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Review

Lactobacillus acidophilus cell structure and application

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Lactic acid bacteria (LAB) are one of the most applied bacteria in the production of fermented foods, from dairy to fruits and vegetables products. They make food durable, improve food safety, flavor, and texture as well as to enhance food physiological and hygienic value due to the presence of viable cells and valuable. Besides, products obtained by LAB fermentation processes are of special importance for functional foods such as probiotics. *Lactobacillus acidophilus* is the best well-known species of this Lactobacillus complex in LAB group and exist in the gastrointestinal tracts of humans and animals. In fermented food, the metabolic activity of this microorganism results in production of flavor, and aroma that cause organoleptic properties of fermented foods and inhibits foods spoilage. *L. acidophilus* effect on modulation of host immunity is proved by clinical evidence but molecular and cellular mechanisms of these effects are not completely clear. Despite the increasing application of this bacterium, little is known about its effects on the gut community, host physiology and immunity. The molecular mechanisms by which *L. acidophilus* exert these effects are not clearly understood. In this regard, recognition of cell structures and the genomic base of this bacterium could be useful. In this paper we try to gather all the information that exists about this bacterium.

Key words: *Lactobacillus acidophilus*, structure, fermented food, probiotic.

INTRODUCTION

For early societies, the transformation of basic food materials into fermented foods was a mystery and a miracle, for they had no idea what caused the usually sudden, dramatic, and welcomed transformation. In ancient times fermentation joined smoking, drying, and freezing as basic and widely practiced food preservation techniques. Wang and Hesseltine (1979) note that "Probably the first fermentation were discovered accidentally when salt was incorporated with the food material, and the salt selected certain harmless microorganisms that fermented the product to give a nutritious and acceptable food (Sanchez, 2008).

Nowadays we know that fermentation is the chemical transformation of organic substances into simpler compounds by the action of enzymes, complex organic catalysts, which are produced by microorganisms such as molds, yeasts or bacteria. The great majority of these microorganisms come from a relatively small number of genera. Lactic acid bacteria (LAB) are one the most applied bacteria in fermentation. These bacteria produce lactic acid as a result of carbohydrate fermentation and are broadly used in the production of fermented foods, from dairy to fruits and vegetables products. The reasons for a widespread use of LAB are to make food durable, to improve its safety, flavor, appearance and texture as well as to enhance its physiological and hygienic value due to the presence of viable cells and valuable metabolites of LAB (Semjonovs et al., 2008).

Elie Metchnikoff was the first scientist who proposed the therapeutic use of LAB for the prevention or treatment of several pathological conditions. At the turn of the 20th century the concept of probiotics was introduced as live microorganisms, which when consumed in adequate amount, confer a health benefit on the host. Probiotics are gaining widespread application for preventing and treatment of disease especially gastro intestinal disease. Mechanisms of probioitics include remodeling of microbial communities and suppression of pathogens, immunomodulation by up-regulation of anti-inflammatory factors, enhancement of immunity, effect on epithelial

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cells differentiation and proliferation, and promoting of intestinal barrier function (Preidis and Versalovic, 2009). Products obtained by LAB fermentation processes therefore are of special importance for functional foods such as proiotics (Semjonovs et al., 2008).

Within the LAB, the subgroup of the Lactobacillus complex is of particular interest due to the fact that many members occupy important ecologic niches in the gastrointestinal tracts of humans and animals and *Lactobacillus acidophilus* (meaning acid-loving milk-bacterium) is probably the best well-known species of this genus (Klaenhammer et al., 2008). It was first isolated in 1900 by Moro from infant feces and is found in the human gastrointestinal (GI) tract and vagina. *L. acidophilus* is important in the fermentation of many foods especially dairy products and fermentation occurs when bacteria break down sugars and carbohydrates to produce alcohol, carbon dioxide and lactic acid (Lindgren and Dobrogosz, 1990; Narendranath et al., 1997). The metabolic activity of this microorganism results in production of flavor, and aroma that cause organoleptic properties of fermented foods and inhibits foods spoilage bacteria (Klaenhammer, 1988; Leroy and De Vuyst, 2004; O'sullivan et al., 2002; Schleifer et al., 1995). Besides dairy products, there is so many commercial probioitc products based on *L. acidophilus*. It is calmed that this bacterium produces healthy by products that protect the stomach, the gut and the reproductive area from harmful bacteria.

Despite the increasing demand and production of such products, two related challenge stand in the way of the widespread adoption of probioitc therapies in the clicnic:

(i) little is known about the effects these agents have on the gut community and host physiology. (ii) Less is known of the molecular mechanisms by which probiotics exert their effects (Sonnenburg and Fischbach, 2011). In another word the exact mechanisms underlying the proposed actions of LAB remain vastly unknown, partly due to the complexity of the gastrointestinal ecosystem in which these biotherapeutic agents interact, and to the increasing variety of strains with potential probiotic characteristics. It must be considered that disruption of immune regulatory functions by an imbalanced microbiota may lead to inflammation and chronic inflammatory diseases.

L. acidophilus effect on modulation of host immunity is proved by clinical evidence but molecular and cellular mechanisms of these effects are not completely clear. Today scientists focus on innate and acquired immunity mechanisms that involved in functional recognition and responsiveness to this bacterium. Antigenic components which mediate the state of immunomodulation and host cell responses to distinct commensal-associated molecular patterns (CAMPs) of these strains must be determined. Besides intestinal epithelial cell responses to *L. acidophilus* structures such as peptidoglycan, lipotechoic acid, S-layer, and adhesion molecules remained to be investigated (Willing and Van Kessel, 2010).

In this regard, recognition of genomic date and induction of especial mutation could be useful in identifying the bioactive components displayed on *L. acidophilus* surface. By this way ultimately the molecular basis of immunogenicity of this bacterium could be clearly understood. In another word the increased use of *L. acidophilus* in production of such products deserves scrutiny of the physiology and molecular structure of this bacterium and determination of host interaction in molecular basis. In this review we focus on molecular structure, interaction with host immunity, and bioitchnological application of *L. acidophilus* molecular structures.

CLASSIFICATION OF *Lactobacillus* sp

LAB are Gram-positive, non-spore forming cocci, coccobacilli or rods (Schleifer and Ludwig, 1995; Schleifer et al., 1995) with a DNA base composition of less than 53 mol% G+C (Stackebrandt and Teuber, 1988). they are able to growth in the presence (aerotolerant anaerobes) and absence of oxygen but generally they grow anaerobically and lack catalase (Condon, 1983; Piard and Desmazeaud, 1991). They possess superoxide dismutase and have alternative means such as peroxidase enzymes to detoxify peroxide radicals, generated through aerobic growth (Condon, 1987; Lin and Yen, 1999).

Most of the lactic acid bacteria have limited biosynthetic ability so they are restricted in environments that are rich in amino acids, vitamins, purines and pyrimidines. The mouth, intestinal tract of animals, plant leaves, milk and decaying organic material are examples of such environments (AXELSSON, 2004; Bottazzi, 1988; Sookkhee et al., 2001). These bacteria obtain energy only from the metabolism of sugars and produce lactic acid as a primary or secondary end product of fermentation (Kandler, 1983; Thompson, 1987). At present, bacterial species from 12 genera are included in LAB group and Lactobacillus is one of the most important of them (He, 2003; Stiles and Holzapfel, 1997).

Lactobacillus genousincludes a heterogenous group of rod-shaped, usually non-motile, facultative anaerobic species that vary widely morphologically and in growth and metabolic characteristics (Schleifer and Ludwig, 1995; Schleifer et al., 1995). Cells vary from very short (almost coccoid) to very long rods, slender or moderately thick, often bent, and can be present as single cells or in short to long chains (Barre, 1978; Collins et al., 1991).

On the basis of their metabolic patterns of hexoses and pentoses, the species of Lactobacillus has been divided into three groups (Baele et al., 2002; Bottazzi, 1988; Holzer et al., 2003): (i) obligately Homofermentative species that produce lactic acid as a major end product

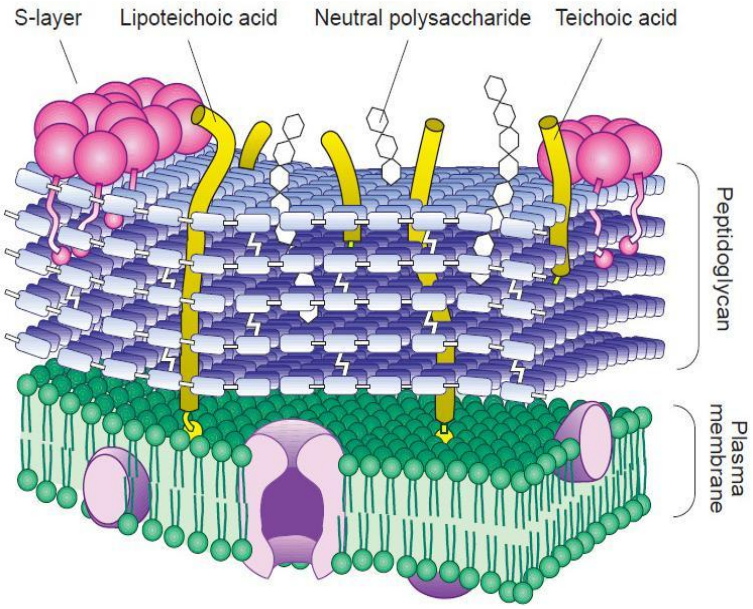


Figure 1. Cell wall structure of *L. acidophilus*. The bilipidicplasma membrane with embedded proteins is covered by a mutilayered peptidoglycan shell decorated with neutral polysaccharides, lipoteichoic acids and teichoic acids, surrounded by an outer envelope of S-layer proteins; for the sake of clarity, cell wall-associated proteins are not depicted.

(>85%) from glucose. *Lactobacillus delbrueckii*, *Lactobacillus helveticus*, *Lactobacillus salivarius* and *L. acidophilus* are the most known examples in this group. The members of this group could growth at 45°C but not at 15°C. (ii) Facultatively heterofermentative species that produce lactic acid as a major of end product but they grow at 15°C and show variable growth at 45°C which represented by *Lactobacillus casei*, *Lactobacillus curvatus*, *Lactobacillus sakei* and *Lactobacillus plantarum*. (iii) Obligately heterofermentative species that produce lactic acid as well as CO_2 and ethanol. Representative species include *Lactobacillus fermentum*, *Lactobacillus brevis* and *Lactobacillus keferi*.

METABOLISM OF *L. acidophilus*

L. acidphilus is an obligately homofermentative LAB that growth in anaerobic condition. This bacterium lacks cytochromes, porphyrins, and respiratory enzymes and as a result is unable to undergo any oxidative phosphorylation or respiration. Because they utilize sugars (e.g. glucose, aesculin, cellobiose, galactose, lactose, maltose, salicin, and sucrose) as their substrates for fermentation, they inhabit environments with high sugar abundance, such as the GI tract in humans and animals (Vijayakumar et al., 2008).

For every one glucose molecule that undergoes fermentation in *L. acidophilus*, the energy yield is two ATPs. As a result, this bacterium, must catabolize large amounts of substrate to generate enough energy for growth (Tamime et al., 2006; Vijayakumar et al., 2008) in *L. acidophilus* transportation of nutrients is carried out by PEP-PTS as well as by permease systems and many of transport proteins involved in carrying nutrient molecules from the outside into the cell and also removing many by-products from the cell into the environment (Rapoport et al., 1996; Singer, 1974; Singer and Nicolson, 1972; Tseng and Montville, 1993).

CELL STRUCTURE OF *L. acidophilus*

Early studies of *L. acidophilus* were performed on strains isolated from fecal material of humans, pigs and chickens. The cell envelope of *L. acidophlus* consists of the cytoplasmic membrane, the overlying cell wall. The wall gives the cell its shape and surround the cytoplasmic membrane, protecting it's from the environment. It also helps to anchor appendage like pili and flagella, which originate in cytoplasmic membrane and protrude through the wall to the outside. These appendages help *L. acidophilus* to move and attach to specific substrate.

Cell wall consists four important components include peptidoglycan, teichoic acids, S-layer, and polysaccharides (Figure 1) (Delcour et al., 1999).

Peptidoglycan

Peptidoglycan (murein) is an essential and specific component of the *L. acidophilus* cell wall (like othe Gram positive bacteria). It is a polymer consisting of sugars and

amino acids that forms a mesh-like layer outside the plasma membrane. The sugar component consists of alternating residues of β-(1, 4) linked N-acetylglucosamine and N-acetylmuramic acid. Attached to the N-acetylmuramic acid is a peptide chain which can be cross-linked to the peptide chain of another strand and form the 3D mesh-like layer.

Peptidoglycan main function is to preserve cell integrity by withstanding the turgor. Indeed, any inhibition of its biosynthesis or its specific degradation during cell growth will result in cell lysis.

Peptidoglycan also contributes to the maintenance of a defined cell shape and serves as a scaffold for anchoring other cell envelope components such as proteins and teichoic acids. It is intimately involved in the processes of cell growth and cell division (Vollmer et al., 2008).

Peptidoglycan recognition in innate immunity

The innate immune system recognizes microorganisms through a series of pattern recognition receptors that are highly conserved in evolution and are specific for common motifs found in microorganisms but not in higher eukaryotes (Dziarski et al., 2003). Because peptidiglycan is a unique and essential cell wall component of virtually all bacteria, it is an excellent target for recognition by the eukaryotic innate immune system (Dziarski and Gupta, 2005; Schiffrin et al., 1997). The most important pattern recognition receptors for peptidoglycan are:

***CD14* and Toll-like receptor 2 (*TLR2*):** *CD14* functions as the macrophage co-receptor (together with TLR4 and MD-2) for LPS from the outer membrane of Gram-negative and peptidoglycan form Gram-positive bacteria (Dziarski and Gupta 2005; Konstantinov et al. 2008). TLR2 is a cell-activating receptor for Gram-positive bacteria and their peptidoglycan and lipoteichoicacid (LTA) components. TLR2 is primarily expressed on monocytes, macrophages, dendritic cells, B cells, and to a lesser extent, on neutrophils and few other cells. The main consequence of interaction of peptidoglycan with TLR2 and CD14 is activation of a signal transduction pathway that results in the activation of NF-κB transcription factor, that is required for the activation of transcription and secretion of several chemokines and cytokines (Dziarski and Gupta, 2005; Vidal et al., 2002).

Intracellular recognition: Nods

Nucleotide-binding oligomerization domain (Nod)-containing proteins are present in the cytoplasm and have structural homology to a large family of plant R (resistance) proteins. In mammals, they include Nod1, Nod2, and several other homologues. They likely function as intracellular regulators of cell activation. Nod1 has ubiquitous expression in several tissues and cell types, and Nod2 is primarily expressed in monocytes, but its expression can be induced in other cells. Nod1 and Nod2 mediate activation of NF-κB through association with a serine-threonine kinase. Nods sense intracellular bacteria by recognizing their peptidoglycan component (Dziarski and Gupta, 2005).

Recognition and effector molecules: PGRPs

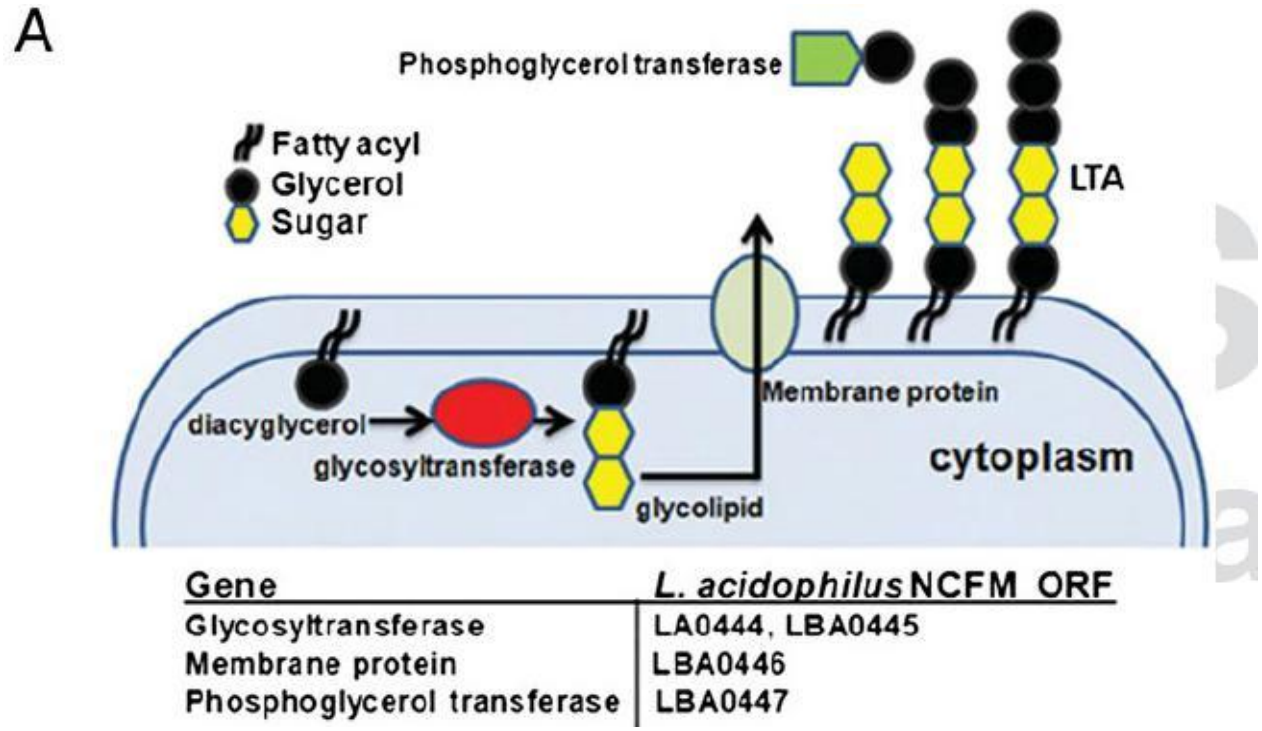
Peptidoglycan recognition proteins (PGRPs) are a family of pattern recognition molecules that were first discovered in insects and then in mammals. Many of the insect PGRPs are expressed in immune competent organs, such as the fat body, gut, and hemocytes (Dziarski and Gupta, 2005; Dziarski et al., 2003).

L. acidophilus peptidoglycan is responsible for certain immune responses induced by this bacterium. Recognition of peptidoglycan supports the natural defenses of the body and stimulates immune responses in the intestinal tract (Fichera and Giese, 1994). Peptidoglycan stimulates a large number of proteins and causes the protective inflammatory response. For example *L. acidophilus* peptidoglycan shows anti-tumoral activity mediated by the stimulation of cellular defence mechanisms.

Teichoic acids (TA) of *L. acidophilus*

The cell wall of L.acidophilus comprises teichoic acids (sensulato) which may account for more than 50% of the weight of the wall. Teichoic acids are quite diverse in structure and abundance, depending on the strain, stage or rate of growth, pH of the medium, carbon source, availability of phosphate, etc (Delcour et al. 1999). Teichoic acids contribute in many respects to the functionality of the cell wall (Delcour et al., 1999) and seems in several forms:(i) teichoic acids (TA) and teichuronic acids (TUA) that are covalently bound to Peptidoglycan, and (ii) lipoteichoic acid (LTA) and lipoglycans (LG) that remain attached to the cytoplasmic membrane, but a fraction of them are found free in the cell wall or even released into the medium (Delcour et al., 1999).

In *L. acidophilus*, LTA consist of a membrane-anchored glycolipid and a polyglycerophosphate chain with covalently linked D-Ala residues (Mohamadzadeh et al., 2011). The current model of LTA biosynthesis in *L. acidophilus* suggests three distinct stages in the expression of LTA, indicating that a glycolipid anchor unit is initially synthesized by action of a glycosyltransferase. Subsequently, the glycolipid is translocated to the exterior of the bacterium by a membrane-associated protein followed by extracellular addition of polyglycerolphosphate to the glycolipid anchor by a phosphoglyceroltransferase (Figure 2) (Mohamadzadeh et al., 2011).



Gene	*L. acidophilus* NCFM ORF
Glycosyltransferase	LA0444, LBA0445
Membrane protein	LBA0446
Phosphoglycerol transferase	LBA0447

Figure 2. LTA biosynthesis in *L. acidophilus.*

LTA plays a vital role in the growth and physiology of *L. acidophilus* and another bacteria as anionic polymers such as: (i) Modulation the activities of autolysins (muramidases), (ii) Scavenging of cations required for enzyme functions in particular Mg, (iii) Donating the electromechanical properties of the cell wall, (ix) Mediating the adhesion to epithelial cells (ECs) through the negative charge that it confers to the bacterial surface, which facilitates the electrostatic binding to surface molecules, (x)Involvement in phage adsorption, and (xi) Acting as a potent immunogens and can be regarded equivalent of the Gram negative lipopolysaccharides. LTA is able to stimulate cytokine synthesis, and this effect is lost upon removal of the D-alanine substituents (Delcour et al., 1999; Mohamadzadeh et al., 2011).

LTA of L. acidophilus and immunity

LTA hasantigenic properties and because of that being able to stimulate specific immune response. LTA may bind to target cells non-specifically through membrane phospholipids, or specifically to CD14 and to Toll-like receptors. Binding to TLR-2 has shown to induce NF-κB expression (a central transcription factor), elevating expression of both pro- and anti-apoptotic genes. Its activation also induces mitogen-activated protein kinases (MAPK) activation along with phosphoinositide 3-kinase activation.

LTA bound to targets also can interact with circulating antibodies and activate the complement cascade to induce a passive immune kill phenomenon. It also triggers the release from neutrophils and macrophages of reactive oxygen and nitrogen species, acid hydrolases, highly cationic proteinases, bactericidal cationic peptides, growth factors, and cytotoxic cytokines, which may act in synergy to amplify cell damage. Therefore, LTA shares many pathogenic similarities with endotoxins (lipopolysaccharide).

Recent data indicate that LTA of lactobacilli stimulate DCs through specific pattern recognition receptors, including Toll-like Receptor 2 (TLR2), resulting in species stimulation of DCs to produce cytokines. The quality and levels of D-Alanine (D-Ala) on LTA are critical for cytokine production, as shown by the synthesis of LTA-deficient in D-Ala (Mohamadzadeh et al., 2011).

Recently It was showed that *L. acidophilus* LTA negative compare to LTA positive, not only down-regulated IL-12 and TNFα but also significantly enhanced IL-10 in DCs and controlled the regulation of costimulatory DC functions, resulting in their inability to induce $CD4^+$ T-cell activation (Mohamadzadeh et al., 2011). Treatment of induced mouse models of colitis showed *L. acidophilus* LTA negative significantly mitigated T cell-induced colitis and effectively ameliorated dextran sulfate sodium-established colitis through a mechanism that involves IL-10 and $CD4^+FoxP3^+$ T-regulatory cells to dampen exaggerated mucosal inflammation (Mohamadzadeh et al., 2011).

It is obvious that inflammatory cytokines (that is, IL-12 and IL-23) plays a pivotal role in inflammatory diseases such as human inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease. In contrast to both of these cytokines, IL-10 exerts regulatory effects on the inflammatory signals. Regarding this results, use of such genetically modified Lactobacilli become more highlighted in regulation of pro and anti-inflammatory cytokines and treatment of diseases (Mohamadzadeh et al., 2011).

S-layer of *L. acidophilus*

L. acidophilus strains, isolated from humans or animals, which belong to the DNA homology groups A are reported to possess an S-layer, while the strains which belong to the DNA homology groups B appear not to have it (Boot et al., 1993).

S-layers are two-dimensional paracrystalline arrays of proteins or glycoproteins (25 to 220 kDa). They are composed of numerous identical, non-covalently bound subunits, which forming a symmetric, porous, lattice-like structurewith oblique (p1, p2), square (p4), or hexagonal (p3, p6) symmetry (Boot et al., 1996a; Hollmann et al., 2010; Mobili et al., 2010; Sleytr et al., 1999).The primary structures of bacterial S-layer proteins are similar in that they are generally rich in acidic, hydrophobic and hydroxyl-containing amino acids, and cysteines are very rarely found. The predicted pI values are usually in a weakly acidic range (rang of 4-6) but for Lactobacilli isoelectric point value is in the range of 9-10 (Hynönen, 2009).

The S-layers play an important role in the maintenance of cellular functions of bacteria. Considering that purified S-layers are stable toward non-physiological pH, radiation, temperature, some kind of proteolysis, high pressures and detergent treatments, a protection role against hostile factors has been proposed for these superficial structures. Another functions or properties that have been ascribed for these layers in *L. actobacillus* sp. Are (Mobili et al., 2010): (i) determination and maintenance of cell shape, (ii) Adhesion to mucus, Extracellular matrix proteins (ECM), and epithelial cells,

(iii) Acting as a shield to cover phage receptors present in the underlying cell wall (Boot et al., 1996a), (ix) Being a molecular sieve, and scaffolding for high-molecular-weight extracellular proteins such as enzymes(Boot et al., 1996b), and (x) Specific interaction with immune cells, and regulating their function through cytokine induction(Hynönen, 2009).

S-layer proteins with adhesive properties could contribute to *L. acidophilus* probiotic activity by the inhibition of the binding of pathogens to host tissues. This can be achieved through direct competition for attachment sites on human intestinal cells, ECM and mucus proteins, or by the blockage of pathogen surface adhesins.

Biosynthesis and applications of S-layer of L. acidophilus

Genomic structure of S-layer of *L. acidophilus* has been studied in detail. The slpAgeneis actively transcribed and encodes the SA-protein which forms the wild-type of S-layer in this bacterium. The slpB is a silent gene near the slpA gene, encoding the SB-protein. The slpA gene is interchanged with the slpB gene through inversion of a chromosomal fragment in a fraction of an *L. acidophilus* culture (0.3% of the cells grown under laboratory conditions). Such a recombination event is expected to lead to the production of a partially different S-protein and S-layer (S-layer variation) (Boot et al., 1996a).The slpX is another gene suspected to contribute to the S-layer complex in some strains such as *L. acidophilus* NCFM which was isolated from human in 1970 at North Carolina state university (Altermann et al., 2005; Gilliland and Walker, 1990; McAuliffe et al., 2005; Sanders and Klaenhammer, 2001). *L. acidophilus* NCFM is able to produce lactacin B, a bacteriocin which is a small peptide with antimicrobial properties. Like most bacteriocins, lactacin B is only capable of inhibiting growth of species closely related to *L. acidophilus* (Altermann et al., 2005; Barefoot et al., 1994).

In this bacterium the slpA and slpB genes are located in a gene cluster in different orientation, whereas slpX is located at a distant chromosomal locus. SlpX shared less than 30% sequence identity with SlpA (26%) and SlpB (24%), and the similarity was confined to the N-terminal and C-terminal regions.

Nonetheless, SlpX is similar in size to SlpA and SlpB (46 to 51 kDa), and all three S-layer proteins shared features, such as a predicted basic isoelectric point of 9.5 and a high proportion of hydrophobic residues (44 to 48%) which are typical characteristics of *L. actobacillus* sp. S-layer proteins(Goh et al., 2009; Sleytr et al., 1999).

Nowadays, application of S-layers in different fields of science is receiving growing attention. Some of these applications are (Goh et al., 2009; Sleytr et al., 1999).

S-Layers as isoporous ultrafiltration membranes. There are special pores in S-layers which are identical in size and morphology in the 2 to 8 nm range. So it can be considered as isoporous ultrafiltration membranes with porosity in the range of 30 to 70%. Hence S-layers could be exploited for the production of ultrafiltration membranes

S-Layers as matrix for the immobilization of functional molecules. The high density and defined position of carboxyl groups located on the surface of S-layer lattices could be used for the immobilization of different molecules such as enzymes, antibodies, protein A, biotin, and avidin.

These immobilized membranes are used in affinity membranes, amperometric or optical biosensors or solid-phase immunoassays. More recently, S-layers proteins have been genetically fused to enzymes, streptavidin, specific antibody fragments, green fluorescent protein

(GFP) and a protein A immunoglobulin-binding domain analogue (Z domain). These fusion proteins, which retain the ability to recrystallize, may find numerous applications varying from biosensors and label-free detection systems to blood detoxification.

S-Layers as supporting structure for functional lipid membranes. Since a great variety of biological processes are membrane mediated, there has always been great interest in the meso and macroscopic reconstitution of biological membranes. Particularly functional trans-membrane proteins have a broad potential for bio-analytical, biotechnological, and biomimetic applications. On the other hand, investigations are primarily impeded by a low stability of artificial planar lipid bilayer systems and liposomes. Consequently, there is a strong demand to develop systems that reinforce such fragile structures without interfering with their function. The stability of lipid membranes can be increased significantly by recrystallization of isolated S-layer (glyco) proteins as coherent monomolecular lattices.

S-Layers as templates in the formation of regularly arranged nanoparticles. The formation of arrays of metal clusters with novel physical properties by colloidal crystallization or monolayer deposition is currently under extensive investigation in the field of molecular electronics and nonlinear optics. S-layers have already demonstrated their application potential as templates in the formation of regularly arranged nanometric metallic or semiconducting point patterns.

S-Layers for vaccine development. Recently, there has been increased interest in the possibility of genetically manipulating lactobacilli, for mucosal vaccines. Crystalline S-layers of *Lactobacilli* could be utilized for vaccine development. Native and cross-linked (Polysaccharides and proteins linked) S-layers are used as combined carrier/adjuvants system either against infection with pathogenic bacteria, in the immunotherapy of cancers, and in the anti-allergic immunotherapy (Sleytr et al., 1999).

S-layer proteins can account for 10 to12% of total cell proteins. This high level of expression facilitates large-scale production of target proteins. More importantly, since S-layer proteins are expressed on the cell surface and are either secreted or can be easily released from the cell surface, recovery and purification of S-layer fusion proteins is relatively simple (Goh et al., 2009; Sleytr et al., 1999).

S-layer of L. acidophilus and immunity

Cell surface components of Lactobacillus sp. resident in the human GI tract could activate the functions of various antigen-presenting cells (APCs) such as Dendritic cells (DCs). DCs are professional APCs that regularly interact with intestinal bacteria at various mucosal sites and along with antigen uptake and processing, functional changes in the DCs initiate both humoral and adaptive immune responses. The immune-regulatory role of DCs is believed to be determined by ligation of pathogen-recognition receptors such as TLRs and CLRs, and signaling pathways induced by these receptors, which can interconnect through a so-called cross-talk but the mechanisms of such immune modulations are largely unknown (Konstantinov et al., 2008).

L. acidophilus NCFM is one of the most widely recognized and commercially distributed probiotic cultures and an array of its mutants are generated. A knockout mutant of *L. acidophilus* NCFM lacking the surface S-layer A protein (SlpA) has a chromosomal inversion leading to dominant expression of a second S layer protein, SlpB (Konstantinov et al., 2008).

Experiments with this mutant demonstrated that the cellular contacts of *L. acidophilus* with Dendritic cells (DCs) involve interactions between DC-SIGN and SlpA. DC-SIGN is DC-specific ICAM-3- grabbing nonintegrin, a CLRs receptor on DCs. In the SlpB-dominant strain, the nature of the interaction of this bacterium with DCs changed dramatically in another word SlpB did not ligate to DC-SIGN. DC-SIGN has specificity for high mannose and fucose, that doesn't exist on SlpB. The experiment results showed that in compare with SlpA dominant strain, the induction of IL-10 (anti-inflammatory cytokine) was significantly reduced in SlpB dominant strain while this strain was more potent in the induction of pro-inflammatory cytokines such as IL- 12, TNF-α, and IL-1. In conclusion, the major S layer protein, SlpA, of *L. acidophilus* NCFM is a bacterial DC-SIGN ligand that is functionally involved in the modulation of DCs and T cells functions. These experiments showed the potentional use of these mutants for treatment of some deases such as colitis (Konstantinov et al., 2008).

Exoploysaccharide *(EPS)* of *L. acidophilus*

The name exopolysaccharides as proposed by Sutherland (1972) provides a general term for various forms of bacterial polysaccharides found outside the cell wall (Lam et al., 2007) which are either associated with the cell surface in the form of capsules or secreted into the extracellular environment in the form of slime. They are referred to as capsular or slime EPS, respectively (Ludbrook et al., 1997; Vuyst and Degeest 1999). Chemical composition, molecular weight, electrical charge, the presence of lateral chains and the rigidity of a molecule of EPS are affected by the conditions of biosynthesis and the microorganism applied (Brzozowski et al., 2009; Çelik, 2007; Cerning, 1995)

EPS in their natural environment are thought to play a role in the protection of the microbial cell against desiccation, phagocytosis and phage attack, antibiotics or toxic compounds (e.g. toxic metal ions, sulfur dioxide ethanol), predation by protozoans, osmotic stress,

adhesion to solid surfaces and biofilm formation, and also in cellular recognition (e.g. via binding to a lectin). It is not likely that EPS serve as a food reserve, since most slime-forming bacteria are not capable of catabolizing the EPS they produce (Vuyst and Degeest, 1999).

Microbial EPS are biothickeners that can be added to a wide variety of food products, where they serve as viscosifying, stabilizing, emulsifying or gelling agents and inhibit syneresis, which is the release of water from processed foods. In the search for a new generation of 'green' food thickeners, much attention is currently being given to EPSs produced by lactic acid bacteria Because these bacteria are food grade and generally regard as safe (GRAS) (Robijn et al., 1996; Senini et al., 2004). In particular for the production of yoghurt, drinking yoghurt, cheese, fermented cream, milk-based desserts, EPS producing LAB especially *L. acidophilus* play a significant role (Lam et al., 2007).

EPS from LAB are subdivided into two groups: (i) Homopolysaccharides: (ii) Heteropolysaccharides that produced by mesophilic and thermophilic Lactic Acid Bacteria (Breedveld et al., 1998; Lam et al., 2007; Marshall et al., 2001; Vincent et al., 2001; Vuyst and Degeest, 1999). Recently, exopolysaccharides biosynthesised from the thermophilic group specially *L. acidophilus* have received the most interest because of its important role in the rheology, texture and mouth feel of fermented milk drinks and products (Chadha, 2009) but usually producing trait in kind of bacteria is unstable (Cerning, 1995).

Health effect of EPS

It is demonstrated that EPS from bacteria specially *Lactobacillus* sp. may contribute to human health, either as non-digestible food fraction or because of their antitumoral, antiulcer, immunomodulating or cholesterol-lowering activity (Ganesh, 2006; Vuyst and Degeest, 1999). EPS has anticarcinogenic ability mediated by the stimulation of the mitogenic activity of B lymphocytes (Ruas-Madiedo et al., 2006; Xu et al., 2010).

It is also speculated that the increased viscosity of EPS containing foods may increase the residence time of ingested fermented milk in the gastrointestinal tract and therefore be beneficial to transient colonization by probiotic bacteria. Another example of a suggested health benefit of EPS from Lactobacilli is, the generation of short-chain fatty acids (SCFAs) upon degradation in the gut by the colonic microflora. SCFAs provide energy to epithelial cells and some have been claimed to play a role in the prevention of colon cancer (Brzozowski et al., 2009; Lam et al., 2007).

The functional properties of polysaccharides are related to their charge, molecular mass and sugar composition. Knowing the environmental and genetic factors regulating expression of the EPS, genetic approaches can be designed which enhance expression of a desired EPS under defined growth or fermentation conditions. Finally, both genetic approaches and enzyme and fermentation technology will increase the number of possibilities for modifying the structure and function of EPS.

This polysaccharide engineering may lead to the development of ‘designer polysaccharides' for applications that may or may not be food related. Although the technology of application of polysaccharides in specific purpose is still in its infancy (Chadha, 2009; Pescuma et al., 2009; Vuyst and Degeest, 1999).

CONCLUSION

Nowadays steady growing demand for foods with health-promoting properties, so called functional foods, requires a goal-directed involvement of appropriate strains and specific non-digestible ingredients selectively enhancing their growth which are generally acknowledged as probiotics and prebiotics, respectively (Semjonovs et al., 2008).The use of any probiotic and prebiotics substance for the enrichment of fermented products provides its delivery into human Gastrointestinal Tract (GIT) and hence, a stimulation of beneficial health effects(Ganesh, 2006).

In this regard importance of some LAB stains belong to the normal microfolra of GIT is completely well-known. Increasing evidence is accumulated on the prophylactic and therapeutic efficiency of *L. acidophilus* of LAB for both human and animals. Due to it, *L. acidophilus* finds extensive technological and commercial application, particularly in dairy fermentations or as a probiotic product.

Health benefits of *L. acidophilus* include providing immune support for infections or cancer, providing a healthy replacement of good bacteria in the intestinal tract following antibiotic therapy, reducing occurrence of diarrhea in humans, aiding in lowering cholesterol and improving the symptoms of lactose intolerance.

L. acidophilus like other prokaryote has a complex structure with defined functions. Cell envelop of this bacterium has a several layers that protect cell againt environmental conditions and help its surviuval. Peptidoglycan, lipteichoic acid, S-layer and EPS are the most important component of this envelope. These components are involved in intestinal epithelial cell responses to bacterial structures and mediate immuno-modulation and even in some case inflammation. It is obvious that disruption of immune system by an imbalanced microbiota is possible that could lead to inflammation and chronic inflammatory diseases such as colitis. So recognition of molecular structurs and mechanisms involved in immune stimulation by *L. acidophilus* is very important.

Moreover, some of *L. acidophilus* cell components have potential use in many different areas of

biotechnology. For example S-layer has gained attention as an ultra filtration membrane, matrix for immobilization and even vaccine development. EPS of *L. acidophilus* has a novel application in preparation of fermented and non-fermented food especially dairy products.

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