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Physicochemical characterization of biosurfactant and its potential to remove oil from soil and cotton cloth

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ABSTRACT

An alkaliphilic bacterium, *Klebsiella* sp. strain RJ-03, produced a biosurfactant, which showed low viscosity with pseudoplastic rheological behavior and exhibited emulsification activity with oils and hydrocarbons. The biosurfactant has excellent oil removing efficiency as compared to chemical surfactants. The isolated biosurfactant has compatibility with detergents and enhanced oil removing efficiency from soil and cotton cloths. It comprised of sugar, uronic acid, protein and sulfate. GC–MS analysis confirmed the presence of six monosaccharides (w/w), glucose (6.65%), galactose (23.98%), rhamnose (14.94%), mannose (17.54%), fucose (9.47%) and 6-O-Me-galactose (1.4%). It is a high molecular weight, thermostable biopolymer showing degradation above 300 °C. Positive ion reflector mode of MALDI TOF-TOF MS analysis revealed series of low and mid range mass peaks (m/z) corresponding to mono-, di-, tri- and oligo-saccharides content. The NMR, FT-IR, EDX-SEM, AFM and PSD analysis confirmed the presence of functional groups, bonds, elements and particle size respectively.

1. Introduction

Oil pollution affects soil ecology, aquatic lives, economy, tourism and leisure activities that results in an increased attention toward the development of new and eco-friendly techniques for its remediation. Bioremediation has been identified as an effective, economical and eco-friendly technology. Prolonged persistence of hydrophobic hydrocarbons in soil and water occurs because of their low solubility and hence, their removal is facilitated by increasing their apparent solubility through treatments by surfactants or sorbents (Barkay, Navon-Venezia, Ron, & Rosenberg, 1999; Mulligan, 2005; Wei, Mather, & Fotheringham, 2005). The use of chemical surfactants is not preferred due to their recalcitrant nature (Rosenberg & Ron, 1999). Use of biosurfactants is ecologically accepted due to their specificity, less toxicity and biodegradable nature. They are also effective in a wide range of extreme conditions including temperature, pH and salinity as compared to chemical (synthetic) surfactants (Banat, Makkar, & Cameotra, 2000; Bramhachari et al., 2007; Mukherjee, 2007; Mulligan, 2005).

Biosurfactants are heterogenous, complex and structurally diverse group of surface active agents, produced by living organisms from different habitats, which either adhere to cell surface or excreted from the cell (Kumar, Mody, & Jha, 2007; Mulligan, 2005; Parikh & Madamwar, 2006). These amphiphilic compounds contain a hydrophobic and a hydrophilic moiety, and have the ability to reduce interfacial and surface tension between different fluid phases (Banat et al., 2000; Bramhachari et al., 2007). Biosurfactants have a wide range of biotechnological applications in dairy, food, beverage, cosmetics, detergent, textile, paint, mining, petroleum, paper pulp and pharmaceutical industries (Banat et al., 2000). Biosurfactant production by extremophiles is reported by a few researchers (Jain, Mody, Mishra, & Jha, 2012; Nicolaus, Kambourova, & Oner, 2010). Extreme environments are valuable resources of microorganisms that may secrete novel biosurfactants having wider adaptability and stability toward adverse environmental conditions (Nicolaus et al., 2010). These properties make them suitable for their applications in bioremediation of oil, hydrocarbon and toxic metals from contaminated soil and wastewaters (Calvo, Manzanera, Silva-Castro, Uad, & Gonzalez-Lopez, 2009; Ruey-an & Wen-gang, 2003; Urum & Pekdemir, 2004).

The present work deals with the isolation and chemical characterization of a biosurfactant produced by an alkaliphilic bacterium, *Klebsiella* strain RJ-03, isolated from oil contaminated waste. The biosurfactant was evaluated for its compatibility with detergents and its efficacy to remove oil from soil and cloths as compared to chemical surfactants like sodium dodecyl sulfate (SDS), Tween80 and laundry detergent powders (Surf Excel, Wheel and Tide).

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2. Materials and methods

2.1. Isolation of alkaliphilic bacteria and screening of biosurfactant producers

Oil industry sludge and wastewater samples were collected from Gondal, GIDC (N 21° 58.95″ E 70° 47.24″), Gujarat (India). Samples were inoculated into Horikoshi medium (HM) broth, pH 10.0 for enrichment. Enriched samples were 10 fold diluted, spread on the Horikoshi agar plates (pH 10) containing (g/l) glucose 10, peptone 5, yeast extract 5, K_2HPO_4 1, $MgSO_4{\cdot}7H_2O$ 0.2, Na_2CO_3 10 and agar 20. Plates were incubated at 30 °C for 24 h and individual colonies were selected and further streaked on Horikoshi agar plate in order to obtain their pure cultures. All isolates were maintained on Horikoshi agar slants at 4 °C for subsequent experiments. Hemolytic assay was performed for screening of biosurfactant producing bacteria (Jain et al., 2012). For this, the isolated alkaliphilic bacteria were streaked onto blood agar plates containing (g/l) trypticase 10, beef extract 3, NaCl 5 and agar 15. After autoclaving, 5% (v/v) sterile human blood was added (pH 10). The plates were incubated at 30 °C for 24 h and visually inspected for zone of clearance, around the colony. Cell free supernatants obtained at different incubation periods and tested for surface active properties by measuring surface tension at 30 °C using du Nouy ring tensiometer (Data physics, Germany). Surface tension of distilled water and SPY medium containing (g/l) sucrose 30, peptone 5 and yeast extract 1, were taken as a control.

2.2. Production, quantification and purification of biosurfactant

A loopful of alkaliphilic bacterium RJ-03 was inoculated into 100 ml HM broth of pH 10 and incubated for 24 h at 30 °C on a rotary shaker (120 rpm) for the preparation of seed culture. Biosurfactant was produced by inoculating 2% (v/v) seed culture (OD_{600} = 2) in 500 ml SPY broth (pH 10) containing (g/l) sucrose 30, peptone 5 and yeast extract 1, and incubated for 168 h at 30 °C on a rotary shaker (120 rpm). After every 24 h, samples were taken and centrifuged at 13,000 × *g* for 20 min in order to remove the bacterial cells. The obtained supernatant was acidified to pH 2.0 using 6 M HCl, kept at 4 °C for 12 h and centrifuged (13,000 × *g*; 20 min; 4 °C). The biosurfactant was recovered from the supernatant by isopropanol precipitation, dried and weighed. The crude product was dialysed for 48 h at 4 °C (12,000 Da cut off dialysis tubing, Sigma) and lyophilized.

2.3. Physico-chemical characterization of biosurfactant

2.3.1. Rheology and emulsification activity assay

Viscosity of the biosurfactant solution (0.5% w/v) was measured using a rheometer (RS1, HAAKE Instruments, Karlsruhe, Germany). Emulsification activity of the biosurfactant solution (0.1% w/v) was determined by measuring the emulsification index (Cooper & Goldenberg, 1987). The oil, emulsion and aqueous layer were measured at every 24 h up to 240 h. The emulsification index was recorded with time and represented accordingly, i.e. the emulsification index after 24 h, 48 h, 72 h, etc. was represented as E_{24}, E_{48}, E_{72}, respectively.

2.3.2. Chemical characterization of biosurfactant

The purified biosurfactant was analyzed spectrophotometrically to determine the total sugar, reducing sugar, sulfate, protein and uronic acid contents (Bradford, 1976; Dodgson & Price, 1963; Knutson & Jeanes, 1968; Morris, 1948; Nelson, 1944).

2.3.3. Molecular mass, MALDI TOF-TOF and GC–MS spectroscopy

The molecular weight of the biosurfactant was determined by Gel Permeation Chromatography (GPC; 7.8 mm ID x 300 mm stainless steel, Model Alliance 2695, Waters, USA) with guard column. About 50 μl of 0.1% product was loaded to GPC column Ultrahydrogel-120 and Ultrahydrogel-500 at 30 °C. The column was calibrated with standard dextran (molecular weight, 5200–668,000 kDa; PSS, USA). Elution was monitored by a refractive index detector (2414).

The biosurfactant solution (5 mg/ml) was prepared in aqueous acetonitrile (50% v/v) and mixed with equal volume of matrix α-Cyano-4-hydroxycinnamic acid (10 mg/ml). MALDI TOF-TOF analysis was carried out on MALDI TOF-TOF analyser (Applied Biosystem 4800, USA) with an Nd-YAG (neodymium-doped yttrium aluminum garnet) laser (355 nm, 200 Hz) operated in an accelerating voltage 20 kV. Each spectrum was collected in positive ion reflector mode with an average of 1400 laser shots per spectrum (Jain et al., 2012; Mishra, Kavita, & Jha, 2011). Reproducibility of the spectrum was checked by triplicate of six spot-sets and the spectra were analyzed after centroid and de-isotoping using Data explorer software (Applied Biosystem, USA). The monosaccharide composition of biosurfactant was determined by analyzing derivatised biosurfactant using GC–MS-QP2010 (Shimadzu, Japan) (Jain et al., 2012; Kavita, Mishra, & Jha, 2011).

2.3.4. Particle size distribution, atomic force microscopy (AFM) and energy dispersive X-ray spectroscopy (EDX)

A sample of the biosurfactant (0.5% w/v) was taken for measurement of particle size distribution by laser diffraction (Malvern Mastersizer 2000, Malvern Ltd., Worcestershire, UK). The sample was prepared on cleaved mica disks for AFM and surface topography (Ntegra-Aura, NT-MDT, Moscow, Russia) were observed under the tapping mode (Seviour, Donose, Pijuan, & Yuan, 2010; Singh et al., 2011). The biosurfactant sample (5–7 mg) attached on an aluminum stuff was used for quantitative elemental analysis using SEM-EDX (SEM-EDX, Oxford Instruments, UK), which revealed the weight and atomic percentage of different elements present (C, O, Na, S, and Ca) in the sample (Singh et al., 2011).

2.3.5. FT-IR and ^1H and ^{13}C NMR analysis

Fourier transform infrared (FT-IR) spectroscopy was used to elucidate the chemical nature of biosurfactant by identifying the types of functional groups. The FT-IR spectrum was recorded in 4000–400 cm^{-1} region on a GX-FT-IR system (Perkin Elmer, USA), where a KBr pellet was used as a background reference. ^1H and ^{13}C NMR spectrum were recorded on a Bruker Avance-II spectrometer, Switzerland at 500 MHz. The biosurfactant was dissolved in D_2O (50 mg/ml) and the spectra were recorded at 70 °C with 5000–5200 accumulations, 5.9 μs pulse duration, 1.2 s acquisition time and 6 μs relaxation delay (Jain et al., 2012).

2.3.6. Thermal gravimetric analysis (TGA) and differential scanning calorimetric (DSC) analysis

TG and DSC analysis of purified biosurfactant were carried out with Mettler Toledo TGA/SDTA System (Greifensee, Switzerland). About 5–8 mg of lyophilized sample was loaded on a platinum pan and its energy level was scanned in the ranges of 30–480 °C and 30–450 °C for TG and DSC analysis respectively, under a nitrogen atmosphere with a temperature gradient of 10 °C/min. Both analyses were performed under gradual increase in temperature by plotting the weight percentage and heat flow against temperature; and the activation energy (E_a) was calculated (Jain et al., 2012; Mishra et al., 2011).

2.3.7. Soil and lubricant oil analysis

The physico-chemical characteristics like pH, particle size, moisture content, porosity, bulk density, cation exchange capacities, total carbon, total nitrogen, elements, surface area and average pore diameter were analyzed for the soil, while the lubricant oil was analyzed for density, specific gravity, physical state and color using standard methods (Jaiswal, 2003; Urum & Pekdemir, 2004; Vogel, 1978).

2.3.8. Removal of lubricant oil from contaminated sandy soil and cotton cloth

Experiments were carried out using 2 kg sandy soil impregnated with 200 ml of lubricant oil. About 35 g of the contaminated sandy soil was transferred into 100 ml of 1% (w/v) of different surfactants solution like biosurfactant, SDS, Tween80, Triton-X100 along with a control flask containing 100 ml distilled water and incubated on a rotary incubator shaker at 30 °C, 100 rpm for 24 h. After incubation, the solution was centrifuged at 7000 rpm for 10 min for separation of aqueous phase from soil containing oil which was further extracted with hexane. The hexane was recovered using a rotary evaporator and the residual lubricant oil was measured gravimetrically (Luna, Sarubbo, & Campos-Takaki, 2009).

Compatibility, stability and efficiency of the biosurfactant to remove oil with respect to commercially available detergents were also studied with a view to establish the potential of biosurfactant as a detergent additive. Detergents and biosurfactant were individually dissolved in water (1% w/v) and their efficiency to remove oil from an oil-contaminated cotton cloth was checked individually as well as in combination with the biosurfactant at a 1:1 ratio. For this, 3 g of lubricant oil was poured on a 25 cm × 25 cm cotton cloth and allowed to dry at 40 °C for 24 h. To test the oil removal capability, each piece of cloth impregnated with oil was soaked in flasks containing 100 ml each of tap water (control), biosurfactant, detergent solutions and biosurfactant: detergent solution (1:1 v/v). The flasks were kept on a shaker at 30 °C, 100 rpm for 60 min. The post-wash water was used to measure the amount of oil removed from the cotton cloth by extracting it with hexane. The extraction process was repeated thrice; the hexane was recovered using a rotary evaporator and the residual lubricant oil was measured gravimetrically (Luna et al., 2009).

2.4. Identification of biosurfactant producer strain RJ-03 by cellular fatty acid profiling and 16S rRNA gene sequencing

The biosurfactant producing bacterium was identified by Fatty acid methyl ester (FAME) analysis (MIDI Sherlock® Microbial Identification System). Molecular identification was done using 16S rRNA gene sequencing method (Jain, Mody, Keshri, & Jha, 2011).

3. Results and discussion

3.1. Isolation, screening and quantitative determination of biosurfactant

A total of 37 bacterial isolates were obtained from the oil industry sludge and wastewater samples, out of which, five isolates showed hemolytic activity on blood agar plates. The strain RJ-03 showed maximum production of biosurfactant with a concentration of 5.1 g/l after 72 h. Cell-free supernatant of RJ-03, showed the highest reduction in surface tension ranging from 69.03 to 44.06 mN/m compared to other isolates (S1 Supporting Information). The reduction of surface tension indicates the ability of a biosurfactant to remove oil from soil by reducing the capillary force responsible for holding the crude oil and soil together (Calvo et al., 2009; Ruey-an & Wen-gang, 2003; Urum & Pekdemir, 2004).

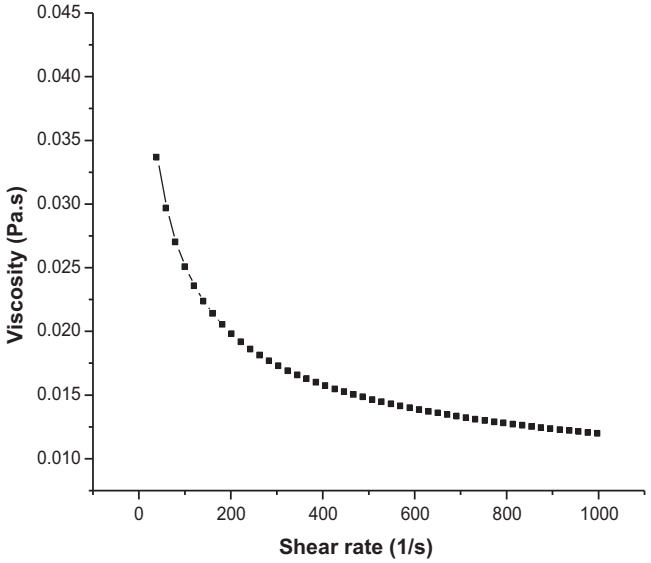

Fig. 1. Effect of shear rate on viscosity of biosurfactant (0.5% w/v) produced by RJ-03.

On the basis of these observations, strain RJ-03 was selected for further study.

3.2. Rheology and emulsification assay

The flow curve (Fig. 1) showed the pseudoplastic characteristics of the biosurfactant solution. The viscosity decreased concomitantly with an increase in the shear rate. The viscosity of the biosurfactant was 0.345 Pa s^{-1} at 0.01 s^{-1} shear rate with 0.00388 Pa shear stress. The purified biosurfactant exhibited emulsification ability with oils, aliphatic and aromatic hydrocarbons (S2 Supporting Information). The development of a stable emulsion was observed with cotton-seed, jojoba, groundnut oil and hydrocarbons like benzene, toluene, carbon tetrachloride and dichloromethane, which was revealed by the high emulsification indices in the range from 100% to 62% up to 240 h. It dissolved easily in water and forms a clear solution at a broad pH range (2–12) and stable emulsion. Some commercial emulsifiers, particularly those with fatty acid components, do not form clear solution and hence limit their applications (Gutierrez, Leo, Walker, & Green, 2009). The presence of proteins and various functional groups present in biosurfactant provides hydrophobicity to enhance emulsification of hydrocarbons and oils (Bramhachari et al., 2007; Gutierrez et al., 2009; Sutherland, 2001).

3.3. Chemical characterization of biosurfactant

Total sugars, reducing sugar, uronic acid, protein and sulfate contents were found to be 52.4, 0.8787, 18.65, 13.6 and 0.281% respectively. The C, H and N analysis confirmed the presence of 33.24% carbon, 9.91% hydrogen and 6.60% nitrogen. Previously it was observed that biosurfactants produced from *Marinobacter* sp., *Vibrio* sp., *Acetobacter* sp., *Halomonas* sp., *Bacillus* sp., *Pseudomonas* sp., *Corynebacterium* sp. and *Halobacter* sp. comprised of carbohydrate, uronic acid, protein and sulfate (Bramhachari et al., 2007; Parikh & Madamwar, 2006). The carboxyl and sulfate groups provided overall negative charge on the biopolymer, thereby supporting binding and adsorptive properties for divalent cations by electrostatic interactions (Bramhachari et al., 2007).

3.4. Molecular mass, MALDI TOF-TOF and GC mass spectroscopy

The GPC chromatogram generated a single peak corresponding to 2427 kDa approximately, with 1.72 polydispersity and 12.65 min retention time (S3 Supporting Information). Biosurfactants are divided in low and high molecular weight, having different chemical structures and surface properties. High molecular weight biosurfactants, produced from *Bacillus* sp., *Acinetobacter* sp. and *Pseudomonas* sp., are used as emulsifiers and flocculants (Banat et al., 2000; Barkay et al., 1999). Matrix assisted laser desorption–ionization mass spectroscopy was used previously for the analysis of biosurfactant (Jain et al., 2012). MALDI TOF-TOF mass spectroscopy of biosurfactant (S4 Supporting Information) represents mass peaks m/z 160.78 and 187.23 corresponding to pentose and hexose sugars respectively. A series of masses m/z 296.02, 328.06 and 366.00 correspond to mass of disaccharides (2 pentose, 1 pentose + 1 hexose and 2 hexose respectively). Besides these, masses m/z 452.06, 484.20, 512.18 and 543.34 were also detected in positive ion reflector mode, assigned to trisaccharides (combination of pentoses and hexoses); however, higher mass peaks m/z ranging 706.9041–1327.4277 (S5 Supporting Information) revealed the presence of oligosaccharide chains consisting of different ratios of pentose and hexose sugars. The heteropolysaccharide nature of the biosurfactant was further confirmed by GCMS with monosaccharide composition of six types of hexose and pentose sugars at varying proportions viz. glucose (6.65%), galactose (23.98%), rhamnose (14.94%), mannose (17.54%), fucose (9.47%) and 6-O-Me galactose (1.4%).

3.5. Particle size distribution, energy dispersive spectroscopy (EDX) and atomic force microscopy (AFM)

The biosurfactant was composed of particles with sizes ranging from 0.930 ($d_{0.1}$) to 56.969 ($d_{0.9}$) μm with an average size of 16.042 ($d_{0.5}$) μm and specific surface area of 1.74185 $m^2\,g^{-1}$ (S6 Supporting Information). The qualitative elemental analysis was performed by SEM-EDX (S7 Supporting Information) which revealed the weight and atomic percentage of different elements present (C, N, O, Na, S, and Ca) in the biosurfactant, produced by the alkaliphilic bacterium RJ-03. An AFM micrograph of the high molecular weight biosurfactant showed irregular biopolymer aggregates of varying particle size in the range of 0.6–1.4 μm and the height distribution profiles of the average surface roughness of 79.87 nm (S8 Supporting Information).

3.6. FT-IR and ^1H and ^{13}C NMR analysis

The FT-IR spectrum showed (Fig. 2) a broad stretched intense peak at around 3428 cm^{-1} characteristic of hydroxyl groups. An asymmetrical C—H stretching vibration of aliphatic CH_2 group attributed at around 2926 cm^{-1} and the peak at around 2107 cm^{-1} designated aliphatic C—H bonds. Peaks observed at 2360 cm^{-1} might be due to CO_2 adsorption or the asymmetric stretching of the —N═C═O— group (Mishra & Jha, 2009). The peak at around 1637 cm^{-1} and the peaks near 1044 cm^{-1} suggested the presence of C═O group present in carboxylate and amide moieties of protein, respectively. The broad stretch of C—O—C, C—O at 1000–1200 cm^{-1} showed the presence of carbohydrates (Mishra & Jha, 2009). A symmetrical stretched peak near 1384 cm^{-1} indicated the presence of carboxyl groups. The absorption at 1252 cm^{-1} could be attributed to the presence of sulfate groups as S═O and C—O—S (Jain et al., 2012; Mishra & Jha, 2009). Absorption peaks in the range around 670–516 cm^{-1} correspond toward stretch of alkyl-halides. The presence of sulfate, acetyl and carboxyl functional groups in the biopolymer proved its anionic nature which could be useful for binding and thus remediating divalent cations (Bramhachari et al., 2007; Gutierrez et al., 2009; Parikh & Madamwar, 2006).

^1H NMR spectra of RJ-03 biosurfactant (Fig. 3) revealed a number of peaks (signals) corresponding to different sugars moities, protein content, sulfated sugar, etc. The presence of chemical shift (δ) value at 1.11–1.20 ppm indicated the methyl group (—CH_3) corresponding to the sugar moiety. The δ value at 1.88 ppm showed the CH-proton of C5/C6 carbon of De-oxy sugar. The presence of sulfate group (sulfate derivative of biopolymer) was proved by the δ value 2.13 ppm. The range of chemical shift (δ) signals from 3.63 to 5.37 possibly indicated a glycosidic linkage of pentose/hexose sugars. The δ value at 4.8–4.9 indicated the protons on β-anomeric carbon of sugar moieties. The presence of NH_2 group of the protein of biopolymer can be confirmed from the δ value of 5.33–5.37 ppm. Signals of five anomeric carbon at δ = 92.4, 94.7, 100.1, 102.6 and 104.6 of repeating monosaccharides units were observed in ^{13}C NMR spectrum analysis of the biosurfactant (Fig. 4). The two peaks δ = 173.2 and 175.4, derived from the carbonyl group (—CO—) were designated to uronic acid while signal in the range of δ = 61.4–70 confirms the presence of acetyl group in the biosurfactant. The signal at δ = 38.1–39.4 range indicated R—CH_2NH_2 of the protein moieties. The C1 signals for β-glucose (94.7) & α-glucose (92.5); C2 signals for β-glucose (75.26), α-glucose (72.8) & β-galactose (73) and C3 signals for β-glucose (76.6) & α-glucose (73.5) were also observed. NMR characteristic spectral peaks of biosurfactant were also observed previously in biopolymers obtained from different sources (Jain et al., 2012; Lin, Minton, Sharma, & Georgiou, 1994; Mishra et al., 2011).

3.7. Thermal gravimetric analysis (TGA) and differential scanning calorimetric (DSC) analysis

The establishment of thermal stability of biosurfactant is an important characteristic with respect to its application. Dynamic thermogravimetry experiment indicated that degradation takes place by two well differentiated steps (Fig. 5). An initial weight loss (8.4%) between 30 and 145 °C was attributed to loss of trapped alcohol and moisture molecules in the structure of the biopolymer followed by second phase of degradation at around 250 °C, where a weight loss of 61.9% was observed with maximum degradation at 300 °C, while complete loss was observed after 350 °C. The transition is an exothermic process from amorphous solid to crystalline solid. The DSC thermogram graph showed (Fig. 6) crystallization temperature (T_c) of 100.21 °C (onset temperature 95.68 °C) and melting point for T_{m1} & T_{m2} peaks were observed at 222.57 °C (onset temperature 218.92 °C) & 280.57 °C (onset temperature 276.72 °C) respectively.

3.8. FAME and molecular identification of biosurfactant producer strain RJ-03

FAME analysis of RJ-03 strain was done using the MIDI Sherlock Microbial Identification System (S9 Supporting Information). A Similarity Index (SI) of 0.712 was observed with *Klebsiella* sp. compared with RTSBA library of Sherlock software. The bacterium used in the present study was further identified as *Klebsiella* sp. based on 16S rRNA gene sequence homology. The strain RJ-03 was found to have closest proximity with *Klebsiella* sp. (AB558499) with 99% query coverage and 98% homology. The 16S rRNA gene sequence has been deposited in NCBI Genbank with the Accession No. JN398669.

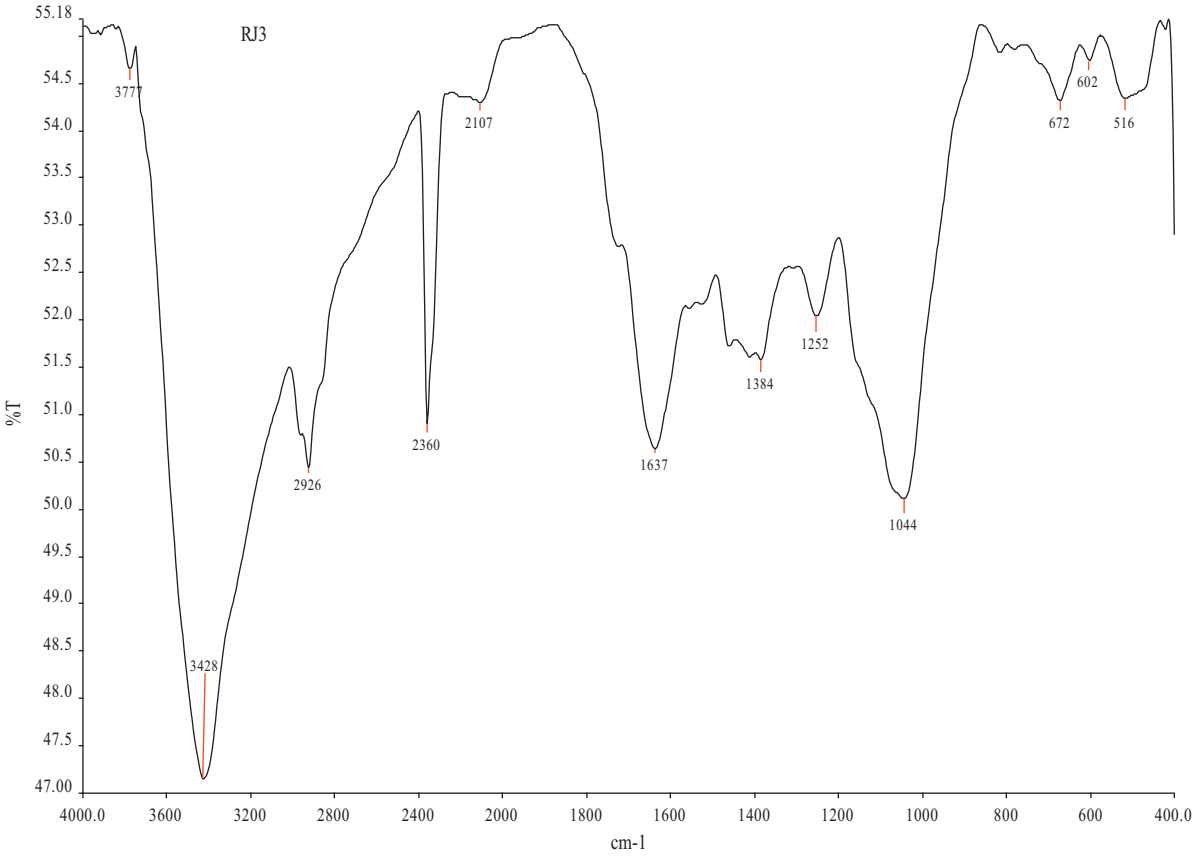

Fig. 2. FT-IR spectrum of purified biosurfactant.

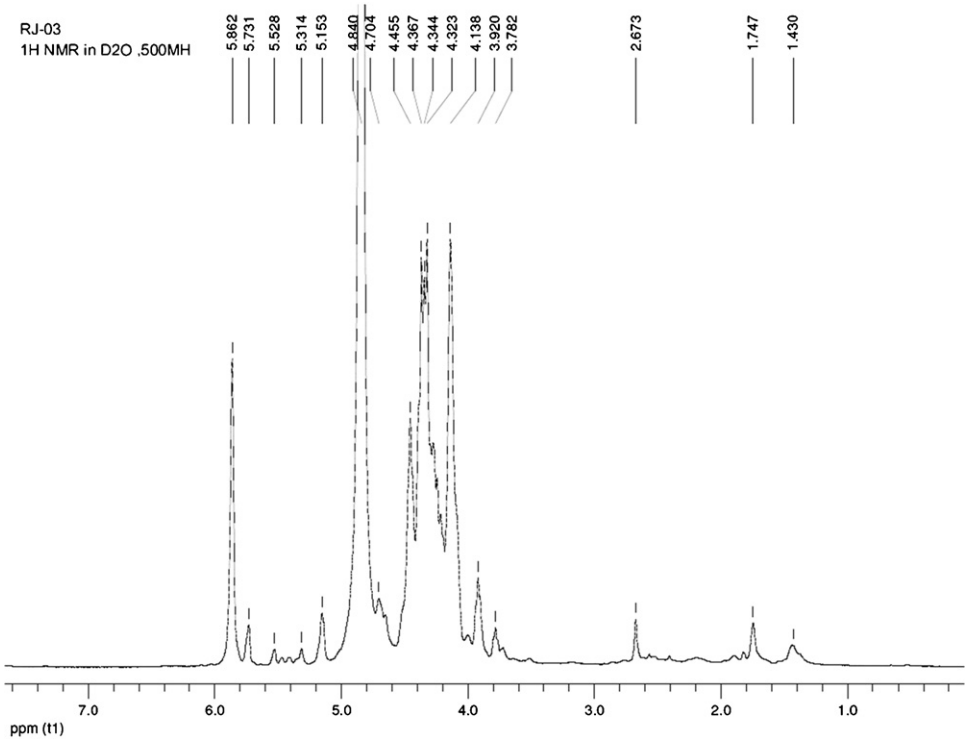

Fig. 3. ^{1}H NMR spectrum of biosurfactant produced by RJ-03.

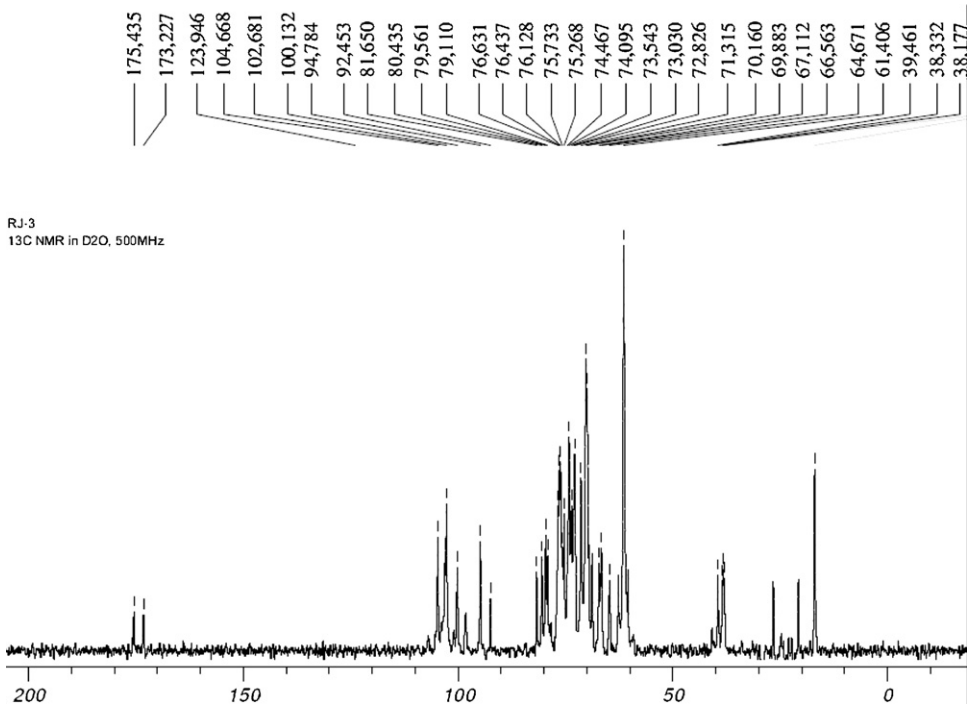

Fig. 4. ^{13}C NMR spectrum of biosurfactant produced by RJ-03.

3.9. Removal of lubricant oil from contaminated soil and cotton cloth

The physico-chemical characteristics of soil and lubricant oil used in the present study were analyzed (Supporting Information S10a–c). The biosurfactant performed much better for the removal of lubricant oil from soil as compared to chemical surfactants (Supporting Information S11a). About >90% of oil was recovered in the presence of biosurfactant compared to 57–67% recovery by chemical surfactants. Biological surfactants have advantages over chemical surfactants as they are more efficient, effective and eco-friendly because they remove oil contaminants without modifying the chemical nature of soil (Lai, Huang, Wei, & Changa, 2009; Urum & Pekdemir, 2004; Whang, Liu, Ma, & Cheng, 2008) by mobilization, due to the reduction of surface and interfacial tension.

Performance of biosurfactant was observed to be excellent as it alone removed 80% oil compared to 55–69% oil removal by commercially available detergents from cotton cloth (Supporting Information S11b). Additionally, the biosurfactant also exhibited compatibility with commercially available detergents at 1:1 ratio (Supporting Information S11b). The results suggest improved wash performance of detergents in the presence of biosurfactant. It was further evidenced that, enhanced oil removal (17–20%) from the cotton cloth during washing by detergents supplemented with the biosurfactant as compared to detergent solution alone. It is known that commercially available detergents contain anionic surfactants, bleaching agents, water softening builders and enzymes

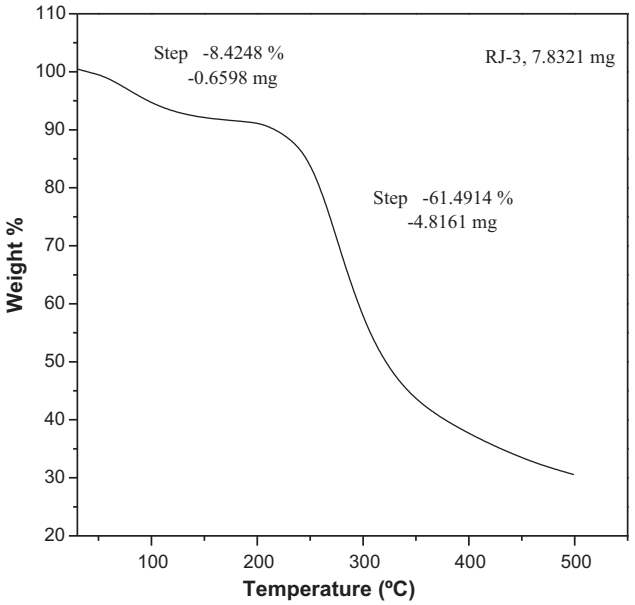

Fig. 5. TG thermogram of biosurfactant obtained from RJ-03.

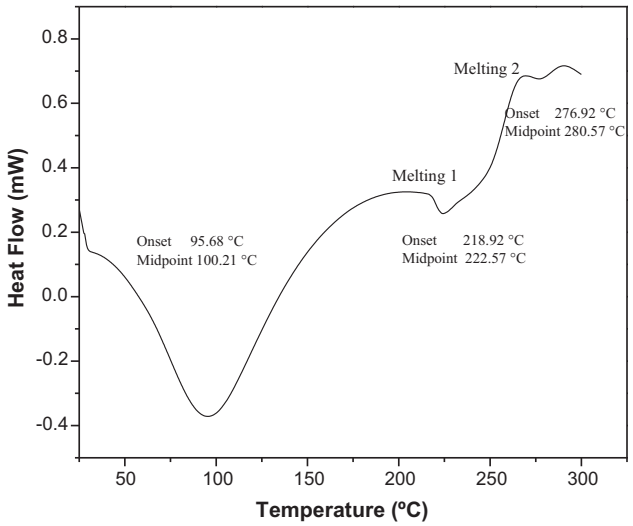

Fig. 6. DSC thermogram of biosurfactant obtained from RJ-03.

(Mukherjee, 2007). The requirement for any substance to be included in the formulation is its stability and compatibility with detergents. The results proved that the biosurfactant showed excellent stability as well as compatibility with commercially available detergents used in the experiments with enhanced washing performance.

An evaluation of the ability of aqueous biosurfactant solutions (aescin, lecithin, rhamnolipid, saponin and tannin) for possible application in washing of crude oil contaminated soil was carried out by Urum and Pekdemir (2004). It was observed that the biosurfactants were able to remove significant amount of crude oil from the contaminated soil at different concentrations for example rhamnolipid and SDS removed 80% oil while lecithin removed about 42% oil. Similarly, 84% oil removal from soil was observed by biosurfactant obtained from *Candida glabrata* UCP 1002 at 2.5% concentration (Luna et al., 2009). Previously 57–82% oil removal was reported from contaminated soil using biosurfactant produced by *Pseudomonas aeruginosa* SP4, *Bacillus subtilis* PT2 and *Rhodococcus ruber* (Lai et al., 2009; Santa-Anna et al., 2007; Whang et al., 2008). Inability of water to remove hydrophobic compounds from sandy soil enhances need of surfactant as they are dual in nature (having both hydrophobic and hydrophilic moieties). Biosurfactants display excellent surface activity in comparison to chemical surfactant due to their bulky molecular weight and complex biodegradable structure. Biosurfactants are not being used enough for the removal of hydrocarbons from contaminated soil and cotton cloth as compared to physical and/or chemical methods (Mukherjee, 2007; Rosenberg & Ron, 1999; Vogel, 1978).

4. Conclusion

The present study demonstrates physico-chemical properties of biosurfactant produced by an alkaliphilic bacterium *Klebsiella* sp. and its importance with respect to remediation of oil from soil as well as cloths. Further, its efficiency, compatibility and stability with various laundry detergents along with their additive interaction with detergents improved the washing performance. This led to conclude its future industrial application as laundry detergent additives.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2012.03.077.

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