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Diversity and acyl-homoserine lactone production among subtidal biofilm-forming bacteria

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ABSTRACT: Bacteria isolated from subtidal biofilms were identified via 16S rRNA gene sequencing and screened for acyl-homoserine lactone (AHL) production. In total, 68 strains were isolated from 1 to 9 d-old subtidal biofilms developed at a coastal fish farm. Identification based on partial 16S rRNA gene sequencing revealed that these isolates were distributed among 3 phylogenetic groups (4 Bacteroidetes, 13 α-Proteobacteria and 51 γ-Proteobacteria), with most isolates belonging to the family *Rhodobacteraceae* and the genera *Thalassomonas*, *Alteromonas*, *Pseudoalteromonas*, *Shewanella* and *Vibrio*. AHL screening was performed using 2 AHL reporter strains, *Agrobacterium tumefaciens* A136 and *Chromobacterium violaceum* CV026. Results showed that 21 strains (31%) produced AHLs, including 3 Bacteroidetes, 5 α-Proteobacteria and 13 γ-Proteobacteria. All the AHL-producing α-Proteobacteria belonged to the family *Rhodobacteraceae*, whereas the AHL-producing γ-Proteobacteria consisted of 6 *Pseudoalteromonas* spp., 6 *Vibrio* spp. and 1 *Thalassomonas* sp. This is the first report of AHL-producing marine bacteria in the genera *Flammeovirga, Pseudoalteromonas* and *Thalassomonas*. The family *Rhodobacteraceae* (11 isolates) and the genera *Vibrio* (15 isolates) and *Pseudoalteromonas* (17 isolates) had the greatest number of AHL-producing isolates. AHL profiling of the AHL-producing isolates was performed by GC-MS. Most AHL-producing isolates produced several different AHLs, many of which were long-chain- and 3-oxo-AHLs. The widespread occurrence of AHL-producing bacteria in subtidal biofilms suggests that AHLs may play a role in the community development in this environment.

KEY WORDS: Subtidal biofilm · Marine bacteria · Acyl-homoserine lactone · AHL

INTRODUCTION

The term marine biofilm refers to surface-attached microbial communities which can include bacteria, diatoms and fungi, etc. (Cooksey & Wigglesworth-Cooksey 1995). Marine biofilms play important roles in various biological processes (Decho 2000). However, these biofilms can also cause biofouling, the undesirable attachment of micro- and macro-organisms on manmade surfaces (Qian 1999, Huggett et al. 2006, Huang et al. 2007b). Bacteria are often the dominant and pioneering microbial taxa during the development of natural marine biofilms (Cooksey & Wigglesworth-Cooksey 1995). Knowledge about marine biofilm-forming bacteria is crucial to understand the development of these special environmental niches.

Bacterial quorum sensing (QS) is a term used to describe intercellular signaling in bacteria (Waters & Bassler 2005). N-acyl-homoserine lactones (AHLs) are the best studied QS signal molecules among gram-negative bacteria (Greenberg 2000). Many phenotypes that are beneficial for bacterial surface colonization are AHL-regulated, including exopolysaccharide production, exo-enzymes, virulence factors, conjugation, biofilm formation and toxin production (Branda et al. 2005, Parsek & Greenberg 2005, Waters & Bassler

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2005). Therefore, we assume that subtidal biofilms may harbor various kinds of AHL-producing strains.

Molecular mechanisms of AHL-driven bacterial QS have been well studied in the lab; however, little work has been done concerning its ecological role in the field (McLean et al. 1997, Parsek & Greenberg 2005). It had been reported that the diffusible AHLs produced by bacterial biofilms increase spore settlement of the green alga *Ulva* sp. (Joint et al. 2002). In a previous study, we reported the direct detection of AHLs in marine subtidal biofilms and found that AHLs affected larval behavior in the polychaete *Hydroides elegans* (Huang et al. 2007a). However, the diversity of AHL-producing bacteria in marine biofilms has not been shown.

In the present study, bacteria were isolated from 1 to 9 d old subtidal biofilms developed in a coastal fish farm of Hong Kong, identified by partial 16S rRNA gene sequencing, and screened for AHL production. AHLs produced by bacteria were identified by gas chromatography–mass spectrometry (GC-MS).

MATERIALS AND METHODS

Development of subtidal biofilms. Subtidal biofilms were developed from 12 to 21 October 2006 at Yung Shue Au fish farm, Hong Kong (22° 25′ 35″ N, 114° 16′ 46″ E). Polystyrene petri dishes were submerged 2 m below the seawater surface. Petri dishes were retrieved from the field after 1, 3, 5, 7 and 9 d and immediately transported back to the laboratory in a heat-proof container filled with *in situ* seawater.

Isolation of bacterial strains from subtidal biofilms. On each sampling day, 5 replicate petri dishes were gently rinsed with autoclaved filtered seawater (AFSW, 0.22 µm pore size), and the biofilms were sampled by scraping the polystyrene surface with autoclaved glass cover slides. The samples were pooled and suspended in 10 ml AFSW, mixed vigorously by vortex and serially diluted (10 to 10^4) with AFSW. From each dilution, duplicate 100 µl aliquots were plated on nutrient agar (0.3% yeast extract, 0.5% peptone, 1.5% agar in AFSW) and marine agar 2216 (Difco) and incubated at 25°C for 48 h.

Agar plates were examined under a dissecting microscope (10×, Leica MZ6) for colony growth after 48 h, and colony color, shape, size, surface topography and the presence of granules were recorded. For Day 1 samples, all different colony types were isolated and purified. For all subsequent samples, new and conspicuous colony types were isolated and purified. Isolates were numbered consecutively with a 'sf' prefix for subtidal films.

PCR amplification of the 16S rRNA gene. Genomic DNA was extracted from isolates using a bacterial genomic DNA extraction kit (TaKaRa). The 16S rRNA gene was then amplified by polymerase chain reaction (PCR) using the primers 27F and 1492R (Table 1). Each PCR reaction contained 1 µl of DNA extract, 1.25 U of rTaq DNA polymerase (TaKaRa), 0.25 mM of dNTPs, 0.1 µM of each primer, and 1X PCR buffer in a 25 µl volume. PCR was performed under the following conditions: 95°C for 3 min, 35 cycles of 95°C for 20 s, 50°C for 30 s, 72°C for 1 min and a final extension at 72°C for 5 min. PCR products were checked on a 1% agarose gel stained with ethidium bromide. Correctly sized PCR products were then purified using a PCR purification mini kit (Watson) for sequencing.

Sequencing 16S rRNA gene fragments. Sequencing of purified PCR products was done using a DYEnamic™ ET terminator sequencing premix (Amersham Biosciences). Primers 39F and 1389R were first used for sequencing. For DNA samples for which primers 39F and 1389R did not work, other primers (Table 1) were used for sequencing. Sequences were read using a MegaBACE™ 500 genetic analyzer (Amersham Biosciences). More than 1 kb of sequence for each isolate was obtained.

Table 1. Primers used for PCR and sequencing of the 16S rRNA gene from subtidal biofilm isolates. The primers are universal for the domain *Bacteria*

Primer	16S rRNA gene nucleotide region (*E. coli* equivalent)	Sequence (5′ to 3′)	Source
27F	8–27	AGAGTTTGATCMTGGCTCAG	Lane (1991)
39F	22–39	GCTCAGGATGAACGCTGG	Present study
519F	519–536	CAGCMGCCGCGGTAATAC	Lane (1991)
805R	785–805	GACTACCAGGGTATCTAATCC	Tanner et al. (1998)
1055R	1055–1074	CACGAGCTGACGACAGCCAT	Lee et al. (1993)
1389R	1389–1408	TGACGGGCGGTGTGTACAAG	Present study
1492R	1492–1510	GGTTACCTTGTTACGACTT	Lane (1991)

Phylogenetic analysis of 16S rRNA gene fragments. Sequences from reference taxa were retrieved from the National Center for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov/BLAST), selecting those with the highest BLASTN scores to the 16S rRNA genes of our isolates. Sequences were aligned to reference taxa using ClustalX (Thompson et al. 1994), and alignments were manually edited using Bioedit (www.mbio.ncsu.edu/BioEdit/bioedit.html). A maximum likelihood tree based on ~1000 bp of the 16S rRNA gene was constructed using PHYML (Guindon & Gascuel 2003, http://atgc.lirmm.fr/phyml) under a general time-reversible substitution model including invariable sites, and gamma distributed among-site rate variation (4 rate categories). Statistical support values for all nodes were obtained by repeating this analysis on 100 bootstrap pseudo-replicates of the data set. Isolates were assigned to genera when their 16S rRNA gene sequence was nested between 16S rRNA sequences from described species of the genus within the phylogenetic tree.

Nucleotide sequence accession numbers. Partial 16S rRNA gene sequences of all isolates were submitted to the GenBank under accession numbers EF587949 to EF588016.

AHL reporter strains. The AHL reporter strains *Agrobacterium tumefaciens* A136 and *Chromobacterium violaceum* CV026, together with AHL over-producers *A. tumefaciens* KYC6 and *C. violaceum* ATCC 31532, were used for the bioassay (McClean et al. 1997, McLean et al. 1997). A136 and KYC6 were grown in Luria–Bertani (LB) broth supplemented with spectinomycin (50 µg ml^{-1}), and A136 was additionally supplemented with tetracycline (4.5 µg ml^{-1}). CV026 and 31532 were grown in LB broth. Prior to the bioassay using *A. tumefaciens* A136 as the reporter strain, the agar plate was covered with 50 µl of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal, 20 mg ml^{-1} stock solution in dimethylformamide).

Screening for AHL-producing bacteria with reporter strains. Subtidal biofilm isolates were screened for AHL production using the cross-feeding, soft agar and extract bioassays (McClean et al. 1997, McLean et al. 1997). In the cross-feeding bioassay, the AHL reporter strain and the tested bacteria were streaked side by side on LB agar plates prepared with AFSW at 11 ppt salinity. For the soft agar bioassay, bacteria were streaked on agar plates prepared with full-strength AFSW (33 ppt). After 7 d of growth, bacterial cells were scratched off and soft agar containing CV026 or A136 plus X-Gal was put on top of the agar plate where bacteria had previously grown. For the extract bioassay, each isolate was grown on 5 agar plates for 7 d. The bacteria and agar were homogenized, then extracted 3 times with 200 ml of ethyl acetate (EA) containing 0.2% (v/v) glacial acetic acid. The 3 fractions of EA extracts were combined, evaporated to dryness and re-dissolved in 0.5 ml of dimethyl sulfoxide (DMSO) holes made in the agar beside streaks of the reporter strains were loaded with 10, 30, and 60 µl of the extracts. Plates were incubated at 28°C for 24 h, when activation of the reporter was recorded.

Identification of AHLs using GC-MS. Isolates that activated a reporter strain in at least one of the bioassays were subjected to GC-MS analysis to identify their AHL(s). AHLs were extracted from agar plates as with the extract bioassay, except that extracts were re-dissolved in 0.5 ml of dichloromethane.

The GC-MS (Varian/CP-3800 and Varian/Saturn 2200) was fitted with a relatively non-polar capillary column (CP-Sil 8 CB-MS, 30 m length, 0.25 µm film thickness, 0.25 mm i.d.). The injection port was held at 220°C. The temperature program was 120 to 275°C at 10°C min^{-1}, followed by a 15°C min^{-1} ramp to 300°C and a hold at 300°C for 5 min, with helium as the carrier gas. AHLs were identified by comparisons to synthetically available AHL standards and also to specific fragmentation patterns reported by Cataldi et al. (2004). AHL standards included *N*-butyryl-, hexanoyl-, octanoyl-, decanoyl-, dodecanoyl-, tetradecanoyl-homoserine lactone (C4-, C6-, C8-, C10-, C12-, C14-HSL), *N*-3-oxo-butyryl-, hexanoyl-, octanoyl-, decanoyl-, dodecanoyl-homoserine lactone (3-oxo-C4-, C6-, C8-, C10-, C12-HSL), and *N*-3-OH-butyryl-, hexanoyl-, octanoyl-, decanoyl-homoserine lactone (3-OH-C4-, C6-, C8-, C10-HSL), which were purchased from the laboratory of Prof. Paul Williams, UK. AHLs with C4- and C6- side chains were grouped into short-chain AHLs. AHLs with C8- and C10- side chains were grouped into medium-chain AHLs, and AHLs with C12- and C14- side chains were grouped into long-chain AHLs.

RESULTS

Diversity of isolates from subtidal biofilms

The 68 subtidal biofilm isolates were distributed among 3 phylogenetic branches, the Bacteroidetes (4 isolates), the α-Proteobacteria (13 isolates) and the γ-Proteobacteria (51 isolates) (Fig. 1). Sixty isolates had ≥97% 16S rRNA gene sequences similar to species deposited in the NCBI database. The other 8 strains had less than 97% similarity with known species, and 5 of them had less than 95%, indicating potentially novel genera or species. It was interesting that half of the Bacteroidetes were potentially novel species, suggesting that this phylum is an unexplored member in marine biofilms.

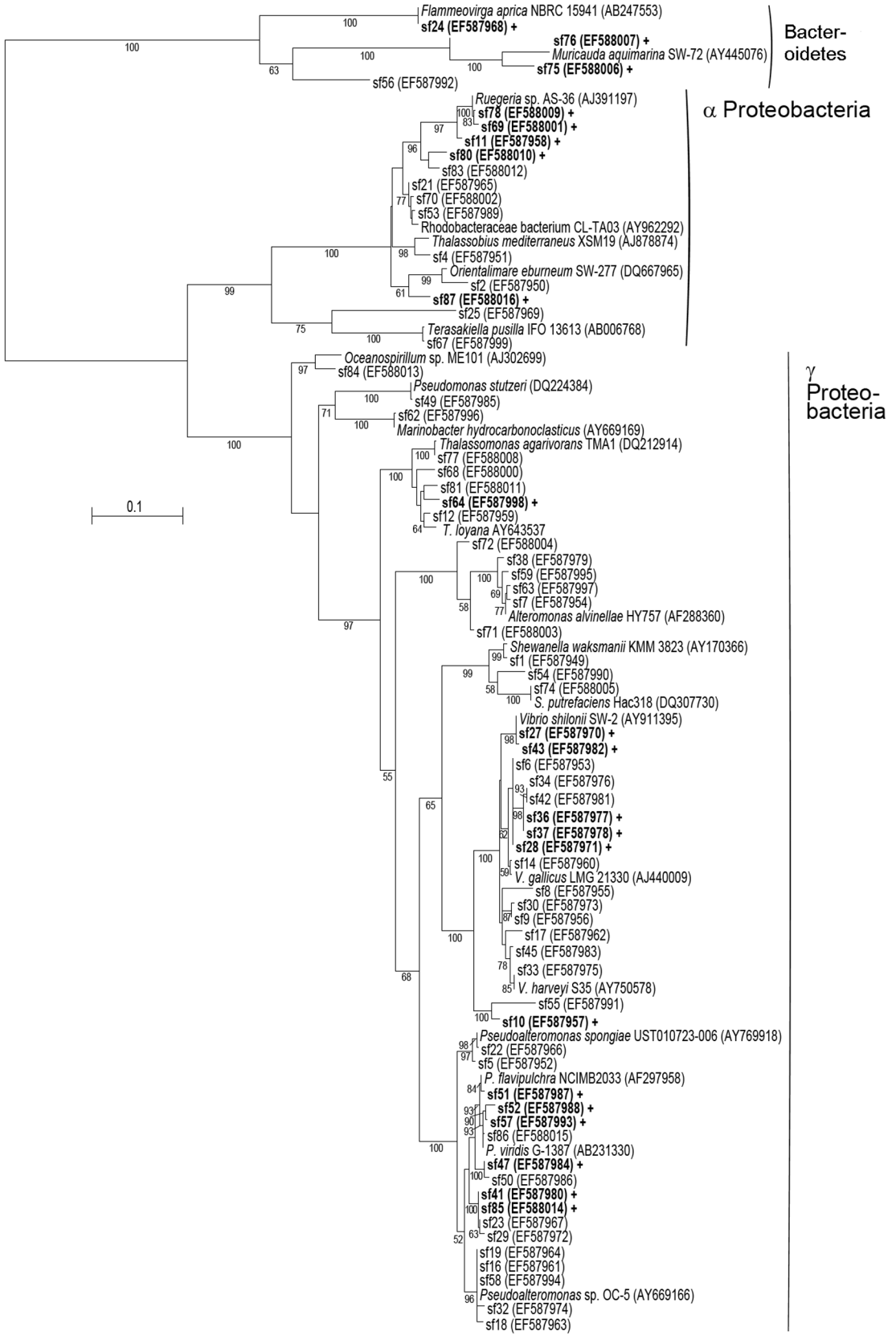

Fig. 1. Phylogeny of subtidal biofilm isolates from the Yung Shue Au fish farm, Hong Kong. This maximum likelihood tree is based on partial 16S rRNA gene sequences of subtidal biofilm isolates (denoted 'sf') and reference taxa. Bootstrap values above 50 are displayed. The number in parentheses found after each taxon name is the GenBank accession number for the representative DNA sequence. Acyl-homoserine lactones (AHLs) were identified from the isolates in bold and marked by a '+'

In the α-Proteobacteria, 11 out of the 13 isolates belonged to the family *Rhodobacteraceae,* and most of them fell within the well-known Roseobacter clade. Among the γ-Proteobacteria, isolates were affiliated to diverse genera such as *Pseudomonas, Shewanella, Thalassomonas, Alteromonas, Marinobacter, Oceanospirillum, Pseudoalteromonas* and *Vibrio,* among which *Pseudoalteromonas* (17 isolates) and *Vibrio* (15 isolates) were the 2 dominant genera.

Screening for AHL-producing bacteria

The AHL bioassay results were not consistent among different screening methods (Table 2). Representative bioassay results are shown in Fig. 2. In the cross-feeding bioassay, 42 out of the 68 subtidal biofilm isolates (62%) could grow on agar plates prepared with 11 ppt ASFW, and 11 of the 42 strains showed AHL production (Table 2). In the soft agar bioassay, 18 out of 68 strains showed AHL production. Finally, in the extract bioassay, 21 out of 68 strains showed AHL production, with some extracts showing very weak coloration or inhibitory effects on the reporter strains. Overall, 21 out of 68 isolates (31%) were AHL-producing bacteria. Most of these active strains induced coloration of the reporter strain *Agrobacterium tumefaciens* A136, but only a few of them, such as sf28, sf41 and sf47, induced coloration of the reporter strain *Chromobacterium violaceum* CV026.

Phylogenetic distribution of AHL-producing strains

The 21 AHL-producing isolates were distributed among the 3 phylogenetic groups, with 3 belonging to the Bacteroidetes, 5 to the α-Proteobacteria and 13 to the γ-Proteobacteria (Fig. 1). While the Bacteroidetes had the overall smallest number of isolates, it had the highest proportion (75%) of AHL-producing isolates. Among the α-Proteobacteria, the proportion of AHL-active bacteria was 38%. All 5 AHL producers belonged to the family *Rhodobacteraceae* and were affiliated to 3 genera: *Silicibacter* (1 isolate), *Ruegeria* (2 isolates) and *Roseobacter* (2 isolates). For the γ-Proteobacteria, 25% of the isolates were AHL-producing, consisting of 1 *Thalassomonas* sp., 6 *Pseudoalteromonas* spp. and 6 *Vibrio* spp.

Identification of AHL molecules in active bacteria

AHLs were identified by their retention time and mass spectra generated by GC-MS (Fig. 3). Most isolates produced more than one AHL (Table 2), which is not uncommon among AHL-producing bacteria. Using the peak areas to approximate the amount of AHL showed that most isolates did not produce large amounts of AHL (Fig. 3). The long-chain AHLs, such as C12- and C14-HSL, and AHLs with substitution of their keto group at the C-3 position, were prevalent (Table 2). AHLs with substitution of their hydroxyl group at the C-3 position were also found (Table 2).

Table 2. Acyl-homoserine lactone (AHL) production among subtidal biofilm isolates detected from bioassays and GC-MS. ng: no growth in either the tested isolates or the reporter strains, nd: not detected, W: weak coloration, +: coloration, –: no coloration

Isolate	Cross-feeding		Soft agar		Extract		GC-MS identification of AHLs
	A136	CV026	A136	CV026	A136	CV026	
sf10	+	–	+	–	+	–	C12-HSL; 3-oxo-C8-HSL
sf11	+	–	+	–	+	–	C12-HSL; 3-oxo-C6-HSL
sf24	ng	ng	+	–	W	W	C14-HSL; 3oxo-C6-, C8-HSL
sf27	+	–	–	–	+	–	C14HSL; 3-oxo-C8-HSL
sf28	+	–	+	+	+	+	C8-, C12-HSL; 3-oxo-C6-HSL
sf36	+	–	–	–	+	–	C10-, C12-HSL; 3-oxo-C10-HSL
sf37	+	–	–	–	+	–	C10-, C12-HSL; 3-oxo-C10-HSL
sf41	+	+	+	+	+	+	C6-, C8-, C12-, C14-HSL; 3-OH-C10-HSL
sf43	+	–	+	–	+	–	C12-HSL; 3-oxo-C4-, C6-HSL
sf47	ng	ng	+	W	+	+	C12-HSL; 3-oxo-C8-HSL, 3-OH-C6-HSL
sf51	ng	ng	+	–	+	–	C10-, C12-HSL; 3-oxo-C4-, C6-, C10-HSL
sf52	ng	ng	+	–	+	–	C8-, C12-HSL; 3-oxo-C10-HSL
sf57	ng	ng	+	–	+	–	C8-, C12-HSL; 3-oxo-C8-, C10-HSL
sf64	ng	ng	+	–	+	–	C8-, C12-, C14-HSL; 3-oxo-C6-, C8-, C10-HSL
sf69	+	–	+	–	+	–	C10-, C12-, C14-HSL; 3-oxo-C6-, C10-HSL; 3-OH-C4-HSL
sf75	ng	ng	+	–	+	–	C14-HSL; 3-oxo-C4-, C8-HSL
Sf76	ng	ng	+	–	W	–	nd
sf78	+	–	+	–	+	ng	C6-, C8-, C14-HSL; 3-oxo-C4-, C6-HSL
sf80	+	–	+	–	+	W	C8-, C12-, C14-HSL; 3-oxo-C4-, C6-HSL
sf85	ng	ng	+	–	+	–	C10-, C12-, C14-HSL; 3-oxo-C4-, C6-, C10-HSL
sf87	ng	ng	+	–	+	W	C6-, C12-, C14-HSL

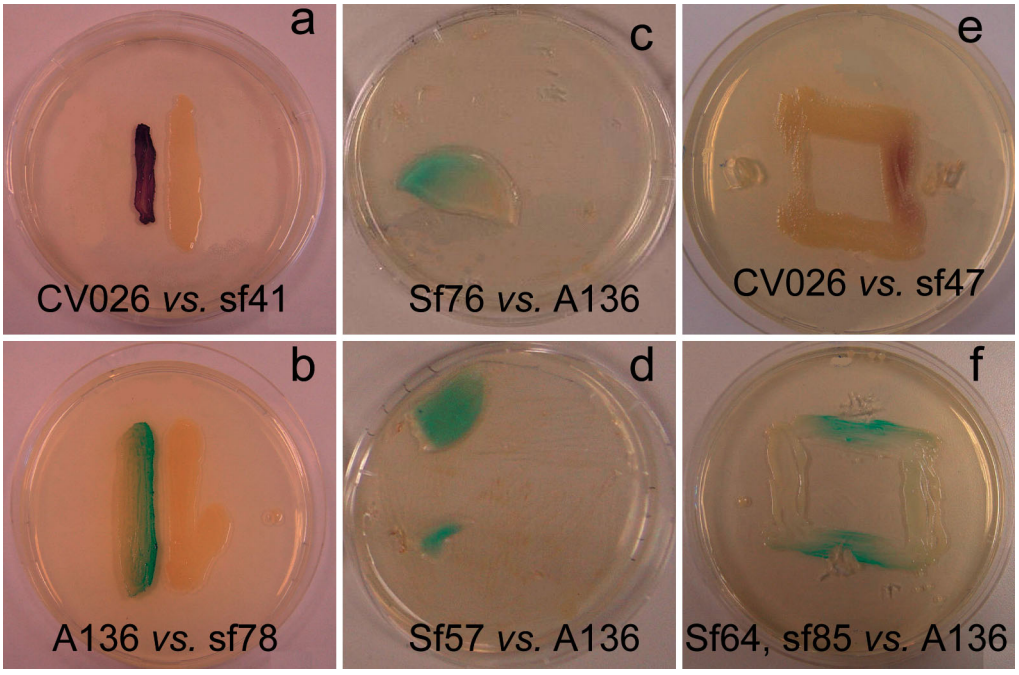

Fig. 2. Representative positive results from the (a,b) cross-feeding bioassay, (c,d) soft agar bioassay, and (e,f) extract bioassay using the reporter strains (a,e) *Chromobacterium violaceum* CV026 and (b,c,d,f) *Agrobacterium tumefaciens* A136

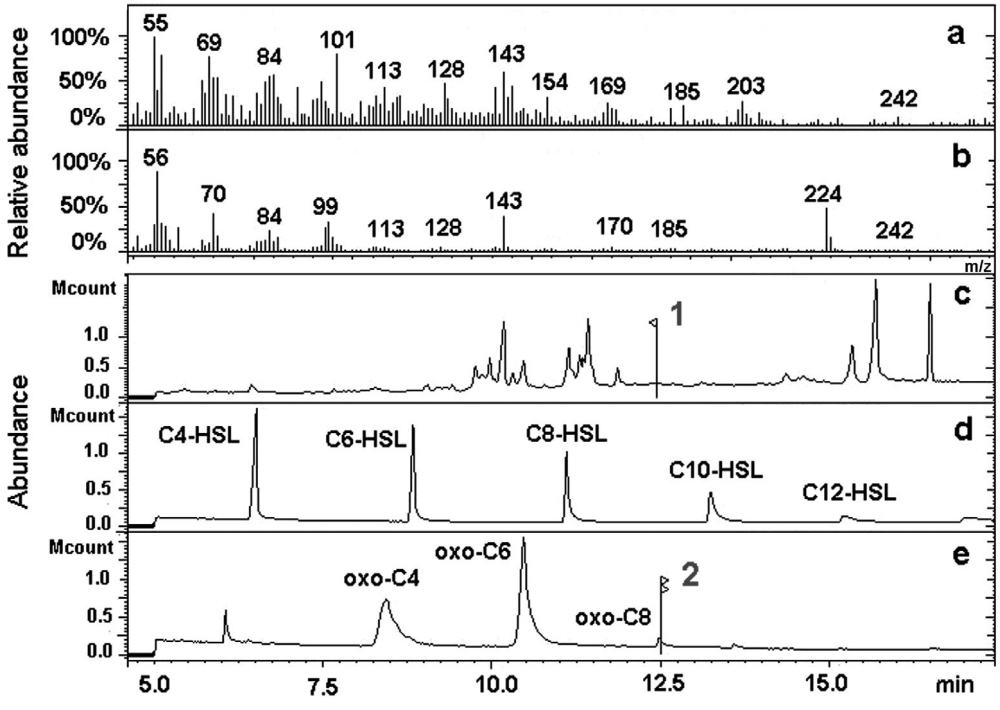

Fig. 3. Identification of 3-oxo-octanoyl-homoserine lactone (3-oxo-C8-HSL) in the strain sf75 extracts by GC-MS. (a,b) Mass spectra of the selected peak indicated by (a) Flag 1 and (b) Flag 2. (c) GC ion chromatogram of the strain sf75 extract. (d) GC ion chromatogram showing the retention times of the standard acyl-homoserine lactones. (e) GC ion chromatogram showing the retention times of the standard 3-oxo-acyl-homoserine lactones. The selected peak 1 (Flag 1) in the sf75 extract has the same retention time and similar mass spectrum as the standard 3-oxo-C8-HSL

DISCUSSION

Phylogenetic diversity of cultivable bacteria in subtidal biofilm isolates

It has long been known that the diversity of microbes from environmental samples is underestimated by the culture-dependent method. Nevertheless, this approach is necessary to study AHL production in individual bacteria from subtidal biofilms, as bioassays are the only method for detecting AHLs and bacteria must be cultured for bioassays.

Most of the 68 bacterial isolates in the present study were related to α-Proteobacteria and γ-Proteobacteria, which are ubiquitous in the marine environment (Dang & Lovell 2000, Gram et al. 2002, Lau et al. 2002, Wagner-Dobler et al. 2005, Mohamed et al. 2008). Eleven of the 13 α-Proteobacteria belonged to the *Rhodobacteraceae* or Roseobacter clade, which is an abundant group found in diverse marine habitats and biofilms on artificial surfaces in seawater (Dang & Lovell 2000, Buchan et al. 2005). The other 2 major groups were *Pseudoalteromonas* spp. and *Vibrio* spp. in the γ-Proteobacteria, which is consistent with the study by Lau et al. (2002). Beside these major groups, we also isolated some minor groups and some potentially new species, suggesting that subtidal biofilms are complex niches that harbor various bacterial species.

Diversity of AHL-producing isolates from subtidal biofilms

Subtidal biofilms are clearly a good source for AHL-producing bacteria. We may have missed some AHL-producing strains due to limitations in our bioassays; still, we observed a larger diversity than what has previously been reported (Gram et al. 2002, Moran et al. 2004, Taylor et al. 2004, Wagner-Dobler et al. 2005, Mohamed et al. 2008). AHL-producing bacteria from subtidal biofilms were distributed among a range of taxa including the Bacteroidetes, the Roseobacter clade within the α-Proteobacteria and the genera *Thalassomonas*, *Vibrio* and *Pseudoalteromonas* within the γ-Proteobacteria. The strains in common with other studies were mainly α-Proteobacteria and *Vibrio* spp. In the present study, 38% of α-Proteobacteria produced AHLs. AHL-producing members in the genus *Ruegeria* were reported for the first time in the present study.

Among the γ-Proteobacteria, 40% of the *Vibrio* spp. and 35% of the *Pseudoalteromonas* spp. produced AHLs (Fig. 1). *Vibrio* spp. are well-known QS species, as QS was first described in *Vibrio fischeri* (Milton 2006). Although the marine *Pseudoalteromonas* spp. was known to produce biologically active compounds (Holmstrom & Kjelleberg 1999), the present study is the first report of AHL production in this genus. AHLs were also detected from isolate sf64 of the genus *Thalassomonas,* which is again the first report of QS from a member of this genus.

Previously, Proteobacteria were thought to be the only group of bacteria capable of AHL-driven QS. However, Wagner-Dobler et al. (2005) reported one Bacteroidetes that activated the AHL reporter strain. In the present study, 3 Bacteroidetes (sf24, sf75 and sf76) activated the AHL reporter strain. For 2 of these isolates, sf24 and sf75, we identified the AHLs by GC-MS analysis (Table 2, Fig. 3). As the production of AHLs by non-Proteobacterial species is rather novel, further characterization of AHL-driven QS in these strains is currently underway. However, the high proportion of AHL-producing isolates in Bacteroidetes found in the present study may not indicate that AHL-driven QS is prevalent among the Bacteroidetes, as only 4 isolates were recovered. Nevertheless, QS could play an important role in surface colonization among the cultivable Bacteroidetes.

Diversity of AHL molecules

The identification of AHL molecules by GC-MS was consistent with bioassay results. For example, the prevalence of long-chained AHL molecules can explain why only a few strains induced coloration of CV026, since the coloration of CV026 is only induced by short- and medium-chain AHLs.

AHLs have a conserved homoserine lactone ring; however, they vary in the acyl side chain length and type or degree of substitution of the acyl side chain. The stability of AHL molecules increases with the length of the side chain (Yates et al. 2002). In the present study, most of the isolates produced more than one kind of AHL molecule, many of which had long acyl side chains. One may argue that marine bacteria produced long-chain AHLs in order to adapt to the alkaline seawater environment, in which the pH value is usually ca. 8.2.

Potential role of AHL in marine biofilms

Biofilm formation and QS are the two hot topics in microbiology. The key challenge in studying bacterial QS and biofilm formation is to determine the functional consequences of QS in mixed-species communities in the natural environment (Parsek & Greenberg 2005). It was presumed that bacteria use QS to give a competitive advantage to the population of QS cells (Zhang & Dong 2004). However, so far there have been no solid

data to support this hypothesis, although QS blockers (Dobretsov et al. 2007) and the presence of AHLs (McLean et al. 2005) were found to change the bacterial community composition. In the present study, we showed that diverse isolates can act as primary colonizers of subtidal biofilms and are capable of producing AHL molecules. We observed that all the *Vibrio* spp. and most of the *Pseudoalteromonas* spp. were isolated from the first-day biofilm, while most of the Roseobacters were isolated from the 7 and 9 d old biofilms. The ability to use QS may be part of the genetic machinery that enables the Roseobacters, *Vibrio* spp. and *Pseudoalteromonas* spp. to dominate this marine niche, and at the same time provides the basic driving force for the succession of the whole bacterial community.

In conclusion, this is the first report of diversity and AHL production among bacteria in marine subtidal biofilms. Several genera of marine bacteria were found for the first time to contain AHL-producing members. The possible role of AHL-driven QS in subtidal biofilm formation deserves further investigation.

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