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Evaluation of Biosurfactant Production by Yeasts from Antarctica

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Microbial surfactants are surface-active metabolites, produced by microorganisms, which have low toxicity, are biodegradable and biocompatible. Furthermore, these molecules are stable in extreme environmental conditions, such as pH, temperature, and salinity changes. The maim factor for the ever increasing biosurfactant use by industries is its capacity to reduce surface tension and interfacial tension of immiscible solutions. The selection of microorganisms capable to produce biosurfactants has been investigated in the last years, and the Antarctic environment has become of great interest due to its extraordinary diversity. The present work reports the evaluation of biosurfactant production by Antarctic isolated yeasts. Four yeasts isolated in the Antarctic environment were evaluated, coded as L69, L87, L104, and L106, and the biosurfactant production was accessed by the emulsification index in kerosene, diesel oil, engine oil and soybean and surface tension. The biosurfactant containing solution was obtained from a five day old fermentation broth, composed by 3 g L-1 yeast extract, 5 g L-1 peptone, and 20 g L-1 glucose, and incubated at 15 ºC and 30 ºC. The emulsification index was accessed by mixing with the oils and the cell free aqueous solution of the fermented broth, in a 1:1 ratio, and the surface tension was obtained using a Tensiometer. The L69 and L104 yeast showed the best results at the tested conditions, whereas the L69 yeast showed 96, 0, 92 and 50% of emulsification index in kerosene, diesel oil, engine oil and soybean, respectively, while L104 showed 100 and 93 % of emulsification index in engine oil and soybean, and zero for the other tested oils. The results showed that these yeasts were capable to produce biosurfactants, however further tests should be performed to characterize these biomolecules.

1. Introduction

Surfactants are amphipathic molecules, e.g., formed by a hydrophobic portion and another hydrophilic. Such feature give these molecules the ability to reduce surface and interfacial tension of immiscible mixtures, its critical micelle concentration and other characteristics that make them the subject of study for application in several industrial processes such as petrochemical, food or beverage, cosmetic, pharmaceutical, mining, metallurgical, agrochemical, fertilizers, environmental, among many others (Ghojavand *et al.*, 2008, Mulligan and Gibbs, 1993, Kosaric, 1992, Banat *et al*., 2000).
Synthetic surfactants are obtained from petroleum or others sources of chemicals (Desai and Banat., 1997) and thus might be toxic, which means it can remain in the environment for a time significantly long before being assimilated by the environment, restricting its use (Sarkar *et al.*, 1989). In order to overcome these factors in the use of synthetic surfactants, biosurfactants has been studied thoroughly but its production is not yet economically viable compared to the production its synthetic equivalent (Nitschke and Costa, 2007).

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Naturally occurring surfactants can be produced by microorganisms, plants and even humans (Bognolo, 1999). Biosurfactants are surfactants produced by microorganisms having virtually all the characteristics of synthetic surfactants but with low or no toxicity. It is also remarkably known to have high biodegradability, biocompatibility and specificity, and been also stable in extreme environmental conditions such as pH changes, high temperature and salinity (Desai and Banat, 1997). These properties make them suitable to replace synthetic surfactants. Since there are a few different types of biosurfactants, their classification is based on the physicochemical properties, being classified as glycolipids, lipopeptides, neutral lipids, fatty acids, phospholipids and polymers (Pacwa-Plociniczak *et al.,* 2011;. Cameotra *et al*., 2010; Amaral *et al.,* 2010).

Industries' increasing interest in the improvement of biosurfactant production has attracted the attention of the research community worldwide, accelerating the study of microorganisms capable of producing biosurfactants. Thus, the selection of isolated microbial cultures of varied ecosystems became a possibility to be explored. Among these ecosystems, Antarctica stood out for being an inhospitable environment for survival and, despite this, has an extraordinary microbial diversity.

However, Antarctica still is one of the geographical areas preferred by microbiologists to collect new microorganisms for biotechnological applications (Buzzini *et al.*, 2012). There, environmental conditions are one of the most extreme conditions on earth: the air is very cold and dry (as well as highly saline), soil is characterized by low humidity and very low organic content, being irradiated highly with UV radiation and also have insufficient water for living organisms (Connell *et al.*, 2008;. Pavlova *et al.*, 2011). The microorganisms, plants and animals living in these conditions are metabolic and physiologically adapted to ensure its existence in this ecosystem (Gerday *et al.*, 2000;. D'Amico *et al.*, 2006). Thus, a large number of bacteria, yeasts, fungi and unicellular algae existing in such cold environment are both scientifically and biologically interesting regard to the production of biologically active substances. Therefore, this paper studied the production of biosurfactant by yeasts from Antarctica.

2. Materials and Methods

2.1 Obtenção dos Microrganismos

Antarctic's yeasts were obtained from the collection maintained by microbial resources division of multidisciplinary centre for chemical, biological and agricultural, which is part of State University of Campinas (CPQBA / UNICAMP). In the present study, four of these yeasts were analysed, which were coded as L69, L87, L104 and L116.

The microorganisms were preserved in penicillin bottles containing glass beads, standard yeast medium and 50% glycerol (v/v). Each flask was inoculated from the same starter culture and contained an average of 25 glass beads, thus enabling the use of the same cell generation throughout the study.

2.2 Fermentation media

One beads and 1 mL of medium, both from the previously prepared cryo-vials, were inoculated into a Erlenmeyer flask containing 100 mL of broth composed by 3 g L^{-1} yeast extract, 5 g L^{-1} peptone and 20 g L^{-1} glucose, which was incubated at 15°C or 30°C for five days, time required for culture growth and make biosurfactant production possible.

2.3 Sample Preparation

Subsequent analyses were performed using 30 mL of the centrifuged fermentation broth (8,000 g for 20 minutes), in order to separate the cells from the aqueous solution. The suspension was stored in 6 penicillin vials, each one containing 5 mL.

2.4 Dry Weight Analysis

After the abovementioned procedure, the precipitated was diluted in distilled water and put to dry for 24 hours in the oven at 37°C in petri dishes. The dry mass was calculated by the difference in weight of the petri dish with and without the solids.

2.5 Emulsification Index

The emulsification index was calculated based on the methodology described by Cai et al. (CAI et al., 2014), which consists of mixing the cell-free aqueous fermentation broth with kerosene (or other oils) in the ratio of 1: 1. The mixture was stirred vigorously in vortex for two minutes at room temperature and allowed to stand for 24 hours. The emulsification index was calculated by dividing the height of the emulsified phase by the overall height and the ratio multiplied by 100, as shown in the following equation (1):

$$\% = \frac{Height_{emulsion}}{Total\ Height_{mixture}} \quad (1)$$

2.6 Superficial Tension

Superficial tension analysis was realised using 10 mL of a aqueous cell-free sample from the fermentation broth, collected as described previously. The ring method was performed as described by du Noüy (1925) with a K6 tensiometer (Krüss GmbH, Hamburg, Germany) at room temperature.

3. Results and Discussion

The following tables present results from dry mass analysis:

Table 1: Results of Dry mass analysis and Precipitate Formation.

Yeast	Dry mass ($g.L^{-1}$)		Precipitate Formation	
	30 ºC	15 ºC	30 ºC	15 ºC
L69	2,95	5,21	S	S
L87	---	---	N	N
L104	---	0,14	N	S
L106	---	---	N	N

S - Precipitate formed. **N –** Absence of Precipitate. **---** Dry mass was zero, since no precipitate was formed.

The following tables and figures show results of emulsification index (%) for kerosene, diesel, motor oil and soy oil. Tables 2-5 shows that the L69 and L104 yeast provided the best values for the emulsification index, being able to emulsify almost all oils tested, while yeasts L87 and L106 emulsified only motor oil. Although L87 and L106 emulsified only one type of oil, the presence of a biosurfactant is proven for all yeasts by the high emulsification indexes achieved (above 80%) but biomolecules produced by L69 and L104 have better emulsifying properties.
The biosurfactant production is usually confirmed by this assay, it is already known that these biomolecules are able to modify cell surface hydrophobicity and promote emulsification or solubilisation of oil solutions that previously would be immiscible (Beal and Betts, 2000).

Table 2: Emulsification Index results kerosene.

Yeast	Emulsification Index	
	30 ºC	15 ºC
L69	96 %	89 %
L87	0 %	0 %
L104	0 %	0 %
L106	0 %	0 %

Table 3: Emulsification Index results for diesel oil.

Yeast	Emulsification Index	
	30 ºC	15 ºC
L69	0 %	0 %
L87	0 %	0 %
L104	0 %	0 %
L106	0 %	0 %

Table 4: Emulsification Index results for engine oil.

Yeast	Emulsification Index	
	30 ºC	15 ºC
L69	92 %	100 %
L87	97 %	100 %
L104	93 %	96 %
L106	100 %	96 %

Table 5: Emulsification Index results for soy oil.

Yeast	Emulsification Index	
	30 ºC	15 ºC
L69	50 %	0 %
L87	0 %	0 %
L104	100 %	100 %
L106	0 %	0 %

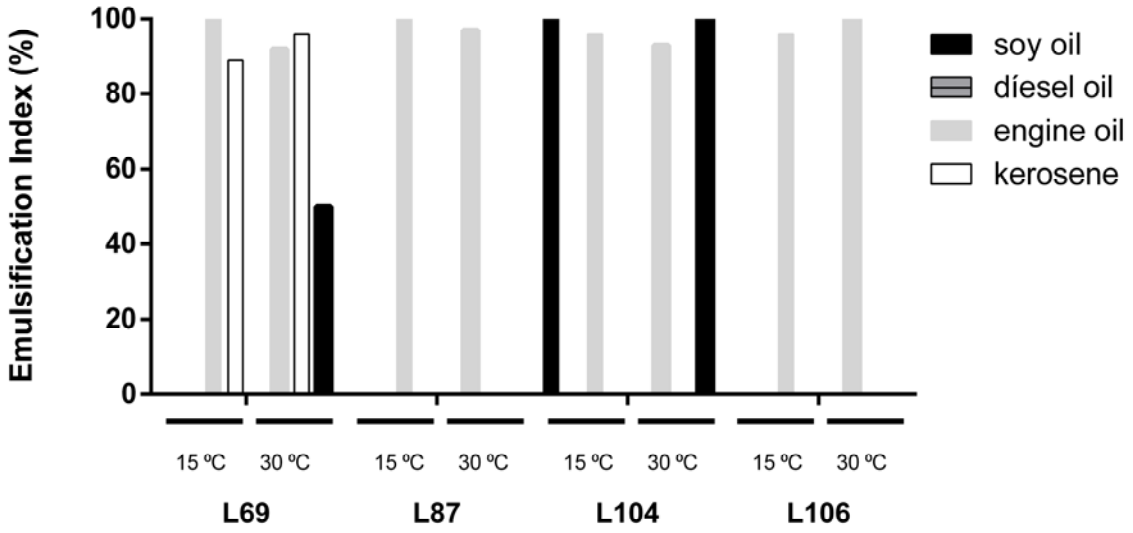

Figure 1: Emulsification Index for Tested Oils.

Table 6: Results of Superficial Tension for all studied yeasts.

Yeast	Superficial Tension (dyne cm^{-1})	
	30 ºC	15 ºC
L69	48,36	49,29
L87	47,88	48,95
L104	40,88	45,06
L106	47,17	47,17

Surface tension analysis is commonly performed to confirm the presence of biosurfactant and analyse its properties. Data presented on Table 6 shows that the studied yeasts have produced no surface-active component, since no decrease in surface tension was observed. Cai et al. (2014) evaluated isolated substances for biosurfactant activity, characterising its surface tension and emulsification index. Their results showed that a decrease in surface tension and the emulsifying capacity were not necessarily correlated, similarly with data presented above.

Cell growth assay results points yeast L69 as the only one that developed at both temperatures, with 2.95 and 5.21 g $.L^{-1}$, at 30 and 15°C respectively. Yeast L104 showed a low growth at 15°C , 0.14 g L^{-1}. Remaining yeasts had developed poorly and thus explain the poor emulsification and tension results.

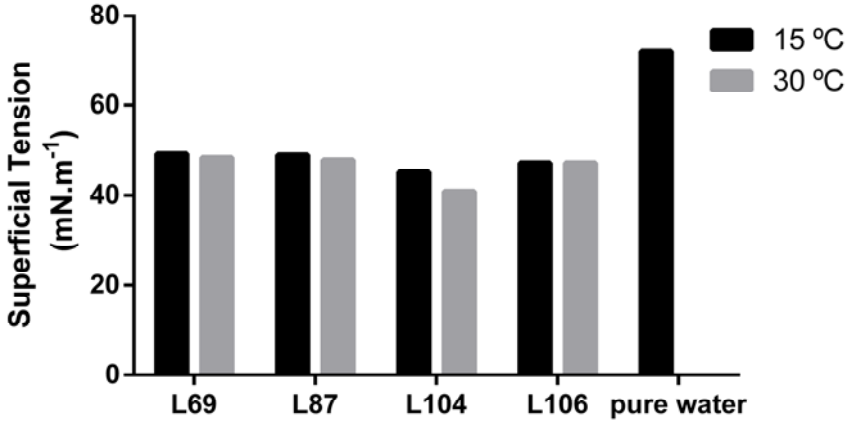

Figure 2: Superficial Tension for all studied yeasts.

By analysing the emulsification index and surface tension results for the produced biosurfactant, it is clear the molecule exhibits emulsification properties but could not decrease superficial tension for any medium. These result shows that the biosurfactant produced by these yeasts have high molecular weight, as characterized by high activity emulsion (Rosenberg and Ron, 1999).
Thus, other tests are necessary to further analysis regarding the characterization of the biomolecule produced, in order to better understand the structure and their properties.

4. Conclusion

The biosurfactant production using yeasts obtained in Antarctica was confirmed by its emulsification indexes, wherein yeasts L69 and L104 yeast provided better emulsifying and emulsified the majority of tested oils. Surface tension assay showed that there was no yeast was capable to produce a surface-active compound.
Therefore, tested yeasts from the Antarctica continent were able to produce biosurfactants, confirmed by the emulsification test. However these biosurfactants showed no ability to reduce surface tension and are classified as high molecular weight. Through these results, studies on the structures and properties of biomolecules will be carried out in order to get a better characterization of biosurfactants produced by microorganisms L69 and L104.
Tests related to product optimization are planned for future experiments, both in the search for cheap substrate, such as agro-industrial or household waste, as well as the best growing conditions, such as pH, temperature, aeration and agitation.
We conclude that different research is needed for a large-scale production of the biosurfactant, but know that microorganisms from extreme environments have the capacity to produce biomolecules of commercial interest is of paramount importance for boosting further research in these areas and attract the attention of researchers to micro-organisms from these habitats.
While both microorganisms and the process must be studied thoroughly before production scale-up, the proof that microorganisms from extreme environments have the capacity to produce biomolecules of commercial interest is essential to stimulate their study and attracts the attention of researchers to microorganisms from these habitats.

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References

Amaral, P.F.F., Coelho, M.A.Z, Marrucho, I.M.J., Coutinho, J.A.P., 2010, Biosurfactants from yeasts: characteristics, production and application, Adv Exp Med Biol, 672, 236–249.
Banat, I.M., Makkar, R.S., Cameotra, S.S., 2000, Potential commercial applications of microbial surfactants. Applied Microbiology and Biotechnology, 53, 495-508.
Beal, R., Betts, W.B., 2000, Role of rhamnolipid biosurfactants in the uptake and mineralization of hexadecane in Pseudomonas aeruginosa, J Appl Microbiol, 89, 158–168.

Bognolo, G., 1999, Biosurfactants as emulsifying agents for hydrocarbons, Colloids and Surfaces A: Physicochemical and Engineering Aspects, 152, 41-52.

Buzzini, P., Branda, E., Goretti, M., Turchetti, B., 2012, Psychrophilic yeasts from worldwide glacial habitats: diversity, adaptation strategies and biotechnological potential, FEMS Microbiology Ecology, 82, 217-241.

Cai, Q., Zhang, B., Chen, B., Zhu, Z., Lin, W., Cao, T., 2014, Screening of biosurfactant producers from petroleum hydrocarbon contaminated sources in cold marine environments, Marine pollution bulletin, 86, 402-410.

Cameotra, S.S., Makkar, R.S., Kaur, J., Mehta, S.K., 2010, Synthesis of biosurfactants and their advantages to microorganisms and mankind, Adv Exp Med Biol, 672, 261–280.

Connell, L., Redman, R., Craig, S., Scorzetti, G., Iszard, M., Rodriguez, R., 2008, Diversity of soil yeasts isolated from South Victoria Land, Antarctica, Microb Ecol, 56, 448–459.

D'Amico, s., Collins, T., Marx, J.C., Feller, G., Gerday, C., 2006, Psychrophilic microorganisms: challenges for life, EMBO reports, 7, 385-389.

Desai, J.D., Banat, I.M., 1997, Microbial production of surfactants and their commercial potential, Microbiol Mol Biol, 61, 47–64.

Du Noüy, P.L., 1925 An Interfacial Tensiometer for Universal Use, The Journal of General Physiology, 625-633.

Gerday, C., Aittaleb, M., Bentahir, M., Chessa, J.P., Claverie, P., Collins, T., D'Amico, S., Dumont, J., Garsoux, G., Georlette, D., Hoyoux, A., Lonhienne, T., Meuwis, M.A., Feller, G., 2000, Cold-adapted enzymes: from fundamentals to biotechnology, Trends in Biotechnology, 18, 103-107.

Ghojavand, H., Vahabzadeh, F., Roayaei, E., Shahraki, A.K., 2008, Production and properties of a biosurfactant obtained from a member of the Bacillus subtilis group (PTCC 1696), Journal of Colloid and Interface Science, 324, 172-176.

Kosaric, N., 1992, Biosurfactants in industry, Pure and Applied Chemistry, 64, 1731-1737.

Mulligan, C.N., Gibbs, B.F., 1993, Factors influencing the economics of biosurfactants. 13, 329-371, Vol. 48: Biosurfactants, Production, Properties, Applications, Eds. Kosaric, N., Marcel Dekker.

Nitschke, M., Costa, S.G.V.A.O., 2007, Biosurfactants in food industry, Trends in Food Science & Technology, 18, 252-259.

Pacwa-Plociniczak, M., Plaza, G.A., Piotrowska-Seqet, Z., Cameotra, S.S., 2011, Environmental applications of biosurfactants: recent advances, Int J Mol Sci, 12, 633–654.

Pavlova, K., Rusinova-Videva, S., Kuncheva, M., Kratchanova, M., Gocheva, M., Dimitrova, S., 2011, Synthesis and Characterization of an Exopolysaccharide by Antarctic Yeast Strain Cryptococcus laurentii AL100, Applied Biochemistry and Biotechnology, 163, 1038-1052.

Rosenberg, E.; Ron, E. Z., 1999, High- and low- molecular-mass microbial surfactants, Appl. Microbiol. Biotechnol., 52, 154-162.

Sarkar, A.K., Sharma, M.M, Goursaud, J.C., Georgiou, G., 1989, A critical evaluation of MEOR processes, In Situ, 13, 207-38, 1989.