Chapter

Probiotic Characteristics and Health Benefits of the Yogurt Bacterium *Lactobacillus delbrueckii* sp. *bulgaricus*

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Abstract

Yogurt is a good source of several micronutrients and has played an important role in human nutrition. Consumption of yogurt has been shown to promote health benefits due to the presence of live bacteria. A number of human studies have demonstrated that yogurt contains viable bacteria, and especially *L. bulgaricus*, improve the health of the host and thus qualifies as a bona fide probiotic in its own right. In this chapter, we review the literature covering attributes of the yogurt bacterium *L. bulgaricus* that confirm its probiotic bacterial characteristics.

Keywords: yogurt, *L. bulgaricus*, probiotic, health benefits

1. Introduction

Yogurt, defined as the product of milk fermentation by *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, has a long history of beneficial impact on the well-being of humans. As starter cultures for yogurt production, lactic acid bacteria (LAB) display symbiotic relations during their growth in milk medium. To meet the National Yogurt Association's criteria for "live and active culture yogurt," the final yogurt product must contain live LAB in amounts $\geq 10^8$ CFU/g at the time of manufacture, and the cultures must remain active up to the end of the stated shelf life. These live cultures are also considered to be probiotics because the cultures provide health benefits to the host when consumed in sufficient amounts [1, 2].

Elie Metchnikoff can be credited as the progenitor of what has now become a highly profitable industry, probiotics. He theorized that health could be improved and senility delayed by colonizing the gut with the host-friendly bacteria found in yogurt. In a market report published by Allied Market Research, the global probiotics market is expected to garner $57.4 billion by 2022, registering a compound annual growth rate (CAGR) of 7.7% during the period 2016–2022. Asia-Pacific was the dominant probiotics market and is expected to be the leading contributor in global revenue due to its high level of adoption of probiotic based food and beverages.

The definition of probiotics has evolved over the years due to some gray areas regarding the characteristics of a typical probiotic. The internationally endorsed definition of probiotics is "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [3]. Modulation of the host's immune system and promotion of host defense are the most commonly supported benefits of probiotics consumption [3]. Most probiotics are lactic acid bacteria *Lactobacillus* sp., *Bifidobacterium* sp., and *Enterococcus* sp.; *Escherichia coli* strain Nissle 1917; the yeast *Saccharomyces boulardii*; some enterococci (*Enterococcus faecium* SF68); *Bacillus* sp., and *Clostridium butyricum* [4, 5, 54].

2. Origin of *Lactobacillus bulgaricus*

Elie Metchnikoff was a Russian biologist and Nobel Prize laureate who attributed the longevity of Bulgarians who were regular consumers of yogurt to the lactobacilli bacteria of yogurt. Metchnikoff's claims attracted wide attention to yogurt at that time. However, it was the Bulgarian graduate student Grigoroff [6] who first isolated and characterized this lactobacilli bacteria from the starter used in producing Kiselo Mlyako (Bulgarian Yogurt). Grigoroff named the bacterium '*Bacillus A*' or what is now recognized as *Lactobacillus bulgaricus* according to the Bergey's classification of bacteria. The origin and natural habitat of commercial *L. bulgaricus* strains may not have a definite answer despite its strong Bulgarian ties as countries like China, Mongolia, Russia, and Turkey also enjoy a long history of naturally fermented dairy products. A study by Song et al. [7] highlighted the uniqueness of *L bulgaricus* strains isolated from traditionally fermented milk products from some of the aforementioned countries.

Moreover, it appears *L. bulgaricus* is on a continuing evolutionary journey as it has adapted itself from a plant source to milk-rich environment [8]. Michaylova et al. [9] have been able to isolate and characterize *L. bulgaricus* from certain plant species (*Cornus mas*) obtained from four regions in Bulgaria. Yılmaz et al. [10] isolated *L. bulgaricus* from raw milk samples collected from different parts of Turkey, while *L. bulgaricus* was one of the isolates from raw milk samples obtained from four races of Algerian goats [11]. A study by Song et al. [7] illustrates the diversity of *L. bulgaricus* and the fact that it might not be an exclusive preserve of Bulgaria.

2.1 *L. bulgaricus* as a probiotic

Elie Metchnikoff is regarded in some quarters as the grandfather of probiotics because of the profound observation he made at the beginning of the twentieth century, a time when the function of the gut flora was completely alien and unknown. Elie Metchnikoff realized that there was a link between regular consumption of lactic acid bacteria in fermented milk products to longevity and enhanced health in a certain group of Bulgarian people. He attributed this beneficial effect to the colonization and implantation of the Bulgarian bacillus which is now characterized as *L. bulgaricus.* Elie Metchnikoff believed that aging and diseases were caused by putrefaction of protein in the bowel by intestinal bacteria and that LAB were capable of inhibiting the growth of these putrefactive bacteria. He was so committed to the fact that fermented products could beneficially alter the microflora of the gut and prolong life that he drank sour milk fermented by lactic acid bacteria every day until his death [12–14].

L. bulgaricus has all the attributes of standard probiotic bacteria. It is crucial for probiotic strains to be able to colonize the intestine and survive passage through the upper gastrointestinal tract (GI) in order confer health benefits [15].

However, there are doubts about the adhesion and survival of *L. bulgaricus* after passage through the human gut. For example, *L. bulgaricus* are not native flora of mammals plus they do not have enough bile salt hydrolase genes and cannot synthesize mucin-binding proteins, all of which are important for survival in the GI [8]. However, regular consumption of yogurt fermented by *L. bulgaricus* may facilitate the colonization of these bacteria in the gut. Elli et al. [16] investigated the recovery of viable *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* from the fecal samples of 20 healthy volunteers who were fed commercial yogurt for 1 week and found these bacteria present in the samples, suggesting that the bacteria, can survive transit in the gastrointestinal tract. Similarly, Mater et al. [17] had earlier established the survival of *L. bulgaricus* and *Streptococcus thermophilus* after passage through the gastrointestinal tract. A total of 37 out of 39 stool samples retrieved from 13 healthy subjects over a 12-day period yogurt intake contained viable *L. bulgaricus*. An encapsulated mixture of *L. bulgaricus* and *S. thermophilus* in chitosan and sodium alginate also survived in a simulated gastrointestinal tract [18].

In a study involving 61 elderly volunteers who were randomly assigned to receive either placebo or probiotics, Moro-Garcia et al. [19] evaluated the immunomodulatory capacity of *Lactobacillus delbrueckii* subsp. *bulgaricus* 848, strain isolated from a region of Bulgaria (Stara Planina) known for the longevity of its population [20]. A positive effect on the immune system was recorded in that study. Blood samples were taken at the beginning of the study and again after 3 and 6 months for the researcher to characterize the cell subpopulation, measured cytokines, quantified T cell receptor excision circles (TREC), and determined human β-defensin-2 (hBD-2) concentrations and human cytomegalovirus (CMV). The group that received *Lactobacillus delbrueckii* subsp. *bulgaricus* 848 had an increase in the percentage of NK cells, an improvement in the parameters defining the immune risk profile (IRP), and an increase in T cell subsets that are less differentiated. There was also a reduced concentration of pro-inflammatory cytokine interleukin-8 but an increase in the antimicrobial peptide hBD-2. In a similar study comparing the consumption of yogurt with milk in elderly subjects, yogurt fermented with *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1, a polysaccharide-producing lactic acid bacterial strain, was more effective in reducing the risk of catching the common cold. *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1 has been proven to have better effects on the immune system than other lactic acid bacteria. For example, the cell body and the immunostimulatory polysaccharides of these bacteria were identified responsible for the activation of biological defense mechanisms against pathogens such as viruses [21]. A recent study by Yamamoto et al. [22] corroborated the immunomodulatory effect of *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1. Thirty-seven elderly persons residing in a single nursing had their immunoglobulin A (IgA) levels increased after ingesting 112 g of the yogurt every morning for 12 weeks. IgA plays a critical role in the defense of mucous membranes against foreign antigens and pathogens, directly neutralizing the infectivity of pathogens and their toxins.

Today's health-conscious consumers are increasingly aware of food content which is a driving force in the market for organic foods. For example, some consumers are motivated to take extra steps to avoid foods that contain chemical preservatives. Despite the fact that chemical preservatives are generally regarded as safe, the long-term side effects are unknown. Focus is thus being shifted to bio-preservation as an alternative. The use of LAB strains as a probiotic and bioprotective culture in fermented products has been widely studied. LAB have a major potential for use in biopreservation to extend shelf

life and enhance the safety of foods [23]. For example, metabolites and antimicrobial products obtained from LAB have inhibitory effect against spoilage microorganisms. Most importantly, LAB produce organic acid such as lactic acid that reduced the pH of the food, thereby inhibiting the growth of other microflora [24, 25]. In the case of yogurt, drop in pH alters the yogurt environment resulting in an unfavorable medium for the development of some pathogens and spoilage microorganisms [26]. For example, strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* isolated from Turkish homemade yogurt had inhibitory effects on *Escherichia coli* and *Listeria monocytogenes* [27].

The use of antimicrobial peptides such as bacteriocins that are produced by LAB will help to significantly minimize the use of chemical preservatives and could thus be used in hurdle technology to produce a more naturally preserved food. LAB produce bacteriocins, bioactive peptides or proteins, and bacteriocin-like inhibitory substances that are antimicrobial compounds that possess bacteriocin capacities requisites but have not been characterized for their amino acid sequence [24, 25]. Some *L. bulgaricus* strains which were isolated from yogurts had an antibacterial effect on *Vibrio cholerae* and *E. coli* due to significant bacteriocin production [28]. A study by Boyanova et al. [29] suggested that the bacteriocin-like inhibitory effects of GLB strains of *L. bulgaricus* could be valuable in the control of *Helicobacter pylori* infections. Clinical benefits were also reported in Thailand, where the addition of *L. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* either before or before and after a 1-week tailored triple therapy regimen significantly improved eradication rates in *H. pylori infection* treatment [30].

Another area of diet concern in which probiotics come into play is the issue of lead (Pb) poisoning. Food and supplements or methods of food preparation may be a source of lead exposure that can have devastating effects on human health, and it remains a public health concern. *L. bulgaricus* KLDS1.0207 that has been isolated from traditional dairy products in Sinkiang Province, China has been evaluated for its protective effects against acute lead toxicity in mice. The high Pb-binding ability and high resistance to Pb in *L. bulgaricus* KLDS1.0207 offered protective effects from acute Pb toxicity in mice. The results in vivo demonstrated that this particular strain of *L. bulgaricus* can relieve renal pathological damage, reduce mortality rates, and enhance the antioxidant index in the liver and kidney making it a potential probiotic against lead toxicity [31, 32].

Another claimed health benefit linked to probiotics is an improvement in lactose metabolism ([33, 34]). It is widely agreed that fermented milk products such as yogurt can help with lactose digestion in lactose malabsorbers and therefore can be well tolerated by most lactose-intolerant subjects. Yogurt preparation using the traditional *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* are even more effective due to their higher β-galactosidase activity. Lactose intolerance is a β-galactosidase deficiency resulting in the inability to digest lactose into the monosaccharides glucose and galactose. People with lactose intolerance develop diarrhea, abdominal discomfort, and flatulence after consumption of milk or milk products. Numerous studies have shown better lactose digestion and consequently less hydrogen exhalation in lactose malabsorbers who consumed yogurt with live cultures rather than with milk or pasteurized yogurt [35, 36].

All of these documented benefits and characteristics of probiotics, in general, provide an equally compelling argument for the effectiveness of *L. bulgaricus* as a probiotic. Yogurt remains one of the most important vehicles for the delivery of probiotic bacteria.

Table 1 lists *L. bulgaricus* strains that are beneficial for a range of health-related issues.

***L. bulgaricus* strain**	**Probiotic activity**	**References**
RTF	Antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas fragi*, *Micrococcus flavus*	[37]
7994	Inhibitory effect on *Achromobacter liquefaciens*, *S. aureus*, *P. fragi*	[38]
848	Immunomodulatory function	[20]
OLL1073R-1	Reduces risk of catching common cold, anti-influenza virus activity	[21, 22, 39]
KLDS1-0207	Protects against lead toxicity	[31, 32]
BB18	Production of bacteriocin (bulgaricin BB18); bactericidal against *Helicobacter pylori*	[40]
ATCC 11 842, LBL-23, LBL-12, LBL-22, LBL-6, LBL-10, LBL-13, LBL-83, LBL-42, LBL-9, LBL-11	Inhibitory action against periodontal pathogen; *Aggregatibacter actinomycetemcomitans*	[41]
B-30892	Inhibits *Clostridium difficile*-mediated cytotoxicity on Caco2 cells	[42]
Commercial yogurt isolate	Inhibitory action against periodontal pathogens; *Porphyromonas gingivalis*, *A. actinomycetemcomitans*, and *Prevotella nigrescens*	[43]
Commercial yogurt isolate	Bacteriocin production inhibitory against *Vibrio cholerae* and *E. coli*	[28]
Commercial yogurt isolate	Inhibitory effect on *E. coli* O157:H7	[44]
NCTC 12197 Tat, DSMZ 20080 T	Inhibitory effects on *Salmonella* spp., *Pseudomonas aeruginosa*, *E. coli*, *S. aureus*	[45]
TLB06FT	Antibacterial activity against *E. coli*, *S. aureus*, *P. aeruginosa*, *Listeria monocytogenes*	[46]
CRL 454	Aids digestion of allergenic β-lactoglobulin	[47]
CRL 871	Production of folate; an alternative to folic acid fortification	[48]
761 N	Free radical scavenging ability; antiviral ability	[49]
GLB	Antimicrobial; control of *H. pylori*	[29]
GB N1 (48)	Hypolipidemic and protective cardiovascular effect	Doncheva et al. (2002)
D6R; PTCC 1332	Inhibitory effects on *S. aureus* and *E. coli*	[27, 50]
F5R	Inhibitory effects on *Bacillus coagulans*, *B. cereus*, *P. fluorescens*, *K. pneumoniae*, *L. monocytogenes*, *S. aureus* and *E. coli*	[27]
DSM 20081	Inhibitory effect on *E. coli*	[51, 52]
DWT1	Inhibits tumor growth	[53]

Table 1.
Probiotic strains of Lactobacillus bulgaricus.

3. Conclusion

Because of the documented health benefits conferred by *L. bulgaricus*, consumer demand for yogurt and yogurt-related products has recently become the fastest growing dairy category in the global market. Our literature review showed that *L. bulgaricus* clearly qualifies as a probiotic in its own right. This growing popularity is not surprising in light of the fact that Nobel Laureate Metchnikoff linked the health and longevity of the Bulgarian people to their high consumption of yogurt containing *L. bulgaricus*. Consequently, increased yogurt intake should be a promising addition to a healthy dietary regimen that led to health promotion and well-being. Future work should be directed to understand the metabolites produced by *L. bulgaricus* and their health benefits. With a more comprehensive understanding on the functional properties of *L. bulgaricus*, we could advocate the importance of yogurt consumption and its impact on our well-being.

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