

TITLE: CHARACTERIZATION OF A BIOSURFACTANT PRODUCED BY A THERMOHALOPHILIC *BACILLUS* STRAIN ISOLATED FROM AN OIL RESERVOIR ROCK SAMPLE.

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Abstract:

Biosurfactants (BS) are surface-active microbial compounds potentially applicable in several industrial activities, such as microbial enhanced oil recovery processes (MEOR), in the oil industry. The BS efficiency in MEOR is presumably dependent on its stability in reservoir conditions of temperature and salinity. Previous studies with the strain Ar70C7-2, a thermophilic and halophilic *Bacillus* isolated from an offshore reservoir rock sample, have shown its biosurfactant to be stable throughout wide ranges of pH, temperature and salinity. Considering that, a new study was performed to characterize the Ar70C7-2 biosurfactant. BS was produced from cultivation in Mineral Medium using glycerol and ammonium nitrate as sources of carbon and nitrogen, at rate 2/1, with 70g/L of NaCl and incubated at 55°C in static culture. Approximately 2.7 g of crude BS were obtained by acid precipitation (6M HCl) from 10 liters of culture. The crude BS was further subjected to extraction using chloroform followed by purification by column adsorption chromatography coupled to the Äkta purifier (GE Healthcare) system, yielding 19 mg of purified BS. Thus, total protein content in the purified BS was quantified by the colorimetric method with bicinchoninic acid using the commercial Pierce® BCA Protein Assay Kit (Thermo Fisher Scientific). Mass spectrometry was performed from the BS purified, using microTOF-QII mass spectrometer (Bruker Daltonics) with addition of formic acid to the sample. The fatty acids analysis was made from the crude extract, by gas chromatography-mass spectrometry (GC-2010 Shimadzu). The results indicated the presence of 17% of protein, and fatty acid chains varying from 13 to 18 carbon atoms, with the C16 homolog being the most abundant. Preliminary structural analysis indicates that the biosurfactant produced by Ar70C7-2 belongs to the class of lipopeptides. Further studies comprising the analysis of amino acid composition will allow establishing a relationship between the chemical structure and the high stability presented by the BS.

Keywords: *Bacillus*, Biosurfactant, Oil, Thermophile, Lipopeptide.

Financial support: Brazilian Federal Agency for the Support and Evaluation of Graduate Education (CAPES); Brazilian National Agency of Petroleum, Natural Gas and Biofuels (ANP); PETROBRAS S/A.