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Journal of Basic Microbiology
Environment · Health · Techniques

Review

Biosurfactant/s from Lactobacilli species: Properties, challenges and potential biomedical applications

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Lactic acid bacteria are generally believed to have positive roles in maintaining good health and immune system in humans. A number of Lactobacilli spp. are known to produce important metabolites, among which biosurfactants in particular have shown antimicrobial activity against several pathogens in the intestinal tract and female urogenital tract partly through interfering with biofilm formation and adhesion to the epithelial cells surfaces. Around 46 reports are documented on biosurfactant production from *Lactobacillus* spp. of which six can be broadly classified as cell free biosurfactant and 40 as cell associated biosurfactants and only approximately 50% of those have reported on the structural composition which, in order of occurrence were mainly proteinaceous, glycolipidic, glycoproteins, or glycolipopeptides in nature. Due to the proteinaceous nature, most biosurfactant produced by strains of *Lactobacillus* are generally believed to be surlactin type with high potential toward impeding pathogens adherence. Researchers have recently focused on the anti-adhesive and antibiofilm properties of Lactobacilli-derived biosurfactants. This review briefly discusses the significance of Lactobacilli-derived biosurfactants and their potential applications in various fields. In addition, we highlight the exceptional prospects and challenges in fermentation economics of *Lactobacillus* spp.-derived biosurfactants' production processes.

Keywords: Biosurfactant / Biofilm / Biomedical / Lactobacilli / Surlactin

Received: March 8, 2016; accepted: June 6, 2016

DOI 10.1002/jobm.201600143

Introduction

Probiotic lactic acid bacteria have an important role in most dairy-based fermentation processes and *Lactobacillus* is one of its most important genera [1]. *Lactobacillus* spp. together with *Streptococcus* are often used in combination in many dairy products for their acid and flavor production capacity. It is important to note that both of this bacterial spp. are known to displace adhering uropathogenic bacteria such as *Enterococcus faecalis* from hydrophobic and hydrophilic substrata in a parallel-plate flow chamber which may be through biosurfactant/s production [2]. We are mostly concerned with the safe role of the genus *Lactobacillus* in relation to food and health issues. Among several metabolites, the food industries have extensively exploited the usage of lactic acid produced by Lactobacilli strains [3]. It is important, however, to emphasize that not all Lactobacilli strains are beneficial and harmless; some may be infective in patients suffering from human immunodeficiency virus [4].

Members of the Lactobacilli spp. have been long known as one of the potential biosurfactant producers although their biosurfactant products have not been completely characterized. A multi component mixture

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with various proportions of protein and polysaccharides-based biosurfactant with exceptional medical applications has been reported [2, 5–7]. In addition to biomedical application considerations, the biosurfactants have been utilized for biodegradation of polluting hydrocarbons. For example, Thavasi et al. [8] reported biosurfactant production by *L. delbrueckii* cultured on peanut oil cake up to 5.35 mg ml^{-1} where the biosurfactant product is used for bioremediation purposes and authors also reported enhanced emulsification with biodegradation potential of hydrocarbon pollutants. Even in the crude form of extracts, the lactic acid bacteria derived biosurfactants certainly find suitability for environmental applications.

Several review papers have discussed Lactobacilli spp. for their potential biomedical applications while few articles converse for lactic acid bacteria originated biosurfactants production with structural details. The work combining Lactobacilli producing biosurfactants with their biomedical potential has not been adequately reported in literature. However, this is the first review article discussing the complete chemical composition wise details on *Lactobacillus*-derived biosurfactants. In this review, we endeavor to highlight possible advantages and benefits of biosurfactant producing Lactobacilli strains in some products and technologies.

Biosurfactant producing Lactobacilli spp.

Lactobacilli are known to produce a variety of metabolic by-products in addition to biosurfactants, some of which have antimicrobial activity including lactic acid, hydrogen peroxide, bacteriocins, and bacteriocin-like substances (Fig. 1) which has imperative medical-related advantages [9]. Biosurfactants for example can play a crucial role in reducing the adherence capacity of several pathogens which is a necessary step for biofilm proliferation and formation [10]. Antimicrobial activities and ability to interfere with pathogens adhesion to the urogenital and intestinal tracts epithelial cells leads to an ability to act as antibiofilm agent. Such biofilms are quite common on surgical wounds, silicone-based devices [11], catheters, intravenous catheters, and cardiac devices and other prostheses [12, 13]. Studies carried out by Gan et al. [14] highlighted the utility of Lactobacilli and its biosurfactant in the prevention of surgical implant infection *in vivo*.

Detailed studies on several mechanisms of interference of pathogen adherence and biosurfactant have been previously reported by several researchers [15–18]. The mechanism of competitive exclusion has been demonstrated by quite a lot of Lactobacilli spp. where their surface protein namely the co-aggregation-promoting factor (Cpf) mediates co-aggregation with the human pathogenic microorganisms. This co-aggregation mechanism inhibits the adherence of pathogens to the epithelial cells of the host tissue effectively creating a barrier that prevents colonization by pathogens [19]. Auto-aggregation of probiotic strains is obligatory for adherence to epithelial cells and surfaces of mucus, which consequently supports their accumulation and growth in those environments. This phenomenon has been well documented by studies mostly carried out in oral cavity and the urogenital tract [20].

"Biosurfactants" are products known to reduce surface tension and interfacial tension and to have activity at interfaces. Numerous classes of biosurfactants have so far been described according to their chemical structure, producing strain and mode of action [21]. At present, we are aware of several potential

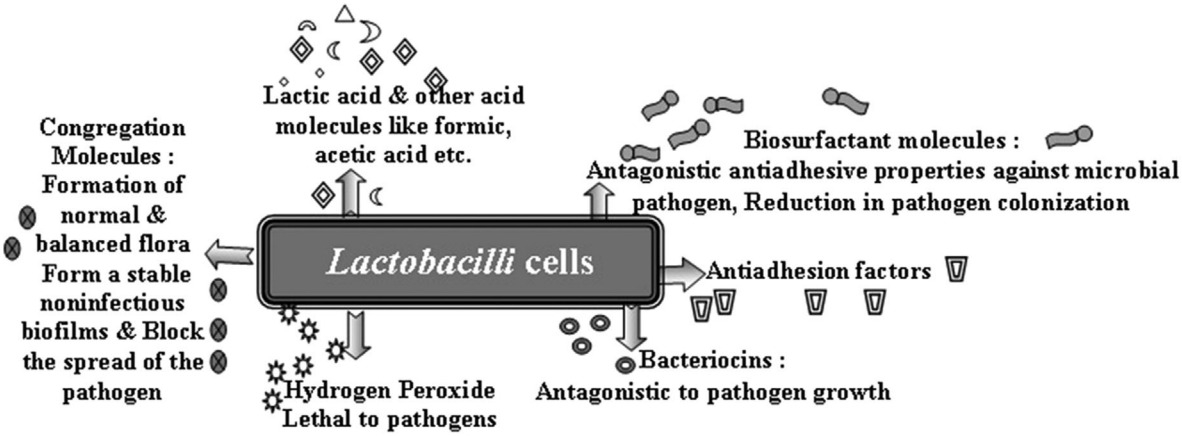

Figure 1. Secretion of various antimicrobial compounds by Lactobacilli cells.

applications of biosurfactants in the surfactant industry [22]. Glycolipids, lipopeptides, protein-like substances, phospholipids, fatty acids, and lipopolysaccharide produced by Lactobacilli spp. have been characterized by several researchers [23–25]. About 46 research outputs reported Lactobacilli spp. biosurfactant production, 6 of which are cell free biosurfactant and 40 cell associated biosurfactant. Based on the preliminary studies, two reports including Ceresa et al. [11] and Fracchia et al. [26] have proposed the presence of cell free biosurfactant as multi-component complex that can show presence of sugar moieties while single report by Thavasi et al. [8] describes a glycolipid-type cell free biosurfactant. The other three research articles available on cell free biosurfactant without revealing much specification on chemical characterization [27–29].

In comparison, among the 40 cell associated biosurfactant reports, about half (50%) do not disclose any structural details most likely due to the complex structures that are difficult to elucidate. It is important to highlight that surlactin/and or proteinaceous cell associated biosurfactant (32.5%) appears to be most frequently produced by Lactobacilli spp. Glycolipid (5%) type of biosurfactants are also produced from probiotic bacterial cultures [24, 30]. Few reports mentioned glycoproteins (7.5%) [23, 31, 32] and glycolipopeptide (5%) production [25, 33].

Most of the focus for cell associated biosurfactant usage appears toward their anti-adhesive and anti-biofilm activities [12, 34]. Few reports do mention their antimicrobial activity of cell associated biosurfactant [15, 16, 35]. Recently, we have discussed the general role of several biosurfactants molecules in biofilms formation and inhibition [10, 36]. Biosurfactants molecules offer many advantages over synthetic surfactants including biodegradability and lower toxicity which makes them supreme candidates for various biomedical applications [21, 37–40]. Their great diversity offers varying properties leading to a number of different applications in various fields [39, 41–46]. Biosurfactant support the microbial entities to grow on hydrophobic or water-immiscible substrates through various mechanisms like lowering the surface tension and interfacial at the phase boundary [43]. Other properties such as wetting, foaming, emulsification affects the adhesion of microbial cells to the organic substrates. A wide variety of Lactobacilli spp. produces varied types of biosurfactants substances as presented in Table 1.

To date a large number of researchers have investigated biosurfactant producing Lactobacilli spp. including *L. casei* sub spp. *rhamnosus* 36 and ATCC 7469, *L. fermentum* B54 and *L. acidophilus* RC14. These strains produce biosurfactants during their mid-exponential (4–5 h) and stationary growth phases (18 h) lowering surface tension [5]. The role of such biosurfactants by Lactobacilli in their natural environment appears to be mainly related to the reduction of adhesion of numerous uropathogens to epithelial cells [9]. Biosurfactants have also been used frequently against microbes and infections in the urinary, vaginal, gastrointestinal tracts, and skin [15]. Often the activity of biosurfactant is mostly related to an inhibition of pathogen adhesion rather than a direct antimicrobial activity or inhibition of the cell growth [48]. Anti-adhesive properties of biosurfactants have a significant role in preventing the adhesion of pathogenic bacteria [34] in addition to the rate of bacterial deposition as well as biofilm development [32, 54]. Biosurfactants also have a great potential in preventing microbial colonization on food contact surfaces [24, 55, 56] and also used in the formulations from food-based industry to reduce pathogens adhesion to human epithelial cell receptors [49].

It should be noted that the composition of protein and polysaccharide fractions of glycoproteins biosurfactant from Lactobacilli are affected by the composition of the medium, time, pH, temperature of incubation, inoculums volume, and the growth phase of bacteria [47]. Yeast extract is essential for bacterial growth, while peptone is crucial for biosurfactant synthesis. Gudiña et al. [17] stated that the use of peptone and meat extract yields a higher production as compared to the standard medium like de Man, Rogosa, and Sharpe medium [57]. The presence of magnesium and manganese were also reported to be essential for bacterial growth and surlactin (protein rich biosurfactant produced by Lactobacilli spp.) production [26]. Other environmental parameters like pH, temperature also determines the activity of biosurfactant [16]. The de Man, Rogosa, and Sharpe medium has been usually used for growth and production of various types of biosurfactants from Lactobacilli spp.

Proteinaceous biosurfactants have high binding affinities to the various materials such as catheters, medical devices [5, 6]. It is also important to note that factors other than biosurfactant do interfere with uropathogenic biofilm formation [44]. *Lactobacillus* spp.-derived biosurfactant can also inhibit biofilm formed by *Candida albicans* [11, 26]. Recent report on the biosurfactant derived from *L. brevis* isolate (CV8LAC) has proved its effectiveness as an anti-adhesive product for several medical devices such as catheters, stents, and prosthesis leading to the reduced colonization and prevention of *C. albicans* infections [11].

Table 1. Brief description on types, production, and characterization of Lactobacilli-derived biosurfactants along with their potential applications.

Lactobacilli spp.	Type of biosurfactant (BS)	Production and characterization	Potential application	Ref.
• *L. acidophilus* RC14 • *L. casei* 70 • *L. casei* subsp, *rhamnosus* GR-1	RC14 and B54 produce BS rich in protein and less content of polysaccharide, phosphate	• Freeze-drying • FTIR • X-ray PS	Protein like BS from B54 and RC14 effectively inhibit the adhesion of *E. faecalis* to glass surface	[5]
• *L. plantarum* RC6 & RC20 (isolated from urogenital tract of healthy woman) • *L. casei* subsp. *rhamnosus* 36 (isolated from a woman with a history of urogenital infections)	36 and B54 produce BS rich in protein with phosphate, polysaccharide, presence of ester carbonyl group			
• *L. acidophilus* T13, *L. fermentum* B54 (poultry isolate) • *L. casei* subsp, *rhamnosus* 81 (dairy isolate)	All 15 *Lactobacillus* isolates produce protein like BS in mid-exponential and stationary growth phase			
• *L. casei* subsp., *rhamnosus* 36 (isolated from a woman with a history of urogenital infections) • ATCC 7469 (American Type Culture Collection) • *L. fermentum* B54 (poultry isolate) • *L. acidophilus* RC14 (isolated from urogenital tract of healthy woman)	Surlactin: the protein rich BS, shows the presence of lipoteichoic acid with molecular weight from 14.4 to >94 kDa	• Freeze-drying • FTIR • X-ray PS • AAA • SDS–PAGE	Protein rich BS that may interfere with uropathogen adhesion	[6]
• *L. acidophilus* RC14	Surlactin: the protein rich BS	• Centrifugation • Filtration • Dialysis	Inhibit initial adhesion of uropathogenic *E. faecalis*. Important for development of anti-adhesive biological coatings for catheter	[2]
• *L. acidophilus* R14	Surlactin: protein, polysaccharides possibly bound to phosphate groups	• Centrifugation • Filtration • Dialysis	Inhibit initial adhesion of few uropathogens	[7]
• *L. fermentum* RC-14	Composition not stated	• Centrifugation • Filtration • Dialysis	Inhibit adhesion of *E. faecalis* and other urophathogens	[42]
• *L. fermentum* B54 • *L. rhamnosus* 36	Produce anti-adhesive, proteinaceous BS Do not release anti-adhesive BS	Not stated Not stated	Inhibit initial adhesion of *E. faecalis*	[43]

(*Continued*)

Table 1. (*Continued*)

Lactobacilli spp.	Type of biosurfactant (BS)	Production and characterization	Potential application	Ref.
• *L. rhamnosus* ATCC 7469^T (American Type Culture Collection)	Do not release anti-adhesive BS	Not stated		
• *L. fermentum* RC-14 • *L. casei* Shirota • *L. rhamnosus* GR-1 • *L. rhamnosus* GR-36	Number of collagen-binding proteins in the crude BS	• Centrifugation • Filtration • Dialysis • Lyophilization • SELDI WCX-1 Protein Chip technology • Collagen cross-linked PS-1 Protein Chip arrays • AAA	Surface-enhanced laser desorption/ ionization (SELDI) – Protein – Rapid characterization of proteins and protein–protein interactions	[44]
• *L. fermentum* RC-14 • *L. rhamnosus* GR 1	Composition not stated	• Filtration	Inhibit implant infections caused by *S. aureus*	[14]
• *L. casei* CECT-5275 • *L. rhamnosus* CECT-288 • *L. pentosus* CECT-4023 • *L. coryniformis* subsp. *Torquens* CECT-25600 (Obtained from the Spanish Collection of Type Cultures, Valencia, Spain)	Crude BS Composition not stated	•Centrifugation •Extraction	*L. pentosus* CECT-4023, strong BS producer, Cheese whey – alternative medium for BS production	[13]
• *L. fermenti* 126 • *L. acidophilus* PG 8/4 • *L. rhamnosus* CCM 1825	Composition not stated	• Centrifugation	Anti-adhesive property	[45]
• *L. acidophilus* H-1 • *L. acidophilus* 336 • *L. acidophilus* Ch-2 Rhodia Food (Biolacta Company, Olsztyn, Poland)	Glycoprotein type: the protein content 8.7 mg (LAA H-1), 4.5 mg (LAA 336), 9.1 mg (LAA Ch-2), per g of dry mass	• Dialysis • Freeze-drying	Inhibitors of *S. aureus*, *S. epidermidis* adhesion and biofilm development	[34]
• *L. casei 8/4* (A culture collection of the Department of Industrial and Food Microbiology, University of Warmia and Mazury in Olsztyn, Poland)	Glycoproteins with additional phosphoric groups	• Centrifugation • Filtration • Dialysis • FTIR • NuPAGE electrophoresis	Exhibit antimicrobial properties may be applied in food stuffs, which is likely to result in a reduction of pathogenic microflora count	[32]
• *L. paracasei* ssp. *paracasei* A20 (Portuguese dairy plant, Portugal)	Crude BS Composition not stated	• Membrane • Filtration • Freeze-drying	Antimicrobial and anti-adhesive	[15]

(*Continued*)

Table 1. (*Continued*)

Lactobacilli spp.	Type of biosurfactant (BS)	Production and characterization	Potential application	Ref.
• *L. paracasei* ssp. *paracasei* A20 (Portuguese dairy plant, Portugal)	Crude BS Composition not stated	• Centrifugation • Membrane filtration • Dialysis • Freeze-drying	Antimicrobial, anti-adhesive activities against several pathogens	[16]
• *L. acidophilus* (Vaginal swabs of healthy women, Kamal Al-Samaraei and Al-Alweia) (Maternity Hospitals in Baghdad)	Surlactin: glycoprotein Molecular weight 60–80 kDa	• Centrifugation • Membrane filtration • Gel filtration • Dialysis • Freeze-drying	Crude surlactin in stationary phase of growth. Highly stable at pH 6 and high temperature conditions	[47]
• *Lactobacillus* spp. CV8LAC (Fresh fruits and vegetables Italy)	Mixture of various components including presence of sugar	• Filtration • Ultrafiltration • TLC	The anti-adhesive properties against *C. albicans* biofilm	[26]
• *L. paracasei* ssp. *paracasei* A20 • *L. plantarum* A14 (Portuguese dairy plant)	Composition not stated	• Centrifugation • Supernatant • Extraction	*L. paracasei* ssp. *paracasei* A20 as a promising BS-producer	[17]
• *L. fermenti* 126 • *L. rhamnosus* CCM 1825 (Culture Collection of the Chair of Industrial and Food Microbiology (CCCIFM)), (University of Warmia and Mazury (UWM)) in Olsztyn, Poland	Protein, polysaccharide and phosphate in different ratio	• FTIR • Capillary gel electrophoresis	Good anti-adhesive properties against *Enterobacteriaceae*	[48]
• *L. delbrueckii* (Marine waters, Tuticorin Port, Tamil Nadu, India)	Glycolipid with carbohydrate and lipid combination of 30%:70% (w/w)	• FTIR • MS	BS alone can promote biodegradation to a large extent without adding fertilizers	[8]
• *L. acidophilus* Leibniz Institute DSMZ-(German Collection of Microorganisms and Cell Cultures)	Protein-like component with presence of polysacchardies and phosphate fractions	• Centrifugation • Filtration • Dialysis • FTIR	Interfere in the adhesion, biofilm formation of the *S. mutans* to glass	[49]
10 Lactobacilli species (traditional Egyptian dairy products collected from the CairoMarkets, Egypt)	Crude BS: composition not stated	• Centrifugation • Acidification • Extraction • Evaporation	Distinct antimicrobial, anti-adhesive activities against pathogens	[35]
• *L. pentosus*	Mostly protein rich fraction	• Not stated	The adsorption properties of BS onto sediments present it as a potential foaming agent in froth flotation	[50]

(*Continued*)

Table 1. (*Continued*)

Lactobacilli spp.	Type of biosurfactant (BS)	Production and characterization	Potential application	Ref.
• *L. pentosus* CECT-4023 T (ATCC-8041) (Spanish Collection of Type Cultures Valencia, Spain)	Glycoproteins, or glycolipopeptide	• Centrifugation • Filtration	Bioremediation of hydrocarbon-contaminated soil	[25]
• *L. plantarum* CFR 2194 (Isolated from kanjika, rice-based ayurvedic fermented product)	Glycoprotein – protein, polysaccharide fractions	• Centrifugation • Filtration • Dialysis • Freeze-drying	Anti-adhesive property against food-borne pathogens	[23]
• Lactobacilli strain	Glycolipid (lipid and sugar fractions) closely similar to xylolipids	• Centrifugation • Filtration • Gel filtration • Dialysis • Freeze-drying • FTIR, NMR • TGA	Proposed application of BS for oral consumption and biomedical applications	[24]
• *L. casei* MRTL3 (raw milk)	Glycolipids – mixture of lipid and sugar	• Centrifugation • TLC, FTIR • NMR	Antibacterial against 8 cultures	[30]
• *L .jensenii* • *L. rhamnosus*	Composition not stated	• TEM	Antimicrobial, anti-adhesive, anti-biofilm activities against *A. baumannii*, *E. coli*, *S. aureus*	[51]
• *L. rhamnosus* (isolated from vagina of Iraqi healthy women was studied)	Crude biosurfactant	• Centrifugation • Acidification • Extraction • Evaporation	Inhibitory effect on adherence and biofilm formation of *E. coli*, *S. aureus*, *K. pneumoniae*, *B. cepacia*	[29]
• *L. reuteri* DSM20016 (Probiotic source)	Cell associated BS Composition not stated	• Centrifugation • Cell collection • Resuspension • Supernatant • Filtration • Dialysis • Freeze-drying	Inhibitor of the *glucosyltransferases* and *fructosyltransferase* strain of *S. mutans* (ATCC35668) affect initial adhesion to the tooth surface	[52]
• *L. pentosus*	Cell associated glycolipopeptide type composed of C:18 and C:16 fatty acids	• Centrifugation • Extraction	Higher emulsion volumes and stable emulsions than polysorbate 20	[33]
• *L. brevis* CV8LAC (fresh cabbage obtained from a producer of biological fruit, vegetables in a rural area of Piedmont, Italy)	BS with mixture of components including sugar as one of the fractions	• Centrifugation • Acidification • Extraction • Evaporation	Inhibition of adhesion, biofilm formation of *C. albicans* on medical-grade silicone elastomeric disk (SEDs)	[11]

(*Continued*)

Table 1. (*Continued*)

Lactobacilli spp.	Type of biosurfactant (BS)	Production and characterization	Potential application	Ref.
• *L. agilis* CCUG31450	Glycoprotein	• Centrifugation • Filtration • Gel filtration • Dialysis • Freeze-drying • FTIR	Anti-adhesive activity against *S. aureus*, and antimicrobial activity against *S. aureus*, *S. agalactiae*, *P. aeruginosa*	[31]
• *L. acidophilus* ATCC 4356	Proteinaceous BS with presence of polysaccharides and phosphate fractions	• Centrifugation • Filtration • Gel filtration • Dialysis • Freeze-drying • FTIR	The inhibitory effect on biofilm forming *S. marcescens*	[46]

BS, biosurfactant; FTIR, Fourier transform infrared spectroscopy; HPLC, high performance liquid chromatography; AAA, amino acid analysis; MS, mass spectroscopy; NMR, nuclear magnetic resonance; TGA, thermal gravimetric analysis; TLC, thin layer chromatography; ASP, ammonium sulphate precipitation; X-ray PS, X-ray photoelectron spectroscopy; TEM, transmission electron microscopy.
Note: Information available on the chemical structure of biosurfactants produced by genus Lactobacilli is not adequate. Researchers, namely, Velraeds et al. [2, 5–7, 43], Howard et al. [44] demonstrated biosurfactant production from *Lactobacillus* strains which were isolated previously by Reid et al. [53].

Lactobacilli lipopeptides – composition, chemical structures, and mechanisms of action

It is well known that biosurfactant molecules can be quite complex and often composed of carbohydrates, proteins, and lipids mixtures. To date, glycolipids, glycolipopeptide, glycoprotein with or without additional phosphoric groups have been reported to be produced by Lactobacilli's thorough utilizing the several analytical techniques such as Fourier transform infrared spectroscopy, nuclear magnetic resonance (^{1}H and ^{13}C), gas chromatography mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), high performance liquid chromatography (HPLC) among others. From the literature survey, it appears that protein-based biosurfactants has been reported very frequently from Lactobacilli spp. in comparison with glycolipopeptide/glycolipoprotein. Often the hydrophobic chain of biosurfactant is composed of lipids, whereas the hydrophilic chain is mostly composed of proteins or sugar moieties conferring unique properties. Sometimes the biosurfactant molecules are complex and difficult to determine, see Table 1 for common biosurfactants produced by Lactobacilli strains. Many such complex structures have been reported for either anti-adhesive, antibiofilm, or as antimicrobial agents against several pathogenic strains. Currently, cell free biosurfactant (only 13% reports available) and cell associated biosurfactant (~87% reports) have been obtained from Lactobacilli spp.

The success story of surlactin

Researchers have classified biosurfactants broadly as low molecular weight (glycolipids, short chain containing lipopetides) and high molecular weight (bioemulsifier-based polymeric and lipopeptides). Among which rhamnolipids and surfactin represents the most extensively characterized low molecular weight biosurfactants. Production of rhamnolipid (due to the presence of rhamnose moiety) from *Pseudomonas pyocyanea* (currently known as *P. aeruginosa*) was documented by Bergström et al. [58]. Since 1968 surfactin (term coined due to strong surfactant activity greater than synthetic surfactant namely sodium lauryl sulphate), a crystalline lipopeptide-type biosurfactant which is routinely isolated from cell free supernatant produced by *Bacillus subtilis* [59]. Today, rhamnolipid and surfactin have been widely exploited for various industrial purposes. Initially, all surfactin compounds were considered as antimicrobial agents which later on subsequent studies proved them as surface active agents. Similarly, about in mid-1990s

researchers started hunting for health associated benefits of lactic acid bacteria, probiotics and their metabolites. The biosurfactants produced by Lactobacilli spp. are believed to interfere the pathogenicity confer by microbes through different mechanisms, where it is believed that biosurfactant plays a critical role [12]. The equation like rhamnolipid only from *Pseudomonas* spp., surfactin type only from *Bacillus* spp., and surlactin type only from *Lactobacillus* spp. can be considered authentically.

Velraeds et al. [5] started talking about biosurfactant from various Lactobacilli strains where *L. acidophilus* exhibited production of protein rich cell associated biosurfactant with presence of small fractions of polysaccharides and phosphates. Parallel studies reported by same researchers [6] coined the terminology namely "Surlactin" for the first time. They described the surlactin as a protein rich cell associated biosurfactant, released by specific strains of *Lactobacillus* spp. during stationary phases and enough competent to interfere the adhesion of uropathogens. Further contribution in the subsequent year by same researchers [2] could demonstrate the inhibitory effect of *L. acidophilus*-derived surlactin-type biosurfactant against uropathogenic *E. faecalis* on silicone-based surface rather than glass surface. Subsequently, fourth report appeared from research group of Velraeds et al. [7] confirmed the role of "surlactin" obtained from *L. acidophilus* RC14 for inhibition of initial adhesion of uropathogens in addition to two yeast strains on silicone rubber. Surlactin again proved to have a marked inhibitory effect against tested pathogens including both strains of *Candida*. From the overview of literature, it is imperative to emphasize that greatest contribution about surlactin is put forward by the authors from Velraeds research group.

In the year 2010, Fouad and coworkers [47] described the surlactin as a glycolipoprotein complex produced using *L. acidophilus*. Authors characterized glycolipoprotein with a molecular weight of 60–80 kDa through gel filtration studies and also highlighted that presence of magnesium (0.04%) and manganese sulfate (0.01%) are essential for the growth of the strain as well as for the production of surlactin.

An interesting piece of work is contributed by Munira and coworkers [60] on surlactin derived from *L. acidophilus* using different strains. Authors have commented that different strains of Lactobacilli produce varied type of surlactin and therefore, it is obvious to behave differently to display the mechanism of action. Their efforts were toward investigating the biological applications of surlactin to inhibit the adhesion of biofilm forming pathogenic strain like *P. aeruginosa* on contact lenses. Their results demonstrated the capability of surlactin to inhibit the adhesion of pathogens up to 60% without any antibacterial activity. The surlactin proved to be effective for treating the infection in rabbits' eyes with *P. aeruginosa*. Furthermore, their studies also proved that infection with *P. aeruginosa* (administrated to rabbits' eyes) can be prevented by using surlactin. However, no satisfactory results were found against *Staphylococcus aureus* culture. Recent studies appeared from Vecino et al. [50] proved the chemical composition of surlactin through using analytical techniques like Fourier transform infrared spectroscopy, to indicate presence of protein rich fractions.

Overall cell associated biosurfactant produced by certain Lactobacilli strains having high proteinaceous (could be a complex of glycoprotein or glycolipoprotein) can be claimed as surlactin-type biosurfactant. Based on Velraeds et al. [2] terms and observation, a definition for surlactin is advised by Fouad et al. [47] claiming that surface active agents produced by *Lactobacillus* strains or surface lactin. The fact that the term surlactin is appeared due to the complexity in chemical structure which can be compared with mucoproteins which has abilities to adhere to the surfaces. Few researchers have reported cell associated biosurfactant as a combination of proteins with the presence of sugar or lipid moieties. However, we shed a light suggesting that there are researchers who have not used the terminology "surlactin" for biosurfactant isolated by them instead they described only as proteinaceous-type biosurfactant [46, 48, 49].

Biosurfactant obtained from cell free supernatant

Most of the low molecular weight biosurfactants of microbial origin (rhamnolipid, surfactin, cyclic lipopeptides, iturins, fengycins) have been reported to be released extracellularly, and have occupied global market including, transplantation, devices manufacturing units. Handling of these compounds for commercial purposes seems to be uncomplicated where many coating formulations have been designed to protect implant materials. Closer observation on available literature about genus *Lactobacillus* put forward that secretion of cell free biosurfactant is not reported much frequently. Chemical composition wise, generally cell free biosurfactant from a small number of *Lactobacillus* spp. are produced as mixture of various compounds, however, distinct work contributed by Thavasi et al. [8] suggests that cell free biosurfactant can be of glycolipid type. They reported production of cell free biosurfactant *L. delbrueckii* of having a combination of carbohydrate

(30%) and lipid (70%) evident through Fourier transform infrared spectroscopy analysis suggesting the presence of significant bands at 2962, 2924, and 2854 cm^{-1} (for the CH aliphatic stretching), 1061 cm^{-1} (PII band: polysaccharides), 1793 cm^{-1} (for the C═O ester bond), 3388 and 3696 cm^{-1} (for OAH bonds), and 766, 700 cm^{-1}(for the CH_2 group). The overall analytical observations illustrated the presence of different fractions in biosurfactant which can be claimed as glycolipid type. Mass spectroscopic analysis (m/z = 326.5 and at 663.4 for lipid and glycolipid moieties) also confirm the data obtained by Fourier transform infrared spectroscopy.

Even though, cell free biosurfactant possessing the mixture of various components (including sugars) do have a noteworthy key role in biomedical applications. Studies by Ceresa et al. [11] demonstrated the production of cell free biosurfactant (structure not determined) from *L. brevis* (CV8LAC) which prevents the adhesion of *C. albicans* on medical-grade silicone elastomeric disks. Thus authors have proved cell free biosurfactant as hopeful aspirant for biomedical approaches. At a concentration of 2000 $\mu g\,ml^{-1}$, cell free biosurfactant also reduced biofilm formation by *C. albicans* by 89 and 90% after 24 and 72 h of incubation. Similarly, Fracchia et al. [26] reported on the antibiofilm activity of CV8LAC cell free biosurfactant against *Candida* cultures which has many appealing applications.

Augustin and Hippolyte [27] characterized cell free biosurfactant (without any structure details) from *Lactobacillus* spp. which was isolated from pendidam, a fermented milk product (local brand, Ngaoundere, Cameroon). The strain TM1 showed biosurfactant production potential in drop collapse (7.30 mm of diameter), best emulsification ability (56.80%), and also good reduction in interfacial tension values (45.09 mN/m). The cell free biosurfactant obtained also has broad spectrum of antimicrobial activity against bacterial strains like *Escherichia coli*, *B. cereus*, *Salmonella* spp., and *E. faecalis*. Augustin and Hippolyte [27] proposed that antimicrobial activity of cell free biosurfactant represents as excreted factors and not as cell associated or cell surface components. No loss in the activity of cell free biosurfactant even after treatment at different conditions and therefore such surface active substances can be utilized in food preservation procedures to prevent their spoilage. On similar background, in the succeeding year, Augustin et al. [28] again reported cell free biosurfactant (no composition revealed) production from three strains of *Lactobacillus* spp. and showed good stability over a wide range of pH (6.0–12.0) and salinity (5.0–15.0%). The cell free biosurfactant have huge potential for their antibacterial activities. Salman and Alimer [29] conducted experimental work to compare the cell free biosurfactant in crude and partially purified cell free biosurfactant to check the inhibitory effect against urinary tract infection causing bacteria like *K. pneumonia*, *S. aureus*, and *B. cepacia*. The researchers suggested that surface activity of cell free biosurfactant is good when it is in the crude form to impede the adherence as well as biofilm formed by those pathogenic bacteria.

Cell bound or associated biosurfactant

Proteinaceous composition. Many antibiotics, antimicrobial agents do possess protein as one of the major functioning fractions of the entire molecule. The proteinaceous nature or peptides do contribute toward antimicrobial activity and have tremendous potential for treating and/or preventing the infectious diseases. Risk of microbial resistance can be reduced certainly with the help of such proteinaceous molecules. Protein rich with and without polysaccharide, phosphate fractions in cell bound or cell associated biosurfactant originated from *Lactobacillus* spp. have undoubtedly fulfilled this expectations proving to combat pathogens. Several researchers could successfully document the potentiality of cell associated biosurfactant as antimicrobial, antibiofilm, and anti-adhesive agents.

Velraeds et al. [5, 6] reported on *L. acidophilus* RC14 and *L. fermentum* B54 strains producing proteinaceous rich biosurfactant where smaller fractions of polysaccharide and phosphate were also detected using Fourier transform infrared spectroscopy. Further their work on other strains namely *L. Casei* sub spp. *Rhamnosus* 36 and *L. rhamnosus* ATCC7469 showed production of protein, polysaccharide, and phosphate containing biosurfactant along with additional ester carbonyl group. The bands detected were at 2932 cm^{-1} (CH band:CH2—CH3), 1652 cm^{-1} (AmI band:CAO stretching in proteins), 1537 cm^{-1}(AmII band: NOH bending in proteins). In addition bands at 1234 cm^{-1}(PI band: phosphates) and at 1066 cm^{-1}(PII band: polysaccharides) were also detected. Velraeds et al. [2] also reported surlactin-type protein rich biosurfactant from *L. acidophilus* RC14 which is a proficient ideal candidate for developing anti-adhesive biological coatings for catheter-like medical devices. The same group also reported protein and polysaccharides bound with phosphate fraction biosurfactant from *L. acidophilus* R14 [7].

Recent work by Shokouhfard et al. [46] reports protein rich containing polysaccharide-type biosurfactant from *L. acidophilus* ATCC 4356 which was detected by Fourier transform infrared spectroscopy analysis. They detected 2929 cm^{-1} (CH band: CH2—CH3 stretching), 1655 cm^{-1} (AmI band: CAO stretching) bands which indicates the

presence of proteins fractions. Polysaccharides with phosphate fractions were also confirmed with bands at 1402 cm^{-1} (AmII band: NOH), 1260 cm^{-1} (PI band: phosphates) and 1056 cm^{-1} (PII band: polysaccharides). The authors proposed the predominance of protein in glycopeptides biosurfactant having anti-adhesive against biofilms developed by *S. marcescens* strains.

Brzozowski et al. [48] also reported biosurfactant production by *L. fermenti* 126 and *L. rhamnosus* CCM 1825 having proteinaceous biosurfactant with an existence of polysaccharide and phosphates biosurfactant obtained *L. rhamnosus* CCM 1825 possessed more proteins and phosphates as compared with *L. fermenti* 126. The *L. fermenti* 126 produced biosurfactant had an excitations at the wavelengths of 3285, 1653, and 1549 cm^{-1}, with a typical of stretching bonds >N—H, CO—N (AmI protein band) and N—H (AmII protein band) supporting the occurrence of proteinaceous components. *L. rhamnosus* CCM 1825-derived biosurfactant also shows excitation at similar wavelengths, i.e., 3287, 1656, and1547 cm^{-1}. The signal received for biosurfactants from *L. fermenti* 126 and *L. rhamnosus* CCM 1825 at the band of 2964, 2929, and 1458 cm^{-1}, and 2961, 2936, and 1453 cm^{-1}, respectively, corresponding to C—H bonds of —CH3, —CH2—, and >CH2 groups of aliphatic chains. Brzozowski et al. [48] commented that the occurrence of a polysaccharide fraction in both biosurfactants is evident in the wavelength range from 1200 to 1000 cm^{-1}. While the excitation spectrum at 1078 and 1083 cm^{-1} (PII polysaccharide band) is representative of bond vibrations in the C—O—C group. The absorbance at 1237 and 1240 cm^{-1} (PI phosphate bond) and 935 and 932 cm^{-1} were equivalent to the stretching bonds formed by phosphorus and oxygen atoms (P—O—C) in aromatic and aliphatic molecules. The absorbance coefficients detected for AmI, AmII, PI, and PII bands, counted as the ratio to the C—H band for the biosurfactant resulted from *L. fermenti* 126 which are equal to 2.0, 1.1, 0.9, and 1.7, respectively. In case of *L. rhamnosus* CCM 1825 biosurfactant showed absorbance coefficients for bands AmI/CH, AMII/CH, PI/CH, and PII/CH with higher values of equal to 2.5, 1.4, 1.2, and 2.1, respectively. Researchers [48] also compared absorbance coefficients and concluded that minor differences are present in the chemical structure of both types of biosurfactants.

Fascinating report on biosurfactant production from *L. fermentum* RC-14, *L. rhamnosus* GR-1 and 36, *L. casei* Shirota which contained proteins with a capacity to bind to both collagen types. These studies by Howard et al. [44] were supported through surface-enhanced laser desorption ionization – time of flight mass spectrometry. Among all three types, biosurfactants from RC-14 predominately indicated the presence of higher number collagen-binding proteins. With these techniques, authors tried to report the use of surface-enhanced laser desorption ionization like system for rapid characterization of complex biosurfactants solutions.

In the oral cavity, *S. mutans* is one of the known gram-positive bacterium predominantly responsible for formation of dental biofilm. The polymers namely glucans and fructans, an extra cellular polysaccharides facilitates the adherence of these cocci-shaped bacteria on tooth surface. Due to unusual capacity of *S. mutans* to adhere on tooth and initiate development of biofilms ultimately leading to dental caries. The gene cassette *gtfB*, *gtfC*, and *gtfD* encoding the synthesis of polymer secretions and therefore, is an impending target for defence against oral cavity caries [61]. Single attempt is recorded on this aspect by Tahmourespour et al. [49] showing the production of protein-like cell associated biosurfactant from *L. acidophilus* DSM 20079 which interferes in the adhesion and also *S. mutans* biofilm formation. The cell associated biosurfactant could make streptococcal chains shorter. Authors tried to verify the data through real time reverse transcription polymerase chain reaction (RT-PCR) quantitation and showed the evidence of decrease in expression of *gtfB* and *gtfC* genes in the presence of cell associated biosurfactant. Tahmourespour et al. [49] attempted to analyze that cell associated biosurfactant through Fourier transform infrared spectroscopy technique pointing out the dominance of protein components with major bands at 2933 cm^{-1} (CH band: CH2—CH3 stretching), 1653 cm^{-1} (AmI band: CAO stretching in proteins), 1480 cm^{-1} (AmII band: NOH bending in proteins) with 1248 cm^{-1} (PI band: phosphates) and 1099 cm^{-1} (PII band: polysaccharides). As a result authors anticipated the presence of protein as one of the major components in addition to a polysaccharide and phosphate in the biosurfactant produced by *L. acidophilus*. Thus on the basis of their findings, cell associated biosurfactant can effectively hamper with adhesion processes of *S. mutans* on teeth surfaces. These studies are momentous to depict the role of cell associated biosurfactant as antimicrobial, anti-adhesive, and antibiofilm agent and affecting the expression level of extracellular enzymes of glucosyl transferases (GTFs) in *S. mutans* biofilms.

Glycolipid. The production of glycolipid complex containing carbohydrate (mono or oligo saccharide) and lipid moiety with surface active properties is widely accepted in case of *Pseudomonas* spp. Whereas, in case of *Lactobacillus* spp. the majority of the literature appears to be protein-based biosurfactant. There are few exceptions

where glycolipid-type biosurfactant obtained from Lactobacilli spp. Sharma and Singh Saharan [30] used *L. casei* MRTL3 as biosurfactant producing strain and reported glycolipid-type biosurfactant analyzing through thin-layer chromatographic studies. The presence of lipid and sugar moieties in biosurfactant was confirmed using ^{1}H-Nuclear magnetic resonance spectroscopy. The presence of methyl esters glycolipid biosurfactant was correlated to an increased hydrophobicity and, as a result enhancing not only the biosurfactant surface activity but also hemolytic and antifungal activities. Sharma et al. [24] reported again glycolipid-type biosurfactant from Lactobacilli spp. having mixture of sugar and lipid fractions which was claimed to be similar to xylolipid. Fourier transform infrared spectroscopy and nuclear magnetic resonance analysis confirmed the presence of glycolipid with hexadecanoic fatty acid (C16) chain. Fourier transform infrared spectroscopy technique has been proved as one of the most significant technique routinely followed to investigate the functional groups of unknown compound [30].

Glycoprotein. As mentioned previously in the above paragraphs, proteinaceous biosurfactants have been generally reported by Lactobacilli spp. In Fourier transform infrared spectroscopy analysis, glycoprotein-type biosurfactant are observed in the typical absorbance maxima at wavelengths ranges between 3500 and 1500 cm^{-1} with a characteristic of stretching →N—H bonds and CO—N and N═O bonds, confirming the incidence of proteins in the sample under analysis. The absorption peak around 3000 corresponds to the presence of bonds occurring in aliphatic chains (—CH3, —CH2—). The indication of a spectrum over wavelength range of 1200–1000 cm^{-1} signifies the polysaccharide fraction of BS while very strong absorption at wavelength of 1087.2 cm^{-1} indicates C—O—C bonds. Recent contribution by Madhu and Prapulla [23] observed protein and polysaccharide fractions typical of glycoprotein in BS isolated from *L. plantarum* CFR 2194. The Fourier transform infrared spectroscopy revealed a nonhomogeneous structure consisting of protein and polysaccharide fractions. Madhu and Prapulla [23] also suggested that the composition of biosurfactant complex is definitely affected by the various components present in the media used in the fermentation process as well as phase of growth of the biosurfactant synthesizing organism. Glycoprotein-based biosurfactant extracted from *L. agilis* CCUG31450 reduces surface tension of water to 42.5 mN m^{-1}, had a critical micelle concentration (*cmc*) of 7.5 mg ml^{-1} with high emulsifying activity (E_{24} = 60%) [31].

Gołek et al. [32] reported on a *L. casei 8/4* producing glycoprotein type BS rich in proteinaceous nature with polysaccharide as one of the major fractions. A characteristic Fourier transform infrared spectroscopy excitation spectra at wavelengths of (1546 and 1653 cm^{-1}), (1547 and 1653 cm^{-1}), and (1549 and 1655 cm^{-1}) confirms protein fractions and excitation spectra for polysaccharides fraction were detected at wavelengths of 1066, 1068, and 1073 cm^{-1}. The evidence for phosphoric groups was observed by excitations spectra that occurred at wavelengths 1236, 1238, and 1240 cm^{-1}, respectively. Authors put forward the similar opinion in agreement with Madhu and Prapulla [23] explaining the importance of media composition and growth condition with respect to determination of structural characteristics of proteins in glycoproteins. Gołek et al. [32] also reported biosurfactant (without structure elucidation) from *L. fermenti* 126, *L. acidophilius* PG 8/4, *L. casei rhamnosus* CCM 1825 and showed their anti-adhesion activities against *Klebsiella pneumonia* on intestinal epithelial cells (using Caco-2 cell line).

Glycolipopeptide. The cell associated biosurfactant complexes of having chemical composition of glycolipopetide are rarely cited. Since huge structural complexity is associated with glycolipopetide-type biosurfactants, very few species of Lactobacilli are known for their production. Vecino et al. [33] reported the production cell associated from *L. pentosus* with a fatty acid (in hydrophobic chain portion) based composition including linoelaidic acid, oleic, elaidic acid, palmitic acid, and stearic acid. The authors proposed that fatty acid chains in cell associated biosurfactant are very much similar to the fatty acid containing fractions detected in rhamnolipid biosurfactant (originated from *Pseudomonas* strains). Moldes et al. [25] characterized glycoprotein or a glycolipopeptide-type biosurfactant produced from *L. pentosus* using Fourier transform infrared spectroscopy analysis showing occurrence of OH and NH groups, C═O stretching of carbonyl groups and NH bending (peptide linkage) and also CH_2—CH_3 and C—O stretching indicating the presence of lipid fractions. Such characteristic spectra of biosurfactant have been reported from several lactic acid bacteria.

Cell associated biosurfactant with no structure details. Literature survey illustrates that the majority of work on cell associated biosurfactant explored is mainly for their antimicrobial, anti-adhesive, and antibiofilm characteristics. However, huge basin remains untouched without revealing the detailed structural and chemical composition related to cell associated biosurfactant. Basically the structural complexity may the most probable hindrance to reveal the unexposed information. Since 1996 up to

1999, the very limited literature had discussed the production of BS. In the year of 1999, Reid et al. [42] added valuable work in this regards. Authors used *L. fermentum* RC14 strain for biosurfactant production in de Man, Rogosa, and Sharpe medium and showed that those complex molecules can inhibit adhesion of commonly found uropathogens in female urinary tract. Velraeds et al. [43] worked with several strains of Lactobacilli including *L. fermentum* B54, *L. rhamnosus* 36, and *L. rhamnosus* ATCC 7469^T and reported proteinaceous kind biosurfactant without much elucidation of structural components. Rodrigues et al. [13] extensively worked toward media optimization for biosurfactant production from various Lactobacilli spp.

Walencka et al. [34] also reported a biosurfactant produced by *L. acidophilus* (no description of structural characteristics) which inhibits biofilms development in *S. aureus* and *S. epidermidis* affecting initial adhesion, biofilm formation, and cells dispersal. It was suggested that the addition of biosurfactant to preformed mature biofilms leads to rapid dispersion and alters the morphological changes of biofilm structures due to altering cell-surface hydrophobicity of the tested bacteria. This can ultimately hamper the deposition rate as well as development of biofilm.

Sambanthamoorthy et al. [51] investigated BS production by *L. jensenii* and *L. rhamnosus* and carried out *in vitro* studies on antimicrobial, anti-adhesive, and antibiofilm abilities of the cell-bound BS (structure not described) against various pathogens such as multidrug resistant *E. coli*, *S. aureus*, and *Acinetobacter baumannii*. On similar aspects Gomaa [35] reported cell free biosurfactant and cell associated biosurfactant production (without any detailed description) using *L. acidophilus*, *L. brevis*, *L ruteri*. Researchers namely, Gan et al. [14], Gudiña et al. [15–17], Kermanshahi et al. [62], Moldes et al. [54] reported several Lactobacilli spp. for production of cell free biosurfactant and cell associated biosurfactant with antimicrobial and anti-adhesive properties. However, it is important to note that no structural details are available on those biosurfactants.

Very few studies have been contributed toward understanding the role of *Lactobacillus*-derived BS on gene expression conferring the virulence properties to biofilm forming bacteria. For example, Tahmourespour et al. [49] initiated studies (discussed previously) and later demonstrated by Salehi et al. [52] indicating the effects of cell associated biosurfactant purified from *L. reuteri* (DSM20016) on the gene expression profile of essential adhesion genes (gftB/C and *ftf*,) in *S. mutans* (ATCC35668). The cell associated biosurfactant (structure not described) has been proved as potential inhibitor of the *glucosyltransferases* and *fructosyltransferase* in *S. mutans* (ATCC35668). It is very important to note that the inhibition is predominantly advantageous due to its selectivity in action and does not hamper other microbiota inside the mouth other than *S. mutans*. *L. rhamnosus*-derived crude cell associated biosurfactant inhibit biofilms produced by potential pathogenic bacteria viz., *S. aureus* and *P. aeruginosa*. Cell associated biosurfactant work effectively to affect the growth and antibacterial and anti-adhesive in association with polyvinyl alcohol–biosurfactant mixture in glass and plastic plates [52].

Biomedical-related role of Lactobacilli-derived biosurfactant

One of the main physiological roles of the Lactobacilli spp. in the gastrointestinal tract is the prevention of the proliferation of harmful pathogenic bacteria. However, this may not be true when they are associated with dental caries [63]. Several *Lactobacillus* spp. are part of the human and animal commensal intestinal flora. They are considered to be protective organisms which prevent the growth of pathogenic organisms through the production of lactic acid and other metabolites creating an acidic environment which inhibits the growth of some harmful bacteria. Various anti-infective properties of Lactobacilli spp. are illustrated in Fig. 2.

Isolating members of Lactobacilli can be difficult. Regular media namely nutrient broth or Luria broths are commonly used for the growth and maintenance of bacterial cultures may not usually support the growth of this genus. Growing Lactobacilli need special nutrient provision (like de Man, Rogosa, and Sharpe medium) and sometimes extended incubation periods and members are likely to get wrongly identified due to their morphological resemblance to other bacterial genera such as *Corynebacterium*, *Streptococcus*, and *Clostridium* [64]. Even though, species of Lactobacilli is generally considered to be protective organism in healthy humans, some critical issues regarding potential pathogenicity have been explained in the immune-compromised patients [4]. Some researchers have suggested that possible infections can be caused by *Lactobacillus* spp. in the acquired immune deficiency syndrome patients having neutropenia or following organ transplantation [65]. Endocarditis, bacteremia, neonatal meningitis, dental caries, abscesses, and chorioamnionitis are all types of clinical infections reported so far by Lactobacilli [63]. Post-adhesion competitions against uropathogens are the main significant features that are contributed by Lactobacilli. Major inhibitory effects on

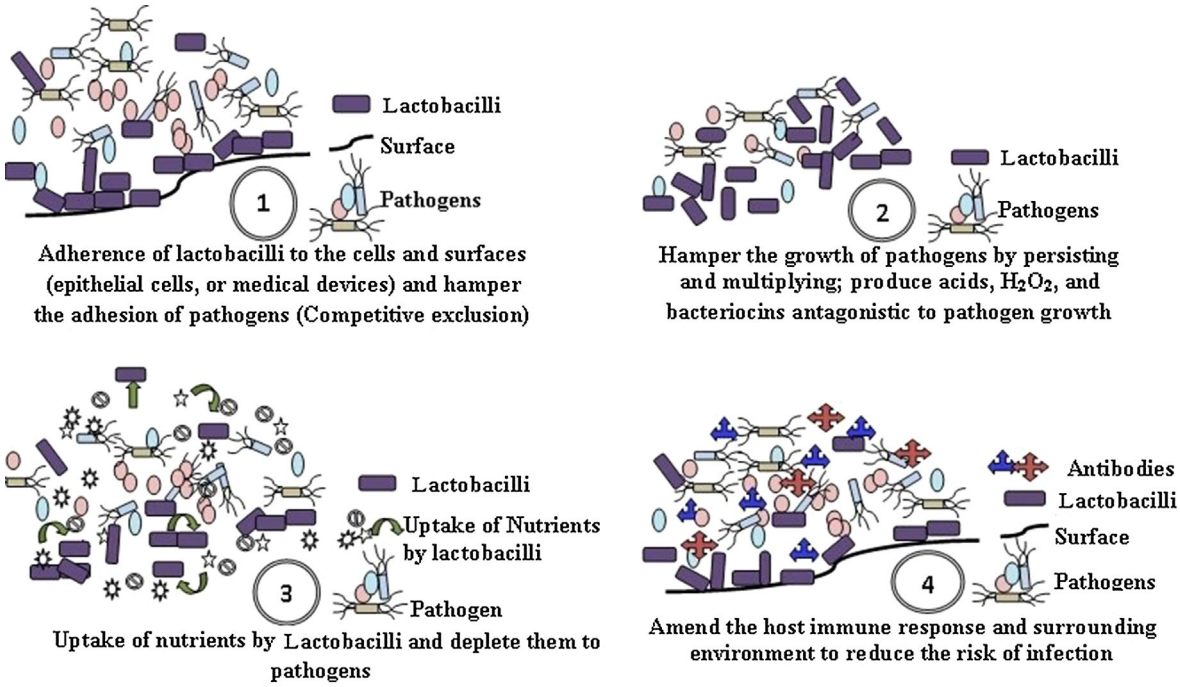

Figure 2. Illustration of four different ways for anti-infective properties of Lactobacilli spp.

the initial deposition and adhesion of some uropathogens have been demonstrated by Velraeds et al. [2, 5, 6]. However, some Lactobacilli spp. may not show initial inhibition of uropathogenic *E. faecalis* [65].

Cytotoxicity ultimately leads to loss of cell membrane integrity resulting in cell lysis or necrosis. Most biologically produced secondary metabolites such as antibiotics, polypeptides, proteins, etc. may have cytotoxic effects to some extent and have to be tested to establish suitability for health-related applications. Lack of cytotoxic effects is therefore important for consideration of application related to human health, from a safety point of view [24]. Literature suggests that in addition to antimicrobial, anti-adhesive, and antibiofilm properties, biosurfactant obtained from *Lactobacillus* spp. do possess cytotoxic effect. This is supported by Sharma et al. [24] who investigated the cytotoxic effect of *L. helveticus*-derived biosurfactant using a mouse fibroblast (ATCC L929) cell line. They reported that as the concentration of biosurfactant increased (6.25–25 mg ml^{-1}) there was a gradual decreased in cell viability. Cell viability of 30.9% was determined at the concentration of 25 mg ml^{-1} and was similar to commonly used rhamnolipid (as positive control) which showed 32.87% of cell viability at similar concentration.

Sambathammorthy et al. [51] also determined cytotoxicity of biosurfactant derived from two strains *L. jensenii* and *L. rhamnosus* using human lung epithelial cell line (A549). The cytotoxicity of the crude biosurfactant was evaluated in two ways, firstly by the release of lactate dehydrogenase and secondly by total cell number assay. No toxicity was observed at the concentrations of 25–100 mg ml^{-1} and low toxicity levels were observed at 200 mg ml^{-1} by both biosurfactant extracts. In conclusion, the limited literature available to this subject appears to confirm that biosurfactants originated from *Lactobacillus* spp. has low cytotoxic effect comparable to commercially available rhamnolipids which are generally considered nontoxic products. This makes them potential safe candidates for use in biomedical application particularly as topical delivery products.

Role of low-cost fermentative media in biosurfactant production

The use of various cheaper renewable substrates such as distillery wastes, animal fat, molasses, plant oils, oil wastes, and starchy substances, lactose containing whey and oil industries are common in the fermentation industries. Three major aspects are needed to be considered to increase the biosurfactant production at commercial scale. The first is improvement in the fermentation technology followed by use of cheaper, renewable substrates and their continued reliable supply. Table 2 presents a brief listing of the variety

Table 2. Summary for various raw substrates used for production of different types of biosurfactant from Lactobacilli spp.

Name of the organisms	Cheaper substrates used in the production process	Type/composition of biosurfactant (BS)	Yield of biosurfactant	References
L. pentosus CECT-4023	Cheese whey and molasses	Crude BS	$P\max = 1.4\,g\,L^{-1}$ and $rp/X = 0.093\,g\,L^{-1}$ per h	[13]
L. agilis CCUG31450	Cheese whey	Glycoprotein	$960\,mg\,L^{-1}$	[31]
L. pentosus	Grape marc after supplementation with corn steep liquor ($10\,g\,L^{-1}$) and yeast extract ($10\,g\,L^{-1}$)	Intracellular BS	$4.8\,mg\,L^{-1}$	[66]
L. pentosus	Grape marc	BS Composition not mentioned	$5.9\,g\,L^{-1}$	[67]
L. pentosus	• Hemicellulosic sugar hydrolyzates obtained from trimming vine shoots • Barley bran husk hydrolyzates	BS Composition not mentioned	• 0.71 g of BS per g of biomass • 0.28 g of BS per g of biomass	[54]
L. pentosus	• Sugars from agricultural distilled grape marc hydrolyzates • Low-cost feedstock agricultural residues as substrates: hazelnut shells, distilled grape marc, walnut shells	BS Protein fractions probably associated with bound phosphate	Not mentioned	[68]
L. delbrueckii	Peanut oil cake	Glycolipid with carbohydrate and lipid combination of 30%:70% (w/w)	$5.35\,mg\,ml^{-1}$	[8]
L. pentosus	Vineyard pruning waste	Glycolipopeptide	Not mentioned	[25]
L. pentosus	Vineyard pruning waste	BS rich in protein content	Ranging between 0.29 and $1.35\,mg\,L^{-1}$	[50]

Note: *P*max, maximum concentration of biosurfactant ($g\,L^{-1}$); *r*p, initial volumetric rate of biosurfactant formation($g\,L^{-1}\,h^{-1}$).

of suitable cheap raw substrates used in biosurfactants production. Low yield of biosurfactant at industrial level is the main problem faced by industries in addition to the high cost inputs essential to run large scale fermentations. Usage of renewable substrates could provide an alternative solution toward advancement of the process. A variety of renewable substrates that can be used for large scale production including agro-industrial waste, animal fat waste, coffee processing residues, dairy industry, distillery industry industrial effluents, food processing industry, fruit processing industry, oil processing mills were all reported [69]. Cheese whey has been exploited as an alternative medium at commercial level [70] where *L. pentosus* CECT-4023 reported to be a very strong biosurfactant producing strain [13]. Enhanced yield of glycoprotein (from 84 up to 960 $mg\,L^{-1}$) has been achieved from Lactobacilli spp. [31].

Agricultural residues are one of the abundant and easily accessible carbon sources for biosurfactant production, most, however, need some pre-treatments. A lignocellulosic material often needs acid hydrolysis and thermal treatment followed by a clarifying step. Portilla-Rivera et al. [66–68] and Paradelo et al. [71] used such media for BS production from *L. pentosus* and obtained products comparable to surfactin produced by *Bacillus subtilis* in terms of hydrocarbon emulsification abilities and potential uses in bioremediation. Paradelo et al. [71] concluded that *L. pentosus* grown on grape marc hydrolysates for BS production, it can reduce the water repellence of hydrophobic material, which is very much better in comparison to chemical-based surfactants. Large-scale production of biosurfactants has become possible by the usage of hemicellulosic sugars from vineyard pruning waste.

Other examples, where hemicellulosic sugars from vineyard pruning waste were utilized for biosurfactant production using *Lactobacillus* have been reported as steps toward reducing environmental impact of waste disposal [25]. Comparative studies on the kinetics of sediment sorption on biosurfactant obtained from *L. pentosus* and two chemical surfactants viz., Tween 20 and sodium dodecyl sulfate have been carried out. Their studies showed that no sodium dodecyl sulfate is

adsorbed onto the sediments, whereas Tween 20 and biosurfactants from *L. pentosus* are absorbed after a few minutes. In addition to agricultural waste substrates, diary-based products have a crucial role in biosurfactant industries. Since, most of the *Lactobacillus* strains used for biosurfactant production are either isolated from dairy based products or of human origin. Various oils have been proved as best substrates for biosurfactants production from genus *Lactobacillus*. Thavasi et al. [8] tried to grow *L. delbrueckii* in peanut oil cake supplemented media and yielded 5.35 mg ml^{-1} of biosurfactant. Their studies have a great impact on demonstrating that biosurfactants alone have the capacity to enhance the biodegradation of crude oil up to greater extent in absence of fertilizers. Even though biosurfactant production from Lactobacilli spp. work has been documented in the literature since long time, inadequate scientific reports are available on biosurfactant production from Lactobacilli spp. using cheap and renewable substrates. It is important to highlight that few reports do discuss on biosurfactant production using *Lactobacillus* spp. by using agriculture residues [71]. In spite of the availability of huge number of renewable substrates, hardly any work has been investigated on this aspect. Biosurfactant derived from lactic acid bacteria has tremendous scope in industrial sectors and therefore, need to explore on broader scale [72].

Future prospects

Lactobacilli produced biosurfactants appear to have great biomedical potential applications. In the field of biosurfactants and related production technology, we are actively in search of novel strains capable of utilizing cheaper, renewable substrates, greater yields, and novel applications. Several numbers of industries are vigorously seeking suitable surface active molecules with advanced applications. Huge possible opportunities are available today in this field for designing new biosurfactant-based formulations which may have high market demand. The composition of most of the biosurfactants has not been fully elucidated. The diverse competence of biosurfactants produced by probiotic bacteria toward therapeutic approaches can be highly significant. It has been suggested that utilizing these surface active molecules in preventing and/or dealing with hospital-acquired infections may be an important undertaking. Other promising applications include inhibiting microbial biofilm formation and the prevention of urogenital infection in mammals in addition to use as an adjuvants to conventional antibiotics in the treatment of hospital-acquired diseases or infections. This needs much more attention so that those surface active molecules can be utilized for several applications in diverse fields.

Acknowledgments

Dr. Surekha K. Satpute is highly grateful to Department of Science and Technology (DST), Government of India, Ministry of Science and Technology, New Delhi, India for financial support (SR/WOS-A/LS-1076/2014(G)). Without DST's financial support, it would not have been possible to conduct this work. Constant support from the Director, School of Basic medical Sciences, Department of Physics, Savitribai Phule Pune University is also acknowledged.

Conflict of interest

The authors report no conflicts of interest.

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