Chapter 2
Biosurfactants of Probiotic Lactic Acid Bacteria

Abstract According to the definition of Food and Agriculture Organization of the Unites Nations and World Health Organization (FAO/WHO) "Probiotics are the live microbial preparation, when consumed, confer the health benefits to the consumer." Lactic acid bacteria are recognized to produce various antimicrobial compounds such as bacteriocin, biosurfactants organic acids, carbon peroxide, diacetyl, low molecular weight antimicrobial substances, and hydrogen peroxide, which prevent the growth of potential pathogens. The use and possible application of biosurfactants in the biomedicals had increased in past decade. Their antimicrobial properties make them appropriate molecules for combating many pathogens and as therapeutic agents. Furthermore, their application as antibiofilm agents against commonly known pathogens specifies their effectiveness as appropriate anti-adhesive coating agents for biomedical insertional equipment. The present chapter covers all the biomedical aspects of biosurfactants of lactic acid bacteria in medical and therapeutic perceptions.

Keywords Biosurfactant · Antibiofilm · Antimicrobial · Probiotics and biomedical surfaces

Introduction

Lactic acid bacteria (LAB) include widespread genera comprising an extensive number of species involved in fermentation of dairy products. Along with the potential in dairy fermentation, LAB are extensively found as the part of animal and human intestinal commensal flora (Vaughan et al. 2005). The roles of LAB have been recognized in maintaining homeostasis within active ecosystems such as the urinary tract and gastrointestinal tract to control colonization by various pathogens (Boris and Barbes 2000). The research on microorganism of probiotics origin has gained attention in current biomedical applications worldwide. Research observations advises that probiotic microorganisms may have a vital role in declining the occurrence of antibiotic-related diarrhoea, prevention of vaginal candidiasis, improved

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immunological defense responses, and urinary tract infections (Falagas et al. 2006; Falagas and Makris 2009). Mainly biosurfactant-producing microorganisms are pathogenic in nature and difficult to handle in industrial formulations (Sharma et al. 2015; Toribio et al. 2010; Saharan and Nehra 2011). The application of biosurfactants derived from pathogenic microorganism in industrial formulations is predominantly objectionable precisely in the food processing, cosmetics formulations, and pharmaceuticals (Saharan et al. 2011; Banat and Desai 1997).

Probiotics are: "Preparation of Live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO 2002). Probiotics have been reported to have encouraging effects on the maintenance of human and animal health (Gupta and Garg 2009). Awareness in probiotics research and formulations have extended pronounced significance due to the rise in antimicrobial resistance worldwide. Probiotics microorganisms are recognized to produce various antimicrobial compounds such as bacteriocin, biosurfactants organic acids, carbon peroxide, diacetyl, low molecular weight antimicrobial substances, and hydrogen peroxide, which prevent the growth of potential pathogens (Pascual et al. 2008; Merk et al. 2005; Ceresa et al. 2015) (Fig. 2.1). Various studies reported the prospective of LABS as biosurfactant producers and their potential role in public health and food processing (Velraeds et al. 1996; Heinemann et al. 2000; Rodrigues et al. 2004; Servin 2004; Rodrigues et al. 2006, Falagas and Makris 2009; Thavasi et al. 2011; Gudiña et al. 2011; Rodríguez-Pazo et al. 2013; Moldes et al. 2013; Sharma et al. 2014, 2015) (Table 2.1).

Furthermore, probiotics have long been acknowledged also for the potential to restrict the adhesion and development of biofilms of pathogens to the various biological (epithelial cells of urogenital and intestinal tracts) and inanimate (food

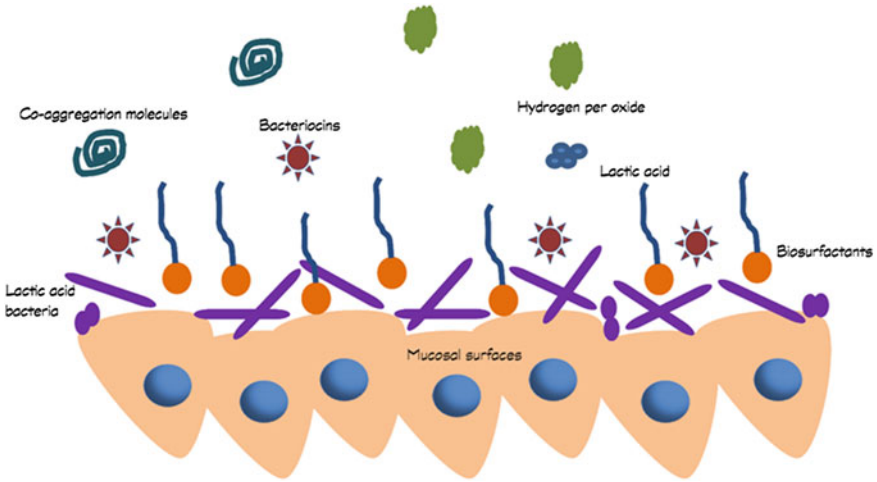

Fig. 2.1 Production of various compounds by LAB on mucosal membrane

Table 2.1 Strains of LAB reported for biosurfactant production and their application

S. No.	LAB Strain	Source of strain	Application	Study
1.	*Lactobacillus* spp.	Urogenital tract of healthy women	Antiadhesive against the uropathogenic *Enterococcus faecalis*	Velraeds et al. (1996)
2.	*Streptococci thermophilus*	Heat exchanger plate of pasteurizer	Antiadhesive activity against *Candida* sp.	Busscher and Van der Mei (1997)
3.	*Streptococci mitis*	Human oral cavity	Inhibition of *Streptococci mutans*	Van Hoogmoed et al. (2006)
4.	*Lactobacillus fermentum* RC-14	Urogenital isolate of a healthy woman	Inhibits adhesion of *Enterococcus faecalis* 1131	Heinemann et al. (2000)
5.	*Lactococcus lactis* 53	–	Antibiofilm activity	Rodrigues et al. (2004)
6.	*Lactobacillus casei* CECT 525, *Lactobacillus rhamnosus* CECT 288, *Lactobacillus pentosus* CECT 4023 *and Lactobacillus coryniformis* subsp. *torquens* CECT 25600	Spanish culture collection center	Kinetics of biosurfactants production	Rodrigues et al. (2006)
7.	*Streptococcus thermophiles* A	NIZO	Antimicrobial and antiadhesive properties	Rodrigues et al. (2006)
8.	*Lactobacillus pentosus*	–	Biosurfactant production	Rivera et al. (2007)
9.	*Lactobacillus pentosus*	CECT 4023T	Biosurfactant production	Moldes et al. (2013)
10.	*Lactobacillus acidophillus*	–	Antiadhesive activity against *Staphylococcus aureus*	Walencka et al. (2008)
11.	*Lactobacillus acidophillus*	CECT 419	Biosurfactant production	Portilla et al. (2008)
12.	*Lactococcus lactis*	Curd sample	Antibacterial activity against the multidrug resistant pathogens	Sarvanakumari and Mani (2010)
13.	*Lactobacillus paracasei*	Portuguese dairy industry	Antimicrobial and antiadhesive properties	Gudiña et al. (2010)
14.	*Lactococcus lactis*	CECT 4434	Simultaneous extraction of biosurfactant and bacteriocin	Rodríguez et al. (2010)

(continued)

Table 2.1 (continued)

S. No.	LAB Strain	Source of strain	Application	Study
15.	*Lactococcus paracasei* subsp. *Paracasei* A20	Portuguese dairy industry	Antimicrobial and Antiadhesive properties	Gudiña et al. (2010)
16.	*Lactobacillus delbrueckii* sbusp. *delbruckii*	DSMZ	Biosurfactant inhibition against *Candida albicans*	Fracchia et al. (2010)
17.	*Lactobacillus delbrueckii*	–	Biosurfactant production and structural characterization	Thavasi et al. (2011)
18.	*Lactobacillus acidophilus*	DSM	Effect on GTFB and GTFC expression level	Tahmourespour et al. (2011)
19.	*Lactobacillus fermentii* and *Lactobacillus rhamnosus*	CCCIFM, Polnad	Antiadhesive properties against the *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *E. coli*	Brzozowski et al. (2011)
20.	*Lactobacillus* spp.	Yogurt, Cheese and Silage	Antagonistic activity	Kermanshahi et al. (2012)
21.	*Lactobacillus plantarum*	–	Structural characterization	Sauvageau et al. (2012)
22.	*Lactobacillus reutri*	DSM	Effect of biosurfactant on gene expression of essential adhesion genes (gtfB, gtfC and ftf) of *Streptococcus mutans*	Salehi et al. (2014)
23.	*Lactobacillus* spp.	Pendidam	Antibacterial activity	Augustin et al. (2014)
24.	*Lactobacillus pentosus* and *Lactobacillus plantarum* co-culture	CECT 4023 CECT 221	Production and antimicrobial activity	Rodriguez-Pazo et al. (2013)
25.	*Lactobacillus* spp.	Egyptian dairy product	Antimicrobial activity	Gomaa (2013)
26.	*Lactobacillus pentosus*	–	Foaming agents	Vecino et al. (2013)
27.	*Lactobacillus plantarum* CFR2194	Kanjika (rice based ayurvedic fermented product)	Antimicrobial and Antiadhesive activity	Madhu and Prapulla (2014)
28.	*Lactobacillus pentosus*	–	Fatty acid characterization	Vecino et al. (2014)
29.	*Lactobacillus pentosus*	–	Structural properties	Vecino et al. (2014)

(continued)

Table 2.1 (continued)

S. No.	LAB Strain	Source of strain	Application	Study
30.	*Lactobacillus brevis*	Fresh cabbage	Antifungal activity	Ceresa et al. (2015)
31.	*Lactobacillus jensenii* and *Lactobacillus rhamnosus*	American type culture collection (ATCC)	Biofilm dispersal and antimicrobial	Sambanthamoorthy et al. (2014)

processing area, biomedical surfaces, and food equipment) (Reid et al. 2001; Saharan et al. 2011; Sharma et al. 2015). LAB interfere with pathogen colonization by different mechanisms. Biosurfactant production is one of their mechanisms to prevent the colonization (Santos et al. 2013). Probiotic organisms probably interfere the adhesion by the release of biosurfactant molecules (Sharma et al. 2015; Gudiña et al. 2010; Rodrigues et al. 2006). Production of lipopeptides by *Bacillus* probiotics prevent the growth of pathogens existing in the gastrointestinal tract (Hong et al. 2005). Likewise, antagonism with other pathogens for adherence to the epithelial cells along with biosurfactants secretion is a well recognized mechanisms used by lactic acid bacteria to obstruct vaginal pathogens (Cribby et al. 2009; Falagas et al. 2007).

LAB-Derived Biosurfactants in Biomedical

Biofilm in microbial system is a community attached to either biotic or abiotic planes rooted by an extra-polymeric matrix for endurance under unfavorable conditions (Donlan and Costerton 2002). Microbial biofilms were first described in 1943 (Zobell 1943), but the problem is still persisting in an extensive kind of extents, particularly in the food (Veran et al., 2010) and biomedical sector (Sihorkar and Vyas 2001). Often frequently, microbial biofilm structures are a regular challenge confronted by the food processing sector. The predominance of biofilms is a substantial problem in food formulation and food processing (Murphy et al. 2006; Gandhi and Chikindas 2007). In food industries, a range of microorganisms colonize the food contact surfaces and form biofilm microbial communities. Once established, microbial biofilms are a substantial source of contamination of food products.

Biofilms sheltering multi-antibiotic-resistant microorganisms are predominantly established at astonishing levels on hospital surfaces and leads to the risk of nosocomial infection transmission. Biofilms in hospital environments are frequently associated with plastic medical tubing of various equipment. The incidence of multidrug resistant pathogens being sheltered inside these biofilms are problematic in biomedical surfaces (Vandecandelaere and Coenye 2015).

Numerous reports have pointed out that probiotic microorganism's derived biosurfactants may combat the growth of hospital acquired pathogens on inanimate surfaces and biomedical surfaces (Sharma et al. 2015; Rodrigues et al. 2004, 2006; Walencka et al. 2008). Biosurfactants derived from the LAB has a valuable application as antiadhesive agents to combat the colonization of pathogenic microorganisms. Application of biosurfactant to any surface modifies its hydrophobicity, interfering in microbial adhesion. Contribution of biosurfactants in microbial adhesion has been extensively defined, and establishes a potential approach to reduce microbial adhesion by pathogens, not merely in the biomedical applications, but also in other capacities, such as the food processing industry (Falagas and Makris 2009). Various observations have been made regarding the antiadhesive nature of biosurfactants derived from the LAB. Reduction of pathogen colonization has been reported in glass (Velraeds et al. 1996), silicon, rubber prostheses (Busscher and Van der Mei 1997; Velraeds et al. 1998; Van Hoogmoed et al. 2006; Rodrigues et al. 2004, 2006) metal (Meylheuc et al. 2006), and other inanimate surfaces (Heinemann et al. 2000; Gudiña et al. 2010; Fracchia et al. 2010).

Falagas and Makris (2009) studied in vitro tests on the significant role of LAB-derived biosurfactants in the inhibition of fungal and bacterial colonization on various surfaces such as urinary catheters and other voice prostheses or surgical implants made of silicone rubber (Rodrigues et al. 2004, 2006). The application of biosurfactant mainly falls in two categories; majorly pre-coating of the surfaces with microbial surfactants, or directly added to biosurfactant-producing LAB strains to inspect biofilm development. Additional exciting application region that is gaining increased attention relates to probiotics use in avoiding oral infections (Meurman 2005; Meurman and Stamatova 2007; Vujic et al. 2013; Toiviainen 2015). Biosurfactant derived from *Streptococcus mitis* inhibited the adhesion of tooth decaying bacteria *Streptococcus sobrinus* and *Streptococcus mutans* to enamel, whereas *S. mitis* biosurfactant was capable to constrain the adhesion of *S. sobrinus* to salivary pellicles (Van Hoogmoed et al. 2004). Novel biosurfactant molecules derived from LAB are reported from different dairy products for anti-biofilm properties (Walencka et al. 2008; Sharma and Saharan 2014; Sharma et al. 2014, 2015). Furthermore, biosurfactant accumulation to redeveloped, settled biofilms enhanced their dispersal and changed the biofilm morphology.

Biosurfactant produced by *Lactococcus lactis* (Xylolipid), isolated from a fermented dairy preparation exhibited significant antibacterial activity for clinical pathogens (Saravanakumari and Mani 2010). Considering their significance for human and animal health with acknowledged safety, LAB symbolizes a nontoxic and effective interference for pathogen control. Biosurfactant isolated from probiotics could be applied to biomedical equipment, such as silicone tubes and catheters, to combat microbial colonization of these surfaces by hospital-acquired pathogens (Falagas and Makris 2009).

It was usually reported that probiotic LAB mainly, *Streptococcus thermophilus* and *Lactobacillus* spp. strains derived surfactants antagonize the growth of certain

pathogens like *Staphylococcus aureus*, *Streptococcus* spp., *Enterococcus faecalis*, *Candida albicans* (Van Hoogmoed et al. 2004). Rodrigues et al. (2004) confirmed that the biosurfactant derived from the *Lactococcus lactis* 53 was competent to constrain the adhesion of various pathogens to silicone tubing. Biosurfactant from probiotic strains significantly reduced the pathogenic microorganism's populations on voice prostheses (Rodrigues et al. 2004). Velraeds et al. (1996) also reported the control of biofilm developed by enteric pathogen with biosurfactant derived from *Lactobacillus* strain and later demonstrated that the biosurfactant triggered significant dose-related control of the preliminary deposition rate of *E. coli* adherent on hydrophobic and hydrophilic layers.

LAB-Derived Biosurfactants as Antimicrobials

The use and effective application of microbial surfactants in the biomedical sector has increased during the past couple of years. Biosurfactant derived from various microorganisms have been reported for antimicrobial properties (Sharma et al. 2015; Díaz De Rienzo et al. 2015; Cameotra and Makkar 2004). Recently, biosurfactants derived from the LAB have been specified to display antimicrobial properties. Various compounds produced by LAB have application in the production of newer generation antimicrobials (Reid et al. 2001; Rodrigues et al. 2004). Gram-positive bacteria are more profound against the biosurfactants than gram-negative bacteria, which were moderately inhibited. The biological properties of biosurfactant depend on the molecular structure of cell. In broad-spectrum, they influence the permeability of cellular plasma membranes.

The mechanism of biosurfactant antimicrobial activity still remains unclear, but certain hypotheses are proposed which are sustained by specific evidence displaying the loss of membrane integrity.

- Antimicrobial activity of the microbial surfactants is an outcome of the adhesion property of these surface active agents to the cell surfaces instigating decline of cell membrane integrity leads to subsequent collapse of the nutrition cycle (Inès and Dhouha 2015).
- The fatty acid moieties of biosurfactants inserting into the cell membrane instigating a proliferation of membrane size and ultra-structural changes (Gomaa 2013).
- Biosurfactants are also able to form pores and disrupt the plasma membrane (Inès and Dhouha 2015) (Fig. 2.2).
- Addition of the smaller acyl tails of the biosurfactant into the plasma membrane triggering disruptions of the plasma membrane, countenancing the plasma membrane to lift away from the cytoplasmic matter (Desai and Banat 1997).

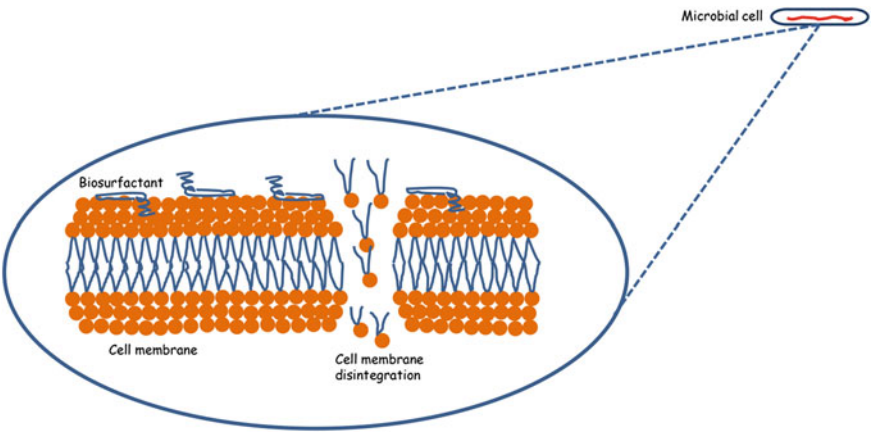

Fig. 2.2 Mode of action of biosurfactants

- Disruption of cell membranes from side to side buildup of intra membranous elements in the cells increasing the electrical conductance of the plasma membrane
- Increasing cell membrane sponginess through the disruption of the plasma membrane phospholipids (Carrillo et al. 2003; Sotirova et al. 2009).
- Biosurfactant exposure to the cells changed the fatty acids contents in the cell membrane due to the disturbance in plasma membrane permeability. Biosurfactant directly interact with the lipids, which trigger inhibition of the membrane-confined enzyme and outflow of intracellular cytoplasmic components. In other study, Cameotra and Makkar (2004) described that the antimicrobial potential of biosurfactant could intrude the plasma membrane structure while interacted with phospholipids and other membrane proteins.
- Treatment of *S. aureus* cells with the biosurfactants resulted in the reduction of lipids content and also had a relation with the disruption of proteins present in the plasma membrane. The phenomenon may probably be accredited to the property of increasing membrane protein initiating the conformational changes of lipid and protein molecules.

Several biosurfactants are recognized to have therapeutic claims as antifungal, antibacterial, and antiviral complexes (Inès and Dhouha 2015). Antimicrobial potential of biosurfactants makes them appropriate complexes for applications in contending to numerous diseases and act as a therapeutic agents (Table 2.2). For illustration, the antimicrobial potential of two biosurfactants moieties derived from probiotic bacteria, *Lactococcus lactis* 53 and *Streptococcus thermophilus* A, against various pathogenic bacteria and yeast strains colonizing voice prostheses were assessed (Rodrigues et al. 2004).

Biosurfactant producing lactobacilli with certain health benefits have been frequently isolated from the urinary tract and intestinal tracts (Reid et al. 2001). There

Table 2.2 Antimicrobial properties of biosurfactants derived from LAB

S. No.	Strain	Antimicrobial activity	References
1.	*Streptococcus thermophiles* A	Antimicrobial activity against the *Candida tropicalis*	Rodrigues et al. (2006)
2.	*Lactobacillus casei*	Antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis,* and *Micrococcus roseus*	Gołek et al. (2009)
3.	*Lactococcus lactis*	Antimicrobial activity of biosurfactant (Xylolipids) against the multidrug resistant *Staphylococcus aureus* and *E. coli*	Sarvanakumari and Mani (2010)
4.	*Lactobacillus paracasei*	Growth inhibition of *E. coli, S. agalactiae,* and *S. pyogenes* with a concentration of 25 mg/ml	Gudiña et al. (2010)
5.	*Lactobacillus paracasei* A20	Antimicrobial activity against various gram-positive and gram-negative microorganisms at various concentration ranging from 3.12 mg/ml to 50 mg/ml	Gudiña et al. (2010)
6.	*Lactobacillus casei* MRTL3	Antimicrobial activity against *Staphylococcus aureus* ATCC 6538P, *S. epidermidis* ATCC 12228, *Bacillus cereus* ATCC 11770, *Listeria monocytogenes* MTCC 657, and *L. innocua* ATCC 33090, *Shigella flexneri* ATCC 9199, *Salmonella typhi* MTCC 733	Sharma and Saharan (2014)

is a high incidence of pathogens associated with the surgical implants mainly caused by the *S. aureus*. Biosurfactants produced by the probiotic strain of *Lactobacillus fermentum* RC-14 reduced the population and adherence of *S. aureus* to the surgical implants (Gan et al. 2002). Rodrigues et al. (2006) demonstrated biosurfactant production by *S. thermophiles* inhibit the growth of *Candida tropicalis* at the concentration of 2.5 g/l. The crude biosurfactant showed significant antimicrobial properties against the *E. coli, S. agalactiae*, and *S. pyogenes* with a concentration of 25 mg/ml (Gudiña et al. 2010). Biosurfactant derived from the *Lactobacillus paracasei* A20 showed antimicrobial activity against various gram-positive and gram-negative microorganisms at various concentrations ranging from 3.12 mg/ml to 50 mg/ml. The biosurfactant derived from *Lactobacillus casei* MRTL3 isolated from raw milk showed significant antimicrobial properties against various pathogens, including *Staphylococcus aureus*, *S. epidermidis*, *Bacillus cereus*, *Listeria monocytogenes* and *L. innocua, Shigella flexneri*, *Salmonella typhi*, and *Pseudomonas aeruginosa* (Sharma and Saharan 2014).

Conclusion

Biosurfactant derived from the genus *Lactobacillus* are of biomedical and food interests. Biosurfactants derived from LAB are advantageous as antibacterial and antifungal agents, and they also have the potential for use as antibiofilm agents in

biomedical and food processing. Encouraging substitutes to conventional antibiotics with less toxicity and broad spectrum may be exploited for their biomedical importance. Moreover, biosurfactants of lactic acid bacteria have the potential to be used as antibiofilm biological coatings for biomedical equipment for decreasing nosocomial pathogens. Biosurfactant of lactic acid bacteria may also be integrated into probiotic formulations to combat urinary tract infections. Although there is immense potential of biosurfactants of lactic acid bacteria in the biomedical and food processing, their application still remains inadequate, conceivably due to high production cost, toxicity, and inadequate evidence on their structural details. Further research investigations on their toxicity and structural attributes are desired to authenticate the use of LAB-derived biosurfactants in various biomedical and food formulations.

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