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THE USE OF NEW LOW-COST SUBSTRATES FOR BIOSURFACTANT PRODUCTION

Rym Salah-Tazdaït[1*], Djaber Tazdaït[1,2], Djura Delhoum[1], Samia Mouffok[1], Fatma Kabouche[1], Imene Keddou[2], Nadia Abdi[2], Hocine Grib[2], Nabil Mameri[2]

1 Department of Biochemistry and Microbiology, Faculty of Biological and Agronomical Sciences, Mouloud Mammeri University of Tizi-Ouzou, P.O. Box 17 RP 15000 Hasnaoua, Tizi-Ouzou, Algeria.
2 Laboratory of Bioengineering and Process Engineering, National Polytechnic School, Avenue Hacen Badi, El-Harrach, Algiers, Algeria.
**Corresponding author. Emails: rym.tazdait@ummto.dz;rymsalah4@gmail.com*

Introduction and study objectives

Proper treatment and disposal of petroleum hydrocarbons, which are the common groundwater contaminants is required to protect both environment and human health. These goals can be met by the implementation of bioremediation, which involves the integration of environmental microbiology with engineering techniques. Microorganisms use different strategies for increasing the solubility of hydrocarbons in water, including the direct interfacial accession, which involves adhesion of microorganisms to hydrocarbon droplets and production of extracellular biosurfactants leading to the enhancement of biodegradation efficiency. The term "biosurfactants" refers to a vast structural diversity of surface-active compounds produced by many microorganisms that can reduce surface tension at the air-water interface.

In literature, several carbon sources were used for biosurfactant production. Most of these studies are focused on the use of conventional carbon sources such as glucose, fructose, pyruvate, citrate, etc., but there are very few reports on biosurfactants production using inexpensive raw materials as substrates (complex carbon or nitrogen sources) which can considerably reduce production costs in most biotechnological processes.

As mentioned above, it is well established that the cost of biosurfactant production can significantly be reduced by substituting conventional carbon substrate (glucose, fructose, citric acid, etc.) with low-cost waste substrates. To the best of our knowledge, no data are available on the use of animal by-products as a sole source of nutrient for biosurfactant production. In this study, biosurfactant production by a pure bacterial culture was studied using an agro-industrial waste: prickly

pear fruits of (*Opuntia ficus-indica*) peels, and two animal by-products: sardine (*Sardina pilchardus*) heads and chicken (*Gallus gallus domesticus*) feet, since they are discarded as a waste.

Methodology

The fuel-contaminated soil samples used for isolation of microbial strains were collected in five different locations at a gas station located in Boumerdès, Algeria. The mineral salt medium (MSM) used for isolation of biosurfactant-producing bacteria has the following composition: 1.6 g of K_2HPO_4, 0.4 g of KH_2PO_4, 0.2 g of $MgSO_4 \cdot 7\ H_2O$, 15 g of NaCl, 3 g of NH_4NO_3, 0.02 g of $CaCl_2$, 0.01 g of $ZnSO_4$, 0.05 g of $FeSO_4 \cdot 7\ H_2O$, 0.008 g of $MnSO_4 \cdot H_2O$, 0.004 g of $CuSO_4 \cdot 5\ H_2O$, 0.0026 g of $Co(NO_3)$, 1 L of distilled water. The medium was amended with 0.3% (w/v) glucose and autoclaved at 120°C for 20 min.

The bacterial strains capable of producing biosurfactants was isolated by selective enrichment culture technique, which promotes the growth of microorganisms containing in the soil sample by providing them the essential nutrients.

The bacterial strains isolated in this study were characterized and identified using morphological test, and biochemical tests: Gram staining, API 10 S test kit (API system, BioMérieux, Marcy, France), and UriSelectTM 4 (Bio-Rad laboratories, CA, USA).

To demonstrate the ability of strains to produce biosurfactants, different tests were carried out : test of the emulsification index E_{24}, test of the blood agar, test of the drop collapse and test of spreading of the hydrophobic phase

Prickly pear peels were obtained from a local farm in Béjaïa (North of Algeria). Sardine heads were collected from restaurants located in Algiers, and chicken feet were collected from a slaughterhouse, which is also located in Algiers. These three organic by-products were first washed several times, and then ground, immersed in heated distilled water (75-80°C) for 45 min (1 Kg/4 L distilled water) under continuous agitation, before being filtered through gauze tissue.

The subcultures were conducted in 100 mL Erlenmeyer flasks containing 50 mL of each of the three media, inoculated with 1 mL of the cell suspension corresponding to an inoculum of 1.5×10^7 colony-forming units (CFU)/mL, and incubated at room temperature (22.7°C, max. deviation ± 1°C) for 48h at 50 rpm and pH 7.0. The cultures were conducted at room temperature at 150 rpm in 500 mL

Erlenmeyer flasks containing 200 mL of fresh medium, the pH was adjusted to 7.0. Each flask was subsequently inoculated with 50 mL subculture of each of the three media. Samples were removed at regular time intervals (8h) during 5 days and were checked for Optical Density (OD) at 600 nm and E_{24} (after centrifugation at 5000 rpm for 30 min to remove cells). The biosurfactant concentrations (g/L) in each culture medium were also determined.

The chemical characterization of the biosurfactant was performed using FTIR technique.

Results and conclusions

In this study, five biosurfactant-producing *Pseudomonas aeruginosa* strains (S1-S5) were isolated. *Pseudomonas aeruginosa* S1 was selected as the best biosurfactant producer. The results showed that prickly pear peels medium yielded the highest biosurfactant production (13.30 g/L), and gave the highest E_{24} (64%) and cleaning activity (80%) values, while chicken feet gave the highest foaming activity (59%). However, extensive research is needed to establish the suitability of these two low-cost substrates in industrial-level biosurfactant production process. The FTIR analysis suggested that the obtained biosurfactant is rhamnolipidic in nature, but further investigation is required to confirm this chemical identity.