SEVEN publicações acadêmicas

Bioemulsifier production by *Penicillium citrinum* UCP 1183 and microstructural characterization of emulsion droplets

Crossref doi
https://doi.org/10.56238/Connexpemultidisdevolpfut-168

Everton Ricardo Carneiro Costa Graduating in
Biological Sciences Catholic University of Pernambuco
Rua do Príncipe, 526 – Boa Vista, 50050-900 Recife – PE.
E-mail: everton.2020204204@unicap.br ORCID: https://orcid.org/0000-0002-4132-9350

Adriana Ferreira Souza
Center for Research in Environmental Sciences and Biotechnology
Catholic University of Pernambuco
Rua do Príncipe, 526 – Boa Vista, 50050-900 Recife – PE.
E-mail: adriana.souza@unicap.br
ORCID: https://orcid.org/0000-0002-9527-2206

Galba Maria de Campos Takaki
Full Professor at ICAN-TECH School
Center for Research in Environmental Sciences and Biotechnology, Catholic University of Pernambuco, Rua do Príncipe, 526, Boa Vista, 50050-900 Recife – PE.
E-mail: galba.takaki@unicap.br
ORCID: https://orcid.org/0000-0002-0519-0849

Rosileide Fontenele da Silva Andrade
Center for Research in Environmental Sciences and Biotechnology,
Catholic University of Pernambuco
Rua do Príncipe, 526 – Boa Vista, 50050-900 Recife – PE.
E-mail: rosileide.andrade@unicap.br
ORCID: https://orcid.org/0000-0001-8526-554X

ABSTRACT

Bioemulsifying compounds are biomolecules of high molecular weight produced by microorganisms and have as their main action the ability to emulsify and stabilize emulsions. In this context, the objective of this work was to investigate the potential of the fungus Penicillium citrinum in the production of bioemulsifier from substrates of renewable origin (corn steep liquor and whey) and to characterize by optical microscopy the droplets of the emulsion formed. For this purpose, Penicillium citrinum was grown in sabouraud medium at 28ºC until the mycelial carpet was obtained. After growth, 20 discs of 8mm were used as inoculum in the production medium containing different concentrations of whey and corn steep liquor according with factorial design of 22. The statistical analysis of the residue concentrations was evaluated by the Pareto diagram, while the visualization of the microstructures of the emulsion droplets was visualized by optical microscopy with a 40x magnification. The results showed a maximum emulsification index of 95.8% and surface tension of 47.5 mN/m in condition 4 of the planning consisting of industrial residues (corn steep liquor 5% and whey 5%). However, in all conditions of factorial design there was significant production of bioemulsifier. The Pareto diagram showed that the independent variable that most influenced the increase in the emulsification index was corn steep liquor followed by whey and its respective association. The type of emulsion formed was water-in-oil. The present study revealed that Penicillium citrinum has high biotechnological potential in production of active biomolecule such as bioemulsifier produced from the metabolization of renewable industrial waste, suggesting potential use in cosmetics industries.

Keywords: Fungus, Biomolecule, Whey, Corn steep liquor, Surface tension, Emulsification index.

1 INTRODUCTION

Bioemulsifiers are amphiphilic molecules of high molecular weight produced microorganisms such as fungi and bacteria. Structurally, these biomolecules have a hydrophilic and hydrophobic end that has the ability to stabilize oil-in-water emulsions (CHOTARD, M. et al., 2022). The main advantages of this biomolecule are the low toxicity and efficiency in extreme environments of temperature, pH and salinity (BARBOSA, F. G. et al., 2022).

The most commercialized and available in the market, among the bioemulsifiers is the emulsan patented by Gutnick et al. (1980). This is a protein-associated extracellular lipopolysaccharide and was produced by the bacterium Acinetobacter calcoaceticus RAG-1 ATCC 31012, with high molecular weight (around 1000 kDa). Among its main characteristics is the inefficiency in reducing interfacial tension and protecting oil droplets from coalescence (ROSENBERG et al., 1979; FANWA, M. N. et al., 2023; GUILLÉN-NAVARRO, K. et al., 2023).

The main properties of bioemulsifiers are as foaming agents and stabilizers and have potential use in the food industry, cosmetics, among others (BIRDILLA et al., 2018; THRAEIB, J. et al., 2022; ESSGHAIER, B. et al., 2023).

The main difficulty of scaling up bioemulsifiers occurs in terms of productivity. However, some microorganisms have the potential to metabolize industrial waste used as the main raw material (corn steep liquor as a source of nitrogen and whey as a carbon source, among others). This is a sustainable and less toxic alternative to the environment and health (ATAKPA, E. O et al., 2022; KUMAR, P. S. et al., 2022)

Bioemulsifiers are less effective in reducing surface tension since they do not present significant changes in surface tension. Jagtap et al., (2010) indicate the methods of emulsification index (E24) and/or emulsification activity to determine the emulsification capacity of any active molecule with various hydrophobic substrates. These properties make it of great importance in the pharmaceutical and cosmetic industries (TAVARES, J. et al., 2021).

Penicillium citrinum is a fungus that has potential of active biomolecules production, such as bioemulsifiers. This fungus can be found in various habitats, ranging from soil (VISAGIE et al., 2014). It is a ubiquitous genus with worldwide distribution and can be found in rotting organic matter (ABDEL-AZEEM, A. M. et al., 2021).

Morphologically the conidia of Penicillium sp. form a brush-shaped structure and their cells are found at the apex of the conidiophore called the metula (ABDEL-AZEEM, A. M. et al., 2021; COSTA, E. R. C. et al., 2022).

Therefore, the objective of this work was investigate the potential of Penicillium citrinum in the production of active biomolecule as the bioemulsifier from renewable industrial waste (whey and corn steep liquor) and the evaluation of microstructural characteristics of the droplets of emulsions formed.

2 METHODOLOGY

2.1 MICROORGANISM

The fungus Penicillium citrinum isolated from mangrove sediments of the State of Pernambuco (contaminated with oil) was used in this study. This fungus is kept in the Culture Bank of the Center for Research in Environmental Sciences and Biotechnology (NPCIAMB) of the Catholic University of Pernambuco, registered in the World Federation Culture Collection-WFCC.

2.2 INDUSTRIAL WASTE

The agro-industrial residues used for the growth of the microorganism and production of the bioemulsifier were corn steep liquor from the Corn Products industry (Cabo de Santo Agostinho – PE, Brazil) and whey obtained from local processing industry.

2.3 GROWING CONDITIONS

The cultivation of Penicillium citrinum was carried out in Sabouraud medium containing peptone 10g/L, glucose 40g/L and agar 15g/L. Then, Petri dishes containing the microorganism were packed in an oven at 28ºC until the mycelial mat was obtained. After obtaining the young culture, discs were used as inoculum in the bioemulsifier production medium.

2.4 PRODUCTION OF THE BIOEMULSIFIER

To produce of the bioemulsifier by Penicillium citrinum, 20 discs of 8mm were used as inoculum in the production medium in Erlenmayer flasks of 250mL containing different concentrations of agro- industrial residues (corn steep liquor and whey) and distilled water.

2.5 FACTORIAL PLANNING

The factorial design (Table 1) was performed to evaluate the conditions of the variables of corn steep liquor and whey in the production of the bioemulsifier. Statistical analysis of planning data was performed using the software package STATISTICA version 8.0 (StatSoft Inc., Tulsa, OK, USA).

Table 1: Levels of factorial design 22 to produce bioemulsifier by Penicillium citrinum in medium containing industrial residues

FACTORS	LEVELS		
	-1	**0**	**+1**
Corn Steep Liquor (%)	3	4	5
Whey (%)	3	4	5

2.6 DETERMINATION OF SURFACE TENSION

Surface tension was measured in the spore-free metabolic fluid in a KSV Sigma 70 tensiometer (Finland) using the NUOY ring by immersing the platinum ring in the metabolic fluid and recording the force required to pull it through the air-liquid and liquid/liquid interface. The values obtained were expressed in mN/m (KUYUKINA et al., 2005).

2.7 DETERMINATION OF EMULSIFICATION INDEX

The emulsification index was performed with the metabolic liquid using different types of hydrophobic substrates (engine oil, burnt motor oil, soybean oil, corn oil, canola oil and post-frying soybean oil). The samples were transferred to graduated tubes in a ratio of 1:1 (v/v), agitated in the vortex for 2 minutes, following the methodology of Cooper Goldenberg (1987). After 24 hours, the emulsion was measured and the results were applied the following formula:

(Eq.1)

$$El_{24}\ (\%) = \text{emulsion height} / \text{total height} \times 100.$$

2.8 EMULSION CHARACTERIZATION

The characterization of the emulsion formed by the bioemulsifier of Penicillium citrinum, produced in the best condition of the factorial design, was evaluated as the size of the bubbles, the type of emulsion and characteristics of polydispersion, flocculation and coalescence seen by light field microscopy in the objective with an increase of 100x according to Souza et al., (2016). Therefore, for the formation of the emulsion, the metabolic fluid and the burnt engine oil were added in the ratio of 1:1 (v/v), homogenized in vortex for 2 minutes. Then, the aliquot of the emulsion was removed and transferred to a lamina covered with coverslips. The visualization of the bubbles was recorded on a digital camera.

2.9 BIOEMULSIFIER PRODUCTION BY Penicillium citrinum UCP 1183 EVALUATED BY SURFACE TENSION AND EMULSIFICATION INDEX

Penicillium citrinum, proved to be a microorganism with promising potential for bioemulsifier production in condition 4 of the planning consisting of industrial waste (corn steep liquor 5% and whey 5%).

In this selected condition, the surface tension (47.5 mN/m) and the emulsification index (95.8%) were high confirming the production of the bioemulsifier molecule. In the other assays, the production of bioemulsifier also occurred, proven by the high values of surface tension and emulsification index according to (Table 2).

In studies conducted by Bhaumik et al., (2020) obtained bioemulsifier production resulting in surface tension of 54% mN/m and emulsification index of 58%. Thus, these data show that the high surface tension and high percentage of emulsification indicate that the microorganism is a producer of bioemulsifier. Also in this context, Cicatello, et al (2019) state that Penicillium chrisogenum is a promising bioemulsifier-producing microorganism (Surface tension 55 mN/m and emuslification index of 70%), as well as Phoma dimorpha (surface tension of 61.50 mN/m and emulsification index 83.3%) Luft, L. et al., (2021).

Table 2: Factorial planning for bioemulsifier production by Penicillium citrinum UCP 1183 evaluated from surface tension and emulsification index

Tests	**Corn steep liquor (%)**	**Whey (%)**	**Surface tension (mN/m)**	**Emulsification index EI_{24} (%)**
1	-1 (3)	-1 (3)	52.0	73.9
2	+1 (5)	-1 (3)	47.0	85.7
3	-1 (3)	+1 (5)	48.2	76.0
4	+1 (5)	+1 (5)	47.5	95.8
5	0 (4)	0 (4)	42.5	69.2
6	0 (4)	0 (4)	42.0	69.0
7	0 (4)	0 (4)	52.5	69.7
8	0 (4)	0 (4)	53.5	69.4

3 INFLUENCE OF WHEY AND CORN STEEP LIQUOR IN BIOEMULSIFIER PRODUCTION BY Penicillium citrinum UCP 1183

Whey contains high nutritional content because it is rich in carbohydrates and can be used as an excellent source of carbon for microorganisms (GOMES et al., 2021). On the other hand, corn steep liquor is a source of nitrogen rich in proteins, vitamins and amino acids that makes it a great nutritional component (GUDIÑA et al., 2023). In this context, in the present study it was possible to prove that Penicillium citrinum used whey as the main source of carbon and corn steep liquor as the main source of nitrogen capable of reducing surface tension to 47.5 mN/m and obtaining the emulsification index to 95.8% (Table 3).

In a comparative analysis, Table 3 shows a comparison of the results obtained in this study with the literature data related to surface tension and emulsification index using different carbon sources to produce bioemulsifiers using filamentous fungi.

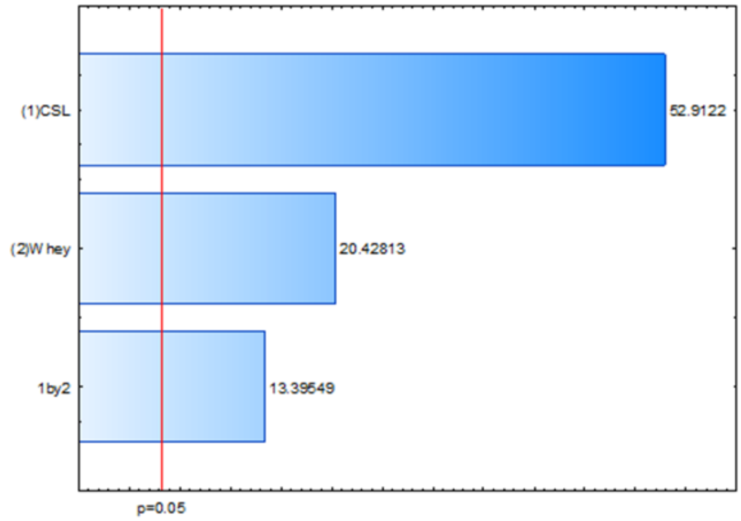
Table 3: Comparative analysis of the data obtained in this study with the literature data for the production of bioemulsifiers using different carbon sources

Microorganism	**Carbon sources**	**Surface tension (mN/m)**	**Emulsification index EI 24 (%)**	**References**
Penicillium citrinum	Whey	47.5	95.8	This study
Phoma dimorpha	Rice bran	61.5	83.3	Luciana et al (2021)
Penicillium crustosum	Orange skin	67.0	17.0	Landa et al (2020)
Mucor hiemalis	Crude Petroleum	40.0	96.0	Ferreira etal. (2020)
Aspergillus flavus	Crude Petroleum	39.8	51.5	Al-Zabam etal. (2020)
Aspergillus terreus	Crude Petroleum	56.3	83.0	Pitocchi et al. (2020)
Chaetomum madrascence	post frying soy oil	36.0	67.5	Aboelmagd, (2019)
Penicillium chrysogenum	Crude Petroleum	55.0	70.0	Cicatello et al.,(2019)

3.1 PARETO DIAGRAM FOR ANALYSIS OF THE INFLUENCE OF WHEY AND CORN STEEP LIQUOR IN BIOEMULSIFIER PRODUCTION BY Penicillium citrinum UCP 1183

The Pareto diagram was used to analyze the influence of whey and corn steep liquor concentrations on the production of bioemulsifier by Penicillium citrinum UCP 1183. According to Figure 1, whey (1), corn steep liquor (2) and the association between the variables whey and corn steep liquor (1by2) showed a statistically significant effect on the increase in emulsifying capacity. However, the independent variable that most influenced the increase in the emulsification index was the corn steep liquor (1). The estimated significant effects are evidenced by the dashed vertical line p.

Figure 1: Pareto diagram for evaluation of the independent variables whey and corn steep liquor in the production of bioemulsifier by Penicillium citrinum



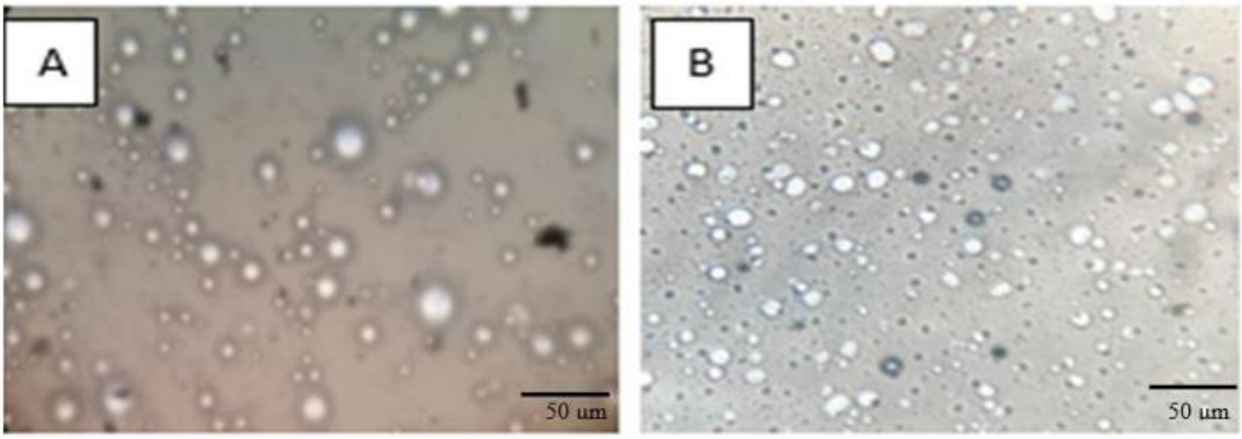
3.2 CHARACTERIZATION OF THE DROPLETS OF THE EMULSION OF THE BIOEMULSIFIER OF *Penicillium citrinum* ANALYZED BY LIGHT MICROSCOPY

The emulsions analyzed under optical microscope were of the bioemulsifier present in the metabolic fluid, obtained from the best planning condition (condition 4) emulsified with burnt engine oil.

The droplets observed (Figure 2A) show a globulous aspect with variable sizes from 2 to 10 μm. Few fields with smaller droplets were observed in flocculation, which suggests that the formation of larger droplets was a result of coalescence of the smaller droplets. Figure 2B shows the droplets of the emulsion formed with Triton X (non-ionic surfactant) with droplets of 2 to 3 μm. However, the presence of several fields with flocculation and polydispersity indicates that it can destabilize the emulsion with coalescence. Both emulsions formed were of the water-in-oil type.

An oil-in-water emulsion is a mixture in which an oily phase is dispersed in water or another aqueous liquid. In the study by Maia et al., (2018) the emulsion formed in engine oil burned with the bioemulsifier produced by B. subtilis reached sizes ranging from 0.5 to 20 μm with an emulsification index of 95%. Souza et al., (2016) when analyzing the droplets formed from the emulsion with motor oil and the synthetic surfactant sodium dodecyl sulfate (SDS), verified average sizes ranging from 2.5 to 12 μm and an emulsification index of 100%. In both situations stability was achieved due to monodispersion of the droplets.

Figure 2: Microscopic analysis of the droplets formed by the bioemulsifier produced by Penicillium citrinum UCP 1183: (A) droplets of non-ionic surfactant (Triton-X) and (B) droplets of the bioemulsifier of P. citrinum in burnt motor oil



4 CONCLUSION

The present study shows that Penicillium citrinum has high biotechnological potential in the production of active biomolecules such as bioemulsifier, produced from the metabolization of renewable industrial waste (whey and corn steep liquor), a fact that contributes to sustainable development. In addition, it is suggested that the bioemulsifier of P. citrinum has potential use in the cosmetics industry for its proven action as foaming agent.

ACKNOWLEDGMENTS

FACEPE (Foundation for the Support of Science and Technology of Pernambuco), CNPq (National Council for Scientific and Technological Development) and UNICAP (Catholic University of Pernambuco) for the availability of the laboratories of NPCIAMB-UNICAP.

REFERENCES

ABOELMAGD, H. I. Potential biosurfactant production by a promising Chaetomium madrascence AUMC 9376 isolated from Egyptian soil. The egyptian journal of experimental biology (Botany), v. 15, n. 1, p. 111-118, 2019.

AL-ZABAN, M. I. et al. Bioremediation of crude oil by Rhizosphere fungal isolates in the presence of silver nanoparticles. International journal of environmental research and public health, v. 17, n. 18, p. 6564, 2020.

ABDEL-AZEEM, A. M. et al. Industrially important fungi for sustainable development. Springer International Publishing, 2021.

ATAKPA, Edidiong Okokon et al. Improved degradation of petroleum hydrocarbons by co-culture of fungi and biosurfactant-producing bacteria. Chemosphere, v. 290, p. 133337, 2022.

BIRDILLA S. D; Mariathason et al. Haererehalobacter sp. JS1, a bioemulsifier producing halophilic bacterium isolated from Indian solar salt works. Journal of basic microbiology, v. 58, n. 7, p. 597-608, 2018.

BHAUMIK, M. et al. Production, partial purification and characterization of a proteoglycan bioemulsifier from an oleaginous yeast. Bioprocess & Biosystems Engineering, v. 43, n. 10, 2020.

BARBOSA, F. G. et al. Production, Physicochemical and Structural Characterization of a Bioemulsifier Produced in a Culture Medium Composed of Sugarcane Bagasse Hemicellulosic Hydrolysate and Soybean Oil in the Context of Biorefineries. Fermentation, v. 8, n. 11, p. 618, 2022.

COOPER, D. G; GOLDENBERG, B. G. Surface-active agents from two Bacillus species. Applied and environmental microbiology, v. 53, n. 2, p. 224-229, 1987.

CICATIELLO, P; STANZIONE, I. Characterization of a surface-active protein extracted from a marine strain of Penicillium chrysogenum. International journal of molecular sciences, v. 20, n. 13, p. 3242, 2019.

CHOTARD, M. et al. Biosurfactant-producing mucor strains: selection, screening, and chemical characterization. Applied Microbiology, v. 2, n. 1, p. 248-259, 2022.

COSTA, E. R. C. et al. Biodegradação de óleo diesel por Penicillium citrinum UCP 1183 isolado de sedimentos de mangue. Research, Society and Development, v. 11, n. 10, p. e573111033071-e573111033071, 2022.

ESSGHAIER, B. et al. Production and Characterization of New Biosurfactants/Bioemulsifiers from Pantoea alhagi and Their Antioxidant, Antimicrobial and Anti-Biofilm Potentiality Evaluations. Molecules, v. 28, n. 4, p. 1912, 2023.

FERREIRA, et al. Biosurfactant and bioemulsifier as promising molecules produced by Mucor hiemalis isolated from Caatinga soil. Electronic Journal of Biotechnology, v. 47, p. 51-58, 2020.

GOMES, J. V. P. et al. Comparison of bioactive compounds and nutrient contents in whey protein concentrate admixture of turmeric extract produced by spray drying and foam mat drying. Food Chem., 345, 2021.

GUDIÑA, E. J. et al. Sustainable exopolysaccharide production by Rhizobium viscosum CECT908 using corn steep liquor and sugarcane molasses as sole substrates. Polymers, v. 15, n. 1, p. 20, 2023.

FANWA, M. N. et al. Triumfetta cordifolia Gum as a Promising Bio-Ingredient to Stabilize Emulsions with Potentials in Cosmetics. Polymers, v. 15, n. 13, p. 2828, 2023.

GUILLÉN-NAVARRO, K. et al. Atividade antifúngica de amplo espectro, biossurfactante e bioemulsionante de Bacillus subtilis subsp. spizizenii—Um potencial agente de biocontrole e biorremediação na agricultura. Plantas, v. 12, n. 6, pág. 1374, 2023.

KUYUKINA, M. S. et al. Effect of biosurfactants on crude oil desorption and mobilization in a soil system. Environment international, v. 31, n. 2, p. 155-161, 2005.

KUMAR, P. S. et al. Sustainable production of biosurfactants via valorisation of industrial wastes as alternate feedstocks. Chemosphere, p. 137326, 2022.

LUFT, L. et al. Production of bioemulsifying compounds from Phoma dimorpha using agroindustrial residues as additional carbon sources. Biocatalysis and Agricultural Biotechnology, v. 35, p. 102079, 2021.

LIU, et al. Production, characterization and application of biosurfactant produced by Bacillus licheniformis L20 for microbial enhanced oil recovery. Journal of Cleaner Production, v. 307, p. 127193, 2021.

MAIA, et al. An efficient bioemulsifier-producing Bacillus subtilis UCP 0146 isolated from mangrove sediments. Colloids and Interfaces, v. 2, n. 4, p. 58, 2018.

PITOCCHI, et al. Cerato-platanins from marine fungi as effective protein biosurfactants and bioemulsifiers. International journal of molecular sciences, v. 21, n. 8, p. 2913, 2020.

ROSENBERG, E. et al. Emulsificante de Arthrobacter RAG-1: isolamento e propriedades emulsificantes. Microbiologia Aplicada e Ambiental , v. 37, n. 3, pág. 402-408, 1979.

SOUZA, et al. Waste soybean oil and corn steep liquor as economic substrates for bioemulsifier and biodiesel production by Candida lipolytica UCP 0998. International journal of molecular sciences, v. 17, n. 10, p. 1608, 2016

TAVARES, et al. Design and validation of an expeditious analytical method to quantify the emulsifying activity during biosurfactants/bioemulsifiers production. Colloids and Surfaces B: Biointerfaces, v. 208, p. 112111, 2021.

THRAEIB, Jaffar Z. et al. Production and characterization of a bioemulsifier derived from microorganisms with potential application in the food industry. Life, v. 12, n. 6, p. 924, 2022.

VISAGIE, et al. Identification and nomenclature of the genus Penicillium. Studies in mycology, v. 53, n. 1, p. 53-62, 2005.