Full Length Research Paper

Production and characterization of biosurfactant isolated from Candida glabrata using renewable substrates

Roberto A. Lima¹,⁴, Rosileide F. S. Andrade¹,⁴, Dayana M. Rodríguez¹,⁴, Helvia W. C. Araújo², Vanessa P. Santos³,⁴ and Galba M. Campos-Takaki⁴*

¹Federal University of Pernambuco, 50670-901 Recife, Pernambuco, Brazil.
²Department of Chemistry, State University of Paraíba, 58429-500 Campina Grande, Paraíba, Brazil.
³Catholic University of Pernambuco, 50.050-900 Recife, Pernambuco, Brazil.
⁴Nucleus Research in Environmental Sciences and Biotechnology, Catholic University of Pernambuco, 50050-590, Recife, Pernambuco, Brazil.

Received 18 October, 2016; Accepted 23 January, 2017

The world market for biosurfactants has grown gradually. However, the lack of competitiveness with chemical surfactants due to high cost of production remains a concern. Considering the need to reduce the costs of production, the aim of this work was to study the production and structural characterization of a biosurfactant produced by a strain of yeast Candida glabrata UCP 1556. The low-cost medium containing agro-industrial wastes whey 40% (v/v) and 20% (v/v) corn steep liquor were used as substrates in submerged fermentation. Biosurfactant production was detected by surface tension, oil displacement test and Critical Micelle Concentration (CMC). The structural characterization was performed by Fourier transform infrared (FT-IR), gas chromatography-mass spectrometry (GC-MS) and ionic profile. The stability of emulsions and potential in reducing the viscosity also were investigated. The results showed that the biosurfactant reduced the surface tension to 28.8 mN/m with CMC of 2% and showed anionic profile. Additionally, the biosurfactant formed stable emulsions at temperature (0 to 120°C), pH (2 to 12) and NaCl (2 to 12%), reduced the viscosity of soybean oil (21.0 to 18.5 Cp), cotton (39.5 to 26.4 Cp) and canola (34.8 to 29.3 Cp) and has oil dispersing capacity of 81.54%. The chemical structure of biosurfactant exhibited absorption in peaks characteristic of lipids represented at 1393 cm⁻¹ (chain of fatty acids) and the presence of peptides was confirmed by the presence of the peak at 1662 cm⁻¹. The biosurfactant is constituted by 58% of lipids and 36% of protein. The fatty acids of the hydrophobic portion of biosurfactant were capric acid (4.1%), palmitoleic acid (33.6%), steric acid (28.4%), oleic acid (22.6%) and linoleic acid (5.7%). The results demonstrated that C. glabrata can produce an anionic lipopeptide biosurfactant in low-cost medium with promising conditions for the production in large scale.

Key words: Candida glabrata, lipopeptide, agro-industrial wastes, physicalchemical characterization.

INTRODUCTION

New prospects for industrial production are focused on biotechnological processes that use agro-industrial wastes as substrates in formulating alternative medium in fermentation processes for producing secondary
Surfactants are used to reduce the tensions between two insoluble surfaces such as between liquids and solids/gases and enhance the solubility of insoluble substrates in aqueous solutions (Benincasa et al., 2004; Cameotra and Makkar, 2010; Araujo et al., 2016). Use of biosurfactants instead of synthetic surfactants can significantly reduce the manufacturing costs by using low-cost substrates and efficient microorganisms to produce biosurfactants (Deleu and Paquot, 2004; Rufino et al., 2016). Biosurfactants are amphipathic molecules comprising one hydrophobic part and one hydrophilic part; there is frequently a hydrocarbon chain in the nonpolar portion, while the polar portion may be ionic, anionic, cationic and non-ionic or amphoteric. The main classes of biosurfactants include glycolipids, lipopeptides, phospholipids, neutral lipids, hydroxylated fatty acids (Bialkova and Šubík, 2006; Nayak et al., 2009; Li et al., 2016). Biosurfactants offer several advantages over synthetic surfactants, such as lower toxicity, higher biodegradability, stability over a wide range of pH and temperatures, and its ecological acceptability. These properties make them suitable for a wide range of industrial applications such as detergency, emulsification, lubrication, foaming, wetting, and solubilizing/dispersing insoluble substrates (Banat et al., 2000; Mulligan, 2005; Shahaby et al., 2015).

Herein, we studied the efficiency of using agro-industrial wastes as substrates to produce biosurfactants with the help of yeasts. Among the yeasts, Candida glabrata has been reported as a microorganism with a high biotechnological potential for producing secondary metabolites and has the potential to meet the demand of this market (Bialkova and Šubík, 2006; Luna et al., 2009; Nhlanchla et al., 2016). Thus we used agro-industrial wastes such as whey (WH) and corn steep liquor (CSL) as carbon and nitrogen sources and C. glabrata UCP 1556 as yeast strain to produce a biosurfactant using low-cost medium followed by physiochemical characterization of biosurfactant.

MATERIALS AND METHODS

Microorganism and culture conditions

C. glabrata UCP 1556 was isolated from Caatinga soil from Pernambuco, kindly supplied from the culture collection of Catholic University of Pernambuco (UCP), registered in World Federation for Culture Collection (WFCC). The C. glabrata was grown in Yeast Mold Agar (YMA) according to the composition: yeast extract 0.3%, malt extract 0.3%, tryptone 0.5%, glucose 1%, agar 5.0%, and distilled water 100 ml. The inoculum was standardized by transferring the cells of C. glabrata to Yeast Mold Broth (YMB), same composition of YMA without agar, incubated for 24 h on a shaker at 150 rpm at 28°C to obtain 10^7 cells/ml.

Agro-industrial substrates

The production medium composed of agro-industrial wastes: corn steep liquor (CSL), a byproduct of corn processing industry and whey (WH) from the dairy industry of São Bento do Una, Pernambuco, Brazil.

Biosurfactant production

Biosurfactant was produced in medium containing WH (40%), CSL (20%) and minerals salts (calcium carbonate 0.12, magnesium sulfate 0.03). Erlenmeyer flasks containing 100 ml of medium were inoculated with 5% of inoculum of C. glabrata suspension of 10^7 CFU/ml and incubated for 72 h at 28°C in orbital shaker at 150 rpm. After this period, the sample was centrifuged at 10.000 g for 15 min and then filtered using Whatman Number 1 filter paper (Sigma Aldrich, 2033 Westport Center Dr, St. Louis, MO 63146, EUA).

Determination of surface tension and the CMC

The surface tension of the cell-free metabolic liquid was monitored for 0, 4, 8, 12, 24, 48, 72, 96, 120, 144 until 168 h using a tensiometer (model Sigma 70, KSV Instruments Ltd., Finland) under the Du Nouy ring method at temperature (±28°C) (Kuyukina et al., 2001). The CMC was determined with the isolated biosurfactant that was solubilized in water at different concentrations (0.001, 0.01, 0.03, 0.05, 0.1, 1, 1.5, 2 and 2.5%) and then their surface tensions were measured. The upper limit of the CMC (Critical concentration of biosurfactant required for micelles formation) range was deemed to have been reached after a constant value of the surface tension was observed.

Growth kinetics

The kinetics of the growth of C. glabrata was assessed during incubation at 150 rpm and temperature at 28°C for 168 h. Duplicates aliquots from cultures were collected in the following intervals, 0, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 h. The viability of yeasts was estimated by enumerating the number of yeasts by the Pour-Plate technique using YMA, and the results were expressed in Log number colony forming units per ml of culture (Ln CFU/ml) (Vance-Harrop et al., 2003).

Determination of glucose consumption and pH of the metabolic liquid

The amount of glucose consumed in the cell-free metabolic liquid...
Determination of the emulsification index

The cell-free metabolic liquid was analyzed in accordance with the methodology described by Cooper and Goldenberg (1987). The hydrophobic substrates tested were vegetable oils, soybean, coconut and canola. The emulsification index was calculated by dividing the measured height of the emulsion layer by the total height of the mixture and multiplying by 100. Hexane was used as control of the hydrophobic substrate for emulsification.

Stability studies

Studies of stability were carried out using 50 ml of cell-free metabolic liquid at different temperatures (0, 5, 70, 100 and 120°C), pH (2 to 12) and NaCl (2 to 12%) after the emulsification index was measured.

Isolation and purification of the biosurfactant

The biosurfactant produced by *C. glabrata* was isolated by the precipitation method using acetone 1:1 (*v*/*v*) added to the cell-free metabolic liquid (Parasziewicz et al., 2002). The purification process was initiated with the crude biosurfactant (500 mg), solubilized in 4 ml of deionized water. Then, biosurfactant in aqueous solution was re-precipitated using acetone, and the process was repeated three times. The isolated biosurfactant was submitted to dialysis (dialysis bags width 25 mm and 16 mm diameter) against deionized water for 72 h at 5°C, and each was changed every 3 h. The purified biosurfactant was collected and freeze dried.

Characterization of the biosurfactant

The protein concentration in the isolated biosurfactant was estimated by using the total protein test kit from Labtest Diagnostica S.A., Brazil. The total carbohydrate content was estimated by the phenol-sulphuric acid method (Dubois et al., 1956). For the lipids (Manocha et al., 1980), the fatty acids content on the hydrophobic portion of the biosurfactant was identified by converting it to methyl esters (Durham and Kloos, 1978). The methyl esters of fatty acids were suspended in *n*-hexane and analyzed by gas chromatography.

Biosurfactant characterization by thin-layer chromatography

The biosurfactant sample was dissolved in chloroform:methanol (1:1 *v*/*v*) and a 100 µl sample was applied on a TLC silica gel plate (Si 60 F254, 0.25 mm, Merck) and developed in a solvent system containing chloroform:methanol:acetic acid (65:15:2 *v*/*v*/*v*) (George and Jayachandran, 2009). The constituents of the biosurfactant were detected after spraying the TLC plate with ninhydrin, rhodamine and anthrone reagents. The ninhydrin reagent 0.05% *w*/w (methanol and water 1:1 *v*/*v*) heated at 100°C for 4 min used to detect amino acids and rhodamine 0.25% *w*/w (in 70% ethanol) to detect the presence of lipids. The anthrone reagent (1 g in 5 ml of sulfuric acid mixed with 95 ml of ethanol) was used to detect the presence of yellow spots which are indicative of glycolipid biosurfactant (Yin et al., 2009). The visualization of bands corresponding to the biosurfactant constituents were visualized in UV light (Yu et al., 2002; Yin et al., 2009).

Analysis of the functional groups of the biosurfactant and ionic charge

The biosurfactant obtained was analyzed using a Fourier transform (FT-IR) spectrometer, recorded on Bruker IFS 66 apparatus, using potassium bromide (KBr) pellets and expressed in wave numbers (cm\(^{-1}\)) ranging from 4000 to 400 cm\(^{-1}\). The ionic charge of the biosurfactant was determined using a DG-ZM3 meter, model Zeta Meter system 3.0+.

Effect of biosurfactant on viscosity

The effect of the viscosity of biosurfactant on vegetable oils such as soybean, cotton and canola was tested after adding 6 ml of each of these oils and 2 ml of 1% biosurfactant solution. The mixtures were vortexed for 2 min and after 20 min of rest the emulsions were read in a viscometer Model Brookfield TC-500 at 25°C using spindel No. 41 at 100 rpm (Jara et al., 2013).

Determination of the potential of biosurfactant in dispersing oil

The identification of the production of the biosurfactant was performed with the test oil dispersion. 10 ml of deionized water and, subsequently, 0.5 ml of the engine oil were placed on Petri dishes of 10 cm diameter. 1 ml of cell-free metabolic liquid containing the biosurfactant was added to the center of the Petri dish containing oil and water. The halo formation (mm) characterized by a clear zone which indicates the presence of surface-active compounds and their ability to disperse hydrophobic compounds (Morikawa et al., 2000).

RESULTS AND DISCUSSION

Biosurfactant production

The biosurfactant production occurred in agro-industrial wastes (40% W and 20% CSL) based medium with excellent reduction of surface tension (28.8 mN/m) after 72 h of cultivation. Our finding is consistent with previous studies were *C. glabrata* can be used as an organism to produce biosurfactants from agro-industrial wastes (Luna et al., 2009; Andrade et al., 2015). In addition, the data from this study were promising considering that Mulligan (2005) and Mesbaiah et al. (2016) affirm that efficient biosurfactants are capable of reducing surface tension to values below 30 mN/m.

Evaluation of the surface tension and CMC

The CMC is the surfactant concentration at which an increase in surface tension is observed. Thus, regardless of the surfactant concentration, a further decrease in the surface tension will not be observed once the CMC has been reached (Desai and Banat, 1997; Rufino et al., 2014). In this study, the biosurfactant of *C. glabrata* was
able of maintain stable values of surface tension after the addition of 2% of the sample. The biosurfactant produced in this work showed a smaller CMC value than those of other biosurfactants from yeasts described in the literature, as values of 2.5% (w/v) were found for biosurfactant from *C. glabrata* (Sarubbo et al., 2006).

**Kinetics of the growth profile of C. glabrata UCP 1556**

Figure 1 shows the maximum growth of *C. glabrata* in the medium containing renewable agro-industrial wastes (40% W and 20% CSL) after 48 h of cultivation in exponential phase and pH 6. On the other hand, the glucose consumption in production medium was detected after the first 24 h of cultivation of *C. glabrata*. For biosurfactant production after 72 h, occurred the maximum production in the stationary phase of growth with surface tension reduction to 28.8 mN/m (Figure 1). Our data confirmed that the biosurfactant are secreted into the culture medium in the stationary phase (Rammnani et al., 2005; Kuyukina et al., 2016).

**Yield of the biosurfactant and purification**

The yield of the crude biosurfactant produced by *C. glabrata* was 8 g/L after 72 h of cultivation. After the first purification, the yield reduced to 6.80 g/L and after the second purification to 5.60 g/L. The yield of the crude biosurfactant produced by *C. glabrata* in this study is in agreement with values reported by other studies (Morikawa et al., 2000; Rufino et al., 2007; Yadav et al., 2016).

**Emulsifier property**

The results showed that the biosurfactant of *C. glabrata* was able of form consistent emulsions using soybean oil, cotton, canola and hexane with values of 75, 90, 80, and 82%, respectively. Therefore, these results are very promising by characterizing the biosurfactant of *C. glabrata* as an excellent emulsifier once Alvarez et al. (2015) consider emulsifying agents those that are capable to form emulsions by Emulsification Index (IE 24) values above 50%.

**Stability studies**

Environmental factors such as pH, salinity and temperature also affect the activity and stability of biosurfactants. Therefore, it is important to study the influence of these parameters when considering specific applications for these compounds (Mulligan, 2005; Rufino et al., 2016). The biosurfactant produced by *C. glabrata* in the renewable agro-industrial wastes (40% W and 20% CSL) medium was stable in all different temperatures (Figure 2A), range of pH (Figure 2B), and in different
Figure 2. Influence of pH (A), temperature (B) and NaCl (C) on emulsifying capacity of biosurfactant produced by *C. glabrata* grown in W (40%) and CSL (20%) as substrates.

NaCl concentrations (Figure 2C) using hydrophobic substrates (soybean, cotton and canola oils) for emulsification index values. The biosurfactant produced by *Candida lipolytica* (Rufino et al., 2007) and a biosurfactant produced by *C. glabrata* (Sarubbo et al., 2006) cultivated with industrial residue have shown similar thermal stability in the temperature range tested from the results obtained in this study. The biosurfactant
produced in this work by C. glabrata have the potential application in several industrial products by maintaining its emulsifying properties proven by the maintenance of its activity after temperature exposure and high saline concentration and acid and alkaline conditions.

**Characterization of the biosurfactant**

The analysis demonstrated that the biosurfactant isolated from C. glabrata consists of 58% lipids and 36% protein. The presence of both protein units and lipids indicates that the sample is a lipoprotein. Similar results were observed using C. lipolytica, in medium supplemented with soybean oil, refinery residue, glutamic acid and yeast extract. This was able to produce a biosurfactant of lipoprotein type (50% protein and 20% lipid) (Yu et al., 2002; Luna et al., 2009; Luna et al., 2016). The presence of these compounds was confirmed by FT-IR and by thin-layer chromatography (TLC), visualized with specific reagents for identification bands. The results demonstrated positive reactions to amino groups are using ninhydrin reagents and to lipids using rhodamine reagents, but negative to glycolipids using anthrone reagents.

Analysis of the FT-IR spectrum of biosurfactant produced by C. glabrata (Figure 3) revealed absorption peaks at 2926 cm\(^{-1}\) indicating the presence of a C-H stretch containing compounds with fatty acids and lipids (Lambert et al., 1998). The peak at 1745 cm\(^{-1}\) is the stretching vibration of C = O of the ester functional group in lipids. The presence of peptides was confirmed by the peak 1662 cm\(^{-1}\) corresponding to stretching C = O (Forato et al., 2013). The peak 1393 cm\(^{-1}\) corresponds to the symmetrical stretching vibrations of -COO- group side chain of amino acids and fatty acids (Naumann, 2000).

According to the analysis conducted using a Zeta Potential Meta 3.0 plus, biosurfactant showed an anionic character, -12.8 nV. Other biosurfactants produced by Candida species also display an anionic character when submitted to the same test (Luna et al., 2013). The anionic surfactants most widely used commercially are subdivided into carboxylate esters and sulfated (esters) widely used in household cleaners and cosmetics (Karray et al., 2016).

The fatty acids present in the hydrophobic portion of the biosurfactant produced by C. glabrata were identified by conversion to methyl esters and are reported in Table 1.

The study composition of the fatty acid of the isolated biosurfactant produced by C. lipolytica analysed by Gas Chromatography revealed the presence of C12:0 (75.34%), C8:0 (7.96%), C18:1 (6.36%), C16:1 (4.23%), C14:0 (3.85%) and C16:0 (2.25%) (Luna et al., 2013).
Table 1. Composition of fatty acids of biosurfactant of C. glabrata (UCP 1556).

| Composition of fatty acids | UCP 1556 (%) |
|---------------------------|--------------|
| Capric acid (C8:0)        | 4.1          |
| Palmitoleic acid (C16:0)  | 33.6         |
| Stearic acid (C18:0)      | 28.4         |
| Oleic acid (C18:1)        | 22.6         |
| Linoleic acid (C18:2)     | 5.7          |

Viscosity reduction

The biosurfactant produced by C. glabrata reduced the viscosity of soybean (21.0 to 18.5 Cp), cotton (39.5 to 26.4 Cp) and canola (34.8 to 29.3 Cp) oils. The effect of reducing the viscosity of oils and the high potential to form emulsions of our biosurfactant can be applied to manufacturing cosmetics (Bhardwaj et al., 2016). However, further studies are required to assess the suitability of this to be used in cosmetics. The hexane was used as control of the hydrophobic substrate.

Oil spreading determination

Spreading oil tests were conducted to confirm the presence of the surfactant due to its dispersion ability. The biosurfactant produced by C. glabrata in medium constituted by agro-industrial wastes (40% Whey and 20% corn steep liquor) showed oil dispersion of 81.54% and formation of the halo with 53 mm diameter. The control used in this study was the triton-X (chemical surfactant) that promoted 95.38% dispersion of water in engine oil resulting in the formation of a halo of 62 mm diameter. Thus, the data demonstrate excellent capacity dispersant of oil by the biosurfactant produced by C. glabrata in this study.

Conclusion

The biosurfactant produced by C. glabrata UCP 1556 yeast from whey and corn steep liquor is an anionic biosurfactant with great surface tension reduction, emulsification and dispersion properties of petroleum derivatives. The promising future of biosurfactant production using renewable agro-industrial substrates represents 30% of low-cost for final bioprocess. In addition, more studies need to enhance the lipopeptide biosurfactant property of this product especially in cosmetic industry.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

ACKNOWLEDGEMENTS

The authors are grateful for the Support of Foundation of Science and Technology of the State of Pernambuco (FACEPE) for the grant, to the National Council for Scientific and Technological Development (CNPq), and R.A.L for post-doctoral in Northeast Network for Biotechnology (RENOBIO), Federal Rural University of Pernambuco, and the Nucleus of Research in Environmental Sciences and Biotechnology (NPCIAMB) of Catholic University of Pernambuco, Brazil.

REFERENCES

Accosinini FR, Mutton MJR, Lemos EGM, Benincasa M (2012). Biosurfactants production by yeasts using soybean oil and glycerol as low cost substrate. Braz. J. Microbiol. 43(1):116-125.

Alvarez VM, Jurelevic N, Marques JM, de Souza PM, de Araújo LV, Barros TG, Souza R, Freire DMG, Seldin L (2015). Bacillus amyloliquefaciens TSBSO 3.8, a biosurfactant-producing strain with biotechnological potential for microbial enhanced oil recovery. Colloids Surf. B Biointerf. 136:14-21.

Andrade R, Antunes AA, Lima RA, Araújo HW, Resende-Stoianoﬀ MA, Franco LO, Campos-Takaki GM (2015). Enhanced Production of an Glycolipid Biosurfactant Produced by Candida glabrata UCP/WFCC1556 for Application in Dispersion and Removal of Petroderivatives. Int. J. Microbiol. Appl. Sci. 4(7):563-576.

Antunes AA, de Araújo HWC, da Silva CAA, da Costa Albuquerque CD, Campos-Takaki GM (2013). Produção de biossurfactante por Chromobacterium violaceum ATCC 12472 utilizando milho e água de água pós-frita como nutrientes. Acta Inst. Biol. 80(3):334-341.

Araujo LV, Guimarães CR, da Silva Marquita RL, Santiago VM, de Souza MP, Nitschke M, Freire DMG (2016). Rhamnolipid and surfactin: Anti-adhesion/antibiofilm and antimicrobial effects. Food Control 63:171-178.

Banat IM, Makkar RS, Cameotra SS (2000). Potential commercial applications of microbial surfactants. Appl Microbiol. Biotechnol. 53(5):495-508.

Benincasa M, Abalos A, Oliveira I, Manresa A (2004). Chemical structure, surface properties and biological activities of the biosurfactant produced by Pseudomonas aegingosa LBI from soapstock. Antonie Van Leeuwenhoek 85(1):1-8.

Bhardwaj G, Cameotra SS, Chopra HK (2016). Biosurfactant from Lysinibacillus chungkukjangi from Rice Bran Oil Sludge and Potential Applications. J. Surfactants Deterg. 19(6):957-966.

Bialkova A, Šubík J (2006). Biology of the pathogenic yeast Candida glabrata. Folia Microbiol. 51(1-3):20.

Cameotra SS, Makkar RS (2010). Biosurfactant-enhanced bioremediation of hydrophobic pollutants. Pure Appl. Chem. 82(9):97-116.

Cooper DG, Coldenberg BG (1987). Surface-active agents from two Bacillus species. Appl. Environ. Microbiol. 53(2):224-229.

Deleu M, Paquot M (2004). From renewable vegetable resources to microorganisms: new trends in surfactants. C. R. Chimie 7(6):641-646.

Desai JD, Banat IM (1997). Microbial production of surfactants and their commercial potential. Microbiol. Mol. Biol. Rev. 61(1):47-64.

Dubois MK, Gilles JK, Hamilton JK, Rebers PA, Smith F (1956). A colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.

Durham DR, Kloo WS (1978). Comparative Study of the Total Cellular Fatty Acids of Staphylococcus Species of Human Origin. J. Int. Syst. Evol. Microbiol. 28(2):223-228.

Forato LA, Britto DD, Scramin JA, Colnago LA, Assis OB (2013). Mechanical and wetting properties of zein films extracted from corn gluten meal. Polímeros 23(1):42-48.

George S, Jayachandran K (2009). Analysis of rhamnolipid biosurfactants produced through submerged fermentation using
orange fruit peels as sole carbon source. Appl. Biochem. Biotechnol. 158(3):694-705.

Jara AM, Andrade RF, Campos-Takaki GM (2013). Physicochemical characterization of tensio-active produced by Geobacillus stearothermophilus isolated from petroleum-contaminated soil. Colloids Surf. B Biointerf. 101:315-318.

Karray F, Mezghani M, Mhiri N, Djelassi B, Sayadi S (2016). Scale-down studies of membrane bioreactor degrading anionic surfactants wastewater: Isolation of new anionic-surfactant degrading bacteria. Int. Biodeterior. Biodegradation 114:14-23.

Kuyukina MS, Iivshina IB, Krivoruchko AV, Motta Silveira GN, Luna JM, Sarubbo LA (2016). Possible correlation with virulence of Pseudomonas aeruginosa. AMB Express 6(1):6-14.

Lambert JB, Shurvell HF, Lightner DA, Cooks RG (1998). Organic Structural Spectroscopy Prentice-Hall, Inc. New Jersey.

Li J, Deng M, Wang Y, Chen W (2016). Production and characteristics of biosurfactant produced by Bacillus pseudomycoideus BS6 utilizing soybean oil waste. Int. Biodeterior. Biodegradation 112:72-79.

Luna JM, Rufino RD, Sarubbo LA, Campos-Takaki GM (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 Surf B Biointerf. 102:202-209.

Luna JM, Santos Filho AS, Rufino RD, Sarubbo LA (2016). Production of Biosurfactant from Candida bombicola URM 3718 for Environmental Applications. Chem. Eng. 49:583-588.

Luna JMD, Sarubbo L, Campos-Takaki GM (2009). A new biosurfactant produced by Candida glabrata UCP 1002: characteristics of stability and application in oil recovery. Braz. Arch. Biol. Technol. 52(4):785-793.

Manocha MS, San-Blas G, Centeno S (1980). Lipid composition of Paracoccidioides brasiliensis: possible correlation with virulence of different strains. Microbiology 117(1):147-154.

Mesbaia FZ, Eidouaouda K, Badis A, Chebbi A, Bentati D, Sayadi S, Chamkha M (2016). Preliminary characterization of biosurfactant produced by a PAH-degrading Paenibacillus sp. under thermophilic conditions. Environ. Sci. Pollut. Res. 23:14221-14230.

Morikawa M, Hirata Y, Imanaka T (2000). A study on the structure–function relationship of lipopeptide biosurfactants. Biochim. Biophys. Acta 1488(3):211-218.

Mulligan CN (2005). Environmental applications for biosurfactants. Environ. Pollut. 133(2):183-198.

Naumann D (2000). Infrared spectroscopy in microbiology. Encyclopedia of Analytical Chemistry. P 102.

Nayak AS, Vijaykumar MH, Karegoudar TB (2009). Characterization of biosurfactant produced by Pseudoxanthomonas sp. PKN-04 and its application in bioremediation. Int. Biodeterior. Biodegrad. 63(1):73-79.

Nhlanhla T, Patel M, Francis DM (2016). Examination of Candida albicans strains from South Africa for the production of gliotoxin and other cytotoxic secondary metabolites. J. Yeast Fungal Res. 7(3):19-28.

Paraszkiewicz K, Kanwal A, Dlugoszki J (2002). Emulsifier production by steroid transforming filamentous fungus Curvularia lunata. Growth and product characterization. J. Biotechnol. 92(3):287-294.

Ramani P, Kumar SS, Gupta R. (2005). Concomitant production and downstream processing of alkaline protease and biosurfactant from Bacillus licheniformis RG1: Bioformulation as detergent additive. Process Biochem. 40(10):3352-3359.

Rufino RD, Luna JM, Campos-Takaki GM, Sarubbo LA (2014). Characterization and properties of the biosurfactant produced by Candida lipolytica UCP 0988. Electronic J. Biotechnol. 17(1):1-6.

Rufino RD, Motta Silveira GN, Luna JM, Sarubbo LA (2016). Conservation of the Biosurfactant Produced by Pseudomonas aeruginosa for Environmental Applications. Chem. Eng. 49:535-540.

Rufino RD, Sarubbo LA, Campos-Takaki GM (2007). Enhancement of stability of biosurfactant produced by Candida lipolytica using industrial residue as substrate. World J. Microbiol. Biotechnol. 23(5):729-734.

Santos DKF, Rufino RD, Luna JM, Santos VA, Sarubbo LA (2016). Biosurfactants: Multifunctional Biomolecules of the 21st Century.Int. J. Mol. Sci. 17(3):401-432.

Sarubbo LA, Luna JM, Campos-Takaki GM (2006). Production and stability studies of the bioemulsifier obtained from a new strain of Candida glabrata UCP 1002. Electron. J. Biotechnol. 9(4):400-406.

Shahaby AF, Alharthi AA, El-Tarras AE (2015). Bioremediation of Petroleum Oil by Potential Biosurfactant-Producing Bacteria using Gravimetric Assay. Int. J. Curr. Microbiol. Appl. Sci. 4(5):390-403.

Yadav AK, Mann S, Pandiyam K, Singh A, Kumar M, Chakdar H, Kashyap PL, Srivastava AK (2016). Isolation and characterization of biosurfactant producing Bacillus sp. from diesel fuel-contaminated site. Microbiology 85(1):56-62.

Yasin F, Abdullah M, Sethi AA, Saleem H, Narmeen A, Ansari A, Khan SA, Ul Gader SA (2016). Solid state fermentation: a cost effective approach for production of starch liquefying fungal amylase using agro industrial wastes. Sci. Int. 28(3):2703-2706.

Yin H, Qiang J, Jia Y, Peng H, Qin H, Zang N, He B (2009). Characteristics of biosurfactant produced by Pseudomonas aeruginosa S6 isolated from oil-containing wastewater. Process Biochem. 44(3):302-308.

Yu GY, Sinclair JB, Hartman GL, Bertagnolli BL (2002). Production of iturin A by Bacillus amyloliquefaciens suppressing Rhizoctonia solani. Soil Biol. Biochem. 34(7):955-963.