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Hypocholesterolaemic Effects of Probiotics

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1. Introduction

There is growing interest in the use of nutraceutical products which includes probiotics, prebiotics and related metabolites with cholesterol-lowering properties to prevent cardiovascular diseases (CVDs) [1]. Probiotics are beneficial bacteria that influence the health of the host by improving their microbial balance. Modifications of intestinal flora have been shown to be beneficial on lipid metabolism in mice [2-6], rats [7-11], guinea pigs [12] and pigs [13]. In contrast, studies in humans [14,15] indicated that the role of fermented milk products as hypocholesterolaemic agents were inconsistent but more reliable effects were documented in the recent clinical studies [16-19]. The cholesterol-lowering effect of probiotics was found to be highly strain-specific as different strains exhibited different levels of cholesterol-lowering activity [20-22]. Therefore, it is important to identify probiotic strains that exhibit excellent cholesterol-lowering ability.

Cholesterol-reducing mechanism(s) by probiotics remain to be elucidated. Deconjugation of bile salt by the bile salt hydrolase (BSH) enzyme and subsequent co-precipitation of cholesterol at acidic pH is one of the models frequently used to explain hypocholesterolaemic effects of probiotics [21,23]. Other studies have shown reduction of cholesterol through assimilation of cholesterol into bacterial cell membrane [24-28], adhesion of cholesterol onto bacterial cell surface [28] and through the binding of bile acids to bacterial exocellular polysaccharides [29]. A recent study showed the ability of probiotics to be able to produce protein(s) with cholesterol-lowering effect [30]. Probiotics are able to grow in prebiotics (indigestible carbohydrate) producing short chain fatty acids (SCFAs). Butyrate, a SCFA has the ability to inhibit liver cholesterol synthesis [31]. The role of probiotics as hypocholesterolaemic agents should be further explored.

2. Cardiovascular disease and treatments

Hypercholesterolemia is the major cause of coronary diseases. Diseases related to hypercholesterolemia have been projected to be the number one leading cause of death in the world by 2020 [32]. In fact, Roth *et al.* [33] concluded that the global burden of CVDs requires immediate action based on analysis of health examination survey of eight countries. An ideal strategy to control this disease is to lower cholesterol through a combination of lifestyle and pharmacologic approaches.

Cholesterol-lowering drugs that are available have different mechanisms of actions. Statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) are generally able to inhibit cholesterol synthesis in the liver and peripheral tissues. They have been extensively studied and found to possess better therapeutic effects than other lipid lowering drugs [34]. A recent meta-analysis that involved 14 studies with over 90000 patients for 5 years showed that statins reduced the risks of major cardiovascular events and overall mortality [35]. Yet, another meta-analysis of 11 studies showed no reduction in mortality with the use of statins [36]. A similar conclusion was observed in the recent Cochrane review [37]. However, Blaha *et al.* [38] emphasize that statins are critical in patients with increased cardiovascular risk as opposed to low-risk patients. This is probably due to the adverse effects related to the use of this class of drugs, which include myopathy [39] and cognitive impairment [40-42]. Other pharmacological agents that are used in the management of hypercholesterolemia are bile acid sequestrants, cholesterol absorption inhibitors, niacin, and fibrates. Nevertheless, these drugs have also been associated with many adverse effects that limit treatment compliance as well as quality of life.

Non-pharmacological treatment serves as a supportive therapy to reduce cardiovascular risk in otherwise healthy people. The common recommendations are dietary modifications, exercise and weight control. Modification of diet will allow lower drug doses that will reduce the adverse effects of drugs. Clifton *et al.* [43] reported that although dietary intervention to lower cholesterol had been effective it was underutilized. The idea of preventing and lowering hypercholesterolemia using 'functional foods' has emerged recently. Functional foods are broadly defined as foods that provide additional physiological benefits to the consumer beyond basic nutrition [44]. Functional foods that are commercially available with health claims of reducing cholesterol levels include oat bran fibre, soy protein, fish oil fatty acids, plant sterols and stanols, probiotics and prebiotics [44].

3. Definitions of probiotics

Over the years, probiotics have been defined in several ways. The term probiotic was coined by Lily and Stillwell [45] to describe growth-promoting factors produced by microorganisms. Parker [46] subsequently defined it as organisms and substances, which contribute to intestinal microbial balance. However, Fuller [47] pointed out that this definition was too broad and redefined probiotic as a live microbial feed supplement, which beneficially affects the health of the host animal by improving its intestinal microbial

balance. Havenaar *et al.* [48] considered the definition given by Fuller [47] to be restricted to feed supplements, animals and the intestinal tract, and described probiotics as mono or mixed cultures of live microorganisms which, when applied to animal or human, beneficially affected the host by improving the properties of the indigenous microflora. This definition does not restrict probiotic activities to intestinal microflora only, but also includes microbial communities at other sites of the body; the probiotic may consist of more than one bacterial species and that it can be applied to both human and animal. Salminen *et al.* [49] defined probiotics as microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host. This definition implies that probiotics do not necessarily need to be viable, and are limited to human only. Finally, FAO/WHO [50] described probiotics as live microorganisms which when administered in adequate amount confer a health benefit on the host.

4. Characteristics and health benefits of probiotics

Evidence is emerging that the intestinal flora does not exist as an entity by itself, but is constantly interacting with the environment, the central nervous system, the endocrine and the immune system [51-54]. There is growing scientific evidence to support the concept that maintenance of gut microflora may prevent or treat intestinal disorders [55-57]. Therefore, attempts have been made to improve health status by modulating the indigenous intestinal microbiota through probiotics [58,59]. However, for probiotics to be effective the selection of strains must be based on criteria that are coherent with the claim the probiotic is used for. Rational selection and validation of promising microbial strains should be based on scientific evidence obtained from *in vitro* models followed by *in vivo* studies. The important criteria that have been put forward by FAO/WHO [60] in the selection of food probiotics include identification of strains using state-of-the-art techniques, ability to tolerate gastric juice and bile, maintain stability and most importantly prove to be safe and beneficial to the consumer. The most common microorganisms in probiotic preparations that possess health benefits are lactic acid bacteria (LAB), mainly lactobacilli and bifidobacteria. Probiotics have been reported to improve intestinal tract health, enhance the immune system, reduce symptoms of lactose intolerance, decrease the prevalence of allergy, treat colitis and lower serum cholesterol levels [61-63].

5. Hypocholesterolaemic effects of probiotics

There has been considerable interest in the beneficial effects of lactobacilli and bifidobacteria on lipid metabolism since the discovery that fermented milk containing a wild *Lactobacillus* strain has hypocholesterolaemic effect in humans [64]. This study is often quoted as the basis for much of the animal and human studies subsequently carried out. The hypocholesterolaemic effects of probiotic *Lactobacillus* and *Bifidobacteria* strains on mice, rat, and human are summarised in Table 1. Taranto *et al.* [2,3] observed that administration of *L. reuteri* to mice reduced the serum total cholesterol (TC) by 20% and increased the ratio of HDL-C to LDL-C by 17%. Mice fed *L. plantarum* PH04 significantly ($P < 0.05$) reduced serum

	Probiotic strains	Effects	References
Mice	<i>L. reuteri</i>	Serum TC lowered	Taranto <i>et al.</i> (1998, 2003)
		Serum TC lowered	Ngyuen <i>et al.</i> (2007)
		Serum LDL-C lowered	Jeun <i>et al.</i> (2010)
	<i>L. fermentum</i> SM-7	Serum TC and LDL-C lowered	Pan <i>et al.</i> (2011)
Rats	A mixture of probiotic microorganisms or <i>L. acidophilus</i>	Serum TC lowered	Fukushima and Nakano (1995, 1996, 1999)
	<i>B. longum</i> SPM1207	Serum TC and LDL-C lowered	Usman and Hosono (2000)
	<i>B. longum</i> SPM1207(sonication killed)	Serum TC and LDL-C lowered	Lee <i>et al.</i> (2009)
	<i>L. plantarum</i> Lp91	Plasma TC and LDL-C lowered	Shin <i>et al.</i> (2010)
		Plasma HDL-C increased	Kumar <i>et al.</i> (2011)
		Serum TC and LDLC lowered	Xie <i>et al.</i> (2011)
Human	Probiotic-fermented milk product	Serum Total Cholesterol	Taylor and Williams (1998)
		Variable data from 1974-1997	
		Variable data from 1988-1998	De Roos and Katan (2000)
	<i>L. acidophilus</i> L1	Serum TC lowered	Anderson & Gilliland, 1999
	<i>B. longum</i> BL1	Serum TC lowered	Xiao <i>et al.</i> , 2003
<i>L. acidophilus</i>	No changes in blood lipid parameters	Lewis and Burmeister (2005)	

Probiotic strains	Effects	References
<i>L. fermentum</i>	No changes in blood lipid parameters	Simons <i>et al.</i> (2006)
<i>L. acidophilus</i> and <i>B. lactis</i>	Serum TC	Ataie-Jafari <i>et al</i> (2009)
<i>L. reuteri</i> NCIMB 30242	Serum HDL-C increased Serum TC and LDL-C	Jones <i>et al.</i> (2012)

Table 1. Hypocholesterolaemic effects of probiotic *Lactobacillus* and *Bifidobacteria* strains in mice, rats and humans

cholesterol by 7% as compared to the control group [4]. The *L. plantarum* PH04 strain was isolated from infant faeces and reported to be able to produce bile salt hydrolase enzyme *in vitro*. In another study, Jeun *et al.* [5] demonstrated that LDL-C was significantly ($P < 0.05$) lower (by 42%) in mice fed *L. plantarum* KCTC3928 and fecal bile acid excretion was accelerated (45%). They also found expressions of the LDL-receptor and 3-hydroxy-3-methylglutaryl coenzyme A reductase were marginally affected by feeding *L. plantarum* KCTC3928 but interestingly the gene expression and protein levels of CYP7A1 were significantly upregulated [5]. The gene CYP7A1 encodes for cholesterol 7- α -hydroxylase, the rate-limiting enzyme in the bile acid biosynthetic pathway in liver and thus controls cholesterol and bile acid homeostasis [65]. The increase in cholesterol 7- α -hydroxylase may explain the increase in fecal bile excretion and low serum cholesterol level observed by Jeun *et al* [5] since bile acid formation is a major pathway of cholesterol excretion from the body. Recently, Pan *et al.* [6] isolated *L. fermentum* SM-7 from a fermented milk drink (koumiss) and it was found to exhibit acid and bile tolerance and exhibited antimicrobial activity against *E. coli* and *S. aureus in vitro*. In mice, *L. fermentum* SM-7 significantly reduced serum TC and LDL-C but did not increase HDL-C significantly [6]. The study also showed that there was no bacterial translocation in the liver, spleen, or kidney of the treated mice indicating safety of the *Lactobacillus* strain[6].

In a series of experiments in rats, Fukushima and Nakano [7,8], and Fukushima *et al.* [9] showed that *L. acidophilus* or a mixture of probiotic microorganisms consistently reduced the serum TC. Usman and Hosono [10] also observed a significant reduction in TC and LDL-C in rats fed *L. gasseri*. In a more recent study, *B. longum* SPM1207 isolated from healthy adult Koreans reduced serum TC and LDL-C significantly ($p < 0.05$), and slightly increased serum HDL-C in rats [11]. In another study, Shin *et al.* [10] demonstrated that *B. longum* SPM1207, although killed by sonication, could significantly reduce serum TC and LDL-C, but with no significant improvement in HDL-C when fed to rats for 3 weeks [66]. Kumar *et al.* [67] revealed a 23% reduction in plasma TC, 38% reduction in LDL-C and 19% increase in HDL-C of rats fed with *L. plantarum* Lp91, a bile salt hydrolase producing strain. The faecal excretion of cholic acid was also found to be significantly higher in the probiotic-fed rats [6].

The latest study also consistently showed significant reduction by about 25% and 33% of serum TC and LDL-C respectively in rats fed *L. fermentum* 9-41-A. This strain was also isolated from faeces of healthy adults and selected for its probiotic characteristics [6].

In contrast to animal models, studies in humans conducted between 1974 to 1997 (reviewed by Taylor and Williams [14]) and from 1988 to 1998 (reviewed by de Roos and Katan [15]) indicate that the role of fermented milk products as hypocholesterolaemic agents was equivocal, as the clinical studies performed gave variable results and no firm conclusions could be drawn. The contradictory results observed were mainly related to the experimental designs especially the use of inadequate sample size and variations in the baseline levels of blood lipids [68]. Anderson and Gilliland [16] conducted a randomised, placebo-controlled, crossover 10-week study that involved 48 hypercholesterolaemic subjects. The subjects consumed milk fermented containing *L. acidophilus* L1 twice daily. The serum TC of subjects who consumed the fermented milk was significantly reduced when compared to those who consumed placebo. Xiao *et al.* [17] used a randomised, single-blind, parallel study for 4 weeks amongst 32 subjects. Subjects who consumed yoghurt containing *B. longum* BL1 had significantly reduced serum TC and LDL-C as compared to those given placebo yoghurt. Lewis and Burmeister [69] however reported that freeze-dried *L. acidophilus* supplementation had no effect on elevated cholesterol subjects using a randomised, placebo-controlled, crossover 6-week study. Simons *et al.* [70] in a double blind, placebo-controlled, parallel design trial also reported once again no beneficial effects on blood lipids after supplementation of *L. fermentum* for 10 weeks in volunteers with TC of about 4 mmol/L. Ataie-Jafari *et al.* [18] reported that *L. acidophilus* and *B. lactis* exhibited cholesterol-lowering effect on hypercholesterolemic subjects. They used a placebo controlled, randomised, crossover trial for 6 weeks, in healthy subjects with serum TC of 5.17–7.76 mmol/l. The most recent study was that conducted by Jones *et al.* [19] on 114 hypercholesterolaemic adults who consumed yoghurt formulated to contain microencapsulated BSH-active *L. reuteri* NCIMB 30242 twice daily for 6 weeks. This double-blind, placebo-controlled, randomised, parallel, multi-centre study showed significant reductions in serum LDL-C (9%), TC (5%) and non-HDL-C (6%) over placebo but the serum concentrations of HDL-C remained unchanged.

A meta-analysis based on six studies was conducted by Agerhol-Larsen *et al.* [72] on the hypocholesterolaemic effect of fermented dairy product on plasma cholesterol levels. The short term intervention study showed reductions in TC (-8.51 mg/dL) and LDL-C (-7.74 mg/dL) in subjects who consumed the fermented dairy product when compared to the control [72]. Later, Guo *et al.* [71] conducted another meta-analysis of randomised controlled trials that evaluated the effects of probiotics consumption on blood lipids. Their study was based on 13 trials of 485 participants with high, borderline high and normal cholesterol levels. The results showed that subjects who received probiotics had significantly lower TC (-6.40 mg/dL) and LDL-C (-4.90 mg/dL) as compared to those taken placebo. Both the meta analyses resulted in a similar observation. It seems that the hypocholesterolaemic effects of probiotics in human clinical trials in the recent years have been more consistent.

6. Proposed mechanisms of cholesterol reduction by lactic acid bacteria *in vitro*

Although studies have shown that some lactic acid bacterial strains have a hypocholesterolaemic effect on the host, the mechanism(s) involved is not fully understood. Different hypotheses have been advanced to explain the hypocholesterolaemic effect of lactic acid bacteria *in vitro*. There was little or no information on the direct action of cultured milk products in reducing cholesterol until Gilliland *et al.* [24] and Walker and Gilliland [25] reported that cholesterol removal *in vitro* could be due to the active up-take or assimilation of cholesterol by the bacterial cell. From then on, the cholesterol assimilation model was frequently used to explain the *in vivo* hypocholesterolaemic effects.

Based on their findings, Gilliland *et al.* [24] proposed that the uptake of cholesterol by *Lactobacillus acidophilus* strains occurred only when the cultures were grown in the presence of bile under anaerobic conditions. They also found that uptake of cholesterol increased with increasing concentrations of bile salts in the media, and the uptake appeared to level off at oxgall concentrations greater than 0.4%. Noh *et al.* [26] noted that cholesterol removed from the culture supernatant of *L. acidophilus* incubated in the presence of bile salt was accompanied by an increase in the amount of cholesterol in the cell pellet. Noh *et al.* [26] noticed that cells that were grown in the presence of cholesterol micelles and bile salts were more resistant to lysis by sonication, suggesting that assimilation of cholesterol into the cellular membrane, resulted in sturdier bacterial cells. This lends support to the idea that cell membrane alteration occurred in the presence of both bile salts and cholesterol. They also observed that assimilation occurred both at pH 6.0 and without pH control. Similar results have been reported for bifidobacteria [73] and lactococci [28]. Kimoto *et al.* [28] observed that both live and heat-killed *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* N7 were able to remove cholesterol from growth media. However, the amount of cholesterol removed by live cells was significantly higher than that removed by dead cells. They found that cell density and dry weight were higher when the cells were grown in the presence of cholesterol, and the rate of cholesterol removal was more rapid during their exponential growth phase. Since only living cells can possibly uptake cholesterol into their membranes, they concluded that the mechanisms of cholesterol removal by the live strain were due to cholesterol assimilation and binding, while removal of cholesterol by dead cells was only due to binding onto bacterial cell surface. They also observed a difference in the fatty acid distribution pattern for *Lactococcus* grown with or without cholesterol. Later Taranto *et al.* [74] reported modifications in the lipid profile of *L. reuteri* grown with cholesterol, while Liong and Shah [75] also observed alteration in the fatty acid profiles of lactobacilli grown in the presence of cholesterol in the growth medium. Pigeon *et al.* [29] suggested that cholesterol removal by *L. delbrueckii* and *Streptococcus thermophilus* strains were due to binding of free bile acids to their cell membranes through exocellular polysaccharide (EPS). They found that the strains, which produced the most EPS, bound the greatest amount of bile acids, while the strains that produced the least amount of EPS only bound minimal amounts of bile acids and conjugated bile acids did not bind to the EPS. They hypothesized

that the strains reduced serum cholesterol levels by binding to enhance excretion of free bile acids via the faeces. Tol and Aslim [76] also demonstrated a correlation between cholesterol removal and EPS production of *L. delbrueckii* spp.

However, Klaver and Van der Meer [23] proposed another mechanism of cholesterol reduction by lactic acid bacteria. They pointed out that the experimental set up to prove the cholesterol assimilation as hypothesized by Gilliland *et al.* [24] and Walker and Gilliland [25] did not take into account the effects of bacterial deconjugation of bile salts. They suggested that the removal of cholesterol was due to the disruption of the cholesterol micelles caused by bile salt deconjugation and co-precipitation of cholesterol with free bile salts as the pH of the medium dropped because of acid production during growth of lactobacilli and bifidobacteria. This conclusion was based largely on their observation that no cholesterol was removed when the growth medium was maintained at pH 6.0, a pH at which free bile acids would remain in solution and prevent the precipitation of free bile salts. They associated these results to the decreased solubility of free bile acids under acidic conditions which in turn reduced the solubility of cholesterol (thus termed co-precipitation of cholesterol). Taranto *et al.* [77] and Ahn *et al.* [21] also stressed that removal of cholesterol was closely related to bile salt deconjugation at low pH. Brashears *et al.* [27] demonstrated that strains of *L. acidophilus* were able to deconjugate bile salts and remove cholesterol when grown at both pH 6.0 and without pH control. On the other hand, strains of *L. casei* grown at pH 6.0 removed very little cholesterol compared to the same strains grown at uncontrolled pH. However, examination of cellular membranes of *L. casei* grown under both conditions revealed no cholesterol deposits. Therefore, the authors concluded that removal of cholesterol by *L. casei* was most likely due to co-precipitation of cholesterol with deconjugated bile salts at pH less than 6.0, while removal of cholesterol by *L. acidophilus* was due to assimilation of cholesterol into cellular membranes. Cholesterol reduction may be strain specific.

In an attempt to determine the validity of the hypothesis of assimilation and/or precipitation of cholesterol by *Lactobacillus* and *Bifidobacterium* species, Grill *et al.* [78] cultured a strain of each species in a medium containing different bile salts. They found that the cholesterol removing ability of bifidobacteria varied according to the type of bile salts. In the presence of taurocholic acid, the removal of cholesterol was due to bacterial uptake and co-precipitation, but in the presence of oxgall, only co-precipitation was observed. It seems that the composition of bile salt is another important factor in determining the amount of cholesterol removed through co-precipitation. Many studies, hypothesised that lactic acid bacterial strains are able to remove cholesterol through a combination of two or more mechanisms which includes, assimilation of cholesterol during growth, binding of cholesterol to cellular membrane and deconjugation of bile salts [27,75,79-86].

More recently, another mechanism was hypothesized by Kim *et al.* [30] who found the cell-free supernatant of *L. acidophilus* ATCC 43121 to contain proteins that were able to significantly reduce cholesterol levels even after heat-treated or controlled at pH 6.0. The extract exhibited greatest cholesterol-reducing activity when maintained at pH 4.0.

Subsequent analysis identified the up-regulated proteins to be associated with stress response, translation, and metabolic processes and also have functions related to the cell membranes. Huang and Zheng [87] reported that soluble factors produced by *L. acidophilus* have the ability to inhibit cholesterol absorption in Caco-2 cells by down-regulating the gene expression of Niemann-Pick C1-like 1 (NPC1L1). NPC1L1 protein has been identified as a key player in cholesterol absorption and a promising target for cholesterol-lowering mechanisms [88]. These studies suggest the possibility to alter gut microbiota through supplementation of probiotics for reduction of cholesterol absorption. Lee *et al.* [89] investigated cholesterol reducing activity of lactobacilli using genetic and proteomic analysis and reported that *ccpA* which encodes the catabolite control protein to play an important role in cholesterol reducing activity of probiotics. They also hypothesized that membrane associated proteins play an important role in probiotic cholesterol reduction.

7. Cholesterol reduction by lactic acid bacteria *in vivo*

It has been suggested that assimilation of cholesterol during growth of probiotic lactic acid bacteria and binding of cholesterol to their cellular membrane would result in less cholesterol available for absorption, leading to reduced serum cholesterol of the host [24]. Fukushima and Nakano [7] suggested that the hypocholesterolaemic effects could also be due to the ability of probiotic organisms to inhibit hydroxymethylglutaryl coenzyme A (HMG CoA) reductase. It is well documented that suppression of HMG CoA reductase is correlated with the inhibition of cholesterol synthesis. Short chain fatty acids (SCFA) have also been implicated to be involved in the reduction of cholesterol. Hara *et al.* [90] observed that a dietary SCFA mixture and SCFA produced by the fermentation of sugar beet fibre significantly reduced plasma cholesterol levels in rats. They suggested that absorbed SCFA might have suppressed the cholesterol synthesis rate in the liver and were involved in the cholesterol-lowering effect. Fukushima *et al.* [9] also suggested that the lowering of cholesterol level in rats fed probiotics was probably due to specific SCFA metabolites such as propionic acid and butyric acid. Propionate and butyrate are able to reduce hepatic cholesterol synthesis [91]. However, researchers acknowledge that deconjugation of bile salts by probiotic strains could be an important factor in lowering serum cholesterol through interference with the enterohepatic absorption of bile salts and cholesterol.

8. Enterohepatic circulation of bile acids

Bile acids are synthesised from cholesterol in the liver and stored in the gall bladder. The steroid is conjugated with an amide bond at the carboxyl C₂₄ position to one of two amino acids, glycine and taurine [92], before it is excreted into the small intestine. The conjugated bile salts are amphipathic in nature and form micelles that facilitate digestion, emulsification and absorption of lipids from the small intestine [93]. The conjugated bile salts are readily absorbed in the gastrointestinal tract by active transport mechanisms and are returned to the liver; this process is known as enterohepatic circulation. A large pool of bile acids accumulates and undergoes a number of enterohepatic cycles daily [94].

The conversion of cholesterol to bile acids is the major route by which cholesterol is metabolized. To date, only two studies have shown the ability of probiotics strains [5,95] to be able to up-regulate CYP7A1 an enzyme that catalyzes the conversion of cholesterol to bile acids. An increase in CYP7A1 leads to reduction in hepatic cholesterol levels [5,95] and increase fecal cholesterol [95] and bile acids [5] excretion in hamsters and mice respectively. However, in the intestine, the bile salts may also be deconjugated by probiotics strains through bile salt hydrolase activity resulting in free bile salts. Free bile salts are more likely to be excreted via the faeces than the conjugated ones [96-98].

9. Significance of bile salt deconjugation by probiotic strains

The peptide-like bond between the bile acid and taurine or glycine is not cleaved by most proteolytic enzymes, but it is by bile salt hydrolase (BSH), an intracellular enzyme [99]. Deconjugation of bile salts *in vitro*, has been demonstrated by intestinal bacteria, such as *Clostridium* [100], *Lactobacillus* [101], *Streptococcus* [101], and *Bifidobacterium* [100]. The BSH enzyme catalyses the hydrolysis of conjugated bile acid to produce free bile salt and the corresponding amino acid. The biological function of BSH in these microorganisms remains unclear. However, in recent years, BSH has received attention because of its potential therapeutic benefits for reducing cholesterol. Upon hydrolysis, the physico-chemical properties of bile salts change drastically. Deconjugated bile acids are less soluble at low pH and less absorbed in the intestine and are more likely to be excreted in the faeces [102]. Excretion of free bile acids via the faeces is the primary route of elimination of cholesterol from humans and other animals [5]. To maintain bile salt homeostasis, more bile acids need to be synthesised and this in turn will reduce cholesterol in the body pool as cholesterol is the precursor for bile acids. The hypocholesterolaemic effect of lactic acid bacteria in this manner is comparable with that of cholestyramine treatment, which like other bile salt sequestrants, binds bile salts and prevents them from being reabsorbed [97]. Intestinal microflora plays a major role in interfering with the reabsorption of bile acid from the intestine, thus, promoting their excretion [97]. Increased faecal bile acid concentrations have also been observed in probiotic-fed mice [5,10], rats [7,67] and in human [19]. These studies strongly suggest that the deconjugating activity of BSH-active lactic acid bacteria may be associated with the hypocholesterolaemic effect.

The significance of BSH activity other than its hypocholesterolaemic effect, is far from understood. However, it has been suggested that certain BSH-active bacteria are able to utilise the amino portion of the deconjugated bile salt. Van Eldere *et al.* [103] reported that bacteria that are able to produce BSH may be able to use the amino acid, taurine, as an electron acceptor which can improve growth. De Smet *et al.* [97] suggested that deconjugation may be a detoxification mechanism which is of vital importance to the *Lactobacillus* cell. It has also been suggested that BSH is a detergent shock protein that protects the bacteria from the toxicity of bile acids in the gastrointestinal tract [104]. Moser and Savage [105] reported that BSH activity may be important for the bacteria to survive and colonise the gastrointestinal tract. Given the potential importance of the enzyme, genes

encoding it may be important targets for genetic manipulation. Although lactobacilli are able to deconjugate bile salts to unconjugated primary bile acids, they do not further transform the unconjugated bile acids into deoxycholate (secondary bile acids) [21]. This is a good probiotic trait because formation of secondary bile acids, which are usually produced by intestinal bacteria, may contribute to colon cancer and gallstones. Ooi and Liong [106] reported that there has been no study that specifically evaluated the detrimental effects of BSH of probiotics in humans and that further studies are required.

10. Conclusion

The consumption of probiotics is gaining popularity especially in the maintenance of health and prevention of disease. In particular, the role of probiotics as a hypocholesterolaemic agent has been explored extensively. Progress has been made in the recent years on the selection, identification and characterization of strains that actually fulfill the criteria of true probiotic microorganisms and that are able to exert cholesterol reducing effects *in vitro*. However, much remains to be done on the mechanism of action as it has not been very well understood. The potential of probiotics in reducing cholesterol levels in animal models have been quite consistent in the recent years but but larger and better controlled human trials with scientifically substantiated evidence of the benefits are required.

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