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Study of *Streptomyces* Diversity in Arid and Semi-Arid Soil of India

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A screening program was carried out to study *Streptomyces* diversity in red soil from arid and semi-arid regions (Kadapa and Bellary) of India and their ability to grow on unconventional substrates such as petrol and diesel. Of the 38 isolates obtained, five main classes were observed based on aerial mycelium color - gray, white, yellow, orange and red, with white being the predominant class (55%). Fourteen isolates (37%) displayed promising antimicrobial activity. Twelve isolates (32%), some with anti-microbial activity, were tolerant to petrol and diesel and grew on media supplemented with yeast extract to which 2% petrol or diesel was added. Numerical taxonomy study revealed two major clusters broadly comprising the A and B series respectively. Six isolates were identified using Probabilistic Identification of Bacteria software and thirteen placed in specific major clusters. Nearly complete 16S rDNA and partial γ-variable sequence analysis of isolate B-14, showing promising antimicrobial activity with ability to grow on diverse carbon substrates and tolerate petrol, showed close resemblance to *S. rochei* (99%) though with distinctive morphological and physiological characteristics. The strain has been assigned the GenBank accession number GQ426322**. Though isolated from soil not exposed to petrol/diesel, the ability of a noteworthy proportion (37%) of streptomycetes to grow in the presence of petrol/diesel fuels suggests possibility of either biodegradation or better tolerance to petrol/diesel toxicity in the presence of suitable nutrients. This indicates that inherent metabolic pathways in streptomycetes can be expressed by biostimulation which can possibly facilitate in identifying more novel isolates.**

Key words: *Streptomyces*, India, arid/semi-arid soil, unconventional substrates.

Abbreviations: M1: Maintenance medium; M1a: M1 medium without glucose and yeast extract; M1b: M1 medium without glucose.

Streptomyces species are well-known for their capacity to produce bioactive molecules (antibiotics and anti-tumor compounds) and industrially important metabolites comprising herbicides, enzymes, enzyme inhibitors and insecticides[1,2].

They also play an important role in degradation of organic matter[3]. A few published papers report screening of novel *Streptomyces* isolates from southern[1,4] and northern[5] India. However, when assessed in conjunction with their industrial and biotechnological importance, the documentation on soil *Streptomyces* diversity in India is comparatively less. Being a biodiversity hotspot, it is reasonable to expect that Indian soil may potentially harbor vast *Streptomyces* diversity, providing an opportunity for the discovery of useful metabolites.

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Streptomyces are nutritionally flexible with the ability to degrade complex substrates such as pectin, lignin and aromatic compounds. In this study, we have attempted to screen and characterize *Streptomyces* isolates from red soil with low nutrient status, in selected arid and semi-arid zones - Bellary and Kadapa - in southern India. Bellary falls in the northern dry zone of Karnataka state (http://www.ces. iisc.ernet.in/ energy/paper/ TR109/tr109_ std2.htm) while Kadapa falls in the semiarid-arid zone (http:// cuddapah.ap.nic.in/s_features. htm). The diversity of this genus in such soils has not been studied previously.

In the present work, apart from the stipulated nutrient status to characterize *Streptomyces* isolates, we have also studied the ability of the isolates to grow on the unconventional substrates, petrol and diesel. Contamination with commonly used refinery products such as petrol and diesel fuels leads to soil degradation with altered physicochemical properties. These fuels are a major cause of pollution due to increasing urbanization and mechanization. Earlier reports have shown *Streptomyces* sp. to be effective in biodegradation of petroleum-based products[6,7]; however, in their studies the isolates were obtained from petroleum-contaminated sources. Our isolates were obtained from soil which has not been utilized for agricultural or industrial activities and thus not exposed to either of these fuels.

MATERIAL AND METHODS

Streptomyces isolation

Soil samples were collected from a depth of approximately 10 cm from red soil of Bellary and Kadapa. Serial dilutions prepared in 0.9% NaCl solution were plated on Starch Casein Nitrate medium[8], amended with 100 µg ml^{-1} cyclo-heximide and 20 µg ml^{-1} tetracycline to prevent growth of fungi and solidified with 1.5% agar. The plates were incubated at 37°C for two weeks.

Morphological characterization

Morphological and cultural characterizations were broadly performed according to standard protocols[9,10]. Colonies with typical actinomycetes morphology were Gram stained and observed under the microscope. Representative Gram positive colonies were scored for the following features: color of aerial and substrate mycelia, shape of colony (raised/ flat), texture and presence of diffusible pigments. The spore chain morphology and spore area (μm^2) were measured using Olympus Trinocular microscope (model BX-51) with Magnus Pro Image Analysis software. Pure cultures of isolates were subcultured on maintenance medium (M1) containing (in g L^{-1}): glucose 10.0, yeast extract 1.0, potassium nitrate 1.0, potassium mono-hydrogen phosphate 0.1 and solidified with 1.5% agar. Pure colonies appeared in 3-4 days and diffusible pigments were observed in 6-7 days. The isolates were designated by alphanumerical codes- 'A' for isolates obtained from Kadapa and 'B' for those from Bellary.

Physiological and biochemical characterization

The ability of the isolates to utilize selected carbohydrates as sole carbon source was tested using raffinose, mannitol, xylose and lactose[9]. To study ability to utilize/tolerate petrol and diesel as unconventional substrates, two sets of experiments were set up. In one set, isolates were streaked on M1a medium (M1 medium lacking glucose and yeast extract) with addition of 2% filter-sterilized petrol or diesel. The other experiment was identical except that M1b medium (M1 medium lacking only glucose) was used. Catalase production was tested for formation of bubbles by adding a drop of hydrogen peroxide to an inoculum of the colonies taken on a glass slide. Nitrate reduction was assessed by red color development in 5 min after adding 1 ml of 0.8 % w/v sulphanilic acid and 1 ml of 0.5 % w/v α-napthylamine[1].

Cultures were grown for 7-10 days in 5 ml of M1 broth and kept at 130 rpm at 30ºC. The culture filtrate thus obtained was used for studying melanin production and antimicrobial activity. Melanin was estimated by mixing 2 ml of the culture filtrate with 1 ml of 0.4% L-tyrosine and incubating at 37ºC for 30 minutes. If color formation was not observed, incubation was carried out for a further 60 minutes[11,12]. Red coloration due to dopachrome formation was read spectrophotometrically at 480 nm using Systronics UV-Vis Spectrophotometer (Systronics India).

Antibacterial and antifungal activity of the isolates was tested by the Bauer-Kirby disk

diffusion method[13]. Bacterial (*Escherichia coli* [MTCC 1673], *Pseudomonas putida* [MTCC 2445], *Micrococcus luteus* [MTCC 106]) and fungal (*Aspergillus niger* [MTCC 281] and *Rhizopus oryzae* [MTCC 554]) test strains were obtained from Microbial Type Culture Collection, Chandigarh, India. Anti-bacterial activity was observed by spreading 100 µl of respective bacterial strain on Mueller-Hinton agar and placing sterile filter paper disks (5 mm) containing 10 µl of the culture filtrate. Discs with uninoculated sterile broth served as control. Anti-fungal activity was observed by making a straight-line streak of fungal strains on Czapek Dox agar.

Experimental and control discs were placed in a similar manner on the streaked areas. The zone of inhibition was measured and expressed in mm. Each experiment was repeated thrice.

Numerical taxonomy

Each isolate was examined for 83 unit characters. Qualitative characters existing in one of two mutually exclusive states were scored either 1 (present) or 0 (absent). Other traits which had multiple states (color of aerial and substrate mycelia and production of diffusible pigments) were coded as several independent characters and were scored 1 (present) for the displayed character state and 0 (absent) for all alternatives. Hierarchical cluster analysis was performed using the Canberra metric option in NTSYS v.2.1 software[14]. The coefficients were clustered by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm and a dendrogram was generated using TREE module. Cophenetic correlation was calculated to know whether the hierarchical clustering procedures yielded good representations of the taxonomic structure inherent in sorted similarity matrices.

Preliminary identification of isolates

The data generated was used to identify the isolates using the software package, Probabilistic Identification of Bacteria for Windows, version 1.9.2[15]. A Willcox probability of 0.95 was considered as the minimum identification threshold and was used to identify taxa in the matrix generated using published data on major and minor clusters of streptomycetes[16].

16S rRNA gene sequence

The genomic DNA of B-14, with better anti-microbial profile and ability to grow on all substrates except diesel fuel, was isolated using QIAamp DNA Mini Kits according to manufacturer's instructions. 16S rDNA fragment was amplified by PCR using 16S rDNA universal primers: 8F (5'-AGAGTTTGATCMTGG-3') and 1492R (5'-ACCTTGTTACGACTT-3') *E. coli* numbering[17].

The conditions used for thermal cycling were as follows: Initial denaturation at 94°C for 5 min followed by 30 cycles consisting of denaturation at 94°C for 45 sec, primer annealing at 52°C for 1 min and primer extension at 72°C for 2 min. At the end of the cycles, the reaction mixture was kept at 72°C for 10 min and then cooled to 4°C. A single discrete band of about 1.5 Kb was observed when resolved by agarose gel electrophoresis.

The PCR amplicon was purified using Exosap-IT as per manufacturer's guidelines, to remove contaminants. DNA sequencing of PCR amplicon was carried out using BigDye® Terminator v3.1 Cycle sequencing kit following manufacturer's instructions on ABI 3730xl Genetic Analyzer (Applied Biosystems, USA). Sequence data was processed for quality check and consensus sequence of 16S gene was generated and used for analysis.

The 16S rDNA sequence was compared with those of Gram positive bacteria maintained in the GenBank database using BLAST (http://www. ncbi. nlm.nih.gov) and aligned against corresponding sequences of *Streptomyces* members obtained from the GenBank/DDBJ/EMBL/RDP databases. The software Mega 4.0[18] was used to construct phylogenetic trees using maximum likelihood[19], maximum parsimony[20] and neighbor-joining[21] treeing algorithms. Evolutionary distances were computed according to Jukes-Cantor (1969)[22].

The bootstrap consensus tree inferred from 1000 replicates was evaluated. Partial nucleotide sequences comprising 120 base pairs of the variable γ-region of the 16S gene was also compared and aligned with the corresponding nucleotide sequences of more than 470 *Streptomyces* strains retrieved from GenBank. Phylogenetic tree was constructed based on these partial nucleotide sequences using neighbor-joining algorithm[21]. The nearly complete 16S

rDNA sequence (1,418 nucleotides) of strain B-14 has been deposited in the GenBank database under accession number **GQ426322** which can be accessed at http://www.ncbi.nlm.nih.gov/nuccore/256682111.

RESULTS AND DISCUSSION

Morphological characterization

Our study led to detection of 38 distinct *Streptomyces* (Table 1). They were classified into five major groups: gray, white, yellow, orange and red series[9,23]. The white series was predominant comprising 55% of isolates. Gray isolates comprised 32%, red and orange series 5% each and yellow included 3%. In this grouping, there were many isolates showing intermediate shades and have been categorized under a series based on the principal color displayed. Gray series included those which were whitish gray to dark gray, and those with maroon and pink tinges. The white series included pure white, pearl white, cream and white with greenish tinge. The red series had isolates that were light and dark pink and with tinges of white. The morphological data indicates that there were many common traits between the isolates such as color or spore chain morphology but these isolates showed distinctive differences in diffusible pigment production, color of mycelium, carbon source preference or antimicrobial profile.

The area (μm^2) of the spores ranged from 0.008 (A-7) to 0.1 (A-8). There was no specific correlation between the color series and spore size (Table 1). The majority of isolates (50%) were of rectiflexibe type. There was no specific pattern of spore chain type distribution, implying that environment does not play an explicit role in selecting specific spore chain configurations. The variations in morphological features point to the rich diversity of such unexplored environments.

Physiological and biochemical characterization

Group B isolates showed more versatility in carbon utilization (Table 2). Raffinose, lactose, xylose and mannitol were more preferred by these isolates (from Bellary) as compared to those from group A (from Kadapa). A-6, B-7 and B-14 were able to grow well on all the tested carbon sources. It was notable that more than 50% of B isolates grew well on at least three sole carbon sources.

Much diversity was observed amongst the isolates when grown on petrol or diesel amended media (Table 2). None of the isolates grew on M1a (without yeast extract and glucose), which indicates that the concentration of these fuels used was probably toxic to the isolates and inhibited their growth. However, on M1b (containing yeast extract but no glucose) supplemented with petrol, 11 isolates (A-2, A-3, A-4, A-10, B-2, B-4, B-5, B-11, B-12, B-14 and B-15) formed distinct colonies. On M1b supplemented with diesel, only A-6 was able to grow. A-6 also exhibited versatility in use of other carbon sources. These isolates have been able to tolerate the presence of petrol or diesel on M1b medium supplemented with yeast extract, though they have been taken from soils not previously exposed to either of these fuels.

It is also significant that more number of B isolates could tolerate the presence of petrol in M1b. Our study indicates that in medium supplemented with yeast extract, the isolates are able to better tolerate petrol or diesel toxicity. They possibly utilize the extra supplementation as nutrient source. It is known that addition of nitrogen and phosphorus can enhance degradation of oil[24,25]. A recent report by Ijah *et al.*[26] substantiates that amendment of oil polluted soil with chicken droppings and NPK fertilizers facilitated crude oil biodegradation up to 87.5% when compared to control (57.3%). In natural environments, oil spills lead to nutritional imbalance, which makes their biodegradation a slow process.

Microbes capable of breaking down the components of oil utilize the additional nutrients (nitrogen and phosphorus) provided in the form of fertilizers and exhibit enhanced degradation capabilities[27,28]. Our study shows that the ability to utilize hydrocarbons may be widespread, even in environments not subjected to high levels of hydrocarbon pollution[29].

All isolates were catalase positive and nitrate reduction was observed in majority (92%) of the isolates. Higher melanin production was observed in 19% of the isolates. An earlier study showed that only 5% of *Streptomyces* isolates from southern India produced melanin[12]. Nevertheless, the low figure is indicative of this feature being an important key for classification of streptomycetes. The anti-microbial profile (Table 3) shows that some isolates show better anti-fungal rather than

Table 1. Morphological characteristics of *Streptomyces* isolates obtained from arid and semi-arid soil of India

Isolate	Aerial Mycelium	Substrate Mycelium	Colony Elevation	Colony Texture	Diffusible Pigment	Spore (μm^2) Area	Spore Arrangement
A-1	White	Pale yellow	Raised	Chalky	None	0.015±0.01	Rectiflexible
A-2	Whitish dark gray	Brown	Raised	Crystalline	Bluish brown	0.015±0.00	Rectiflexible
A-3	Grayish white	Red	Raised	Chalky	Red	0.003±0.01	Retinaculum apertum
A-4	Grayish white	Pale yellow	Raised	Chalky	None	0.019±0.01	Retinaculum apertum
A-5	White	Pale yellow	Raised	Chalky	None	0.01±0.01	Rectiflexible
A-6	White	Dark red	Raised	Chalky	Dark red	0.016±0.00	Retinaculum apertum
A-7	Orange	White	Flat	Leathery	None	0.008±0.00	Spiral
A-8	Grayish white	Pale yellow with red edges	Raised	Chalky	None	0.10±0.01	Rectiflexible
A-9	Pearl white	Pale yellow	Raised	Crystalline	None	0.0114± 0.00	Rectiflexible
A-10	Darkpink	Pale yellow	Raised	Chalky	None	0.010±0.01	Rectiflexible
A-11	Gray	Red	Raised	Chalky	Light red	0.01±0.00	Rectiflexible
A-14	Pale yellow	Pale yellow	Flat	Leathery	None	0.019±0.01	Rectiflexible
A-16	Grayish white	Pale yellow	Raised	Crystalline	None	0.012±0.01	Indeterminate
A-18	White	Light orange	Raised	Chalky	None	0.04±0.00	Indeterminate
A-19	Maroon gray	Pale yellow	Raised	Crystalline	None	0.012±0.00	Rectiflexible
A-20	White	Light brown	Raised	Chalky	None	0.013±0.01	Rectiflexible
A-21	Gray	Dark brown	Raised	Chalky	Brown	0.008±0.01	Rectiflexible
B-1	Grayish white	Dark brown	Flat	Chalky	Black	0.036±0.00	Indeterminate
B-2	Cream	Pale yellow	Raised	Chalky	None	0.044±0.00	Rectiflexible
B-3	Light to dark pink	Dark pink	Raised	Chalky	Pink	0.022± 0.01	Indeterminate
B-4	Whitish gray	Orange	Raised	Chalky	Orange	0.049±0.01	Rectiflexible
B-5	Light gray	Burnt orange	Raised	Chalky	Very light orange	0.044±0.01	Indeterminate
B-6	Pinkish gray	Pale yellow	Raised	Chalky	None	0.011±0.01	Indeterminate
B-7	White, gray at turns	Pale orange	Raised	Chalky	Orange	0.043±0.01	Indeterminate
B-8	Grayish white	Pale yellow with pink tinge	Raised	Chalky	None	0.045±0.01	Indeterminate
B-9	Grayish white	Pale yellow	Raised	Chalky	None	0.033±0.01	Spiral
B-10	Gray	Pale yellow	Raised	Chalky	None	0.026± 0.00	Indeterminate
B-11	Gray	Burnt orange	Raised	Chalky	Faint orange	0.021±0.01	Rectiflexible
B-12	Light gray	Burnt orange	Raised	Chalky	Light orange	0.024±0.01	Indeterminate
B-13	Grayish white to cream	Pale yellow	Raised	Chalky	Light orange	0.011±0.01	Indeterminate
B-14	Cream	Orange	Raised	Chalky	Orange	0.026±0.01	Rectiflexible
B-15	Gray	Burnt orange	Raised	Chalky	Orange	0.027±0.01	Rectiflexible
B-16	White with greenish tinge	Dark green	Raised	Chalky	Grayish green	0.026±0.01	Spiral
B-17	Gray	Greenish yellow with red patches	Raised	Chalky	Faint green	0.028±0.00	Rectiflexible
B-18	Whitish gray	Pale yellow	Raised	Chalky	None	0.023±0.01	Indeterminate
B-19	Light orange	Bright orange	Raised	Chalky	Orange	0.023±0.01	Indeterminate
B-20	Grayish white	Burnt orange	Raised	Chalky	Burnt orange	0.020±0.01	Indeterminate
B-21	Cream	Pale yellow	Raised	Chalky	None	0.019±0.01	Indeterminate

anti-bacterial activity. Eight of the isolates exhibited an inhibition zone of ≥15 mm against one or both the fungi. A-2 and B-5 showed broad - spectrum activities while A-21 and B-14 were active against four out of the five test microbes. A-16 was most effective against both test fungi. Promising antibacterial activity was displayed only by B-14 (against *E. coli*). Non-production of inhibition zones could be due to antimicrobials to which the test bacteria are resistant[30]. Other media may help in production of metabolites with higher potency or quantity from these isolates, which needs to be investigated further.

Numerical taxonomy

Using the Canberra metric in NTSYS software, two major clusters could be delineated, one predominantly comprising the A series and the other the B series (Fig. 1). The high cophenetic correlation value of 0.91 implies agreement between the dendrogram and those of the original similarity matrix. Similar morphological and physiological parameters have been used for grouping unidentified *Streptomyces* strains[31].

Preliminary identification

The software Probabilistic Identification of Bacteria for Windows is a functional tool to place unidentified isolates in specific groups. The identification threshold of 0.95 was reached with isolates A-2, A-3, A-21, B-1 and B-3 (Table 4). A-2, A-3, A-21 and B-1 were identified as *S. purpureus* but A-21 and B-1 had a higher modal score. The characteristics studied agree well with the published information[32] about *S. purpureus*. Members produce anti-fungal compounds and in our study, A-21 has shown better anti-fungal activity. A-2 and A-3 could be strains that show differences in morphology due to the niche environmental conditions of their habitat or could be novel isolates.

Other isolates with comparatively high identification threshold scores (≥0.8) were A-6, A-20, B-18 and B-19. The most likely taxa would be *S. fulvissimus*, *S. albidoflavus*, *S. violaceusniger* and *S. exfoliatus* respectively. *S. fulvissimus* produces valinomycin. In our study, the antimicrobial profile of A-6 was not remarkable but its ability to grow on diesel-containing media and utilize diverse carbon substrates shows that it can be a candidate for study of biodegradation of complex substrates. *S. albidoflavus* produces chitinolytic enzymes with potential anti-larval activities[33] though we have not tested this property in A-20.

Most strains of *S. violaceusniger* group produce antimicrobial compounds[34], while in our study only B-18 has shown antibacterial activity.

In the present study, some isolates could not be definitively identified pending further tests. However, the other isolates with ID score ≥6[35] were A-1 and A-16 (*S. antibioticus*), A-8 (*S. anulatus*), A-9 and B-15 (*S. atroolivaceus*), A-10, A-11 and B-17 (*S. exfoliatus*), and B-10 (*S. violaceusniger*). If they belong to these major clusters, there is possibility of their being sources of bioactive metabolites. *S. antibioticus* produces actinomycin, a transcription inhibitor and anti-neoplastic agent, which also inhibits HIV-1[36,37]. A-16 showed an exceptional antifungal profile and the nature of the metabolite produced is being investigated further. *S. anulatus* strains are notable protease producers. *S. albidoflavus* (A-20) and *S. anulatus* (A-8) are close phenotypic relatives[38] and are found in Cluster B. This is in accordance with the results of Williams *et al.*[39] who reported similarity level of 77.5%. All members of *S. exfoliatus* group (A-10, A-11, B-3, B-17 and B-19) cluster together, though not all isolates presented the typical pink spore color characteristic of this group[40]. Overall A-16, A-21 (identified) and B-14 (unidentified) proved to be interesting candidates for further study.

16S rRNA gene analysis of B-14

Almost complete 16S rRNA gene sequence (1418 nucleotides) was determined. From the primary sequence analysis, it was confirmed that B-14 was closely related to other species of the genus *Streptomyces* (Fig. 2). High sequence similarity value of 99.7% was obtained with *Streptomyces* sp. YDG17 (EU621883) corresponding to 4 nucleotide differences in 1416 sites and with *S. rochei* CTF20 (EU294136) in 1414 sites. These formed a definite sub-clade (bootstrap value of 75), which was also recovered in the analyses using maximum composite likelihood and maximum parsimony treeing algorithms though with low bootstrap values. Partial sequence analysis of γ-variable region (Fig. 3) showed that B-14 was grouped into a branch with *S. rochei* (D44296), *S. vinaceusdrappus* (D44214), *S. plicatus* (D44194), *S. flavogriseus*

Table 2. Physiological characteristics of *Streptomyces* isolates obtained from arid and semi-arid soil of India.

Isolate	Carbon source utilization				Growth on M1b medium		Melanin production (OD_{480})	Nitrate Reduction
	Raffinose	Lactose	Mannitol	Xylose	Petrol	Diesel		
A-1	-	+	+	-	-	-	0.057	+
A-2	-	-	-	+	+	-	0.043	+
A-3	-	-	-	+	+	-	0.030	+
A-4	-	-	-	-	+	-	0.063	+
A-5	-	-	+	+	-	-	0.711	+
A-6	+	+	+	+	-	+	0.032	-
A-7	-	-	+	-	-	-	0.040	+
A-8	-	-	+	+	-	-	0.885	+
A-9	+	-	+	-	-	-	0.035	+
A-10	-	-	-	+	+	-	0.072	+
A-11	-	-	-	-	-	-	0.375	+
A-14	-	-	-	+	-	-	0.114	+
A-16	-	-	-	-	-	-	0.036	+
A-18	-	-	-	+	-	-	0.082	+
A-19	-	-	-	+	-	-	0.303	-
A-20	-	-	-	-	-	-	0.067	-
A-21	-	-	-	+	-	-	0.022	+
B-1	-	+	+	+	-	-	0.072	+
B-2	-	+	+	-	+	-	0.077	+
B-3	-	+	-	-	-	-	0.633	+
B-4	+	+	+	-	+	-	0.098	+
B-5	-	+	+	-	+	-	0.752	+
B-6	-	+	+	+	-	-	0.105	+
B-7	+	+	+	+	-	-	0.024	+
B-8	+	+	+	-	-	-	0.040	+
B-9	+	+	+	-	-	-	0.083	+
B-10	+	+	+	-	-	-	0.036	+
B-11	+	+	+	-	+	-	0.025	+
B-12	-	+	+	-	+	-	0.017	+
B-13	-	+	+	-	-	-	0.715	+
B-14	+	+	+	+	+	-	0.043	+
B-15	+	+	+	-	+	-	0.047	+
B-16	-	+	+	+	-	-	0.051	+
B-17	-	+	-	-	-	-	0.046	+
B-18	+	+	+	-	-	-	0.111	+
B-19	-	-	-	-	-	-	0.096	+
B-20	-	+	+	+	-	-	0.053	+
B-21	-	+	+	+	-	-	0.077	+

(D44024) and *S. lavendulae* (D44250) with 100% relatedness value. Combined 16S rDNA and partial γ-variable region studies show that B-14 could be most related to unidentified isolate YDG17 or *S. rochei*. Morphological and physiological data support congruency with *S. rochei* (though with a very low ID score of 0.13). Gray aerial and yellow substrate mycelia on salts-starch agar[41], lack of production of melanoid pigments and low/no growth on raffinose are characteristic of *S. rochei*. B-14 showed gray aerial mycelia only on International Streptomycetes Project-5 (ISP5) medium but produced cream colored aerial mycelia and orange diffusible pigment on M1 medium and grew profusely on raffinose as sole carbon source. Hence B-14 shows features which are distinct from that of *S. rochei*.

Table 3. Antibiogram profile of selected *Streptomyces* isolates with inhibition zone greater than 15 mm (against fungi) and 10 mm (against bacteria)

Isolate	Zone of inhibition (mm)				
	R. oryzae	*A. niger*	*E. coli*	*M. luteus*	*P. putida*
A-2	17.0	13.0	8.0	7.5	8.0
A-3	14.0	15.0	8.0	0.0	0.0
A-4	16.0	10.0	8.0	0.0	0.0
A-14	17.0	0.0	0.0	0.0	0.0
A-16	21.0	23.0	0.0	0.0	0.0
A-20	0.0	17.0	10.0	0.0	0.0
A-21	19.0	19.0	0.0	8.0	8.5
B-5	13.0	13.0	8.0	8.0	10.0
B-7	0.0	19.0	8.0	0.0	9.0
B-13	0.0	11.0	9.0	0.0	10.5
B-14	11.0	10.0	15.0	0.0	8.0
B-17	0.0	0.0	0.0	10.0	0.0
B-18	0.0	0.0	10.0	8.0	9.0
B-21	0.0	19.0	8.0	0.0	8.0

Table 4. Assessment of selected *Streptomyces* isolates (with ID score ≥0.60) using the software package Probabilistic Identification of Bacteria for Windows[15]

Isolate	Taxon	ID Score	ID Modal Score
A-21	*S. purpureus*	0.99995	0.33333
A-2	*S. purpureus*	0.99940	0.00112
A-3	*S. purpureus*	0.99849	0.00037
B-1	*S. purpureus*	0.99602	0.11337
B-3	*S. exfoliatus*	0.99400	0.05155
A-20	*S. albidoflavus*	0.94365	0.11111
B-19	*S. exfoliatus*	0.91596	0.00259
A-6	*S. fulvissimus*	0.88118	0.00014
B-18	*S. violaceusniger*	0.84714	1.02030
B-15	*S. atroolivaceus*	0.76774	0.00015
A-11	*S. exfoliatus*	0.76083	0.01922
B-10	*S. violaceusniger*	0.74971	0.50254
A-16	*S. antibioticus*	0.74715	0.23148
A-10	*S. exfoliatus*	0.69225	0.00052
B-17	*S. exfoliatus*	0.68410	0.19685
A-8	*S. anulatus*	0.66694	0.00123
A-9	*S. atroolivaceus*	0.62063	0.28205
A-1	*S. antibioticus*	0.60297	0.09259

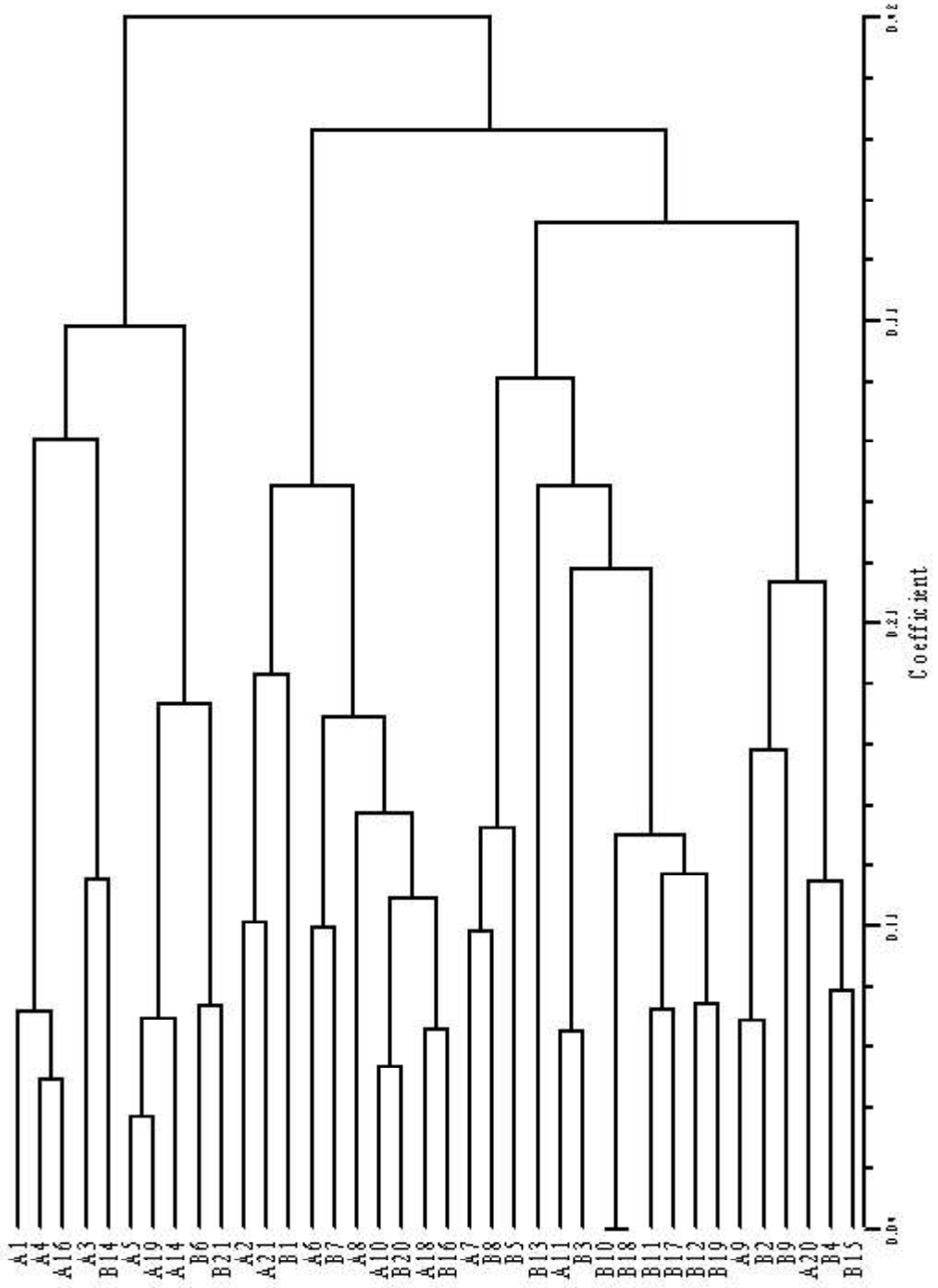

Fig. 1: Dendrogram showing the relationships between the A and B isolates based on Canberra metric and UPGMA analysis

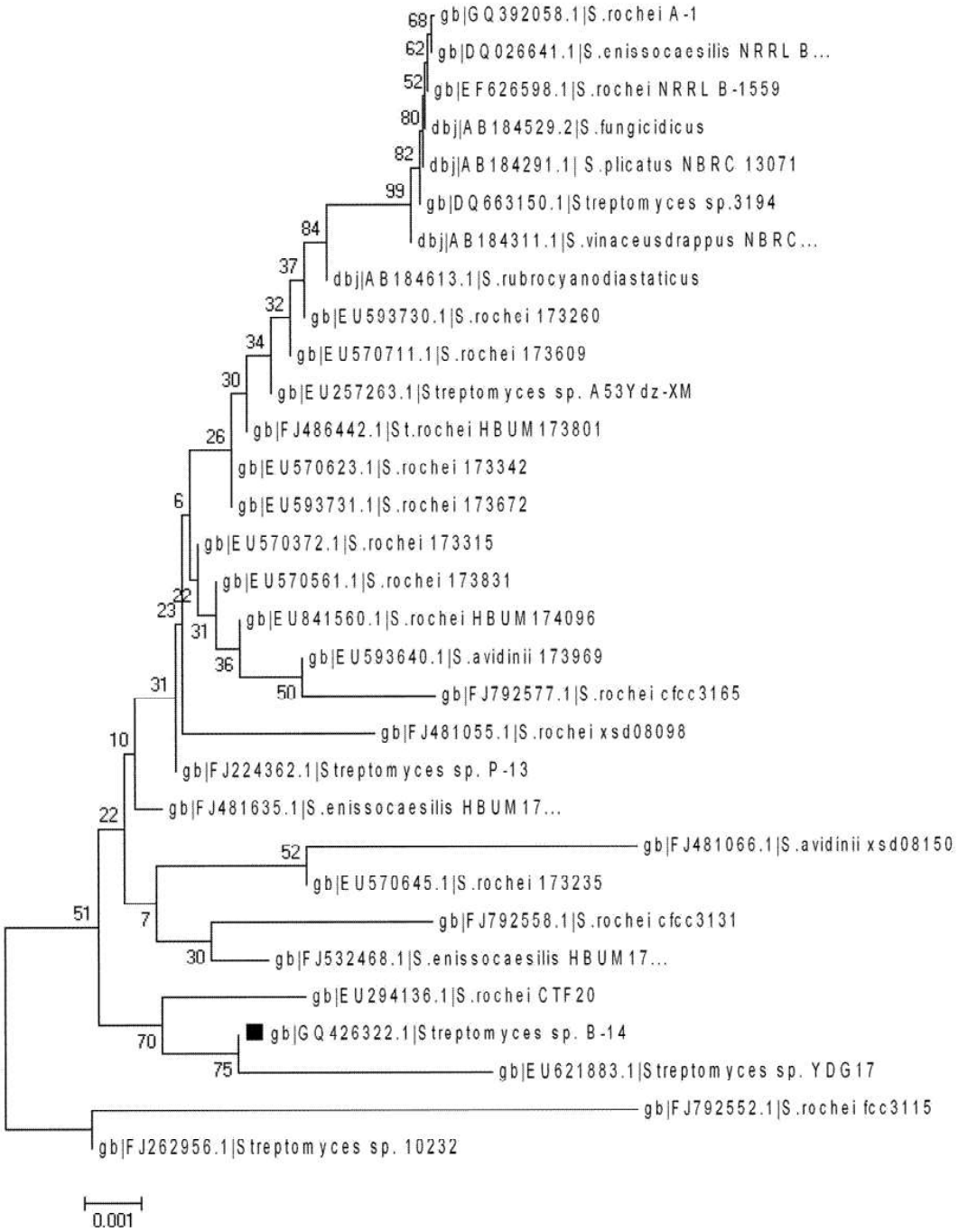

Fig. 2. Neighbour-joining tree[21] based on nearly complete 16S rDNA sequences showing the position of B-14 and related Streptomyces species, computed using the Jukes-Cantor method[22]. Branches of B-14 clade were also recovered using maximum likelihood[19] and maximum parsimony[20] treeing algorithms. Bootstrap values are indicated at the nodes (1000 replications). Phylogenetic analyses were conducted in MEGA4[18]. Bar, 0.001 substitutions per nucleotide position

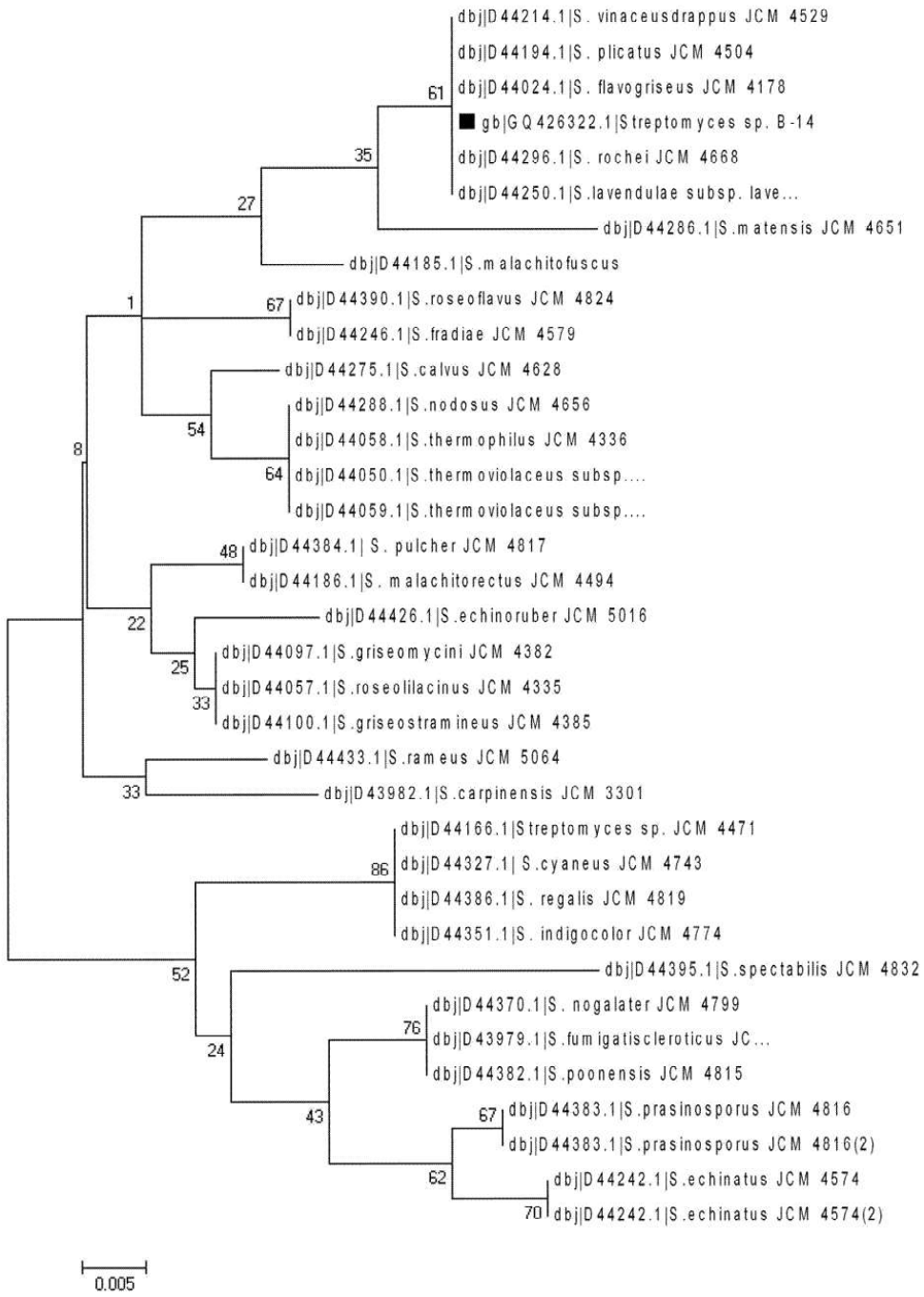

Fig. 3. Unrooted neighbour-joining phylogenetic tree[21] of thirty five *Streptomyces* species with strain B-14 based on the γ-variable region (158–277) of 16S rDNA sequences. Bootstrap values are indicated at the nodes (1000 replications). The evolutionary distances were computed according to Jukes-Cantor method[22]. Phylogenetic analyses were conducted in MEGA4[18]. Bar, 0.005 substitutions per nucleotide position

CONCLUSIONS

From the 38 isolates, six have been definitely identified and thirteen have been tentatively placed in specific major clusters. Further tests can help in identifying the others. This habitat remains to be explored further since it potentially harbors interesting and uncharacterized strains. Isolates such as A-2 (*S. purpureus*), A-16, A-21 and B-14 (showing exceptional antibiogram profile) and A-6 (*S. fulvissimus*), B-4, B-11, B-14 and B-15 (exhibiting ability to tolerate diesel/ petrol) have shown promising features and they are being studied further in our lab. Our preliminary work opens up prospects to study the ability of *Streptomyces* to tolerate/break down such complex hydrocarbon contaminants which are not present in their native habitat. It can provide alternative eco-friendly approaches for biodegradation of petroleum contaminants using native isolates with the capability of utilizing complex and unconventional substrates. Bioprospection and investigations of such locally available microbial biodiversity[42] provide the possibility of extending the spectrum of known producers of bioactive compounds and those with potential for bioremediation.

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