Characterization of Biosurfactant Produced by Bacterial Isolates from Engine Oil Contaminated Soil

R. Thenmozhi, A. Sornalaksmi, D. Praveenkumar, A. Nagasathya

ABSTRACT

In the present study the biosurfactant produced by Pseudomonas aeruginosa PDKT-2 and Serratia marcescens PDKT-1, B. licheniformis PDKT-5 when grown on mineral salt medium with engine oil as sole carbon source was investigated. The produced biosurfactant was separated by acetone precipitation. Their amphipathic structures were established by biochemical and spectroscopy method and they were confirmed as rhamnolipid and lipopeptide structure respectively.

Key words: Biosurfactant, Bacterial isolates, Engine oil

Introduction

Surfactants are amphipathic molecules which reduce surface and interfacial tensions and widely used in pharmaceutical, cosmetic, food and petroleum industries. Biosurfactants are the structurally diverse group of surface-active molecules synthesized by microorganisms. The major classes of low mass surfactants include glycolipids, lipopeptides, lipoprotein and phospholipids, whereas high mass includes polymeric and particulate surfactants [34]. Most biosurfactants are either anionic or neutral and the hydrophobic moiety is based on long chain fatty acids or fatty acid derivatives whereas the hydrophilic portion can be a carbohydrate, aminoacid, lipid, phosphate or cyclic peptide. There are several advantages for biosurfactants in contrast with chemical surfactants, such as lower toxicity; higher biodegradability; better environmental compatibility; higher foaming; high selectivity and specific activity at extreme temperatures, pH, and the ability to be synthesized from renewable feed-stock [7,26]. In some instances, these compounds have antibiotic properties which may serve to disrupt membranes of microorganisms competing for food [33]. Some of their superior, such as absence of toxicity, biodegrade ability, and their specificity, make these microbial products both attractive for specific industries and environmentally acceptable. Most of the emphasis to date has been on the application of biosurfactants in petroleum-related activities and industries. They offer attractive products for use in enhanced oil recovery, in cleaning oil spills, in oil emulsification, and in breaking industrially derived oil-in-oil emulsions.

In this paper, the production of biosurfactants by Serratia marcescens PDKT-1, Pseudomonas aeruginosa PDKT-2, B. licheniformis PDKT-5, and some of its properties were determined by using physicochemical methods.
Materials and methods

Test organism:

The strains Serratia marcescens PDKT-1, Pseudomonas aeruginosa PDKT-2, B. licheniformis PDKT-5 which were isolated in our previous study [37] was used in this present work. The strains were streaked on the surface of nutrient agar plates. After incubation at 37°C, distinct colonies were isolated [19].

Media and Cultivation Condition:

The biosurfactant experiment were carried out into 250 ml of Erlenmeyer flask containing 100ml of sterilized mineral salt medium production with 1% engine oil and 1% over night culture was inoculated to the medium. The production work was carried out with a control and duplicate at 37°C for period of 7 days.

Biosurfactant Recovery:

The culture broth was centrifuged at 10000 rpm for 15 minutes, to remove the cells and thereafter sterilized with Millipore membrane filter. The clear sterile supernatant served as the source of the crude biosurfactant. The biosurfactant was recovered from the cell free culture supernatant by cold acetone precipitation. Three volumes of chilled acetone was added and allowed to stand for 10 h at 4°C. The precipitate was collected by centrifugation and evaporated to dryness to remove residual acetone after which it was re-dissolved in sterile water. [30].

Biosurfactant Characterization:

Biochemical Analysis:

Test for Sugar:

Benedict’s test:

To 8 drops of culture supernatant added with 5 ml of benedict’s reagent and heated in a boiling water bath for 15 mins. A reddish orange precipitate was formed in positive result.

Barfoed’s test:

To 5 ml of culture supernatant added with 5 ml of barfoed’s reagent and heated it in a boiling water bath for 3 mins. A reddish brown precipitate was formed in positive result.

Test for protein

Ninhydrin test

To 4 ml of culture supernatant was added, 4 ml of 1% freshly prepared ninhydrine solution and boiled for 10 minutes and cooled. A violet coloured solution was obtained in positive result.

Test for lipid

Solubility test

To the 2ml of culture supernatant was added to water, ethanol and chloroform. Insoluble in water, sparingly soluble in ethanol, extremely soluble in chloroform indicate positive results.

Fourier Transform Infrared Spectroscopy [FTIR] [36].

The biosurfactant produced by the bacterial isolates PDKT-1, PDKT-2, PDKT-5 were extracted from the 2 ml of supernatant fluid with 2ml chloroform, dried with Na2SO4 and evaporated on a rotary evaporator. The IR spectra were recorded by thin film technique using a Spectrum RXI, FT-IR Spectrometer, in the 4000-400cm-1 spectral region at a resolution 2 cm-1, 100 scans for each spectrum, using a 0.23 mm KBr liquid cell.

Result and discussion

All the three strains PDKT-1, PDKT-2, PDKT-5 produced biosurfactant (white precipitate) by using used engine oil as substrate. The isolated biosurfactant was analyzed chemically for the presence of amino acids, carbohydrate and lipids in the extracted biosurfactant (Table.1). The strains PDKT-1, PDKT-5 showed positive result for ninhydrin test, there was violet-blue complex formation which indicated the presence of amino acids. Absence of colour formation was observed in PDKT-2. In anthrone test for carbohydrates, there was a colour change to bluish green which indicates the presence of carbohydrates was observed only in strain PDKT-2. Also, there was the absence of blue or reddish brown complex in iodine test and the formation of red precipitate in Barfoeds tests within 2-5 min, was observed that it indicates the absence of polysaccharides and the presence of monosaccharides respectively . No colour formation in the strains PDKT-1, PDKT-5 revealed the absence of carbohydrates. Biol’s test for pentose sugars, the formation of blue-green coloured complex was observed. It confirms the presence of pentose sugar in the isolated biosurfactant of PDKT-2. In the solubility test for lipids, the tested biosurfactants from three strains were insoluble in water, but soluble in alcohol and chloroform. In saponification test for lipids, NaOH saponifies the lipid, which is present in the biosurfactant that indicates the presence of lipid in the isolated biosurfactant of three strains (PDKT-1, PDKT-2, and PDKT-5). In achrolin test for glycerol the tested biosurfactant, does not produce pungent smell. It indicated the absence of glycerol in PDKT-1, PDKT-2, PDKT-5. The above
results revealed that the isolated biosurfactant of PDKT-1, PDKT-5 and PDKT-2 belong to lipopeptide and rhamnolipid type respectively.

Following chemical screening, the molecular structure of the extracted biosurfactants from the strains (PDKT-1, PDKT-2, and PDKT-5) was confirmed by FTIR spectroscopy. Infrared spectrum of the extracted biosurfactant from PDKT-1 (Fig.-1) showed strong bands at 3430 cm⁻¹, indicating the presence of a peptide component resulting from the N-H stretching mode and at 1655cm⁻¹ resulting from the stretching mode of CO-N bond. The bands at 2960 cm⁻¹ to 2860 cm⁻¹ and1470 cm⁻¹ resulting from the C-H stretching mode suggest the presence of aliphatic chain. These results revealed that the isolated biosurfactant from PDKT-1 was a lipopeptide.

Analysis of the extracted biosurfactant from PDKT-2 showed positive test for sugar and rhamnose. FT-IR Spectrometry strongly suggested a broad absorption valley at 3400 cm⁻¹ indicating the presence of OH groups in the molecules. Strong absorption valleys observed in the range from 2725 to 2813 cm⁻¹ demonstrated typical CH stretching variation in the alkaline chain. Absorption valleys at 1607 cm⁻¹ indicated stretching vibration of C-O and C = O bonds in carboxyl esters. Scissoring vibration of a CH₂ group adjoining a carboxyl ester was also observed at 1351 cm⁻¹. The peak in the region of 1104 cm⁻¹ indicates C-O-C stretching in the rhamnose (Fig-2). These results confirm that the extracted biosurfactant from PDKT-2 belonged to rhamnolipid type.

Strong bands at 3430 cm⁻¹, indicating the presence of a peptide component resulting from the N-H stretching mode and at 1655cm⁻¹ resulting from the stretching mode of CO-N bond observed in the biosurfactant extracted from PDKT-5 (Fig-3). The bands from 3000 cm⁻¹ to 2000 cm⁻¹ resulting from the aliphatic bonds CH₃, CH₂ and C–H stretching bands from 3000 cm⁻¹ to 2000 cm⁻¹ demonstrated typical CH stretching variation in the alkaline chain. Absorption valleys at 2960 cm⁻¹ to 2813 cm⁻¹ and 1470 cm⁻¹ resulting from the C-H stretching mode suggest the presence of aliphatic chain. These results revealed that the isolated biosurfactant from PDKT-2 was a glycolipid.

Although the identification and characterization of microbial surfactants produced by various microorganisms have been extensively studied, only few reports available on biosurfactant producing microorganisms on used engine oil. In this study, isolated biosurfactant from PDKT-2 was analyzed chemically, the presence of carbohydrates, lipids was confirmed. That particular carbohydrate was found to be a pentose sugar and the glycerol was absent in the lipid, hence this indicates that the isolated biosurfactant was a glycolipid. Glycolipids containing sugar and lipid component and do not containing glycerol (Table.1). The sugar constitutes most prevalently glucose, galactose, mannose and glycocoyamine have all been identified (Sawhney and Singh, 2000). Chemical analysis confirmed the presence of lipid and amino acid component in PDKT-1 and PDKT-5.Similar result on chemical analysis of biosurfactant was reported by Mahesh et al., [21].

In the present investigation, infrared spectrum of biosurfactant from Serratia marcescens PDKT-1 belonged to lipopeptide type. This result was in agreement with previous findings of Matsuyama et al., [24] who reported that serrawettins, surface – active exolipids, nonionic biosurfactants was produced by Serratia marcescens. Serratia rubidaea produces rubiwtettin R1, linked D-3-hydroxy fatty acids and RG1, β-glucopyranosyl linked D-3-hydroxy fatty acids. [23]. Serratia produces surface active cyclodepsipeptides known as serrawettin W1, W2 and W3 [22,23]. Different strains of Serratia marcescens produce different serrawettins. Serrawettin W1 was produced by strains 274 and ATCC 13880 or NS 38, W2 was produced by strain NS 25 and W3 was produced by strain NS 45. Besides this Serratia liquefaciens produces serrawettin W2. [23]. Recently studies on Lipopeptide biosurfactant production by Serratia marcescens NSK-1 strain isolated from petroleum-contaminated soil by Anyanwu [3] which coincides the present study results.

Rhamnolipids produced by Pseudomonas aeruginosa were the most studied biosurfactants due to their potential applications in a wide variety of industries and the high levels of their production [38]. Rhamnolipids, in which one or two molecules of rhamnose are linked to one or two molecules of β-hydroxy-decanoic acid, are the best-studied glycolipids. Production of rhamnose-containing glycolipids was first described in Pseudomonas aeruginosa [17]. L-Rhamnosyl-L-rhamnosyl-β-hydroxydecanoylβ-hydroxydecanoate and L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate, referred to as rhamnolipid 1 and 2, respectively, are the principal glycolipids produced by P. aeruginosa [9,14,16]. The formation of rhamnolipid types 3 and 4 containing one β-hydroxydecanoic acid with one and two rhamnose units, respectively (Syldatk et al., 1985), methyl ester derivatives of rhamnolipids 1 and 2 [13] and rhamnolipids with alternative fatty acid chains [29,31] has also been reported.In the present investigation the biosurfactant produced by the strain Pseudomonas aeruginosa PDKT-2 was characterized as rhamnolipid by FT IR technique. According to the results of the IR spectra, the rhamnolipids produced by belong to Pseudomonas aeruginosa PDKT-2 the glycolipid group, which is made up of aliphatic acid and ester. The adsorption bands obtained were consistent with
Fig. 1: Shows FT-IR spectrum of extracted biosurfactant from the isolate PDKT-1

Fig. 2: Shows FT-IR spectrum of the extracted biosurfactant from the isolate PDKT-2
the report of Guo et al. This result was similar to recent findings. For example Rahman et al., 2010 characterized the biosurfactant produced by the strain Pseudomonas aeruginosa DS10-129 by FTIR technique and reported that it belonged to rhamnolipid type. Recently Da Rosa et al., (2010) reported that the rhamnolipid type biosurfactant was produced by Pseudomonas aeruginosa LBM10 which supports the present study results.

Biosurfactants are easily biodegradable and thus they are particularly suited for environmental applications such as bioremediation and the dispersion of oil spills. Among the many classes of biosurfactants, lipopeptides are particularly interesting because of their high surface activities for example, surfactin, a well-studied lipopeptide produced by Bacillus subtilis, was a very effective biosurfactant [7]. The present study revealed that the biosurfactant from B. licheniformis 86 by Horowitz and Griffin [15] and another B. licheniformis strain isolated by Jenny et al., [18] have been shown to produce lipopeptides with peptide moieties containing C-terminal amino acid residues different from those of surfactin. Lin et al., [20] reported that the surface-active compound from B. licheniformis JF-2 was a lipopeptide. The lipopeptide type biosurfactant producing strain, B. licheniformis F2.2 and Bacillus licheniformis PTCC 1595 which were isolated by [36,28] respectively correlates the present study findings.

References


