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ORIGINAL ARTICLE

Kocuria marina BS-15 a biosurfactant producing halophilic bacteria isolated from solar salt works in India

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Abstract Biosurfactant screening was made among the eight halophilic bacterial genera isolated from Kovalam solar salt works in Kanyakumari of India. After initial screening, *Kocuria* sp. (Km), *Kurthia* sp. (Ku) and *Halococcus* sp. (Hc) were found to have positive biosurfactant activity. Biosurfactant derived from *Kocuria* sp. emulsified more than 50% of the crude oil, coconut oil, sunflower oil, olive oil and kerosene when compared to the other strains. Further, *Kocuria marina* BS-15 derived biosurfactant was purified and characterized by TLC, FTIR and GC–MS analysis. The TLC analysis revealed that, the purified biosurfactants belong to the lipopeptide group. The IR spectrum results revealed that functional groups are $R_2C{=}N{=}N$, alkenes and N–H. The GC–MS analysis confirmed the compound as Nonanoic acid and Cyclopropane with the retention time of 12.78 and 24.65, respectively.

1. Introduction

Biosurfactants are amphiphilic compounds produced on living surfaces, mostly on microbial cell surfaces or excreted extracellularly and contain hydrophobic and hydrophilic moieties. They reduce surface tension and interfacial tension between individual molecules at the surface and interface respectively (Karanth et al., 1999). The microbial surfactants (MS) are complex molecules covering a wide range of chemical types including peptides, fatty acids, phospholipids, glycolipids, antibiotics, lipopeptides, etc (Cooper and Zajic, 1980). These properties resulted in detergency, emulsifying, foaming and disparity traits as well as increase in solubility and mobility of hydrophobic organic compounds (singh et al., 2007).

Research in the area of biosurfactants has expanded quite a lot in recent years due to its potential use in different areas. In recent years increasing global environmental awareness has led

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to much more interest in microbial surfactants compared to their chemical counter parts. It is due to the unique properties of biosurfactants including biodegradability, low toxicity, mild production conditions, and environmental acceptability, lower critical micelle concentration, higher selectivity as well as better activity at extreme temperature, pH and salinity (Das and Mukherjee, 2007).

Biosurfactant producing organisms are very diverse and have been isolated from a wide variety of environments, including soil, sea water, marine sediments, oil fields (Bodour et al., 2003) and even extreme environments (Cameotra and Makkar, 1998). The synthesis of these surface active molecules takes place by *de novo* pathway and/or assembly from substrates (Syldatk and Wagner, 1987). This wide range of structural diversity results in a broad spectrum of potential industrial applications including production of food, cosmetics, and pharmaceuticals, agriculture, mining, enhanced oil recovery, transportation of crude oil, cleaning oil storage tanks and pipelines and soil remediation (Pastewski et al., 2006).

The search for novel biosurfactants in extremophiles seems to be particularly promising since they have particular adaptations to increased stability in adverse environments and the microbial products are highly stable and important in medical biotechnology. In the solar salt works of southern India, there is a wide diversity of moderate and extreme halo bacterial sp. having pharmacologically important compounds including biosurfactants (Donio et al., 2013). There are very few reports on biosurfactant producers in hypersaline environments and in the recent years, there has been a greater increase in interest and importance in halophilic bacteria for biomolecules. Halotolerant or halophilic microorganisms, able to live in saline environments, offer a multitude of actual or potential applications in various fields of biotechnology (Margesin and Schinner, 2001). Halophiles, which have a unique lipid composition, may have an important role to play as surface-active agents. The archae bacterial ether-linked phytanyl membrane lipid of the extremely halophilic bacteria has been shown to have surfactant properties (Post and Collins, 1982). Yakimov et al. (1995) reported the production of biosurfactant by a halotolerant Bacillus species and its potential in enhanced oil recovery; *Bacillus licheniformis* strain BAS 50 was able to grow and produce a lipopeptide surfactant when cultured on a variety of substrates at salinities up to 13% NaCl.

Individual wastes alone have resulted in high yields of biosurfactants by new efficient microbial isolates viz. *Kocuria turfanesis* strain BS-J and *Pseudomonas aeruginosa* strain BS-P (Dubey et al., 2012). Microbial communities like Acinetobacter, Arthrobacter, Pseudomonas, Halomonas, Bacillus, Rhodococcus, Enterobacter and yeast have been reported to produce biosurfactants (BS)/bioemulsifiers (BE) (Das et al., 2008a). About 56 reports including 35 for bioemulsifier, 12 for glycolipid and 9 for other types are available on different types of BS/BE produced by marine microorganisms. The present study intends screening of the biosurfactants from the eight halobacterial species which were isolated from the solar salt works in Kanyakumari district. Among the eight bacterial isolates that were screened for potential biosurfactant producer(s), *K. marina* BS-15 was found to be able to produce biosurfactant by various screening methods.

2. Materials and methods

2.1. Sampling and isolation of biosurfactant producing bacteria

Brine water had the salinity of 230‰ and 9 pH was collected from the condenser pond of Kovalam (8°05′04.35″ N 77°31′17.07″ E) solar salt works in India. The samples were serially diluted and the decimal dilutions spread on nutrient agar medium containing 5%, 10%, 15% of NaCl with a thin film of crude oil (Himedia, Mumbai, India). Bacterial colonies surrounded by an emulsified halo were identified as a biosurfactant producer, after 37 °C incubation (Morikawa et al., 1992). The biosurfactant producing bacterial colonies were picked up and inoculated into the nutrient broth containing 10% of NaCl. After 24 h, the bacterial cells were harvested by centrifugation at 5000 rpm and were subjected to different biosurfactant screening assays.

2.2. Screening assays for biosurfactant production

The oil displacement test is a method used to determine the surface activity by measuring the diameter of the clear zone, which occurs after dropping a surfactant-containing solution on a thin layer of oil on water. The binomial diameter allows an evaluation of the surface tension reduction efficiency of a given biosurfactant (Rodrigues et al., 2006). Drop-collapse test by adding mineral oil in 96-well microtitre plates (Jain et al., 1991); Emulsification activity by adding kerosene and equal volume of cell free supernatant (Cooper and Goldenberg, 1987) and hemolytic activity in 5% blood agar plate.

2.3. Biosurfactant detection by methylene blue method

Assay was carried out using the method of Jones and Esposito (2000) with some modifications. One milliliter of mineral salt medium culture was vigorously shaken for 30 s with 0.003% methylene blue, and then an equal amount of chloroform was added to the sample. The mixture was left for 20 min to extract the methylene blue anionic surfactant ion pair into chloroform layer. At this point, it is necessary to note that all the blue dye has migrated into the chloroform layer. The tube was centrifuged at 3000 rpm for 5 min. After the extraction with chloroform, the absorbance of each sample was measured at 625 nm against a reference of pure grade chloroform.

2.4. Emulsification activity

The emulsification activity index was measured using the method described by Cooper and Goldenberg (1987) in which 2 ml of the kerosene was added to equal volume of cell free supernatant and homogenized in a vortex at high speed for 2 min. The emulsification stability was measured after 24 h and the emulsification index was calculated by dividing the measured height of the emulsion layer by the total height of the liquid layer and multiplying by 100. The emulsification activity of the strain was compared with the standards including SDS and Tween 80.

$$\text{E24}\ (\%) = \frac{\text{Total height of the emulsified layer}}{\text{Total height of the liquid layer}} \times 100$$

2.5. Phenotypic and genomic identification of BS-15

Based on the higher biosurfactant activity, the BYS2 bacterial strain was identified based on Gram staining, motility, and other biochemical tests according to Bergey's Manual of Systematic Bacteriology (Holt et al., 1994).

One hundred nano gram of genomic DNA was extracted from the BS-15 bacterial strain and the 16S rRNA gene was amplified using the universal primer with standard PCR protocol. The PCR products were purified by Gel extraction kit (Medox Biotech India Pvt. Ltd) and sequenced (Chromos Biotech Pvt. Ltd, Bangalore). The nucleotides of the 16S rRNA sequence were matched with the other microbes in the NCBI database using BLAST program. The construction of phylogenetic tree carried out by Geneious Basic software and evolutionary history were inferred using the neighbor-joining method (Sneath and Sokal, 1973).

2.6. Extraction and partial purification of biosurfactants produced by K. marina BS-15

After the removal of the bacterial cells from the culture media by centrifugation, the obtained supernatant was treated by acidification to pH 2.0 using 6 M HCl solution, and the acidified supernatant was left overnight at 4 °C for the complete precipitation of the biosurfactant. After centrifugation at 8500 rpm for 20 min, the precipitate was dissolved in distilled water at pH 7.0, followed by the biosurfactant extraction step with a solvent having a 65:15 chloroform-to-methanol ratio at room temperature. Then the organic phase was transferred to a round-bottom flask connected to a rotary evaporator to remove the solvent, yielding biosurfactant product. About 1.97 mg of the biosurfactant was extracted per liter of culture medium. The components in the extracted biosurfactant were separated on silica gel 60 plates (Merck) using a solvent system having a 65:25:4 chloroform-to methanol-to-water ratios. The separated components were detected by iodine vapor and UV light exposure (Nitschke and Pastore, 2006).

2.7. Partial characterization

2.7.1. Fourier transmission infra red spectroscopy (FT-IR)

The basic functional groups of the purified Biosurfactants from halophilic *K. marina* BS-15 were analyzed qualitatively by the Fourier transform infra red (FTIR) method described by Kemp (1991).

Table 1 Biosurfactant screening of halophilic bacterial sp. isolated from solar salt works in India.

Halophilic bacterial sp.	Screening methods		
	Drops collapse	Oil displacement	Hemolytic activity
Proteus sp. (Pr)	–	–	–
K. marina BS-15	+ + +	+ +	+ + +
Photobacterium sp. (Pb)	–	–	–
Aerococcus sp. (Ac)	–	–	–
Kurthia sp. (Ku)	+ + +	+ +	+
Coprococcus sp. (Cp)	+ +	+ +	–
Clavibacter sp. (Cb)	–	+	–
Halococcus sp. (Hc)	+ +	+ + +	+ +

+ + +, higher activity; + +: medium activity; +, less activity; –, no activity.

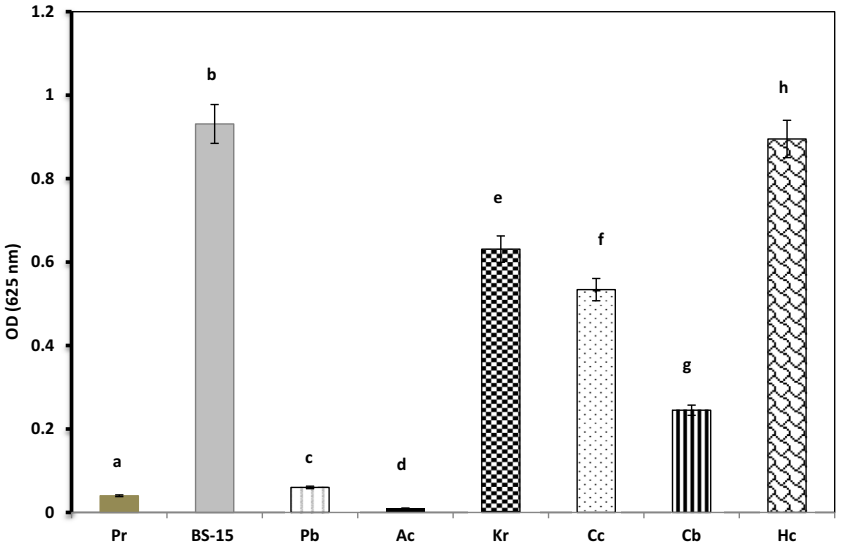


Figure 1 Methylene blue method detection of biosurfactants from *halobacterial* sp. Means with the same superscript do not significantly ($P <= 0.001$) each other's – One Way ANOVA.

2.7.2. Gas chromatography–mass spectrometry (GC–MS)

GC–MS analysis of partially purified biosurfactants was analyzed individually using Agilent GC–MS 5975 Inert XL MSD (United States) gas chromatography equipped with J and W 122–5532G DB-5 ms 30 × 0.25 mm × 0.25 μm and mass detector (EM with replaceable horn) that operated in EMV mode. Helium was used as carrier gas with the flow rate of 1.0 ml min^{-1}. The injection port temperature was operated at 250 °C. The column oven temperature was held at 80 °C for 2 min then programmed at 10 °C min^{-1} to 250 °C, which was held for 0 min, and then at 5 °C min^{-1} to 280 °C which was held for 9 min. Electron impact spectra in positive ionization mode were acquired between m/z 40 and 450.

3. Results and discussion

3.1. Biosurfactant screening from halophilic bacteria

Seven halophilic bacterial sps isolated from the solar salt works were screened for biosurfactant production. Among the strains, *K. marina* BS-15 was highly positive for drop collapse test, oil displacement test and hemolytic activity. The other strain, *Kurthia* sp. (Ku) was highly positive for drop collapse test and *Halococcus* sp. (Hc) was highly positive for oil displacement test. The other strains such as *Proteus* sp. (Pr), *Photobacterium* sp. (Pb), *Aerococcus* sp. (Ac), *Clavibactor* sp. (Cb) failed to produce biosurfactants (Table 1). Satpute et al. (2008) suggested that more screening methods are essential to identify different types of biosurfactants from potential biosurfactant producers. The test conducted in our earlier works (Donio et al., 2013) like drop collapse test, Oil spreading technique and hemolytic activity helped to screen the lipopeptides and polymeric type of biosurfactants from the *Halomonas* sp. BS4. It is also essential to perform different biosurfactant screening methods for the different biosurfactant producing microbes, because biosurfactants are a heterogeneous nature of secondary metabolites with surface active properties. The drop collapse and oil displacement methods are the most effective tools to prove the biosurfactant production in many of the bacterial strains.

3.2. Biosurfactant detection by methylene blue method

The reactivity between biosurfactants and methylene blue is given in Fig. 1. The OD at 625 nm revealed that, the strains of *K. marina* BS-15, *Kurthia* sp. (Ku), *Coprococcus* sp. (Cc) and *Halococcus* sp. (Hc) were able to produce biosurfactants. The strains of *Proteus* sp. (Pr), *Photobacterium* sp. (Pb) and *Clavibacter* sp. (Cb) were unable to produce biosurfactants. Among the bacterial strains, *K. marina* BS-15 and Halococcus sp. had a highly significant ($P <= 0.001$) biosurfactant production when compared to the others. Methylene blue detection is one of the efficient methods to detect anionic surfactants and the biosurfactants produced from microbes react with methylene blue and form anionic surfactant ion pair (Siegmund and Wagner, 1991). This was migrated into the chloroform layer and confirmed the production of biosurfactants in the production medium. Aparna et al. (2012) detected the irhamnolipid type of biosurfactants from *Pseudomonas* sp. 2B using the CTAB–Methylene blue agar medium based method.

Table 2 Emulsification activity (%) of crude biosurfactant from halobacterial strains against oils.

Oils used	Control				Emulsification activity (%)					
	SDS	Tween 80	Pr	BS-15	Pb	Ac	Kr	Cc	Cb	Hc
Crude oil	50.06 ± 0.50	56.04 ± 0.05	27.19 ± 0.26	52.06 ± 0.05	23.15 ± 0.21	25.21 ± 0.33	50.08 ± 0.10	19.15 ± 0.21	21.23 ± 0.20	37.28 ± 0.44
Coconut oil	65.1 ± 0.1	59.27 ± 0.45	32.05 ± 0.04	63.27 ± 0.45	18.12 ± 0.15	17.22 ± 0.33	46.1 ± ' 0.1	22.21 ± 0.33	15.05 ± 0.05	43.05 ± 0.05
Sunflower oil	63.17 ± 0.28	61.24 ± 0.39	29.16 ± 0.20	68.02 ± 0.02	26.29 ± 0.49	28.24 ± 0.39	34.1 ± 0.26	18.30 ± 0.51	27.15 ± 0.22	56.05 ± 0.05
Olive oil	71.25 ± 0.38	68.21 ± 0.33	25.28 ± 0.44	50.43 ± 0.37	15.25 ± 0.38	30.08 ± 0.10	29.30 ± 0.51	27.1 ± 0.16	12.15 ± 0.21	65.12 ± 0.15
Kerosene	52.46 ± 0.41	53.2 ± 0.26	31.15 ± 0.21	37.09 ± 0.10	32.15 ± 0.21	14.17 ± 0.28	43.19 ± 0.26	29.22 ± 0.32	31.17 ± 0.28	70.90 ± 0.27

The values are significantly differed each other's ($F = 18.37$; $P <= 0.001$) – Two Way ANOVA.
Pr, *Proteus* sp.; Km, *K. marina* BS-15; Pr, *Photobacterium* sp.; Ac, *Aerococcus* sp.; Kr–*Kurthia* sp.; Cp, *Coprococcus* sp.; Cb, *Clavibacter* sp.; and Hc, *Halococcus* sp.

3.3. Emulsification activity of biosurfactant

The emulsification activities of the halophilic bacterial strains are given in Table 2. The better emulsification activities (E24) like 70.90% by *Halococcus* sp. (Hc) against kerosene, 68.02% by *K. marina* BS-15 against sunflower oil, 65.12% by *Halococcus* sp. (Hc) against olive oil, 63.27% by *K. marina* BS-15 against coconut oil, 56.06% by *K. marina* BS-15 against crude oil and 56.05% by *Halococcus* sp. (Hc) S8 against sunflower oil were observed. Two way ANOVA revealed that, the values significantly differed from each other's ($F = 18.37$; $P <= 0.001$). The results indicated that the biosurfactant was capable of effectively emulsifying both aromatic and aliphatic hydrocarbons. The present results also revealed that, the hydrocarbon enriched oils and plant oils were the suitable source for oil degradation and biosurfactant production respectively. Emulsification potential of *Rhodococcus* sp. TA6 was studied successfully by Shavandi et al. (2011) using several hydrocarbon substrates as a sole carbon source including pentane to light motor oil. Prieto et al. (2008) have reported that soybean oil, diesel oil, gasoline and cyclohexane are good substrates for emulsification by rhamnolipid-type biosurfactant of *P. aeruginosa* while n-hexane and fish oil result in poor emulsification.

3.4. Phenotypic identification of biosurfactant producing halophilic bacteria

The morphological, biochemical and physiological tests revealed that, the eight strains isolated from solar salt works were *Proteus* sp. (Pr), *Kokuria* sp. (Km), *Photobacterium* sp. (Pb), *Aerococcus* sp. (Ac), *Kurthia* sp. (Ku), *Coprococcus* sp. (Cp), *Clavibacter* sp. (Cb) and *Halococcus* sp. (Hc) etc (Table 3). Among these bacterial strains, *Kokuria* sp. (Km) was highly positive for biosurfactant production whereas *Kurthia* sp. (Ku) and *Halococcus* sp. (Hc) were moderately positive for biosurfactant production. *Kokuria* sp. (Km) was a Gram positive cocci, non motile, positive for indole, methyl red and VP tests. Also they ferment glucose, sucrose and fructose etc. *Kocuria* spp. are the members of the *Micrococcaceae* family that are frequently found in the environment (Lee et al., 2009). Kim et al. (2004) isolated and identified the *K. marina* KMM 3905T from Troitsa Bay marine sediment. This strain grows in the temperature range of 4–43 °C, tolerates maximum of 15% NaCl, positive for urease and nitrate reduction, negative for oxidase and alkaline phosphatase, and negative for acid production from glucose, lactose, or sucrose. At present the genus *Kocuria* comprises of 18 species with validly published names (http://www.bacterio.cict.fr/k/kocuria.html) and all these species have been isolated from different environmental sources including *K. rosea*, *K. varians* and *K. kristinae* (Stackebrandt et al., 1995), *K. himachalensis* (Mayilraj et al., 2006), *K. halotolerans* (Tang et al., 2009) and *K. assamensis* (Kaur et al., 2011).

3.5. Genomic identification of K. marina BS-15

Phylogenetic and evolutionary analysis of the 16S rRNA sequence revealed that, *K. marina* BS-15 shared high similarity to other *K. marina* strains. *K. marina* BS-15 also had high similarity to the strains of *K. marina* RB-210, *Kocuria* sp. QW12 and *K. marina* S48 (Fig. 2). The strain was deposited in NCBI database. The strain name and GenBank accession number are *K. marina* BS-15, KC594576.1 respectively. *K. marina* is also most closely related to *K. rhizophila* DSM 11926, *K. varians* DSM 20033, and *K. carniphila* CCM 132 based on the report by Kim et al. (2004) and constructing phylogenetic analysis using 16S rRNA gene sequences. However, *K. marina* forms an independent phylogenetic lineage within *Kocuria* (Tvrzova et al., 2005).

Table 3 Phenotypic identification of biosurfactant producing *Halobacterial* sp. from Kovalam solar salt works.

Test	Isolates							
	Pr	BS-15	Pb	Ac	Kr	Cc	Cb	Hc
Gram staining	–	+	–	–	+	–	+	+
Cell shape	Long rod	Cocci	Shot rod	Cocci	Rod	Cocci	Cocci	Cocci
Motility	Motile	Non motile	Non motile	Non motile	Motile	Non motile	Non motile	Non motile
Indole	+	+	–	–	–	–	–	+
Methyl red	+	+	–	+	+	+	+	+
VP	+	+	–	–	–	+	–	+
Citrate	+	+	–	+	+	–	–	+
Oxidase	+	–	–	–	+	–	–	–
Catalase	–	+	–	–	+	–	–	+
Nitrate	–	+	–	–	–	+	–	+
Urease	+	+	–	–	+	+	–	–
TSI	+	+	+	+	+	–	–	–
Gelatin	–	–	–	–	–	–	–	–
Starch	–	–	–	–	–	–	–	–
CHO fermentation								
Glucose	–	+	–	–	–	+	+	+
Sucrose	–	+	–	–	–	+	+	–
Galactose	–	–	–	–	+	+	–	+
Maltose	–	–	–	–	+	–	–	–
Fructose	–	+	–	–	–	–	–	+

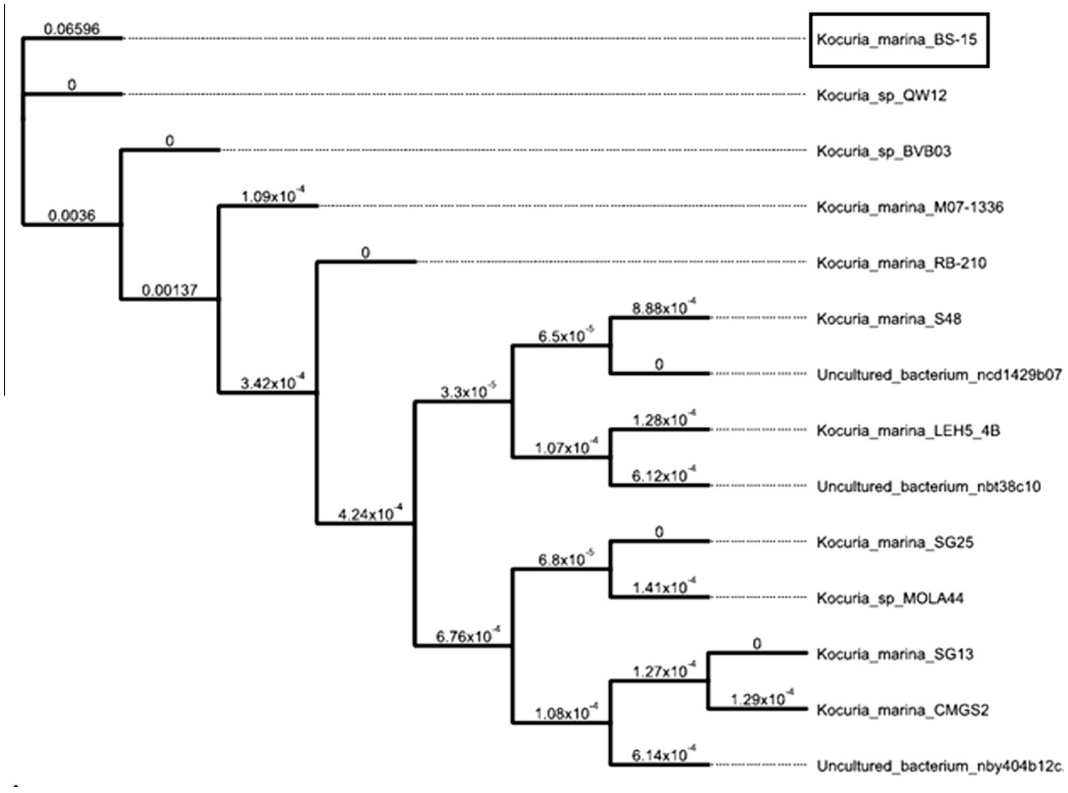


Figure 2 Graphical phylogenetic tree analysis of *Kocuria marina* BS-15 based on 16S rRNA gene sequence data compare with other sp.

3.6. Purification of K. marina BS-15 yielding biosurfactan

Thin layer chromatography (TLC) data revealed a single spot with R_f value of 0.65 under UV detection. Based on the R_f value, the spot was concluded as a lipid moiety containing the compound of lipopeptide (Fig. 3). This preliminary result suggests that the partially purified biosurfactant produced by *K. marina* BS-15 should contain a lipopeptide. Anyanwu et al. (2011) confirmed in their study the TLC data with the R_f value of 0.68 and 0.70 after iodine treatment as lipopeptide. Our earlier study Donio et al. (2013) also confirmed that the biosurfactant extracted from halophilic Bacillus BS-3 had the R_f value of 0.68 as lipopetide type. Study conducted by Vater et al., 2002 also substantiated one surfactant with the R_f values of 0.62 as lipopeptide.

3.7. Partial characterization of biosurfactant

The FTIR analysis (Fig. 4) revealed that the peak at 491 cm^{-1} is due to C–I (Carbon–Iodine) bond. The peak at 605 cm^{-1} and the peaks at 651 and 671 cm^{-1} confirm the presence of C-Br. The peaks at 2084 and 3147 cm^{-1} are due to the presence of cumulated system $R_2C{=}N{=}N$ in the sample. Also, an absorption band at 993 cm^{-1} showed stretching mode of the $RCH{=}CH_2$ indicating the presence of alkenes. The stretch, 3429 and 2360 cm^{-1} denoted as the N–H group. The transmittance around at 1400 cm^{-1} referred to the aliphatic chain of the C–H group. An intense stretching peak 1159, 1537 and 1626 cm^{-1} indicates the presence of RNO_2 groups. The availability of all these functional groups firmly substantiated that

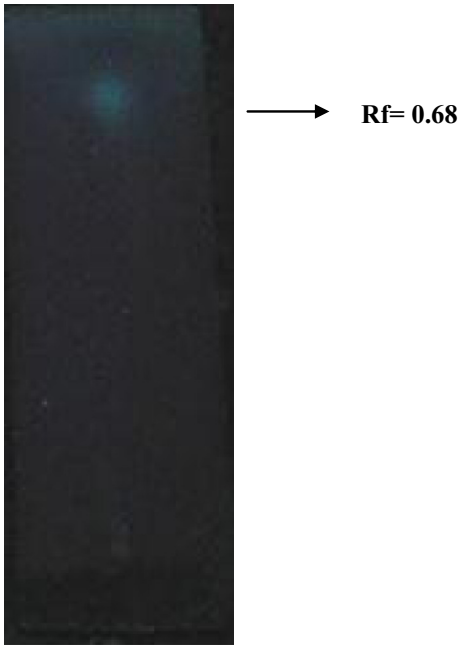


Figure 3 Thin layer chromatography analysis of *Kocuria marina* BS-15 biosurfactants.

the biosurfactant is a peptide nature. Our previous work (Donio et al., 2013a) also confirmed that, the lipopeptide type of biosurfactant isolated from halophilic *Bacillus* sp. BS-3 had the IR stretch of 3429 cm^{-1} donated N–H group and the other peak at 2084 cm^{-1} corresponds to cumulated system like $R_2C{=}N{=}N$ in the sample.

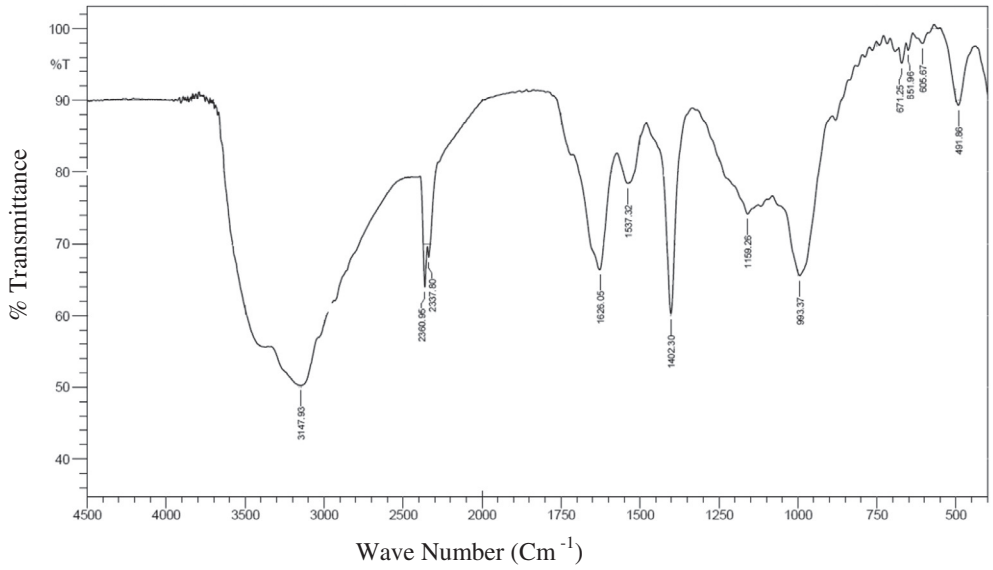


Figure 4 FT-IR spectrum of the partially purified biosurfactant produced by the halophilic bacteria *Kocuria marina* BS-15.

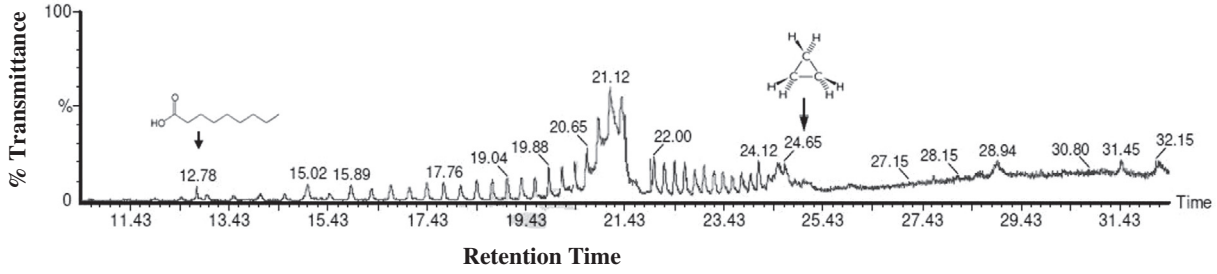
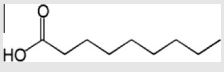
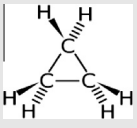


Figure 5 GC analysis of partially purified biosurfactant from halophilic *Kocuria marina* BS-15.

The GC–MS analysis (Fig. 5 and Table 4) characterized, two lipid based compounds that had the biosurfactant properties. The peak at the retention time of 12.78 was confirmed as Nonanoic acid with the molecular weight of 148 and formula of $C_9H_{18}O_2$. Findings by Kiran et al. (2010a) supported that the surface active compound produced by *Nocardiopsis lucentensis* MSA04 was characterized by GC–MS analysis as glycolipid with a hydrophobic non-polar hydrocarbon chain (nonanoic acid methyl ester) and hydrophilic sugar, 3-acetyl 2,5 dimethyl furan reported by Kiran et al. (2010a). Lipopeptide families of biosurfactant including nonanoic acid, 9-oxo-, methyl ester and brevifactin were characterized by GC–MS analysis in *Brevibacterium aureum* MSA13 (Kiran et al., 2010b). The lipid based compound Cyclopropane (molecular weight 42.08; formula C_3H_6) was also detected with the peak having the retention time of 24.65. Cyclopropane ring-containing lipids, especially in phospholipids and glycolipids, were reported for many bacteria (Mizoguchi et al., 2013). *Escherichia coli*, whose cell membranes were dominantly composed of cyclopropane ring containing phospholipids and

Table 4 Biosurfactant characterized from *Kocuria marina* BS-15 by GC–MS analysis.

Retention time	Name of the compounds	Molecular formula	Molecular weight	Molecular structure
12.78	Nonanoic acid	$C_9H_{18}O_2$	158	
24.65	Cyclopropane	C_3H_6	42.08	

fatty acids (Brown et al., 1997). Two polycyclopropane fatty acid derivatives, FR-900846 (2) and U-106305 (3) were isolated from *Streptoverticillium fervens* and *Streptomyces* (Grogan and Cronan, 1997). Dubey et al. (2012) also confirmed the biosurfactant production in *K. turfanesis* strain-J using curd whey as substrates. The present study revealed that, the search for novel biosurfactants in extremophiles seems to be particularly promising since they have particular adaptations like increased stability in adverse environments and the microbial products are highly stable and important in various fields. These halo bacterial biosurfactants from solar salt works will help to develop more valuable eco friendly pharmacological products to the pharmacological industries.

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