Antibacterial and Cholesterol Reducing Lactic Acid Bacteria from Silk Worm (Bombyx mori) Gut Environment – A Review

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ABSTRACT
The human body is colonized by an enormous population of bacteria (microbiota) that provides the host with coding capacity and metabolic activities. Among the human gut microbiota are health-promoting indigenous species (probiotic bacteria) that are commonly consumed as live dietary supplements. Recent studies are starting to provide insights into how probiotic bacteria sense and adapt to the gastrointestinal tract environment. In this Review, the application of lactic acid bacteria as probiotics using the well-recognized model probiotic bacterial genera Lactobacillus from gut of silk worm Bombyx mori has been discussed as examples. Recent researches have demonstrated that probiotics can prevent pathogen colonization of the gut and reduce the incidence or relieve the symptoms of various diseases caused by dysregulated immune responses. Therefore, probiotics, through their effects on the host immune system, might ameliorate diseases triggered by disordered immune responses. Caveats remain and, because the beneficial effects of probiotics can vary between strains, the selection of the most suitable ones will be crucial for their use in the prevention or treatment of specific diseases.

INTRODUCTION
Lactic acid bacteria
Lactic acid bacteria (LAB) are a group of Gram-positive, non-sporulating bacteria that includes species of Lactobacillus, Leuconostoc, Pediococcus and Streptococcus. Dietary LAB refers to those species and strains that are used in food and feed fermentation processes. The term LAB does not reflect a phyletic class, but rather a group of organisms that are defined by their ability to produce a common end product lactic acid from the fermentation of sugars. LAB have limited biosynthetic abilities, and require pre-formed amino acids, B vitamins, purines, pyrimidines and, usually a sugar as a carbon and energy source. These nutritional requirements restrict their habitats to those in which the required compounds are abundant (Oh et al. 2000). Nevertheless, LAB occupies a range of niches, including milk, plant surfaces and the oral cavity, gastrointestinal tract and vagina of vertebrates. Although many genera of bacteria produce lactic acid as a primary or secondary end product of fermentation, the term Lactic Acid Bacteria (LAB) is conventionally reserved for genera in the Order Lactobacillales, which includes Lactobacillus, Leuconostoc, Pediococcus, Lactococcus and Streptococcus, in addition to Carnobacterium, Enterococcus, Oenococcus, Tetragenococcus, Vagococcus, and Weissella. Because they obtain energy only from the metabolism of sugars, lactic acid bacteria are restricted to environments in which sugars are present. They have limited biosynthetic ability, having evolved in environments that are rich in amino acids, vitamins, purines and pyrimidines, so they must be cultivated in complex media that fulfil all their nutritional requirements (Sandra Nell et al. 2010). Most are free-living or live in beneficial or harmless associations with animals, although some are opportunistic pathogens. They are found in milk and milk products and in decaying plant materials. Lactic acid bacteria are among the most important groups of microorganisms used in food fermentations. They contribute to the taste and texture of fermented products and inhibit food spoilage bacteria by producing growth-inhibiting substances and large amounts of lactic acid. They are normal flora of humans in the oral cavity, the intestinal tract and the vagina, where they play a beneficial role (Adrienne et al. 2006). The differential characteristics of lactic acid bacteria based on morphology and physiology are given in Table 1.

Silkworm (Bombyx mori)
The silkworm is the larva or caterpillar of the domesticated silk moth, Bombyx mori (Fig. 1) belonging to the Family Bombycidae. It is an important economic insect since it is the producer of silk. A silkworm’s preferred food is white mulberry leaves, but it may also eat the leaves of any other mulberry tree (i.e., Morus rubra or Morus nigra) as well as the Osage orange. It is entirely dependent on humans for its reproduction and no longer occurs naturally in the wild (Shan Wu et al. 2010).
GUT AND GUT FLORA OF SILK WORM BOMBYX MORI AS PROBIOTIC APPROACH

The gut of silkworm is shown in Fig. 2. The gut is colonised with a large number of bacteria. Probiotics are the live microbial food supplements beneficially affecting the host by improving the microbial balance. Several researchers have reported about beneficial role played by use of probiotics in humans, ruminants, aquaculture and insects. Therefore, products containing probiotic bacteria are gaining popularity, increasing the importance of their accurate speciation (Sonnenburg et al. 2006). Similarly, in mulberry silkworm (Bombyx mori) presence of different types of bacteria in the gut have been reported. Most of the species belonging to Genus Streptococcus are found to be pathogenic to Bombyx mori larvae while bacteria from Genus Pediococcus, Leuconostoc and Lactobacillus did not produce any infected silkworm. The precise mechanism of beneficial effect on host or interaction among the different bacterial strains present as microflora is not known. Different species of lactic acid bacteria have been extensively studied and found to be beneficial as probiotics (Shan Wu et al. 2010).

Probiotics formulations are commercially used for humans, for aquaculture while there are no reports on the availability of probiotics formulations specifically designed for silkworms. Although, it was observed improvement in larval body weight, cocoon weight, shell weight and pupation percentage of silkworm larvae when fed on mulberry leaves treated with a commercial probiotic formulation containing Lactobacillus plantarum. However, the extent of colonization of L. plantarum in the gut of silkworm is to be ascertained by using genobiotic strains. As probiotic cultures used in the present study was originally isolated from an indigenous silkworm breed, it readily colonized the gut of silkworm when applied as probiotic formulation (Singh et al. 2003).

Functional analysis of lactic acid bacteria isolated from silkworm breeds revealed their antibiotic potential against a range of Gram positive and Gram negative bacteria and it was found to inhibit the germination of conidia of entomopathogens B. bassiana and M. anisopliae in vitro. Considering the sensitivity of antibiotics and the proven antibacterial activity of lactic acid bacteria, isolated from silkworm breeds, it is prudent to go for probiotic application of lactic acid bacteria for ecofriendly management of silkworm diseases (Sonnenburg et al. 2006).

Role of Nonpathogenic Interactions of Gut Bacteria of Silkworm in Improving Cocoon Production by Bombyx mori, A Molecular Approach

The diversity of the Insecta is reflected in the large and varied microbial communities inhabiting the gut. Studies, particularly with gut flora of silk worm, have focused on the nutritional contributions of gut bacteria in insects living on suboptimal diets. The indigenous gut bacteria, however, also play a role in withstanding the colonization of the gut by non-indigenous species including pathogens. Gut bacterial consortia adapt by the transfer of plasmids and transconjugation between bacterial strains present as microflora is not known. Different species of lactic acid bacteria have been extensively studied and found to be beneficial as probiotics (Shan Wu et al. 2010).

Table 1: Differential characteristics of lactic acid bacteria based on morphology and physiology.

<table>
<thead>
<tr>
<th>Genus Characteristic</th>
<th>Lactobacillus</th>
<th>Enterococcus</th>
<th>Lactococcus</th>
<th>Leuconostoc</th>
<th>Pediococcus</th>
<th>Streptococcus</th>
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<tbody>
<tr>
<td>Morphology</td>
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<td>CO2 from glucose</td>
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<td>Growth</td>
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<td>at 10°C</td>
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<td>at 45°C</td>
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<td>in 6.5% NaCl</td>
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<td>at pH 4.4</td>
<td>±</td>
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<td>-</td>
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<tr>
<td>at pH 9.6</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>D, L, DL</td>
<td>L</td>
<td>L</td>
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<td>configuration</td>
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</tbody>
</table>

+ positive; - negative; ± varies between species, *test for homo or heterofermentation of glucose: - homofermentation, + heterofermentation

New Paradigm in Silkworm Disease Management Using Probiotic Application of Lactic Acid Bacteria

Diseases in silkworm cause 30 to 40 per cent loss in
sericultural productivity. Antibiotics are widely used in sericulture industry as a component of bed disinfectants and as therapeutic applications against bacterial diseases. Several studies indicated that gut microflora is sensitive to the antibiotics and the loss of which causes adverse effects on the physiological system of the insects. Decreased survival of mulberry silk worms, impairing reproductive systems, weight reduction and reduced amylase activity due to elimination of microorganisms by antibiotics such as tetracycline, streptomycin and chloramphenicol have been reported (Hooper & Gordon 2001). Administration of antibiotics was reported to have detrimental effect on intestinal microflora of silkworms. Chloromycetin administration cause general reduction in gut bacterial population of silkworms. It was observed that population of endogenous gut bacteria viz., Micrococci, Streptococci and Flavobacteria were reduced in number with concomitant increase in the number of coliforms (Mohanraj & Subramanian 2009).

Synthetic antibiotics are widely used in sericulture units for disease control. Studies have shown that overuse of antibiotics causes annihilation of gut microbes which are functionally important for carrying out several physiological processes in silkworm. Considering the sensitivity of gut flora to antibiotics, studies were carried out on probiotic application of lactic acid bacteria, isolated from silkworm on the endogenous gut microflora and its antimicrobial activity. Probiotic applications of lactic acid bacteria have resulted in increase of endogenous actinomycetes population. The culture filtrates of lactic acid bacteria were having strong antibacterial activity against a wide range of Gram positive and Gram negative bacteria (Chikara Kaito et al. 2005).

The usage of probiotics is more common in human and veterinary medical sciences. It was taken up to explore the positive influence of probiotic application of lactic acid bacteria, isolated from Indian silkworm breeds on endogenous gut microflora and their antibacterial activity against bacterial pathogens. Application of probiotics has paved way for ecofriendly management of silkworm disease management (Mohanraj & Subramanian 2009).

**Evidence for the Beneficial Effects of Probiotics**

Probiotics are live bacteria that can survive in the human intestine. The gut microflora is a complex ecosystem regulated by nutrients and physical conditions such as temperature, acidity and the gaseous composition of the intestinal lumen. The indigenous gut microflora is a ‘cohabiting’ partner to the host from birth to death. It fills a distinct ecological niche that can resist the colonization of exogenous pathogenic microorganisms. Moreover, normal immune system development can occur in response to stimuli from gut microflora (Bernard Dugas et al. 2008). Therefore, individuals whose normal gut microflora are destabilized might in turn exhibit disrupted immune function and and/or become vulnerable to infectious diseases. Probiotics can assist in the recovery of gut microflora disturbed by a variety of causes and are expected to prevent or ameliorate certain diseases, at least in part, by modulation of the host immune system (Erika Isolauri et al. 2001).

One of the beneficial effects exerted by probiotics is their defence against pathogenic infection. Because probiotics are usually resistant to gastric acid and bile salts, they can traverse the stomach and survive within the ileum. They compete with pathogenic microbes for nutrients, and their metabolites (short chain fatty acids) can make the gut environment unsuitable for pathogens (Sandra Nell et al. 2010).

Fig. 3. shows three hypothetical pathways by which probiotics can trigger and modulate immune function in the intestine. (a) Specialized epithelial cells called M (microfold) cells in the follicle-associated epithelium covering Peyer’s patches or in the villi can take up probiotics directly by transcytosis. Macrophages (Mfs) or dendritic cells (DCs) are present immediately below M cells and then engulf probiotics and trigger immune responses. (b) DCs in the intestinal lamina propria have been found to extend their dendrites between intestinal epithelial cells (IECs) and might directly sample and process probiotics in the gut lumen. (c) Probiotics directly affect IECs to secrete an array of cytokines, which in turn modulate the immune functions of DCs, T cells and B cells in the gut-associated lymphoid tissue (GALT) (Kankainen et al. 2009).
Mechanisms of Immunomodulation by Probiotics

The gut immune system has the challenge of responding to pathogens while remaining relatively unresponsive to food antigens and the commensal microflora. In the developed world, this ability appears to be breaking down, with chronic inflammatory diseases of the gut commonplace in the apparent absence of overt infections. In both mouse and man, mutations in genes that control innate immune recognition, adaptive immunity, and epithelial permeability are all associated with gut inflammation. This suggests that perturbing adaptive immunity, and epithelial permeability are all associated with gut inflammation. This ability appears to be breaking down, with chronic inflammatory diseases of the gut commonplace in the apparent absence of overt infections. In both mouse and man, mutations in genes that control innate immune recognition, adaptive immunity, and epithelial permeability are all associated with gut inflammation. This suggests that perturbing homeostasis between gut antigens and host immunity represents a critical determinant in the development of gut inflammation and allergy (Bernard Dugas et al. 2008).

As mentioned above, probiotics serve to not only stabilize the gut microflora but can potentially modulate the function of immune cells. Microorganisms in the gut lumen are recognized and processed by the immune system through several routes. (i) They can attach to intestinal epithelial cells (IECs) and modulate their function directly. (ii) M (microfold) cells localized in the follicle-associated epithelium overlying Peyer’s patches can transport them to the immune cells in the subepithelial dome region immediately underneath. (iii) DCs in the lamina propria (LP) actively extend dendrites to sample microbes in the gut lumen. Although there is still far from complete understanding, probiotics might exert their immune-modulating functions through a similar set of pathways (Fig. 3).

Various Health Benefits from Probiotics Consumption—Bacteriocins of Lactic Acid Bacteria

The health benefits of probiotics are shown in Fig. 4. Numerous bacteriocins have been isolated from LAB over the last three decades, and vary in size from small (<3 kDa), heavily post-translationally modified peptides to large heat labile proteins (Fredrik Bäckhed et al. 2005). The continual emergence of new bacteriocins has necessitated a continual updating on the classification of bacteriocins. Bacteriocins produced by LAB are commonly divided into three main groups. Class I consists of small, post-translationally modified peptides which are characterized by the presence of modified thioether amino acids such as lanthionine, β-methylanthionine and α-unsaturated amino acids such as dehydroalanine and dehydrobutyryl and are usually referred to as lantibiotics. On the basis of alignment of mature peptides, lantibiotic peptides from LAB can be subdivided into six subgroups. Nisin A and nisin Z make up a single group, while lactacin 481 and lactacin J49 belong to the lactacin 481 group; plantaricin C, LtnA1 (one component of the two-peptide lactacin 3147) and Plwα belong to the mersacidin group; LtnA2 (the second component of lactacin 3147) and Plw belong to the LtnA2 group; the two peptides, Cyll1 and Cylls from the two-peptide cytolsin, form a group of their own; while lactocin S is also grouped separately. Such criteria as the stability, inhibition spectrum and mode of action are important when considering lantibiotic peptides for particular applications as these will influence the efficacy of the bacteriocin in different environments (Shinichi Kawamoto & Jun Shima 2004).

Class II comprises of a very large group of heat-stable unmodified peptide bacteriocins which can also be further subdivided. Class IIa includes Pediocin-like Listeria-active peptides with a conserved N-terminal sequence YGNGV-C. Undoubtedly, the most documented and well characterized bacteriocin of the LAB is nisin. Nisin is a 34 amino acid peptide with a pentacyclic structure. It has a broad spectrum of inhibition and kills a wide range of Gram-positive bacteria. This heat-stable bacteriocin is active at physiological pH and has been shown to be effective in non-acidic environments such as on the surface of mould ripened cheese. It is highly active in a variety of food environments and is particularly effective at the low pH common in fermented dairy products (Fredrik Bäckhed et al. 2005). Nisin is encoded on a self transmissible transposon and therefore can be transferred in a food grade manner between strains. Another example of a potentially useful lantibiotic produced by a strain of L. lactis is lactacin 3147 S (Dimov et al. 2005). This bacteriocin, like nisin, inhibits a wide range of Gram-positive bacteria. This heat-stable bacteriocin is active at physiological pH and has been shown to be effective in non-acidic environments such as on the surface of mould ripened cheese. It is composed of two peptides, LtnA1 and LtnA2, both of which are required for full biological activity. Cultures which produce lactacin 3147 have the important advantages over nisin producing strains, in that they are generally good acid producers and thus can substitute for com-

commercial starters in food fermentations (Francisco Diez-Gonzalez 2002). Moreover, the genetic determinants for the bacteriocin are encoded on a 60 kb conjugative plasmid, pMRC01, which can be transferred in a food grade manner to other strains (Jerry et al. 2008).

The prospects of having a bank of efficient bacteriocin producing lactic acid bacteria means that it is possible to produce bacteriocins in a variety of food products with concomitant improvements in quality and safety, and these bacteriocins are most effective in killing pathogens such as Listeria monocytogenes and Clostridium botulinum (Oh et al. 2000).

Properties of Some Well Characterized Bacteriocins: Lantibiotics

Many species of lactic acid bacterial genera such as Lactobacillus, Lactococcus, Leuconostoc, Streptococcus, and Carnobacterium among others are capable of producing small peptides that can inhibit a broad range of Gram-positive bacteria. Most LAB bacteriocins inhibit bacteria by forming pores in the cell membrane and dissipating the proton motive force. Gram negative bacteria are protected from the lethal effect of LAB bacteriocins by the outer membrane.

Many different types of LAB bacteriocins have been studied and characterized, but the most widely known are nisin, lactacin, enterocin, pediocin and plantaricin (Oh et al. 2000). These have been extensively studied for their application in foods, but just a few of them have been used in livestock.

Lantibiotics are bacteriocins produced by LAB that contain lanthionine rings in their molecules and are typically classified as Class I bacteriocins. There are several LAB species capable of producing lantibiotics. The types of lantibiotics that have been more frequently identified and characterized are nisin and lacticin. Nisin is typically produced by Lactococcus lactis strains and lacticin can be produced by Lac. lactis and Lac. sake. These antimicrobial peptides have between 23 to 25 amino acid residues. Nisin is probably the best characterized LAB bacteriocin and is the only antimicrobial peptide approved for use in foods (Francisco Diez-Gonzalez 2002).

Nisin: The use of nisin in foods has been approved for cheeses, but there is an enormous amount of information about its application to inhibit a variety of pathogenic and spoilage bacteria in many food products. One of the most promising applications of nisin is on the control of Listeria monocytogenes in ready-to-eat meats. Despite the widespread use of nisin, its application in livestock has been largely limited. One of the few uses that this bacteriocin has been investigated for is as part of a germicidal preparation for cows teats. The germicidal preparation was capable of reducing the population of Staphylococcus aureus by more than 3 log CFU/g and has been commercialized. Monensin is an ionophore that has been widely used in cattle production as a growth promoter because of its multiple beneficial effects on rumen fermentation (Dimov et al. 2005).

Other lantibiotics: LAB strains that produce lacticin 3147...
are considered as generally recognized as safe and can be used for food production, but approval to the purified bacteriocin preparation has not been granted. Significant evidence has shown that lacticin is capable of inhibiting a variety of Gram-positive bacteria for food applications, but similar to nisin its application in live domestic animals has been rather scarce. Because of its ability to inhibit *Staphylococcus aureus* and *Streptococcus dysgalactiae*, lacticin 3147 has also been used to disinfect cow’s teats and to treat mastitis with relative success. Incorporation of lacticin 3147 into a teat seal preparation reduced approximately 10-fold the prevalence of mastitis causing bacteria in animals that were inoculated with *S. dysgalactiae* (Shinichi Kawamoto & Jun Shima 2004).

### Bacteriocins: Mode of Action

Lactic acid bacteria (LAB) bacteriocins can be grouped on the basis of structure, but also on the basis of mode of action (Fig. 5). Some members of the class I (or lantibiotic) bacteriocins, such as nisin, have been shown to have a dual mode of action. They can bind to lipid II, the main transporter of peptidoglycan subunits from the cytoplasm to the cell wall, and therefore prevent correct cell wall synthesis, leading to cell death. Furthermore, they can use lipid II as a docking molecule to initiate a process of membrane insertion and pore formation that leads to rapid cell death. A two-peptide lantibiotic, such as lacticin 3147, can have these dual activities distributed across two peptides, whereas mersacidin has only the lipid-II-binding activity, but does not form pores. In general, the class II peptides have an amphiphilic helical structure, which allows them to insert into the membrane of the target cell, leading to depolarisation and death. Large bacteriolytic proteins (here called bacteriolysins, formerly class III bacteriocins), such as lysostaphin, can function directly on the cell wall of Gram-positive targets, leading to death and lysis of the target cell (Shinichi Kawamoto & Jun Shima 2004).

### Commensal Bacteria in Epithelial/Immune Cell Function in the Gut

Probiotic bacteria have multiple potential health effects, including blocking gastroenteric pathogens, neutralizing food mutagens produced in the colon, enhancing the immune response, lowering serum cholesterol and stopping intestinal dysfunction. In general, probiotic bacteria must colonize the gastrointestinal tract (GIT) of the host, have acid-and bile salt-tolerance, and block putrefactive bacteria in the GIT. Lactic acid bacteria, especially *Lactobacillus* spp. and *Bifidobacterium* spp. are important GIT residents and are used as probiotic strains to improve health. *Lactobacillus* and *Bifidobacterium* have been used in fermented foods for several centuries without adverse effects and are classified as Generally Recognized as Safe (GRAS) because of their long history of safe use, particularly in dairy food.

Over the past decade, there has been increasing interest in the use of LAB as mucosal delivery vehicles. This stems from the long-term scientific quest for effective strategies to deliver vaccine antigens, microbiocides and therapeutics to the mucosal tissues, specifically through the intranasal, oral or genital mucosal surfaces. Mucosal delivery of therapeutics or vaccines for chronic diseases and infections of mucosal origin could enhance their potency and specificity, but also reduce the potential side effects of systemic routes of administration. In this respect, the intrinsic advantages of LAB represent an attractive alternative to the use of other mucosal delivery systems such as liposomes, micro particles and attenuated pathogens (Alander et al. 1999).

Given that a probiotic drink might contain only a billion live bacteria, compared with the one hundred trillion bacteria that are estimated to be present in the human gut, some researchers argue that probiotics must have negligible effects on the human metabolic system. However, new research published in ‘Molecular Systems Biology’ indicates that probiotics have substantial effects on metabolism, not only by increasing the number of friendly bacteria in the gut, but also by influencing the functional ecology of other gut bacteria. Probiotics are thought to reduce the numbers of potentially harmful bacteria in the intestine, such as pathogenic *Escherichia coli* and Clostridia spp. by increasing the sizes of populations of friendly bacteria, which ferment carbohy-
drates and have reduced proteolytic activity (Adrienne et al. 2006). Probiotics have therefore been used to treat numerous conditions, such as irritable bowel syndrome, and to attempt to prevent allergies in children (Erika Isolauri et al. 2001). It is known that the host response to probiotic ingestion and the biochemical mechanisms that might be involved, using a novel top-down systems-biology approach that allowed all the bacterial and host metabolic interactions to be resolved. Therefore, it suggests that probiotics affect how much fat the body can absorb this demonstrate that direct and beneficial effects of probiotics on both the composition of gut microbial populations and metabolic processes (Dimov et al. 2005).

Cholesterol combating activity of LAB probiotics: Lactic acid bacteria are beneficial probiotic organisms that contribute to improved nutrition, microbial balance and immunoenhancement of the intestinal tract, as well as lower cholesterol (Liong & Shah 2004). Reduction in serum cholesterol levels after consumption of probiotic product fermented with a wild Lactobacillus strain, there has been considerable interest in the beneficial effects of fermented milk products containing lactobacilli and bifidobacteria on human lipid metabolism. Several human studies have suggested a moderate cholesterol-lowering action of probiotics products fermented with certain strains of LAB (Perdigo´n et al. 2002). However, role of probiotic products as hypcholesterolemic agents in humans is still equivocal. From several in vitro studies a number of mechanisms have been proposed for the purported cholesterol-lowering action of probiotic bacteria. These include physiological actions of the end products of short-chain fatty acid fermentation (especially propionate), cholesterol assimilation by the bacteria, cholesterol binding to the bacterial cell wall, and enzymatic deconjugation of bile acids. These hypotheses need to be confirmed in animal and human studies, and the exact mechanisms of action of probiotic bacteria on cholesterol reduction remains unclear (Dora et al. 2002).

Cardiovascular disease is the most important cause of death. Hypercholesterolemia is strongly associated with coronary heart disease and arteriosclerosis, and decreasing serum cholesterol is an important treatment option. HDL-cholesterol can prevent arteriosclerosis by removing cholesterol from the blood stream, whereas LDL-cholesterol causes accumulation of cholesterol in blood vessels. Every 1% reduction in body cholesterol content lowers the risk for cardiovascular diseases by 2% (Marco Ventura et al. 2009). Therapeutic lifestyle changes including dietary interventions, in particular a reduction of saturated fat and cholesterol, are established as a first line therapy to reduce LDL-cholesterol. A change in dietary habits, such as eating fermented products containing lactic acid bacteria, can reduce cholesterol. Since cholesterol-lowering potential of lactic acid bacteria such as Lactobacillus and Bifidobacterium is commonly studied in vitro or in vivo (experimental animals and human subjects) (Bernard Dugas et al. 2008). Cholesterol reduction by lactic acid bacteria can be explained by five mechanisms:

a. Fermentation products of lactic acid bacteria inhibit cholesterol synthesis enzymes and thus reduce cholesterol production.

b. The bacteria facilitate the elimination of cholesterol in faeces.

c. The bacteria inhibit the absorption of cholesterol back into the body by binding with cholesterol.

d. The bacteria interfere with the recycling of bile salt (a metabolic product of cholesterol) and facilitate its elimination, which raises the demand for bile salt made from cholesterol and thus results in body cholesterol consumption.

e. Due to the assimilation of lactic acid, lactic acid bacteria have anti-tumour effects and block harmful intestinal enzyme activities, a recognized risk factor for colon cancer (Dora et al. 2002).

Consumption of L. rhamnosus GG decreased the activity of β-glucuronidase, nitroreductase and cholyglycine hydrolase. Consumption of milk enriched with L. casei for four weeks temporarily decreased β-glucuronidase activity in 10 healthy men but not in 10 healthy control subject. Consumption of milk fermented with a Bifidobacterium species decreased β-glucuronidase activity compared with baseline but did not affect faecal pH or the activity of nitrate reductase, nitroreductase and azoreductase. Consumption of fermented milk with L. acidophilus, B. bifidum, Streptococcus lactis, and Streptococcus cremoris for three weeks decreased nitroreductase activity but not β-glucuronidase and azoreductase (Fredrik Bäckhed et al. 2005).

CONCLUDING REMARKS AND PERSPECTIVES

The gastrointestinal tract is the site where the divergent needs of nutrient absorption and host defence collide. The former requires a large surface area and a thin epithelium that has the potential to compromise host defence. Many infectious diseases involve the gut, and the investment by the gut in protecting itself is evident in the abundant lymphoid tissue and immune cells it harbours. Most infectious diseases of the gut are largely under control, yet gastrointestinal food allergies and idiopathic inflammatory conditions have dramatically increased; in other words, we now have inflammation without infection (Lorena et al. 2009). Although the
reason for this remains unknown, a prevailing notion is that the absence of overt gut infection has upset the balance between the normal bacteria that colonize the healthy gut and the mucosal immune system. Further studies should be focused on the mechanisms of action of LAB within the gastrointestinal tract and in the immune system which stimulate the in vivo immunity effects (Perdigo´n et al. 2002).

Probiotics has dramatically highlighted some important caveats associated with their clinical application, whether a probiotic mixture (three *Lactobacillus* strains, two Bifidobacterium strains and one *Lactococcus* strain) administered to severe acute pancreatitis and IBD patients could prevent the incidence of opportunistic infection and improve clinical symptoms. The probiotics showed no significant impact on the incidence of opportunistic infections but more worryingly showed a higher mortality in the probiotic-treated group. Probiotics might not be wholly without risk, and the selection of safe but effective probiotic strains will be crucial for their widespread clinical application (Jerry et al. 2008).

In this review, we evaluated the in vitro cholesterol-lowering effects and anti bacteriopathogenic strains of lactic acid bacteria of silk worm origin. We hope this study provides further background and new improved strains for the understanding of the purported action of probiotic bacteria on cholesterol reducing and anti-bacteriopathogenic levels.

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