B	R S
Chloramphenicol S	Dihydrostrep. S	Penicillin G	R
SLS growth reduced <.1	growth inhibited .1		

PREDATION

E. coli -	Ps. aeruginosa -	Arthrobacter -	S. marcescens -
B. subtilis -	Chlorella ?	Actino 32 -	Actino 41 ?
Yeast -	Rhizopus -	Penicillium -	Sclerotinia -

PROFILE OF ORGANISM: 495 *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 2-30 μ Shape Rods Arrangement Single
Width 0.5 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Rough	Smooth	Smooth
Edge	Undulate	Erose	Erose
Elevation	Effuse/Flat	Effuse/Raised	Effuse/Convex
Opt. Props.	Transparent	Translucent	Translucent
Colour	2.5 Y 6/6	2.5 Y 6.5/6	2.5 Y 5/4
WSP	-	-	-

GENERAL WSP - Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring + or Pellicle Colour Offwhite

PHYSIOLOGY

TEMP°C	Min. 10	Opt. 35	Max. 35
PH	Min. 5	Opt. 5-10	Max. 10

O_2 Growth Anaerobic - Candle Jar + Air + Best Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose O Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY- NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis ++
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ?- NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 - NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. + Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 + Polymyxin B I S
Chloramphenicol S Dihydrostrep. R Penicillin G R
SLS growth reduced .01 growth inhibited .1

PREDATION
E.coli - Ps.aeruginosa ? Arthrobacter + S.marcescens -
B.subtilis + Chlorella + Actino 32 + Actino 41 +
Yeast ± Rhizopus + Penicillium + Sclerotinia -

PROFILE OF ORGANISM: AL-1 (i) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-20 μ Shape Rods Arrangement Single
Width 0.3 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Rough/Smooth	Smooth	Smooth
Edge	Lobate	Erose	Entire
Elevation	Raised	Effuse/Raised	Convex
Opt. Props.	Translucent	Translucent	Translucent
Colour	5 YR 8/4 WSP -	5 Y 7.5/4 -	2.5 Y 5/6 +wsp 5 Y 6/6 no wsp

GENERAL WSP often Subsurface - Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring Colour C

PHYSIOLOGY
TEMP°C Min. 5 Opt. 30 Max. 35
PH Min. 7 Opt. 7-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 1% Partial inhibition 2% Total inhibition 3%

CHO
SUGARS OF Glucose No action Glucose ?+ Sucrose - Lactose ?+ slow Cb ?+ slow
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE ?- ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis ++
Casamino A use + ammonif. + Casitone use + ammonif. -
Casein lysis + ammonif. + Penassay use + ammonif. -

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor + Cystein $\rightarrow H_2S$ -
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 - NH_4 - Urea - Glutamate + Asparaginate + Tryptone +
Casitone ?+ Gelatin + Casein + SM + acetate + Prototrophic
Stim. by Y.E. + Needs Y.E. - chitin

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B R S
Chloramphenicol R Dihydrostrep. R Penicillin G R
SLS growth reduced .01 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa ? Arthrobacter + S.marcescens -
B.subtilis + Chlorella + Actino 32 + Actino 41 +
Yeast + Rhizopus + Penicillium + Sclerotinia +

PROFILE OF ORGANISM: AL-1 (ii) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-7 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Smooth	Smooth	Smooth
Edge	Undulate	Entire	Entire
Elevation	Raised	Convex	Convex
Opt. Props.	Translucent	Opaque	Translucent
Colour	5 Y 7/6 WSP -	5 Y 7.5/4 -	5 Y 6.5/6 no wsp = 5 Y 7/6 + wsp

GENERAL WSP often Subsurface - Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring Colour C

PHYSIOLOGY
TEMP°C Min. 5 Opt. 30 Max. 35
PH Min. 6 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose no action Glucose ?+ Sucrose - Lactose ?+ slow Cb ?+ slow
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE ?- ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis +
Casamino A use + ammonif. + Casitone use + ammonif. -
Casein lysis + ammonif. + Penassay use + ammonif. -

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ -
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 - Urea - Glutamate + Asparaginate + Tryptone +
Casitone ?+ Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. - glu + chitin Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B I S
Chloramphenicol R Dihydrostrep. R Penicillin G R
SLS growth reduced .01 growth inhibited .1

PREDATION
E.coli + Ps.aeruginosa - Arthrobacter + S.marcescens -
B.subtilis + Chlorella + Actino 32 + Actino 41 +
Yeast + Rhizopus + Penicillium + Sclerotinia +

PROFILE OF ORGANISM: AL-1 (iii) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-8 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Irregular
Surface	Smooth	Smooth	Smooth
Edge	Erose	Undulate	Entire
Elevation	Effuse/Raised	Raised	Convex
Opt. Props.	Transparent	Translucent	Translucent
Colour	5 Y 8/5 WSP -	5 Y 8/4 -	5 Y 6/6 no wsp -2.5 Y 5/6 + wsp

GENERAL WSP often Subsurface - Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring Colour C

PHYSIOLOGY
TEMP°C Min. 5 Opt. 30 Max. 35
PH Min. 6 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose No action Glucose ?+ Sucrose - Lactose ?+ slow Cb ?+ slow
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis ++
Casamino A use + ammonif. + Casitone use + ammonif. -
Casein lysis + ammonif. + Penassay use + ammonif. -

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ -
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 - NH_4 - Urea - Glutamate + Asparaginate + Tryptone +
Casitone ?+ Gelatin + Casein + SM + acetate + Prototrophic
Stim. by Y.E. + Needs Y.E. - chitin

ANTIBIOTIC etc. SENSITIVITIES

Actinomycin D	S	NO_2 -	Polymyxin B R S
Chloramphenicol	(?S) R	Dihydrostrep. R	Penicillin G R
SLS growth reduced	<.1	growth inhibited	.1

PREDATION

E. coli -	Ps.aeruginosa ?	Arthrobacter +	S.marcescens -
B.subtilis +	Chlorella +	Actino 32 +	Actino 41 +
Yeast +	Rhizopus +	Penicillium +	Sclerotinia +

PROFILE OF ORGANISM: *CYTOPHAGA JOHNSONAE* ATCC 21123 *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-9 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	≃ Circular	≃ Circular	≃ Circular
Surface	Smooth/Rough	Smooth	Smooth
Edge	Erose	Erose	Entire
Elevation	Effuse/Raised	Effuse/Convex	Convex
Opt. Props.	Transparent	Translucent	Transparent
Colour	5Y 7/6	2.5Y 7.5/6	2.5Y 5/6

GENERAL WSP - Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring Colour Dirty LC

PHYSIOLOGY
TEMP°C Min. 10 Opt. 30-35 Max. 35
PH Min. 5 Opt. 5-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Air
NaCl Good growth up to 2% Partial inhibition 3% Total inhibition >3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE ?+ ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis ++
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor + Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea + Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. + Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B RS
Chloramphenicol I Dihydrostrep. R Penicillin G R
SLS growth reduced .1 growth inhibited >.1

PREDATION
E.coli - Ps.aeruginosa - Arthrobacter + S.marcescens +
B.subtilis + Chlorella + Actino 32 + Actino 41 +
Yeast + Rhizopus + Penicillium + Sclerotinia +

PROFILE OF ORGANISM: 18 L Y *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-6 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	≏ Circular	≏ Circular	≏ Circular
Surface	Smooth	Rough	Smooth
Edge	Undulate	Erose	Entire
Elevation	Convex	Effuse/Convex	Convex
Opt. Props.	Transparent	Translucent	Translucent
Colour	10 YR 6/8	10 YR 6.5/5 -	2.5 Y 6/6 -

GENERAL WSP SA broth WSP SA+SLS Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous - Ring + Colour Dirty LC

PHYSIOLOGY
TEMP°C Min. 10 Opt. 30 Max. 40
PH Min. 6 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Air
NaCl Good growth up to 1% Partial inhibition 2% Total inhibition >3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol + STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis ++
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor + Cystein $\rightarrow H_2S$ -
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
$NO_3 + NH_4$ + Urea + Glutamate+ Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. + Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D NO_2 - Polymyxin B
Chloramphenicol I Dihydrostrep. R Penicillin G R
SLS growth reduced < .1 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa ? Arthrobacter - S.marcescens -
B.subtilis - Chlorella - Actino 32 + Actino 41 +
Yeast + Rhizopus - Penicillium + Sclerotinia ?

PROFILE OF ORGANISM: 18 L W *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-6 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	≏ Circular	Irregular	≏ Circular
Surface	Smooth	Rough/Smooth	Smooth
Edge	Erose	Erose	Undulate
Elevation	Effuse/Convex	Effuse/Convex	Convex
Opt. Props.	Transparent	Opaque	Opaque
Colour	5 Y 7/4	2.5 Y 7/4	5 Y 6.5/6

GENERAL WSP - WSP sometimes Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring + Colour Dirty Pale Yc

PHYSIOLOGY
TEMP°C Min. 10 Opt. 30 Max. 40
PH Min. 5 Opt. 5-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Air
NaCl Good growth up to 1% Partial inhibition 2% Total inhibition >3%

CHO
SUGARS OF Glucose O Glucose + Sucrose + Lactose + Cb.+
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis ++
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor + Cystein $\rightarrow H_2S$ -
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea + Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. + (?+chitin) Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D NO_2 - Polymyxin B R S
Chloramphenicol (S) Dihydrostrep. R Penicillin G R
SLS growth reduced <.1 growth inhibited .1

PREDATION
E.coli - Ps.aeruginosa ? Arthrobacter + S.marcescens +
B.subtilis + Chlorella + Actino 32 + Actino 41 +
Yeast + Rhizopus - Penicillium + Sclerotinia +

PROFILE OF ORGANISM: 4553 (i) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-4 μ Shape Rods Arrangement Single
Width 0.4-0.5 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	≐ Circular	≐ Circular	Circular
Surface	Smooth	Smooth	Smooth
Edge	Undulate	Entire	Erose
Elevation	Raised	Convex	Convex
Opt. Props.	Translucent	Translucent	Opaque
Colour	5 Y 7/4	5 Y 8/4 -	2.5 Y 6/6 -

GENERAL WSP often Subsurface - Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence ± Silky +
Viscous + Ring + or Pellicle Colour Offwhite

PHYSIOLOGY
TEMP°C Min. 10 Opt. 30 Max. 35
PH Min. 5 Opt. 5-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 1% Partial inhibition 2% Total inhibition >3%

CHO
SUGARS OF Glucose O Glucose+ Sucrose + Lactose + Cb.+
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis ++
Casamino A use + ammonif. + Casitone use+ ammonif. +
Casein lysis + ammonif. + Penassay use+ ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. + glu - Chitin Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B R S
Chloramphenicol R Dihydrostrep. R Penicillin G R
SLS growth reduced < .1 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter + S.marcescens +
B.subtilis + Chlorella + Actino 32 + Actino 41 +
Yeast + Rhizopus + Penicillium + Sclerotinia ?

PROFILE OF ORGANISM: 4553 (ii) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1.5-3 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	Circular
Surface	Rough	Rough	Smooth
Edge	Undulate	Undulate	Entire
Elevation	Effuse/Raised	Effuse/Raised	Convex
Opt. Props.	Transparent	Translucent	Translucent
Colour	10 YR 6/6	7.5 YR 5.5/6	2.5 Y 6/6

WSP - Subsurface - Slime Layers - Spreading S
GENERAL WSP often

LIQUID MEDIUM
Turbidity ++ Flocculence ± Silky +
Viscous + Ring + Colour C

PHYSIOLOGY
TEMP°C Min. 10 Opt. 25 Max. 35
PH Min. 6 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 1% Partial inhibition 2% Total inhibition > 3%

CHO
SUGARS OF Glucose O Glucose + Sucrose + Lactose + Cb.+
ALCOHOLS Glycerol - Mannitol - STARCH SSY- NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE+ CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis +
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$?+
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
$NO_3 + NH_4$ + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. + glu - Chitin Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B I S
Chloramphenicol R Dihydrostrep. R Penicillin G R
SLS growth reduced < .1 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter - S.marcescens +
B.subtilis - Chlorella + Actino 32 ? Actino 41 +
Yeast + Rhizopus + Penicillium ? Sclerotinia +

PROFILE OF ORGANISM: 4554 *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 2-5 μ Shape Rods Arrangement Single
Width 0.5 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	≙ Circular	≙ Circular	Circular
Surface	Smooth	Smooth	Smooth
Edge	Undulate	Erose	Undulate
Elevation	Effuse/Raised	Effuse/Raised	Convex
Opt. Props.	Translucent	Translucent	Opaque
Colour	2.5 Y 7/6	10 YR 6/6	2.5 Y 6/6

GENERAL WSP often WSP - Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring Pellicle Colour Offwhite

PHYSIOLOGY
TEMP°C Min. 10 Opt. 35 Max. 40
PH Min. 5 Opt. 5-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 1% Partial inhibition 2% Total inhibition > 3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose+ Cb.+
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis ++
Casamino A use + ammonif.+ Casitone use + ammonif. +
Casein lysis + ammonif.+ Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3+ NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. - Needs Y.E.-

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2^- Polymyxin B R S
Chloramphenicol (S) Dihydrostrep. R Penicillin G R
SLS growth reduced < .1 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter + S.marcescens +
B.subtilis + Chlorella + Actino 32 + Actino 41 +
Yeast + Rhizopus + Penicillium + Sclerotinia +

PROFILE OF ORGANISM: 4555 (i) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-3 μ Shape Rods Arrangement Single
Width 0.5 μ Motility - Flexing - Gram -

COLONIES	#1.	SA.	PC.
Form	Circular	Circular	Circular
Surface	Smooth	Smooth	Smooth
Edge	Entire	Entire	Entire
Elevation	Effuse/Raised	Convex	Convex
Opt. Props.	Translucent	Opaque	Opaque
Colour	5 Y 7/4 WSP -	7.5 Y 8/4 -	5Y 7/5 no wsp 5Y 6/6 + wsp

GENERAL WSP often Subsurface - Slime Layers + Spreading (S)

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring Pellicle Colour Offwhite

PHYSIOLOGY
TEMP°C Min. 10 Opt. 25 Max. 40
PH Min. 5 Opt. 5-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 1% Partial inhibition 2% Total inhibition 3%

CHO
SUGARS OF Glucose 0 Glucose+ Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis+ Haemolysis ++
Casamino A use + ammonif. + Casitone use + ammonif.+
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$?+ $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase+

N SOURCES
NO_3+ NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate+ Prototrophic +
Stim. by Y.E. - Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B R S
Chloramphenicol S Dihydrostrep. R Penicillin G R
SLS growth reduced <.1 growth inhibited .1

PREDATION
E.coli + Ps.aeruginosa - Arthrobacter + S.marcescens +
B.subtilis + Chlorella + Actino 32 + Actino 41 +
Yeast + Rhizopus + Penicillium + Sclerotinia +

PROFILE OF ORGANISM: 4555 (ii) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 2-6 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	≃Circular	Circular	Circular
Surface	Smooth	Smooth	Smooth
Edge	Undulate	Entire	Entire
Elevation	Effuse/Flat	Convex	Convex
Opt. Props.	Translucent	Opaque	Opaque
Colour	5 Y 7.5/6	WSP - 5 Y 7.5/4 -	5 Y 7/4 -

GENERAL WSP often Subsurface - Slime Layers + Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring + Colour Offwhite

PHYSIOLOGY
TEMP°C Min. 10 Opt. 25 Max. 35
PH Min. 5 Opt. 5-10 Max. 10
O_2 Growth Anaerobic - Candle Jar+ Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose+ Cb.+
ALCOHOLS Glycerol - Mannitol - STARCH SSY- NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE ?+ ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq.+ Skim milk lysis + Haemolysis ++
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$?+ $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein$\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3+ NH_4 + Urea - Glutamate+ Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic+
Stim. by Y.E. ± glu - chitin Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B R S
Chloramphenicol (S) Dihydrostrep.R Penicillin G R
SLS growth reduced <.1 growth inhibited .1

PREDATION
E.coli - Ps.aeruginosa - Arthrobacter+ S.marcescens ?
B.subtilis + Chlorella + Actino 32 + Actino 41 +
Yeast + Rhizopus + Penicillium + Sclerotinia +

PROFILE OF ORGANISM: 4556 (i) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-5 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	≏Circular	≏Circular	Circular
Surface	Smooth	Smooth	Smooth
Edge	Erose	Erose	Entire
Elevation	Effuse/Raised	Raised	Convex
Opt. Props.	Translucent	Opaque	Opaque
Colour	5 Y 7/6	3 Y 7/4 -	5 Y 6.5/6 -

GENERAL WSP often WSP- Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous - Ring + Colour C

PHYSIOLOGY
TEMP°C Min. 5 Opt. 30-35 Max. 35
PH Min. 5 Opt. 5-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE ?+ ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis ++
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$?+ $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. + glu - chitin Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B R S
Chloramphenicol R Dihydrostrep. R Penicillin G R
SLS growth reduced .1 growth inhibited .1

PREDATION
E.coli ? Ps.aeruginosa ? Arthrobacter + S.marcescens +
B.subtilis + Chlorella + Actino 32 + Actino 41 +
Yeast + Rhizopus + Penicillium + Sclerotinia +

PROFILE OF ORGANISM: 4556 (ii) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1.5-4 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing - Gram -

COLONIES	#1.	SA.	PC.
Form	Circular	Circular	Circular
Surface	Smooth	Rough	Rough
Edge	Erose	Erose	Erose
Elevation	Flat	Flat	Raised
Opt. Props.	Transparent	Translucent	Translucent
Colour	2.5 Y 7/8	7.5 YR 6/6 -	2.5 Y 6/6 -

GENERAL WSP sometimes Subsurface - Slime Layers - Spreading (S)

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring + or Pellicle Colour C

PHYSIOLOGY
TEMP°C Min. 10 Opt. 30 Max. 35
PH Min. 6 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 1% Partial inhibition 2% Total inhibition 3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE ?+ ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis +
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$?+
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
$NO_3 + NH_4$ + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. - Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B I S
Chloramphenicol R Dihydrostrep. R Penicillin G R
SLS growth reduced .1 growth inhibited > .1

PREDATION
E.coli + Ps.aeruginosa - Arthrobacter - S.marcescens +
B.subtilis - Chlorella + Actino 32 - Actino 41 +
Yeast + Rhizopus + Penicillium ? Sclerotinia ?

PROFILE OF ORGANISM: 4557 (i) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-5 μ Shape Rods Arrangement Single
Width 0.3-0.5 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	≏Circular	≏Circular	Circular
Surface	Smooth	Rough	Smooth
Edge	Erose	Undulate	Entire
Elevation	Effuse/Raised	Effuse/Convex	Convex
Opt. Props.	Translucent	Opaque	Opaque
Colour	5 Y 7/4	5 Y 8/4 -	5 Y 6.5/5 ±

GENERAL WSP often Subsurface - Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring + Colour Offwhite

PHYSIOLOGY
TEMP°C Min. 5 Opt. 30 Max. 35
PH Min. 5 Opt. 5-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose 0 Glucose+ Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY- NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE + CHITIN+

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis ++
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. ± glu chitin Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B I S
Chloramphenicol S Dihydrostrep. R Penicillin G R
SLS growth reduced <.1 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter + S.marcescens +
B.subtilis + Chlorella + Actino 32 + Actino 41 +
Yeast + Rhizopus + Penicillium + Sclerotinia +

PROFILE OF ORGANISM: 4557 (ii) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 2-5 μ Shape Rods Arrangement Single
Width 0.5 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Punctiform	Circular	Circular
Surface	Smooth	Rough	Smooth
Edge	Entire	Undulate	Entire
Elevation	Flat	Raised	Convex
Opt. Props.	Transparent	Translucent	Opaque
Colour	10 YR 6/8	10 YR 6/6 -	2.5 Y 6/6 -

GENERAL WSP often Subsurface - Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence + Silky +
Viscous + Ring + Colour C

PHYSIOLOGY
TEMP°C Min. 10 Opt. 30 Max. 35
PH Min. 6 Opt. 7-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose 0 Glucose+ Sucrose+ Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis -
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. - Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B I S
Chloramphenicol R Dihydrostrep. R Penicillin G R
SLS growth reduced .1 growth inhibited >.1

PREDATION
E.coli + Ps.aeruginosa - Arthrobacter - S.marcescens +
B.subtilis - Chlorella + Actino 32 - Actino 41 +
Yeast ± Rhizopus - Penicillium + Sclerotinia -

PROFILE OF ORGANISM: 4558 (i) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 2-6 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	≏ Circular	≏ Circular	Circular
Surface	Smooth	Smooth	Smooth
Edge	Undulate	Undulate	Entire
Elevation	Effuse/Flat	Raised	Convex
Opt. Props.	Translucent	Opaque	Opaque
Colour	4 Y 6.5/4	WSP - 5 Y 7.5/4 -	5 Y 5.5/4 ±

GENERAL WSP often Subsurface - Slime Layers + Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring Pellicle Colour Offwhite

PHYSIOLOGY
TEMP°C Min. 5 Opt. 30 Max. 40
PH Min. 6 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC+ AGAR Pits - Gelase +
POLYPECTATE - ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis ++
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ -
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. - Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B R I
Chloramphenicol R Dihydrostrep. R Penicillin G R
SLS growth reduced <.1 growth inhibited .1

PREDATION
E. coli - Ps. aeruginosa - Arthrobacter + S. marcescens +
B. subtilis + Chlorella + Actino 32 + Actino 41 +
Yeast + Rhizopus + Penicillium + Sclerotinia ?

PROFILE OF ORGANISM: 4558 (ii) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-4 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing - Gram -

COLONIES	#1.	SA.	PC.
Form	Circular	Circular	Circular
Surface	Smooth	Rough	Smooth
Edge	Erose	Undulate	Erose
Elevation	Effuse/Flat	Raised	Raised
Opt. Props.	Translucent	Translucent	Opaque
Colour	10 YR 6/6	10 YR 7/6	2.5 Y 6/6

GENERAL WSP often WSP - Subsurface - Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous ± Ring + Colour C

PHYSIOLOGY

TEMP°C	Min. 10	Opt. 35	Max. 40
PH	Min. 7	Opt. 7-10	Max. 10

O_2 Growth Anaerobic - Candle Jar + Air + Best Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb.+
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE + CHITIN +

PROTEINS

Tryptone +	Gelatin liq. +	Skim milk lysis +	Haemolysis +
Casamino A use +	ammonif. +	Casitone use +	ammonif. +
Casein lysis +	ammonif. +	Penassay use +	ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. - Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES

Actinomycin D S	NO_2 -	Polymyxin B I S
Chloramphenicol R	Dihydrostrep. R	Penicillin G R
SLS growth reduced .01	growth inhibited .1	

PREDATION

E. coli -	Ps.aeruginosa -	Arthrobacter -	S.marcescens +
B.subtilis -	Chlorella +	Actino 32 ?	Actino 41 ?
Yeast +	Rhizopus ?	Penicillium ?	Sclerotinia -

PROFILE OF ORGANISM: 4559 (i) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-6 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Circular	≏Circular	Circular
Surface	Smooth	Rough	Smooth
Edge	Erose	Undulate	Entire
Elevation	Effuse/Flat	Raised	Convex
Opt. Props.	Translucent	Opaque	Opaque
Colour	5 Y 7/5	2.5 Y 7.5/4 -	4 Y 7/6 -

GENERAL WSP sometimes Subsurface - Slime Layers + Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous - Ring Pellicle Colour Offwhite

PHYSIOLOGY
TEMP°C Min. 10 Opt. 35 Max. 40
PH Min. 6 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper- CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq.+ Skim milk lysis + Haemolysis ++
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein$\rightarrow H_2S$ +
Indole - Phosphatase + Catalase+ Oxidase +

N SOURCES
NO_3+ NH_4 + Urea- Glutamate + Asparaginate+ Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. + (+? chitin) Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B R S
Chloramphenicol R Dihydrostrep. R Penicillin G R
SLS growth reduced <.1 growth inhibited .1

PREDATION
E.coli + Ps.aeruginosa - Arthrobacter + S.marcescens ?
B.subtilis + Chlorella + Actino 32 + Actino 41 +
Yeast + Rhizopus + Penicillium + Sclerotinia ?

PROFILE OF ORGANISM: 4559 (ii) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-5 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Circular	≃ Circular	≃ Circular
Surface	Smooth	Rough	Smooth
Edge	Erose	Undulate	Undulate
Elevation	Effuse/Raised	Raised	Convex
Opt. Props.	Translucent	Translucent	Translucent
Colour 10 YR 7/6	WSP -	10 YR 6.5/5 -	3 Y 6/6 -

GENERAL WSP sometimes Subsurface - Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring + Colour Offwhite

PHYSIOLOGY
TEMP°C Min. 10 Opt. 25-35 Max. 40
PH Min. 6 Opt. 7-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose 0 Glucose+ Sucrose + Lactose + Cb.+
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE ?+ ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis +
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. + (?+ chitin) Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B R S
Chloramphenicol R Dihydrostrep. R Penicillin G R
SLS growth reduced <.1 growth inhibited .1

PREDATION
E.coli - Ps.aeruginosa - Arthrobacter + S.marcescens +
B.subtilis - Chlorella ? Actino 32 ? Actino 41 +
Yeast + Rhizopus + Penicillium + Sclerotinia -

PROFILE OF ORGANISM: 4560 (i) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-7 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Circular	≃Circular	≃Circular
Surface	Smooth	Rough	Smooth
Edge	Undulate	Undulate	Erose
Elevation	Effuse/Raised	Raised	Umbonate
Opt. Props.	Translucent	Opaque	Opaque
Colour	6 Y 7.5/4	10 YR 7/6	2.5 Y 6/8 no wsp -2.5 Y 3/4 + wsp
GENERAL	WSP often	Subsurface +	Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring + Colour Offwhite

PHYSIOLOGY
TEMP°C Min. 10 Opt. 30-35 Max. 40
PH Min. 6 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 1% Partial inhibition 2% Total inhibition > 3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb.+
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase+
POLYPECTATE - ALGINATE+ CHITIN+

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis+ Haemolysis ++
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea - Glutamate + Asparaginate + Tryptone+
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E.+(?+chitin) Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B I S
Chloramphenicol S Dihydrostrep. R Penicillin G R
SLS growth reduced <.1 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter + S.marcescens +
B.subtilis + Chlorella + Actino 32 + Actino 41 +
Yeast + Rhizopus + Penicillium + Sclerotinia +

PROFILE OF ORGANISM: 4560 (ii) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-7 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing - Gram -

COLONIES	#1.	SA.	PC.
Form	Circular	Circular	≃ Circular
Surface	Smooth	Smooth	Smooth
Edge	Entire	Entire	Erose
Elevation	Raised	Convex	Umbonate
Opt. Props.	Translucent	Opaque	Opaque
Colour	5 Y 7.5/6 WSP -	2.5 Y 7.5/6 -	4 Y 6/6 no wsp ≃ 4 Y 4/6 + wsp

GENERAL WSP often Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring + or Pellicle Colour Offwhite

PHYSIOLOGY
TEMP°C Min. 10 Opt. 20 Max. 40
PH Min. 7 Opt. 7-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition >3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE ?- ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq.+ Skim milk lysis + Haemolysis ++
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$?+ $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. + (?+ chitin) Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B I S
Chloramphenicol S Dihydrostrep. R Penicillin G R
SLS growth reduced <.1 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter + S.marcescens +
B.subtilis + Chlorella + Actino 32 + Actino 41 +
Yeast + Rhizopus + Penicillium + Sclerotinia -

PROFILE OF ORGANISM: 4560 (iii) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-5 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing - Gram -

COLONIES	#1.	SA.	PC.
Form	≏ Circular	Circular	Circular
Surface	Smooth	Rough	Smooth
Edge	Erose	Erose	Entire
Elevation	Flat	Flat	Raised
Opt. Props.	Translucent	Transparent	Translucent
Colour	10 YR 6/6	10 YR 6/7 -	2.5 Y 6/6 -

GENERAL WSP once, SA Broth Subsurface - Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence + Silky +
Viscous + Ring Pellicle Colour LC

PHYSIOLOGY
TEMP°C Min. 10 Opt. 25-35 Max. 40
PH Min. 7 Opt. 7-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition >3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE ?- ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis +
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$?+ $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. - glu + chitin Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B I S
Chloramphenicol R Dihydrostrep. R Penicillin G R
SLS growth reduced .1 growth inhibited >.1

PREDATION
E. coli - Ps. aeruginosa ? Arthrobacter - S. marcescens +
B. subtilis - Chlorella ? Actino 32 - Actino 41 ?
Yeast ± Rhizopus - Penicillium ? Sclerotinia -

PROFILE OF ORGANISM: 4561 (1) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-4 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Circular	Circular	Circular
Surface	Smooth	Rough	Smooth
Edge	Erose	Erose	Erose
Elevation	Raised	Convex	Convex
Opt. Props.	Translucent	Opaque	Opaque
Colour	5 Y 7.5/5	5 Y 8.5/4 -	2.5 Y 6/6 -

GENERAL WSP sometimes Subsurface - Slime Layers + Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring + Colour C

PHYSIOLOGY
TEMP°C Min. 10 Opt. 25 Max. 40
PH Min. 6 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis ++
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. ?+ -glu chitin Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B R I
Chloramphenicol R Dihydrostrep. I Penicillin G R
SLS growth reduced <.1 growth inhibited .1

PREDATION
E. coli - Ps. aeruginosa ? Arthrobacter + S. marcescens +
B. subtilis + Chlorella ? Actino 32 + Actino 41 +
Yeast + Rhizopus + Penicillium + Sclerotinia +

PROFILE OF ORGANISM: 4561 (ii) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-4 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	≃ Circular	≃ Circular
Surface	Smooth	Rough	Smooth
Edge	Undulate	Undulate	Erose
Elevation	Effuse/Raised	Effuse/Convex	Convex
Opt. Props.	Translucent	Opaque	Opaque
Colour	5 Y 7.5/6	5 Y 7.5/5 -	5 Y 6/6 -

GENERAL WSP sometimes Subsurface - Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring + Colour C

PHYSIOLOGY
TEMP°C Min. 10 Opt. 35 Max. 35
PH Min. 7 Opt. 7-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis ++
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. ? + glu chitin Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B R S
Chloramphenicol R Dihydrostrep. I Penicillin G R
SLS growth reduced .1 growth inhibited >.1

PREDATION
E.coli + Ps.aeruginosa ? Arthrobacter + S.marcescens ?
B.subtilis + Chlorella - Actino 32 + Actino 41 +
Yeast + Rhizopus + Penicillium + Sclerotinia +

PROFILE OF ORGANISM: 4561 (iii) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-8 μ Shape Rods Arrangement Single
Width 0.3 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	≃ Circular	Circular	Circular
Surface	Smooth	Smooth	Smooth
Edge	Entire	Erose	Entire
Elevation	Flat	Effuse/Raised	Convex
Opt. Props.	Transparent	Translucent	Translucent
Colour	2.5 Y 6/8	10 YR 7/6	2.5 Y 6/8

WSP - Subsurface Slime Layers Spreading
GENERAL WSP -

LIQUID MEDIUM
Turbidity Flocculence Silky
Viscous Ring + Colour

PHYSIOLOGY
TEMP°C Min. Opt. Max. 40
PH Min. Opt. Max.
O_2 Growth Anaerobic Candle Jar Air Best
NaCl Good growth up to Partial inhibition Total inhibition

CHO
SUGARS OF Glucose Glucose Sucrose Lactose Cb.
ALCOHOLS Glycerol Mannitol STARCH SSY NB Potato
CELLULOSE Filter Paper CMC AGAR Pits Gelase
POLYPECTATE ALGINATE CHITIN

PROTEINS
Tryptone Gelatin liq. Skim milk lysis Haemolysis
Casamino A use ammonif. Casitone use + ammonif. +
Casein lysis ammonif. Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor Cystein-> H_2S
Indole Phosphatase Catalase Oxidase

N SOURCES
NO_3 NH_4 Urea Glutamate Asparaginate Tryptone
Casitone + Gelatin Casein SM + acetate Prototrophic
Stim. by Y.E. Needs Y.E.

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B R S
Chloramphenicol S Dihydrostrep. R Penicillin G R
SLS growth reduced growth inhibited

PREDATION
E. coli Ps.aeruginosa Arthrobacter S.marcescens
B.subtilis Chlorella Actino 32 Actino 41
Yeast + Rhizopus Penicillium Sclerotinia

PROFILE OF ORGANISM: 4562 (i) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-7 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	≏ Circular	≏ Circular	≏ Circular
Surface	Smooth	Rough	Smooth
Edge	Undulate	Undulate	Erose
Elevation	Effuse/Flat	Raised	Raised
Opt. Props.	Translucent	Opaque	Translucent
Colour	5 Y 7.5/4	5 Y 7/4 -	5 Y 6/6 -

GENERAL WSP sometimes Subsurface - Slime Layers+ Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring + Colour C

PHYSIOLOGY
TEMP°C Min. 10 Opt. 20-30 Max. 40
PH Min. 7 Opt. 7-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose 0 Glucose+ Sucrose + Lactose + Cb.+
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE ?- ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis ++
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif.+

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor + Cystein $\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3+ NH_4 + Urea - Glutamate + Asparaginate+ Tryptone +
Casitone + Gelatin+ Casein + SM + acetate + Prototrophic+
Stim. by Y.E. - Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2- Polymyxin B R I
Chloramphenicol I Dihydrostrep. R Penicillin G R
SLS growth reduced <.1 growth inhibited .1

PREDATION
E.coli + Ps.aeruginosa ? Arthrobacter - S.marcescens -
B.subtilis + Chlorella - Actino 32 + Actino 41 +
Yeast + Rhizopus + Penicillium + Sclerotinia+

PROFILE OF ORGANISM: 4562 (ii) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-7 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	≏ Circular	Circular	Circular
Surface	Rough/Smooth	Smooth	Rough
Edge	Lobate	Erose	Erose
Elevation	Effuse/Raised	Effuse/Raised	Raised
Opt. Props.	Transparent	Translucent	Translucent
Colour	10 YR 6.5/6 WSP -	8.5 YR 6/6 -	2.5 Y 5/6 -

GENERAL WSP often Subsurface - Slime Layers+ Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring Pellicle Colour C

PHYSIOLOGY
TEMP°C Min. 10 Opt. 25 Max. 40
PH Min. 6 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose 0 Glucose+ Sucrose+ Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE ?- ALGINATE+ CHITIN +

PROTEINS
Tryptone + Gelatin liq.+ Skim milk lysis + Haemolysis +
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis - + ammonif. +Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor + Cystein$\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3+ NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate+ Prototrophic +
Stim. by Y.E. ?+chitin Needs Y.E. - glu

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B R S
Chloramphenicol S Dihydrostrep. R Penicillin G R
SLS growth reduced <.1 growth inhibited .1

PREDATION
E.coli - Ps.aeruginosa ? Arthrobacter - S.marcescens ?
B.subtilis ? Chlorella - Actino 32 + Actino 41 +
Yeast + Rhizopus - Penicillium + Sclerotinia +

PROFILE OF ORGANISM: 4563 (i) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-5 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	≃ Circular	≃ Circular	Circular
Surface	Smooth	Rough	Rough
Edge	Erose	Erose	Entire
Elevation	Convex	Convex	Convex
Opt. Props.	Translucent	Opaque	Opaque
Colour	5 Y 7/4	5 Y 7.5/4 -	5 Y 6/6 -

GENERAL WSP often Subsurface - Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring Pellicle Colour C

PHYSIOLOGY
TEMP°C Min. 10 Opt. 30 Max. 40
PH Min. 6 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE ?- ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis ++
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$?+ $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor + Cystein $\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. ?+ glu chitin Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B R I
Chloramphenicol (S) Dihydrostrep. R Penicillin G R
SLS growth reduced <.1 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter + S.marcescens +
B.subtilis + Chlorella + Actino 32 + Actino 41 +
Yeast + Rhizopus + Penicillium + Sclerotinia +

PROFILE OF ORGANISM: 4563 (ii) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-5 μ Shape Rods Arrangement Single
Width 0.5 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	≃ Circular	≃ Circular	≃ Circular
Surface	Rough/Smooth	Rough	Smooth
Edge	Lobate	Undulate	Undulate
Elevation	Effuse/Raised	Convex	Convex
Opt. Props.	Transparent	Opaque	Opaque
Colour	2.5 Y 6/6	2.5 Y 6.5/6 -	2.5 Y 6/6 -

GENERAL WSP often Subsurface - Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring Pellicle Colour C

PHYSIOLOGY
TEMP°C Min. 10 Opt. 30 Max. 35
PH Min. 6 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose O Glucose + Sucrose+ Lactose + Cb.+
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis+ Haemolysis ++
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$?+ $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor + Cystein$\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3+ NH_4 + Urea - Glutamate + Asparaginate+ Tryptone+
Casitone + Gelatin + Casein + SM + acetate + Prototrophic+
Stim. by Y.E. ± glu + chitin Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B I S
Chloramphenicol R Dihydrostrep. R Penicillin G R
SLS growth reduced .1 growth inhibited >.1

PREDATION
E. coli - Ps.aeruginosa ? Arthrobacter + S.marcescens +
B.subtilis + Chlorella ? Actino 32 + Actino 41 +
Yeast + Rhizopus ? Penicillium + Sclerotinia ?

PROFILE OF ORGANISM: 4564 (i) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-6 μ Shape Rods Arrangement Single
Width 0.3 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	≈ Irregular	Circular
Surface	Smooth	Rough	Smooth
Edge	Erose	Erose	Erose
Elevation	Effuse/Flat	Convex	Raised
Opt. Props.	Translucent	Opaque	Transparent
Colour	5 Y 7.5/6	5 Y 7.5/4 -	5 Y 6/6 -

GENERAL WSP often Subsurface - Slime Layers+ Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring + Colour C

PHYSIOLOGY

TEMP°C	Min. 10	Opt. 25	Max. 40
PH	Min. 6	Opt. 6-10	Max. 10

O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose O Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE ?- ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis ++
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$?+ $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor + Cystein $\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E.+ (? + chitin) Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B I S
Chloramphenicol R Dihydrostrep. R Penicillin G R
SLS growth reduced <.1 growth inhibited .1

PREDATION
E.coli - Ps.aeruginosa ? Arthrobacter + S.marcescens -
B.subtilis + Chlorella - Actino 32 + Actino 41 +
Yeast + Rhizopus + Penicillium + Sclerotinia +

PROFILE OF ORGANISM: 4564 (ii) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-3 μ Shape Rods Arrangement Single
Width 0.3 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Circular	± Circular	Circular
Surface	Smooth	Smooth	Smooth
Edge	Entire	Erose	Entire
Elevation	Raised	Raised	Convex
Opt. Props.	Transparent	Translucent	Translucent
Colour	2.5 Y 6.5/6	2.5 Y 7/6	2.5 Y 6/6

WSP - GENERAL WSP often Subsurface - Slime Layers- Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring + Colour C

PHYSIOLOGY
TEMP°C Min. 10 Opt. 25 Max. 40
PH Min. 6 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol- Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq.+ Skim milk lysis + Haemolysis ++
Casamino A use+ ammonif. + Casitone use + ammonif. +
Casein lysis+ ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$?+ $NO_2 \rightarrow$ gas or ?- NO_3 as e^- acceptor - Cystein$\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3+ NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. ?+glu chitin Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B R S
Chloramphenicol R Dihydrostrep. R Penicillin G R
SLS growth reduced <.1 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa ? Arthrobacter + S.marcescens -
B.subtilis + Chlorella - Actino 32 + Actino 41 +
Yeast ± Rhizopus + Penicillium + Sclerotinia ?

PROFILE OF ORGANISM: 4565 (i) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-4 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	≃ Circular	≃ Circular	≃ Circular
Surface	Smooth	Rough	Smooth
Edge	Erose	Undulate	Undulate
Elevation	Effuse/Flat	Raised	Convex
Opt. Props.	Translucent	Opaque	Opaque
Colour	5 Y 6/4	4 Y 7/4 -	5 Y 7/6 -

GENERAL WSP often WSP ± Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring + Colour C

PHYSIOLOGY
TEMP°C Min. 10 Opt. 10-35 Max. 40
PH Min. 7 Opt. 7-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition >3%

CHO
SUGARS OF Glucose O Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis ++
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$?+ $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
$NO_3 + NH_4$ + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. + glu chitin Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B I S
Chloramphenicol (S) Dihydrostrep. R Penicillin G R
SLS growth reduced <.1 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter + S.marcescens +
B.subtilis + Chlorella + Actino 32 + Actino 41 +
Yeast + Rhizopus + Penicillium + Sclerotinia +

PROFILE OF ORGANISM: 4565 (ii) *(LYSOBACTER ENZYMOGENES)*

CELLS
Length 1-11 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Circular	≃Circular	≃ Circular
Surface	Smooth	Smooth	Smooth
Edge	Entire	Entire	Entire
Elevation	Raised	Raised	Convex
Opt. Props.	Transparent	Translucent	Translucent
Colour	10 YR 6/8	7.5 YR 6/7 -	1.5 Y 6/6 -

GENERAL WSP often Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring + Colour C

PHYSIOLOGY
TEMP°C Min. 10 Opt. 25-30 Max. 40
PH Min. 7 Opt. 7-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 1% Partial inhibition 2% Total inhibition >3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb.+
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE + CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis++
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$?+ $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein$\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3+ NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. ± glu chitin Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B I S
Chloramphenicol R Dihydrostrep. R Penicillin G R
SLS growth reduced <.1 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter + S.marcescens +
B.subtilis + Chlorella + Actino 32 + Actino 41 +
Yeast ± Rhizopus + Penicillium + Sclerotinia +

PROFILE OF ORGANISM: 13B *(L.ENZOMOGENES var. COOKII)*

CELLS
Length 3-13 μ Shape Rods Arrangement Single
Width 0.5 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	± Circular
Surface	Rough	Rough	Smooth
Edge	Erose	Erose	Entire
Elevation	Effuse	Effuse/Umbonate	Convex
Opt. Props.	Transparent	Transparent	Translucent
Colour	7.5 YR 6/8	7.5 YR 6/6	2.5 Y 6/6

GENERAL WSP - Subsurface + Slime Layers + Spreading WSP sometimes S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring + Colour Offwhite

PHYSIOLOGY
TEMP°C Min. 8 Opt. 30 Max. 35
PH Min. 5 Opt. 5-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato +
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE ?+ ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis +
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ -
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 - NH_4 + Urea + Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic -
Stim. by Y.E. ± glu - chitin Needs Y.E. + glu - chitin

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 + Polymyxin B I S
Chloramphenicol S Dihydrostrep.R Penicillin G R
SLS growth reduced .01 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter + S.marcescens -
B.subtilis + Chlorella + Actino 32 + Actino 41 +
Yeast + Rhizopus + Penicillium + Sclerotinia -

PROFILE OF ORGANISM: 3C *(LYSOBACTER ANTIBIOTICUS)*

CELLS
Length 1-2.5 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Circular	Circular	≏Circular
Surface	Rough/Smooth	Rough/Smooth	Smooth
Edge	Entire	Erose	Erose
Elevation	Effuse/Convex	Effuse/Convex	Convex
Opt. Props.	Transp/Opaque	Transp/Opaque	Opaque
Colour	7.5 YR 2.5/4	7.5 YR 4/6 +	10 YR 4/3 ++

GENERAL WSP always Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring Colour Salmon pink

PHYSIOLOGY
TEMP°C Min. 10 Opt. 25 Max. 40
PH Min. 5 Opt. 5 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 1% Partial inhibition 2% Total inhibition 3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose - Lactose - Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY- NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq. ? Skim milk lysis + Haemolysis ?
Casamino A use + ammonif. - Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. -

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ?- NO_3 as e^- acceptor ?+ Cystein $\rightarrow H_2S$
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
$NO_3 + NH_4$ + Urea + Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin ?- Casein + SM + acetate + Prototrophic +
Stim. by Y.E. + Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D NO_2 - Polymyxin B IS
Chloramphenicol R Dihydrostrep. R Penicillin G R
SLS growth reduced <.1 growth inhibited .1

PREDATION
E. coli + Ps.aeruginosa- Arthrobacter + S.marcescens +
B.subtilis + Chlorella + Actino 32 ? Actino 41 +
Yeast + Rhizopus - Penicillium + Sclerotinia ?

PROFILE OF ORGANISM: 7-1 (*FLEXIBACTER* sp.)

CELLS
Length 1-30 μ Shape Rods Arrangement Single
Width 0.3 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	≏Circular	≏Circular	Circular
Surface	Smooth	Smooth	Smooth
Edge	Entire	Entire	Entire
Elevation	Flat	Flat	Raised
Opt. Props.	Transparent	Translucent	Translucent
Colour	10 YR 6/12 WSP -	10 YR 6/12 -	10 YR 5/8 -

GENERAL WSP - Subsurface+ Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous - Ring Colour OY

PHYSIOLOGY
TEMP°C Min. 0 Opt. 30 Max. 40
PH Min. <6 Opt. ?6 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 3% Partial inhibition>3% Total inhibition>3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose - Cb. +
ALCOHOLS Glycerol + Mannitol + STARCH SSY + NB ?+ Potato -
CELLULOSE Filter Paper - CMC ?- AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN -

PROTEINS
Tryptone + Gelatin liq. ? Skim milk lysis + Haemolysis -
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. Penassay use + ammonif. -

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor + Cystein$\rightarrow H_2S$ +
Indole - Phosphatase + Catalase ? Oxidase +

N SOURCES
NO_3 ?- NH_4 + Urea + Glutamate Asparaginate Tryptone +
Casitone + Gelatin ?- Casein + SM + acetate + Prototrophic
Stim. by Y.E.-glu +chiti Needs Y.E. + chitin

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D NO_2 - Polymyxin B R S
Chloramphenicol S Dihydrostrep. I Penicillin G (S)
SLS growth reduced .01 growth inhibited .1

PREDATION
E.coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis - Chlorella + Actino 32 - Actino 41 -
Yeast - Rhizopus - Penicillium ? Sclerotinia ?

PROFILE OF ORGANISM: 15 D *(CYTOPHAGA COMPACTA)*

CELLS
Length 1-60 μ Shape Coccobacilli Arrangement Single
Width 0.6 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Circular	Circular	Circular
Surface	Smooth	Smooth	Smooth
Edge	Entire	En tire	Entire
Elevation	Raised	Convex	Convex
Opt. Props.	Translucent	Translucent	Opaque
Colour	5 YR 6/10	5 YR 5.5/10 -	5 YR 5/10 -

GENERAL WSP - Subsurface + Slime Layers + Spreading R

LIQUID MEDIUM
Turbidity + Flocculence + Silky +
Viscous - Ring + or Pellicle Colour COY

PHYSIOLOGY
TEMP°C Min. 8 Opt. 25-30 Max. 35
PH Min. 5 Opt. 6-9 Max. 10
O_2 Growth Anaerobic + Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose No action Glucose + Sucrose ?+ slow Lactose - Cb. -
ALCOHOLS Glycerol - Mannitol - STARCH SSY + NB + Potato ?
CELLULOSE Filter Paper - CMC - AGAR Pits - Gelase +
POLYPECTATE ?+ ALGINATE - CHITIN -

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis +
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? + NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ +
Indole + Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 - NH_4 + Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic -
Stim. by Y.E. ± glu - chitin Needs Y.E. +

ANTIBIOTIC etc. SENSITIVITIES

Actinomycin D	S	NO_2 -	Polymyxin B R I
Chloramphenicol	R	Dihydrostrep. R	Penicillin G R
SLS growth reduced	.01	growth inhibited	.1

PREDATION

E. coli -	Ps.aeruginosa -	Arthrobacter -	S.marcescens -
B.subtilis -	Chlorella -	Actino 32 ?	Actino 41 ?
Yeast ±	Rhizopus -	Penicillium ?	Sclerotinia -

PROFILE OF ORGANISM: 18H *(CYTOPHAGA COMPACTA)*

CELLS
Length 3-75 μ Shape Coccobacilli Arrangement Single
Width 0,7 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Circular	Circular	Circular
Surface	Smooth	Smooth	Smooth
Edge	Entire	Entire	Entire
Elevation	Raised	Convex	Convex
Opt. Props.	Transparent	Translucent	Translucent
Colour	6 YR 6/12	5 YR 5/12 -	5 YR 5/12 -

GENERAL WSP - Subsurface + Slime Layers + Spreading (R)

LIQUID MEDIUM
Turbidity + Flocculence + Silky +
Viscous - Ring + Colour OY

PHYSIOLOGY
TEMP°C Min. 8 Opt. 25 Max. 35
PH Min. 5 Opt. 5-9 Max. 10
O_2 Growth Anaerobic + Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 3%

CHO
SUGARS OF Glucose no action Glucose + Sucrose - Lactose - Cb.-
ALCOHOLS Glycerol - Mannitol - STARCH SSY + NB + Potato ?
CELLULOSE Filter Paper - CMC - AGAR Pits - Gelase +
POLYPECTATE ?+ ALGINATE - CHITIN -

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis +
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? + NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ +
Indole + Phosphatase + Catalase+ Oxidase +

N SOURCES
NO_3+ NH_4 + slow Urea - Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic +
Stim. by Y.E. ± glu ± chitin Needs Y.E. - glu + chitin

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B R I
Chloramphenicol R Dihydrostrep. R Penicillin G R
SLS growth reduced <.1 growth inhibited .1

PREDATION
E.coli - Ps.aeruginosa - Arthrobacter + S.marcescens -
B.subtilis + Chlorella ? Actino 32 ? Actino 41 +
Yeast ± Rhizopus - Penicillium + Sclerotinia -

PROFILE OF ORGANISM: *FLAVOBACTERIUM ESTEROAROMATICUM* NCIB 8186 (not *FLAVOBACTERIUM.*

CELLS
Length 1-2.5 μ Shape Coccobacilli Arrangement Single
Width 0.3 μ Motility - Flexing - Gram +

COLONIES	#1.	SA.	PC.
Form	Punctiform	Punctiform	Circular
Surface	Smooth	Smooth	Smooth
Edge	Entire	Entire	Entire
Elevation	Convex	Convex	Raised
Opt. Props.	Transparent	Translucent	Transparent
Colour	7.5 Y 7.5/4	7.5 Y 7.5/8 -	5 Y 6/7 -

GENERAL WSP - Subsurface + Slime Layers - Spreading R

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky -
Viscous - Ring - Colour LC

PHYSIOLOGY
TEMP°C Min. 15 Opt. 25-40 Max. 40
PH Min. 5 Opt. 5-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 2% Partial inhibition 3% Total inhibition > 3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol + Mannitol - STARCH SSY + NB + Potato ?
CELLULOSE Filter Paper - CMC - AGAR Pits - Gelase +
POLYPECTATE ?+ ALGINATE - CHITIN -

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis -
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ +
Indole - Phosphatase ± Catalase ?- Oxidase +

N SOURCES
$NO_3 - NH_4$ + Urea + Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic -
Stim. by Y.E. + glu - chitin Needs Y.E. +

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B R I
Chloramphenicol R Dihydrostrep. S Penicillin G I
SLS growth reduced < .1 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis - Chlorella - Actino 32 - Actino 41 ?
Yeast ± Rhizopus - Penicillium ? Sclerotinia -

PROFILE OF ORGANISM: *FLAVOBACTERIUM FLAVESCENS* NCIB 8187 (not *FLAVOBACTERIUM*)

CELLS
Length 0.6-2.4 μ Shape Coccobacilli Arrangement Single
Width 0.3 μ Motility + Flexing - Gram +

COLONIES	#1.	SA.	PC.
Form	Punctiform	Punctiform	Circular
Surface	Smooth	Smooth	Smooth
Edge	Entire	Entire	Entire
Elevation	Convex	Convex	Convex
Opt. Props.	Transparent	Translucent	Translucent
Colour	5 Y 7.5/6	5 Y 7/6	4 Y 6.5/6
GENERAL	WSP -	Subsurface +	Slime Layers - Spreading R

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky ?+ or-
Viscous - Ring - Colour LC

PHYSIOLOGY
TEMP°C Min. 15 Opt. 30-35 Max. 40
PH Min. 5 Opt. 5-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 2% Partial inhibition 3% Total inhibition >3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb.+
ALCOHOLS Glycerol + Mannitol - STARCH SSY + NB + Potato ?
CELLULOSE Filter Paper - CMC - AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN -

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis +
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase ?- Oxidase +

N SOURCES
NO_3- NH_4 + Urea + Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic-
Stim. by Y.E. + glu - chitin Needs Y.E. +

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B R I
Chloramphenicol I Dihydrostrep. S Penicillin G I
SLS growth reduced <.1 growth inhibited .1

PREDATION
E.coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis - Chlorella - Actino 32 - Actino 41 ?
Yeast ± Rhizopus - Penicillium ? Sclerotinia -

PROFILE OF ORGANISM: *FLAVOBACTERIUM SUAVEOLANS* NCIB 8188a (not *FLAVOBACTERIUM*)

CELLS
Length 0.5-1 μ Shape Coccobacilli Arrangement Single
Width 0.2 μ Motility + Flexing - Gram - → + 1 day

COLONIES	#1.	SA.	PC.
Form	Circular	Circular	Circular
Surface	Smooth	Smooth	Smooth
Edge	Entire	Entire	Entire
Elevation	Convex	Convex	Convex
Opt. Props.	Translucent	Opaque	Opaque
Colour	7.5 Y 7.5/4	6 Y 8/10	5 Y 7/8
WSP	-	-	-

GENERAL WSP- Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky ±
Viscous - Ring Colour Deep LC

PHYSIOLOGY

TEMP°C	Min. 10	Opt. 35	Max. 40
PH	Min. 7	Opt. 7-10	Max. 10

O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 1% Partial inhibition 2% Total inhibition > 3%

CHO
SUGARS OF Glucose O Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol + Mannitol - STARCH SSY + NB ?+ Potato +
CELLULOSE Filter Paper - CMC ?- AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN -

PROTEINS
Tryptone + Gelatin liq. + slow Skim milk lysis + Haemolysis -
Casamino A use + ammonif. + Casitone use + ammonif. -
Casein lysis + ammonif. + Penassay use + ammonif. -

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ +
Indole - Phosphatase ± Catalase ?- Oxidase +

N SOURCES
NO_3 - NH_4 + Urea + Glutamate + Asparaginate - Tryptone +
Casitone ?+ Gelatin + slow Casein + SM + acetate + Prototrophic -
Stim. by Y.E. + glu - chitin Needs Y.E. + glu - chitin

ANTIBIOTIC etc. SENSITIVITIES

Actinomycin D	S	NO_2 -	Polymyxin B R I
Chloramphenicol	I	Dihydrostrep.	S Penicillin G I
SLS growth reduced	<.1	growth inhibited	.1

PREDATION

E. coli -	Ps.aeruginosa -	Arthrobacter -	S.marcescens -
B.subtilis -	Chlorella -	Actino 32 ?	Actino 41 ?
Yeast -	Rhizopus -	Penicillium ?	Sclerotinia ?

PROFILE OF ORGANISM: *FLAVOBACTERIUM SUAVEOLANS* NCIB 8188b (not *FLAVOBACTERIUM*)

CELLS
Length 0.5-1 μ Shape Coccobacilli Arrangement Single
Width 0.2 μ Motility + Flexing - Gram − → + 1 day

COLONIES	#1.	SA.	PC.
Form	Circular	Circular	Circular
Surface	Smooth	Smooth	Smooth
Edge	Entire	Entire	Entire
Elevation	Flat	Convex	Convex
Opt. Props.	Translucent	Translucent	Translucent
Colour	5 Y 7.5/4	5 Y 8/8	5 Y 6/6
WSP	-	-	-

GENERAL WSP - Subsurface + Slime Layers - Spreading R

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky ±
Viscous - Ring Colour Pale LC

PHYSIOLOGY
TEMP°C Min. 10 Opt. 20-40 Max. 40
PH Min. 6 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 1% Partial inhibition 2% Total inhibition > 3%

CHO
SUGARS OF Glucose O Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol + Mannitol - STARCH SSY+ NB ?+ Potato +
CELLULOSE Filter Paper - CMC ?- AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN -

PROTEINS
Tryptone + Gelatin liq.+ slow Skim milk lysis + Haemolysis -
Casamino A use + ammonif. + Casitone use + ammonif. -
Casein lysis + ammonif. + Penassay use + ammonif. -

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ +
Indole - Phosphatase ± Catalase ?- Oxidase +

N SOURCES
NO_3- NH_4 + Urea + Glutamate + Asparaginate - Tryptone +
Casitone ?+ Gelatin+slow Casein + SM + acetate + Prototrophic -
Stim. by Y.E. + Needs Y.E. + glu - chitin

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B R S
Chloramphenicol I Dihydrostrep. S Penicillin G I
SLS growth reduced < .1 growth inhibited .1

PREDATION
E.coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis - Chlorella - Actino 32 ? Actino 41 ?
Yeast - Rhizopus - Penicillium ? Sclerotinia ?

PROFILE OF ORGANISM: *FLAVOBACTERIUM DEVORANS* NCIB 8195

CELLS
Length 1-9 μ Shape Rod Arrangement Stars
Width 0.8-1.0μ Motility + Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Circular	Circular	Circular
Surface	Smooth	Smooth	Rough
Edge	Entire	Entire	Entire
Elevation	Convex	Convex	Convex
Opt. Props.	Opaque	Opaque	Opaque
Colour	2.5 Y 7/10 WSP -	2.5 Y 7/10 -	2.5 Y 6/8 -

GENERAL WSP - Subsurface - Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous + Ring + Colour Dirty YC

PHYSIOLOGY
TEMP°C Min. 10 Opt. 35-40 Max. 40
PH Min. 7 Opt. 7-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 3% Partial inhibition >3% Total inhibition > 3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB ?+ Potato ?+
CELLULOSE Filter Paper - CMC - AGAR Pits - Gelase -
POLYPECTATE ?- ALGINATE - CHITIN -

PROTEINS
Tryptone + Gelatin liq. - Skim milk lysis - Haemolysis +
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis ? ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ -
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 - NH_4 + Urea + Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin - Casein + SM + acetate + Prototrophic -
Stim. by Y.E. + Needs Y.E. +

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B R R
Chloramphenicol S Dihydrostrep. R Penicillin G I
SLS growth reduced .01 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis - Chlorella ? Actino 32 ? Actino 41 ?
Yeast - Rhizopus - Penicillium ? Sclerotinia ?

PROFILE OF ORGANISM: *FLAVOBACTERIUM AQUATILE* NCIB 8535 (not *FLAVOBACTERIUM*)

CELLS
Length 1-3 μ Shape Coccobacilli Arrangement Single
Width 0.3 μ Motility + Flexing - Gram +

COLONIES	#1.	SA.	PC.
Form	Punctiform	Punctiform	Circular
Surface	Smooth	Smooth	Smooth
Edge	Entire	Entire	Entire
Elevation	Convex	Convex	Raised
Opt. Props.	Transparent	Translucent	Translucent
Colour	5 Y 7/6	WSP - 5 Y 7/8 -	2.5 Y 6/8 -

GENERAL WSP - Subsurface + Slime Layers - Spreading R

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous - Ring - Colour LC

PHYSIOLOGY
TEMP°C Min. 15 Opt. 25-35 Max. 40
PH Min. 6 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 2% Partial inhibition 3% Total inhibition >3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol + Mannitol - STARCH SSY + NB + Potato ?
CELLULOSE Filter Paper - CMC - AGAR Pits - Gelase +
POLYPECTATE ?- ALGINATE - CHITIN -

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis +
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + ammonif. Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase ?- Oxidase +

N SOURCES
NO_3 - NH_4 + Urea + Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin + Casein + SM + acetate + Prototrophic -
Stim. by Y.E. ± glu - chitin Needs Y.E. +

ANTIBIOTIC etc. SENSITIVITIES

Actinomycin D	S	NO_2 -	Polymyxin B R R
Chloramphenicol	R	Dihydrostrep. S	Penicillin G I
SLS growth reduced	<.1	growth inhibited	.1

PREDATION

E. coli -	Ps.aeruginosa -	Arthrobacter -	S.marcescens -
B.subtilis -	Chlorella -	Actino 32 -	Actino 41 ?
Yeast ± weak	Rhizopus -	Penicillium ?	Sclerotinia -

PROFILE OF ORGANISM: *FLAVOBACTERIUM RESINOVORUM* NCIB 8767 *(CYTOPHAGA* sp.)

CELLS
Length 1-15 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Circular	Circular	Circular
Surface	Smooth	Smooth	Rough
Edge	Entire	Entire	Erose
Elevation	Raised	Convex	Raised
Opt. Props.	Transparent	Translucent	Translucent
Colour 7.5 YR	6/10	7.5 YR 6/10	7.5 YR 5/10
WSP	-	-	-

GENERAL WSP - Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous - Ring Colour Dirty OY

PHYSIOLOGY

TEMP°C	Min. 5	Opt. 15-20	Max. 25
PH	Min. 6	Opt. 6-10	Max. 10

O_2 Growth Anaerobic - Candle Jar + Air + Best Air
NaCl Good growth up to 1% Partial inhibition <2% Total inhibition 2%

CHO
SUGARS OF Glucose O+ slow F Glucose + Sucrose - Lactose - Cb.+
ALCOHOLS Glycerol - Mannitol - STARCH SSY + NB ?+ Potato +
CELLULOSE Filter Paper - CMC - AGAR Pits - Gelase +
POLYPECTATE ?- ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq. + slow Skim milk lysis + Haemolysis -
Casamino A use + ammonif. + Casitone use + ammonif. -
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 - NH_4 + Urea + Glutamate + Asparaginate + Tryptone+
Casitone ?+ Gelatin +slow Casein + SM + acetate + Prototrophic -
Stim. by Y.E. + Needs Y.E. +

ANTIBIOTIC etc. SENSITIVITIES

Actinomycin D	S	NO_2	-	Polymyxin B	I S
Chloramphenicol	S	Dihydrostrep.	S	Penicillin G	R
SLS growth reduced	<.1	growth inhibited	.1		

PREDATION

E. coli -	Ps. aeruginosa -	Arthrobacter -	S. marcescens -
B. subtilis -	Chlorella -	Actino 32 +	Actino 41 -
Yeast -	Rhizopus -	Penicillium -	Sclerotinia +

PROFILE OF ORGANISM: *FLAVOBACTERIUM PROTEUS* NCIB 8771

CELLS
Length 1-40 μ Shape Rods Arrangement Single
Width 0.5 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	≐ Circular	Punctiform	Irregular
Surface	Smooth	Smooth	Rough
Edge	Entire	Entire	Undulate
Elevation	Convex	Raised	Umbonate
Opt. Props.	Translucent	Translucent	Translucent
Colour	5 Y 8/2	10 YR 7/4 -	10 YR 6/3 -

GENERAL WSP - Subsurface - Slime Layers - Spreading (S)

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous - Ring Pellicle Colour Dirty OY

PHYSIOLOGY
TEMP°C Min. 15 Opt. 25 - 35 Max. 35
PH Min. 6 Opt. 6 - 10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition 1% Total inhibition 2%

CHO
SUGARS OF Glucose F Glucose + Sucrose - Lactose - Cb ?+ slow
ALCOHOLS Glycerol + Mannitol + STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN -

PROTEINS
Tryptone + Gelatin liq. ? Skim milk lysis - Haemolysis -
Casamino A use + ammonif. - Casitone use + ammonif. +
Casein lysis - ammonif. Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor ++ Cystein $\rightarrow H_2S$ +
Indole - Phosphatase ± Catalase + Oxidase -

N SOURCES
NO_3 - NH_4 + Urea + Glutamate - Asparaginate - Tryptone +
Casitone + Gelatin ?- Casein - SM + acetate - Prototrophic -
Stim. by Y.E. + Needs Y.E. +

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B I S
Chloramphenicol S Dihydrostrep. I Penicillin G R
SLS growth reduced >.1 growth inhibited ?

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter - S.marcescens ?
B.subtilis - Chlorella - Actino 32 + Actino 41 ?
Yeast - Rhizopus - Penicillium - Sclerotinia -

PROFILE OF ORGANISM: *FLAVOBACTERIUM SUAVEOLANS* NCIB 8992

CELLS
Length 1-2 μ Shape Rods Arrangement Single
Width 0.3 μ Motility - Flexing - Gram -

COLONIES	#1.	SA.	PC.
Form	Circular	Circular	Circular
Surface	Smooth	Smooth	Smooth
Edge	Entire	Entire	Entire
Elevation	Raised	Convex	Convex
Opt. Props.	Translucent	Opaque	Opaque
Colour	2.5 Y 7/6	7.5 YR 6/12	10 YR 6/8
WSP	-	-	-

GENERAL WSP - Subsurface - Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous - Ring Colour OY

PHYSIOLOGY
TEMP°C Min. 10 Opt. 30 Max. 35
PH Min. 7 Opt. 7-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 1% Partial inhibition 2% Total inhibition 3%

CHO
SUGARS OF Glucose F Glucose + Sucrose - Lactose - Cb.+
ALCOHOLS Glycerol - Mannitol - STARCH SSY +poorNB ?+ Potato +
CELLULOSE Filter Paper - CMC - AGAR Pits - Gelase +
POLYPECTATE ?- ALGINATE- CHITIN -

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis -
Casamino A use +slow ammonif.+slow Casitone use + ammonif. -
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein$\rightarrow H_2S$ +
Indole + Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 - NH_4 +slow Urea - Glutamate - Asparaginate - Tryptone +
Casitone ?+ Gelatin + Casein + SM + acetate + Prototrophic -
Stim. by Y.E. + Needs Y.E. +

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2^- Polymyxin B I S
Chloramphenicol S Dihydrostrep. I Penicillin G R
SLS growth reduced .01 growth inhibited .1

PREDATION
E.coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis - Chlorella - Actino 32 ? Actino 41 ?
Yeast - Rhizopus - Penicillium - Sclerotinia ?

PROFILE OF ORGANISM: *FLAVOBACTERIUM RHENANUM* NCIB 9157a

CELLS
Length 2-3 μ Shape Rods Arrangement Single
Width 0.5 μ Motility + Flexing - Gram -

COLONIES	#1.	SA.	PC.
Form	≐ Circular	≐ Circular	≐ Circular
Surface	Smooth	Smooth	Smooth
Edge	Entire	Entire	Entire
Elevation	Raised	Convex	Convex
Opt. Props.	Transparent	Translucent	Translucent
Colour	2.5 Y 7/8	WSP - 2.5 Y 7/8 -	10 YR 5/6 -

GENERAL WSP- Subsurface + Slime Layers - Spreading (S)

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky -
Viscous - Ring Colour Dirty OY

PHYSIOLOGY
TEMP°C Min. 10 Opt. 10-25 Max. 35
PH Min. 6 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 1% Partial inhibition 2% Total inhibition 3%

CHO
SUGARS OF Glucose F Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol + Mannitol + STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE ?- ALGINATE - CHITIN -

PROTEINS
Tryptone + Gelatin liq. - Skim milk lysis - Haemolysis -
Casamino A use + ammonif. + Casitone use + ammonif. -
Casein lysis - ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor ++ Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
$NO_3 - NH_4$ + Urea + Glutamate + Asparaginate + Tryptone +
Casitone ?+ Gelatin - Casein + SM + acetate - Prototrophic -
Stim. by Y.E. + Needs Y.E. +

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B R S
Chloramphenicol S Dihydrostrep. I Penicillin G R
SLS growth reduced .1 growth inhibited >.1

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis ? Chlorella - Actino 32 ? Actino 41 ?
Yeast - Rhizopus - Penicillium - Sclerotinia ?

PROFILE OF ORGANISM: *FLAVOBACTERIUM RHENANUM* NCIB 9157b

CELLS
Length 2-9 μ Shape Ovoid Rods Arrangement Single
Width 0.5 μ Motility + Flexing - Gram -

COLONIES	#1.	SA.	PC.
Form	Circular	Circular	Irregular
Surface	Smooth	Rough	Rough/Smooth
Edge	Erose	Erose	Erose
Elevation	Flat	Raised	Raised
Opt. Props.	Translucent	Translucent	Translucent
Colour	2.5 Y 7/8 WSP -	2.5 Y 7/7 -	2.5 Y 6/6 -

GENERAL WSP - Subsurface + Slime Layers - Spreading (S)

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky -
Viscous - Ring + Colour Dirty OY

PHYSIOLOGY
TEMP°C Min. 10 Opt. 35 Max. 35
PH Min. 6 Opt. 7-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 1% Partial inhibition <2% Total inhibition 2%

CHO
SUGARS OF Glucose F Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol + Mannitol + STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN -

PROTEINS
Tryptone + Gelatin liq. - Skim milk lysis - Haemolysis +
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis - ammonif. Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor ++ Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase + Oxidase -

N SOURCES
NO_3 - NH_4 + Urea + Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin - Casein ?- SM + acetate - Prototrophic -
Stim. by Y.E. + Needs Y.E. +

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B R S
Chloramphenicol S Dihydrostrep. 1 Penicillin G R
SLS growth reduced .1 growth inhibited >.1

PREDATION
E.coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis ? Chlorella - Actino 32 ? Actino 41 ?
Yeast - Rhizopus - Penicillium - Sclerotinia +

PROFILE OF ORGANISM: *FLAVOBACTERIUM HEPARINUM* NCIB 9290 *(FLEXIBACTER HEPARINUS)*

CELLS
Length 1-9 μ Shape Rods Arrangement Single
Width 0.3 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	Irregular	Irregular	≏Circular
Surface	Rough/Smooth	Rough/Smooth	Smooth
Edge	Lobate	Lobate	Entire
Elevation	Umbonate	Umbonate	Umbonate
Opt. Props.	Transparent	Translucent	Translucent
Colour	2.5 Y 7/4	10 YR 7/4	10 YR 6/4

GENERAL WSP - Subsurface - Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity + Flocculence - Silky +
Viscous - Ring Colour Offwhite

PHYSIOLOGY
TEMP°C Min. 10 Opt. 20-35 Max. 35
PH Min. 7 Opt. 7-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 2% Partial inhibition 3% Total inhibition >3%

CHO
SUGARS OF Glucose O + slow Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol O+ slow Mannitol + STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC - AGAR Pits - Gelase +
POLYPECTATE ?- ALGINATE - CHITIN -

PROTEINS
Tryptone + Gelatin liq. - Skim milk lysis - Haemolysis +
Casamino A use +slow ammonif. + slow Casitone use + ammonif. -
Casein lysis ? ammonif. + Penassay use + ammonif. -

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor + Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 - NH_4 + Urea + Glutamate - Asparaginate - Tryptone +
Casitone ?+ Gelatin - Casein + SM + acetate - Prototrophic
Stim. by Y.E. -glu +chitin Needs Y.E. + chitin

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B I S
Chloramphenicol R Dihydrostrep. R Penicillin G R
SLS growth reduced .01 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis - Chlorella ? Actino 32 + Actino 41 ?
Yeast - Rhizopus - Penicillium - Sclerotinia -

PROFILE OF ORGANISM: *FLAVOBACTERIUM LUCECOLORATUM* NCIB 9324 (not *FLAVOBACTERIUM*)

CELLS
Length 1-2 μ Shape Rods Arrangement Single
Width 0.3 μ Motility + Flexing - Gram - → +

COLONIES	#1.	SA.	PC.
Form	Punctiform	Punctiform	≃ Circular
Surface	Smooth	Smooth	Smooth
Edge	Entire	Entire	Entire
Elevation	Pulvinate	Pulvinate	Convex
Opt. Props.	Translucent	Translucent	Translucent
Colour	5 Y 7/5	WSP - 7.5 Y 8.5/2 -	2.5 Y 6.5/6 -

GENERAL WSP Potato, little Subsurface + Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence + Silky +
Viscous - Ring + Colour Deep LC

PHYSIOLOGY
TEMP°C Min. 10 Opt. 30-35 Max. 35
PH Min. 7 Opt. 7-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 3% Partial inhibition >3% Total inhibition >3%

CHO
SUGARS OF Glucose O+ slow F Glucose + Sucrose + Lactose + Cb.+
ALCOHOLS Glycerol + Mannitol + STARCH SSY - NB ?+ Potato -
CELLULOSE Filter Paper - CMC - AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN -

PROTEINS
Tryptone + Gelatin liq. + Skim milk lysis + Haemolysis -
Casamino A use + slow ammonif. + slow Casitone use + ammonif. -
Casein lysis + ammonif. + Penassay use + ammonif. -

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 - NH_4 + slow Urea + Glutamate + Asparaginate + Tryptone +
Casitone ?+ Gelatin + Casein + SM + acetate + Prototrophic -
Stim. by Y.E. + Needs Y.E. +

ANTIBIOTIC etc. SENSITIVITIES

Actinomycin D	S	NO_2 -	Polymyxin B R R
Chloramphenicol	R	Dihydrostrep. R	Penicillin G I
SLS growth reduced	<.1	growth inhibited	.1

PREDATION

E.coli -	Ps.aeruginosa -	Arthrobacter -	S.marcescens -
B.subtilis -	Chlorella -	Actino 32 ?	Actino 41 ?
Yeast -	Rhizopus -	Penicillium -	Sclerotinia ?

PROFILE OF ORGANISM: *CYTOPHAGA SUCCINICANS* RL 8 (contaminant, not *C.SUCCINICANS*)

CELLS
Length 2-4 μ Shape Rods Arrangement Single
Width 0.6 μ Motility + Flexing - Gram -

COLONIES	#1.	SA.	PC.
Form	Circular	Circular	Irregular
Surface	Smooth	Smooth	Rough
Edge	Entire	Entire	Curled
Elevation	Raised	Convex	Pulvinate
Opt. Props.	Translucent	Opaque	Opaque
Colour	4 YR 5/10	2.5 YR 6/12 -	2.5 YR 5/12 -

GENERAL WSP - Subsurface + Slime Layers - Spreading R

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky +
Viscous - Ring Colour Dirty C

PHYSIOLOGY

TEMP°C	Min. 10	Opt. 15-30	Max. 35
PH	Min. 6	Opt. 7-10	Max. 10

O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 3% Partial inhibition>3% Total inhibition >3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol ?+ slow Mannitol ?+ slow STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC - AGAR Pits - Gelase +
POLYPECTATE ?- ALGINATE - CHITIN -

PROTEINS
Tryptone + Gelatin liq. - Skim milk lysis - Haemolysis -
Casamino A use + ammonif. - Casitone use + ammonif. -
Casein lysis - ammonif. Penassay use - ammonif. -

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ + $NO_2 \rightarrow$ gas or ? ng. in Penassay NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ +
Indole - Phosphatase - Catalase + Oxidase +

N SOURCES
NO_3 - NH_4^+ slow Urea +? Glutamate + Asparaginate - Tryptone +
Casitone - Gelatin - Casein - SM + acetate - Prototrophic -
Stim. by Y.E. + Needs Y.E. +

ANTIBIOTIC etc. SENSITIVITIES

Actinomycin D	S	n.g. in NO_2 Penassay	Polymyxin B I S
Chloramphenicol	S	Dihydrostrep. S	Penicillin G R
SLS growth reduced <.1		growth inhibited .1	

PREDATION

E. coli -	Ps.aeruginosa -	Arthrobacter -	S.marcescens -
B.subtilis -	Chlorella -	Actino 32 ?	Actino 41 ?
Yeast -	Rhizopus -	Penicillium -	Sclerotinia -

PROFILE OF ORGANISM: *FLAVOBACTERIUM ARBORESCENS* NCIB 8185 (not *FLAVOBACTERIUM*)

CELLS
Length 0.5-1.5 μ Shape Rods Arrangement Single
Width 0.2 μ Motility + Flexing - Gram - → +

COLONIES	#1.	SA.	PC.
Form	Punctiform	Circular	Circular
Surface	Smooth	Smooth	Smooth
Edge	Entire	Entire	Entire
Elevation	Raised	Convex	Convex
Opt. Props.	Translucent	Translucent	Translucent
Colour 2.5 YR	6/10	5 YR 6/12	5 YR 5.5/10

GENERAL WSP - Subsurface + Slime Layers - Spreading R

LIQUID MEDIUM
Turbidity ++ Flocculence + Silky +
Viscous - Ring + Colour Dirty OY

PHYSIOLOGY
TEMP°C Min. 10 Opt. 35 Max. 40
PH Min. 6 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 3% Partial inhibition >3% Total inhibition >3%

CHO
SUGARS OF Glucose F Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol + Mannitol + STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN -

PROTEINS
Tryptone + Gelatin liq. ? Skim milk lysis + Haemolysis -
Casamino A use + ammonif. - Casitone use + ammonif. +
Casein lysis + ammonif. Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ +
Indole - Phosphatase - Catalase + Oxidase +

N SOURCES
NO_3 - NH_4 + Urea + Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin ?- Casein + SM + acetate + Prototrophic -
Stim. by Y.E. + Needs Y.E. +

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D V.H.S. NO_2 - Polymyxin B R R
Chloramphenicol S Dihydrostrep. (?S) Penicillin G (S)R
SLS growth reduced .01 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis ? Chlorella - Actino 32 + Actino 41 -
Yeast ± Rhizopus - Penicillium - Sclerotinia ?

PROFILE OF ORGANISM: *FLAVOBACTERIUM AURANTIACUM* NCIB 8204 *(? FLAVOBACTERIUM)*

CELLS
Length 1-2 μ Shape Coccobacilli Arrangement Single
Width 0.5 μ Motility - Flexing - Gram -

COLONIES	#1.	SA.	PC.
Form	Punctiform	Punctiform	Circular
Surface	Smooth	Smooth	Smooth
Edge	Erose	Entire	Entire
Elevation	Raised	Pulvinate	Pulvinate
Opt. Props.	Opaque	Opaque	Opaque
Colour	Paler than 2.5 YR 6/8	Paler than 2.5 YR 5/8	Redder than 2.5 YR 5/10

GENERAL WSP - Subsurface - Slime Layers + Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky - Colour Pinky Orange
Viscous ± Ring

PHYSIOLOGY
TEMP°C Min. 10 Opt. 20-35 Max. 40
PH Min. 5 Opt. 5-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 3% Partial inhibition >3% Total inhibition >3%

CHO
SUGARS OF Glucose No action Glucose + Sucrose + Lactose - Cb. -
ALCOHOLS Glycerol ?+ slow Mannitol + STARCH SSY ?+ NB ?+ Potato -
CELLULOSE Filter Paper - CMC - AGAR Pits - Gelase +
POLYPECTATE ?- ALGINATE - CHITIN -

PROTEINS
Tryptone + Gelatin liq. - Skim milk lysis - Haemolysis -
Casamino A use - ammonif. - Casitone use + ammonif. -
Casein lysis - ammonif. ?- Penassay use + ammonif. -

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ +
Indole - Phosphatase - Catalase + Oxidase -

N SOURCES
NO_3 - NH_4 + slow Urea + Glutamate + Asparaginate + Tryptone +
Casitone ?+ Gelatin - Casein ?- SM + acetate - Prototrophic -
Stim. by Y.E. + Needs Y.E. +

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D V.H.S. NO_2 - Polymyxin B R I
Chloramphenicol S Dihydrostrep. S Penicillin G S
SLS growth reduced <.1 growth inhibited .1

PREDATION
E.coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis - Chlorella - Actino 32 ? Actino 41 ?
Yeast - Rhizopus - Penicillium - Sclerotinia ?

PROFILE OF ORGANISM: 5-9 *(MYXOCOCCUS)*

CELLS
Length 7-75 μ Shape Rods Arrangement Single
Width 0.6 μ Motility - Flexing + Gram -

COLONIES	#1.	SA.	PC.
Form	≏Circular	≏Circular	≏Circular
Surface	Smooth	Smooth	Smooth
Edge	Entire	Entire	Entire
Elevation	Raised	Convex	Convex
Opt. Props.	Transparent	Translucent	Translucent
Colour	Pinker than 2.5 YR 6/8	2.5 YR 6/6	2.5 YR 5/8

WSP - Subsurface - Slime Layers - Spreading (S)

LIQUID MEDIUM
Turbidity + Flocculence - Silky +
Viscous - Ring Colour Pink

PHYSIOLOGY
TEMP°C Min. 10 Opt. 30 Max. 40
PH Min. 6 Opt. 6-8 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 1% Partial inhibition < 2% Total inhibition 2%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose + Lactose + Cb. +
ALCOHOLS Glycerol - Mannitol - STARCH SSY + NB + Potato ?+
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE ?- ALGINATE - CHITIN -

PROTEINS
Tryptone + Gelatin liq. ? Skim milk lysis - Haemolysis -
Casamino A use + ammonif. + Casitone use + ammonif. +
Casein lysis + weak ammonif. Penassay use + ammonif. -

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ +
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea + Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin ?- Casein ?+ SM + acetate ?- Prototrophic +
Stim. by Y.E. - glu + chitin Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D HS NO_2 + Polymyxin B (I)R (S)R
Chloramphenicol R Dihydrostrep. R Penicillin G 1
SLS growth reduced .01 growth inhibited .1

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis ? Chlorella - Actino 32 - Actino 41 -
Yeast - Rhizopus - Penicillium - Sclerotinia ?

PROFILE OF ORGANISM: 14 *(? VIBRIO)*

CELLS
Length 3-6 μ Shape Curved Rods Arrangement Single
Width 0.4 μ Motility - Flexing - Gram -

COLONIES	#1.	SA.	PC.
Form	Circular	≏ Circular	≏ Circular
Surface	Smooth	Smooth	Smooth
Edge	Entire	Entire	Entire
Elevation	Convex	Convex	Convex
Opt. Props.	Translucent	Translucent	Translucent
Colour	Redder than 2.5 YR 4/10 WSP -	2.5 YR 6/8 -	2.5 YR 5/8 -

GENERAL WSP - Subsurface + Slime Layers - Spreading (S)

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky -
Viscous + Ring - Colour Pale pink

PHYSIOLOGY
TEMP°C Min. 10 Opt. 20-30 Max. 35
PH Min. 6 Opt. 6 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Candle/Air
NaCl Good growth up to 0% Partial inhibition <1% Total inhibition 1%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose - Lactose - Cb. -
ALCOHOLS Glycerol - Mannitol - STARCH SSY + NB + Potato ?+
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN -

PROTEINS
Tryptone + Gelatin liq. ? Skim milk lysis - Haemolysis -
Casamino A use + ammonif. - Casitone use + ammonif. +
Casein lysis ? ammonif. Penassay use - ammonif. -

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ n.g. $NO_2 \rightarrow$ gas or ? in Penassay n.g. NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ -
Indole - Phosphatase + Catalase + Oxidase +

N SOURCES
NO_3 + NH_4 + Urea + Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin ?- Casein ?+ SM + acetate - Prototrophic +
Stim. by Y.E. + glu - Chitin Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - n.g. in Penassay Polymyxin B R I
Chloramphenicol S Dihydrostrep. R Penicillin G R
SLS growth reduced < .01 growth inhibited .01

PREDATION
E. coli - Ps.aeruginosa - Arthrobacter - S.marcescens -
B.subtilis - Chlorella - Actino 32 - Actino 41 -
Yeast - Rhizopus - Penicillium - Sclerotinia ?

PROFILE OF ORGANISM: 402 *(LYSOBACTER GUMMOSUS)*

CELLS
Length 0.5-2 μ Shape Rods Arrangement Single
Width 0.4 μ Motility - Flexing - Gram ?+ -

COLONIES	#1.	SA.	PC.
Form	Circular	Circular	Circular
Surface	Smooth	Smooth	Smooth
Edge	Entire	Entire	Entire
Elevation	Pulvinate	Pulvinate	Pulvinate
Opt. Props.	Translucent	Opaque	Opaque
Colour	5 Y 8/2	5 Y 8/2 -	5 Y 7/4 -

GENERAL WSP- Subsurface - Slime Layers - Spreading S

LIQUID MEDIUM
Turbidity ++ Flocculence - Silky to ?- too viscous to tell
Viscous + Ring + or Pellicle Colour Offwhite

PHYSIOLOGY
TEMP°C Min. 10 Opt. 20 Max. 40
PH Min. 6 Opt. 6-10 Max. 10
O_2 Growth Anaerobic - Candle Jar + Air + Best Air
NaCl Good growth up to 1% Partial inhibition 2% Total inhibition 3%

CHO
SUGARS OF Glucose 0 Glucose + Sucrose ?+ slow Lactose + Cb ?+ slow
ALCOHOLS Glycerol - Mannitol - STARCH SSY - NB - Potato -
CELLULOSE Filter Paper - CMC + AGAR Pits - Gelase +
POLYPECTATE - ALGINATE - CHITIN +

PROTEINS
Tryptone + Gelatin liq. ? Skim milk lysis + Haemolysis +
Casamino A use + ammonif. - Casitone use + ammonif. +
Casein lysis + ammonif. + Penassay use + ammonif. +

BIOCHEMISTRY
$NO_3 \rightarrow NO_2$ - $NO_2 \rightarrow$ gas or ? - NO_3 as e^- acceptor - Cystein $\rightarrow H_2S$ -
Indole - Phosphatase + Catalase Oxidase +

N SOURCES
NO_3 + NH_4 + Urea + Glutamate + Asparaginate + Tryptone +
Casitone + Gelatin ?- glu Casein + SM + acetate + Prototrophic +
Stim. by Y.E. + chitin Needs Y.E. -

ANTIBIOTIC etc. SENSITIVITIES
Actinomycin D S NO_2 - Polymyxin B I S
Chloramphenicol (R) Dihydrostrep. R Penicillin G R
SLS growth reduced .1 growth inhibited >.1

PREDATION
E.coli - Ps.aeruginosa - Arthrobacter + S.marcescens -
B.subtilis + Chlorella + Actino 32 + Actino 41 +
Yeast + Rhizopus ? Penicillium + Sclerotinia +