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Antimicrobial and Anti-Adhesive Potential of a Biosurfactants Produced by *Candida* Species

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Additional information is available at the end of the chapter

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1. Introduction

Several compounds with tensioactive properties are synthesized by living organisms, from plants (e.g. *saponins*) to microorganisms (e.g. *glycolipids*) and humans (e.g. surface-active lipoprotein complex), being considered natural surfactants [1]. Additionally, these compounds have been produced through biotechnological processes broadening their diversity and potential applications [2]. 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 stationary growth on water-immiscible substrates. Generally, biosurfactants are secondary 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 [1].

Physical and chemical properties, surface tension reduction, and stability of the emulsion formed are important characteristics in 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 [3]. Biosurfactants have several advantages over chemical surfactants, such as lower toxicity, higher biodegradability and effectiveness at extreme temperatures or pH values [4]. 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 have been carried out [5]. Despite their potential and biological origin only a few studies have been carried out on applications related to the biomedical field [6]. Some biosurfactants are suitable alternatives to synthetic medicines and antimicrobial agents and may be used as safe and effective therapeutic agents [6].

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 [7]. 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 [8,9].

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*, *S. enterica*, *Escherichia coli* and *Proteus mirabilis* [10]. 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 [11].

Microbial surfactants are not yet competitive with those produced by the chemical industry, but efforts should be made on the different production aspects to find suitable and economic substrates and to develop new strategies to increase the volumetric productivity. We have shown that the co-utilization of ground-nut oil refinery residue and corn steep liquor is an attractive choice for biosurfactant production. The biosurfactant adhesive mechanism is based in the inhibition of microorganisms to different surfaces can interact with interfaces of the molecule. In this sense, they are an alternative to synthetic surface-active agents because of their low toxicity and biodegradability [7,12].

Considering the lack of studies with yeasts biosurfactants for medical purposes, and is an attractive characteristics showed by produced by the *Candida lipolytica* (Rufisan) and *Candida sphaerica* (Lunasan). In this sense the revision shown the role and applications of rufisan and lunasan biosurfactant as a antimicrobial and antiadhesive activities were investigated against pathogenic and nonpathogenic microorganisms, and indicated the therapeutic perspectives.. Results gathered in the current work showed the potential of those molecules in this field of application; however, its use still remains limited, possibly as due to high production costs, as well as on their toxicity towards human systems.

2. Adhesion and microbial biofilms

The interest of biosurfactants as alternative medicines and antimicrobial agents increased considering the safe use as effective therapeutic agent on human and animal cells [12]. In consequence, the microbial adhesion is mediated by specific interactions between cells surface structures and molecular mass on the substratum surface, or by non-specific interaction forces, including electrostatic forces, acid-base interactions and Vander Waals forces [13].

during exponential growth, presumably as a result of increased cell wall hydrophobicity during this growth phase

The conditioning film on the biomaterial surface (and on the bacterial cell surface) plays an important role, as it changes the physicochemical properties of the interacting surfaces. Albumin is a strong adhesion inhibitor, for unknown reasons, although changes in hydrophobicity and sterical hindrance are proposed as mechanisms [14].

The adhesion of microorganisms to a surface is one of the first stages in the development of a biofilm and is believed to be influenced by a number of factors. As the substrate is essential in the development of a biofilm, an understanding of how substrate properties affected the adherence of bacterial cells may assist in designing or modifying substrates inhibitory to bacterial adhesion. Many of these molecules are proteinaceous constitution, such as serum albumin, fibrogen and collagen, and some have been shown to affect subsequent bacterial adhesion [13].

The formation of infectious biofilm on biomaterial appeared to involve several sequential steps. Immediately after exposure of a device to body fluids, such as blood, saliva, or urine, macromolecular components adsorb to form a conditioning film [15]. The most microbial surfactants are complex molecules, comprising different structures that include peptides, glycolipids, glycopeptides, fatty acids and phospholipids, as reviewed recently. Among the many classes of biosurfactants, lipopeptides are particularly interesting because their high surface activities and antibiotic potential. Lipopeptides are molecules act as antibiotics, antiviral and antitumoral agents, and enzyme inhibitors. Those molecules enhance or decrease the bacterial surface hydrophobicity following that the surface is less or more hydrophobic [16]. Morikawa et al. [17] identified and characterized a biosurfactant, arthrofactin, produced by *Arthrobacter*.

Glycolipids are the most common class of biosurfactants of which the most effective from the point of view of surface active properties are the trehalose lipids of *Mycobacterium* and related bacteria, the rhamnolipids of *Pseudomonas* sp and the sophorolipids of yeasts [18]. Otto et al. [19] described the production of sophorose lipids from deproteinized whey concentrate by a two-stage process. Several antimicrobial, immunological and neurological properties have been attributed to mannosylerythritol lipid (MEL), a yeast glycolipid biosurfactant, produced from vegetable oils by *Candida* strains.

3. Antimicrobial activity of biosurfactant

Several biosurfactants which exhibit antimicrobial activity against various microorganisms have been previously described. They include surfactin and iturin produced by *Bacillus subtilis* strains [20], rhamnolipids from *Pseudomonas* species [21], mannosylerythritol lipids from *C. antarctica* [22] and biosurfactants produced by some fungi [23].

Among the genus *Bacillus*, *B. subtilis* produces a broad spectrum of bioactive lipopeptides which have a great potential for biotechnological and biopharmaceutical applications. The

characteristic structure of lipopeptides is a fatty acid combined with an amino-acid moiety. Several lipopeptides have potent antibiotic activity and have been the subject of several studies on the discovery of new antibiotics. The list includes surfactin, produced by *B. subtilis*, the most powerful of biosurfactant known to date. These compounds have many pharmacological activities: antibacterial, antifungal, antiviral, and antimycoplasma properties; inhibition of the fibrin clot formation and hemolysis; formation of ion channels in lipid bilayer membranes [24]; antitumour activity against Ehrlich's ascites carcinoma cells; and inhibition of the cyclic adenosine 3,5-monophosphate phosphodiesterase [25].

The evaluation of the antimicrobial activity of these compounds was carried out against 29 bacteria. *Enterococcus faecalis* (11 strains), *Staphylococcus aureus* (6 strains) and *Pseudomonas aeruginosa* (7 strains) and *Escherichia coli* CI 18 (1 strain) displayed a profile of well defined drug resistance. All strains were sensitive to the surfactants, in particular *Enterococcus faecalis*. The results demonstrated that lipopeptides have a broad spectrum of action, including antimicrobial activity against microorganisms with multidrug-resistant profiles [26].

The antimicrobial activity of the crude biosurfactant isolated from *Candida lipolytica* UCP 0988 was determined by measuring the growth inhibition percentages obtained for several microorganisms (Table 1).

| Microorganism | Growth inhibition (%) | | | | |
|-----------------------------------|-----------------------|-------------|-------------|-------------|-------------|
| | Biosurfactant mg/L | | | | |
| | 0.75 | 1.5 | 3.0 | 6.0 | 12.0 |
| <i>Lactobacillus casei</i> | 4.5 ± 0.02 | 9.1 ± 0.07 | 15 ± 0.02 | 27.2 ± 0.08 | 28.4 ± 0.05 |
| <i>Lactobacillus casei</i> 72 | 4.5 ± 0.03 | 14.2 ± 0.08 | 18.1 ± 0.06 | 32.9 ± 0.06 | 33.7 ± 0.03 |
| <i>Lactobacillus reuteri</i> 104R | 5.9 ± 0.07 | 9.7 ± 0.01 | 15.5 ± 0.01 | 24.4 ± 0.05 | 25.4 ± 0.08 |
| <i>Lactobacillus reuteri</i> ML1 | 8.2 ± 0.06 | 11.3 ± 0.02 | 16.3 ± 0.02 | 31.1 ± 0.02 | 32.1 ± 0.02 |
| <i>Escherichia coli</i> | 0 | 0 | 3.0 ± 0.01 | 5.0 ± 0.01 | 5.0 ± 0.01 |
| <i>Streptococcus agalactiae</i> | 0 | 10.9 ± 0.03 | 35.3 ± 0.03 | 35.8 ± 0.02 | 35.5 ± 0.02 |
| <i>Streptococcus mutans</i> NS | 15.6 ± 0.07 | 20.1 ± 0.04 | 23.8 ± 0.13 | 46.0 ± 0.1 | 46.4 ± 0.01 |
| <i>Streptococcus sanguis</i> 12 | 13.7 ± 0.07 | 21.5 ± 0.04 | 31.7 ± 0.05 | 48.1 ± 0.08 | 48.0 ± 0.01 |
| <i>Streptococcus mutans</i> | 22.5 ± 0.02 | 34.8 ± 0.01 | 44.6 ± 0.01 | 58.3 ± 0.01 | 58.0 ± 0.06 |
| <i>Streptococcus oralis</i> J22 | 12.8 ± 0.04 | 13.2 ± 0.03 | 14.2 ± 0.03 | 18.7 ± 0.08 | 62.8 ± 0.06 |
| <i>Streptococcus mutans</i> HG985 | 41.8 ± 0.02 | 43.1 ± 0.01 | 55.6 ± 0.04 | 64.6 ± 0.03 | 64.9 ± 0.01 |
| <i>Pseudomonas aeruginosa</i> | 0 | 7.9 ± 0.02 | 10 ± 0.01 | 11.6 ± 0.01 | 16.5 ± 0.04 |
| <i>Staphylococcus aureus</i> | 0 | 0 | 1.57 ± 0.06 | 3.15 ± 0.06 | 15.1 ± 0.03 |
| <i>Staphylococcus epidermidis</i> | 10.1 ± 0.01 | 12.9 ± 0.07 | 14.9 ± 0.07 | 18.1 ± 0.01 | 18.0 ± 0.06 |
| <i>Candida albicans</i> | 0 | 0 | 3.1 ± 0.03 | 5.95 ± 0.01 | 6.0 ± 0.02 |

Table 1. Percentages of growth inhibition obtained with the biosurfactant Rufisan isolated from *Candida lipolytica* at different concentrations (mg/l). Results are expressed as means ± standard deviations of values obtained from triplicate experiments

The biosurfactant was effective against the microorganisms tested, albeit to different degrees. The highest anti-adhesive percentages were obtained for a biosurfactant

concentration of 12 mg/l or 4xCMC. Non-pathogenic species associated with the oral cavity of *Streptococcus* were used (*S. mutans* HG – 64.9%; *S. oralis* J22 – 62.8%; *S. mutans* – 58%; *S. sanguis* 12 – 48%; *S. mutans* NS – 46%). On the other hand, the biosurfactant did not show an effective antimicrobial activity against the *Lactobacillus* strains studied. It inhibited only 32.1% of the growth of *L. reuteri* ML1 at the maximum concentration tested (12 mg/l).

The growth of the other microorganisms tested was poorly inhibited. Percentages of 5%, 5%, 15%, 16% and 18% were observed for *C. albicans*, *E. coli*, *S. aureus*, *P. aeruginosa* and *S. epidermidis*, respectively.

The antimicrobial activity of the biosurfactant isolated from *Candida sphaerica* was determined by measuring the growth inhibition percentages obtained for several microorganisms (Table 2).

| Microorganism | Growth inhibition (%) Biosurfactant mg/L | | | | |
|-----------------------------------|---|------------|------------|------------|------------|
| | 0.625 | 1.25 | 2.5 | 5.0 | 10.0 |
| <i>Lactobacillus casei</i> | 5.5 ± 0.2 | 12.0 ± 0.3 | 15.0 ± 0.1 | 30.0 ± 0.4 | 40.4 ± 0.2 |
| <i>Lactobacillus casei</i> 72 | 5.5 ± 0.3 | 11.0 ± 0.2 | 13.0 ± 0.4 | 25.0 ± 0.3 | 43.3 ± 0.1 |
| <i>Lactobacillus reuteri</i> 104R | 10.0 ± 0.1 | 17.3 ± 0.3 | 22.0 ± 0.5 | 4.40 ± 0.3 | 46.5 ± 0.1 |
| <i>Lactobacillus reuteri</i> ML1 | 8.5 ± 0.3 | 11.0 ± 0.2 | 16.0 ± 0.2 | 27.0 ± 0.2 | 49.0 ± 0.2 |
| <i>Escherichia coli</i> | 7.3 ± 0.2 | 10.0 ± 0.3 | 11.0 ± 0.3 | 35.0 ± 0.2 | 46.0 ± 0.2 |
| <i>Streptococcus agalactiae</i> | 14.0 ± 0.1 | 17.8 ± 0.1 | 22.6 ± 0.1 | 38.3 ± 0.1 | 40.2 ± 0.6 |
| <i>Streptococcus mutans</i> NS | 14.2 ± 0.3 | 15.6 ± 0.4 | 20.0 ± 0.3 | 23.8 ± 0.1 | 36.0 ± 0.1 |
| <i>Streptococcus sanguis</i> 12 | 22 ± 0.2 | 33.1 ± 0.1 | 45.6 ± 0.3 | 46.0 ± 0.4 | 48.0 ± 0.1 |
| <i>Streptococcus mutans</i> | 10.3 ± 0.6 | 15.4 ± 0.1 | 28.0 ± 0.1 | 32.2 ± 0.5 | 42.5 ± 0.2 |
| <i>Streptococcus oralis</i> J22 | 13.6 ± 0.3 | 15.0 ± 0.4 | 15.5 ± 0.5 | 28.0 ± 0.8 | 39.0 ± 0.1 |
| <i>Streptococcus mutans</i> HG985 | 11.0 ± 0.4 | 13.2 ± 0.3 | 15.2 ± 0.3 | 30.7 ± 0.4 | 68.0 ± 0.2 |
| <i>Pseudomonas aeruginosa</i> | 8.3 ± 0.1 | 13.5 ± 0.2 | 25.0 ± 0.2 | 42.0 ± 0.1 | 57.6 ± 0.3 |
| <i>Staphylococcus aureus</i> | 10.6 ± 0.1 | 20.0 ± 0.2 | 27.3 ± 0.3 | 32.2 ± 0.2 | 43.9 ± 0.1 |
| <i>Staphylococcus epidermidis</i> | 7.7 ± 0.1 | 8.0 ± 0.1 | 12.5 ± 0.1 | 13.6 ± 0.4 | 47.0 ± 0.2 |
| <i>Candida albicans</i> | 12.5 ± 0.2 | 17.3 ± 0.3 | 32.0 ± 0.3 | 44.2 ± 0.1 | 57.0 ± 0.2 |

Table 2. Percentages of growth inhibition obtained with the crude biosurfactant Lunasan isolated from *Candida sphaerica* at different concentrations (mg/l). Results are expressed as means ± standard deviations of values obtained from triplicate experiments

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) [27].

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* [28]. 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 choleraesuis* 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* [18]. Other examples have been reported by Rodrigues and co-workers [8, 27]. Crude biosurfactants isolated from *Lactococcus lactis* 53 and *Streptococcus thermophilus* A showed antimicrobial activity against *C. tropicalis* GB in low concentrations.

Some biosurfactants are able, even in low concentrations, to destabilize the microorganism's membranes, killing them or disabling their growth [29, 30]. 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 [31]. Gram-positive bacteria are more sensitive to biosurfactants than Gram-negative bacteria, which are weakly inhibited or not inhibited at all [32]. *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 [33]. Other glycolipids inhibit not only the growth of Gram-positive bacteria, but also Gram-negative ones, such as *E. coli* and *S. marcescens* [31]. Kitamoto et al. [34] reported in their work an antimicrobial activity against *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans* for a mannosylerythritol reduced 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 [35].

4. Anti-adhesive activity of biosurfactant

Involvement of biosurfactants in microbial adhesion and desorption has been widely described, and adsorption of biosurfactants to solid surfaces might constitute an effective strategy to reduce microbial adhesion and combating colonization by pathogenic microorganisms, not only in the biomedical field, but also in other areas, such as the food industry [36, 37].

Biosurfactants have been found to inhibit the adhesion of pathogenic organisms to solid surfaces or to infection sites, thus prior adhesion of biosurfactant to solid surfaces might constitute a new and effective means of combating colonization by pathogenic microorganisms [12]. Precoating vinyl urethral catheters by running a surfactin solution through them before inoculation with media resulted in a decrease of the amount of biofilm formed by *Salmonella typhimurium*, *Salmonella enteric*, *Escherichia coli* and *Proteus mirabilis* [38]. 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 to the antimicrobial properties, the anti-adhesive activity of the biosurfactant was evaluated against a variety of bacterial and fungal strains. The biosurfactant showed anti-adhesive activity against most of the microorganisms tested, but the anti-adhesive effect depends on the concentration and the micro-organism tested (Table 3).

| Microorganism | Anti-adhesive activity (%) | | | | | PBS (control) |
|-----------------------------------|----------------------------|--------|--------------------|--------|--------|------------------|
| | 0.75 | 1.5 | Biosurfactant mg/L | | 12.0 | |
| | | | 3.0 | 6.0 | | |
| <i>Lactobacillus casei</i> | 91±0.1 | 91±0.1 | 99±0.1 | 99±0.1 | 99±0.1 | 0 |
| <i>Lactobacillus casei</i> 72 | 81±0.0 | 87±0.0 | 89±0.0 | 91±0.0 | 95±0.0 | 0 |
| <i>Lactobacillus reuteri</i> 104R | 84±0.2 | 93±0.2 | 94±0.2 | 95±0.2 | 97±0.2 | 0 |
| <i>Lactobacillus reuteri</i> ML1 | 81±0.0 | 82±0.0 | 84±0.0 | 87±0.0 | 89±0.0 | 0 |
| <i>Escherichia coli</i> | 8±0.1 | 17±0.1 | 18±0.1 | 25±0.1 | 27±0.1 | 0 |
| <i>Streptococcus agalactiae</i> | 80±0.0 | 81±0.0 | 81±0.0 | 84±0.0 | 96±0.0 | 0 |
| <i>Streptococcus mutans</i> NS | 91±0.1 | 95±0.1 | 96±0.1 | 98±0.1 | 99±0.1 | 0 |
| <i>Streptococcus sanguis</i> 12 | 61±0.0 | 62±0.0 | 68±0.0 | 70±0.0 | 77±0.0 | 0 |
| <i>Streptococcus mutans</i> | 76±0.1 | 84±0.1 | 85±0.1 | 88±0.1 | 97±0.1 | 0 |
| <i>Streptococcus oralis</i> J22 | 73±0.0 | 85±0.0 | 87±0.0 | 89±0.0 | 90±0.0 | 0 |
| <i>Streptococcus mutans</i> HG985 | 76±0.1 | 76±0.1 | 81±0.1 | 83±0.1 | 85±0.1 | 0 |
| <i>Pseudomonas aeruginosa</i> | 13±0.0 | 26±0.0 | 33±0.0 | 41±0.0 | 49±0.0 | 0 |
| <i>Staphylococcus aureus</i> | 88±0.0 | 91±0.0 | 92±0.0 | 97±0.0 | 98±0.0 | 0 |
| <i>Staphylococcus epidermidis</i> | 2±0.0 | 5±0.0 | 6±0.0 | 16±0.0 | 21±0.0 | 0 |
| <i>Candida albicans</i> | 8±0.0 | 8±0.0 | 16±0.0 | 36±0.0 | 51±0.0 | 0 |

Table 3. Anti-adhesive properties of crude biosurfactant isolated from *Candida lipolytica*. 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. Results are expressed as means ± standard deviation of results from triplicate experiments

The crude biosurfactant showed anti-adhesive activity against most of the microorganisms tested from the minimum concentration used (0.75 mg/l). The anti-adhesive property was proportional to the concentration of the biosurfactant. For the microorganisms of the *Lactobacillus* anti-adhesive values around 81% were observed at the minor concentration tested (0.75 mg/l). The major anti-adhesive specificity was observed against *L. casei* with

values of 91% and 99% with the minimum concentration used. Low inhibitions were observed for *S. epidermidis* and *E. coli*, with values of 27% and 21%, respectively, at the maximum biosurfactant concentration. For the other microorganisms, the anti-adhesive activity was above 45%.

Gudina et al. [24] observed an anti-adhesive activity for the biosurfactant from *Lactobacillus paracasei* against several pathogenic microorganisms such as *S. aureus*, *S. epidermidis* and *S. agalactiae*. However, this biosurfactant showed low anti-adhesive activity against *E. coli*, *C. albicans* and *P. aeruginosa*, in contrast with the antimicrobial activity exhibited against these strains at the same biosurfactant concentrations.

The use and potential commercial applications of biosurfactants in the medical field has increased considerably in the last years. Their antimicrobial and anti-adhesive properties make them relevant molecules for use in combating many diseases and infections and as therapeutic agents [18].

| Microorganism | Anti-adhesive activity (%) | | | | |
|-----------------------------------|----------------------------|--------------------|----------|----------|-----------|
| | 0.625 | Biosurfactant mg/L | | 5.0 | 10.0 |
| | | 1.25 | 2.5 | | |
| <i>Lactobacillus casei</i> | 53 ± 0.1 | 53 ± 0.2 | 56 ± 0.1 | 67 ± 0.3 | 90 ± 0.2 |
| <i>Lactobacillus casei</i> 72 | 59 ± 0.3 | 61 ± 0.2 | 65 ± 0.4 | 70 ± 0.2 | 72 ± 0.1 |
| <i>Lactobacillus reuteri</i> 104R | 41 ± 0.1 | 42 ± 0.1 | 45 ± 0.4 | 50 ± 0.2 | 55 ± 0.1 |
| <i>Lactobacillus reuteri</i> ML1 | 26 ± 0.2 | 28 ± 0.1 | 30 ± 0.3 | 34 ± 0.2 | 40 ± 0.2 |
| <i>Streptococcus agalactiae</i> | 80 ± 0.1 | 86 ± 0.2 | 88 ± 0.2 | 92 ± 0.3 | 100 ± 0.2 |
| <i>Streptococcus mutans</i> | 58 ± 0.1 | 64 ± 0.1 | 67 ± 0.1 | 80 ± 0.2 | 100 ± 0.1 |
| <i>Streptococcus mutans</i> NS | 60 ± 0.3 | 65 ± 0.2 | 68 ± 0.2 | 80 ± 0.3 | 100 ± 0.2 |
| <i>Streptococcus mutans</i> HG | 41 ± 0.2 | 42 ± 0.1 | 44 ± 0.2 | 47 ± 0.2 | 50 ± 0.1 |
| <i>Streptococcus pyogenes</i> | 33 ± 0.3 | 40 ± 0.1 | 42 ± 0.1 | 47 ± 0.5 | 49 ± 0.2 |
| <i>Streptococcus sanguis</i> 12 | 80 ± 0.3 | 83 ± 0.4 | 87 ± 0.1 | 98 ± 0.2 | 100 ± 0.1 |
| <i>Streptococcus oralis</i> J22 | 77 ± 0.1 | 84 ± 0.1 | 88 ± 0.3 | 95 ± 0.4 | 97 ± 0.2 |
| <i>Streptococcus salivarius</i> | 92 ± 0.4 | 93 ± 0.2 | 95 ± 0.4 | 97 ± 0.4 | 100 ± 0.2 |
| <i>Staphylococcus epidermidis</i> | 11 ± 0.1 | 12 ± 0.1 | 13 ± 0.2 | 19 ± 0.1 | 22 ± 0.3 |
| <i>Staphylococcus aureus</i> | 75 ± 0.2 | 82 ± 0.3 | 85 ± 0.3 | 90 ± 0.2 | 92 ± 0.1 |
| <i>Escherichia coli</i> | 89 ± 0.4 | 93 ± 0.2 | 96 ± 0.1 | 97 ± 0.2 | 99 ± 0.2 |
| <i>Pseudomonas aeruginosa</i> | 80 ± 0.2 | 82 ± 0.1 | 83 ± 0.3 | 89 ± 0.2 | 92 ± 0.2 |
| <i>Candida albicans</i> | 52 ± 0.3 | 56 ± 0.2 | 57 ± 0.1 | 64 ± 0.2 | 71 ± 0.2 |
| <i>Candida tropicalis</i> | 80 ± 0.3 | 85 ± 0.2 | 87 ± 0.1 | 98 ± 0.3 | 100 ± 0.1 |
| <i>Rothia dentocariosa</i> | 55 ± 0.3 | 57 ± 0.2 | 60 ± 0.4 | 68 ± 0.3 | 72 ± 0.2 |

Table 4. Anti-adhesive properties of crude biosurfactant isolated from *Candida sphaerica*. 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

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 [22]. 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 4).

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%). For the pathogenic bacteria studied (*Streptococcus agalactiae*, *Streptococcus mutans*, *Streptococcus mutans* NS, *Streptococcus sanguis* 12, *Streptococcus salivarius* GB and *Echerichia coli*), 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. tropicalis* GB at a biosurfactant concentration of 10 mg ml⁻¹. The highest percentages of adhesion inhibition were obtained for *P. aeruginosa* (92%), *Staphylococcus aureus* (92%), *Streptococcus oralis* J22 (97%) and *Rothia dentocariosa* GBJ (72%), 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 *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 salivarius* GB and *Echerichia coli*. These results were higher to that obtained with the biosurfactants isolated from *Lact. paracasei* ssp A20 [24]. A role of biosurfactants as defense weapons in competition with post-adhesion has been suggested for biosurfactants produced by *Streptococcus mitis* and *S. mutans* [39].

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* [40]. 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 [41]. Furthermore, Velraeds et al. [42] reported the inhibition of adhesion of pathogenic enteric bacteria by a biosurfactant produced by *Lactobacillus fermentum* RC-14. The authors suggested the use of this anti-adhesive agent in catheters aiming at decreasing biofilm formation. Falagas and Makris [37] 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.

5. Conclusion

In conclusion, in this work we have demonstrated the antimicrobial and anti-adhesive properties of the crude biosurfactant isolated from *C. lipolytica* UCP0988 against several pathogenic and nonpathogenic microorganisms, including bacteria, yeasts and filamentous fungi. 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, making it a suitable alternative to conventional antibiotics.

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