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DIVERSITY OF BIOSURFACTANT-PRODUCING *STREPTOMYCES* ISOLATES FROM HYDROCARBON-CONTAMINATED SOIL

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ABSTRACT

Actinomycetes are unsurpassed as producers of bioactive metabolites, primarily those with antimicrobial/anticancer properties; however, their capacity for producing other natural products such as biosurfactants, has been less explored. Biosurfactants are heterogeneous surface active compounds and can be potentially deployed for bioremediation of hydrocarbons and as emulsifying agents in agriculture, pharmaceutical and food industries. In this study, *Streptomyces* was selectively isolated and characterized from hydrocarbon-contaminated soil. The isolates were dereplicated based on morphology and antimicrobial properties and representative isolates screened for biosurfactant production using drop-collapse test, emulsification assay and bacterial adherence to hydrocarbons (BATH) test. *Streptomyces* sp. PN-18 exhibited high antimicrobial activity, significant cell-surface hydrophobicity (>95%) and emulsification activity (>70 EU mL^{-1}). The best production medium was found to be tryptone soy broth in which emulsification activity increased to 110 EU mL^{-1} with emulsification index (E_{24}) of 65%. Nearly complete 16S rDNA sequence analysis (GenBank accession GQ856644) of PN-18 showed similarity to *S. rochei* (97.9%), though with significant differences in morphological and physiological characteristics. The study demonstrates *Streptomyces* diversity in hydrocarbon-impacted environments and the possibility of exploiting PN-18 for bioremediation.

KEYWORDS: ***Streptomyces,*** **biosurfactant, hydrocarbon-contaminated soil, bioremediation**

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INTRODUCTION

Oil spills are one of the main environmental disasters globally. Major areas contaminated due to fuel spillage include marine habitats, areas near refineries and petrol stations or during transportation[1]. Crude oil and its derivatives are composed mainly of aliphatic and aromatic hydrocarbons, naphthalenes and polycyclic aromatic hydrocarbons. There is deterioration of biochemical and physico-chemical properties of contaminated soils which also adversely affects growth of plants[2]. Reclamation of such soils can be carried out using physical, chemical and biological methods[1]. The first two methods are not cost-effective and can lead to incomplete decomposition. Biologically, petroleum hydrocarbons can be degraded by microorganisms involving mainly two mechanisms. The first mechanism involves adhesion of cells to large oil drops and the second method is pseudosolubilization involving the cellular assimilation of emulsified small oil droplets mediated by biosurfactants[3]. Biosurfactants, a heterogeneous group of surface active compounds produced by many organisms, reduce surface and interfacial tension at the hydrocarbon-water interface resulting in pseudosolubilization. They belong to various classes such as glycolipids, lipopeptides, fatty acids, phospholipids, neutral lipids and lipopolysaccharides. These compounds have garnered attention due to biodegradability, low toxicity, and production from renewable resources as well as being functionally active even under extreme conditions[4].

Reports on biosurfactant production have been increasing since 1980s, with more than 150 publications in 2010 on the Thomson Reuters ISI Web of Knowledge search platform. While the most prolific microbial producers of natural products have been actinomycetes (*Streptomyces* in particular) and fungi and the former has been exploited for production of antibiotics and anti-tumour compounds, comparatively less data are available on their biosurfactant-production capability. *Streptomyces* are generally saprophytic soil-dwellers and non-pathogenic except for *S. somaliensis* which is known to cause actinomycetoma[5]. This feature, in combination with the production of bioactive metabolites has made these organisms attractive for industrial production of such products. *Streptomyces* strains isolated from the Kuwait Burgan oil field have been shown to utilise *n*-hexadecane, *n*-octadecane, kerosene, and crude oil as sole carbon and energy sources[5]. *Rhodococcus erythropolis, R. aurantiacus* and *Nocardia erythropolis* have been reported to produce surface-active lipids[6]. *S. rochei,* isolated from bitumen soil, could degrade 3–4 ring poly aromatic hydrocarbons (PAH) and also produced biosurfactant[7]. Biosurfactant from marine *Nocardiopsis* B4[8] reduced surface tension to 29 mN/m and showed emulsification index of 80% in 6-9 days. There is necessity to screen for new biosurfactants with strong interfacial activity, lower critical micelle concentration (CMC), better emulsion capacity, and activity in a broad pH/temperature range and discover production strains with high yields[9]. In conjunction with these aims, the current study was undertaken to select and characterize biosurfactant-producing *Streptomyces* isolates from local hydrocarbon-contaminated soil (near petrol station) and evaluate emulsification properties of the produced biosurfactant(s).

MATERIALS AND METHODS

1. Physicochemical characteristics of soil sample

Soil near two petrol pumps in Noida, Uttar Pradesh, contaminated due to frequent spillage or dumping of excess petrol/diesel, was chosen as a potential site for selective isolation of *Streptomyces* isolates. Soil samples were collected from the top 10 cm using a sterile scoop, placed in sterile polycarbonate bottles and sealed. These samples were subsequently pooled together and stored at 4^{o}C before further analysis. 2 g of the soil sample was mixed with 10 ml of distilled water, shaken vigorously, allowed to

stand for 10 min and filtered. pH, conductivity, salinity and Total Dissolved Solids (TDS) measurements of the filtrate were taken on Eutech CON 2700.

2. *Selective isolation and characterization of putative Streptomyces*

Serial dilutions of the soil samples (10^{-1} to 10^{-2}) were prepared in 0.9% NaCl solution and 150 µL plated on Starch Casein Nitrate (SCN) medium[10] as well as on modified SCN media containing 1% petrol or diesel instead of starch. The media were augmented with cycloheximide (100 µg mL^{-1}) and tetracycline (20 µg mL^{-1}) and solidified with 1.5% agar. Petrol/diesel was added to the plate by two methods - either spread on the surface of the solidified media or Whatman filter paper soaked with petrol/diesel were put on the lid of the inverted petriplate containing solidified media spread with soil dilutions[11]. The plates were incubated for two weeks at 37°C. Appropriate biosafety procedures were followed. The broad work flow is presented in figure 1.

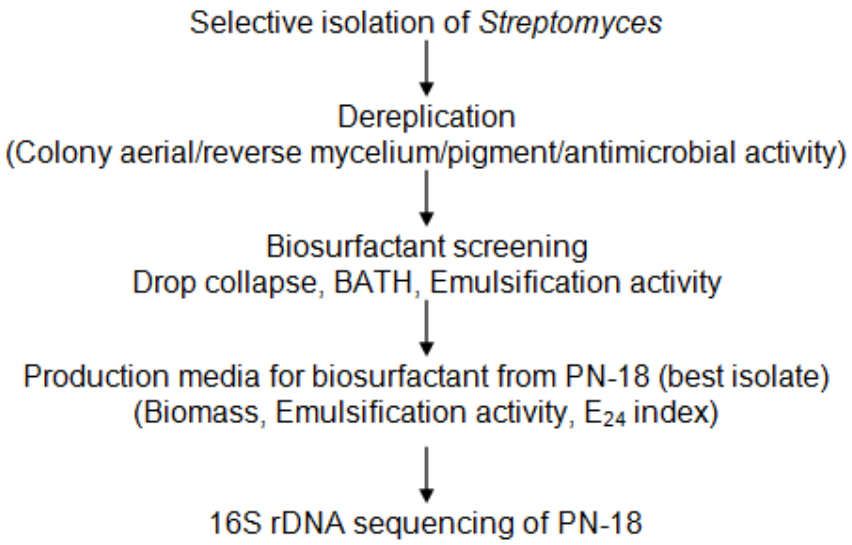

Figure 1
Work flow and methodology of current study.

Colonies with typical actinomycetes morphology[12] were Gram stained and observed under the microscope. Representative Gram positive colonies were scored for the following characteristics: colour of aerial and reverse substrate mycelia, texture (chalky, leathery), shape of the colony (raised, flat), presence of diffusible pigments, any moist secretion and clear zone formed around colonies in original plates indicating inhibition of other bacteria/fungi. Pure cultures were maintained by streaking putative *Streptomyces* colonies on Bennett's medium (in g L^{-1} beef extract 1.0, yeast extract 1.0, glucose 10.0, peptone 2.0, agar 15.0). The isolates were identified by alphanumerical coding. Anti-microbial activity, in the context of biosurfactant screening[9,37], was assessed based on modified Kirby-Bauer's method[13]. Plugs of 6 mm diameter were collected from 14 days old *Streptomyces* cultures using a cork borer and placed on Mueller-Hinton agar seeded with target microorganisms. *Bacillus subtilis* [MTCC 121], *Escherichia coli* [MTCC 1673], *Pseudomonas putida* [MTCC 2445], *Agrobacterium rhizogenes* [MTCC 532], *Agrobacterium tumefaciens* [MTCC 431] and *Micrococcus luteus* [MTCC 106] were the target bacteria; *Rhizopus oryzae* [MTCC 554] and *Penicillium funiculosum* [MTCC 2552] were the target fungi. Plates were first kept at

4°C for 2 hours to facilitate diffusion of any produced antimicrobial compounds and then incubated at 30°C. Inhibition zones were determined after 24 hours.

3. Numerical taxonomy

The isolates were dereplicated on the basis of colony characteristics and anti-microbial profile using numerical taxonomy software NTSys v.2.1[14]. Twenty unit characters (qualitative and quantitative) for each isolate were coded, hierarchical cluster analysis was performed, coefficients clustered by Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm and dendrogram were generated[15].

4. Screening for biosurfactant production

Representative isolates (at least one) from each cluster were screened for biosurfactant production. One culture plug of each representative isolate, grown on Bennett's medium, was inoculated in 5 ml of Bennett's broth and incubated at 30°C at 150 rpm in shaker for 7 days, centrifuged at 10,000 rpm and cell-free supernatant obtained was used for biosurfactant production tests. Positive controls used were 0.1% SDS and cell-free supernatant of *Bacillus subtilis* (MTCC 2423) and *Pseudomonas putida* (MTCC 2445), which are known to produce biosurfactant. The following screening tests were performed:

a. Drop Collapse Test: Cell-free supernatant (20 µl) was mixed with 5 µl of methylene blue, spotted onto Parafilm, analyzed for destabilization[16] and quantitatively assessed for diameter of the drops after 1 min[17]. Positive result for biosurfactant production was indicated when the drop diameter was at least 1 mm larger than that produced by negative control (deionized water)[4].

b. Bacterial Adhesion to Hydrocarbons (BATH) Test: Cell surface hydrophobicity, to investigate the affinity of cells to a hydrophobic solvent, was measured[18]**.** Cultures of *B. subtilis* and *P. putida* grown for 48 hour were used as positive controls.

c. Emulsification activity: The amounts of biosurfactants in cell-free supernatants were determined[6]. Sterile medium was used as a blank. An absorbance of 0.01 units at 400 nm was considered as one unit of emulsification activity per mL (EU mL^{-1}).

d. Emulsification capacity (E_{24} index): Emulsification index (E_{24}) of cell-free supernatant was determined according to Cooper and Goldenberg[19].

5. Production of biosurfactants in various media formulations

Based on the results of screening tests, isolate PN-18 was used to investigate suitability of various media formulations for optimal production of biosurfactant. PN-18 was grown individually for 14 days in nutrient broth ((NB1), tryptone soya broth (TSB1) and yeast extract-malt extract broth (YM1), and each of the above media supplemented with 0.1% glycerol (NB2, TSB2 and YM2) individually. Nutrient broth was selected for comparison as it is a general purpose medium while the other two media have been reported for production of biosurfactant/surfactant-like molecules[20-21]. Glycerol has been reported to stimulate sporulation and secondary metabolite production and hence was added to the above media[22]. After 14 days, the culture was centrifuged and mycelial biomass (dry weight) estimated. Supernatant collected was used to test emulsification activity and E_{24} index using petrol, diesel and n-heptane as described above.

6. 16S rRNA gene sequence

The genomic DNA of PN-18 was isolated and 16S rDNA fragment was amplified by PCR. Primers and reaction conditions were as per Shanker et al. (2010)[15]. Consensus sequence of 16S gene was compared and aligned with those of Gram positive bacteria (type strains only) in the Ribosomal Database Project. Software Mega 4.0[23] was used to construct phylogenetic trees using neighbour-joining treeing algorithm[24]. Bootstrap consensus tree inferred from 1000 replicates was evaluated. Nearly complete 16S rDNA sequence (1,405 nucleotides) of strain PN-18 has been deposited in the GenBank database under accession number GQ856644

(http://www.ncbi.nlm.nih.gov/nuccore/GQ856644).

7. Statistical analyses

All data presented are mean values of three determinations. Statistical comparisons, where presented, were done using Student's T-test with significance assumed if $P<0.05$ using software COSTAT. Each experiment was repeated thrice.

RESULTS AND DISCUSSION

1. Physico-chemical characteristics of soil sample

Soil around two fuel stations in NOIDA, contaminated with petrol/diesel, was collected and analysed for physico-chemical parameters (pH, conductivity, salinity and TDS). The physico-chemical data indicated that the soil sample was slightly alkaline in nature (Table 1). Electrical conductivity of 13 mS indicates high salinity and elevated concentration of ions. Interpreting these physico-chemical data in overall correlation with detection of biosurfactant-producing microorganisms, it is known that organic acids and biosurfactants from microbes can also contribute to increase in conductance[25], while fresh hydrocarbon-contaminated ground water can show lowered conductivity due to the resistivity of the unaltered hydrocarbon[26]. The TDS values reported in this study (7743 mg L^{-1}) are much higher than that reported in other studies (800 mg L^{-1})[27] but are consistent with earlier reports of overall higher TDS in hydrocarbon-contaminated aquifers[28] and with conductivity values obtained in this report. Taken together, the high conductivity, salinity and TDS values possibly indicate that continual hydrocarbon contamination of soil could be concomitantly associated with increased weathering of minerals resulting from biodegradation by associated soil microflora[27].

Table 1
Physico-chemical characteristics of hydrocarbon-contaminated soil sample.

Characteristic	Value (Mean ± SD) n=3
pH	8.02 ± 0.01
Conductivity (mS)	13.83 ± 0.11
Salinity (%)	7.96 ± 0.05
Total Dissolved Solids (mg L^{-1})	7743.33 ± 51

2. Selective isolation and characterization of Streptomyces sp.

Soil samples were serially diluted and plated on petrol/diesel media, incubated for two weeks and putative actinomycete isolates used for further studies. We obtained around 100 isolates out of which 20 representative isolates, were selected on the basis of aerial/reverse mycelia characteristics and production of diffusible pigments, moist secretions, and/or any inhibition zone against other non-specific microorganisms growing in respective parent plates during the isolation step. In some cases, morphologically similar isolates were also selected but these were obtained from different dilution plates and thereby presumed to be potentially different clones and coded accordingly (Table 2). They were classified into two major groups: gray and white[29] comprising 55% and 45% respectively of the total isolates. Most of these isolates had raised colonies with a chalky texture.

Table 2
***Characteristics of Streptomyces isolates obtained from hydrocarbon-contaminated* soil.**

Colony	Aerial mycelium color	Substrate mycelium Color	Raised / Flat Colony	Texture of Colony	Diffusible Pigments	Moist Secretion	Zone of inhibition
PN-1	White	Pale yellow	Raised	Chalky	None	None	None
PN-2	White	Pale Yellow	Flat	Leathery	None	None	None
PN-3	White	Pale yellow	Raised	Chalky	None	None	None
PN-4	Gray	Dark brown	Raised	Chalky	None	None	None
PN-5	White	Pale Yellow	Flat	Leathery	None	None	None
PN-6	Gray	Off-white	Raised	Chalky	None	Yes	None
PN-7	Gray	Dark brown	Raised	Chalky	None	None	None
PN-8	Gray	Dark brown	Raised	Chalky	None	None	None
PN-9	Gray	Dark brown	Raised	Chalky	None	None	None
PN-10	Gray	Dark brown	Raised	Chalky	None	None	None
PN-11	Gray	Dark brown	Raised	Chalky	None	None	None
PN-12	White	Grayish white	Raised	Chalky	None	Yes	None
PN-13	White	Grayish white	Raised	Chalky	None	Yes	None
PN-14	Gray	Dark brown	Raised	Chalky	None	None	Yes
PN-15	Gray	Dark brown	Raised	Chalky	None	None	Yes
PN-16	Gray	Dark brown	Raised	Chalky	None	None	Yes
PN-17	Gray	Dark brown	Raised	Chalky	None	None	Yes
PN-18	Gray	Dark brown	Raised	Chalky	None	None	Yes
PN-19	Gray	Dark brown	Raised	Chalky	None	None	None
PN-20	Gray	Dark Brown	Flat	Leathery	None	None	None

Antimicrobial activity against specific target microorganisms was tested using culture plugs of isolates. Results showed that 45% of the isolates showed anti-bacterial activity (Table 3). This included isolates PN-1, 4, 8 and 13, which did not produce inhibition zones in parent plates. Most of the target organisms were sensitive to PN-14. PN-1 and PN-4 displayed only anti-fungal activity, while PN-15, 16 and 17 exhibited only anti-bacterial activity. Isolates PN-14 to PN-18 were morphologically similar but demonstrated variations in their activities against target microorganisms.

Table 3
***Antimicrobial activity of the Streptomyces isolates*.**

Isolate	Zone of Inhibition (cm) Mean ± SD							
	E. coli	***B. subtilis***	***P. putida***	***A. rhizogenes***	***A. tumefaciens***	***M. luteus***	***R. oryzae***	***P. funiculosum***
PN-1	0	0	0	0	0	0	0	1.8±0.01
PN-4	0	0	0	0	0	0	1.5±0.12	
PN-8	0	1.6±0.01	2.0±0.02	0	0	1.70±0.12	0	2.4±0.03
PN-13	0	2.0±0.02	0	0	0	0	0	0
PN-14	1.47±0.06	1.74±0.2	1.96±0.07	1.05±0.04	0	2.30±0.13	3.1±0.03	0
PN-15	2.13±0.06	1.89±0.33	1.34±0.13	0.96±0.02	1.9±0.1	0	0	0
PN-16	2.33±0.06	1.5±0.07	1.11±0.06	1.18±0.03	0	0	0	0
PN-17	2.43±0.13	1.76±0.1	1.53±0.12	1.05±0.04	1.23±0.09	0	0	0
PN-18	2.45±0.09	1.29±0.05	1.4±0.01	0	2.50±0.04	0	2.0±0.03	0

3. Dereplication

Based on morphology and anti-microbial properties, data was analysed with software NTSys. These twenty isolates were clustered into four distinct groups as seen from Figure 2. While dereplication of *Streptomyces* based on colour grouping has repeatedly shown congruence[30-31], narrowing down of isolates from a large population sample based only on colony morphology in the absence of other properties (biochemical tests and/or bioactivity) could be misleading. As seen from the results of this study, inclusion of bioactivity profile as additional criterion is recommended since these organisms are pre-eminent as producers of natural products.

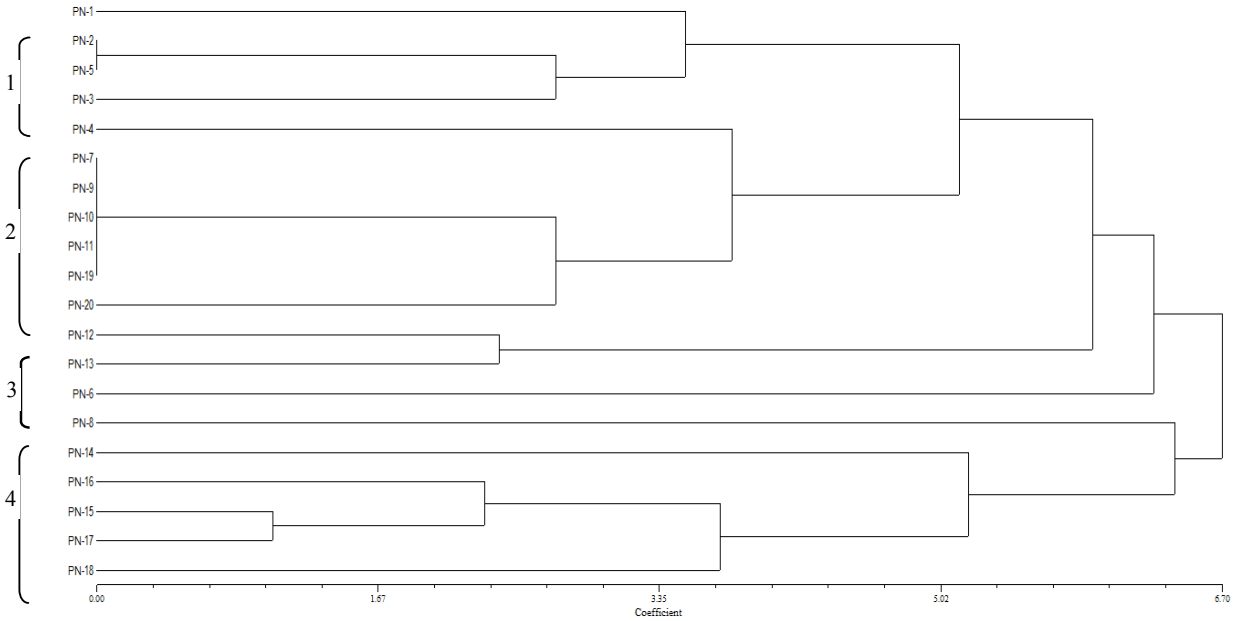

Figure 2

Dendrogram of Streptomyces isolates obtained from hydrocarbon-contaminated soil showing the relationships between isolates based on UPGMA analysis.

4. Screening for biosurfactant production

Representative isolates tested for biosurfactant production showed positive results. Crude cell-free supernatant from all the isolates except PN-6 showed collapsing of drops (Table 4). The values ranged from 0.41 to 0.49 cm, comparable to those of bacterial positive controls (*B. subtilis* - 0.49 and *P. putida* – 0.46). In the BATH assay using different hydrocarbons (petrol, diesel and heptane), all the isolates displayed varying degrees of hydrophobicity towards hydrocarbons thereby showing affinity of microbial cells for the hydrocarbon substrate to possibly facilitate its bioavailability. PN-18 showed significantly higher cell surface hydrophobicity towards all the tested hydrocarbons followed by PN-12. PN-6 also showed relatively high hydrophobicity values but no collapsing of drops. While the BATH test has been reported to be effective as an indirect screening method for biosurfactant-producing microbes[9] we have observed a correlation between isolates showing hydrophobicity and biosurfactant production.

Table 4
Screening for biosurfactant production based on drop collapse and cell surface hydrophobicity (BATH test).

Isolate	Diameter of drops (cm) Mean±SD	Hydrophobicity (%) Mean±SD		
		Petrol	Diesel	Heptane
PN-4	0.46±0.01^{b}	78.48±0.17^{d}	96.89±0.11^{a}	73.73±0.21^{e}
PN-6	0.3±0.01^{a}	79.88±0.83^{c}	93.80±0.28^{b}	96.70±0.14^{b}
PN-8	0.48±0.02^{b}	43.72±1.18^{f}	10.06±2.79^{g}	92.99±0.22^{c}
PN-12	0.49±0.01^{b}	95.44±0.65^{b}	93.18±0.07^{b}	96.69±0.14^{b}
PN-14	0.48±0.03^{b}	52.57±0.27^{e}	41.59±0.78^{d}	57.80±0.62^{f}
PN-15	0.45±0.10^{b}	22.75±0.42^{h}	12.23±0.42^{f}	90.62±0.94^{d}
PN-16	0.42±0.20^{b}	37.62±0.54^{g}	14.48±0.41^{e}	55.96±0.12^{g}
PN-17	0.44±0.02^{b}	78.57±0.10^{d}	90.85±1.18^{c}	58.62±0.13^{f}
PN-18	0.46±0.01^{b}	97.89±0.47^{a}	96.66±0.61^{a}	98.20±0.17^{a}
B. subtilis	0.49±0.01^{b}	22.32±0.71^{h}	42.17±1.01^{d}	45.53±1.27^{h}
P. putida	0.46±0.20^{b}	7.69±0.74^{i}	40.62±0.88^{d}	23.83±1.02^{i}
SDS	0.60±0.20^{c}	-	-	-
Deionized water	0.3±0.01^{a}	-	-	-

Means within a column followed by same superscript are non-significant at p<0.05.

Further, the emulsification activity of these isolates was tested in presence of petrol, diesel and n-heptane (Figure 3). PN-18 showed uniformly high emulsification activity against all tested hydrocarbons (more than 70 EU mL^{-1}) while PN-6 and PN-12 demonstrated values above 70 EU mL^{-1} for selected hydrocarbons only. Other isolates showed an average value less than 60 EU mL^{-1}. In a screening study, Satpute et al.[32] had shown that marine bacteria (grown on Zobell Marine Media) emulsify petrol and xylene with values from as low as 2.5 up to 187.5 EU mL^{-1}. The emulsification assay helps in selection of biosurfactant/bioemulsifier producer strains and has been recommended for screening potential producers[33].

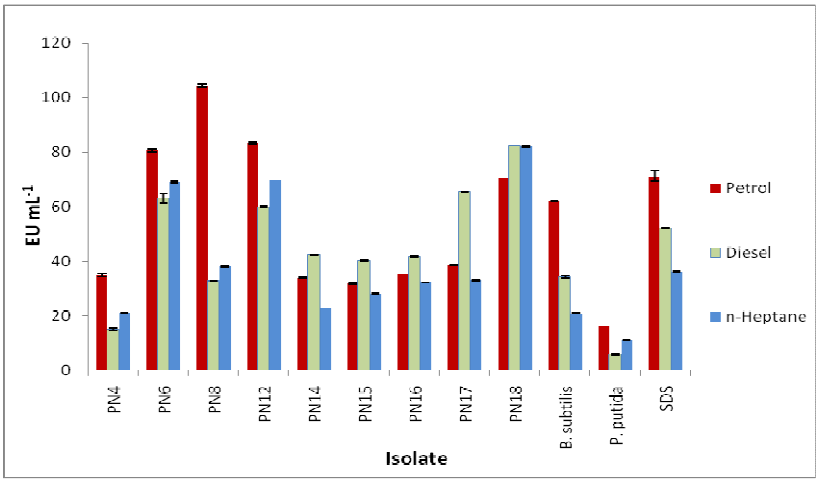

Figure 3
Emulsification activity (EU mL^{-1}) of Streptomyces isolates obtained from hydrocarbon-impacted soil. Error bars represent respective standard deviation values.

5. Biosurfactant production media

Isolate PN-18 showed highest hydrophobicity and emulsification activity and hence was chosen for further studies. General purpose media (nutrient broth) and media reported for biosurfactant production (tryptone soy broth and yeast extract-malt extract broth) were used for this purpose. Growth was not observed in YM2. Tryptone Soy Broth (TSB1) was found to elicit best emulsification activity (Table 5) when tested with various hydrocarbons. Emulsification index after 24 h (E_{24}), indicating the stability of the formed emulsified layer, was also highest (65.9%) in this medium. According to recent reports, *Bacillus* and *Nocardiopsis* isolates showed more than 2-fold variation in emulsification activities (less than 100 to above 200 EU mL^{-1}) depending on medium composition[34,8]. Biomass data, when compared with emulsification activity, showed that least biomass increase was seen in TSB1 concomitant with maximum emulsification activity, thereby indicating the diversion of biosynthetic pathways towards biosurfactant production. Hence we conclude that emulsification activity depends primarily on eliciting conditions, which is chiefly the medium composition.

Table 5

Change in biomass in relation to emulsification activity of Streptomyces sp. PN-18 in various media after 14 days.

Medium[#]	Biomass (g) Mean ± SD	Emulsification activity (EU mL^{-1}) Mean ± SD			E_{24} index (%) with n-heptane[*] Mean ± SD
		Petrol	Diesel	n-heptane	
NB1	0.198±0.01[b]	8.5±0.01[k]	51.7±0.1[g]	34.0±0.4[h]	48.00±0.1[c]
NB2	0.174±0.01[c]	34.1±0.1[h]	76.7±0.1[e]	27.2±0.1[i]	47.37±0.1[c]
TSB1	0.129±0.01[e]	110.1±0.4[a]	101.2±0.2[b]	82.6±0.02[d]	65.90±0.2[a]
TSB2	0.143±0.01[d]	87.7±0.1[c]	65.8±0.01[f]	69.0±0.1[f]	61.36±0.1[b]
YM1	0.226±0.002[a]	80.3±0.2[d]	23.1±0.2[l]	19.9±0.1[J]	50.00±0.1[c]

Means within a column followed by same superscript are non-significant at p<0.05.

[#]NB1: Nutrient Broth; NB2: Nutrient Broth + 0.1% glycerol; TSB1: Tryptone Soy Broth; TSB2: Tryptone Soy Broth + 0.1% glycerol; YM1: Yeast Malt Extract.

[*]Control was 0.1% SDS showing E_{24} index of 60%.

6. 16S rRNA gene analysis of PN-18

To identify isolate PN-18, 16S rRNA gene sequence (1,405 nucleotides) was determined. From the primary sequence analysis, PN-18 showed the maximum sequence similarity value of 97.9% with *Streptomyces rochei.* PN-18 formed a definite sub-clade (bootstrap value of 58%) with *S. rochei* and *S. vinaceusdrappus* (Figure 4). Morphological and physiological data showed that PN-18 shares some similar features with *S. rochei*[35]. Gray aerial and yellow substrate mycelia on salts-starch agar, lack of production of melanoid pigments and low/no growth on raffinose are characteristic of *S. rochei*. PN-18 exhibited certain differences from *S. rochei* such as dark brown substrate mycelia and growth on raffinose as sole carbon source. Moreover, biosurfactant production is not a characteristic feature of *S. rochei* and has been documented in only one report[7], where the isolate was obtained from bitumen soil and produced biosurfactant with emulsification index of 55%. Further information on this carbohydrate biosurfactant was not detailed. PN-18 has been isolated from hydrocarbon-impacted soil and produces biosurfactant with higher emulsification activity of 110 EU mL^{-1} and E_{24} index of 65%. Further studies (Fatty Acid Methyl Ester profile and DNA-DNA hybridization) can conclusively suggest taxonomic placement of PN-18.

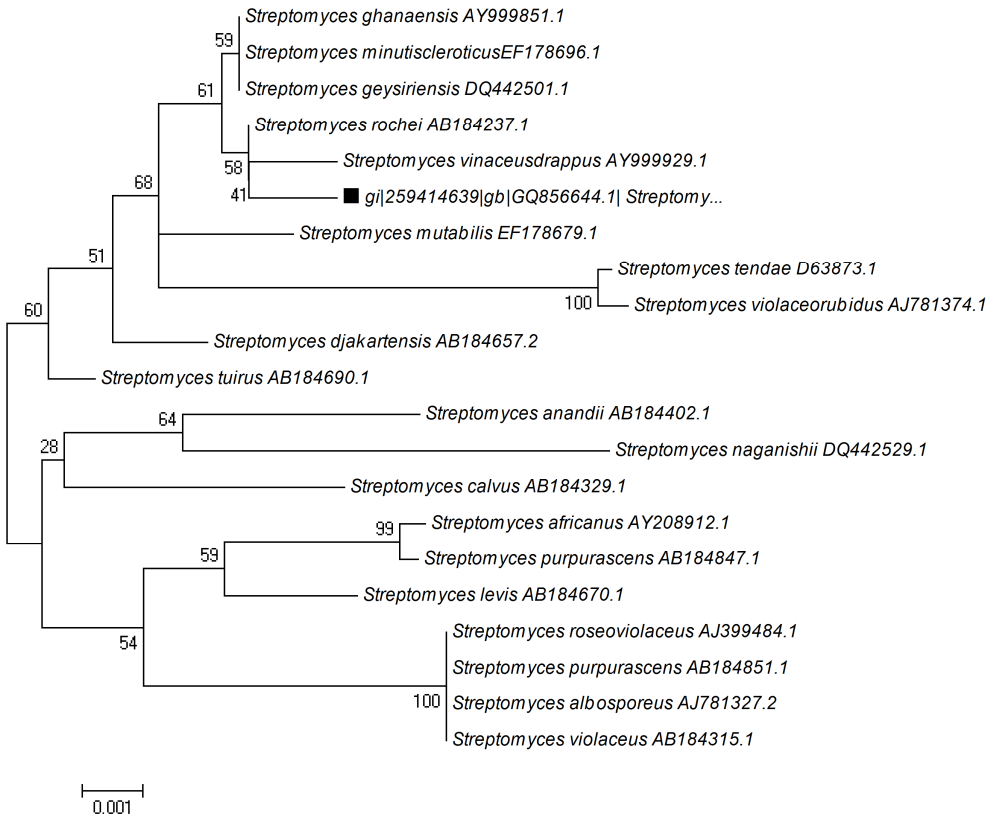

Figure 4

Neighbour-joining phylogenetic tree of PN-18 conducted using MEGA 4.0. Comparison has been made with type strains only. Numbers at nodes indicate levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets; only values >50% are given. Bar, 0.001 nucleotide substitutions per site.

CONCLUSIONS

While many organisms have been documented to be biosurfactant-producers[4], few members of *Actinomycetes*, pre-eminent natural product producers, have been extensively characterized for biosurfactants. Many reports continue to be published on bioactivity of actinomycetes from niche habitats[36]; however, to our knowledge there have been only two other reports of *Streptomyces* members isolated from oil-contaminated sites[5,7] and one of them (*S. rochei*) was studied for biosurfactant production. Our work shows that the diversity of *Actinomycetes* exists in such adverse habitats as hydrocarbon-impacted sites also. Many of the isolates studied in this work have shown promising antimicrobial activity[9,37]. Biosurfactant from PN-18 has shown high emulsification activity and shows promise for bioremediation of hydrocarbons. It can also be potentially used as an emulsifying agent in agriculture (pesticide/herbicide) and food industries after appropriate toxicity testing. Moreover, its 16S rDNA has been sequenced and shown to be 97.9% related to *S. rochei*, a species not reported for biosurfactant production. Further assessment on the type of biosurfactant produced can determine its application in industry, many of which increasingly prefer biodegradability of products to meet stringent environmental norms.

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