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Sustainable production of biosurfactant by *Issatchenkia orientalis* UCP 1603 using renewable substrates

Produção sustentável de biossurfactante por *Issatchenkia orientalis* UCP 1603 usando substratos renováveis

Producción sostenible de biosurfactante por *Issatchenkia orientalis* UCP 1603 utilizando sustratos renovables

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Abstract
Biological surfactants are amphipathic molecules produced by microorganisms and are considered multifunctional compounds of the 21st century. The current work aimed to use low-cost renewable substrates for economic production of biosurfactant by *Issatchenkia orientalis* UCP 1603. Fermentations were carried out at 28°C and 150 rpm for 72 h, using agro-industrial by-products (cassava wastewater, corn steep liquor and post-frying soybean oil) as substrates, according to a 2^3 full-factorial design (FFD) to identify their influence on biosurfactant production. The results showed the ability of the yeast to produce biosurfactant in all conditions of FFD, standing out the condition 4 due to the greatest reduction of surface tension (from 72 to 29.7 mN/m). The statistical analyses evidenced the significative influence of cassava wastewater and corn steep liquor on biosurfactant production. The tensoactive properties of the biomolecule were confirmed by parafilm test and emulsification index. This study evidenced *I. orientalis* as promising biosurfactant-producing yeast, with excellent ability and higher biotechnological potential for transformation of agro-industrial by-products.
Keywords: Microbial tensioactive; Agro-industrial by-products; *Issatchenkia orientalis.*

Resumo
Os surfactantes biológicos são moléculas anfipáticas produzidas por micro-organismos e são considerados compostos multifuncionais do século XXI. O presente trabalho teve como objetivo utilizar substratos renováveis de baixo custo para a produção econômica de biossurfactante por *Issatchenkia orientalis* UCP 1603. As fermentações foram realizadas a 28°C e 150 rpm por 72 h, utilizando subprodutos agroindustriais (manipueira, milhocina e óleo de soja pós-fritura) como substratos, de acordo com um planejamento fatorial completo de 2^3 para identificar sua influência

na produção de biossurfactante. Os resultados mostraram a capacidade da levedura em produzir biossurfactante em todas as condições do planejamento, destacando-se a condição 4 devido à maior redução da tensão superficial (de 72 para 29,7 mN/m). As análises estatísticas evidenciaram a influência significativa da manipueira e milhocina na produção de biossurfactante. As propriedades tensoativas da biomolécula foram confirmadas por teste de parafilme e índice de emulsificação. Este estudo evidenciou *I. orientalis* como uma levedura promissora produtora de biossurfactante, com excelente capacidade e elevado potencial biotecnológico de transformação de subprodutos agroindustriais.
Palavras-chave: Tensioativo microbiano; Subprodutos agroindustriais; *Issatchenkia orientalis*.

Resumen
Los tensioactivos biológicos son moléculas anfipáticas producidas por microorganismos y se consideran compuestos multifuncionales del siglo XXI. El presente trabajo tuvo como objetivo utilizar sustratos renovables de bajo costo para la producción económica de biosurfactante por *Issatchenkia orientalis* UCP 1603. Las fermentaciones se realizaron a 28°C y 150 rpm durante 72 h, utilizando subproductos agroindustriales (agua residual de yuca, licor de maíz macerado y aceite de soja post fritura) como sustratos, según un diseño factorial completo de 2^3 para identificar su influencia en la producción de biosurfactante. Los resultados mostraron la capacidad de la levadura para producir biosurfactante en todas las condiciones del diseño, destacándose la condición 4 debido a la mayor reducción de la tensión superficial (de 72 a 29,7 mN/m). Los análisis estadísticos evidenciaron la influencia significativa de agua residual de yuca y licor de maíz macerado en la producción del biosurfactante. Las propiedades tensoactivas de la biomolécula fueron confirmadas por prueba de parafilme e índice de emulsificación. Este estudio evidenció a *I. orientalis* como una levadura promisora productora de biosurfactante, con excelente capacidad y elevado potencial biotecnológico para la transformación de subproductos agroindustriales.
Palabras clave: Tensioactivo microbiano; Subproductos agroindustriales; *Issatchenkia orientalis*.

1. Introduction

The growing search for natural surfactants or biosurfactants is related to their biodegradability properties, low toxicity, and lower environmental impact, when compared to those of synthetic origin. Biosurfactants are metabolites of an amphipathic nature, produced mainly by bacteria and yeasts, and more rarely by filamentous fungi. They have promising and multifunctional properties, such as stability to different environmental conditions of temperature, pH, and salinity (Santos, et al., 2016; Araújo, et al., 2019; Muñoz, et al., 2022).

Although biosurfactants have promising industrial applications, chemical surfactants derived from petroleum are the most available on the market. The large-scale production of microbial surfactants is still limited, considering the high cost of production, mainly caused by the value of nutrients in conventional culture media. In this context, agro-industrial by-products and waste can be used as renewable sources to ensure an economic and sustainable production process (Santiago, et al., 2021; Valencia, et al., 2021).

The yeast *Issatchenkia orientalis* (syn. *Pichia kudriavzevii*) has been isolated from different substrates as described from cocoa bean (Dandi, et al., 2013), as well as corns talk, sweet sorghum stalk, and rice straw (Kwon, et al., 2011). In addition, it was described as fermenter of monosaccharides as glucose, fructose and mannose as substrates for ethanol production (Hisamatsu, et al., 2006), and ethanol from rice straw via simultaneous saccharification (Oberoi, et al., 2012). The first report of biosurfactant production by *I. orientalis* SR4 using xylene in the medium composition as unique carbon source was described by Katemai, et al. (2008). The authors were isolated and characterized of the biosurfactant produced by *I. orientalis* using the emulsification activity test surface activity and suggest composition as an authentic oleic acid.

In this context, this study is the first to formulate a new culture medium based on agro-industrial residues to evaluate the production of biosurfactant by the yeast *Issatchenkia orientalis* UCP 1603, isolated from the Caatinga biome (Pernambuco, Brazil). In addition, this is a biotechnological strategy that will contribute to the reduction of production costs, as well as an important path to environmental protection and sustainability, in order to replace the widely commercialized chemical surfactants.

2. Methodology

2.1 Microorganism and maintenance

The microorganism used in this study was the yeast *I. orientalis* UCP 1603, isolated from Caatinga soil of the state of Pernambuco, Brazil, and identified by morphological, biochemical, and molecular methods. The strain was deposited on Culture Collection UCP (Universidade Católica de Pernambuco), which is registered at number 927 at the World Federation for Culture Collection (WFCC). The yeast was maintained at 5°C in Yeast Malt Agar (YMA) medium, with the following composition (w/v): yeast extract 0.3%, malt extract 0.3%, tryptone 0.5%, D-glucose 1% and agar 5%, dissolved in distilled water (100 ml) and pH 6.0. The subculture was performed every four months to maintain cell viability.

2.2 Agro-industrial substrates

In this study, three agro-industrial by-products was used for the formulation of biosurfactant production medium. Cassava wastewater (CWW) was kindly supplied by indigenous village of Pankará, in Carnaubeira da Penha, Pernambuco, Brazil, and was obtained from cassava press for the manufacture of powder flour. Corn steep liquor (CSL), a residue from the corn processing industry, was kindly provided by Ingredion Industries Ltd, municipality of Cabo de Santo Agostinho, Pernambuco, Brazil. Post-frying soybean oil (PFSO) was kindly provided by a local food trade in the city of Recife, Pernambuco, Brazil,

2.3 Biosurfactant production

Fermentations were carried out in 250 ml-Erlenmeyer flasks containing 100 ml of saline solution (KH_2PO4 0.2 g/L and $MgSO4.7H_2O$ 0.2 g/L, pH 5.0) supplemented with different concentrations of CWW, CSL and PFSO, according to a 2^3 full-factorial design (FFD). Production media were sterilized in autoclave and then, inoculated with a cell suspension (10^7 cells/ml). The flasks were incubated in an orbital shaker at 150 rpm and 28°C for 72 h. After this period, the cultures were subjected to centrifugation at 6000 *g* for 15 min to obtain cell-free metabolic liquids, which were used to perform the following analyses: determination of surface tension and emulsification index, as well as parafilm test.

2.4 Full-factorial design (FFD)

A 2^3 FFD was carried out in order to determine the effect of each independent factor (concentration of CWW, CSL and PFSO) and the interaction between them, using surface tension as response variable. A set of eight assays with four replicates in the central points was performed, and each independent variable was investigated at high (+1), center (0) and low (−1) levels, according to Table 1. Statistical analysis of the data obtained from the experiments was executed using STATISTICA software package version 10.0 (StatSoft Inc., Tulsa, OK, USA), and the significance of the results was determined ($p \leq 0.05$).

Table 1. Variables and levels of the 2^3 full-factorial design (FFD) applied for biosurfactant production by *I. orientalis* UCP 1603.

Variables	**Levels**		
	-1	**0**	**+1**
Cassava wastewater (%, *v/v*)	2.5	5.0	7.5
Corn steep liquor (%, *v/v*)	1.0	3.0	5.0
Post-frying soybean oil (%, *v/v*)	1.0	3.0	5.0

Source: Authors.

2.5 Determination of surface tension

Surface tension was measured in triplicate on cell-free metabolic liquids using an automatic tensiometer model Sigma 70 (KSV Instruments Ltd., Finland), by the Du Noüy ring method at room temperature (± 28°C) (Kuyukina, et al., 2001). The measurement of surface tension on distilled water was used as control (surface tension of water = 72 mN/m).

2.6 Parafilm M test

Parafilm M test was carried out placing the cell-free metabolic liquid (25 µl) of on hydrophobic surface of the parafilm M strip. The shape of the drops on the surface were examined after 1 min and their diameters were measured using a caliper. The spreading of drops on the hydrophobic surface was considered an indicator of the presence of the biosurfactant (Hasani, et al., 2018). Distilled water and SDS 2% were used as negative and positive control, respectively.

2.7 Emulsification index

Emulsification index (EI_{24}) was determined in triplicate using the methodology described by Nitschke & Pastore (2004). Briefly, 2 ml of the cell-free metabolic liquid from each condition of FFD were mixed separately with 2 ml of hydrophobic compound (canola oil, soybean oil, PFSO and burned motor oil) in a test tube and vortexed thoroughly for 2 min at room temperature (28°C). EI_{24} was determined after 24 h as the percentage of the height of the emulsion layer divided by the total height of the liquid column.

3. Results and Discussion

3.1 Biosurfactant production

The determination of surface tension has often been used as a rapid method to detect the BS production in the culture medium (Araújo, et al., 2019; Sharma, 2021). Promising biosurfactant-producing microorganisms are considered those that reduce surface tension to values less than 40 mN/m (Rahman, et al., 2019). In this context, Table 2 presents the experimental results of values of surface tension obtained by *I. orientalis* UCP 1603 in low-cost media, showing its ability to produce BS in all conditions of the FFD. The greatest reduction in surface tension (72 to 29.9 mN/m) was verified in condition 4, in medium containing 7.5% CWW, 5% CSL and 1% PFSO.

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Table 2. Results of surface tension, parafilm test and emulsification index obtained by *I. orientalis* UCP 1603 cultivated in agro-industrial substrates, according to the 2^3 full-factorial design.

Conditions	CWW	CSL	PFSO	Surface tension (mN/m)		Parafilm test (mm)	Emulsification index* (%)
				Experimental	Predicted		
1	-1	-1	-1	33.9	33.70	7.0	40.0
2	+1	-1	-1	34.7	34.50	8.0	70.0
3	-1	+1	-1	33.8	33.60	6.0	35.0
4	+1	+1	-1	29.9	29.70	10.0	62.0
5	-1	-1	+1	36.7	36.50	6.0	47.5
6	+1	-1	+1	34.7	34.50	7.0	40.2
7	-1	+1	+1	31.2	31.00	6.0	60.0
8	+1	+1	+1	31.2	31.00	6.0	65.0
9	0	0	0	32.4	33.06	7.0	70.2
10	0	0	0	32.7	33.06	8.0	70.0
11	0	0	0	32.9	33.06	8.0	70.0
12	0	0	0	32.6	33.06	7.0	73.0

*Results of emulsification index using burned motor oil. Source: Authors.

Previously, research on the production of biosurfactant by yeasts of the genus *Issatchenkia* have been performed (Thaniyavarn, et al., 2008; Johny, 2013; Aragã, et al., 2014). However, only the work of Nwaguma, et al. (2019) reported the production of this biomolecule by strains of *I. orientalis* isolated from palm saps grown in olive oil and yeast extract. However, these authors only presented results of rapid methods for detection of biosurfactant such as hemolytic activity, oil displacement and emulsifying activity. Hence, the present work shows innovation on the production of biosurfactant by *I. orientalis* isolated from the soil of the Caatinga biome, Brazil, and cultivated in agro-industrial substrates. Other yeasts have been investigated for their ability to produce biosurfactants in media based on alternative substrates and by-products, as summarized in Table 3.

Table 3. Comparison of result of surface tension obtained by *I. orientalis* UCP 1603 in this study, with other yeasts cultured in alternative substrates for biosurfactant production.

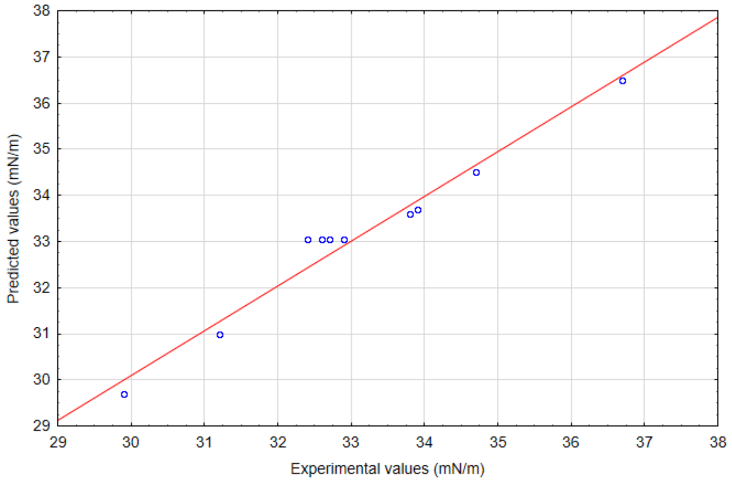
Yeasts	Substrates	Surface tension (mN/m)	References
Issatchenkia orientalis UCP 1603	CWW, CSL and PFSO	29.9	Present study
Yarrowia lipolytica *Rhodotorula mucilaginosa*	Glucose and soybean oil Glucose and motor oil	34.7 41.5	Yalçin, et al. (2018)
R. glutinis	Whey and CSL	26.2	Andrade, et al. (2018)
Candida tropicalis UCP 1613	Whey, CWW and PFSO	28.8	Rubio-Ribeaux, et al. (2017)
C. glabrata UCP 1556	Whey and CSL	28.8	Lima, et al. (2017)
Pichia caribbica	Xylose and oleic acid	35.9	Joshi-Navare, et al. (2014)
Cyberlindnera samutprakarnensis	Glycerol Soybean oil	35.8 39.9	Poomtien, et al. (2013)

Source: Authors.

3.2 Effects of agro-industrial substrates on biosurfactant production

In this study, the production of biosurfactant by *I. orientalis* UCP 1603 was improved using the 2^3 FFD proposed in Table 1, to determine the relationship and influence between independent and dependent variables on the process. To analyze the mathematical models, adjustments to the points were made by nonlinear regression methods and Table 2 shows the experimental and predicted values of surface tension of the model. According to Figure 1, the obtained experimental values of surface tension were distributed close to the straight line, which indicates that such values were close to predicted values in all conditions evaluated. In addition, the ANOVA revealed that the regression model had a high coefficient of determination (R^2=0.969), indicating that 96.9% of the variation in the process was explained by the independent variables (concentration of agro-industrial substrates) and that only 3.1% was not explained by the model. Reproducibility of the experimental data was confirmed by the low pure error (0.043) and value of the adjusted determination coefficient (Adj. R^2=0.9173). Thus, the model demonstrated to be suitable to predict the biosurfactant production under the experimental conditions.

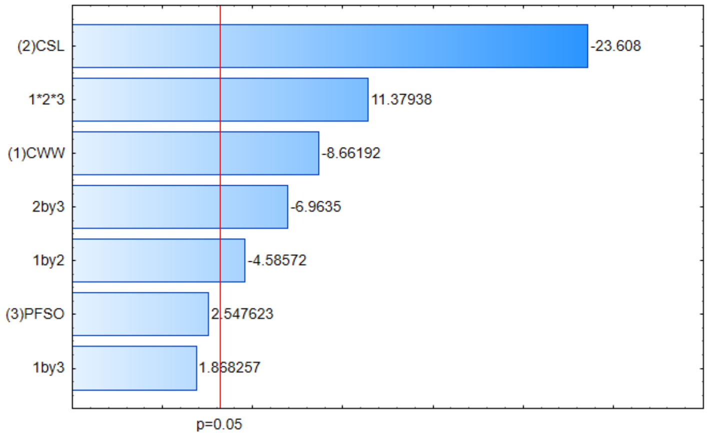
Figure 1. Predicted and experimental values of surface tension according to the full-factorial design applied to biosurfactant production by *I. orientalis* UCP 1603.



Source: Authors.

In addition, surface tension was used as response variable, and the effects of independent variables (CWW, CSL and PFSO concentrations), and the interactions between them, were analyzed by estimated effects represented in Pareto diagram (Figure 2). According to it, only CSL, CWW, the interaction between them as well as the interaction between CSL and PFSO showed negative influence on surface tension. This means that an increase in concentrations of these substrates led to lower surface tension, suggesting the production of biosurfactant in the culture medium. Previously, several studies reported the effectiveness of CSL and CWW as inductors for microbial surfactants production (Maia, et al., 2018; Araújo, et al., 2019; Cândido, et al., 2022). The elemental composition of these agro-industrial by-products (Table 4) confirms their suitability as alternative substrates to the conventionally used carbon and nitrogen sources, guaranteeing microbial growth and the production of biosurfactants.

Figure 2. Pareto diagram obtained from the statistical analysis of the 2^3 full-factorial design applied to the production of biosurfactant by *I. orientalis* UCP 1603. The point at which the effect estimates were statistically significant (p = 0.05) is indicated by dashed line.



Source: Authors.

Table 4. Elemental composition of agro-industrial substrates used for formulation of biosurfactant production medium by *I. orientalis* UCP 1603.

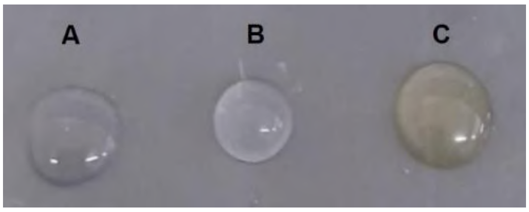
Substrate	Elemental composition (%)				Reference
	Carbon	Nitrogen	Hydrogen	Sulfur	
Cassava wastewater	33.35	2.04	6.74	0	Souza et al. (2020)
Corn steep liquor	34.84	7.06	6.59	1.18	Souza et al. (2020)
Post-frying soybean oil	75.96	0.29	11.05	0.93	Galindo et al. (2021)

Source: Authors.

3.3 Parafilm test

The parafilm test is commonly used as a preliminary assay to select biosurfactant-producing microorganisms (Yaraguppi, et al., 2020; Shatila, et al., 2021). In current study, it was used to compare with the results of surface tension and to confirm the surfactant properties in the metabolic liquids obtained in the FDD (Table 2). A correlation between the surface tension results and the droplet diameter was evidenced, showing that the greater the reduction in surface tension (greater production of biosurfactant or presence of more effective biosurfactant), the greater the scattering of the droplets, in agreement with Pele, et al. (2018). Consequently, the best result in the parafilm test was obtained in condition 4 of the FFD, where the drop had a diameter of 10.0 mm, compared to SDS 2% (8.0 mm) and distilled water (5.0 mm) (Figure 3). As observed, when the metabolic liquid contains biosurfactant, the surface tension between the drop containing the biosurfactant and the hydrophobic surface was reduced, resulting in the spreading of the liquid droplet over the surface. Similar results were previously described by Pele, et al. (2018) and Primeia, et al. (2020), who reported droplet diameter values between 8.0-12.0 mm for biosurfactant-producing microorganisms.

Figure 3. Parafilm M test using SDS 2% (A), distilled water (B) and cell-free metabolic liquid containing biosurfactant produced by *I. orientalis* UCP 1603.



Source: Authors.

3.4 Emulsification index

Emulsifying property commonly is evaluated by the determination of EI_{24}, as the ability to maintain at least 50% of the original emulsion after 24 hours of formation (Lima, et al., 2017). Therefore, the biomolecule produced by *I. orientalis* UCP 1603 demonstrated promising emulsifying property with burned motor oil, with EI_{24} of 70-73% obtained in central point of the FFD (Table 2). Previously, in the study carried out by Marcelino, et al. (2019), strains of the genus *Issatchenkia* cultivated in hydrolysate of detoxified bagasse reached an emulsification index in kerosene of 44.7-57.8%. In addition, lowest values of EI_{24} were obtained with canola oil (13-52%), soybean oil (34-62.5%) and PFSO (35-59%) (data not shown), suggesting that the emulsifying activity depends on the affinity of the bioemulsifier/biosurfactant with the hydrocarbon substrates.

4. Conclusion

This study showed the biotechnological potential of *Issatchenkia orientalis* UCP 1603 as a new biosurfactant producer using low-cost agro-industrial by-products as alternative and renewable substrates. The biosurfactant production by the yeast was associated with higher levels of organic carbon and nitrogen sources. The results obtained were promising for several applications of this biomolecule and gives a broader strategy in the process media-optimization, using statistic methodology.

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References

Andrade, R. F., Silva, T. A., Ribeaux, D. R., Rodriguez, D. M., Souza, A. F., Lima, M. A., ... & Campos-Takaki, G. M. (2018). Promising biosurfactant produced by *Cunninghamella echinulata* UCP 1299 using renewable resources and its application in cotton fabric cleaning process. *Advances in Materials Science and Engineering*, *2018*. https://doi.org/10.1155/2018/1624573

Aragã, V. O., & Martins, C. M. (2014). *Pichia* spp. yeasts from Brazilian industrial wastewaters: Physiological characterization and potential for petroleum hydrocarbon utilization and biosurfactant production. *African Journal of Microbiology Research*, 8(7), 664-672.

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. https://doi.org/10.1186/s12934-018-1046-0

Cândido, T. R. S., Mendonça, R. S., Lins, U. M. D. B. L., de Souza, A. F., Rodriguez, D. M., de Campos-Takaki, G. M., & da Silva Andrade, R. F. (2022). Production of biosurfactants by Mucoralean fungi isolated from Caatinga bioma soil using industrial waste as renewable substrates. *Research, Society and Development*, *11*(2),. http://dx.doi.org/10.33448/rsd-v11i2.25332

Dandi, N. D., Dandi, B. N. & Chaudhari, A. B. (2013) Bioprospecting of thermo- and osmo-tolerant fungi from mango pulp-peel compost for bioethanol production. *Antonie van Leeuwenhoek*,103, 723–736.

Galindo, H. M., Souza, A. F. Melo, E. J. V. et al. 2021. "Sustainable chitosan production by mucoralean fungi using waste post-frying oils and corn steep liquor as substrates", *International Journal of Development Research*, 11, (01), 43185-43194. https://doi.org/10.37118/ijdr.20748.01.2021

Hasani, Z. P., Moghimi, H., & Hamedi, J. (2018). Biosurfactant production by *Mucor circinelloides*: Environmental applications and surface-active properties. *Engineering in Life Sciences*, *18*(5), 317-325.

Hisamatsu, M., Furubayashi, T., Karita, S., Mishima, T., Isono, N. (2006) Isolation and identification of a novel yeast fermenting ethanol under acidic conditions. *Journal Applied Glycoscience*, 53: 111–113.

Johny, J. M. (2013). Screening, gene sequencing and biosurfactant production from *Pichia fermentans* isolated from dairy effluents. I*OSR J Environ Sci Toxicol Food Technol*, 6(5), 2319-2402.

Joshi-Navare, K., Singh, P. K., & Prabhune, A. A. (2014). New yeast isolate *Pichia caribbica* synthesizes xylolipid biosurfactant with enhanced functionality. *European Journal of Lipid Science and Technology*, 116(8), 1070-1079.

Katemai, W., K., Maneerat,S., Kawai,K., Kanzaki, H., Nitoda, T.' & H-Kittikun, A. (2008), Purification and characterization of a biosurfactant produced by *Issatchenkia orientalis* SR4. *Journal Geeral Applied Microbiology*, 54, 79–82.

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

Kwon, Y.J., Ma, A.Z., Li, Q., Wang, F./ Zhuang, G. Q. & Liu, C. Z. (2011) Effect of lignocellulosic inhibitory compounds on growth and ethanol fermentation of newly-isolated thermotolerant *Issatchenkia orientalis*. *Bioresource Technology*, 102: 8099–8104.

Lima, R. A., Andrade, R. F., RodrÃguez, D. M., Araujo, 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. https://doi.org/10.5897/AJMR2016.8341

Maia, P. C., Santos, V. P., Fereira, A. S., Luna, M. A., Silva, T. A., Andrade, R. F., & Campos-Takaki, G. M. (2018). An efficient bioemulsifier-producing *Bacillus subtilis* UCP 0146 isolated from mangrove sediments. *Colloids and Interfaces*, 2(4), 58. https://doi.org/10.3390/colloids2040058

Marcelino, P. R. F., Gonçalves, F., Jimenez, I. M., Carneiro, B. C., Santos, B. B., & da Silva, S. S. (2020). Sustainable production of biosurfactants and their applications. *Lignocellulosic biorefining technologies*, 159-183.

Muñoz, S. S., Balbino, T. R., Alba, E. M., Barbosa, F. G., de Pier, F. T., de Almeida, A. L. M., ... & da Silva, S. S. (2022). Surfactants in biorefineries: Role, challenges & perspectives. *Bioresource Technology*, 345, 126477.

Nitschke, M., & Pastore, G. M. (2004). Biosurfactant production by *Bacillus subtilis* using cassava-processing effluent. *Applied Biochemistry and Biotechnology*, 112(3), 163-172.

Nwaguma, I. V., Chikere, C. B., & Okpokwasili, G. C. (2019). Isolation and molecular characterization of biosurfactant-producing yeasts from saps of *Elaeis guineensis* and *Raphia africana*. *Microbiology Research Journal International*, 1-12.

Oberoi, H. S., Babbar, N., Sandhu, S. K., Dhaliwal, S. S., Kaur, U., Chadha, B. S., et al. (2012) Ethanol production from alkali-treated rice straw via simultaneous saccharification and fermentation using newly isolated thermotolerant *Pichia kudriavzevii* HOP-1. *Journal Industrial Microbiology Biotechnology*, 39: 557–566.

Pele, M. A., Montero-Rodriguez, D., Rubio-Ribeaux, D., Souza, A. F., Luna, M. A., Santiago, M. F., ... & Campos-Takaki, G. M. (2018). Development and improved selected markers to biosurfactant and bioemulsifier production by *Rhizopus strains* isolated from Caatinga soil. *African Journal of Biotechnology*, *17*(6), 150-157. https://doi.org/10.5897/AJB2017.16230

Poomtien, J., Thaniyavarn, J., Pinphanichakarn, P., Jindamorakot, S., & Morikawa, M. (2013). Production and characterization of a biosurfactant from *Cyberlindnera samutprakarnensis* JP52T. *Bioscience, biotechnology, and biochemistry*, 77(12), 2362-2370.

Primeia, S., Inoue, C., & Chien, M. F. (2020). Potential of biosurfactants' production on degrading heavy oil by bacterial consortia obtained from tsunami-induced oil-spilled beach areas in Miyagi, Japan. *Journal of Marine Science and Engineering*, 8(8), 577.

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.

Rubio-Ribeaux, D., da Silva Andrade, R. F., da Silva, G. S., de Holanda, R. A., Pele, M. A., 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. https://doi.org/10.5897/AJMR2017.8486

Santiago, M. G., Lins, U. M. D. B. L., de Campos Takaki, G. M., da Costa Filho, L. O., & da Silva Andrade, R. F. (2021). Produção de biossurfactante por *Mucor circinelloides* UCP 0005 usando novo meio de cultura formulado com cascas de jatobá (*Hymenaea courbaril* L.) e milhocina. *Brazilian Journal of Development*, 7(5), 51292-51304. https://doi.org/10.34117/bjdv.v7i5.30166

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.

Shatila, F., Uyar, E., & Yalçın, H. T. (2021). Screening of biosurfactant production by *Yarrowia lipolytica* strains and evaluation of their antibiofilm and anti-adhesive activities against *Salmonella enterica* ser. *enteritidis* biofilms. *Microbiology*, 90(6), 839-847.

Singh, P., & Cameotra, S. S. (2004). Potential applications of microbial surfactants in biomedical sciences. *TRENDS in Biotechnology*, 22(3), 142-146.

Souza, A. F., Galindo, H. M., de Lima, M. A. B., Ribeaux, D. R., Rodríguez, D. M., da Silva Andrade, R. F., ... & de Campos-Takaki, G. M. (2020). Biotechnological strategies for chitosan production by mucoralean strains and dimorphism using renewable substrates. *International Journal of Molecular Sciences*, *21*(12), 4286. https://doi.org/10.3390/ijms21124286

Thaniyavarn, J., Chianguthai, T., Sangvanich, P., Roongsawang, N., Washio, K., Morikawa, M., & Thaniyavarn, S. (2008). Production of sophorolipid biosurfactant by *Pichia anomala*. *Bioscience, biotechnology, and biochemistry*, 72(8), 2061-2068.

Valencia, GA, Andrade, CJD, Ienczak, JL, Monteiro, AR, & Gutiérrez, TJ (2021). Valorização dos resíduos agroalimentares. Em Biovalorização de Resíduos (pp. 111-132). Springer, Singapura.

Yalçın, H. T., Ergin-Tepebaşı, G., & Uyar, E. (2018). Isolation and molecular characterization of biosurfactant producing yeasts from the soil samples contaminated with petroleum derivatives. *Journal of basic microbiology*, 58(9), 782-792.

Yaraguppi, D. A., Bagewadi, Z. K., Muddapur, U. M., & Mulla, S. I. (2020). Response surface methodology-based optimization of biosurfactant production from isolated *Bacillus aryabhattai* strain ZDY2. *Journal of Petroleum Exploration and Production Technology*, 10(6), 2483-2498.