

***Bacillus* species in food fermentations: an under-appreciated group of organisms for
safe use in food fermentations**

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Abstract

Fermented foods are consumed throughout the world and substantially contribute to food security. Many fermented products rely on the participation of *Bacillus* species but their contribution to food quality is not well documented. The purpose of this review is to highlight metabolic properties that relate to food quality and food safety. Most fermented foods that include *Bacillus* species use legumes or tubers as substrate and are traditionally prepared in East Asia, Africa and South America. In food fermentations, *Bacillus* species produce amylases and proteases, extracellular polysaccharides and polypeptides, and lipopeptides with antimicrobial activity. The metabolic traits of bacilli also provide opportunities for use in food fermentations in which they do not traditionally occur.

Keywords: *Bacillus*; food fermentations; microbial enzymes; antimicrobial lipopeptides; probiotic; *Bacillus cereus*

1 **Introduction**

2 Food fermentations utilize the activity of microorganisms for conversion of food components
3 to improve shelf-life and safety, as well as sensory and nutritional properties of food. In
4 addition, the presence of live microorganisms in fermented foods is increasingly recognized to
5 improve gastrointestinal health [1–3]. The assembly of communities of fermentation microbes
6 is determined by the fermentation conditions, i.e., the ingredients, temperature, pH, moisture
7 content, and using back-slopping or starter cultures (5). Most fermentations include yeasts
8 and/or lactic acid bacteria (LAB) as major fermentation microbes but mycelial molds, acetic
9 acid bacteria, staphylococci, propionibacteria, *Enterobacteriaceae* and bacilli also play
10 dominant roles in some fermented foods [4]. The contribution of *Bacillus* to food quality is not
11 nearly as well documented as the contribution of yeasts and lactobacilli.

12 *Bacillus* spp. are isolated from soil, water and plants, and are almost ubiquitously present in
13 foods [5]. They form endospores as a key element of their ecology [6]. *Bacillus* endospores
14 resist adverse conditions including heat, pressure, desiccation, and chemical assaults [6,7]. The
15 resistance of spores to high temperature and high pressure and thus the survival in ingredients
16 that are cooked or steamed is further increased by the *spoVA*^{2mob} operon [8,9]. The ecology of
17 *Bacillus* spp. and their relevance for plant agriculture and food production is shown in Figure
18 1. The presence of *Bacillus* spores in plants including cereals, pulses and cassava relates to
19 their occurrence as endophytes [10–13]. Because spores survive even cooking steps [14],
20 cooked or uncooked cereal grains, pulses and cassava generally harbor endospores of *Bacillus*
21 species [15,16].

22 *Bacillus* species are recruited for traditional food fermentations of pulses, tubers or cereals in
23 Asia, Africa and South America (Figure 2) [3,17–19]. This review aims to provide an overview
24 on the role of *Bacillus* in food fermentations by presenting traditional fermented foods that

25 include *Bacillus* species as major fermentation organisms, their specific contribution to food
26 quality and safety, and explore the potential of using these organisms in non-conventional
27 fermentation processes.

28 **The presence of *Bacillus* in food fermentations**

29 An overview on food fermentations that include *Bacillus* species as major fermentation
30 organisms is shown in Table 1. Major processing and fermentation steps are also indicated in
31 Table 1 to inform on major processing steps and fermentation conditions that select for *Bacillus*
32 species. The overview is likely incomplete because not all fermented foods are documented in
33 the scientific literature. Moreover, the overview in Table 1 does not reflect that fermented foods
34 are produced in numerous varieties that share major processing and fermentation steps but may
35 differ in flavor and appearance [3].

36 **Fermented products from soybean and pulses**

37 Consumption of soybean and soybean derived fermented products has a long history in many
38 East and South Asian countries (Table 1) [19]. *Bacillus* spp. frequently occur in alkaline food
39 fermentations, which use soybeans as substrate [17]; Several examples are presented in more
40 detail below.

41 ***Natto***

42 *Natto* is a traditional Japanese fermented food produced from soybeans fermented by *natto*
43 starter strains of *Bacillus subtilis* var. *natto* [20]. To make *natto*, soybeans are soaked, steamed,
44 inoculated with *B. subtilis* var. *natto* and fermented at 40 °C for 24 h. The *natto* is cooled and
45 aged in refrigerator for up to 1 week allowing the development of stringiness (Table 1). The
46 fermentation process supports secretion of proteases by bacilli to degrade soy proteins, the
47 production and racemization of L- and D-glutamic acids, and the synthesis and secretion of
48 poly- γ -glutamate [21].

49 ***Cheonggukjang***

50 *Cheonggukjang* is a Korean soybean paste made from cooked soybeans fermented with
51 *Bacillus* species at around 40 °C for 2-3 days (Table 1) [22]. Fermentation with *Bacillus*
52 hydrolyses soybean proteins and polysaccharides. In addition, isoflavones are converted to the
53 corresponding aglycones during fermentation [22].

54 ***Doenjang***

55 *Doenjang* is traditionally manufactured by fermentation of *meju*, the fermented soybean block.
56 *Meju* is traditionally prepared by soaking, steaming, crushing, and then fermented for 2 to 3
57 months with *B. subtilis*, *Rhizopus* spp., and *Aspergillus* spp. The fermented *meju* is separated
58 into two parts; the supernatant liquid part is filtered to prepare soy sauce and precipitated solid
59 part is used for further ripening for over 2 months to make *doenjang* (Table 1) [23]. In a
60 modified, commercialized method, *meju* fermentation is solely inoculated by *B. subtilis* to
61 make *koji*, and *koji* is further ripened by *Aspergillus oryzae*, additional grains (e.g., wheat and
62 barley), and/or other flavor enhancing materials. Therefore, the use of controlled
63 microorganisms and standardized processes can boost the production efficiency while
64 maintaining the flavor consistency of *doenjang* [23].

65 ***Gochujang***

66 *Gochujang* is a traditional Korean fermented paste made from *meju*, red pepper powder, and
67 glutinous rice. The characteristic flavor of *gochujang* is a combination of hot taste from red
68 pepper, sweet taste from sugars, umami taste from amino acids, and salty taste from NaCl.
69 *Gochujang* is fermented with *Aspergillus* spp., *Bacillus* spp. and *Rhizopus* spp. for several years
70 in large earthen pots by mixing glutinous rice powder, salt, and red pepper powder with *meju*
71 powder (Table 1) [24].

72 *Hawaijar*

73 *Hawaijar* is a fermented soybean product in Manipur, India, with a distinct flavour and
74 stickiness [25]. It is consumed commonly in the local diet as a staple source of high protein
75 food [26]. Whole soybeans are soaked overnight, washed and boiled. After draining excess
76 water, the cooked soybeans are washed with hot water, wrapped with cotton cloth or banana
77 leaves and packed tightly in a basket with a lid. The basket is wrapped with cloth and kept in
78 the sun, near a stove, or buried in paddy straw for fermentation for 4 to 5 days (Table 1). The
79 final product has a pH of 8.0 to 8.2 and is of brown colour with a sticky slimy white appearance
80 and a light ammonia odour [26,27]. In *hawaijar* fermentation, no starter culture is added during
81 its preparation. *Bacillus* strains that dominate the fermentation originate from raw soybeans
82 [11] or are acquired from other materials used for fermentation [27].

83 *Sufu*

84 *Sufu* is a Chinese fermented made from cubes of soybean curd (tofu) by mold ripening [28].
85 Four steps are involved in *sufu*-making; preparing tofu from boiled soymilk; brining; preparing
86 *pehtze* (soybean curd overgrown with fungal mycelium) with a fungal starter culture; salting;
87 and ripening for 2-3 months in a dressing mixture. During brining, the tofu adsorbs the salt
88 until the salt content of tofu reaches about 6.5 %, which takes about 2 days. *Pehtze* is prepared
89 by inoculating pure culture of *Bacillus* spp. or *Micrococcus* spp. to tofu and incubated at 30-
90 38 °C for about 1 week. *Pehtze* is dried at 50-60 °C for 12 h prior to subsequent fermentation
91 in the dressing mixture. The most common types are mold-fermented *sufu*, and bacteria-
92 fermented *sufu*, which includes *Bacillus* and/or *Micrococcus* as major members of fermentation
93 microbiota (Table 1) [29].

94 **Roots, tubers, and vanilla fermentation products**

95 Tubers including cassava, yams and potatoes are second only to cereals in importance as a
96 global source of carbohydrates. In most African and Asian countries, the traditional diets of a
97 majority of people rely largely on cassava and yam, which are poor in other nutrients,
98 particularly proteins, essential amino acids, vitamins and minerals, but contain significant
99 amounts of starch and dietary fiber [30]. Cassava also contains the cyanogenic glycosides
100 linamarin and lotaustralin that release cyanide during digestion unless the β -glucosidic bonds
101 are hydrolysed during food processing [31]. An overview on fermented tubers and the
102 organisms that occur in the fermentation is provided in Table 1; several examples are presented
103 in more detail below.

104 ***Ntoba Mbodi***

105 Ntoba Mbodi is a popular alkaline fermented food in Congo [31]. It is made by fermenting
106 cassava leaves in the following way: the leaves are harvested, wilted for 2-3 days, cleaned, cut
107 into small pieces, wrapped in small portions into large leaves, and allowed to ferment at
108 ambient temperature for 2-4 days (Table 1). Fermentation also eliminates cyanogenic
109 glucosides [31,32]. A rise in pH of up to 10 is observed during the process. The main
110 microorganisms responsible for the fermentation are *Bacillus* spp. [31].

111 ***Cassava tape***

112 Cassava tape (fermented cassava) is an Indonesian traditional food made by fermentation of
113 cassava. It is made from steamed cassava mixed with a starter commonly known as “ragi tape”.
114 In general, the cleaned and peeled cassava tubers are steamed, cooled, and placed in basket.
115 Powdered ragi is sprinkled over the cassava. The cassava is covered with banana leaves and
116 incubated at room temperature for 2-3 d (Table 2.1). The quality of cassava tape depends on
117 many conditions, including the quality of cassava, preparation method, and microbes [33].

118 Several *Bacillus* spp. including *B. subtilis*, *B. amyloliquefaciens* and *B. thuringiensis* have been
119 isolated from cassava tapes [33].

120 ***Elubo***

121 Yams (*Dioscorea* spp.) are processed and fermented to the traditional West African dried yam
122 flour *elubo*. Yam slices are blanched at for 10 min 60 °C and fermented for 24 h at 30 °C.
123 During the first 24 h of spontaneous fermentation, the microbial population grows as the pH
124 falls from 6.2 to 5.4. Back-slopping at a rate of 10% (w/v) is used to accelerate natural lactic
125 fermentation by using the preceding fermentation batch as an inoculum (Table 1). Fermentation
126 experiment of blanched samples with pure cultures of the isolates indicated that
127 *Lactiplantibacillus plantarum*, *Levilactobacillus brevis* and *B. subtilis* are the main species in
128 the reconstituted flour paste [34].

129 ***Taruba***

130 Indigenous populations in the Amazon region of South America produce fermented cassava
131 beverages for daily consumption from cassava alone, or from cassava with addition of corn or
132 potatoes. Cassava roots are generally washed and soaked, followed by crushing and sieving,
133 cooking or toasting, and fermentation for one or several days. Bacilli including *B. subtilis* and
134 *B. amyloliquefaciens* were consistently identified as members of fermentation microbiota,
135 which also includes *Lp. plantarum*, *Lv. brevis* and pediococci [35,36].

136 ***Vanilla***

137 Vanilla flavoring obtained from cured *Vanilla planifolia* (Andrews) beans is widely used in
138 food, beverages, and cosmetics [37]. The characteristic vanilla flavor is formed during a curing
139 process that yields the character impact compound vanillin. The conventional curing processes
140 involves four steps: blanching, sweating, drying, and conditioning. Mature fresh vanilla beans
141 are blanched and then stored at high humidity and temperature. Sweating retains a sufficiently

142 high moisture content for the enzyme-catalyzed reaction. The vanilla beans are dried by sun or
143 air to inhibit mold growth and stored in a closed box for several months for formation of
144 vanillin [38,39]. Thermophilic bacilli develop during sweating (Table 1) [40]. *Bacillus* isolated
145 from vanilla beans produced β -D-glucosidase, which hydrolyses glucovanillin to vanillin [39].

146 **Cereal fermented product**

147 *Daqu*

148 *Daqu* is a spontaneous solid-state cereal fermentation that is used as a saccharification starter
149 to initiate the alcoholic mash fermentation for production of cereal liquors and vinegars in
150 China (Figure 3) [41,42]. The liquor starter is prepared with different grains including wheat,
151 rice, sorghum, that are shaped in blocks and fermented with controlled temperature and
152 humidity [43]. The bacterial microbiota composition of *daqu* is diverse and includes *Bacillus*
153 spp., *Enterobacteriaceae* and LAB (Table 1) [4,44]. Fungal organisms include *Aspergillus* spp.,
154 *Mucor* spp., and *Penicillium* spp. [44,45]; the most frequently isolated yeasts belong to the
155 genus *Saccharomyces* [44]. In China, *daqu* is also used as a starter culture for sourdough
156 fermentation but bacilli were not identified as members of microbiota of the corresponding
157 sourdough [46].

158 **Fish sauce**

159 Fish sauce is a condiment that is traditionally consumed in Southeast Asia but becomes
160 increasingly popular in other places of the world [47]. It is produced by mixing fish, such as
161 anchovies, with salts and fermenting for 6 to 12 months at room temperature [48]. Fish proteins
162 are hydrolyzed by proteases from the fish and from halotolerant/halophile microorganisms,
163 resulting in umami-tasting peptides and amino acids [49,50]. The fermentation organisms in
164 fish sauces predominantly include halophilic or salt tolerant bacteria including *Bacillus* species
165 (Table 1) [51,52].

166 **The role of *Bacillus* in food fermentation**

167 *Bacillus* species have a variety of desirable characteristics, including their ability to form
168 endospores, and several strains or species have been awarded GRAS (Generally Regarded as
169 Safe) status by the Food and Drug Administration [53] and QPS (Qualified Presumption of
170 Safety) by the European Food Safety Authority [54]. Figure 4 provides an overview on the
171 presence of desirable or undesirable metabolic traits in type strains of the genus *Bacillus*. The
172 role of *Bacillus* as source of industrial enzymes has been well reviewed elsewhere [55,56]. In
173 this section, the role of enzymes produced by *Bacillus* during food fermentations are discussed.

174 **Role of amylases**

175 The amylolytic system of *Bacillus* species includes glucan branching enzymes, extracellular
176 amylases including α -amylases, β -amylases, pullulanases and glucoamylases, and intracellular
177 oligosaccharide hydrolases. Most type strains of the *B. cereus* and *B. subtilis* groups produce
178 multiple amylases (Figure 4). Yams and cassava have a low content of fermentable
179 carbohydrates and low amylase activity [57]. Therefore, extracellular amylases produced by
180 *Bacillus* spp. are particularly important for the hydrolysis of starch [58].

181 Thermostable amylases of *Bacillus* are also a key element of *daqu* fermentations [59]. Strains
182 of the *B. subtilis* group that are present in *daqu* fermentations have a high starch degrading
183 ability including thermotolerant α -amylase activities [53]. Amylase activity in *daqu* was
184 significantly increased by inoculation with *B. licheniformis* [59]. Amylases produced by
185 *B. licheniformis* in *daqu* fermentation yielded maltose, maltotriose, and maltodextrins as major
186 products from starch [60].

187 **Role of β -glucosidases, phytase and α -galactosidase**

188 β -Glucosidase hydrolyzes β -glucosidic linkages between carbohydrate residues in aryl-amino-
189 or alkyl- β -D-glucosides, cyanogenic glucosides, short chain oligosaccharides and

190 disaccharides under different physiological conditions [61]. The gene that produce BglA is
191 encoded by most type strains of the *B. subtilis* group (Figure 4).

192 Isoflavones are abundant in soybeans and have various health benefits related to their
193 oestrogenic activities [62,63]. They have bioactivity only when the glycosides that are present
194 in soybeans are hydrolysed to the isoflavone aglycones [62,63]. During the fermentation of soy
195 with *Bacillus*, glucoside conjugates of isoflavones are converted into aglycones by *Bacillus*
196 β -glucosidase [64].

197 The β -glucosidase of *Bacillus* species also hydrolyzes cyanogenic glycosides in cassava.
198 Linamarin accounts for 80 % of the cyanide content of cassava and is known to cause severe
199 disease upon continual consumption [65]. β -Glucosidase activity of bacilli detoxifies cassava
200 cyanides without compromising other nutrients [66].

201 Vanillin is the main aromatic constituent in cured vanilla pods [67]. In fresh vanilla beans,
202 vanillin is present as glucovanillin, an odorless β -D-glucoside of vanillin. One of the most
203 important aspects of curing is the conversion of glucovanillin to vanillin by β -glucosidase [68].
204 *Bacillus* in vanilla fermentation produce β -glucosidase which hydrolyse glucovanillin [39]. Its
205 formation may be due to the action of thermo-tolerant bacteria such as *B. subtilis* because of
206 their dominance in vanilla curing up to 65 °C.

207 Fermentation is also considered a necessary and accepted method to reduce the anti-nutritive
208 phytic acid and raffinose family oligosaccharides in pulses (73). Most of total seed phosphorus
209 (P) in legumes and cereal grains is present as phytate, which chelates minerals and decrease
210 their bioavailability to humans and monogastric animals (74). Most fermented soy products
211 involve a cooking step prior to fermentation. This cooking step inactivates plant enzymes and
212 phytate hydrolysis during fermentation is dependent on microbial enzymes. Species of the

213 *B. subtilis* group are effective producers of phytases, which hydrolyze phytate and increase the
214 bioavailability of minerals in cereals and legumes (Figure 4) (75).

215 Raffinose family oligosaccharides (RFOs), i.e., raffinose, stachyose, verbascose, are abundant
216 in soybean and legumes. They are composed of one or more galactose residues joined by
217 α -(1 \rightarrow 6)-glycosidic bonds to sucrose. These indigestible carbohydrates can lead to flatulence
218 and other gastrointestinal disorders [69]. α -Galactosidases degrade RFOs and improve the
219 nutritional value of soy and legumes [70]. *Bacillus* species, particularly species of the
220 *B. subtilis* group also encode for α -galactosidases, which hydrolyse raffinose-family
221 oligosaccharides in soybean and legumes (Figure 4).

222 **Role of proteases**

223 Type strains of the *B. subtilis* group produce multiple extracellular proteinases (Figure 4).
224 Because of their broad pH and temperature activity and stability range, proteases from *Bacillus*
225 play an essential role in soybean and fish fermentations as they are tolerant to alkaline
226 environments and high temperature.

227 Proteases are secreted by *B. subtilis* during food fermentations. Degradation of proteins to
228 peptides and amino acids impacts the texture and flavor of the products, may generate bioactive
229 peptides, and provides precursor amino acids for conversion to flavor volatiles [1,71–73].

230 Fermented fish sauces are particularly rich in soluble taste-active peptides, and amino acids
231 [49,50]. They are produced during proteolytic degradation by proteases in the muscles or
232 digestive tracts of fish, and various microorganisms existing in the fermentation broth [74].

233 Proteases produced by *Bacillus* play an essential role in the degradation process. A halotolerant
234 protease produced by *B. licheniformis* has been identified in Thai fish sauce with a high
235 enzymatic activity, even under high salt conditions (30 % NaCl) [48].

236 **Role of polyglutamate and glutaminase**

237 Glutaminase of *Bacillus* convert glutamine to the umami tastant glutamate. Glutaminases of
238 *Bacillus* spp. are tolerant to high temperature and high salt conditions; at alkaline conditions,
239 glucaminases of *Bacillus* also synthesize kokumi-active γ -glutamylpeptides [71,75,76].
240 Glutamate accumulates in the food matrix and contributes to the umami taste but is also
241 converted to poly- γ -glutamic acid (γ -PGA). PGA is synthesized by polymerizing D- and L-
242 glutamate to an anionic biopolymer comprised of only glutamic acid residues. It is water
243 soluble, biodegradable, and has good thickening capacity and excellent absorbability [77]. γ -
244 PGA is produced by *Bacillus* spp. to form part of the mucilage of *natto* and *cheonggukjang*
245 (Figure 4) [78,79]. The stringy consistency of *natto* is mainly due to γ -PGA produced by *B.*
246 *subtilis* [79]. The production of γ -PGA from *natto* starters were reduced in medium containing
247 NaCl greater than 3 % [80]. A salt tolerant *B. subtilis* strain was found in a *cheonggukjang*
248 fermentation, which also produces γ -PGA [78].

249 **Role of antimicrobial lipopeptides**

250 *Bacillus* species produce lipopeptides, including surfactins, fengycins and iturins [81,82] with
251 a broad-spectrum antimicrobial activity [82,83]. The non-ribosomal peptide synthases that
252 synthesize these lipopeptides are present almost exclusively in strains of the *B. subtilis* group
253 (Figure 4).

254 Surfactins show antibacterial activity but, with some exceptions, no marked antifungal activity
255 [81]. Iturins display a strong antifungal action against a wide variety of yeast and fungi but
256 only limited antibacterial activity. For example, iturin from *B. pumilus* HY1 inhibited *A. flavus*
257 and *A. parasiticus* with an minimum inhibitory concentration of 50 mg/L [84]. This
258 fungitoxicity of iturins has been attributed to membrane permeabilization [82]. Fengycins also
259 have a strong antifungal activity, specifically against filamentous fungi [81]. *In vitro* studies

260 indicate that the ability of *B. subtilis* inhibiting aflatoxin-producing fungi was substantially
261 greater than the inhibitory effect of lactic acid bacteria [85]. Lipopeptides produced by *Bacillus*
262 were detected in *cheonggukjang*, *daqu*, and other solid-state fermentations [86–88]. *In situ*
263 antifungal activity of lipopeptides produced by *B. velezensis* and *B. amyloliquefaciens* was
264 demonstrated in model *daqu* fermentations [89]

265 **Safety and health benefits of *Bacillus* species**

266 **Assessment of the safety of *Bacillus* species and their toxins**

267 *Bacillus* species are also of concern for food safety. Toxins are produced by certain strains of
268 the *B. cereus* group (Figure 4). The *B. cereus* group includes *B. cereus*, *B. anthracis*,
269 *B. thuringiensis*, *B. mycooides*, which includes strains previously designated as
270 *B. weihenstephanensis*, *B. pseudomycooides*, *B. cytotoxicus* and *B. toyonensis* (Figure 4) [90].
271 Toxins produced by these organisms such as non-haemolytic enterotoxin (*nhe*); haemolysin
272 BL (*hbl*); cytotoxin K (*cytK*), cereulide, and the lethal anthrax toxin (Figure 4). The
273 lipopeptides surfactin and lichenysin are biosurfactants with *in vitro* hemolytic and cytolytic
274 activity [82] but are not known to contribute to human (foodborne) disease [90], and are not
275 produced by pathogenic *Bacillus* species (Figure 4). The non-haemolytic enterotoxin,
276 haemolysin BL, and single protein cytotoxin K have been linked to the diarrheal type of *B.*
277 *cereus* food poisoning [91]. Nearly all *B. cereus* strains harbor the *nhe* genes, while *hbl* and
278 *cytK* are detected in about 30-70 % of isolates. The emetic toxin cereulide is produced is
279 produced by a non-ribosomal peptide synthase, which is present only in *B. cereus* and related
280 species (Figure 4) [92]. Cereulide produced in food results in rapid onset of vomiting after
281 ingestion but is rarely lethal. *B. anthracis* is the causative agent of anthrax, an acute lethal
282 disease in animals and in humans [93]. Bipartite exotoxins produced by *B. anthracis* include
283 protective antigen-lethal factor (PA-LF) and PA-edema factor (PA-EF) [90].

284 Because *Bacillus* strains other than strains of the *B. cereus* group rarely produce toxins, bacilli
285 were included in the QPS list, with the qualification of "absence of toxigenic potential" [54,94].
286 Figure 4 documents that the potential for toxin production is closely linked to phylogeny.

287 **Use of *Bacillus* species as probiotics**

288 Probiotics are defined as “live microorganisms which confer a health benefit on the host when
289 administered in adequate amounts” [95]. The role of LAB as animal and human probiotics is
290 well documented [96,97]. Endospores of *Bacillus* species have been proposed as heat resistant
291 alternatives [98] and their probiotic activity is increasingly documented in clinical trials [99].
292 Several clinical trials support claims that *Bacillus* probiotics reduce chronic gastrointestinal
293 symptoms, and increase the resistance to infections [99]. The occurrence of pathogenic strains
294 or species in the genus *Bacillus*, however, impedes consumer acceptance of *Bacillus* probiotics
295 [99]. Moreover, it remains unclear whether *Bacillus* fermented foods deliver a sufficient dose
296 to viable bacterial cells or spores to exert probiotic effects [100].

297 **Conclusions**

298 *Bacillus* species are an under-appreciated group of bacteria that are prevalent in numerous food
299 fermentations, particularly in Asia and Africa. Strains of the *Bacillus subtilis* group produce
300 extracellular hydrolytic enzymes, potent antifungal lipopeptides and extracellular polymeric
301 compounds. These properties that are only rarely found in other dominant groups of food
302 fermenting bacteria such as lactic acid bacteria, acetic acid bacteria, or propionic acid bacteria.
303 Toxigenic bacilli and those species that possess functional traits cluster in two separate
304 phylogenetic groups. With the better understanding and further study of *Bacillus*, the
305 fundamentally positive characteristics of the *Bacillus* species will make them the preferred
306 starter cultures for many novel and improved fermented foods.

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662 **Figure legends**

663 **Figure 1.** A simplified view of the lifestyle of *Bacillus* in different environmental niches.
664 Mutualistic interactions of *Bacillus* with plants or humans are highlighted in red letters;
665 pathogenic interactions are in blue letters. Soil is the largest reservoir of *Bacillus*. *Bacillus* spp.
666 form stable symbiotic associations with plants and occur in the rhizosphere or as endophytes
667 [12,13]. The plants provide nutrients to *Bacillus*; in turn, *Bacillus* strains help the plant defend
668 against harmful microbes by production of antimicrobial peptides [101]. *B. thuringiensis*
669 additionally produces an insecticidal spore protein, which is used commercially as biopesticide
670 [102]. *Bacillus* spp. are generally present in plant foods and may cause food spoilage [14] or
671 improves food quality as a food fermenting organism [3,17]. In vertebrate hosts, pathogenic
672 *Bacillus* species of the *B. cereus* group cause emetic or diarrheal illness cause illness or death
673 [90], whereas some non-pathogenic *Bacillus* spp. are used as probiotics to promote host health
674 [99].

675 **Figure 2.** Geographic location of *Bacillus* species associated food fermentation. **Panel A,**
676 cereal fermented foods; **Panel B,** fermented soybeans and legumes; **Panel C,** fermented
677 cassava or yams. The maps were generated with a template file from www.freeworldmaps.net.

678 **Figure 3.** Flow chart of traditional Chinese liquor brewing process including the process of the
679 *daqu* production (left) and the liquor production (right). The stacking fermentation is used for
680 production of few but not all liquors [103,104].

681 **Figure 4** Core genome phylogenetic tree of type strains in the genus *Bacillus* (left) and genes
682 coding for useful enzymes, or toxins, in these type strains of *Bacillus* spp. (right). Genome
683 sequences of type strains of the genus *Bacillus* were downloaded from NCBI Genome database
684 (Supplementary Table S1) using NCBI-genome-download (version 0.3.1). Gene annotation was
685 performed by Prokka (version 1.14.6) in PGCGAP module (version 1.0.20) to obtain the GFF
686 format annotation. The pan and core protein clusters of all these genomes were constructed using

687 FastOrtho. The phylogenetic tree of all *Bacillus* type strains was established by IQ-TREE
688 (version 2.0.3) with the model selection of MFP.

689 Query protein sequences were downloaded from UniprotKB database according to the Entry
690 ID and searched against the Prokka-annotated translations of *Bacillus* genomes by BLASTP
691 with an e-value cutoff of 10^{-5} . Furthermore, shell script was used to screen the blastp results
692 with identity more than 0.60 and R script was used to create the heatmap matrix. Finally, the
693 phylogenetic tree and the blastp results were depicted in iTOL.

694 Shown in different colors are (from left to right): the amyolytic system, other glycosyl
695 hydrolases, glutaminases, polyglutamate synthesis, proteases, antimicrobial lipopeptides,
696 enterotoxins of *B. cereus*, and the anthrax toxin.

697 **Amyolytic system:** GlgB (B7JDF1), branching enzyme; AmyE (P00691), α -amylase; AmyS
698 (P06279), heat stable α -amylase; BbmA (O06988), β -amylase; SpoII (P36924), β -amylase;
699 AmyX (C0SPA0), pullulanase or debranching enzyme; MdxE (WP_163131464), maltose
700 binding proteins.

701 **Other glycosyl hydrolases:** BglA (P22073), β -glucosidase; GanA and GanB (O07012 and
702 O07013), exo- and endo- β -galactosidases/ β -galactanases; YesZ (O31529), β -galactosidase;
703 LplD (P39130), α -galactosidase; AguA (Q09LY5), thermostable xylanase; PhyC (O31097),
704 phytase.

705 **Glutaminases:** GlsA1 and GlsA2 (O31465 and O07637), glutaminases.

706 **Polyglutamate synthesis:** PghL (A0A6M3ZBI1), poly- γ -glutamate hydrolase, PgsABCR
707 (A0A6M3ZGL0, E0U4Z3, A0A6M4JPT9, and Q45449), poly- γ -glutamate synthetase and
708 regulatory genes.

709 **Proteolytic system:** HtrA and HtrB (O34358 and Q9R9I1), envelope-associated serine
710 proteases; YyxA (P39668), uncharacterized serine protease.

711 **Antimicrobial lipopeptides:** FenABCDE (A0A7G7U8D7, H9TE69, A0A7G7U8D5,
712 A0A7T5SHH4 and A0A7G7U8D6), fengycin synthase; ItuABCD (Q93I56, Q93I55, Q93I54
713 and X5F8R7), iturin synthase; MycABC (Q9R9J1, Q9R9J0 and Q9R9I9), mycosubtilin
714 synthase; PpsBDE (A0A6H2JR43, A0A6H2JR81 and O31827), plipastatin synthase (doi:
715 10.6026/97320630007384); SrfAA SrfAB, SrfAC and SrfAD (P27206, Q04747, Q08787 and
716 Q08788), surfactin synthase; *sfp* (P39135), surfactin synthetase-activating enzyme.

717 **Toxins:** CesH (Q20CJ2), cereulide synthetase; CytK (Q63EQ2), cytotoxin K; HblACD
718 (A0A2A8M4Y0, Q9L4L8 and Q9REG7), Hemolysin BL; NheABC (A0A8F1XQI7,
719 A0A8I1GLU0 and A0A8F1XT31), Nhe enterotoxin; Anthrax toxin (TYC44981).

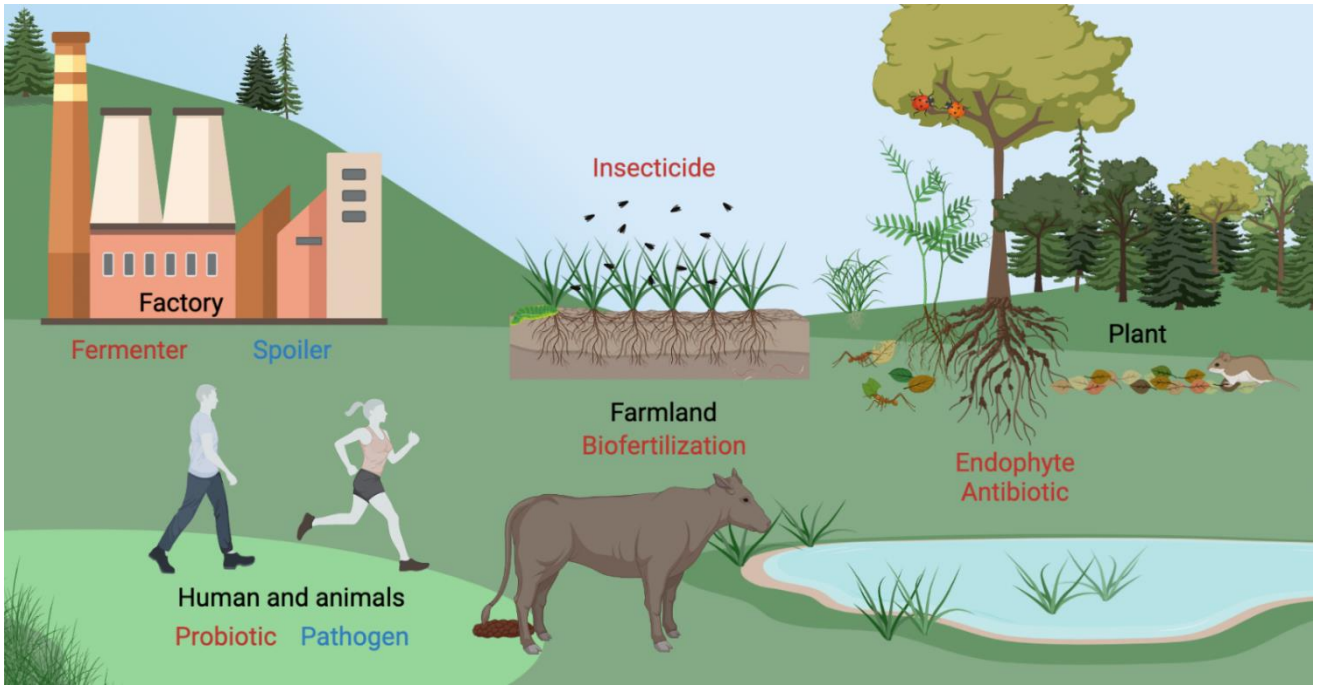
720 The genome sequences of type strains that were used to generate this heatmap is provided as
721 Online Supplementary Table 1.

722

Table 1. *Bacillus* species associated fermented foods. With exception of fish sauces, all fermentations are solid state fermentations.

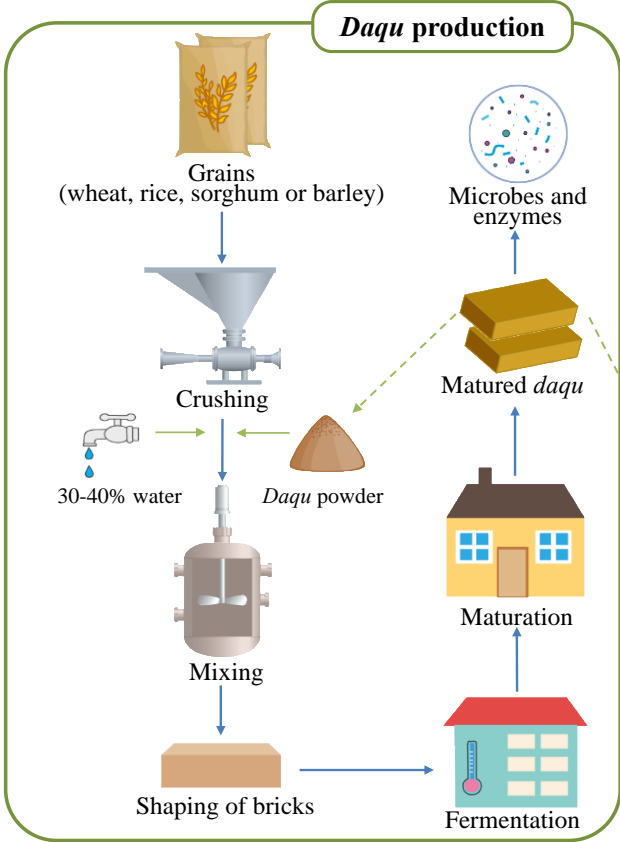
Product	Substrate	Main microorganisms	Ingredients and main processing steps. Fermentations are spontaneous fermentations unless back-slopping or use of starter culture is indicated.	Region of origin; food use
Soybeans or soy protein and legumes				
<i>Natto</i>	Soybeans	<i>B. subtilis</i> var. <i>natto</i>	Soybeans or pulses are soaked and steamed, inoculated with spores of <i>B. subtilis</i> var. <i>natto</i> and fermented for 15-24 h [20].	Japan; main course as meat substitute
<i>Cheonggukjang</i>	Soybeans, local pulses	<i>B. subtilis</i> , <i>B. amyloliquefaciens</i> and <i>Rhizopus oligosporus</i>	Soybeans or pulses are soaked and steamed, inoculated with spores of <i>Bacillus</i> and fermented for 1-3 d [22].	Korea; main course as meat substitute
<i>Doenjang</i>	Soybeans, local pulses	<i>B. subtilis</i> , <i>Rhizopus</i> spp., and <i>Aspergillus</i> spp.	<i>Meju</i> is made by soaking, steaming and crushing pulses and fermentation for 2 to 3 months. The solid fraction of <i>meju</i> is used for further ripening for over 2 months to make <i>doenjang</i> [23].	Korea; condiment
<i>Gochujang</i>	Soybeans, rice, pepper	<i>Bacillus</i> spp., including <i>B. subtilis</i> , <i>B. amyloliquefaciens</i> ; <i>Aspergillus</i> spp.; and <i>Rhizopus</i> spp.	The ingredients, 25 % red pepper powder, 22.2 % glutinous rice, 5.5 % <i>meju</i> , 12.8 % salt, 5 % malt and 29 % water, are fermented for 6 months to 1 year [24].	Korea; condiment
<i>Hawaijar</i>	Whole soybeans	<i>B. subtilis</i> , <i>B. licheniformis</i> , <i>S. sciuri</i> , <i>Alkaligenes</i> spp., <i>Providencia rettgeri</i>	The soybeans are soaked, boiled, washed, wrapped with clean cotton cloth/healthy leaves, and packed tightly in a bamboo basket with a lid. The basket is kept warm for fermentation for 4-5 d [27].	India up (Asia); staple
<i>Sufu</i>	Tofu (soybean curd)	<i>Bacillus</i> spp. or <i>Micrococcus</i> spp.	<i>Sufu</i> is produced in four steps: preparation of tofu; brining, inoculation with a fungal starter culture to prepare <i>pehtze</i> ; and ripening in dressing mixture [29].	China up (Asia); side dish
<i>Dawadawa</i>	Locust bean and local pulses	<i>Bacillus</i> spp., including <i>B. subtilis</i> , <i>B. pumilus</i> , <i>B. licheniformis</i> and <i>B. subtilis</i> var. <i>natto</i>	The locust beans are cleaned, boiled, pounded and separated the seed coat from the cotyledons. The cotyledons are re-boiled and packed into baskets or perforated pots and allowed to ferment spontaneously for about 48 h. Before fermentation, ash, maize, or millet flour is sprinkled on the cotyledons [105].	West and central Africa; meat substitute
<i>Ugba</i>	Locust bean and local pulses	<i>Bacillus</i> spp., including <i>B. subtilis</i> , <i>B. licheniformis</i> , <i>B. megaterium</i> , <i>B. pumilus</i> ; <i>Staphylococcus</i> spp.; and <i>Micrococcus</i> spp.	Soybeans or pulses are boiled, the cotyledons are sliced off. The sliced cotyledons are washed in water, soaked overnight and fermented for 3 to 5 d [106].	Nigeria; side dish
Tubers and roots				
<i>Ntoba Mbodi</i>	Cassava leaves	<i>Bacillus</i> spp., including <i>B. subtilis</i> , <i>B. licheniformis</i> , <i>B. amyloliquefaciens</i> , <i>B. pumilus</i> , <i>B. sphaericus</i> , and <i>B. xylanilyticus</i>	Cassava leaves are wilted for 2-3 days, cleaned, cut into small pieces, washed with water, distributed into small portions, and wrapped in large leaves, and allowed to ferment at ambient temperature for 2-4 d [32,107].	Congo

Product	Substrate	Main microorganisms	Ingredients and main processing steps. Fermentations are spontaneous fermentations unless back-slopping or use of starter culture is indicated.	Region of origin; food use
<i>Agbelima</i>	Cassava	<i>Bacillus</i> spp., including <i>B. subtilis</i> , <i>B. licheniformis</i> and <i>B. pumilus</i> ; LAB; and yeasts.	Cassava root are peeled, grated and mixed with a traditional inoculum which is also prepared from cassava roots. The grated mash is packed into plastic sacks and fermented with weights placed on top of the sacks to dewater the mash [108,109].	West Africa; staple
<i>Tape</i>	Cassava	<i>Bacillus</i> spp., including <i>B. subtilis</i> , <i>B. amyloliquefacie</i> , and <i>B. thuringiensis</i>	Cassava roots are cleaned, peeled steamed, cooled, and placed in a basket. Powdered ragi is sprinkled over the cassava. The cassava is covered with banana leaves and incubated at room temperature for 2-3 d [33].	Indonesia; dessert or ingredient for baking
<i>Elubo (yams)</i>	Yams	<i>Lactiplantibacillus</i> , <i>Levilactobacillus</i> , <i>B. subtilis</i>	Yam slices are blanched at 60 °C for 10 min and then fermented at 30 °C for 24 h [34].	West African, staple
<i>Taruba</i>	Cassava	<i>L. plantarum</i> , <i>L. brevis</i> and <i>B. amyloliquefaciens</i>	Cassava is crushed and pressed, toasted for 30 min and fermented at ambient temperature for 12 d [35,36].	Amazon, beverage
Vanilla				
<i>Vanilla</i>	Seed pods of <i>Vanilla planifolia</i>	<i>Bacillus</i> spp., including <i>B. subtilis</i> , <i>B. fusiformis</i> , and <i>B. pumilus</i>	Mature vanilla beans are blanched by immersing in hot water for 3-5 min to destroy the cell tissue structure. Then, the blanching vanilla beans are treated under conditions of high humidity and temperature. The sweating vanilla beans are sun- or air dried to inhibit mold growth and stored in a closed box for few months [38,39].	Madagascar, Indonesia, Mexico, others; condiment
Cereals				
<i>Daqu</i>	Grains (wheat, rice, sorghum and barley)	<i>Bacillus</i> spp., including <i>B. subtilis</i> , <i>B. amyloliquefaciens</i> , <i>B. velezensis</i> , and <i>B. licheniformis</i> ; LAB; <i>Enterobacteriaceae</i> ; <i>Aspergillus</i> spp.; <i>Rhizopus</i> spp.; <i>Saccharomyces</i> ; and <i>Saccharomycopsis</i>	Grains are ground and mixed with water to ~35 %. The mixture is shaped to bricks and then fermented for 2 months and matured for 2 months [41,42].	China; starter culture for liquor and vinegar
Fish				
<i>Fish sauce</i>	Fish	<i>Filobacillus</i> , <i>Bacillus</i> , <i>Micrococcus</i> , <i>Virgibacillus</i> , <i>Halobacillus</i> , <i>Halococcus</i> ; <i>Tetragenococcus halophilus</i>	Fish are mixed with salts and fermented for 6 to 12 months at room temperature [51,52].	Southeast Asia up; condiment



A**B****C**

Daqu production



Liquor production

