Characterization of Novel Biosurfactants Produced by the Strain *Fusarium oxysporum*

Rema Santhappan[1*] **and M Rajasekara Pandian**[2]

[1]*Department of Biochemistry, MGR College, Hosur, Tamil Nadu, India*

[2]*Department of Zoology, Arignar Anna Government Arts College, Namakkal, Tamil Nadu, India*

[*]**Corresponding author**: Rema Santhappan, Department of Biochemistry, MGR College, Hosur, Tamil Nadu, India, Tel: 04344-261020; E-mail: remasenthil@gmail.com

Rec date: September 26, 2017; **Acc date:** October 16, 2017; **Pub date:** October 18, 2017

Abstract

A biosurfactant-producing strain, *Fusarium oxysporum*, was isolated from crude oil polluted soil sample collected from automobile service station of Hosur, India. The biosurfactant was extracted from the culture sample and the purified extract weighed 1.02 gm. The extract when analyzed with TLC produced brown spots with iodine vapors indicating that the surfactant is of lipid composition. The FTIR spectra recorded for the compounds produced by fungal species *Fusarium* indicated the presence of functional groups –COO- and CH_2 groups. The NMR spectral studies of the compounds produced by Fusarium were identified to be two major esters namely ester of 25-methyl-heptaF

Crude oil; Fusarium; Biodegradation; Biosurfactants; NMR; GC-MS

Abbreviations: NMR: Nuclear Magnetic Resonance Spectroscopy; GC-MS: Gas Chromtograpy-Mass Spectroscopy; FTIR: Fourier Transform Infrared Spectroscopy

Introduction

Biosurfactants are molecules that are capable of interacting at interfaces and reduce surface and interfacial tension [1]. At present, surfactants are derived from petroleum. To reduce production costs, the choice of inexpensive raw materials is important. The production of biosurfactant has been reported in different microorganisms like Bacillus, [2] Pseudomonas [3] Acientobacter and Arthrobacter species [1,4]. In recent years considerable attention has been given to isolate these biosurfactant-producing microorganisms because of their potential commercial utilization [5]. The present study focuses on the biosurfactant production by *Fusarium oxysporum*, on which studies are limited.

All surfactants have two distinct components in their molecular structure. A hydrocarbon part, which is poorly soluble in water, is called the hydrophobic or lipophilic group. Usually the hydrophobic moiety is C_8 to C_{22} alkyl chain or alkyl aryl group where the alkyl chain can be linear or branched. The other part of the molecule, which is water soluble, is known as the hydrophilic group. Biosurfactants

olubilization.

mical properties and molecular size uced by the microbes are complex

different structures. They include glycolipids, phospholipids, fatty acids, peptides and glycopeptides. The results of the spectroscopy analyses by IRTF and NMR has settled down the chemical structures of the biosurfactant from *P. aeruginosa* BIOMI E5 strain, which confirmed to be rhamnolipids. In the present investigation with *Fusarium oxysporum*, purification and structural studies involving HPLC, FTIRR, GC and NMR biosurfactants produced were confirmed to be as fatty acids.

Material and Methods

Preparation of culture extract

Three litters of Czepak's culture broth taken in a five liter conical flask was induced with 3 ml of crude oil and inoculated with the attenuated spore of *Fusarium oxysporum* strain and was allowed to grow for 9 days in an orbital shaker at 120 rpm at 30°C. The cells were harvested on 9th day. The culture was centrifuged at 10,000 g for 15 minutes at 4°C and the pellet were discarded. The supernatant was refrigerated overnight to remove the sediments and centrifuged at 10,000 rpm and was used for further processing.

Residual crude oil fraction was removed by slight cyclohexane treatment. The resulting supernatant was taken in a separating funnel and equal volume of chloroform: methanol was added and shaken vigorously. The methanol: chloroform layer was separated from

aqueous layer and collected in a small round bottom flask and the extract was air dried in an evaporatory rotor under vacuum at 30°C.

The dried extract was dissolved in Chloroform: methanol mixture

Figure 4: FTIR Spectra of partially puriÊed biosurfactant.

^{1}H-NMR analysis

HXe ^{1}H-NMR spectrum of the extract from *Fusarium oxysporum* is shown (Figures 5-7). In the downÊeld region at delta 4.83 ppm and delta 4.52 ppm, the methylene protons were observed with high intensity. HXe observed two signals for methylene protons were due to the attachment of methylene groups to more I ewect carbonyl group on one side and terminal methane group on other side of the fatty acid. HXe signal at delta 1.95 ppm was assigned to one methane proton and the moderate intensity signal at delta 3.30 ppm was used to reveal the presence of methyl protons in ester group. HXe low intensity signal at delta 2.63 ppm was taken to identify the terminal methyl protons in fatty acid. HXe very low intensity signal at delta 7.95 ppm may be due to the presence of aromatic impurity in tht x o 8r8u Ö ^ï D = igure 4

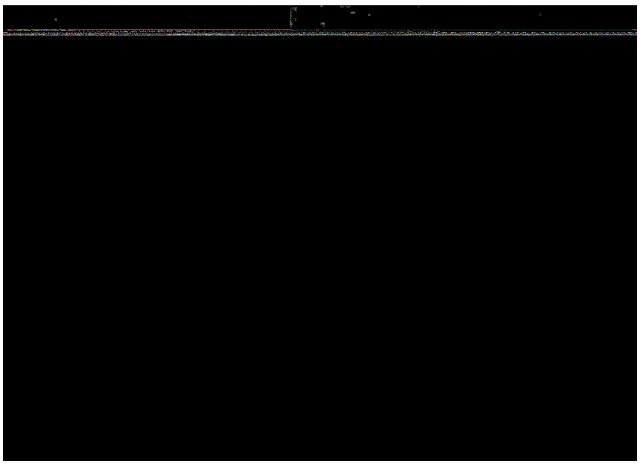

Figure 8: ^{13}C-NMR Spectra of the puriẐed biosurfactant.

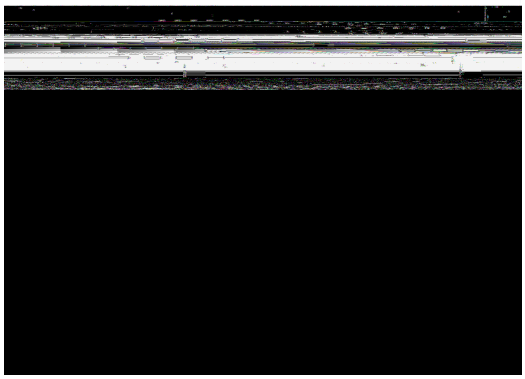

Figure 9: ^{13}C-NMR Spectra of the puriẐed biosurfactant.

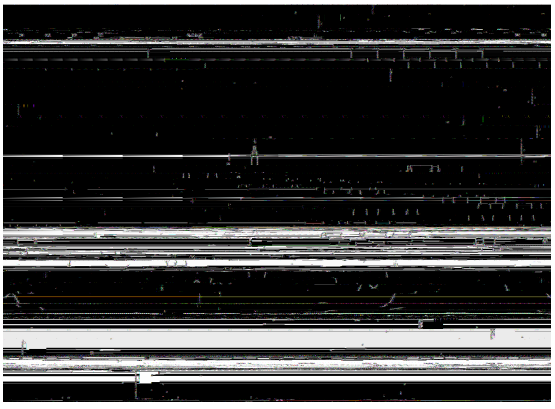

Figure 10: ^{13}C-NMR Spectra of the puriẐed biosurfactant.

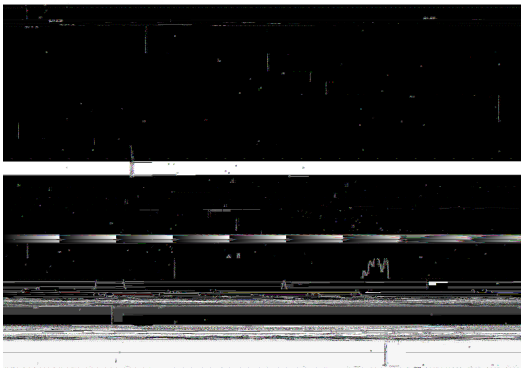

Figure 11: ^{13}C-NMR Spectra of the puriẐed biosurfactant.

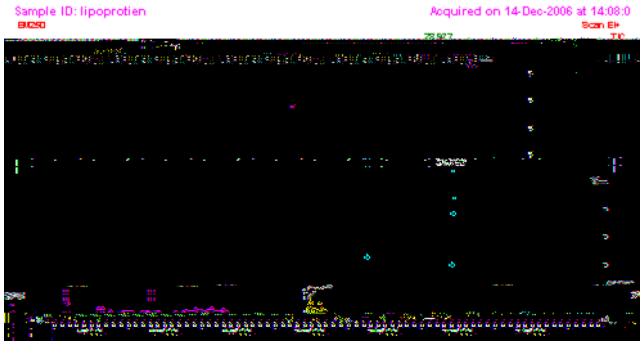

Figure 12:

5. Ellaiah P, Prabhakar T, Sreekanth M, HXkaer TA, Bhima RP, et al. (2002) Production of glycolipids containing biosurfactant by Pseudomonas species. Indian Journal of Experimental Biology 40: 1083-1086.
6. Van Ginkel CG (1996) Complete degradation of xenobiotic surfactants by consortia of aerobic microorganism. Biodegradation 7: 151-164.
7. Gerson DF (1993) HXe biophysics of microbial surfactants: growth on insoluble substrate. In: Surfactant Science Series, Biosurfactants: Production, Properties, applications. Marcel Dekker, New York, USA, pp: 269-286.
8. Han KS, Lim EJ, Lee SO, Lee JD, Lee TH (2000) PuriẐcation and characterization of biosurfactants from Nocardia sp. L-417. Biotechnol Appl Biochem 31: 249-153.
9. Jayaraman S (1981) Laboratory manual in Biochemistry. New Age International Publishers, New Delhi, India, pp: 171-172.
10. Manneerat S (2005) Biosurfactants from marine microorganism Song klanakarin. J Sci Technol 27: 1263-1272.
11. Rehn HJ, Reiw I (1981) Mechanism and occurrence of microbial oxidation of long chain alkanes. Adv Biochem Eng 19: 175-216.
12. Cooper DG, Zajic JE, Denis C (1981) Surface active properties of a biosurfactant from Corynebacterium lepus. J Am Oil Chem Soc 58: 77-80.
13. Macdonald CR, Cooper DG, Zajic JE (1981) Surface-active lipids from Nocardia erythropolis grown on hydrocarbons. Appl Environ Microbiol