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Antimicrobial applications of sophorolipid from *Candida bombicola*: A promising alternative to conventional drugs

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ABSTRACT

Sophorolipids (SLs) are extracellular glycolipids, produced mainly by yeast *Candida bombicola*, composed of a disaccharide sophorose (O-β-D-glucopyranosyl-2-1-β-D-glucopyranose) linked by a glycosidic bound to the terminal or sub-terminal carbon of a fatty acid chain. Due to these structural characteristics, SLs have been reported with several applications, which are directly related to the predominance of their acidic and lactonic forms. SLs are the most promising and attractive biosurfactant, highlighting its antimicrobial action against Gram-positive and Gram-negative bacteria. The antimicrobial activities of SLs are due to the mechanism of changes or rupture in the cellular membrane, inducing the outpouring of their cytoplasmic contents, and the consequent death of the pathogen. This surfactant can be used as an alternative for the substitution of conventional drugs.

1. INTRODUCTION

The consumer concerns about the use of synthetic antimicrobials to improve the quality of life led to a search for biodegradable compounds of natural origin [1]. The importance of biofilms control and the potential use of biosurfactants, as an antimicrobial agent, have enhanced the interest in these compounds, which are molecules with surfactant characteristics, produced by microorganisms and, although the similarity with petroleum-based surfactants, they are considered superior and more advantageous, because of their ecological and sustainable nature [2]. Structurally, they are amphiphilic molecules, whereas the hydrophobic moiety is a long chain of fatty acid, hydroxy acid, or α-alkyl β-hydroxy-acid, and the hydrophilic moiety is generally a carbohydrate, amino acid, cyclic peptide, phosphate, carboxylic acid, or alcohol [3].

Sophorolipids (SLs) are secondary metabolites classified as extracellular glycolipids, primary produced by yeast *Candida bombicola*, from carbohydrates and lipids, being excreted as a mixture of related chemical structures [4]. They are composed of a disaccharide sophorose (O-β-D-glucopyranosyl-2-1-β-D-glucopyranose) linked by a glycosidic bound between the carbon 1' and the terminal (ω) or sub-terminal (ω-1) carbon of a fatty acid chain of 16 or 18 carbons [5]. They have no cytotoxicity and are accepted and approved by the Food and Drug Administration. Currently, they are the most applied biosurfactants in the industry, and the products are available in commercial level.

These metabolites are produced in two principal structural forms, acidic and lactonic [6], which results in changes in the physical-chemical and biological properties, responsible for the different applicabilities of these compounds [7]. In relation to the producing microorganisms, there are several species of yeasts that synthesize different profiles of SLs, highlighting *C. bombicola*, because of the high yields, which mainly produces SLs in the lactonic diacetylated form (6', 6") with monounsaturated fatty acids (C16 and C18), and in a minor extent, acidic non-acetylated or monoacetylated forms (6") [8]. Therefore, because of these structural characteristics, SLs have been reported with several applications, which are directly related to the composition of their acidic and lactonic forms. These applications are highlighted in agriculture, food, cosmetic, bioremediation, and biomedicine with antimicrobial activity [9,10].

2. ANTIMICROBIAL ACTIVITY OF SLs

The antimicrobial activity of SLs is related to the synergistic effect of their sugar and lipid portions (surfactant effect) [9,11]. This mechanism is characterized by changes or rupture in the cellular membrane, inducing the outpouring of their cytoplasmic contents and the consequent release of intracellular enzymes, for instance, malate dehydrogenase indicating the interaction of SLs with the cellular membrane [12,13]. Although the mechanism of action of biosurfactants is not well known, an activity of altering charge-charge properties is

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hypothesized, which may decrease the chances for bacteria to acquire antibiotic resistance [14].

The interactions between carbohydrates and bacterial membranes have been studied for years [15]; however, only recently, studies have attempted to show the impact of mono and disaccharides on the structure of membranes [16] such as the sophorose disaccharide present in the SL molecule, which are effective as a bactericidal agent, regardless if its lipid content is acidic or lactonic, being capable of inducing death of planktonic cells and biofilms of both Gram-positive and Gram-negative bacteria although the negative group presents a more complex cellular envelope both can be damaged by sophorose [17].

The peptidoglycan layer of Gram-positive bacteria is covered by polysaccharides, neutral acids, and proteins. The surface of the Gram-negative bacteria is constituted by lipopolysaccharides with neutral pH, but when the carboxylic and phosphate groups are ionized, they confer anionic charges. These negative charges make the bacterial membrane more hydrophilic [18] compared to Gram-positive bacteria. SLs, due to their amphiphilic characteristics, decrease the hydrophobicity of both bacterial groups, but because of the majority composition in fatty acids, they exhibit a greater tendency to hydrophobicity, leading to a more significant performance in hydrophobic microorganisms (Gram-positive) [19,20].

The antimicrobial activity of SLs depends on the concentration, treatment time, composition of fatty acids, and the predominance of acidic and lactonic forms [21,22], as well as the sugar group of the molecule [23]. Lactonic forms have better surface tension properties and antimicrobial activity [7]. Furthermore, it is known that the acetylated forms have better biological and physical-chemical properties [24]. Some of the studied bacteria tested along with the use of the glycolipid are mentioned in Table 1; it is possible to observe the differences in the minimal inhibitory concentration (MIC) depending on the target bacteria and the SL composition, which makes this MIC very variable in the literature because of these many factors.

SLs from *C. bombicola* produced in palmitic, stearic, and oleic acids were applied to Gram-positive bacteria (*Enterococcus faecium, Aerococcus viridans, Staphylococcus xylosus, Staphylococcus cohnii,* and *Staphylococcus equorum*) Gram-positive endospore forming (*Bacillus licheniformis, Bacillus pumilus,* and *Bacillus mycoides*) and Gram-negative bacteria (*Pseudomonas luteola, Enterobacter cloacae, Enterobacter sakazakii,* and *Vibrio fluvialis*), obtaining MIC from 4.88 µg/mL to 19.5 µg/mL, demonstrating effect in all bacteria studied [25].

Similar studies by different authors have shown that SLs from *C. bombicola* were also able to reduce *Escherichia coli* O157:H7 population. Applications with 0.5% and 1.0% of SL-oleic and SL-palmitic reduced planktonic cell cultures after 1–2 h of incubation. AWhile the use of only 0.1% of SL-stearic was sufficient to reduce the same bacteria after 2 h [26].

SLs produced by *C. bombicola* on coconut and corn oils were tested against *Staphylococcus aureus* and *E. coli*. The synthesized from corn oil was more efficient for *E. coli,* and coconut oil for *S. aureus* [21]; this demonstrates the varied action mechanism of different SLs as an antimicrobial agent in the various pathogenic strains. It was also tested by other authors in *Bacillus subtilis* and *Pseudomonas aeruginosa*, obtaining a MIC of 5.0 and 10.0 mg/mL, respectively [20].

Enterococcus faecalis and *P. aeruginosa*, bacteria responsible for nosocomial infections, were inhibited by purified acidic SLs from *C. bombicola*, predominantly non-acetylated (C18), at ≥5 mg/mL. At 20 mg/mL, an inhibitory effect on the growth of *E. faecalis* was observed, with no formation of colonies [22]. On the other hand, the mixture of SLs without purification, containing 75% of lactonic and 25% of acidic was effective against *E. coli* at 1 mg/mL and *S. aureus* at 15–150 µg/mL [27].

SLs produced from glucose, and lauryl alcohol was tested in Gram-negative bacteria (*E. coli* ATCC 8739 and *P. aeruginosa* ATCC 9027), Gram-positive (*S. aureus* ATCC 6358 and *B. subtilis* ATCC 6633), and yeast *Candida albicans* ATCC 2091 [28]. The results showed complete inhibition when compared to SL-oleic and SL-linolenic. The inhibition was 30 µg/mL for *E. coli* and 1 µg/mL for *P. aeruginosa* at 2 and 4 h, respectively, for *S. aureus* was 6 µg/mL, *B. subtilis* was 1 µg/mL, and *C. albicans* was 50 µg/mL, after 4 h of treatment.

Table 1: Minimum inhibitory concentrations of different types of SL against Gram-positive and Gram-negative bacteria (µg/ml)

Microorganisms	SLs types	MIC	References
S. aureus	75% lactonic and 25% acidic; diacetylated lactonic (C18:0, C18:1 or C18:2)	400 µg/ml; 50 µg/ml	Joshi-Navare *et al*., 2013; Pontes *et al*., 2016
S. epidermidis	Non-acetylated acidic	50 µg/ml	Valotteau *et al*., 2017
E. faecalis	Non-acetylated acidic	50 µg/ml	Valotteau *et al*., 2017
L. ivanovii	Non-acetylated acidic	50 µg/ml	Valotteau *et al*., 2017
S. pyogenes	Non-acetylated acidic	50 µg/ml	Valotteau *et al*., 2017
S. mutans; S. salivarius and *S. sobrinus*	Lactonic	≥50 µg/ml	Solaiman *et al*., 2017
L. acidophilus and *L. fermentum*	Lactonic	1.000 µg/ml	Solaiman *et al*., 2017
E. coli	Non-acetylated acidic; 75% lactonic and 25% acidic; diacetylated lactonic (C18:0, C18:1 or C18:2)	50 µg/ml; 1.000 µg/ml; 750 µg/ml	Valotteau *et al*., 2017; Joshi-Navare *et al*., 2013; Pontes *et al*., 2016
P. aeruginosa	Non-acetylated acidic; acidic (C18:1)	50 µg/ml; 5.000 µg/ml	Valotteau *et al*., 2017; Lydon *et al*., 2017
S. typhimurium	Non-acetylated acidic	50 µg/ml	Valotteau *et al*., 2017

SL: Sophorolipid, MIC: Minimal inhibitory concentration, *S. aureus: Staphylococcus aureus, S. epidermidis: Staphylococcus epidermidis, E. faecalis: Enterococcus faecalis, L. ivanovii: Listeria ivanovii, S. pyogenes: Streptococcus pyogenes, S. mutans: Streptococcus mutans, S. salivarius: Streptococcus salivarius, S. sobrinus: Streptococcus sobrinus, L. acidophilus: Lactobacillus acidophilus, L. fermentum: Lactobacillus fermentum, E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa, S. typhimurium: Salmonella typhimurium*

The acidic and lactonic forms of SLs from *Rhodotorula babjevae* YS3 presented antifungal action against *Colletotrichum gloeosporioides*, *Fusarium verticillioides*, *Fusarium oxysporum*, *Corynespora cassiicola,* and *Trichophyton rubrum* verified by MIC of 62 µg/mL, 125 µg/mL, 125 µg/mL, ≥2000 µg/mL, and ≥1000 µg/mL, respectively [29].

Synergistic actions of diacetylated lactonic SLs (SL-oleic) with cefaclor and tetracycline have been described, demonstrating that the activity of conjugated antibiotics was enhanced for *E. coli* ATCC 8739 and *S. aureus* ATCC 29737 [27]. SL conjugated with caprylic acid (0.8%) increased the inhibition of *P. aeruginosa* PAO1, *B. subtilis* NCTC 10400, *S. aureus* ATCC 9144, and *E. coli* NCTC 10418 [30]. In another study, the same authors verified the combination of SLs and rhamnolipids (0.04%/0.01%) against biofilms of *P. aeruginosa* ATCC 15442, *S. aureus* ATCC 9144, and a mixed culture of both, obtaining positive results about the synergism of this molecule with different compounds [31].

The activity of SLs from *C. bombicola* was compared with thiamine dilauryl sulfate (TDS) in the presence of alcohol against *Salmonella* spp. and *Listeria* spp. The lactonic SLs presented superior antimicrobial activity in *Listeria* spp. than in *Salmonella* spp. The populations of *Listeria* spp. were reduced from 7.2 log CFU/mL to an undetectable level after treatment of 1 min with 0.1% (w/v) of single-layer perceptron and TDS in the presence of ethanol (20%). TDS was more effective than SLs against *Salmonella* spp. and *Listeria* spp., but both are capable of causing cell lysis; demonstrating that SLs and TDS in the presence of ethanol can be used to inactivate pathogens, especially Gram-positive bacteria [12].

3. CONCLUSION

This review presented the potentials of this glycolipid and their applications as an antibacterial and antifungals agent. SLs can be used to repair infectious diseases, as therapeutic agents, sanitizers, and germicides in several sectors, highlighting the main bacteria of foodborne illness and contamination, both Gram-positive and Gram-negative, can be inhibited by antimicrobial activity of SLs produced by *C. bombicola*. Considering the significance of the development of new sustainable strategies, combined with the importance of controlling the formation of biofilms and being a non-toxic product, SLs present a promising perspective for an excellent antimicrobial agent.

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