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Trends in the application of *Bacillus* in fermented foods

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Bacillus species such as *Bacillus subtilis* and *Bacillus amyloliquefaciens* are widely used to produce fermented foods from soybeans and locust beans in Asian and West African countries, respectively. Genomic information for *B. subtilis* strains isolated from Asian *Bacillus*-fermented foods (BFFs) has been gathered, and the chemical components of fermented products were defined with metabolomic approaches, facilitating the development of new starter strains and the evaluation of health claims. On the other hand, although advanced studies have been performed for some commercially produced BFFs, home-manufactured products still remain to be characterized in rural areas. In West Africa, the microbial flora of BFFs was examined in detail, leading to the isolation of candidates of the starter that produced bacteriocin against *Bacillus cereus* contaminating the products. These studies may provide a choice of *Bacillus* strains in food application and increase opportunities for further usage of *Bacillus* in foods.

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Introduction

In 1985, more than 300 researchers from Asia, Europe, North America, and West Africa gathered at the Asian Symposium on Non-salted Soybean Fermentation to exchange knowledge and experiences about *Bacillus*-fermented food (BFF) [1]. The process of fermentation with *Bacillus* spp. is also referred to as alkaline fermentation. This symposium encouraged cooperative relationships between applied microbiologists and food industries and has affected the trends in the application of *Bacillus* in fermented foods. Since then, state-of-the art methods have been employed to characterize BFFs: genomes of bacteria and bacteriophages involved in BFFs have been sequenced [2,3,4••,5,6], and metabolites and flavors developed during fermentation have been examined in detail [7,8,9•,10–12]. In some cases, experiments with animal models provided supporting evidence for the health benefits of BFFs [13,14,15•,16,17]. The current trends in application study for BFFs are summarized in Figure 1.

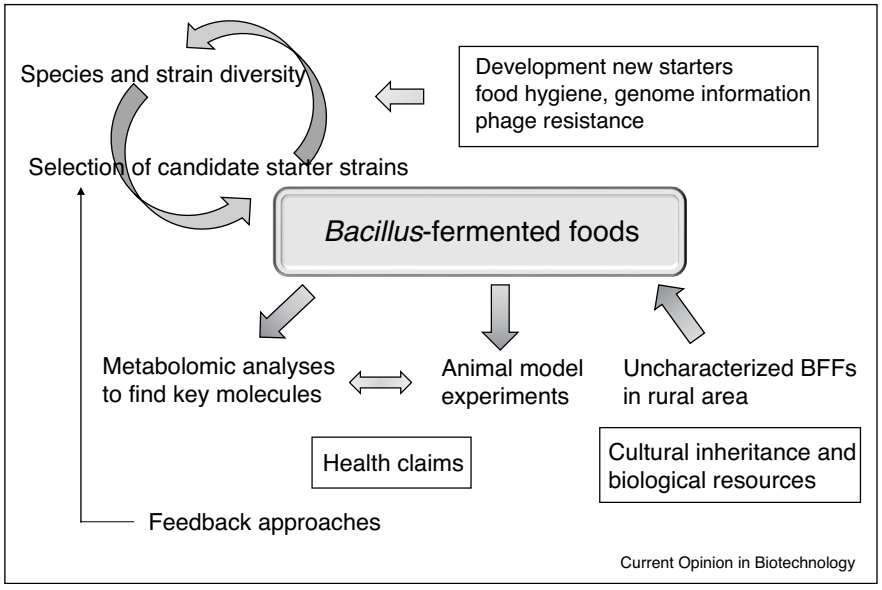
BFFs including natto (Japan), chungkookjang (Korea), kinema (Nepal), dawadawa (Ghana and Nigeria), and sumbara (Burkina Faso) are geographically distributed across Asia (Bhutan, China, India, Indonesia, Japan, Korea, Myanmar, Nepal, Thailand, and Taiwan) and West Africa (Benin, Burkina Faso, Ghana, Nigeria, Sierra Leone, and Sudan) and have different names in these countries despite similarities in the manufacturing process and appearance [18••,19,20]. The diversity of global fermented foods, including those produced with *Bacillus* species, was reviewed recently by Tamang *et al.* [18••]. In this review, more than 25 legumes fermented with *Bacillus* have been listed. Different *Bacillus* spp. have also been found in fermented food products that use as raw material other vegetables and roots, like bamboo shoots and Cassava [18••].

Although advanced studies have been performed for BFFs, home-manufactured BFFs from rural area still remain to be characterized [21]. Recently, the cultural and social backgrounds of these foods were explored in Southeast Asia [21]. In this review, trends in the application of *Bacillus* in fermented foods are reviewed with emphasis on research achievements in recent years (2–3 years) on non-salted/alkaline fermented foods.

Bacteria and bacteriophage strains and their genomes

Bacillus spp. isolated from BFFs include *B. subtilis*, *B. amyloliquefaciens*, *Bacillus licheniformis*, *Bacillus circulans*, *Bacillus pumilus*, and *Bacillus brevis* [18••]. This diversity of *Bacillus* spp. may, in most cases, reflect the lack of a specific starter strain; instead, the bacteria naturally residing on the surface of plant leaves and/or source raw materials are used for fermentation. The only exception, being natto in Japan, in which the starter strain *B. subtilis* (natto) is used exclusively in automated natto-producing factories [22]. In Korea, candidate starter strains to produce chongkukjang (also referred as cheonggukjang) were evaluated by a metabolomics study of the fermented product and experiments with animal models [7,14].

Figure 1



Scheme of application study of *Bacillus* in food industry.
Arrows indicate workflow and important concepts and idea are boxed.

Thus, a commercial starter for chongkukjang will be used in near future.

Only some *Bacillus* strains are involved in soybean fermentation [4••,22]. A comparative genome analysis was performed for *B. subtilis* strains isolated from fermented soybean products from six different Asian countries (Japan, Korea, Laos, Myanmar, Nepal, and Thailand) [4••]. The assembled genome sequences were compared with a non-fermenting laboratory strain (*B. subtilis* 168) and a fermenting natto starter strain (*B. subtilis* BEST195). Various genetic variations related to the fermentation process were identified [4••]. For example, all analyzed *B. subtilis* strains, KorC1 (Korea), LaoA1 (Laos), MyaA2 (Myanmer), ThaB (Thailand), NepD5 (Nepal), and the Japanese commercial starter strain commmonly had a large deletion in *bioF* gene that is essential for biotin synthesis [4••]. The deletion of *bioF* gene results in biotin auxothrophy which could be related to the accumulation of glutamate in the cells and increased synthesis of poly-gamma-glutamic acid (γPGA) [22]. The *degQ* gene is known to regulate the synthesis of extracellular proteases and γPGA [23,24]. The six soybean-fermenting strains had the same single nucleotide polymorphism. The single nucleotide polymorphism alters the -10 TAT box in the *degQ* promoter, which most likely results in *degQ* overproduction, leading to elevated production of extracellular proteases and γPGA. Swarming motility is required for *B. subtilis* cells to form large colonies. The six strains of *B. subtilis* isolated from the Asian BFFs were swarming positive. In the genome of these strains an adenine nucleotide deletion was found in *swrAA* (*yvzD*) orf (two consecutive adenine nucleotides were deleted in LaoA1), making the frame shift mutation, results in rapid spread of cells on the surface of soybean grains by swarming [25,26]. In other word, the swarming negative laboratory strain (*B. subtilis* 168) has an adenine nucleotide insertion in the *swrAA*. Rapidly swarming wildtype natto starter (NAFM5) formed ordered bundles of elongated string-like cells at the growing edge of colonies [27] (Figure 2). In contrast, swarming negative mutant cells of natto starter showed smaller colonies than that of the wild type and had disordered colony structure: the swarming negative mutants, NAFM15 (*ctpB*::Spc^r), NAFM16 (*yvjD*::Spc^r), and NAFM185 (*sigK*::Spc^r), produced the string-like cells as well as wild type strain but they were in both separated and tangled forms (Figure 2 and Kimura, unpublished results). Interestingly, these swarming negative mutants are not able to produce γPGA. It is worth noting that strains (ThaB and the Japanese starter) that showed obvious γPGA production on petri dish commonly have a deletion in *fliF*, which encodes the flagellar basal-body M-ring protein [4••,28,29]. This deletion appears to truncate FliF, producing a dysfunctional protein. Indeed, *B. subtilis* (natto) starter swims poorly in liquid medium. Flagellar rotation is known to be negatively correlated with γPGA production, although the mechanism explaining this phenomenon is not fully understood [28,29]. The frequent occurrence of a certain insertion sequence, a mobile genetic element, among *B. subtilis* strains found in Asian BFFs suggests that the strains share ecological niches, enabling horizontal transfer of the insertion sequence [4••,30,31].