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Promising biosurfactant produced by a new *Candida tropicalis* UCP 1613 strain using substrates from renewable-resources

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The current work aimed to use agro-industrial wastes as a strategy to obtain biosurfactant from a new *Candida tropicalis* UCP 1613 isolated from mangrove sediments of Rio Formoso, Pernambuco State, Brazil. The yeast was identified based on 18S rRNA sequencing method and blast homology search. The promising strain showed the ability to use an optimal composition of a cheap medium containing whey (3%), cassava wastewater (7%) and soybean post frying oil (10%) during 96h which reduced significantly the surface tension from 70 to 28.8 mN/m. The yield of the biosurfactant obtained was 4.9 g/L and the minimum value of critical micelle concentration was 1.5%. In addition, this was isolated and characterized as an anionic polymeric molecule, composed of proteins (51%), lipids (37%) and carbohydrates (11%), and confirmed by Fourier transform infrared spectroscopy (FTIR) analysis. Also, biosurfactant was capable in forming stable emulsions at different ranges of temperature, pH and salinity. Alternatively, the biosurfactant displayed no toxicity against the different vegetable evaluated seeds *Brassica oleracea, Lactuca sativa* L. and *Solanum lycopersicum.* The antimicrobial activity of this biosurfactant was verified showing the main efficacy against Gram-positive bacteria besides inhibiting the antifungal activity against the yeast tested. The results obtained suggest its potential application to pharmaceutical, as well as environmental area.

Key words: *Candida tropicalis,* mangrove sediment, oil dispersion, biosurfactant.

INTRODUCTION

Nowadays the presence of surfactants in the daily activities of man makes life easier. These compounds are mainly obtained through chemical synthesis from oil and possess in their composition both hydrophilic and hydrophobic structural moieties. Thus, this duality gives them the ability to lower the surface tension, critical

micelle concentration, and interfacial tension between liquid–liquid/liquid–solid systems (Marchant et al., 2012; Sharma et al., 2014). In contrast, biosurfactants are the main classes of natural surfactants produced by fungi, yeast and bacteria.

According to their chemical structures, they are grouped into five major classes of lipopeptides, glycolipids, phospholipids, neutral lipids, and polymeric compounds (Desai and Banat, 1997; Khopade et al., 2012). Microbial surfactants as also known, have numerous advantages when compared to their chemically synthesized counterparts due to their lower toxicity, higher biodegradability, and better environmental compatibility, ability to be synthesized from renewable resources, higher foaming, higher selectivity and specific activity at extreme temperature, pH and salinity (Zheng et al., 2012; Rufino et al., 2014). These properties confer them commercial importance evidenced through the biotechnological applications in pharmaceutical, biomedical, cosmetic, petroleum, and food industries (Nitschke and Costa, 2007; Banat et al., 2010).

However, the benefit of these molecules contrasts with its high costs of the production associated with inefficient methods of recovery turning with distant reality for industry (Makkar et al., 2011). In this context, biocircular economy brings an innovative approach as a demand for global sustainability. Thus, efforts toward the recovery and reuse of waste from agro industrial origin through bioprocesses allow in valorizing the microbial synthesis of biobased products as biosurfactants (Mohan et al., 2016).

In addition, other significant aspects to be explored are the absence of toxicity and antimicrobial activity. These properties are suitable for the biotechnological applications of biomolecules such as biosurfactants (Sobrinho et al., 2013). With this in mind, the present paper was focused on the characterization of the biosurfactant produced by *Candida tropicalis* UCP 1613 isolated from mangrove sediment in Northeast of Brazil. The biosurfactant was extracted and characterized through compositional analyze, ion charge and Fourier transform infrared spectroscopy (FTIR). The stability of the biosurfactant under different conditions of pH, temperature and salinity was also studied and verified as its efficacy in the phytotoxicity assay and antimicrobial activity.

MATERIALS AND METHODS

Mangrove sediments collection

The samples isolated from mangrove sediments of Rio Formoso (localized at 08°39' 50" S 35°09' 32"), Pernambuco state, Brazil, were collected in July, 2015. The samples collected from mangrove ecosystem were previously marked in the area, collected in sterilized bottle, and immediately conducted to the Nucleus of Research in Environmental Sciences and Biotechnology (NPCIAMB), of the Catholic University of Pernambuco.

Agro-industrial substrates

The production medium was composed by agro-industrial wastes: cassava wastewater, a waste from cassava (*Manihot esculenta* Crantz) processing, if released directly into the environment before proper treatment, which could be a source of pollution. The whey was kindly supplied from the dairy industry of São Bento do Una, PE, Brazil, and waste cooking oil from informal commerce in Recife, PE, Brazil.

Yeast Isolation

The fresh mangrove sediment 1g was added to Erlenmeyer flasks of 250 mL containing 100 mL of Yeast Mold Broth (YMB) with the following composition (w/v): yeast extracts (0.3%), malt extract (0.3%), tryptone (0.5%), d-glucose (1.0%).

A 200 mg/L of chloramphenicol was supplemented after sterilization to minimize bacterial growth. The flasks were incubated at 150 rpm and 28°C for 48 h. After this period, serial dilutions from 10^{-1} to 10^{-5} were prepared and aliquots of 0.1 mL of each dilution were added to Petri dishes containing Yeast Mold Agar (YMA) with the following composition (w/v): yeast extract (0.3%), malt extract (0.3%), tryptone (0.5%), d-glucose (1.0%) and agar (5%). These plates were then incubated at 30°C for 72 h.

Identification of the isolated yeast

The colonial morphology of yeast was identified according to Accorsini et al. (2012) by color, borders and size of the colonies, texture, surface appearance and elevation. The molecular characterization was modified from the methodology performed by White (1990). DNA extraction was performed using Wizard ®Genomic DNA Purification Kit (Promega).

From the internal transcribed spacer region (ITS), which separates the genes 18, 5.8 and 28S rDNA, each sample were amplified using the pair of oligonucleotide primers ITS1 (5' TCC GTA GGT GAA CCT GCT GG 3') and ITS4 (5' TCC TCC GCT TAT TGA TAT GC 3'). The polymerase chain reactions (PCR) were performed with a final volume of 25 uL containing: 50 ng DNA, 10pm of each primer, 0.2 mM dNTPs, 1U Taq DNA polymerase, 1.5 mM $MgCl_2$ and buffer 1X (20 mM Tris-HCl pH 8.4; 50 mM KCl). The following parameters were used in the thermocycler (Eppendorf Mastercycler pro, model 6325): initial denaturation 95°C for 4 min, 40 cycles of denaturation, annealing and extension (92°C/1 min, 55°C/1 min and 72°C/2 min, respectively) and finally, the extension at 72°C for 5 min.

Then, amplicons were purified and sequenced by capillary electrophoresis on the ABI3130 platform (Life Technologies) which allows sequencing to 900 pb quality by the company Myleus Biotechnology. The chromatograms generated were subjected to

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Phred-Phrap software to read quality check of the base, during the sequencing step. The consensus sequences were submitted for analysis of similarity with sequences deposited in Gene Bank (Genbank) from the National Center for Biotechnology Information (NCBI), using the online tool basic local alignment search nucleotides (Blastn). The hits that had the highest percentage of similarity with the sequence under study, considering the best combination of "score" and "e-value" were regarded as identical.

Culture conditions

Cell grown of yeast on a slant were transferred to 50 mL of YMB. The culture was incubated in an orbital shaker at 150 rpm and 28°C for 24 h. Erlenmeyer's flask containing medium with the following composition: $MgSO_4$ 0.2 g/L, K_2HPO_4 1.0 g/L, KH_2PO_4 1.0 g/L, $CaCl_2$ 0.02 g/L, $FeCl_3$ 0.05 g/L and NH_4NO_3 1.0 g/L, supplemented with whey (3%), cassava wastewater (7%) and soybean post frying oil (10%) were adjusted to pH 5.3. The medium was sterilized by autoclaving at 121°C for 20 min.

After this period, 0.5% of YMB culture containing 10^4 cells/mL was used to initiate the growth. The culture was incubated at 28°C in an orbital shaker at 150 rpm for 96 h in triplicate and at regular intervals, every 24 h samples was taken to determinate surface tension and biomass.

Biomass determination

For biomass determination, 5 mL samples were mixed in pre-weighed tubes with chilled distilled water and centrifuged at 5000 rpm for 20 min. After two washing cycles, the cell pellet was dried in an oven 90°C for 24 h. All the assays were carried out in triplicate (Rufino et al., 2014).

Determination of surface tension

The determination of surface tension was carried out in the cell-free broth, obtained by centrifugation of the cultures at 5000 rpm for 20 min at room temperature, using a Sigma 700 digital surface tensiometer (KSV Instruments LTD, Finland), working on the principle of the Du Nuoy ring method (Gilanyi et al., 1976).

Isolation of biosurfactant

The biosurfactant was isolated by the precipitation method using the cell-free metabolic liquid with acetone 1:1 (v/v). The precipitate was allowed to stand for 24 h at 4°C, and after this period it was centrifuged at 5000 rpm for 20 h, at 5°C.

The supernatant was discarded and the isolated biosurfactant was submitted to dialysis against deionized water, which was changed every 3 h, for 96 h at 5°C. The yield of the isolated product was calculated as g/L and the analyses were performed in triplicate. The biosurfactant was collected and freeze-dried (Shavandi et al., 2011).

Critical micelle concentration (CMC)

The concentration at which micelles began to form was defined Critical Micelle Concentration (CMC). From known amounts of crude precipitate resuspended in distilled water, these determine the critical micelle concentration (CMC).

These measurements were realized using DuNouy Tensiometer model Sigma 70 (KSV Instruments LTD, Finland) at room temperature. The CMC was reached by measuring the surface tension until observing a constant value of the surface tension. All experiments were performed in triplicate (Rufino et al., 2014).

Compositional analysis

The protein content in the isolated biosurfactant was determined using the total protein test kit from Labtest Diagnóstica S.A., Brazil. The phenol-sulphuric acid method allowed in determinating the total carbohydrate content (Dubois et al., 1956). In the case of the lipid content 0.5 g of the isolated material was extracted with chloroform: methanol in different proportions (1:1 and 1:2, v/v). The organic extracts were then evaporated under vacuum and the lipid content was determined by gravimetric estimation (Manocha et al., 1980).

Ionic charge

The ionic charge of the biosurfactant was determined by using a Zeta potentiometer model ZM3-D-G, Zeta Meter System 3.0+, with direct images to the video of the Zeta Meter, San Francisco, CA, USA.

The top row was filled with a pure compound of a known ionic charge. The substance is known as anionic sodium dodecyl sulfate (SDS) at a concentration of (0.02 M) cationic substance and barium chloride (0.05 M). Petri dish was kept at room temperature for 48 h. The result was calculated when the precipitation lines appear as per Meylheuc et al. (2001) (Silva et al., 2014).

Fourier transform infra-red (FTIR) analysis

The identification of functional groups in the isolated biosurfactant was determined using a FTIR spectrophotometer (Bruker IFS 66) with KBr in the pestle. The FTIR spectrum was obtained at a frequency range of 4000 - 400 cm^{-1} (Smith, 1996).

Stability assay

In order to demonstrate biosurfactant stability under different conditions, the superficial tension of its partially purified solution (1 g/L) was examined. Samples were heated at 0, 5, 28, 70, and 100°C and cooled to room temperature, after which the surface tension was measured.

In the case of pH, this was adjusted to various pH values (2 to 12) by adding HCl or NaOH to a solution at room temperature and the surface was measured. The effect of NaCl concentrations (2 to 12%) was also a determinate. The tests were performed in triplicate (Tados, 2005).

Phytotoxicity assay

The phytotoxicity of the biosurfactant was evaluated based on seed germination and root elongation of cabbage (*Brassica oleracea)*, lettuce *(Lactuca sativa* L.) and tomato (*Solanum lycopersicum*), according to Tiquia et al. (1996).

Solutions of the isolated biosurfactant were prepared with distilled water at concentrations of 1, 1.5 and 2%. Toxicity was determined in sterilized Petri dishes (1 × 10 cm) containing Whatman N°1 filter paper. The seeds were pre-treated with sodium hypochlorite. Ten (10) seeds were inoculated in each Petri dish with 5 mL of the test solution at 27°C. After five days of incubation in the dark, seed germination, root elongation (≥5 mm) and the

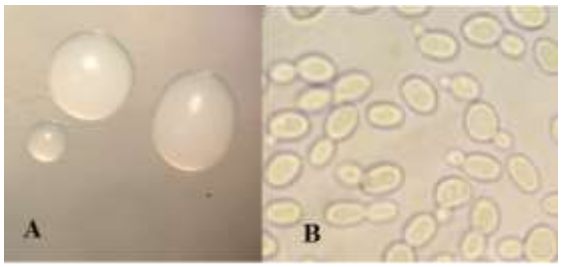

Figure 1. Colonies of yeast strain RF8 on yeast mold agar medium (A) and observation at optic microscopy (40X) (B).

germination index (a factor of relative seed germination and relative root elongation) were determined as follows:

Relative seed germination (%) = (number of seeds germinated in the extract/number of seeds germinated in the control) x 100

Relative root length (%) = (mean root length in the extract/mean root length in the control) x 100

Germination index = [(% of seed germination) x (% of root growth)]/100%.

Controls were prepared with distilled water to replace the biosurfactant solutions. Mean and standard deviation values of triplicate samples were calculated for each concentration.

Antimicrobial assay

The isolated biosurfactant was evaluated by the agar disc diffusion method (Bauer et al., 1996). Sterile discs (0.6 cm) soaked with the biosurfactant solution in methanol was assayed on the surface of and nutrient agar and malt extract medium for bacteria and yeast, respectively inoculated with the tested microorganism.

After incubation period for 24 h at 37± 2°C and 48 h at 25 ± 2°C for bacteria and yeast, respectively, the diameter of inhibition zones was measured (Bradshaw, 1992). Negative controls were prepared using the same solvents as employed to obtain the extract. Positive controls, ofloxacin (5 µg, Oxoid) was used for Gram-positive bacteria, cefaperazone–sulbactam (10 µg, Oxoid) for Gram-negative bacteria and amphotericin B (30 µg, Sigma) for *Candida albicans*. All the experiments were conducted in triplicates.

RESULTS

Morphological and molecular identification of the yeast producer of biosurfactant isolated from mangrove sediment

According to morphological observations of strain, yeast showed a characteristic of cream, smooth, glossy colonies whereas microscopic analysis revealed cells with small and medium sized with budding and no filaments characteristic. These traits allow presumptively the classifying of the isolate as *Candida* sp. (Figure 1).

Additionally, the molecular analyses of the sequences of ITS region were compared with the nucleotide database using the NCBI- blast tool. *Candida* sp. showed highest genetic agreement with *C. tropicalis* with a similarity of 99%. Thus the isolated was identified as *C. tropicalis* UCP 1613.

Kinetics of growth, biosurfactant production and biosurfactant yield

In this study, *C. tropicalis* UCP 1613 was cultivated in medium containing the agro-industrial substrates whey, cassava wastewater and soybean post frying oil during 96 h at 28°C. As shown in Figure 2, the growth of microorganism started rapidly after inoculation and remained increasing to 96 h of cultivation, when biomass reached 7.7 g/L. The surface tension dropped rapidly from 70 to 32.9 mN/m in the first 24 h and continued decreasing to 28.5 mN/m at 48 h of growth, indicating excellent surface-active properties.

Biosurfactant production started in the early stages of the exponential growth phase, simultaneously to the surface tension reduction which increased significantly until the end of cultivation, which attained the maximum biomass yield. The yield of the crude biosurfactant produced by *C. tropicalis* UCP 1613 was 4.9 g/L after 96 h of cultivation in medium containing 3% whey, 7% cassava wastewater and 10% soybean post frying oil (Figure 3).

Critical micelle concentration (CMC)

Thus in this study the biosurfactant obtained from the use of agro-industrial wastes showed a great surface tension reduction capacity, since the water surface tension was reduced from 70 to 28.5 mN/m with the increase of the biosurfactant concentration up to CMC of 1.5%, and then remained constant (Figure 4).

Preliminary characterization of biosurfactant

The determination of biochemical composition of the biosurfactant analyzed revealed the presence of 51% proteins, 37% lipids and 11% carbohydrates, suggesting its polymeric nature. Furthermore, an anionic profile with −57.4 ZPmv, 3, 4 µS/cm at 25.3°C was detected. In addition, the FTIR analysis was also used as complementary in this study.

According to the spectrum showed in Figure 4, stretching bonds of functional groups N–H between 3,514-3,257cm^{-1} indicated the presence of a peptide component. Also, a complex sequence of peaks due to the stretching vibration mainly C-C and C-O-P of oligo and polysaccharides (starches) were detected at an interval of 1200 to 1000 cm^{-1}. In addition, functional

Figure 2. Phylogenetic tree based on the ITS1 primer used for the identification of *Candida tropicalis* UCP 1613.

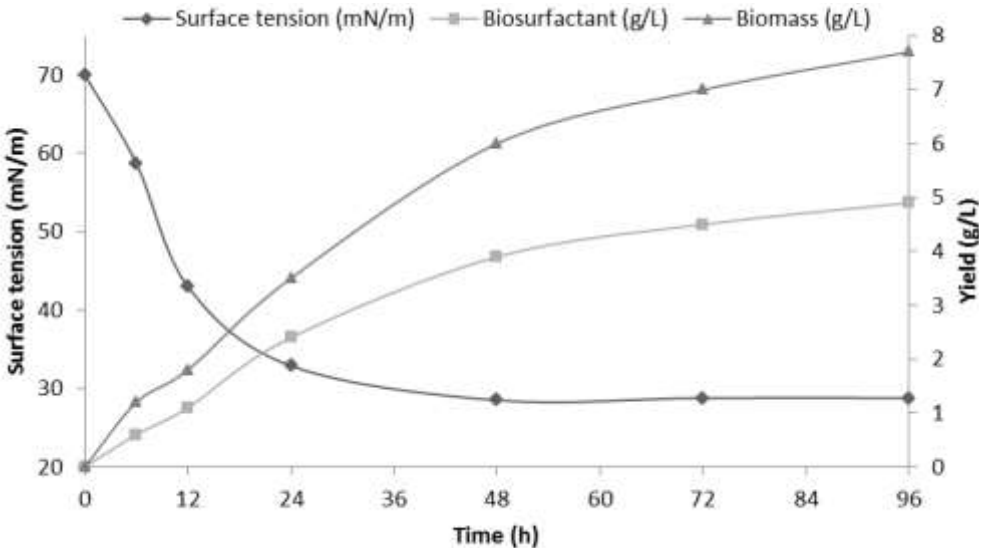

Figure 3. Growth, surface tension and yield of biosurfactant isolated from *C. tropicalis* UCP 1613 cultured in mineral medium supplemented with 3% whey, 7% cassava wastewater and 10% soybean post frying oil.

groups -CH=CH_2 and CH_3 were identified at 1448.27 cm^{-1} corresponding to fatty acids (Figure 5). Bands at approximately 1756.31 cm^{-1} showed the presence C=O consistent with ester functional group in lipids (Verma et al., 2015).

Biosurfactant stability

The behavior of the biosurfactant produced by *C. tropicalis* UCP 1613 was tested in different conditions of temperature, pH and NaCl concentrations. As it is shown in Figure 6, it was noticeable how the surface tension of the biosurfactant remained stable over a wide range of temperature, pH and NaCl concentrations.

Biosurfactant phytotoxicity

The phytotoxicity effect was evaluated through the germination index (GI) which combines measures of relative seed germination and relative root elongation. Seeds of vegetables *Brassica oleracea*, *Lactuca sativa* L. and *Solanum gilo* were used with different concentrations

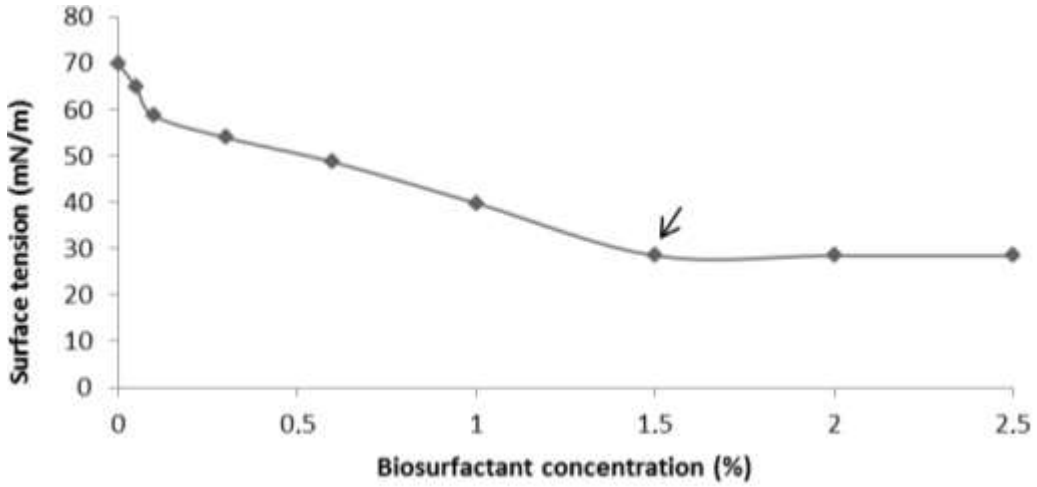

Figure 4. Surface tension versus concentration of the biosurfactant isolated from *C. tropicalis* UCP 1613 cultured in mineral medium supplemented with 3% whey, 7% cassava wastewater and 10% soybean post frying oil.

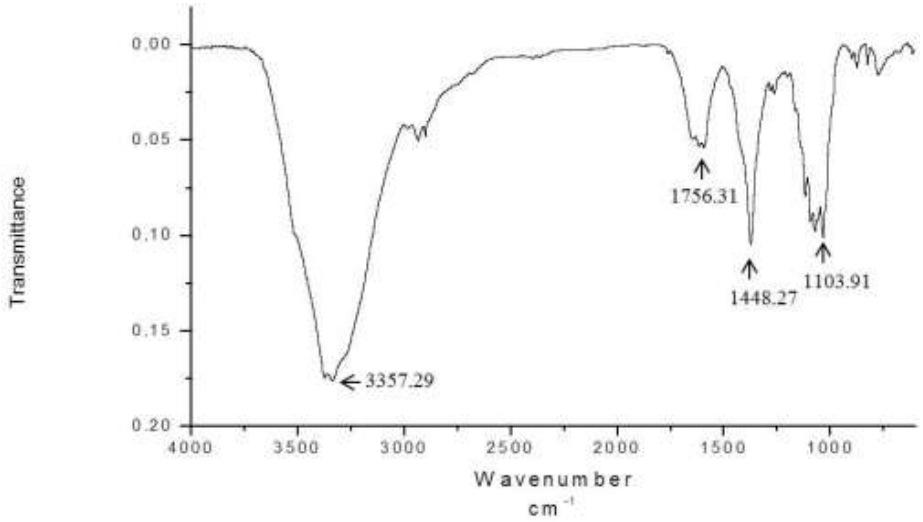

Figure 5. FTIR spectrum of extracted biosurfactant produced from *C. tropicalis* UCP 1613 in mineral medium supplemented with 3% whey, 7% cassava wastewater and 10% soybean post frying oil.

of the biosurfactant isolated from *C. tropicalis* UCP 1613.

In this regard, the Table 1 which displays that this metabolite did not show a phytotoxic effect against seeds tested. Also, it was interesting, the proportional relation was observed between the different germination index (GI) and the concentrations of biosurfactant.

Antimicrobial activity

In the present study, the polymeric biosurfactant produced by *C. tropicalis* UCP 1613 exhibited interesting antimicrobial activities. Table 2 shows that, Gram-positive bacteria tested mainly *Lactobacillus* sp. displayed susceptibility to the biosurfactant, albeit to different degrees. In contrast, Gram-negative bacteria were less sensitive as well as yeast.

DISCUSSION

The interest on *Candida* species has been increased in recent years due to its diverse biotechnological role. Specifically, *C. tropicalis* has been recovered from

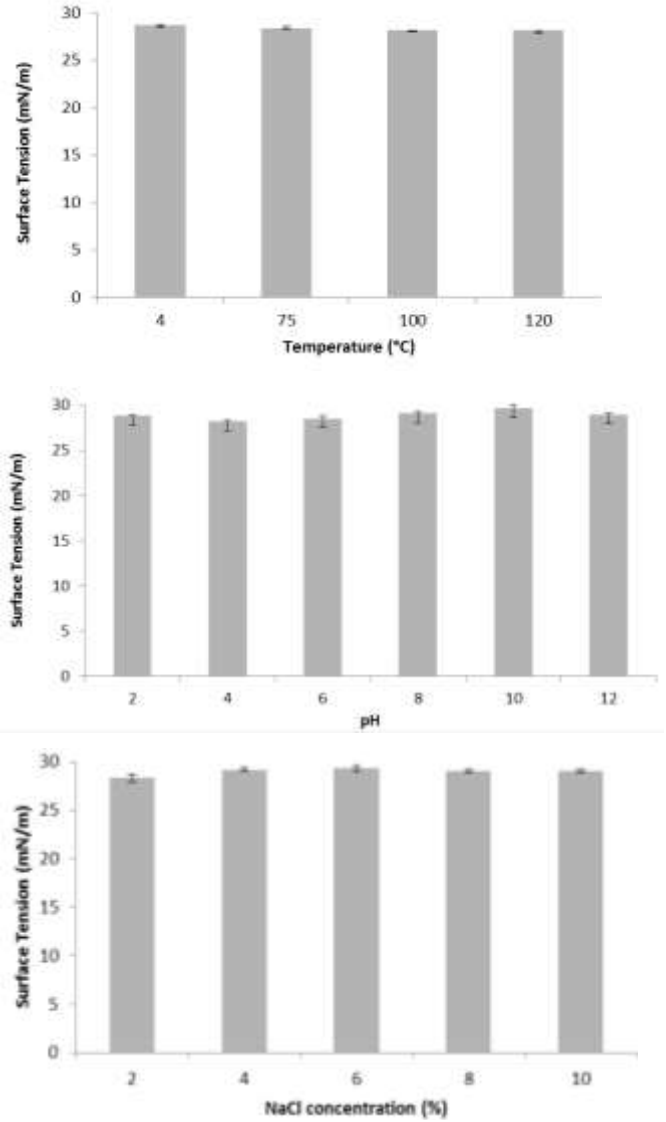

Figure 6. Stability of surface tension of biosurfactant produced by *Candida tropicalis* UCP 1613 in mineral medium supplemented with 3% whey, 7% cassava wastewater and 10% soybean post frying oil. Influence of temperature (A), pH (B) and sodium chloride concentrations (C) on surface tension stability.

seawater, sediments, mangrove plants, mud flats, marine algae and shrimp, indicating its wide distribution in tropical and subtropical marine environment (Luna et al., 2011; Yadav et al., 2012). The use of agro-industrial residues for the production of biosurfactant by members of the genus *Candida* have been previously described by several researchers (Luna et al., 2011; Rufino et al., 2014; Brasileiro et al., 2015). The ability of these microorganisms to grow and produce biosurfactants in wastes-based medium has been used to reduce the high costs of production of surface active compounds of biotechnological interest.

Table 1. Phytotoxicity of biosurfactant isolated from *Candida tropicalis* UCP 1613 cultured in mineral medium supplemented with 3% whey, 7% cassava wastewater and 10% soybean post frying oil against *Brassica oleracea*, *Lactuca sativa* L, *Solanum lycopersicum*.

Germination index (%)	Biosurfactant concentration (%)		
	1	1.5	2
Brassica oleracea	100±0.5	121±0.4	135±0.2
Lactuca sativa L.	100±0.2	102±0.2	118±0.1
Solanum lycopersicum	132±0.2	145±0.3	157±0.4

Experiments were performed in triplicate and the results represent means ± standard deviations of the three independent experiments.

In this study, the biosurfactant showed a growth-associate production, similar to the ones produced for *Candida sphaerica* cultivated in distilled water supplemented with ground-nut oil refinery residue and corn steep liquor as substrates (Sobrinho et al., 2008) and for *Candida lipolytica* cultivated in soybean oil refinery residue and glutamic acid (Rufino et al., 2014).

Although the biosurfactants produced by bacteria are more effective in reducing the surface tension, with values up to 25 to 26 mN/m, several yeasts biosurfactants described in the last few decades have shown similar values to those obtained by bacteria (Luna et al., 2011). One example is the biosurfactant produced by *C. tropicalis* in this study, which reduced the surface tension of medium to 28.5 mN/m after 48 h of cultivation. This value is lower than other biosurfactants produced by *C. tropicalis*: 35 mN/m (Haba et al., 2000); 35.44 mN/m (Coimbra et al., 2009), 37.86 mN/m (Campos et al., 2014) and 29.98 mN/m (Almeida et al., 2017).

By other side, the biosurfactant yield of *C. tropicalis* UCP 1613 was 4.9 g/L, which was better than others, previously reported for *C. tropicalis* strains: 3.0 g/L by *C. tropicalis* UCP 0996 cultured for 120 h in medium containing molasses, corn steep liquor and waste canola oil (Almeida et al., 2015) and 3.61 g/L by *C. tropicalis* cultivated in waste frying oil (Batista et al., 2010). However, other *Candida* members showed highest biosurfactant yields, for example: *C. lipolytica* (8.0 g/L) cultivated during 72 h in optimized medium containing soybean oil refinery residue and glutamic acid (Rufino et al., 2014) and *C. sphaerica* (9.0 g/L) cultured during 144 h in medium composed by ground-nut oil refinery residue and corn steep liquor (Luna et al., 2016).

In addition, *Candida utilis* achieved 12.52 g/L after 88 h of cultivation in waste canola frying oil and ammonium nitrate (Campos et al., 2014). These results demonstrate that the medium composition and culture conditions influence on yields of biosurfactants is produced by *Candida* strains. Also, the suitability of whey, cassava wastewater and soybean post frying oil was confirmed as alternative substrates for biosurfactant production (Silva

Table 2. Antimicrobial activity of the biosurfactant produced by *Candida tropicalis* UCP 1613.

Microorganism	Inhibition diameter (mm)		
	Biosurfactant concentration (%)		
	1	1.5	2
Pseudomonas sp.	10±0.3	12±0.3	14±0.4
Salmonella typhimurium UCP 6017	11±0.4	13±0.3	15±0.2
Serratia marcescens UCP 1549	12±0.2	14±0.4	15±0.1
Escherichia coli	11±0.3	13±0.2	14±0.3
Lactobacillus sp.	18±0.3	21±0.2	23±0.3
Staphylococcus aureus	16±0.2	18±0.2	19±0.5
Staphylococcus epidermidis	14±0.3	17±0.4	18±0.2
Bacillus licheniformis UCP 1013	15±0.4	16±0.2	18±0.5
Candida albicans	10±0.1	11±0.5	13±0.2
Candida lipolytica	11±0.2	13±0.4	14±0.5

et al., 2014; Andrade et al., 2015). Another important characteristic of biosurfactants is the critical micelle concentration (CMC) which is defined as the minimal concentration of the compound, required to yield maximal surface tension reduction of water which initiate micelle formation (Santos et al., 2013). The biosurfactant produced showed a greater capacity to reduce surface tension in comparison to biosurfactants from *Candida glabrata* (31 mN/m) (Luna et al., 2009), and *C. lipolytica* (32 mN/m) (Verma et al., 2015) (33 mN/m) (Santos et al., 2013). But also, this displayed a lower value of CMC than other biosurfactants reported, considering rates of 2.5% such as the biosurfactant from *C. glabrata* (Luna et al., 2009) and *C. lipolytica* (Sarubbo et al., 2007).

The compositional information of microbial surfactants allows the determining of the heterogeneous nature of these compounds. Thus, these are classified according to their molecular structure into glycolipids, lipopeptides, phospholipids, fatty acids, lipopolysaccharides, protein complexes, neutral lipids and polymers (Aparna et al., 2012). The analytical evidence confirmed the presence of carbohydrates and proteins in hydrophilic region of the molecule and also, the existence of lipids in the region hydrophobic of the biosurfactant. Similarly to the biosurfactant obtained in this study from *C. tropicalis* UCP 1613, *C. lipolytica* UCP 1002 has been also reported as producer of a heteropolymer composed 45% protein, 20% lipid and 10% carbohydrate (Sarubbo et al., 2006). However, surfactants produced by this genus can differ widely from one species to another (Rufino et al., 2014). In addition, the zeta potential determines the function of the surface charge of the particle which serves to predict and control the stability of colloidal suspensions and emulsions. The higher values obtained indicate good stability by repulsion between hydrophilic particles, as per the literature (Pornsunthorntawee et al., 2008; Satpute et al., 2010). Other biosurfactants produced by *Candida* species also show an anionic character (Sobrinho et al., 2008; Andrade et al., 2015; Luna et al., 2013).

Different environmental factors such as pH, salinity and temperature affect biosurfactants activity and stability. Therefore, it is important to study the influence of these parameters when considering specific applications for these compounds (Velmurugan et al., 2015). These results obtained suggest the feasibility of application in industries which works under extreme conditions of salinity, temperature and pH, as it was informed for other biosurfactants from *Candida* species (Gusmão et al., 2010; Luna et al., 2014) In this regard, the absence of toxicity is also fundamental for possible application of biosurfactant in the environment. Eco-toxicity bioassays are analytical methods which evaluate toxic effect of the chemical substances.

The exposure of living organisms employed as bio-indicators to these substances constitutes a valuable tool (Fletcher, 1991). The use of plants in toxicity tests offers several advantages among them is low maintenance cost and rapid results, with a special benefit assessment of the potential eco-toxic compounds in terrestrial environments (Farré and Barceló, 2003). In general, in this study was observed that from the different solutions of biosurfactant tested there was a positive effect on the growth seeds (Table 1). Also from the observation of leaves and the elongation of secondary, it was suggested a growth stimulating effect which was verified considering that GI higher than 80%, indicate the absence of phytotoxicity (Tiquia et al., 1996). Similar results were observed by Luna et al. (2013) and Rufino et al. (2014), who investigated the phytotoxic potential of the biosurfactant produced by *C. sphaerica* UCP 0995 and *C. lipolytica* UCP 0988, respectively. The same observations were confirmed by Krawczyńska et al. (2012), Alsohim et

al. (2014) and Silva et al. (2015) which detected the positive effect of biosurfactants in the seedling development.

Alternatively one useful property of many biosurfactants that has been reviewed recently is their antimicrobial activity (antibacterial, antifungal and antiviral) (Banat et al., 2014; Rienzo et al., 2015; Borsanyiova et al., 2016). In this study, the yeasts were less sensitive than Gram-negative bacteria. Some studies confirm that biosurfactants, even in low concentrations, may destabilize the microorganism's membranes and finally inhibit their growth (Carrillo et al., 2003; Calvo et al., 2009). In this regard, it has been observed that Gram-positive bacteria are more susceptible to biosurfactants than Gram-negative bacteria (Elving et al., 2000), which are not inhibited at all or present a slight inhibition. This evidence is in agreement with the results obtained in this study. Similarly, biosurfactants produced by *C. sphaerica* and C. *lipolytica* have also demonstrated antimicrobial activity against different species of fungi and bacteria, suggesting the use of these biomolecules as an alternative antimicrobial agents in the medical field (Luna et al., 2011; Rufino et al., 2011).

Conclusions

In the present study with the new yeast isolated from mangrove, sediments were identified as *C. tropicalis* UCP 1613. The optimal carbon and nitrogen sources was a combination of whey (3%), cassava wastewater (7%) and soybean post frying oil (10%) resulting significantly reduction of the surface tension.

The biosurfactant produced was non-cytotoxic which showed stable surface tension to high temperature, acid to alkaline pH, and all concentrations of electrolyte (up to 12%). Besides, the new biosurfactant is characterized as anionic and polymeric molecule. The tension-active molecule showed properties to effective antimicrobial activity, Gram-positive and negative bacteria, and yeasts. This study strongly suggested the biosurfactant produced by *C. tropicalis* UCP 1613 which play a promising role in biomedical application due to its non-toxicity, stability and higher antimicrobial activity. The use of renewable resources as agro-industrial waste from circular bio-economy approach allowed the obtaining of biosurfactant as a value added product.

CONFLICT OF INTERESTS

The authors declared no conflict of interest.

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