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Eco-friendly production of thermostable, halotolerant and pH wide range biosurfactant by *Issatchenkia orientalis* UCP 1603

Produção ecológica de biossurfactante termoestável, halotolerante e ampla faixa de pH por *Issatchenkia orientalis* UCP 1603

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Abstract
Microbial surfactants are amphiphilic molecules with attractive industrial prospects due to their advantages over commonly commercialized chemical surfactants, such as low toxicity, high biocompatibility and biodegradability, and efficiency at extreme conditions. However, the large-scale production of biosurfactants still becomes uncompetitive due to the low yields and onerous costs, being considered the use of renewable substrates as a viable strategy. In this sense, the present study aimed to investigate the sustainable production of biosurfactant by the yeast *Issatchenkia orientalis* UCP 1603 in 2 L of salt-based medium supplemented with 7.5% cassava wastewater, 5% corn steep liquor and 1% post-frying soybean oil. Fermentation was carried out in 2.8 L-Fernbach flasks for 72 h, at 28°C and 150 rpm, and reduction in surface tension to 30.1 mN/m was verified. The highest yield of the biosurfactant produced (4.02 g/L) was observed after extraction with 70% ethanol (2:1, *v/v*) and the isolated biosurfactant reduced the surface tension of the medium to 28.7 mN/m, the interfacial tension against n-hexadecane to 16.6 mN/m and has a CMC of 800 mg/L. The biocompound showed anionic and polymeric nature and did not present toxicity against cabbage (*Brassica oleracea* var. *capitata*) seeds. The stability in the range of pH 4-10, salinity 5-25% and temperature 5-100°C, evidenced a highly stable biosurfactant, with promising potential of application in several industrial activities or environmental processes in adverse conditions.
Keywords: *Issatchenkia orientalis*; Microbial surfactant; Renewable substrates; Higher stale tensoactive.

Resumo
Os surfactantes microbianos são moléculas anfifílicas com perspectivas industriais atraentes devido às suas vantagens em relação aos surfactantes químicos comumente comercializados, como baixa toxicidade, elevada biocompatibilidade e biodegradabilidade, e eficiência em condições extremas. No entanto, a produção em larga escala de biossurfactantes ainda se torna pouco competitiva devido aos baixos rendimentos e custos onerosos, considerando o uso de substratos

renováveis como uma estratégia viável. Neste sentido, o presente estudo teve como objetivo investigar a produção sustentável de biossurfactante pela levedura *Issatchenkia orientalis* UCP 1603 em 2 L de meio a base de sais suplementado com manipueira 7,5%, milhocina 5% e óleo de soja pós-fritura 1%. A fermentação foi conduzida em frascos de Fernbach de 2,8 L por 72 h, a 28°C e 150 rpm, verificando-se a redução da tensão superficial a 30,1 mN/m. O maior rendimento do biossurfactante produzido (4,02 g/L) foi constatado após extração com etanol 70% (2:1, *v/v*) e o biossurfactante isolado diminuiu a tensão superficial do meio a 28,7 mN/m, a tensão interfacial frente a n-hexadecano a 16,6 mN/m e possui CMC de 800 mg/L. O biocomposto mostrou natureza aniônica e polimérica, e não apresentou toxicidade frente a sementes de repolho (*Brassica oleracea* var. *capitata*). A estabilidade na faixa de pH 4-10, salinidade 5-25% e temperatura 5-100°C, evidenciaram um biossurfactante altamente estável, com potencial promissor de aplicação em diversas atividades industriais ou processos ambientais em condições adversas.
Palavras-chave: *Issatchenkia orientalis*; Surfactante microbiano; Substratos renováveis; Estabilidade térmica; Tolerância à salinidade elevada.

Resumen

Los tensioactivos microbianos son moléculas anfifílicas con atractivas perspectivas industriales debido a sus ventajas sobre los tensioactivos químicos comúnmente comercializados, tales como como baja toxicidad, alta biocompatibilidad y biodegradabilidad, y eficiencia en condiciones extremas. Sin embargo, la producción a gran escala de biosurfactantes aún se vuelve poco competitiva debido al bajo rendimiento y los costos onerosos, considerándose el uso de sustratos renovables como una estrategia viable. En ese sentido, el presente estudio tuvo como objetivo investigar la producción sostenible de biosurfactante por la levadura *Issatchenkia orientalis* UCP 1603 en 2 L de medio a base de sales suplementado con agua residual de yuca 7,5%, licor de maíz macerado 5% y aceite de soya post fritura 1%. La fermentación se llevó a cabo en matraces Fernbach de 2,8 L durante 72 h, a 28°C y 150 rpm, con una reducción de la tensión superficial de 30,1 mN/m. El mayor rendimiento del biosurfactante producido (4,02 g/L) se observó después de la extracción con etanol al 70 % (2:1, *v/v*) y el biosurfactante aislado redujo la tensión superficial del medio a 28,7 mN/m, la tensión interfacial frente al n-hexadecano a 16,6 mN/m y tiene una CMC de 800 mg/L. El biocompuesto mostró naturaleza aniónica y polimérica, y no presentó toxicidad contra semillas de col (*Brassica oleracea* var. *capitata*). La estabilidad en el rango de pH 4-10, salinidad 5-25% y temperatura 5-100°C, evidenciaron un biosurfactante altamente estable, con prometedor potencial de aplicación en diversas actividades industriales o procesos ambientales en condiciones adversas.
Palabras clave: *Issatchenkia orientalis*; Tensioactivo microbiano; Sustratos renovables; Estabilidad térmica; Tolerancia a alta salinidad.

1. Introduction

Surfactants are chemical compounds whose main characteristic is their amphipathic nature. Therefore, they have properties such as the ability to reduce surface and interfacial tension and form stable emulsions between immiscible liquids (Lima, et al., 2017; Ferreira, et al., 2020; Maia, et al., 2022). Most chemical surfactants have petrochemical origin, a non-renewable source whose use can cause environmental problems, due to its high toxicity and low biodegradability (Rubio-Ribeaux, et al., 2017; Kashif, et al., 2022). In the last decades, environmental legislations have been emerging and with that, the research for the search for products of natural origin has been increasing, mainly of biomolecules derived from microorganisms, such as biosurfactants (Freitas, et al., 2022).

Microbial surfactants, when compared to synthetic surfactants, have several advantages due to their low toxicity, biocompatibility, high biodegradability, and functionality under extreme conditions of pH, temperature, and salinity (Pele, et al., 2019; dos Santos, et al., 2021; Montero-Rodríguez, et al., 2022). However, the large-scale production of biosurfactants is still disadvantageous due to costly costs and low yields. An economically viable alternative is the use of renewable substrates and by-products as constituents of the production medium. Thus, the process of obtaining biosurfactants for different industries becomes competitive and, later, their commercial use (Araujo, et al., 2019; Mendonça, et al., 2021; Sarubbo, et al., 2022).

Biosurfactants can be produced by several microbial groups, including bacteria, filamentous fungi, and yeasts. In the case of the latter, most works report the use of representatives of the *Candida* genus (Luna, et al., 2015; Lima, et al., 2017; Rubio-Ribeaux, et al., 2020; Pinto, et al., 2022). The yeast *Issatchenkia orientalis*, previously known as *Pichia kudriavzevii*, *Candida krusei* or *C. glycerinogenes* (Douglass, et al., 2018; Zwirzitz, et al., 2021), has shown promising potential for the production of

surfactant molecules (Katemai, et al., 2008; Csutak, et al., 2012). Our recent study showed *I. orientalis* UCP 1603, isolated from the Caatinga biome, with the ability to produce biosurfactant from agro-industrial by-products, in 100 ml of alternative medium (Fonseca, et al., 2022). Hence, the present work aimed to produce this biomolecule on a larger scale (2 L), as well as its extraction and preliminary characterization, with special interest in the evaluation of stability under extreme conditions of temperature, pH and salinity.

2. Methodology

2.1 Microorganism and inoculum preparation

The yeast *Issatchenkia orientalis* UCP 1603 used in this work was kindly provided by the Culture Collection of the Nucleus for Research in Environmental Sciences and Biotechnology (NPCIAMB), of the Catholic University of Pernambuco (UNICAP). The microorganism was kept at 5°C in YMA medium (Yeast Malt Agar; 3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, 10 g/L glucose and 20 g/L agar). The yeast was cultivated in YMB medium (Yeast Malt Broth; 3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone and 10 g/L glucose) at 28°C and 150 rpm for 24 h, and after this time, a cell suspension containing 10^7 cells/ml was prepared and used as inoculum.

2.2 Agro-industrial substrates

The agro-industrial by-products used in this study as alternative substrates were cassava wastewater (CWW), kindly provided by the Pankará indigenous village residing in Carnaubeira da Penha (Pernambuco, Brazil), obtained after processing cassava; corn steep liquor (CSL), residue from corn processing, donated by the company Ingredion Industries Ltd, from the municipality of Cabo de Santo Agostinho (Pernambuco, Brazil); and post-frying soybean oil (PFSO), obtained from a local food trade in the city of Recife (Pernambuco, Brazil) (Fonseca, et al., 2022).

2.3 Biosurfactant production in Fernbach flasks

Biosurfactant production was carried out in triplicate in 2.8 L capacity Fernbach flasks with 2.0 L of medium containing saline base (KH_2PO_4 0.2 g/L and $MgSO_4.7H_2O$ 0.2 g/L) supplemented with 7.5% CWW, 5% CSL and 1% PFSO, according to our previous study (Fonseca, et al., 2022). The pH of the medium was adjusted to 6.0 and the flasks were autoclaved at 121°C for 15 min. The flasks were inoculated with 10% of the cell suspension and incubated for 72 h at 28°C, under orbital shaking (150 rpm). After this period, the cultured medium was centrifuged at 6000 *g* for 15 min and filtered, and the cell-free metabolic liquid was used for the analyzes described below.

2.4 Determination of surface and interfacial tension

Surface tension was determined in cell-free metabolic liquid by the Du Noüy ring method (Kuyukina, et al., 2001), using an automatic tensiometer model Sigma 701 (Biolin Scientific, São Paulo, Brazil), at room temperature (± 25ºC). The surface tension value was obtained as an average of ten measurements, and distilled water was used as control (surface tension = 70 mN/m). Interfacial tension was measured by the Du Noüy ring method using cell-free metabolic liquid and n-hexadecane (dos Santos, et al., 2021).

2.5 Isolation of biosurfactant

The biosurfactant was isolated from the cell-free metabolic liquid by extraction with 70% ethanol, using different ratios of the metabolic liquid and the solvent, as described in Table 1. The samples were incubated overnight at 5°C and then centrifuged

at 6000 *g* and 15 min. After centrifugation, the supernatants were discarded and the precipitates obtained were centrifuged twice with distilled water and subjected to lyophilization (Advantage Plus EL-85 lyophilizer, SP Scientific, USA). Subsequently, the samples were kept in a desiccator until constant weight, determined by gravimetry, and the yields expressed in g/L. Then, aqueous solutions (1 g/L) of each precipitate were prepared, and the surface tension was determined, as previously described (item 2.4), in order to verify the surfactant properties of each isolate. From the results, the isolation method of the biosurfactant produced by *I. orientalis* UCP 1603 was selected.

Table 1 - Methodologies of extraction with 70% ethanol used for the isolation of the biosurfactant produced by *I. orientalis* UCP 1603.

Method	Ratio (metabolic liquid: solvent)	Reference
Extraction with 70% ethanol	1:1	Nogueiras, et al. (2020)
	2:1	Bueno, et al. (2010)
	3:1	Present study
	1:3	Present study
	2:3	Present study

Source: Authors.

2.6 Critical Micellar Concentration (CMC)

To determine the critical micelle concentration (CMC), the isolated biosurfactant was solubilized in distilled water at different concentrations (10-2000 mg/L) and then, the surface tension was determined, as described in item 2.4. The surface tension values *vs.* biosurfactant concentration were plotted on a graph and the inflection point of the curve, where the surface tension value started to remain constant, was considered as the value of the biosurfactant CMC.

2.7 Preliminary characterization of the biosurfactant

The biochemical composition of the isolated biosurfactant was determined using specific commercial kits for quantification of total proteins and enzymatic glucose (In Vitro Diagnóstica Ltda; Itabira-MG, Brazil). The total lipid content was obtained after extraction with chloroform and methanol, according to the methodology of Manocha, et al. (1980). In addition, the functional groups present in the biosurfactant molecule were identified by Fourier transform infrared (FTIR) spectroscopy in the Shimadzu equipment (model IR-TRACER 100), using attenuated total reflectance (ATR) accessory consisting of a mixed crystal "diamond/ZnSe".

The ionic charge of the biosurfactant was determined using the Zeta-Meter system 3.0 + ZM3-DG potentiometer (Direct Imaging, Zeta Meter, Inc., USA).

2.8 Biosurfactant stability

The stability of the biosurfactant produced by *I. orientalis* was investigated, after dissolving the isolated biosurfactant in distilled water at the CMC, previously determined. The solution was separated into small volumes, which were separately subjected to different temperatures (5, 10, 20, 40, 80 and 100ºC) adjusted to different pH values (2, 4, 6, 8, 10 and 12) or concentrations of NaCl (5, 10, 15, 20 and 25%). After 1 h in the different treatments, the solutions were allowed to stand at room temperature and the surface tension was determined, as previously described (item 2.4) (Liang, et al., 2014).

2.9 Biosurfactant phytotoxicity

The phytotoxicity of the isolated biosurfactant was evaluated under static conditions, through seed germination and root growth on cabbage (*Brassica oleracea* var. *capitata*), following the methodology of Tiquia et al. (1996). First, the seeds were disinfected in a 1% sodium hypochlorite solution; and then, they were washed with sterile distilled water to remove excess hypochlorite and left at room temperature until dry. A test solution of the biosurfactant was prepared in distilled water at the CMC. The assay was carried out in triplicate in sterile Petri dishes (15 cm) containing discs of Whatman No. 1 filter paper, to which 10 treated seeds were symmetrically added, under aseptic conditions. The plates were inoculated with 3 ml of the test solution or distilled water as a control, and incubated at 28ºC for 120 h. After this period, seed germination, root growth (≥ 5 mm) and germination index (GI) were determined:

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

Relative root length (%) = (average root length in test solution/average root length in control) x 100

Germination index = [(% seed germination) x (% relative root length)]/100%.

3. Results and Discussion

3.1 Production of biosurfactant in 2 L of waste-based medium

Most of the work aimed at the production of microbial surfactants has been carried out in Erlenmeyer flasks containing small volumes of medium (Krieger, et al., 2010; Sarubbo, et al., 2022). However, the large-scale production and application of these biomolecules requires studies to scale production to obtain higher yields and make the process economically viable (Almeida, et al., 2017; de Medeiros, et al., 2022). Despite this, several authors have reported a significant variation in the reduction of surface tension by some biosurfactants, when they increased the scale of production. For example, Almeida et al. (2017) reported the production of biosurfactant by yeast *Candida tropicalis* UCP 0996 and verified the reduction of surface tension to 29.98 mN/m, when cultivated in Erlenmeyer flasks with 100 ml of medium, and of 34.12 and 35.6 mN/m, when the fermentation was carried out in 2 and 50 L, respectively. However, the production of biosurfactant by *I. orientalis* performed in 2 L of medium in the present work, showed no significant differences in the reduction of surface tension (30.1 ± 0.2 mN/m), when compared with fermentation carried out in 100 ml of medium (29.9 mN/m) (Fonseca, et al., 2022).

On the other hand, the reduction of interfacial tension is considered as another parameter that indicates the production of biosurfactant (dos Santos, et al., 2021). In this sense, interfacial tension of 16.6 mN/m was verified and it was considered as an acceptable value, since in the literature high values of interfacial tension are referred to as those above 18 mN/m (Luna, et al., 2013; Pinto, et al., 2022). Previously, Luna et al. (2013) and Ribeiro et al. (2020) reported interfacial tension values of 12.5 and 13.99 mN/m for biosurfactants produced by *C. sphaerica* UCP 0995 and *Saccharomyces cerevisiae* URM 6670, respectively.

3.2 Isolation of the biosurfactant

After the production of the biosurfactant, the next important step is the recovery of the surfactant from the fermentation medium, followed by purification to make it available for a variety of industrial applications. However, this step is still limited due to the high cost of recovery, purification processing or both, constituting approximately 60% of total production costs (Geetha, et al., 2018; Sarubbo, et al., 2022). In this context, several strategies to reduce production costs have been studied, especially the use of by-products or agro-industrial wastes as low-cost substrates. However, these renewable resources contaminate or hinder the extraction and purification process and, consequently, the characterization of biosurfactants (Mohanty, et al., 2021; Gaur, et al., 2022). Several techniques have been used for the recovery of biosurfactants, such as extraction with

water-miscible solvents.

In this work, 70% ethanol was used to isolate the biosurfactant produced by *I. orientalis* evaluating different ratios of the metabolic liquid and the solvent, as described in Table 1. The results shown in Table 2 verified the ratio of 2:1 (*v/v*) as the most efficient methodology for the isolation of the biosurfactant (yield 4.02 g/L), considering the higher yield of the precipitate obtained and the surface tension of 28.7 mN/m, after dilution in distilled water. Similar biosurfactant yields have been reported previously for *C. lipolytic* and *C. sphaerica* (4.5 g/L) (Rufino, et al., 2007; Sobrinho, et al., 2008), and *C. gloebosa* (4.04 g/L) (Cardoso, et al., 2020).

Table 2 - Results of the extraction with 70% ethanol used for the isolation of the biosurfactant produced by *I. orientalis* UCP 1603.

Ratio (Metabolic liquid: 70% ethanol; *v/v*)	**Yield (g/L)**	**Surface tension (mN/m)**
1:1	2.57	43.5
2:1	4.02	28.7
3:1	2.02	42.0
1:3	2.24	42.2
2:3	1.61	45.0

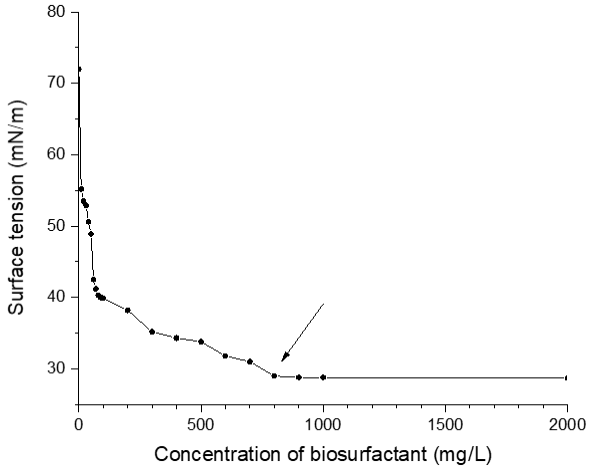
Source: Authors.

3.3 CMC

One of the characteristics common to all surface-active compounds is the property of forming aggregates (micelles) in aqueous solution, from a certain concentration. In this sense, CMC is defined as the minimum concentration necessary for a surfactant to initiate the formation of micelles, reducing the surface tension of the medium as much as possible, and is commonly used as a measure of the efficiency of a biosurfactant (Bezerra; et al., 2018; Sarubbo, et al., 2022). The CMC of biosurfactants can vary between 1 and 2000 mg/L; however, a CMC less than 1000 mg/L is desired to be considered a biosurfactant as efficient (Rocha e Silva, et al., 2018; Lira, et al., 2020).

In the present study, the surface tension was gradually reduced from 72 to 28.8 ± 0.2 mN/m, with increasing concentration of the isolated biosurfactant (10 to 2000 mg/L). However, from 800 mg/L, no significant reduction in surface tension was observed, indicating that the CMC was reached (Figure 1). This CMC value coincides with those previously reported for yeast biosurfactant *C. guilliermondii* UCP 0992 (700 mg/L) (Lira, et al., 2020) and *S. cerevisiae* URM 6670 (800 mg/L) (Ribeiro, et al., 2020). Furthermore, the biosurfactant produced by *I. orientalis* showed better efficiency than the chemical surfactant sodium dodecyl sulfate (SDS), which has a CMC of around 2000 mg/L, with a minimum surface tension of 35.41 mN/m (Liang, et al., 2014; Kusuma, et al., 2021).

Figure 1 - Critical micellar concentration of the biosurfactant produced by *I. orientalis* UCP 1603.



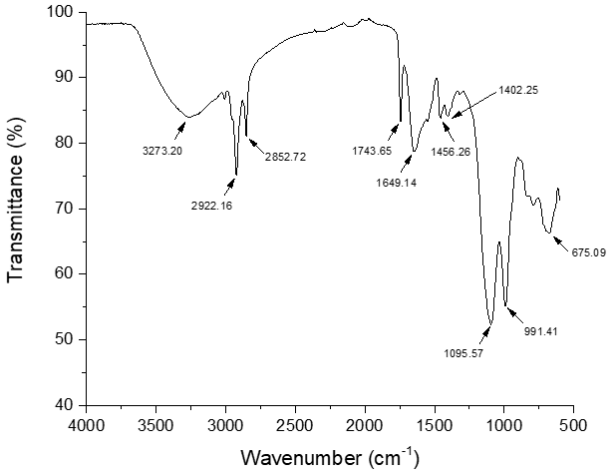
Source: Authors.

3.4 Preliminary characterization of the biosurfactant

The Zeta potential determines the ionic charge of the particle, which serves to predict and control the stability of colloidal suspensions and emulsions. According to the analysis performed in the present study, the biosurfactant produced by *I. orientalis* UCP 1603 showed anionic character (-14.10 mV). Anionic surfactants are widely used in the formulation of cleaning products and cosmetics (Karray, et al., 2016; Lima, et al., 2017).

On the other hand, the biochemical composition of the biosurfactant indicated its polymeric nature (lipids 32.9%, proteins 16.4% and carbohydrates 50.7%), which was confirmed by the functional groups identified in the infrared spectrum. According to the spectrum shown in Figure 2, the broadband observed at 3273.2 cm^{-1} corresponded to the O-H stretch, while the peak at 2922.16 cm^{-1} was associated with the stretching vibration of the C-H bond of the constituent sugar residues (Jain, et al., 2012). The absorption around 2852.72 cm^{-1} was attributed to the symmetrical stretch (CH) of the $–CH_2$ and $-CH_3$ groups of aliphatic chains and the C=O stretch was observed at 1743.65 cm^{-1}. In the literature, biosurfactants produced by *C. lipolytica* UCP 0988 (Sarubbo, et al., 2007) and *C. tropicalis* (Batista, et al., 2010) were also cited as carbohydrate-protein-lipid complexes. Recently, the biosurfactant produced by *C. utilis* UFPEDA 1009 was classified as a carbohydrate-protein-lipid complex, as the biochemical composition showed 65.88% of lipids, 7.10% of protein and 6.39% of sugars (Campos, et al., 2019).

Figure 2 - Infrared spectrum of the biosurfactant produced by *I. orientalis* UCP 1603.

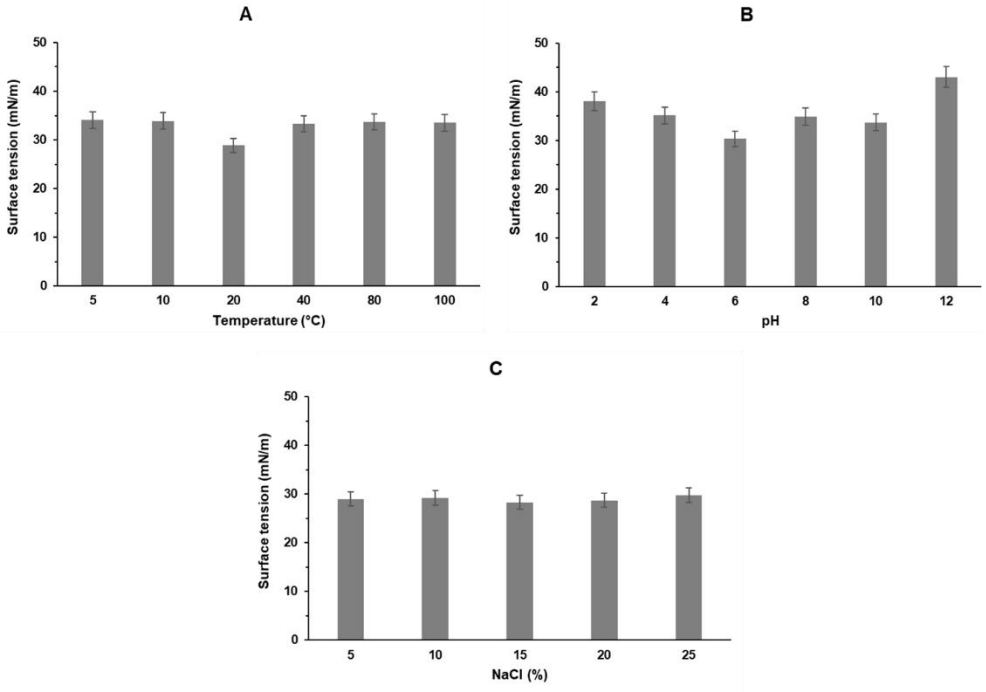


Source: Authors.

3.5 Stability of the biosurfactant

The stability of microbial surfactants is an advantage that these biomolecules have, when compared with those of synthetic origin, and constitutes an essential feature that allows their application in various industrial sectors (Pele, et al., 2019; dos Santos, et al., 2021; Sarubbo, et al., 2022). In this sense, stability studies of the biosurfactant produced by *I. orientalis*, after isolation, were carried out and the results are shown in Figure 3.

Figure 3 - Stability of the surface tension of the biosurfactant produced by *I. orientalis* UCP 1603 under the influence of temperature (A), pH (B) and salinity (C).



Source: Authors.

According to the results, the biosurfactant showed a slight increase in surface tension when it was subjected to

temperatures of 5, 10, 40, 80 and 100°C, compared to 20°C (Figure 3A). Despite this, it was considered that it remained stable, since the variation did not exceed 5 mN/m (Fai, et al., 2015), thus proving the efficiency of this biomolecule at extreme temperatures. Similar behavior was observed at pH 4, 8 and 10, when compared to pH 6 (Figure 3B). However, at pH 2 and 12, it showed a more accentuated increase in surface tension (variation ≥ 8 mN/m), proving to be sensitive to very acidic or alkaline environments. This change in surface tension along the pH scale was also verified for the biosurfactant produced by *C. bombicola* (Pinto, et al., 2018), which can be justified by the fact that acidic and basic media influence the solubility of the biomolecule and, therefore, its ability to reduce surface tension.

According to the literature, biosurfactants commonly tolerate saline concentrations of up to 10% (*w/v*), while the synthetic surfactants are inactivated at ≥ 2% NaCl (Santos, et al., 2016; Pele, et al., 2019). However, the biosurfactant produced by *I. orientalis* proved to be halotolerant, showing efficiency in the reduction of surface tension up to 25% of NaCl (Figure 3C). Previously, some studies highlighted the stability up to 25% of NaCl of the biosurfactants produced by *Bacillus subtilis* HOB2 (Haddad, et al., 2009), *Pseudozyma tsukubaensis* (Fai, et al., 2015) and *Serratia marcescens* UCP 1549 (dos Santos, et al., 2021), where the authors suggested the usefulness of these biomolecules in industrial or environmental processes with high ionic strength.

3.6 Biosurfactant phytotoxicity

Phytotoxicity assays using vegetable seeds are commonly performed to investigate the toxic potential of microbial surfactants (Pele, et al., 2019; Durval, et al., 2021). In the present work, the germination index was used to evaluate the toxicity of the biosurfactant for cabbage (*Brassica oleracea* var. *capitata*) seeds, considering that the GI value ≥ 80% is an indicator of the absence of phytotoxicity (Tiquia, et al., 1996). Thus, the results obtained indicated that the biosurfactant produced by *I. orientalis*, at the concentration of CMC, did not show inhibitory effect on seed germination or root elongation, reaching GI= 115.7%. The biocompound was then considered as non-toxic, and its positive effect on inducing germination of cabbage seeds was also evidenced, when compared with distilled water as an abiotic control. Similar results have been previously described for biosurfactants produced by *C. tropicalis* UCP 1613 (Rubio-Ribeaux, et al., 2017) and *C. guilliermondii* UCP 0992 (Lira, et al., 2022); but, to our knowledge, this is the first study to describe the absence of toxicity for a biosurfactant produced by *I. orientalis*.

4. Conclusion

The present study confirmed the biotechnological potential of *I. orientalis* UCP 1603 in the ecological production of non- toxic and efficient biosurfactant in extreme conditions of pH, temperature and salinity, properties that constitute promising characteristics for its application in several industrial sectors. In addition, the suitability of the use of renewable by-products as low-cost substrates was confirmed, being an economically viable and sustainable alternative for biotechnological processes.

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References

Araújo, H. W., Andrade, R. F., Montero-Rodríguez, D., Rubio-Ribeaux, D., Alves da Silva, C. A., & Campos-Takaki, G. M. (2019). Sustainable biosurfactant produced by *Serratia marcescens* UCP 1549 and its suitability for agricultural and marine bioremediation applications. *Microbial Cell Factories*, *18*(1), 1-13.

Batista, R. M., Rufino, R. D., Luna, J. M., de Souza, J. E. G., & Sarubbo, L. A. (2010). Effect of medium components on the production of a biosurfactant from *Candida tropicalis* applied to the removal of hydrophobic contaminants in soil. *Water Environment Research*, *82*(5), 418-425.

Campos, J. M., Stamford, T. L. M., & Sarubbo, L. A. (2019). Characterization and application of a biosurfactant isolated from *Candida utilis* in salad dressings. *Biodegradation*, *30*(4), 313-324.

Cardoso, S., Dantas, R., Costa, C., Campos, E., & Tambourgi, E. (2020). Evaluation of the medium fermentative parameters for the production of biosurfactant using the *Candida gloebosa*. *Chemical Engineering Transactions*, *79*, 475-480.

Csutak, O., Stoica, I., & Vassu, T. (2012). Evaluation of production, stability and activity of biosurfactants from yeasts with application in bioremediation of oil-polluted environment. *Rev Chim*, *63*(10), 973-7.

Douglass, A. P., Offei, B., Braun-Galleani, S., Coughlan, A. Y., Martos, A. A., Ortiz-Merino, R. A., & Wolfe, K. H. (2018). Population genomics shows no distinction between pathogenic *Candida krusei* and environmental *Pichia kudriavzevii*: one species, four names. *PLoS pathogens*, *14*(7), e1007138. https://doi.org/10.1371/journal.ppat.1007138

Durval, I., Rufino, R., & Sarubbo, L. (2021). Biosurfactant as an Environmental Remediation Agent: Toxicity, Formulation, and Application in the Removal of Petroderivate in Sand and Rock Walls. *Biointerface Res. Appl. Chem*, *12*, 34-48.

Fai, A. E. C., Simiqueli, A. P. R., de Andrade, C. J., Ghiselli, G., & Pastore, G. M. (2015). Optimized production of biosurfactant from *Pseudozyma tsukubaensis* using cassava wastewater and consecutive production of galactooligosaccharides: an integrated process. *Biocatalysis and Agricultural Biotechnology*, *4*(4), 535-542.

Ferreira, I. N. S., Rodríguez, D. M., Campos-Takaki, G. M., & da Silva Andrade, R. F. (2020). Biosurfactant and bioemulsifier as promising molecules produced by *Mucor hiemalis* isolated from Caatinga soil. *Electronic Journal of Biotechnology*, *47*, 51-58.

Fonseca, T. C. de S., Souza, A. F., Santos, P. N., Silva, P. H., Rodriguez, D. M., Costa, L. O., & Campos-Takaki, G. M. Sustainable production of biosurfactant by *Issatchenkia orientalis* UCP 1603 using renewable substrates. Research, Society and Development, 11(4), e16111427174, 2022. https://doi.org/10.33448/rsd-v11i4.27174

Freitas, E. L., et al. Avaliação do fungo *Penicillium sclerotiorum* UCP 1040 na produção de biossurfactante utilizando óleo pós-fritura e milhocina. Research, Society and Development, 11(5), e0411527502-e0411527502, 2022.

Gaur, V. K., Sharma, P., Sirohi, R., Varjani, S., Taherzadeh, M. J., Chang, J. S., & Kim, S. H. (2022). Production of biosurfactants from agro-industrial waste and waste cooking oil in a circular bioeconomy: An overview. Bioresource technology, 343, 126059.

Haddad, N. I., Wang, J., & Mu, B. (2009). Identification of a biosurfactant producing strain: *Bacillus subtilis* HOB2. Protein and Peptide letters, 16(1), 7-13.

Jain, R. M., Mody, K., Mishra, A., & Jha, B. (2012). Isolation and structural characterization of biosurfactant produced by an alkaliphilic bacterium *Cronobacter sakazakii* isolated from oil contaminated wastewater. *Carbohydrate Polymers*, *87*(3), 2320-2326.

Kashif, A., Rehman, R., Fuwad, A., Shahid, M. K., Dayarathne, H. N. P., Jamal, A., Aftab, M. N., Mainali, B., & Choi, Y. Current advances in the classification, production, properties and applications of microbial biosurfactants – A critical review. Advances in Colloid and Interface Science, 306(1), 102718, 2022. https://doi.org/10.1016/j.cis.2022.102718

Katemai, W., Maneerat, S., Kawai, F., Kanzaki, H., & Nitoda, T. (2008). Purification and characterization of a biosurfactant produced by *Issatchenkia orientalis* SR4. *The Journal of General and Applied Microbiology*, *54*(1), 79-82. https://doi.org/10.2323/jgam.54.79

Kusuma, S. H., Meitha, K., & Suhandono, S. (2021). Characterization of di-rhamnolipid biosurfactant in recombinant *Escherichia coli*. In *Key Engineering Materials* (Vol. 874, pp. 107-114). Trans Tech Publications Ltd.

Kuyukina, M. S., Ivshina, I. B., Philp, J. C., Christofi, N., Dunbar, S. A., & Ritchkova, M. A. (2001). Recovery of *Rhodococcus* biosurfactants using methyl tertiary-butyl ether extraction. *Journal of Microbiolology Methods*, 46,109-120. https://doi:10.1016/s0167-7012(01)00259-7

Liang, T. W., Wu, C. C., Cheng, W. T., Chen, Y. C., Wang, C. L., Wang, I. L., & Wang, S. L. (2014). Exopolysaccharides and antimicrobial biosurfactants produced by *Paenibacillus macerans* TKU029. *Applied biochemistry and biotechnology*, *172*(2), 933-950.

Lima, R. A., Andrade, R. F., Rodríguez, D. M., Araújo, H. W., Santos, V. P., & Campos-Takaki, G. M. (2017). Production and characterization of biosurfactant isolated from *Candida glabrata* using renewable substrates. *African Journal of Microbiology Research*, *11*(6), 237-244.

Lira, I. C. A. S., da Silva Santos, E. M., Guerra, J. M. C., Meira, H. M., Sarubbo, L. A., & de Luna, J. M. (2022). Microbial Biosurfactant: Production, Characterization and Application as a Food Emulsions. *Research, Society and Development*, 11(5), e44111528339-e44111528339.

Lira, I. R. A. S., Santos, E. M. D. S., Filho, A. A. S., Farias, C. B. B., Guerra, J. M. C., Sarubbo, L. A., & de Luna, J. M. (2020). Biosurfactant production from *Candida guilliermondii* and evaluation of its toxicity. *Chemical Engineering*, *79*.

Luna, J. M., Rufino, R. D., Jara, A. M. A., Brasileiro, P. P., & Sarubbo, L. A. (2015). Environmental applications of the biosurfactant produced by *Candida sphaerica* cultivated in low-cost substrates. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *480*, 413-418.

Luna, J. M., Rufino, R. D., Sarubbo, L. A., & Campos-Takaki, G. M. (2013). Characterisation, surface properties and biological activity of a biosurfactant produced from industrial waste by *Candida sphaerica* UCP0995 for application in the petroleum industry. *Colloids and surfaces B: Biointerfaces*, *102*, 202-209.

Mendonça, R. S., Sá, A. V. P., Rosendo, L. A., dos Santos, R. A., do Amaral Marques, N. S. A., Souza, A. F., & de Campos Takaki, G. M. (2021). Produção de biossurfactante e lipídeos por uma nova cepa de *Absidia cylindrospora* UCP 1301 isolada do solo da Caatinga usando subprodutos agroindustriais de baixo custo. *Brazilian Journal of Development*, *7*(1), 8300-8313.

Mohanty, S. S., Koul, Y., Varjani, S., Pandey, A., Ngo, H. H., Chang, J. S., & Bui, X. T. (2021). A critical review on various feedstocks as sustainable substrates for biosurfactants production: a way towards cleaner production. Microbial cell factories, 20(1), 1-13.

Montero-Rodríguez, D., de Souza Mendonça, R., de Souza, A. F., da Silva Ferreira, I. N., Andrade, R. F. S., & Campos-Takaki, G. M. (2022). Solid-state fermentation for low-cost production of biosurfactant by promising *Mucor hiemalis* UCP 1309. *Research, Society and Development*, *11*(6), e25211628817-e25211628817.

Pinto, M. I. S., Campos Guerra, J. M., Meira, H. M., Sarubbo, L. A., & de Luna, J. M. (2022). A Biosurfactant from *Candida bombicola*: Its Synthesis, Characterization, and its Application as a Food Emulsions. *Foods*, *11*(4), 561.

Pinto, M. I., Ribeiro, B., Guerra, J. M. C., Rufino, R., Sarubbo, L., & Luna, J. (2018). Production in bioreactor, toxicity and stability of a low-cost biosurfactant. *Chemical Engineering Transactions*, *64*, 595-600.

Rahman, P. K., Mayat, A., Harvey, J. G. H., Randhawa, K. S., Relph, L. E., & Armstrong, M. C. (2019). Biosurfactants and bioemulsifiers from marine algae. In The Role of Microalgae in Wastewater Treatment (pp. 169-188). Springer, Singapore.

Ribeiro, B. G., dos Santosb, M. M., da Silvac, I. A., Meirad, H. M., de Oliveirad, A. M., Guerrab, J. M., & Sarubbod, L. A. (2020). Study of the biosurfactant production by *Saccharomyces cerevisiae* URM 6670 using agroindustrial waste. *Chem Eng*.

Rocha e Silva, F. C. P., Rocha e Silva, N. M. P., Luna, J. M., Rufino, R. D., Santos, V. A., & Sarubbo, L. A. (2018). Dissolved air flotation combined to biosurfactants: A clean and efficient alternative to treat industrial oily water. *Reviews in Environmental Science and Bio/Technology*, *17*(4), 591-602.

Rubio-Ribeaux, D., De Oliveira, C. V. J., Marinho, J. D. S., Lins, U. D. B. L., Do Nascimento, I. D. F., Barreto, G. C., & Campos-Takaki, G. (2020). Innovative production of biosurfactant by *Candida tropicalis* UCP 1613 through solid-state fermentation. *Chemical Engineering Transactions*, *79*, 361-366.

Rubio-Ribeaux, D., Andrade, R. F. S., Silva, G. S., Rodrigo, A. D. H., Milagre, A. P., Nunes, P., & Campos-Takaki, G. M. (2017). Promising biosurfactant produced by a new *Candida tropicalis* UCP 1613 strain using substrates from renewable resources. *African Journal of Microbiology Research*, *11*(23), 981-991.

Santos, D. K. F., Rufino, R. D., Luna, J. M., Santos, V. A., & Sarubbo, L. A. (2016). Biosurfactants: multifunctional biomolecules of the 21st century. *International journal of molecular sciences*, *17*(3), 401.

Sarubbo, L. A., Farias, C. B., & Campos-Takaki, G. M. (2007). Co-utilization of canola oil and glucose on the production of a surfactant by *Candida lipolytica. Current Microbiology*, *54*(1), 68-73.

Sarubbo, L. A., Maria da Gloria, C. S., Durval, I. J. B., Bezerra, K. G. O., Ribeiro, B. G., Silva, I. A., & Banat, I. M. (2022). Biosurfactants: Production, properties, applications, trends, and general perspectives. *Biochemical Engineering Journal*, 108377.

Singh, A. K., & Sharma, P. (2020). Disinfectant-like activity of lipopeptide biosurfactant produced by *Bacillus tequilensis* strain SDS21. *Colloids and Surfaces B: Biointerfaces*, *185*, 110514.

Tiquia, S. M., Tam, N. F. Y., & Hodgkiss, I. J. (1996). Effects of composting on phytotoxicity of spent pig-manure sawdust litter. *Environmental pollution*, *93*(3), 249-256.

Zwirzitz, A., Alteio, L., Sulzenbacher, D., Atanasoff, M., & Selg, M. (2021). Ethanol production from wheat straw hydrolysate by *Issatchenkia orientalis* isolated from waste cooking oil. *Journal of Fungi*, 7(2), 121. https://doi.org/10.3390/jof7020121