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Production of Rhamnolipids by *Pseudomonas aeruginosa* AP029-GLVIIA and Application on Bioremediation and as a Fungicide

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Rhamnolipids are biosurfactants synthesized by different species of microorganisms. In this study, the influence of carbon/nitrogen ratio (C/N) and percentage of inoculum onrhamnolipid production by *Pseudomonas aeruginosa* AP029-GLVIIA using glucose as substrate was evaluated. The critical micellar concentration (CMC) and surface tension were analyzed for the highest biosurfactant concentration, which presented values of 49.63 mg/L and 29.5 mN/m, respectively. Emulsification rates were determined for different solvents and showed the bioproduct's ability to form stable emulsions for up to 90 days. The efficiency of the biosurfactant in removing petroleum present in the sand was 16.8%and the antimicrobial activity of the rhamnolipid against fungal species was determined, showing its potential to inhibit fungi of the species *Candida tropicalis* and *Candida albicans*.

Keywords: Rhamnolipid, *Pseudomonas aeruginosa*, Emulsification, Antifungal activity, bioremediation.

Surfactants are an important class of chemical compounds synthesized in large part by petroleum derivatives(Gudiña *et al*., 2015a; Padilha et al., 2015)They are formed by hydrophobic and hydrophilic portions, which are distributed at the interface between liquid phases causing the decrease of surface and interfacial tensions(Akbari *et al*., 2018;Ehinmitola *et al*., 2018;Gudiña *et al*., 2016; Grüninger *et al*., 2019; Mondal *et al*., 2015; Mondal *et al*., 2016, Mondal et al., 2017a). The surfactant production is expected to increase to 24 million tons and be worth approximately $ 120 million by 2020(Jiang *et al*., 2020).

Currently, the studies about biosurfactants have been expanded due to the high environmental impact caused by some chemical surfactants. In addition to having similar properties to chemical surfactants, these amphiphilic bioproducts have advantages such as biodegradability, low toxicity and stability under extreme conditions of pH, temperature and salinity(França *et al*., 2015). Surfactants have potential to be applied in

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numerous products or fields, such as, detergents, paints, paper products, pharmaceuticals, cosmetics, petroleum, food, and watertreatment(Costa *et al*., 2010; Mondal et al., 2017b).

Biosurfactants can be produced by different strains of microorganisms (bacteria, filamentous fungi and yeasts) using renewable raw materials with low cost as a substrate(Abdel-Mawgoud *et al*., 2010; Araújo *et al*., 2013). These molecules are classified into five major groups: lipopeptides, glycolipids, fatty acids, phospholipids and polymeric biosurfactants(Geetha *et al*., 2018).

Among the glycolipids there are the rhamnolipids, composed of rhamnose molecules and one or two units of â-hydroxydecanoic acid, which are present mainly in four isoforms(Mulligan, 2005). The production of these molecules occurs predominantly by *Pseudomonas aeruginosa* and the z is classified as mono and di-rhamnolipids according to the amount of rhamnose present in the structure.In addition, the proportion of these two forms can be influenced by nutritional and environmental conditions of microbial growth(Oluwaseun et al., 2017; Varjani and Upasani, 2017). Mono-rhamnolipids congeners show increase emulsification and antimicrobial properties in comparison to di-rhamnolipids(Sood *et al*., 2020). Other species of *Pseudomonas* have also been reported as producing rhamnolipids, such as *P. chlororaphis*, *P. plantarii*, *P. putida* and *P. fluorescens*(Randhawa and Rahman, 2014). The main characteristics of these biosurfactants are related to their ability to reduce the surface tension of water to between 28 and 30 mN/m, reduce interfacial tension between water and hydrocarbons, and have a critical micelle concentration(CMC) between 10 and 200 mg/L(França *et al*., 2015; Gudiña *et al*., 2015a).

Although can be applied in different areas, the production of biosurfactant on a large scale is not yet totally feasible, since the cost with the production and recovery of this product is relatively high(Souza *et al*., 2018). However, an alternative to reduce production costs would be the use of low-cost raw materials such as frying oils, sugarcaneand beet molasses and cassava wastewater(Banat *et al*., 2014). But, even with some limitations it is estimated that in 2023 approximately 524 tons of biosurfactant will be traded, which will be responsible for a turnover of US$ 2.7 billion(Felipe and Dias, 2017).

In this context, the objective of this study was to evaluate the production of rhamnolipids by *Pseudomonas aeruginosa* AP029-GLVIIA by varying the carbon/nitrogen ratio (C/N), based on a simple and affordable source of carbon and energy (glucose), and the percentage of inoculum. Thus, the produced rhamnolipids were characterized in terms of CMC, emulsification index, bioremediation and antifungal activity against the species *Candida albicans* and *Candida tropicalis*.

MATERIAL AND METHODS

Chemical

The main chemicals used during this study were corn oil (Cargil Co. - SP, Brazil), D-glucose (Synth Co. - SP, Brazil), hexadecane (Sigma Co., USA), iron sulfate II heptahydrate(Synth Co. - SP, Brazil), kerosene(Líder Co. - RN, Brazil),magnesium sulfate heptahydrate(Synth Co. - SP, Brazil), monobasic potassium phosphate(Synth Co. - SP, Brazil), motor oil (Petronas Co.-MG, Brazil), peptone (BD Co. - SP, Brazil), sodium chloride (Cinética Co. - PR, Brazil), sodium nitrate (Cinética Co. - PR, Brazil), sodium phosphate dibasic heptahydrate(Synth Co. - SP, Brazil), soybean oil (Bunge Co. - SP, Brazil), and yeast extract (BD Co. - SP, Brazil) they were all of analytical grade.

Microorganism and maintenance

Pseudomonas aeruginosa AP029-GLVIIA was isolated from an oil well in the city of Mossoró(Rio Grande do Norte, Brazil) and deposited in the culture collection of the Department of Antibiotics in the Federal University of Pernambuco (UFPE - Brazil). The microorganism was maintained in petri dish with PCA (Plate Count Agar) at 5 °C(Araújo *et al*., 2017).

Inoculum and culture medium

For inoculum the microorganism was transferred from the petri dish to 250 mL conical flasks containing 100 mL of medium consisting of 3.0 g/L yeast extract, 5.0 g/L sodium chloride and 5.0 g/L peptone at pH 7.0 then after 24 hours of cultivation at 38 °C and 200 rpm, aliquots were transferred to the production medium(Peng

et al., 2012).The production medium (pH 6.5) consisted of a saline solution of $MgSO_4.7H_2O$ (1.0 g/L), $Na_2HPO_4.7H_2O$ (1.1 g/L), KH_2PO_4 (1.5 g/L), $NaNO_3$ (2.0 g/L), $FeSO_4.7H_2O$ (0.1 g/L) and glucose. The influence of glucose on the production ofrhamnolipids was evaluated by varying its concentration for 10.0, 18.0and 26.0 g/L.

Five runs were performed in order to evaluate different culture conditions. The percentage of inoculum was 3.0, 10.0 and 17.0% (v/v) and the C/N ratio was 5, 9 and 13.All experiments were assayed in duplicate at 38 °C and 200 rpm for 72 hours using 100 mL of solution (production medium and inoculum) in 250 mL flasks.The pH of the crude broth was measured by potentiometer mPA 210 (Tecnopon, Brazil) and adjusted to 8.0. Then the medium containing the rhamnolipids was centrifuged (centrifuge 5804 R, Eppendorf, USA) at 1370 x g for 10 minutes and the supernatant obtained was used for further analysis.

Analytical Methods

Determination of biomass

The biomass quantification was performed by the dry mass method as described byBezerra et al. (2012). The crude broth was centrifuged (centrifuge 5415 D, Eppendorf, Germany) at 15700 x g for 15 minutes. Each point was measured in triplicate and the cell concentration (g/L) was estimated according to Equation 1:

$C_{biomass}$=(((mass of the tube with biomass-Empty tube mass))/2) x 1000 ...(1)

Determination of glucose

Glucose quantification was evaluated by the 3,5 dinitro-salicylic acid (DNS) method according toMiller (1959). The analyses were performed in triplicate.

Avaliation of total proteins

Measurement of total proteins was performed according to Bradford (1976). The assays were performed in duplicate.

Recovery and quantification of the rhamnolipids

The recovery of the rhamnolipidswas performed first by acid precipitation of the supernatant I obtained from the centrifugation as commented in topic 2.2.The cell-free broth was acidified to pH 2.0 using HCl (6M) and stored at 4 °C overnight.The sample was then centrifuged at 1370 x g for 10 minutes. The supernatant from that centrifugation was discarded and 5 mL of distilled water and petroleum ether in the ratio of 1: 1 (v/v) were added to the precipitate.This procedure was repeated three times and at each repetition the emulsion formed by the ether and the rhamnolipidswere removed and stored.Finally, the organic phase obtained from the last step was taken to the rotary evaporator V-850 (Büchi, Switzerland).Ten mL of distilled water was added to the obtained concentrate and stored(Peng et al., 2012).The quantification of the rhamnolipids was performed by the thioglycolic colorimetric method according toOliveira *et al*.(2013).

Properties of the biosurfactant

Critical Micellar Concentration (CMC)

Different dilutions of a 100 mg/L crude rhamnolipids mixture (1.65, 4.96, 6.20, 9.92, 24.82, 33.08, 49.63 and 100 mg/mL) were performed to determine the CMC. The surface tension for each defined concentration was measured using the Phoenix 150 SEO tensiometer and the CMC values were obtained in triplicate(Araújo *et al*., 2017).

Emulsification index

The emulsification index was determined by the method ofCooper and Goldenberg (1987). In this case 2.0 mL of supernatant I was added to a tube containing 2.0 mL of the working solvent: hexadecane, toluene, kerosene, soybean oil, corn oil and motor oil.After 24 hours, the emulsification index (E) was measured according to Equation 2, described byWei *et al*. (2005). These measurements were repeated every 15 days until completing 90 days and were performed in triplicate.

E (%)=((height of the emulsion)/(total) height) x100 ...(2)

Assessment of potential for Bioremediation

The evaluation for oil recovery was carried out using sand from a beach (Praia do Meio) of Natal (RN) - Brazil, containing 10% (w/w) of oil in Erlenmeyers of 250 mL. The mixture was allowed to stand for 24 hours and, subsequently, 40 mL of rhamnolipids (1.0 g/L) were added to each flask.Samples were incubated at 40 °C and 100 rpm for 24 h. Then the water/oil mixture was centrifuged at 5000 rpm for 25 minutes in order to quantify the mass of purified oil. The control assay was performed using distilled water under the same conditions and all experiments were performed in

triplicate(Gudiña *et al*., 2015a; Pereira *et al*., 2013).

Evaluation of antifungal activity

The tests to evaluate the antifungal activity of the biosurfactant were carried out following the methodology described by the Clinical and Laboratory Standards Institute (CLSI) with modifications(Cockerill *et al*., 2012). The antifungal action of purified and unpurified rhamnolipids was evaluated against two yeast strains:*Candida albicans*ATCC 90028 and *Candida tropicalis*ATCC 13803. In a 96-well plate, 50.0 ìL of the fungal suspension with 10^5 CFU/mL in Müeller Hinton broth (MH), supplemented with 0.2% glucose, were added to the rhamnolipids (7.425, 3.71, 1.85 , 0.93 and 0.46 ìg/mL) and fluconazole (0.58 ìg/mL) and then incubated at 35.0 ± 2.0 °C, under agitation of 200 rpm. The optical density at 595 nm was evaluated usinga microplate reader (Epoch Biotek, Winooski) at zero time and after 24 hours.

Statistical analysis

The analysis of the emulsification index and the antifungal activity were performed in triplicate and evaluated by the Tukey test using the software Statistica 7.0 (StatSoft Co, USA) and GraphPad Prism 5.0 (La Jolla California, USA).

RESULTS AND DISCUSSION

Production of rhamnolipids

Rhamnolipid production by *Pseudomonas aeruginosa*AP029-GLVIIA using glucose as substrate was evaluated by changing the C/N ratio and the percentage of inoculum added to the culture medium. In the five conditions studied, the concentrations of biomass, glucose, rhamnolipid and total proteins were analyzed, as well as pH variation.

According to Table 1, it can be seen that as the C/N ratio increased there was an increase in both biomass formation and rhamnolipid production. Indeed, it is known that these metabolitesformation is favored under nitrogen limiting conditionsSantos *et al*. (2002). The highest production of biosurfactant occurred for a ratio C/N of 13 with percentage of inoculum of 3.0%. It should be highlighted that Sousa *et al*. (2014) found a similar result producing rhamnolipids using glycerol as the carbon source. But, comparing the runs 2, 4 and 5, in which there was an increase in the amount of inoculum, it was observed that the product and the biomass had their values decreased and increased, respectively.The decrease in the amount of biosurfactant produced may be associated with the Quorum Sensing (QS) shown by *Pseudomonas aeruginosa*. The QS consists of a bacterial communication system capable of coordinating functions as motility and virulence agents, as well as controlling the levels of important compounds for biofilms formation, such as rhamnolipids, lectin A and siderophores(Kariminik *et al*., 2017). In general, the production of rhamnolipids was favored by using ahigher C/N ratio and alower percentage of inoculum.

During the cultivation, glucose consumption varied from 82.5 to 90.4%, then showing good assimilation of the substrate by the microorganism. In addition, the highest consumption occurred for the first 24 hours of each experiment. The values are of the same magnitude as shown by Bezerra *et al*. (2012); Sousa *et al*. (2014)that obtained substrate uptake of 91.9 and 50.8%, respectively,when usedcassava wastewater and glycerol as substrate.Different carbon sources have been used for the production of rhamnolipids, for instance, Ramírez *et al*. (2015)investigated the olive-mill waste andVarjani and Upasani (2016)evaluated crude oil, nonane, decane, dodecane, N-paraffins, kerosene, diesel, xylene, glucose and glycerol, with glucose being the substrate that presented the highest yield in production of rhamnolipids.Additionally, Mondal *et al*. (2017)used different carbon sources and observed that glucose provided the best result for the biosurfactant synthesis.

According to Table 1, the concentration of total proteins increased in proportion to the increase in the amount of rhamnolipid, probably because these metabolites are capable of increasing the permeability of the cell membrane, and consequently, the concentration of proteins in the medium(Shao *et al*., 2017). However, the decrease in the protein concentration as shown in the runs 4 and 5 may be associated with the production of proteases but the rhamnolipids production was almost unchanged due to the QS(Bouyahya *et al*., 2017).

With regard to pH during cultivation it ranged from 5.88 to 8.20 when considering all runs performed, however for the maximum

rhamnolipids concentration it ranged between 6.28 and 6.58.Similar results were shown by Varjani and Upasani (2017)that reported that rhamnolipids synthesis by *Pseudomonas sp*. is favored by pH between 6.0 and 6.5. In addition, the production of total proteins showed an interesting relationship with pH. It can be seen that as protein concentration increases there was an increase in the pH value, as shown in Figure 1, indicating metabolism for proteins formation with ammonium formation that affected pH by increasing it(Santos *et al*., 2002).

Characterization of biosurfactant

Critical Micellar Concentration (CMC)

In the present study the CMC of the unpurified (crude) rhamnolipids produced by *P. aeruginosa* AP029-GLVIIA was evaluated. The CMC determination was performed by measuring the surface tension of the cell free broth corresponding to the point of greatest rhamnolipid concentration (24 hours,run 3). The rhamnolipids producedwere able to reduce the surface tension of water from 71.94 ± 1.07 to 29.42 ± 1.41 mN/mwith a CMC of 49.63 mg/L.It is emphasized that the CMC depends on the pH, temperature, ionic strength and surfactant structure. But, as the rhamnolipids were synthesized in ionic medium, the influence of pH will be more significant when compared to the other mentioned parameters. An interesting fact concerns the variation in the value of CMC when considering the different

Table 1. Effect of C/N ratio and percentage of inoculum on rhamnolipid production, cell growth, pH and total protein production

Run	C/N Ratio	Inoculum (%)	Biomass (g/L)	Time[1] (h)	Rhamnolipid (g/L)	Time[2] (h)	pH[3]	Total proteins(g/L)
1	5	3	1.33 ± 0.02	12	0.30 ± 0.00	4	6.52 ± 0.03	0.149 ± 0.01
2	9	3	1.57 ± 0.06	24	0.78 ± 0.01	60	6.28 ± 0.05	0.248 ± 0.01
3	13	3	2.50 ±0.04	24	0.84 ± 0.06	24	6.44 ± 0.05	0.426 ± 0.00
4	9	10	2.03 ± 0.20	12	0.39 ± 0.03	12	6.58 ± 0.03	0.233 ± 0.00
5	9	17	1.93 ± 0.14	10	0.40 ± 0.01	48	6.46 ± 0.00	0.123 ± 0.00

[1,2]Time at which maximum values of biomass and product were reached, respectively.

[3] pH corresponding to the highest concentration of rhamnolipids obtained in the assay.

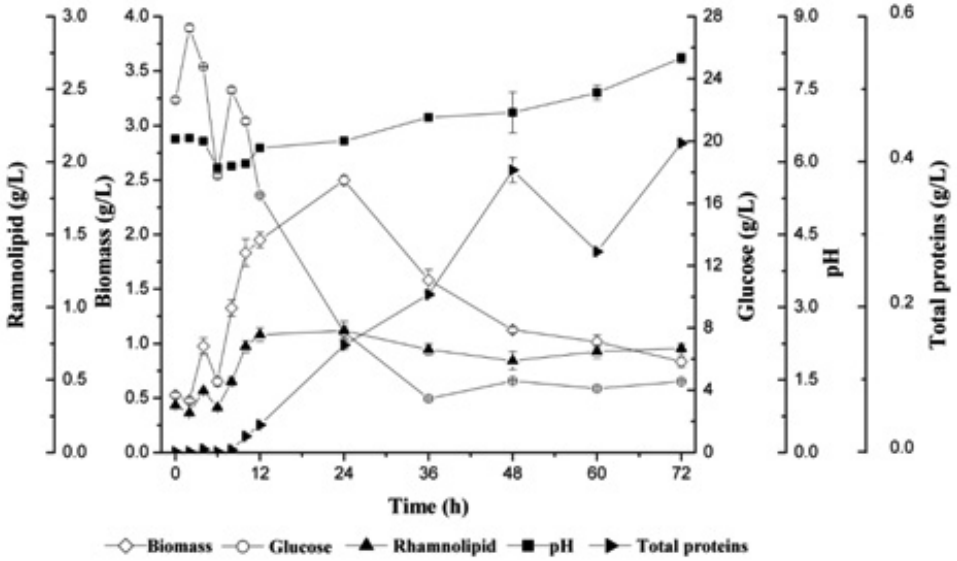

Fig. 1. Cell growth profile, substrate consumption, rhamnolipid production and pH as a function of time for run 3 (C/N of 13 and 3% inoculum)

Table 2. Comparison of emulsification indexes of different biosurfactants

Microorganisms	Carbon Sources	Solvents	Emulsification index	References
Pseudomonas aeruginosa AP029/GLVIIA	Glucose	Hexadecane, toluene, kerosene, soybean oil, corn oil and motor oil	57.47 to 77.55%	This study
Pseudomonas aeruginosa LBI	Natural oils	Keroseneandtoluene	70 to 100%	Costa *et al*. (2006)
Pseudomonas aeruginosa AP029/GLVIIA	Cassava	Kerosene	65%	(Bezerra *et al*. (2012)
Pseudomonas aeruginosa #112	Corn steep liquor and molasse	Hexadecane	60%	Gudiña *et al*. (2016)
Pseudomonas aeruginosa UCP0992	Cornsteepliquor	Soy, corn and motor oil	62.5 to 100%	Rufino *et al*. (2016)
Pseudomonas aeruginosa NCIM 5514	Glucose	Hidrophobicsolvents	17.1 to 82.3%	Varjani and Upasani (2016)
Bacillus subtilis ICA56	Glucose	Motor oil	79%	França *et al.* (2015)

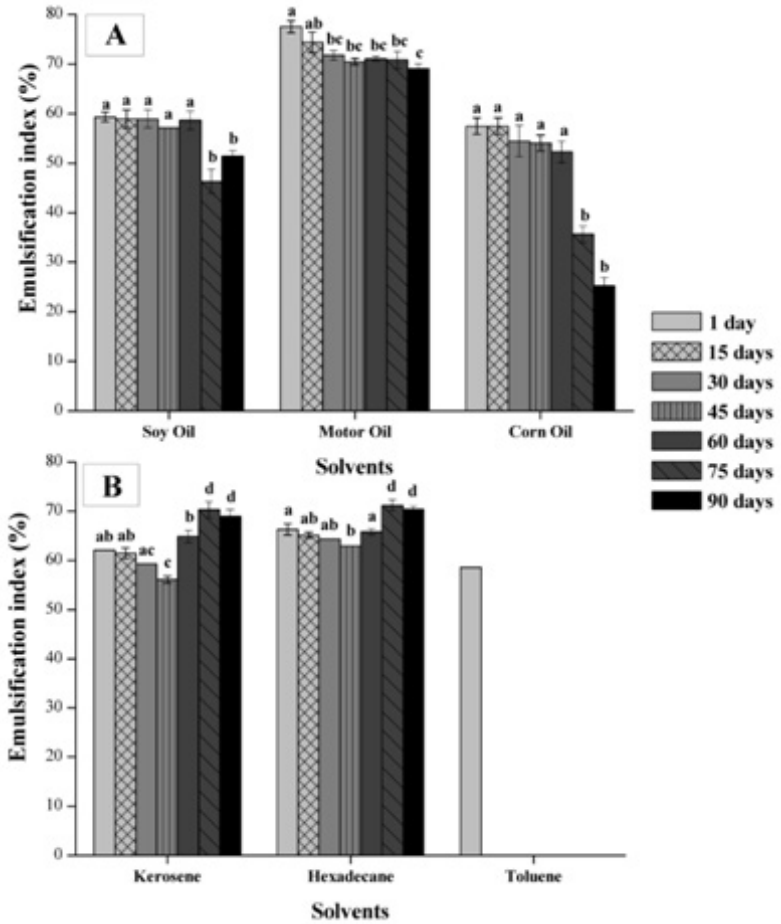

Fig. 2. Emulsification index determined at different times in oils (A) and hydrocarbons (B). Letters or set of equal letters did not present a statistically significant difference ($p<0.05$)

isoforms adopted by rhamnolipids(K³osowska-Chomiczewska *et al*., 2017). Samadi *et al*. (2012) observed that fora mixture of rhamnolipids (RLs), CMCwas 22 mg/L and surface tension of 26 mN/m. However, when it was applied only mono-rhamnolipids (RL1), the CMC decreased to 15 mg/L while the tension reached 25 mN /m. Finally, for a mixture of di-rhamnolipids (RL2) the CMC reached 30 mg/L and tension of 29.5 mg/L.Gogoi *et al*., (2016)when studying rhamnolipid production obtained CMC values of 110 and 72 mg/L for crude and purified rhamnolipid, respectively. Regarding the surface tension, the purified rhamnolipid reached 29.5 mN/m. In contrast, Sodium Dodecyl Sulphate (SDS), a chemical surfactant widely used in industry, has CMC values of up to 2890 mg/L and surface tension of 37 mN/m. Thus, when comparing these values with those of the rhamnolipids, it can be seen that the the latter has higher efficiency, since the values obtained are smaller(Bognolo, 1999).

Emulsification index

The formation of the emulsion occurs when a liquid phase is dispersed in the form of droplets in a continuous liquid phase(Desai and Banat, 1997). Emulsification tests were performed with the cell-free supernatant (24 hours, run 3) and they were determined using six organic solvents: hexadecane, toluene, kerosene, soybean oil, corn oil and motor oil. In order to evaluate the stability of the emulsion formed, the indices were measured every 15 days until to complete 90 days.

Figure 2 shows the results of the emulsion formed in the first 24 hours: corn oil (57.47%), toluene (58.62%), soybean oil (59.32%), kerosene (62.07%), hexadecane (66.30%) and motor oil (77.55%).There were oscillations over time, but the emulsification index values remained above 50% for the hydrocarbons, except for the toluene which kept the emulsion for only 24 hours.In relation to the oils only the corn was unable to maintain the emulsion higher than 50% in the last 30 days. In all solvents the emulsion formed at the top of the system, indicating that the rhamnolipidsare responsible for forming water-in-oil (W/O) emulsions(Nguyen and Sabatini, 2011)

Assessment of potential for Bioremediation

In this study, a previous test for the recovery of contaminated sand oil was carried out. From the experiment it was possible to determine that the rhamnolipids were able to remove 16.8 ± 1.6% of the petroleum when compared to the control test (sand/ petroleum/distilled water).When studying different surfactins produced by species of *Bacillus subtilis,*Pereira et al. (2013)achieved oil removal results between 19.0 and 22.0% using a 1.0 g/L rhamnolipid solution. In similar work, Gudiña *et al*. (2015b)obtained values of 15.0, 26.3 and 25.1% when using 1.0, 2.5 and 5.0 g/L surfactin.On the other hand, Gudiña *et al*. (2015a)

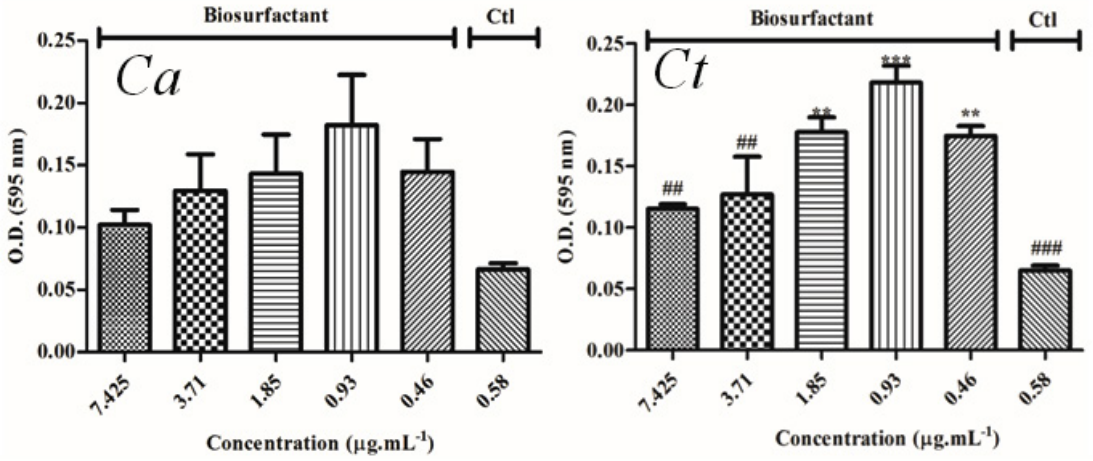

Fig. 3. Evaluation of the antifungal activity of the purified rhamnolipid incubated against the fungi *Candida albicans* (Ca) and *Candida tropicalis* (Ct). Ctl corresponds to the control of antifungal activity, fluconazole. *** $p<0.001$ and ** $p<0.01$ compared to the control group (Ct1); ### $p<0.001$ and $p<0.05$ compared to the biosurfactant at the concentration of 0.93 mg

produced rhamnolipids and used them to removal of petroleum showing values of 22.1; 43.7 and 55.0% for the same concentrations of rhamnolipids presented in the present study. Recently, Das and Kumar (2019)demonstrated that biosurfactant was able to recover 46.5% of the crude oil present at a sand pack column.

Evaluation of antifungal activity

Analyses of antimicrobial activity were performed using purified and unpurified rhamnolipids, however, only the purified one was able to inhibit the growth of the microorganisms *Candida albicans*ATCC 90028 and *Candida tropicalis*ATCC 13803. Figure 3 shows the inhibition of fungi versus the concentrations of rhamnolipids (7.42, 3.71, 1.85, 0.93 and 0.46 ìg/mL) and the applied control, fluconazole, (0.58 ìg / mL).According to the results, the concentration of rhamnolipid showing higher antifungal activity was 7.42 ìg/mL for the two yeasts assayed. In addition, to *Candida tropicalis*the biosurfactantconcentration of 3.71 ìg/mLdid not show statistical difference when compared with the control (fluconazole) while for yeast*Candida albicans* all tested concentrations have similar action to fluconazole.

The present study demonstrates that the rhamnolipids produced by *P. aeruginosa* AP029-GLVIIA have potential to act as antifungal agents.

Abalos *et al*. (2001)used rhamnolipids to inhibit the growth of the following microorganisms: *Aspergillusniger* and *Gliocadiumvirens* (16 ìg/mL), *Chaetomiumglobosum*, *Penicilliumchrysogenum* and *Aureobasidiumpullulans* (32 ìg/mL), *Botrytis cinerea* and *Rhizoctoniasolani* (18 ìg/mL). The values presented in parentheses correspond to the Minimum Inhibitory Concentration (MIC).

In addition to antifungal activity, rhamnolipids also have a high potential to inhibit bacterial growth.Tedesco *et al*. (2016)applied biosurfactants against the bacteria *Staphylococcus aureus* and *Burkholderiacepacia* obtaining values MIC of 1.56 and 3.12 ìg/mL.Oluwaseun et al. (2017) evaluated the antimicrobial activity of rhamnolipids, produced by *Pseudomonas aeruginosa* C1501, against various microorganisms (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Saccharomyces cerevisiae*, *Aspergillusflavus* and *Aspergillusniger*). The results showed that this bioproduct has the capacity to be used at industrial, food and biomedical applications. On the other hand, Ndlovu et al. (2017) studied the antibacterial and antifungal activity of biosurfactant extracts by *Bacillus amyloliquefaciens* and *Pseudomonas aeruginosa* against antibiotic resistant (*Staphylococcus aureus*,*Escherichia coli*) and fungal pathogens (*Candida abicans*,*Cryptococcus neoformans*). The biosurfactant presented antimicrobial action about all microorganisms analyzed.

Recently, Ferreira *et al.* (2019) investigated the antimicrobial activity of rhamnolipids against Gram-positive and Gram-negative food pathogens (*Bacillus cereus*, *Listeria monocytogenes*and *Staphylococcus aureus*) under different pH. The study suggests that the biosurfactant can be enhanced in acid food.

CONCLUSION

The *Pseudomonas aeruginosa* AP029-GLVIIA was able to produce rhamnolipids using glucose as the carbon source. Thebest conditionfor rhamnolipids production and biomass formation was using a C/N ratio and inoculum percentage of 13.0 and 3.0%, respectively. The rhamnolipids were able to form stable emulsions in different organic solvents, besides presenting satisfactory responses in relation to surface tension (29.42 ± 1.41 mN/m) and critical micellar concentration (49.63 mg/L). In addition, the tests of oil removal and antifungal activity showed that this kind of biosurfactant has potential for interesting biotechnological applications.

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