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## ACCEPTED MANUSCRIPT

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## Microbial assisted (*pseudomonas sp.*) production of novel bio-surfactant rhamnolipids and its characterisation by different spectral studies

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## Abstract

The production and characterisation of rhamnolipids bio-surfactant obtained by the strain P19 of *pseudomonas sp.* was investigated. With regard to the carbon and nitrogen sources, several media were investigated to enhance the production of the bio-surfactant. Primarily the bacteria was isolated from the soil by using mineral salt (MS) broth[Peptone – 0.5g, KH<sub>2</sub>PO<sub>4</sub> – 0.7g, NaH<sub>2</sub>PO<sub>4</sub> – 0.9g, NaNO<sub>3</sub> – 2g, MgSO<sub>4</sub>.7H<sub>2</sub>O – 0.4g, CaCl<sub>2</sub>. 2H<sub>2</sub>O – 0.1g ; trace elements: 2 ml solution of a mixture containing FeSO<sub>4</sub>.7H<sub>2</sub>O – 2g, MnSO<sub>4</sub> – 1.5g, (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>. 4H<sub>2</sub>O – 0.6g in 1 Litre of distilled water; along with 2g/L D-glucose as carbohydrate source. The production of rhamnolipids by the bacteria was confirmed by C-TAB- methylene blue agar plate assay. The selected strain was identified on the basis of Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) of the ribosomal protein. The isolated bio-surfactant was characterised by HRMS and NMR spectroscopic studies. The CMC of purified rhamnolipids was calculated using the UV-Vis method and found to be 34.2mgL<sup>-1</sup>. The optical micrograph and HR-TEM images invoked the formation of miceller nano-aggregates of the bio-surfactant in aqueous solution.

Keywords Bio-surfactant; Rhamnolipids; Production; characterisation; CMC.

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