

# Antimicrobial Applications of Sophorolipid from *Candida bombicola*: a Promising Alternative to Conventional Drugs



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## Abstract

Sophorolipids are extracellular glycolipids, produced mainly by yeast *Candida bombicola*, composed of a disaccharide sophorose (O  $\beta$ -D-glucopyranosyl-2-1- $\beta$ -D-glucopyranose) linked by a glycosidic bond to the terminal or sub-terminal carbon of a fatty acid chain. Because of these structural characteristics, sophorolipids have been reported with several applications, which are directly related to the predominance of their acidic and lactonic forms. Sophorolipids are the most promising and attractive biosurfactant, highlighting its antimicrobial action against Gram-positive and negative bacteria. The antimicrobial activities of sophorolipids is due 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.

## 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, has 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  $\alpha$ -alkyl  $\beta$ -hydroxy-acid and the hydrophilic moiety is generally a carbohydrate, amino acid, cyclic peptide, phosphate, carboxylic acid or alcohol [3].

Sophorolipids 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  $\beta$ -D-glucopyranosyl-2-1- $\beta$ -D-glucopyranose) linked by a glycosidic bond between the carbon 1' and the terminal ( $\omega$ ) or sub-terminal ( $\omega$ -1) carbon of a fatty acid chain of 16 or 18 carbons [5]. They have no cytotoxicity and are accepted and approved by the FDA (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 sophorolipids, highlighting *C. bombicola*, because of the high yields, which mainly produces sophorolipids in the lactonic di-acetylated 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, sophorolipids 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].

## Antimicrobial activity of sophorolipids

The antimicrobial activity of sophorolipids 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 sophorolipids 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 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 sophorolipid molecule, which is 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. The sophorolipids, 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 sophorolipids 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 [24]. Furthermore, it is known that the acetylated forms have better biological and physical-chemical properties [25].

Sophorolipids from *C. bombicola* produced in palmitic, stearic and oleic acids were applied to Gram-positive bacteria (*Enterococcus faecium*, *Aerococcus viridans*, *Staphylococcus xylosum*, *S. cohnii* and *S. equorum*) Gram-positive endospore-forming (*B. licheniformis*, *B. pumilus* and *B. mycoides*) and Gram-negative bacteria (*Pseudomonas luteola*, *Enterobacter cloacae*, *E. sakazakii* and *Vibrio fluvialis*), obtaining MIC from 4.88 µg.mL<sup>-1</sup> to 19.5 µg.mL<sup>-1</sup>, demonstrating effect in all bacteria studied [26]. Similar studies by different authors have shown that sophorolipids (SL) 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 to 2 hours of incubation. Although, only 0.1% of

SL-stearic was sufficient to reduce colonies of *E. coli* O157: H7 after 2h, or its eradication at concentrations of 0.5 to 1.0% [27].

Sophorolipids produced by *C. bombicola* on coconut and corn oils were tested against *S. 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 sophorolipids as an antimicrobial agent in the various pathogenic strains. It was also tested by other authors in *B. subtilis* and *Pseudomonas aeruginosa*, obtaining a MIC of 5.0; 10.0mg.mL<sup>-1</sup>, respectively [20].

*Enterococcus faecalis* and *P. aeruginosa*, bacteria responsible for nosocomial infections, were inhibited by purified acidic sophorolipids from *C. bombicola*, predominantly non-acetylated (C18), at ≥5 mg.mL<sup>-1</sup>. At 20 mg.mL<sup>-1</sup>, an inhibitory effect on the growth of *E. faecalis* was observed, with no formation of colonies [22]. On the other hand, the mixture of sophorolipids without purification, containing 75% of lactonic and 25% of acidic was effective against *E. coli* at 1 mg.mL<sup>-1</sup> and *S. aureus* at 15-150 µg.mL<sup>-1</sup>, [28]. Sophorolipids produced from glucose and lauryl alcohol were 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 *C. albicans* ATCC 2091 [29]. The results showed complete inhibition when compared to SL-oleic and SL-linolenic. The inhibition was 30 µg.mL<sup>-1</sup> for *E. coli* and 1 µg.mL<sup>-1</sup> for *P. aeruginosa* at 2 and 4h, respectively; for *S. aureus* was 6 µg.mL<sup>-1</sup>; *B. subtilis* 1 µg.mL<sup>-1</sup> and *C. albicans* 50 µg.mL<sup>-1</sup>, after 4h of treatment.

The acidic and lactonic forms of sophorolipids from *Rhodotorula babjevae* YS3 presented antifungal action against *Colletotrichum gloeosporioides*, *Fusarium verticillioides*, *Fusarium oxysporum*, *Corynespora cassicola* and *Trichophyton rubrum* verified by MIC of 62 µg.mL<sup>-1</sup>, 125 µg.mL<sup>-1</sup>, 125 µg.mL<sup>-1</sup>, ≥2,000 µg.mL<sup>-1</sup> and ≥ 1,000 µg.mL<sup>-1</sup>, respectively [30]. Synergistic actions of diacetylated lactonic sophorolipids (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 [31]. Sophorolipid conjugated with caprylic acid (0.8%) increased the inhibition of *P. aeruginosa* PAO1, *Bacillus subtilis* NCTC 10400, *S. aureus* ATCC 9144 and *E. coli* NCTC 10418 [32]. In another study, the same authors verified the combination of sophorolipids 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 [33].

The activity of sophorolipids from *C. bombicola* was compared with thiamine dilauryl sulfate (TDS) in the presence of alcohol against *Salmonella spp.* and *Listeria spp.* The lactonic sophorolipids presented superior antimicrobial activity in *Listeria spp.* than in *Salmonella spp.* The populations of *Listeria spp.* were reduced from 7.2 log CFU.mL<sup>-1</sup> to an undetectable level after treatment of 1min with 0.1% (w/v) of SLP and TDS

in the presence of ethanol (20%). TDS was more effective than sophorolipids against *Salmonella spp.* and *Listeria spp.*, but both of them are capable of causing cell lysis; demonstrating that sophorolipids and TDS in the presence of ethanol can be used to inactivate pathogens, especially Gram-positive bacteria [34].

### Conclusion

This review presented the potentials of this glycolipid and their applications as an antimicrobial and antifungals agent. Sophorolipids can be used to repair infectious diseases, as therapeutic agents, sanitizers and germicides in several sectors, highlighting the main bacteria of food borne illness and contamination, both Gram-positive and Gram-negative, can be inhibited by antimicrobial activity of sophorolipids produced by *Candida 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, sophorolipids present a promising perspective for an excellent antimicrobial agent.

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### References

1. Zhang H, Wu J, Guo X (2016) Effects of antimicrobial and antioxidant activities of spice extracts on raw chicken meat quality, Food Science and Human Wellness. Beijing Academy of Food Sciences 5(1): 39-48.
2. Chen M, Dong C, Penfold J, Thomas RK, Smyth TJ, et al. (2011) Adsorption of sophorolipid biosurfactants on their own and mixed with sodium dodecyl benzene sulfonate, at the air/water interface. Langmuir 27(14): 8854-8866.
3. Desai JD, Banat IM (1997) Microbial production of surfactants and their commercial potential, Microbiol Mol Biol Rev 61(1): 47-64.
4. Cooper DG, Paddock DA (1984) Production of a Biosurfactant from *Torulopsis-bombicola*. Appl Environ Microbiol 47(1): 173-176.
5. Asmer HJ, Lang S, Wagner, F, Wray V (1988) Microbial production, structure elucidation and bioconversion of sophorose lipids, Journal of the American Oil Chemists' Society 65(9): 1460-1466.
6. Morya VK, Park JH, Kim TJ, Jeon S, Kim EK (2013) Production and characterization of low molecular weight sophorolipid under fed-batch culture. Bioresour Technol 143: 282-288.
7. Paulino BN, Pessôa MG, Mano MC, Molina G, Neri-Numa IA, et al. (2016) Current status in biotechnological production and applications of glycolipid biosurfactants. Appl Microbiol Biotechnol 100(24): 10265-10293.
8. Minucelli T, Renato MRV, Dinosio B, Galdino A, Martha Viviana, et al. (2017) Sophorolipids production by *Candida bombicola* ATCC 22214 and its potential application in soil bioremediation. Waste and Biomass Valorization 8(3): 743-753.
9. Glover RE, Smith RR, Jones MV, Jackson SK, Rowlands CC (1999) An EPR investigation of surfactant action on bacterial membranes FEMS Microbiology Letters 177(1): 57-62.
10. Oliveira MR, Magri A, Cristaini B, Maria A (2015) Review: Sophorolipids A Promising Biosurfactant and it's Applications, International Journal of Advanced Biotechnology and Research(IJBR) 6(2): 161-174.
11. Lang S, Katsiwela E, Wagner F (1989) Antimicrobial effects of biosurfactants. Lipid / Fett 91(9): 363-366.
12. Zhang X, Ashby R, Solaiman DK, Uknalis J, Fan X (2016) Inactivation of *Salmonella spp.* and *Listeria spp.* by palmitic, stearic, and oleic acid sophorolipids and thiamine dilauryl sulfate. Front Microbiol 7: 2076.
13. Kulakovskaya E, Baskunov B, Zvonarev A (2014) The Antibiotic and Membrane-damaging Activities of Cellobiose Lipids and Sophorose Lipids, J Oleo Sci 63(7): 701-707.
14. Díaz De Rienzo MA, Banat IM, Dolman B, Winterburn J, Martin PJ (2015) Sophorolipid biosurfactants: Possible uses as antibacterial and antibiofilm agent. N Biotechnol 32(6): 720-726.
15. Quiocho FA (1986) Carbohydrate-Binding Proteins: Tertiary Structures and Protein-Sugar Interactions. Annu Rev Biochem 55(1): 287-315.
16. Moiset G, López CA, Bartelds R, Syga L, Rijpkema E, et al. (2014) Disaccharides impact the lateral organization of lipid membranes. J Am Chem Soc 136(46): 16167-16175.
17. Valotteau, C, Banat IM, Mitchell CA, Lydon H, Marchant R, et al. (2017) Antibacterial properties of sophorolipid-modified gold surfaces against Gram positive and Gram negative pathogens. Colloids Surf B Biointerfaces 157: 325-334.
18. Abu-Lail NI, Camesano TA (2003) Role of lipopolysaccharides in the adhesion, retention, and transport of *Escherichia coli* JM109. Environ Sci Technol 37(10): 2173-2183.
19. Pontes C, Alves M, Santos C, Ribeiro MH, Gonçalves L, et al. (2016) Can Sophorolipids prevent biofilm formation on silicone catheter tubes? Int J Pharm 513(1-2): 697-708.
20. Hoa NLH (2017) Production and characterization of sophorolipids produced by *Candida bombicola* using sugarcane molasses and coconut oil. Asia-Pacific Journal of Science and Technology 22(2): 66-75.
21. Morya VK, Park, JH, Kim TJ, Jeon S, Kim EK (2013) Production and characterization of low molecular weight sophorolipid under fed-batch culture. Bioresour Technol 143: 282-288.
22. Lydon HL, Baccile N, Callaghan B, Marchant R, Mitchell CA (2017) Adjuvant antibiotic activity of acidic sophorolipids with potential for facilitating wound healing. Antimicrob Agents Chemother 61(5): e02547-16.
23. Shah V, Badia D, Ratsep P (2007) Sophorolipids having enhanced antibacterial activity. Antimicrob Agents Chemother 51(1): 397-400.
24. Paulino BN, Pessôa MG, Mano MC, Molina G, Neri-Numa IA, et al. (2016) Current status in biotechnological production and applications of glycolipid biosurfactants. Appl Microbiol Biotechnol 100(24): 10265-10293.
25. Lang S, Brakemeier A, Heckmann R, Spockner, Rau U (2000) Production of native and modified sophorose lipids, Chim Oggi 18: 76-79.
26. Solaiman DKY, Ashby, RD, Bier Bier M and Caglayan P (2016) Antibacterial Activity of Sophorolipids Produced by *Candida bombicola* on Gram-positive and Gram-negative Bacteria Isolated from Salted Hides, Jalca, 111(November): 358-364.
27. Zhang X, Ashby RD, Solaiman DKY, Liu Y, Fan X (2017) Antimicrobial activity and inactivation mechanism of lactic and free acid sophorolipids against *Escherichia coli* O157:H7, Biocatalysis and Agricultural Biotechnology. Elsevier Ltd, 11: 176-182.
28. Joshi-Navare K, Khanvilkar P, Prabhune A (2013) Jatropha oil derived sophorolipids: Production and characterization as laundry detergent additive, Biochemistry Research International 2013(2013): 1-11.
29. Denge-Pulate, V, Chandorkar, P, Bhagwat, S. and Prabhune, A.A. (2014) Antimicrobial and SEM studies of sophorolipids synthesized using

- lauryl alcohol', *Journal of Surfactants and Detergents*, 17(3): 543-552. doi: 10.1007/s11743-013-1495-8.
30. Sen, S, Borah, S, N, Bora, A. and Deka, S. (2017) Production, characterization, and antifungal activity of a biosurfactant produced by *Rhodotorulababjevae* YS3, *Microbial Cell Factories*. BioMed Central, 16(1): 95. doi: 10.1186/s12934-017-0711-z.
31. Joshi-Navare, K. and Prabhune, A. (2013) A biosurfactant-sophorolipid acts in synergy with antibiotics to enhance their efficiency, *BioMed Research International*, 2013.
32. Díaz De Rienzo MA, Stevenson, PS, Marchant, R, Banat IM (2016) Effect of biosurfactants on *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms in a BioFlux channel. *Applied Microbiology and Biotechnology* 100(13): 5773-5779.
33. Díaz De Rienzo MA, Stevenson PS, Marchant R, Banat IM (2016) *Pseudomonas aeruginosa* biofilm disruption using microbial surfactants. *Journal of Applied Microbiology* 120(4): 868-876.
34. Zhang, X, Fan X (2016) Inactivation of *Escherichia coli* O157:H7 *in vitro* and on the surface of spinach leaves by bio based antimicrobial surfactants, *Food Control*. Elsevier Ltd 60: 158-165.



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