Minireview

Hypocholesterolemic potential of probiotics: Concept and mechanistic insights

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Cardiovascular diseases (CVDs) have become one of the leading causes of global mortality. The raised serum cholesterol levels and its progressive accumulation in arterial walls results in development of atherosclerosis that leads to most of the heart attacks and strokes. Use of probiotics has been implicated for several health benefits including their cholesterol lowering potential and hence management of CVDs. The current review aims to describe the association of gut microbiota, probiotics and their potential mechanisms responsible for hypocholesterolemic effects. Probiotics execute hypocholesterolemic effects through several mechanisms such as bile salt hydrolase activity (BSH), deconjugation of bile salts, cholesterol assimilation, coprecipitation of cholesterol with deconjugated bile salts, removal of cholesterol with cellular surfaces through physical forces, incorporation of cholesterol in the cell membrane of the probiotics, intestinal conversion of cholesterol in coprostanol, and inhibition of Niemann–Pick C1 like 1 (NPC1L1) cholesterol transporter in the enterocytes. However, the health benefits including the hypocholesterolemic effects appear to be a strain specific phenomenon. Further studies are necessary for better understanding of the in-depth molecular mechanisms governing the hypocholesterolemic effects and to establish the probiotics as a potential non-pharmaceutical approach for management of CVDs.

Keywords: Bile salt hydrolase, Cardiovascular diseases, Gut microbiota, Short chain fatty acids

Gut microbiota, its role and other health benefits

Mammalian gastrointestinal tract (GIT) hosts highly diverse type of microorganisms referred to as gut microbiota. The complex and abundant gut microbiota reaches as high as $10^{13}–10^{14}$ microorganisms and shapes a symbiotic relationship with the host. The symbiotic relationship assists in maintaining homeostasis in the host by performing essential tasks, such as release of vital nutrients, immune system balance, and selective exclusion of pathogens from gut. Certain infections, drug intake, stress, pollution, disease state, and inflammation may lead to gut dysbiosis. Dysbiosis leads to diseases, such as Crohn’s disease, ulcerative colitis, and irritable bowel syndrome (IBS), colorectal cancer, inflammatory bowel diseases (IBD) and colon cancer. Other disorders associated with gut dysbiosis include diabetes, obesity, hypertension, immune disorders, and metabolic syndrome. From last few decades, there is an increasing interest in exploiting the benefits of the gut microbiota, and modulating its composition in order to alleviate various intestinal pathologies and disorders to maintain the health status of host. Further, gut microbiota results in production of various enzymes which promote bioconversion of cholesterol and bile acids.

The complex, dynamic, and diverse intestinal microbiota is essential for maintaining the overall health of the host. However, changes in the composition of the microbiota may lead to several metabolic disorders, such as autoimmune, allergies, obesity, inflammatory bowel disease (IBD) and diabetes. Gut microbiota plays a vital regulatory role in cholesterol and bile acid metabolism pathways. A study showed that germ free rats excreted unmodified cholesterol indicating that cholesterol was modified in the colon by colonic bacteria. In human colon, the microbes convert cholesterol into a non-absorbable sterol called coprostanol, which is usually excreted via feces. Also, the primary bile acids i.e., cholic and chenodeoxycholic acids are converted into different secondary bile acids.

Diet rich in fats alters the species composition of intestinal microbiota and plays a crucial role in development of obesity, insulin resistance, and other disorders linked to metabolic syndrome. Differences have been observed in composition of gut microbiota.
of lean and obese animals. Generally, bacteria belonging to the Bacteroidetes phylum (Gram positive bacteria (low G+C content) were reduced in number while the number of Firmicutes phylum (Gram negative bacteria) members increased proportionately.12

Some recent studies specified the alterations in gut microbiota composition could lead to development of hypercholesterolemia.10 The germ-free and conventionally raised ApoE-deficient mice fed on chow diet showed that lack of gut microbiota caused a significant increase in the plasma and hepatic cholesterol levels compared with conventionally grown ApoE/- mice.13 A significant increase in serum total cholesterol (TC), triglycerides (TG), and low density lipid-cholesterol (LDL-C) levels was observed in subjects with hypercholesterolemia compared with normcholesterolemic controls, and hypercholesterolemic subjects show lack of richness and diversity of bacterial communities.14 Gut microbiota also plays a strong role in development of inflammatory bowel diseases (IBD), such as Crohn’s disease (CD) and ulcerative colitis (UC) with characteristic dysbiosis, and pouchitis.15

Among healthy subjects, the microbiota is proficiently separated from the mucosal immune system by the gut barrier formed of highly specialized single layer of epithelial cells. Such specialized cells are equipped with innate immune functions that help in controlling the access of bacterial antigens to the mucosal immune cells.16 Numerous clinical and experimental studies suggest that dysbiosis may be averted by intervention of probiotics.12 The current article presents the health benefits of probiotics in general, and their role in prevention/management of cholesterol associated CVD.

Probiotics and diet

The increased awareness among consumers has related diet directly to individual’s good health and well being. Concept of nutrition is moving from energy needs of individual to promotion of health, and it has led to demand of foods that contain some health promoting factors that are beyond traditional nutrient value.17 Diet provides sufficient nutrients to meet the metabolic requirements of the individual, however, the science of nutrition have evolved over a past few decades, and now consumers look for additional health benefits of diet. Diet is additionally taken to modulate certain specific functions in the body.18 Therefore, in this context probiotics are viewed as natural remedies that can confer additional benefits over conventional nutrition. Probiotics are defined by the Food and Agriculture Organization of the United Nations (FAO/WHO) as “live microorganisms which when administered in adequate amounts confer a health benefit on the host”19 Long before the scientific knowhow and health benefits of probiotics, fermented food products, such as bread, yoghurt, kefir, kumis, cheese, beverages, beer, and wine were consumed in the most parts of the worlds, and were considered very healthy foods of high nutritional as well as therapeutic value.20

History of probiotics

The history of probiotics dates back to evolution of human beings. Egyptians hieroglyphs writing systems showed that traditional fermented yak milk was used by Tibetan nomads during their long treks21 and the apparent health benefits of fermented milk products were documented in early 1800s. Louis Pasteur was the first to identify that the bacteria and yeast were able to carry out process of fermentation, however, could not link such microbes to the various health benefits.22 Later in 1905, Nobel laureate Elie Metchnikoff studied the rationale of longevity among peasants of Bulgaria and related it to the health benefits of the probiotic lactobacilli used in fermented yogurt.23 Henry Tissier, a French pediatritian observed that children with diarrhea lacked particular bacteria otherwise abundant in stool of healthy children and the bacterium was later identified as Bifidobacterium spp. (a potent probiotic)23 During World War I, Alfred Nissle isolated Escherichia coli Nissle 1917 from the stools of soldiers who did not suffered from diarrhea, and such strain was used to treat ulcerative colitis24. The word ‘probiotic’ was introduced by the German scientist Werner Kollath in 1953. He defined probiotics as, ‘active substances that are essential for a healthy development of life.’ Later on in 1965, Lilly and Stillwell defined probiotics as, “substances secreted by one organism which stimulate the growth of another”. In 1992, Fuller has described probiotics as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance.”25

Probiotics as per definition

In 2001, for the first time FAO/WHO conducted an expert consultation on evaluation of health and nutritional properties of probiotics in food in Córdoba, Argentina from 1-4 October, 2001. The expert committee debated the emerging uses of
probiotics and came up with guidelines to set out a systematic approach for the evaluation of probiotics in food leading to the substantiation of health claims on recommendations of Argentinean government. Consequently, probiotics were defined as, “live microorganisms which when administered in adequate amounts confer a health benefits on the host”, and it is the most widely accepted definition. A recent meeting was organized by the International Scientific Association for Probiotics and Prebiotics (ISAPP) on 23 October 2013 to revisit the concept of probiotics and prebiotics. The panel recommended a more grammatically correct definition as, “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host”.

**Lactic acid bacteria**

A large number of lactic acid bacteria (LAB), bifidobacteria and yeast (*Saccharomyces cerevisiae, S. boulardii*) from fermented milks have been used for centuries as probiotics. Some of the important criteria for selection of LAB include their ability to survive under harsh gut conditions especially acidic pH, tolerance to high bile salt concentrations, and resistance to digestive enzymes. Typical LAB are Gram positive bacteria with low G+C content, mostly acid- and aerotolerant, organotrophic, strictly fermentative rod or coccus and they produce lactic acid as their key end product. Since probiotic bacteria have got ‘generally regarded as safe’ (GRAS) status, therefore, probiotics must be non-haemolytic and non-gelatinolytic, antibiotic sensitive, non-toxic, must not produce any biogenic amine, and must not cause any allergic reactions.

On the basis of cellular morphology, mode of glucose fermentation, optimum temperature, sugar utilization pattern, LAB are classified into major genera *Lactobacillus, Lactococcus, Pediococcus, Enterococcus, Streptococcus, Leuconostoc, Aerococcus, Alloococcus, Carnobacterium, Oenococcus, Tetragenococcus, Vagococcus and Weissella*. *Lactobacillus* represents the largest genus which includes above 100 species. *Lactobacillus acidophilus, L. salivarius, L. rhamnous, L. brevis, and L. casei* have been characterized in various studied conducted so far. Probiotic LAB have been isolated from diverse sources such as fermented dairy products, fermented meat and fish, cereals, beets, pickles, dead and decaying plant material and animal wastes, sourdough, infant feces, fermented beverages, fermented juices, poultry gut, human breast milk, camel milk, *kalarei* and others. Also, probiotics have been isolated from oral cavity, colon, ileum, and vaginal cavity of human beings.

**Health benefits of Probiotics**

Probiotics exercise multiple health beneficial effects on the host via mechanisms mediated including interference with potential pathogens, improvement in gut barrier function, production of neurotransmitters, and immunomodulation. However, the health promoting spectrum of probiotics is expanding fast. Other major health benefits include production of bacteriocins, improving the nutritional and microbial balance in the gut, anti-mutagenic effects, prevention and treatment of various types of diarrhea, alleviation of lactose intolerance, modulation of microbiota, alleviation of allergies and other atopic diseases. Furthermore, probiotics are being used for management of obesity, type 2 diabetes, improving the bowel function of patients suffering with colorectal cancer, mood enhancing effects, and as antidepressants. Additionally, probiotics have been reported to show fibrolytic, antioxidative and anti-aging effects. Some novel applications of probiotics include their usage as drug delivery vehicles and therapeutics for mental and emotional well-being of humans. However, probiotics differ widely with respect to their health benefitting properties. Some of the widely studied health benefits conferred by probiotics include prevention and treatment of diarrheal diseases, anticancer, anti-mutagenic, anti-diabetic, and management of hypercholesterolemia among others. Some of the important and widely studied health benefits of probiotics are summarized in Fig. 1.

Several cross-sectional reports suggested that alterations in the intestinal microbiota are associated not only for overall gut health but also with extra-
intestinal organs and systems. Several studies have been focused to evaluate the role of gut microbiota in regulating the gut-brain axis and its influence on mood, cognitive impairments associated with obesity and other mental disorders. Gut-brain axis engages a complex bidirectional communication arbitrated by immunological, hormonal and neural signals between gut and the brain. Any imbalance in gut-brain axis communication may lead to development of metabolic disorders such as diabetes and psychiatric disorders. Such disorders often lead to development of gut dysbiosis which may contribute to disturbance in molecular coordination between the gut and brain. Gut microbiota modulate the production of cytokines, chemokines, neuroactive metabolites and other endocrine secretions including neuroactive metabolites and endocrine secretions and their corresponding receptors. Some of the major health benefits of probiotics are summarized in Table 1.

### Hypocholesterolemic potential of probiotics

The role of probiotics is being investigated in treatment and management of complex metabolic disorders caused by a cluster of interrelated factors that increase the risk of cardiovascular diseases (CVD). According to the estimation of WHO, CVD particularly heart attacks and strokes are responsible for 17.5 million deaths annually. Furthermore, the number of deaths may grow to 23.6 million by 2030. Significant number of such fatalities is usually attributed to abnormal blood lipid profile which increases the chances of heart attack by three times in people with hypercholesterolemia when compared to individuals with normal blood lipid profiles. Diet rich in high salt, saturated fats, and devoid of complex carbohydrates, fruits and vegetables could increase the risk of CVD development. Some additional modifiable risk factors including raised LDL-C, increased TG rich lipoproteins, and low levels of high-density lipoprotein cholesterol (HDL-C) are reported to be the most significant contributors of CVDs. The other parallel risk factors include overweight and obesity, certain inflammatory markers e.g., hr-C-reactive protein, and tumor necrosis factor-α.

A WHO report pointed out that 10% reduction in serum cholesterol in men of age 40 years could decrease the heart diseases incidence by 50% within 5 years. Several psychological, physical, pharmacological and dietary interventions have been proposed for management of CVDs. Both drug therapy and non-pharmacologic approaches are commonly used strategies to manage the raised serum cholesterol levels. Despite the proven hypocholesterolemic potential of certain pharmacological agents, undesirable side effects including gastrointestinal discomfort have been

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| Probiotic formulation-EcoVag®      | Treatment of bacterial vaginosis (BV) and recurrent | Anti-inflammatory activity and improves the gut barrier function |
| capsules                            | vulvovaginal candidiasis                           | Decrease microbial translocation and inflammation parameters (IL-6) seen  |
| Sascharomyces boulardii             | Effect on HIV patients on antiretroviral therapy    | Improves insulin sensitivity, inflammatory markers and improve metabolic markers associated with type 2 diabetes patients |
reported. The cost factors of hypocholesterolemic drugs and ineffectiveness of several non-pharmacologic alternatives have made the probiotic application for management of CVDs as one of the most focused area of research.

Although cholesterol is vital and basic block for body tissues, however, raised serum cholesterol levels are considered as a major risk factor for development of coronary heart diseases (CHD). In human body, cholesterol is used for synthesis of bile, a yellow-green aqueous solution primarily composed of bile acids, phospholipids, biliverdin pigment along with cholesterol. Primary bile acids are formed from dietary cholesterol, and then conjugated with either glycine or taurine by amide bond and which results in formation of glycocholic acid (cholylglycine) or taurocholic acid during de novo synthesis of bile salts. Bile is synthesized in liver, stored and concentrated in the gall bladder and after food intake bile is released into the duodenum where it acts as a biological detergent which causes emulsifications of fats. At physiological pH, conjugated bile acids are ionized and termed as bile salts. Bile salts are amphipathic in nature and can solubilize lipids to form mixed micelles. Conjugated bile salts are hydrolyzed by bile salt hydrolase (BSH), also known as cholyglycine hydrolase, into amino acid residues (glycine or taurine) and free bile acids. Thus, BSH active probiotic bacteria and products containing such probiotics may be used for management of hypercholesterolemia. Furthermore, influence of probiotic bacteria on serum cholesterol levels have been thoroughly studied by various in vitro and in vivo trials.

**Association of high cholesterol level with onset of CVD**

Cholesterol is essential to cell membrane structure and function and is used to synthesize hormone and vitamin in mammals. However, raised serum cholesterol level increases the incidence of atherosclerotic diseases in adults, while the association is not well established among elderly people. Atherosclerosis is accompanied by dyslipidaemia, which is characterized by prominent increase in LDL-C and decreased HDL-C, which are known as the major risk factor for development and progression of atherosclerosis. In 1950s, an inverse correlation was ascertained that HDL-C (13.9%), TC (37.6%), TAG (53.9%), and a significant decrease in the TC concentration, LDL, TG, and thiobarbituric acid reactive substance in the serum and livers of hamsters fed on high-fat and high-cholesterol diet. An in vivo cholesterol lowering study using probiotic *Pediococcus pentosaceus* KID7 given to atherogenic diet-fed hypercholesterolemic mice (C57BL/6J) showed a considerable lowering in serum TC levels. Furthermore, mRNA expression of genes associated with lipid metabolism in liver,

Saeed et al. observed that remnant-like particle cholesterol, LDL-triglycerides, leads to incident cardiovascular disease development, however among animal models only LDL-triglycerides was associated with CHD and heart strokes. Cholesterol lowering drugs like statins have shown well characterized decrease in rate of myocardial infarction incidence and mortality among patients of older age. However, various side effects have been reported among young subjects, and such drugs are quite expensive. Therefore, search for alternate natural therapeutic interventions for lowering the serum cholesterol level, and hence CVDs has been a continuous research practice.

**In vivo and in vitro hypocholesterolemic studies of probiotics**

Various experimental and clinical studies have suggested that probiotic intake has beneficial effects on total lipid profile and serum cholesterol levels. Probiotic strain *Lactobacillus plantarum* Lp3, isolated from traditional Tibetan yak milk, showed high cholesterol lowering rate of 73.3% in vitro. Administration of *L. plantarum* Lp3 to rats fed a high-cholesterol diet exhibited a remarkable reduction in serum and liver cholesterol, triglycerides level, and reduced rate of lipid deposition in the cytoplasm of rat’s liver tissue. Costabile et al. (2017) observed that daily intake of encapsulated probiotic *Lactobacillus plantarum* ECGC 13110402 (2×10⁹ CFU twice daily) showed statistically significant reduction in LDL-C (13.9%), TC (37.6%), TAG (53.9%), and a significantly increased HDL-C (14.7%) in the subjects (>60 years of age; 6–12 week). Probiotic strains *L. plantarum* EM and *L. acidophilus* ATCC 43121 cells in living, resting and dead state showed significant in vitro cholesterol lowering potential; therefore, the adjunct culture may be used for lowering the serum cholesterol level regardless of its viability.

A probiotic product PROBIO S-23 (*Pediococcus acidilactici* NBHK002, *Bifidobacterium adolescentis* NBHK006, and *L. rhamnosus* NBHK007) exhibited significant decrease in the TC concentration, LDL, TG, and thiobarbituric acid reactive substance in the serum and livers of hamsters fed on high-fat and high-cholesterol diet. An in vivo cholesterol lowering study using probiotic *Pediococcus pentosaceus* KID7 given to atherogenic diet-fed hypercholesterolemic mice (C57BL/6J) showed a considerable lowering in serum TC levels. Furthermore, mRNA expression of genes associated with lipid metabolism in liver,
such as LDL-receptor, cholesterol-7α-hydroxylase and apolipoprotein was significantly increased in the group fed on probiotic supplements67.

In another study, probiotic bacteria L. fermentum NCIMB 2797 and NCIMB 5221 showed cholesterol assimilation of 70.30±8.85% under in vitro conditions66. Supplementation of multispecies probiotic product Ecologic® Barrier for 12 weeks in a randomized, placebo-controlled, double-blind intervention, positively affected the cardiometabolic parameters and gut permeability among pregnant women in a dose dependent manner7. In vivo studies showed that a novel probiotic strain Lactobacillus plantarum DMDL 9010 (dose of 10⁹ cells per day) significantly reduced the serum TC, LDL-C and atherosclerosis index by 23.03, 28.00 and 34.03%, respectively, however, no significant reduction was observed in the levels of TG, and serum HDL-C69. Another study substantiated these results, and reported that the probiotics mixture comprising of three species of Bifidobacterium (B. longum, B. lactis, and B. breve) and two species of Lactobacillus (L. reuteri and L. plantarum) significant increased the HDL-C levels with remarkable reduction in serum TC, TG, and LDL-C among hypocholesterolemic rats. Additionally histological evaluation of liver tissues indicated considerable decrease in the lipid accumulation70. Lactobacillus plantarum LRCC 5273 improved the diet-induced hypercholesterolemia in C57BL/6 mice through activation of hepatic and intestinal liver X receptor-α (LXR-α) which resulted in increased fecal cholesterol and bile acids excretion via feces71.

Though enough evidences are available based on in vitro and in vivo studies which substantiate the hypocholesterolemic potential of probiotics. But the exact cholesterol lowering mechanisms are still obscure, and needs more in-depth studies. Cholesterol lowering potential of probiotics is determined by several complex and intricate factors viz. the strain specific effects of probiotics, dosage of probiotics, metabolic and enzymatic potential of probiotics, carrier (food matrices etc.) of probiotics, viable numbers, and above all the discreet and specific physiology and metabolism of the individual hosts and their responsiveness to probiotic mediated health benefits in general and cholesterol lowering ability in particular. Some of the well studied cholesterol lowering mechanisms includes bile salt hydrolase (BSH) action, cholesterol assimilation, deconjugation of bile salts mediated by BSH activity70, binding of cholesterol to cellular surface66, coprecipitation of cholesterol with deconjugated bile salts, incorporation of cholesterol into probiotic cell membranes18, production of short chain fatty acids (SCFA) from dietary carbohydrates, conversion of cholesterol to coprostanol58, cholesterol removal by probiotic metabolites such as exopolysaccharides (EPS)5, and numerous amino acids and peptidoglycan in probiotic cell walls58.

Additional hypocholesterolemic mechanisms explored includes stimulation of liver X receptors (LXRs) which down regulates the Niemann-Pick C1-like 1 (NPC1L1) receptor expression72, down regulation of some key genes involved in intestinal cholesterol biosynthesis73, inhibition of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, which catalyses the rate limiting step of cholesterol synthesis58 and up regulation of certain essential genes involved in lowering the total lipid profile (Fig. 2). Some of the most important mechanisms are discussed below.

**Bile salt hydrolase (BSH) activity**

The bile salt hydrolase (BSH) activity of probiotics has being associated with their cholesterol lowering potential through interaction with host bile salt metabolism18. BSH active probiotics have an advantage to survive and colonize the small intestine where the enterohepatic cycle takes place, and therefore, BSH activity is considered as an important colonization factor as well55. Probiotics come across significant amount of conjugated bile salts in a mammalian gut which have been suggested to suppress the bacterial growth either through direct antimicrobial effects, up-regulation of host mucosal defenses, or synergistic action of both the mechanisms18. However, probiotics which have BSH
activity may deconjugate the bile salts into free bile acids and amino acids that are eventually excreted through faeces. Due to faecal excretion of bile salts, smaller amount of bile salts are transported back to the liver by the enterohepatic circulation. Shortage of bile salts increases the demand for de novo cholesterol synthesis in the liver. Hence, in the liver the expression of the LDL-α receptor is increased, which results in increased hepatic uptake of LDL-C, thereby, LDL-C and TC concentrations are lowered from the circulation.

Deconjugation of bile via BSH activity

Serum cholesterol lowering has been considered as one of the key benefits of probiotic consumption, and such benefits may be ascribed to enzymatic deconjunctive activity of bile acids. Deconjugated bile salts are least soluble and less efficiently reabsorbed from the gut lumen compared to their conjugated counterparts. Furthermore, free bile salts are less efficient to cause emulsification, and hence absorption of lipids in the gut. In order to maintain a steady physiological state, bile salts that have escaped the enterohepatic circulation ought to be replaced by synthesis of new bile salts in the liver using cholesterol as a precursor and consequently decreasing the serum cholesterol level. Therefore, the deconjugation of bile acids leads to hypocholesterolemic effects either by increasing the demand of cholesterol for de novo synthesis of bile acids or by reducing the cholesterol solubility and, thereby, absorption of cholesterol throughout the intestinal surface (Fig. 3). Several animal and human studies support that the cholesterol lowering potential of probiotics could be attributed to bile salt deconjugation action of BSH.

Additionally deconjugated bile acids have higher binding affinity towards nuclear receptors such as farsenoid X receptor (FXR), leading to a suppressed transcription of the cholesterol 7-alpha hydroxylase (7AH), an enzyme which catalyzes bile acid synthesis from cholesterol, thereby, influencing the cholesterol synthesis. The bile acid–FXR complex is formed by binding of bile acids to FXR, which in turn bind to the promoter region of the Cyp7a gene that is responsible for transcription of the rate limiting enzyme 7AH, leading to reduced bile acid synthesis. The FXR-α and membrane GPCR TGR-5 are specifically activated by bile acids which causes feedback inhibition of bile acid synthesis and regulation of lipid, as well as of glucose and energy metabolism in the host. The activation of receptor FXR-α has been found to reduce LDL-C level.

Cholesterol assimilation by probiotics

Probiotic strains have been reported to exert significant cholesterol lowering potential under in vivo and in vitro conditions. One of the leading mechanisms adopted by probiotics is cholesterol assimilation that helps them to lower the serum cholesterol levels. The cholesterol assimilation in GIT would allow lowering of cholesterol absorption by enterocytes. Assimilation of cholesterol by growing probiotic cells could reduce the cholesterol that is readily available in intestines for absorption. Transcriptomic analysis of Bifidobacterium bifidum PRL2010 cells grown in presence of cholesterol showed a significant increase in transcription level of genes encoding for putative transporters and reductases that might act during cholesterol assimilation. In another study, Lactobacillus acidophilus ATCC 43121 grown in presence of cholesterol micelles and bile salts showed resistance to lysis by sonication, which was ascribed to hardening of bacterial cells due to assimilation of cholesterol into the cellular membrane. Thus, assimilation of cholesterol by growing probiotic cells could lower the amount of cholesterol available for absorption from the intestinal lumen.

Binding of cholesterol to cellular surface of probiotics

Cholesterol may be removed by probiotic cells through binding onto their cell surfaces, thus making the cholesterol unavailable for absorption leading to hypocholesterolemic effects and it may also hinder the formation of intestinal cholesterol micelle. The formation of micelle needs bile salts, phospholipids and cholesterol molecules, and any shortage in cholesterol may lead to formation of...
Co-precipitation of cholesterol with deconjugated bile salts

Co-precipitation of cholesterol in presence of deconjugated bile salts is adopted by probiotics for cholesterol lowering. Cholic acid released due to deconjugation of bile salts, causes lowering of pH (<5.0) thus, leading to the co-precipitation of cholesterol in the acidic environment. A strong correlation was reported between deconjugation ability of Lactobacillus strains and assimilation of cholesterol from micelles into their cellular membranes and co-precipitation of cholesterol in the presence of cholic acid. Another study substantiated an appropriate correlation between glycocholate/taurocholate deconjugation and cholesterol co-precipitation by the selected Lactobacillus strains. Cholesterol co-precipitation to the extent of 9% was observed due to the action of selected Lactobacillus strains. Different strains showed varied level of coprecipitation action. Furthermore, cholesterol co-precipitation was reported to be the major factor for hypocholesterolemic effects due to its dependency on low pH (3.8–4.9), and cholic acid being in its protonated form (non-ionized). However, the intestinal pH is not likely to be lower than 6.0. Thus, co-precipitation of cholesterol represents one of the key means that might be used to lower the raised serum cholesterol levels.

Conversion of cholesterol to 5β-coprostanol

Both dietary and endogenous cholesterol is excreted through trans-intestinal efflux. In the intestinal lumen, cholesterol is converted to an insoluble compound 5β-coprostanol (5β-cholestan-3β-ol) and in minor amount of coprostanone. Such metabolites are less soluble, therefore, show least intestinal absorption, and are eliminated with the faeces, which leads to a decrease in the intestinal absorption of cholesterol. Several studies have been conducted to investigate the possible mechanism of cholesterol conversion into coprostanol by probiotic bacteria. Chiang et al. (2008) reported that bacteria Sterolibacterium denitrificans produce cholesterol dehydrogenase/isomerase which catalyzes the biohydrogenation of cholesterol to cholest-4-en-3-one, an intermediate cofactor in the conversion of cholesterol to 5β-cholestan-3β-ol. Several of the probiotic isolates were reported to produce cholesterol reductase. Furthermore, cholesterol lowering was observed with a gradual increase in intracellular and extracellular coprostanol levels. Transcriptomic analysis Bifidobacterium bifidum KDK411 grown in presence of cholesterol demonstrated the significant increase in the transcription of genes encoding for putative transporters and reductases that are used in cholesterol assimilation and its conversion to coprostanol. Similarly, high reductase activity of probiotic Leuconostoc mesenteroides subsp. mesenteroides KDK411 was associated with the increased fecal excretion of coprostanol. Thus, conversion of cholesterol to 5β-cholestan-3β-ol might be responsible for hypocholesterolemic effects mediated by probiotics in hypocholesterolemic subjects.

Role of short chain fatty acids (SCFA) in hypocholesterolemic potential of probiotics

The non-digestible carbohydrates, including cellulose, xylan, resistant starch and inulin, present in diet are fermented by colonic anaerobic bacteria to yield energy for their microbial growth. In this process, SCFA are produced as the end products of
non-digestible carbohydrates metabolism. Therefore, formation of SCFA is an outcome of a complex interaction between diet and the gut microbiota within the hosts’ gut. The SCFA appears to be natural ligands to various cell surface receptors such as free fatty acid receptor 2 and 3 (FFAR2/3), across a range of cell (entero-endocrine and immune cells) and tissue types. SCFA correspond to a major carbon flux from the diet mediated by action of gut microbiota and support the regulatory role of SCFA in local, intermediary and peripheral metabolism. Recently, evidences have emerged for regulation of cholesterol metabolism by SCFA production and the major SCFA produced by microbial fermentation in gut includes acetate, propionate and butyrate. Although probiotic metabolites such as SCFA can be used as energy sources by the host, however, SCFA mainly acts as regulators of energy intake and energy metabolism.

The SCFA such as acetate, propionate, and butyrate play vital role in glucose, cholesterol, and lipid metabolism. Studies have suggested that butyrate inhibits activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, the rate limiting enzyme in endogenous cholesterol production, and decreases the transformation of primary to secondary bile acids as a result of colonic acidification. HMG CoA reductase is suppressed by cholesterol derived from internalized degradation of LDL-C, and its inhibition induces the expression of LDL-receptors in liver, which in turn enhances the catabolism of LDL-C.

On a daily basis, 100-450 mM SFCA are produced in the large intestine with relative proportions of acetate, propionate, and butyrate in ratio of 60:20:15 depending on the substrate, where acetate appears to increase TC, propionate increases glucose in the blood and reduces hypercholesterolemia response caused by acetate. Propionate does that by decreasing its usage by the liver, for cholesterol and fatty acids synthesis. In addition to inhibiting cholesterol synthesis, SCFA also hinders the synthesis of fatty acid (FA) and lipolysis, and causes stimulation of the oxidation of FA and thermogenesis in the body. Several cholesterol lowering drugs such as statins also target the enzyme HMG CoA reductase by competitively inhibiting it during conversion of HMG-CoA to mevalonate which is an early rate limiting step in cholesterol synthesis. Thus, SCFA produced by probiotics may be a novel strategy to regulate the cholesterol biosynthesis.

Hypocholesterolemic effect of probiotics by downregulation of Niemann-Pick C1-like 1 (NPC1L1) genes

Hypocholesterolemic effect of probiotics is mediated through excretion of cholesterol either through biliary tract from the liver or excretion through enterocytes. Cholesterol in the plasma is excreted by ATP-binding cassette sub-family G members 5/8 (ABCG5/G8). The activation of LXR-α (transcription factor) increases expression of ABCG5/G8, which is essential for intestinal cholesterol excretion. Activation of nuclear receptor LXR-α downregulates the expression of Niemann-Pick C1-Like 1 (NPC1L1), a gene associated with cholesterol absorption in intestines. Several studies have shown that probiotics potentially suppress the cholesterol uptake, and promote its efflux in enterocytes. Probiotic strains Lactobacillus rhamnosus BFE5264 and L. plantarum NR74 have been shown to affect the cholesterol absorption through down-regulation of NPC1L1 expression. LXRα negatively regulate the genes of cholesterol biosynthesis enzymes, farnesyl diphosphate farnesyl transferase and lanosterol 14α-demethylase. Therefore, LXRα play a key role in regulating the cholesterol biosynthesis. Therefore, role of NPC1L1 and the LXR underscores the basis for the use of probiotics in managing hypercholesterolaemia. Thus, downregulation of NPC1L1 may help in lowering the cholesterol uptake in the intestines and re-uptake in the liver.

Conclusion

Probiotics have received much attention during past few decades due to their proclaimed health benefits including the cholesterol lowering potential. The association of high cholesterol level with onset of CVD diseases has been well established and the role of probiotics in lowering the raised serum cholesterol has been targeted. Probiotics help in lowering raised cholesterol level both by physiological processes and at molecular level by increasing the expression of gene products and receptors involved in cholesterol utilization and excretion. Probiotics mediated multiple hypocholesterolemic mechanisms help lowering the CVD risk factors and subsequent global mortality. Meta analysis must be carried out to understand the probiotic mediated cholesterol lowering mechanisms and effects thereof. Probiotics confer wide range of health benefits, including hypocholesterolemia in a strain specific manner. Probiotics have the strong potential to provide natural and safe alternative to pharmacological approaches for managing CVDs.
Future perspectives

The exact mechanism for cholesterol removal is poorly understood, thus different in vivo- and human clinical studies must be carried out to substantiate such health claims. Both probiotics and their derivatives such as exopolysaccharides exhibit the cholesterol lowering benefits, thus, a conglomeration containing both probiotics and their derivatives might be crucial for CVD management. Since probiotics mediate hypcholesterolemic effect in a strain/specie specific manner, thus, the mechanism governing the cholesterol lowering must be studied in depth at a strain level/specie level. The safety studies of cholesterol lowering bacteria and their products must also be studied in detail. Furthermore, molecular studies pertaining to expression of genes involved in hypocholesterolemia must be executed.

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