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Evaluation Antimicrobial and Antiadhesive Properties of the Biosurfactant Lunasan Produced by *Candida sphaerica* UCP 0995

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Abstract Different groups of biosurfactants exhibit diverse properties and display a variety of physiological functions in producer microorganisms; these include enhancing the solubility of hydrophobic/water-insoluble compound, heave metal binding, bacterial pathogenesis, cell adhesion and aggregation, quorum sensing and biofilm formation. *Candida sphaerica* was grown in a low cost medium, consisting of distilled water supplemented with 9% refinery residue of soybean oil and 9% corn steep liquor, for 144 h at 28°C and 150 rpm. The cell-free supernatant obtained at the end of the experiments was submitted to extraction, and afterward the biosurfactant was isolated using methanol with a yield of 9 g l^{-1}. The critical micelle concentration of the biosurfactant was found to be 0.25 mg ml^{-1} with a surface tension of 25 mN m^{-1}. Several concentrations of the biosurfactant (0.625–10 mg ml^{-1}) were used to evaluate its antimicrobial and antiadhesive activities against a variety of microorganisms. The biosurfactant showed antimicrobial activity against *Streptococcus oralis* (68%), *Candida albicans* (57%), and *Staphylococcus epidermidis*(57.6%) for the highest concentration tested. Furthermore, the biosurfactant at a concentration of 10 mg ml^{-1} inhibited the adhesion between 80 and 92% of *Pseudomonas aeruginosa, Streptococcus agalactiae, Streptococcus sanguis12.* Inhibition of adhesion with percentages near 100% occurred for the higher concentrations of biosurfactant used. Results gathered in this study point to a potential use of the biosurfactant in biomedical applications.

Introduction

Several compounds with tensoativos properties are synthesized by living organisms, from plants (e.g., *saponins*) to microorganisms (e.g., *glycolipids*) and humans (e.g., pulmonary surfactant), being considered natural surfactants [5, 31]. In addition, these compounds have been produced through biotechnological processes broadening their diversity and potential applications [27]. Surfactants are usually organic compounds that are amphiphilic, meaning they contain both hydrophobic groups ("tails") and hydrophilic groups ("heads"), and that act preferably in the interface of fluid phases with different levels of polarity and bridges of hydrogen, such as oil/water or air/water interfaces. Many microbes appear to produce a complex mixture of biosurfactants, particularly during their growth on water-immiscible substrates. In general, biosurfactants are microbial metabolites with the typical amphiphilic structure of a surfactant, where the hydrophobic moiety is either a long-chain fatty acid, hydroxyl fatty acid, or α-alkyl-β-hydroxy fatty acid and the hydrophilic moiety can be a carbohydrate, an amino acid, a cyclic peptide, a phosphate, a carboxylic acid, or alcohol, among others [26]. Physical and chemical properties, surface tension reduction, and stability of the emulsion formed are

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important characteristics in a biosurfactant that make possible its use in countless biological applications. Most work on biosurfactant applications has been focused on their use in environmental applications owing to their diversity, environmentally friendly nature, suitability for large-scale production and selectivity [6]. Biosurfactants have several advantages over chemical surfactants, such as lower toxicity, higher biodegradability, and effectiveness at extreme temperatures or pH values [9, 30]. Many of the potential applications that have been considered for biosurfactants depend on whether they can be produced economically; however, much effort in process optimization and at the engineering and biological levels has been carried out [34]. Despite their potential and biological origin only a few studies have been carried out on applications related to the biomedical field [4]. Some biosurfactants are suitable alternatives to synthetic medicines and antimicrobial agents and may be used as safe and effective therapeutic agents [28, 46].

Furthermore, biosurfactants have been found to inhibit the adhesion of pathogenic organisms to solid surfaces or to infection sites hampering biofilm formation that is the cause of many diseases, as for example cystic fibrosis [2, 12, 35]. Therefore, prior adhesion of biosurfactants to solid surfaces might constitute a new and effective means of combating colonization by pathogenic microorganisms and subsequent biofilm formation [14, 38, 40, 41, 46].

Pre-coating vinyl urethral catheters by running a surfactin solution through them before inoculation with media resulted in a decrease in the amount of biofilm formed by *Salmonella typhimurium*, *Salmonella enterica*, *Escherichia coli*, and *Proteus mirabilis* [29]. Given the importance of opportunistic infections with *Salmonella* species, including urinary tract infections of AIDS patients, these results have great potential for practical applications. In addition, the use of lactobacilli as a probiotic for the prevention of urogenital infections has been widely studied [7].

The aim of this study was to isolate and characterize the medical main functional properties of the crude biosurfactant produced by *Candida sphaerica*. Characterization included the determination of the surface tension and critical micelle concentration. The antimicrobial and antiadhesive activities of this biosurfactant were assayed against a group of pathogenic and non-pathogenic microorganisms.

Materials and Methods

Microorganisms and Culture Conditions

Candida sphaerica UCP0995, isolated from soil contaminated with metal and obtained from the culture collection of the Universidade Católica de Pernambuco (Brazil), was used for the production of the biosurfactant Lunasan. The microrganism was maintained at 5°C on Yeast Mold Agar (YMA) (OXOID, Basingstoke, England) slants containing (w/v): yeast extract (0.3%), malt extract (0.3%), tryptone (0.5%), D-glucose (1.0%), and agar (5.0%). Transfers were conducted to fresh agar slants each month to maintain viability.

Several strains that commonly colonize prostheses and medical devices were used to test the antimicrobial and antiadhesive properties of the biosurfactant. *Lactobacillus casei* 36, *Lact. casei* 72, *Lactobacillus reuteri* 104R, and *Lact. reuteri* ML1 were cultured in De Man, Rogosa, and Sharpe (MRS broth) slants containing (w/v): peptone (1%), meat extract (0.8%), yeast extract (0.4%), glucose (2%), sodium acetato trihydrate (0.5%), polysorbate 80 (0.1%), dipotassium hydrogen phosphate (0.2%), magnesium sulfate heptahydrate (0.02%), manganese sulfate heptahydrate (0.05%), and agar (1.0%). *Streptococcus mutans* NS, *Strept. mutans* HG985, *Streptococcus oralis* J22, and *Streptococcus sanguis* 12 were cultured in Todd Hewitt Broth (THB) slants containing (w/v): heat infusion (0.3%), peptone (2.0%), dextrose (0.2%), sodium bicarbonate (0.2%), sodium chloride (0.2%), and disodium phosphate (0.04%). *P. aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae* and, *Streptococcus pyogenes* were cultured in Trypticase Soy Broth (TSB) (OXOID, Basingstoke, England) slants containing (w/v): tryptose (2.0%), dextrose (0.1%), disodium phosphate (0.2%), potassium nitrate (0.1%), and agar (1.0%). *Candida albicans* was grown in Yeast Mold Agar (YMA) All the strains were grown at 37°C, with the exception of *C. albicans* (31°C). All media were obtained from Oxoid. Strains were stored at −80°C in the appropriate medium containing 15% (v/v) glycerol solution until they were used. Whenever required, frozen stocks were streaked on agar plates and incubated overnight at the optimum growing temperature for each strain for further culturing. Working stock cultures were kept at 4°C for up to 2 weeks [20].

Growth Conditions

The inoculum of *C. sphaerica* was prepared by transferring cells grown on a slant to 50 ml of Yeast Mold broth (YMB). The seed culture was incubated for 24 h at 28°C C and agitated at 150 rpm. The yeast was cultivated in submerged culture with shaking in a New Bruswick C-24 shaker. The production of the Lunasan biosurfactant was performed in distilled water-based medium with 9% of refinery residue of soybean oil and 9% of corn steep liquor. The medium was sterilized by autoclaving at 121°C for 20 min. The final pH of the medium was 5.3 and the surface tension before inoculation was 50 mN m^{-1}. The

inoculum (1% v/v) was introduced in the amount of 10^4 cells ml^{-1} to cool medium yeast. Cultivation was carried out in Erlenmeyer flasks at 27°C with shaking at 150 rpm for 144 h. At regular intervals, samples were withdrawn for analyses. All the assays were carried out in triplicate and did not vary more than 5%.

Isolation of Biosurfactant

After 144 h cultivation of *C. sphaerica* in the above-described conditions, the cell-free supernatant (9% of refinery residue of soybean oil and 9% of corn steep liquor) was submitted to an extraction process. The pH was adjusted to 2 with HCl 6 M and precipitated with two volumes of methanol. After resting for 24 h at 4°C, samples were *centrifuged* at 5000×*g* for 30 min, washed twice with cold methanol, and dried in an incubator at 37°C for 24–48 h, until constant weight. Afterward, the samples were kept in desiccators to reach the current weight and the biosurfactant yield ($g\ l^{-1}$) was determined. Known amounts of crude precipitate were resuspended in distilled water and used for measurement of the critical micelle concentration (CMC). All experiments were conducted in triplicate.

Determination of Superficial Tension and Critical Micelle Concentration (CMC)

The surface tension was measured by the ring method using a DuNouy Tensiometer model Sigma 70 (KSV Instruments LTD, Finland) at room temperature. The concentration at which micelles began to form was represented as the CMC. The CMC was automatically determined by measuring the surface tensions of the purified biosurfactant in distilled water up to a constant value of surface tension [24].

Determination of Antimicrobial Activity of Biosurfactant

The antimicrobial activity of the crude biosurfactant against several microbial strains was determined by the microdilution method [36, 39] in 96-well flat-bottom plastic tissue culture plates (Greiner Bio-One GmbH, Frickenhausen, Germany). For each strain, appropriate medium and temperature were used (as previously described); briefly, 125 µl of sterile, double-strength culture medium were placed into the first column of the 96-well microplate and 125 µl of sterile, single-strength culture medium in the remaining wells. Subsequently, 125 µl of biosurfactant solution (concentrations from 0.625 to 10 mg ml^{-1}) in PBS—phosphate- buffered (100 mg ml^{-1}) were added to the first column of the microplate and mixed with the medium; this results in a biosurfactant concentration of 50 mg ml^{-1} serially, 125 µl were transferred to the subsequent wells, discarding 125 µl of the mixture in the tenth column, so that the final volume for each well was 125 µl. This process results in twofold serial dilutions of the biosurfactant in the first 10 columns (10–0.625 mg ml^{-1}). Columns 11 and 12 did not contain biosurfactant and served as negative and growth controls, respectively. All the wells (except for the 11th column) were inoculated with 25 µl of an overnight culture at the defined optimum conditions, diluted to 10^8 CFU ml^{-1}). Microplates were covered and incubated for 48 h under the appropriate growth conditions for each microorganism. Triplicate assays were performed at all the biosurfactant concentrations for each strain. After 48 h of incubation, the absorbance at 600 nm (A600) was determined for each well. The growth inhibition percentages at different biosurfactant concentrations for each microorganism were calculated as:

$$\%\ \text{Growth inhibition}_c = [1 - (A_c/A_0)] \times 100$$

where A_c represents the absorbance of the well with a biosurfactant concentration c and A_0 the absorbance of the control well (without biosurfactant) [19].

Determination of Antiadhesion of Biosurfactant

The antiadhesive activity of the crude biosurfactant isolated from *Candida sphaerica* against several microbial strains was quantified according to the procedure described by Heinemann et al. [21]. Briefly, the wells of a sterile 96-well flat-bottomed plastic tissue culture plate (Greiner Bio-One GmbH) were filled with 200 µl of the crude biosurfactant. Several biosurfactant concentrations that were tested ranging from 0.625 to 10 mg ml^{-1} plate were incubated for 18 h at 4°C and subsequently washed twice with PBS. Control wells contained PBS buffer only. An aliquot of 200 of a washed bacterial or yeast suspension (10^8 CFU ml^{-1}) was added and incubated in the wells for 4 h at 4°C. Unattached microorganisms were removed by washing the wells three times with PBS. The adherent microorganisms were fixed with 200 µl of methanol (99% purity) per well, and after 15 min, the plates were emptied and left to dry. Then the plates were stained for 5 min with 200 µl of 2% crystal violet used for Gram staining per well. Excess stain was rinsed out by placing the plate under running tap water. Subsequently, the plates were air-dried, the dye bound to the adherent microorganisms was resolubilized with 200 µl of 33% (v/v) glacial acetic acid per well, and the absorbance of each well was measured at 595 nm. The microbial inhibition percentages at different biosurfactant concentrations for each microorganism were calculated as:

$$\% \text{ Microbial inhibition}_c = [1 - (A_c/A_0)] \times 100$$

where A_c represents the absorbance of the well with a biosurfactant concentration c and A_0 the absorbance of the control well. The microtitre-plate antiadhesion assay estimates the percentage of microbial adhesion reduction in relation to the control wells, which were set at 0% to indicate the absence of biosurfactant and therefore of its antiadhesion properties. In contrast, negative percentage results indicate the percentage increase in microbial adhesion at a given surfactant concentration in relation to the control. The microtitre-plate antiadhesion assay allows the estimation of the crude biosurfactant concentrations that are effective in decreasing adhesion of the microorganisms studied [20].

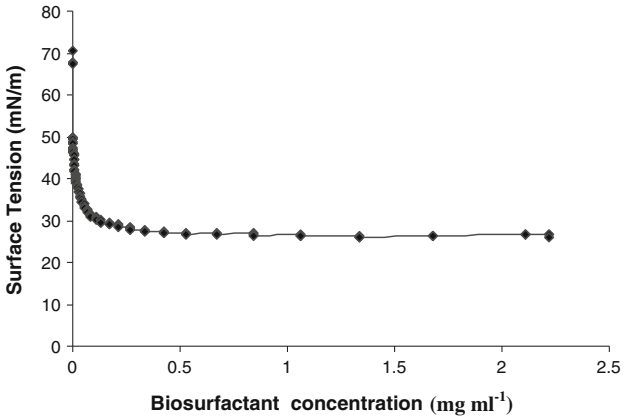

Fig. 1 Surface tension versus concentration of isolated biosurfactant produced by *Candida sphaerica* grow in distilled water supplemented with 9.0% of refinery residue of soybean oil and 9% corn steep liquor

Results and Discussion

The yield of the biosurfactant produced by *C. sphaerica* was 9 g l^{-1} after 144 h of experiment, which is in accordance with the values previously reported in the literature [26]. Sarubbo et al. [43] reported a yield of 8 g l^{-1} for a biosurfactant produced by *C. lipolytica* using canola oil and glucose as substrates. Also, Rufino et al. [42] obtained a yield of 8 g l^{-1} for the biosurfactant from *C. lipolytica* using yeast extract and soybean oil refinery residue is substrates. Furthermore, studies conducted by Sobrinho et al. [47] using two industrial refinery residue of soybean oil and corn steep liquor as carbon sources indicated a yield of 4.5 g l^{-1} of biosurfactant produced by *C. sphaerica.*

An important property of a biosurfactant is its ability to act in the formation of micelles which are aggregates of amphipathic molecules [3, 22]. Surface tension decreases as the surfactant concentration in an aqueous medium increases and micelles are formed. The critical micelle concentration (CMC) is the minimum biosurfactant concentration necessary to reduce the surface tension to the maximum extent. The biosurfactant from *C. sphaerica* showed a great surface tension reduction capacity since the water surface tension was reduced from 70 to 25 mN m^{-1} with the increase of the biosurfactant concentration up to CMC of 0.25 mg ml^{-1} (Fig. 1). From this point the increase of biosurfactant concentration did not lead to further reductions in water surface tension, indicating that the CMC had been reached. Results show that the biosurfactant produced by *C. sphaerica* possesses an increased capacity to reduce tension as compared to the biosurfactants from *C. lipolytica* (32 mN m^{-1}) [42], *C. glabrata* (31 mN m^{-1}) [44], *C. antarctica* (35 mN m^{-1}) [1], and *Yarrowia lipolytica* (50 mN m^{-1}) [18]. Furthermore, the biosurfactant produced in this study also showed a CMC that is much lower than the CMCs reported for other yeast surfactants, considering the rates of 2.5% for *C. glabrata* [27] biosurfactants, 1% for *C. lipolytica* biosurfactant grown in refinery waste [41], and 0.8 mg ml^{-1} for *C. sphaerica* [47].

The antimicrobial activity of the biosurfactant isolated from *Candida sphaerica* was determined by measuring the growth inhibition percentages obtained for several microorganisms (Table 1). The tested biosurfactant presented antimicrobial activity against all microorganisms used, although, depending on the microorganism, the biosurfactant presents different effective concentrations. The highest concentration of biosurfactant tested (10 mg ml^{-1}) showed high percentages of inhibition for *Streptococcus oralis* J22 (68%), *C. albicans* (57%), and *Staphylococcus epidermidis* (57.6%). The antimicrobial activity of the crude biosurfactant isolated from *Candida sphaerica* with concentrations between 5 and 10 mg ml^{-1} against *C. albicans, Staph. aureus* and *Staph. epidermidis* was less to that obtained with the biosurfactants isolated from *Lact. paracasei* ssp A20, which completely inhibited the growth of those microorganisms with concentrations between 25 and 50 mg ml^{-1}) [20]. The crude biosurfactant showed antimicrobial activity against a broad range of microorganisms, including Gram-positive and Gram-negative bacteria and yeasts. Biosurfactants antimicrobial activity has been described, as for example surfactin, a *cyclic* lipopeptide produced by *Bacillus subtilis* [32]. The antimicrobial activity of surfactin was tested against several microbes. All tested bacteria, except for *B. subtilis*, showed susceptibility to surfactin. *P. aeruginosa* was the most sensitive Gram-negative bacteria, while *E. coli*, *Salmonella choterasius*, and *Serratia marcescens* were inhibited in a lower level. Also, the lipopeptide affected the growth of Gram-positive bacteria, especially *Micrococcus luteus* and *Bacillus cereus* [35]. Other examples have been reported by Rodrigues et al. [35, 37, 38]. Crude biosurfactants

Table 1 Percentages of growth inhibition obtained with the crude biosurfactant isolated from *Candida sphaerica* at different concentrations (mg ml^{-1})

Microorganism	Biosurfactant (mg ml^{-1})				
	0.625	1.25	2.5	5	10
Lactobacillus casei	5.5 ± 0.2	12 ± 0.3	15 ± 0.1	30 ± 0.4	40.4 ± 0.2
Lactobacillus casei 72	5.5 ± 0.3	11 ± 0.2	13 ± 0.4	25 ± 0.3	43.3 ± 0.1
Lactobacillus reuteri 104R	10 ± 0.1	17.3 ± 0.3	22 ± 0.5	4.4 ± 0.3	46.5 ± 0.1
Lactobacillus reuteri ML1	8.5 ± 0.3	11 ± 0.2	16 ± 0.2	27 ± 0.2	49 ± 0.2
Streptococcus agalactiae[a]	7.3± 0.2	10 ± 0.3	11± 0.3	35 ± 0.2	46 ± 0.2
Streptococcus mutans	14 ± 0.1	17.8 ± 0.1	22.6 ± 0.1	38.3 ±0.1	40.2 ± 0.6
Streptococcus mutans NS	14.2 ± 0.3	15.6 ± 0.4	20 ± 0.3	23.8 ± 0.1	36 ± 0.1
Streptococcus mutans HG	22 ± 0.2	33.1 ± 0.1	45.6 ± 0.3	46± 0.4	48± 0.1
Streptococcus pyogenes	10.3 ± 0.6	15.4 ± 0.1	28 ± 0.1	32.2 ± 0.5	42.5 ± 0.2
Streptococcus sanguis 12	13.6± 0.3	15 ± 0.4	15.5 ± 0.5	28 ±0.8	39 ± 0.1
Streptococcus oralis J22	11 ± 0.4	13.2 ± 0.3	15.2 ± 0.3	30.7 ± 0.4	68 ± 0.2
Staphylococcus epidermidis[a]	8.3 ± 0.1	13.5 ± 0.2	25 ± 0.2	42± 0.1	57.6 ± 0.3
Staphylococcus aureus[a]	10.6± 0.1	20 ± 0.2	27.3 ± 0.3	32.2± 0.2	43.9± 0.1
Pseudomonas aeruginosa	7.7 ± 0.1	8.0 ± 0.1	12.5 ± 0.1	13.6 ±0.4	47 ± 0.2
Candida albicans[a]	12.5± 0.2	17.3 ± 0.3	32 ± 0.3	44.2 ± 0.1	57 ± 0.2

Results are expressed as means ± standard deviations of values obtained from triplicate experiments

[a] Pathogenic microorganisms

isolated from *Lactococcus lactis* 53 and *Streptococcus thermophilus* A showed antimicrobial activity against *C. troplicalis* GB in low concentrations. Some biosurfactants are able, even in low concentrations, to destabilize the microorganisms membranes, killing them or disabling their growth [10, 11]. The interest in biosurfactants was first expressed due to its potential antimicrobial properties, being the first reported and actually the most studied biosurfactants, rhamnolipid, and surfactin [45]. Gram-positive bacteria are more sensitive to biosurfactants than Gram-negative bacteria, which are weakly inhibited or not inhibited at all [15]. *C. bombicola* and *C. apicola* were reported to produce a glycolipid-type biosurfactant (sophorolipid) that inhibit the growth of *B. subtilis*, *S. epidermidis*, and *Streptococcus faecium* in concentrations between 6 and 29 mg l^{-1} [25]. Other glycolipids inhibit not only the growth of Gram-positive bacteria, but also Gram-negative ones, such as *E. coli* and *S. marcescens* [46]. Kitamoto et al. [23] reported in their study an antimicrobial activity against *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans* for a mannosylerythritol produced by *C. antarctica*, a sophorolipid produced by *C. apicola*, and a rhamnolipid produced by *P. aeruginosa.* Several biosurfactants that exhibit antimicrobial activity have been previously described. However, there are few reports about the antimicrobial activity of biosurfactants isolated from *Candida;* only biosurfactants obtained from *S. thermophilus* A and *L. lactis* 53 showed significant antimicrobial activity against several bacterial and yeast strains isolated from explanted voice prostheses [25].

Adhesion to surfaces and subsequent biofilm formation consist in a surviving strategy used by microorganisms in several hostile environments, protecting them from dehydration, predators, biocides and extreme conditions [13]. The antiadhesive activity of this biosurfactant was evaluated against a variety of bacterial and fungal strains. The biosurfactant showed antiadhesive activity against most of the microorganisms tested, but the antiadhesive effect depends on the concentration and the microorganism tested (Table 2).This biosurfactant was effective against all the microorganisms tested, albeit to different degree. With regard to the *Lactobacillus* strains, the antiadhesive activity was higher against *Lact. casei* (90%), *Lact. casei* 72 (72%), *Lact. reuteri* 104R (55%) and *Lact. reuteri* ML1 (40%). The pathogenic bacteria studied (*Streptococcus agalactiae*, *Staphylococus epidermidis, Staphylococus aureus*) a complete inhibition of adhesion was also achieved with biosurfactant concentrations of 10 mg ml^{-1}. Regarding the yeast, a total inhibition of adhesion was also observed for *C. albicans* at a biosurfactant concentration of 10 mg ml^{-1}. The highest percentages of adhesion inhibition were obtained for *P. aeruginosa* (100%), *Staphylococcus aureus* (100%), *Streptococcus oralis* J22 (97%), while low activity was obtained for *Streptococcus mutans* HG 985(50%) and *Staphylococcus epidermidis* GB (22%). The antiadhesive activity of the crude biosurfactant isolated from

Table 2 Antiadhesive properties of crude biosurfactant isolated from *Candida sphaerica*

Microorganism	Biosurfactant (mg ml^{-1})				
	0.625	1.25	2.5	5	10
Lactobacillus casei	53 ± 0.1	53 ± 0.2	56 ± 0.1	67 ± 0.3	90 ± 0.2
Lactobacillus casei 72	59 ± 0.3	61 ± 0.2	65 ± 0.4	70 ± 0.2	72 ± 0.1
Lactobacillus reuteri 104R	41 ± 0.1	42 ± 0.1	45 ± 0.4	50 ± 0.2	55 ± 0.1
Lactobacillus reuteri ML1	26 ± 0.2	28 ± 0.1	30 ± 0.3	34 ± 0.2	40± 0.2
Streptococcus agalactiae[a]	80± 0.1	86 ± 0.2	88± 0.2	92 ± 0.3	100 ± 0.2
Streptococcus mutans	58± 0.1	64 ± 0.1	67 ± 0.1	80 ±0.2	100± 0.1
Streptococcus mutans NS	60 ± 0.3	65 ± 0.2	68 ± 0.2	80 ± 0.3	100± 0.2
Streptococcus mutans HG	41 ± 0.2	42 ± 0.1	44± 0.2	47± 0.2	50± 0.1
Streptococcus pyogenes	33 ± 0.3	40 ± 0.1	42 ± 0.1	47± 0.5	49 ± 0.2
Streptococcus sanguis 12	80± 0.3	83 ± 0.4	87 ± 0.1	98 ±0.2	100± 0.1
Streptococcus oralis J22	77 ± 0.1	84 ± 0.1	88 ± 0.3	95 ± 0.4	97 ± 0.2
Staphylococcus epidermidis[a]	11 ± 0.1	12 ± 0.1	13 ± 0.2	19± 0.1	100± 0.3
Staphylococcus aureus[a]	75± 0.2	82± 0.3	85 ± 0.3	90± 0.2	100± 0.1
Pseudomonas aeruginosa	80 ± 0.2	82 ± 0.1	83± 0.3	89 ±0.2	92 ± 0.2
Candida albicans[a]	52± 0.3	56± 0.2	57 ± 0.1	64 ± 0.2	100 ± 0.2

Negative controls were set at 0% to indicate the absence of biosurfactant. Positive percentages indicate the reductions in microbial adhesion when compared to the control

[a] Pathogenic microorganisms

C. sphaerica completely inhibited the adhesion with a concentration of 10 mg ml^{-1} against *Streptococcus agalactiae, Streptococcus mutans, Streptococcus mutans* NS, *Streptococcus sanguis* 12, *Streptococcus.* These results were higher to that obtained with the biosurfactants isolated from *Lact. paracasei* ssp A20 [19]. A role of biosurfactants as defense weapons in competition with post-adhesion has been suggested for biosurfactants produced by *Streptococcus mitis* and *S. mutans* [8]. Besides possessing *antifungal, antibacterial and antiviral activities*, biosurfactants have also proved to be great inhibitors of microbial adhesion and of biofilm formation. For example, the biosurfactant released by *S. mitis* was found to reduce the adhesion of *Streptococcus. mutans* [33]. Similarly, *Lactobacillus fermentum* RC-14 releases surfactant compounds that can inhibit the adhesion of uropathogenic bacteria, including *Enterococcus faecalis*. The adsorption of a biosurfactant on surface was found to change its hydrophobicity, which might caused interference in the adhesion and desorption processes [17]. Furthermore, Velraeds et al. [48] reported the inhibition of adhesion of pathogenic enteric bacteria by a biosurfactant produced by *Lactobacillus fermetum* RC-14. The authors suggested the use of this antiadhesive agent in catheters aiming at *decreasing* biofilm formation. Falagas and Makris [16] have proposed the application of biosurfactants isolated from probiotic bacteria to patient care equipments (such as catheters and other medical insertional devices) in hospitals, with the aim of decreasing colonization by microorganisms responsible for nosocomial infections.

This study we have demonstrated the antimicrobial and antiadhesive properties of the new biosurfactant isolated from *C. sphaerica* against several pathogenic and non-pathogenic microorganisms. The results obtained suggest the possible use of this biosurfactant as an alternative antimicrobial agent in the medical field for applications against microorganisms responsible for diseases and infections in the urinary, vaginal, and gastrointestinal tracts, as well as in the skin, making it a suitable alternative to conventional antibiotics. Furthermore, due to its anti-adhesive activity, the biosurfactant can potentially be used as a coating agent for several medical devices, an application area not explored yet for biosurfactants obtained from yeasts.

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