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Novel insight on probiotic *Bacillus* subtilis: Mechanism of action and clinical applications

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Abstract

Probiotics are the living microorganisms that provide health benefits to the recipient. *Lactobacillus* and *Bifidobacterium* genera have been used since long for the competitive exclusion of pathogens from the gut. However, their limitations such as sensitivity to gastric acid, temperature, slow growth, and specific stability conditions lead to search for a novel probiotic that is stable through its shelf-life as well as during gastrointestinal transit; hence, offering better efficacy. *Bacillus* bacteria have strong scientific data which substantiates the validity of the use as preferred probiotics. In recent times, there has been significant progress in scientific evaluation and studies on probiotic *Bacillus subtilis*, revealing possible mechanisms of action like antimicrobial effect by synthesis of antimicrobial substances, antidiarrheal effect, immunostimulatory effect, competitive exclusion of pathogens, prevention of intestinal inflammation, and normalization of intestinal flora. Numerous preclinical and clinical studies on *B. subtilis* have shown its promising efficacy in the treatment and prevention of diarrhea of various etiologies. *B. subtilis* is certified as generally recognized as safe by Food and Drug Administration and features in the European Food Safety Authority Qualified Presumption of Safety, hence suggesting as safe for human use. All of these beneficial attributes make *B. subtilis* the most attractive probiotic species for various clinical conditions.

Key Words: Antidiarrheal effect, antimicrobial substances, *Bacillus subtilis*, immunomodulation

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INTRODUCTION

The term "probiotics" was derived from the Greek word, meaning "for life."[1] According to the WHO/FAO, probiotics are: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host."[2] Various factors such as environmental, nutritional and/or metabolic changes favor pathogen proliferation and that disturb equilibrium between beneficial and pathogenic flora leading to disease conditions. Synthetic antibiotics were the first option to control pathogenic overgrowth; however, the unregulated use of these compounds induced multi-drug resistance in pathogenic bacteria. Hence, antibiotic utilization needs to be well regulated. In this sense, the utilization of beneficial bacteria (probiotics) has emerged as an alternative based on the beneficial good results obtained with it.[3] *Lactobacillus*

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and *Bifidobacterium* genera have been used almost exclusively for the competitive exclusion of pathogens from the gut of humans and animals. However, there are some major challenges related to manufacturing probiotic formulations containing lactobacilli and *Bifidobacteria spp.,* as below:

- These microorganisms are microaerophilic or strict anaerobic, therefore, their handling and production is complex and could be a challenge
- Both microorganisms are slow growers
- Sensitive to temperature hence product must be maintained at low temperatures and shelf-life in general is short
- Many of lactobacilli and *Bifidobacteria* are sensitive to gastric juice during gastrointestinal (GI) tract transit.[4]

Bacillus bacteria are highly diverse group of microorganisms, known for more than 100 years. There are strong scientific data which substantiates the validity of the use of *Bacillus* bacteria as probiotics.[5] Only a few of the 100 species contained in the *Bacillus* genus are used as probiotics in humans such as *Bacillus subtilis, Bacillus licheniformis, Bacillus coagulans, Bacillus cereus var. toyoi, Bacillus natto (subtilis), Bacillus clausii, Bacillus pumilus,* and *Bacillus cereus.*[6] In recent decades, scientific studies on probiotic *B. subtilis* have made significant progress to elucidate the activity spectrum which makes this bacterium the most attractive probiotic for clinical use. In this review, we have present data from experimental and clinical research that may allow making an impression of the therapeutic potential of *B. subtilis.*

BACILLUS SUBTILIS BACTERIA IN NATURE

Bacillus constitutes a diverse group of rod-shaped, Gram-positive bacteria, characterized by their robust spore-forming ability.[3] *Bacillus* genus bacteria are most widespread microorganisms in nature. *Bacillus* species are predominant in soil and also have been isolated from water, air, and food products like wheat, grain, wholemeal, soya beans, and milk microflora. Bacilli consistently enter the GI and respiratory tracts of healthy people with food, water, and air. Isolation of *B. subtilis* from the human GI tract was reported for healthy adults and children. The number of bacilli in the gut can reach 10^7 CFU/g, comparable to lactobacilli count. Thus, researchers considered *Bacillus* to be one of the dominant components of the normal gut microflora.[5] The *B. subtilis* genome is totally sequenced, leading to the generation of great amount of basic knowledge and also developments of molecular and genetic methodologies.[3]

BACTERIAL SPORE FORMATION

Bacterial spores are formed as a means to survive extreme environmental conditions for long-term survival otherwise that conditions kill vegetative bacteria. Sporulation is very much dependent upon the nutrients availability in the immediate vicinity of the live cell. According to Cutting *et al.* (2009), decrease in nutrients is sensed by the bacterium and it enters an irreversible program of development that results in the production of spores as shown in Figure 1.[7] Bacterial endospore contains a condensed and inactive chromosome at its core. Surrounding the spore, layer of peptidoglycan-rich cortex and one or more layers of proteinaceous material is present referred as the spore coat. During the lack of nutrient condition, growing vegetative cell (VC) undergoes a series of morphological changes that forms a forespore (F) within the mother cell (MC) of the sporangium and then after some time spore (S) is released by lysis of the MC.[7]

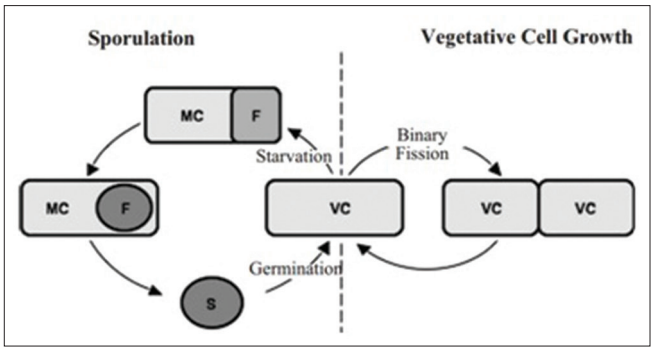

Figure 1: Sporulation life cycle of bacterial spore formers

BACILLUS SUBTILIS SPORE FORMATION AND ITS ADVANTAGES

B. subtilis spores are highly resistant to temperature, extreme pH, gastric acid; bile and solvents, hence keep viability in the gut.[3,5] *B. subtilis* can be stored for long time periods without refrigeration.[3]

BACILLUS SUBTILIS SPORE GERMINATION AND PROLIFERATION IN GASTROINTESTINAL TRACT

Immunological data showed that *B. subtilis* spores germinate in the mouse gut and proliferate and able to grow and resporulate.[8] Casula and Cutting observed germination of *B. subtilis* spores as VCs in the mouse jejunum and ileum using a competitive reverse transcription polymerase chain reaction targeting a genetically engineered chimeric gene, ftsH-lacZ, which is only expressed by VCs.[9] Ozawa *et al.* found that spores of *B. subtilis* strain BN germinate and multiply to some extent in the GI tract of pigs.[10] Leser *et al.* showed that *B. subtilis* germinate and grow in proximal part of the pig GI tract.[11]

MECHANISM OF ACTION OF PROBIOTIC *BACILLUS SUBTILIS*

Possible mechanisms of action include antimicrobial effect by synthesis of antimicrobial substances, antidiarrheal effect,

immunostimulatory effect, competitive exclusion of pathogens, prevention of intestinal inflammation, and stimulation of growth of intestinal normal flora.[11] *B. subtilis* has unique properties such as spore formation, versatility of growth nutrients utilization, high level of enzymes production, fast growth rate, and growth in aerobic and anaerobic conditions.[3]

Synthesis of antimicrobial agents

Bacillus bacteria play a significant role in the gut because of their high metabolic activity. The activity of *Bacillus* is mainly determined by their ability to produce antibiotics. *B. subtilis* is the most productive species which devotes 4%–5% of genome to antibiotic synthesis and produce 66 antibiotics. *Bacillus* antibiotics have different structure and spectrum of antimicrobial activity.[5] The potential of *B. subtilis* to produce antibiotics has been recognized for 50 years. Antimicrobial agents synthesized and secreted by *B. subtilis* are listed in Table 1.[12-14] *B. subtilis* synthesized antimicrobial substances have antimicrobial effects against broad spectrum of pathogens. These substances are natural part of human antimicrobial defense system hence the possibility of developing pathogen resistance or unwanted side effects is less. Hence, probiotic *B. subtilis* is ideal therapeutic option because of their broad spectrum of activity and specific and rapid killing activity against various pathogens.[14] *B. subtilis* probiotic properties are strain-specific.[3] Pectinolytic enzymes have been isolated from *B. subtilis*. Bacilli produce amino acids, including essential amino acids and vitamins.[5]

Immunostimulatory effect

B. subtilis enhances the protection against pathogens by stimulating nonspecific and specific immunity. A number of studies in humans and animal models have provided strong evidence that oral administration of *Bacillus* spores stimulates the immune system. Spores of *B. subtilis* trigger specific humoral and cell-mediated immune responses. The interaction between *B. subtilis* spores and macrophages plays an important role in development of both innate and adaptive immune responses of the host. Numerous studies have demonstrated that *B. subtilis* leads to macrophage activation. *B. subtilis* B10, *B. subtilis* BS02, and *B. subtilis* (*natto*) B4 spores may possess immunomodulatory activities by the induction of pro-inflammatory cytokines and exerts probiotic activities through activated macrophage functions. In addition, *B. subtilis* showed no apparent cytotoxicity against RAW 264.7 cells and thought to be safe.[15-17] *B. subtilis* MBTU PBBM1 spores administration leads to augmentation of antibody response (antibodies IgG and IgA) and also proliferation of the T lymphocytes which indicates *B. subtilis* MBTU PBBM1 spores have the potential to improve both the humoral and cellular immunity in mice.[18] Commensal bacteria play an important role in the development of the gut-associated lymphoid tissue (GALT) and important for both innate and adaptive immunity. *B. subtilis* promotes active lymphocyte proliferation within GI tract. *B. subtilis* administration in appendix of germfree rabbits has been shown to promote GALT development. This evidence showed that *Bacillus* species are important for development of robust gut-associated lymphoid system (GALT) and promote a potent immune response.[19] The effect of lymphocyte activation by *B. subtilis* spores was both quantitatively and qualitatively similar to mitogens phytohaemagglutinin and Concanavalin A. *B. subtilis* spores stimulated cytokine production *in vitro* and after oral administration in mice.[5] Oral treatment with *B. subtilis* spores increased expression of activation markers on lymphocytes in dose-dependent manner in healthy volunteers.[20] *B. subtilis* spores-induced systemic antibody response to tetanus toxoid fragment C and ovalbumin in mice. These data suggest that *B. subtilis* spores are an efficient mucosal and systemic adjuvant for enhancing humoral immune responses.[21]

Maintenance of intestinal homeostasis and prevention of intestinal inflammation

B. subtilis derived quorum-sensing pentapeptide, competence and sporulation factor (CSF) activate key survival pathways including p38 MAP kinase and protein kinase B (Akt) in intestinal epithelial cells of the host. CSF also induces heat shock proteins, which protect intestinal epithelial cells against injury and loss of barrier function and hence provide the ability to maintain intestinal homeostasis.[22] *B. subtilis* quorum sensing molecule CSF reduced epithelial injury caused by intestinal inflammation and improved the survival rate of mice with lethal colitis. This indicates that *B. subtilis* are potentially useful for treating intestinal inflammation. *B. subtilis* is beneficial for maintaining intestinal homeostasis and host health and can be utilized to treat antibiotics-induced colitis, inflammatory bowel disease (IBD) (including Crohn's disease and ulcerative colitis) and necrotizing enterocolitis.[23] *Bacillus* species (*B. subtilis, Bacillus firmus, Bacillus megaterium,* and *B. pumilus*) have been shown to convert genotoxic compounds to unreactive products *in vitro*.[24] Orally administered *B. subtilis* spores were effective in decreasing infection and enteropathy in suckling mice infected with *Citrobacter rodentium* (a model for the traveler's diarrhea pathogen enterotoxigenic *Escherichia coli*) which is known to cause epithelial lesions, crypt hyperplasia, and mortality.[7,25] Intestinal mucosal barrier dysfunction associated with IBD. Mucosal biopsies taken from IBD patients showed loss of key epithelial tight junction (TJ) proteins such as claudin-1, occludin, junctional adhesion molecule (JAM)-A, and zona occludens (ZO)-1. Effects of *B. subtilis* on epithelial TJs and intrinsic regulatory mechanisms of the intestine were studied in dextran sulfate sodium-induced colitis in Balb/c mice. *B. subtilis* significantly reduced disease activity index scores and graded histologic damage. *B. subtilis* improved

Table 1: Antimicrobial agents synthesized and secreted by *Bacillus subtilis*

Category of antimicrobial agents	Antimicrobial agents	Mechanism of action	Spectrum of antimicrobial activity
Ribosomal synthesized peptides Bacteriocins: Type A lantibiotics	Subtilin, ericin A, ericin S	Voltage-dependent pores into the cytoplasmic membrane	Gram-positive bacteria, antiviral, and antimycoplasma activities
Ribosomal synthesized peptides: Type B lantibiotics	Mersacidin	Inhibition of cell wall synthesis	Gram-positive bacteria, including methicillin-resistant strains of *S. aureus* and vancomycin resistant strains of *Enterococci*
Unusual lantibiotics	Subtilosin A	Antimicrobial activity by interacting with membrane-associated receptors	Gram-positive bacteria, strong bactericidal activity against *L. monocytogenes*
	Sublancin 168	-	Gram-positive bacteria, pathogens such as *B. cereus*, *S. pyogenes* and *S. aureus*
	Bacillocin 22	-	-
Nonribosomal synthesized peptides	Surfactin	Powerful biosurfactant - it exerts a detergent-like action on biological membranes	Antiviral and antimycoplasma activities, inhibit biofilm formation of human pathogen *S. enterica*
	Bacilysin	Inhibits glucosamine synthase involved in synthesis of nucleotides, amino acids and coenzymes and resulting in lysis of microbial cells	*S. aureus* and *C. albicans*
	Iturine lipopeptides: Mycosubtilin, iturines, bacillomycins	-	Strong antifungal and hemolytic and limited antibacterial activities
	Fengycin (plipastatin)	-	Antimicrobial and fungicidal action
	Rhizocticins	-	Antifungal activity
	Mycobacillin (B3)	-	Antibacterial and antifungal activities
	Corynebactin (bacillibactin), 3,3'-neotrehalosadiamine (168), difficidin, TL-119 (A-3302-B)	-	-
Miscellaneous antibiotic compounds	Polyketides: Difficidin, oxydifficidin, bacillaene	-	Antibacterial activity against both aerobic and anaerobic organisms
	Amicoumacin	-	Antibacterial activities, *S. aureus* and *H. pylori*
	Bacilysocin	-	-
	BSAP-254	-	Antagonistic effect against the food borne pathogens
	Entianin	-	Strong antibacterial activity against *S. aureus*, *E. faecalis*, and other Gram-positive pathogens
	Subpeptin JM4-A, subpeptin JM4-B	-	Active against broad spectrum of bacteria, including *Salmonella*, *B. cereus*, *S. aureus*
	Antifungal protein B29I	-	Inhibitory activity on mycelial growth in *F. oxysporum*, *Rhizoctonia solani* and other fungi
	Bacteriocin-like substances		Gram-positive and Gram-negative bacteria
	AMP IC-1	-	Antagonistic to *B. cereus*
	AMPNT-6	-	Active against marine food borne pathogen
	Bac 14B	-	Useful for seed disinfection

-: Not yet identified, *S. aureus*: *Staphylococcus aureus, L. monocytogenes*: *Listeria monocytogenes, S. pyogenes*: *Streptococcus pyogenes, S. enterica*: *Salmonella enterica, C. albicans*: *Candida albicans, H. pylori*: *Helicobacter pylori, E. faecalis*: *Enterococcus faecalis, B. cereus*: *Bacillus cereus, F. oxysporum*: *Fusarium oxysporum, R. solani*: *Rhizoctonia solani*

barrier function by upregulating expression of epithelial TJs proteins (claudin-1, occludin, JAM-A, and ZO-1) and reduced intestinal epithelial damage by downregulating cytokine expression (interleukin-6 [IL-6], IL-17, IL-23, and tumor necrosis factor-α).[26]

Maintenance of intestinal normal flora

B. subtilis positive effect on the maintenance of the normal intestinal flora has been demonstrated in many studies. *B. subtilis* 3 strain showed efficacy against pathogenic cultures of *E. coli* and *Campylobacter* species during treatment of

experimental infections in mice and maintained normal microflora in the animals during receipt of antibiotic therapy. *In vitro* studies of *B. subtilis*, 3 showed a wide spectrum of antagonistic activity toward the tested pathogens and did not inhibit normal microflora.[27] *B. subtilis* MA139 significantly increased the number of *Lactobacillus* and reduced the content of *E. coli* in the intestines and feces in pigs.[28] *B. subtilis* KN-42 significantly increased lactobacilli counts and reduced *E. coli* counts and improved the growth performance and GI health of piglets.[29] *B. subtilis* KD1 improved intestinal flora by significantly increasing lactobacilli counts and reducing *E. coli* counts and improve the growth performance in broilers.[30] *B. subtilis var. natto* in mice influenced the fecal microflora, specifically *Bacteroides* and *Lactobacillus* species. Mice fed with an egg white diet showed decrease in numbers of *Lactobacillus* spp., while *B. subtilis var. natto* spores supplemented diet stabilized it. Using a casein diet, the numbers of Bacteroidaceae increased. This result indicated that *B. subtilis var. natto* could be beneficial in maintaining the natural microflora.[24] Therefore, an increase in *Lactobacillus* counts and decrease in *E. coli* counts may result in a lower diarrhea incidences.[29] *Salmonella* is a major foodborne pathogen which can cause severe illness in humans such as enteric fever, bacteremia, focal infection, and enterocolitis. *B. subtilis* NC11 exhibits strong inhibition activity against *Salmonella enteritidis* infection to intestinal epithelial cells.[31] *B. subtilis* CU1 effects on intestinal mucosal immune system, and microbial balance were evaluated in antibiotic-induced dysbiosis mouse model. *B. subtilis* CU1 spores (3×10^9 spores/day/mouse) administered before and during the antibiotic treatments. Treatment with the *B. subtilis* CU1 strain decreases the antibiotic-induced intestinal inflammation. *B. subtilis* CU1 shown to normalize the B220+MHCII+B-cells in mesenteric lymphoid node and F4/80+ macrophages in Peyer's patches in the antibiotic group. *B. subtilis* CU1 treatment reduced antibiotic-induced alterations in the gut microbiota. This result suggests that *B. subtilis* CU1 may contribute to the reduction of antibiotic-induced inflammation through normalization of mucosal immune responses and intestinal microbiota.[32] Taking into account beneficial properties of *B. subtilis*, this bacterium is a potential probiotic candidate to be considered for various clinical conditions.

CLINICAL TRIALS OF *BACILLUS SUBTILIS*

B. subtilis therapy was highly effective in treatment of various infectious pathologies in patients.[33] Clinical efficacy of *B. subtilis* was summarized as an antidiarrheal agent, used in different counties. *B. subtilis* is one of the most important microorganisms for the treatment and prophylaxis of intestinal disorders in humans.[34] *B. subtilis* was more effective in treatment of acute diarrhea than lactobacilli.[5]

Regularity of bowel movements

Labellarte *et al.*, carried out randomly assigned, double-blind placebo-controlled trial of *B. subtilis* (approximately 1.9×10^9 CFU/capsule) in 40 healthy male and female adults for an average of 20 days. The study showed that daily consumption of *B. subtilis* was effective in promoting regularity of bowel movements and well tolerated.[35]

Survival in the gastrointestinal tract

Hanifi *et al.*, carried out randomized, double-blind, placebo-controlled trial of *B. subtilis* R0179 in 81 healthy adults (18–50 years old). Subjects received *B. subtilis* R0179 at dose of 0.1, 1 or 10×10^9 CFU/capsule/day or placebo for 4 weeks. Fecal viable counts of *B. subtilis* R0179 showed a dose-dependent GI survival response and fecal viable counts were 0.1×10^9 (4.6 ± 0.1 $\log_{10}$ CFU/g), 1×10^9 (5.6 ± 0.1 $\log_{10}$ CFU/g) and 10×10^9 (6.4 ± 0.1 $\log_{10}$ CFU/g) respectively ($P < 0.0001$). *B. subtilis* R0179 survives passage through the human GI tract and is safe and well tolerated in healthy adults at intake from 0.1 to 10×10^9 CFU/day.[36]

Diarrhea and antibiotic-associated diarrhea

Clinical efficacy of *Bacillus* bacteria in the treatment of GI infections has been reported. Mazza (1994) summarized results of numerous studies and concluded that *B. subtilis* are one of the most important microorganisms for the treatment and prophylaxis of intestinal disorders in humans.[34] In clinical study, *B. subtilis* and *B. licheniformis* (2×10^9 microbial cells; Biosporin) were administered to the patients with acute enteric infections. Results showed the pronounced curative effect of *Bacillus* probiotics manifested by the rapid normalization of stool, abdominal pain relief, and decrease in intestinal dysbiosis. *Bacillus* probiotics found to be safe and well tolerated.[37] *B. subtilis* and *B. licheniformis* (Biosporin) has been also evaluated for effect on intestine microflora in acute digestive disorders and dysbacterioses in 53 newborn children with perinatal pathology. Results showed high therapeutic and prophylactic efficiency for dysbacterioses and diarrheas in the newborn children without side effects.[38] One of the most common side effects of antibiotic therapy is antibiotic-associated diarrhea (AAD). The frequency of AAD depends on the type of antibiotic used and varies from 25% to as high as 44%. The route of antibiotic administration (oral or parenteral) does not affect the rate of AAD, and no difference has been found in the frequency of AAD with respect to age and gender. The severity of AAD may vary from uncomplicated diarrhea to *Clostridium difficile*-associated pseudomembranous colitis. The main mechanism for the development of AAD is significant changes in the composition and quantity of the gut microbiota during the treatment with antibiotics. AAD may be caused by different enteric pathogens such as *Salmonella* spp., *Staphylococcus aureus, Candida albicans, Clostridium*

perfringens, and *Klebsiella* spp. *Bacillus* bacteria have attracted the growing attention of researchers as effective probiotics for the treatment and prevention of enteric infections. Research study showed high efficacy of the *Bacillus* probiotic *B. subtilis* 3 and *B. licheniformis* 31 (predominant amount of *B. subtilis* 3 in 50:1 parts; Biosporin) in the treatment of acute intestinal infections.[39] In clinical trial, *B. subtilis* spores (4×10^9) administered to 11 children aged 3–24 months for 5 days along with antibiotic and alone antibiotic was given to 8 subjects. Results showed that number of stools increased in alone antibiotic group while in *B. subtilis* along with antibiotic group no such changes were observed. Bacteriotherapy along with antibiotic also increased saccharolytic flora, aerobic and anaerobic flora.[40] Horosheva *et al.* carried out a randomized, double-blind, placebo-controlled clinical trial on outpatients aged ≥45 years who were prescribed ≥1 oral or intravenous antibiotics for at least 5 days. One group of patients ($n = 90$) received probiotic *B. subtilis* 3 (2×10^9 CFU/vial), 2 times a day from beginning 1 day before initiation of antibiotic therapy and ending 7 days after discontinuation of antibiotic. Results showed that AAD developed in 25.6% (23/90) patients in placebo group while in significantly lower AAD rate 7.8% (7/90) patients reported in *B. subtilis* 3 group ($P < 0.001$). *B. subtilis* 3 significantly reduced the incidence of nausea, vomiting, bloating, and abdominal pain.[39] There have been 23 clinical trials involving over 1800 patients for probiotic preparation containing a combination of *B. subtilis* R0179 and other probiotic. It has been used in the improvement of symptoms associated with chronic diarrhea and irritable bowel syndrome, as a coadjuvant therapy with sulfasalazine and mesalazine to improve remission times in mild to moderate ulcerative colitis and to improve compliance with conventional triple therapy for *Helicobacter pylori* eradication.[41]

Stimulation of immune responses

Lefevre *et al.* (2015) carried out randomized, double-blind, placebo-controlled, parallel-arms trial on 100 elderly subjects and allocated to receive *B. subtilis* CU1 (2×10^9 spores daily) or placebo for short course of 10 days followed by 18 days of treatment break per month and this scheme was repeated for 4 times during the trial (4 months). Biological sample analysis in subset of 44 subjects showed that *B. subtilis* CU1 significantly increased secretory IgA level in saliva and stools compared to the placebo as shown in Figures 2 and 3.[42] SIgA is a key element in the maintenance of gut microbiota homeostasis and in the protection of GI and respiratory tracts against pathogens. *B. subtilis* CU1 had been shown to increase IgA producing B cells in Peyer's patches in mice (Racedo SM and Urdaci MC, unpublished observations), it can be postulated that *B. subtilis* CU1 strengthens the generation of α4β7+IgA+B-cells in the Peyer's patches of the small intestine. The homing of IgA producing B cells to the intestinal mucosa and the salivary glands leads to high SIgA levels in saliva and stools. *B. subtilis* CU1 significantly increased serum interferon-γ (IFN-γ) and stimulated systemic immune response. IFN-γ plays an important role in the host defense against several infectious diseases including viral infection and has a variety of immune functions such as stimulation of macrophages and natural killer cells. A *post hoc* analysis in subset of 44 subjects showed a decreased frequency of respiratory infections in the *B. subtilis* CU1 group compared to the placebo group. This result suggests that *B. subtilis* CU1 may be an effective and safe way to stimulate immune responses.[42]

SAFETY OF *BACILLUS SUBTILIS*

According to European Scientific Committee on Animal Nutrition, *B. subtilis* was tested and showed no evidence of toxicity. Acute and chronic toxicity studies in animals also indicated safety of these strains. *B. subtilis* is generally recognized as safe by the Food and Drug Administration (FDA), meaning it is not harmful to humans.[3] *B. subtilis* species is considered safe by the European Food Safety Authority (EFSA) Qualified Presumption of Safety. Thus, *B. subtilis* strain may be considered as nonpathogenic and safe for human consumption.[43] *B. subtilis* could be considered as a perfect multifunctional probiotic bacterium for humans.[3]

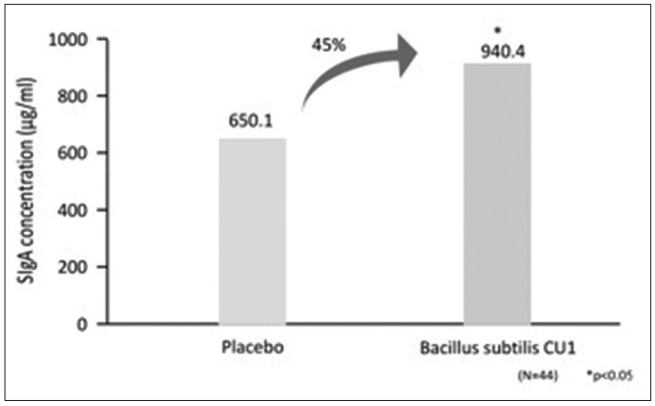

Figure 2: Increase in secretory IgA level in saliva

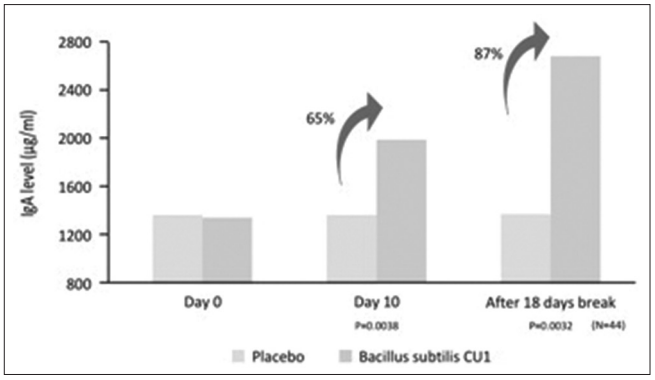

Figure 3: Increase in secretory IgA level in stools

SUMMARY

The world market for probiotics supplements has been growing over the last two decades based on their important clinical merits. Lactobacillus and Bifidobacterium are the most used genera, mainly for their ability to exclude pathogens. However, they do not have multifunctional probiotic capacities as B. subtilis. Bacillus bacteria are increasingly attracting attention of researchers as promising probiotics due to their strong antimicrobial, antidiarrheal and immunostimulatory effects, ability to stimulate growth of natural flora and prevent intestinal inflammation; besides having an excellent stability profile in otherwise unfavorable conditions. Moreover, it has established efficacy and safety in numerous randomized, double-blind clinical trials, as validated and approved by authorities like FDA and EFSA. In this sense, B. subtilis has the potential to emerge as the "perfect multifunctional probiotic bacteria" for various clinical conditions in humans.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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