Abstract

The use of biosurfactant is a promising alternative over the chemical surfactant as they are better biodegradable and do not pollute the environment. To date, there is little information on the biosurfactant producing microorganisms belonging to different Rhizobium and Bradyrhizobium species. In this study, the emulsifying properties of both culture media and a substantially cell-free medium from bacterial cell cultures in which B. elkanii SEMIA 587 or mutant strains cultivated in defned media supplemented with sucrose were determined by measuring the emulsifying activity, which determined the ability of biosurfactant in forming oil-water emulsion, and emulsifying index (E_{24}), which determined the capacity of surfactant in forming emulsions on different hydrophobic substrates (soybean oil, sunfower oil or diesel oil). The performance of strains as bio emulsifer were very contrasting as shown by their specifc emulsifcation indexes with different substrates, beginning at 24 hours. Regarding emulsifying index for cells, best result of E_{24} was obtained by using sunfower oil for wild type and mutants with exception for 587::TnphoA31 that witch got better results in soybean oil. However, analysing using cell-free medium have shown higher E_{24} values mostly in soybean oil except for 587::TnphoA31 and 587::TnphoA50 which have performed better in diesel oil. This fnding indicates a probable production of bioemulsifcants that adhere to the cell wall of this bacterium and are extracellular. The same E_{24} value (79.17%) was observed in the case of soybean oil with culture medium from both the wild-type and the mutant strain 587::TnphoA50. The emulsifcation capacity was very sensitive to the temperature, pH and NaCl concentration changes. These results demonstrate that the Bradyrhizobium strains could be attractive for use in biosurfactants

Keywords: Bioemulsi er; Bradyrhizobium elkanii; Emulsifying activity; Hydrophobic substrate

Abbreviations: Exopolysaccharide; E24: Emulsi cation Index; TY: Tryptone Yeast; YMA: Yeast Mannitol Agar; RDM: De ned Medium for Rhizobia

Introduction

Biosurfactants were rst discovered as extracellular amphiphilic compounds of fermentation bacteria [1]. Initial interest in these compounds was due to their ability to increase the solubility of insoluble or poorly soluble hydrocarbons. However, the increased popularity of using renewable resources in industry (especially in food and pharmaceutical industries) has led to increased interest in identifying and applying the use of natural surfactants, mainly biosurfactants [2].

During the last decade, biosurfactants have been investigated as potential replacements for synthetic surfactants and are expected to have many potential industrial and environmental applications related to emulsi cation, foaming, detergency, wetting, dispersion and solubilization of hydrophobic compounds [3,4]. Among the various potential replacements for synthetic surfactants, biosurfactants, bioemulsi ers and exopolysaccharides are attracting interest and attention due to their structural and functional diversity [5]. Some investigations showed that surface activity of biosurfactants is comparable with surface activity of synthetic surfactants. Due to their physicochemical properties, low toxicity, excellent surface activity, high speci city, e ectiveness under extreme conditions and biodegradability, bioemulsi ers and biosurfactants are widely applied in environmental protection techniques, e.g., water and soil remediation, oil spill removal, etc. [6,7]. ese properties of bioemulsi ers and biosurfactants re ect their potential for commercial applications [8].

Bioemulsi ers are microbiological in origin and appear to be made either by the cells themselves or by the production of extracellular emulsifying agents [9]. e outer structure of many bacteria is surrounded by extracellular polysaccharides (EPSs) that occur either as discrete capsules or a thick slime layer. A wide variety of microorganisms present this structure, such as Aeribacillus pallidus [10] species of Candida [11], Pseudomonas and Bacillus [12].

Microorganisms producing biosurfactants can be e ectively used for the removal of hydrocarbons as well as heavy metals [13]. As biosurfactants are known to enhance bioavailability and carry out biodegradation of hydrophobic compounds, di erent technologies, such as soil washing technology and clean up combined technology, employ biosurfactants for the e ective removal of hydrocarbons and metals, respectively [14,15]. ese biosurfactants can be widely exploited in agricultural areas for the enhancement of biodegradation of pollutants to improve the quality of agriculture soil, for indirect plant growth promotion through antimicrobial activity and to increase the plant-microbe interaction that is bene cial for plants [16].

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587::TnphoA50) strains displayed lower emulsi cation index values for soybean oil, sun ower oil and diesel oil, except the 587::TnphoA50 strain with soybean oil (47.83%) (Table 1). Both the cell-free supernatant of wild-type and mutant strains showed low levels for diesel. Diesel oil was not emulsi ed e ectively.

Based on Table 1, the E_{24} of cell-free medium from a bacterial cell culture in which mutant strains 587::TnphoA24

agents, in addition to their use in sustainable economic processes. e ndings of this study contribute signi cantly toward understanding applications of the use of the Bradyrhizobium spp. in environmental biotechnology and bioremediation.

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