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# Enzymatic Screening and Antimicrobial Activity of Various Probiotic Bacteria

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**Abstract:** Subtilis microbe is mostly found in soil and produces proteases on nitrogen and carbon-containing sources and increases the fertility rate by degrading nitrogenous organic materials.The pre-sent study was aimed to develop hyper producing mutant strain of *Bacillus subtilis* for the production of proteases, to improve the process variables by the response surface methodology. The Enzyme produced by retained strains have optimum activity at high temperature. *B. subtilis* is considered a benign organism as it does not possess traits that cause disease. It is not considered pathogenic or toxigenic to humans, animals, or plants. The potential risk associated with the use of this bacterium in fermentation facilities is low. The identity of the selected strains was established on the basis of the morphological, biochemical characteristics and phylogenetic position as determined by 16S Ribosomal DNA gene sequencing.

**Keywords:** Protease, rDNA gene, *Bacillus subtilis*, Probiotics, Process

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## Introduction

Probiotics are made up of good bacteria that helps keep the body healthy and working well. This good bacteria helps in many ways, including fighting off bad bacteria when there is too much of it. They can be found in yogurt and other fermented foods, dietary supplements, and beauty products. The main job of probiotics, or good bacteria, is to maintain a healthy balance in the body.

There are several potential health or nutritional benefits possible from some species of lactic acid bacteria. Among these are: improved nutritional value of food, control of intestinal infections, improved digestion of lactose, control of some types of cancer, and control of serum cholesterol levels. Good bacteria work to fight off the bad bacteria and restore the balance within the body. Probiotics are the living microorganisms that are administered/consumed in ample amount which produces beneficial effects on the host. Lactic Acid Bacteria (LABs) are considered to be the utmost studied probiotic microorganisms because of their dynamic nutritional value; confer

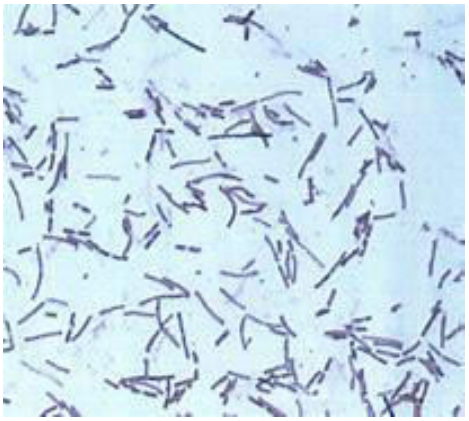

Fig. 1: *Bacillus subtilis*.

Fig. 2: *Lactobacillus.*

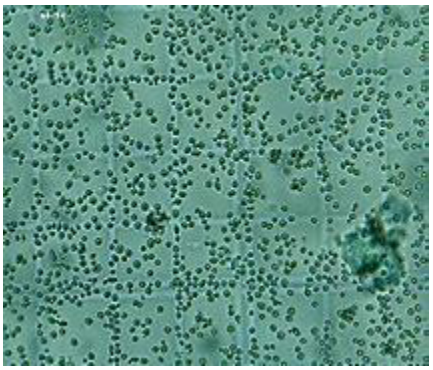

Fig. 3: *Boulardii lactobacillus.*

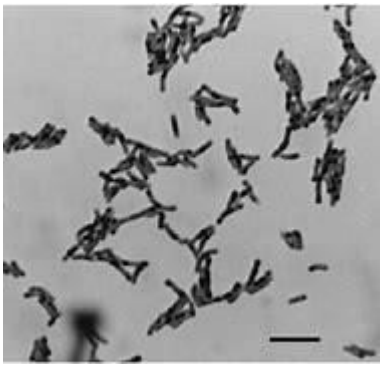

Fig. 4: *Bifidobacterium.*

several health benefits, modulating host immune responses, inhibiting the growth of the food-borne and other harmful pathogen. LABs are commonly present in fermented milk products, fermented foods, and beverages. However, for the assortment of probiotic LAB strains for commercialization, a number of aspects have to be measured which includes: Safety, Stability and Feasibility, Functional and Technological aspects, and proper Quality control. Hence, the current review comprehends the scenario of LABs and its Probiotic characteristics, selection criteria and their beneficial impacts on Human health along with the present guidelines and code of practice. Good bacteria keeps the body healthy by supporting immune function and controlling inflammation.

Certain types of good bacteria can also help the body to digest food, keep bad bacteria from getting out of control, help support the cells that line the gut to prevent bad bacteria that may have entered (through food or drinks) from entering your blood and breakdown and absorb medications. Yogurt is one of the best sources of probiotics, the friendly bacteria that can improve health. Bananas are beneficial for the gut and contain naturally occurring fibers that help increase good bacteria and reduce bloating. The most common are bacteria that belong to groups called *Lactobacillus* and *Bifidobacterium*. The antimicrobial compounds produced by the LAB, interact with the cell membranes of the harmful pathogens (*Listeria monocytogens*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Helicobacter pylori*, *Candida albicans*, etc.) and inhibit their growth by intracellular acidification (lowering the pH) and denaturing the proteins of the membrane which leads to the dysfunction of

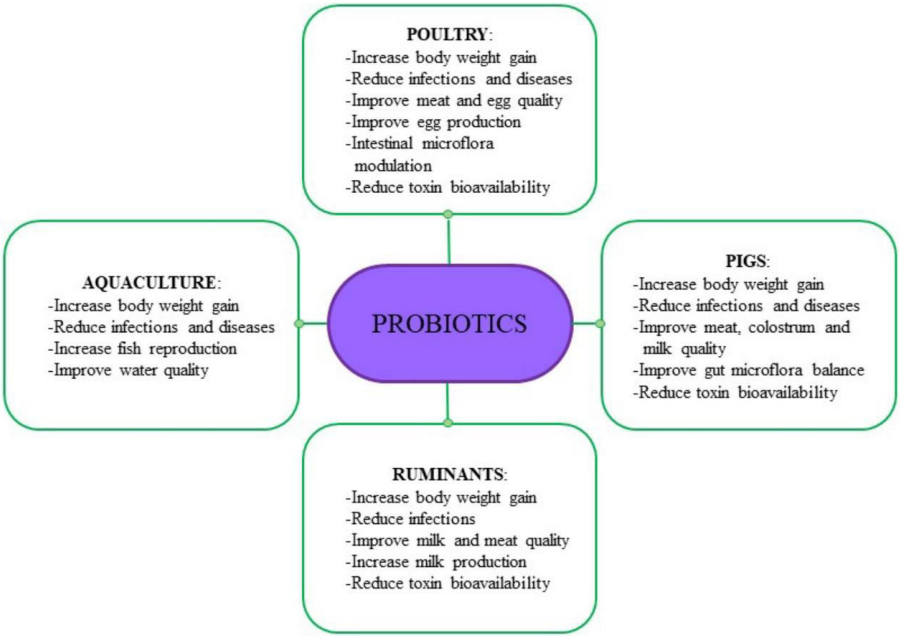


Fig. 5: Potential applications of probiotics in various fields.

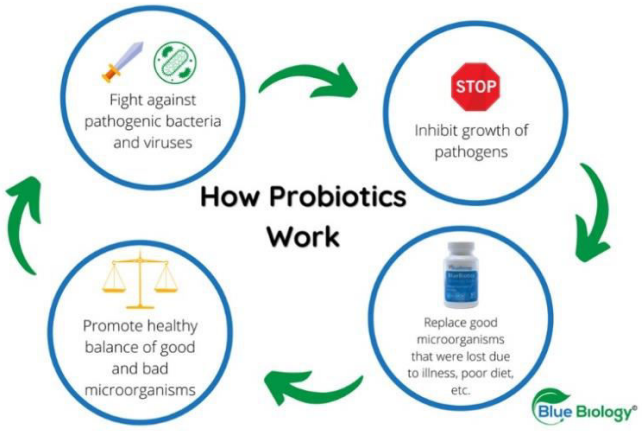


Fig. 6: Mechanism of probiotics.

the membrane permeability. This ability of LAB led to its use as a starter culture in many food fermentation processes because it protects the lifespan of many foods by inhibiting the growth of other harmful pathogens. Other bacteria may also be used as probiotics, and so may yeasts such as *Saccharomyces boulardii*. *Lactobacillus* GG may help prevent or treat infectious diarrhea in children and adults. Probiotics are most effective in treating Rotavirus in children and Campylobacter infections in adults. Diarrhea in children can be serious. *Bifidobacteria* help modulate the gut microbiota, prevent inflammation and protect from many diseases, including colorectal cancer, enteric infections (of the intestines) and diarrhoea, inflammatory bowel diseases (Crohn's ulcerative colitis), and even depression.

*Antimicrobial activity*:

It has been well documented that probiotic LAB shows very good anti-bacterial, anti-fungal and anti-viral properties by producing numerous anti-microbial compounds which include lactic acid, acetic acid, propionic acid, alcohol, and diacetyl. These antimicrobial compounds produced by the LAB, interacts with the cell membranes of the harmful pathogens and inhibits their growth by

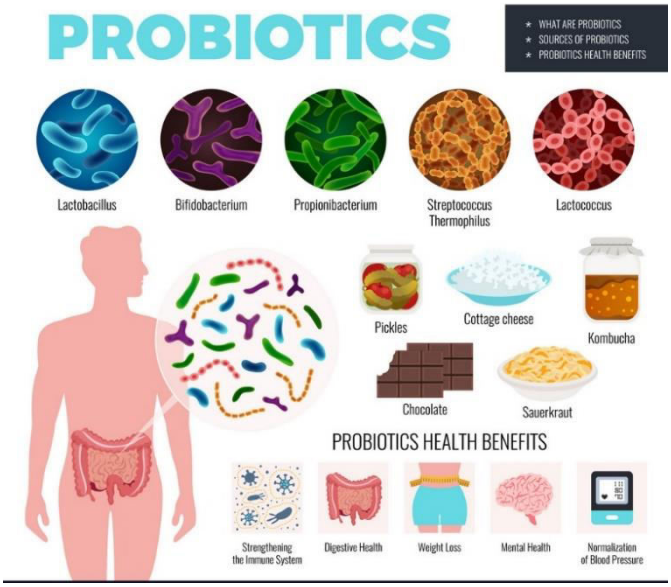


Fig. 7: Role of probiotics in human health.

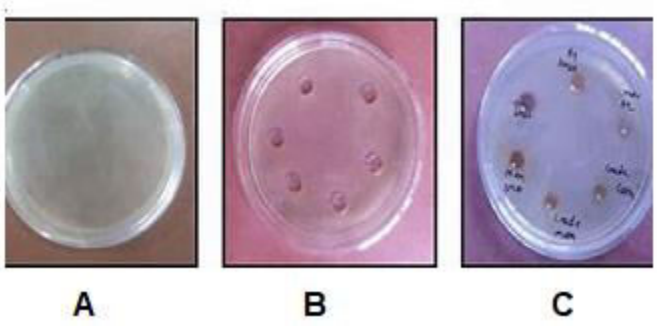


Fig. 8: Antibiotic Resistance Pattern and Stability/ Transferability.

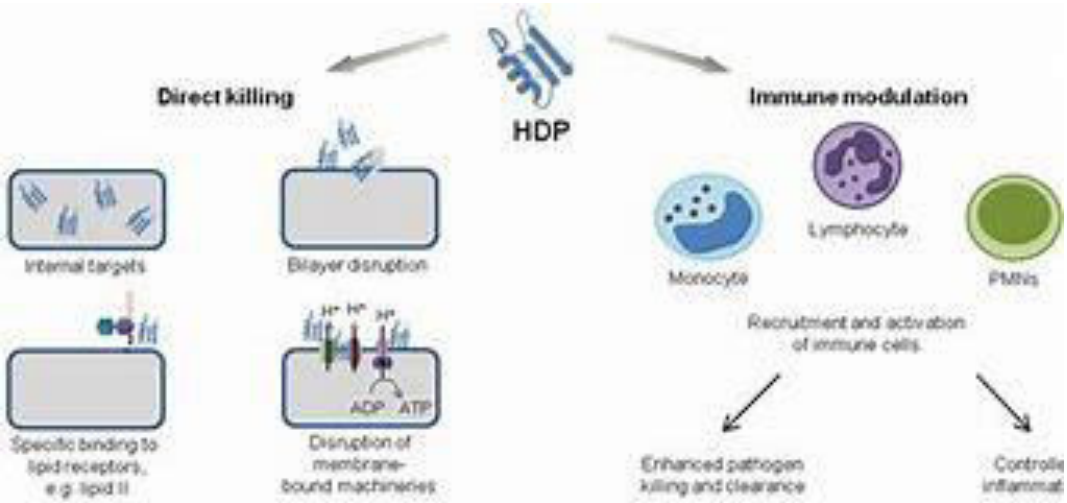


Fig. 9: Immune response of the probiotics.

intracellular acidification (lowering the pH) and denaturing the proteins of the membrane which leads to the dysfunction of the membrane permeability. In addition to these antimicrobial compounds, LAB also produces Bacteriocins which are ribosomally synthesized proteinaceous toxins which inhibit the growth of similar or closely related bacteria by making pores in the membrane of the pathogenic bacteria and thus, disrupting the permeability of the membrane

*Determining the Antibiotic Susceptibility Test with The commonly used antibiotics for each LAB strains*:

- Using standard PCR (Polymerase Chain Reaction) method;
- Determining the expression of the target gene by using Real-Time Quantitative PCR (RTqPCR);
- Using Genome sequencing and Microarray methods.

*PCR Kits for Microbiology*:

There are two broad types of detection method: End-point PCR detection takes place, as the name suggests, when the amplification process is complete. Typically, agarose gel electrophoresis, followed by staining with fluorescent ethidium bromide is used to detect the amplified DNA fragments.

There are different *in vitro* methods for determining the antimicrobial activity of a substance. In the case of probiotics, it is possible to determine a direct antagonism between a probiotic culture and that of a pathogenic strain or to determine the antimicrobial activity of a probiotic extract (planktonic cells) (Fijan, 2016). When the purpose of the analysis is merely to discover the antagonism of one microorganism in relation to another, then microbial antagonism assays on solid media are most appropriate (Tagg *et al*., 1976, Balouiri *et al*., 2016). This approach involves the detection of growth inhibition of an indicator strain caused by the test culture. In this section, we make a critical analysis of the main methods currently available for *in vitro* evaluation of the antimicrobial activity of probiotics. The Agar Spot Test was described by several authors (Tagg *et al.*, 1976, Tharmaraj and Shah, 2009, Choi and Chang, 2015, Macaluso *et al*., 2016), with several modifications over time. We describe here the variation of this method that is mostly indicated to determine the antimicrobial activity of probiotics. There are two variations of this method that are commonly used, namely: simultaneous (or direct) and deferred antagonism. In the direct assay, the test and indicator cultures are grown simultaneously, and the demonstration of antagonism depends on the release of a diffusible inhibitor at the beginning of the test culture growth (Tagg *et al*., 1976). In deferred antagonism, the probiotic microorganism under test is grown on agar media for a certain period and then inactivated; next, an overlap of the indicator strain is placed on the surface of the molten agar. This method is considered more sensitive and allows an independent variation of time and incubation conditions of test and indicator cultures (Tagg *et al*., 1976). After incubation, the antimicrobial activity is expressed either as inhibition zone (mm) or as arbitrary units (AU/ml). The Agar Well Diffusion assay can be used to determine the antagonistic effects of cell-free supernatants. Different nutrients, selective or differential media, are prepared. The plates are inoculated with the indicator microorganism. Subsequently, 6-mm or 7-mm wells are prepared in each plate. The supernatant of the probiotic microorganism is centrifuged and diluted in aliquots at different concentrations and then pipetted into the wells. After incubation, the antimicrobial activity is expressed as an inhibition zone or as arbitrary units (AU/ml) (Tagg *et al*., 1976, Parente *et al*., 1995). We do not recommend using the disk diffusion method for this purpose because of standardization issues due to variations between the viscosity of the test substance and the physical differences of the discs (Hoelzer *et al.*, 2011, Balouiri *et al*., 2016).

Spot test analysis, or spot test is a chemical test, a simple and efficient technique where analytic assays are executed in only one, or a few

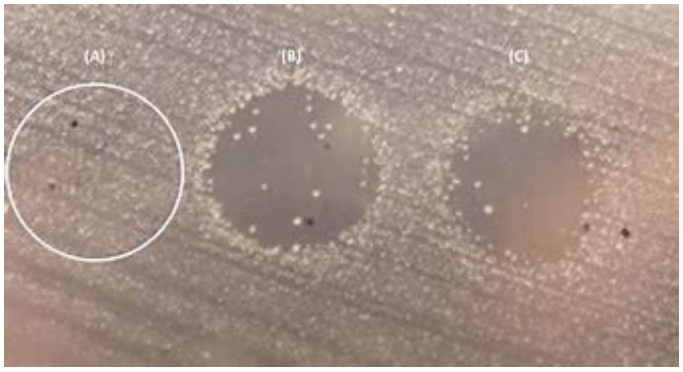


Fig. 10: Spot test analysis.

drops, of a chemical solution, preferably on a piece of filter paper**,** without using any sophisticated instrumentation.

Synbiotic yoghurt is fermented with a probiotic starter culture and prebiotics, both of which are considered to provide health benefits. It has been reported that new lactulose-derived prebiotic oligosaccharides (OsLu) are chemically stable throughout fermentation and cold storage. This study looked at how physical qualities change when OsLu and lactulose are added in proportions acceptable for fermented milk consumption (2% and 4%). After prebiotic supplementation, 66 swirled yoghurts were prepared from skimmed milk (0.1% fat) as high-heated (95 °C, 256 s). Protein content (4.5-5.2%) was determined using skim milk powder (SMP) or total milk protein (TMP). Lactose and maltodextrin were inert carbohydrate controls. OsLu or lactulose significantly reduced fermentation regardless of concentration, indicating substrate inhibition. Dairy foods, particularly those derived from cattle, are the most common carriers of probiotics and bacteria that produce conjugated linoleic acid (CLA). The current study discovered probiotic bacteria in raw Lahaul valley cow milk that can produce cis-9, trans-11 CLA isomers, as well as antioxidant properties and food formulation abilities. Preliminary probiotic screening yielded 34 strains, 21 of which qualified for all probiotic properties and passed safety tests. The CLA production (16.87-28.01 g/ml) of the seven most statistically promising probiotics was studied further, and GC-MS corroborated cis-9, trans-11 CLA isomer formation. To establish genomic evidence for safety study, whole genome sequencing of *Enterococcus faecalis* LJM:05 was undertaken, resulting in the elimination of antibiotic-resistance and virulence genes (lower identity scores).

## Conclusion

This study examined the antibacterial activity and enzymatic screening of several probiotic bacteria, revealing useful information about their functional characteristics and possible uses. Our knowledge of the mechanisms by which probiotic bacteria exert their positive benefits and their potential as natural antibacterial agents is aided by the findings. The enzymes that probiotic bacteria make could be recognised and described using enzymatic screening. These enzymes are essential for the utilisation and degradation of nutrients as well as the synthesis of antibacterial substances. The creation of specialised probiotic formulations with improved functional qualities can result from a better understanding of the enzymatic activities of probiotic bacteria.

The promise of probiotic bacteria as a natural substitute for synthetic antimicrobial drugs was highlighted by research into their antibacterial activity against a variety of diseases. The ability of probiotic bacteria to compete for resources and adhesion sites, as well as their production of

antimicrobial compounds, all contribute to their inhibitory effects on pathogenic microbes. Probiotic bacteria's antibacterial properties offer hope for the creation of cutting-edge medicinal approaches to treat microbial infections.

It is significant to note that probiotic bacteria's antibacterial activity can vary depending on the strain and the particular infection being attacked. To clarify the processes underlying the antimicrobial actions and to improve the settings for optimal efficacy, more study is required.

Overall, this work broadens our understanding of probiotic microorganisms and the prospective uses for them in the food and pharmaceutical industries, among other areas. The findings support continuing initiatives to improve human health and create sustainable, organic antibacterial methods by utilizing the advantageous traits of probiotic microorganisms. Probiotic bacteria will continue to be effectively used in a variety of applications as more research is done into their enzymatic abilities and antibacterial potential.

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