Arch Microbiol (2016) 198:933–939
DOI 10.1007/s00203-016-1251-3

ORIGINAL PAPER

Microvirga pakistanensis sp. nov., a novel bacterium isolated from desert soil of Cholistan, Pakistan

Arshia Amin[1,2,3] · Iftikhar Ahmed[2,4] · Neeli Habib[1] · Saira Abbas[2,4] · Fariha Hasan[3] · Min Xiao[5] · Wael N. Hozzein[7] · Wen-Jun Li[1,5,6]

Received: 7 March 2016 / Revised: 27 May 2016 / Accepted: 31 May 2016 / Published online: 11 June 2016

Abstract A Gram-negative, non-spore-forming, non-pigmented, strictly aerobic and non-motile short rod bacterium, designated NCCP-1258^T, was isolated from Cholistan desert soil, Bahawalpur, Pakistan. Growth of strain NCCP-1258^T was observed at pH range 6.5–9.5 (optimum 7.5–8.5) and temperature range 20–45 °C (optimum 40 °C), and it tolerated 0–2 % NaCl (optimum 0.5 %, w/v). Phylogenetic analysis based on 16S rRNA gene sequence comparison revealed that strain NCCP-1258^T belongs to genus *Microvirga* and is most closely related to *Microvirga lotononidis* (98.0 %), *Microvirga vignae* (97.4 %), *Microvirga lupini* (97.2 %), *Microvirga zambiensis* (97.2 %) and *Microvirga flocculans* (97.1 %). Analysis of the concatenated sequences of four housekeeping gene loci (*dnaK*, *gyrB*, *recA* and *rpoB*) also confirmed the placement of strain NCCP-1258^T within the genus *Microvirga*. DNA–DNA relatedness values of NCCP-1258^T with above-mentioned type strains were less than 42 %. The DNA G+C content of strain NCCP-1258^T was 64.3 mol%. Chemotaxonomic data (predominant menaquinone system was Q-10; major fatty acids were $C_{16:0}$, $C_{18:1}$ ω7*c* and $C_{19:0}$ cyclo ω8*c*; the polar lipid profile contained diphosphatidylglycerol, phosphatidylcholine, phosphatidyl dimethyl ethanolamine and phosphatidyl ethanolamine) also supported the affiliation of strain NCCP-1258^T to the genus *Microvirga*. On the basis of physiological and biochemical characteristics, phylogenetic analyses and DNA–DNA relatedness, strain NCCP-1258^T can be distinguished from the closely related taxa and thus represents a novel species of the genus *Microvirga*, for which the name *Microvirga pakistanensis* sp. nov. is proposed with the type strain NCCP-1258^T (=CGMCC 1.15074^T = KCTC 42496^T).

Communicated by Erko Stackebrandt.

Electronic supplementary material The online version of this article (doi:10.1007/s00203-016-1251-3) contains supplementary material, which is available to authorized users.

✉ Iftikhar Ahmed
iftikharnarc@hotmail.com

✉ Wen-Jun Li
liwenjun3@mail.sysu.edu.cn

[1] Yunnan Institute of Microbiology, Yunnan University, Kunming, People's Republic of China

[2] Institute of Microbial Culture Collection of Pakistan (IMCCP), National Agricultural Research Centre (NARC), Park Road, Islamabad 45500, Pakistan

[3] Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan

[4] PARC Institute of Advance Studies in Agriculture (PIASA), National Agricultural Research Centre (NARC), Park Road, Islamabad 45500, Pakistan

[5] State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-Sen University, Guangzhou 510275, People's Republic of China

[6] Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Ürűmqi 830011, People's Republic of China

[7] Bioproducts Research Chair (BRC), College of Science, King Saud University, Riyadh 11451, Kingdom of Saudi Arabia

Keywords *Microvirga pakistanensis* · Cholistan desert · Moderately thermotolerant

Introduction

The genus *Microvirga* was established by Kanso and Patel (2003), which describes aerobic, Gram-negative, non-sporulating and rod-shaped bacterium, which required yeast extract for growth. The first species described in the genus *Microvirga* was *Microvirga subterranea* (Kanso and Patel 2003); the genus was assigned to the class *Alphaproteobacteria*, phylum *Proteobacteria*. At the time of writing this manuscript, the genus *Microvirga* comprised nine species (LPSN, http://www.bacterio.net/microvirga.html). Members of *Microvirga* have been isolated from various habitats, e.g., Japanese hot spring (Takeda et al. 2004), Australian geothermal waters (Kanso and Patel 2003), Chinese rice field soil (Zhang et al. 2009), Korean atmospheric samples (Weon et al. 2010), cow pea grown in semiarid Brazil (Radl et al. 2014), and nitrogen fixing *Lupinus texensis* from Texas, USA (Ardley et al. 2012). Recently, another one as-yet not validly named new species, *Microvirga massiliensis* sp. nov., was isolated in Marseille from a stool sample collected in Senegal, and it had the human commensal with the largest genome (Caputo et al. 2016).

During investigation of the microbial diversity of desert soil of Cholistan, Bahawalpur, Pakistan, several strains including pink colored strain designated NCCP-1258^T were isolated. Based on 16S rRNA gene sequence analysis, strain NCCP-1258^T was most closely related to *Microvirga lotononidis* (type strain WSM3557^T) (Ardley et al. 2012), which was isolated from native legumes *Listia angolensis* (in Zambia) and *Lupinus texensis* (Texas, USA). In this study, a bacterium, designated NCCP-1258^T, was characterized by polyphasic taxonomic approach to delineate its exact taxonomic position. Further, phenotypic and biochemical characterization was performed along with phylogenetic relationships of 16S rRNA gene and four housekeeping genes.

Materials and methods

Isolation, morphology and phenotypic characterization

During a study of bacterial diversity from desert soil of Cholistan, Bahawalpur, Pakistan, (lat/lon 29°23′43″N 71°41′1″E) a soil sample was serially diluted in sterilized water, and inocula from 10^{-3} to 10^{-4} dilutions were spread on R2A agar medium (containing yeast extract 0.1 %, peptone 0.1 %, casein hydrolysate 0.1 %, soluble starch 0.1 %, glucose 0.1 %, sodium pyruvate 0.06 %, KH_2PO_4 0.06 % and bacto agar 1.5 %) and incubated at 40 °C. During isolation, a pink-colored colony of strain NCCP-1258^T was recovered after 3 days of incubation on R2A agar medium at 40 °C. For further purification, the strain was streaked repeatedly. The purified cells of strain NCCP-1258^T were maintained on R2A medium and stored in glycerol stocks at –80 °C as well as in lyophilized ampules. The type strains of closely related species, *Microvirga lotononidis* WSM3557^T (=LMG 26455^T), *M. lupini* Lut6^T (=LMG 26460^T), *M. zambiensis* WSM3693^T (=LMG 26454^T) and *M. flocculans* ATCC BAA-817^T (=JCM 11936^T) were used as reference strains in all the characterization experiments unless otherwise mentioned.

Growth of strain NCCP-1258^T was tested on various media, such as ISP 2, oat meal agar (ISP 3), TSA, R2A and nutrient agar media at 40 °C. The colony morphology of strain NCCP-1258^T was observed on R2A agar at 40 °C after 3 days of incubation. Cells grown on R2A agar for 24 h were observed using phase-contrast microscopy (BH-2; Olympus) and further detailed morphology under scanning electron microscopy (QUANTA 200; FEI). Gram staining was carried out using the standard Gram reaction (Gregersen 1978). Growth at various temperatures (4, 10, 15, 20, 28, 30, 33, 37, 40, 45, 50, 55 and 60 °C) was observed on R2A agar for 1 week. The pH range for growth was tested at pH between 4.0 and 11.0 (with 0.5 pH value increments) using the buffer system described by Xu et al. (2005) at 40 °C for 4 days in R2A broth and the growth was determined using a spectrophotometer at OD_{600} nm. Tolerance to NaCl (0–20 %, w/v, with 1 % increment) was investigated on R2A agar by incubation at 40 °C for 10 days. Catalase and oxidase activities were determined as described previously (Kovacs 1956). Growth under anaerobic conditions was determined on R2A agar supplemented with or without 0.1 % nitrate by using the GasPak Anaerobic Systems (BBL) according to the manufacturer's instructions.

The biochemical and enzymatic activities and utilization of sole carbon and nitrogen sources were determined using API 20E, API 50CH, API 20NE, and API ZYM strips according to the manufacturer's instructions (bioMérieux, France). Further physiological and biochemical features of strain NCCP-1258^T were determined using Biolog GN III microplate™ by incubating at 40 °C according to the manufacturer's instructions.

Phylogenetic analyses

PCR amplification and sequencing of 16S rRNA gene of strain NCCP-1258^T were performed using the protocol described previously (Li et al. 2007). The phylogenetic position of strain NCCP-1258^T was identified based on 16S rRNA gene sequence and by comparison with the sequences

of type species on EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/ (Kim et al. 2012) and BLAST search on the DDBJ/NCBI servers. To clarify the taxonomic status of the strain, housekeeping genes [gyrase subunit B (*gyrB*), RNA polymerase beta subunit (*rpoB*), deoxyribonucleic acid subunit K (*dnaK*) and recombination protein subunit A (*recA*)] were also amplified and sequenced using the primers and annealing temperature conditions as described by Ardley et al. (2012). The sequences were submitted to DNA Data Bank of Japan (http://www.ddbj.nig.ac.jp/) and are listed in respective dendrograms.

Phylogenetic analyses were performed using MEGA 6 software package (Tamura et al. 2013) based on 16S rRNA gene sequences of strain NCCP-1258^T and its closely related taxa. Phylogenetic trees were constructed using three algorithms: maximum parsimony (MP), neighbor joining (NJ), and maximum likelihood (ML) methods. The phylogenetic relationship was also confirmed using housekeeping loci. The sequence similarities of the housekeeping genes were estimated with the available sequences of closely related validly published species using the Kimura 2-parameter model. The concatenated data set was created by combining the nucleotide sequences of the four housekeeping genes, and phylogenetic trees were reconstructed using this concatenated data set with the concatenated sequences of related species of the genus *Microvirga* and other closely related genera. The stability of the relationship was assessed using bootstrap analysis for all the phylogenetic trees with 1000 re-samplings for the tree topology.

DNA base composition, DNA–DNA hybridization

DNA–DNA hybridization was performed between strain NCCP-1258^T and the reference strains. Total genomic DNA was extracted using a combination of the protocols of Marmur (1963) as described previously (Goris et al. 1998). DNA–DNA hybridizations were performed with biotin-labelled probes in microwell plate (NUNC) according to the method of Ezaki et al. (1989) with modifications by Goris et al. (1998) and fluorescence measurements were conducted using Bio Assay Reader (model HTS7000, PerkinElmer). The hybridization was performed at 40 °C with eight replications. The DNA G+C content of the genomic DNA of strain NCCP-1258^T was determined on reversed-phase HPLC of enzymatically degraded DNA of *Escherichia coli* DH5α as the reference (Mesbah et al. 1989).

Chemotaxonomy

Chemotaxonomic characteristics of strains NCCP-1258^T and its reference strains were determined under the same experimental conditions. Cellular fatty acid analysis was performed by growing strain NCCP-1258^T and all reference strains on TSA at 33 °C for 3 according to the recommendation given by Sasser (1990) using Microbial Identification System (Sherlock version 6.1; MIDI database: TSBA6). Menaquinones were extracted and analyzed using HPLC by following the procedures of Collins et al. (1977) and Kroppenstedt (1982). Polar lipids were extracted and identified by two-dimensional thin-layer chromatography by following procedures of Minnikin et al. (1979) and Collins and Jones (1980).

Results and discussion

Morphology and phenotypic characterization

The 3-day-old cells of strain NCCP-1258^T were pleomorphic with round ends, which occurred singly, in pairs or in small chains or clusters (Supplementary Figure 1a–e). Optimum growth of cells was observed in R2A medium with pH 7.5–8.5 (range 6.5–9.5), at 40 °C (range 20–45 °C) and could tolerate 0–2 % NaCl (optimum 0.5 %, w/v). Cells of strain NCCP-1258^T were non-motile, which differentiated strain NCCP-1258^T from the closely related reference species: *M. flocculans*, *M. zambiensis* and *M. lotononidis* that are reported to be motile by polar flagella (Takeda et al. 2004; Ardley et al. 2012). Strain NCCP-1258^T was positive for oxidase and urea hydrolysis, unlike *M. flocculans* and *M. subterranean* which did not hydrolyze urea, *M. zambiensis*, *M. lupini*, *M. lotononidis*, and *M. subterranean* were oxidase negative. NCCP-1258^T was positive for tryptophan deamination, whereas the closely related strains were either weakly positive or negative (Kanso and Patel 2003; Takeda et al. 2004; Ardley et al. 2012). Strain NCCP-1258^T hydrolyzed esculin and gelatine (weak) and produce acid from arbutin and D-melibiose, but the closely related strains did not (Ardley et al. 2012). Other detailed reactions which differentiated strain NCCP-1258^T from the closely related species of *Microvirga* are enlisted in Table 1 and/or described in species description.

Phylogenetic analysis, DNA base composition and DNA–DNA hybridization

The comparison of 16S rRNA gene sequence (1499 nucleotides; DDBJ/EMBL/GenBank accession number LC065285) of strain NCCP-1258^T showed the highest similarity of 98.0 % with *Microvirga lotononidis* WSM3557^T, 97.4 % with *M. vignae* BR3299^T, 97.2 % with *M. lupini* Lut6^T and *M. zambiensis* WSM3693^T and 97.1 % with *M. flocculans* ATCC BAA-817^T and less than 97 % with the other species of genus *Microvirga* and other taxa of the related genera on the EzTaxon Server database. A neighbor

joining phylogenetic trees based on 16S rRNA gene and concatenated sequences of four housekeeping genes (Fig. 1, Supplementary Figure 2) revealed that strain NCCP-1258^T fell within the radiation of a cluster comprised of *Microvirga lotononidis*, *M. vignae*, *M. lupini*, *M. zambiensis* and *M. flocculans* with a bootstrap value of 72 %. A similar tree topology was also observed when the phylogenetic analyses were performed using MP and ML algorithms (Fig. 1). The sequence similarity value of the *gyrB*, *rpoB*, *dnaK* and *recA* housekeeping genes was also highest with *M. lotononidis* (93.6, 91.4, 88.2 and 83.4 %, respectively). These low sequence similarities of the four housekeeping genes also supported the hypothesis that strain NCCP-1258^T belongs to a novel species.

Since the 16S rRNA gene sequence similarity of strain NCCP-1258^T is higher than 97 % with four closely related type strains DDH was carried out as suggested by Stackebrandt and Goebel (1994). The results revealed that DDH values of strain NCCP-1258^T were 41.6 % with the type strain *Microvirga lotononidis* WSM3557^T, 39.2 % with *M. lupini* Lut6^T, 33.6 % with *M. zambiensis* WSM3693^T, and 15.4 % with *M. flocculans* ATCC BAA-817^T. These values are less than the 70 % threshold that is indicative of the presence of new species (Wayne et al. 1987). The DNA

Table 1 Differentiating phenotypic and biochemical characteristics of strain NCCP-1258^T with the type strains of closely related species of genus *Microvirga*

Characteristics	NCCP-1258^T	*M. lotononidis* WSM3557^T	*M. lupini* Lut6^T	*M. zambiensis* WSM3693^T	*M. flocculans* ATCC BAA-817^T
Motility	Non-motile	Motile, polar flagella	Non-motile	Motile, polar flagella	Motile, polar flagella
NaCl optimum	0.5	0–1.0	0–0.5	0–0.5	0
Range (w/v)	0–2.0	0–2.0	0–1.5	0–2.0	0–1.0
Temperature optimum	40	40	40	35	40–45
Range (°C)	20–45	15–45	10–43	15–35	20–45
pH optimum	7.5–8.5	7.0–8.5	7.0–8.5	7.0– 8.5	7.0
Range	6.5–9.5	5.5–9.5	5.5–9.5	6.0–9.5	6.0–9.0
Oxidase	+	−	−	−	+
Urease	+	+	+	+	−
Tryptophane deaminase	+	W+	W+	−	−
Acetoin production	−	W+	W+	W+	−
Nitrate reduction	+	+	+	+	−
Hydrolysis of					
Gelatin	W+	−	−	−	+
Esculin	+	−	−	−	+
Acid production from					
Arbutin, D-melibiose	+	−	−	−	−
Salicin	W+	+	+	+	−
D-cellobiose	−	+	+	+	−
Glucose fermentation	+	+	+	+	−
Assimilation of					
Mannose	+	−	−	−	−
D-arabinose	+	+	+	+	−
L-arabinose	−	+	+	+	−
D-mannitol	−	+	+	+	−
Enzyme activity					
Esterase (C 4)	+	−	−	−	−
Esterase lipase (C 8)	+	−	−	+	−
Lipase (C 14)	+	−	−	−	+
Leucine arylamidase, α–chymotrypsin	−	−	−	+	−
Napthol-As-BI-phosphohydrolase	−	−	−	+	−

Table 1 continued

Characteristics	NCCP-1258^T	*M. lotononidis* WSM3557^T	*M. lupini* Lut6^T	*M. zambiensis* WSM3693^T	*M. flocculans* ATCC BAA-817^T
Carbon sources utilized					
D-fructose	+	−	+	−	−
L-fucose	+	−	W+	−	−
L-glutamic acid	+	+	+	+	−
Acetic acid	−	+	+	+	−

All strains are negative for lysine and ornithine decarboxylases, citrate utilization, H_2S production, indole production, and arginine dihydrolase. No acid is produced from glycerol, erythritol, D-ribose, D- and L-xylose, D-adonitol, methyl-β-D-xylopyranoside, D-galactose, L-sorbose, L-rhamnose, dulcitol, D-sorbitol, methyl-α-D-mannopyranoside, methyl-α-D-glucopyranoside, amygdalin, D-maltose, D-lactose, D-trehalose, inulin, D-melezitose, D-raffinose, amidon (starch), glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D- and L-arabitol, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, D-mannitol, inositol, D-sorbitol, L-rhamnose, D-sucrose and amygdalin. All strains are negative for assimilation of *N*-acetyle glucosamine, maltose, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate and phenyl acetic acid (API 50CH, API 20NE and API20E bioMérieux, France)

Strongly positive enzyme reaction was observed for alkaline phosphatase whereas negative for valine arylamidase, cystein arylamidase, trypsin, acid phosphatise, α-galactosidase, β-galactosidase, β-glucoronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase (API ZYM, bioMérieux, France)

All strains were observed to utilize succinic acid and succinamic acid as carbon source but do not utilize dextrin, glycogen, Tweens 40 and 80, L-erythritol, D-galactose, α-D-lactose, lactulose, methyl pyruvate, mono-methyl-succinate, cis-aconitic acid, citric acid, formic acid, α-β-γ-hydroxy butyric acid, α-keto butyric acid, α-ketoglutaric acid, α-ketovaleric acid, D- and L-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, bromo succinic acid, L-alaninamide, D- and L-alanine, L-alanyl-glycine, L-asparagine, L-glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-, L-serine, L-threonine, D- and L-carnitine, γ-amino butyric acid, urocanic acid, inosine, phenyl ethylamine, putrescine, D- and L-α-glycerol phosphate, glucose-1 and glucose-6-phosphate (Biolog GN III microplates™)

All data are from current study

+, positive; –, negative; W+, weakly positive

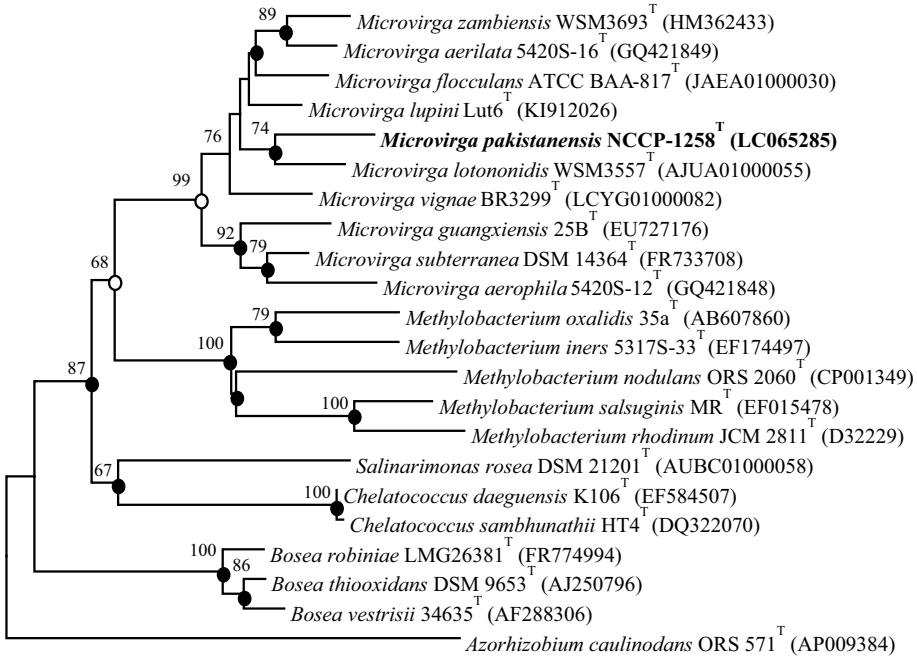

Fig. 1 Neighbor joining phylogenetic tree inferred from 16S rRNA gene sequences (1304 bp) showing inter-relationship of strain NCCP-1258^T with the members of genus *Microvirga* and other closely related genera. Bootstrap values (>60 %) expressed as percentages of 1000 replications, are shown at the branch points. *Solid circles* represents nodes, which were recovered by three algorithms (NJ, MP and ML), whereas *empty circles* represents nodes, which were recovered by any of two algorithms. *Azorhizobium caulinodans* ORS 571^T (AP009384) was rooted as outgroup. The *length of the bar* represents 1 % nucleotide sequence divergence

Table 2 Cellular fatty acid profile (%) of strain NCCP-1258^T in comparison with closely related type strains of the genus *Microvirga*

Characteristics	NCCP-1258^T	*M. lotononidis* WSM3557^T	*M. lupini* Lut6^T	*M. zambiensis* WSM3693^T	*M. flocculans* ATCC BAA-817^T
$C_{12:0}$	0.9	0.6	1.9	1.8	0.7
$C_{14:0}$	1.1	2.8	0.9	3.1	3.2
$C_{14:0}$ 3-OH	6.6	22.4	28.8	22.3	11.0
$C_{16:0}$	14.5	10.6	10.4	9.0	13.2
$C_{17:1}$ ω8*c*	0.7	0.6	1.7	1.8	0.8
$C_{17:0}$	2.3	0.9	0.5	1.7	1.2
$C_{17:0}$ cyclo	6.4	1.2	2.6	2.3	1.4
$C_{18:0}$	3.3	2.0	1.0	1.1	–
$C_{18:1}$ ω9*c*	–	2.1	2.5	3.3	7.3
$C_{18:1}$ ω7*c*	54.0	60.0	55.4	55.1	53.1
$C_{19:0}$ cyclo ω8*c*	21.4	27.6	7.6	22.4	22.8
Summed features 2	10.2	12.3	14.8	14.4	12.0
Summed features 3	2.8	2.8	6.1	7.8	3.6

Summed feature 2 comprised one or both of $C_{14:0}$ 2OH/iso-$C_{16:1}$ I and Summed feature 3 comprised one or both of $C_{16:1}$ ω6*c*/$C_{16:1}$ ω7*c*, which cannot be separated by MIDI system

G+C content of strain NCCP-1258^T was determined to be 64.3 mol%, which is within the range of the members of genus *Microvirga* (Weon et al. 2010; Ardley et al. 2012).

Chemotaxonomic analyses

The cellular fatty acid profile of strain NCCP-1258^T comprised predominantly of $C_{18:1}$ ω7*c* (54.0 %), $C_{19:0}$ cyclo ω8*c* (21.4 %), and $C_{16:0}$ (14.5 %), was similar to the profiles obtained for the reference strains, although significant variation in the values of these components clearly differentiates our strain from the closely related reference strains (Table 2). It was noted that $C_{17:0}$ cyclo and $C_{18:1}$ ω7*c* were present in higher amounts, while $C_{18:1}$ ω9*c* is absent in the profile of strain NCCP-1258^T. Strain NCCP-1258^T contained Q-10 as a sole menaquinone system. The closely related type strains were also reported to have Q-10 as the major menaquinone, Q-8 and Q-9 were present as minor components in these closely related reference strains but absent in strain NCCP-1258^T. The polar lipids profile comprised of diphosphatidyl glycerol (DPG), phosphatidyl choline (PC), phosphatidyl dimethyl ethanolamine (PDE) and phosphatidyl ethanolamine (PE) (Supplementary Figure 3). Polar lipid profiles of the closely related strains *M. lotononidis* WSM3557^T, *M. lupini* Lut6^T, and *M. zambiensis* WSM3693^T were also reported to contain these four components (Ardley et al. 2012). However, phosphatidyl glycerol (PG) is absent in strain NCCP-1258^T which differentiated this isolate from the closely related taxa. On the basis of physiological, chemotaxonomic, phylogenetic, and genomic data, strain NCCP-1258^T is considered to be a new member of the genus *Microvirga.*

Description of *Microvirga pakistanensis* sp. nov

Microvirga pakistanensis (pa.kis.tan.en'sis. N.L. fem. adj. pakistanensis is pertaining to Pakistan, where the type strain was isolated).

Cells are Gram-negative, aerobic, non-motile, and non-spore-forming short rods (~2 μm in length). Colonies are small, round with entire margins, shiny surface, concave, and pink in color. The optimum temperature, pH, and NaCl concentration for growth are 40 °C, 7.5–8.5 and 0.5 % (w/v), respectively. Positive for tryptophan deamination, nitrate reduction, oxidase, and hydrolysis of esculin, urea, and gelatine (weak), and fermentation of glucose but negative for Voges–Proskauer test. Acid was produced from arbutin, D-melibiose, salicin (weak), but not from D-cellobiose. Positive for assimilation of D-mannose, D-arabinose but negative for assimilation of L-arabinose and D-mannitol. Strong enzyme activity for esterase (C4), esterase lipase (C8) and lipase (C14) but negative for leucine arylamidase, α-chymotrypsin and napthol-As-BI-phosphohydrolase. D-fructose, L-fucose, and L-glutamic acid were utilized as carbon sources but negative for utilization of acetic acid. The major cellular fatty acids are $C_{16:0}$, $C_{18:1}$ ω7*c*, $C_{19:0}$ cyclo ω8*c*, and summed feature 2 ($C_{14:0}$ 2-OH/iso-$C_{16:1}$ I). The predominant menaquinone system is Q-10. The polar lipid profile comprises of diphosphatidylglycerol (DPG), phosphatidylcholine (PC), phosphatidyl dimethyl ethanolamine (PDE), and phosphatidyl ethanolamine (PE). The DNA G+C content is 64.3 mol%.

The type strain NCCP-1258^T (=CGMCC 1.15074^T = KCTC 42496^T) was isolated from desert soil of Cholistan, Bahawalpur, Pakistan. GenBank accession numbers for type strain NCCP-1258^T are LC065285 (16S

rRNA gene); LC085517 (*rpoB* gene), LC085516 (*gyrB* gene), LC085515 (*recA* gene) and LC085514 (*dnaK* gene).

Acknowledgments This work was supported by the Key Project of International Cooperation of Ministry of Science and Technology (MOST) (No. 2013DFA31980) and the Deanship of Scientific Research at King Saud University for funding this work through the research group no. RGP-1436–27. W.-J. Li was also supported by the Hundred Talents Program of Chinese Academy of Sciences and Guangdong Province Higher Vocational Colleges and Schools Pearl River Scholar Funded Scheme (2014). We are greatly thankful to Dr Julie K. Ardley from Centre for Rhizobium Studies, Murdoch University Australia for providing us type strains *M. lotononidis* WSM3557^T, *M. lupini* Lut6^T and *M. zambiensis* WSM3693^T.

References

Ardley JK, Parker MA, De Meyer SE, Trengove RD, O'Hara GW, Reeve WG, Yates RJ, Dilworth MJ, Willems A, Howieson JG (2012) *Microvirga lupini* sp. nov., *Microvirga lotononidis* sp. nov. and *Microvirga zambiensis* sp. nov. are alphaproteobacterial root-nodule bacteria that specifically nodulate and fix nitrogen with geographically and taxonomically separate legume hosts. Int J Syst Evol Microbiol 62:2579–2588

Caputo A, Lagier JC, Azza S, Robert C, Mouelhi D, Fournier PE, Raoult D (2016) *Microvirga massiliensis* sp.nov., the human commensal with the largest genome. Microbiologyopen 5:307–322

Collins M, Jones D (1980) Lipids in the classification and identification of coryneform bacteria containing peptidoglycans based on 2, 4-diaminobutyric acid. J Appl Bacteriol 48:459–470

Collins MD, Pirouz T, Goodfellow M, Minnikin DE (1977) Distribution of menaquinones in *Actinomycetes* and *Corynebacteria*. J Gen Microbiol 100:221–230

Ezaki T, Hashimoto Y, Yabuuchi E (1989) Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. Int J Syst Bacteriol 39:224–229

Goris J, K-i Suzuki, Vos PD, Nakase T, Kersters K (1998) Evaluation of a microplate DNA–DNA hybridization method compared with the initial renaturation method. Can J Microbiol 44:1148–1153

Gregersen T (1978) Rapid method for distinction of Gram-negative from Gram-positive bacteria. Eur J Appl Microbiol Biotechnol 5:123–127

Kanso S, Patel BK (2003) *Microvirga subterranea* gen. nov., sp. nov., a moderate thermophile from a deep subsurface Australian thermal aquifer. Int J Syst Evol Microbiol 53:401–406

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721

Kovacs N (1956) Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature 178:703

Kroppenstedt RM (1982) Separation of bacterial menaquinones by HPLC using reverse phase (RP18) and a silver loaded ion exchanger as stationary phases. J Liq Chromatogr 5:2359–2367

Li WJ, Zhang YG, Zhang YQ, Tang SK, Xu P, Xu LH, Jiang CL (2007) *Georgenia ruanii* sp. nov., a novel actinobacterium isolated from forest soil in Yunnan (China), and emended description of the genus *Georgenia*. Int J Syst Evol Microbiol 57:1424–1428

Marmur J (1963) A procedure for the isolation of deoxyribonucleic acid from microorganisms. Method Enzymol 6:726–738

Mesbah M, Premachandran U, Whitman WB (1989) Precise measurement of the G+C content of deoxyribonucleic-acid by high-performance liquid-chromatography. Int J Syst Bacteriol 39:159–167

Minnikin D, Collins M, Goodfellow M (1979) Fatty acid and polar lipid composition in the classification of *Cellulomonas, Oerskovia* and related taxa. J Appl Bacteriol 47:87–95

Radl V, Simões-Araújo JL, Leite J, Passos SR, Martins LM, Xavier GR, Rumjanek NG, Baldani JI, Zilli JE (2014) *Microvirga vignae* sp. nov., a root nodule symbiotic bacterium isolated from cowpea grown in semi-arid Brazil. Int J Syst Evol Microbiol 64:725–730

Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. MIDI technical note 101. MIDI Inc, Newark

Stackebrandt E, Goebel BM (1994) Taxonomic note: A place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Evol Microbiol 44:846–849

Takeda M, Suzuki I, Koizumi J (2004) *Balneomonas flocculans* gen. nov., sp. nov., a new cellulose-producing member of the alpha-2 subclass of *Proteobacteria*. Syst Appl Microbiol 27:139–145

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA 6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE, Stackebrandt E, Starr MP, Trüper HG (1987) Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37:463–464

Weon HY, Kwon SW, Son JA, Jo EH, Kim SJ, Kim YS, Kim BY, Ka JO (2010) Description of *Microvirga aerophila* sp. nov. and *Microvirga aerilata* sp. nov., isolated from air, reclassification of *Balneimonas flocculans* Takeda et al, 2004 as *Microvirga flocculans* comb. nov. and emended description of the genus *Microvirga*. Int J Syst Evol Microbiol 60:2596–2600

Xu P, Li WJ, Tang SK, Zhang YQ, Chen GZ, Chen HH, Xu LH, Jiang CL (2005) *Naxibacter alkalitolerans* gen. nov., sp. nov., a novel member of the family '*Oxalobacteraceae*' isolated from China. Int J Syst Evol Microbiol 55:1149–1153

Zhang J, Song F, Xin YH, Zhang J, Fang C (2009) *Microvirga guangxiensis* sp. nov., a novel alphaproteobacterium from soil, and emended description of the genus *Microvirga*. Int J Syst Evol Microbiol 59:1997–2001