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# *Bacillus* strains as human probiotics: characterization, safety, microbiome, and probiotic carrier

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**Abstract** Both spore and vegetative forms of *Bacillus* species have been used as probiotics, and they have high stability to the surrounding atmospheric conditions such as heat, gastric conditions, and moisture. The commercial *Bacillus* probiotic strains in use are *B. cereus*, *B. clausii*, *B. coagulans*, *B. licheniformis*, *B. polyfermenticus*, *B. pumilus*, and *B. subtilis*. These strains have antimicrobial, anticancer, antioxidant, and vitamin production properties. However, *Bacillus* probiotics can also produce toxins and biogenic amines and transfer antibiotic resistance genes; therefore, their safety is a concern. Studies on the microbiome using probiotic *Bacillus* strains are limited in humans. Most microbiome research has been conducted in chicken, mouse, and pig. Some *Bacillus* probiotics are used as fermentation starters in plant and soybean and dietary supplement of baking foods as a probiotic carrier. This review summarizes the characterization of *Bacillus* species as probiotics for human use and their safety, microbiome, and probiotic carrier.

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## Introduction

Intestinal microbiota plays an important role in the maintenance of host health, as they are involved in nutritional, immunologic, and physiological functions (Hooper and Gordon, 2001). Dysbiosis of the intestinal microbiota can cause many chronic diseases such as inflammatory bowel disease, obesity, cancer, and autism (Zhang et al., 2015). Probiotics are defined as live microorganisms that are administered to hosts in adequate amounts for improving the host health (FAO/WHO, 2006). Probiotics have been used as natural therapeutic agents with health benefits. Probiotic potential has been mainly investigated in the *Lactobacillus* and *Bifidobacterium*. Several strains of *Saccharomyces cerevisiae*, *Aspergillus niger*, and *Bacillus* have also been investigated.

The fundamental characteristics of probiotics include decreasing gastrointestinal (GI) complications in hosts. Several studies have shown that orally ingested *Bacillus* spores can proliferate into the intestine for a certain period (Duc et al., 2004; Park et al., 2003). Probiotic adherence is related to stability, such as survival of strains that have been exposed to the GI tract, autoaggregation, and hydrophobicity (Lee et al., 2015). Fecal analysis revealed that commercial *Bacillus* probiotics (*B. cereus*, *B. clausii*, and *B. pumilus*) could persist in mouse GI tract for up to 16 days (Duc et al., 2004). Probiotics contain antimicrobial substances, including bacteriocin, short chain fatty acids, and organic acids, and they modulate GI disorders by antimicrobial and antiadhesion effect against pathogen strains (Lee et al., 2015).

Probiotics are also known to have immune-modulatory roles, anticancer effects, and promote the lowering of cholesterol. Their function depends on their metabolites such as bacteriocin, biosurfactant, exopolysaccharide, and siderophore (Kanmani et al., 2013). Recently, the potential effects of psychobiotics on brain cells or emotions have been investigated (Sarkar et al., 2016). These mechanisms are based on modulation of the intestinal microbiome, which mediates its effect through the generation of anti-inflammatory cytokines that influence cognitive function, causing hyperglycemia.

*Bacillus* strains are widespread in nature and are found in soil, air, fermented foods, and the human gut (Sorokulova, 2013). *Bacillus* probiotics in the spore form can survive in extreme environmental conditions, enabling long-term survival in conditions that could otherwise kill vegetative bacteria (Nicholson et al., 2000). It has been demonstrated that spores of *Bacillus* probiotics germinate, grow, and resporulate in the GI tract (Casula and Cutting, 2002; Hoa et al., 2001). Recent studies have investigated various *Bacillus* strains, including *B. clausii*, *B. pumilus*, *B. polyfermenticus*, and *B. subtilis* (Green et al., 1999; Hoa et al., 2000). The main disadvantages of using *Bacillus* stains as probiotics are their ability to transfer genes related to antimicrobial resistance and production of enterotoxins and biogenic amines (BA). *Bacillus* probiotics have been shown to temporarily reside as symbiotic organisms within the host (Dong et al., 2009).

This review summarizes the use of representative *Bacillus* species as human probiotics, their safety including antibiotic resistance, toxigenic potential, and BA production, microbiome, and application in food.

## Characterization of *Bacillus* probiotics

Most commercial *Bacillus* probiotics consist of *B. subtilis*, *B. polyfermenticus*, *B. clausii*, some *B. cereus*, *B. coagulans*, *B. pumilus*, and *B. licheniformis* (Table 1). The spores of *Bacillus* species are considered to be responsible for germination or persistence in the small intestine and modulation of the intestinal conditions (Bernardeau et al., 2017) (Fig. 1). These probiotic characteristics are strain-specific and vary among strains.

### *Bacillus cereus*

*Bacillus cereus* is an important food pathogen and has also been used as a probiotic in humans and livestock (Zhu et al., 2016). Commercial *B. cereus* strains for human use are available; these include Biosubtyl$^{DL}$ (*B. cereus*), Bactisubtil (*B. cereus* IP 5832), and Subtyl (*B. cereus* var. *vietnami*) (Table 1) (Duc et al., 2004; Hoa et al., 2001). Some studies have investigated their safety including identification, toxin production, and transferable antibiotic resistance (Zhu et al., 2016). However, *B. cereus* strains can survive longer in the GI tract than can *B. clausii* and *B. subtilis* because *B. cereus* spores can efficiently adhere to human epithelial cells owing to their hydrophobicity (Andersson et al., 1998).

**Table 1** List of commercial *Bacillus* probiotics for human use

| Strain | Products | References |
|---|---|---|
| *B. cereus* | Bactisubtil® (Marion Merrell Dow Laboratories, France; Hoechst, France; Aventis Pharma, France; Cassella-Med, Germany); Biovicerin® (Geyer Medicamentos S.A. Porto Alegre, Brazil); Subtyl (Mekophar, Vietnam) | Hong et al. (2005) |
| *B. clausii* | Domuvar® (BioProgress SpA., Italy); Enterogermina® (Flora-Balance, USA); Lactopure (Pharmed Medicare, India); Lactospore (Sabinsa Corp., USA); Neolactoflorene® (Newpharma S.r.l., Italy); Sustenex® (Ganeden Biotech Inc., USA) | Cutting (2011), Mandel et al. (2010) |
| *B. polyfermenticus* SCD | Bispan® (Binex Co. Ltd., South Korea) | Ma et al. (2010), Paik et al. (2005) |
| *B. pumilus* | Biosubtyl (Biophar Company, Vietnam) | Duc et al. (2004) |
| *B. subtilis* | Bio-Kult® (Protexin, UK); Lactipan Plus (Instituto Biochimico Italiano SpA, Italy); Bibactyl (Tendiphar Corporation, Vietnam); Biosubtyl DL (IVAC, Vietnam); Bidisubtilis (Bidiphar Binh Dinh Pharmaceutical and Medical Equipment Company, Vietnam); Pastylbio (Pasteur Institute of Ho Chi Minh City, Vietnam); Medilac (Hanmi Pharmaceutical Co., Ltd., Korea); Nature's First Food® (Nature's First Law, USA); Neolactoflorene (Newpharma S.r.l., Italy) | Hoa et al. (2000), Sanders et al. (2003) |
| | Primal Defense™ (Garden of Life® Palm Beach, USA) | |
| Mixed type *B. licheniformis* and *B. subtilis* | Biosporin® (Biofarma, Ukraine; Garars (Russia); MegaSporeBiotic (Microbiome Labs, USA) | Elshaghabee et al. (2017) |

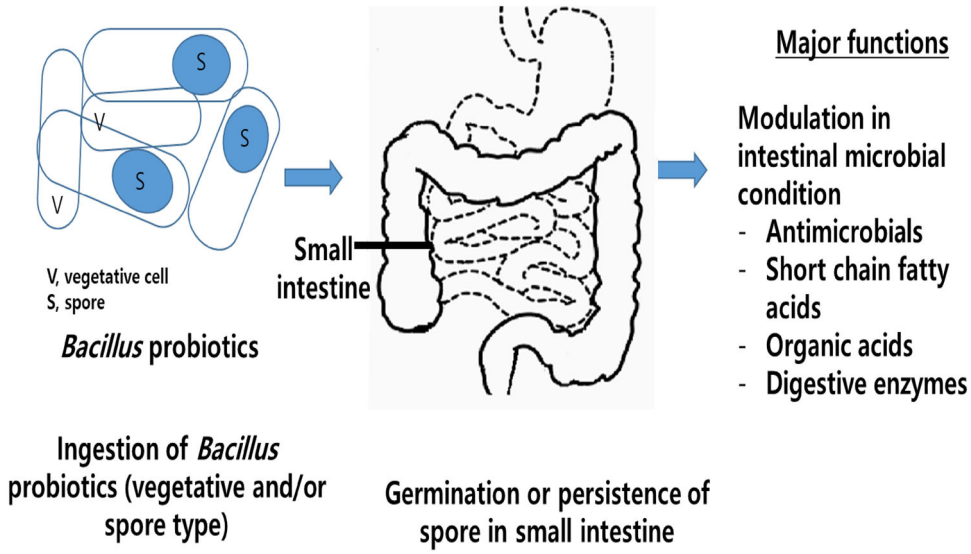


**Fig. 1** Pictorial representation of *Bacillus* probiotics for available human use and their functions. The *Bacillus* probiotics germinate and persist in the small intestine

### *Bacillus clausii*

*Bacillus clausii* strains are used as probiotics mainly because of their immune-modulatory and antimicrobial properties (Ciffo, 1984; Marseglia et al., 2007). Commercial over-the-counter (OTC) *B. clausii* strains available as probiotics for human use include Tufpro, Ecogro, Enterogermina, Entromax, and Ospor (Table 1) (Abbrescia et al., 2014; Patrone et al., 2016). The effects of *B. clausii* strains on immune responses include alleviating nasal symptoms during allergic reactions (Marseglia et al., 2007), anti-inflammatory effect against the side effects of antibiotic-based *Helicobacter pylori* therapy (Nista et al., 2004), and therapeutic effect against urinary tract infection (Fiorini et al., 1985). *B. clausii* strains demonstrate increased interferon production, mitogenic T cell proliferation, and mitogen-induced lymphokine production in ex vivo and in vivo models (Ciprandi et al., 1986; Muscettola et al., 1992). *B. clausii* ATCC 9799 has been used in combination with antibiotics (Ciffo, 1984; Mazza, 1994). In addition, *B. clausii* strains (DSM 8716, ATCC 21536, and ATCC 21537) have been shown to be safe as they are unable to transfer the macrolide resistance gene owing to the inherent presence of the *erm* gene (Bozdogan et al., 2004).

### *Bacillus coagulans*

*Bacillus coagulans* have been often mislabeled as *Lactobacillus sporogenes* because they are lactic acid producing spore-forming bacteria (Hong et al., 2005). This strain can produce coagulin, a bacteriocin, which has antimicrobial effect against a broad spectrum of enteric microbes (Hyronimus et al., 1998). In addition, this strain has been generally recognized as safe (GRAS) by the FDA. Commercial *B. coagulans* strains include Lactopure/Pharmed Medicare (India), Neolactoflorene®/Newpharm S.r.l. (Italy), and Sustenex®/Ganeden Biotech Inc. (USA) (Table 1) (Mandel et al., 2010). The coagulin produced by *B. coagulans* I4 is plasmid-linked, heat-stable, and protease-sensitive, and exhibits both bactericidal and bacteriolytic activities (Le Marrec et al., 2000). In humans, *B. coagulans* reduces the blood lipid concentration (Mohan et al., 1990). Reduction in total cholesterol (330–226 mg%) and LDL (267–173 mg%) has been reported, but no dietary control has been conducted.

### *Bacillus licheniformis*

*Bacillus licheniformis* is founded mainly in the soil but has been reported on probiotic strains isolated from traditional fermented foods (Lee et al., 2010). *Bacillus licheniformis* strains have been mainly used as combined-type probiotics (Table 1). The probiotic characteristics of this strain have been mainly investigated in aquaculture of shrimp, fish, and livestock (Swapna et al., 2015). *B. licheniformis* fermented soybean paste has been shown to have anti-obesity and anti-diabetic properties and also reduced accumulation of β-amyloid in the brain hippocampus (Yang et al., 2015).

### *Bacillus polyfermenticus*

*Bacillus polyfermenticus* SCD, also known as Bispan® strain (Binex Co., Ltd.), was first isolated from an air sample by Dr. Terakado in 1933 (Table 1) (Paik et al., 2005). It produces various enzymes, which aid digestion in humans. *B. polyfermenticus* SCD has cholesterol-reducing and antioxidant activities in mice and humans. Recently, *B.*

*polyfermenticus* strains were isolated from meju (Jung et al., 2012) and kimchi (Lee et al., 2015) and their probiotic application was investigated. *Bacillus polyfermenticus* produces polyfermenticin SCD, a bacteriocin, which has antimicrobial effect against *S. aureus* KCCM 32359, *Clostridium perfringens* ATCC 3624, and *H. pylori* (Kim et al., 2004; Lee et al., 2001). The cholesterol-lowering effect and antioxidant metabolism of *B. polyfermenticus* SCD were verified in rats (Paik et al., 2005). The anticarcinogenic effect of *B. polyfermenticus* SCD was investigated in human colon cancer cells including HT-29, DLD-1, and Caco-2 cells (Ma et al., 2010). Its mechanism of action found to be based on reduction of ErbB2 and ErbB3 protein expression and mRNA levels.

### *Bacillus subtilis*

*Bacillus subtilis* is a widespread microorganism in nature and a GRAS substance. *B. subtilis* can grow efficiently in low-cost carbon and nitrogen sources (Olmos and Paniagua-Michel, 2014). Some *B. subtilis* strains demonstrated their potential as dominant species by isolation in GI tracts, secretion of quorum-sensing pentapeptide, competence, and sporulation factor (Sorokulova, 2013). Commercial *B. subtilis* strains include Bio-Kult® (Protexin Health Care), Biosporin® (Biofarma, Ukraine); Garars (Russia), Lactipan Plus (Instituto Biochimico Italiano SpA, Italy), Bibactyl (Tendiphar Corporation, Vietnam), Biosubtyl DL (IVAC, Vietnam), Bidisubtilis (Bidiphar Binh Dinh Pharmaceutical and Medical Equipment Company, Vietnam), and Biobaby® (Ildong Pharmaceutical Co. Ltd., Korea) (Table 1) (Hoa et al., 2000; Pinchuk et al., 2001).

Probiotic *B. subtilis* strains have been investigated for having antimicrobial, antiviral, and anticancer effects. *B. subtilis* strains have been used as single and mixed type commercial probiotics. *B. subtilis* P223 inhibits the adhesion of *Salmonella enteritidis*, *Listeria monocytogenes*, and *E. coli* to the HT-29 cells (Jeon et al., 2017). *Bacillus subtilis* 3 can produce antibiotics including amicoumacin A and nonamicoumacin against *H. pylori* (Pinchuk et al., 2001). A *B. subtilis* recombinant strain exhibited antiviral activity against influenza virus, herpes virus, and equine encephalomyelitis virus in in vitro and experimental animal models, respectively (Chudnovskaya et al., 1995). *Bacillus subtilis* 3 demonstrated an antiviral effect in animal models and against their antiviral substances owing to the expression of peptide P18 (Starosila et al., 2017). The probiotic *B. subtilis* 3 and peptide P18 together demonstrated their antiviral effect against the influenza virus A/FM/1/47 (H1N1) in Madin-Darby canine kidney (MDCK) cells and mice at a concentration of $10^6$ CFU/well and $10^7$ CFU/mouse, respectively. In mice, the lethal rate decreased to 30% for 14 days while the control, without *B. subtilis* 3, died on day 8. The probiotic *B. subtilis* ATCC 6051 was reported to have γ-aminobutyric acid (GABA) producing ability (Wang et al., 2019). GABA has many well-known functions including anxiety inhibition, sleep promotion, reducing blood pressure, and enhancement of immune response (Sarkar et al., 2016).

## Safety on *Bacillus* probiotics

*Bacillus* strains are regularly consumed inadvertently through fermented foods. Risks of *Bacillus* probiotics include enterotoxin production, transfer of antibiotic resistance genes, cytotoxicity against normal cells, and production of BA. Specifically, *B. cereus* has a hazard probability associated with enterotoxin production as human pathogen (Stenfors Arnesen et al., 2008). However, several *B. cereus* strains have been used as probiotics (Hoa et al., 2000). Therefore, probiotic strains must be investigated to ensure safety of their phenotypes and genotype characteristics.

## Toxigenic potential of *Bacillus* species

*Bacillus* strains except *B. anthracis* and *B. cereus* have a long history of safe use in fermented foods such as natto and doenjang (Sorokulova, 2013). Some *Bacillus* species are a greater risk to human health, and there are reports of serious local and opportunistic systemic infections and abortions caused by these microorganisms (SCAN, 2000). The use of *B. cereus* probiotics has posed the risk of enterotoxin production and diarrhea-type disease or emetic-type disease. The following genes further emphasize the need for safe evaluation of these bacteria: enterotoxin genes such as hemolysin (*hblD/A*), non-hemolytic enterotoxin (*nheB*), enterotoxin FM (*entFM*), and emetogenic toxin (*ces*) (Table 2). Several *B. cereus* probiotics such as Bactisubtil, Biosubtyl$^{DL}$, and Subtyl, have been reported for their enterotoxin production (Granum and Lund, 1997; Kotiranta et al., 2000; Zhu et al., 2016). In addition, *B. cereus* for human use, *B. licheniformis,* and *B. subtilis* have been reported in cases of food borne diarrheal illness, toxin production, and vomiting (Hong et al., 2005). However, *B. coagulans*, *B. subtilis* PY79 and BS3, *B. licheniformis* BL31, and *B. indicus* did not show toxicity (Endres et al., 2009; Hong et al., 2008; Sorokulova et al., 2008).

## Antibiotic resistance of *Bacillus* species

Antibiotic resistance is of main interest in view of evaluating the safety of a strain, because the resistance may be conjugatively transferred to other strains. *Bacillus subtilis*

**Table 2** Gene expression related to toxin production by *Bacillus* probiotics

| Target gene | Sequence (5′ → 3′) | References |
|---|---|---|
| *bceT*[a] | TTACATTACCAGGACGTGCTT | Matarante et al. (2004) |
| (Enterotoxin T) | TGTTTGTGATTGTAATTCAGG | |
| *ces*[b] | GGTGACACATTATCATATAAGGTG | Kim et al. (2015) |
| (cereulide) | GTTTTCTGGTAACAGCGTTCTAC | |
| *cytK*[a] | ACAGATATCGG(GT)CAAAATGC | Kim et al. (2015) |
| (Cytotoxin K) | TCCAACCCAGTT(AT)(GC)CAGTTC | |
| *entFM*[a] | ATGAAAAAAGTAATTTGCAGG | Matarante et al., 2004 |
| (Enterotoxin FM) | TTAGTATGCTTTTGTGTAACC | |
| *hblC*[a] | GATACTCAATGTGGCAACTGC | Chon et al. (2012) |
| (L2 subunit of HBL) | TTGAGACTGCTCGTCTAGTTG | |
| *hblD*[a] | ACCGGTAACACTATTCATGC | Chon et al. (2012) |
| (L1 subunit of HBL) | GAGTCCATATGCTTAGATGC | |
| *hblA*[a] | AAGCAATGGAATACAATGGG | Chon et al. (2012) |
| (B subunit of HBL) | AGAATCTAAATCATGCCACTGC | |
| *hblD/A*[a] | GGAGCGGTCGTTATTGTTGT | Matarante et al. (2004) |
| (hemolysin BL enterotoxin) | GCCGTATCTCCATTGTTCGT | |
| *nheA*[a] | GTTAGGATCACAATCACCGC | Guinebretiere et al. (2002) |
| (A subunit of NHE[b]) | ACGAATGTAATTTGAGTCGC | |
| *nheB*[a] | CTATCAGCACTTATGGCAG | Matarante et al. (2004) |
| (B subunit of NHE[b]) | ACTCCTAGCGGTGTTCC | |
| *nheC*[a] | TGGATTCCAAGATGTAACG | Chon et al. (2012) |
| (C subunit of NHE[b]) | ATTACGACTTCTGCTTGTGC | |
| *nprA* | GTATACGGAGATGGTGATGG | Hisieh et al. (1999) |
| | GGATCACTCATAGAGCGAAG | |

*HBL* Hemolysin BL, *NHE* nonhemolytic enterotoxin

[a]Enterotoxin genes (*hblA*, *hblC*, *hblD*, *nheA*, *nheB*, *nheC*, *cytK*, *bceT*, *entFM*)

[b]Emetogenic toxin (*ces*)

BS3 and *B. licheniformis* BN31 strains do not carry plasmids (Sorokulova et al., 2008). Antibiotic resistance has been investigated using microbiological cut-off values, quantitative methods of the MIC (minimum inhibitory concentration), or via genetic basis of resistance following the method as per the guideline of Clinical and Laboratory Standards Institute (CLSI) and European Food Safety Authority (EFSA) (Zhu et al., 2016). The antibiotics of LAB strains that have been used are ampicillin, vancomycin, gentamicin, kanamycin, streptomycin, clindamycin, tetracycline, and chloramphenicol. However, *Bacillus* strains have been listed as resistant/susceptible to all antibiotics except ampicillin (EFSA, 2012). Four probiotic strains of *B. clausii* are marketed as an OTC medical supplement and have been investigated for antibiotic resistance by antibiotic susceptibility assay, PCR amplification, and DNA sequencing (Abbrescia et al., 2014). However, their antibiotic resistance was considered a useful characteristic when compared to the wild type strain.

## Biogenic amine (BA) production

BAs are important compounds for metabolic and physiological functions in all living organisms and they are naturally found in many foods owing to the microbial decarboxylation of precursor amino acids via substrate-specific enzymes (Calzada et al., 2013). BAs are classified into aliphatic (putrescine, cadaverine, spermine, and spermidine), aromatic (tyramine, phenylethylamine, and serotonin), and heterocyclic (histamine and tryptamine) amines. Some BAs have an important function as modulators of hormones and neurotransmitters (Spano et al., 2010). However, histamine and tyramine are chiefly studied owing to their potential physiological actions and toxicological effects. Specifically, histamine and tyramine are toxic to the HT-29 intestinal cell-line at a high concentration (Linares et al., 2016). In the presence of nitrites, BAs can be potential carcinogens when converted to nitrosamines (Naila et al., 2010). When ingested in high concentrations, BAs have considerable toxicological risks

**Table 3** Biogenic amine gene expression in *Bacillus* probiotics

| Target gene | Sequence (5′ → 3′) | References |
|---|---|---|
| *AGD1* | GAACGACTAGCAGCTAGTTAT | Lucas et al. (2007) |
| (Agmatine deiminase) | CCAATAGCCGATACTACCTTG | |
| *Cad2* | CAYRTNCCNGGNCAYAA | Landete et al. (2005) |
| (Lysine decarboxylase) | GGDATNCCNGGNGGRTA | |
| *Hdc* | GATGGTATTGTTTCKTATGA | Coton and Coton (2005) |
| (Histidine decarboxylase) | CAAACACCAGCATCTTC | |
| *Odc* | TGCA CTTCCATATCCTCCAG | Granchi et al. (2006) |
| (Ornithine decarboxylase) | GAATTTCTGGAGCAAATC CA | |
| *OD* | CATCAAGGTGGACAATATTTCCG | Granchi et al. (2006) |
| (Putrescine) | CCGTTCAACAACTTGTTTGGCA | |
| *TD* | ACATAGTCAACCATRTTGAA | Coton et al. (2004) |

such as headache, respiratory distress, heart palpitations, hyper- or hypotension, and several allergic disorders. BA production by probiotic strains is considered to be useful as probiotic or fermentation starter. BA production can be confirmed using glycerol-containing decarboxylase media (Jeon et al., 2018), HPLC analysis of the fermented product, or molecular analysis by PCR (Table 3). In addition, *B. licheniformis* cy2, *B. polyfermenticus* CJ9, *B. licheniformis* KCTC 1918, and *B. subtilis* KCTC 1028 reported BA production of putrescine, cadaverine, spermidine, phenylethylamine, and spermine (Chang and Chang, 2012). However, *B. subtilis* P229 did not produced BA (Jeon et al., 2018).

## Microbiome study on *Bacillus* probiotics

Microbiome can be influenced during the life of the host in response to a variety of factors such as diet, environment, medical interventions, and disease conditions. Therefore, the influence of probiotics has been reported on the GI microbiome of humans and mammals (Thursby and Juge, 2017). The gut microbiota is dominated by two divisions of bacteria that influence the host immune systems: (i) Bacteroidetes and (ii) Firmicutes (Kawai et al., 2018). The symbiotic relationship between the gut microbiota and the host was demonstrated by Guianane and Cotter (2013). The study of gut microbiome in humans focused on the use of

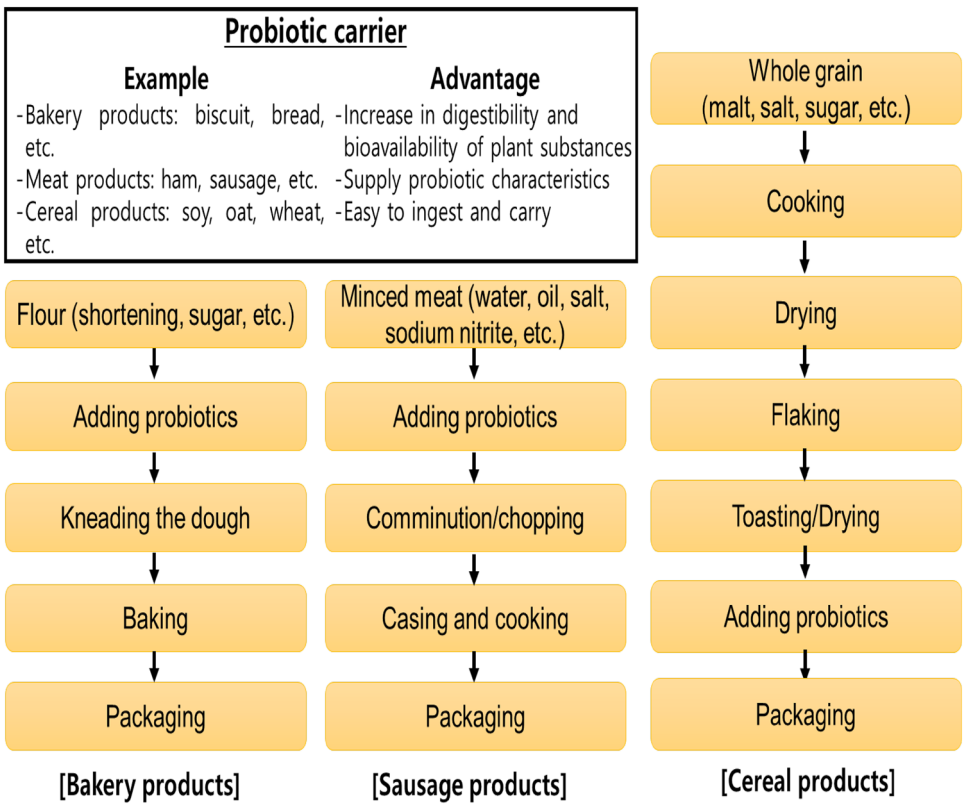


**Fig. 2** Concise schematic of *Bacillus* probiotics used in the food industry within various products used as probiotic carriers

probiotics, and main probiotic strains used were lactic acid bacteria such as *Lactobacillus casei* and *Lactobacillus rhamnosus* (Kawai et al., 2018; Pirker et al., 2013).

The effect of *Bacillus* strains on gut microbiome was evaluated in broiler chicken and pig (Jacquier et al., 2019; Ma et al., 2018; Poulsen et al., 2018). The treatment with *B. subtilis* led to increase in concentration of *Ruminococcus*, *Lachnoclostridium*, and *Anaerostipes* at the cecal level in 500 male broiler chicken (Jacquier et al., 2019). These results suggested that there was immune modulation by butyrate production with the potential change in the microbiome. In another study, *B. subtilis* influenced the increase of abundance of Christensenellaceae and Caulobacteraceae, and decrease in the concentration of *Vampirovibrio*, *Escherichia/Shigella,* and *Parabacteroides* in broiler chickens (Ma et al., 2018). In suckling and newly weaned piglets, treatment with (1) control (n = 72); (2) gentamicin (n = 71); (3) *Bacillus* spores (n = 63); (4) gentamicin and *Bacillus* spores (n = 71), the spore of *Bacillus* spp. and gentamicin was confirmed diversity of the microbial composition (Poulsen et al., 2018). However, administration of *Bacillus* spores decreased the diversity of microflora and did not affect dysbiosis.

## Probiotic carrier on *Bacillus* probiotics

Probiotic foods using lactic acid bacteria have been mainly used as dairy foods such as yogurt, cheese, ice cream, etc. However, *Bacillus* probiotics applied in vegetable, cereal, and meat products are suggested to undergo processing in some food systems (Fig. 2). LAB and yeast have challenged encapsulation using maltodextrin, skim milk, or alginate for food application (Gul and Athalar, 2019; Suvarna et al., 2018), while *Bacillus* probiotics have advantage of their high survival due to their spore formation. The application of probiotic strain has lead affinity and receptibility to consumer retaining probiotic characteristics. In addition, the bioavailability of polyphenol substances can increase the β-glucosidase production (Lee and Paik, 2017).

Fermented soybean foods are an example of probiotic carriers for *Bacillus*. Fermented soybean foods are diverse such as *doenjang* (Korea), *kanjang* (Korea and Japan), *cheonggukjang* (Korea), *natto* (Japan), Rabadi (India, Pakistan), Gari (Africa), Tapaiubi (Malaysia), Douchi (China), Soibum (India), Ugba (Nigeria), etc. (Elshaghabee et al., 2017). *Natto* has been shown to reduce blood clotting by fibrinolysis (Elshaghabee et al., 2017). The use of probiotic *Bacillus* strain is limited to soybean, bake, cereals, and meat owing to disadvantage of unique smell. Therefore, for their application in the food industry, there is a demand to reduce the unique smell.

In conclusion, this review summarized the use of *Bacillus* species as probiotics, their function, safety in humans, and modulation of microbiome. Probiotic therapy has been increasingly used as an alternative to antibiotics, immune-modulators, and microbiome regulators. However, the functional probiotic properties are strain specific. Probiotic *Bacillus* strains have the merit of stability as they can be used in their spore form in storage conditions unlike LAB. Therefore, probiotic *Bacillus* strains require scientific investigation with regard to their toxin production, antibiotic resistance, and BA production, in order to be labeled safe for use. In addition, the study of gut microbiome using *Bacillus* probiotics is needed for the development of novel probiotics for changing the microflora.

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