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Modern Status and Perspectives of *Bacillus Bacteria* as Probiotics

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Introduction

Probiotics are identified as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" [1]. Although different microorganisms are claimed today as probiotics, usually they represent two main genera - *Lactobacillus* and *Bifidobacterium*. Other probiotic bacteria have received limited attention of researchers. Among them are *Bacillus* bacteria - highly diverse group of microorganisms, known more than 100 years.There are strong scientific data substantiating the validity of the use of these bacteria as probiotics.

Bacillus Bacteria in Nature

Bacteria of the *Bacillus* genus are among the most widespread microorganisms in nature. *Bacillus* spp. are predominant in soil [2, 3]. These bacteria have also been isolated frequently from water[4] and air [5]. Being ubiquitous in soil, air and water they find their way easily into food products. The *Bacillus* counts in wheat, grain and wholemeal, is reported to be 10^6 CFU/g [6, 7]. Due to the high resistance of bacilli spores to heat they can survive the baking process and therefore be found in bread and bakery products [8].*Bacillus* are often present in milk microflora, remain after pasteurisation and can be the predominant microflora in pasteurised milk products [9]. *Bacillus*spp. are the major microflora of soya beans and are implicated in fermentation for preparation of soya food products and condiments [10, 11]. *Bacillus subtilis*and *B. Licheniformis* are the dominant species and their counts in fermented products are 10^8 CFU/g[12]. In Japan a pure culture of *B. Subtilis* var. *Natto* is used to produce natto – a popular food made by fermenting of cooked soybeans [13].

So, bacilli consistently enter the gastrointestinal and respiratory tracts of healthy people with food, water and air. Isolation of *Bacillus* bacteria from the gut was reported for healthy adults [14]and children [15]. The number of bacilli in the gut can reach 10^7 CFU/g [16], comparable to *Lactobacilli* count. Thus some researchers considered *Bacillus* to be one of the dominant components of the normal gut microflora [17]. Bacilli are resistant to acid and bile and keep viability in the gut [18, 19]. Under strict anaerobic conditions bacilli can use nitrate as an electron acceptor and grow anaerobically [20, 21].

Probiotic activity of *Bacillus* bacteria

Bacillus bacteria could play a significant role in the gut because of their high metabolic activity. Activity of *Bacillus* is largely determined by their ability to produce antibiotics. There were identified 795 antibiotics from *Bacillus* bacteria [22]. The most productive species is *B. subtilis*, which devotes 4-5% of genome to antibiotic synthesis and produce 66 antibiotics [23]. *Bacillus* antibiotics differ in the structure and spectrum of activity.Most of the antibiotics, produced by bacilli are peptides, but some strains produce antibiotics, belonging to other chemical classes. For example, an amini glycoside butirosin from *B. circulans*; proticin, a phosphours-containing triene from *B. licheniformis*[13]; an isocoumarin derivatives baciphelacin from *B. thiaminolyticus* [24]; amicoumacin A from *B. subtilis*[25].*Bacillus* antibiotics vary in the spectrum of antimicrobial activity. Some *Bacillus* strains synthesize bacteriocines, which are effective only against bacteria of the same species, others produce antibiotics against Gram-negative or Gram-positive bacteria and still other strains have a wide spectrum of antibiotic activity (including antifungal and antiprotozoan). These antibiotics can be synthesized under aerobic and anaerobic conditions.

Production of lytic enzymes could also contribute to antimicrobial activity of *Bacillus* bacteria. Lytic enzyme from *B. subtilis*YU-1432 was effective against *Porphyromonas gingivalis*, which cause periodontal disease [26]. Fibrinolytic enzyme, produced by *B. vallismortis* showed high efficacyin lysis of *Streptococcus mutants*[27]. Some *Bacillus*lytic enzymes are active against Gram-negative bacteria [28], others have broad spectrum of activity, including Gram-positive and Gram-negative bacteria [29, 30]and yeasts [31].

Bacillus bacteria could support digestive function of the gut producing essential enzymes. High amylolytic activity is known for bacilli with the production of acid-resistant enzymes [32]. Pectinolytic enzymes are often isolated from *Bacillus* bacteria, especially from *B. subtilis* [33]. Some *Bacillus* strains have lipolitic and cellulolytic activity [34, 35]. Bacilli are characterized by high proteolytic activity [36]. *Bacillus* proteolytic enzymes stimulate regeneration processes [37]and enhance the fibrinolyticactivity in the plasma even after oral administration [38]. Proteolytic enzymes of *Bacillus* contribute to normal digestion by degradation of the anti nutritional factors [39] and the allergenic compounds [40]. *Bacillus* enzymes could stimulate the normal microflora of the gut. Thus subtilisin and catalase, produced by *B. subtilis* (*natto*) enhance the growth and viability of lactobacilli [41]. *Bacillus*enzymes were found to be active in live and in dead cells [42].

Bacilli produce amino-acids, including essential amino-acids[43, 44]and vitamins [45, 46]. Some *Bacillus*strains effectively degrade cholesterol *in vitro*[47]and significantly reduceplasma low-density-lipoprotein cholesterol, hepatic total cholesterol, and triglycerides after oral administration in animal studies [48].

Bacilli could significantly influence the immunological status of the host. Oral treatment with *B. subtilis*spores increased expression of activation markers on lymphocytes in dose-dependent manner [49]. The effect of lymphocyte activation by *B. subilis*spores was both quantitatively and qualitatively similar to that induced by the mitogens phytohaemagglutinin (PHA) and Concanavalin A (ConA) [50]. *B. subtilis*spores stimulated cytokine production *in vitro*and after oral

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administration in mice [51, 52]. Adjuvant properties of *Bacillus* bacteria were confirmed in many studies. *B. subtilis*spores induced systemic antibody response to tetanus toxoid fragment Cand ovalbumin in mice [53]. Killed *B. subtilis* spores enhanced the levels of systemic IgG and mucosal sIgA specific to the influenza when intra-nasally were administered to mice [54].

The quorum-sensing pentapeptide, competence and sporulation factor (CSF) of *B. subtilis* activate key survival pathways in intestinal epithelial cells of the host [55]. It was shown, that CSF induces heat shock proteins, which protect intestinal epithelial cells against injury and loss of barrier function. Thus *Bacillus* bacteria may provide the host with the ability to maintain intestinal homeostasis.

Presented data show the possibility to isolate highly effective probiotic strains among *Bacillus* bacteria. However, probiotic activity is strain-specific.For example, analyze of various*Bacillus* probiotic strains for their antimicrobial activity against clinical multi-resistant strains of pathogens, showed that only one *B. subtilis*3 strain was effective *in vitro* studies and in animals testing [56, 57]. Thus every selected *Bacillus* bacterium should be studied individually to confirm probiotic activity.

Safety of *Bacillus* bacteria

Humans have been in contact with these bacteria during their entire existence as a species.Evidence of this is the presence of bacilli in samples of permafrost soil, as old as 10^5years [58], isolation of *Bacillus* spp. from Dominican amber, 25-40 million years old [59, 60], discovery of spores in salt crystal, 250 million years old [61]. Some scientists consider the *Bacillus*spores to be the longest-lived cellular structures known [62, 63].

Bacillus bacteria have a long history of safe use in foods. Over a period of many centuries these bacteria have been used for preparation of alkaline-fermented foods where they are dominated microflora [64]. Humans are constantly exposed to the *Bacillus*species from the environment with no evidence of infections outbreak due to these bacteria (except *B.anthracis* and *B. cereus*) or apparent ill effects. Some cases of infection associated with“nonpathogenic” *Bacillus* species are described [65]. But frequency of such cases is low and comparable with the frequency of infections known for other bacteria of normal microbiota, such as *Lactobacillus* [66] and *Bifido bacteria* [67]. It is well known that bacterial pathogenicity is strain-specific, so every bacterial strain, promising as probiotic, should be tested individually. Pathogenic potential of some *Bacillus* strains is known, so the European Scientific Committee on Animal Nutrition proposed a scheme for the testing of toxin production in *Bacillus*bacteria intended for use as feed additives [68]. Several *Bacillus*strains - *B. subtilis*and *B. licheniformis* [69], *B. subtilis*and *B. indicus* [70], were tested according to this scheme and showed no evidence of toxicity. Additional testing in animals, including acute and chronic toxicity studies, also indicated safety of these strains. *B. subtilis*RO179 was safe *in vitro* toxicity study and in chronic oral toxicity challenges, performed in rats[71]. Safety of *B. coagulans* was demonstrated in acute and sub-chronic oral toxicity [72] and in chronic one-year oral toxicity [73]. Results of these studies indicated that treatment of animals with *Bacillus* bacteria even in the high doses caused no signs of toxicity or any other adverse effects, related to tested cultures. Toxicity data, obtained for *Bacillus* strains [69] were in accordance with the safety records for *Lactobacillus* and *Bifidobacteria* [74, 75].

Current Status of *Bacillus* Probiotics

First clinical data about effective treatment of gastrointestinal infections with *Bacillus* bacteria were published in France [76]. Additionally, spores of *Bacillus*sp. IP 5832used during therapy with antibiotics, prevented dysbiosis in patients [77]. *B. subtilis*therapy was highly effective in treatment of various infectious pathologies in patients [78]. Mazza [79] summarized the main results about clinical efficacy of *B. subtilis*as an antidiarrheal agent, used in different counties. Author concluded that *B. subtilis*is one of the most important microorganisms for the therapy and prophylaxis of intestinal disorders in humans. *B. subtilis* bacteria were more effective in treatment of acute diarrhea, than lactobacilli [80].

Clinical efficacy of *B. coagulans* has been shown in treatment of diarrhea, dysbiosis and as adjunct therapy for rheumatoid arthritis [81]. Anti-diarrheal activity of *B. clausii* was proved in clinical trials, resulted in significant decrease of diarrhea and pain duration in patients [82].

Resistance of *Bacillus* bacteria to environmental conditions makes them attractive for use as food supplements [83,84].

Several *Bacillus* probiotic strains have been approved for human use. *B. subtilis* 3 and*B. licheniformis*31 (Biosporin) received a drug status by the national authorities of Russia and Ukraine and are used in these countries for prophylaxis and treatment of acute intestinal infections [80,85]. *B. clausii* (Enterogermina) and *B. cereus* (Bactisubtil) were approved for medical use in Europe. Recently, *B. coagulans* (GanedenBC30) obtained self-affirmed GRAS status in USA. *B. subtilis* (*natto*) OUV23481 was approved in Japan as FOSHU (food for specified health use) [86].

Perspectives of *Bacillus* Probiotics

Probiotic therapy gains more attention of researchers as alternative approach to conventional antibiotic therapy, especially because of emerging of new multi-resistant pathogens. Efficacy of probiotics against pathogenic bacteria was well documented in many scientific reports. But the variety of pathogens and the limited spectrum of specific activity of existing probiotic bacteria raise the question about improvement of probiotics. The recent advances in the genetic engineering can be used to modify probiotic cultures both to strengthen their existing activity and to create new strains with the desired properties. So it is possible to influence the mechanism of probiotics’ action.

Bacillus bacteria are promising system for developing of new recombinant probiotics. They are genetically well studied and used as a model for cloning of different pro- and eukaryotic genes.Recombinant *Bacillus* strains are known as commercial producers of biologically active compounds (enzymes, antibiotics, etc.) [87]. *Bacilli* do not colonize mucous membranes permanently, so the amount of these bacteria and recombinant protein can be controlled by the different doses and schemes of administration. Additionally, this reduces the possibility of gene transfer or any adverse effects dealing with such transfer.

It is known that bacilli are able to secrete large amount of proteins into the culture medium, thus it opens the opportunity to develop *Bacillus* strains with extracellular production of recombinant proteins. This approach has been used for construction of *B. Subtilis* strain with antiviral activity. *Bacillus subtilis* probiotic strain was transformed with plasmid DNA containing the chemically synthesized gene of human alfa-2 interferon. This plasmid was stable in the bacterial cell even after sporulation and repeated germination. The recombinant bacteria produced interferon extracellularly in an amount of 10^4 – 10^5 IU/mL [88]. Antiviral activity of *B. subtilis*recombinant strain against influenza virus, herpes virus and equine encephalomyelitis virus was shown *in vitro* and in experimental animal infections [89]. Efficacy of animal protection against 10 LD_{50}dose of influenza virus by oral administration of recombinant strain was 70%, against 100 LD_{50}dose – 50% [90].Oral treatment of mice with *B.subtilis* recombinant strain significantly increased cytotoxic activity of macrophages and inhibited the growth of Lewis lung carcinoma [91]. Safety of *B. subtilis* recombinant strain (Subalin)was demonstrated *in vitro*, in animals [92]and in healthy volunteers [93]. Subalin was effective in combined treatment of meningoencephalitis[94]and hepatitis A and B [95]. Recombinant *B. subtilis* strain (Subalin) is approved as a drug by the national authority of Ukraine.

B. brevis strain was transformed with the plasmid, carrying a synthetic gene of a single chain human insulin precursor [96]. Authors demonstrated that recombinant product, obtained from the culture supernatant, had the biological activity, similar to human insulin. Proinsulin (PI) was efficiently overproduced and secreted into the culture medium by recombinant *B. subtilis*strain [97]. The maximum concentration of PI in supernatant has reached 1 mg/mL.

Plasmid DNA, coding for the intracellular production of the human IL-1 receptor antagonist (IL-Ra) was used to transform *B. subtilis*strain [98]. After treatment of rats and rabbits with recombinant bacteria, human IL-1Ra protein was found intact and biologically active in the intestinal lavage and in the serum of animals.

Bacillus spores can be engineered to express heterologous antigens. *B. subtilis* is one of the most studied spore surface display system [99]. Structural coat proteins CotB, CotC and CotG were used to anchor the heterologous passenger proteins on the spore surface. The spore display approach was applied to construction of *B. subtilis*-based tetanus vaccine [100, 101], rotavirus vaccine [102], anthrax vaccine [103], vaccine against trematode *Clonorchissinensis* [104]. Other proteins were successfully exposed on the spore surface by using this method: green fluorescent protein GFP_{UV} [105]; proinsulin [106]; human serum albumin [107].

Conclusion

Bacillus bacteria are attracting increasing attention of the researchers as promising probiotics.

More scientific data indicate the valuable role of these bacteria in the environment and in the host organism. *Bacillus* bacteria are ubiquitous in nature; they are normal microflora of foods. Bacilli consistently enter the gastrointestinal and respiratory tract of humans and animals with food, water and air and they are component of the normal gut microflora. These bacteria are known as highly effective producers of biotechnologically important products – antibiotics, enzymes, vitamins, amino acids. Strains with unique activity can be isolated among *Bacillus* bacteria. Bacilli have a great potential for developing of new recombinant probiotics with desired properties. Currently several approaches have been applied to construct recombinant bacilli, producing heterologous proteins for treatment of pathological conditions or for vaccine delivery.Further studies of the mechanisms of *Bacillus* probiotics' action will lead to elaboration of new probiotics, based on new selected strains or genetically modified strains with predetermined properties.

References

1. FAO/WHO (2001) Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report.
2. Garbeva P, van Veen JA, van Elsas JD (2003) Predominant *Bacillus* spp. In agricultural soil under different managment regimes detected via PCR-DGGE. Microb Ecol 45: 302-316.
3. Nicholson WL (2004) Ubiquity, longevity, and ecological roles of *Bacillus* spores. In Bacterial spore formers: Probiotics and emerging applications, E. Ricca, A.O. Henriques, andS.M. Cutting, eds. (Norflok, Horison Bioscience), pp 1-15.
4. Ivanova EP, Vysotskii MV, Svetashev VI, Nedashkovskaya OI, Gorshkova NM, et al. (1999) Characterization of *Bacillus* strains of marine origin. Int Microbiol 2: 267-271.
5. Andersson AM, Weiss N, Rainey F, Salkinoja-Salonen MS (1999) Dust-borne bacteria in animal sheds, schools and children's day care centres. J Appl Microbiol 86: 622-634.
6. Rogers R (1978) *Bacillus* isolated from refrigerated doughs, wheat flour and wheat. Cereal Chem 55: 671 - 674.
7. Pepe O, Blaiotta G, Moschetti G, Greco T, Villani F (2003) Rope-producing strains of *Bacillus* spp. from wheat bread and strategy for their control by lactic acid bacteria. Appl Environ Microbiol 69: 2321-2329.
8. Sorokulova IB, Reva ON, Smirnov VV, Pinchuk IV, Lapa SV, et al. (2003) Genetic diversity and involvement in bread spoilage of *Bacillus* strains isolated from flour and ropy bread. Lett Appl Microbiol 37: 169-173.
9. Pendurkar SH, Kulkarni PR (1990) Heat resistance of *Bacillus* spores exposed to food processing conditions. Nahrung 34: 177-180.
10. Ray, P, Sanchez C, O'Sullivan DJ, and McKay LL (2000) Classification of a bacterial isolate, from pozol, exhibiting antimicrobial activity against several gram-positive and gram-negative bacteria, yeasts, and molds. J Food Prot 63: 1123-1132.
11. Inatsu Y, Nakamura N, Yuriko Y, Fushimi T, Watanasiritum L, et al. (2006) Characterization of *Bacillus subtilis* strains in Thua nao, a traditional fermented soybean food in northern Thailand. Letters in Applied Microbiology 43: 237-242.
12. Omafuvbe BO, Abiose SH, Adaraloye OO (1999) The production of «Kpaye» - a fermented condiment from *Prosopis africana* (Guill and Perr) Taub. seeds. Int J Food Microbiol 51: 183-186.
13. Katz E, Demain AL (1977) Peptide antibiotics of *Bacillus* - chemistry, biogenesis, and possible functions. Bacteriological Reviews 41: 449-474.
14. Hong HA, Khaneja R, Tam NMK, Cazzato A, Tan S, et al. (2009) *Bacillus subtilis* isolated from the human gastrointestinal tract. Research in Microbiology 160: 134-143.
15. Ellis-Pegler RB, Crabtree C, Lambert HP (1975) The faecal flora of children in the United Kingdom. The Journal of Hygiene 75: 135-142.
16. Benno J, Mitsuoka T (1986) Bifidobacteria and microflora. Development of intestinal microflora in human and animals 5: 13-25.
17. Finegold SM (1986) Normal human intestinal flora. Ann Ist Super Sanita 22: 731-737.
18. Hoa TT, Duc LH, Isticato R, Baccigalupi L, Ricca E, et al. (2001) Fate and dissemination of *Bacillus subtilis* spores in a murine model. Appl Environ Microbiol 67: 3819-3823.
19. Duc LH, Hong HA, Cutting SM (2003) Germination of the spore in the gastrointestinal tract provides a novel route for heterologous antigen delivery. Vaccine 21: 4215-4224.
20. Hoffmann T, Troup B, Szabo A, Hungerer C, Jahn D (1995) The anaerobic life of *Bacillus subtilis* - cloning of the genes encoding the respiratory nitrate reductase system. FEMS Microbiol Lett 131: 219-225.
21. Nakano MM, and Hulett FM (1997) Adaptation of *Bacillus subtilis* to oxygen limitation. FEMS Microbiol Lett 157: 1-7.
22. Berdy J (2005) Bioactive microbial metabolites - A personal view. J Antibiot 58: 1-26.

23. Stein T (2005) *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. Mol Microbiol 56: 845-857.

24. Okazaki H, Kishi T, Beppu T, Arima K (1975) A new antibiotic, baciphelacin. J Antibiot 28: 717-719.

25. Pinchuk IV, Bressollier P, Verneuil B, Fenet B, Sorokulova IB, et al. (2001) *In vitro* anti-*Helicobacter pylori* activity of the probiotic strain *Bacillus subtilis* 3 is due to secretion of antibiotics. Antimicrobial Agents & Chemotherapy 45: 3156-3161.

26. Lee HS, Lee H (2011) Purification and Biochemical Characterization of Bacteriolytic Enzyme from *Bacillus subtilis* YU-1432 Active against *Porphyromonas gingivalis*. J Korean Soc Appl Biol Chem 54: 600-605.

27. Kim JB, Jung WH, Ryu JM, Lee YJ, Jung JK, et al. (2007) Identification of a fibrinolytic enzyme by *Bacillus vallismortis* and its potential as a bacteriolytic enzyme against *Streptococcus mutans*. Biotechnol Lett 29: 605-610.

28. Ryazanova LP, Ledova LA, Tsurikova NV, Stepnaya OA, Sinitsyn AP, et al. (2005) Effect of the proteolytic enzymes of *Bacillus licheniformis* and the lysoamidase of *Lysobacter sp* XL1 on *Proteus vulgaris* and *Proteusmirabilis* cells. Appl Biochem Microbiol 41: 490-494.

29. Biziulevichius GA, Arestov IG (1997) In vivo studies on lysosubtilin. I. Efficacy for prophylaxis and treatment of gastrointestinal disorders in newborn calves. Vet Res 28: 19-35.

30. Gupta M, Kaur M, Gupta KG (1992) Lytic effect of *Bacillus subtilis* elastase on grem-positive and negative bacteria. Indian Journal of Experimental Biology 30: 380-383.

31. Shibasaki S, Okada J, Nakayama Y, Yoshida T, Ueda M (2008) Isolation of bacteria which produce yeast cell wall-lytic enzymes and their characterization. Biocontrol Sci 13: 91-96.

32. Sharma A, Satyanarayana T (2013) Microbial acid-stable alpha-amylases: Characteristics, genetic engineering and applications. Process Biochem 48: 201-211.

33. Khan M, Nakkeeran E, Umesh-Kumar S (2013) Potential Application of Pectinase in Developing Functional Foods. In Annual Review of Food Science and Technology, Vol 4, M.P. Doyle and T.R. Klaenhammer, eds. (Palo Alto, Annual Reviews), pp 21-34.

34. Guncheva M, Zhiryakova D (2011) Catalytic properties and potential applications of *Bacillus* lipases. J Mol Catal B-Enzym 68: 1-21.

35. Maki M, Leung KT, Qin WS (2009) The prospects of cellulase-producing bacteria for the bioconversion of lignocellulosic biomass. Int J Biol Sci 5: 500-516.

36. Gupta R,Beg QK, Lorenz P (2002) Bacterial alkaline proteases: molecular approaches and industrial applications. Appl Microbiol Biotechnol 59: 15-32.

37. Kogan AS, Salganik RI, Efimov IM, Morozov SA, Sobotovich VF (1982) Use of immobilized bacterial proteases to prolong enzyme therapy of purulent surgical diseases. Vestnik khirurgii imeni IIGrekova 128: 60-63.

38. Sumi H, Hamada H, Nakanishi K, Hiratani H (1990) Enhancement of the fibrinolytic activity in plasma by oral administration of nattokinase. Acta haematologica 84: 139-143.

39. Rojas JBU, Verreth JAJ, Amato S, Huisman EA (2003) Biological treatments affect the chemical composition of coffee pulp. Bioresour Technol 89: 267-274.

40. Phromraksa P, Nagano H, Boonmars T, Kamboonruang C (2008) Identification of proteolytic bacteria from Thai traditional fermented foods and their allergenic reducing potentials. J Food Sci 73: M189-M195.

41. Hosoi T, Ametani A, Kiuchi K, Kaminogawa S (2000) Improved growth and viability of lactobacilli in the presence of *Bacillus subtilis* (*natto*), catalase, or subtilisin. Canadian Journal of Microbiology 46: 892-897.

42. Tan TC, Qian ZR (1996) Activity of the enzyme system in thermally killed *Bacillus* cells. Enzyme Microb Technol 19: 150-156.

43. Smirnov VV, Reznik SR, Kudriavtsev VA, Osadchaia AI, Safronova LA (1992) Extracellular amino acids of aerobic spore-forming bacteria. Mikrobiologiia 61: 865-872.

44. Chattopadhyay SP, Banerjee AK (1978) Production of valive by a *Bacillus* sp. Zeitschrift Fur Allgemeine Mikrobiologie 18: 243-254.

45. Walter W, Bacher A (1977) Biosynthesis of thiamin in *Bacillus* subtilis. Isolation of mutants accumulating 4-amino-5-hydroxymethyl-2-methylpyrimidine phosphate. Journal of general microbiology 103: 359-366.

46. Bentley R, Meganathan R (1982) Biosynthesis of vitamin K (Menaquinone) in bacteria. Microbiological Reviews 46: 241-280.

47. Kim KP, Rhee CH, Park HD (2002) Degradation of cholesterol by *Bacillus subtilis* SFF34 isolated from Korean traditional fermented flatfish. Letters in Applied Microbiology 35: 468-472.

48. Paik HD, Park JS, Park E (2005) Effects of *Bacillus polyfermenticus* SCD on lipid and antioxidant metabolisms in rats fed a high-fat and high-cholesterol diet. Biol Pharm Bull 28: 1270-1274.

49. Caruso A, Flamminio G, Folghera S, Peroni L, Foresti I, et al. (1993) Expression of activation markers on peripheral-blood lymphocytes following oral administration of *Bacillus subtilis* spores. Int J Immunopharmacol 15: 87-92.

50. Fais S, Pallone F, Nava C, Magnani M (1987) Lymphocyte activation by *B. subtilis* spores. Bollettino dell'Istituto sieroterapico milanese 66: 391-394.

51. Huang JM, La Ragione RM, Nunez A, Cutting SM (2008) Immunostimulatory activity of *Bacillus* spores. FEMS Immunol Med Microbiol 53: 195-203.

52. Huang Q, Xu X, Mao YL, Huang Y, Rajput IR, et al. (2013) Effects of *Bacillus subtilis* B10 spores on viability and biological functions of murine macrophages. Anim Sci J 84: 247-252.

53. Barnes AGC, Cerovic V, Hobson PS, Klavinskis LS (2007) *Bacillus subtilis* spores: A novel microparticle adjuvant which can instruct a balanced Th1 and Th2 immune response to specific antigen. Eur J Immunol 37: 1538-1547.

54. Song M, Hong HA, Huang JM, Colenutt C, Khang DD, et al. (2012) Killed *Bacillus subtilis* spores as a mucosal adjuvant for an H5N1 vaccine. Vaccine 30: 3266-3277.

55. Fujiya M, Musch MW, Nakagawa Y, Hu S, Alverdy J, et al. (2007) The *Bacillus subtilis* quorum-sensing molecule CSF contributes to intestinal Homeostasis via OCTN2, a host cell membrane transporter. Cell Host Microbe 1: 299-308.

56. Sorokulova IB (1997) A comparative study of the biological properties of Biosporin and other commercial *Bacillus*-based preparations. Mikrobiolohichnyi Zhurnal 59: 43-49.

57. Sorokulova IB, Kirik DL, Pinchuk IV (1997) Probiotics against *Campylobacter* pathogens. J Travel Med 4: 167-170.

58. Nicholson WL, Munakata N, Horneck G,Melosh HJ, Setlow P (2000) Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. Microbiol Mol Biol Rev 64: 548-572.

59. Cano RJ, Borucki MK (1995) Revival and identification of bacterial spores in 25- to 40-million-year-old Dominican amber. Science 268: 1060-1064.

60. Cano RJ, Borucki MK, Higby-Schweitzer M, Poinar HN, Poinar GO Jr, et al. (1994) *Bacillus* DNA in fossil bees: an ancient symbiosis? Appl Environ Microbiol 60: 2164-2167.

61. Vreeland RH, Rosenzweig WD, Powers DW (2000) Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal. Nature 407: 897-900.

62. Nicholson WL (2002) Roles of *Bacillus* endospores in the environment. Cell Mol Life Sci 59: 410-416.

63. Fajardo-Cavazos P, Nicholson WL (2006) *Bacillus* endospores isolated rom granite: close molecular relationships to globally distributed *Bacillus* spp. from endolithic and extreme environments. Appl Environ Microbiol 72: 2856-2863.

64. Wang J, Fung DYC (1996) Alkaline-fermented foods: A review with emphasis on pidan fermentation. Crit Rev Microbiol 22: 101-138.

65. Oggioni MR, Pozzi G,Valensin PE, Galieni P, Bigazzi C (1998) Recurrent septicemia in an immunocompromised patient due to probiotic strains of *Bacillus subtilis*. J Clin Microbiol 36: 325-326.

66. Cukovic-Cavka S, Likic R, Francetic I, Rustemovic,N, Opacic M, et al. (2006) *Lactobacillus acidophilus* as a cause of liver abscess in a NOD2/CARD15-positive patient with Crohn's disease. Digestion 73: 107-110.

67. Borriello SP, Hammes WP, Holzapfel W, Marteau P, Schrezenmeir J, et al. (2003) Safety of probiotics that contain lactobacilli or bifidobacteria. Clinical Infectious Diseases 36: 775-780.

68. EU (2000) Opinion of the scientific committee on animal nutrition on the safety of use of *Bacillus* species in animal nutrition.

69. Sorokulova IB, Pinchuk IV, Denayrolles M, Osipova IG, Huang JM, et al. (2008) The safety of two *Bacillus* probiotic strains for human use. Dig Dis Sci 53: 954-963.

70. Hong HA, Huang JM, Khaneja R, Hiep LV, Urdaci MC, et al. (2008) The safety of *Bacillus subtilis* and *Bacillus indicus* as food probiotics. J Appl Microbiol 105: 510-520.

71. Tompkins TA, Hagen KE, Wallace TD, Fillion-Forte V (2008) Safety evaluation of two bacterial strains used in asian probiotic products. Canadian Journal of Microbiology 54: 391-400.

72. Endres JR, Clewell A, Jade KA, Farber T, Hauswirth J, et al. (2009) Safety assessment of a proprietary preparation of a novel probiotic, *Bacillus coagulans*, as a food ingredient. Food and Chemical Toxicology 47: 1231-1238.

73. Endres JR, Qureshi I, Farber T, Hauswirth J, Hirka G, et al. (2011) One-year chronic oral toxicity with combined reproduction toxicity study of a novel probiotic, *Bacillus coagulans*, as a food ingredient. Food and Chemical Toxicology 49: 1174-1182.

74. Sims W (1964) A pathogenic *Lactobacillus*. J Path Bacteriol 87: 99-105.

75. Momose H, Igarashi M, Era T, Fukuda Y, Yamada M, et al. (1979) Toxicological studies on *Bifidobacterium longum* BB536. Appl Pharmacol 17: 881-887.

76. Henry R, Mielle F, Mohr H (1950) *Bacillus subtilis* in intestinal therapy. Gazette medicale de France 57: 537-541.

77. Work E (1959) The action of a lytic enzyme from spores of a bacillus on various species of bacteria. Annales De L Institut Pasteur 96: 468-480.

78. Pham Ngoc T, Vu Thi C, Nguyen Thi H (1968) *Bacillus subtilis* in therapy and prevention of disease. Revue d'immunologie et de therapie antimicrobienne 32: 53-65.

79. Mazza P (1994) The use of *Bacillus subtilis* as antidiarrhoeal microorganism. Bollettino Chimico Farmaceutico 133: 3-18.

80. Gracheva NM, Gavrilov AF, Solov'eva AI, Smirnov VV, Sorokulova IB, et al. (1996) The efficacy of the new bacterial preparation biosporin in treating acute intestinal infections. Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii: 75-77.

81. Jurenka JS (2012) *Bacillus coagulans*. Altern Med Rev 17: 76-81.

82. Sudha MR, Bhonagiri S, Kumar MA (2013) Efficacy of *Bacillus clausii* strain UBBC-07 in the treatment of patients suffering from acute diarrhoea. Benef Microbes 4: 211-216.

83. Cutting SM (2011) *Bacillus* probiotics. Food Microbiol 28: 214-220.

84. Permpoonpattana P, Hong HA, Khaneja R, Cutting SM (2012) Evaluation of *Bacillus subtilis* strains as probiotics and their potential as a food ingredient. Benef Mirbobes 3: 127-135.

85. Smirnov VV, Reznik SR, Sorokulova IB (1994) Highly effective probiotic Biosporin. Likarska Sprava: 133-137.

86. Shimizu T (2003) Health claims on functional foods: the Japanese regulations and an international comparison. Nutr Res Rev 16: 241-252.

87. Liu L, Liu YF, Shin HD, Chen RR, Wang NS, et al. (2013) Developing *Bacillus* spp. as a cell factory for production of microbial enzymes and industrially important biochemicals in the context of systems and synthetic biology. Appl Microbiol Biotechnol 97: 6113-6127.

88. Sorokulova IB, Beliavskaia VA, Masycheva VA, Smirnov VV (1997) Recombinant probiotics: problems and prospects of their use for medicine and veterinary practice. Vestnik Rossiiskoi Akademii Meditsinskikh Nauk: 46-49.

89. Chudnovskaya NV, Ribalko SL, Sorokulova IB, Smirnov VV, Belyavskaya VA (1995) Antiviral activity of *Bacillus* probiotics. Dopovidi Nac Acad Nauk Ukraini: 124-126.

90. Beliavskaia VA, Cherdyntseva NV, Bondarenko VM, Litviakov NV (2003) Biological effects of interferon, produced by recombinant bacteria of the probiotic preparation subalin. Zhurnal mikrobiologii, epidemiologii, i immunobiologii: 102-109.

91. Litviakov NV, Cherdyntseva NV, Beliavskaia VA, Malinovskaia EA, Il'inykh IS, et al. (2001) [Role of macrophages in the antitumor action of the recombinant probiotic Subalin]. Voprosy Onkologii 47: 86-89.

92. Beliavskaia VA, Kashperova TA, Bondarenko VM, Il'ichev AA, Sorokulova IB, et al. (2001) Experimental evaluation of the biological safety of gene-engineered bacteria using a model strain *Bacillus subtilis* interferon-producing strain. Zhurnal mikrobiologii, epidemiologii, i immunobiologii: 16-20.

93. Sorokulova IB (1998) The safety and reactogenicity of the new probiotic subalin for volunteers. Microbiologichny Zhurnal 60: 43-46.

94. Berestovaya TG, Rudenko AA, Sorokulova IB, Muravskaya LV, Hunas IV, et al. (1999) Clinical efficacy of subalin at meningoenzefalitis. Farmacologichniy visnik: 24-28.

95. Sorokulova IB, Rybalko SL, Rudenko AA, Berestovaya TG, Legeza KN, et al. (2006) Probiotic Subalin – New Approach to Treatment of Bacterial and Viral Infections.(DIA).

96. Koh M, Hanagata H, Ebisu S, Morihara K, Takagi H (2000) Use of *Bacillus brevis* for synthesis and secretion of Des-B30 single-chain human insulin precursor. Biosci Biotechnol Biochem 64: 1079-1081.

97. Olmos-Soto J, Contreras-Flores R (2003) Genetic system constructed to overproduce and secrete proinsulin in *Bacillus subtilis*. Appl Microbiol Biotechnol 62: 369-373.

98. Porzio S, Bossu P, Ruggiero P, Boraschi D, Tagliabue A (2004) Mucosal delivery of anti-inflammatory IL-1Ra by sporulating recombinant bacteria. BMC Biotechnol 4: 16.

99. Knecht LD, Pasini P, Daunert S (2011) Bacterial spores as platforms for bioanalytical and biomedical applications. Anal Bioanal Chem 400: 977-989.

100. Isticato R, Cangiano G, Tran HT, Ciabattini A, Medaglini D, et al. (2001) Surface display of recombinant proteins on *Bacillus subtilis* spores. Journal of Bacteriology 183: 6294-6301.

101. Amuguni JH, Lee S, Kerstein KO, Brown DW, Belitsky BR, et al. (2011) Sublingually administered *Bacillus subtilis* cells expressing tetanus toxin C fragment induce protective systemic and mucosal antibodies against tetanus toxin in mice. Vaccine 29: 4778-4784.

102. Lee, S, Belitsky BR, Brinker JP, Kerstein KO, Brown DW, et al. (2010) Development of a *Bacillus subtilis*-Based Rotavirus Vaccine. Clin Vaccine Immunol 17: 1647-1655.

103. Duc LH, Hong HA, Atkins HS, Flick-Smith HC, Durrani Z, et al. (2007) Immunization against anthrax using *Bacillus subtilis* spores expressing the anthrax protective antigen. Vaccine 25: 346-355.

104. Zhou ZW, Xia HM, Hu XC, Huang Y, Li YW, et al. (2008) Oral administration of a *Bacillus subtilis* spore-based vaccine expressing *Clonorchis sinensis* tegumental protein 22.3 kDa confers protection against *Clonorchis sinensis*. Vaccine 26: 1817-1825.

105. Kim JH, Roh C, Lee CW, Kyung D, Choi SK, et al. (2007) Bacterial surface display of GFP(UV) on *Bacillus subtilis* spores. J Microbiol Biotechnol 17: 677-680.

106. Feng F, Hu P, Chen L, Tang Q, Lian CQ, et al. (2013) Display of Human Proinsulin on the *Bacillus subtilis* Spore Surface for Oral Administration. Curr Microbiol 67: 1-8.

107. Mao LY, Jiang ST, Li GH, He YQ, Chen L, et al. (2012) Surface Display of Human Serum Albumin on *Bacillus subtilis* Spores for Oral Administration. Curr Microbiol 64: 545-551.

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