Role of Lactobacilli in Gastrointestinal Ecosystem

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Abstract


Lactobacilli are normal and important inhabitants of the gastrointestinal tract of human and animals. They constitute an integral part of healthy gastrointestinal microecology and have received considerable scientific attention. The role that lactobacilli play in the ecology of the gastrointestinal tracts of humans and animals may be of major importance, but it is as yet poorly understood.

The present review summarizes the current state of knowledge regarding the role of lactobacilli in gastrointestinal ecosystem. Topics covered include: nutritional and therapeutic benefits of lactobacilli; deconjugation of bile acids; neutralization of toxins and other intestinal reactions; protection against intestinal pathogens by production of antibacterial substances such as organic acids, hydrogen peroxide, bacteriocins and etc.; stabilization of intestinal microflora, excluding colonization of enteropathogenic bacteria by adhesion to the intestinal wall and competition for nutrients; reduction of the cholesterol level in blood serum by cholesterol assimilation; decrease risk of colon cancer by detoxification of toxic, mutagenic and carcinogenic substances, and modulation of fecal procarcinogenic enzymes such as b-Glucuronidase, azoreductase and nitroreductase; tumor suppression by stimulation of non-specific and specific immune response.

Key words: Lactobacilli, gastrointestinal microflora, gastrointestinal ecosystem, antagonistic, antimutagenic, anticarcinogenic, immunological effects
Abbreviations: GIT - Gastrointestinal tract; GIE - Gastrointestinal ecosystem; GIM - Gastrointestinal microflora; CE - Competitive exclusion; IT – Intestinal tract; LAB - Lactic acid bacteria; BSH - Bile salt hydrolase

Introduction

Gastrointestinal ecosystems (GE) of humans and animals are complex, open, integrated, interactive units containing many microbial species, which are divided in two major groups: autochthonous (indigenous) which exist in close association with the gut epithelium and allochthonous (non-indigenous) which occurs free in the...
gut lumen. It is, however, difficult to make a clear distinction between the two groups. In regard to autochthonous microorganisms, Savage (1977, 2001) has suggested a useful definition. According to Savage the autochthonous microflora must:

- Be capable of growing anaerobically;
- Be always present in the gut of normal adult animals;
- Colonize the various parts of the digestive tract;
- Establish their own habitats during succession (i.e., sequential establishment of the various microorganisms) in infant animals;
- Maintain a stable population in normal adults;
- Associate intimately with the epithelial mucosa of the tract of the gut they colonize.

Its high population density, wide diversity, and complexity of interactions characterize the microbial community inhabiting the gastrointestinal tract (GIT). It has been estimated that there are in the gut $10^{14}$ bacterial cells whereas the number of eukaryotic cells comprising the human body amounts to only $10^{13}$ (Luckey, 1972). Within this population there are about 500 different bacterial species (Savage, 1984; Ewing and Cole, 1994; Bengmark, 1998; Bird et al., 2000; Miguel, 2001; Cole et al., 2003), although 40 types comprise 99% of the microflora (Tannock, 1988). The human body contains 10 times more protective indigenous flora than do eukaryotic cells (Tancrede, 1992). The unique biotic and abiotic components of the GIT (specialized chemical environment and appropriately adapted microbes) permit the definition of the “gastrointestinal ecosystem” (GIE) (Savage, 1977, 2001). The systems are established during postnatal life, when various microbial species find their way into the intestinal tract. When germfree animals are conventionalized, i.e. colonized with conventional flora, similar ecosystems are established. Results of studies on ex-germfree animals have demonstrated that some microbial species may act synergistically and others may act antagonistically with each other. The exact mechanisms involved in these interactions, however, have not been elucidated (Dueluzeau et al., 1984; Midtvedt et al., 1987; Savage, 2001).

The numerically dominant inhabitants of the GIE are obligate anaerobes. In the neonatal mammal, such as the human infant, piglet and calf the intestinal tract is sterile at birth (Haenel, 1970). It is rapidly colonized from the mouth and rectum, usually initially with the mother’s vaginal and perianal flora. In detail of wide variety of young animals showed that at birth the GIT becomes flooded with multiplying bacteria: coliforms, bifidobacteria, streptococci, including enterococci, clostridia, bacteroides, and other anaerobes, followed rapidly by lactobacilli.

The metabolic activities of the gastrointestinal microflora (GIM) are extremely complex and diverse. Many investigations have shown that intestinal bacteria play a major part in the enzyme activities of intestine; production of b-glucuronidase (Hawksworth et al., 1971), azoreductase (Brown, 1981; Raffii et al., 1990), nitroreductase (Zachariah and Juchau, 1974), 7a-dehydroxylase (Hirano et al., 1981), 7a-dehydrogenase (Wilkins and Tassell, 1983), toxic, carcinogenic or mutagenic metabolites from diet (Rowland et al., 1985; Goldin et al., 1996); deconjugation of bile acids (Aries and Hill, 1970; Floch et al., 1971, 1972; Gilliland and Speck, 1977a; Tannock et al., 1994); detoxification of dietary toxicants; enterohepatic circulation of drugs, food additives and ste-
roids; alteration in susceptibility of host tumor induction etc. For review see Drasar and Hill (1974), Yuguchi et al. (1992), Denev (1996, 1997); Bird et al. (2000); Denev et al. (2000); Savage (2001).

The composition and metabolic activities of GIM is very complex and strictly determined by the local environmental conditions (Croucher et al., 1983; Edwards et al., 1985; Seddon, 1989), physiological interactions (Floch et al., 1971, 1972), diet (Winitz et al., 1970; Maier et al., 1974; Moore and Holdeman, 1975, Denev, 1993a; Savage, 2001) and another factors (Hill et al., 1982; Kim, 1989; Seddon, 1989; Mitsuoka, 1990, Denev, 1996, 1997). The size of the microbial population in different regions of the tract also varies between animal species too (Savage, 1977; Tannock, 1984, 2004).

Microbiological interest in the role of lactobacilli inhabiting the GIT dates, from the time of Metchnikoff (1903, 1907) when it was observed that the natural fermentation of milk by lactic acid bacteria (LAB) prevented the growth of non-acid-tolerant types of microbe. It was proposed that, if lactic fermentation prevented the putrefaction of milk, it should have a similar effect in the digestive tract. The implantation of lactobacilli in the GIT would suppress the growth of “putrefactive” bacteria, thus reducing the amount of toxic substances generated in the digestive tract. Although Metchnikoff’s hypothesis was popular at the beginning of that century it was unsupported by experimental or clinical proof, partly due to the lack of adequate experimental techniques at that time. However, the remarkable developments in techniques for microbiological research and for raising germ-free animals in recent years, have produced data showing the important role played by intestinal bacteria in animals, leading to a fresh reassessment of Metchnikoff’s hypothesis.

The balance of the GIM is then controlled by acid secretion in the stomach (although this is buffered to some extent by the milk imbibed) and ingestion of immunoglobulins and other protective factors in the mother’s milk, so that lactobacilli become the dominant organisms and other groups rapidly decline (Reiter, 1978). Lactobacilli predominate in the stomach and small intestine, but in the jejunum and large intestine strict anaerobes such as bacteroides are the majority flora and lactobacilli constitute only 0.07-1.0% of the total flora. This desirable predominance of lactobacilli in the upper intestine, which is established on suckling, help to prevent the potentially lethal diarrhea or scouring that occurs in young animals when enteropathogenic coliforms proliferate in the upper GIT. The mechanisms by which lactobacilli suppress the rest of the bacterial flora is not fully understood but is probably partly due to lactic acid production and partly to inhibitory systems present in the raw milk, including the lactoperoxidase system, which can be activated by H$_2$O$_2$-producing lactobacilli (Reiter, 1978). In the young animals, lactobacilli usually become the principal component of stomach and small intestine, developing from about 10$^4$ to 10$^9$/g intestinal content, highest numbers being obtained in the lower part of the small intestine (Smith, 1971).

*Lactobacillus* spp. are important in creating a balanced microbial population in GIE (Sandine et al., 1972; Shahani and Ayebo, 1980; Bianchi-Salvadory, 1986; Denev et al., 2000; Varnam, 2002; Tannock, 2004). The interest in their existence in different areas of the body and their role in the host and microflora-related physiochemical conditions in the organism
has become one of the subjects of a comparatively new research field - microbial ecology (Haenel et al., 1957, 1957a, 1980; Dubos, 1966; Reuter, 1975; Goldin and Gorbach, 1984; Gorbach, 1990; Prins, 1996; Mackie et al., 1999).

The therapeutic and nutritive attributes of LAB particularly the lactobacilli have been actively researched since then (Speck, 1976; Sandine et al., 1972; Sandine, 1979; Shahani and Ayebo, 1980; Garg and Mital, 1992). Thereafter, many investigations have shown that the presence of lactobacilli in the intestinal tract (IT) is helpful in maintaining good health, restoring body vigor, stimulating host immune mechanisms, productivity, and combating intestinal and other disease disorders (Rettger et al., 1935; Gilliland and Speck, 1977; Gilliland, 1979; Friend and Shahani, 1984; Perdigon et al., 1987; Denev, 1993; Denev et al., 2000; Morishita, 2003; Sandholm and M. Saarela, 2003).

Subsequent research has emphasized that the lactobacilli to be used as dietary adjunct must be able to survive the hostile environment in the GIT and proliferate (Hawley et al., 1959; Gilliland et al., 1978; Hargrove and Alford, 1978). Several investigations have shown that *Lactobacillus acidophilus* is not only capable of establishing in the complex ecosystem of the gut (Kopeloff, 1926; Myers, 1931; Stark et al., 1934; Rettger et al., 1935) but also of imparting many health benefits (Gilliland, 1989; Mital and Garg, 1992; Sellars, 1992; Mital et al., 1995). These include stabilization of intestinal microflora, antagonistic action toward intestinal and food-borne pathogens, control of serum cholesterol, prevention of colon cancer, and enhanced availability of nutrients (Shah, 2001).

There are many other reports, beginning with Metchnikoff suggesting that LAB particularly the lactobacilli are a numerically dominant and important group of microorganisms colonizing the GIT and of the female reproductive tract of humans (Drasar and Hill, 1974; Mitsuoka et al., 1975; Conway, 1989; Mikelsaar and Mandar, 1993; Varnam, 2002) and animals (Roach et al., 1977; Savage, 1975, 1977; Sharpe, 1981; Worthington and Fulghum, 1988; Tannock et al., 1990; Denev et al., 2000; Tannock, 1992, 2004).

Beyond these roles, some *Lactobacillus* species have been exploited as “live microbial supplements”, intended to beneficially affect the host by balancing the natural microflora of the GIT. Probiotics, literally meaning “for life” (Fuller, 1992) have been the subject of considerable scientific and commercial attention over the past two decades (for review see Denev, 1996; 1997; Fuller, 1989, 1997; Klaenhammer, 1995, 1997, 1997a,b; Denev et al., 2000; Shah, 2001; Tannock, 2002, 2004).

Due to their many beneficial properties lactobacilli have attracted the greatest attention of researchers. In addition, the mechanisms involved in the expression of these activities are not clearly understood. Therefore, an attempt has been made to review the pertinent literature on these aspects critically.

**MAJOR CHARACTERISTICS OF THE LACTOBACILLI**

Lactobacilli are members of the LAB, a broadly defined group characterized by the formation of lactic acid as a sole or main end product of carbohydrate metabolism (Tannock, 2004). There are 44 species of lactobacilli listed in *Bergey’s Manual of Systematic Bacteriology*.
Eighty species of lactobacilli are recognized at present (Satokari et al., 2003). Many Lactobacillus spp. have been detected in GT of vertebrate animals and humans (Table 1).

Members of the genus Lactobacillus constitute an extremely diverse group of bacteria that provide considerable benefits to humans and animals as natural inhabitants of the GIT. They are Gram-positive, non-sporeforming, rods or coccobacilli with a G+C content usually below 50 mol%. The mol% G+C of the DNA ranges from 32-53. They are strictly fermentative, aerotolerant (microaerophilic) or anaerobic and have complex nutritional requirements (carbohydrates, amino acids, peptides, fatty acid esters, salts, nucleic acid derivatives, vitamins). Various compounds (e.g. citrate, malate, tartarate, quinolinate, nitrate, nitrite, etc.) may be metabolized and used as energy source (Kandler and Weiss, 1986; Hammes and Vogel, 1995). Using glucose as a carbon source, lactobacilli may be either homofermentative (producing more than 85% of fermentative products as lactic acid) or heterofermentative (producing lactic acid, carbon dioxide, ethanol, and/or acetic acid in equimolar amounts). The nutritional requirements of lactobacilli are reflected in their habitats, which are rich in carbohydrate-containing substrates: they are found on plants or material of plant origin, in fermented or spoiled food, or in association with the bodies of animals. Lactobacilli are catalase and cytochrome negative, usually non-motile, that does not usually reduce nitrate. Gelatin not liquefied. Casein not digested but small amounts of soluble nitrogen produced by most strains. Indole and H2S2 not produced. Lactobacilli are aciduric with optimal pH 5.5-6.2. Growth temperature range of Lactobacilli are 2-53°C - optimum generally 30-40°C (Hammes and Vogel, 1995). The classification of lactobacilli relies on biochemical-physiological criteria include three groups (Table 2).


COLONIZATION OF LACTOBACILLI TO EPITHELIAL SURFACES

In recent years much attention has been given to the study of the administration of living microbial preparations to farm animals (probiotics), which would to assure improvement in nutrition and in the animal’s resistance to disease. Since the report of Metchnikoff (1907) many reports have indicated that Lactobacillus strains are important in creating balanced microbial populations in the GIT. Colonization of intestinal tissue by lactobacilli is a natural phenomenon which arises earlier and more intensely in animals reared on traditional farms than those in factory-farms, where colonization may be detained or upset by acceleration of the rearing cycles, and above all by the often indiscriminate use of antibiotics at subtherapeutic levels.

For the administration of Lactobacillus culture to animals to be of practical
<table>
<thead>
<tr>
<th>Species</th>
<th>References</th>
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<tbody>
<tr>
<td>L. acidophilus</td>
<td>Axelsson and Lindgren (1987); Tannock et al. (1990); Arihara et al. (1996); Holzapfel et al. (2001)</td>
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<tr>
<td>L. agilis</td>
<td>Baele et al. (2001)</td>
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<tr>
<td>L. animalis</td>
<td>Roach et al. (1977)</td>
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<tr>
<td>L. aviarius</td>
<td>Fujisawa et al. (1984)</td>
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<tr>
<td>L. aviarius subsp. aviaires</td>
<td>Gusils et al. (1999)</td>
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<tr>
<td>L. aviarius subsp. araffinosus</td>
<td></td>
</tr>
<tr>
<td>L. brevis</td>
<td>Russell (1979)</td>
</tr>
<tr>
<td>L. casei</td>
<td>Kandler and Weiss (1986); Salminen et al. (1993); Ahne et al. (1998); Holzapfel et al. (2001)</td>
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<tr>
<td>L. crispatus</td>
<td>Tannock et al. (1990); Du Toit et al. (2001)</td>
</tr>
<tr>
<td>L. delbruecki</td>
<td>Russell (1979)</td>
</tr>
<tr>
<td>L. coryniformis</td>
<td>Lin and Savage (1984)</td>
</tr>
<tr>
<td>L. curvatus</td>
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<tr>
<td>L. fermentum subsp. cellobiosus</td>
<td>Gusils et al. (1999)</td>
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<tr>
<td>L. animalis</td>
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</tr>
<tr>
<td>L. gallinarum</td>
<td>Fujisawa et al. (1992)</td>
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<td>L. johnsonii</td>
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<tr>
<td>L. gasseri</td>
<td>Klein et al. (1998); Fujiwara et al. (2001)</td>
</tr>
<tr>
<td>L. fermentum</td>
<td>Fuller et al. (1978); Barrow et al. (1980); Morishita (1982); Hautefort et al. (1996)</td>
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<tr>
<td>L. intestinalis</td>
<td>Du Toit et al. (2001)</td>
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<tr>
<td>Lactobacillus gastricus</td>
<td>Roos et al. (2005)</td>
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<td>Lactobacillus antri</td>
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<tr>
<td>Lactobacillus kalixensis</td>
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<tr>
<td>Lactobacillus ultunensis</td>
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<tr>
<td>L. murinus</td>
<td>Ma et al. (1990)</td>
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<tr>
<td>L. plantarum</td>
<td>Russell (1979); Bengmark (1998); Du Toit et al. (2001)</td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td>Holzapfel et al. (2001)</td>
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<tr>
<td>L. reuteri</td>
<td>Tannock et al. (1982); Sara et al. (1985); Molin et al. (1992, 1992a); Holzapfel et al. (2001)</td>
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<tr>
<td>L. ruminis</td>
<td>Sharpe et al. (1973)</td>
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<tr>
<td>L. salivarius</td>
<td>Fuller (1973); Barrow et al. (1980); Sara et al. (1985); Hoetefort et al. (1996)</td>
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<tr>
<td>Lactobacillus thermotolerans</td>
<td>Niamsup et al. (2003)</td>
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<tr>
<td>L. vitiulus</td>
<td>Sharpe et al. (1973); Tannock et al. (1982)</td>
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use, the following conditions are essential: a) host specificity of the strains used, and b) their ability to form stable populations in the GIT. Attachment of the strain to the intestinal mucosa is one of the selection criteria for probiotic microorganisms. Adhesion or close association of potentially probiotic LAB, including lactobacilli to epithelial cells may further contribute to competitive exclusion (CE) (Gusils et al., 2002a).

Some strains of lactobacilli inhabiting the GIT of animals exhibit a particularly intimate association with their hosts. The lactobacilli colonize the proximal region of the human small intestine at population levels that are minor (<10⁹ g⁻¹) when compared to the colonic flora (10¹⁰ g⁻¹) where bifidobacteria are predominant members (10⁹ g⁻¹). Tissue-associated lactobacilli have been found on the gastrointestinal surfaces of many animal species. Lactobacilli adhere to and multiply on the stratified squamous epithelial cells of the proximal small intestine in animals (rodents, pigs and fowl), and are continually shed into the lower regions of the tract (Pedersen and Tannock, 1989; Tannock, 1990). In mice, for example, lactobacilli inhabit the tissue surface the esophagus and fore-stomach (Tannock, 1992). In rats, lactobacilli colonize the fore-stomach (Brownlee and Moss 1961, Savage, 1977). In fowl, they inhabit the epithelial surface of the crop (Fuller and Turvey, 1971; Fuller, 1973, 1975, 1978). In pigs, lactobacilli colonize the epithelial surface of the esophagus and of a small area of tissue in the

Table 2
Classification of Lactobacillus spp.

<table>
<thead>
<tr>
<th>Group I:</th>
<th>Obligately homofermentative - (15 species)</th>
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<tbody>
<tr>
<td></td>
<td>Hexoses are almost exclusively (&lt;85%) fermented to lactic acid by the Embden-Meyerhov-Parnas (EMP) pathway. The organisms possess fructose 1,6-bisphos-phate-aldolase but lack phospho- ketolase, and therefore, neither gluconate nor pentoses are fermented</td>
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<th>Group II:</th>
<th>Facultative heterofermentative - (11 species)</th>
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<td></td>
<td>Hexoses are almost exclusively fermented to lactic acid by the RMP pathway. The organisms possess both aldolase and phosphoketolase, and therefore, not only ferment hexose but also pentoses (and often gluconate). In the presence of glucose, the enzyme</td>
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<tr>
<th>Group III:</th>
<th>Obligately heterofermentative - (18 species)</th>
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<tr>
<td></td>
<td>Hexoses are fermented by the phosphogluconate pathway yielding lactate, ethanol (acetic acid) and CO₂ in equimolar amounts. Pentoses enter this pathway and may be fermented.</td>
</tr>
</tbody>
</table>

* Kandler and Weiss (1986); Du Toit et al. (2001)
stomach - pars esophagea (Fuller et al., 1978; Barrow et al., 1980). Under natural conditions, lactobacilli colonize epithelial surfaces in mice, rats, fowl and pigs soon after birth (hatching). Lactobacilli attain high population levels on the epithelial surface (of the order of $10^8$ bacteria per gram of sample) and a layer of lactobacilli, sometimes several cells thick, is present on the tissue surface throughout the host’s life. The lactobacilli are shed from the colonized surface and can be detected throughout the remainder of the animal’s digestive tract (Tannock, 1990).

**Specificity of colonization.** There are several indications in the literature that the colonization ability of lactobacilli is host specific (Lin and Savage, 1984; Tannock and Archibald, 1984; Conway and Kjelleberger, 1989; Gusils et al., 2002). The host specificity of epithelium-associating strains has been demonstrated in both *in vitro* and *in vivo* experiments (Fuller, 1973; Suegara et al., 1975; Kotarski and Savage, 1979; Wesney and Tannock, 1979; Barrow et al., 1980; Fuller and Brooker, 1980; Tannock et al., 1982). Mitsuoka (1969) observed differences between the adhesion characteristics of *L. acidophilus* strains isolated from man and animals. Morishita et al. (1971) found that *L. acidophilus* isolated from a human IT could not be implanted in the IT of a chick. Gilliland et al. (1980) compared two *L. acidophilus* strains for their use as a dietary adjunct for young calves. They found that the *L. acidophilus* strain of calf origin was more effective as a dietary adjunct in calf than the strain of human origin. According to Tannock (1990), several factors are likely to be involved in this phenomenon:

- Lactobacilli isolated from fowl adhere only to crop epithelial cells, not to cells from the rat forestomach in *in vitro* experiments in which the bacteria have been cultivated in a standard laboratory medium. Rat isolates of lactobacilli cultured in the same standard medium adhere only to epithelial cells from rats. Bacterial strains from different hosts cultured under the same physiological conditions thus exhibit host specificity, which suggests that interactions occur between specific adhesins and receptors on bacterial and host cells.
- The stratified, squamous epithelia lining the proximal digestive tracts of fowl, pigs, and rodents differ in chemical composition. The murine forestomach, for example, has a keratinized epithelium, whereas the chicken crop does not. Lactobacilli may have to be nutritionally adapted to inhabit certain types of epithelial surfaces.
- Physiological conditions, including the nature of the diet, may influence the colonization of epithelial surfaces by lactobacilli. Although clearly marked differences in diet affect the composition of the normal microflora of the gastrointestinal tract, investigation of subtle physiological differences on intestinal microbes has proved technologically difficult.

This indicates that a *Lactobacillus* strain isolated from indigenous microflora of one animal species will not necessarily colonize the same site in another animal species. Beside the animal species specificity for adhesion, one can also recognize strain specificity within the bacterial species. Barrow et al. (1980) demonstrated that the degree of adherence to squamous epithelial cells of the stomach of pigs was different for various strains within the same species of lactobacilli. This may be caused by the fact that some *Lactobacillus* spp. do not consist of genetically homologous group. For example, according to DNA-hybridization techniques, *L. acidophilus* forms a heterogeneous group of bacteria (Johnson et al., 1987). Host-species specificity of the intestinal microflora has also
become clear from characterization studies (differences in biotypes, serotypes and plasmid patterns). In a study by Convay et al. (1987), the tested lactic acid bacteria showed comparable adhesion patterns for human and pig ileal cells. On the other hand, it is not surprising that Mayram-Makinen et al. (1983) showed that lactobacilli from plant and cultured milk and cheese did not adhere in vitro to epithelial cells of pigs and calves.

Although many strains of lactobacilli do not associate with epithelial surfaces and many animal species do not demonstrate the phenomenon of epithelial colonization by microbes, the tissue-associating lactobacilli provide a useful focus for further discussion of the microecology of gastrointestinal lactobacilli.

**Mechanisms of colonization.** In recent years there has been increasing interest in the phenomenon of microbial attachment to surfaces as an ecological determinant (Tannock, 1990; Salminen et al., 1996). Adhesion of lactobacilli to epithelial surfaces in the beginning of the colonization process for some strains of lactobacilli in the GIT. Anchored to the surface of an epithelium, these lactobacilli are able to resist the flushing action of the relatively rapid flow of digest passing through the proximal digestive tract. Successful colonization by any strain of lactobacillus, however, depends on the metabolic ability of the microbe to function in the GE (Tannock, 1990, 2002).

Adhesion can be non-specific, based on physicochemical factors, or specific involving adhesin molecules on the surfaces of adhesive bacteria and receptor molecules on epithelial cells (Salminen et al., 1996). Earlier studies using animal systems shown that lactobacilli colonize intestinal surfaces by adsorption to gastric epithelial cells (Fuller, 1973; Savage, 1979; Kotarski and Savage, 1979). This physical association with epithelial surfaces is an important criterion in determining the ability of the organism to colonize the gut. Many investigations have reported the presence of a surface layer in certain lactobacilli exterior to the cell wall and its involvement in the adherence of these organisms to the gastrointestinal tract (Brooker and Fuller, 1975; Masuda and Kawata, 1980, 1981; Hood and Zottola, 1987). Subsequent research has shown that this layer is composed of protein subunits that bind to the neutral polysaccharide moiety of the wall, other than peptidoglycan or teichoic acid, probably through H-bonding (Masuda and Kawata, 1980, 1981; Sleytr and Messner, 1983; Hood and Zottola, 1987). Being on the surface, these proteins are presumably directly involved in the interactions between the cell and its environment.

Bhowmik et al. (1985) demonstrated the presence of a surface protein layer in some *L. acidophilus* strains and partially characterized it. The surface protein was resistant to hydrolysis by several proteolytic enzymes, suggesting that the layer may have a protective function. *L. acidophilus* exhibited in high degree of hydrophobicity, and this property was depending on the surface protein. Therefore, they theorized that the surface layer might have a role in the attachment of these organisms to the intestinal mucosa. Schneitz et al. (1993) observed that the surface layer formed the outermost part of the cell wall in strongly adherent *L. acidophilus* strains, whereas this layer was covered with polymerized material or was absent in strains that lacked the ability to adhere or those with reduced adherence.
Fuller and Brooker (1980) suggested that the adherence of lactobacilli to epithelial cells is a multistage process from the initial phase of electrostatic attraction to the formation of fibrils and microcapsules. They theorized that the outermost surface layer is a potential mediator of the initial steps in adherence. Further, adherence is not only a mechanical tie between epithelial cells and bacteria but are also an active process that probably elicits a metabolic response in both (Hoepelman and Tuomanen, 1992).

Coconnier et al. (1992) proposed a model for the adherence of *L. acidophilus* BG2 F04 to human intestinal cells. The mechanism involves two factors: the bacterial cell-wall factor and the adhesion-promoting factor. The bacterial cell-wall factor is polysaccharide in nature and is responsible for the interaction between the bacterium and the extracellular adhesion-promoting factor. Earlier, Brooker and Fuller (1975) had also reported that the surface layer was rich in carbohydrates. Later, Hood and Zottola (1987) observed that the adherence was mediated by a polysaccharide located at the bacterial surface. The second factor, adhesion-promoting factor, is an extracellular proteinaceous component produced by adhering lactobacilli and provides a divalent bridge that links the bacteria to the intestinal enterocyte. This classic work has provided conclusive evidence about the factors involved in the adherence of lactobacilli to the IT and their chemical nature. For more information about adherence ability of lactobacilli see Tuomola and Salminen (1998); Tuomola et al. (2001); Tannock (2002); Gusils et al. (2002).

**FUNCTIONS OF LACTOBACILLI IN THE GASTROINTESTINAL TRACT**

The activities of LAB, especially lactobacilli in the GT have interested microbiologists for at least 90 years, since the time of Metchnikoff (1903, 1907). Since then, there has been much interest and research activity focused on the functions of lactobacilli in the GIT, and on their potential health and nutritional benefits. Several reviews and books related to this topic have been published (Gilliland, 1979, 1990; Sandine, 1979; Shahani and Ayebo, 1980; Nakazawa and Hosono, 1992; Mital and Gard, 1993, 1996; Tannock, 1995, 2002). The most important nutritional and therapeutic effects of lactobacilli (Gurr, 1977; Sandine, 1979; Shahani and Ayebo, 1980; Fernandes et al., 1987; Gilliland, 1990; Mital and Gard, 1995; Denev, 1996, 1997; Denev et al., 2000; Tannock, 2002) can be summarized as follows:

- Stimulation of the overall metabolism by producing vitamins (e.g., folic acid) and enzymes (e.g., lactase);
- Stabilization of the intestinal microflora, excluding colonization by pathogenic bacteria such as *Staphylococcus aureus*, *Salmonella* spp., *Shigella* spp. and enteropathogenic *E. coli* strains, via adhesion to the intestinal wall and competition for nutrients;
- Control of growth of undesirable organisms in the intestinal tract and protection against intestinal infections by production of antibacterial substances and competitive exclusion;
- Deconjigation of bile acids;
- Reduction of the cholesterol level in blood serum by cholesterol assimilation, bile salt hydrolysis and/or modulation of the ratio of high-density to low-density lipoproteins/cholesterol;
• Decreased risk of colon cancer by detoxification of toxic, mutagenic and carcinogenic compounds and modulation of fecal procarcinogenic enzymes such as β-glucuronidase, azoreductase and nitroreductase;
• Tumor suppression via aspecific stimulation of the immune system.

Control of Growth of Undesirable Organisms in the Intestinal Tract

Antagonistic Effect

It is well known that the presence of lactobacilli is important for the maintenance of the intestinal microbial ecosystem (Denev et al., 2000). The potential control of intestinal pathogens by LAB has received much attention for the past 90 years. Since Metchnikoff (1903, 1907) proposed a role of lactobacilli in suppressing undesirable intestinal microflora, numerous researchers have investigated the antagonistic activities of lactobacilli (reviewed by Lindgren and Dobrogosz, 1990; Klaenhammer, 1988, 1993; James et al., 1992; Juven et al., 1992; Piard and Desmazeaud, 1992; Ray and Daeschel, 1992; Hoover and Steenson, 1993; Nettles and Barefoot, 1993; Sudirman et al., 1993; Benoit et al., 1994; De Vuyst and Vandamme, 1994; Dodd and Gasson, 1994; Desmazeaud, 1994; Larpent, 1994; Kanatani et al., 1995; De Vuyst et al., 1996; Tahara et al., 1996; Takatoshi et al., 1996; Thanaruttikannont and Rengpipat, 1996; Van der Vossen et al., 1996; Ouwehand, 1998; William, 1999; Denev, 1996, 1997; Denev et al., 2000; Dunne et al., 2001). This antibacterial activity may result not only from decreasing the pH and redox potential, competition for nutrients and adhesion sites, but also because of the formation of specific antimicrobial compounds against Gram-negative and Gram-positive organisms.

Several investigations have shown that Lactobacillus spp. exerts antagonistic action against intestinal and food-borne pathogens and other related organisms (Grossowics et al., 1947; Wager, 1948; Wheater et al., 1951, 1952; Polonskaya, 1952; Vincent et al., 1959. De Klerrk and Coetze, 1961; Tramer, 1966; Reddy and Shahani, 1971; Hamdan and Mikolajcik, 1973; Mikolajcik and Hamdan, 1975; Tagg et al., 1976; Shahani et al., 1976, 1977; Lascar, 1995; Singh et al., 1995; Denev et al., 2000; Bird et al., 2002). They have been shown to possess inhibitory activity toward the growth of pathogenic bacteria such as Listeria monocytogenes (Harris et al., 1989), E. coli, Salmonella spp. (Drago et al., 1997), and others (Coconnier et al., 1997).

Gilliland and Speck, 1977 were among the first investigators reporting on antibacterial activity associated with Lactobacillus spp. Numerous reviews and books addressing this field have recently been published (McCormick and Savage, 1983; Barefoot and Klaenhammer, 1983, 1984; Reddy et al., 1984; Joerger and Klaenhammer, 1986; Muriana and Klaenhammer, 1987; Wong and Chen, 1988; Daeschel, 1989; Geis, 1989; Klaenhammer, 1988, 1993; James et al., 1992; Juven et al., 1992; Piard and Desmazeaud, 1992; Ray and Daeschel, 1992; Hoover and Steenson, 1993; Nettles and Barefoot, 1993; Sudirman et al., 1993; Benoit et al., 1994; De Vuyst and Vandamme, 1994; Dodd and Gasson, 1994; Desmazeaud, 1994; Larpent, 1994; Kanatani et al., 1995; De Vuyst et al., 1996; Tahara et al., 1996; Takatoshi et al., 1996; Thanaruttikannont and Rengpipat, 1996; Van der Vossen et al., 1996; Ouwehand, 1998; William, 1999; Denev, 1996, 1997; Denev et al., 2000; Dunne et al., 2001). This antibacterial activity may result not only from decreasing the pH and redox potential, competition for nutrients and adhesion sites, but also because of the formation of specific antimicrobial compounds against Gram-negative and Gram-positive organisms.

The intestinal lactobacilli play a major role in the regulation of bacterial population and the control of various enteropathogens in the GE. They suppress the multiplication of the pathogenic and putrefactive bacteria that cause intestinal problems, diarrhea and digestive upsets, etc., and contribute to the maintenance of health. Lactobacilli are consumed by hu-
mans and fed to animals of commercial value in efforts to control a variety of diseases and to maintain a balanced microflora by reduction of potential pathogens residing in the gut.

It is not the intent of this paper to review all of the literature on control of intestinal pathogens by lactobacilli however a few examples will be given. Certain *Lactobacillus* strains inhibit *E.coli* adhesion to porcine enterocytes and attachment to porcine mucus (Ouwehand and Conway, 1996). Furthermore, metabolic end products of lactobacilli present in cell-free culture supernatant fluids inhibit adhesion or invasion by pathogenic bacteria. Probiotic *Lactobacillus* strains also inhibit mucosal adherence of EPEC and *S. typhimurium* in vitro (Bernet et al., 1994). In addition to prevent colonization by pathogens, Lactobacilli may strengthen the epithelial barrier, thereby preventing pathogenic translocation of the epithelium by promoting accelerated epithelial repair. For example, oral administration of *Lactobacillus acidophilus* can ameliorate diarrhea in patients undergoing pelvic radiotherapy, perhaps by preventing radiation-induced damage to the intestinal epithelium (Kaila et al., 1995). Furthermore, enhanced macromolecule intestinal permeability and translocation of intact proteins induced by cow milk was reversed in suckling rats fed milk supplemented with *L. casei* GG (Salminen et al., 1988).

The exact mechanism whereby lactobacilli may inhibit intestinal pathogens is not clear. Antagonistic factors that may contribute to colonization and continuous presence of lactobacilli in the digestive tract of animals are the presence of specific antimicrobial compounds. The antagonism could be due to the production of inhibitory compounds such as organic acids, hydrogen peroxide, bacteriocins and broad-range antibiotic-like substances, or to competitive adhesion to the epithelium and etc.

**Bacteriocins and bacteriocin-like substances.** The discovery of bacteriocins dates back to 1925, when Gratia observed the inhibition of *E. coli* by *E. coli* V. The antibacterial substances produced by *E. coli* were named colicins (Fredericq, 1948). The first report of production of antagonistic substances other than metabolic end products by LAB was made in 1928 by Rogers. He observed antagonistic activity for *L. lactis* subsp. *lactis* against *L. delbrueckii* subsp. *bulgaricus*. The substance was determined to be a polypeptide (Whitehead, 1933) and subsequently named nisin (Mattick and Hirsch, 1947). De Klerk and Coetzee (1961) first isolated bactericidal substances from 11 homofermentative strains and one heterofermentative strain of *Lactobacillus*, that were only active against other *Lactobacillus* spp. and thus could be defined as bacteriocins. Among the Gram-positive bacteria, the lactobacilli in particular produce a wide variety of antimicrobial proteins including peptide antibiotics, antibiotic-like substances, bacteriocins and bacteriocin-like substances.

Bacteriocins, as defined by Tagg et al. (1976), are proteinaceous compounds produced by bacteria that have bactericidal activity against closely related species. They form a heterogeneous group with respect to producing bacterial species, molecular size, physical and chemical properties, stability, antimicrobial spectrum, mode of action, and so on.

Bacteriocins of LAB have been the subject of many studies over the past 10 years (De Vuyst and Vandamine, 1994;
De Vuyst, 1994, 1996; Cenatiempo et al., 1996). Investigations on bacteriocins have historically focused on four general areas that include: bacteriocin expression, production and mode of action; characterization of plasmids encoding bacteriocin production and immunity and their use as vehicles for gene transfer; identification and description of novel bacteriocins produced by bacteria not previously recognized as bacteriocinogenic; and taxonomic characterization of bacteria based on bacteriocin production or susceptibility (Klaenhammer, 1988).

Since the late 1920s and early 1930s, when the discovery of nisin initiated the investigation of proteinaceous antimicrobial compounds from LAB, a large number of chemically diverse bacteriocins have been identified and characterized, particularly in recent years. Nonetheless, we can observe common traits that justify their classification into just a few classes (Klaenhammer, 1993). On a sound scientific basis, three defined classes of bacteriocins in LAB have been established, class I: the lantibiotics; class II: the small heat-stable non-lantibiotics and class III: large heat-labile bacteriocins (Nes et al., 1996). In the past decades a large number of reports have been published describing Lactobacillus spp. that is claimed to produce bacteriocin and bacteriocin-like components. A selection is listed in Table 3.

Bacteriocins and bacteriocin-like compounds play an important role in controlling the composition of indigenous microflora and the regulation of population dynamics in various bacterial ecosystems. Since, bacteriocins usually are only active against organisms closely related to the producer, their role in inhibiting intestinal pathogens may be limited. Bacteriocins of Gram-positive organisms usually possess a somewhat broader inhibitory spectrum and are also active against other Gram-positive species. However, they may be very important in enabling a selected strain of Lactobacillus in competing with other LAB in the intestines. Some bacteriocins as acidolin, acidophilin, bulgarican and reuterin have been reported to possess a much broader inhibitory spectrum: many Gram-positive and Gram-negative bacteria were said to be inhibited by the purified products (Shahani et al., 1977; Fernandez et al., 1987; Talarico et al., 1988; Axelsson, 1989; Chung et al., 1989; Talarico and Dobrogosz, 1989). Certainly these types of bacteriocins may be very effective in helping to control the growth of intestinal pathogens (Gilliland, 1990). For more information about general characteristics, mechanisms of action, genetics and biosynthesis of bacteriocins see Fuller (1992), Klaenhammer (1993), De Vuyst and Vandamme (1994), Mishra and Lambert (1996), Nes et al. (1996), Tannock (2002).

Recently, bacteriocins have been studied intensively from every possible scientific angle: microbiology, biochemistry, molecular biology and food technology. Knowledge, especially about bacteriocins from lactobacilli, is accumulating very rapidly. Future investigations of bacteriocins have provided new information in each of these areas.

Other antagonistic compounds. Antagonism by lactobacilli has also been associated with major end products of their metabolism. Several by-products of lactobacillus metabolism are capable of antagonism in vitro and in vivo. The best knows of these compounds are lactic and acetic acids, hydrogen peroxide, diacetyl and etc.

Organic acids such as lactic and ace-
tic which are responsible for significant pH changes in gut-sufficient to antagonize many microorganisms, including patho-
genic Gram-negative organisms (Adams and Hall, 1988). While pH per se is a factor in such inhibitions, lower pH values also potentate the activities of these acids because undissociated forms are more bac-
tericidal than dissociated ones. This phe-
nomenon may be attributed to the ability of undissociated acids to penetrate the bacterial cell (Kashet, 1987). Further, the volatile acids are especially antimicrobial under the low oxidation-reduction potentials that lactobacilli help maintain in the intestine (Goepfert and Hicks, 1969; Tannock, 2002). Organic acids decrease or kill harmful bacteria, and as a result sup-

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<tr>
<th>Species</th>
<th>Bacteriocin</th>
<th>References</th>
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<tbody>
<tr>
<td>L. acidophilus</td>
<td>Lactocidin</td>
<td>Vincent et al. (1959)</td>
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<td></td>
<td>Acidophilin</td>
<td>Vakil and Shahani (1965)</td>
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<td></td>
<td>Acidolin</td>
<td>Hamdan and Mikolajcik (1973)</td>
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<td></td>
<td>Lactacin B</td>
<td>Barefoot and Klaenhammer (1983)</td>
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<td>Klaenhammer et al. (1993)</td>
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<td></td>
<td>Bacteriocin M46</td>
<td>Ten Brink et al. (1990)</td>
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<td></td>
<td>Acidophilucin A</td>
<td>Toba et al. (1991)</td>
</tr>
<tr>
<td></td>
<td>Acidocin A</td>
<td>Kanatani et al. (1992, 1995)</td>
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<tr>
<td>L. brevis</td>
<td>Lactobrevin</td>
<td>Kavasnikov and Sudenko (1967)</td>
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<td></td>
<td>Brevicin</td>
<td>Rammelsberg and Radler (1990)</td>
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<tr>
<td>L. casei</td>
<td>Caseicin 80</td>
<td>Rammelsberg et al. (1990)</td>
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<td></td>
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<td>Disks et al. (1992)</td>
</tr>
<tr>
<td>L. delbrueckii subsp. lactis UO004</td>
<td>Bacteriocin UO004</td>
<td>Boris et al. (2001)</td>
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<tr>
<td>L. fermentum</td>
<td>Bacteriocin 466</td>
<td>De Klerk and Coetzee (1961)</td>
</tr>
<tr>
<td>L. gasseri</td>
<td>Gassericin A</td>
<td>Toba et al. (1991a)</td>
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<td></td>
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<td>Takatoshi et al. (1996)</td>
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<tr>
<td>L. plantarum</td>
<td>Lactolin</td>
<td>Kodama (1952)</td>
</tr>
<tr>
<td></td>
<td>Plantacin A</td>
<td>Daeschel et al. (1990)</td>
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<td></td>
<td>Plantacin B</td>
<td>West and Warner (1988)</td>
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<td></td>
<td>Plantacin S</td>
<td>Jumenez-Diaz et al. (1993)</td>
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<td></td>
<td>Plantacin D</td>
<td>Aymerich et al. (2000)</td>
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<td></td>
<td>Plantacin T</td>
<td>Jumenez-Diaz et al. (1993)</td>
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<td></td>
<td>Plantacin Y</td>
<td>Chin et al. (2001)</td>
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<tr>
<td>L. reuteri</td>
<td>Reutericin 6</td>
<td>Toba et al. (1991b)</td>
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<tr>
<td>L. salivarius</td>
<td>Salivaricin B</td>
<td>Ten Bring and Holo (1996)</td>
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Table 3
Bacteriocins and bacteriocin-like substances of some intestinal Lactobacillus species
press the production of harmful substances such as amines, phenols, indole, scatole and hydrogen sulphide which are produced by these harmful bacteria. Because many of these harmful bacteria have a neutral optimum pH and are not acid-tolerant, the lowering of pH by organic acid metabolites of lactobacilli, such as lactic acid, has a bacteriostatic or bactericidal effect on these acid-sensitive bacteria (Fuller, 1992).

Hydrogen peroxide ($\text{H}_2\text{O}_2$) is one of the primary metabolites produced by lactobacilli. The lactobacilli have the ability to generate hydrogen peroxide during growth by several different mechanisms (Table 4).

The accumulation of hydrogen peroxide in growth media is shown to occur because lactobacilli do not possess the catalase enzyme (Kandler and Weiss, 1986). The antimicrobial activity of hydrogen peroxide is well recognized and documented. This compound is cytotoxic because of its capacity to generate reactive cytotoxic radicals that are strongly oxidative (Halliwell, 1978). Lactobacilli usually produce hydrogen peroxide by direct reduction of oxygen. Aside from the generation of hydroxyl radicals, the in vivo bactericidal effects of hydrogen peroxide may be relevant to the activation of a peroxidase-hydrogen peroxide system, commonly referred to as lactoperoxidase system. A well-studied lactoperoxidase system is described in milk, where bactericidal activity against enteric Gram-negative bacteria is associated with thiocyanate (Reiter and Harnulv, 1984). This mechanism may also occur in the intestines (Retire et al., 1980; Fervidness et al., 1987). Lactobacilli stimulated the lactoperoxidase thiocyanate system in the intestines, which reduced the ability of $E. \text{coli}$ and other enteropathogens to survive in the gut (Fernandes and Shahani, 1989).

Hydrogen peroxide production by lactobacilli will usually occur by direct reduction of atmospheric oxygen catalysed by

### Table 4

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<th>Substrates, reaction</th>
<th>Catalyzed by</th>
<th>End Products</th>
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<tr>
<td>Pyruvate + $O_2$ + phosphate</td>
<td>Pyruvate oxidase</td>
<td>Acetyl phosphate + $CO_2$ + $H_2O_2$</td>
<td>Gotz et al. (1980)</td>
</tr>
<tr>
<td>Lactate + $O_2$</td>
<td>L-lactate oxidase</td>
<td>Pyruvate + $H_2O_2$</td>
<td>Kandler (1983)</td>
</tr>
<tr>
<td>Lactate + $O_2$</td>
<td>NAD-independent D-lactate dehydrogenase</td>
<td>Pyruvate + $H_2O_2$</td>
<td>Sedewitz et al. (1983)</td>
</tr>
<tr>
<td>Saturated fatty acids + $O_2$</td>
<td>Fatty acyl-CoA</td>
<td>$H_2O_2$</td>
<td>De Kairuz et al. (1988)</td>
</tr>
<tr>
<td>$\alpha$-Glycerophosphate + $O_2$</td>
<td>$\alpha$-Glycerophosphate oxidase</td>
<td>Dihydroxyacetone phosphate + $H_2O_2$</td>
<td>Condon (1987)</td>
</tr>
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</table>
either pyruvate-, NADH, alpha-glycero-phosphate-oxidase (Condon, 1987) or lactate-oxidase. In phosphate buffer with glucose and pyruvate added, resting cell of a strain of L. acidophilus, isolated from the chicken GT, produced 280 nmol hydrogen peroxide per hour per milligram of cell dry weight. Concentrations of oxygen high enough for significant amounts of hydrogen peroxide to be produced are likely to exist only in the upper portions of the GIT of live animals. That might be one of the explanations for the antagonistic effects of lactobacilli towards salmonellas observed in the crop of chicks whereas none or very limited antagonism was found in their caeca (Juven et al., 1991). For more information about antimicrobial compounds produced by lactobacilli and their structures and properties see Yang (2000).

COMPETITIVE EXCLUSION OF PATHOGENIC BACTERIA BY LACTOBACILLI

One of the physiological effects of lactobacilli in the host has proposed to be the antagonism against pathogens. Competitive exclusion (CE) by the lactobacilli is another mechanism that has been suggested as being important in controlling intestinal infections (Watkins and Miller, 1983a; Edens et al., 1997; Edelman, 2005). CE involves the ability of the lactobacilli to occupy binding sites on the intestinal wall, thereby preventing attachment and growth of enteric pathogens.

Nurmi and Rantala (1973) first recognized the importance of early establishment of protective microflora in young chicks. Originally, these workers treated chicks with a suspension of crop and intestinal tract materials obtained from adult birds; in subsequent studies, cecal content or feces cultures anaerobically in a liquid medium were used for treatment. These preparations of unknown bacterial composition were administered orally to day-old chicks, which were challenged 24 or 48 h later with a standardized dose of Salmonella. Results showed that such treated chicks were more resistant to infection than control birds. This prophylactic approach, known as the “Nurmi concept” or “competitive exclusion”, opened new horizons for the control of Salmonella in poultry.

The advent of CE technologies for the control of pathogenic enteric bacteria in poultry as well as humans has received considerable interest during the past two decades. Intensive efforts by groups of USDA scientists (Bailey, 1988; Ziprin et al. 1991; Corrier et al., 1994, 1995a, 1995b; Hollister et al., 1995) signal the importance of this technology and its application to the poultry industry. The mechanisms used by one species of bacteria to exclude or reduce the growth of another species are varied, but Rolfe (1991) determined that there are at least four major mechanisms of CE. These mechanisms are: 1) creation of microecology that is hostile to other bacterial species; 2) elimination of available bacterial receptor sites; 3) production and secretion of antimicrobial metabolites, and 4) selective and competitive depletion of essential nutrients.

The effect of CE lactobacillus cultures on the growth of undesirable organisms in the IT has been well documented the last decade. Watkins et al. (1979, 1982) reported that broiler chicks inoculated with L. acidophilus were more resistant to the pathogenic effect of E. coli. The addition of L. acidophilus acidified the crop, cecum and colon of the inoculated chicks, and this acidification appeared to increase the competitiveness of the L. acidophi-
lus against the other intestinal microflora. Watkins and Miller (1983a) suggested that *L. acidophilus* increases competitive gut exclusion against harmful organisms (*S. typhimurium, Staphylococcus aureus*, and *E. coli*) in the intestinal tract. Watkins and Miller (1983b) demonstrated that *L. acidophilus* colonized the epithelial cells of the GIT through a close relationship and through actual physical attachment.

With the discovery that *L. reuteri* is a major component of the intestinal microflora of humans (Gibson et al., 1997), chicks and other animals (Sarra et al., 1985; Dobrogosz et al., 1991; Edens et al., 1993; 1997) it appeared that the population dynamics of *L. reuteri* might be involved in establishing a favorable microecology in the IT via its ability to secrete lactic and acetic acid, and reuterin, which act as modulators of intestinal tract microbial populations (Dobrogosz et al., 1991).

According to Soerjadi et al. (1981a, 1981b) members of the *Lactobacillaceae* family are responsible for CE of pathogens from the gut of chicks. Schneitz et al. (1993) demonstrated the importance of lactobacilli with the ability to adhere as CE microorganisms in poultry. Mulder et al., (1997) also reported that *Lactobacillus* species are very important in establishing a stable GIM which is able to prevent colonization of potentially pathogenic microorganisms. Therefore, the use of probiotics and other CE products containing intestinal *Lactobacillus* spp. may be also exert a positive effect against this colonization.

Lactobacilli have been shown to affect the prevalence of several intestinal pathogens *in vitro* and *in vivo* (Cocconier et al., 1998; Jin et al., 1996; Pascual et al., 1999; Mack et al., 1999; La Ragione et al., 2004). The detailed molecular mechanisms underlying this phenomenon remain poorly characterized, and the reports vary in the degree of successful inhibition depending on the strains used as CE agents, the pathogens as well as the methods of assessment. The importance of adhesion in pathogen exclusion at chicken ileal epithelia has been shown *in vitro* with *L. acidophilus* against *Salmonella pullorum* (Jin et al., 1996) but *L. acidophilus* failed to exclude *Salmonella enterica* serovar Enteritidis and *Salmonella enterica* serovar *typhimurium* and APEC (Jin et al., 1996, 1998). Also, exclusion of *E. coli* and salmonella by lactobacilli in human and piglet mucus have been reported (Lee et al., 2003), but lactobacilli failed to inhibit the adherence of *Salmonella* spp. to chicken mucus (Gusils et al., 2003). *L. crispatus* and its collagen binding S-layer protein inhibit the adherence of *E. coli* to the components of basement membrane (Horie et al., 2002) and a 29-kDa surface protein from *L. fermentum* inhibited adhesion of uropathogenic *Enterococcus faecalis* to polystyrene (Heinemann et al., 2000). For more information about CE properties of lactobacilli see (Edens et al., 1997; Mulder et al., 1997; Edelman, 2005). Improving our understanding of the development and the regulatory mechanisms of the lactobacillus flora in GE is essential for the development of effective CE products for humans and animals.

NEUTRALIZATION OF TOXINS AND OTHER INTESTINAL REACTIONS

Among the intestinal bacteria there are some which act on food components and digestive secretions like bile acids to produce harmful putrefactive products such as enterotoxins, ammonia, amines, phenols, indole and hydrogen sulphide, or
other toxic and carcinogenic substances. If these harmful metabolites are produced in large quantities, they promote a decrease in liver function and the development of cancer.

Intestinal lactobacilli have been shown to inhibit the growth of pathogenic bacteria, decrease the metabolic activity of harmful bacteria, including production of enzymes which contribute to the production of toxic and cancer-related substances (Mitsuoka, 1984; Honma, 1986). Intestinal lactobacilli can also suppress the production (Honma et al., 1987) and breakdown of nitroazines, which have been shown to be connected with the development of stomach cancer and cancer of the small intestine (Kanbe, 1992).

Investigations of *L. bulgaricus* in pigs showed that the organism produces a metabolite thought to neutralize the effect of enterotoxin released from coliforms. Piglets fed a *L. bulgaricus* culture, rendered non-viable with lactic acid, and artificially infected with *E. coli*, grew faster and suffered less diarrhea compared with control animals. These studies showed that some lactobacilli especially *L. bulgaricus* was able to neutralize *E. coli* enterotoxin (Mitchel and Kenworthy, 1976). Although the neutralizing substance has yet to be identified, further support for anti-enterotoxic activity has also been obtained from experiments with rats and calves. Cell free extracts of *L. casei* and *L. acidophilus* have also been shown in vitro to inhibit the growth of *E. coli*. Scheline (1972) tested a number of different substrates and intestinal microorganisms to determine the type of reactions specific *Lactobacillus* species catalyze. In the study cited above, these investigators found that a *Lactobacillus* species could reduce the double bond in hydroxycinnamic acid, reduce the nitro group of 4-nitrobenzoic acid and reduce the azo bond found in methyl red and acid yellow. In more recent publication Pradham and Majumdar (1986) reported that *L. acidophilus* cleaves the azo bond of sulfasalazine, also known as azulfidine, a drug used to treat patients with ulcerative colitis. These investigators also found that *L. acidophilus* degraded 17.6% of the antimicrobial agent phthalylsulfathiazole and 8% of the antibiotic chloramphenicol palmitate. These investigators also confirmed the previous study and demonstrated that *L. acidophilus* could rapidly hydrolyze the azo bond of tartrazine and methyl red. *L. helveticus* and *L. salivarius* also had similar but lower activity when compared to *L. acidophilus*. Lactobacilli have also been shown to reduce the double bond of 3-hydroxy cinnamic acid and cinnamic acid (Whiting and Carr, 1970) to produce respectively 3-hydroxy phenylproponic acid and phenylproponic acid. A *Lactobacillus* species has been shown to be capable of amino acids (Melnoykowycz and Johansson, 1955). A *L. acidophilus* isolates from the stomach of rat was shown to exhibit histidine decarboxylase activity (Horakova et al., 1971).

Nitrosamines have been shown to be potent carcinogens (Magee, 1971). The nitrosamines found in foods are dimethyl-nitrosamine, diethylnitrosamine, N-nitrosopyrrolidine, and N-nitro-sopiperidine. These are formed when nitrites react with secondary amines in an acidic medium (Hawksworth and Hill, 1971). Nitrites are derived from nitrates, which are found in green plants, vegetables, cured meats, and some cheese products (Ender and Ceh, 1968). Nitrites may also be present in drinking water in higher concentrations (Correa et al., 1970). A relat-
tionship between gastric cancer and high nitrate intake has also been suggested (Correa et al., 1970). *Lactobacillus* species could degrade nitrosamines and thus mitigate their toxical and carcinogenic effect (Rowland and Grasso, 1975; Kanbe, 1992).

Some lactobacilli (*L. acidophilus*) have been shown to suppress the production of harmful substances such as ammonia, indole and hydrogen sulfide, which are hazardous to the body. This suppressing effect may help to decrease the amount of toxins going to the liver and injuring it (Yamamoto et al., 1986; Yamashita et al., 1987).

Lactobacilli reduced activities of fecal bacterial enzyme b-glucuronidase, nitroreductase, and azoreductase (Goldin and Gorbach, 1984) that are involved in pro-carcinogen activation and reduced excretion of mutagens in feces and urine (Lidbeck et al., 1992).

These studies demonstrated the potential of lactobacilli as toxin neutralizers, inhibitors of cancer promoting enzyme activity, destructors of fecal nitrosamines and other drugs, harmful, mutagenic and carcinogenic substances in the GIT.

### DECONJUGATION OF BILE ACIDS

Bile acids are synthesized from cholesterol in the liver, conjugated to either glycine or taurine, and then released into the intestines, where they facilitate fat absorption by the epithelial cells (Hofmann and Mehjian, 1973). These acids are deconjugated, dehydroxylated, dehydrogenated, and desulfated in the intestines by microbial enzymes (Hylemon and Glass, 1983). The capacity to deconjugate bile acids (hydrolyze the amide bond between the steroid nucleus and amino acid) is widespread among members of the autochthonous gastrointestinal microflora (Hayakawa, 1973; Midtvedt, 1974), including species of the genera *Bacteroides* (Masuda, 1981), *Bifidobacterium* (Ferrari et al., 1980; Grill et al., 1995), *Clostridium* (Masuda, 1981), *Lactobacillus* (Gilliland and Speck, 1977a; Christiaens et al, 1992; Tannock et al., 1994), *Fusobacterium* (Shimada et al., 1969), *Streptococcus* (Kobashi et al., 1978) and others species (Midtvedt, 1974). The reaction is catalyzed by the enzyme bile salt hydrolase (BSH) (Lundeen and Savage, 1990).

BSH catalyze the cleavage of an amino acid from the steroid nucleus of conjugated bile salts. It is not clear why lactobacilli produce an enzyme with this property, because they would not gain energetically from the deconjugation process, but it may be an essential property enabling the bacteria to survive transit through the small bowel, into which relatively high concentrations of conjugated bile acids are released (De Boever and Verstraete, 1999).

The deconjugating activity of the lactobacilli could be important to the host, because deconjugated bile salts are less effective in emulsification of dietary lipids and micelle formation. Thus, the BSH activity of lactobacilli in the small bowel could impair lipid digestion and absorption by the host and could have implications in the poultry and pig industries, where rapid growth and efficient feed conversion are required for profitability (Tannock, 2004).

BSH has been purified from three organisms, *Bacteroides fragilis* (Stellwag and Hylemon, 1976), *Clostridium perfringens* (Gopal-Srivastava and Hylemon, 1988; Coleman and Hudson, 1995) and *Lactobacillus ssp.* (Lundeen and Savage, 1990, 1992). Lactobacilli have been shown to be the predominant pro-
ducers of BSH activity in the mouse gut. Gastric lactobacilli contribute approximately 86% of the total BSH activity and the 74% in the cecum of mice (Tannock et al., 1989). However, the enzymes catalyzing the reaction in these bacteria have not been extensively studied (Tannock, 2004). Hill and Drasar (1968) and Aries et al. (1969) reported that *Lactobacillus* ssp. isolated from human feces were unable to deconjugate taurocholic acid. Midtvedt and Norman (1968) reported that *L. arabinosus* deconjugated both taurocholic and glycocholic acids, whereas *L. brevis* deconjugated only glycocholic acid. *L. acidophilus, L. casei* and *L. delbrueckii* did not deconjugate either of the two bile acids. Floch et al. (1972) isolated *Lactobacillus* species from human feces, which deconjugated glycocholic, glycodeloxycholic, and glycocchenodeoxycholic acids. The species of lactobacillus were not identified.

Gilliland and Speck (1977a) examined a number of lactobacilli from human feces for their ability to deconjugate bile acids. They found that all of them possessed this capacity but acted on either a tauro- or glycoconjugate. However, only *L. buchneri* among the lactobacilli tested acted upon both.

The bile acids excreted in feces are entirely deconjugated. They represent about 5% of the enterohepatically circulated pool and consist of a variety of secondary bile acids in addition to small quantities of primary bile acids (Mallory et al., 1973). The deconjugated bile acids exert a greater inhibitory action against bacteria than conjugated forms (Dunne et al., 2001).

Deconjugated bile acids, particularly dehydroxy acids (deoxycholic and chenodeoxycholic acids) inhibit the growth of a variety of intestinal bacteria (Binder et al., 1975). Further, dihydroxy bile acids are more inhibitory than trihydroxy one-cholic acids (Floch et al., 1972). Percy-Robb and Collee (1972) observed that the in vitro antibacterial actions of bile acids were strongly pH dependent. They suggested that bile acids might be involved in a self-regulatory mechanism that prevented microbial colonization of the upper bowel.

Bacterial metabolism of bile acids has been postulated to play an important role in etiology of colon cancer (Hill, 1986). It is suggested that the secondary bile acids (i.e. those resulting from bacterial metabolism) can act as promoters of the tumorigenic process in the colon. In addition, Hill has proposed that dehydrogenation of the steroid nucleus to produce delta 1 and delta 4 bonds in association with 3-keto groups is particularly important in relation to colon cancer. Certain strains can perform this reaction *in vitro* although the significance of the reaction *in vivo* is questionable (Lombardi et al., 1978).

Deconjugated bile acids by lactobacilli play a significant role in maintaining ecological equilibrium in the intestine (Hoch et al., 1972; Mital and Garg, 1995; Tannock, 2004) They are more inhibitory to bacteria than conjugated ones (Percy-Robb and Collee, 1972); therefore, deconjugation of bile acids may enhance antagonistic actions of intestinal lactobacilli toward intestinal pathogens, thus helping to maintain a favorable balance among species present in the intestinal tract. Several reports have been published on this topic during the last years (Charteris et al., 1998; Boever and Verstaete, 1999; Grill et al., 2000; Shinoda et al., 2001; Tannock, 2004). Understanding of the structural, enzymatic, and regulatory properties of the hydrolases from lactobacilli will be impor-
tant to understanding of the role of these organisms in bile acid transformations in the GIT.

Much attention has recently been paid to the phylogeny of the gut microbiota, but little has been paid to the microbial physiology of complex bacterial communities or their individual components (Lu et al. 2003). It is time that this imbalance was rectified. Lactobacilli could provide model bacteria for such physiological studies because their relationship with the farm animal host (chickens, pigs) is much better defined than that of other members of the microbiota.

ANTIMUTAGENIC ACTIVITY

Bruce et al. (1977) was the first to report the presence of mutagenic compounds in human feces. Since then, many researchers have investigated the occurrence and levels of fecal mutagens in different populations (Ehrich et al., 1979; Reddy et al., 1980; Ferguson et al., 1985). Mutagenic activity in feces has been reported to be promoted by bile, enhanced by anaerobiosis, and inhibited by exposure to oxygen (Lederman et al., 1980; Van Tassell et al., 1982a).

Studies with pure cultures have shown that *Bacteroides fragilis*, *B. ovatus*, *B. uniformis*, and *B. thetaiotaomicron* are capable of producing mutagens (Van Tassell et al., 1982b). The fact that these organisms are common inhabitants of the intestinal tract and that only a relatively small percentage of individuals produce the mutagen indicates that a precursor is the determining factor in their production. It has been demonstrated that bacteria from feces that do not contain the mutagen are capable of producing it in the presence of a precursor. The precursor is either a product of other intestinal bacteria, a result of a diet, or a metabolite derived from the host. Further, it has been shown that cell-free extracts of bacteroides can produce the mutagen in the presence of bile. The enzyme system involved is not oxygen sensitive (Mital and Gard, 1995). However, the mutagen is only produced anaerobically (Wilkins et al., 1981).

It is well known that carcinogenesis is initiated by mutation induced in animal and human cells with carcinogen. Several compounds displaying mutagenic properties have been identified in numerous foods (Hosono, 1992; Thyagaraja and Hosono, 1993). According to McCann et al. (1975) mutagenicity and carcinogenicity show good correlation.

Recently, attention has been focused on the biological transformation of substances to carcinogens by intestinal microflora. Studies on the conditions that enhance or decrease the activation or inactivation of environmental mutagens are important for the control of their risks to human (Thyagaraja and Hosono, 1994). There is evidence that LAB, especially lactobacilli, influence the mutagenicity of intestinal content and the levels of fecal microbial enzymes. The same strain lactobacilli have been shown to significantly decrease mutagenicity in healthy volunteers consuming fried ground beef. They decreased fecal *E.coli* levels in colon cancer patients and reduced the fecal enzymes (Goldin et al., 1992; Ling et al., 1994; Morotomi, 1996).

Hosono et al. (1986a) were the first to report that milks fermented with *L.delbrueckii subsp. bulgaricus* IFO 3533, *L. lactis subsp. lactis* IFO 12546 and *Enterococcus faecalis* IFO 12964 exhibited antimutagenic activity against 4-nitroquinoline-N-oxide (4NQO). Hosono et
al. (1986b) have also described the anti-mutagenic activity of milks fermented with Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus again all the mutagens.

Nishioka et al. (1989) have reported that with respect to 49 strains of Lactobacillus three exhibited strong antimutagenic activity against 4NQO and 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2).

Hosoda et al. (1996) investigated the antimutagenic effect of milk fermented with L. acidophilus. They concluded that L. acidophilus decreased fecal mutagenicity in the human intestine.


HYPOCHOLESTEROLEMIC EFFECT

High-serum cholesterol levels are considered to be a major risk factor for coronary heart disease, and also a factor inducing colon cancer in addition to high dietary fat and low fiber (Pekkanen et al., 1990; Lichtenstein and Goldin, 1993; Law et al., 1994). Several reports have indicated that serum cholesterol levels can be reduced by the consumption of certain cultured dairy products (Suzuki, 1995). In addition, intestinal microflora has been implicated in influencing the serum cholesterol level. Germ-free animals on an elevated cholesterol diet accumulate approximately twice as much cholesterol in blood as do conventional animals on a similar diet (Eyssen, 1973). The latter excrete much higher levels of cholesterol in the feces than germ-free animals. This suggests that the intestinal microflora interfere with the cholesterol absorption from the intestines.

Several studies have suggested that lactobacilli and especially intestinal strains of L. acidophilus have the potential of decreasing serum cholesterol levels (Danielson et al., 1989; Walker and Gilliland, 1993; De Rodas et al., 1996). However, not all strains of L. acidophilus were active in this regard (Gilliland et al., 1985; Lin et al., 1989). On the other hand L. acidophilus had the ability to assimilate cholesterol in in vitro studies, but only in the presence of bile and under anaerobic conditions.

Gilliland et al. (1985) found that some strains of L. acidophilus could remove cholesterol from the laboratory medium only in the presence of bile and under anaerobic conditions. Because these conditions also occur in the intestine, they tested the manifestation of such an action in vitro using young pigs and L. acidophilus strains of pig origin. The results showed that the feeding of pigs with L. acidophilus possessing the ability to grow in the presence of bile and to remove cholesterol from laboratory medium, significantly inhibited the increase in their serum cholesterol levels on a high cholesterol diet.

The lactobacilli are the primary contributors to bile salt hydrolase activity in the ileum and cecum of the mouse (Tanock et al., 1989). If strains of Lactobacillus ssp. exhibiting a high degree of bile salt hydrolase activity could be incorporated into the intestinal tract, it is possible that the amount of deconjugation activity in the intestine could be increased.
Some species of lactobacilli that are present in the intestinal tract can deconjugate taurocholic and glycocholic acids in anaerobic conditions Gilliland and Speck (1977a). This deconjugation activity is important because deconjugated bile acids do not function as well as conjugated bile acids in the intestinal absorption of cholesterol (Eyssen, 1973). Increased deconjugation of bile acids could also result in greater excretion of bile acids from the intestinal tract because free bile acids are less likely to be reabsorbed in the intestines (Chickai et al., 1987). Increased excretion of bile acids stimulates the synthesis of replacement bile acids from cholesterol, thus providing the potential to reduce cholesterol levels in the body (Gilliland, 1990). The synthesis of bile acids is homeostatically regulated by the amount of bile acids returning to the liver (Danielsson and Sjovall, 1975). Also, cholesterol absorption into the blood from the intestines is not supported as well by deconjugated bile acids as it is by conjugated bile acids.

According to Walker and Gilliland (1993) there is no correlation between bile tolerance and cholesterol uptake ability of the L. acidophilus. They also have evaluated four strains of L. casei and observed considerable variation among strains in their ability to assimilate cholesterol. Additionally they have shown that L. plantarum and bifidobacteria possess the ability to assimilate cholesterol. Thus, it may be possible to use other of intestinal lactobacilli or related organisms in exerting beneficial influences on serum cholesterol levels. Additional research will be required to confirm whether or not this is a possibility.

Imaizumi et al. (1992) found many strain differences and that which microbial could draw no clear conclusions on the general mechanism activity within the gut might regulate serum cholesterol levels.

Bile tolerance, the ability to deconjugate bile salts, and the ability to assimilate cholesterol are factors that may affect the potential of some strains of L. acidophilus to help control human serum cholesterol concentration when used as dietary adjuncts (Lys and Gilliland, 1994). Future research is needed to determine the mechanism of cholesterol uptake and to determine whether or not the ingestion of cells of a selected strains of lactobacilli and especially L. acidophilus could decrease serum cholesterol levels in adult humans with primary hypercholesterolemia. For more information about hypocholesterolemic effect of LAB, including lactobacilli see Denov et al. (2000), Shah (2001), Lovegrove and Jackson (2003).

ANTICARCINOGENIC EFFECTS

Since the first observation by Bogdanov et al. (1962) that L. bulgaricus produced substances, which were active against tumor development there, have been a number of reports in the same vein. During the last two decades, several experiments and reviews indicating that LAB play a significant role in suppressing carcinogenesis have been published (Sandine, 1979; Friend et al., 1982; Reddy et al., 1983; Deeth, 1984; Friend and Shahani, 1984a; Gurr, 1987; Fernandes et al., 1987, 1988; Hosono, 1988a; Kanbe, 1988; Gilliland, 1989; Wada and Hayakawa, 1990; Guilliams, 1999; Wollowski et al., 2001; Mercenier et al., 2002).

Epidemiological evidence and dietary studies have shown that consumption of dairy products fermented by lactobacilli may reduce the risk of colon cancer in both
animals and humans. Numerous studies have suggested that intestinal and other lactobacilli possess anticarcinogenic activity (Mitsuoka, 1981; Goldin and Gorbach, 1980, 1983; Shahani et al., 1983; Friend and Shahani, 1984; Shimizu et al., 1987; Fernandes and Shahani, 1990; Adachi, 1992; Salminen, 1996).

Parenteral administration of some strains lactobacilli, especially *Lactobacillus casei* Shirota strain has had antitumor and immunostimulating activities on experimentally implanted tumors (Morotomi, 1996; Miguel, 2001). Similar effects have been verified by oral administration. In a recent study *Lactobacillus GG* was shown to cause a dramatic reduction in DMH induced tumor formation in rats. It was reported that *Lactobacillus GG* inhibited the initiation and early phase promotion on the tumorigenesis process (Goldin et al., 1996). However, it was reported that the organism was not able to prevent the growth of tumors once they had been established (Goldin et al., 1996). The fact that *Lactobacillus GG* is an organism of human origin capable of surviving in the GT and that it inhibits tumor initiation or early promotion in the colon provides a basis for further studies of these factors in humans. In a similar manner, *L. casei* Shirota strain has been shown to have inhibitory properties on chemically induced tumors in animals (Kato et al., 1994; Morotomi, 1996). In a clinical study, prophylactic effects of oral administration of *L. casei* Shirota strain on initiation or early promotion in the colon provides a basis for further studies of these factors in humans. In a similar manner, *L. casei* Shirota strain has been shown to have inhibitory properties on chemically induced tumors in animals (Kato et al., 1994; Morotomi, 1996). In a clinical study, prophylactic effects of oral administration of *L. casei* Shirota strain on the recurrence of superficial bladder cancer has been reported in two Japanese studies (Aso and Akazan, 1992; Aso et al., 1995). LAB has also been indicated in colonic fermentation producing butyrate or butyric acid in the colon. This alteration in intestinal metabolism may be due to changes in the intestinal microecology following probiotic intake or the direct metabolism of slowly absorbable components by orally administered colonizing LAB (Salminen et al., 1996). Butyrate has been shown in vitro studies to slow the growth of cultured colon cancer cells. Young (1996) has proposed that butyrate may be a diet-regulated, natural antitumor compound at least partly responsible for the antitumor effect of dietary components and also dietary probiotics. When these studies are related to animal data on several *Lactobacillus* strains and colon cancer related parameters, it is important to assess the potential of probiotic lactobacilli for cancer chemoprevention (Salminen, 1996; Wollowski et al., 2001).

Since intestinal microorganisms have been postulated to play an important role in conversion of chemical procarcinogens to carcinogens in the gut, the activity of certain fecal enzymes (azoreductase, nitroreductase, β-Glucuronidase β-Glucosidase and etc.) has been used to monitor colon carcinogenesis (Golden and Gorbach, 1976; Wollowski et al., 2001). β-Glucuronidase plays a role in the generation of carcinogenic aglycones, such as 3-hydroxybenzopyrene from 3-hydroxybenzopyrene-p-glucuronide (Kinoshita and Gelboin, 1978) in colon, from glucuronides formed in the liver. Nitroreductase and azoreductase are related to the formation of aromatic amines which can be
converted to nitroso- and N-hydroxycompounds in many tissues. These products are known carcinogens (Cerniglia et al., 1982; Manning et al., 1985). Steroid-7-dehydroxylase is responsible for the formation of lithocholic acid from chenodeoxycholic acid which is primary bile acid formed from cholesterol in the liver. Lithocholic acid, a secondary bile acid formed by the bacterial enzyme in the colon, is a co-carcinogenic substance (Carey, 1973).

Experiments performed in animal and human models showed that some lactobacilli could decrease the levels of enzymes azoreductase, nitroreductase, β-Glucuronidase and β−Glucosidase, responsible for activation of some procarcinogens, and decrease the risk or the speed of tumor development (Goldin and Gorbach, 1992). The results of some studies are shown in Table 5.

Several investigations have shown an influence of the intake of LAB and especially Lactobacillus ssp. fermented-milk products on the gut flora, enzyme activities and colon carcinogenesis (Wollowski et al., 2001). According to Friend and Shahani (1984) the lactobacilli inhibit carcinogenesis by (a) inactivating or inhibiting formation of carcinogenic compounds in the gastrointestinal tract; and (b) suppressing promotion of cancer through stimulation or enhancement of the immune properties of the host. Fernandes and Shahani (1990) observed that LAB, including lactobacilli, inhibits carcinogenesis by (a) prevention of cancer initiation by means

### Table 5

**Effect of Lactobacilli on fecal bacterial enzymes**

<table>
<thead>
<tr>
<th>Species</th>
<th>Azoreductase</th>
<th>Nitroreductase</th>
<th>Glucuronidase</th>
<th>Glucosidase</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. acidophilus</em> d*</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td></td>
<td>Goldin and Gorbach (1977, 1984)</td>
</tr>
<tr>
<td><em>L. acidophilus</em> d</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td></td>
<td>Goldin et al. (1980)</td>
</tr>
<tr>
<td><em>L. acidophilus</em> d</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td></td>
<td>Auebo et al. (1980)</td>
</tr>
<tr>
<td><em>L. acidophilus</em> d</td>
<td>d</td>
<td>d</td>
<td></td>
<td></td>
<td>Pedrosa et al. (1990)</td>
</tr>
<tr>
<td><em>L. acidophilus</em> d</td>
<td>d</td>
<td>d</td>
<td></td>
<td></td>
<td>Marteau et al. (1990)</td>
</tr>
<tr>
<td><em>L. acidophilus</em> d</td>
<td>d</td>
<td></td>
<td></td>
<td></td>
<td>Lidbeck et al. (1991)</td>
</tr>
<tr>
<td><em>L. acidophilus</em> d</td>
<td>d</td>
<td>d</td>
<td></td>
<td></td>
<td>Bouhnik et al. (1996)</td>
</tr>
<tr>
<td><em>L. acidophilus</em> (NCFM)</td>
<td>d</td>
<td>d</td>
<td></td>
<td></td>
<td>Cole et al. (1989)</td>
</tr>
<tr>
<td><em>L. casei</em> strain GG</td>
<td>d</td>
<td></td>
<td></td>
<td></td>
<td>Goldin et al. (1992)</td>
</tr>
<tr>
<td><em>L. acidophilus</em> d</td>
<td>d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. reuteri</em></td>
<td>d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. casei</em> Shirota</td>
<td>d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. johnsonii</em></td>
<td>d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. rhamnosus</em> GG</td>
<td>d</td>
<td>d</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(d)* Decrease
of direct and indirect reduction of pro-carcinogens and (b) suppression of initiated cancer.

Another possible explanation for the preventive effect of lactobacilli on carcinogenesis is their effect on other bacteria in the intestine. Lactobacilli might suppress the growth of bacteria that convert procarcinogens into carcinogens, thereby reducing the amount of carcinogens in the intestine. The activity of the enzymes that convert procarcinogens into carcinogens is often used as an indicator of the effect of probiotics on the intestinal microflora (Roos and Katan, 2000).

The mechanism of anticarcinogenic action has not yet been fully elucidated. Several studies have suggested that anti-carcinogenic activity of Lactobacillus spp. may be attributed to:

- Activation of the host’s immune system to antitumourigenesis (McIntosh, 1996);
- Improvement (quantitatively/qualitatively) of the intestinal microflora, reducing the putative producers of carcinogens and cancer promoters (improvement of the intestinal microecology: e.g. more bile-acid degrading bacteria; less bacteria producing the enzymes azoreductase, nitroreductase, beta-glucuronidase, beta-glucosidase, etc. (Mercenier et al., 2002);
- Production of compounds that inhibit the proliferation of tumor cells (Reddy et al., 1973);
- Antagonistic action against the organisms that convert procarcinogens to carcinogens by enzyme activity (Auebo et al., 1980; McIntosh, 1996; Roos and Katan, 2000);
- Bind to mutagenic compounds in the intestine and decreasing the absorption of these mutagens (Orrhage et al., 1994; Young, 1996; Guilliams, 1999; Roos and Katan, 2000);
- Direct suppression of the carcinogens and/or procarcinogens by binding, blocking, removing (McIntosh, 1996);
- Degradation of the carcinogens formed (Rowland and Grasso, 1975);
- Alteration of colonic motility and transit time (McIntosh, 1996).

During the last five years an impressive and useful reviews in the field of anti-carcinogenic properties of lactobacilli are provided by Wollowski et al. (2001); Herich et al. (2002); Norat and Riboli (2003); Gill and Rowland (2003).

**IMMUNE SYSTEM STIMULATION**

The immune system consist of a number of organs and several different cell types that recognize accurately and specifically foreign antigens on microorganisms and thereby, eliminate those organisms. The organs of the immune system are bone marrow, thymus, spleen, Peyer’s patches, and lymph nodes. The cells of this system are the leukocytes, or white blood cells. There are two categories of leukocytes; 1) phagocytes, including neutrophils, monocytes, and macrophages, which form part of the innate immune system and provide nonspecific immunity, and 2) the lymphocytes, which mediate adaptive, or specific immunity. The specific immune response can produce cellular immunity mediated by specifically sensitive immune cells and the humoral immune response mediated by the antibody production (Perdigon et al., 1995; Cebra, 1999).

A large proportion of the immune cells of the body are associated with the gut. In the healthy host, the presence of the microbiota is tolerated by the immune system, although the mechanisms involved are not precisely known. Nevertheless, it can be inferred that tolerance toward the microbiota exists, because human patients with inflammatory bowel diseases and experimental animals with dysfunctional im-
mune systems suffer from chronic, immune-mediated inflammation of the bowel mucosa (Podolsky, 2002). Much evidence points to the presence of the microbiota as the fuel for this smoldering inflammation. The autochthonous microbe-immune system relationship in healthy animals must therefore be one of tolerance and requires mechanistic investigation. The allochthonous microbe-immune system relationship is presumably quite different, at least initially, because the immune system will experience novel antigenic complexes with each encounter with a different bacterial strain. Continuous close encounters with the same strain, either serendipitous (food microbiota) or intentional (probiotic), could, one supposes, eventually engender tolerance (Tannock, 2004).

The gut microflora is an important constituent in the intestine’s defense barrier, as shown by increased antigen transport across the gut mucosa in the absence of an intestinal microflora. This notion further supported by a demonstration that the gut microflora elicit specific immune responses at a local and a systemic level (Kailua et al., 1992; Isolauri et al., 2001; Perdigon et al., 2001). It has been hypothesized that LAB may have some potential role in augmenting the immune system of the host (Turjman et al., 1982). This hypothesis was proposed on the observation that LAB suppressed peritoneally and subcutaneously implanted tumors in mice in short-term studies. LAB did not directly suppress the tumor, but the suppression appeared to be mediated indirectly through the host’s immune system.

The effects of LAB on the immune system could have theoretical applications for tumor suppression, and anti-infective activity. None of these potential effects has been in man, and LAB do not yet have a place among human cancer therapies (Marteau and Rambaud, 1993). However, very active and interesting research is developing in this field.

During the past ten years, interactions between lactobacilli and immunocompetence have been studied extensively in vitro and in vivo. These investigations have revealed that lactobacilli not only constitute an integral part of the healthy gastrointestinal microecology, but are also involved in the host’s protective mechanisms by increasing its specific and non-specific immune mechanisms (Renner, 1995). The intestinal microflora has a profound influence on the immunological state of the host. Live indigenous bacteria or their antigens can, in fact, penetrate the epithelial barrier of the intestine and directly stimulate the immunocompetent cells. The germs present in the intestinal lumen favour the production of suppressor cells, the lymphocytic differentiation or even the “helper” cell activity.

The intestinal lactobacilli can stimulate the immune system in two ways. They can either migrate thought the gut wall as viable cells and multiply to a limited extent or antigens released by the dead organisms can be absorbed and stimulate the immune system directly. A third school of thought suggests that the lactobacilli are acting indirectly through an effect on the other components of the gut flora. It is a product of this change that induces the immune response. At present, which of these methods is, being used is not certain, but there appears to be some relationship between the ability of a strain to translocate and the ability to be immunogenic.

The improved immunity induced by lactobacilli may be manifested in three ways:

- Increased macrophage activity shown by the
enhanced ability to phagocytose microorganisms or carbon particle;
- Increased production of systemic antibody usually of the immunoglobulin classes IgG, IgM and interferon;
- Increased local antibody at mucosal surfaces such as the gut wall. These are usually IgA.

The first (and possibly the only) contact of lactobacilli with the immune system occurs in the gut-associated lymphoid tissue. Perdigon et al. (1990) showed that oral administration of *L. casei*, *L. acidophilus*, *L. bulgaricus* and *S. thermophilus* increased the levels of immunoglobulins in the intestinal fluid. However, only *L. casei* pretreated mice had increased secretion of specific antibodies against *Salmonella* when challenged with *Salmonella*. De Simone et al. (1987a, 1988) reported that yogurt or heated yogurt administered to mice increased the percentage of B-lymphocytes in the Peyer’s patches, and the proliferative responses of these cells to stimulants. When those mice challenged with *Salmonella*, antibody production and thus resistance to infection was increased by living yogurt.

A large number of experiments have demonstrated that LAB, especially lactobacilli could exert immunostimulatory effects (Adachi, 1992; Tomioka and Saito, 1992; Malin et al., 1996; Perdigon et al., 1999, 2001; Koop-Hoolihan, 2001) and enhance host resistance against experimental infections (Perdigon and Alvarez, 1992). The effects seem mainly due to the activation of the macrophages and non-specific immunity by cell wall components of the lactobacilli (Kato et al., 1984; Sato, 1984; Yokokura et al., 1986; Sato et al., 1988a, 1988b; Ohashi et al., 1989; Moineau and Goulet, 1991; Miettinen et al., 1996; Fuller and Perdigon, 2000; Perdigon et al., 2001).

The non-specific defence mechanisms of the host include phagocytosis, which is effected by macrophages, polymorphonuclear leucocytes, histiocytes and mononuclear phagocytic cells that are part of the mononuclear phagocytic system, which removes foreign antigens. In this system, the most important cells are macrophages. The state of activation of these is a measure of the non-specific immune response of the host (Perdigon and Alvarez, 1992).

Changes in monocytes’ and lymphocytes’ activities, a measure of cell-mediated immunity, have also been studied following short-term feeding of viable LAB and yogurt to mice. Mice were fed viable *L. acidophilus*, *L. casei subsp. casei*, *L. delbrueckii subsp. bulgaricus* and *S. thermophilus*, and changes in macrophage enzymes and *in vivo* phagocytic function of reticuloendothelial system were measured (Perdigon et al., 1986a). Feeding of *L. acidophilus* and *L. casei subsp. casei* increased all three enzymatic activities (Perdigon et al., 1986b, 1987). The feeding of *L. delbrueckii subsp. bulgaricus* also increased β-glucuronidase and β-galactosidase activities. (Perdigon et al., 1986a, 1987).

The effect of lactobacilli on the cellular and the humoral immune response has been investigated by Perdigon et al. (1986c, 1988a, b). They found that the oral administration of *L. casei*, *L. acidophilus* and *L. delbrueckii subsp. bulgaricus* enhanced both the cellular and the humoral immune response. *L. casei* and *L. acidophilus* were tested singly and together by Perdigon (1986b) for stimulation of phagocytic activity. It was found that the mixture was more effective than the strains given singly. These suggest that a mixture
of lactobacilli was more effective and the individual effects of the component strains may be additive.

In feeding experiments with mice where $1.2 \times 10^9$ cells of LAB per day were administered, the following results were obtained (Perdigon et al., 1993): *L. acidophilus* increased IgA production only temporarily, but increased IgG production throughout the feeding period; *L. casei* enhanced the proliferation of IgA-producing cells; yoghurt feeding increased the number of plasma cells, lymphocytes and macrophages. *L. casei* feeding given to mal-nourished animals slightly increased the number of circulating leukocytes and the phagocytic activity of peritoneal macrophages. This feeding also restored the strict anaerobic population in the small intestine, but failed to prevent bacterial translocation. *L. casei* induced an increase in the number of IgA-producing cells as well as the level of IgM (Perdigon et al., 1995). The above results suggested that: (a) *L. casei*, *L. acidophilus*, *L. delbrueckii subsp. bulgaricus* and certain doses of *S. thermophilus* are capable of activating the cells involved in the non-specific immune response; (b) the LAB capable of survival and growth in the intestinal tract, such as *L. casei* and *L. acidophilus*, are more efficient in the activation.

De Simone et al (1986, 1987b) showed that several LAB including yogurt bacteria stimulated in vitro production of interferon-g (IFN-g) by human blood lymphocytes. Several *in vivo* studies in man showed then that ingested yogurt bacteria at last in very large doses ($10^{13}$ and $3 \times 10^{12}$ day$^{-1}$) stimulated IFN-g production (De Simone et al., 1991; Solis and Lemonnier, 1991). Kishi et al. (1996) observed that *L. brevis subsp. coagulans* significantly increased a-interferon production. A beneficial consequence of interferon stimulation could be an increase in resistance to some infections. However, interferon can exert both beneficial and detrimental effects to man (Murray, 1988). More experiments, including dose-response studies, and trials with clinical end-points are needed.

Perdigon et al. (1993) present the following hypothesis for the immunomodulating effects of LAB: not all lactobacilli induce synthesis of IgA; for those that do, the dose administered plays a critical role; the first effect of lactobacilli on the immune system occurs at the local level on the gut-associated lymphoreticular tissue, which may or may not send signals that would permit the activation of the immune system in general. Many other studies have shown that oral administration of lactobacilli could influence parameters of the systemic immunity (Morissete et al., 1991; Perdigon and Alvarez, 1992; Marteau, 1995; Sotirov et al., 2000, 2001; Isolauri et al., 2001). Although many of these results were obtained using mice as experimental models, they are not easily transferred to human beings. For the use of lactobacilli as immunomodulatory agents, experimental models are absolutely necessary to determine that effects of lactobacilli on the host are innocuous and to select those bacteria that most effectively enhance the immune response. However, the exploitation of that knowledge for therapeutic purposes is still limited (Perdigon et al., 1995). For more information about immunologic effects of lactobacilli see Guilliams (1999), Matsuzaki and Chin (2000), Isolauri et al. (2001; Perdigon et al. (2001); Kaur et al. (2002).

Lactobacilli clearly offer microbiologists exciting research prospects, both for
biomedical applications and for acquiring fundamental knowledge of how bacterial cells function in the gut ecosystem. As model gut bacteria, they may provide lessons in the molecular mechanisms that define autochthony as well as in understanding bacterial physiology in relation to host welfare. For these reasons, lactobacilli are set to remain the fond favorites of many microbiologists.

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