Mechanisms responsible for the hypocholesterolaemic effect of regular consumption of probiotics

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Abstract

CVD affect a large proportion of the world's population, with dyslipidaemia as the major risk factor. The regular consumption of both probiotic bacteria and yeast has been associated with improvement in the serum lipid profile. Thus, the present review aims to describe and discuss the potential mechanisms responsible for the hypocholesterolaemic effect of regular consumption of probiotic bacteria and yeast. Regarding the hypocholesterolaemic effect of probiotic bacteria, the potential mechanisms responsible include: deconjugation of bile salts; modulation of lipid metabolism; and decreased absorption of intestinal cholesterol through co-precipitation of intestinal cholesterol with the deconjugated bile salts, incorporation and assimilation of cholesterol in the cell membrane of the probiotics, intestinal conversion of cholesterol in coprostanol, and inhibition of the expression of the intestinal cholesterol transporter Niemann–Pick C1 like 1 (NPC1L1) in the enterocytes. The potential mechanisms responsible for the hypocholesterolaemic effect of probiotic yeasts include: deconjugation of bile salts; co-precipitation of intestinal cholesterol with the deconjugated bile salts; incorporation and assimilation of cholesterol in the cell membrane; and inhibition of hepatic cholesterol synthesis. The regular consumption of probiotic bacteria and yeast, as a non-pharmaceutical approach to help manage cardiovascular risk, holds promise, according to the beneficial hypocholesterolaemic effects described herein. However, the hypocholesterolaemic effects vary according to the strains used, the physiological state of the host, and the type of diet to which the probiotics are added. Further studies are necessary to fill the gaps with regard to the knowledge related to this topic.

Key words: Dyslipidaemias: CVD: Lipid metabolism: Probiotics

Introduction

CVD are the leading cause of morbidity and mortality worldwide[1]. Although there are multiple risk factors for the development of CVD, dyslipidaemia remains the major risk factor. Epidemiological and clinical studies have observed a strong association between high serum concentrations of total cholesterol (TC) and LDL-cholesterol, with increased risk of developing CVD[2].

According to Lahti *et al.*[3], the overall lipid content in human serum can be influenced by the metabolic activity of the intestinal microbiota. The intestinal microbiota is composed of a complex, dynamic, and diverse collection of micro-organisms that inhabit the gastrointestinal tract. These micro-organisms are essential for maintaining the health state of the host, whereas changes in the composition of the microbiota have been considered a risk factor for the development of some chronic diseases, such as CVD[4].

The composition of the intestinal microbiota can be modulated by the regular consumption of probiotics, defined as, 'live microorganisms, which when administered in adequate amounts confer a health benefit on the host'[5]. Accordingly, it had been suggested that the regular consumption of probiotics can decrease the serum cholesterol concentration (hypocholesterolaemic effect)[2].

Mann & Spoerry[6] were the first researchers to report the hypocholesterolaemic effect of regular consumption of milk fermented by *Lactobacillus acidophilus* in Maasai tribesmen. Since then, many studies have been performed to evaluate the hypocholesterolaemic effect of probiotic food and micro-organisms (Tables 1 and 2). However, the results of these studies vary from a significant decrease in serum TC concentration, an unchanged serum concentration, and an increase in the serum concentration. These contradictory results can be a consequence of different experimental designs, amounts of probiotics consumed, the strains of probiotics used, the viability of the probiotic micro-organism, the content of lipids and cholesterol in the diet, the animal model used, the intervention time, and an inadequate sample size[7,8].

According to Manson[9] a 1 % reduction in serum TC concentration yields a 2 to 3 % reduction in the risk of coronary disease. Thereby, identification of probiotics that exhibit a hypocholesterolaemic effect is of great interest since they are safe and does not cause the accumulation of toxic substances in the body, as therapeutic drugs do. Additionally, probiotics are cheaper in comparison with therapeutic drugs[10,11].

Table 1. Hypocholesterolaemic effect of regular consumption of probiotics in experimental studies

Probiotic strains	Animals and diet	Probiotic intake	Duration	Effects	References
Lactobacillus acidophilus ATCC 4356	Sprague–Dawley rats, hypercholesterolaemic diet	10^9 CFU/d	4 weeks	↓ TC, LDL, TAG = HDL	Huang *et al.* (2010)[77]
L. acidophilus A4	Male Sprague–Dawley rats, hypercholesterolaemic diet	–	4 weeks	↓ TC, LDL, HDL	Lee *et al.* (2010)[78]
Sonication-killed *Bifidobacterium longum* SPM1207	Sprague–Dawley rats, high-cholesterol diet	10^8–10^9 CFU/ml	3 weeks	↓ TC, LDL = HDL	Shin *et al.* (2010)[79]
L. plantarum 9-41-A and *L. fermentum* M1-16	Male Sprague–Dawley rats, hypercholesterolaemic diet	10^9 CFU/ml/d	6 weeks	= HDL ↓ TC, LDL, TAG	Xie *et al.* (2011)[80]
Yoghurt containing *B. pseudocatenulatum* G4 and *B. longum* BB536	Male Sprague–Dawley rats, hypercholesterolaemic diet	Fermented with 1 % of each strain	8 weeks	↓ TC, LDL, VLDL, TAG ↑ HDL	Al-Sheraji *et al.* (2012)[81]
L. reuteri ATCC 4659, DSM and L6798	Male ApoE-/- mice, Western diet supplemented with 0·2 % cholesterol	10^9 CFU/d	12 weeks	= TC, TAG	Fäk & Bäckhed (2012)[82]
Pediococcus parvulus 2·6 and its exopolysaccharides	Female LDL-receptor-deficient mice, hypercholesterolaemic diet	2 % added to the diet	6 weeks	= TC, TAG	Lindström *et al.* (2012)[83]
Dahi prepared by *L. plantarum* Lp9	Male Wistar rats, hypercholesterolaemic diet	20×10^8 CFU/g	120 d	↓ TC, LDL, VLDL, TAG ↑ HDL	Mohania *et al.* (2013)[84]
Cheddar cheese manufactured with *L. plantarum* K25	Male Kunming mice, high-cholesterol diet	2·0 g cheese/kg body weight	4 weeks	↓ TC, LDL = HDL, TAG	Zhang *et al.* (2013)[85]
L. casei Shirota YIT9029	Male ApoE-/- mice, AIN-93G purified diet	10^8, 10^{10} or 10^{12} CFU/ml	16 weeks	↓ TC, LDL, TAG, ↑ HDL	Tang *et al.* (2014)[86]
B. bifidum PRL2010	Male ApoE-/- mice, AIN-93G purified diet	10^9 CFU/d	20 d	↓ TC = HDL, TAG	Zanotti *et al.* (2015)[37]

ATCC, American Type Culture Collection; CFU, colony-forming units; ↓, decreased; TC, total cholesterol; LDL, LDL-cholesterol; =, did not change; HDL, HDL-cholesterol; VLDL, VLDL-cholesterol; ↑, increased; AIN, American Institute of Nutrition.

Table 2. Hypocholesterolaemic effect of regular consumption of probiotics in clinical trials

Probiotic strains	Subjects	Trial design	Probiotic intake	Duration	Effects	References
Yoghurt containing *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12	Female adults with TC < 6·2 mmol/l, TAG < 2·3 mmol/l and a BMI < 30 kg/m^2	Randomised, triple-blind	300 g/d of the yoghurt ($3·9 \times 10^7$ CFU of both strains)	6 weeks	= TAG, LDL ↓ TC ↑ HDL	Sadrzadeh-Yeganeh *et al.* (2010)[87]
Kefir enriched with an antioxidative probiotic *L. fermentum* ME-3	Healthy adults with mean BMI 30 ± 5 kg/m^2	Randomised, double-blind, placebo-controlled	200 ml of enriched *kefir* (2×10^8 CFU/g *kefir*)	2 weeks	↓ Oxidised LDL, TAG = TC, LDL ↑ HDL	Kullisaar *et al.* (2011)[88]
L. acidophilus La5 and *B. animalis* Bb12	Pregnant women at their third trimester, primigravida, aged 18–30 years	Randomised single-blind, placebo-controlled	200 g/d of a yogurt with a total of 10^7 CFU/ml	9 weeks	↓ TC, LDL, HDL, TAG	Asemi *et al.* (2012)[89]
L. rhamnosus GG (ATCC 53103)	Healthy adults with a mean age of 42 years and a mean BMI of 24 kg/m^2	Randomised, double-blind, placebo-controlled	250 ml of a milk containing $6·2 \times 10^7$ CFU/ml	3 weeks	= TC, LDL, HDL, TAG	Lahti *et al.* (2013)[3]
L. plantarum	Patients with the metabolic syndrome	Randomised, cross-over	80 ml of fermented milk/d (10^7 CFU/g)	90 d	↓ TC, glucose, homocysteine levels	Barreto *et al.* (2014)[90]
B. animalis subspecies *lactis* MB 2409, *B. bifidum* MB 109 and *B. longum* subspecies *longum* BL04	Hypercholesterolaemic children aged 6–18 years	Randomised, double-blind, placebo-controlled, cross-over	One capsule/d (10^9 CFU/g of each strain)	32 weeks	↓ TC, LDL, TAG ↑ HDL	Guardamagna *et al.* (2014)[91]
Yogurt containing *L. acidophilus* La5 and *B. lactis* Bb12	Adults with type 2 diabetes who had LDL ≥ 100 mg/dl	Randomised, double-blind controlled trial	300 g yogurt/d twice per d	8 weeks	↓ LDL ↑ HDL = TC, TAG	Mohamadshahi *et al.* (2014)[92]
VSL#3	Healthy adults with overweight (BMI > 25 kg/m^2)	Randomised, placebo-controlled trial	$112·5 \times 10^9$ CFU/capsule of all strains	6 weeks	↓ LDL, TAG = TC, HDL, VLDL	Rajkumar *et al.* (2014)[93]

TC, total cholesterol; CFU, colony-forming unit; =, did not change; LDL, LDL-cholesterol; ↓, decreased; ↑, increased; HDL, HDL-cholesterol; VLDL, VLDL-cholesterol.

Some mechanisms responsible for the hypocholesterolaemic effect of probiotics have been suggested from *in vitro* and experimental studies, although they are not fully known[3]. The mode of probiotic action is likely to be multifactorial and strain-specific, but usually includes aspects of microbial physiology, microbial ecology and host physiological response[12].

Therefore, the present review aims to describe and discuss the potential mechanisms suggested by the scientific literature as being responsible for the hypocholesterolaemic effect of regular consumption of probiotic bacteria and yeasts.

Mechanisms responsible for the hypocholesterolaemic effect of regular consumption of probiotic bacteria

Cholesterol homeostasis in our body is regulated primarily by the liver and also by the intestine. The liver regulates the *de novo* synthesis of cholesterol, the production of bile salts, which are the principal form of cholesterol excretion, the secretion of lipids in the bloodstream in the form of VLDL, the synthesis and storage of cholesterol, and also modulates the expression of the cellular receptor responsible for the uptake of blood lipoproteins by the liver (LDL receptor). Regarding the intestine, it regulates the absorption and excretion of dietary cholesterol, and the excretion of bile salts in the faeces[13]. Thus, for treatment with probiotics to provide a hypocholesterolaemic effect, these micro-organisms must act mainly on the liver and intestine.

Probiotic bacteria can regulate the host's cholesterol metabolism although it is important to consider that different strains may have unique clinical effects since each strain may use different mechanisms, alone or in combination, to cause the hypocholesterolaemic effect[3,14]. Then, we will describe and discuss the potential mechanisms responsible for the hypocholesterolaemic effect of regular consumption of probiotic bacteria (Fig. 1).

Deconjugation of bile salts

In a typical Western diet, dietary cholesterol intake is approximately 300–450 mg/d. This amount of cholesterol will complement the 800–1400 mg of endogenous cholesterol in the bile. In this manner, about 1000–2000 mg/d of cholesterol reaches the intestinal lumen[15]. After playing their role in the digestion process, bile salts are reabsorbed from the intestine by passive diffusion along the gut and by active transport in the terminal ileum (enterohepatic circulation). This process is highly efficient; however, it is estimated that 400 to 800 mg of the bile salts remain in the intestinal lumen, becoming a substrate for microbial activity in the large intestine[16]. Thus, bile salts can be deconjugated by the intestinal microbiota[17].

The bile salts are deconjugated by the action of the enzyme bile salt hydrolase (BSH)[17]. Many of the bacteria that comprise the intestinal microbiota are BSH-positive, including some species of the genera *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Clostridium* and *Bacteroides*. Although the activity of BSH is low or not detected in those bacteria that are not commensal in the gastrointestinal tract, the *bsh* gene can be acquired horizontally among different micro-organisms. The activity of the BSH enzyme provides an advantage for these micro-organisms to survive and colonise the small intestine since the conjugated bile salts have an anti-bacterial effect[18].

BSH hydrolyses the amide bond (C-24N-acyl amide) of the bile salts with its conjugated amino acids, releasing glycine or taurine from the steroid nucleus. The steroid nucleus is now called a deconjugated or unconjugated bile salt. In the deconjugated form, the bile salts have low solubility and are absorbed in lower amounts by the enterocytes than the conjugated form; therefore, they are eliminated with the faeces[18]. With the increasing faecal excretion of bile salts, fewer of them are carried back to the liver by the enterohepatic circulation, which increases the demand for cholesterol for *de novo* synthesis of bile salts in the liver. Thus, the liver increases the expression of the LDL receptor, increasing the hepatic uptake of LDL-cholesterol from the circulation[15]. Thereby, serum LDL-cholesterol and TC concentrations are reduced.

This mechanism has been considered to be mainly responsible for the hypocholesterolaemic effect caused by the regular consumption of probiotic bacteria since cholesterol and bile salt metabolism are closely related[19]. On account of this, probiotic bacteria positive for the BSH enzyme have been evaluated for their hypocholesterolaemic effect in experimental and clinical studies.

In this manner, in an experimental study with hypercholesterolaemic rats treated, during 30 d, with three strains of *Bifidobacterium* (*B. bifidum* MB 109, *B. breve* MB 113 and *B. animalis* subsp. *lactis* MB 2409; 0.33×10^9 colony-forming units (CFU)/d of each strain) capable of deconjugating the bile salts glycocholic and taurodeoxycholic, a significant decrease in serum TC and LDL-cholesterol concentrations was observed[10]. This hypocholesterolaemic effect was assigned to the increased activity of BSH; however, the concentration of bile salts in the faeces was not determined. Also, rats fed a cholesterol-enriched diet and treated with different doses (10^8 or 10^9 CFU/ml) of *L. casei* F0822, for 3 weeks, exhibited a significant decrease in serum LDL-cholesterol and TC concentrations when compared with the control group. Nonetheless, serum HDL-cholesterol concentration was not changed by probiotic treatment. Additionally, it was observed that there was a negative correlation (r −0.83; $P < 0.05$) between daily faecal bile salt excretion and serum cholesterol concentration[20]. Thus, the hypocholesterolaemic effect was attributed to the deconjugation of bile salts.

In a clinical study conducted on hypercholesterolaemic subjects, who consumed yogurt containing microcapsules of *L. reuteri* NCIMB 30242, for 10 weeks, a reduction in serum TC, non-HDL-cholesterol and LDL-cholesterol concentrations was observed, in comparison with the placebo group. However, the group that consumed the probiotic did not excrete more bile salts in the faeces than the placebo group, despite *L. reuteri* NCIMB 30242 being BSH-positive[21]. However, when the same strain was consumed in the encapsulated form (2.9×10^9 CFU/capsule, twice per d) by hypercholesterolaemic subjects, during 13 weeks, there was an increase in the serum deconjugated bile acid concentration. This was negatively associated (r −0.369; $P = 0.003$) with the hypocholesterolaemic effect caused by the treatment with these probiotic bacteria[22].
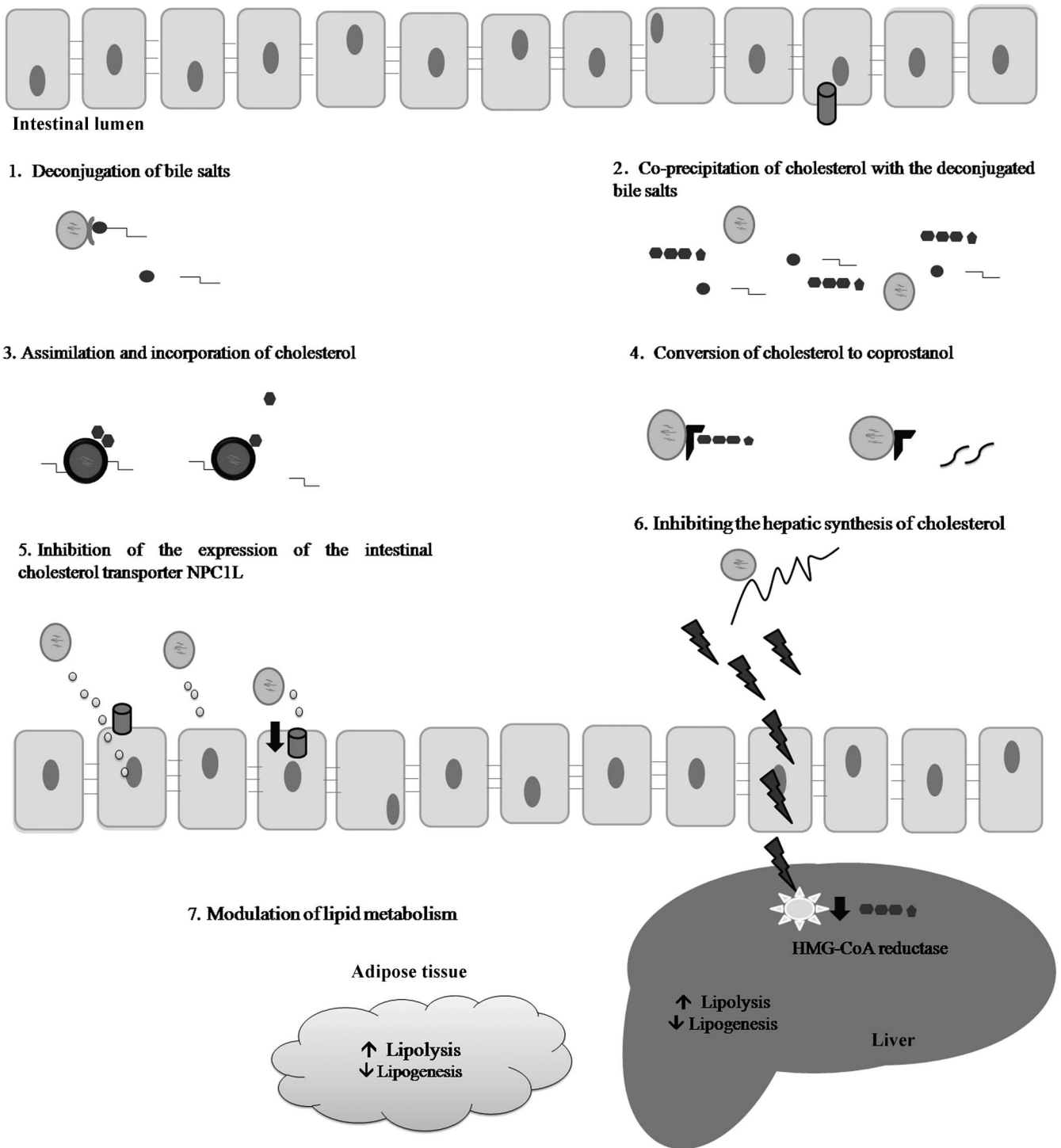
Fig. 1. Main mechanisms responsible for the hypocholesterolaemic effect of regular consumption of probiotic bacteria. Some probiotic bacteria are able to (1) deconjugate bile salts by the action of the bile salt hydrolase enzyme. Also, (2) co-precipitation of cholesterol with deconjugated bile salts, (3) assimilation and incorporation of cholesterol into the cell membranes, (4) conversion of cholesterol to coprostanol by the cholesterol reductase enzyme and (5) inhibition of the gene expression of the intestinal cholesterol transporter Niemann–Pick C1 like 1 (NPC1L) are some of the mechanisms by which probiotic bacteria reduce the absorption of intestinal cholesterol and lipids. The (6) fermentation of indigestible carbohydrates by probiotic bacteria leads to the production of propionate, which inhibits the activity of the liver enzyme 3-hydroxy-methyl-3-glutaryl-CoA (HMG-CoA) reductase. Additionally, these bacteria can modulate lipid metabolism (7), contributing to the hypocholesterolaemic effect.

Therefore, we can see here a clear example of how different experimental designs can influence the hypocholesterolaemic effect associated with a strain.

Studies are being conducted to evaluate the hypocholesterolaemic effects of an isolated BSH enzyme from microorganisms since there is a great interest in the pharmaceutical

industry for the use of this isolated enzyme for the treatment of hypercholesterolaemia[23]. Thus, Sridevi *et al.*[24] observed that hypercholesterolaemic rats treated with 10 IU/kg of a BSH enzyme isolated from *L. buchneri* American Type Culture Collection (ATCC) 4005, showed a 50 % reduction in serum TC and a reduction of 15 % in TAG concentration. Furthermore, treatment with 20 IU/kg of BSH resulted in a reduction of 58 and 45 % in serum TC and TAG concentrations, respectively. Thus, isolated use of the BSH enzyme also shows promising results with regard to the hypocholesterolaemic effect.

According to Miremadi *et al.*[25] when choosing a BSH-positive probiotic, it is preferable that the probiotics are able to deconjugate glycoconjugated bile salts instead of tauro-conjugated bile salts. Since, during the deconjugation of tauroconjugated bile salts, hydrogen sulfide is produced, which is highly toxic to the host, increasing the colonocyte turnover, which can lead to the development of inflammatory bowel disease and colorectal cancer. Also, glycoconjugated bile salts are the predominant components (the ratio of glycoconjugated bile salts to tauroconjugated bile salts is 3:1) of the bile salts in the human intestine, so it is postulated that probiotics that prefer this type of bile salt can be more effective in lowering serum cholesterol concentrations.

With an increase in the excretion of bile salts in the faeces, an increase in the production of bile salts by the liver is expected. However, few studies have investigated whether this change really occurs. Park *et al.*[26] observed that Sprague–Dawley rats fed a cholesterol-enriched diet and treated with *L. acidophilus* ATCC 43121 (2×10^6 CFU/d), for 3 weeks, showed an increase in the expression of LDL receptor mRNA in the liver, as compared with the control group. The increased expression of this receptor increased the uptake of LDL particles, leading to decreased serum TC and LDL-cholesterol concentrations. The captured cholesterol was probably utilised for the synthesis of bile salts since the treatment with *L. acidophilus* ATCC 43121 increased the faecal excretion of bile salts.

It has also been observed that treatment with a probiotic is capable of increasing expression of the 7α-hydroxylase enzyme (also known as CYP7A1), which is a rate-limiting enzyme in the synthesis process of bile salts in the liver[13]. Mice fed a high-fat diet and treated with *L. plantarum* KCTC3928 (10^9 CFU/d), for 4 weeks, exhibited an 80 % increase in the amount of mRNA of the *Cyp7a1* gene and an increase of 60 % in CYP7A1 protein expression. Those animals also exhibited a significant decrease in serum TC, LDL-cholesterol and TAG concentrations, and an increase in the faecal excretion of bile salts[27]. Recently, Degirolamo *et al.*[28] observed that after 21 d of treatment with the VSL#3 probiotic (50×10^9 CFU/d) the amount of bile salts that were secreted into the intestine was increased; this effect was a consequence of an increased hepatic expression of the genes *Cyp7a1* and *Cyp8b1*. Also, it was observed that this whole process of excretion and *de novo* synthesis of bile salts induced by the treatment with VSL#3 is dependent on the down-regulation of the gut–liver axis FXR–FGF15 (farnesoid X receptor–fibroblast growth factor 15).

The increased excretion of cholesterol into the deconjugated bile salts leads to an increase in the synthesis of bile salts by the host, which may cause a reduction in serum cholesterol concentration. As will be discussed below, the ability of probiotic bacteria to deconjugate bile salts may influence the occurrence of other mechanisms suggested as responsible for the hypocholesterolaemic effect of these micro-organisms, which demonstrates the importance of this mechanism.

Decreased absorption of intestinal lipids

There are typically two sources of cholesterol entering the intestinal tract: (1) dietary cholesterol and lipids; and (2) biliary cholesterol. Thus, the small-intestinal absorption of lipids helps to maintain cholesterol homeostasis[15]. In this manner, if the quantity of absorbed lipids decreases, the body starts to use the serum and stored lipids, which can contribute to the improvement in serum cholesterol concentration[18]. Therefore, it is now being investigated whether this mechanism is the one responsible for the hypocholesterolaemic effect caused by the regular consumption of probiotic bacteria.

Thus, hamsters fed a cholesterol-enriched diet and treated with adlay-based milk fermented with *L. plantarum* or *L. paracasei*, for 8 weeks, exhibited a decrease in serum TC, LDL-cholesterol, HDL-cholesterol and TAG concentrations, compared with the control group. Additionally, probiotic treatment increased the faecal excretion of cholesterol and TAG[29]. Therefore, the authors attribute this hypocholesterolaemic effect to a decrease in the absorption of the intestinal lipids. Also, in a clinical study where hypercholesterolaemic subjects consumed capsules of *L. reuteri* NCIMB 30242 (2.9×10^9 CFU/capsule, twice per d) for 13 weeks, the hypocholesterolaemic effect observed was attributed to a reduction in intestinal lipid absorption[21].

Further mechanisms have been suggested to be responsible for the reduction of intestinal cholesterol absorption caused by the regular consumption of probiotics; among them there are: (1) the co-precipitation of cholesterol with the deconjugated bile salts; (2) the assimilation and incorporation of cholesterol into the cell membranes of the micro-organisms, (3) the conversion of cholesterol to coprostanol; and (4) the inhibition of the expression of intestinal cholesterol transporter in the enterocytes. These mechanisms will be discussed further.

Co-precipitation of cholesterol with the deconjugated bile salts

Intestinal cholesterol needs to be emulsified by the bile salts in order to be absorbed since it is a hydrophobic substance. Thus, the absorption of intestinal cholesterol is dependent on the action of the bile salts[17].

However, as previously discussed, some probiotic bacteria are capable of deconjugating bile salts in the intestinal lumen. Furthermore, in the deconjugated form, bile salts are less effective as emulsifiers than conjugated bile salts and do not form stable micelles[30]. Additionally, deconjugated bile salts at acidic pH are protonated and precipitated, and, consequently, intestinal cholesterol can be co-precipitated with deconjugated bile salts and will not be absorbed by the host[12,31].

Klaver & Meer[32], by means of an *in vitro* study, were one of the first to note that the cholesterol co-precipitation process is dependent on the presence of bile salts in the deconjugated form. Ahn *et al.*[31] observed a significant positive correlation between the ability of deconjugation of the bile salts and precipitation of cholesterol by *L. acidophilus* SNUL020, SNUL01 and FM01. Also, it was noticed that *L. plantarum* Lp91 co-precipitates greater amounts of cholesterol compared with *L. plantarum* Lp21 and NCDO82. These strains exhibited a lower ability to deconjugate sodium glycocholate compared with Lp91[33]. Thus, the presence of bile salts in the deconjugated form is essential for the occurrence of this mechanism.

Nevertheless, the co-precipitation of cholesterol with deconjugated bile salts is highly dependent on the pH of the medium. First, the enzyme BSH needs a slightly acidic pH, usually between 5 and 6, to have optimum performance[2]. Second, deconjugated bile salts precipitate at a pH less than 6. Moreover, the precipitation of cholesterol is co-dependent on the precipitation of these bile salts[32]. Third, when the pH of the medium reaches a value of 7, cholesterol can re-dissolve in the medium[34].

Thus, it is believed that the co-precipitation of cholesterol with deconjugated bile salts may not significantly contribute to the decrease in the absorption of intestinal cholesterol *in vivo* since the physiological pH of the intestinal lumen varies over a range of neutrality and alkalinity. However, in *in vitro* studies, this mechanism is listed as being responsible for the decreased absorption of intestinal cholesterol by the regular consumption of the probiotic bacteria.

Assimilation and incorporation of cholesterol into the cell membrane of the probiotics

The amino acids that comprise the peptidoglycan of the cell walls of the probiotic bacteria and the exopolysaccharides secreted by these micro-organisms are primarily responsible for the strong link between the cholesterol in the medium and the cell surface of the probiotic bacteria. Subsequently, this cholesterol is assimilated into the cell membrane of the bacteria and eliminated with them through the faeces[35].

Currently, it is known that the ability to assimilate the cholesterol present in the medium is highly strain-specific[30]. Thus, in a study conducted with thirty-four strains of *Bifidobacterium* (*B. animalis*, *B. bifidum*, *B. longum*, *B. adolescentis* and *B. pseudocatenulatum*), it was observed that these bacteria were capable of assimilating 4 to 81 mg cholesterol/g dry biomass. The strains *B. bifidum* MB 109 and *B. bifidum* MB 107 were able to assimilate the highest amounts among the different strains studied[10]. Also, among the different species of lactic acid bacteria isolated from dairy products (ewe milk, traditional yoghurt, and sour buttermilk) traditionally eaten in Iran, it was observed that the *L. brevis* strains were able to assimilate more cholesterol (80 % of the cholesterol after 9 h of incubation) than the other strains of lactic acid bacteria[36].

This strain specificity could be the consequence of the differences in the capacity of production of exopolysaccharides since there is a correlation between the amount of exopolysaccharide produced and the quantity of cholesterol assimilated by the strain[14]. It is likely that the composition and structure of the peptidoglycan of the bacterial cell walls will also influence the ability of each strain to assimilate the cholesterol.

Additionally, differences in the expression of genes involved in intracellular cholesterol transport could be responsible for the differences observed between the strains. Recently, it was observed that the high ability exhibited by *B. bifidum* PRL2010 to assimilate cholesterol was due to the increased expression of the genes encoding the ABC-type carriers. When maintained in medium containing cholesterol the expression of these genes increased up to two times compared with the bacteria of the same strain maintained in a cholesterol-free medium. It was also observed that none of the genes related to cholesterol catabolism was up-regulated[37].

As a consequence of the cholesterol assimilation process, the fatty acid composition of the bacteria membrane is modified. Generally, there is an increase in the concentration of SFA and unsaturated fatty acids in the cell membranes[25,37–39]. This modification in the composition is required since the membrane must maintain its integrity and fluidity after the cholesterol is assimilated. Also, this modification can lead to changes in the tensile strength and the charge of those membranes, which allow these bacteria to be more resistant to adverse conditions present in the intestinal lumen, preventing the occurrence of cell lysis[18,39]. Perhaps, that is why Miremadi *et al.*[25] observed that there was a positive correlation between the tolerance to the bile salt and the overall content of the assimilated cholesterol (r 0·808; $P < 0·05$). Then, those strains that exhibited the greatest bile salt tolerance showed higher overall cholesterol assimilation in comparison with those strains that had a low tolerance.

In vivo studies were performed so that the hypocholesterolaemic effect of the regular consumption of probiotic bacteria that are able to assimilate cholesterol *in vitro* could be evaluated. In this manner, it was observed that Sprague–Dawley rats fed a hypercholesterolaemic diet and treated for 2 weeks with *B. longum* SPM1207 (10^8–10^9 CFU/ml) showed a significant reduction in serum TC and LDL-cholesterol concentration[40]. Also, Kumar *et al.*[33] selected the strains of *L. plantarum* Lp91 and Lp21 after performing an *in vitro* study, where those strains assimilated larger amounts of cholesterol in de Man, Rogosa and Sharpe (MRS) broth supplemented with Thio-0·3 % ox-bile in comparison with *L. plantarum* NCDO82. Thus, Sprague–Dawley rats fed a hypercholesterolaemic diet supplemented with *L. plantarum* Lp91 and Lp21 (10^8 CFU/g, for 21 d) showed a reduction in serum TC, LDL-cholesterol, VLDL and TAG concentrations.

Nevertheless, some *in vivo* studies have found conflicting results. Park *et al.*[41] did not attribute this mechanism to the hypocholesterolaemic effect observed by treatment with *L. acidophilus* ATCC 43121 (3×10^7 CFU/d) since the concentration of cholesterol in the faeces of these animals did not differ from that of the control group. Also, the treatment with *L. rhamnosus* LC705 (two capsules/d containing 2×10^{10} CFU, during 4 weeks), a strain capable of assimilating cholesterol *in vitro*, did not affect the serum TC, LDL-cholesterol, HDL-cholesterol and TAG concentrations of adult subjects, with moderate elevation in serum cholesterol[42]. However, this disagreement between the results of *in vitro* and *in vivo*

studies is expected since the *in vitro* studies do not reproduce the real environmental conditions of the gastrointestinal tract[43].

A limitation observed by us during the revision of this mechanism consists of the lack of quantification of the cholesterol excreted in the faeces by the subjects treated with probiotic bacteria. The result of this quantification could help to confirm the occurrence of the assimilation of cholesterol by the probiotics tested *in vivo*. Thus, studies that evaluate the occurrence of this mechanism should quantify the cholesterol excreted in the faeces.

According to Miremadi *et al.*[25], there is a negative correlation between the residual cholesterol in the medium and the growth of probiotic bacteria, which indicates that cholesterol assimilation is growth-dependent. Although probiotic bacteria that are non-growing (stationary phase) or that are dead are also able to assimilate cholesterol, it is only in smaller amounts, when compared with the bacteria that are in the growth phase[30,44]. Thus, the reduction of cholesterol concentration in the medium occurs as a consequence of the assimilation of growing cells and the adhesion of cholesterol to the surface of the dead cells[25].

Thus, we can conclude that the ability of bacteria to assimilate the cholesterol in the medium appears to be dependent on the growth of the strain and is strain-specific. Then, if the strain is able to assimilate cholesterol, but does not survive the passage through the gastrointestinal tract, the occurrence of the hypocholesterolaemic effect can be compromised.

The presence of bile salts in the deconjugated form plays an important role in the process of assimilation of cholesterol by the probiotic bacteria. The bile salts in the deconjugated form increase the assimilation of cholesterol into the cell membrane of the probiotic bacteria since it increases the permeability, as well as the fluidity and porosity of the membranes, allowing the incorporation of cholesterol[30]. However, unlike what happens with the mechanism of co-precipitation of cholesterol, the presence of bile salts in the deconjugated form is not essential for the assimilation of cholesterol[14].

Despite being little studied, probiotic bacteria are also capable of binding bile salts present in the medium to their cell surface[30]. Pigeon *et al.*[45] observed that the strains of *L. delbrueckii* subsp. *bulgaricus* LB-18 and LB-10442 were capable of binding higher amounts of cholic acid than the other strains of *Streptococcus thermophiles* studied. According to these authors, the strains that produced the largest amounts of exopolysaccharides were capable of binding larger amounts of bile salts.

Similar to what happens with assimilated cholesterol, the bile salts will be eliminated along with the bacteria through the faeces. In this manner, endogenous cholesterol will be redirected to the production of more bile salts, which may improve the cholesterol serum profile of the host. Therefore, more studies to evaluate this mechanism should be conducted. Subsequently, after the identification of the strains capable of binding the bile salts, *in vivo* studies should be performed for the evaluation of the hypocholesterolaemic effect of this mechanism.

Conversion of cholesterol to coprostanol

In the intestinal lumen dietary cholesterol and endogenous cholesterol excreted via the transintestinal cholesterol efflux can be metabolised by the colonic microbiota. Thus, cholesterol can be reduced to coprostanol (5β-cholestan-3β-ol) and in minor amounts to coprostanone. These metabolites have low intestinal absorption and are eliminated with the faeces, which leads to a decrease in the intestinal absorption of cholesterol[17].

The efficiency of cholesterol conversion to coprostanol is mainly the result of the activity and abundance of cholesterol-reducing bacteria in the intestinal microbiota[17]. Thus, numerous attempts have been made to identify and isolate the cholesterol-reducing bacteria, so it can be used in the prevention and treatment of hypercholesterolaemia.

The cholesterol-reducing bacteria must have the cholesterol reductase enzyme. This enzyme can be found in some strains of probiotic bacteria, like *L. acidophilus* ATCC 314, *L. acidophilus* FTCC 0291, *L. bulgaricus* FTCC 0411, *L. casei* 1311 FTDC ATCC 393, *B. bifidum* PRL2010, and *Eubacterium coprostanoligenes* ATCC 51222[17,37,39]. There is little information in the scientific literature about the strains positive for this enzyme; however, the activity of this enzyme is strain-specific.

Recently, it has been observed that the cholesterol reductase enzyme can be modulated by the medium in which the bacteria are located. Thus, the cells of *B. bifidum* PRL2010 cultivating in the medium containing cholesterol have an increased expression of the gene *BBPR_0519* in comparison with the cells cultivated in the absence of cholesterol. The *BBPR_0519* gene encodes the enzyme aldo/keto reductase, which converts cholesterol in coprostanol[37].

Some *in vivo* studies were designed to investigate the hypocholesterolaemic effect of the treatment with cholesterol-reducing bacteria. In this manner, the treatment (2×10^7 cells/ml) with *E. coprostanoligenes* ATCC 51222 increased the coprostanol: cholesterol ratio in the contents of the digestive tracts of experimental rabbits, in comparison with the controls. Subsequently, this result was assigned to the hypocholesterolaemic effect observed in the animals treated[46].

However, some studies have failed to prove that the hypocholesterolaemic effect observed was a result of an increase in the conversion of cholesterol to coprostanol. In this manner, Sprague–Dawley rats fed a cholesterol-enriched diet and treated with *L. acidophilus* ATCC 43121 (2×10^6 CFU/d), for 3 weeks, showed faecal excretion of coprostanol similar to that of the control group[26]. A similar result was found in the study conducted by Guo & Li[20], where treatment with *L. casei* F0822 (10^8 or 10^9 CFU/ml, for 3 weeks) did not change the faecal concentration of coprostanol. Also, healthy subjects who had consumed 100 ml of milk fermented with *L. acidophilus* SNUL01 (10^8 CFU/ml, during 3 weeks) exhibited faecal excretion of coprostanol similar to that of the placebo group[31]. However, none of the studies mentioned above reported whether those probiotic bacteria have the cholesterol reductase enzyme.

The activity of the cholesterol reductase enzyme is affected by the presence of bile salts and by the type of bile salts present in the medium. Thus, the conversion of cholesterol in coprostanol is reduced in the medium supplemented with oxgall and taurocholic acid[39]. Therefore, the utilisation of a strain positive for the cholesterol reductase enzyme is not sufficient to increase

the conversion of cholesterol to coprostanol, as the environmental conditions in the gastrointestinal tract, such as the presence/absence of cholesterol and bile salts, can influence the expression and activity of this enzyme.

Inhibition of the expression of intestinal cholesterol transporter Niemann–Pick C1 like 1 in the enterocytes

Through transporters present in the membrane of enterocytes, the cholesterol present in the intestinal lumen is absorbed. In this way, the transporter Niemann–Pick C1 like 1 (NPC1L1) is indispensable for the absorption of dietary and biliary cholesterol. Thus, NPC1L1 knockout (NPC1L1-/-) mice absorb 69 % less cholesterol than the NPC1L1 knockin (+/+) mice. Moreover, they are resistant to the hypercholesterolaemic effect induced by treatment with cholesterol-enriched diets[47,48].

Based on the importance of the NPC1L1 cholesterol transporter, an investigation of the effect of the regular consumption of probiotics on the expression of these transporters in the gut of their consumers was initiated.

In an *in vitro* study with Caco-2 enterocytes, treatment with *L. plantarum* Lp27 was able to reduce the expression of the cholesterol transporter NPC1L1, as well as reducing the absorption of cholesterol in those cells[49]. Huang & Zheng[7] observed that Caco-2 enterocytes treated with *L. acidophilus* ATCC 4356 expressed lower amounts of mRNA and protein of the cholesterol transporter NPC1L1 than the control. Additionally, heat-killed ATCC 4356 was unable to inhibit NPC1L1 expression, although the conditioned medium of this strain was able to reduce NPC1L1 expression even after being inactivated by heat. Thus, the authors concluded that ATCC 4356 secreted some sort of heat-stable molecules, for example, small oligopeptides or lipids, which were responsible for inhibiting the expression of this NPC1L1 transporter.

Clinical trials that evaluated this mechanism are scarce since it is necessary to perform a biopsy for tissue sample collection. Therefore, an experimental study conducted with hamsters that were fed with a hyperlipidaemic diet and treated with a mix of *L. acidophilus* La5 and *B. animalis* subsp. *lactis* Bb12 (2×10^9 of each strain, for 5 weeks), it was observed that those expressed lower amounts of the transporter NPC1L1 in the gut when compared with the animals that were fed with a hyperlipidaemic diet (positive control) and those that were not fed with the hyperlipidaemic diet (negative control). The treatment with the probiotic was also able to decrease serum TC, oxidised LDL-cholesterol and TAG concentration, as well as the hepatic TC and TAG concentration[11].

Similar results were observed in rats treated with *L. plantarum* Lp27 (10^9 CFU/d), isolated from *kefir* grains, for 4 weeks. These animals expressed minor amounts of the transporter NPC1L1 in the duodenal and jejunal regions and exhibited a reduction in serum TC, LDL-cholesterol and TAG concentrations in comparison with the control group. The serum HDL-cholesterol concentration was unchanged; also, a reduction in liver TC and TAG concentrations was observed[49]. According to Altmann et al.[47], the transporter NPC1L1 is mainly expressed on the surface of the enterocytes located in the jejunal region.

In this manner, when it becomes less expressed in this region, cholesterol absorption is compromised.

The molecular mechanisms used by the probiotic bacteria to reduce NPC1L1 transporter expression are not fully understood. Huang & Zheng[7] observed that when liver X receptors (LXR) are depleted by small interfering RNA in Caco-2 cells, NPC1L1 expression is no longer decreased by treatment with *L. acidophilus* ATCC 4356, and no reduction in cholesterol uptake is observed. In this manner, it has been proposed that some molecules produced by the probiotic bacteria will be able to connect and stimulate the LXR, down-regulating NPC1L1 protein expression in the small intestine.

Modulation of lipid metabolism

The intestinal microbiota is capable of modulating the metabolism of its host[50]; thus, this could be a potential mechanism responsible for the hypocholesterolaemic effect of the regular consumption of probiotics, as will be discussed below.

Inhibition of hepatic synthesis of cholesterol

Inhibition of the activity of the hepatic enzyme 3-hydroxymethyl-3-glutaryl-CoA (HMG-CoA) reductase, a limiting enzyme in the process of hepatic cholesterol synthesis, is another mechanism described in the literature, as responsible for the hypocholesterolaemic effect of the regular consumption of probiotic bacteria. The inhibition of the hepatic activity of HMG-CoA reductase occurs mainly as a consequence of the action of SCFA[51].

SCFA are the endproducts of bacterial fermentation of non-digestible carbohydrates by the host. Due to the low pKa (<4·8) of these acids and the pH of about 7 present in the colonic region, SCFA are usually found in the form of anions[52]. The major SCFA produced are acetate, propionate and butyrate; other bacterial endproducts produced in minor amounts include lactate, succinate, formate, valerate, caproate, isobutyrate, 2-methyl-butyrate and isovalerate[53].

Each SCFA plays a different role in our body, and some of them play opposite roles. In the inhibition of the activity of HMG-CoA reductase, SCFA propionate and acetate are the main ones involved in the process[12]. After being absorbed, acetate reaches the liver, where it is converted to acetyl-CoA by the activity of the enzyme acetyl-CoA synthetase. Acetyl-CoA is then used for the synthesis of cholesterol by the activity of the enzyme HMG-CoA reductase. Thus, the SCFA acetate increases serum cholesterol concentration[54]. On the other hand, the SCFA propionate acts as an inhibitor of lipogenesis and cholesterogenesis. Propionate is able to inhibit the incorporation of acetate into the molecules of steroids since it inhibits the activity of HMG-CoA reductase[55]. Furthermore, propionate is able to stimulate the hepatic synthesis of bile salts, by increasing the activity of the 7α-hydroxylase enzyme[56]. Therefore, propionate has a hypocholesterolaemic effect, while acetate presents a hypercholesterolaemic effect.

Thus, for the probiotic micro-organisms to have a hypocholesterolaemic effect, sufficient amounts of propionate must be produced to offset the effects of acetate in cholesterogenesis.

Therefore, several *in vivo* studies were designed to investigate the hypocholesterolaemic effect of the SCFA produced by the probiotic micro-organisms. In this manner, Sprague–Dawley rats fed a cholesterol-enriched diet and treated with *L. plantarum* MA2 (10^{11} cells/d, for 5 weeks) exhibited higher caecal concentrations of propionate than the control group. Additionally, the probiotic treatment lowered the serum TC, LDL-cholesterol and TAG concentrations, as well as the liver TC and TAG concentrations[57]. Moreover, the hypocholesterolaemic effect of the treatment with different doses (10^8 or 10^9 CFU/ml; for 3 weeks) of *L. casei* F0822 was associated with an increase in caecal concentrations of propionate and total SCFA. Additionally, the propionate:acetate ratio was increased in the group treated with the probiotic in comparison with the control group[20]. Thus, part of the hypocholesterolaemic effect observed in these studies may be attributed to the increased production of the SCFA propionate.

However, conflicting results have been found in clinical trials. It was seen that healthy, mildly hypercholesterolaemic male subjects, who consumed the fermented milk *kefir* (500 ml/d), for 4 weeks, did not exhibit any changes in serum TC, LDL-cholesterol, HDL-cholesterol or TAG concentrations in comparison with baseline. Also, the faecal concentration of total SCFA and propionate was increased in those subjects supplemented with *kefir*[58]. Nonetheless, male subjects with atherosclerotic plaques in the carotid wall, who had ingested an oat-based drink fermented by *L. plantarum* DSM 9843 (10^{11} CFU/d) for 4 weeks, exhibited a similar faecal concentration of total SCFA, acetate and propionate, compared with the placebo group. Additionally, serum TC, LDL-cholesterol, HDL-cholesterol and TAG concentrations were not affected by treatment with the fermented drink[8].

The lack of association between the hypocholesterolaemic effect and the increased faecal concentration of the SCFA propionate in the clinical trials could be a consequence of the nature of the sample utilised for the determination of these acids. It is known that a greater production of SCFA occurs in the caecum; from this region toward the distal colon, the concentration of these acids decreases progressively (up to 95 %). Along the route, the SCFA are rapidly absorbed by the colonocytes. Furthermore, in the distal colon, the production of SCFA is small, due to the low availability of substrate[59]. In this manner, when the concentration of SCFA is evaluated in the faeces, the results will not reflect the actual concentration of the SCFA produced by the intestinal microbiota. Moreover, the experimental studies usually evaluated the concentration of the SCFA in the caecum content, where most of the SCFA is produced.

Another possible limitation of those studies is the fact that the bacteria of the genus *Lactobacillus* are not capable of producing SCFA with more than two carbons. Therefore, the increase in propionate production observed in all studies that utilised this genus of bacteria would be a consequence of the metabolic activity of the other bacteria present in the intestinal microbiota of the host. Those micro-organisms would convert the lactate produced by *Lactobacillus* into propionate[20]. Therefore, this increase in propionate production is affected by the initial composition of the intestinal microbiota of the host.

The increase in the total amount of SCFA produced may decrease intracolonic pH. As seen previously, at low pH values the bile salts are protonated and precipitated. Thus, the increase in the production of the SCFA contributed to the occurrence of the co-precipitation of cholesterol with the deconjugated bile salts[32]. Furthermore, the decrease in intracolonic pH could favour an increase in the production of the BSH enzyme by the bacteria, which would consequently lead to an increase in the excretion of cholesterol by the host[25].

In conclusion, the hypocholesterolaemic effect of the SCFA depends on the amount of propionate and acetate that is produced by the probiotic bacteria in the intestinal lumen since they have opposite effects on the hepatic synthesis of cholesterol. Thus, to have the hypocholesterolaemic effect, it is necessary to consume probiotic bacteria that produce more propionate than acetate during the fermentation process. Additionally, the production of SCFA can be increased when the host consumes a prebiotic.

Reduction of body fatness

Considering that subjects with high body fatness are more likely to develop dyslipidaemia, and the intestinal microbiota is capable of modulating the body fatness of its host[50], changes in the intestinal microbiota composition by the regular consumption of probiotics could be an important mechanism to reduce body fatness[60] and indirectly improve the serum lipid profile.

In this manner, it has been suggested that some strains of probiotic bacteria could increase lipolysis and reduce the lipogenesis process of their host, consequently improving the serum lipid profile[61,62]. Those strains would be capable of down-regulating the expression of genes related to the lipogenesis process such as the enzymes fatty acid synthase and acetyl CoA carboxylase, as well as LXR-α and sterol regulatory element-binding protein 1. Also, it could also up-regulate the expression of genes related to the lipolysis process, such as the enzyme carnitine palmitoyltransferase-1, and PPAR-α[62–64].

Although the molecular mechanism by which these micro-organisms mediate this modulation is not fully known, it is likely that this effect is a result of the interaction between the probiotic bacteria or their metabolites (SCFA and bioactive peptides) and the host[50]. Thus, more studies that evaluate how the regular consumption of probiotics can reduce body fatness should be performed for a better understanding of how this mechanism influences the hypocholesterolaemic effect of probiotic bacteria.

Mechanisms responsible for the hypocholesterolaemic effect of regular consumption of probiotic yeasts

Yeasts have diverse fermentative activities, and therefore have been used for the production of many kinds of fermented foods[65]. Although many strains of yeasts are used in the food industry, there are few studies evaluating the hypocholesterolaemic effect of the regular consumption of probiotic yeast. Thus, little is known about the mechanisms responsible for the hypocholesterolaemic effect of regular consumption of probiotic yeast (Fig. 2).
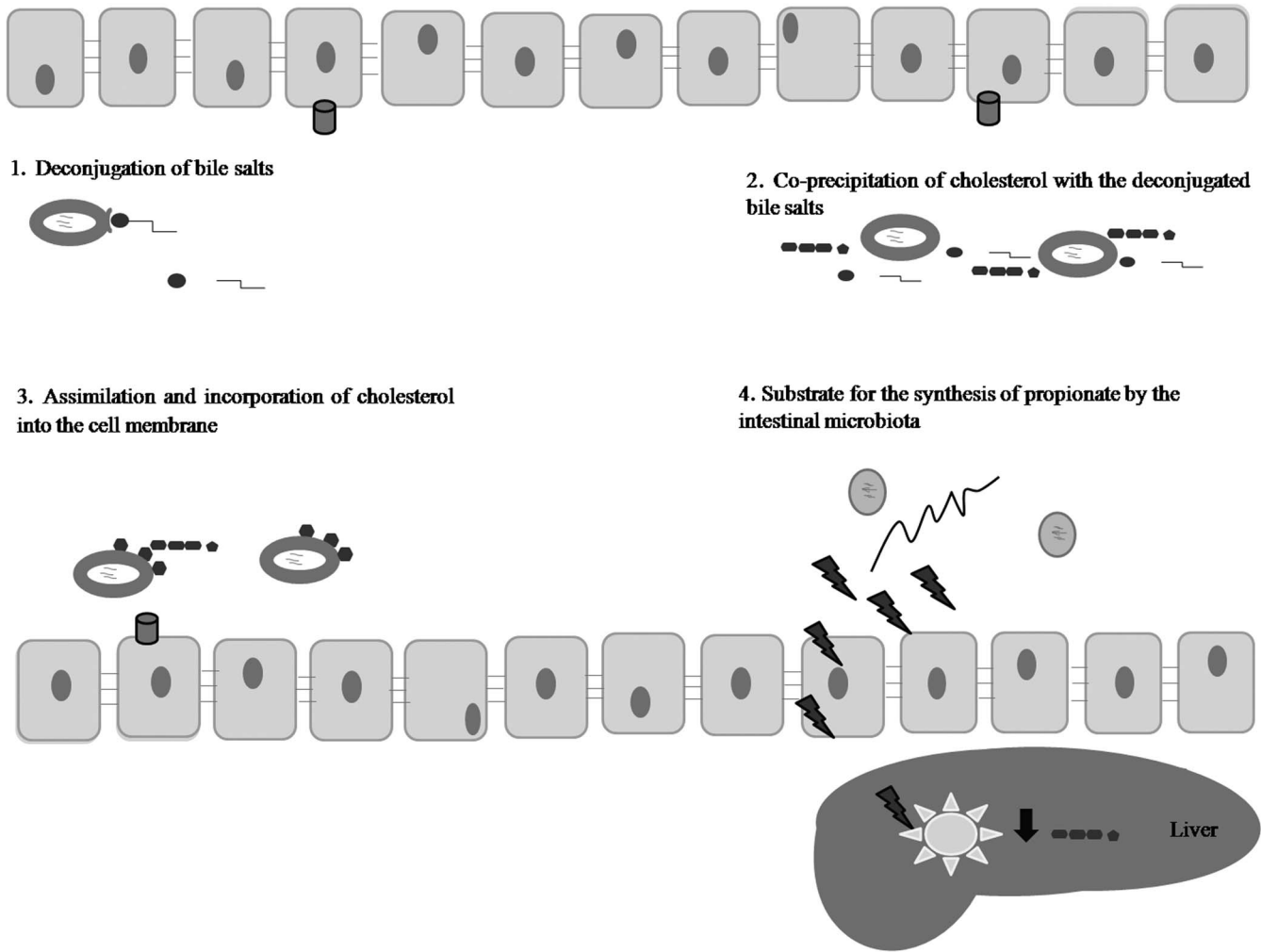
Fig. 2. Main mechanisms responsible for the hypocholesterolaemic effect of regular consumption of probiotic yeasts. Some probiotic yeasts are able to (1) deconjugate bile salts by the action of the bile salt hydrolase enzyme. The (2) co-precipitation of cholesterol with deconjugated bile salts and (3) assimilation and incorporation of cholesterol into the cell membranes are some of the mechanisms by which probiotic yeasts reduce the absorption of intestinal cholesterol and lipids. Additionally, (4) consumption of the polysaccharides extracted from the cell wall of the yeast can be used as substrate by the intestinal microbiota, which may lead to an increase in the production of propionate, which inhibits the activity of the liver enzyme hydroxy-methyl-3-glutaryl-CoA reductase.

The hypocholesterolaemic effect of probiotic yeasts is strain-specific, as reported by Yoshida et al.[65]; among eighty-one strains of yeasts evaluated, only forty-eight were able to decrease the serum TC concentration when administered as a dietary admixture at a concentration of 10 % ad libitum, for 7 d. This variability observed between the strains has been attributed to the differences in the composition of the cell wall. The cell wall of yeasts is mainly composed of β-glucan and α-mannan, two types of dietary fibres; however, the structures of those polysaccharides differ according to the strain studied[66].

As consequence of the high concentration of nucleic acid in yeast, which could increase consumer's serum uric acid concentration, as well as the high doses of yeast polysaccharides tested, the hypocholesterolaemic effect of polysaccharides present in the cell wall of yeast has been studied in the isolated form[67]. Thus, Sprague–Dawley rats fed a cholesterol-enriched diet, supplemented with β-glucan (30 g/kg of diet; for 8 weeks) isolated from the baker's yeast Saccharomyces cerevisiae,

exhibited a reduction in serum TC and LDL-cholesterol concentrations, while HDL-cholesterol and TAG concentrations were not affected by the treatment[68]. Similar results were observed in the study by Wilson et al.[69], where Syrian golden hamsters fed on a hypercholesterolaemic diet supplemented with 10 g of yeast β-glucan/100 g of diet, for 12 weeks, showed a reduction in serum TC, non-HDL-cholesterol and TAG concentrations. Additionally, treatment with yeast β-glucan reduced the aortic fatty streak area as compared with the control group.

The hypocholesterolaemic effect of these polysaccharides has also been observed in clinical trials. In this manner, the cell wall extract of Kluyveromyces marxuanus YIT 8292 (2 and 4 g/d, for 4 weeks) when consumed by mildly hypercholesterolaemic subjects reduced serum TC and LDL-cholesterol concentrations. A similar effect was observed when normal and hypercholesterolaemic subjects consumed a yogurt supplemented with 3 or 4 g/d of polysaccharides extracted from the cell wall of K. marxuanus YIT 8292, for 8 weeks[70].

The hypocholesterolaemic effect caused by the consumption of the polysaccharides extracted from the cell wall of the yeast may be due to an increase in the faecal excretion of bile salts, which increases the demand for cholesterol for the hepatic synthesis of bile salts. Moreover, these polysaccharides can be used as a substrate by the intestinal microbiota, which may lead to an increase in the production of SCFA, especially propionate, which as previously described can decrease the hepatic synthesis of cholesterol[66].

Similar to probiotic bacteria, some strains of yeasts are capable of producing the BSH enzyme, and consequently deconjugating bile salts[71]. This would lead to an increase in the excretion of endogenous cholesterol, which would stimulate the hepatic synthesis of bile salts, and consequently reduce the amount of absorbed intestinal cholesterol; hence the formation of micelles will be compromised[18].

Liu et al.[71] observed that the BSH activity of K. marxianus K1 and M3 is proportional to the amount of cholesterol that is removed from the culture medium. Also, partly, the decrease in the concentration of cholesterol is due to its co-precipitation with the deconjugated bile salts. Thus, the mechanism of co-precipitation of cholesterol carried out by yeasts resembles that made by bacteria. In this manner, Wistar rats fed a high-cholesterol diet and treated with different doses (5, 10 and 20 g/kg body weight) of K. marxianus M3 isolated from Tibetan mushrooms, for 7 weeks, showed a reduction in serum TC, LDL-cholesterol and TAG concentrations, and an increase in serum HDL-cholesterol concentration[72].

Yeasts are also able to assimilate the cholesterol present in the medium; however, this mechanism is highly strain-specific and growth-dependent. Thus, from the nine strains of yeasts evaluated, only Saccharomyces cerevisiae KK1, Saccharomyces cerevisiae 832, Saccharomyces boulardii and Isaatchenkia orientalis KK5.Y.1 were able to assimilate significant amounts of cholesterol present in the culture medium, in comparison with the other strains[73].

Psomas et al.[73] observed that the yeast cells that were grown in the medium containing both oxgall and cholesterol were more resistant to lysis by sonication. After enzymic lysis, the authors concluded that the assimilated cholesterol is incorporated into the membrane or cell wall and is not metabolised by them. Therefore, the cholesterol assimilation mechanism of probiotic yeasts is similar to that of probiotic bacteria.

In an experimental study, the consumption of Saccharomyces boulardii (12×10^{10} CFU/kg of diet; for 14 d) was able to prevent and treat hypercholesterolaemia and hypertriacylglycerolaemia in golden Syrian hamsters fed on a diet enriched with 0·1% cholesterol[74]. The hypocholesterolaemic effect of S. boulardii may be a consequence of the assimilation of cholesterol[73]. Also, Aloğlu et al.[75] attributed the hypocholesterolaemic effect observed by them to the high cholesterol assimilation capacity (73·33%) of Cryptococcus humicola M5-2. Thus, Wistar rats fed a cholesterol-enriched diet supplemented with 0·1% of C. humicola M5-2 lyophilised (1×10^7 cells/ml) had a significant reduction in serum TC and TAG concentrations in comparison with the control.

In a clinical study, Ryan et al.[76] attributed the decrease in serum remnant lipoprotein particle concentrations in hypercholesterolaemic adults treated with Saccharomyces boulardii ($1·4 \times 10^{10}$ CFU/capsule, eight capsules/d, for 8 weeks) to the ability of this strain to assimilate the cholesterol present in the medium. However, serum TC, LDL-cholesterol and HDL-cholesterol concentrations were not altered by the treatment with the yeast.

Further studies are needed to evaluate the hypocholesterolaemic effect of probiotic yeasts, as the results are promising, although clinical trials are scarce. Through more studies, it will also be possible to identify more mechanisms through which regular consumption of yeast can cause the hypocholesterolaemic effect.

Conclusion

The hypocholesterolaemic effect of probiotic bacteria and yeasts has been observed in some in vitro, experimental and clinical studies. This effect on the lipid profile provided by probiotics is still modest if compared with statins. However, this does not prevent the use of these micro-organisms for the prevention, as well as for the treatment, of dyslipidaemia in association with statins.

The potential mechanisms described as responsible for the hypocholesterolaemic effect of probiotic bacteria and yeasts are highly strain-specific, and some are co-dependent. These mechanisms usually include the increase in faecal excretion of cholesterol as bile salts; the decrease in absorption of the intestinal cholesterol and lipids; and the modulation of lipid metabolism.

However, some conflicting results have been found, mainly as a consequence of the different experimental designs. It is necessary to point out that there exists a gap in the knowledge about the hypocholesterolaemic effect of probiotics, as the mechanisms involved in this effect are not fully understood and some have never been observed in humans. In this context, further studies are necessary, particularly with respect to probiotic yeasts, which have been poorly studied.

Additionally, experimental and clinical studies do not use other biomarkers besides TC, LDL-cholesterol, HDL-cholesterol and TAG. These biomarkers are no longer considered by many investigators as independent risk factors for CVD. In this manner, we suggest calculation of the HDL-cholesterol-related ratios (TAG:HDL-cholesterol, TC:HDL-cholesterol and LDL:HDL-cholesterol) and the apoB100:apoA1 ratio, as they help to predict the cardiovascular risk.

Acknowledgements

References

1. World Health Organization (2011) Cardiovascular Disease. Fact sheet no. 317. Geneva, Switzerland: WHO. http://www.who.int/mediacentre/factsheets/fs317/en/ (accessed January 2015).
2. Guo Z, Liu XM, Zhang QX, *et al.* (2011) Influence of consumption of probiotics on the plasma lipid profile: a meta-analysis of randomized controlled trials. *Nutrition, Metabolism and Cardiovascular Diseases* **21**, 844–850.
3. Lahti L, Salonen A, Kekkonen RA, *et al.* (2013) Associations between the human intestinal microbiota, *Lactobacillus rhamnosus* GG and serum lipids indicated by integrated analysis of high-throughput profiling data. *PeerJ* **1**, e32.
4. DiRienzo DB (2013) Effect of probiotics on biomarkers of cardiovascular disease: implications for heart-healthy diets. *Nutrition Reviews* **72**, 18–29.
5. Food and Agriculture Organization of the United Nations/ World Health Organization (2001) *Probiotics in Food. Health and Nutritional Properties and Guidelines for Evaluation, FAO Food and Nutrition Paper no. 85*. Rome and Geneva: Food and Agriculture Organization of the United Nations/ World Health Organization.
6. Mann GV & Spoerry A (1974) Studies of a surfactant and cholesteremia in the Maasai. *American Journal of Clinical Nutrition* **27**, 464–469.
7. Huang Y & Zheng Y (2010) The probiotic *Lactobacillus acidophilus* reduces cholesterol absorption through the down-regulation of Niemann–Pick C1-like 1 in Caco-2 cells. *British Journal of Nutrition* **103**, 473–478.
8. Karlsson C, Ahrné S, Molin G, *et al.* (2010) Probiotic therapy to men with incipient arteriosclerosis initiates increased bacterial diversity in colon: a randomized controlled trial. *Atherosclerosis* **208**, 228–233.
9. Manson JE, Tosteson H, Ridker PM, *et al.* (1992) The primary prevention of myocardial infarction. *New England Journal of Medicine* **21**, 1406–1416.
10. Bordoni A, Amaretti A, Leonardi A, *et al.* (2013) Cholesterol-lowering probiotics: *in vitro* selection and *in vivo* testing of bifidobacteria. *Applied Microbiology and Biotechnology* **97**, 8273–8281.
11. Stancu CS, Sanda GM, Deleanu M, *et al.* (2014) Probiotics determine hypolipidemic and antioxidant effects in hyperlipidemic hamsters. *Molecular Nutrition and Food Research* **58**, 559–568.
12. Fava F, Lovegrove JA, Gitau R, *et al.* (2006) The gut microbiota and lipid metabolism: implications for human health and coronary heart disease. *Current Medicinal Chemistry* **13**, 3005–3021.
13. Zhao C & Wright KD (2010) Liver X receptor in cholesterol metabolism. *Journal of Endocrinology* **204**, 233–240.
14. Tok E & Aslim B (2010) Cholesterol removal by some lactic acid bacteria that can be used as probiotic. *Microbiology and Immunology* **54**, 257–264.
15. Lecerf JM & Lorgeril M (2011) Dietary cholesterol: from physiology to cardiovascular risk. *British Journal of Nutrition* **106**, 6–14.
16. Ridlon JM, Kang DJ & Hylemon PB (2006) Bile salt biotransformations by human intestinal bacteria. *Journal of Lipid Research* **47**, 241–259.
17. Gérard P (2014) Metabolism of cholesterol and bile acids by the gut microbiota. *Pathogens* **3**, 14–24.
18. Kumar M, Nagpal R, Kumar R, *et al.* (2012) Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases. *Experimental Diabetes Research* **2012**, 902917.
19. Ishimwe N, Daliri EB, Lee BH, *et al.* (2015) The perspective on cholesterol-lowering mechanisms of probiotics. *Molecular Nutrition and Food Research* **59**, 94–105.
20. Guo CF & Li JY (2013) Hypocholesterolaemic action of *Lactobacillus casei* F0822 in rats fed a cholesterol-enriched diet. *International Dairy Journal* **32**, 144–149.
21. Jones ML, Martoni CJ & Prakash S (2012) Cholesterol lowering and inhibition of sterol absorption by *Lactobacillus reuteri* NCIMB 30242: a randomized controlled trial. *European Journal of Clinical Nutrition* **66**, 1234–1241.
22. Jones ML, Martoni CJ, Parent M, *et al.* (2012) Cholesterol-lowering efficacy of a microencapsulated bile salt hydrolase-active *Lactobacillus reuteri* NCIMB 30242 yoghurt formulation in hypercholesterolaemic adults. *British Journal of Nutrition* **107**, 1505–1513.
23. Bi J, Fang F, Lu S, *et al.* (2013) New insight into the catalytic properties of bile salt hydrolase. *Journal of Molecular Catalysis B: Enzymatic* **96**, 46–51.
24. Sridevi N, Vishwe P & Prabhune A (2009) Hypocholesteremic effect of bile salt hydrolase from *Lactobacillus buchneri* ATCC 4005. *Food Research International* **49**, 516–520.
25. Miremadi F, Ayyash M, Sherkat F, *et al.* (2014) Cholesterol reduction mechanisms and fatty acid composition of cellular membranes of probiotic *lactobacilli* and *bifidobacteria*. *Journal of Functional Foods* **9**, 295–305.
26. Park YH, Kim JG, Shin YW, *et al.* (2007) Effect of dietary inclusion of *Lactobacillus acidophilus* ATCC 43121 on cholesterol metabolism in rats. *Journal of Microbiology and Biotechnology* **17**, 655–662.
27. Jeun J, Kim S, Cho SY, *et al.* (2010) Hypocholesterolemic effects of *Lactobacillus plantarum* KCTC3928 by increased bile acid excretion in C57BL/6 mice. *Nutrition* **26**, 321–330.
28. Degirolamo C, Rainaldi S, Bovenga F, *et al.* (2014) Microbiota modification with probiotics induces hepatic bile acid synthesis via downregulation of the Fxr–Fgf15 axis in mice. *Cell Reports* **7**, 12–18.
29. Wang CY, Wu SC, Ng CC, *et al.* (2009) Effect of *Lactobacillus*-fermented adlay-based milk on lipid metabolism of hamsters fed cholesterol-enriched diet. *Food Research International* **43**, 819–824.
30. Lye HS, Rahmat-Ali GR & Liong MT (2010) Mechanisms of cholesterol removal by lactobacilli under conditions that mimic the human gastrointestinal tract. *International Dairy Journal* **20**, 169–175.
31. Ahn YT, Kim GB, Lim KS, *et al.* (2003) Deconjugation of bile salts by *Lactobacillus acidophilus* isolates. *International Dairy Journal* **13**, 303–311.
32. Klaver FAM & Meer RVD (1993) The assumed assimilation of cholesterol by *lactobacilli* and *Bifidobacterium bifidum* is due to their bile salt-deconjugating activity. *Applied and Environmental Microbiology* **59**, 1120–1124.
33. Kumar R, Grover S & Batish VK (2011) Hypocholesterolaemic effect of dietary inclusion of two putative probiotic bile salt hydrolase-producing *Lactobacillus plantarum* strains in Sprague–Dawley rats. *British Journal of Nutrition* **105**, 561–573.
34. Tahri K, Grill JP & Schneider F (1996) Bifidobacteria strain behavior toward cholesterol: coprecipitation with bile salts and assimilation. *Current Microbiology* **33**, 187–193.
35. Lye HS, Kuan CY, Ewe JA, *et al.* (2009) The improvement of hypertension by probiotics: effects on cholesterol, diabetes, renin, and phytoestrogens. *International Journal of Molecular Sciences* **10**, 3755–3775.
36. Iranmanesh M, Ezzatpanah H & Mojgani N (2014) Antibacterial activity and cholesterol assimilation of lactic acid bacteria isolated from tradicional Iranian dairy produts. *LWT – Food Science and Technology* **58**, 355–359.
37. Zanotti I, Turroni F, Piemontese A, *et al.* (2015) Evidence for cholesterol-lowering activity by *Bifidobacterium bifidum*

PRL2010 through gut microbiota modulation. *Applied Microbiology and Biotechnology* **99**, 6816–6829.

38. Liong MT & Shah NP (2005) Acid and bile tolerance and the cholesterol removal ability of bifidobacteria strains. *Bioscience Microflora* **24**, 1–10.

39. Lye HS, Rusul G & Liong MT (2010) Removal of cholesterol by lactobacilli via incorporation and conversion to coprostanol. *Journal of Dairy Science* **93**, 1383–1392.

40. Lee DK, Jang S, Baek EH, et al. (2009) Lactic acid bacteria affect serum cholesterol levels, harmful fecal enzyme activity, and fecal water content. *Lipids in Health and Disease* **8**, 21.

41. Park YH, Kim JG, Shin YW, et al. (2008) Effects of *Lactobacillus acidophilus* 43121 and a mixture of *Lactobacillus casei* and *Bifidobacterium longum* on the serum cholesterol level and fecal sterol excretion in hypercholesterolemia-induced pigs. *Bioscience, Biotechnology and Biochemistry* **72**, 595–600.

42. Hatakka K, Holma R, El-Nezami H, et al. (2008) The influence of *Lactobacillus rhamnosus* LC705 together with *Propionibacterium freudenreichii* ssp. *shermanii* JS on potentially carcinogenic bacterial activity in human colon. *International Journal of Food Microbiology* **128**, 406–410.

43. Madani G, Mirlohi M, Yahay M, et al. (2013) How much *in vitro* cholesterol reducing activity of lactobacilli predicts their *in vivo* cholesterol function? *International Journal of Preventive Medicine* **4**, 404–413.

44. Sirilun S, Chaiyasut C, Kantachote D, et al. (2010) Characterisation of non human origin probiotic *Lactobacillus plantarum* with cholesterol-lowering property. *African Journal of Microbiology Research* **4**, 994–1000.

45. Pigeon RM, Cuesta EP & Gililliand SE (2002) Binding of free bile acids by cells of yogurt starter culture bacteria. *Journal of Dairy Science* **85**, 2705–2710.

46. Li L, Buhman KK, Hartman PA, et al. (1995) Hypocholesterolemic effect of *Eubacterium coprostanoligenes* ATCC 51222 in rabbits. *Letters in Applied Microbiology* **20**, 137–140.

47. Altmann SW, Davis HR Jr, Zhu LJ, et al. (2004) Niemann–Pick C1 like 1 protein is critical for intestinal cholesterol absorption. *Science* **303**, 1201–1204.

48. Davis HR, Zhu L, Hoos LM, et al. (2004) Niemann–Pick C1 like 1 (NPC1L1) is the intestinal phytosterol and cholesterol transporter and a key modulator of whole-body cholesterol homeostasis. *Journal of Biological Chemistry* **279**, 33586–33592.

49. Huang Y, Wu F, Wang X, et al. (2013) Characterization of *Lactobacillus plantarum* Lp27 isolated from Tibetan *kefir* grains: a potential probiotic bacterium with cholesterol-lowering effects. *Journal of Dairy Science* **96**, 2816–2825.

50. Bäckhed F (2011) Programming of host metabolism by the gut microbiota. *Annals of Nutrition and Metabolism* **58**, 44–52.

51. Pavlović N, Stankov K & Mikov M (2012) Probiotics – interactions with bile acids and impact on cholesterol metabolism. *Applied Biochemistry and Biotechnology* **168**, 1880–1895.

52. Guilloteau P, Martin L, Eeckhaut V, et al. (2010) From the gut to the peripheral tissues: the multiple effects of butyrate. *Nutrition Research Reviews* **23**, 366–384.

53. Vipperla K & O'Keefe SJ (2012) The microbiota and its metabolites in colonic mucosal health and cancer risk. *Nutrition in Clinical Practice* **27**, 624–635.

54. Henningsson A, Bjiirck I & Nyman M (2001) Short-chain fatty acid formation at fermentation of indigestible carbohydrates. *Scandinavian Journal of Nutrition* **45**, 165–168.

55. Wolever TMS, Spadafora PJ, Cunnane SC, et al. (1995) Propionate inhibits incorporation of colonic [$^{1,2-13}$C]acetate into plasma lipids in humans. *American Journal of Clinical Nutrition* **61**, 1241–1247.

56. Arora T, Sharma R & Frost G (2011) Propionate. Anti-obesity and satiety enhancing factor? *Appetite* **56**, 511–515.

57. Wang Y, Xu N, Xi A, et al. (2009) Effects of *Lactobacillus plantarum* MA2 isolated from Tibet *kefir* on lipid metabolism and intestinal microflora of rats fed on high-cholesterol diet. *Applied Microbiology and Biotechnology* **84**, 341–347.

58. St-Onge MP, Farnworth ER, Savard T, et al. (2002) *Kefir* consumption does not alter plasma lipid levels or cholesterol fractional synthesis rates relative to milk in hyperlipidemic men: a randomized controlled trial. *BMC Complementary and Alternative Medicine* **2**, 1.

59. Kumar M, Nagpal R, Verma V, et al. (2012) Probiotic metabolites as epigenetic targets in the prevention of colon cancer. *Nutrition Reviews* **71**, 23–34.

60. Aronsson L, Huang Y, Parini P, et al. (2010) Decreased fat storage by *Lactobacillus paracasei* is associated with increased levels of angiopoietin-like 4 protein (ANGPTL4). *PLoS ONE* **5**, e13087.

61. Cheik NC, Rossi EA, Guerra RL, et al. (2008) Effects of a ferment soy product on the adipocyte area reduction and dyslipidemia control in hypercholesterolemic adult male rats. *Lipids in Health and Disease* **7**, 50.

62. Moon YJ, Baik SH & Cha YS (2014) Lipid-lowering effects of *Pediococcus acidilactici* M76 isolated from Korean traditional *makgeolli* in high fat diet-induced obese mice. *Nutrients* **6**, 1016–1028.

63. Park JE, Oh SH & Cha YS (2014) *Lactobacillus plantarum* LG42 isolated from *gajami sik-hae* decreases body and fat pad weights in diet-induced obese mice. *Journal of Applied Microbiology* **116**, 145–156.

64. Miyoshi M, Ogawa A, Higurashi S, et al. (2014) Anti-obesity effect of *Lactobacillus gasseri* SBT2055 accompanied by inhibition of pro-inflammatory gene expression in the visceral adipose tissue in diet-induced obese mice. *European Journal of Nutrition* **53**, 599–606.

65. Yoshida Y, Yokoi W, Wada Y, et al. (2004) Potent hypocholesterolemic activity of the yeast *Kluyveromyces marxuanus* YIT 8292 in rats fed a high cholesterol diet. *Bioscience, Biotechnology and Biochemistry* **68**, 1185–1192.

66. Yoshida Y, Yokoi W, Ohishi K, et al. (2005) Effects of the cell wall of *Kluyveromyces marxuanus* YIT 8292 on the plasma cholesterol and fecal sterol excretion in rats fed on a high cholesterol diet. *Bioscience, Biotechnology and Biochemistry* **69**, 714–723.

67. Waszkiewicz-Robak B (2013) Spent brewer's yeast and β-glucans isolated from them as diet components modifying blood lipid metabolism disturbed by an atherogenic diet. In *Lipid Metabolism*, chapter 12 [R Valenzuela Baez, editor]. InTech. http://www.intechopen.com/books/lipid-metabolism/spent-brewer-s-yeast-and-beta-glucans-isolated-from-them-as-diet-components-modifying-blood-lipid-me.

68. El-Arab AE, Foheid S & El-Said M (2009) Effect of yeast and botanical β-glucan on serum lipid profile and cecum probiotic bacteria using rats fed cholesterol diet. *Polish Journal of Food and Nutrition Sciences* **59**, 169–174.

69. Wilson TA, Barbato DA & Nicolosi RJ (2013) β-Glucan fiber from spent brewer's yeast reduces early atherosclerosis greater than psyllium in hypercholesterolemic Syrian golden hamsters. *Food and Nutrition Sciences* **4**, 566–571.

70. Yoshida Y, Naito E, Ohishi K, et al. (2007) Effect of *Kluyveromyces marxuanus* YIT 8292 crude cell wall fraction on serum lipids in normocholesterolemic and mildly hypercholesterolemic subjects. *Bioscience, Biotechnology, and Biochemistry* **71**, 900–905.

71. Liu H, Xie Y, Xiong L, et al. (2012) Effect and mechanism of cholesterol-lowering by *Kluyveromyces* from Tibetan *Kefir*. *Advanced Materials Research* **343**, 1290–1298.

72. Xie Y, Zhang H, Liu H, et al. (2015) Hypocholesterolemic effects of *Kluyveromyces marxianus* M3 isolated from Tibetan mushrooms on diet-induced hypercholesterolemia in rat. *Brazilian Journal of Microbiology* **46**, 389–395.

73. Psomas EJ, Fletouris DJ, Litopoulou-Tzanetaki E, et al. (2003) Assimilation of cholesterol by yeast strains isolated from infant feces and feta cheese. *Journal of Dairy Science* **86**, 3416–3422.

74. Girard P, Pansart Y & Verleye M (2014) Anti-hypercholesterolemic effect of *Saccharomyces boulardii* in the hamster. *Pharmacology* **94**, 239–244.

75. Aloğlu HŞ, Özer ED, Öner Z, et al. (2015) Investigation of a probiotic yeast as a cholesterol lowering agent on rats fed on a high cholesterol enriched diet. *KafKas Universitesi veteriner faKUltesi Dergisi* **21**, 685–689.

76. Ryan JJ, Hanes DA, Schafer MB, et al. (2015) Effect of the probiotic *Saccharomyces boulardii* on cholesterol and lipo-protein particles in hypercholesterolemic adults: a single-arm, open-label pilot study. *Journal of Alternative and Complementary Medicine* **21**, 288–293.

77. Huang Y, Wang J, Cheng Y, et al. (2010) The hypocholesterolaemic effects of *Lactobacillus acidophilus* American Type Culture Collection 4356 in rats are mediated by the down-regulation of Niemann–Pick C1-like 1. *British Journal of Nutrition* **104**, 807–812.

78. Lee J, Kim Y, Yun HS, et al. (2010) Genetic and proteomic analysis of factors affecting serum cholesterol reduction by *Lactobacillus acidophilus* A4. *Applied and Environmental Microbiology* **76**, 4829–4835.

79. Shin HS, Park SY, Lee DK, et al. (2010) Hypocholesterolemic effect of sonication-killed *Bifidobacterium longum* isolated from healthy adult Koreans in high cholesterol fed rats. *Archives of Pharmacal Research* **33**, 1425–1431.

80. Xie N, Cui Y, Yin YN, et al. (2011) Effects of two *Lactobacillus* strains on lipid metabolism and intestinal microflora in rats fed a high-cholesterol diet. *BMC Complementary and Alternative Medicine* **11**, 53.

81. Al-Sheraji SH, Ismail A, Manap MY, et al. (2012) Hypocholesterolaemic effect of yoghurt containing *Bifidobacterium pseudocatenulatum* G4 or *Bifidobacterium longum* BB536. *Food Chemistry* **135**, 356–361.

82. Fåk F & Bäckhed F (2012) *Lactobacillus reuteri* prevents diet-induced obesity, but not atherosclerosis, in a strain dependent fashion in Apoe-/- mice. *PLOS ONE* **7**, e46837.

83. Lindström C, Holst O, Nilsson L, et al. (2012) Effects of *Pediococcus parvulus* 2.6 and its exopolysaccharide on plasma cholesterol levels and inflammatory markers in mice. *AMB Express* **2**, 66.

84. Mohania D, Kansal VK, Shah D, et al. (2013) Therapeutic effect of probiotic *Dahi* on plasma, aortic, and hepatic lipid profile of hypercholesterolemic rats. *Journal of Cardiovascular Pharmacology and Therapeutics* **18**, 490–497.

85. Zhang L, Zhang X, Liu C, et al. (2013) Manufacture of Cheddar cheese using probiotic *Lactobacillus plantarum* K25 and its cholesterol-lowering effects in a mice model. *World Journal of Microbiology Biotechnology* **29**, 127–135.

86. Tang Z, Ma J, Zeng Z, et al. (2014) Inhibition of atherosclerotic plaque formation in ApoE-deficient mice by dietary supplementation with *Lactobacillus casei*. *Functional Foods in Health and Disease* **4**, 147–158.

87. Sadrzadeh-Yeganeh H, Elmadfa I, Djazayery A, et al. (2010) The effects of probiotic and conventional yoghurt on lipid profile in women. *British Journal of Nutrition* **103**, 1778–1783.

88. Kullisaar T, Shepetova J, Zilmer K, et al. (2011) An antioxidant probiotic reduces postprandial lipemia and oxidative stress. *Central European Journal of Biology* **6**, 32–40.

89. Asemi Z, Samimi M, Tabasi Z, et al. (2012) Effect of daily consumption of probiotic yoghurt on lipid profiles in pregnant women: a randomized controlled clinical trial. *Journal of Maternal–Fetal and Neonatal Medicine* **25**, 1552–1556.

90. Barreto FM, Simão ANC, Morimoto HK, et al. (2014) Beneficial effects of *Lactobacillus plantarum* on glycemia and homocysteine levels in postmenopausal women with metabolic syndrome. *Nutrition* **30**, 939–942.

91. Guardamagna O, Amaretti A, Puddu PE, et al. (2014) Bifidobacteria supplementation: effects on plasma lipid profiles in dyslipidemic children. *Nutrition* **30**, 831–836.

92. Mohamadshahi M, Veissi M, Haidari F, et al. (2014) Effects of probiotic yogurt consumption on lipid profile in type 2 diabetic patients: a randomized controlled clinical trial. *Journal of Research in Medical Sciences* **19**, 531–536.

93. Rajkumar H, Mahmood N, Kumar M, et al. (2014) Effect of probiotic (VSL#3) and omega-3 on lipid profile, insulin sensitivity, inflammatory markers, and gut colonization in overweight adults: a randomized, controlled trial. *Mediators of Inflammation* **2014**, 348959.