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ORIGINAL ARTICLE

Antimicrobial and antiadhesive properties of a biosurfactant isolated from *Lactobacillus paracasei* ssp. *paracasei* A20

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Abstract

Aims: The aim of this study was to determine the antimicrobial and antiadhesive properties of a biosurfactant isolated from *Lactobacillus paracasei* ssp. *paracasei* A20 against several micro-organisms, including Gram-positive and Gram-negative bacteria, yeasts and filamentous fungi.

Methods and Results: Antimicrobial and antiadhesive activities were determined using the microdilution method in 96-well culture plates. The biosurfactant showed antimicrobial activity against all the micro-organisms assayed, and for twelve of the eighteen micro-organisms (including the pathogenic *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus agalactiae*), the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were achieved for biosurfactant concentrations between 25 and 50 mg ml^{-1}. Furthermore, the biosurfactant showed antiadhesive activity against most of the micro-organisms evaluated.

Conclusions: As far as we know, this is the first compilation of data on antimicrobial and antiadhesive activities of biosurfactants obtained from lactobacilli against such a broad group of micro-organisms. Although the antiadhesive activity of biosurfactants isolated from lactic acid bacteria has been widely reported, their antimicrobial activity is quite unusual and has been described only in a few strains.

Significance and Impact of the Study: The results obtained in this study regarding the antimicrobial and antiadhesive properties of this biosurfactant opens future prospects for its use against micro-organisms 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.

Introduction

Biosurfactants are amphiphilic compounds produced by micro-organisms with pronounced surface and emulsifying activities. These molecules exhibit a distinct tendency to accumulate at the interface between fluid phases that show different degrees of polarity and hydrogen bonding, such as oil and water or air and water, reducing the surface and interfacial tension (Van Hamme *et al.* 2006).

Different groups of biosurfactants exhibit diverse properties and display a variety of physiological functions in the producer micro-organisms; these include enhancing the solubility of hydrophobic/water-insoluble compounds, heavy metal binding, bacterial pathogenesis, cell adhesion and aggregation, quorum sensing and biofilm formation (Ron and Rosenberg 2001; Singh and Cameotra 2004).

Several biosurfactants exhibit antibacterial, antifungal and antiviral activities, which make them relevant molecules for applications in combating many diseases and infections. Biosurfactants with known antimicrobial activity include surfactin and iturin produced by *Bacillus subtilis* strains (Ahimou *et al.* 2000), mannosylerythritol lipids from *Candida antarctica* (Arutchelvi *et al.* 2008), rhamnolipids from *Pseudomonas aeruginosa* (Benincasa

et al. 2004) and biosurfactants isolated from *Streptococcus thermophilus* A and *Lactococcus lactis* 53 (Rodrigues *et al.* 2004, 2006b,c).

Another valuable application of biosurfactants is their use as antiadhesive agents against pathogens. Adsorption of biosurfactants to a substratum surface modifies its hydrophobicity, interfering in the microbial adhesion and desorption processes (Rodrigues *et al.* 2006a); in that sense, the release of biosurfactants by probiotic bacteria *in vivo* can be considered as a defence weapon against other colonizing strains in the urogenital and gastrointestinal tracts (Van Hoogmoed *et al.* 2004). Biosurfactants produced by lactobacilli have been shown to reduce adhesion of pathogenic micro-organisms to glass (Velraeds *et al.* 1996), silicone rubber (Busscher *et al.* 1997), surgical implants (Gan *et al.* 2002) and voice prostheses (Rodrigues *et al.* 2004, 2006d). Consequently, previous adsorption of biosurfactants can be used as a preventive strategy to delay the onset of pathogenic biofilm growth on catheters and other medical insertional materials, reducing the use of synthetic drugs and chemicals (Rodrigues *et al.* 2006a; Singh *et al.* 2007; Falagas and Makris 2009).

The aim of this study was to determine the antimicrobial and antiadhesive properties of a biosurfactant isolated from *Lact. paracasei* ssp. *paracasei* A20 against a group of pathogenic and nonpathogenic micro-organisms.

Materials and methods

Strains and culture conditions

A lactobacilli strain isolated from a Portuguese dairy plant, *Lact. paracasei* ssp. *paracasei* A20, was used for biosurfactant production. This strain was found to be a biosurfactant-producing strain in a previous work (*data not shown*).

The strain was stored at −80°C in MRS broth (Oxoid, Basingstoke, UK) containing 15% (v/v) glycerol solution until it was used. Whenever required, frozen stocks were streaked on MRS agar plates and incubated overnight at 37°C for further culturing. Working stock cultures were kept at 4°C for up to 2 weeks.

Several strains 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 MRS broth; *Streptococcus mutans* NS, *Strept. mutans* HG985, *Streptococcus oralis* J22 and *Streptococcus sanguis* 12 were cultured in Todd-Hewitt Broth; *Escherichia coli*, *Ps. aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae* and *Streptococcus pyogenes* were cultured in trypticase soy broth; *Candida albicans* was grown in yeast mould broth; the strains *Malassezia* sp., *Trichophyton mentagrophytes* and *Trichophyton rubrum* were cultured in Sabouraud dextrose broth (all media were obtained from Oxoid). All the strains were grown at 37°C, with the exception of *C. albicans* (31°C), *T. mentagrophytes* (26°C) and *T. rubrum* (26°C). 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.

Biosurfactant production and isolation

For crude biosurfactant production by *Lact. paracasei* ssp. *paracasei* A20 in flasks, 600 ml of culture broth were inoculated with 6 ml of an overnight subculture and incubated for 72 h at 37°C and 120 rev min^{-1}. The growth media used for the production of biosurfactant was MRS-Lac medium (standard medium where glucose was replaced by lactose). After 72 h, cells were harvested by centrifugation (10 000 ***g***, 5 min, 10°C), washed twice in demineralized water and resuspended in 100 ml of phosphate-buffered saline (PBS: 0·01 mol l^{-1} KH_2PO_4/K_2HPO_4 and 0·15 mol l^{-1} NaCl, adjusted to pH 7·0). The suspension was kept at room temperature for 2 h with gentle stirring for biosurfactant release (Rodrigues *et al.* 2006b,c). Subsequently, bacterial cells were removed by centrifugation, and the remaining supernatant liquid was filtered through a 0·22-μm pore-size filter (Millipore, Bedford, MA, USA). The supernatant was dialysed against demineralized water at 4°C in a Cellu-Sep© membrane (molecular weight cut-off 6000–8000 Dalton; Membrane Filtration Products, Seguin, TX, USA) and freeze-dried. Once dried, the biosurfactant was stored at −20°C for further studies. To confirm the biosurfactant production, the surface tension was routinely measured using the Ring method as previously described (Kim *et al.* 2000).

Antimicrobial assays

The antimicrobial activity of the crude biosurfactant against several microbial strains was determined by the microdilution method 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 in PBS (100 mg ml^{-1}) were added to the first column of the mi-

croplate 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 two-fold serial dilutions of the biosurfactant in the first 10 columns (50–0·09 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 2·5 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 micro-organism. Triplicate assays were performed at all the biosurfactant concentrations for each strain.

After 48 h of incubation, the absorbance at 600 nm (A_{600}) was determined for each well. The growth inhibition percentages at different biosurfactant concentrations for each micro-organism 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).

The minimum inhibitory concentration (MIC) was determined for each strain as the lowest concentration of biosurfactant that completely inhibits measurable growth ($A_{600} = 0$). To establish the minimum bactericidal concentration (MBC), 100 μl of each well with no visible growth were transferred into a tube with 5 ml of the appropriate medium and were incubated for 5 days under appropriate temperature. After that, the A_{600} of each tube was determined. The lowest concentration of biosurfactant that did not allow growth was considered as the MBC for that strain. Tubes that showed growth were considered to come from wells with bacteriostatic concentration of biosurfactant.

Anti-adhesion assays

The antiadhesive activity of the crude biosurfactant isolated from *Lact. paracasei* ssp. *paracasei* A20 against several microbial strains was quantified according to the procedure described by Heinemann *et al.* (2000). 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 were tested ranging from 3 to 50 mg ml^{-1}. The plate was incubated for 18 h at 4°C and subsequently washed twice with PBS. Control wells contained PBS buffer only. An aliquot of 200 μl 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 micro-organisms were removed by washing the wells three times with PBS. The adherent micro-organisms 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 micro-organisms 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 micro-organism 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 anti-adhesion 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 anti-adhesion assay allows the estimation of the crude biosurfactant concentrations that are effective in decreasing adhesion of the micro-organisms studied.

Results

Antimicrobial activity

The antimicrobial activity of the crude biosurfactant isolated from *Lact. paracasei* ssp. *paracasei* A20 was determined by measuring the growth inhibition percentages obtained for several micro-organisms (Table 1). From those results, the MIC for each micro-organism was determined. Furthermore, whenever possible, the MBC was also determined.

This biosurfactant was effective against all the micro-organisms tested, albeit to different degrees. With regard to the nonpathogenic *Lactobacillus* strains and the *Streptococcus* species associated with the oral cavity (*Strep. sanguis*, *Strep. mutans* and *Strep. oralis*), in all the micro-organisms a complete growth inhibition was observed for biosurfactant concentrations between 25 and 50 mg ml^{-1}, except for cariogenic *Strep. mutans* NS and *Strep. mutans* HG985. Regarding the pathogenic bacteria studied

Table 1. Percentages of growth inhibition obtained with the crude biosurfactant isolated from *Lactobacillus paracasei* ssp. *paracasei* A20 at different concentrations (mg ml^{-1}). Results are expressed as means ± standard deviations of values obtained from triplicate experiments

	[Biosurfactant] (mg ml^{-1})				
Micro-organism	3·12	6·25	12·5	25·0	50·0
Candida albicans	56·3 ± 0·7	65·3 ± 0·6	77·3 ± 0·7	89·9 ± 0·7	100·0 ± 0·0*
Escherichia coli	59·0 ± 0·5	72·8 ± 0·6	89·6 ± 0·8	100·0 ± 0·0	100·0 ± 0·0*
Lactobacillus casei 36	53·5 ± 0·6	69·8 ± 1·3	85·3 ± 0·5	100·0 ± 0·0*	100·0 ± 0·0
Lact. casei 72	61·7 ± 0·8	74·3 ± 0·9	84·3 ± 0·4	100·0 ± 0·0*	100·0 ± 0·0
Lactobacillus reuteri 104R	60·8 ± 0·7	75·5 ± 0·7	83·7 ± 0·5	96·0 ± 0·5	100·0 ± 0·0*
Lact. reuteri ML1	62·8 ± 0·7	76·8 ± 0·4	84·5 ± 0·4	94·5 ± 0·6	100·0 ± 0·0*
Malassezia sp.	40·0 ± 0·3	49·5 ± 0·6	54·3 ± 0·7	62·5 ± 0·4	71·6 ± 1·5
Pseudomonas aeruginosa	50·9 ± 0·8	58·8 ± 0·6	69·3 ± 0·6	83·5 ± 1·1	91·5 ± 0·5
Staphylococcus aureus	63·1 ± 0·4	75·9 ± 1·0	86·4 ± 0·6	95·2 ± 0·5	100·0 ± 0·0*
Staphylococcus epidermidis	71·4 ± 0·7	85·9 ± 0·9	91·0 ± 0·7	96·7 ± 0·6	100·0 ± 0·0*
Streptococcus agalactiae	51·5 ± 0·8	64·3 ± 0·6	76·8 ± 0·8	100·0 ± 0·0	100·0 ± 0·0*
Streptococcus mutans HG 985	48·5 ± 0·9	57·7 ± 0·7	67·5 ± 0·6	74·8 ± 1·3	88·3 ± 0·9
Strep. mutans NS	40·0 ± 1·0	51·8 ± 0·6	64·1 ± 2·2	74·1 ± 0·7	83·9 ± 0·8
Streptococcus oralis J22	44·3 ± 0·6	56·9 ± 1·0	73·6 ± 1·0	100·0 ± 0·0	100·0 ± 0·0*
Streptococcus pyogenes	57·8 ± 0·4	68·8 ± 0·5	81·6 ± 0·4	100·0 ± 0·0	100·0 ± 0·0*
Streptococcus sanguis 12	52·0 ± 0·6	61·7 ± 0·6	75·9 ± 0·6	88·6 ± 0·7	100·0 ± 0·0*
Trichophyton mentagrophytes	41·3 ± 0·6	51·9 ± 0·5	62·7 ± 0·7	70·3 ± 1·1	78·6 ± 0·9
Trichophyton rubrum	47·2 ± 0·4	56·6 ± 0·5	65·3 ± 0·5	76·8 ± 0·7	86·1 ± 0·7

*Indicates the minimum bactericidal concentration (MBC).

(*E. coli*, *Ps. aeruginosa*, *Staph. aureus*, *Staph. epidermidis*, *Strep. pyogenes* and *Strep. agalactiae*), with the exception of *Ps. aeruginosa*, a complete growth inhibition was also achieved for all the micro-organisms with biosurfactant concentrations between 25 and 50 mg ml^{-1}. Regarding the pathogenic yeasts, a total growth inhibition was observed for *C. albicans* with a biosurfactant concentration of 50 mg ml^{-1}, but not for *Malassezia* sp. under any of biosurfactant concentrations tested. The same occurred with the skin-associated pathogenic fungi (*T. mentagrophytes* and *T. rubrum*).

For all the micro-organisms studied, the antimicrobial activity was observed even at low biosurfactant concentrations, and a complete growth inhibition was achieved for 12 of the 18 micro-organisms at the highest biosurfactant concentration assayed (50 mg ml^{-1}). Furthermore, it is noticeable that even when MIC and MBC were not achieved, a high growth inhibition was observed (from 71·6 to 91·5%) with the highest biosurfactant concentration assayed (50 mg ml^{-1}).

Antiadhesive activity

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 micro-organisms tested, but the antiadhesive effect depends on the concentration and the micro-organism tested (Table 2).

With regard to the nonpathogenic *Lactobacillus* strains, the antiadhesive activity was higher against *Lact. reuteri* strains (77·6–78·8% inhibition) than against *Lact. casei* strains (56·5–63·8% inhibition) for a biosurfactant concentration of 50 mg ml^{-1}. For streptococci associated with the oral cavity, the highest antiadhesive percentages were obtained for *Strep. sanguis* 12 (72·9%), whereas the lowest were for *Strep. mutans* HG985 (31·4%). Regarding the pathogenic bacteria, high antiadhesive percentages were obtained for *Staph. aureus* (76·8%), *Staph. epidermidis* (72·9%) and *Strep. agalactiae* (66·6%); on the contrary, low activity was obtained for *Ps. aeruginosa* (21·2%) and *E. coli* (11·8%). In the same way, the antiadhesive activity against the yeasts and fungi strains studied was quite low even at the highest biosurfactant concentrations assayed (between 15·3 and 38·9% inhibition).

Discussion

The crude biosurfactant isolated from *Lact. paracasei* ssp. *paracasei* A20 showed antimicrobial activity against a broad range of micro-organisms, including Gram-positive and Gram-negative bacteria, as well as yeasts and filamentous fungi. For 12 of the 18 micro-organisms studied, the MIC and the MBC were determined for the biosurfactant range of concentrations between 25 and 50 mg ml^{-1}. As far as we know, this is the first compilation of data on antimicrobial activity of biosurfactants obtained from lactobacilli against such a broad group of micro-organisms.

Table 2. Antiadhesive properties of crude biosurfactant isolated from *Lactobacillus paracasei* ssp. *paracasei* A20. 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, and negative percentages indicate increased microbial adhesion. Results are expressed as means ± standard deviation of results from triplicate experiments

	[Biosurfactant] (mg ml^{-1})				
Micro-organism	3·12	6·25	12·5	25·0	50·0
Candida albicans	−23·7 ± 2·2	−15·0 ± 1·6	3·7 ± 0·6	14·9 ± 1·4	29·5 ± 1·6
Escherichia coli	−71·4 ± 1·4	−55·2 ± 1·8	−9·8 ± 2·3	3·1 ± 1·7	11·8 ± 1·6
Lactobacillus casei 36	31·6 ± 2·3	44·8 ± 0·7	53·3 ± 1·3	59·1 ± 0·5	63·8 ± 1·5
Lact. casei 72	11·9 ± 0·5	24·1 ± 1·1	40·2 ± 0·4	50·8 ± 1·8	56·5 ± 0·5
Lactobacillus reuteri 104R	16·5 ± 1·2	56·7 ± 0·7	64·0 ± 1·2	71·4 ± 1·2	77·6 ± 1·4
Lact. reuteri ML1	21·6 ± 1·0	53·6 ± 2·1	65·3 ± 1·0	68·9 ± 1·0	78·8 ± 1·5
Malassezia sp.	−17·3 ± 0·9	6·6 ± 0·7	21·9 ± 2·2	28·9 ± 1·1	38·9 ± 2·6
Pseudomonas aeruginosa	−3·1 ± 1·3	9·6 ± 1·1	14·5 ± 1·3	16·5 ± 0·8	21·2 ± 0·9
Staphylococcus aureus	10·3 ± 1·2	51·1 ± 1·4	62·0 ± 1·1	72·0 ± 1·6	76·8 ± 1·4
Staphylococcus epidermidis	2·1 ± 1·0	41·6 ± 1·0	54·4 ± 1·3	62·1 ± 1·5	72·9 ± 0·3
Streptococcus agalactiae	18·3 ± 1·1	37·6 ± 0·7	48·8 ± 2·3	60·0 ± 0·8	66·6 ± 1·2
Streptococcus mutans HG 985	3·1 ± 1·3	13·2 ± 1·3	16·1 ± 1·3	23·7 ± 2·1	31·4 ± 0·6
Strep. mutans NS	4·5 ± 1·2	13·4 ± 1·5	19·7 ± 1·6	27·5 ± 0·4	38·6 ± 0·8
Streptococcus oralis J22	20·7 ± 1·5	31·0 ± 1·5	34·9 ± 1·7	45·2 ± 1·1	55·5 ± 1·9
Streptococcus pyogenes	2·1 ± 1·4	12·9 ± 0·5	32·2 ± 1·0	38·0 ± 0·1	40·9 ± 1·2
Streptococcus sanguis 12	14·5 ± 0·9	33·4 ± 1·2	54·5 ± 0·5	62·4 ± 1·7	72·9 ± 0·6
Trichophyton mentagrophytes	−26·4 ± 1·1	−18·5 ± 1·5	−3·3 ± 1·9	9·4 ± 1·3	25·7 ± 1·2
Trichophyton rubrum	−25·1 ± 1·9	−17·5 ± 1·0	−6·4 ± 0·9	3·9 ± 1·5	15·3 ± 1·1

Several biosurfactants that exhibit antimicrobial activity have been previously described. However, there are few reports about the antimicrobial activity of biosurfactants isolated from lactobacilli; only biosurfactants obtained from *Strep. thermophilus* A and *L. lactis* 53 showed significant antimicrobial activity against several bacterial and yeast strains isolated from explanted voice prostheses (Rodrigues *et al.* 2004, 2006b,c). In that sense, the antimicrobial activity of the crude biosurfactant isolated from *Lact. paracasei* ssp. *paracasei* A20 against *C. albicans*, *Staph. aureus* and *Staph. epidermidis* was similar to that obtained with the crude biosurfactants isolated from *L. lactis* 53 and *Strep. thermophilus* A, which completely inhibited the growth of those micro-organisms with concentrations between 25 and 100 mg ml^{-1} (Rodrigues *et al.* 2004).

In addition to the antimicrobial properties, the biosurfactant isolated in this study exhibited a considerable antiadhesive activity against most of the micro-organisms tested. Involvement of biosurfactants in microbial adhesion and desorption has been widely described, and adsorption of biosurfactants isolated from lactobacilli to solid surfaces might constitute an effective strategy to reduce microbial adhesion and combating colonization by pathogenic micro-organisms, not only in the biomedical field, but also in other areas, such as the food industry (Rodrigues *et al.* 2006a; Nitschke and Costa 2007; Singh *et al.* 2007; Falagas and Makris 2009).

The antiadhesive activity observed with this biosurfactant against several pathogenic micro-organisms such as *Staph. aureus*, *Staph. epidermidis* and *Strep. agalactiae* are very promising for further studies and applications aiming to reduce microbial colonization on different materials. However, this biosurfactant showed low antiadhesive activity against *E. coli*, *C. albicans* and *Ps. 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 antiadhesive properties make them relevant molecules for use in combating many diseases and infections and as therapeutic agents (Rodrigues *et al.* 2006a). Falagas and Makris (2009) 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 micro-organisms responsible for nosocomial infections.

In conclusion, in this work we have demonstrated the antimicrobial and antiadhesive properties of the crude biosurfactant isolated from *Lact. paracasei* ssp. *paracasei* A20 against several pathogenic and nonpathogenic micro-organisms, 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 micro-organisms

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.

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