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Effects of Probiotics on Cholesterol Metabolism: Biochemical Mechanisms and Clinical Potential

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ABSTRACT:
In recent years, the increasing prevalence of cardiovascular diseases has accelerated the search for natural and effective strategies to regulate cholesterol metabolism. In this context, the hypocholesterolemic potential of probiotic microorganisms has attracted growing scientific attention. Probiotics are live microorganisms that, when consumed in adequate amounts, provide health benefits to the host. They are primarily composed of species from the *Lactobacillus* and *Bifidobacterium* genera.
Numerous studies have demonstrated the ability of probiotic strains to affect cholesterol metabolism. They do so through a variety of underlying biochemical mechanisms. These include bile salt hydrolase activity for bile salt deconjugation, cholesterol assimilation and degradation, precipitation with secondary bile acids, binding to the microbial cell wall or membrane, and the effects of short-chain fatty acids produced during prebiotic fermentation. Several studies have reported that specific strains—particularly *Lacticaseibacillus casei*, *Lactobacillus acidophilus*, and *Lactiplantibacillus plantarum*—can effectively exert these effects. However, as most mechanisms have been investigated under in vitro conditions, their actual efficacy under physiological settings may be limited. Nonetheless, several human clinical trials have reported cholesterol-lowering effects of specific probiotic strains, although findings remain inconsistent and strain-dependent.
This review examines the impact of probiotics on cholesterol metabolism in detail and analyzes the relevant mechanisms based on findings in the literature. The current evidence suggests that probiotics can exert cholesterol-lowering effects in a strain-specific manner. These results suggest that probiotics hold potential as adjunct therapeutic agents for hypercholesterolemia, supporting their possible role in cardiovascular risk reduction. Nevertheless, further controlled and long-term clinical studies are needed to confirm these effects and support their potential role in therapeutic administrations.
Keywords: *Probiotics; cholesterol metabolism; hypercholesterolemia; hypocholesterolemia*

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INTRODUCTION

In the literature, numerous studies have examined the cholesterol-lowering capacity of probiotic bacteria (Al-Muzafar and Amin, 2017; Al-Sheraji et al., 2015; Keleszade et al., 2022; Li et al., 2022). However, the metabolic processes underlying this ability are mostly explained by in vitro studies. The most notable of these processes include bile salt hydrolase (BSH) activity, which enables the deconjugation of bile salts, the assimilation of cholesterol present in the media by probiotic bacteria, and the precipitation of cholesterol by secondary bile acids resulting from deconjugation. Additionally, cholesterol catabolism by enzymes present in some probiotics, the binding of cholesterol to the cell wall, and the incorporation of cholesterol into the bacterial cell membrane are recognized as important mechanisms. Short-chain fatty acids (SCFAs), produced during prebiotic fermentation, also play a significant role (Ooi and

Liong, 2010).

Wang et al. (2018) conducted a meta-analysis of randomized controlled trials to evaluate the effects of products containing either a specific probiotic strain or a mixture of strains on cholesterol parameters. They concluded that groups receiving probiotic supplementation showed a significant decrease in serum total cholesterol (TC) compared to controls, regardless of the probiotic form used. Subgroup analyses revealed that single strains such as Lactiplantibacillus plantarum, Bifidobacterium lactis, and Lactobacillus acidophilus were more effective than commercial mixtures such as VSL#3 (Wang et al., 2018). Another meta-analysis by Sharma et al. (2016) compared probiotic-treated groups with controls. This study indicated that serum TC and LDL cholesterol (LDL-C) levels decreased significantly by an average of 8.40 mg/dl and 6.63 mg/dl, respectively. However, no significant change was observed in HDL cholesterol (HDL-C) or triglyceride (TG) levels (Sharma et al., 2016).

Today, in addition to commercial probiotic mixtures, most probiotic strains isolated from foods or different parts of the human body belong to the Lactobacillus and Bifidobacterium genera. Among these isolates, species such as Lactobacillus johnsonii, Lactobacillus acidophilus, Lacticaseibacillus rhamnosus, Lactobacillus gasseri, Limosilactobacillus reuteri, and Lacticaseibacillus casei are frequently reported (Heller, 2001).

It has been observed that probiotic bacteria—whether applied alone or in mixtures—can produce hypocholesterolemic effects in both in vitro and in vivo environments (Anandharaj et al., 2020). However, despite these promising findings, outcomes across clinical trials remain variable. Such differences may be related to strain-specific effects, study heterogeneity, small sample sizes, or short intervention periods. These gaps underline the need for further well-designed, long-term studies to clarify the role of probiotics in cholesterol management. The aim of this review is to provide a comprehensive analysis of the mechanisms through which probiotic microorganisms exert cholesterol-lowering effects, while also evaluating their clinical potential as adjunct strategies for cholesterol management and cardiovascular risk reduction.

Mechanism of the Hypocholesterolemic Effects of Probiotics

Bile Salt Hydrolase Enzyme Activity

Bile plays a critical role in the digestion and absorption of fats in the small intestine due to its emulsifying properties. This water-soluble fluid is synthesized in the liver and then concentrated and stored in the gallbladder. During digestion, the gallbladder is stimulated by hormones, and bile is released into the small intestine via various metabolic pathways. Bile contains cholesterol, phospholipids, conjugated bile acids, bile pigments, and certain electrolytes (Begley et al., 2006).

Primary bile acids such as cholic acid and chenodeoxycholic acid have very low solubility in blood circulation. Therefore, their solubility must be increased so that these acids can accumulate in the gallbladder and then be transported to the intestines. To achieve this, primary bile acids are conjugated with glycine or taurine in the liver, converting them into bile salts with high water solubility. Under physiological conditions, bile salts constitute approximately 80% of the organic matter in bile fluid. As a result of enzymatic reactions, these bile salts undergo deconjugation and are converted into secondary bile acids such as glycocholic acid, taurocholic acid, and taurocholic acid (Önür and Beyler, 2001). Thanks to the BSH enzyme possessed by some probiotic microorganisms, bile salts undergo deconjugation, resulting in the formation of secondary bile acids. These secondary compounds have low solubility and their absorption by the small intestine is limited. As a result, most secondary bile acids are excreted from the body via feces. This reduction in bile salts stimulates the body to produce more bile. In this new synthesis process, the body uses its cholesterol reserves to produce the necessary bile salts. Consequently, probiotic strains that possess the BSH enzyme exert a cholesterol-lowering effect through this mechanism (Pereira and Gibson, 2002). Various studies in the literature indicate that one of the underlying mechanisms for the hypocholesterolemic effect of probiotics is the BSH enzyme activity found in certain probiotic strains (Ooi and Liong, 2010).

In an in vitro study conducted by Jones et al. (2004),

it was determined that the *Lactiplantibacillus plantarum 80* (pCBH1) strain, which possesses probiotic properties, has BSH activity. In addition, the researchers applied a microencapsulation method to increase the strain's resistance to environmental stress factors. As a result of this process, it was observed that the bacterium's capacity to deconjugate bile salts increased significantly compared to the live cell form, and consequently, the hypocholesterolemic effect also increased (Jones et al., 2004).

Similarly, Hernández-Gómez et al. (2021) evaluated the BSH enzyme activity of probiotic bacteria isolated from various local cheeses, dairy products, and commercial sources. Specifically, five different strains belonging to the genus *Lactobacillus (Lacticaseibacillus casei Shirota*, *Lactiplantibacillus plantarum 299v*, *Lacticaseibacillus rhamnosus ATCC 53103 (LGG)*, *Lactiplantibacillus plantarum DGIA1*, and *Limosilactobacillus fermentum K73*) BSH activity was found to be positive, and the extent to which these strains deconjugated bile salts *in vitro* was also measured. These analyses revealed that the *Lactiplantibacillus plantarum* DGIA1 strain deconjugated bile salts such as sodium glycocholic acid, sodium glycocholic acid, sodium taurocholate, and sodium taurodeoxycholate at rates of 69%, 100%, 81%, and 92%, respectively. These high deconjugating rates indicate that the strain has a hypocholesterolemic effect (Hernández-Gómez et al., 2021).

Tsai et al. (2014) examined approximately 800 different bacteria isolated from animals and plants for BSH activity under in vitro conditions. As a result of this screening, *Pediococcus acidilactici* NBHK002, *Bifidobacterium adolescentis* NBHK006, and *Lacticaseibacillus rhamnosus* NBHK007 were identified as the three probiotic strains with the highest BSH activity. They evaluated the cholesterol-lowering effects of the probiotic product named “PROBIO-S23,” obtained from the combination of these three strains, in an in vivo model. The experimental animals were fed a high-fat, high-cholesterol (HFHC) diet for 6 weeks and administered low (78 mg/kg body weight (bw)/day), medium (390 mg/kg bw/day), and high doses (1950 mg/kg bw/day) of PROBIO-S23 product was administered daily via oral gavage for the same period. After six weeks of administration, significant decreases in serum TC, TG, and LDL-C levels were observed in all groups receiving the three different doses of probiotic mixture compared to the control group fed the HHF diet; however, no significant changes were detected in HDL-C levels (Tsai et al., 2014).

Most evidence suggests that BSH activity is one of the best-characterized mechanisms for the hypocholesterolemic effects of probiotics, though its impact remains strain-dependent. Various probiotic strains have demonstrated BSH activity and induced significant cholesterol-lowering effects, but the extent of this effect varies across different strains and environmental conditions. While the BSH mechanism has shown strong efficacy in vitro and in animal studies, its direct clinical relevance in humans remains uncertain. Further clinical trials are needed to determine how well this mechanism translates to human physiology, particularly in individuals with varying dietary patterns and health conditions.

Cholesterol Precipitation via Secondary Bile Acids

Probiotics can reduce cholesterol absorption through the precipitation of cholesterol by secondary bile acids, which are formed following the deconjugation of bile salts. Some probiotic strains, possessing bile salt hydrolase (BSH) activity, deconjugate primary bile acids, leading to the production of secondary bile acids. These secondary bile acids, having low solubility, are not readily absorbed in the small intestine, which promotes cholesterol excretion via feces. This process effectively reduces cholesterol levels (Begley et al., 2006; Klaver and Meer, 1993).

Shobrahani and Halami (2016) evaluated the cholesterol-lowering potential of three probiotic strains isolated from raw milk. Although these strains lacked BSH activity, the researchers tested their effects in acidic environments (pH 5.0). When bile fluid was added, cholesterol levels decreased by 18-20%. When conjugated bile acids were included, secondary bile acids formed, and cholesterol levels decreased by 28-31%. This showed that secondary bile acids precipitate cholesterol more effectively in acidic environments (Shobrahani and Halami, 2016).

In another in vitro study, *Lacticaseibacillus paracasei* M3, *Lacticaseibacillus casei* M5, and *Lacticaseibacillus paracasei* M7 were analyzed. *L. casei* M5 had the highest cholesterol precipitation capacity (50.16 µg/mL). This strain also showed the highest BSH activity, supporting the link between BSH activity, secondary bile acid production, and cholesterol precipitation (Bhat and Bajaj, 2020).

Despite these findings, the physiological relevance of this mechanism is debated. Cholesterol precipitation occurs optimally at acidic pH (5.0-6.0), yet the human intestinal lumen is neutral to slightly alkaline (pH ~7.0). Since cholesterol solubility increases at near-neutral pH, precipitation with secondary bile acids may be limited in vivo (Reis et al., 2017).

The ability of secondary bile acids to precipitate cholesterol has been well-documented, particularly in acidic environments. However, the relevance of this mechanism in the human intestine, which operates at a neutral pH, remains unclear. Despite this, studies suggest that probiotics can still contribute to cholesterol reduction through secondary bile acid production and precipitation, particularly in controlled, low pH conditions. Although secondary bile acid-induced cholesterol precipitation has been observed in vitro, its clinical relevance in humans is less certain due to the neutral pH of the human intestine. Further clinical investigation is required to assess whether this mechanism can be effectively leveraged in humans and under what conditions (e.g., dietary factors or gut microbiome composition) this effect might be enhanced.

Binding of Cholesterol to the Cell Membrane

One of the mechanisms involved in the cholesterol-lowering effect of probiotic strains is the binding of cholesterol to the probiotic cell wall. Kimoto et al. (2002) tested seven *Lactococcus* strains under different conditions: live and growing cells, live cells with metabolic activity stopped, and heat-killed cells. The highest cholesterol removal occurred with live and actively multiplying strains. Remarkably, some heat-inactivated strains also retained the ability to bind cholesterol to their cell walls, maintaining part of their cholesterol-lowering effect (Kimoto et al., 2002).

Incorporation of Cholesterol into the Structure of the Cell Membrane

Another mechanism considered to be one of the cholesterol-lowering effects of probiotic bacteria is the uptake of cholesterol present in the media into the cell and its integration into the cell membrane structure (Ooi and Liong, 2010).

Lye et al. (2010a) investigated 15 probiotic strains and found that those with the highest hydrophobicity (*L. acidophilus* ATCC 314, *L. acidophilus* FTCC 0291, *L. delbrueckii* subsp. *bulgaricus* FTCC 0411 and FTDC 1311, and *Lacticaseibacillus casei* ATCC 393) had greater ability to incorporate cholesterol. Cells grown in cholesterol-supplemented media showed significant changes in fatty acid profiles compared to cholesterol-free conditions. Fluorescence anisotropy analyses revealed that cholesterol molecules were incorporated into the phospholipid tails, membrane surfaces, and bilayer structures of probiotic membranes. This integration, although strain-dependent, contributes to cholesterol reduction (Lye et al., 2010a).

Cholesterol Assimilation

Another mechanism contributing to the cholesterol-lowering effects of probiotic bacteria is defined as cholesterol assimilation. This property may vary depending on the strain, and the presence of bile salts is required for the assimilation process to occur (Tomaro-Duchesneau et al., 2014). Some probiotic bacteria can assimilate cholesterol through their cell walls, which have a peptidoglycan structure. Furthermore, the literature reports a positive correlation between the amount of exopolysaccharide produced by these bacteria and their cholesterol assimilation capacity. These strains use bile salts while assimilating cholesterol; in the resynthesis of bile salts, they consume cholesterol from the body's cholesterol pool. This process ultimately results in a hypocholesterolemic effect (Reis et al., 2017).

In an *in vitro* study conducted with different probiotic strains belonging to the *Lactobacillus acidophilus* genus, it was determined that cholesterol assimilation rates varied between 11% and 71% depending on the strain in the presence of

various bile salts. The highest cholesterol assimilation ability was found in the *Lactobacillus acidophilus* ATCC 4356 strain (Lin and Chen, 2000).

Cholesterol Catabolism

Another mechanism found in some probiotic strains that contributes to lowering cholesterol levels in the media is cholesterol catabolism. As a result of this process, a large portion of cholesterol is converted into coprostanol, while a smaller portion is converted into coprostanol (Juste and Gérard, 2021). In addition to cholesterol ingested with food and reaching the small intestine, cholesterol synthesized endogenously and transported via transintestinal cholesterol efflux (i.e., the direct passage of circulating cholesterol into the intestinal lumen) can also be catabolized by certain probiotic strains present in the intestine. The enzyme responsible for this conversion is the cholesterol reductase enzyme found in the structure of these probiotics (Reis et al., 2017).

In an in vitro study using lactic acid bacteria, the cholesterol-lowering effects of five different probiotic strains with the highest level of hydrophobicity were investigated. Intracellular and extracellular cholesterol reductase enzyme activity was detected in all these strains. It was stated that these strains create a hypocholesterolemic effect by converting cholesterol present in the media into coprostanol (Lye et al., 2010b).

Short-Chain Fatty Acids Produced by the Fermentation of Prebiotics

One of the reasons behind the cholesterol-lowering effect of some probiotic strains is the SCFAs produced when probiotic bacteria in the gut microbiome ferment prebiotics as a substrate. Among these acids, propionate is particularly noteworthy for its inhibitory effect on cholesterol synthesis in the liver and is known to contribute to the balancing of serum cholesterol parameters (Markowiak-Kopeć and Śliżewska, 2020).

In an in vivo study conducted by Hara et al. (1999), rats used as experimental animals were divided into three different groups. The first group was fed a diet without pulp, the second group was fed a diet enriched with pulp obtained from sugar cane, and the third group was fed a diet prepared with a SCFA mixture and bile salt supplement. At the end of the experiment, tissue samples obtained from the livers and small intestines of the rats were examined; it was determined that the lowest cholesterol synthesis rate was observed in the group consuming the SCFAs mixture (p<0.05) (Hara et al., 1999).

CONCLUSIONS AND RECOMMENDATIONS

This review summarizes the current evidence on the impact of probiotic bacteria on cholesterol metabolism. Various probiotic strains have shown significant cholesterol-lowering potential through multiple mechanisms. One key mechanism is bile salt hydrolase (BSH) activity, which promotes the deconjugation of bile salts, leading to the production of secondary bile acids that reduce cholesterol absorption. Additionally, probiotics contribute to cholesterol assimilation and membrane incorporation, wherein they assimilate cholesterol through their cell walls or incorporate it into their membrane structures, thereby reducing the available cholesterol. Bile acid precipitation is another mechanism in which secondary bile acids formed from probiotics precipitate cholesterol, reducing its absorption in the small intestine. Furthermore, the production of short-chain fatty acids, particularly propionate, inhibits cholesterol synthesis in the liver and helps balance serum cholesterol levels.

Meta-analyses indicate that probiotic strains such as Lactobacillus acidophilus, Lacticaseibacillus casei, and Lactiplantibacillus plantarum can significantly reduce serum cholesterol levels. However, the effects of these strains remain strain-specific and are influenced by various factors, including dosage, duration, host physiology, and environmental conditions. Although in vitro studies provide valuable mechanistic insights, their translation to in vivo conditions is still limited. Therefore, there is a critical need for long-term, controlled clinical trials to fully understand the efficacy of probiotics in human populations.

In terms of future research, strain characterization is essential. Detailed analysis of probiotic strains will help identify those with the most potent cholesterol-lowering effects, allowing for the development of

more targeted and effective interventions. Moreover, multi-strain versus single-strain comparisons should be explored to determine which approach offers the greatest benefits for cholesterol reduction. Clinical trials are also necessary to assess the impact of probiotics on cholesterol metabolism in humans, establishing appropriate dosage and duration guidelines for their effective use. Another significant recommendation is the development of functional foods and supplements. The creation of synbiotics—combinations of probiotics and prebiotics—could enhance the efficacy of probiotics in cholesterol management. Additionally, exploring technologies such as microencapsulation will improve the stability and viability of probiotics during gastrointestinal transit.

Lastly, it is crucial to focus on environmental and host factors. Future studies should examine how factors such as diet, pH, microbiome composition, and metabolic conditions influence the effectiveness of probiotics. This will be key to optimizing their clinical use. In conclusion, probiotics hold significant potential as functional agents for cardiovascular protection. However, further research is necessary to clarify the strain-dependent efficacy of probiotics and to refine their use in clinical settings.

Conflict of Interest

The authors declared that there is no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

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