Effect of Probiotic Supplementation on Modulation of Serum Lipids

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Abstract

An alteration in the intestinal microbiota due to a high-fat diet inherited disorder modulates lipid metabolism leading to cardiovascular diseases. Recently, to improve the lipid profiles, several pharmacological and non-pharmacological therapeutic strategies have been developed which involve the use of probiotics. Researchers noted mechanism wherein probiotic bacteria ferment food-derived indigestible carbohydrates to produce short-chain fatty acids in the gut, causing decline in the systemic levels of blood lipids by inhibiting hepatic cholesterol synthesis and/or cholesterol redistribution from plasma to the liver. In certain bacteria, interference with cholesterol absorption from the gut by deconjugating bile salts through bile salt hydrolase affects the cholesterol metabolism or through direct cholesterol assimilation to stabilize their cell membrane and binding to the cell walls of probiotics in the intestine to convert cholesterol into coprostanol. The animal and human experimental model suggests significant reduction in serum triglycerides, total and LDL-cholesterol along with increased HDL-cholesterol using appropriate strains of lactic acid bacteria and bifidobacteria. At present, few clinical trials yielded conclusive results; therefore, need to have a precise understanding of the underlying mechanism, dosage, efficacy, safety concerns before probiotic consumption and when used in combination with drug therapy which could only be achieved through well-designed clinical trials.

Keywords

Intestinal microbiota · Cardiovascular diseases · Serum lipid profile · Probiotics · Lipid metabolism

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10.1 Overview

The urbanization and change in lifestyle patterns have led to an exorbitant increase in the rate of chronic diseases, particularly in cardiovascular diseases. According to the statistical reports, WHO accounts that approx. 17 million people (~31% worldwide) died from cardiovascular diseases (CVDs) mainly due to acute myocardial infarction (AMI) and stroke (WHO 2017). The WHO has already predicted that by the year-end of 2030, cardiovascular disorders (CVDs) are considered as the major contributing factor in the cause of human death affecting approximately 23.6 million people worldwide with the majority of cases in the South Asia region (Enas et al. 2007). This could lead to considerable economic losses and the requirement of a significant amount of expenditure for the healthcare sector (Gadelha and Bezerra 2019).

10.1.1 Contributing Factors for Cardiovascular Diseases (CVDs)

The risk factor which is contributing significantly is the buildup of visceral fat which is due to complex interactions between genetics and environmental factors and is associated with subclinical systemic inflammation (Luo and Liu 2016). The other contributing factors for high lipid levels that could finally lead to cardiovascular diseases (CVDs) are certain medical conditions such as diabetes, hypothyroidism, alcoholism, kidney disease, liver disease, and stress (Park et al. 2020). The prominent CVDs are hypercholesterolemia and hypertriglyceridemia, which led to an increased level of low-density lipoproteins (LDL) and reduction in high-density lipoproteins (HDL), and are also important targets for attempts to prevent heart-related disorders (Grundy et al. 2005; Sherbet et al. 2013).

To predict cardiovascular risk, lipid profile analysis has almost become a routine test. The major challenges faced to control the risk of CVDs are high epidemic proportions; it develops quietly and is associated with other contributing factors such as certain medical conditions which led to increased risk for cardiovascular diseases (Ezzati et al. 2002; Al-Hamad and Raman 2017; Fortes et al. 2018).

10.1.2 Pharmacological Approach Versus Non-pharmacological Approach

Although several studies indicate that drugs have shown convincing results in lowering cholesterol levels. But the pharmacological approach is associated with certain drawbacks. Certain treatments involved in CVDs have unwanted side effects when used for the long term; involve expensive drug therapy, use of statins (3-hydroxy-3-methylglutaryl coenzyme inhibitors), fibrates, niacin, cholesterol absorption inhibitors, and bile acid sequestrants (Bliznakov 2002). Therefore, the need of an hour is to work on the dietary management of serum cholesterol and triglyceride (TG) levels as the large population is affected by cardiovascular

diseases. In this context, realizing the drawbacks associated with the pharmacological approach, researchers and pharmacologists are working on finding a novel alternative method through non-pharmacological approaches which include lifestyle modification, adherence to low-fat/low saturated fat diet (Taylor and Williams 1998), involving the potential cholesterol- lowering plant-based products such as plant stanols, soy, cinnamon, and use of soluble fibers (WHO 2003). Still, the situation has become very difficult due to its multifactorial origin (Bilen et al. 2016). In this context, the role of intestinal microbiota has been realized and studied.

10.1.3 Role of the Intestinal Microbiota

Scientific evidence has clearly shown that the intestinal microbiota whether intestinal or systemic play a vital role in maintaining good health. Any type of change in the microflora of the intestine leading to gut instability or dysbiosis affects the emergence of many different diseases, especially non-transmissible chronic diseases. It has been reported that individuals with lower bacterial diversity in their intestinal microbiota have hypercholesterolemia as compared to the controls (Rebolledo et al. 2017). Therefore, the virtue of probiotics has already been recognized in terms of general gut health and immunity. Probiotics are defined as "living microbial supplements that beneficially affect the host animals by improving its intestinal microbial balances" or as live microorganisms that when administered in adequate amounts, confer a health benefit on the host (WHO 2003).

A different mechanism of lipid metabolism has been reported in the literature which has been discussed in the later section of this chapter. Several strains of lactic acid bacteria and bifidobacterium are reported to be involved in lipid metabolism wherein (1) bile salt hydrolases are produced which causes deconjugation of bile salts with low absorption and increased excretion. (2) In order to overcome the losses, this led to an increased demand for cholesterol to synthesize new molecules. (3) Inhibition of cholesterol transmembrane transporter expression in enterocytes; (4) The production of short-chain fatty acids led to the inhibition of hepatic synthesis of cholesterol and fatty acids; (5) The cholesterol is incorporated into the cell membrane of microorganism during microbial growth (Ooi and Liong 2010; Reis et al. 2017).

10.2 Hypocholesterolemic Potential of Probiotics: Earlier Research Evidences

Over the years, the use of animal and human models to evaluate the effect of probiotics on serum cholesterol levels has shown certain promising outcomes. To study the effect of new probiotic strains on lipid metabolism in humans, certain animals such as rats, mice, hamsters, guinea pigs, and pigs have been used. These animals have shown similarities with humans in terms of digestive anatomy and

physiology, nutrient requirements, bioavailability and absorption, and metabolic processes. The metabolic processes which are shared with humans are cholesterol and bile acid metabolism, plasma lipoprotein distribution, and regulation of hepatic cholesterol enzymes (Fernandez et al. 2000). Due to these aforesaid metabolic properties, the animals are therefore useful as experimental models for research applications (Patterson et al. 2008). The reliability of results thus obtained through the animal model has been confirmed through the human trial results.

The initial scientific reports of the use of probiotics in lipid metabolism date back more than 40 years and came from the tribes of Samburu and Maasai warriors in Africa wherein reduced serum cholesterol was noted after consumption of large amounts of milk fermented with a wild *Lactobacillus* strain (Shaper et al. 1963; Mann 1974). This led to the realization of the potential hypocholesterolemic effect in fermented milk products containing lactobacilli and/or bifidobacteria through animal and human studies.

10.3 Probiotic Market for Cholesterol Reduction

In the past two decades, the importance of prebiotics and probiotics in lowering blood cholesterol level has been recognized on a global level. In this direction, the European market has taken an initiative to penetrate through products claiming to lower the blood cholesterol level and thereby contributing towards a healthier heart (Young 1998). Danone launched Actimel Cholesterol Control® in Belgium, containing the suggested cholesterol-lowering probiotic *Lactobacillus acidophilus* and the branded prebiotic ACTILIGHT® (Beghin-Say), while, "Mona," a Danish company introduced a cultured dairy-based drink under the brand name Fysiq®. It contains the probiotic *Lactobacillus acidophilus* and the branded bifidogenic dietary fiber, RAFTILINE® (Orafti). On similar lines, MD Foods, a Danish company introduced a yogurt-style product "Gaio." The UK-based life sciences firm OptiBiotix Health has developed a naturally occurring bacterial strain, LP-LDL (*Lactobacillus plantarum* ECGC 13110402), known to lower LDL-cholesterol along with lowering systolic blood pressure. The CEO of OptiBiotix Health, Steve Prescott has preliminary talks with food and beverage brands in the USA for developing yogurt or other functional food having the claimed probiotic. The selected strain has high bile salt hydrolase activity, cholesterol removal potential, resistance to gastric, pancreatic, and bile acids, and a higher surviving rate on freeze-drying. The LP-LDL is shelf-stable at room temperature while it needs to be refrigerated in presence of moisture. UAS labs have developed *Lactobacillus reuteri* NCIMB 30242 (LRC); Kaneka claimed a product Floradapt Cardio containing three strains of *Lactobacillus plantarum* that could reduce LDL along with reduction in blood pressure (Watson 2020).

Still, it is difficult to understand the beneficial effects of these products in terms of blood lipid levels in humans (Pereira and Gibson 2002a, b).

10.3.1 Commercially Available Probiotic Products: Role and Research Evidence in Cholesterol Reduction

One of the fermented milk products launched by the company Gaio® having bacterial cultures, *Enterococcus faecium* and two strains of *Streptococcus thermophilus* (CAUSIDO® culture) were able to reduce plasma cholesterol levels on the intake of appropriate levels. These bacterial strains were isolated from the intestinal flora of inhabitants of Abkhasia (Caucasus), a region known for the longevity of its people and where fermented milk is a major part of their diet (Agerbaek et al. 1995). In one of the study, carried out by Agerbaek and coworkers, the consumption of 200 mL/ day of this fermented milk product for over 6 weeks by Danish middle-aged men (44 years old) with a subject group of 58 male volunteers led the reduction up to 6% of total plasma cholesterol and 10% reduction of LDL-cholesterol (Agerbaek et al. 1995). In this study, no change in serum HDL-cholesterol or plasma triacylglycerol levels was observed. The study was random, double-blind, and controlled. In another study, Richelsen and coworkers noted that there was a marked difference in serum LDL-cholesterol after the consumption of up to 200 mL/day of the Gaio® product for 1 month (Richelsen et al. 1996). However, it was also noted that long-term consumption up to 6 months did not show any marked difference in terms of reduction of LDL-cholesterol with that of placebo product.

Certain researchers concluded that although low-fat milk or fermented milk products may have some hypocholesterolemic effects but are not superior to the placebo milk product since a reduction in cholesterol levels was also noted in the placebo group (Taylor and Williams 1998). A similar trend has also been noted by Agerbaek and coworkers in both test and placebo experimental set (Agerbaek et al. 1995). During in vitro studies, Agerbaek and coworkers noted that the cultures present in the fermented milk play a vital role in determining the reduction in cholesterol levels. It was also inferred that though *Streptococcus thermophilus* is acid sensitive and does not, to any significant degree, survive passage through the small intestine during in vivo studies. So, it is the *Enterococcus faecium* which plays a significant role in possessing the beneficial effect of fermented milk product. It was also observed that reduction in the concentration of *Enterococcus faecium* in the test product could have contributed majorly in lowering of product efficacy and thereby decline in the effectiveness of cholesterol reduction (Agerbaek et al. 1995).

Similarly, Rossi and coworkers tested the ability of certain microorganisms which could reduce in vitro cholesterol levels and can tolerate bile salts. The test microorganisms were *E. faecium*, *L. acidophilus*, *L. jugurti*, *S. thermophilus,* and *L. delbrueckii.* The reduction in the cholesterol levels in the medium containing *E. faecium only* and in the mixture of *E. faecium* plus *L. acidophilus was observed* after 24 h of anaerobic incubation and a significant decline in vitro cholesterol level up to 53 and 65%, respectively, was noted (Rossi et al. 1999). Similar observations were made by Larsen and coworkers, wherein, a significant decline in LDL-cholesterol was reported in the Gaio® product group only (8.4%, $P < 0.05$) after 8 weeks. This decline would correspond to a reduction in the risk factor for

CHD up to 20–30%, which is of clinical relevance (Larsen et al. 2000). The prominent reason for the hypocholesterolemic effect of Gaio® product is related to the CAUSIDO® bacterial culture, particularly *E. faecium.*

In another study, conducted by Schaasfsma and coworkers, the effect on serum total cholesterol level was noted when "Danone" yogurt containing *Lactobacillus acidophilus* and 2.5% (w/v) fructo-oligosaccharides was fed for two treatment periods of 3 weeks separated by a washout period of 1 week. During the treatment periods, 30 male subjects consumed 125 mL of either test or reference product thrice daily. The study was randomized, placebo-controlled, double-blind with a two-way crossover trial. A significant reduction in serum total cholesterol (4.4%) and LDL-cholesterol (5.4%) was noted in test product (*Lactobacillus acidophilus* and 2.5% (w/v) fructo-oligosaccharides, 0.5% (w/v) vegetable oil and 0.5% (w/v) milk fat) against reference (traditional yogurt, containing 1% (w/v) milk fat). It further needs to be evaluated to know whether the hypocholesterolemic effect of the test product is either due to *Lactobacillus acidophilus* strain, or fructo-oligosaccharides (FOS), or both. The dosage and time factor also need to be taken into consideration to determine the effect on serum cholesterol levels (Schaafsma et al. 1998).

OptiBiotix Health conducted clinical studies and claimed that the use of probiotic *Lactobacillus plantarum* ECGC 13110402 led to 1% reduction in serum cholesterol along with lessening of artery disease risk by 2–3%. The clinical study was carried out with subjects having normal blood pressure which led to a significant reduction in systolic blood pressure up to 5.1% after 6–12 weeks. Further, studies need to be conducted using hypertension patients in a larger group for a clear representation of significant effects. Though certain studies were conducted for a third human trial by the University of Roehampton, UK, but it got delayed due to COVID-19. The 2017 study was conducted with a double-blind, placebo-controlled, randomized human intervention for over 12 weeks with 49 normal to mildly hypercholesterolemic adults aged 30–65. The results showed that twice daily ingestion of 2×10 CFU (Colony-Forming Unit) encapsulated LP-LDL led to a statistically significant (13.9%) reduction in LDL-cholesterol in volunteers with baseline total cholesterol of <5 mM during the 0–12 week period and a 13.1% decrease in the group with more elevated LDL. There was a 4.5% increase in HDL ("good") cholesterol for those taking the probiotic in the 6–12-week period, with a more meaningful (14.7%) increase for the over 60s (Watson 2020).

The main objective of this study is to critically review the scientific evidence available regarding the effect of probiotic supplementation in the prevention and treatment of lipid profile abnormalities and to decipher the mechanism lying behind the lowering of cholesterol through probiotics to develop a better formulation for human consumption.

10.4 Animal Studies on Probiotic Supplementation in Lowering Cholesterol Levels

Probiotic supplementation reduces cholesterol concentration in the serum of chickens (Mohan et al. 1995). Fukushima et al. reported that hypercholesterolemic male Fischer rats fed with 30 g/kg of *L. acidophilus*-fermented rice bran significantly showed an improved lipid profile in a four-week study. A reduction in serum total cholesterol and liver cholesterol of 21.3% and 22.9%, respectively, was noted against control (Fukushima et al. 1999). In one of the research studies, it was also reported that the supplementation of probiotics (*Lactobacillus acidophilus*, *Bifidobacterium bifidum,* and *Aspergillus oryzae*) at 100 mg/ kg in the diet of broiler chickens significantly reduced the serum cholesterol concentration. The dietary supplementation of a mixed culture of 12 strains of *Lactobacillus* at 1% in the basal diet of broilers resulted in higher body weight gain and lowered serum cholesterol concentration (Kalavathy et al. 2003). It was noted that administration of *Lactobacillus reuteri* led to the prevention of hypercholesterolemia in mice, there was a significant reduction in total cholesterol (22%) and triglycerides (33%), along with significant increase in the ratio of HDL to LDL up to 17% (Taranto et al. 2000). Arun and coworkers reported that the dietary supplementation of *Lactobacillus sporogenes* (6×10 spore per g) at 100 mg kg diet significantly lowered total cholesterol, VLDL cholesterol, and triglycerides concentrations in the serum of broiler chickens (Arun et al. 2006).

Ichim and coworkers evaluated the effect of DBR on the activity of gut microbiota through in vitro studies in terms of cholesterol metabolism. The Daily Body Restore (DBR) is a proprietary blend composed of 9 probiotic organisms of the genera *Lactobacillus* and *Bifidobacterium*, and 10 digestive enzymes. The Shime® system consisting of sequential colon reactors was supplemented with DBR for analysis of short-chain fatty acid production. After 8 weeks of DBR treatment, LDL concentrations were dramatically reduced by 78%, and HDL was increased by 52% relative to control mice. The addition of DBR to the Shime® system led to significantly increased production of propionate in colon reactors, indicative of microbial production of short-chain fatty acids known to inhibit cholesterol synthesis (Ichim et al. 2016). The cholesterol-lowering potential of *L. fermentum* MTCC 5898 was noted in rats fed with high-fat diet. It was also noted that besides the decline in total cholesterol, a significant reduction in triglycerides, VLDL, and LDL was also observed. An increment in HDL level was also noted in the probiotic treated group (Yadav et al. 2018).

Fazeli et al. noted that the consumption of *L. plantarum* A7 (10^8 CFU mL^{-1}) for 14 days is effective in lowering serum lipid levels in rats (Fazeli et al. 2010). On similar lines, Salaj and coworkers while working with *Lactobacillus plantarum* strains, i.e., *Lactobacillus plantarum* LS/07 and *Lactobacillus plantarum* Biocenol LP96, examined its effects on lipid metabolism and body weight in rats fed with high-fat diet. It was noted that *Lactobacillus plantarum* LS/07 reduced serum cholesterol and LDL-cholesterol, while *Lactobacillus plantarum* Biocenol LP96 decreased triglycerides and VLDL. In both the strains, no change in serum HDL

and liver lipids was noted. Findings also showed that the effect of lactobacilli on lipid metabolism may differ among strains and both the strains involved in the study, could improve lipid profile (Salaj et al. 2013). Aminlari and coworkers also reported that on evaluating two probiotic bacteria, *L. plantarum*, and *Bacillus coagulans,* on lipid panel parameters. It was observed that there was a decline in serum concentrations of total cholesterol, triglycerides, LDL, and VLDL in comparison with the enriched-cholesterol diet group (Aminlari et al. 2018). In support of an earlier study, El-shafie and coworkers also noted the hypocholesterolemic effect of *Lactobacillus plantarum* NRRL B-4524 used as a single or as mixed culture with *Lactobacillus paracasei* in rat diets (El-Shafie et al. 2009). Introduction of *L. paracasei* TD3 in rat's diet could significantly reduce serum cholesterol levels (~9.2%), whereas there was no significant difference between experimental groups for triglycerides, LDL, and HDL levels. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) enzymes were significantly decreased in the probiotic group (Dehkohneh et al. 2019). Recently, Chen and coworkers tried to investigate the role of *L. plantarum* FZU3013, a probiotic isolated from *Hongqu* rice wine via a traditional brewing process in improving the nonalcoholic fatty liver (NAFL) associated with hyperlipidemia in mice fed with a high-fat diet. The results have shown a significant reduction in the HFD-induced body weight gain which inhibits the excessive accumulation of liver lipids, promotes excretion of bile acids from feces. These findings clearly show the potential of *L. plantarum* FZU3013 in improving lipid metabolism disorders by modulating specific intestinal microbial phylotypes and regulate hepatic lipid metabolism related genes, thereby preventing NAFL and hyperlipidemia (Chen et al. 2020). In another study, Yamasaki and coworkers, while working with *Lactobacillus plantarum* 06CC2 (LP06CC2), an isolate from Mongolian dairy product noted the suppression of an increase in liver cholesterol and hepatic damage indices. The mice fed with LP06CC2 have also shown an increase in cecal content and fecal butyrate. A bile acid deconjugation using glycocholate leading to a decrease in bile acid absorption is indicated in presence of LP06CC2. It is evident from results that LP06CC2 is a promising microorganism for the reduction of the cholesterol pool via modulation of bile acid deconjugation (Yamasaki et al. 2020).

Table 10.1 clearly shows the evidence of animal clinical trials conducted to evaluate the effects of probiotics on the lipid profile and other variables.

In the aforesaid studies, the inclusion criteria were limited only to the use of probiotics and not on synbiotics in lowering cholesterol levels. It has also been noted and reported that the cholesterol-lowering effect of probiotics varies from strain to strain.

10.4.1 Limitations of Using Probiotics as Cholesterol-Lowering Adjunct

The evaluation of probiotics as a cholesterol-lowering adjunct has its limitations since the use of high dosage and a large amount was reported on a regular basis

Table 10.1 Research evidences showing human clinical trials conducted to evaluate the effects of probiotics on the lipid profile and other variables

Probiotic strain/s	Animal/subjects	Dose/ duration of the study	Study outcome	Reference
Bifidobacterium longum Bb-46 (fortified in buffalo milk-yogurt)	48 male hypercholesterolemic albino rats	50 g regular basis 35 days	A significant reduction in total cholesterol (TC) level by 50.33%, LDL-cholesterol by 56.3% and triglycerides by 51.2% compared to the control	El-Gawada et al. (2005)
Lactobacillus reuteri (containing bile salt hydrolase) (BSH)	20 pigs	Twice daily 13 weeks	A decline in total, as well as LDL-cholesterol was observed to be 11% and 26%, respectively	De Smet et al. (1998)
L. plantarum CK 102 (healthy human isolate)	32 Sprague-Dawley (SD) male rats; 5 weeks old; induced hypercholesterolemic; mean BW of 129 ± 1 g	5.0×10^7 CFU/mL daily, 6 weeks	A significant decrease in total cholesterol (27.9%), 28.7% decline in LDL-cholesterol, and 61.6% decrease in triglycerides ($P < 0.05$)	Ha et al. (2006)
L. plantarum KCTC3928 (Cellbiotech Co. Ltd., Korea)	21 six-week-old C57BL/6 male mice; induced hypercholesterolemic	1×10^9 CFU/mL of *L. plantarum* KCTC3928, 4 weeks	A significant decrease in total cholesterol up to 33%, 42% decline in LDL-cholesterol, and 32% reduction in triglycerides ($P < 0.05$) An increase in HDL-cholesterol up to 35% was noted ($P < 0.05$)	Jeun et al. (2010)
L. acidophilus (wild chickens & human isolates)	30 Awassi weaning lambs; hypercholesterolemic	1×10^9 CFU/ capsule 2 capsules daily, 120 days	A significant decline in total cholesterol up to 22.6% ($P < 0.05$)	Lubbadeh et al. (1999)
L. plantarum PH04 (isolated from infant feces)	12 male hypercholesterolemic mice	14 days	A significant reduction in total serum cholesterol (~7% reduction) and in triglycerides	Nguyen et al. (2007)

(continued)

Table 10.1 (continued)

Probiotic strain/s	Animal/subjects	Dose/ duration of the study	Study outcome	Reference
			(~10% reduction) against control	
L. gasseri	Rats	Fed milk with probiotic strain	Lowering of total and LDL-cholesterol levels up to 42 and 64%, respectively. Decline in triglyceride levels was also noted.	Usman and Hosono (2000)

(Pereira and Gibson 2002a, b). A dosage level of 10^9 to 112×10^9 CFU/day was reported and it was noted that individuals fed with larger doses are safe in terms of beneficial effects along with no adverse clinical effects. The effect of probiotics is strain-dependent and a combination of different strains gives better results (Rajkumar et al. 2014). Therefore, as per the research carried out by Rajkumar and coworkers, there is a need to test small dosage amounts over a long period. In the current scenario, the thrust area of study must be on a *priority basis*, to examine the proven probiotic strains in lipid metabolism on human subjects and *secondly*, to ensure that the probiotic food additive use should reach the colon alive and have recommended viable numbers (1×10^7 cfu/g) (Ranadheera et al. 2010). The variation in the culture viability depends on the handling of the product (generally kept under cold temperature conditions), *thirdly,* the mechanism suggested so far for cholesterol assimilation in growing cells, bile salt hydrolase enzyme, and the incorporation in the cellular membrane through probiotic bacteria only a few probiotic strains can do so with small effect compared to that of the cholesterol-lowering drugs (Guo et al. 2012; Liong and Shah 2006).

Several studies were conducted wherein divergent results were obtained due to the specificity and combination of the strains employed, the doses administered, the duration of the studies, and other extraneous variables. It is recommended that further studies be conducted, designed to identify the long-term effects and the influence of probiotics when used in combination with drug-based treatment (Gadelha and Bezerra 2019). Research studies indicate that supplementation with probiotics, as investigated in well-controlled studies can be used as an adjuvant to traditional treatments for dyslipidemia.

A synergistic effect was observed when probiotic supplementation was combined with other treatments. In one of the studies, soy isoflavones when combined with probiotics have an additive effect as compared to groups given supplementation only. The combination of physical exercise with probiotic administration stimulates an increase in HDLc. The soy products containing isoflavones exhibited significant reductions in electronegative LDL in hypercholesterolemic individuals (Cavallini et al. 2016). The probiotic supplementation led to the improvement in inflammatory

profile, glycemic control, body mass, and immunological markers, which are generally been considered risk factors for the development of CVDS (Gadelha and Bezerra 2019). On the contrary, there are few research evidences and clinical reports wherein it was noted that all the patients do not respond equally well when probiotic was fed as an adjunct. Therefore, it is advisable to keep these patients on cholesterol-lowering drugs such as statins with slight modifications in lifestyle.

10.5 Studies on Probiotic Supplementation Lowering Cholesterol Levels in Humans

The hypocholesterolemic potential of probiotics has also been evaluated in human subjects. In one of the studies conducted using 48 hypercholesterolemic volunteers for 10 weeks, wherein a daily consumption of 200 g of yogurt containing *L. acidophilus* L1 contributed to a significant reduction in serum cholesterol concentration compared to control (Anderson and Gilliland 1999). Xiao and coworkers examined the effect of yogurt consumption fermented with *L. acidophilus* cultures on 30 Dutch healthy men involved as subjects for several weeks, it was noted that there was a significant reduction in total as well as LDL-cholesterol levels by 4.4 and 5.4%, respectively, when compared with controls (Xiao et al. 2003).

Several interesting data were also obtained wherein a decline in serum cholesterol levels was noted in bottle-fed babies as the number of *Lactobacillus acidophilus* in their stools get increased (Harrison and Peat 1975). In one of the research reports, it has been noted that the consumption of 2 L of whole fat milk and skim milk led to the reduction of serum cholesterol by 5 and 15%, respectively. On the other hand, the same literature report also suggested that if an equivalent amount of milk fat is replaced with butter there was an increase in serum cholesterol by 7% (Howard and Marks 1977). Yogurt has also been reported to cause a decline in serum cholesterol levels in humans (Hepner et al. 1979) and the effect is transient (Rossouw et al. 1981). Ashar and Prajapati (2001) reported the hypocholesterolemic effect of probiotic diet in humans and showed total cholesterol reduction to an extent of 12–21% by feeding on acidophilus milk.

Schaarmann and coworkers also tried to decipher the relationship between the intake of probiotic yogurt and the concentration of cholesterol fractions. The group conducted an experiment using 29 healthy women as subjects. The groups were divided into normal cholesterolemic group (total cholesterol <250 mg/dL) and a hypercholesterolemic group (total cholesterol >250 mg/dL. The experiment consists of three periods (placebo, standard yogurt, and probiotic yogurt), and each lasting for 51 days, the product consumed was having a concentration of 300 g/day. The probiotic yogurt composed of *Lactobacillus acidophilus* and *Bifidobacterium* longum strains, whereas standard yogurt has *Streptococcus thermophilus* and *Lactobacillus lactis* as microbial strains. Results showed a decline in LDL-cholesterol and triacylglycerides after consumption of standard and probiotic yogurts. Though a large decline was noted after intake of probiotic yogurt along with an increase in HDL cholesterol compared to standard yogurt but the difference was not significant

in the hypercholesterolemic group. Therefore, no significant change was observed in the normocholesterolemic and the hypercholesterolemic groups when probiotic yogurt was fed (Schaarmann et al. 2001). In one of the study carried out by Schaafsma et al. wherein 54 volunteers were used as subjects, it was noted that there was a 5–10% decline in serum cholesterol levels after several weeks of moderate consumption of yogurt fermented with *Lactobacillus bulgaricus* and *S. thermophilus* (Schaafsma et al. 1998).

Fuentes and coworkers noted that daily intake of *L. plantarum* in the form of a capsule containing 1.2×10^9 CFU led to lowering of TC and LDL-C concentrations in hypercholesterolemic subjects after 12 weeks of study (Fuentes et al. 2013). In agreement with reports of Fuentes and coworkers, Wu et al. noted that the consumption of lactobacilli strains such as *L. reuteri and L. plantarum* has shown a significant reduction in TC and LDL-C. The researchers also suggested that the consumption of synbiotic food, containing *L. sporogenes* and inulin has a beneficial effect on TG and HDL-C (Wu et al. 2017).

Several scientific reports suggest a significant reduction in the total cholesterol (TC), LDL-cholesterol (LDLc), and triglycerides along with an incremental increase in the levels of HDL-cholesterol (HDLc) in the system when supplemented with probiotics (Ahn et al. 2015; Fuentes et al. 2016). The results are highly heterogenous; therefore, a subset was analyzed and noted that TC more than 200 mg/dL had shown better response to treatment with probiotics.

Cho and Kim conducted 30 random trials; subjects treated with probiotics demonstrated reduced total cholesterol and LDL-cholesterol compared to control while there was no significant effect of probiotics on HDL-cholesterol as well as triglycerides. The major factors determining the significant effect of probiotics on total cholesterol and LDL-cholesterol were greater for higher baseline total cholesterol levels, longer treatment durations, and certain probiotic strains (Cho and Kim 2015). Similar observations have also been reported during studies conducted by Yan and coworkers (Yan et al. 2019).

Sharma et al. reported that meta-analysis of 14 randomized clinical trials with normal, borderline, and borderline high baseline cholesterol levels were noted when fed with probiotics, a significant decline in serum TC (−8.40 mg/dL) and LDL-cholesterol levels (−6.63 mg/dL) observed compared to control (devoid of probiotics). The probiotic strains studied in this meta-analysis were *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus rhamnosus, Bifidobacterium longum, Bifidobacterium lactis, Bifidobacterium animalis,* and *Enterococcus faecium*. Out of these 14 trials, 13 trials showed a trend in the reduction of both serum TC and LDL-cholesterol levels. Out of these 13 trials, only 6 trials showed statistically a significant decline in serum TC levels along with 4 trials wherein a significant decline in serum LDL-cholesterol levels was noted. On the other hand, a significant change in HDL-cholesterol and TG levels was not observed with the use of probiotics (Sharma et al. 2016).

Park and coworkers conducted a double-blind, randomized, placebo-controlled study which includes 70 participants (both sexes), age 20+ having blood triacylglyceride (TG) levels below 200 mg/dL (normal value) in order to investigate

the effect of *Lactobacillus plantarum* Q180 (LPQ180) on postprandial lipid metabolism and the intestinal microbiome environment. It was noted that there was a significant decline in LDL-cholesterol ($p = 0.042$) and apolipoprotein (Apo) B-100 ($p = 0.003$) levels, after 12 week of treatment with LPQ180. Besides this, there was a significant decrease in total indole and phenol levels ($p = 0.019$). Healthy postprandial lipid metabolisms in subjects and a healthy intestinal environment with a higher level of enteric bacteria such as *R. bromii*, *K. alysoides*, *B. intestinihominis*, and *F. plautii* were observed due to ingestion of LPQ180. A higher level of these enteric bacteria led to a higher SCFA content after LPQ180 supplementation for 12 weeks. It could be due to large deviations from small number of subjects in each group and healthy TG levels (Park et al. 2020). Table 10.2 indicates research evidences showing human clinical trial conducted to evaluate the effects of probiotics on the lipid profile and other variables.

The main limitations faced during clinical trial studies were that these were of short duration varying from 15 days to 12 weeks, small sample size, and no analysis of the intestinal microbiota (IM), and the majority of subjects involved were having dyslipidemia.

10.6 Contradictory Results on Probiotic Usage in Both Animal and Human Studies

Several research reports showed a lowering in cholesterol levels in several fermented milk products, while some researchers failed to do so even when supplemented with dietary supplements in both animals and humans, which have led to doubtful results. In the majority of the studies, volunteers were fed with yogurt fermented with *L. acidophilus* as probiotic to study its influence on serum lipids, but no significant change in plasma total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides was noted (De Roo et al. 1998; Lewis and Burmeister 2005; Fabian and Elmadfa 2006; Pawan and Bhatia 2007).

Kikuchi-Hayakawa et al. (2000) while working with *L. casei* strain Shirota (Yakult®) in hamsters clearly states that cholesterol metabolism is strain specific. Although this strain could grow well in presence of mixed lipid micelles containing bile acids and under anaerobic conditions but still could not significantly remove cholesterol (only 11%) from culture broth even after 24 h of incubation. Contrary to the other strains tested such as *L. acidophilus, L. crispatus, L. gasseri* which has shown a significant removal of cholesterol (~80%).

Lewis and Burmeister conducted an experimental study wherein 80 volunteers (20–65 years) consumed two capsules containing freeze-dried *L. acidophilus* (3×10^{10} CFU/2 capsules) three times daily for 6 weeks, in order to determine the effect of *Lactobacillus acidophilus* on human lipid profiles. It was found that *L. acidophilus* capsules did not significantly change plasma total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides of the subjects (Lewis and Burmeister 2005). Fabian and Elmadfa (2006) also observed that the mean concentrations of total, HDL- and LDL-cholesterol in 33 female volunteers showed

Table 10.2 Research evidences showing human clinical trials conducted to evaluate the effects of probiotics on the lipid profile and other variables

Probiotic strain/s	Subjects Age (years)	Dose/duration of the study	Study outcome	Reference
Lactobacillus curvatus HY7601 and *L. plantarum* KY1052	121 non-diabetic people with hypertriglyceridemia	2 grams of powder containing *Lactobacillus sp.* 0.5 × 1010 CFU of each 12 weeks	18.3% reduction in TG and a 15.6% reduction in LDL along with 21.1% increase in apo A-V. TG and apo A-V values were inversely correlated.	Ahn et al. (2015)
Bifidobacterium lactis HN019	51 people with metabolic syndrome (18–60 years)	26 subjects, consumed milk fermented with 2.72×10^{10} CFU of *Bifidobacterium lactis HN019;* 45 days	Significant reductions were observed in TC ($p = 0.009$) and LDL-c ($p = 0.008$).	Bernini et al. (2016)
Lactobacillus casei shirota	30 healthy volunteers (55–74 years)	*Lactobacillus casei shirota* with 1.3×10^{10} CFU/day. 4 weeks	No significant reduction in TC or TG	Dong et al. (2013)
Lactobacillus acidophilus La5 and Bifidobacterium lactis Bb12	60 people with type-2 diabetes	6 week	A significant reduction of 4.54% in total cholesterol and 7.45% reduction in LDL-cholesterol. No change in HDL-cholesterol and triglycerides	Ejtahed et al. (2011)
E. faecium M-74	43 volunteers	56 weeks	Reduction of serum cholesterol concentration by 12%	Hivak et al. (2005)
Lactobacillus acidophilus La5 and *Bifidobacterium animalis*, subspecies *lactis* Bb12	156 people with metabolic syndrome Mean age: 67 years G1: Yogurt, plus placebo	Subjects consuming probiotic ingested at least 3×10^9 CFU/day.	No differences in lipid profile markers between groups ($p < 0.05$)	Ivey et al. (2015)

	capsule; G2: Probiotic capsule plus milk; G3 and G4: Placebos			
L. fermentum ME-3	45 healthy volunteers (50–75 years), with BMI from 24–30 kg/m^2 and borderline risk factors for cardiovascular disease	2 capsules per day containing the probiotic *L. fermentum* ME-3 (6×10^9 CFU/day), plus other compounds. 4 week	Significant reductions in LDL-c, TC, TG, and OX-LDL ($p < 0.05$) and a tendency to improvements in HDL.	Kullisaar et al. (2016)
L. plantarum 299v (ProViva)	36 healthy volunteers with moderately elevated fibrinogen concentrations (>3.0 g/L); 35–45 years old	400 mL of rose-hip drink containing 5.0×10^7 CFU/mL daily, 6 weeks.	A decrease in total cholesterol level up to 2.5% and LDL-cholesterol level up to 7.9%.	Naruszewicz et al. (2002)
Saccharomyces boulardii	11 hypercholesterolemic men (21–69 years)	5.6×10^{10} CFU/day of *Saccharomyces boulardii* 8 weeks	Only remnant lipoprotein (RLP) exhibited a significant reduction ($p < 0.03$)	Ryan et al. (2015)
Lactobacillus acidophilus La-5 and *Bifidobacterium animalis lactis* BB	45 people, 35–60 years with type 2 Diabetes mellitus	120 g/day of milk fermented with probiotics (*Lactobacillus acidophilus* La-5, *Bifidobacterium animalis lactis* BB-12; 10^9 CFU of each). 6 weeks	Reduced LDL-c ($p = 0.03$) and TC ($p = 0.04$)	Tonucci et al. (2017)
B. longum BL1 (fortified low fat yogurt)	32 subjects (aged 28–60 years old)	4 weeks	A significant decline in serum total cholesterol, LDL-cholesterol and triglycerides along with 14.5% increase in HDL-cholesterol	Xiao et al. (2003)

no relevant differences between the two groups, one enriched with *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, and the other served as control (devoid of probiotics). On the similar lines, Simons and coworkers while working with *Lactobacillus fermentum* (2×10^9 CFU per capsule; four capsules daily) in 46 volunteers (aged 30–75 years) noted that there was no change in the lipid profile even after 10 weeks of the study period on the consumption of probiotics (Simons et al. 2006). Hatakka and coworkers studied the hypocholesterolemic effect of probiotics and noted that the administration of *L. rhamnosus* LC705 (10^{10} CFU/g per capsule; two capsules daily) in 38 men with a treatment period of 4 weeks did not show any influence on blood lipid levels (Hatakka et al. 2008).

10.6.1 Attributing Factors Leading to Contradictory Results

The aforesaid results thus obtained could be attributed to several factors, though in vivo trials utilize real-life models with real pathological systems, but these trials are affected by external factors such as different strains of probiotics, administration dosage, analytical accuracy of lipid analyses, clinical characteristic of subjects, duration of the treatment period, inadequate sample sizes, failure to control the nutrient intake and energy expenditure during the experiments variations in the baseline levels of blood lipids, and lack of suitable controls or placebo groups (Liong 2007; Greany et al. 2008).

Literature reports are available on probiotic strains to be developed and tested for serum cholesterol reduction (Shaper et al. 1963; Pereira and Gibson 2002a, b). In case of animal model, a large number of strains have demonstrated the effect of probiotics on serum lipid level with consistent results (Pereira and Gibson 2002a, b). While, on the other hand, when this effect was examined in human studies, inconsistent results were noted wherein some showing positive significant effects while others with no effects (Taylor and Williams 1998; Huang et al. 2013; Wang et al. 2012; Wu et al. 2017). The data variation could be due to differences in the experimental design based on the following factors such as type and quantity of the fermented milk product; age and sex distribution and starting plasma cholesterol levels of the subjects studied; and length of the study period). Therefore, direct comparisons are not possible (Pereira and Gibson 2002a, b).

To overcome the conflicting results, meta-analysis can be conducted wherein in the randomized controlled trials the effect of probiotics on serum lipid levels could be examined. In meta-analysis, changes in the mean and standard deviation of lipid parameters (TC, LDL-cholesterol, HDL-cholesterol, and TGs) were studied (Sharma et al. 2016).

10.6.2 Role of Minimal Effective Probiotic Dosage for Hypocholesterolemic Potential

At present, there is much research evidence that reports the hypocholesterolemic potential of probiotics but the "*minimal effective dosage"* of probiotics needs to be examined to reduce blood cholesterol levels. There are no regulatory standards for probiotic product to produce cholesterol-lowering effect and a tremendous variation is noted in the concentration of probiotics in food products (FAO 2002). It has been noted from the previous research studies that the effective administrative dosage of probiotics varies and is dependent on the strains used, along with the clinical characteristics of subjects, such as lipid profiles. Thus, more studies are needed, to determine the effective dosage of probiotics to exhibit hypocholesterolemic effects. The prescribed dosage should also work for in vivo studies involving the lipid profiles.

10.6.3 Role of Analyzing Lipid Profile

The effect on lipid profile during consumption of fermented milk product was noted in patients suffering from mild to moderate primary hypercholesterolemia by Bertolami et al. (1999). The study conducted was prospective, randomized, double-blinded, and placebo- controlled with a crossover design. In this study, 32 subjects between the age group (36–65 years) were included in the trial for a period (8 weeks). It was observed that fermented milk (Gaio®) caused a statistically significant decline in total serum cholesterol. However, there was some discrepancy in the results, not all the subjects respond to the product, among these three subjects have shown an elevated cholesterol level.

The manipulation of intestinal microbiota with probiotic supplementation aids in several benefits to the host (Lin et al. 2014). The use of probiotics has already been there in human healthcare, for the prevention and treatment of diseases through modulation of intestinal microbiota (Coppola and Gil-Turnes 2014; Kechagia et al. 2013). The probiotics must have the capacity to adhere to the intestinal mucosa, overcoming the barriers imposed by the gastrointestinal tract, primarily the gastric pH, bile salts, and pancreatic enzymes (Soccol et al. 2010).

The existing research evidence from animal and human studies indicates that use of the fermented dairy products has shown moderate lowering action of cholesterol. However, the potential mechanism behind the lowering is still unclear. Herein, we have examined the various mechanisms of action of probiotics reported in the literature for lowering cholesterol levels.

10.7 Mechanism of Action of Probiotics for Cholesterol Metabolism

Several researchers reported through in vitro studies that some of the strains of Lactobacilli (Gilliland et al. 1985; Rasic et al. 1992; Noh et al. 1997) and Bifidobacterium (Tahri et al. 1995, 1996) can assimilate cholesterol in presence of bile acids.

(a) *Role of physiochemical conditions for cholesterol removal:* Gilliland and coworkers while working with *Lactobacillus acidophilus* strains noted that the removal of cholesterol from growth medium could only be possible in presence of bile and under anaerobic conditions (Gilliland et al. 1985). While in vivo cholesterol assimilation by cells or attachment of cholesterol to the surface has been explained by Meei YN Lin based on the ability of six *L. acidophilus* strains noted during in vitro studies. It was noted that when *L. acidophilus* ATCC4356 was grown anaerobically in a medium supplemented with bile acids for 24 h, the maximum uptake of 57% was reported (Lin and Chen 2000). Generally, these are the conditions that occur in the intestine and help in part of the cholesterol ingestion in diet thereby making it impossible for cholesterol to be absorbed in the blood. Among the *L. acidophilus* strains, a considerable variation was found in terms of their ability to grow in presence of bile and to remove cholesterol from laboratory medium (Gilliland et al. 1985; Gilliland and Walker 1990; Walker and Gilliland 1993).

No metabolic degradation via. *Alteration of cell wall/membrane*: The binding ability of intact cells to cholesterol varied widely among strains which could be due to differences in chemical and structural properties of the bacterial cell wall peptidoglycans. Still, it remains unclear whether the variation in cholesterol uptake in different strains is due to differences in their cell membrane or some other cell components. The studies of De Rodas et al. (1996) were supported with research evidence given by Noh et al. (1997) wherein authors reported that assimilation of cholesterol by *L. acidophilus* ATCC43121 was not metabolically degraded. These researchers noted that the cells grown in presence of cholesterol micelles and bile salts were resistant to lysis by sonication and are more resistant to sonic disruption due to the possibility of the alteration of the cell wall or membrane by cholesterol. Kimoto and coworkers also noted a difference in the fatty acid distribution pattern for cells grown in the presence and absence of cholesterol (Kimoto et al. 2002). Lye and coworkers noted that an increased concentration of saturated and unsaturated fatty acids was noted in the cells incorporated with cholesterol. This altered composition in presence of cholesterol also led to an increased membrane strength and subsequently higher cellular resistance toward lysis (Lye et al. 2010). Further evaluation by the same group was conducted to determine the possible location of the incorporated cholesterol within the membrane phospholipid bilayer of probiotic cells. The researchers incorporated fluorescence probes into the membrane bilayer of probiotic cells that were grown in the absence and presence of

cholesterol. It was noted that when probiotic cells were grown in the presence of cholesterol, the incorporation of cholesterol was noted in regions of the phospholipid tails, upper phospholipids, and polar heads of the cellular membrane phospholipid bilayer.

However, certain in vitro studies such as growth performance in bile containing medium as well as the ability to bind to cholesterol were conducted using 28 different strains of *L. gasseri*, by Usman and Hosono (1999). During studies, it was noted that there was greater variation in bile tolerance contrary to the earlier studies (Gilliland et al. 1985; Klaver and van der Meer 1993). The variation in bile tolerance among 28 different strains could be due to differences in growth performance. Later on, the hypothesis of cholesterol removal by probiotic cells during different growth conditions was supported by Kimoto and coworkers. The authors observed that live and growing cells could remove more cholesterol than those which are non-growing (live but suspended in phosphate buffer) and dead (heat-killed). The cholesterol removal from media by non-growing and dead cells indicates that some cholesterol is bound to the cell surface (Kimoto et al. 2002). It has been suggested that in order to assimilate cholesterol in the intestinal tract, the organism must be bile tolerant. However, no correlation between the two has still been noted.

The in vivo studies were conducted using young pigs as experimental models to test the cholesterol assimilation in the intestine (Gilliland et al. 1985). Pigs are considered as an animal model for in vivo studies of cholesterol assimilation in the intestine, since their digestive system, the distribution of coronary arteries, and the atherosclerotic tendencies resemble those of humans (Ratcliffe and Luginbuhl 1971). The in vivo experimental studies when conducted using *L. acidophilus* RP32L significantly inhibit an increase in serum cholesterol when fed with high lipid diet. This is due to cholesterol assimilation by the *L. acidophilus* strain (Gilliland et al. 1985; Rasic et al. 1992). Agerbaek and coworkers noted diverse variation in the hypocholesterolemic effect of yogurt and other fermented milk products which could be due to different bacterial strains used in fermentation in different human studies (Agerbaek et al. 1995). The other important reason for this effect could be the viability of ingested and their ability to colonize in the small intestine, wherein the cholesterol absorption takes place.

(b) *Co-precipitation with deconjugation of bile salts:* Certain bacteria interfere with cholesterol absorption from the gut through enzymatic deconjugation of bile acids by bile salt hydrolase (BSH) and affecting cholesterol metabolism. The bacteria reported hydrolyzing conjugated bile acids are *Bacteroides* spp., Bifidobacteria fusobacteria, Clostridia, Lactobacilli, and Streptococci (Hylemon and Glass 1983). The other mechanism for cholesterol assimilation in the intestine was studied using *Lactobacillus acidophilus* and *Bifidobacterium bifidum* by Klaver and van der Meer (1993), wherein they proposed the hypothesis that cholesterol removal from the culture medium by *L. acidophilus* RP32 and other species could be related to co-precipitation with deconjugated bile salts in an acidic environment and not due to bacterial uptake of cholesterol.

Reports suggest that gut flora not only hydrogenates, dehydrogenates, and oxidizes bile acids, but also cleaves side chains to yield steroids. It was reported that the deconjugated bile acids are less soluble and less likely to get absorbed from the intestinal lumen compared to conjugated bile salts. Therefore, greater excretion of free bile acids was noted from the intestinal tract compared to their conjugated forms. Increased excretion of bile acids results in lowering in serum cholesterol concentration and therefore there will be a decline in the amount of bile acids reaching liver and a further decline in the secretion back into the intestine through enterohepatic circulation. In order to replace the excreted bile acids, more bile acids need to be synthesized from cholesterol in the liver or to reduce the absorption of cholesterol through the intestinal lumen in lipid fed subjects. This hypothesis was supported by the study conducted by Lye and coworkers (Lye et al. 2009). *L. gasseri* SBT0270 has shown the ability to suppress the reabsorption of bile acids into the enterohepatic circulation (by deconjugation) and therefore more cholesterol utilization for de novo bile acid synthesis in homeostatic response enhances the excretion of acidic steroids in feces in vitro (Usman and Hosono 1999) resulting in lowering of serum cholesterol (Begley et al. 2006; Ooi and Liong 2010).

It is still unclear in terms of microbial free bile acid properties whether it could be related to the hypocholesterolemic effects observed in vivo (Gilliland et al. 1985) since pH in the intestinal tract of humans is usually neutral to alkaline. Though it has shown promising results during in vitro studies.

(c) *pH-dependent phenomenon for cholesterol assimilation:* The aforesaid hypothesis mentioned was studied in *Bifidobacterium* species and an intense binding between the cell surface and cholesterol was observed which indicates cholesterol uptake into cells. The pre-requisites to remove cholesterol were dependent on cell growth and the presence of bile salts. The authors also concluded that cholesterol removal from the broth is not only attributed to the co-precipitation of cholesterol with deconjugated bile salts, but rather to conjugation of both effects (Tahri et al. 1995). Later on, Tahri and his team did further research to support this mechanism and studied the effect of pH on cholesterol assimilation in absence of microbial cells. At pH below 5.5, the cholesterol was partially removed when deconjugated bile salts were added. While, as soon as the pH level increased to pH 7, the precipitated cholesterol gets re-dissolved so it indicates that it is a transient phenomenon and is dependent on pH. It was also noted that resting cells of Bifidobacterium did not interact with cholesterol and it is only the growing cells that could assimilate cholesterol in their cell membrane (Tahri et al. 1996). Similar has been noted and reported for probiotics in the intestine to take up and assimilate cholesterol for stabilization of their cell membrane and binding cholesterol to cell walls of probiotics bacteria (Razin 1975; Noh et al. 1997; Tanaka et al. 1999; Lepercq et al. 2004).

(d) *Role of excessive dietary calcium in cholesterol metabolism:* There are certain research hypotheses related to the effect of the presence of dietary calcium. It is clearly stated that excessive dietary calcium binds with bile acids and suppresses

reabsorption into the enterohepatic circulation and lowers down the LDL-cholesterol level.

(e) *Enzymatic conversion of cholesterol to coprostanol:* In one of the proposed mechanisms for cholesterol metabolism, it has been reported that cholesterol is converted in the intestine to coprostanol and excreted in feces. This way there is a decline in the concentration of cholesterol being absorbed. In one of the research study, carried out by Chiang and coworkers, it was noted that *Sterolibacterium denitrificans* produces cholesterol dehydrogenase/isomerase which catalyzes the transformation of cholesterol to cholest-4-en-3-one, an intermediate cofactor in the conversion of cholesterol to coprostanol (Chiang et al. 2008). Thereafter, several researchers worked on this mechanism using strains of probiotic bacteria. The lactobacilli probiotic bacteria such as *Lactobacillus acidophilus, L. bulgaricus,* and *L. casei* ATCC 393 were evaluated for their conversion via fluorometric assays by Lye and coworkers during in vitro studies (Lye et al. 2010). It was also determined that cholesterol reductase is present both intracellular and extracellular in most of the probiotic strains thereby leading to the conversion of cholesterol to coprostanol. A decline in the cholesterol concentration along with an increment of coprostanol was also noted. Further studies need to be undertaken regarding this enzyme cholesterol reductase as it is already been administered to humans to lower blood cholesterol levels.

(f) *Generation of short-chain fatty acids:* As per another set of theory for the mechanism behind the reduction in the systemic levels of blood lipids through probiotics was explained through research evidences. The researchers suggested that the probiotics can ferment the food-derived indigestible carbohydrates and yield short-chain fatty acids (SCFA). The generation of short-chain fatty acids causes a decline in plasma cholesterol concentration either by inhibiting hepatic cholesterol or redistribution of cholesterol from plasma to the liver (Fuller and Gibson 1998). St-Onge and coworkers reported SCFA production in the large intestine at a concentration range of 100–450 mmol/day. The SCFA comprises acetate, propionate, and butyrate with a ratio of 60:20:15. The ratio depends upon the substrate used (St-Onge et al. 2000). It has been noted that generally the presence of acetate in serum increases total cholesterol, while propionate increases blood glucose and lowers hypocholesterolemic response caused by acetate. The SCFA, propionate reduces utilization by the liver for fatty acid and cholesterol synthesis. In one of the studies conducted by Wolever and coworkers, it was reported that sufficient propionate must be generated to overcome the effect of acetate generation which is a precursor for lipid synthesis. The plasma cholesterol concentration is influenced by the proportion of each fatty acid produced during fermentation (Wolever et al. 1996). Figure 10.1 depicts the schematic representation of different proposed mechanisms reported for cholesterol reduction.

Our research and development team, Department of probiotics, Synbiome, while working with *Lactobacillus plantarum* spp. strains MSD1 and MSD2 during in vitro

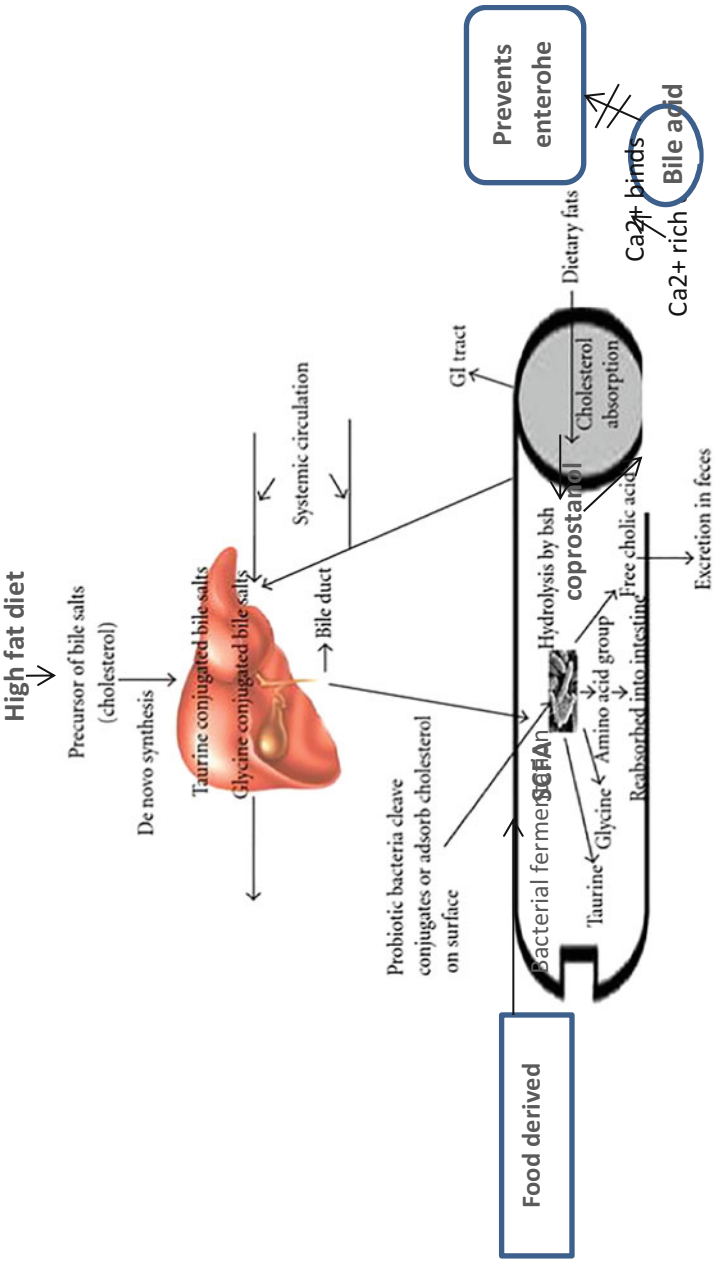

Fig. 10.1 Schematic representation of different proposed mechanisms reported for cholesterol reduction

studies experimented with three different sets for the cholesterol assimilation, wherein, in the first set, MRS medium containing cholesterol, in the second set, cholesterol supplemented with 6 mM taurocholate, and in the third set, cholesterol supplemented with 6 M sodium tauroglycocholate was used. Our findings have shown that both the strains of *L. plantarum* had significant results in terms of the amount of cholesterol assimilation. However, strain MSD1 has a greater capacity to assimilate the amount of cholesterol in all three sets along with a higher percent cholesterol removal compared to other strain MSD2. The amount of cholesterol assimilation by *Lactobacillus* spp. MSD1 in MRS medium, containing cholesterol supplemented with 6 mM sodium tauroglycocholate has led to significant cholesterol assimilation (46.72 μg/mL) with a percent cholesterol removal (66.74%) compared to the other two sets. The strain, *L. plantarum* spp. MSD1 has 2.8 times more cholesterol removal capacity in medium containing cholesterol supplemented with 6 mM sodium tauroglycocholate compared to the medium containing solely cholesterol. The amount of cholic acid released by the action of bile salt hydrolase (BSH) was assayed through the plate assay technique showing precipitation of cholic acid and through TLC technique. It was also noted that *L. plantarum* spp. MSD1 occurred in long chains in presence of bile salts and could grow even at higher concentrations of 14% bile salt till 48 h of incubation(*data not published*).

To date, most of the studies have been conducted in vitro and very few attempts have been made through in vivo trials to evaluate the possible hypocholesterolemic mechanism involved.

10.8 Conclusions

The dairy products fermented with appropriate bacterial probiotic strains could aid in lowering of circulating cholesterol concentration and thereby diminishing the risk of CHD. The fermented dairy products can be considered as functional foods which are involved in lowering of high cholesterol concentration. If the bacterial strains fulfilled certain criteria such as being bile tolerant, can deconjugate bile acids, and bind cholesterol it could lower down blood cholesterol levels. Several mechanisms have been suggested for lowering of cholesterol levels through probiotics. These include the end products of SCFA fermentation, cholesterol assimilation, binding of cholesterol to the bacterial cell wall, and enzymatic deconjugation of bile acids. The major concern was raised with the mechanism of deconjugation of bile acids wherein it was suggested the potential increased risk for colon cancer due to the carcinogenic properties of deconjugated bile acids (Sanders 2000).

However, still, the exact mechanism of action of probiotic bacteria on lowering serum cholesterol is not clear. It could be concluded that there are several mechanisms that have been studied and reported for mediating hypocholesterolemic effect by probiotics. However, all these mechanisms have been reported via in vitro studies and the mechanism is not firmly established for in vivo studies. To date, the products containing live bacteria (yogurt, acidophilus milk, and Kefir) did not retain in the human intestinal tract and are eliminated in feces. It is necessary to consume

probiotic products daily for the long-term effect on metabolism but there are certain reports on animal as well as human dietary studies wherein this concept of daily probiotic consumption has given conflicting results. Further, research studies through proper-designing of in vivo trials may disclose additional understanding and knowledge on defining the exact mechanism for lowering of cholesterol using probiotics, better safety assessment prior to consumption, to improve the strain stability characteristics, and to solve the problem of survivability in large bowl to eliminate the existing controversies on the use of probiotics for regulating lipid metabolism. Besides this, the in vitro cholesterol reduction needs to be confirmed in mixed culture and mixed substrate environment.

The underlying mechanism of cholesterol-lowering effects by probiotics needs to be explored in order to have a better understanding of the mechanisms and better formulations for human consumption.

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