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Study of the Biosurfactant Production by *Saccharomyces Cerevisiae* URM 6670 Using Agroindustrial Waste

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Surfactants are chemical compounds that have the property of being preferably located between phases with different degrees of polarity, which may be of synthetic or natural origin, the latter being called biosurfactants. These have several advantages over chemical surfactants, since they can be produced from agroindustrial substrates and have low toxicity, high biodegradability and compatibility with the environment. Thus, this work had studied the production of a biosurfactant by the yeast *Saccharomyces cerevisiae* URM 6670 using agroindustrial residues. The yeast was grown in six different media, substituting the original sources of carbon and nitrogen from the mineral medium by agricultural wastes (soybean frying oil and corn steep liquor) at 150 rpm for 120 h and temperature 28 °C. After choosing the best production condition by surface and interfacial tension and emulsification index (E_{24}) measurements, the physicochemical composition, CMC and particle size distribution of the biomolecule was determined. The surface and interfacial tensions obtained ranged from respectively 26.45 ± 0.02 to 45.88 ± 0.15 mN/m and 6.87 ± 0.04 to 14.31 ± 0.14 mN/m, with the best results obtained for the biosurfactant produced only in the presence of the residues. For the emulsification index, the results were also more promising from the optimized media, ranging from 3.77 ± 0.10 % to 94.58 ± 2.43 %. Regarding the physicochemical composition, the value obtained for the isolated biosurfactant indicated its glycolipidic nature (19.64 ± 3.66 % of carbohydrates and 80.04 ± 3.86% of lipids). In determining CMC, a value of 0.8 g/L was obtained; and particle size distribution with best result using emulsion solution at 2xCMC concentration. Therefore, the use of agroindustrial residues for the production of biosurfactant by *Saccharomyces cerevisiae* URM 6670 is promising, allowing the reduction of costs associated with the raw material.

1. Introduction

Natural compounds which have the property of preferentially locating between phases with different polarity degrees and hydrogen bonds are known as biosurfactants. These have amphipathic molecules with hydrophilic and hydrophobic moieties, the polar (cationic or anionic), nonionic or amphoteric polar portion, and the nonpolar portion commonly consisting of a hydrocarbon chain (Ribeiro et al., 2019).These characteristics favor the ability of biosurfactants to reduce surface and interfacial tensions as well as to form microemulsions in which hydrocarbons are solubilized in water or vice versa. In addition, they have advantages over synthetic emulsifiers such as low toxicity, high biodegradability, efficacy under different environmental conditions and biocompatibility (Campos; Stamford; Sarubbo, 2019). Biosurfactants can be classified according to different criteria: molecular weight, ionic charges and type of secretion. In the case of surfactants from microorganisms, the main classification criterion is the type of chemical structure, the most common being lipopolysaccharides, lipoproteins and complex biopolymers (high molecular weight), and phospholipids, lipopeptides and glycolipids

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(low weight). molecular). Among these, glycolipids are the group of biosurfactants most studied by several authors, due to their high production and potential applications (Mnif; Ellouz‐Chaabouni; Ghribi, 2017). One of the most relevant functions of a biosurfactant is the ability to reduce the surface tension of a system by forming micelles proportional to its concentration in the medium. And this concentration is related to the Critical Micellar Concentration (CMC), corresponding to the minimum concentration of surfactant or biosurfactant necessary for the surface and interfacial tension to be reduced to the maximum (Santos et al., 2016). Low molecular weight biosurfactants are more efficient in reducing both surface and interfacial tension (Solomon et al., 2017). Several microorganisms can synthesize biosurfactants and must have the ability to grow on both water-immiscible substrates and soluble compounds (Souza et al., 2017). Substrate selection depends on the choice of raw material with adequate nutrient balance for growth and production, and it must contain high carbohydrate and lipid content, essential elements for the production of biosurfactants. However, the costs of using these substrates are high and make it difficult to apply biosurfactants on a commercial scale when compared to the production of chemical surfactants (Radzuan; Banat; Winterburn, 2017). On the other hand, the use of industrial waste as raw material in bioprocesses is recommended, as long as there is a good balance of nutrients to obtain the desired product. Thus, the production of biosurfactants that was previously considered economically unfeasible is now implemented with potential viability through the use of various wastes (Campos; Stamford; Sarubbo, 2014). In this context, new production perspectives with lower costs have been raised in biotechnological processes aiming at the formulation of alternative means, agroindustrial waste compounds in secondary metabolites production processes, since they account for about 50% of the final cost of the product. (Lima et al., 2017). Thus, the objective of this work was to produce a biosurfactant by *Saccharomyces cerevisiae* URM 6670 yeast using agroindustrial residues as the sole carbon and nitrogen source, as well as characterize its structure and evaluate the emulsions formed at different concentrations.

2. Material and methods

2.1 Microrganisms and maintenance

For the production of biosurfactant, *Saccharomyces cerevisiae* URM 6670 yeast was used and maintained in YMA (Yeast Mold Agar) medium composed by: yeast extract (0.3%), malt extract (0.3%), tryptone (0, 5%), D-glucose (1.0%) and agar (5.0%). Monthly sampling was performed to maintain cell viability.

2.2 Medium of inoculum growth and biosurfactant production

For inoculum growth was used YMB (Yeast Mold Broth) medium, same composition as YMA medium, except agar. The biosurfactant production process was carried out in six different culture media, the media being 1, 2, 3 and 4 added minerals: 0.40 % $NaNO_3$, 0.10 % NaCl, 0.10 % KCl, 0.01 % $CaCl_2$, 0.30 % KH_2PO_4, 0.30 % Na_2HPO_4, 0.02 % $MgSO_4$, 0.10 % $FeSO_4$, 0.08 % $CoCl_2$, 0.07 % $CuSO_4.5H_2O$, 0.07 % $MnSO_4$, 0.001 % H_3BO_3, 0.07 % $Fe(SO_4)_3$ and 0.07 % $ZnSO_4$, and supplemented with 1% olive oil (medium 1 and 3) or soybean frying oil (medium 2 and 4) as carbon source, and with 1 % NH_4NO_3 (medium 1 and 2) or corn steep liquor (medium 3 and 4) as nitrogen source. Medium 5 will consist only of 1 % olive oil and 1% corn steep liquor and medium 6 with 1 % soybean frying oil and 1 % corn steep liquor.

2.3 Preparation of inoculum and production of biosurfactant

Inoculum was standardized by transferring yeast from YMA medium to Erlenmeyer flasks containing 50 mL of YMB medium and incubating (28 °C, 200 rpm) for 24 h. After this time, dilutions were made until the desired final cell concentration (10^8 cells/mL) was reached. For biosurfactant production, 2.0 % (v/v) of the inoculum was added to the production media and the incubated media (150 rpm and 28 °C) for 88 h. After the incubation period, the media were subjected to centrifugation under stirring 3500 rpm for 20 minutes to obtain a metabolic fluid (free from biomass).

2.4 Determination of surface and interfacial tension and emulsification index (E_{24})

The surface and interfacial tension of metabolic (cell free) liquids were measured on a Sigma 700 tensiometer (KSV, Finland) using the NOUY ring. The surface tension was measured by immersion of the platinum ring in samples containing cell free metabolic fluid. The interfacial was measured against n-hexadecane in metabolic liquids (after filtration with 0.45 µm diameter filter membrane). The metabolic fluid produced in each medium was also subjected to the determination of the emulsification index (E_{24}), as described by Prasanna, Bell and Grandison (2012), against corn, soybean, canola, sunflower and motor oils.

2.5 Determination of physicochemical composition

After determining the best biosurfactant produced by the surface and interfacial assays and E_{24}, the physical chemical composition was determined by the AOAC (2002) test methodologies with biosurfactant isolated.

The moisture content was determined by gravimetric method in a kiln at 105 ºC (method 985.14). Total protein concentration was obtained using the Kjeldahl method (method 992.15). For the fixed mineral residue (ashes) the gravimetric method was used (method 923.03). To quantify the lipid fraction, the cold extraction method of Bligh-Dyer (1959) was used. The carbohydrate content was calculated from the difference between 100 and the sum of the moisture, protein, lipid and ash determinations.

2.6 Determination of critical micelle concentration (CMC)

In order to obtain CMC, 5 g/L biosurfactant solution, isolated from the best production medium, was submitted to successive dilutions with distilled water, and then the surface tensions of the respective dilutions were quantified with the aid of the NOUY ring technique.

2.7 Ligth microscopy of emulsions

To determine CMC, biosurfactant solutions were initially prepared at concentrations of ½ CMC, CMC and 2XCMC, and emulsions prepared as described by Prasanna, Bell and Grandison (2012) with motor oil and canola oil. The distribution of the particle size of the solutions (60 uL after 24 hours storage at 27-28 °C) was observed with the aid of a light microscope (Nikon Eclipse E-100) under a blade cavity and objective lens magnification of 10 times.

3. Results and Discussion

3.1 Surface and interfacial tension

According to Santos et al. (2016), surface tension, one of the most important properties of a surfactant, is considered as the force of attraction between the molecules of a liquid. The interfacial is the interaction between two distinct phases, an aqueous and a hydrophobic one. The concentration of a surfactant influences micelle formation, which is inversely proportional to the surface and interfacial tension values. The average results of surface and interfacial tension of the media after the biosurfactant production process are presented in Table 1.

Table 1: Surface and interfacial tension after the biosurfactant production process. Experiments were performed in triplicate and the results represent the mean ± standard deviation of the three independent experiments.

Medium	Surface tension (mN/m)	Interfacial tension (mN/m)
1	39.50 ± 0.09^{a}	—
2	44.10 ± 0.13^{b}	14.31 ± 0.14^{a}
3	36.37 ± 0.06^{c}	6.87 ± 0.04^{b}
4	45.88 ± 0.15^{d}	17.32 ± 0.15^{c}
5	26.45 ± 0.02^{e}	13.99 ± 0.18^{da}
6	26.64 ± 0.19^{fe}	9.12 ± 0.29^{e}

Media in the same column with different letters are significantly different ($p \leq 0.05$) according to Tukey's teste.

According to Table 1, it was not possible to quantify the interfacial tension of medium 1, probably due to the value of this voltage being below the detection limit and sensitivity of the equipment. Regarding the surface tension values, both medium 5 and medium 6 presented very similar results (26.45 ± 0.02 and 26.64 ± 0.06 mN/m, respectively), and it is necessary to compare their stress results. the best value was obtained from medium 6 (9.12 ± 0.29 mN/m). This medium, despite presenting interfacial tension higher than 7 mN/m, is the best alternative, since low surface tension values were obtained using only residues as carbon, nitrogen and mineral sources. To be classified as good surfactants, the surface and interfacial tension of the medium must be reduced by the action of biosurfactants to values below 35 mN/m and 1 mN/m, respectively (Luna et al., 2013). In contrast, values below 7 mN/m are also acceptable, being considered high only when values above 18 mN/m are found (Santos et al., 2016).

Surface tension results similar to those of media 1 and 3 were found by Ribeiro et al. (2019) using *C. utilis* UFPEDA 1009 in culture medium supplemented with canola oil (35.33 ± 0.19 mN/m). Regarding media 5 and 6, with the lowest values of surface tension, Santos et al. (2017), who used *C. lipolytica* UCP0988 in a medium containing 5 % animal fat and 2.5 % with steep liquor, presented the closest surface tension values (28.0 mN/m, in 2 L bioreactor and 25.0 mN/m, bioreactor 50 L) to those obtained experimentally, being considered as the best results.

3.2 Emulsification index (E_{24})

Another way to evaluate the properties of a biosurfactant is by its emulsification activity against hydrocarbons and/or water-insoluble compounds. Thus, the emulsification index (E_{24}) is a rapid and qualitative method for determining the emulsifying properties of a surfactant. According to Campos et al. (2015), the stability of these emulsions is evaluated as an indicator of surface activity, although the ability to remain stable is not always associated with a reduction in surface tension. Although the physiological function of biosurfactants has not yet been clarified, the ability to solubilize and emulsify different hydrophobic compounds is known and presented by several authors. Table 2 shows the emulsification index values obtained for each culture medium studied.

Table 2: Emulsification index (%) after the biosurfactant production process. Experiments were performed in triplicate and the results represent the mean ± standard deviation of the three independent experiments.

Medium	Canola oil	Soy oil	Corn oil	Sunflower oil	Motor oil
1	15.24 ± 0.21^{a}	29.81 ± 0.26^{a}	35.83 ± 1.71^{a}	26.11 ± 0.26^{a}	72.75 ± 1.87^{a}
2	30.20 ± 0.81^{b}	28.38 ± 1.12^{ba}	36.54 ± 2.72^{ba}	37.75 ± 1.01^{b}	70.69 ± 2.44^{ba}
3	11.11 ± 0.52^{ca}	14.81 ± 5.24^{c}	9.06 ± 2.34^{c}	35.38 ± 1.09^{cb}	91.07 ± 2.53^{c}
4	11.54 ± 2.72^{dca}	15.71 ± 3.97^{dc}	3.77 ± 0.10^{d}	3.77 ± 0.10^{d}	87.75 ± 2.18^{dc}
5	34.78 ± 3.07^{eb}	37.75 ± 1.96^{e}	38.61 ± 0.73^{eba}	43.56 ± 2.68^{e}	66.09 ± 0.81^{eb}
6	42.86 ± 1.26^{f}	41.07 ± 2.53^{fe}	41.96 ± 1.26^{fe}	37.50 ± 2.53^{fc}	94.58 ± 2.43^{fc}

Media in the same column with different letters are significantly different ($p \leq 0.05$) according to Tukey's teste.

According to Table 2, all media presented E_{24} values with significant differences in relation to the same oil. The medium 6 also presented better result, with the highest percentage of emulsification among the evaluated oils. In relation to vegetable oils, canola oil obtained the highest index (42.86 ± 1.26 %).

The higher the E_{24} percentage, the better the emulsification activity of the biosurfactant with the hydrophobic compound. Comparing with the values presented in the literature, Campos, Stamford and Sarubbo (2014) obtained emulsification indexes with the biosurfactant produced by *C. utilis* UFPEDA 1009, of 43, 73, 73, 33 and 30 % for soybean oils. sunflower, corn, rice and motor, respectively, using medium containing 5.0 % canola frying oil, 6.0 % D-glucose, 0.2 % NH_4NO_3 and 0.3 % yeast extract. Using *C. sphaerica* UCP0995 in medium supplemented with 9.0 % soybean oil residue and 9.0% corn liquor, Luna et al. (2015) presented E_{24} values with motor oil, corn and soybeans, respectively, of 78.12 ± 0.37 %, 21.74 ± 0.41 % and 24.0 ± 0.53 %, close to those obtained with means 1 and 2 facing the same oils.

3.3 Physicochemical composition

For the biosurfactant that presented better results in the surface and interfacial tension and E_{24} tests (yield: 5.84 ± 0.34 g/L), low percentages of moisture (0.01 ± 0.00 %), proteins (0.08 ± 0.00 %) and ashes (0.22 ± 0.19 %). Regarding the lipid content, it was 80.04 ± 3.86 %, while the carbohydrate percentage was 19.64 ± 3.66 %.

According to results found, its structure is basically composed of lipids and carbohydrates, since the percentage sum of these components is greater than 90 %. Selvakumar, Anibabiyans and Madhan (2016), using the methods of antrona and vanillin-phosphoric acid to verify the presence of carbohydrates and lipids, respectively, in a biosurfactant produced by *S. cerevisiae* MTCC 181, obtained the result corresponding to a glycolipid. Thus, from the results obtained, it can be stated that the biosurfactant produced possibly has a glycolipid structure.

3.4 Critical Micelle Concentration (CMC)

For a biosurfactant to be considered a good agent, it must have both efficiency, as measured by CMC (ranging from 0.001 to 2 g/L), and effectiveness, associated with surface and interfacial tensions. Thus, the lower the concentration of the biosurfactant, the greater its efficiency. The Figure 1 showed relationship between surface tension and biosurfactant concentration. As show in Figure 1, the biosurfactant produced from *S. cerevisiae* presented CMC of the 0.8 g/L, it can be inferred that the increase in the biosurfactant concentration does not lead to an additional reduction in surface tension.

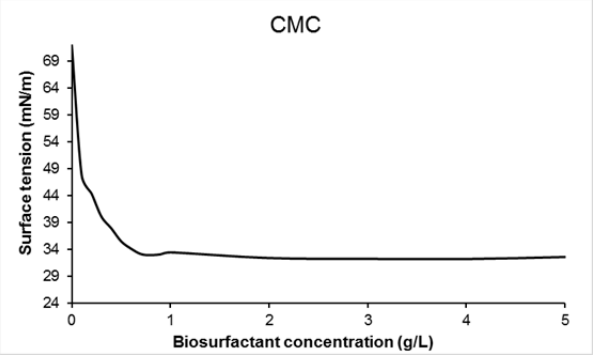

Figure 1: Critical micelle concentration of the biosurfactant produced by S. cerevisiae.

CMC equal to 0.8 g/L was also obtained by Rufino et al. (2014) using *C. lipolytica* and in mineral medium with 6.0 % soy frying oil and 1.0 % glutamic acid. Ribeiro et al. (2019) when producing a biosurfactant by *C. utilis* in mineral medium supplemented with 6.0 % canola frying oil and 6.0 % glucose, found CMC of the 0.6 g/L. Soares da Silva et al. (2017), using a bacterium of the genus *Pseudomonas*, also found CMC of 0.6 g/L of a biosurfactant using 2.0 % canola frying oil and 3.0 % corn steep liquor.

3.5 Particle Size Distribution

The particle size distribution of emulsions allows to observe the emulsion droplet size, one of the physical parameters that can be evaluated in a biosurfactant, being related to processes involving flocculation, for example. For commercial application, it is necessary to study the decrease or increase in concentration of a surfactant with regard to emulsion stability, determined by the emulsion droplet size (Han et al., 2015). The photomicrographs of emulsions of the biosurfactant solutions produced (½ CMC, CMC e 2 CMC) with the canola and motor oils are shown in Figure 2.

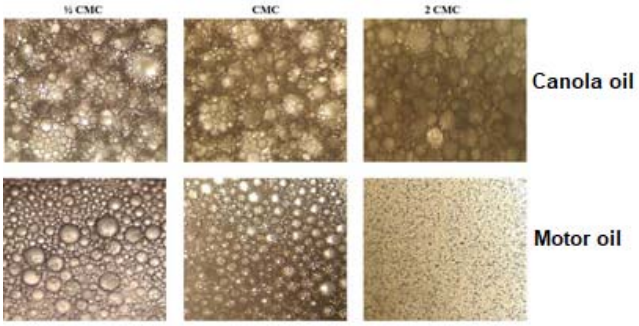

Figure 2: Emulsions prepared with solutions (½ CMC, CMC and 2XCMC) of S. cerevisiae biosurfactant.

The stability of the emulsion being inversely proportional to the size of the droplet, being influenced by factors as interfacial tension and nature of emulsifier. As observed in the Figure 2, smaller particle sizes were found in the motor oil emulsions, in agreement with the E_{24} results (Table 2), as well as more uniform and separate format, resistant to coalescence. Moreover, it can be inferred that the higher the biosurfactant concentration, the smaller the particle size and the greater the stability. A similar study was carried out by Han et al. (2015), who observed the exopolysaccharides emulsions of *Bacillus* species with sunflower oil, compared with commercial guar gum emulsions, resulting in photomicrographs similar to those of motor oil in this work. Ribeiro et al. (2019), performing the same study with biosurfactant emulsion of *C. utilis*, also presented similar photomicrographs, with better results with motor oil.

4. Conclusions

The yeast *Saccharomyces cerevisiae* has potential for biosurfactant production with good tensoactive and emulsifiers activity, and only agroindustrial residues can be used as carbon and nitrogen sources for its production. Thus, the results show that it is possible to reduce the costs associated with the raw materials used for biosynthesis, obtaining a glycolipid with added industrial value.

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References

AOAC. Association of Official Analytical Chemists, 2002, 17 ed., Washington, DC.

Campos, J.M., Stamford, T.L.M., Sarubbo, L.A., 2014, Production of a Bioemulsifier with Potential Application in the Food Industry, Appl. Biochem. Biotechnol., 172, 3234 – 3252.

Campos, J.M., Stamford, T.L.M., Rufino, R.D., Luna, J.M., Stamford, T.C.M., Sarubbo, L.A, 2015, Formulation of mayonnaise with the addition of a bioemulsifier isolated from Candida utilis, Toxicol. Rep., 2, 1164-1170.

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

Han, Y., Liu, E., Liu, L., Zhang, B., Wang, Y., Gui, M., Wu, R., Li, P., 2015, Rheological, emulsifying and thermostability properties of two exopolysaccharides produced by Bacillus amyloliquefaciens LPL061, Carbohydr. Polym., 115, 230–237.

Lima, R.A., Andrade, R.F.S., Rodríguez, D.M., Araújo, H.W.C., Santos, V.P., Campos-Takaki, G.M., 2017, Production and characterization of biosurfactant isolated from Candida glabrata using renewable substrates, Afr. J. Microbiol. Res., 11, 6, 237-244.

Luna, J.M., Rufino, R.D., Jara, A.M.A.T., Brasileiro, P.P.F., Sarubbo, L.A, 2015, Environmental applications of the biosurfactant produced by Candida sphaerica cultivated in low-cost substrates, Col. Surf. A: Physicochem. Eng. Asp., 480, 413–418.

Luna, J.M., Rufino, R.D., Sarubbo, L.A., Campos-Takaki, G. M., 2013, Characterization, 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.

Mnif,I., Ellouz‐Chaabouni, S., Ghribi, D., 2017, Glycolipid Biosurfactants, Main Classes, Functional Properties and Related Potential Applications in Environmental Biotechnology, J. Polym. Environ., 4, 1-15.

Prasanna, P.H.P., Bell, A., Grandison, A.S., 2012, Emulsifying, rheological and physicochemical properties of exopolysaccharide produced by Bifidobacterium longum subsp. infantis CCUG 52486 and Bifidobacterium infantis NCIMB 702205, Carbohydr. Polym., 90, 533–540.

Radzuan, M.N., Banat, I.M., Winterburn, J., 2017, Production and characterization of rhamnolipid using palm oil agricultural refinery waste, Bioresource Technol., 225, 99–105.

Ribeiro, B.G., Santos, M.M., Pinto, M.I.S., Meira, H.M., Durval, I.J.B., Guerra, J.M.C., Sarubbo, L.A., 2019, Production and optimization of the extraction conditions of the biosurfactant from Candida utilis UFPEDA1009 with potential application in the food industry, Chem. Eng. Trans., 76, 1477-1482.

Rufino, R.D., Luna, J.M., Campos-Takaki, G.M., Sarubbo, L.A., 2014, Characterization and properties of the biosurfactant produced by Candida lipolytica UCP 0988, Elect. J. Biotechnol., 17, 34–38.

Santos, D.K.F., Luna, J.M., Rufino, R.D., Santos, V.A., Sarubbo, L.A., 2016, Biosurfactants: Multifunctional Biomolecules of the 21st Century, Int. J. Mol. Sci., 17, 401-430.

Santos, D.K.F., Meira, H.M., Rufino, R.D., Luna, J.M., Sarubbo, L.A, 2017, Biosurfactant production from Candida lipolytica in bioreactor and evaluation of its toxicity for application as a bioremediation agent, Process Biochem., 8, 1-11.

Selvakumar, K., Anibabiyans, R., Madhan, R., 2016, Extracellular glycolipids from Saccharomyces cerevisiae as bioemulsifying agents in food processing, Innov. J. food sci., 4, 1, 3-6.

Soares da Silva, R.C.F.S., Almeida, D.G., Meira, H.M., Silva, E.J., Farias, C.B.B., Rufino R.D., Luna, J.M., Sarubbo, L.A., 2017, Production and characterization of a new biosurfactant from Pseudomonas cepacia grown in low-cost fermentative medium and its application in the oil industry, Biocat. Agric. Biotechnol, 12, 206-215.

Solomon, O.B., Oluwafemi, O.A., Muktar, A.G., Mohammed, S.A., Cletus, I.C., Timothy, A.T., 2017, Production and Characterization of Biosurfactants by Candida boleticola H09 and Rhodotorula bogoriensis H15 for Crude Oil Recovery and Cleaning of Oil Contaminated Fabrics, Int. J. Life Sci.Technol., 10, 15, 109-123.

Souza, K.S.T., Gudina, E.J., Azevedo, Z., Freitas, V., Schwana, R.F., Rodrigues, L.R., Dias, D.R., Teixeira, J.A., 2017, New glycolipid biosurfactants produced by the yeast strain Wickerhamomyces anomalus CCMA 0358, Col. Surf. B: Biointerf., 154, 373–382.