

Full Length Research Paper

# Reclassification of *Solimonas soli* (Kim et al., 2007) and *Singularimonas variicoloris* (Friedrich et al., 2008) as *Sinobacter soli* comb. nov. and *Sinobacter variicoloris* comb. nov. and emended description of the genus *Sinobacter* (Zhou et al., 2008)

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Three types of strains; CW-KD 4<sup>T</sup>, MN28<sup>T</sup> and DCY12<sup>T</sup> which were published by Zhou et al. (2008) as *Sinobacter flavus* gen. nov., sp. nov., Friedrich et al. (2008) as *Singularimonas variicoloris* gen. nov., sp. nov., and Kim et al. (2007) as *Solimonas soli* gen. nov., sp. nov., respectively, were selected and subjected to reclassification analyses using the polyphasic taxonomy. Phylogenetic analysis displayed that the three strains represented a deep-separated lineage within the family, Sinobacteraceae. The 16S rRNA gene sequence similarities among the three strains were 99.7% (CW-KD 4<sup>T</sup> and MN28<sup>T</sup>), 98.0% (CW-KD 4<sup>T</sup> and DCY12<sup>T</sup>) and 98.1 (MN28<sup>T</sup> and DCY12<sup>T</sup>). The isoprenoid quinone of the three strains was Q-8. The major fatty acids of strains CW-KD 4<sup>T</sup> and MN28<sup>T</sup> were C<sub>16:0</sub> and Sum in Feature 5, but the major fatty acids for strain DCY12<sup>T</sup> were anteiso-C<sub>15:0</sub> and anteiso-C<sub>17:0</sub>. The G+C contents of the DNA were 68.1 mol% (MN28<sup>T</sup>), 65.8 mol% (CW-KD 4<sup>T</sup>) and 63.5 mol% (DCY12<sup>T</sup>). The co-predominant polar lipids of the three type strains were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. DNA-DNA hybridization results showed the levels of relatedness of 59.0% (MN28<sup>T</sup>-DCY12<sup>T</sup>), 21.4% (DCY12<sup>T</sup>-CW-KD 4<sup>T</sup>) and 37.5% (MN28<sup>T</sup>-CW-KD 4<sup>T</sup>) among each other. On basis of phenotypic and genotypic characteristics of the present study, the three type strains were reclassified as different species of the same genus *Sinobacter*, and *Sinobacter variicoloris* comb. nov and *Sinobacter soli* comb. nov. were proposed for the strains MN28<sup>T</sup> and DCY12<sup>T</sup>, respectively. The type strains are MN28<sup>T</sup> (=DSM 15731<sup>T</sup>=LMG 22844<sup>T</sup>) and DCY12<sup>T</sup> (=DSM 21787<sup>T</sup>=LMG 24014<sup>T</sup>=KCTC 12834<sup>T</sup>).

**Key words:** *Sinobacter*, polyphasic taxonomy, type strain, reclassification.

## INTRODUCTION

*Solimonas soli* DCY12<sup>T</sup> was published by Kim et al. (2007) and before the genus *Solimonas* validly published, *Sinobacter flavus* gen. nov., sp. nov. had already been accepted by International Journal of Systematic and Evolutionary Microbiology (IJSEM) and validly published in the early of year 2008, and in that publication, the new

family *Sinobacteraceae* was further suggested to cover the new proposed genus *Sinobacter* (Zhou et al., 2008). After the *Sinobacter* and *Sinobacteraceae* validly listed, another novel genus with single species *Singularimonas variicoloris* gen. nov., sp. nov. was also published at the end of the year 2008 (Friedrich et al., 2008). Interesting, during the project to reanalyze the three type strains of the new published genera *Sinobacter*, *Solimonas* and *Singularimonas*, they formed one deep-separated branch together within the family *Sinobacteraceae* and showed the 16S rRNA gene sequence similarities higher

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than 98% among each other (98.0 to 99.7%). Considering the high 16S rRNA gene sequence similarity values among the three species and the identical phylogenetic branch formed within the family *Sinobacteraceae*, the three type species of new published genera were selected and subjected to reclassification analysis using polyphasic taxonomy, and propose their descriptions as different bacterial species of the same genus *Sinobacter*.

## MATERIALS AND METHODS

Strain CW-KD 4<sup>T</sup> was isolated using classic enrichment culture technique from a farmland soil sample, in Jiangsu Province, China (Zhou et al., 2008). Type strains MN28<sup>T</sup> (=DSM 15731<sup>T</sup>) and DCY12<sup>T</sup> (=DSM 21787<sup>T</sup>) were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany).

### Morphological and physiological characteristics investigation

Luria-Bertani (LB) medium was selected for the cultivation of the three strains and the bacterial incubation was performed at 30°C for 5 days and the strains were preserved in a 20% (v/v) glycerol solution in distilled water at -80°C. The morphological characteristics of the strains were observed by light microscopy (model XTL-3400, Olympus) after incubation for 3 days at 30°C on LB agar. The method of Yamaguchi and Yokoe (2000) was followed to determine carbohydrates acid production. The utilization of sole carbon sources was performed as described Zhou et al. (2007). For the various physiological tests, API 20NE and API 50CHB test strips (bioMérieux) were applied and performed according to the manufacturer's instructions, and LB broth was selected as the growth factor in API 20NE microtest system as described by Kim et al. (2007).

### Systematic determination

The 16S rRNA gene sequences which retrieved from GenBank database following BLAST searches were manually aligned with its reference sequences. Multiple alignments were performed by CLUSTAL\_X program (Thompson et al., 1997) and gaps were edited in the BioEdit program (Hall, 1999). Phylogenetic analysis was performed using the software package MEGA version 3.1 (Kumar et al., 2001). Distances (distance options according to the Kimura two-parameter model; Kimura, 1980, 1983) and clustering were based on the neighbour-joining and maximum-parsimony methods. Bootstrap analysis (1000 resamplings) was used to evaluate the topology of the phylogenetic trees (Felsenstein, 1985).

### Chemotaxonomic markers analysis

Biomass for molecular systematic and chemotaxonomic studies was obtained by incubated the strains at 30°C for 5 days in shake flasks (about 180 rpm) with Tryptose Soya Broth (TSB). The fatty acids were extracted, methylated and analysed using the standard MIDI (Microbial Identification) system as described by Sasser (1990). Polar lipids were extracted, examined by two dimensional thin-layer chromatography (TLC) and identified using the procedures of Minnikin et al. (1984). Genomic DNA was prepared for the base composition following the procedure of Marmur (1961). Because the DNA G+C base content of strain DCY12<sup>T</sup> was reported as 40.5 mol% in previous publication (Kim et al., 2007), and the result is

unusually for this group of bacteria, the DNA G+C base content of the three strains was re-examined under the same conditions by HPLC before DNA-DNA hybridization performed. The DNA G+C base content was determined by reverse-phase HPLC according to Mesbah et al. (1989) using the strain *E. coli* DH5 $\alpha$  to emend the standard deviation. DNA-DNA hybridizations were carried out applying the optical renaturation methods (Huss et al., 1983; Jahnke, 1992; De Ley et al., 1970). DNA-DNA hybridizations were performed on three strains for each other, respectively. The hybridization reactions for themselves were also performed to be set as control.

## RESULTS

### Morphological and physiological characteristics

Cells of three type strains are Gram-negative, aerobic, rods, chemo-organotrophic, non-endospore-forming, non-motile and the optimum temperature is about 25–35 °C. Glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, Methyl- $\beta$ -D-xylopyranoside, D-galactose, D-mannose, L-sorbitol, dulcitol, inositol, mannitol, sorbitol, methyl- $\alpha$ -D-mannopyranoside, methyl- $\alpha$ -D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, salicin, D-cellobiose, D-lactose, D-melibiose, D-trehalose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, D-gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate is not assimilated by the three tested strains in API 50CHB examination. None of strains showed indole production, glucose acidification, gelatinase or  $\beta$ -galactosidase, and arabinose, mannitol, citric acid and phenylacetic acid were not assimilated in API 20NE tests. Detailed phenotypic characteristics that differentiate three strains from each other are shown in Table 1 and the *Sinobacter* species description.

### Systematic analysis result

Neighbour-joining phylogenetic tree (Figure 1) and maximum-parsimony phylogenetic tree (Figure 2) constructed from 16S rRNA gene sequence displayed that the three strains belong to family *Sinobacteraceae* and represented one separated branch within the new family. The 16S rRNA gene sequence similarity values among the three strains were 99.7% (CW-KD 4<sup>T</sup> and MN28<sup>T</sup>), 98.0% (CW-KD 4<sup>T</sup> and DCY12<sup>T</sup>) and 98.1% (MN28<sup>T</sup> and DCY12<sup>T</sup>). The lowest 16S rRNA gene sequence similarity among three strains is 98.0% (higher than 97%). Further more, the three strains were clustered as one separated phylogenetic branch. The phylogenetic analysis results displayed above implied that three strains should be allocated in the same bacterial genus, and the DNA-DNA hybridization reactions need to be performed to confirmed the species definition.

**Table 1.** Phenotypic characteristics that differentiate strains MN28<sup>T</sup>, CW-KD 4<sup>T</sup> and DCY12<sup>T</sup> from each other.

Characteristic	MN28 <sup>T</sup>	CW-KD 4 <sup>T</sup>	DCY12 <sup>T</sup>
Cell morphology	Rod	Long rod	Rod
Oxidase	+	+	-
Catalase	(+)	-	+
<b>API 50CHB</b>			
D-glucose	-	-	+
D-fructose	-	-	+
L-rhamnose	+	+	-
Aesculin	+	+	-
D-maltose	-	-	+
D-sucrose	-	-	+
<b>API 20NE</b>			
Nitrate reduction	-	-	+
Nitrite reduction	-	+	-
D-glucose	-	-	+
Arginine dihydrolase	-	-	+
Urease	-	-	+
Aesculin	+	+	-
D-mannose	+	-	-
N-acetylglucosamine	+	-	-
D-maltose	-	-	+
Potassium gluconate	+	-	-
Capric acid	-	+	-
Adipic acid	+	-	-
Malic acid	-	+	-
DNA G+C content (mol%)	68.1	65.8	63.5
Polar lipids	DPG, PG, PE, APL, PI	DPG, PG, PE, APLs	DPG, PG, PE, PGL, PI

Symbols: +, positive; -, negative; (+), weak positive; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; APL, an unknown aminophospholipid; PI, phosphatidylinositol; PGL, phosphoglycolipid.

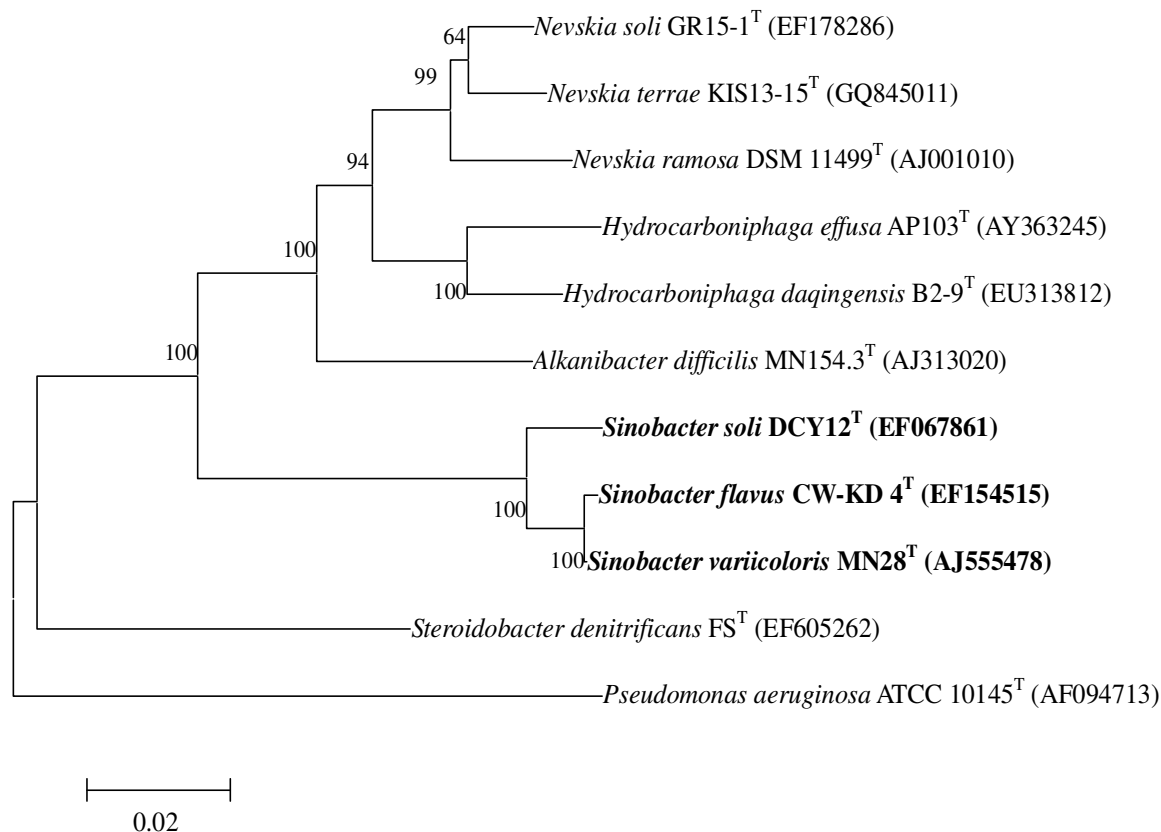
### Chemotaxonomic data comparison

The major fatty acids of strains CW-KD 4<sup>T</sup> and MN28<sup>T</sup> were C<sub>16:0</sub> and Sum In Feature 5, but the major fatty acids of strain DCY12<sup>T</sup> were anteiso-C<sub>15:0</sub> and anteiso-C<sub>17:0</sub>. The detailed fatty acid profiles are displayed in Table 2. The three strains showed the similar predominant polar lipids, and the co-predominant polar lipids contained diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The minor components of strain MN28<sup>T</sup> contained an unknown aminophospholipid and a phosphatidylinositol, the minor components of strain CW-KD 4<sup>T</sup> contained two kinds of unknown aminophospholipids, and other components of strain DCY12<sup>T</sup> contained a phosphoglycolipid and a phosphatidylinositol. The DNA G+C base content of MN28<sup>T</sup>, CW-KD 4<sup>T</sup> and DCY12<sup>T</sup> were 68.1, 65.8 and 63.5%, respectively, with standard deviation minus 1.8%

(emended by *E. coli* DH5α). DNA–DNA hybridization results showed the levels of relatedness of 59.0% (MN28<sup>T</sup>-DCY12<sup>T</sup>), 21.4% (DCY12<sup>T</sup>-CW-KD 4<sup>T</sup>) and 37.5% (MN28<sup>T</sup>-CW-KD 4<sup>T</sup>) among each other.

### DISCUSSION

Polyphasic taxonomy results displayed that three strains showed high 16S rRNA gene sequence similarity, the same phylogenetic branch, the same respiratory quinone (Q-8), the similar G+C mol% base content and the similar predominant polar lipids. The phylogenetic and chemotaxonomic characteristics strongly suggested the three strains are the members of the same genus and the genus *Sinobacter* is proposed for the three strains. Although the three selected strains are proved to be as the same genus *Sinobacter*, they could easily be



**Figure 1.** Neighbour-joining phylogenetic tree based on the 16S rRNA gene sequences of the strains MN28<sup>T</sup>, CW-KD 4<sup>T</sup> and DCY12<sup>T</sup>, and representative members of the family *Sinobacteraceae*. *Pseudomonas aeruginosa* was selected as out-group. Numbers at nodes indicate bootstrap values (%), and only those values greater than 50% are indicated. Bar, 0.02 substitutions per nucleotide position.

distinguished from each other on the basis of phenotypic features (Table 1) and fatty acid profiles (Table 2). Phenotypic characteristics combination with the DNA–DNA hybridization results suggested that the three type strains should be represented as three different species of the genus *Sinobacter*, and the name *Sinobacter variicoloris* comb. nov. is proposed for the type strain MN28<sup>T</sup> and *Sinobacter soli* comb. nov. is suggested for the type strain DCY12<sup>T</sup>.

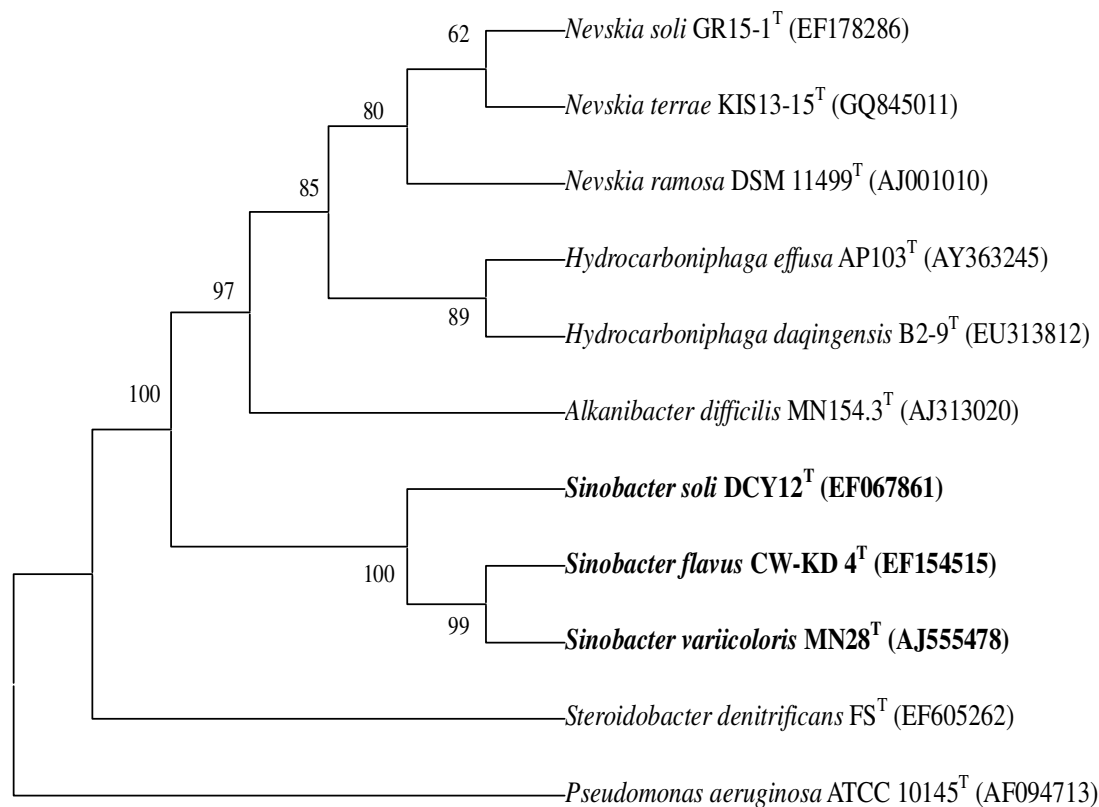
#### Emended description of the genus *Sinobacter* (Zhou et al., 2008)

Cells are Gram-negative, rod, non–endospore-forming and non-motile. Aerobic and chemo-organotrophic and optimum temperature is about 25 to 35°C. Fatty acid profiles of the genus are usually complex, branched chain and/or hydroxy fatty acids are found. The co-predominant polar lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The isoprenoid quinone is mainly composed of Q-8. The DNA G+C content is about 63 to 68 mol%. The type species is

*Sinobacter flavus* Zhou et al. (2008).

#### Emended description of *Sinobacter flavus* Zhou et al. (2008)

The description of the species *Sinobacter flavus* is as given by Zhou et al. (2008), with alterations as described below. L-rhamnose and aesculin are positive in API 50CHB tests. Glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, Methyl-β-D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbitose, dulcitol, inositol, mannitol, sorbitol, methyl-α-D-mannopyranoside, methyl-α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-sucrose, D-trehalose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, D-gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate is negative in API 50CHB examination. Nitrite reduction is positive, aesculin, capric acid and



**Figure 2.** Maximum-parsimony phylogenetic tree based on the 16S rRNA gene sequences of the strains MN28<sup>T</sup>, CW-KD 4<sup>T</sup> and DCY12<sup>T</sup>, and representative members of the family *Sinobacteraceae*. *Pseudomonas aeruginosa* was selected as out-group. Numbers at nodes indicate bootstrap values (%), and only those values greater than 50% are indicated.

malic acid are assimilated in API 20 NE tests. Nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease, gelatinase or  $\beta$ -galactosidase is negative; glucose, arabinose, mannose, mannitol, N-acetylglucosamine, D-maltose, potassium gluconate, adipic acid, citric acid or phenylacetic acid is not assimilated in API 20NE tests. The major fatty acid profile contains C<sub>16:0</sub> (19.43%), Sum In Feature 5 (C<sub>18:1</sub> cis 11/t 9/t 6, 17.3%), iso-C<sub>16:0</sub> (9.29%), cyclo-C<sub>19:0</sub> C11-12 (7.86%) and Sum In Feature 4 (C<sub>18:2</sub> cis 9, 12/C<sub>18:0</sub> a, 7.38%) when cultured on the TSA medium. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine with two kinds of unknown aminophospholipids as the minor compositions. The type strain is CW-KD 4<sup>T</sup>=DSM 18980<sup>T</sup>=KCTC 12881<sup>T</sup>=CCTCC AB 206145<sup>T</sup>. The DNA G+C content is about 65 mol% (HPLC).

#### Description of *Sinobacter variicoloris* comb. nov.

**Basonym:** *Singularimonas variicoloris* (Friedrich et al., 2008)

The description of the species *Sinobacter variicoloris* is as

given by Friedrich et al. (2008) for the description of *Singularimonas variicoloris*, with the below alterations. L-rhamnose and aesculin are positive in API 50CHB tests. Glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, Methyl- $\beta$ -D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbitol, dulcitol, inositol, mannitol, sorbitol, methyl- $\alpha$ -D-mannopyranoside, methyl- $\alpha$ -D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-sucrose, D-trehalose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, D-gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate is negative in API 50CHB examination. Aesculin, mannose, N-acetylglucosamine, potassium gluconate and adipic acid are assimilated in API 20NE determination. Nitrate/nitrite reduction, indole production, glucose acidification, arginine dihydrolase, urease, gelatinase or  $\beta$ -galactosidase is negative; glucose, arabinose, mannitol, D-maltose, capric acid, malic acid, citric acid or phenylacetic acid is not assimilated in API20NE tests. The major fatty acid profile contains C<sub>16:0</sub> (20.67%), Sum In Feature 5 (C<sub>18:1</sub> cis 11/t 9/t 6, 17.69%),

**Table 2.** Cellular fatty acid contents (%) of strains MN28<sup>T</sup>, CW-KD 4<sup>T</sup> and DCY12<sup>T</sup> under same culture conditions.

Fatty acid	MN28 <sup>T</sup>	CW-KD 4 <sup>T</sup>	DCY12 <sup>T</sup>
Iso-C <sub>12:0</sub>	ND	1.13	0.4
C <sub>12:0</sub>	8.68	4.99	1.96
C <sub>12:0</sub> 2-OH	ND	1.06	ND
Iso-C <sub>14:0</sub>	0.56	2.86	1.32
C <sub>14:0</sub>	3.14	2.29	0.8
Iso-C <sub>15:0</sub>	0.34	1.41	7.85
<b>Anteiso-C<sub>15:0</sub></b>	<b>ND</b>	<b>1.58</b>	<b>35.37</b>
Iso-C <sub>16:0</sub>	4.77	9.29	2.15
C <sub>16:1</sub> a	0.89	1.06	0.36
C <sub>16:1</sub> c	5.69	5.86	1.21
C <sub>16:0</sub>	20.67	19.43	5.64
Iso-C <sub>17:0</sub>	0.12	ND	8.40
<b>Anteiso-C<sub>17:0</sub></b>	<b>0.18</b>	<b>ND</b>	<b>12.78</b>
C <sub>18:0</sub>	0.38	0.95	2.00
C <sub>18:1</sub> CIS 9	0.37	3.17	ND
Iso-C <sub>19:0</sub>	0.14	ND	3.50
Anteiso-C <sub>19:0</sub>	ND	ND	3.48
<b>Cyclo-C<sub>19:0</sub> C<sub>11-12</sub></b>	<b>5.83</b>	<b>7.86</b>	<b>1.29</b>
†Sum In Feature 1	8.84	4.19	1.88
†Sum In Feature 2	ND	1.37	0.27
†Sum In Feature 3	6.58	6.47	1.26
†Sum In Feature 4	0.26	7.38	ND
†Sum In Feature 5	17.69	17.3	3.95

Abbreviations: ND, not detected.

† Fatty acids that could not be separated by GC using Microbial Identification System (Microbial ID) software were considered summed features. Summed feature 1 contains C<sub>12:0</sub> alde/ unknown 10.928, Sum In Feature 2 contains C<sub>16:1</sub> ω7c/ Iso-C<sub>15:0</sub> 2OH, Sum In Feature 3 contains C<sub>14:0</sub> 3-OH/ Iso-C<sub>16:1</sub> I, Sum In Feature 4 contains C<sub>18:2</sub> cis 9,12/C<sub>18:0</sub> A, Sum In Feature 5 contains C<sub>18:1</sub> cis 11/t 9/t 6.

Sum In Feature 1 (C<sub>12:0</sub> alde/ unknown 10.928, 8.84%) and C<sub>12:0</sub> (8.68%) when cultured on the TSA medium. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine with an unknown aminophospholipid and a phosphatidylinositol as the minor compositions. The type strain is MN28<sup>T</sup> =DSM 15731<sup>T</sup> =LMG 22844<sup>T</sup>. The DNA G+C content is about 68 mol% (HPLC).

### Description of *Sinobacter soli* comb. nov.

#### Basonym: *Solimonas soli* (Kim et al., 2007)

The description of the species *Sinobacter soli* is as given by Kim et al. (2007) for the description of *Solimonas soli*, with the below alterations. D-glucose, D-fructose,

D-maltose and D-sucrose are positive in API 50CHB tests. Glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, Methyl-β-D-xylopyranoside, D-galactose, D-mannose, L-sorbitose, L-rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl-α-D-mannopyranoside, methyl-α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, D-cellobiose, D-lactose, D-melibiose, D-trehalose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, D-gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate is negative in API 50CHB examination. Nitrate could be reduced to nitrite, arginine dihydrolase and urease activities are positive, D-glucose, and D-maltose are assimilated in API 20NE determination. Nitrate reduction, indole production, gelatinase or β-galactosidase is negative; aesculin, arabinose, mannose, mannitol, N-acetylglucosamine, potassium gluconate, capric acid, adipic acid, malic acid, citric acid or phenylacetic acid is not assimilated in API 20NE tests. The major fatty acid profile contains anteiso-C<sub>15:0</sub> (35.37%), anteiso-C<sub>17:0</sub> (12.78%), iso-C<sub>17:0</sub> (8.4%) and iso-C<sub>15:0</sub> (7.85%) when cultured on TSA medium. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine with a phosphoglycerolipid and a phosphatidylinositol as the minor compositions. The type strain is DCY12<sup>T</sup> =DSM 21787<sup>T</sup> =LMG 24014<sup>T</sup> =KCTC 12834<sup>T</sup>. The DNA G+C content is about 64 mol% (HPLC).

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