Enhanced Knowledge in Sciences and Technology Vol. 2 No. 2 (2022) 060-069
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EKST

Homepage: http://publisher.uthm.edu.my/periodicals/index.php/ekst
e-ISSN : 2773-6385

Review: Isolation and Characterization of Biosurfactant-Producing Lactic Acid Bacteria from Sauerkraut

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DOI: https://doi.org/10.30880/ekst. 2022.02.02.007
Received 03 January 2022; Accepted 08 June 2022; Available online 23 November 2022

Abstract: The lactic acid bacteria (LAB), commonly associated with food and feed fermentation normally be inherent in the mucosal surfaces of healthy humans and animals. Microbial surface-active agents are amphiphilic compounds produced commonly by microorganisms predominately bacteria and yeast on their cell surface, or extracellularly with exceptional surface and emulsifying activities. The physiological function of microbial surfactants in a producer cell is not entirely understood. On the contrary, there has been hypothesis about their involvement in emulsification of water insoluble substrates. Sauerkraut is a conventional fermented foods that contains lots of health benefits. One of the microbes that determines the success of fermentation is lactic acid bacteria. The purpose of this research is to review and assess previous the available literatures from previous studies of isolation and characterization of biosurfactant-producing lactic acid bacteria from sauerkraut. Most of the biosurfactant-producing microorganisms are pathogenic and challenging to handle in commercial formulations. The development of biosurfactant production from nonpathogenic microorganisms such as "Biosurfactant derived from LAB" is a prevailing task that is receiving increased attention in direction to escape pathogenicity.

Keywords: Lactic Acid Bacteria (LAB), Sauerkraut, Biosurfactant

1. Introduction

Sauerkraut is one of the oldest and most popular traditional fermented vegetables that can be prepared by a fermentation process of shredded cabbage with 2.3–3.0% salt for 14-21 days. The ingredients in sauerkraut have been reported to contain vitamins, minerals, probiotic and that could give

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many health benefits [1]. The fermentation process produces lactic acid bacteria through two-step of microbial process involving heterofermentative and homofermentative LAB, with *Leuconostoc* spp. in the initial phase and *Lactobacillus* spp. and *Pediococcus* spp. in the subsequent phase. Lactic acid bacteria (LAB) are a group of Gram-positive bacteria which is the main product of carbohydrates conversion. It also exhibits various kind of functional ingredients including probiotics, starter cultures, antimicrobial agents and vitamins [2]. LAB has been generally believed to have positive roles in maintaining good health and immune system in humans. Several *Lactobacilli* spp. is known to produce important metabolites, including biosurfactants, which 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 [3].

Biosurfactant is a microbially derived of amphiphilic compound comprising of both hydrophilic and hydrophobic segments. Due to these properties, it has the unique characteristic to efficiently reduce the surface and interfacial tension between two immiscible liquids such as oil-water and water-oil. Biosurfactant can be classified into two categories, low molecular weight biosurfactants (lipopeptides, glycolipids, phospholipids) and high molecular weight surface-active polymers (polypeptides, lipopolysaccharides, lipoproteins, proteins). In contrast with chemical surfactants, biosurfactant exhibits numerous advantages including lower toxicity and higher biodegradability, better environmental compatibility, high selectivity, effectiveness at extreme temperatures, salinities, or pH and ability to be produced from inexpensive substrates [4, 5].

Biosurfactants have been demonstrated to have antibacterial and anti-adhesive properties, as well as heavy metal binding, aggregation, quorum sensing, biofilm formation, and solubility in hydrophobic substances, all of which increase substrate uptake into cells. Several studies have reported on the wide range of potential application of biosurfactant in many industries such as petroleum, pharmaceuticals, biomedical, food processing, and agriculture [6]. Due to health and safety concern, most of pathogenic biosurfactant producers are used in petroleum and agriculture industries while the non-pathogenic producers could provide a better alternative in pharmaceuticals, biomedical, and food processing. A study has found that *Pseudomonas* spp. and *Bacillus* spp. are the species mainly used for biosurfactant production [7]. However, recent papers revealed LAB as a good biosurfactant producer, with the most from Lactobacillus strains, Bifidobacterium strain, and others [8, 9, 10, 11, 12]. Meanwhile, the application of biosurfactant derived by LAB in food industry can be seen as food emulsifiers, antioxidants, antibiofilm agents, antimicrobial agents, and antiadhesives. Hence, this research was conducted to review and assess the available literature from the previous studies of isolation and characterization biosurfactant-producing lactic acid bacteria from the sauerkraut.

2. Lactic acid bacteria (LAB)

Lactic acid bacteria (LAB) are gram-positive bacteria that have been classified based on morphological, physiological, and metabolic characteristics. The LAB found on the mucosal surfaces of healthy humans and animals is often related with food and feed fermentation [14, 13]. LAB is recognized for use in the food and health industries because they are generally considered as safe (GRAS) by the US Food and Drug Administration. The Russian microbiologist Elie Metchnikoff was the first to draw attention to the beneficial effects of LAB, demonstrating that the Balkan people's practice of consuming fermented milk products may be responsible for their increased longevity. He thought that the presence of LAB in the gastrointestinal system protects against the deleterious effects of putrefying microorganisms. The lactic acid bacteria genus LAB has a large number of diverse species of lactic acid bacteria. Lactobacillus bacteria are primarily found in the commensal gut flora of humans and animals [16]. In recent decades, there has been a growing appreciation for the importance of LAB in maintaining homeostasis within the gastrointestinal tract environment and preventing pathogen colonization [17]. Lactobacilli have a protective role by creating compounds such lactic acid, bacteriocins, hydrogen peroxides, and biosurfactants, which limit pathogen growth [15].

2.1 Biosurfactant

Biosurfactants are amphiphilic compounds produced in living surfaces, primarily on microbial cell surfaces or excreted extracellular hydrophobic and hydrophilic moieties that confer the ability to accumulate between fluid phases, hence reducing surface and interfacial tension at the surface and interface. Compared to chemical surfactants, these compounds have several advantages such as lower toxicity, higher biodegradability, and effectiveness at extreme temperatures and pH values [4]. Furthermore, biosurfactants can be customized to fit specific applications by altering the manufacturing conditions or altering the genes involved in their biosynthesis.

The physiological function of microbial surfactants in a producer cell is not entirely understood. On the contrary, there has been hypothesis about their involvement in the emulsification of water-insoluble substrates. When microbial cells are cultivated, oils and growth-stimulating substances are frequently accumulated in the production medium. These accumulated substances have roles in emulsifying the substrate, spreading the interfacial area and expediting mass transfer on the surface of cell. The interfacial surface between aqueous phase and oil can be a restrictive factor and emulsification is a natural process advantageous for the microbial cell to absorb the substrate [28, 18].

In other cases, when pathogens infect plants or animals, biosurfactants are considered to function as a dispersing and wetting agent for the surface of the host cells [19, 20]. On the other hand, biosurfactants play a significant role in adhesion of the cells to the interfaces. Adhesion is a physiological mechanism for survival of microbial cells in the environment [21, 22]. Microbial cells can also use their biosurfactants to regulate their cell surface properties like to attach or detach from biotic and abiotic surfaces [26, 27].

Physiologically biosurfactants have been recognized as the components of cellular metabolism, motion, and defense. They have been reported majorly in bacteria, biofilms, as a molecule for quorum-sensing, encouraging the uptake of poorly soluble substrates, lubricants, immune modulators, secondary metabolites, and known antimicrobial substances [24, 23].

Microbial surfactants act as vital molecules for interfacial processes, which conditioned the cell surface, with which the cells interact [25]. Biosurfactants also have significant roles in the dissolution of oil molecules particularly for oil-degrading microorganisms [29]. The role of bacterial biosurfactants has been widely studied in *Pseudomonas* where they are known to stimulate colonization [30].

2.2 Mechanism of biosurfactant

A phase boundary between two phases in a heterogeneous system is called interface. Organic molecules from the aqueous phase tend to immobilize at the solid interface in all interfacial systems. When this happens, they will eventually form a conditioning film which will change the properties of the surfaces. Due to the amphiphilic nature of the biosurfactants, a range of interactions are involved in the possible adsorption of charged biosurfactants to interfaces. Most natural interfaces contain negatively charge but, in some cases, positive charge also can occur. Figure 1 shows the biosurfactants accumulate at the interface between two immiscible fluids or between a fluid and a solid.

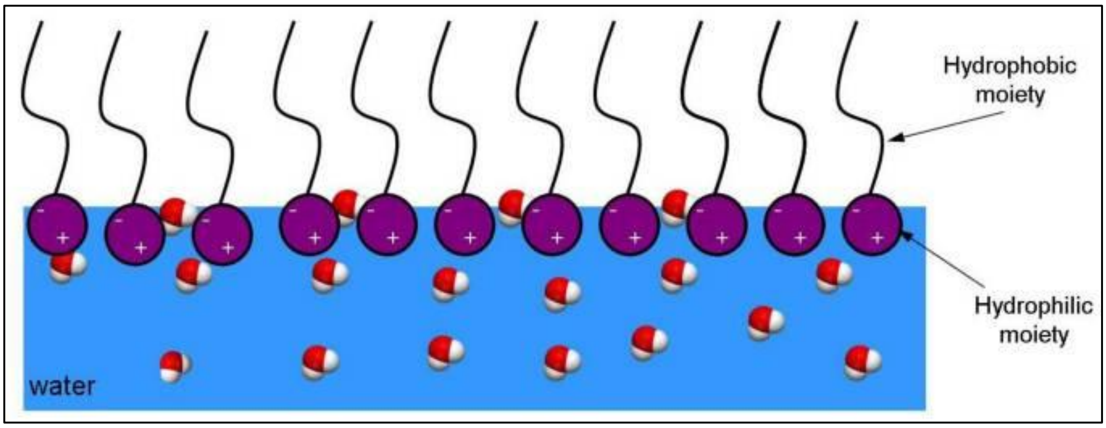

Figure 1: Accumulation of biosurfactant at the interface between liquid and air.

They diminish the repulsive forces between two dissimilar phases by lowering surface (liquid-air) and interfacial (liquid-liquid) tension, allowing them to mix and interact more freely [31]. Until the critical micelle concentration (CMC) is reached, biosurfactant actions are dependent on the concentration of surface-active chemicals. At concentrations above the CMC, biosurfactant molecules will associate to form micelles, bilayers, and vesicles. Micelle formation is the one that is responsible to reduce the surface and interfacial tension as well as increasing the solubility and bioavailability of hydrophobic organic compounds [32].

2.3 Application of biosurfactants

Microbial surfactants have a wide range of potential applications in many industries, including oil, cosmetics, special chemical foods, agriculture, and medicine, due to their low or non-toxicity, high biodegradability, ecological acceptability, high foaming capability, and stability in extreme environments. Since probiotic bacteria are a relatively new development in biotherapeutics, their use has been studied primarily in terms of antibacterial or antiadhesive action. In food processing industry, biosurfactant usually play role as food formulation ingredient and anti-adhesive agents. Biosurfactant is substituted in the food formulation to promote the formation and stabilization of emulsion due to their ability to decrease the surface and interfacial tension. Besides, it is also used to prevent fat globule agglomeration, stabilize aerated systems, improve the texture and shelf life of starch-based products, modify the rheological characteristics of wheat dough, and improve the consistency and texture of fat-based products [35]. LAB derived biosurfactants could be a safer alternative as antimicrobial agents in the food industry. Lactobacillus jensenii and Lactobacillus rhamnosus both produced biosurfactants that inhibit the development of *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* [33].

Many studies found that biosurfactants produced from probiotic microorganisms have been found to inhibit the growth of hospital-acquired infections on inanimate and biomedical surfaces. Besides, a significant application of biosurfactant can be seen as antiadhesive agents to combat the colonization of pathogenic microorganisms. Application of biosurfactant to any surface modifies its hydrophobicity, interfering in microbial adhesion. The role of biosurfactants in microbial adherence has been extensively characterized, and it establishes a potential strategy to limit microbial adhesion by pathogens in a variety sector, including biomedical applications and the food processing industry [34].

Microbial enhanced oil recovery (MEOR) uses the metabolic process of microorganism to increase oil production from marginally producing reservoirs. According to Das & Mukherjee (2007), certain microorganism such Bacillus subtilis is capable in utilizing the crude oil & hydrocarbons as sole carbon sources & can be used for oil spill clean-ups [36].

3. Antimicrobial activity of biosurfactant-LAB

The antimicrobial property of biosurfactants is owing to the potential of molecules to self-associate and create a pore network inside cellular membrane [38]. Biosurfactants can breach into the plasma membrane through hydrophobic edges, thus ultimately manipulating the organization of the hydrocarbon chains and also fluctuating the plasma membrane thickness [37].

Biosurfactant mediated membrane distractions are broad-spectrum in mode of action and are beneficial for disruption of plasma membranes of pathogenic bacteria [41]. Biosurfactants derived from various microorganisms showed potential antimicrobial properties [33, 40].

Rhamnolipids isolated from the *P. aeruginosa* LBI have shown significant antimicrobial properties against various bacterial and fungal pathogens, comprising *B. cereus*, *S. aureus*, *M. luteus*, and *Neurospora crassa* [42].

The antimicrobial property of the biosurfactant obtained from *L. paracasei* ssp. *paracasei* A20 was evaluated by determining the growth inhibition for various microorganisms [40]. The biosurfactant derived from *L. paracasei* ssp. *paracasei* A20 was found effective against various pathogens tested. With respect to the *Lactobacillus* strains and various *Streptococcus* species related to the oral cavity such as *S. sanguis, S. mutans*, and *S. oralis*), broad growth reduction was witnessed for biosurfactant concentration ranges of 25–50 mg/mL. On the other hand, *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. agalactiae*) showed complete reduction with biosurfactant concentration ranges of 25–50 mg/mL.

Various biosurfactants derived from LAB exhibiting antimicrobial properties have been formerly documented (Table 1). Conversely, there are less studies on the antimicrobial properties of biosurfactants derived from various LAB; only biosurfactants derived from *S. thermophilus* A and *L. lactis* 53 showed noteworthy antimicrobial properties against various pathogens isolated from voice prostheses [43, 47].

In another study, the cell-bound biosurfactant derived from *L. agilis* CCUG31450 showed antimicrobial properties at a concentration of 5 mg/mL against *S. aureus, P. aeruginosa, and S. agalactiae*. However, no antimicrobial activity was observed against *E. coli* and *C. albicans*. The cell-bound biosurfactants isolated from probiotic lactobacilli bacteria exhibited antibacterial properties and inhibited the growth of various drug-resistant pathogens. The biosurfactants derived from *L. jensenii* and *L. rhamnosus* were evaluated against clinical isolates of MDR *A. baumannii, E. coli, and S. aureus* and it was found that biosurfactants obtained from *L. jensenii* and *L. rhamnosus* were effective in inhibiting MDR pathogens at the concentration of 50 mg/mL. *L. jensenii* derived biosurfactant exhibited nearly 100 % inhibition against all the pathogens screened. The activity of *L. rhamnosus* oscillated from 96 to 97 % against *A. baumannii* and approx. 72–85 % against *E. coli* [33].

Inhibition of microbial growth was reported against *S. enterica* and *L. monocytogenes* when cell free biosurfactant from *L. plantarum* and *L. Pentosus* were evaluated, displaying the maximal antimicrobial activity of cell free biosurfactants from fermentation [44].

The initiation of antibiotics for inhibiting bacterial pathogens is regarded as one of the vital developments in contemporary medicine. The use and exploitation of antibiotics in the past 10–20 years show that the majority of clinical pathogens have established resistance to multiple antibiotics. As a result, it is necessary to find out novel antimicrobials that are potent to control infectious diseases instigated by multi drug-resistant pathogen microorganisms [46]. Biosurfactants are increasingly seen as an incipient class of novel antimicrobial compounds. They are an appropriate alternative to conventional antibiotics and could be used as safe and potent therapeutic compounds, particularly multi-drug resistant among clinical pathogens [45].

Table 1: Different strains of LAB reported for antibacterial properties.

S. no	Strain	Activity	References
1.	*Lactococcus lactis* 53	Exhibited significant antimicrobial activity against *Staphylococcus epidermidis, Streptococcus salivarius, S. aureus, Candida tropicalis* and *C. albicans*	Rodrigues *et al*. (2004, 2006)
2.	*L. plantarum* CFR2194	*E. coli, S. aureus* and *Yersinia enterocolitica*	Madhu and Prapulla (2014)
3.	*Streptococcus thermophiles* A	Antimicrobial activity against the *Candida tropicalis*	Rodrigues *et al*. (2006)
4.	*Lactobacillus casei*	Antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis* and *Micrococcus roseus*	Golek *et al*. (2009)
5.	*Lactococcus lactis*	Antimicrobial activity of biosurfactant (Xylolipids) against the multi-drug resistant *Staphylococcus aureus* and *E. coli*	Saravanakumari and Mani (2010)
6.	*Lactobacillus paracasei*	Growth inhibition of *E. coli, S. agalactiae* and *S. pyogenes* with a concentration of 25 mg/ml	Gudiña *et al*. (2010)
7.	*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)
8.	*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)
9.	*L. jensenii* and *L. rhamnosus*	MDR *A. baumannii, E. coli* and *S. aureus*	Sambanthamoorthy *et al*. (2014)

4. Conclusion

This study aimed to review the previous studies of the isolation and characterization of biosurfactant-producing lactic acid bacteria from sauerkraut. Biosurfactants are the molecules of choice for the future prospective in food formulations, nanoparticles synthesis, biomedical, and healthcare services. Further research to minimize the production and operational cost of production of biosurfactants will strengthen the demand for surface active agents. Toxicity evaluation and better compatibility assessment of the biosurfactant with human and animal-related material are the need of the hour to develop biosurfactant-mediated processes. Further study and better structural evaluation of the biosurfactant are required for industrial development and for future applications.

Acknowledgement

We would also like to thank Universiti Tun Hussein Onn Malaysia (UTHM) especially the Faculty of Applied Sciences and Technology (FAST) for its support.

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