Journal of Microbiology (2015) Vol. 53, No. 1, pp. 6–13
DOI 10.1007/s12275-015-4462-4

Description of *Pseudomonas asuensis* sp. nov. from biological soil crusts in the Colorado plateau, United States of America[§]

Gundlapally Sathyanarayana Reddy[*#] and Ferran Garcia-Pichel

School of Life Sciences, Arizona State University, Main Campus, Tempe, AZ 85287-4501, USA
[#]Present address: CSIR-Centre for Cellular and Molecular Biology Uppal Road, Hyderabad 500 007, India

(Received Aug 12, 2014 / Revised Oct 20, 2014 / Accepted Oct 22, 2014)

A Gram-negative, aerobic, non spore-forming, non-motile, rod-shaped, yellow pigmented bacterium CP155-2^T was isolated from a biological soil crusts sample collected in the Colorado plateau, USA and subjected to polyphasic taxonomic characterization. Strain CP155-2^T contained summed feature 3 ($C_{16:1}\omega5c$/$C_{16:1}\omega7c$) and $C_{18:1}\omega7c$ as major fatty acids and diphosphatidylglycerol (DPG) along with phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) as major polar lipids. Based on these characteristics CP155-2^T was assigned to the genus *Pseudomonas*. Phylogenetic analysis based on 16S rRNA gene sequence further confirmed the affiliation of CP155-2^T to the genus *Pseudomonas* and showed a 16S rRNA gene sequence similarity of less than 98.7% with already described species of the genus. *Pseudomonas luteola*, *Pseudomonas zeshuii*, and *Pseudomonas duriflava* were identified as the closest species of the genus *Pseudomonas* with 16S rRNA gene sequence similarities of 98.7%, 98.6%, and 96.9%, respectively. The values for DNA–DNA relatedness between CP155-2^T and *Pseudomonas luteola* and *Pseudomonas zeshuii* were 23% and 14% respectively a value below the 70% threshold value, indicating that strain CP155-2^T belongs to a novel taxon of the genus *Pseudomonas* lineage. The novel taxon status was strengthened by a number of phenotypic differences wherein CP155-2^T was positive for oxidase, negative for gelatin hydrolysis, could utilize D-cellobiose, D-raffinose, L-rhamnose, D-sorbitol but not L-aspartic acid and L-glutamic acid. Based on the collective differences strain CP155-2^T exhibited, it was identified as a novel species and the name *Pseudomonas asuensis* sp. nov. was proposed. The type strain of *Pseudomonas asuensis* sp. nov. is CP155-2^T (DSM 17866^T =ATCC BAA-1264^T =JCM13501^T =KCTC 32484^T).

Keywords: *Pseudomonas asuensis*, biological soil crusts, Colorado plateau, United States of America

*For correspondence. E-mail: rukmagsn@gmail.com
[§]Supplemental material for this article may be found at http://www.springerlink.com/content/120956.
GenBank/EMBL accession numbers for the 16S rRNA gene sequence of *Pseudomonas asuensis* sp. nov. (CP155-2^T) is AJ871471.

Introduction

The definition, occurrence and importance of biological soil crusts (BSCs) has been introduced previously by Belnap (1993) and our group (Nagy *et al.,* 2005; Reddy and Garcia-Pichel, 2005, 2006; Reddy *et al.,* 2006). BSCs, besides Cyanobacteria, are dominated by heterotrophic communities such as *Actinobacteria, Proteobacteria, Bacteriodetes*, and low G+C bacteria (Smith *et al.,* 2004; Nagy *et al.,* 2005; Reddy and Garcia-Pichel, 2006). The Proteobacterial communities dominate the BSCs by contributing 16.3% and 13.8% of the total diversity respectively in the BSCs from Colorado plateau and Sonoran deserts (Nagy *et al.,* 2005; Reddy and Garciaichel, 2006) studied so far. Among the members of Proteobacteria, occurrences of *Alphaproteobacteria*, *Betaproteobacteria*, and *Deltaproteobacteria* have been reported by culture independent and culture based methods in BSCs (Garcia-Picehl *et al.*, 2003; Smith *et al.,* 2004; Nagy *et al.,* 2005; Reddy and Garcia-Pichel, 2006). However, no *Gammaproteobacteria* has been reported from BSCs from the arid or semi-arid regions of southwest arid parts of the United States of America though only two molecular sequences were detected from soil crusts from Tengger desert, China (Zhang *et al.*, 2012). In the present study, for the first time, a bacterium belonging to *Gammaproteobacteria* was isolated from BSCs in the Colorado plateau and characterized. Based on the polyphasic taxonomic assessment, the strain CP155-2^T was identified as a novel species of the genus *Pseudomonas.*

The genus *Pseudomonas* belongs to Gram-negative aerobic *Gammaproteobacteria* belonging to the family *Pseudomonadaceae* was originally described by Migula (1894) and presently contains 211 validly described species (http://www.bacterio.net/pseudomonas.html). The genus *Pseudomonas* is highly heterogeneous and some members of the genus have been reclassified to other genera based on phenotypic characteristics (Sneath *et al.,* 1981), DNA–DNA hybridization (Palleroni, 1984), chemotaxonomic data (Oyaizu and Komagata, 1983; Vancanneyt *et al.,* 1996), and 16S rRNA gene sequence similarities (Anzai *et al.,* 2000). The genus is subdivided into two main intra-generic clusters (Anzai *et al.,* 2000), the first cluster comprised of six groups (*Pseudomonas syringae*; *Pseudomonas chlororaphis*; *Pseudomonas fluorescens*; *Pseudomonas putida* and *Pseudomonas aeruginosa*) and the second contained only one group (*Pseudomonas pertucinogena*). Analyses of 16S rRNA gene sequence of CP155-2^T indicated that the isolate was closely related to *Pseudomonas luteola* (Kodama *et al.,* 1985; Holmes *et al.,* 1987) and *Pseudomonas zeshuii* (Feng *et al.,* 2012) of *Pseudomonas aeruginosa/Pseudomonas stutzeri* within the first cluster (Anzai *et al.,* 2000). Based on polyphasic taxonomic

characteristics, strain CP155-2^T was identified as belonging to a novel species of the genus *Pseudomonas* and the name *Pseudomonas asuensis* sp. nov. was proposed.

Materials and Methods

Isolation of the bacterial strain and growth conditions

The bacterium CP155-2^T was isolated from biological soil crust (BSC) samples collected from Colorado plateau, United States of America (38° 09′ 839″ N and 109° 44′ 560″ W) in May 2003. The isolation procedure, maintenance, growth conditions were used previously described by Reddy and Garcia-Pichel (2005). Briefly, 0.5 g of crust sample was suspended in Ringer's solution (Reddy *et al.,* 2006), vortexed and the suspension was allowed to settle. Approximately 100 µl of supernatant was plated on BG11-PGY agar medium (Reddy and Garcia-Pichel, 2005) and incubated at room temperature for 15 days in the dark. A yellow pigmented morphotype was picked, clonally purified by repeated streaking on BG11-10X PGY agar medium (Reddy and Garcia-Pichel, 2005) and maintained on the same medium. Growth at different temperatures (4, 10, 15, 20, 25, 30, 37, 42, 45, and 50°C) was determined using BG11-10X PGY agar medium as described earlier (Reddy and Garcia-Pichel, 2013). Tolerance to different salt concentrations was assayed by adding different concentrations of NaCl (1% interval) to BG11-10X PGY agar medium at 25°C. Growth at various pH levels (4, 5, 6, 7, 8, 9, 10, and 11) was assessed on BG11-10X PGY agar medium at 25°C. The pH of the BG11-10X PGY agar medium was maintained using three buffers (final concentration, 50 mM): acetate buffer (pH 4.0–5.0), phosphate buffer (pH 6.0–8.0) and Tris buffer (pH 9.0–11.0).

Morphological and biochemical characterization

Morphological characteristics were determined using both a phase contrast microscope and transmission electron microscope (TEM). For TEM, cells were freshly grown on BG11-10X PGY agar plates, a single colony was suspended in 4.0% formalin, negatively stained with 0.5% (w/v) uranyl acetate and mounted on Formvar-coated copper grids. The grids were examined in TEM mode on a JEOL JEM-1010 electron microscope operated at 60 kV (Reddy *et al.,* 2006). Gram-staining was performed as described by Gerhardt *et al.* (1994) and motility was determined using BG11-10X PGY agar medium containing 0.4% agar. Growth under anaerobic conditions was tested by culturing CP155-2^T BG11-10X PGY agar plates in GasPak jars at 25°C (Reddy *et al.,* 2006). Oxidase activity was evaluated with oxidase discs supplied by HiMedia (HiMedia Laboratories) and catalase activity was determined applying a 3% (v/v) hydrogen peroxide solution. Aerobic growth on different media was assessed on tryptic soy agar (TSA; MacFaddin, 1985), nutrient agar (NA; Lapage *et al.,* 1970; Atlas and Parks, 1993), Luria-Bertani agar (LB; Bertani, 1952), antarctic bacterial medium (ABM; Shivaji *et al.,* 1988) and Reasoner's 2A agar (R2A agar; Reasoner and Geldreich, 1985). For determination of biochemical tests, the culture was grown at 25°C in BG11-10X PGY medium and tests were performed as described by Lanyi (1987), Smibert and Krieg (1994), and Cowan and Steel (1965). The ability of the culture to utilize a carbon compound as the sole carbon source was checked adding each carbon compound at a final concentration of 0.5% to a base of BG11 medium without citric acid (Reddy and Garcia-Pichel, 2005). In order to avoid the inconsistencies, Hi25 Enterobacteriaceae identification kit (catalogue no. KB003) and the HiCarbo kits parts A, B, and C (catalogue no. KB009 [both from HiMedia]) were used to double check the important phenotypic characteristics of strain CP155-2^T according to the manufacturer's protocol (Reddy *et al.*, 2013). The sensitivity of the culture to different antibiotics was checked using antibiotic discs supplied by Becton Dickinson Microbiological systems, Maryland, USA (Reddy and Garcia-Pichel, 2005; Reddy *et al.,* 2006) on BG11-10X PGY medium. All the incubations (unless specifically mentioned otherwise) were carried out at 25°C for a week.

DNA isolation, 16S rRNA gene sequencing, and DNA-DNA hybridization

For 16S rRNA gene sequencing, DNA from strain CP155-2^T was prepared using the Mo Bio microbial DNA isolation kit (Mo Bio Laboratories Inc.). The 16S rRNA gene was amplified using primers GM3F (5′-AGAGTTTGATCMTGGC-3′) and 16S2 (5′-ACGGCTACCTTGTTACGACTT-3′) (Nübel *et al.,* 1997; Reddy *et al.,* 2000). Fragments of about 1,500 bp were purified from agarose gels using a Qiagen kit and then sequenced using the primers 907R (5′-CCGTCAATTCCT TTRAGTTT-3′) (Nübel *et al.*, 1997), pC* (5′-CCCACTGC TGCCTCCCGTAG-3′), pE (5′-AAACTCAAAGGAATTG ACGG-3′) and 16S2 (Reddy *et al.,* 2000). For the purpose of DNA-DNA hybridization, DNA was isolated and purified from CP155-2^T, *Pseudomonas luteola* (JCM 3352^T) and *Pseudomonas zeshuii* (KACC 15471^T) from 500 ml culture as described earlier (Marmur, 1961). Hybridization was performed as described earlier (Pandey *et al.,* 2002) by filter hybridization method. Approximately 5 µg DNA per well (in five replicates) was immobilized onto a Hybond N+ nylon membrane (supplied by GE Healthcare Bio-sciences) under vacuum using a dot blot apparatus. Hybridization probe of CP155-2^T was prepared by labeling 500 ng of DNA with [α-32P]-dATP using LCK1 nick translation kit according to the protocol described by the manufacturers (Jonaki, BARC). Hybridization was performed overnight at 60°C in a hybridization buffer (0.5 M Phosphate buffer pH 7.3, 1 mM EDTA, and 7% SDS). The membrane filter, after hybridization, was washed with 2X SSC and 0.1% SDS for 15 min at RT followed by 1X SSC and 0.1% SDS for 15 min at 50°C. The filter was dried, radioactive signals were developed using a phosphor imaging device (Fuji FLA3000) and quantified using the Image quage version 4.0.

Phylogenetic analysis

The 16S rRNA gene sequence of strain CP155-2^T was subjected to BLAST and EzTaxon sequence similarity search and closely related 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed with the CLUSTAL-X program (Thompson *et al.,* 1997) and phylogenetic trees were constructed using the MEGA5 program (Tamura *et al.,* 2011). To avoid the 'Fel-

senstein zone' (i.e. retrieving a wrong tree even if it has high bootstrap values), phylogenetic analyses (Felsenstein, 1985) were performed with different phylogenetic algorithms. For this purpose, neighbor joining (NJ), minimum evolution (ME), maximum likelihood (ML), and Maximum parsimony (MP) options of MEGA5 program (Tamura *et al.,* 2011) were used to generate the trees. In each case, bootstrap analysis was performed by employing 1000 replicate data sets in order to assess the stability among the clades. Pair wise distances for the neighbor-joining algorithm (Saitou and Nei, 1987) were calculated according to the Kimura two-parameter model (Kimura, 1980).

Chemotaxonomic characterization

For quantitative analysis of whole cell fatty acids, cells of strain CP155-2^T were grown overnight on tryptic soy agar (TSA) at 25°C and fatty acid methyl esters were prepared, separated and identified (Sherlock ver. 6.01; database TSBA6; MIDI, Inc.) (Sasser, 1990). Polar lipids from cells of CP155-2^T were extracted and analyzed by two-dimensional TLC according to the procedure described by Komagata and Suzuki (1987) using chloroform : methanol : water (65 : 25 : 4, v/v/v) in the first direction and chloroform : methanol : acetic acid : water (80 : 12 : 15 : 4, v/v/v/v) in the second direction as the solvent systems. Total lipids were detected by spraying with molybdophosphoric acid (5%, w/v) in absolute ethanol, phospholipids with the molybdenum blue spray reagent, aminolipids with ninhydrin spray (0.2%, w/v) in acetone and glycolipids with anisaldehyde reagent (Lee *et al.*, 2013; Reddy *et al.,* 2013). Strain CP155-2^T was grown on BG11-10X PGY agar (Reddy and Garcia-Pichel, 2005) for two days, cells were scraped off the plate and suspended in chloroform: methanol (2:1 v/v) and vortexed. The pigmented suspension was filtered, filtrate was dried by passing nitrogen gas and UV–Visible spectrum was recorded from 200 to 1200 nm in methanol (Reddy and Garcia-Pichel, 2005; Reddy and Garcia-Pichel, 2013).

Results and Discussion

Morphological, growth, and phenotypic characteristics

The colonies of CP155-2^T were circular, slightly convex, smooth, yellow pigmented, granular and the size ranged from 3 to 4 mm. Cells of CP155-2^T were Gram-negative, aerobic, non-motile, non-spore forming, rod-shaped, and were approximately 1.0 μm × 2.5 μm (Supplementary data Fig. S1) in size. Aerobic growth on different media indicated that strain CP155-2^T could grow on TSA, NA, LB agar, ABM, and R2A agar and did not grow on BG11-10X PGY agar medium under anaerobic conditions. Strain 155-2^T grew from 5°C to 37°C (but not at 40°C) with an optimum growth temperature of 25°C. The pH range for growth was 6 to 9 with an optimum pH of 7 and can tolerate up to 1% NaCl. Cells were positive for catalase, oxidase, DNAse, phosphatase, hydrolysis of esculin and nitrate reduction, but negative for lipase, β-galactosidase, urease, gelatinase, arginine decarboxylase, lysine decarboxylase, ornithine decarboxylase, phenyl alanine deaminase, and arginine dihydrolase. The other physiological characteristics of strain CP155-2^T are in the species description section and in Tables 1 and 2. Strain CP155-2^T shared most of the physiological characteristics with the phylogenetically closely related species *Pseudomonas luteola* (Kodama *et al.,* 1985; Feng *et al.,* 2012) and *Pseudomonas zeshuii* (Feng *et al.,* 2012) but was different from them with respect to tolerance to sodium chloride, hydrolysis of gelatin and utilization of carbon compounds (Table 1).

Phylogenetic analysis

The 16S rRNA gene sequence of strain CP155-2^T obtained was a continuous stretch of 1,498 nucleotides spanning from 6 to 1513 with respect to *Escherichia coli* 16S rRNA gene sequence (nucleotide sequence accession number J01695). A BLAST (Altschul *et al.,* 1990) similarity search based on

Table 1. Characteristics that differentiates species *Pseudomonas asuensis* sp. nov. CP155-2^T from its closely related species of the genus *Pseudomonas*
Data from 1, *Pseudomonas asuensis* sp. nov. CP155-2^T (present study); 2, *Pseudomonas zeshuii* BY-1^T (Feng *et al.,* 2012); 3, *Pseudomonas luteola* IAM 13000^T (Holmes *et al.,* 1987; Feng *et al.,* 2012); 4, *Pseudomonas duriflava* HR2^T (Liu *et al.,* 2008). -, negative; +, positive; ND, not determined.

Characteristic	1	2	3	4
Growth at 42°C	-	-	+	+
Nitrate reduction	+	+	+	-
Tolerance of 5% NaCl	-	+	+	-
Oxidase	+	-	-	-
Hydrolysis of:				
Gelatin	-	+	+	-
Starch	-	-	-	+
Tween 80	-	+	-	+
Utilization of:				
D-Cellobiose	+	-	-	-
D-Maltose	+	+	-	+
Gluconate	+	+	+	-
D-Glucose	+	+	-	+
Maltose	+	+	+	-
D-Mannitol	+	+	+	-
D-Mannose	+	-	+	-
D-Raffinose	+	-	-	-
D-Ribose	+	-	+	+
L-Rhamnose	+	-	-	-
D-Sorbitol	+	-	-	-
Trehalose	+	+	+	-
D-Xylose	+	ND	+	+
Sodium malonate	-	-	+	-
Sodium acetate	-	+	+	-
L-Alanine	+	+	-	+
Potassium 5-ketogluconate	-	-	+	-
Glycogen	-	-	-	+
L-Serine	-	+	+	-
a-Ketobutyric acid	-	+	-	-
a-Ketovaleric acid	-	+	-	+
Propionic acid	-	+	-	-
L-Proline	+	+	+	-
L-Aspartic acid	-	+	+	+
L-Glutamic acid	-	+	+	+

Table 2. Comparison of the fatty acid content of strain *Pseudomonas asuensis* sp. nov. CP155-2^T and closely related species of the genus *Pseudomonas*
1, *Pseudomonas asuensis* sp. nov. CP155-2$^{T\#}$ (present study); 2, *Pseudomonas zeshuii* BY-1$^{T\$}$; 3, *Pseudomonas luteola* IAM 13000$^{T\$}$. Data from $^{\#}$present study; $^{\$}$Feng *et al.,* 2012); -, negative.

Fatty acid	1	2	3	4
$C_{10:0}$ 3-OH	0.9	2.5	2.0	2.1
$C_{12:0}$	6.0	7.2	6.9	6.6
$C_{12:0}$ 2-OH	2.9	1.5	1.5	0.7
$C_{12:0}$ 3-OH	3.2	4.2	4.3	3.5
$C_{14:0}$	1.4	1.1	1.7	0.8
$C_{16:0}$	10.9	21.9	23.0	11.2
$C_{18:1}$ ($\omega 7c$)	38.4	39.4	35.6	46.3
$C_{18:0}$	1.7	-	0.6	0.7
$C_{19:0}$ cyclo ($\omega 8c$)	2.9	2.3	6.8	4.6
Summed feature 3*	31.8	17.7	15.7	22.8

* Summed features represent groups of two or three fatty acids that could not be separated by GLC using the MIDI system. Summed feature 3 comprises $C_{16:1}\omega 6c$ and/or $C_{16:1}\omega 7c$.

the 16S rRNA gene sequence of strain CP155-2^T (EMBL accession number AJ871471) resulted in the identification of the *P. luteola* strains 4239 (EMBL accession number NR_037134) (Anzai *et al.,* 1997), BSP10 (EMBL accession number KF360062), Marseille (EMBL accession number AY574976), NBRC 103146 (EMBL accession number NR114215), and *P. zeshuii* strain BY-1 (EMBL accession number JN411093) (Feng *et al.,* 2012) as the nearest cultivatable bacteria with 16S rRNA gene sequence similarities of more than 98.5% and the closest non-cultivable identified was clone ELU0063-T430-S-NIPCRAMgANa 000123 (EMBL accession number HQ768311) (Li *et al.,* 2012). Except for the *P. luteola* strain NBRC 103146 (=IAM 13000 =JCM 3352 =DSM 6875 =ATCC 43273) (Kodama *et al.,* 1985) and the *P. zeshuii* strain BY-1 (EMBL accession number JN411093) (Feng *et al.,* 2012) all other cultivable bacteria were not validly described and were therefore ignored from the phylogenetic analyses. The BLAST results indicated that the strain belongs to the genus *Pseudomonas* (Migula, 1894), class *Gammaproteobacteria* and order *Pseudomonadales* of the family *Pseudomonadaceae*. The affiliation of strain CP155-2^T to the genus was further confirmed, based on 16S rRNA gene sequence, by EzTaxon (Kim *et al.,* 2012). The highest degree of sequence similarity of strain CP155-2^T was found with *P. luteola* (Kodama *et al.,* 1985; Holmes *et al.,* 1987), *P. zeshuii* (Feng *et al.,* 2012) and *P. duriflava* (Liu *et al.,* 2008). Based to base comparison of

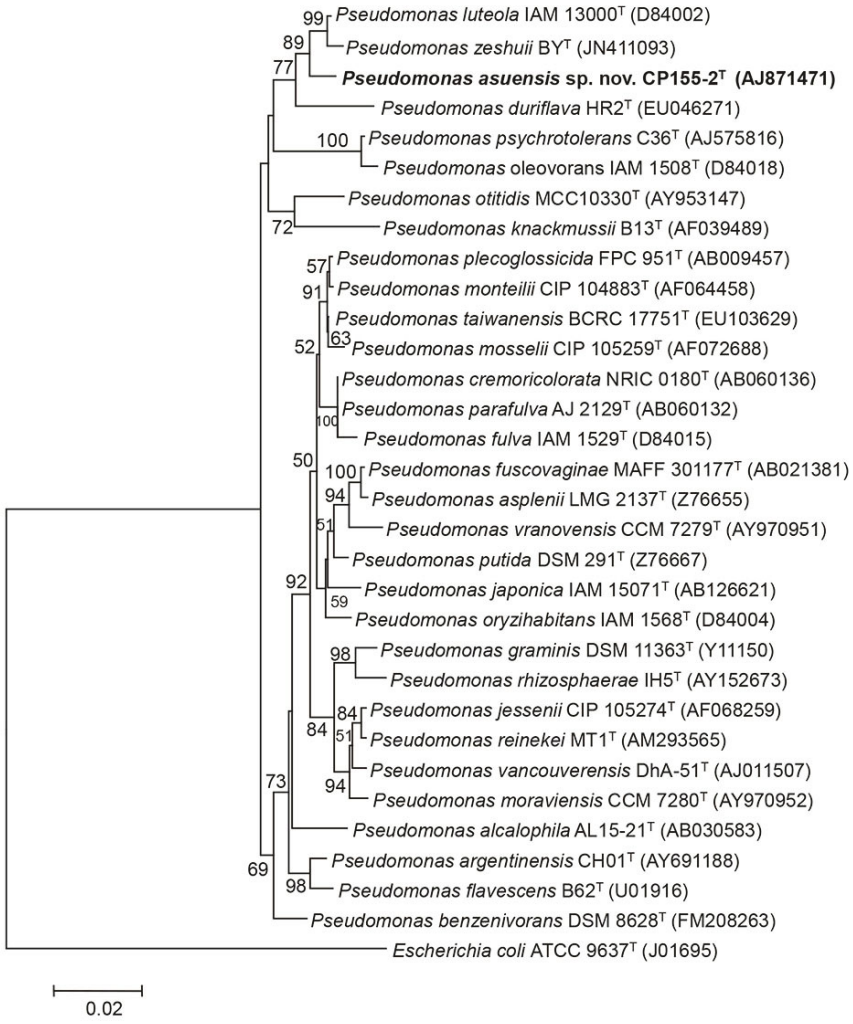

Fig. 1. Neighbour-joining tree, based on 16S rRNA (1458 nucleotide) gene sequence, showing the phylogenetic relationship between *Pseudomonas asuensis* sp. nov. CP155-2^T and other related taxa. Bootstrap values (expressed as percentages of 1,000 replications) greater than 50% are given at nodes. Scale bar: 0.01 substitutions per nucleotide

16S rRNA gene sequence of CP155-2^T with *P. luteola*, *P. zeshuii*, and *P. duriflava* (the three closest species in MEGA5) resulted in identification of a difference of 19/1459 (1 gaps), 20/1458 (1 gaps), and 43/1404 (2 gaps) nucleotides respectively. The 16S rRNA gene sequence similarities thus observed were 98.7%, 98.6%, and 96.9% respectively with *P. luteola*, *P. zeshuii,* and *P. duriflava*. In addition, phylogenetic analyses methods neighbor joining (Fig. 1), minimum evolution, maximum likelihood and maximum parsimony (trees not shown) demonstrated clustering of strain CP155-2^T with *P. luteola* (Kodama *et al.,* 1985; Holmes *et al.,* 1987), *P. zeshuii* (Feng *et al.,* 2012) and *P. duriflava* (Liu *et al.,* 2008) and thus belonged to the genus *Pseudomonas* lineage in the phylogenetic trees (Fig. 1).

Chemotaxonomic characteristics

The predominant cellular fatty acids of strain CP155-2^T were $C_{10:0}$ 3-OH (0.9), $C_{12:0}$ (6.0), $C_{12:0}$ 2-OH (2.9), $C_{12:0}$ 3-OH (3.2), $C_{14:0}$ (1.4), $C_{16:0}$ (10.9), $C_{18:1}$ ($\omega 7c$) (38.4), $C_{18:0}$ (1.7), $C_{19:0}$ cyclo ($\omega 8c$) (2.9), and summed feature 3* (31.8) (Table 2). Strain CP155-2^T had large amounts of $C_{18:1}$ ($\omega 7c$) and summed feature 3* ($C_{16:1}$ $\omega 6c$ and/or $C_{16:1}$ $\omega 7c$), characteristic feature of the genus *Pseudomonas.* Interestingly, compared to the closest species (which exhibited highest 16S rRNA gene sequence similarity of more than 98.5%), strain CP155-2^T had two fold increased amounts of fatty acid summed feature 3* and decreased amounts of $C_{16:0}$ while other fatty acids were comparable. Thus, strain CP155-2^T could be differentiated from *P. luteola* (Kodama *et al.,* 1985) and *P. zeshuii* (Feng *et al.,* 2012) based on differences in the compositions of the two fatty acids (Table 2). Strain CP155-2^T contained a major proportion of diphosphatidylglycerol (DPG) along with phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and various unknown polar lipids such as aminopolar lipid (APL), polar lipid 1 (PL1), and polar lipid 2 (PL2) (Supplementary data Fig. S2). The polar lipid profile of strain CP155-2^T was dominated by DPG, PG, APL, and PE, which were common in both the species *P. luteola* and *P. zeshuii* (Feng *et al.,* 2012). However, strain CP155-2^T lacks a few unknown lipids when compared to the above two closely related species (Feng *et al.,* 2012). The lipid profile supported affiliation of strain CP155-2^T with the genus *Pseudomonas* and differentiated it from its closely related species *P. luteola* and *P. zeshuii.* Strain CP155-2^T was yellow pigmented and the UV-Visible spectrum in methanol showed an absorption maxima at 267 nm, 428 nm, 449 nm, and 476 nm (Fig. 2). The yellow pigment did not show any shift in the absorption maxima upon addition of KOH, indicating that the pigment is not of flexirubin type (Reddy and Garcia-Pichel, 2005). Based on the absorption maximum at 449 nm, the pigment could be assigned to the family of β-carotenoid type (Rodriguez-Amaya, 1999; Kaiser *et al.,* 2007; Arulselvi *et al.,* 2014). Further, the absorption maxima at 428 nm, 476 nm, and 449 nm tentatively indicated that the type of β-carotenoid was *cis*-1-(6-C11:0)-glycosyl-apo-8′-lycopene (Aasen *et al.,* 1969; Perez-Fons *et al.,* 2011). In addition to the above three, notably, the pigment had an absorption maximum in the UV region at 267 nm (Fig. 2) signifying that the strain could be a potentially UV resistant one (Arrage *et al.,* 1993; Zenoff *et al.,* 2006), probably a common feature among the pigmented bacteria isolated from biological soil crusts (Rainey *et al.,* 2005; Reddy and Garcia-Pichel, 2007, 2009, 2013; Reddy *et al.,* 2007).

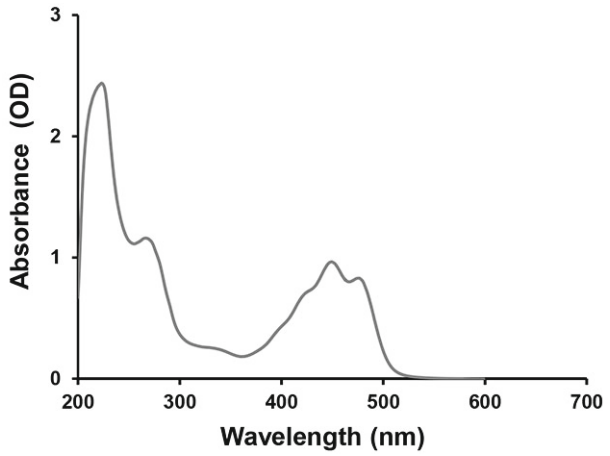

Fig. 2. A typical absorption spectrum of a methanolic pigment extract from *Pseudomonas asuensis* sp. nov. CP155-2^T.

Taxonomic conclusion

The morphological, growth, biochemical, and chemotaxonomic characteristics of strain CP155-2^T are described in previous sections and in the species description part of the manuscript. The sequence similarity search by EzTaxon and BLAST analyses using the almost complete 16S rRNA gene sequence of CP155-2^T identified the genus *Pseudomonas* as its closest relative and its affiliation to the genus was confirmed by the above morphological and chemotaxonomic characteristics. The highest degree of 16S rRNA gene sequence similarity of strain CP155-2^T was found with *P. luteola* (Kodama *et al.,* 1985; Holmes *et al.,* 1987), *P. zeshuii* (Feng *et al.,* 2012), and *Pseudomonas duriflava* (Liu *et al.,* 2008). However, the DNA-DNA homology shared by CP155-2^T with *P. luteola* and *P. zeshuii* was less than 70.0% (Supplementary data Fig. S3), suggesting that strain CP155-2^T belongs to a novel taxon and named as *Pseudomonas asuensis* sp. nov. (Stackebrandt and Goebel, 1994). The species status to CP155-2^T was further supported by phenotypic differences wherein CP155-2^T was positive for oxidase, negative for gelatin hydrolysis, could utilize D-cellobiose, D-raffinose, L-rhamnose, D-sorbitol but not L-aspartic acid and L-glutamic acid. Other phenotypic and chemotaxonomic differences are listed in Tables 1 and 2.

Strain CP155-2^T plays an important role in the ecology of biological soil crusts is evident from its ability to reduce nitrates (aids in ammonium fixation), produce phosphatase to enrich the crusts with phosphate by solubilizing the rocks (Rodríguez and Fraga, 1999; Sharma *et al.,* 2011; Brempong and Aferi, 2014) and utilize oxalate (Chandra and Shethna, 1975), phenanthrene (Samanta *et al.,* 1999; Tao *et al.,* 2007) and various sugars that are part of the composite structure of exopolysaccharide extruded by Cyanobacteria, the most dominant community of soil crusts (Sharma and Gaur, 2008; Yadav *et al.,* 2011). Besides, CP155-2^T could also have a sig-

nificant role in biological soil crust formation as it extrudes exopolysaccharide (Schulz *et al.,* 2013).

Description of *Pseudomonas asuensis* sp. nov.

Pseudomonas asuensis (a.su.en'sis. N.L. fem. adj. asuensis, an adjective arbitrarily derived from Arizona State University).

The colonies of *Pseudomonas asuensis* are yellow, crusti, convex, round, and smooth. Cells stain Gram-negative, non-motile, curved to straight rods, straight to V-shaped and few are beaded rods. They grow from 5°C to 37°C (but not at 40°C), with an optimum growth temperature of 25°C. The pH range for growth is 6 to 9 with an optimum pH of 7 and can tolerate up to 1% NaCl. Cells are positive for catalase, oxidase, DNAse, phosphatase, hydrolysis of esculin, and nitrate reduction, but negative for lipase, β-galactosidase, urease, gelatinase, arginine decarboxylase, lysine decarboxylase, ornithine decarboxylase, phenyl alanine deaminase, and arginine dihydrolase. Cells cannot hydrolyze casein, cellulose or starch. Cells are also negative for the methyl red, Voges-Proskauer and indole tests and did not produce H_2S gas. Cells are positive for Simmon's citrate and are capable of producing acid from D-glucose but not from L-arabinose, D-fructose, D-galactose, lactose, D-maltose, D-mannitol, sucrose, D-sorbitol or D-xylose. They can ferment L-arabinose, D-fructose, D-galactose, D-glucose, lactose, D-maltose, D-mannose, mellibiose, D-rhamnose, sucrose, and D-xylose but not D-sorbitol. Cells are positive for utilization of adonitol, L-arabinose, D-cellobiose, dextrose, dulcitol, D-fructose, fumaric acid, D-glucose, glycerol, *meso*-inositol, inulin, D-levulose, lactose, lactic acid, D-maltose, D-mannitol, D-mannose, D-raffinose, D-ribose, L-rhamnose, saccharose, succinate, sucrose, D-sorbitol, L-sorbose, D-trehalose, L-alanine, L-arginine, L-aspargine, L-cystiene, L-glutamine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-proline, L-threonine, L-valine, oxalate, phenanthrene as sole carbon source but not acetate, citrate, dextran, ethanolamine, D-galactose, D-mellibiose, pyruvate, D-xylose, L-aspartic acid, L-glycine, L-glutamic acid, L-histidine, L-phenylalanine, L-serine, L-tryptophane, L-tyrosine, adenine, cytosine, guanine, thymidine, nicotinic acid, indole, and tartaric acid. Cells are sensitive to (μg/disc) amikacin (30), chloramphenicol (30), ciprofloxacin (5), doxycyclin (10), erythromycin (15), kanamycin (30), lomefloxacin (30), nalidixic acid (30), nitrofurantoin (300), norfloxacin (10), rifampicin (30), streptomycin (10), tetracycline (30), tobramycin (30), vancomycin (30), and polymyxin B (300 units/disc), but resistant to ampicilin (10), cephazolin (30), cefoperazone (75), cefuroxime (30), cephotaxime (30), colistin (10), co-trimoxazole (25), lincomycin (30), and penicillin G (10). The pigment present is a β-carotenoid type with absorption maxima at 267 nm, 428 nm, 449 nm, and 476 nm. The fatty acids and their percent contribution are listed in Table 2. The polar lipids present are phosphatidyl ethanolamine (PE), phosphatidyl glycerol (PG), diphosphatidyl glycerol (DPG or cardiolipin), aminophospholipid (APL), and unknown phosphor lipids PL1 and PL2. The type strain of *Pseudomonas asuensis* is CP155-2^T (=ATCC BAA-1264^T =JCM13501^T =KCTC 32484^T). The 16S rRNA gene accession number is AJ871471. Strain CP155-2^T was isolated from biological soil crusts collected from the Colorado Plateau, USA.

Acknowledgements

This research was funded by the National Science Foundation Biotic Surveys and Inventories grant 0206711 to F. G-P.

References

Aasen, A.J., Francis, G.W., and Liaaen-Jensen, S. 1969. Bacterial Carotenoids XXIX. The carotenoids of two yellow halophilic cocci including a new glycosidic methyl apo-lycopenoate. *Acta. Chem. Scand.* **23**, 2605–2615.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215**, 403–410.

Anzai, Y., Kim, H., Park, J.Y., Wakabayashi, H., and Oyaizu, H. 2000. Phylogenetic affiliation of the *Pseudomonads* based on 16S rRNA sequence. *Int. J. Syst. Evol. Microbiol.* **50**, 1563–1589.

Anzai, Y., Kudo, Y., and Oyaizu, H. 1997. The phylogeny of the genera *Chryseomonas*, *Flavimonas*, and *Pseudomonas* supports synonymy of these three genera. *Int. J. Syst. Bacteriol.* **47**, 249–251.

Arrage, A.A., Phelps, T.J., Bennot, R.E., and White, D.C. 1993. Survival of surface microorganisms exposed to UV radiation and hydrogen peroxide. *Appl. Environ. Microbiol.* **59**, 3545–3550.

Arulselvi, P.I., Umamaheswari, S., Sharma, R.G., Karthik, C., and Jayakrishna, C. 2014. Screening of yellow pigment producing bacterial isolates from various eco-climatic areas and analysis of the carotenoid produced by the isolate. *J. Food Process Technol.* **5**, 1.

Atlas, R.M. and Parks, L.C. 1993. Handbook of Microbiological Media. CRC Press, Inc. London.

Belnap, J. 1993. Recovery rates of cryptobiotic crusts: inoculant use and assessment methods. *Great Basin Naturalist* **53**, 89–95.

Bertani, G. 1952. Studies on Lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli*. *J. Bacteriol.* **62**, 293–300.

Brempong, A.S. and Aferi, N.K. 2014. Isolation of phosphate solubilizing bacteria from tropical soil. *Global Advanced Research Journal of Agricultural Science* **3**, 8–15.

Chandra, T.S. and Shethna, Y.I. 1975. Isolation and characterization of some new oxalate-decomposing bacteria. *Antonie van Leeuwenhoek* **41**, 101–111.

Cowan, S.T. and Steel, K.J. 1965. Manual for the identification of medical bacteria. London: Cambridge University Press.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**, 783–791.

Feng, Z., Zhang, J., Huang, X., Zhang, J., Chen, M., and Li, S. 2012. *Pseudomonas zeshuii* sp. nov., isolated from herbicide-contaminated soil. *Int. J. Syst. Evol. Microbiol.* **62**, 2608–2612.

Garcia-Pichel, F., Johnson, S.L., Youngkin, D., and Belnap, J. 2003. Small-scale vertical distribution of bacterial biomass and diversity in biological soil crusts from arid lands in the Colorado plateau. *Microb. Ecol.* **46**, 312–321.

Gerhardt, P., Murray, R.G.E., Wood, W.A., and Krieg, N.R. 1994. Methods for general and molecular bacteriology. American Society for Microbiology, Washington, D.C., USA.

Holmes, B., Steigerwalt, A.G., Weaver, R.E., and Brenner, D.J. 1987. *Chryseomonas luteola* comb. nov. and *Flavimonas oryzihabitans* gen. nov., comb. nov., *Pseudomonas*-like species from human clinical specimens and formerly known, respectively, as groups Ve-1 and Ve-2. *Int. J. Syst. Bacteriol.* **37**, 245–250.

Kaiser, P., Surmann, P., Vallentin, G., and Fuhrmann, H. 2007. A small-scale method for quantitation of carotenoids in bacteria and yeasts. *J. Microbiol. Methods* **70**, 142–149.

Kim, O.S., Cho, Y.J., Lee, K., Yoon, S.H., Kim, M., Na, H., Park, S.C.,

Jeon, Y.S., Lee, J.H., Yi, H., *et al.* 2012. Introducing EzTaxon: a prokaryotic 16S rRNA Gene sequence database with phylotypes that represent uncultured species. *Int. J. Syst. Evol. Microbiol.* **62**, 716–721.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16**, 111–120.

Kodama, K., Kimura, N., and Komagata, K. 1985. Two new species of *Pseudomonas*: *P. oryzihabitans* isolated from rice paddy and clinical specimens and *P. luteola* isolated from clinical specimens. *Int. J. Syst. Bacteriol.* **35**, 467–474.

Komagata, K. and Suzuki, K. 1987. Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol.* **19**, 161–207.

Lanyi, B. 1987. Classical and rapid identification methods for medically important bacteria. *Methods Microbiol.* **19**, 1–67.

Lapage, S., Shelton, J., and Mitchell, T. 1970, Media for isolation-cultivation-identification-maintenance of medical bacteria, Vol. I, Williams and. Wilkins, Baltimore. Methods in Microbiology, *In* Norris, J. and Ribbons, D. (eds.), Vol. 3A, Academic Press, London.

Lee, L.J., Lee, H.J., Jang, G.S., Yu, J.M., Cha, J.Y., Kim, S.J., Lee, E.B., and Kim, M.K. 2013. *Deinococcus swuensis* sp. nov., a gamma-radiation-resistant bacterium isolated from soil. *J. Microbiol.* **51**, 305–311.

Li, E., Hamm, C.M., Gulati, A.S., Sartor, R.B., Chen, H., Wu, X., Zhang, T., Rohlf, F.J., Zhu, W., Gu, C., *et al.* 2012. Inflammatory bowel diseases phenotype, *C. difficile* and NOD_2 genotype are associated with shifts in human ileum associated microbial composition. *PLoS One* **7**, E26284.

Liu, R., Liu, H., Feng, H., Wang, X., Zhang, C.X., Zhang, K.Y., and Lai, R. 2008. *Pseudomonas duriflava* sp. nov., isolated from a desert soil. *Int. J. Syst. Evol. Microbiol.* **58**, 1404–1408.

MacFaddin, J.F. 1985. Media for isolation, cultivation, identification, maintenance of medical bacteria, Vol.1, Williams & Wilkins, Baltimore.

Marmur, J. 1961. Procedure for the isolation of deoxyribonucleic acid from microorganisms. *J. Mol. Biol.* **3**, 208–218.

Migula, W. 1984. Über ein neues system der bakterien. arb bakteriol *Inst Karlsruhe* **1**, 235–238 (in German).

Nagy, M., Pérez, A., and Garcia-Pichel, F. 2005. The prokaryotic diversity of biological soil crusts in the Sonoran desert (Organ Pipe Cactus National Monument, AZ). *FEMS Microb. Ecol.* **54**, 233–245.

Nübel, U., Garcia-Pichel, F., and Muyzer, G. 1997. PCR primers to amplify 16S rRNA genes from cyanobacteria. *Appl. Environ. Microbiol.* **63**, 3327–3332.

Oyaizu, H. and Komagata, K. 1983. Grouping of *Pseudomonas* species on the basis of cellular fatty acid composition and the quinone system with special reference to the existence of 3-hydroxy fatty acids. *J. Gen. Appl. Microbiol.* **29**, 17–40.

Palleroni, N.J. 1984. Genus I. *Pseudomonas* Migula 1894. Bergey's Manual of Systematic Bacteriology, vol. 1, pp. 141–199. *In* Krieg, N.R. and Holt, J.G. (eds.) Baltimore: Williams & Wilkins.

Pandey, K.K., Mayilraj, S., and Chakrabarti, T. 2002. *Pseudomonas indica* sp. nov., a novel butane utilizing species. *Int. J. Syst. Evol. Microbiol.* **52**, 1559–1567.

Perez-Fons, L., Steiger, S., Khaneja, R., Bramley, P.M., Cutting, S.M., Sandmann, G., and Fraser, P.D. 2011. Identification and the developmental formation of carotenoid pigments in the yellow/orange *Bacillus* spore-formers. *Biochim. Biophys. Acta.* **1811**, 177–185.

Rainey, F.A., Ray, K., Ferreira, M., Gatz, B.Z., Nobre, M.F., Bagaley, D., Rash, B.A., Park, M.J., Earl, A.M., Shank, N.C., *et al.* 2005. Extensive diversity of ionizing-radiation-resistant bacteria recovered from Sonoran desert soil and description of nine new species of the genus Deinococcus obtained from a single soil sample. *Appl. Environ. Microbiol.* **71**, 5225–5235.

Reasoner, D.J. and Geldreich, E.E. 1985. A new medium for the enumeration and subculture of bacteria from potable water. *Appl. Environ. Microbiol.* **49**, 1–7.

Reddy, G.S.N., Aggarwal, R.K., Matsumoto, G.I., and Shivaji, S. 2000. *Arthrobacter flavus* sp. nov., a psychrophilic bacterium isolated from a pond in McMurdo Dry Valley, Antarctica. *Int. J. Syst. Evol. Microbiol.* **50**, 1553–1561.

Reddy, G.S.N. and Garcia-Pichel, F. 2005. *Dyadobacter crusticola* sp. nov., from biological soil crusts in the Colorado plateau, USA and emended description of the genus *Dyadobacter* Chelius and Tripplett 2000. *Int. J. Syst. Evol. Microbiol.* **55**, 1295–1299.

Reddy, G.S.N. and Garcia-Pichel, F. 2006. The community and phylogenetic diversity of biological soil crusts in the Colorado plateau studied by molecular fingerprinting and intensive cultivation. *Microb. Ecol.* **52**, 345–357.

Reddy, G.S.N. and Garcia-Pichel, F. 2007. *Sphingomonas mucosissima* sp. nov., and *Sphingomonas dessicabilis* sp. nov. isolated from biological soil crusts in the Colorado plateau, USA. *Int. J. Syst. Evol. Microbiol.* **57**, 1028–1034.

Reddy, G.S.N. and Garcia-Pichel, F. 2009. Description of *Patulibacter americanus* sp. nov., isolated from biological soil crusts, emended description of the genus *Patulibacter* Takahashi *et al.,* 2006 and proposal of *Solirubrobacterales* ord. nov. and *Thermoleophilales* ord. nov. *Int. J. Syst. Evol. Microbiol.* **59**, 87–94.

Reddy, G.S.N. and Garcia-Pichel, F. 2013. Description of *Hymenobacter arizonensis* sp. nov. from the southwestern arid lands of the United States of America. *Antonie van Leeuwenhoek* **103**, 321–330.

Reddy, G.S.N., Manasa, B.P., Singh, S.K., and Shivaji, S. 2013. *Paenisporosarcina indica* sp. nov., a psychrophilic bacterium from Pindari glacier of the Himalayan mountain ranges and reclassification of *Sporosarcina antarctica* Yu *et al.,* 2008 as *Paenisporosarcina antarctica* comb. nov. and emended description of the genus *Paenisporosarcina. Int. J. Syst. Evol. Microbiol.* **63**, 2927–2933.

Reddy, G.S.N., Nagy, M., and Garcia-Pichel, F. 2006. *Belnapia moabensis* gen. nov., sp. nov., an α-*proteobacterium* from biological soil crusts in the Colorado Plateau, USA. *Int. J. Syst. Evol. Microbiol.* **56**, 51–58.

Reddy, G.S.N., Potrafka, R., and Garcia-Pichel, F. 2007. *Modestobacter versicolor* sp. nov., an actinobacterium from biological soil crusts that produces melanins under oligotrophy, with emended descriptions of the genus *Modestobacter* and *M. multiseptatus* (Mevs *et al.,* 2000). *Int. J. Syst. Evol. Microbiol.* **57**, 2014–2020.

Rodríguez, H. and Fraga, R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol. Adv.* **17**, 319–339.

Rodriguez-Amaya, D.B. 1999. A guide to analysis in foods, pp. 14–15. ILSI Res, Washington, D.C., USA.

Saitou, N. and Nei, M. 1987. The Neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406–425.

Samanta, S.K., Chakraborti, A.K., and Jain, R.K. 1999. Degradation of phenanthrene by different bacteria: evidence for novel transformation sequences involving the formation of 1-naphthol. *Appl. Microbiol. Biotechnol.* **53**, 98–107.

Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Technical Note 101. MIDI Inc., Newark, DE, USA.

Schulz, S., Brankatschk, R., Dümig, A., Kögel-Knabner, I., Schloter, M., and Zeyer, J. 2013. The role of microorganisms at different stages of ecosystem development for soil formation. *Biogeosciences* **10**, 3983–3996.

Sharma, H.K. and Gaur, H.S. 2008. Hatch inhibition of *Meloidogyne incognita* by aqueous extracts and exudates of five species of cyanobacteria. *Nematol. Medit.* **36**, 99–102.

Sharma, S., Kumar, V., and Tripathi, R.B. 2011. Isolation of phos-

phate solubilizing microorganism (PSMs) from soil. *J. Microbiol. Biotech. Res.* **1**, 90–95.

Shivaji, S., Rao, N.S., Saisree, I., Sheth, V., Reddy, G.S.N., and Bhargava, P.M. 1988. Isolation and identification of *Micrococcus roseus* and *Planococcus* sp. from Schirmacher oasis, Antarctica. *J. Biosci.* **13**, 409–414.

Smibert, R.M. and Krieg, N.R. 1994. Phenotypic characterization. Methods for general and molecular bacteriology, pp. 607–654. *In* Gerhardt, P., Murray, R.G.E., Wood, W.A., and Krieg, N.R. American Society for Microbiology, Washington, D.C., USA.

Smith, S.M., Abed, R.M.M., and Garcia-Pichel, F. 2004. Biological soil crusts of sand dunes in Cape Cod National Seashore, Massachusetts, USA. *Microb. Ecol.* **28**, 200–208.

Sneath, P.H.A., Stevens, M., and Sackin, M.J. 1981. Numerical taxonomy of *Pseudomonas* based on published records of substrate utilization. *Antonie van Leeuwenhoek* **47**, 423–448.

Stackebrandt, E. and Goebel, B.M. 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.* **44**, 846–849.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**, 2731–2739.

Tao, X.Q., Lu, G.N., Dang, Z., Yang, C., and Yi, X.Y. 2007. A phenanthrene-degrading strain *Sphingomonas* sp. GY2B isolated from contaminated soils. *Process Biochem.* **42**, 401–408.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25**, 4876–4882.

Vancanneyt, M., Witt, S., Abraham, W.R., Kersters, K., and Fredrickson, H.L. 1996. Fatty acid content in whole-cell hydrolysates and phospholipid fractions of *Pseudomonads*: a taxonomic evaluation. *Syst. Appl. Microbiol.* **19**, 528–540.

Yadav, S., Sinha, R.P., Tyagi, M.B., and Kumar, A. 2011. Cyanobacterial secondary metabolites. *Int. J. Pharma Bio Sciences* **2**, 144–167.

Zenoff, V., Heredia, J., Ferrero, M., Sineriz, F., and Farias, M.E. 2006. Diverse UV-B resistance of culturable bacterial community from high-altitude wetland water. *Curr. Microbiol.* **52**, 359–362.

Zhang, W., Zhang, G., Liu, G., Dong, Z., Chen, T., Zhang, M., Dyson, P.J., and An, L. 2012. Bacterial diversity and distribution in the southeast edge of the Tengger Desert and their correlation with soil enzyme activities. *J. Environ. Sci.* (China). **24**, 2004–2011.