Bioactivity of glycolipopeptide cell-bound biosurfactants against skin pathogens

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ABSTRACT

The antimicrobial and anti-adhesive activities of the cell-bound biosurfactants, produced by *Lactobacillus pentosus* (PEB), characterized as glycolipopeptide macromolecules, were evaluated against several microorganisms present in the skin microflora, envisaging its potential use as a "*natural*" ingredient in cosmetic and personal care formulations. Their performance was compared with another cell-bound biosurfactants also characterized as glycolipopeptides produced by *Lactobacillus paracasei* (PAB). At concentrations of 50 mg/mL, the PEB showed an important antimicrobial activity against *Pseudomonas aeruginosa* (85% when extracted with phosphate buffer (PB) and 100% when extracted with phosphate buffer saline (PBS)), *Streptococcus agalactiae* (100% for both extracts), *Staphylococcus aureus* (67% when extracted with PBS and 100% when extracted with PB), *Escherichia coli* (72% when extracted with PB and 89% when extracted with PBS), *Streptococcus pyogenes* (about 85% for both extracts) and *Candida albicans* (around 70% for both extracts), comparable with that obtained for the PAB. However, at lower concentrations the PAB exhibited in general higher antimicrobial activities. Biosurfactants produced by both microorganisms also showed significant anti-adhesive properties against all the microorganisms under study, except for *E. coli* and *C. albicans* (less than 30%). Overall, these cell-bound biosurfactants could be used as potential antimicrobial and anti-adhesive agents in cosmetic and pharmaceutical formulations.

Keywords: Lactobacilli; antimicrobial; anti-adhesive.

1. INTRODUCTION

Human skin is the largest tissue of the human body and is composed by resident, temporarily resident and transient microbial species, being the Gram-positive bacteria from the genera *Propionibacterium*, *Staphylococcus*, *Micrococcus*, *Corynebacterium* and *Acinetobacter* the main resident microorganisms [1]. Among the *Staphylococcus*, *Staphylococcus aureus* is a common transient specie, which causes skin infections, whereas *Staphylococcus epidermidis* is a resident bacteria of skin microflora that protects the human skin from certain types of infection [2].

The microflora generates inhibitory substances, namely bacteriocins, enzymes and low molecular weight inhibitors, which contribute to keep the balance of resident microbial populations, and prevent its colonization by pathogens [1]. Beauty and personal care products incorporate some anti-bacterial preservatives towards harmful microorganisms as triclosan, methylparaben or bronopol, among others. Although these anti-bacterial preservatives are currently used, there is a growing demand for cosmetics free of synthetic preservatives [3,4]. In this sense, biosurfactants from lactic acid bacteria (LAB), which are "Generally Recognized As Safe" (GRAS) by the American Food and Drug Administration (FDA), are natural compounds that exhibit antimicrobial activity and cleaning abilities that could therefore be used as an alternative to the chemically synthetized preservatives [5-8]; but also because at the same time they are non-toxic, biodegradable and environmentally friendly [9-11]. For instance, interesting results have been reported when using a rhamnolipid formulation (25% of biosurfactant and 75% of water) as an antimicrobial and surface-active agent in soak toothbrush holders, hairbrushes and infant plastic toys [12].

Synthetic surfactants can cause skin irritation and allergic reactions by interaction with proteins such as keratin (cytoskeletal proteins) or collagen and elastin (extracellular matrix proteins); also they promote the removal of lipids from the epidermal surface and

affect the living cells in the skin [13]. Contrarily, biosurfactants are composed of lipid and proteins that are compatible with the skin cells membrane [14,15].

The aim of the current study is to evaluate the antimicrobial and anti-adhesive activities of the cell-bound biosurfactants produced by *Lactobacillus pentosus* against skin pathogens, in comparison with the cell-bound biosurfactants produced by *Lactobacillus paracasei* both characterized as glycolipopeptide macromolecules. The corresponding biosurfactants were extracted using two different methodologies and both extracts were evaluated.

2. MATERIALS AND METHODS

2.1. Strains and standard culture conditions for biosurfactant production

L. pentosus CECT-4023T (ATCC-8041) was obtained from the Spanish Type Culture Collection (CECT) (Valencia, Spain), while *L. paracasei* was isolated from a Portuguese dairy industry [5].

Both strains were grown for 24 h in Petri dishes containing complete medium, so-named by its inventors (de Man, Rogosa and Sharpe), MRS Agar, at 31°C and 37°C, respectively. Inocula were prepared by solubilizing all cells from plates with 5 mL of culture media. Then, cells were incubated at 150 rpm, at the optimum temperature for each microorganism in 250 mL Erlenmeyer flasks containing the rest of culture media (100 mL as total volume).

2.2. Production and extraction of the biosurfactants from Lactobacilli strains

The fermentation medium for *L. pentosus* contained 11 g/L of glucose and 18 g/L of xylose. This strain is a hetero-fermentative facultative lactic acid bacterium able to metabolize pentoses, whereas the fermentation medium for *L. paracasei,* a homo-fermentative strain, was formulated with 33 g/L of glucose. Both media were

supplemented with 10 g/L of corn steep liquor and 10 g/L of yeast extract as nitrogen source, sterilized (121°C during 15 min) and used directly as fermentation media.

The fermentations were carried out in a 2 L Applikon fermenter, at 200 rpm, with a working volume of 1.5 L, at 31°C, during 48 h for *L. pentosus* and at 37°C, during 24 h, for *L. paracasei*. The pH was adjusted to 6 for both strains.

Afterwards, the fermentation medium was centrifuged, the biomass was washed twice with distilled water and re-suspended in 250 mL of phosphate buffer saline (PBS) (10 mM KH_2PO_4/K_2HPO_4 with 150 mM NaCl) or phosphate buffer (PB) (10 mM KH_2PO_4/K_2HPO_4 without salt). The biomass/liquid ratio used for the extraction was 6:1. The extraction with PBS was carried at room temperature (25°C) during 2 h at 150 rpm [16]; whereas the extraction with PB was established at 65°C during 1.5 h at 150 rpm according to a previous study [17]. The solutions containing the cell-bound biosurfactants were dialyzed against demineralized water at 4ºC in a Cellu-Sep© membrane (molecular weight cut-off 6000–8000 Dalton; Membrane Filtration Products, Inc., USA) for 48 h, and then the biosurfactants were lyophilized using a lyophilizer CHRIST® Alpha 1-4 LD plus (Germany).

Four different cell-bound biosurfactant extracts were obtained depending on the Lactobacilli strain and the methodology used for their extraction, namely the biosurfactants produced by *L. pentosus* (PEB) extracted with PBS and PB; and the biosurfactants obtained from *L. paracasei* (PAB) extracted with PBS and PB.

2.3. Cell-bound biosurfactants characterization

Different surfactant properties such as critical micellar concentration (CMC) and surface tension reduction (ST), as well as protein, carbohydrate and lipid contents of the cell-bound biosurfactants were evaluated following the protocols established in previous works [18,19]. Therefore, total carbohydrate content in the biosurfactant extracts was determined by the phenol-sulfuric acid method using D-glucose as a standard [20]; total

protein content was calculated by multiplying the total nitrogen content of the biosurfactant extracts by a conversion factor of 6.25 [21] and lipid content was analyzed by Gas Chromatography coupled to a Mass Spectrometer (GC-MS-MS).

The fatty acid methyl esters (FAMEs) separation was performed on a Model Scion 451 GC (Bruker) equipped with a PTV 1019 universal capillary injector (1 µL of sample was injected using a splitless mode) and a DB-WAX column (30 m long, 0.25 mm i.d., 0.25 µm film thickness) using an oven temperature gradient as follows: 50°C for 2 min, then raised to 220°C at a rate equal to 4 °C/min and then maintained for more 15 min. Helium was used as carrier gas at a constant flow rate of 1 mL/min. The temperature of both injector inlet and the transfer line of the detector was set at 240ºC.

The mass spectra were obtained using a mass-selective detector under electron impact ionization at a voltage of 70 eV and data were acquired over an m/z range 50-400. The software used to process the peak areas was MS Data Review (version 8.1).

FAMEs were identified using a mass spectra library supplied with the GC-MS-MS system and by comparison of retention times and mass spectra of a FAME standard mix (Supelco 37 Component FAME Mix: 10 mg/mL of the FAME reference standard mix in methylene chloride, Sigma-Aldrich) injected under the same conditions.

2.4. Strains and standard culture conditions for antimicrobial and anti-adhesive assays

The following strains, kindly provided by the Faculty of Pharmacy, University of Porto (Portugal), were used in the antimicrobial and anti-adhesive assays: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, S*treptococcus pyogenes* and *Candida albicans*. These strains were grown overnight in Trypticase Soy Broth (TSB) medium at 37ºC in aerobic conditions. The composition of TSB medium was: 17 g/L casein peptone (pancreatic), 3 g/L soya peptone (papain digest.), 5 g/L sodium chloride, 2.5 g/L di-potassium hydrogen phosphate and 2.5 g/L glucose.

All strains were stored at -80ºC in appropriate medium supplemented with glycerol (20% (v/v)) until use.

2.5. Antimicrobial assay

The antimicrobial activity of the biosurfactants from *Lactobacillus* strains against skin pathogens was determined according to the procedure described elsewhere [5]. Briefly, a micro-dilution method in 96-well flat-bottom plastic tissue culture plates (Orange Scientific, Belgium) was used. A 125 µL of sterile double strength growth TSB medium was placed in the well 1 of the microplate, together with 125 µL of biosurfactant solution at 100 mg/mL. Serially, 125 µL from well 1 was transferred to the subsequent wells, adding 125 µL of sterile single strength growth TSB medium. After the consecutive dilutions the biosurfactant concentration in the wells ranged between 50-0.10 mg/mL. Following, 2.5 µL of a pre-culture of the evaluated microorganism, grown overnight in TSB medium at 37°C and diluted to an optical density of 0.6, where added to each well, except well 11, that was used as negative control, containing only TSB medium (125 µL). In addition, well 12 was used as positive control, containing only TBS medium (125 µL) and the microorganism inoculum (2.5 µL).

The microplates were covered, incubated for 48 h at 37°C and the optical density of each well were measured at 600 nm in a microplate reader (Biotech Synergy HT). The growth inhibition percentages at different biosurfactant concentrations for each pathogen were calculated following Equation 1:

$$Growth\ inhibition_c(\%) = \left[1 - \frac{(OD_c)}{(OD_0)}\right] x\ 100 \qquad \text{Equation (1)}$$

where OD_c represents the optical density of the well with a biosurfactant concentration c and OD_0 is the optical density of the control well (without biosurfactant). Triplicate assays were performed at all biosurfactant concentrations for each strain.

2.6. Anti-adhesive assay

The anti-adhesive activity of biosurfactants from *Lactobacillus* strains was tested against the same pathogens described in the antimicrobial assay. Wells of a sterile 96-well flat-bottom plastic tissue culture plate were filled with 200 µL of crude biosurfactant solution in PBS or PB following the methodology reported elsewhere [5]. Several biosurfactant concentrations were tested ranging from 0.02 to 25 mg/mL. The plate was incubated for 18 h at 4°C and subsequently washed twice with PBS or PB. Control wells contained only PBS or PB. A 200 µL aliquot of a washed bacterial suspension in PBS or PB, adjusted to an optical density of 0.6, was added to each well and incubated for 24 h at 4°C. Unattached microorganisms were removed by washing the wells three times with PBS or PB; whereas the attached microorganisms were fixed with 200 µL of 99% methanol per well during 15 min, then the plates were emptied and left to dry. Afterwards, the plates were stained for 5 min with 200 µL of 2% crystal violet per well (used for Gram staining). The excess of 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 re-solubilized with 200 µL of 33% (v/v) glacial acetic acid per well and the optical density was measured at 595 nm. The microbial inhibition percentages at different biosurfactant concentrations for each microorganism were determined according to Equation 2:

$$Microbial\ inhibition_c(\%) = \left[1 - \frac{(OD_c)}{(OD_0)}\right] x\ 100 \qquad \text{Equation (2)}$$

where OD_c represents the optical density of the well with a biosurfactant concentration c and OD_0 is the optical density of the control well (without biosurfactant). Triplicate assays were performed at all biosurfactant concentrations for each strain.

3. RESULTS AND DISCUSSION

Biosurfactants are promising macromolecules for cosmetic, pharmaceutical or biomedical uses [9,11,22]. They are biocompatible molecules that reduce the surface tension in aqueous solutions allowing the solubilization of hydrophobic active principles. Comparing to their chemical counterparts, the biosurfactants exhibit a number of advantages and specifically for applications that involve contact with the skin, they could be regarded as prebiotic ingredients, protecting the skin as they prevent the growth of pathogenic microorganisms and stimulate the establishment of a beneficial microflora. In this work, the antimicrobial and anti-adhesive capacity of two different cell-bound biosurfactants produced by two probiotic Lactobacilli strains was studied. Additionally, it is remarkable that more than 90% of the biosurfactant-related works about theses biological activities refer that biosurfactants are produced extracellularly and only a few report the use of cell-bound biosurfactants. However, some of the cell-bound biosurfactants that have been reported are produced by probiotic bacteria and therefore, are quite interesting as they can potentially exhibit prebiotic properties [9].

Table 1 shows the composition of the biosurfactants herein studied that were extracted from the cell membrane using two different approaches. The biosurfactants extracted with PBS were found to possess a higher content in lipids than those extracted with PB, whereas the content in carbohydrates was higher in the extracts obtained using PB. Additionally, a higher protein content was found in the biosurfactants produced by *L. paracasei* mainly when these were extracted with PB. Regarding the Lactobacilli strains, *L. paracasei* produced biosurfactants with a lower content of lipids than those produced by *L. pentosus*.

In addition, **Figure 1** shows the GC-MS spectra of the *L. pentosus* and *L. paracasei* biosurfactants illustrating their fatty acid profile. The biosurfactants were composed by C15 (myristic acid), C16 (palmitic acid), C17 (palmitoleic acid) and C18 (stearic, oleic, linoleic and α-linoleic acids) fatty acid chains. A high percentage of C16 and C18 fatty

acids was observed, being the most abundant the palmitic acid (22.1-43.9%) and stearic acid (26.1-41.6%). Moreover, differences in the fatty acids content were observed depending on the Lactobacilli strain and the methodology used for their extraction. For instance, the PEB contained a higher percentage of oleic acid (25.2-28.6%) than the PAB (1.2-7.4%); whereas palmitoleic acid was only present in the biosurfactants obtained from *L. paracasei*. Moreover, the biosurfactants extracted with PBS contained a higher percentage of stearic and oleic acids and a lower content in palmitic acid than those extracted with PB.

3.1. Antimicrobial activity

Figure 2 to **Figure 4** show the antimicrobial activities of the four cell-bound biosurfactants evaluated against skin pathogenic: Gram-negative bacteria, Gram-positive bacteria and fungi, respectively.

In order to discuss the antimicrobial activities of the biosurfactant extracts, only the concentrations that showed an antimicrobial activity higher than 50% will be considered. It is interesting to notice that at the highest concentration assayed (50 mg/mL), the cell-bound PEB exhibited 100% growth inhibition against *S. agalactiae* and about 70% against *C. albicans* (**Figure 3c** and **Figure 4**, respectively). Regarding the extraction method used, it was found that the biosurfactant produced by *L. pentosus* extracted with PBS possessed a higher antimicrobial activity against the Gram-negative microorganisms *E. coli* (89%) (**Figure 2a**) and *P. aeruginosa* (100%) (**Figure 2b**) as compared to the one extracted with PB (72% and 85% respectively). Contrarily, the biosurfactants extracted with PB showed a higher antimicrobial activity against *S. aureus* (100%) (**Figure 3a**) and *S. pyogenes* (87%) (**Figure 3d**) as compared to the ones extracted with PBS (67% and 83%, respectively). In the case of *S. agalactiae* (**Figure 3c**) and *C. albicans* (**Figure 4**) the antimicrobial activity was 100% and 71%, respectively, using PBS and PB

extraction methods; while for *S. epidermidis* (**Figure 3b**) the values were lower than 50%.

Regarding the antimicrobial properties of the biosurfactants from *L. paracasei* at the highest concentration tested (50 mg/mL), it was observed the same antimicrobial activity against *E. coli*, *P. aeruginosa*, *S. epidermidis*, *S. agalactiae*, *S. pyogenes* and *C. albicans* (all 100%) by PAB extracted with PBS and PB; and only in the case of *S. aureus* the PAB extracted with PB exhibited 100% antimicrobial activity, whereas in PBS was 83%.

On the other hand, at a lower biosurfactant concentration (25 mg/mL), PAB generally showed different antimicrobial activities depending on the extraction method. The effect observed on *S. aureus* was in accordance with that noticed using the biosurfactants from *L. pentosus*. Indeed, for this pathogenic microorganism a better antimicrobial activity was found for the extracts obtained with PB **(Figure 3a)**. As well, similar antimicrobial performance was observed against *S. epidermidis* at 25 mg/mL using PB **(Figure 3b)**. On the other hand, the use of PBS rendered a highest antimicrobial effect in comparison with the extract obtained with PB against *S agalactiae* **(Figure 3c)**, *S. pyogenes* (**Figure 3d)** and *C. albicans* (**Figure 4**). However, in the case of *E. coli* it was found that the procedure used to extract the biosurfactants from *L. paracasei* did not affect its antimicrobial activity, contrarily to the extracts obtained from *L. pentosus* **(Figure 2a)**.

Based on these results, it can be speculated that the antimicrobial activity of the biosurfactants depend on the strain used for its production, regardless of being from the same genus, and also depend on the methodology used for their extraction.

Table 1S (see in the supplementary information) gathers information on the minimum doses of biosurfactants that led to antimicrobial activities higher than 50% or equal to 100%. PEB, at concentrations of 25 mg/mL, were able to reduced 50% the growth of *P. aeruginosa*, *S. agalactiae* and *S. pyogenes,* whenever extracted with PBS; whereas on *E. coli*, *S. aureus* and *C. albicans*, 50% of growth inhibition was obtained at concentration

of 50 mg/mL. Moreover, at the same concentration (50 mg/mL) 100% antimicrobial inhibition against *P. aeruginosa* and *S. agalactiae* was found.

Regarding the biosurfactant from *L. pentosus* extracted with PB, it was found that a concentrations of 25 mg/mL could only reduce 50% the growth of *P. aeruginosa* and *S. aureus*; whereas using this extract, at the highest concentration (50 mg/mL), 100% inhibition was observed for *S. aureus* and *S. agalactiae*.

In general, the dose of PAB required to obtain 50% of growth inhibition, was lower than that needed for the PEB. At concentrations of 12.5 mg/mL, the PAB, reduced 50% the growth of *E. coli,* when extracted with PBS*;* and the growth of *S. agalactiae,* when extracted with PB. Additionally, 100% of growth reduction was observed for all the pathogenic strains, at doses of 50 mg/mL, except for *S. aureus* in the case of the biosurfactant from *L. paracasei* extracted with PBS.

The antimicrobial activity of biosurfactants has sparked an increased interest in researchers and promoted additional efforts to further characterize these promising substances for biomedical, pharmaceutical, food or cosmetic applications. For instance, Sharma and Saharan [7] evaluated the antimicrobial ability of the glycolipid biosurfactant produced by *Lactobacillus helveticus* MRTL91 against *E. coli* (90%), *P. aeruginosa* (76%), *S. aureus* (92%) or *S. epidermidis* (98%) at 25 mg/mL, observing a higher antimicrobial activity against *E. coli* and against *S. epidermidis* in comparison with the data observed in the current work.

Additionally, Gudiña and collaborators [6] showed that the glycoprotein biosurfactant from *Lactobacillus agilis* CCUG31450 inhibited the growth of *S. aureus* (20%), *P. aeruginosa* (13.5%) and *S. agalactiae* (11%) at 5 mg/mL, however it did not present an antimicrobial activity against *E. coli* and *C. albicans* under the same conditions. These results are in good agreement with the current study showing similar inhibitory capacities at 5 mg/mL, for *S. aureus*, *P. aeruginosa* and *S. agalactiae*. Nevertheless, PAB and PEB

extracts at similar concentrations as the ones used by Gudiña et al. [6] (5 mg/mL), showed slightly antimicrobial inhibition (less than 25%) against *E. coli* and *C. albicans*. Furthermore, the same authors studied the antimicrobial properties of the glycoprotein biosurfactant produced by *L. paracasei* when grown in MRS Lac medium (standard MRS medium where glucose was replaced by lactose) [5,23]. Gudiña and co-workers [5] found a complete growth inhibition of *E. coli*, *S. agalactiae* and *S. pyogenes* at 25 mg/mL. Those growth inhibition values were slightly higher than those herein obtained using an extract produced by the same strain. However, it is important to notice that in the current work, a different biosurfactant was produced, namely a glycolipopeptide, as the strain was grown using glucose as carbon source, whereas Gudiña et al. [5] used lactose as carbon source.

It is well known that a same strain can produce different biosurfactants depending on the carbon source and fermenting conditions used [19,24-26]. For example, Singh et al. [25] reported that *Bacillus amylofaciens* strain AR2 could produce different types of surfactins depending on the carbon source used. In fact, the strain produced lipopeptides as a mixture of surfactin, iturin and fengycin when the minimal salt medium was supplemented with dextrose, sucrose and glycerol; whereas using maltose, lactose and sorbitol as carbon sources only iturin was produced.

Additionally, Shah et al. [27] evaluated different carbon sources (e.g. glucose, fructose, xylose, ribose, lactose, mannose, arabinose and galactose) for the production of sophorolipids and also studied their effect as antimicrobial agents. The authors suggested that the biosurfactant structures were different in the hydrophilic fraction (carbohydrate chain) but not in the hydrophobic side (fatty acid chain). The change on the carbon source led to different antibacterial activities. For instance, the sophorolipids produced when arabinose-containing medium was used were more effective against three of the four Gram-positive bacteria studied and against the *Moraxella* sp. (Gram-negative

bacteria) as compared to the sophorolipids obtained when using glucose-based medium. Also, the sophorolipids obtained from cultures grown on arabinose showed no inhibition of the growth of *E. coli*; whereas the most effective sophorolipids against *Bacillus subtilis* was the ones obtained using lactose-based medium.

3.2. Anti-adhesive activity

Figure 5 to **Figure 7** illustrate the anti-adhesive properties of biosurfactants from *L. pentosus* and from *L. paracasei* at concentrations up to 25 mg/mL. Additionally, **Table 2S** (see in the supplementary information) summarizes the lowest concentration of biosurfactant extracts required to obtain anti-adhesive percentages of 50% and 100%. Generally, the biosurfactants obtained from both Lactobacilli strains exhibited similar anti-adhesive activities. For instance, at 25 mg/mL, biosurfactants produced by *L. pentosus* and extracted with PBS or PB inhibited around 63%, 73% and 77% the highest adhesion of *P. aeruginosa* (**Figure 5b**), *S. aureus* (**Figure 6a**) and *S. agalactiae* (**Figure 6c**), respectively. Moreover, it was found that the biosurfactant from *L. pentosus* extracted with PB led to higher anti-adhesive activity against *S. epidermidis* (57%) (**Figure 6b**) and *S. pyogenes* (69%) (**Figure 6d**) comparing to those extracted with PBS (38% and 52%, respectively).

On the other hand, biosurfactants from *L. paracasei* extracted with PBS showed a more pronounced anti-adhesive effect on all the Gram-positive pathogens tested (such as *S. aureus, S. epidermidis, S. agalactiae* and *S. pyrogenes*) (**Figure 6a** to **Figure 6d**), and against *P. aeruginosa* (Gram-negative) comparing to the biosurfactants extracted with PB (**Figure 5b**). Furthermore, PAB inhibited the highest adhesion against *S. agalactiae* around 81% and 70% depending if the biosurfactant extracts were extracted with PBS or PB, respectively (**Figure 6c**).

Moreover, the inhibitory effect of all biosurfactant extracts (extracted with PBS and PB) on the adhesion of *E. coli* (**Figure 5a**) and *C. albicans* (**Figure 7**) was less than 30% at the highest concentration tested (25 mg/mL).

The anti-adhesive activity of biosurfactants is a relevant feature if their use as coatings of biomedical materials is envisaged. Indeed, many studies suggest that biosurfactants play an important role avoiding biofilm formation on different surfaces such as silicone rubber [28-30], titanium surface [31], polystyrene plates [32], among others. Sharma and Saharan [7] found that *L. helveticus* MRTL91, at 25 mg/mL, considerably inhibited the adhesion of *S. aureus* (83%) and *S. epidermidis* (85%), although lower inhibitions were found for *E. coli* (50%), *P. aeruginosa* (49%) and *C. albicans* (data not provided). This poor inhibition obtained for *E. coli and C. albicans* was also found in the current study using the biosurfactant extracts obtained from *L. pentosus* and *L. paracasei*. However, the anti-adhesive capacities of the glycolipopeptide biosurfactants obtained from these strains against *P. aeruginosa* were slightly better (63% and 72%, respectively) in comparison with the glycolipid biosurfactant produced by *L. helveticus*.

Shokouhfard et al., [33] evaluated the anti-adhesive properties of a biosurfactant isolated from *Lactobacillus acidophilus* ATCC 4356 (biosurfactant composed by high protein content compared to other components such as polysaccharides and phosphates) on *S. marcescens* strains. The results showed good anti-adhesive activities, up to 60%, for the different types of *S. marcescens* tested using 2.5 mg/mL of biosurfactant extract.

Gudiña et al. [6] showed that the glycoprotein biosurfactant from *L. agilis* CCUG31450 inhibited the adhesion of *S. aureus* around 60% at concentrations between 5 and 10 mg/mL and around 50% at concentrations between 1 and 2.5 mg/mL. The same behavior was observed for the glycolipopeptide biosurfactants used in the current work at the same concentrations (an anti-adhesion average of 64%), except for the one produced by *L.*

paracasei, extracted with PB and grown in glucose-based medium, that exhibited slightly lower values (less than 50% at 6.25 mg/mL).

Madhu and Prapulla [34] evaluated a glycoprotein biosurfactant from *Lactobacillus plantarum* CFR 2194 that successfully inhibited the adhesion of *S. aureus* (67%) at 25 mg/mL. It is important to notice that this inhibition was lower than the one herein obtained for glycolipopeptide biosurfactants from *L. pentosus* (76%) and *L. paracasei* (72%) both extracted with PBS.

In addition, Gudiña et al. [5] used 25 mg/mL of a glycoprotein biosurfactant from *L. paracasei,* grown on lactose and extracted with PBS, and found good anti-adhesive activities against *S. aureus* (72%), *S. epidermidis* (62%) and *S. agalactiae* (60%), whereas a poor activity was observed for *P. aeruginosa* (16.5%) and *E. coli* (12%). Using the same concentration as Gudiña and co-workers [5], the glycolipopeptide biosurfactants from *L. paracasei* grown on glucose-based medium, showed higher anti-adhesive properties against *S. agalactiae* (70-81%), *P. aeruginosa* (57-72%) depending if the biosurfactant extracts were extracted with PB or PBS respectively, and *E. coli* (30% extracted with PBS and PB). In addition, in the case of *S. aureus* and *S. epidermidis* the results obtained in the current work (PAB in PBS) were similar to the anti-adhesive activity obtained by Gudiña et al. [5], being 72% and 55%, respectively.

4. CONCLUSIONS

The cell-bound biosurfactants produced by *L. pentosus*, showed 100% of antimicrobial activity against *P. aeruginosa* (when extracted with PBS), *S. aureus* (when extracted with PB) and *S. agalactiae* (extracted with PBS or PB) at concentration of 50 mg/mL. In the case of cell-bound biosurfactants produced by *L. paracasei* using both extraction methods, 100% of growth inhibition was found for all pathogens evaluated, except for *S. aureus* when extracted in PBS (83%).

Regarding the biosurfactants anti-adhesive activities, relevant values were obtained with all biosurfactant extracts evaluated against *P. aeruginosa* (between 57% for PAB extracted with PB to 72% for PAB extracted with PBS), S. *aureus* (between 60% for PAB extracted with PB to 76% for PEB extracted with PBS) and *S. agalactiae* (between 70% for PAB extracted with PB to 81% for PAB extracted with PBS). However, for *E. coli* and *C. albicans* these values were lower than 30%.

Based on the results herein gathered, it can be speculated that small changes in the carbohydrates, lipids and proteins percentages, of the polymeric fraction of biosurfactants, can play an important role on their biological activities and accordingly on their applications in the cosmetic industry.

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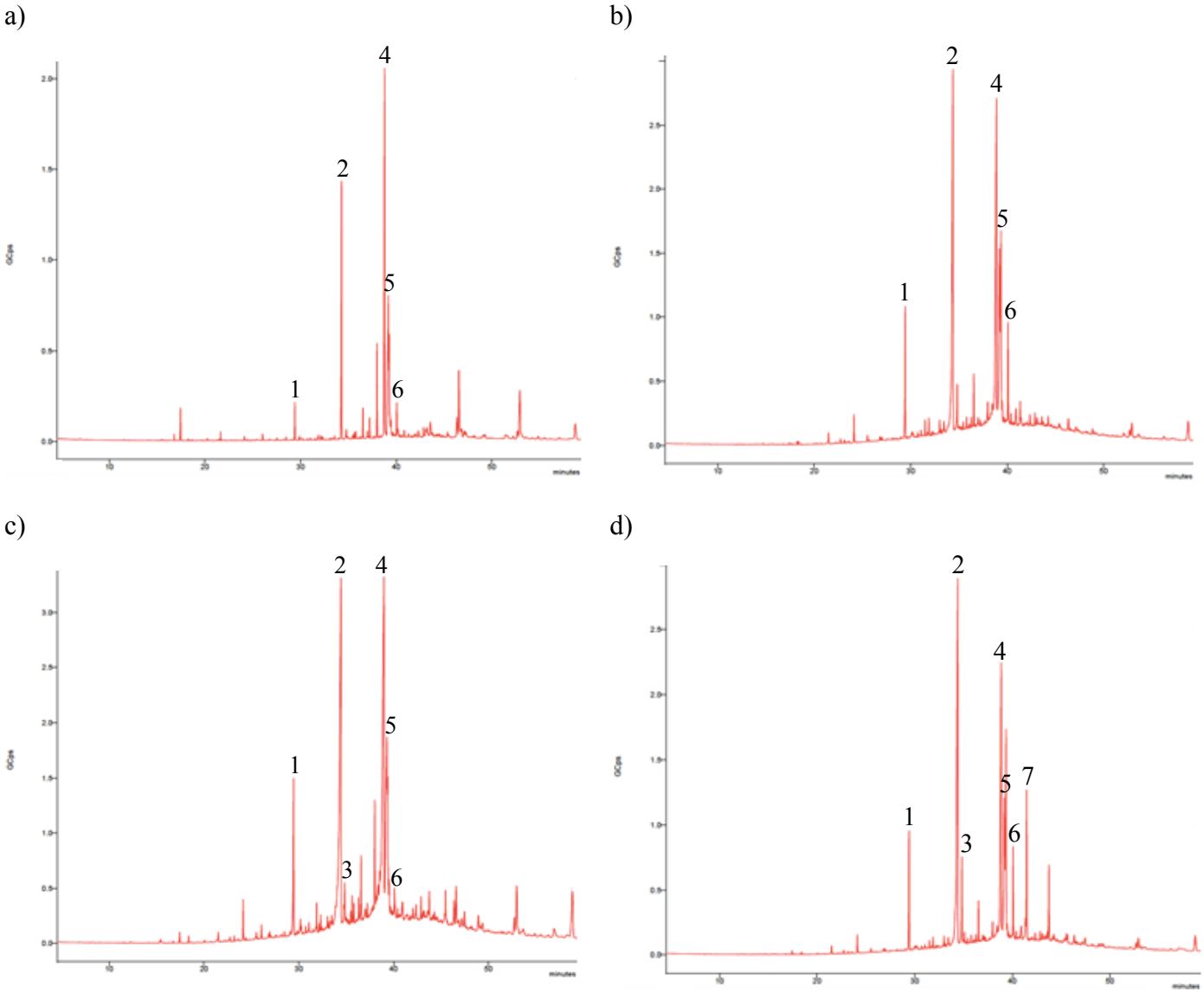


Figure 1. Fatty acids profile of the biosurfactants produced by *Lactobacillus pentosus* (a, b) and *Lactobacillus paracasei* (c, d) extracted using phosphate buffer saline (PBS) (a, c) and phosphate buffer (PB) (b, d) respectively. The numbers denote the major relative fatty acids in the biosurfactants extracts as follows: 1= myristic acid (methyl ester); 2= palmitic acid; 3= palmitoleic acid; 4= stearic acid, 5= oleic acid, 6= linoleic acid, 7= α-linoleic acid.

a) b)

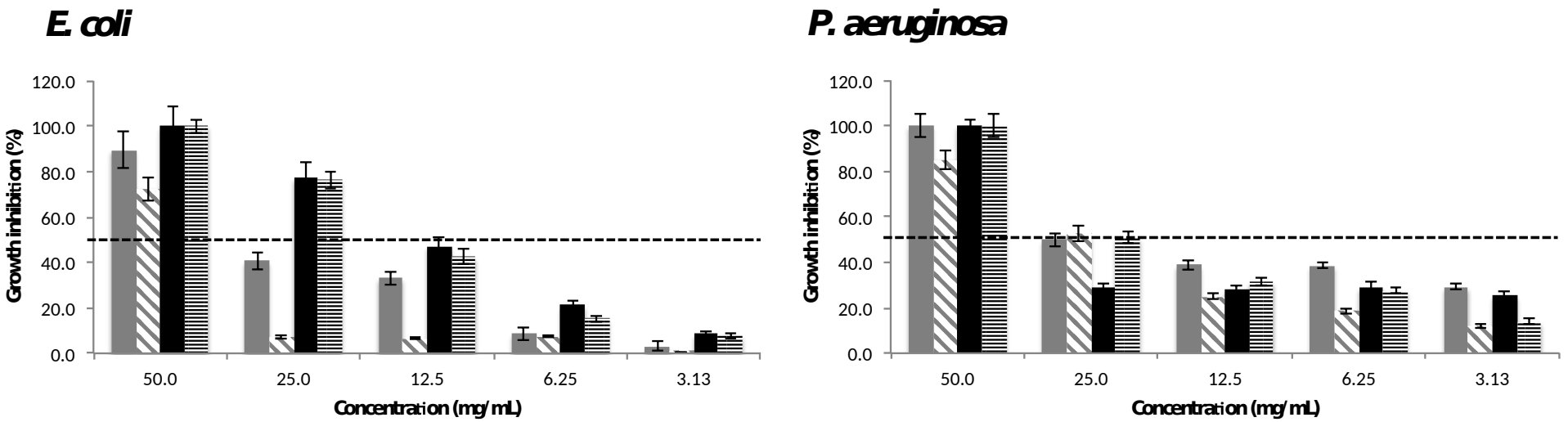


Figure 2. Antimicrobial activity of the biosurfactants produced by *Lactobacillus pentosus* (PEB) and *Lactobacillus paracasei* (PAB) against Gram-negative microorganisms. PEB and PAB were extracted using phosphate buffer saline (PBS) and phosphate buffer (PB) respectively (■ PEB in PBS; ▨ PEB in PB; ■ PAB in PBS; ▤ PAB in PB**)**. The results represent the average of triplicate experiments ± standard deviation.

a) b)

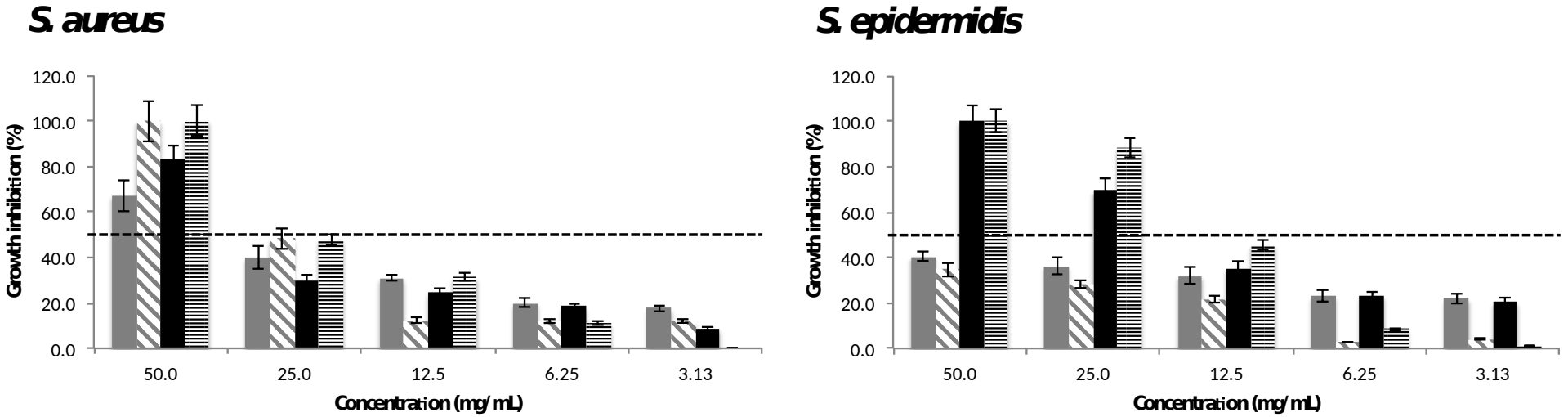
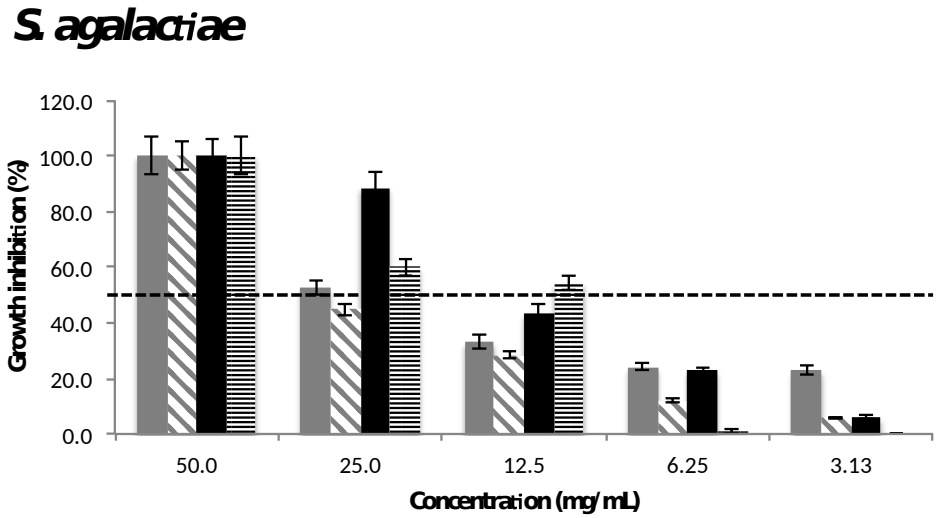


Figure 3. Antimicrobial activity of the biosurfactants produced by *Lactobacillus pentosus* (PEB) and *Lactobacillus paracasei* (PAB) against Gram-positive microorganisms. PEB and PAB were extracted using phosphate buffer saline (PBS) and phosphate buffer (PB) respectively (■ PEB in PBS; ▨ PEB in PB; ■ PAB in PBS; ▤ PAB in PB**)**. The results represent the average of triplicate experiments ± standard deviation.

c)



d)

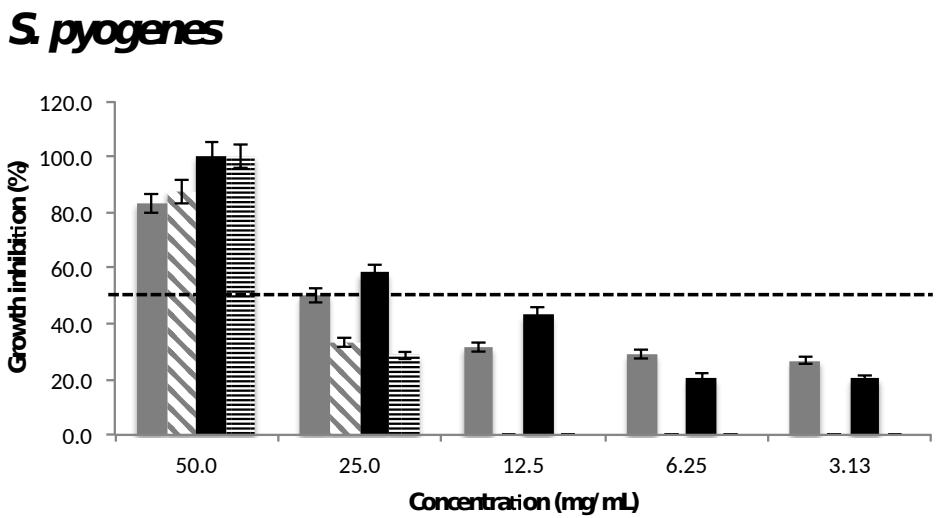


Figure 3 (Continuation). Antimicrobial activity of the biosurfactants produced by *Lactobacillus pentosus* (PEB) and *Lactobacillus paracasei* (PAB) against Gram-positive microorganisms. PEB and PAB were extracted using phosphate buffer saline (PBS) and phosphate buffer (PB) respectively (▒ PEB in PBS; ▒ PEB in PB; ■ PAB in PBS; ▒ PAB in PB**)**. The results represent the average of triplicate experiments ± standard deviation.

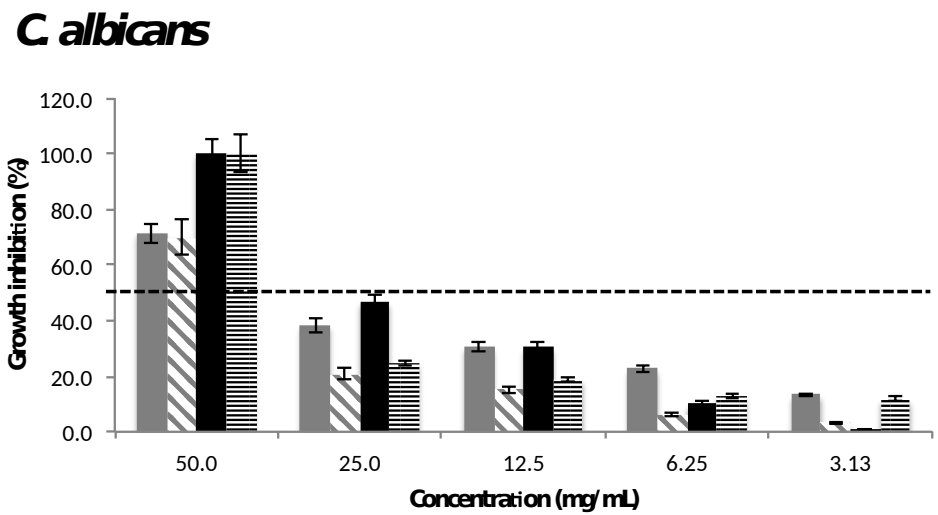


Figure 4. Antimicrobial activity of the biosurfactants produced by *Lactobacillus pentosus* (PEB) and *Lactobacillus paracasei* (PAB) against fungi microorganisms. PEB and PAB were extracted using phosphate buffer saline (PBS) and phosphate buffer (PB) respectively (■ PEB in PBS; ▨ PEB in PB; ■ PAB in PBS; ▤ PAB in PB**)**. The results represent the average of triplicate experiments ± standard deviation.

a) b)

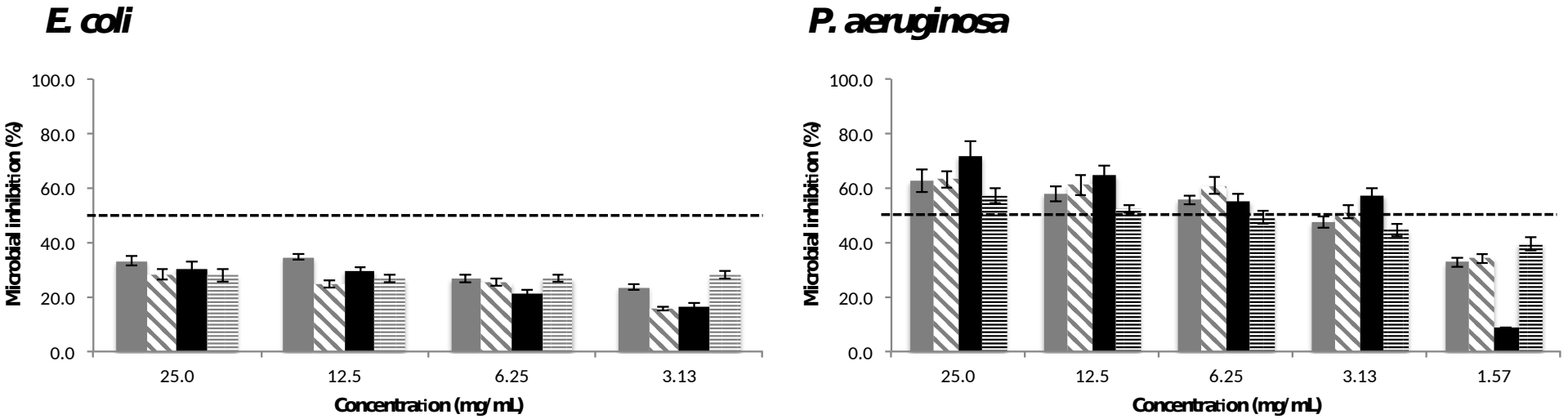


Figure 5. Anti-adhesive activity of the biosurfactants produced by *Lactobacillus pentosus* (PEB) and *Lactobacillus paracasei* (PAB) against Gram-negative microorganisms. PEB and PAB were extracted using phosphate buffer saline (PBS) and phosphate buffer (PB) respectively (▒ PEB in PBS; ▨ PEB in PB; ■ PAB in PBS; ▤ PAB in PB**)**. The results represent the average of triplicate experiments ± standard deviation.

a) b)

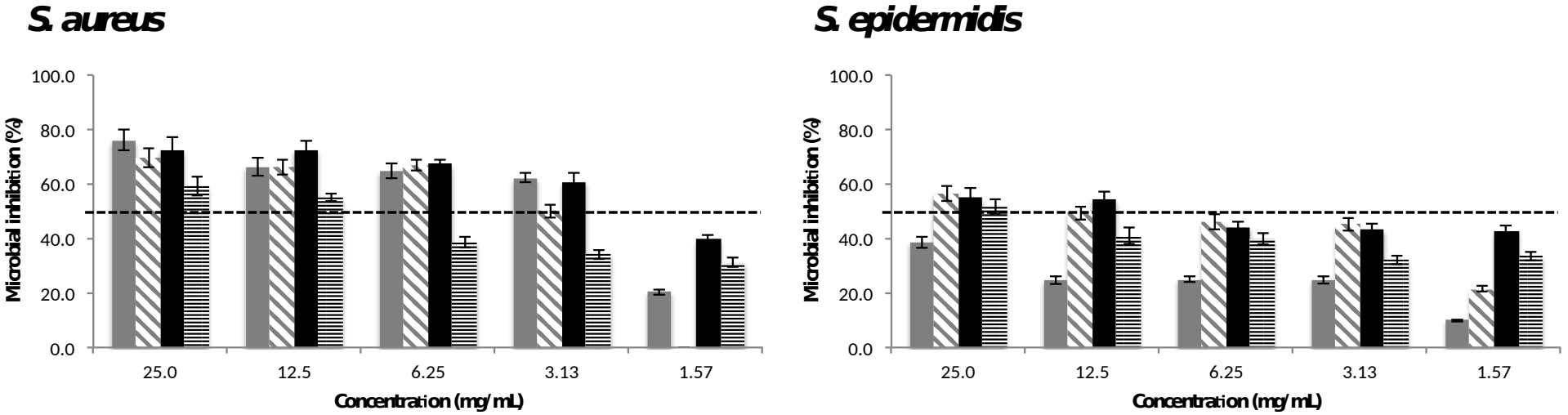
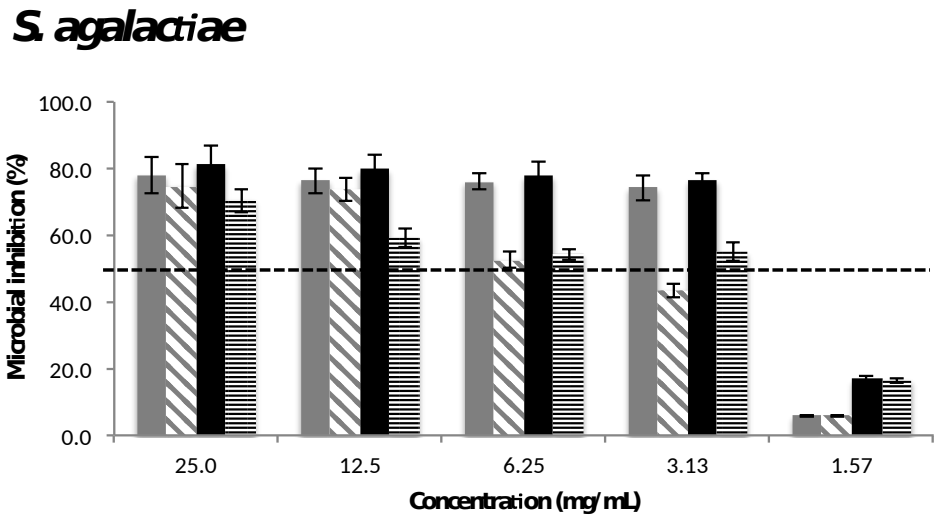


Figure 6. Anti-adhesive activity of the biosurfactants produced by *Lactobacillus pentosus* (PEB) and *Lactobacillus paracasei* (PAB) against Gram-positive microorganisms. PEB and PAB were extracted using phosphate buffer saline (PBS) and phosphate buffer (PB) respectively (■ PEB in PBS; ▨ PEB in PB; ■ PAB in PBS; ▤ PAB in PB**)**. The results represent the average of triplicate experiments ± standard deviation.

c)



d)

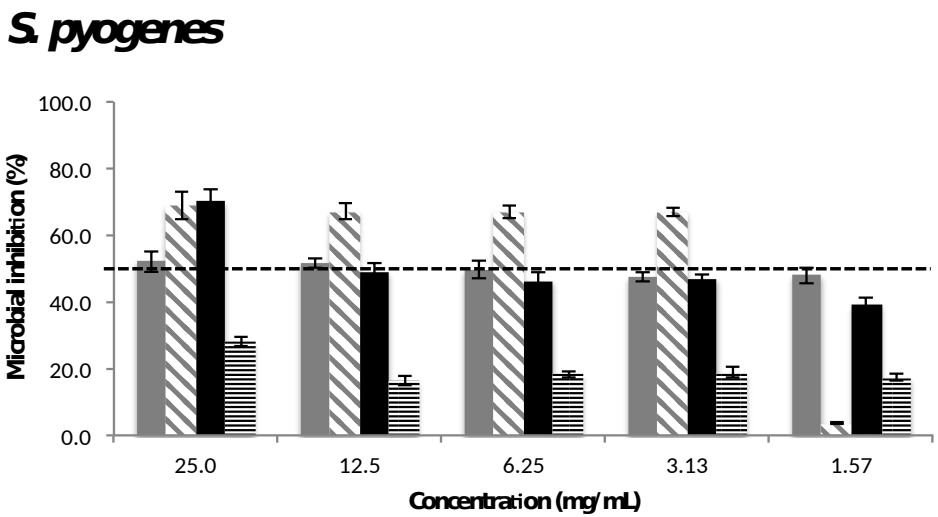


Figure 6 (Continuation). Anti-adhesive activity of the biosurfactants produced by *Lactobacillus pentosus* (PEB) and *Lactobacillus paracasei* (PAB) against Gram-positive microorganisms. PEB and PAB were extracted using phosphate buffer saline (PBS) and phosphate buffer (PB) respectively (■ PEB in PBS; ▨ PEB in PB; ■ PAB in PBS; ▤ PAB in PB**)**. The results represent the average of triplicate experiments ± standard deviation.

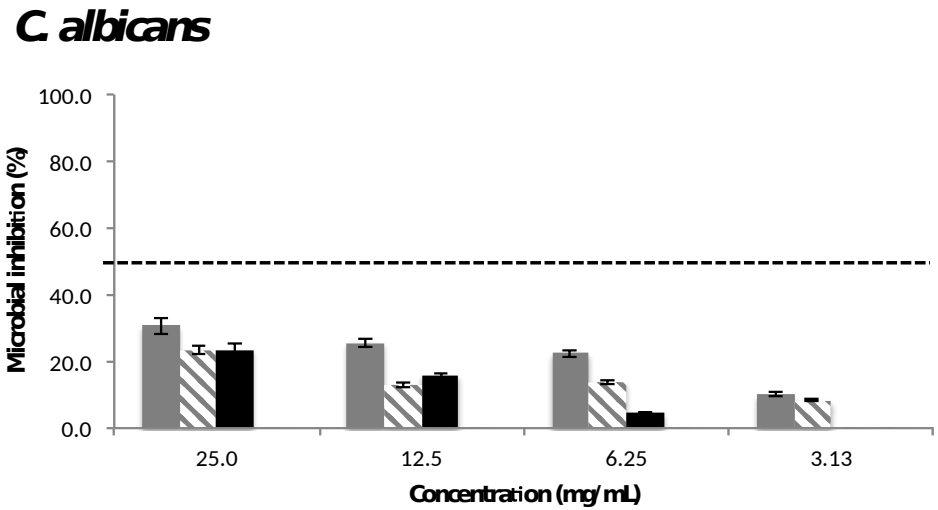


Figure 7. Anti-adhesive activity of the biosurfactants produced by *Lactobacillus pentosus* (PEB) and *Lactobacillus paracasei* (PAB) against fungi microorganisms. PEB and PAB were extracted using phosphate buffer saline (PBS) and phosphate buffer (PB) respectively (▩ PEB in PBS; ▨ PEB in PB; ■ PAB in PBS; ▤ PAB in PB**)**. The results represent the average of triplicate experiments ± standard deviation.

TABLE 1. Chemical composition and surfactant properties of the cell-bound biosurfactants produced by the Lactobacilli strains under study.

Cell-bound biosurfactant	**PEB in PBS**	**PEB in PB**	**PAB in PBS**	**PAB in PB**
CMC (mg/mL)	1.26±0.11	0.81±0.08	1.35±0.13	1.26±0.11
ST reduction (mN/m)	19.2±0.57	19.7±0.22	25.1±0.49	20.9±0.41
Protein content (%)	12.6±1.07	30.7±1.54	21.19±0.18	58.22±3.14
Carbohydrate content (%)	7.7±0.57	19.5±1.17	5.47±1.19	14.24±3.81
Lipid content (%)	50.5±2.27	41.8±2.51	24.40±1.15	13.66±1.22

PEB and PAB: biosurfactants produced by *L. pentosus* and *L. paracasei* and extracted using phosphate buffer saline (PBS) and phosphate buffer (PB) respectively.

Supplementary information

for

Bioactivity of glycolipopeptide cell-bound biosurfactants against skin pathogens

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TABLE 1S. Biosurfactant concentration that led to 50% and 100% of growth inhibition between the values assayed.

	PEB in PBS		**PEB in PB**	
Microorganisms	Dose to achieve 50% growth inhibition (mg/mL)	Dose to achieve 100% growth inhibition (mg/mL)	Dose to achieve 50% growth inhibition (mg/mL)	Dose to achieve 100% growth inhibition (mg/mL)
Gram-negative pathogens				
E. coli	50	ND	50	ND
P. aeruginosa	25	50	25	ND
Gram-positive pathogens				
S. aureus	50	ND	25	50
S. epidermidis	ND	ND	ND	ND
S. agalactiae	25	50	50	50
S. *pyogenes*	25	ND	50	ND
Fungi				
C. albicans	50	ND	50	ND
	PAB in PBS		**PAB in PB**	
	Dose to achieve 50% growth inhibition (mg/mL)	Dose to achieve 100% growth inhibition (mg/mL)	Dose to achieve 50% growth inhibition (mg/mL)	Dose to achieve 100% growth inhibition (mg/mL)
Gram-negative pathogens				
E. coli	12.5	50	25	50
P. aeruginosa	50	50	25	50
Gram-positive pathogens				
S. aureus	50	ND	25	50
S. epidermidis	25	50	25	50
S. agalactiae	25	50	12.5	50
S. *pyogenes*	25	50	50	50
Fungi				
C. albicans	25	50	50	50

ND: not inhibition at the concentrations assayed; PEB and PAB: biosurfactants produced by *L. pentosus* and *L. paracasei* and extracted using phosphate buffer saline (PBS) and phosphate buffer (PB) respectively.

TABLE 2S. Biosurfactant concentration that gave 50% or 100% of anti-adhesive capacity between the values assayed.

	PEB in PBS		**PEB in PB**	
Microorganisms	Dose to achieve 50% microbial inhibition (mg/mL)	Dose to achieve 100% microbial inhibition (mg/mL)	Dose to achieve 50% microbial inhibition (mg/mL)	Dose to achieve 100% growth inhibition (mg/mL)
Gram-negative pathogens				
E. coli	ND	ND	ND	ND
P. aeruginosa	3.13	ND	3.13	ND
Gram-positive pathogens				
S. aureus	3.13	ND	3.13	ND
S. epidermidis	ND	ND	6.25	ND
S. agalactiae	3.13	ND	6.25	ND
S. *pyogenes*	1.57	ND	3.13	ND
Fungi				
C. albicans	ND	ND	ND	ND
	PAB in PBS		**PAB in PB**	
	Dose to achieve 50% microbial inhibition (mg/mL)	Dose to achieve 100% microbial inhibition (mg/mL)	Dose to achieve 50% microbial inhibition (mg/mL)	Dose to achieve 100% microbial inhibition (mg/mL)
Gram-negative pathogens				
E. coli	ND	ND	ND	ND
P. aeruginosa	3.13	ND	6.25	ND
Gram-positive pathogens				
S. aureus	3.13	ND	12.5	ND
S. epidermidis	12.5	ND	25	ND
S. agalactiae	3.13	ND	3.13	ND
S. *pyogenes*	6.25	ND	ND	ND
Fungi				
C. albicans	ND	ND	ND	ND

ND: not inhibition at the concentrations assayed; PEB and PAB: biosurfactants produced by *L. pentosus* and *L. paracasei* and extracted using phosphate buffer saline (PBS) and phosphate buffer (PB) respectively.