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ORIGINAL PAPER

Sphingobacterium pakistanensis sp. nov., a novel plant growth promoting rhizobacteria isolated from rhizosphere of *Vigna mungo*

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Abstract The taxonomic status of a bacterium, strain NCCP-246^T, isolated from rhizosphere of *Vigna mungo*, was determined using a polyphasic taxonomic approach. The strain NCCP-246^T can grow at 16–37 °C (optimum 32 °C), at pH ranges of 6–8 (optimum growth occurs at pH 7) and in 0–4 % (w/v) NaCl. Phylogenetic analysis based upon on 16S rRNA gene sequence comparison revealed that strain NCCP-246^T belonged to genus *Sphingobacterium*. Strain NCCP-246^T showed highest similarity to the type strain of *Sphingobacterium canadense* CR11^T (97.67 %) and less than 97 % with other species of the genus. The DNA–DNA relatedness value of strain NCCP-246^T with *S. canadense* CR11^T and *Sphingobacterium thalpophilum* JCM 21153^T was 55 and 44.4 %, respectively. The chemotaxonomic data revealed the major menaquinone as MK-7 and dominant cellular fatty acids were summed feature 3 [$C_{16:1}$ ω7c/$C_{16:1}$ ω6c] (37.07 %), iso-$C_{15:0}$ (28.03 %), $C_{16:0}$ (11.85 %), $C_{17:0}$ cyclo (8.84 %) and $C_{14:0}$ (2.42 %). The G+C content of the strain was 39.2 mol%. On the basis of DNA–DNA hybridization, phylogenetic analyses, physiological and, biochemical data, strain NCCP-246^T can be differentiated from the validly named members of genus *Sphingobacterium* and thus represents as a new species, for which the name, *Sphingobacterium pakistanensis* sp. nov. is proposed with the type strain NCCP-246^T (= JCM18974 ^{T} = KCTC 23914^T).

Keywords *Sphingobacterium* · *nif*H gene · *Vigna mungo* · Antibiotic resistance · P-solubilization

Iftikhar Ahmed and Muhammad Ehsan have contributed equally in the experiments.

The DDBJ/EMBL/GenBank accession number for the 16S rRNA gene sequence of strain NCCP-246^T (=JCM18974^T = KCTC 23914^T), is AB610802.

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Introduction

The genus *Sphingobacterium* was first described by Yabuuchi et al. (1983) due to the presence of large content of sphingolipids (Shivaji et al. 1992) in the family *Sphingobacteriaceae* and initially comprised of three species *Sphingobacterium spiritivorum, Sphingobacterium multivorum* and *Sphingobacterium mizutae*. At present, this genus contains 27 validly named species, and characterized as Gram-negative rods that are positive for catalase and oxidase, negative for heparinase, gelatinase and indole production; contain iso-$C_{15:0}$, iso-$C_{15:0}$ 2-OH, $C_{16:1}$ ω7*c* and $C_{17:0}$ 3-OH as the main fatty acids (Takeuchi and Yokota 1992; Steyn et al. 1998) and menaquinone 7 (MK-7) as the predominant isoprenoid quinone (Lee et al. 2013). The range of DNA G+C content is approximately 35–44 mol% (Liu et al. 2008; He et al. 2010). The members of this genus are widely distributed in various soils (Schmidt et al. 2012; Marqués et al. 2012; Duan et al. 2009; Shivaji et al. 1992), compost (Yoo et al. 2007; Kim et al. 2006), activated sludge (Sun et al. 2013), Lichen (Lee et al. 2013), rhizosphere (Mehnaz et al. 2007), faeces (Takeuchi and Yokota 1992), lakes (Albert et al. 2013), food sources (Schmidt et al. 2012; Takeuchi and Yokota 1992) and various other sources.

During investigation of microbial diversity of legumes (*Vigna mungo*) and rhizospheric soil, strain NCCP-246^T was recovered on tryptic soy agar (TSA, Difco). The purified strain was subjected to phenotypic and phylogenetic characterization experiments. Type strains of closely related taxa, *Sphingobacterium canadense* CR11^T and *Sphingobacterium thalpophilum* JCM 21153^T were also studied as reference strains in all of these experiments under the same laboratory conditions unless otherwise mentioned. On the basis of results, strain NCCP-246^T represented a novel species in the genus *Sphingobacterium*.

Materials and methods

Isolation, morphology and phenotypic characterization

Strain NCCP-246^T was isolated from rhizosphere of *Vigna mungo* on tryptic soy agar (TSA, Difco) by dilution plate method. The samples of roots and rhizospheric soil were collected from the research farm of Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan. The purified strain was maintained on agar medium and also stored in 35 % glycerol (w/v) at −80 °C. Colonial morphology of the strain NCCP-246^T was observed on well separated colonies grown on TSA for 2 days at 30 °C. The cell morphology was observed by phase-contrast microscopy with a Nikon Optiphot-2 light microscope and further detailed on a scanning electron microscope (S4300N, Hitachi) following previously described procedure (Jung et al. 2012). Gram staining was performed using commercial kit according to the instructions (bioMérieux, France). The optimum and range of pH for growth of cells was determined at 30 °C in tryptic soy broth (TSB; Difco) by adjusting to a range of pH 4.0–10.0 (at increment of 1 pH unit) and by monitoring OD_{600} using a spectrophotometer (IMPLEN, Germany). The pH values adjusted by 1 N HCl or 1 N Na_2CO_3 were verified after autoclaving. The temperature range for growth of cells was determined on TSA (pH 7.0) by incubating at different temperatures (4, 10, 16, 22, 28, 32, 37, 45, 50 °C) for 6 days. Tolerance to NaCl was determined using mTGE medium (Difco), which contains (per litre): beef extract (6 g), tryptone (10 g), dextrose (2 g); agar (15 g) and supplemented with various concentration of NaCl (0–7 %) and incubation at 30 °C for 2–3 days. Relation to oxygen was determined on TSA by incubation in an anaerobic chamber (Mitsubishi Gas Chemicals Co., Inc.) at 30 °C for 10 days.

Physiological and biochemical characteristics were determined using API 20E and API 50CH galleries (bioMérieux, France). Resistance to antibiotics was assessed with an ATB-Vet strip (bioMérieux, France) and enzyme activities were determined with an API-ZYM strip (bioMérieux, France). Additional metabolic feature of strain NCCP-246^T in comparison to the reference strains were assessed by using Biolog GN2 microplate characterization system (Biolog, USA). Catalase and oxidase activities were determined by using API Color Catalase and API Oxidase Reagent (bioMérieux, France), respectively. Motility of the cells was determined by microscopy. All commercial kits were used according to the manufacturers' protocols.

Chemotaxonomic analysis

For whole-cell fatty acids analysis, NCCP-246^T and the reference strains were grown on tryptic soy agar

(TSA, Difco) at 30 °C for 24 h. The cellular fatty acid methyl esters were prepared (Sasser 1990) and were analyzed on GC (6890N; Agilent, USA) according to the standard protocol of the Sherlock Microbial Identification System (MIDI Sherlock version 4.5, MIDI database TSBA40 4.10). Respiratory quinone of NCCP-246^T and the reference strains were analyzed from 300 mg lyophilized cells grown on tryptic soy broth (TSB, Difco) at 37 °C for 24 h as described by Minnikin et al. (1984). Isoprenoid quinones were examined by TLC and HPLC.

DNA base composition, DNA–DNA hybridization and phylogenetic analysis

For DNA G+C content analysis and DNA–DNA hybridization, DNA of strain NCCP-246^T and the reference strains were isolated using Qiagen Genomic-tip 500/G (Qiagen, Germany) following the manufacturer's protocol, with a minor modification in which RNase T_1 was also used in addition to RNase A. DNA–DNA hybridization was performed with five replications of each sample, at 40 °C with photobiotin-labelled DNA and microplates as described by Ezaki et al. (1989), using an Fluoroskan Ascent Fluorescent plate reader (Thermo Life Sciences, USA) for fluorescence measurements.

Nearly complete 16S rRNA gene was amplified as previously described (Ahmed et al. 2007). The purified PCR product was sequenced using universal forward 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′), 518F (5′-CCA GCA GCC GCG GTA ATA CG-3′), and reverse 800R (5′-TAC CAG GGT ATC TAA TCC-3′), 1492R (5′-ACC TTG TTA CGA CTT-3′), 1510R primers from Macrogen, Korea (http://dna.macrogen.com/en). The contig sequences obtained were assembled using BioEdit software to get the consensus sequence. The strain was identified using the sequence of 16S rRNA gene on Ez-Taxon Server (http://eztaxon-e.ezbiocloud.net) and BLAST search on DDBJ/NCBI servers. Sequences of closely related validly published type strains were retrieved from database of EzTaxon Server for constructing the phylogenetic trees. Molecular evolutionary analyses were performed as described earlier (Roohi et al. 2012) using MEGA 5.10 and phylogenetic trees were constructed based on a comparison of 1348 nucleotides by the Kimura 2-parameter model using three algorithms: neighbor joining (NJ), maximum parsimony (MP) and maximum likelihood (MLH). Ambiguous positions and gaps were excluded in calculations. The stability of the relationship was assessed by bootstrap analysis (Felsenstein 2005), by performing 1,000 re-sampling for the tree topology.

Amplification of *nif*H gene, analyses for phosphate solubilization and indole production activity

The *nif*H gene analysis was carried out to check the nitrogen fixation ability of strain NCCP-246^T by PCR amplification of the gene as described by Poly et al. (2001) using universal forward PolF^b (5′-TGC GAY CCS AAR GCB GAC TC-3′) and reverse PolR^b (5′-ATS GCC ATC ATY TCR CCG GA-3′) primers.

The strain NCCP-246^T was tested for qualitative and quantitative phosphorus solubilization activity. The qualitative mineral phosphate solubilization assay was performed by measuring the halo zone around bacterial colonies on Pikovskaya agar medium (Hayat et al. 2013). The quantitative phosphate solubilization capacity of strain NCCP-246^T was determined in Pikovskaya broth containing 0.5 % tri-calcium phosphate (pH 7.0) on rotary shaker for 8 days at 30 °C (Hayat et al. 2013). The drop in pH of the medium recorded and the available phosphorus was analysed using the protocol of Watanabe and Olsen (1965) and solubilization index and solubilization efficiency were calculated.

Strain NCCP-246^T was tested for production of indole acetic acid (IAA) following the procedure described earlier (Hayat et al. 2013). Strain NCCP-246^T was inoculated in LB medium with or without adding tryptophan (500 μg mL^{-1}). Bacterial culture was placed for 48 h on incubating shaker at 30 °C. Fully grown culture was centrifuged at 6000 rpm for 10 min. The supernatant (2 mL) was mixed with two drops of orthophosphoric acid (10 mM) and 4 mL of the Salkowski reagent (50 mL, 35 % of perchloric acid, 1 mL 0.5 M $FeCl_3$ solution). Development of pink color indicated IAA production, which is measured on spectrophotometer at 530 nm (Bric et al. 1991).

Results and discussion

Morphology and phenotypic characterization

Strain NCCP-246^T formed circular, entire, whitish-yellow colonies, which have smooth surface with

Table 1 Differentiating phenotypic characteristics of strain NCCP-246^T in comparison to the reference type strains of closely related members of genus *Sphingobacterium*

	NCCP-246^T	*Sphingobacterium canadense* CR11^T	*Sphingobacterium thalpophilum* JCM 21153^T
Growth at			
Temp. (°C) range (optimum)	16–37 (32)	22–45[a] (32)	16–45 (32)
pH range (optimum)	5–8 (7)	5–9[a] (7–8)	5–8 (7)
Oxidase	−	+	+
Hydrolysis of gelatin	−	+	−
Voges-Proskauer reaction	+	+[b]	−
Acid from			
D-Arabinose	+	w+	w+
L-Arabinose	w+	+	w+
L-Sorbose	−	w+[a]	−
L-Rhamnose	−	w+[a]	−[c]
Methyl-αD-mannopyranoside	+	+[a]	w+
Amygdalin	w+	+[a]	+
Arbutin	w+	+[a]	+
Salicin	w+	+	+
D-Celiobiose	+	+	w+
D-Lactose	+	+	w+
D-Melibiose	+	+	−
D-Trehalose	+	+[a]	−
Inulin	+	+	w+
D-Melezitose	w+	w+	−
D-Rafinose	+	+	w+
Gentibiose	w+	−	−
D-Turanose	w+	w+	−
L-Fucose	w+	−	−
Oxidation/fermentation of:			
α-Cyclodextrin	+	+	−
Pyruvic acid methyl ester	+	+	−
Succinic acid mono-methyl ester	+	+	−
L-Alanyl-glycine	+	+	−
L-Serine	+	−	−
L-Threonine	+	+[a]	−
Tween 80	+	−	−
L-Fucose	+	−	−
α-Ketobutyric acid	+	+	−
D,L-Lactic acid	+	−	−
D,L,α-Glycerol phosphate	+	+	−
Glycyl-L-glutamic acid	−	+	−
N-Acetyl-D-galactosamine	−	+	+
L-Rhamnose	−	+[a]	−
D-Galacturonic acid	−	+	−
Uridine	−	+	+

Table 1 continued

	NCCP-246^T	*Sphingobacterium canadense* CR11^T	*Sphingobacterium thalpophilum* JCM 21153^T
α-D-Glucose-1-phosphate	−	+	−
D-Glucose-6-phosphate	−	+	−
L-Aspartic acid	−	−	+
L-Glutamic acid	−	−	+
Inosine	−	−	+
Enzyme activity:			
Esterase (C 4)	−	w+	w+
Leucine arylamidase	−	+	+
α-Galactosidase	w+	+	+
β-Galactosidase	–	+	+
β-Glucoronidase	−	w+	–[c]
α-Glucosidase	w+	+	+
β-Glucosidase	+	+	w+
α-Fucosidase	w+	w+	+
Resistance to (μg ml^{-1}):			
Amox-clav.acid ((4/2)	S	R	S
Tetracycline (4)	R	R	R^w
Cotrimoxazol (2/38)	S	S	R
Sulfamethizol (100)	R	R	S
Oxolinic acid (2)	R	S	S
Enrofloxacin (0.5)	S	S	R
Rifamcin (4)	S	S	R
G+C content, mol% (as analyzed on HPLC)	39.2	37.2	39.8

All strains were positive for production of acid from D-glucose, D-fructose, D-mannose, esculin, D-maltose, D-saccharose (sucrose), amidon (starch), glycogen (weak), and methyl-αD-glucopyranoside; but negative for glycerol, erythritol, D-ribose, L-xylose, D-adonitol, methyl-*β*-D-xylopyranoside, dulcitol, inositol, D-mannitol, D-sorbitol, xylitol, D-lyxose, tagatose, D-fucose, D, L-arabitol, potassium gluconate, potassium 2-keto-gluconate, and potassium 5-keto-gluconate. All strains were positive for urease, catalase, ONPG (2-nitrophenyl-*β*-D galactopyranoside), and nitrate reduction; but negative for arginine dihydrolase, lysine and ornithine decarboxylases, citrate utilization, H_2S production, tryptophane deaminase and indole production (bioMérieux, France). All strains are positive for oxidation/reduction reaction (Biolog, USA) for the substrates: dextrin, *N*-acetyl-D-glucosamine, D-cellobiose, D-fructose, genitiobiose, α-D-glucose, α-D-lactose, lactulose, maltose, D-mannose, D-melibiose, *β*-Methyl-D-glucoside, sucrose, D-trehalose, turanose, acetic acid, glycerol, L-alabinose, D-galactose, D-raffinose, L-asparagine and L-proline. Positive enzyme activity observed in all the strains for alkaline phosphatase, acid phosphatase, *N*-acetyl- *β*-glucosaminidase, napthol-As-BI-phosphohydrolase, valine arylamidase, esterase lipase (C 8), α-mannosidase, whereas negative for lipase (C 14), cystine arylamidase, trypsin and α–chymotrypsin. All strains were resistant (μg mL^{-1}) to penicillin (0.25), amoxycilin (4), oxacillin (2), cephalothin (8), cefoperazon (4), streptomycin (8), spectinomycin (64), kanamicin (8), gentamicin (4), apramycin (16), chloramphenicol (8), tetracycline (4), erythromycin (1), lincomycin (2), pristinamycin (2), tylosin (2), metronidazol (4) but sensitive for flumequin (4), and fusidic Acid (2)

+ positive, − negative, *w*+ weakly positive, *R* resistant, *S* sensitive, R^w weakly resistant. All data are from this study

[a] Data that were different to those found in previous studies (Mehnaz et al. 2007)

[b] Data that was different to that reported by Marques et al. (2012) but in agreement with Sun et al. (2013)

[c] Data that were different to those found in previous studies (Sun et al. 2013)

slightly convex elevation and opaque. The colonies grow to 2–3 mm in diameter after 24–48 h on TSA. The cells were non-motile, short rods (Supplementary Fig. 1), strictly aerobic and Gram-stain negative. Growth was observed at 16–37 °C (optimum 32 °C), pH 5–8 (optimum 7) and in concentrations of 0–4 %

NaCl (optimum 0–1 %). The phenotypic characteristics of strain NCCP-246^T in comparison to the reference strains, *S. thalpophilum* JCM 21153^T, *S. canadense* CR11^T are presented in Table 1 and summarized in the species description. Strain NCCP-246^T shared many phenotypic features with the closely related taxa, *S. thalpophilum* JCM 21153^T, *S. canadense* CR11^T; however, it also differed from these species in certain physiological and biochemical characteristics (Table 1).

Strain NCCP-246^T was positive for phosphorus solubilization (54.4 ± 1.2 μg mL^{-1}) and decreased the pH of medium from 7.0 to 4.8 compared to control (phosphorus solubilization 12.1 ± 2.2 μg mL^{-1}), where there was no significant decrease in pH (6.5). The solubilization index and solubilization efficiency of strain NCCP-246^T were 2.7 and 166.7, respectively. The *nif*H gene was successfully amplified in strain NCCP-246^T. These results correlate the growth promoting activity of strain NCCP-246^T. However, there was observed a negligible production of IAA (0.32 ± 0.03 μg mL^{-1}) in LB medium with or without addition of tryptophan. These growth promoting characters (i.e. phosphorus solubilization, negligible production of IAA) also differentiate the strain from closely related species *S. canadense* CR-11^T, which has been reported negative for phosphorus solubilization and positive for IAA production (Mehnaz et al. 2007).

Phylogenetic analysis, DNA–DNA hybridization and DNA base composition

An almost complete 16S rRNA gene sequence (1460 nucleotides) of strain NCCP-246^T was compared with sequences of the closely related type strains on EzTaxon Server database. The highest similarity of 16S rRNA gene sequence of strain NCCP-246^T was found as 97.67 and 96.98 % with *S. canadense* CR11^T (AY787820) and *S. thalpophilum* JCM 21153^T (AJ438177), respectively, and less than 97 % with other species of genus *Sphingobacterium*. Strain NCCP-246^T clustered with *S. thalpophilum* JCM 21153^T (AJ438117) in the maximum-likehood phylogenetic tree with 52 % bootstrap support (Fig. 1). The monophyletic node of this cluster also appeared, when phylogenetic trees were constructed using NJ and MP algorithms (Supplementary Figs. 2, 3), suggesting a relationship of strain NCCP-246^T with *S. thalpophilum* JCM 21153^T (AJ438117). Strain NCCP-246^T is closely related to *S. thalpophilum* JCM 21153^T and *S. canadense* CR11^T in many other characteristics, although the data presented here also exhibited differences from these two and the type species of genus *Sphingobacterium* (Table 1).

The DNA–DNA relatedness of NCCP-246^T was 55 % with *S. canadense* CR11^T and 44.4 % with *S. thalpophilum* JCM 21153^T. These values are less than the 70 % threshold needed to assign the strain to a novel species (Stackebrandt and Goebel 1994). DNA G+C content of strain NCCP-246^T was analyzed as 39.2 mol%, whereas a slightly different values observed for *S. canadense* CR11^T (37.2 mol%) and *S. thalpophilum* JCM 21153^T (39.8 mol%) than reported by Mehnaz et al. (2007). These values are, however, in the range as described earlier for the members of genus *Sphingobacterium.*

Chemotaxonomic analysis

The cellular fatty acid profile for strain NCCP-246^T consisted predominantly of summed feature 3 (comprised one or more of $C_{16:1}$ ω7c/$C_{16:1}$ ω6c; 37.07 %), iso-$C_{15:0}$ (28.03 %), $C_{16:0}$ (11.85 %), $C_{17:0}$ cyclo (8.84 %), $C_{16:0}$ 3-OH (4.81 %) iso-$C_{15:0}$ 3-OH (2.70 %) and $C_{14:0}$ (2.42 %) (Table 2). This profile of strain NCCP-246^T is similar to those of the reference strains analyzed under the same conditions except small difference of values. MK-7 was found to be the major isoprenoid quinone in strain NCCP-246^T, which is in agreement with the reference strains, *S. thalpophilum* JCM 21153^T, *S. canadense* CR11^T.

On the basis of morphological, physiological, phylogenetic, chemotaxonomic and genomic characteristics which we determined, strains NCCP-246^T is considered to be a new member of genus *Sphingobacterium* and thus its description is given as follows:

Description of *Sphingobacterium pakistanensis* sp. nov

Sphingobacterium pakistanensis (pa.kis.tan.en'sis. N.L. masc. adj. pakistanensis pertaining to Pakistan, where the organism was isolated)

Cells are Gram negative, strictly aerobic, non-motile, sometime occurs in pairs and short rod (1.7–3.3 μm) in appearance. The colonies are round with entire margin,

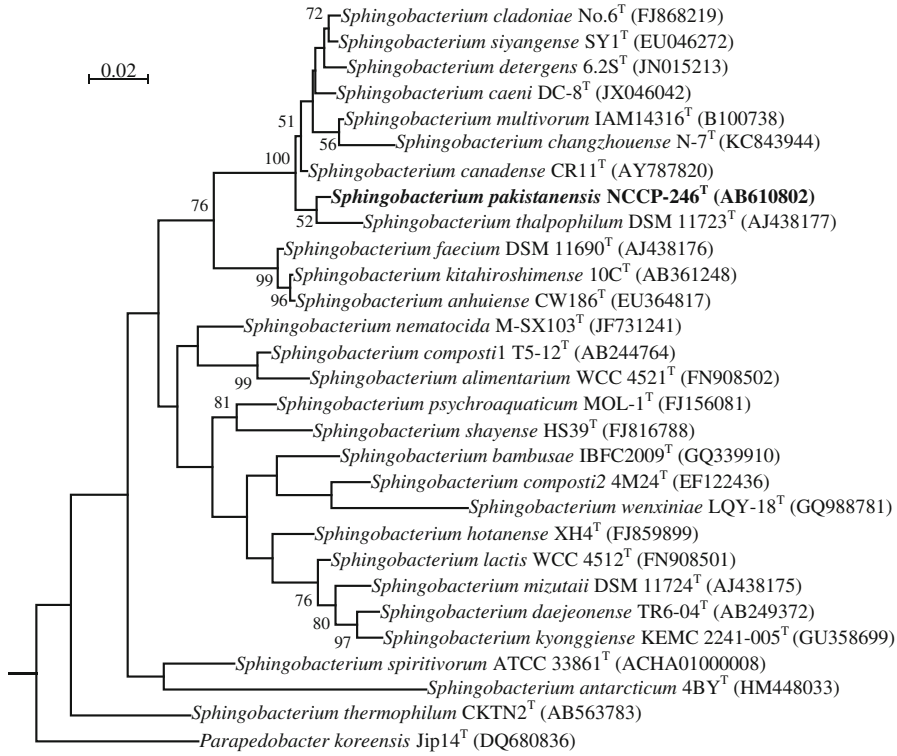

Fig. 1 Phylogenetic tree generated using maximum-likehood algorithm showing inter-relationship of strain NCCP-246^T with the closely related type strains of genus *Sphingobacterium* inferred from sequences of 16S rRNA gene. *Parapedobacter koreensis* Jip14^T (DQ680836) is used as an out group. Bootstrap values (only >50 % shown), expressed as a percentage of 1,000 replications, are given at the *branching points*. The accession number of each type strain is shown in *parentheses*

slightly convex in elevation, having opaque surface and off white in color, which turns yellowish white after few days. Two days old culture on TSA agar plates produce colonies of 2–3 mm diameter and have butyrous (butter like) texture. Cells grow on TSA agar plates at 16–37 °C (optimum 32 °C) and in TSB medium with a pH ranges of 5–8 (optimum growth occurs at pH 7). It can tolerate 0–4 % (w/v) NaCl but no growth was observed with 5 % NaCl. It is negative for IAA and positive for *nif*H gene and can solubilize mineral phosphorus (54.35 ± 1.21 μg mL^{-1}) from tri-calcium phosphate, which is relative insoluble. Positive for urease, catalase, Voges-Proskauer reaction, ONPG (2-nitrophenyl-β-D galactopyranoside), and can reduce nitrate; but negative for oxidase, hydrolysis of gelatin, arginine dihydrolase, lysine and ornithine decarboxylases, citrate utilization, H_2S production, tryptophane deaminase and indole production. No fermentation of D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, D-sucrose, D-malibiose, amygdalin, L-arabinose. Acid is produced from D-glucose, D-fructose, D-mannose, esculin, D-maltose, D-arabinose, D-saccharose (sucrose), D-rafinose, amidon (starch), D-celiobiose, D-lactose, D-melibiose, D-trehalose, inulin, glycogen (weak), L-arabinose (weak), amygdalin (weak), arbutin (weak), salicin (weak), D-melezitose (weak), gentibiose (weak), D-turanose (weak), L-fucose (weak), *N*-acetyle glucosamine (weak), D-xylose (weak), D-galactose (weak), methyl-αD-mannopyranoside and methyl-αD-glucopyranoside; but negative for acid production from glycerol, erythritol, D-ribose, L-xylose, D-adonitol, methyl-β D-xylopyranoside, dulcitol, inositol, D-mannitol, D-sorbitol, xylitol, D-lyxose, tagatose, D-fucose, D, L-arabitol, L-sorbose, L-rhamnose, potassium gluconate, potassium 2-keto-gluconate, and potassium 5-keto-gluconate. Positive for oxidation/reduction activity for the substrates: α-cyclodextrin, dextrin, *N*-acetyl-D-glucosamine, D-cellobiose, D-fructose, genitiobiose, α-D-glucose, α-D-lactose, lactulose, maltose, D-mannose, D-melibiose, β-methyl-D-glucoside, sucrose, D-trehalose, turanose, pyruvic acid methyl ester, succinic acid mono-methyl ester, acetic acid, L-alanyl-glycine, L-serine, L-threonine, glycerol, tween 80, L-alabinose, L-fructose, D-galactose, D-raffinose, α-keto-butyric acid, D,L-lactic acid, L-asparagine, L-proline, and D,L,α-glycerol phosphate; but negative for the substrates: glycyl-L-glutamic acid, *N*-acetyl-D-galactosamine, L-rhamnose, D-galacturonic acid, uridine, α-D-glucose-1-phosphate, D-glucose-6-phosphate, L-asparatic acid, L-

Table 2 Cellular fatty acid profiles (%) of strain NCCP-246^T and closely related type strains of members of genus *Sphingobacterium*

Fatty acid	NCCP-246^T	*Sphingobacterium canadense* CR11^T	*Sphingobacterium thalpophilum* JCM 21153^T
$C_{14:0}$	2.42	2.41	3.98
iso-$C_{15:0}$	28.03	24.83	24.48
$C_{16:0}$	11.85	10.49	11.79
iso-$C_{15:0}$ 3-OH	2.70	2.75	3.69
$C_{16:0}$ 2-OH	tr	tr	2.89
$C_{16:0}$ 3-OH	4.81	5.46	5.67
$C_{17:0}$ cyclo	8.84	7.70	8.81
Sum in feature[a]			
Sum in feature 3[b]	37.07	41.63	36.43

All data are obtained this study. Values are percentages of total fatty acid detected tr, trace amount (<1.0 %)

[a] Fatty acids that could not be separated by GC using the microbial Identification System (Microbial ID) software were considered summed features

[b] Summed feature 3 ($C_{16:1}$ ω7c/$C_{16:1}$ ω6c), which could not have been separated by MIDI system

glutamic acid, and inosine. Resistant (μg mL^{-1}) to penicillin (0.25), amoxycilin (4), oxacillin (2), cephalothin (8), cefoperazon (4), streptomycin (8), spectinomycin (64), kanamicin (8), gentamicin (4), apramycin (16), chloramphenicol (8), tetracycline (4), doxycycline (4), erythromycin (1), lincomycin (2), pristinamycin (2), tylosin (2), colistin (4), sulfamethizol (100), oxolinic acid (2), metronidazol (4), nitrofurantoin (25) but sensitive to flumequin (4), fusidic acid (2) rifamcin (4) enrofloxacin (0.5), cotrimoxazol (2/38), and amox-clav.acid ((4/2). Strongly positive enzyme activity is observed for alkaline phosphatase, acid phosphatase, napthol-As-BI-phosphohydrolase, *N*-acetyl-*β*-glucosaminidase; positive for valine arylamidase, α-mannosidase, *β*-glucosidase, esterase lipase (C-8), weak enzyme activity for α-glucosidase, α-galactosidase, α-fucosidase, whereas negative for all other enzymes of API-Zym (bioMérieux, France). Major cellular fatty acids are summed feature 3 ($C_{16:1}$ ω7c/$C_{16:1}$ ω6c or $C_{16:1}$ ω6c/$C_{16:1}$ ω7c; 37.07 %), iso-$C_{15:0}$ (28.03 %), $C_{16:0}$ (11.85 %), $C_{17:0}$ cyclo (8.84 %), $C_{16:0}$ 3-OH (4.81 %), iso-$C_{15:0}$ 3-OH (2.70 %) and $C_{14:0}$ (2.42 %). The major respiratory quinone is MK-7. The DNA G+C content of the type strain is 39.2 mol%.

Strain NCCP-246^T (= JCM18974 ^{T} = KCTC 23914^T) is the type strain, isolated from *Vigna mungo* roots and rhizospheric soil sample collected from Research Farm area of Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan.

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