



Species of *Bacillus* as Potential Probiotics

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ABSTRACT

Species of *Bacillus* are Gram-positive, sporulating, aerobic or facultative anaerobic rod-shaped bacteria. They are ubiquitous in nature, widely found in soil, air and water. Most species of *Bacillus* are saprophytes. Those species of *Bacillus* that survive in presence of stomach acid, bile, proteolytic enzymes and adhere to the colonocytes, they are termed as probiotic. Probiotic *Bacillus* species include *Bacillus subtilis*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus licheniformis*, *Bacillus polyfermenticus* and *Bacillus pumilus*. Probiotic *Bacillus* spp. can be grown in whey medium as well on urd dhal or black gram dhal (*Vigna mungo*) to obtain good biomass to use directly or can be incorporated in foods for obtaining therapeutic benefits. *Bacillus* species used as probiotics for the last five decades has the advantage over non-spore forming *Lactobacillus* spp. is that they exhibit higher acid tolerance; better stability during heat processing and low temperature storage.

Key words: Black gram dhal, Whey medium, *Bacillus*,

I INTRODUCTION

Microorganisms may be Prokaryotes or Eukaryotes. Prokaryotes included Bacteria and Archaea. Eukaryotes include Protozoa, Fungi and Algae. Bacteria are classified based on the role as beneficial bacteria, saprophytes and pathogens. Human body contains 10⁷ microorganisms at various locations. Mammalian gastro-intestinal tract has good bacteria having therapeutic benefits termed as probiotic. Probiotics were first discovered by Russian scientist Elie Metchnikoff. Probiotics are 'live microorganisms, which when administered in adequate amounts confer health benefits to the host'. Probiotic formulations are being developed and standardized for both human and animal consumption. Lactobacilli and Bifidobacteria, the two globally recognized groups of bacteria are being consumed for their potential health benefits [1]. *Bacillus* species used as probiotics for the last five decades has the advantage over non-spore forming *Lactobacillus* spp is that they exhibit higher acid tolerance; better stability during heat processing and low temperature storage. Additionally, they have shown to possess pathogen exclusion as they synthesize antimicrobial compounds. Probiotic *Bacillus* species are used to relieve symptoms of irritable bowel syndrome (IBS), inflammatory bowel disease and anti - *H. pylori* antibiotic therapy [2].

II LITERATURE REVIEW

Microorganisms used in probiotic products around the world

<i>Lactobacillus</i> species	<i>Bifidobacterium</i> species	<i>Bacillus</i> species	Others	References
<i>Lactobacillus acidophilus</i>	<i>Bifidobacterium bifidum</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	[3][4]
<i>Lactobacillus casei</i>	<i>Bifidobacterium longum</i>	<i>Bacillus clausii</i>	<i>Saccharomyces boulardii</i>	
<i>Lactobacillus reuteri</i>	<i>Bifidobacterium breve</i>	<i>Bacillus coagulans</i>	<i>Clostridium butyricum</i>	
<i>Lactobacillus johnsonii</i>	<i>Bifidobacterium infantis</i>	<i>Bacillus licheniformis</i>	<i>Propionibacterium freudenreichii</i>	

Isolation of Probiotic *Bacillus* spp.

Probiotic *Bacillus* spp. can be obtained from Soil, dung, fermented milk products, meat, egg etc. Through serial dilution, heating to 80°C/10 mins selects the spore formers and forms colonies on acidified bile (0.3 %) containing nutrient agar when incubated at 37 °C/24 h.

Characteristics of Probiotic *Bacillus* sp.

Bacillus spp formed dry, flat, rhizoid colonies on nutrient agar. Genus *Bacillus* are Gram-positive, sporulating, aerobic or facultative anaerobic rod-shaped bacteria. Special characteristics of probiotic *Bacillus* spp include heat resistant, synthesis of antimicrobial compounds, lactic acid synthesis [5].

Biomass production of Probiotic *Bacillus subtilis* in whey medium

Natural medium such as whey rich in Lactose 4.9%, whey protein 0.8%, fat 0.3%, minerals 0.5% can be used to grow probiotic *Bacillus* species. Cell pellets obtained by centrifugation can be preserved by freeze drying [6].

Biomass production of Probiotic *Bacillus subtilis* on Urd dhal (*Vigna mungo*) medium

Solid state substrate like Urd dhal rich in carbohydrate 59%, Protein 25%, Fat 1.6%, and Minerals 1.3% with 1 part water, 0.2-part tomato juice used for growth factor manganese sulphite and pantoic acid. 0.1-part SMP supported growth of probiotic *Bacillus* species. Fermented dhal dried in dehumidifier at 25°C/12 h can be made into powder and packed in plastic pouch. It can be used in the form of tablets, capsules or directly incorporated into food as the cells are in concentrated form [7].

Major probiotic mechanisms of Probiotic *Bacillus* spp. Include

➤ Enhancement of the epithelial barrier:

Intestinal barrier is a major defense mechanism used to maintain epithelial integrity and protect organism from environment. Probiotics promote mucous secretion to improve barrier function and exclusion of pathogens. Several probiotic *Bacillus* spp. increase mucin (mucins containing glycosylated proteins +50–80% carbohydrate) expression in human intestinal cell [8]. Piglets of 90 numbers divided into 3 groups for 28 days study. G1 containing 150 mg/kg of aureomycin (chlortetracycline- $C_{22}H_{23}ClN_2O_8$); G2 containing 75 mg/kg aureomycin and 1×10^8 cfu/kg of *Bacillus amyloliquefaciens* SCO6; G3 containing 2×10^8 cfu/kg *Bacillus amyloliquefaciens* SCO6 without antibiotic were given in the diet. G2 and G3 significantly improved the villus height (450 μ m) compared to G1 (300 μ m). G2 and G3 enhanced the length, integrity, tightness of intestinal microvillus and G3 had superior intestinal epithelial tight junction [9].

➤ Competitive Exclusion of Pathogenic Enteropathogens

E. coli is a pathogenic strain it causes gastroenteritis. *B. subtilis* 3 strain effective against pathogenic *E. coli* in human maintained normal microflora during antibiotic therapy. Patients of 90 numbers aged ≥ 45 years, given ≥ 1 oral or intravenous antibiotics for 5 days. The antibiotic contained *B. subtilis* 3 at the rate of 2×10^9 cfu/vial was administered 2 times a day during antibiotic therapy to 7 days after antibiotic treatment. Among the patients, 25.6 % patients in placebo group increased AAD (Antibiotic Associated Diarrhea) and significantly lower AAD rate in 7.8 % patients reported in *B. subtilis* 3 group. *B. subtilis* 3 significantly reduced incidence of nausea, vomiting, bloating, and abdominal pain. *B. subtilis* 3 significantly increased number of *Lactobacillus* (Pyridoxine - Vitamin B6 produced by *B. subtilis*) and reduced *E. coli* in intestines. Increase in *Lactobacillus* counts and decrease in *E. coli* counts it may result in a lower diarrhea incidence [10].

Salmonella enteritidis is a foodborne pathogen it causes severe illness in humans Salmonellosis. *B. subtilis* NC11 out of 4 strains exhibited strong inhibition activity against *Salmonella enteritidis*. Native chicks (12–24 weeks old). Among bacterial isolates of 240 numbers obtained from GI tract of chicks, 117 were *Bacillus* spp. The screening for antibacterial activity using agar well diffusion assay method revealed that, out of 117 *Bacillus* isolates, only 15 isolates inhibited growth of *Salmonella enteritidis* DMST 15676 and *Bacillus subtilis* NC11 showed highest of 12mm inhibitory zone.

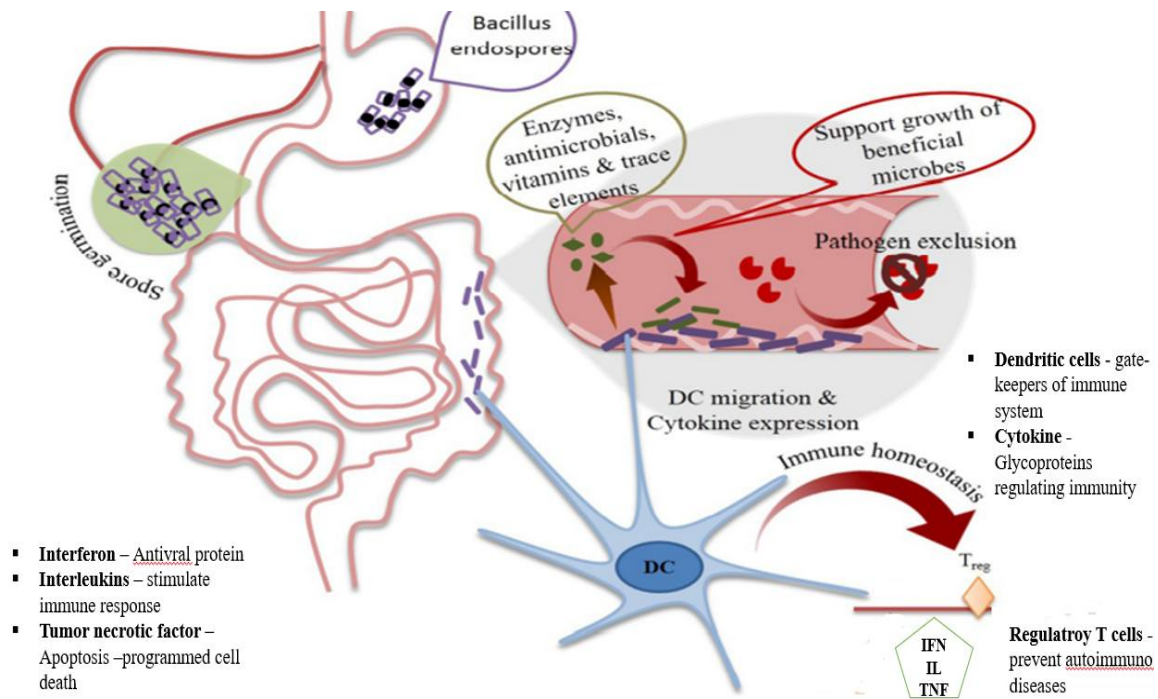
➤ Production of antimicrobial substances

Candida albicans cause candidiasis. It mainly causes in old age, diabetes, hypertension, long antibiotic therapy. It is known as white fungus and affected Covid patients. The symptoms included nausea, vomiting, abdominal cramp, diarrhea, weight loss, blood in stool. *Bacillus subtilis* var. *natto* produced inhibitory substances like Subtilin, Surfactin and Bacilysin. Subtilin is a peptide it having MW 3321 Daltons. Surfactin is a cyclic lipopeptide having MW 1036 Daltons, a powerful biosurfactant and exerted detergent like action on cell membrane by making pores. Bacilysin is a dipeptide antibiotic having MW 270 Daltons and inhibited glucosamine synthase involved in synthesis of nucleotides, amino acids and resulting in lysis of microbial cells. Patient's male and female adults (20+20) having candidiasis were administered *B. subtilis* var. *natto* 10^9 cfu/capsule for 20 days that regularized bowel movements & reduced candidiasis symptoms [8].

Mechanism of Health Benefits of Spore forming Probiotic *Bacillus*

Daily intake of *Bacillus* endospores in the form of tablet or powder or liquid form. The endospores enter the stomach and in the presence of gastric juices and bile in the stomach led to germination of spores. Germinated spores reach the intestine produce enzymes like proteinase, amylase and lactase as well antimicrobials like Subtilin, Surfactin, Bacilysin and vitamin like pyridoxin. They make biofilm, extended the therapeutic benefits by evacuating the pathogen and supporting the growth of beneficial microbes. Probiotic *Bacillus* could proliferate different immune cells like dendritic cell migration (DC are gate keepers of immune system) and cytokine expression (cytokine are glycoproteins it regulating immunity) to maintain immune

homeostasis by regulatory T cells that stimulated interferon (Antiviral protein), interleukins (stimulate immune response) and tumor necrotic factor (that supported apoptosis which is programmed cell death) [11].



Mechanism of Health Benefits of Spore forming Probiotic *Bacillus*

Role of Probiotic *Bacillus*

➤ Irritable bowel syndrome (IBS):

Irritable bowel syndrome is a chronic disorder that affects normal function of the intestines. It is a chronic immune disorder mediated inflammation (cytokines) of the gut mucosa. IBS symptoms include fever, vomiting, headache, bloating, diarrhea. Stress, post bacterial infection (members of family Enterobacteriaceae); protozoan infections (*Amoeba*, *Giardia*); genetics are the causes of IBS. *Bacillus subtilis* and *Bacillus coagulans* reduce abdominal pain and diarrhea in IBS. Symbiotic tablet Lactol containing 1.5×10^8 - *Bacillus Coagulans* + *B. subtilis* Spores and 100mg of FOS (Fructooligosaccharide), a prebiotic was administered for 85 adult suffering from IBS for 12 weeks. The diarrheal frequency increased with score of 5 in patients of control group (without symbiotic tablet) while it decreased with score of 2 in symbiotic tablet group [12].

➤ Inflammatory Bowel Disease (IBD):

Inflammatory bowel disease is a chronic disorder of intestine, relating mainly to ulcerative colitis (inflammation and sores or ulcers along lining of colon and rectum) and Crohn's disease (inflammation of lining as well deeper layers of digestive tract) characterized by intestinal symptoms including abdominal pain, fever, weight loss, anaemia, blood in stool, reduced appetite, arthritic pain and impairment of liver function. It is caused in old age, and in those who smoke and take more and heavy doses of anti-inflammatory medications. *Bacillus coagulans* (GBI-30) in tablet form was given to 61 IBD patients for 4-weeks. The subjects in probiotic group showed significant improvements in IBD with reduction in abdominal pain [13].

➤ Anti-*H. pylori* antibiotic therapy:

Helicobacter pylori (*H. pylori*), a curved or spiral-shaped, flagellated, microaerophilic, Gram-negative bacteria. *H. pylori* causes chronic inflammation (gastritis) by invading the lining of the stomach and producing cytotoxin thus can lead to ulcer formation. The main symptoms include burning pain in abdomen, nausea, loss of appetite, burping, bloating, weight loss. Most recommended treatment for eradication of *H. pylori* is standard triple therapy of omeprazole (proton pump inhibitor), amoxicillin (β -lactam AB), clarithromycin (macrolide AB). *Bacillus clausii* reduced side-effects related to anti-*H. pylori* antibiotic therapy. *Bacillus clausii* (2×10^9 spores) containing tablets given to 120 patients suffering from symptoms of antibiotic therapy for *H. pylori* for 14 days treatment. Incidences of nausea, diarrhea and epigastric pain in patients treated with *B. clausii* were significantly reduced [14].

Safety of Probiotic *Bacillus* spp.

Bacillus subtilis is used for preparation of East Asian fermented foods such as Natto (traditional Japanese food made by soybeans). *Bacillus* strains either as single or mixed with *Lactobacillus* strains are used as an alternative to conventional antibiotics. Risk of transferring antibiotic resistance genes of microflora to gut of humans and animals is possible. Enterogermina contains spores of four strains of *Bacillus clausii* which are resistant towards chloramphenicol and tetracycline. Medicinal purpose *Bacillus clausii* of 2×10^9 cfu/ml along with antibiotics used in treatment of infantile diarrhea.

III CONCLUSION

Species of *Bacillus* probiotics possess excellent health promoting record. *Bacillus* spp are spore former, heat resistant, acid and bile tolerance having antibiotic resistance. They help in biofilm formation, maintains homeostasis of intestine. Some of the species of *Bacillus* produce antimicrobial compounds like Subtilin, Surfactin. Probiotic natured *Bacillus* spp. prevent Irritable Bowel Syndrome, Inflammatory Bowel Disease and Anti - *H. pylori* antibiotic therapy and therapeutic benefits need complete establishment.

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