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Pedobacter namyangjuensis sp. nov. Isolated from Soil and Reclassification of *Nubsella zeaxanthinifaciens* Asker *et al.* 2008 as *Pedobacter zeaxanthinifaciens* comb. nov.

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A Gram-stain-negative, non-motile, strictly aerobic, yellow-pigmented bacterium, designated strain 5G38^T, was isolated from a field cultivated with Chinese cabbage in Korea. The strain grew at 5–40°C and at pH 6.0–8.0. 16S rRNA gene sequence analysis revealed that strain 5G38^T represented a distinct lineage within the family *Sphingobacteriaceae* and showed the highest 16S rRNA gene sequence similarity of 95.2% with *Pedobacter koreensis* WPCB189^T, followed by *Pedobacter agri* PB92^T (94.6%), *Pedobacter suwonensis* 15-52^T (94.4%), *Pedobacter rhizosphaerae* 01-96^T (94.4%), *Pedobacter sandarakinus* DS-27^T (94.4%), and *Nubsella zeaxanthinifaciens* TDMA-5^T (94.3%). Strain 5G38^T formed monophyletic clade with *Nubsella zeaxanthinifaciens* in the cluster comprised of species of the genus *Pedobacter*. Chemotaxonomic characteristics of the novel strains, including DNA G+C content of genomic DNA (37.0 mol%), the predominant respiratory quinine (MK-7), and the major fatty acids which were iso-$C_{15:0}$, summed feature 3 (comprising $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH) and iso-$C_{17:0}$ 3-OH, are similar to those of the genus *Pedobacter*. However, the novel strains can be distinguished from the other species of *Pedobacter* by physiological properties. The name *Pedobacter namyangjuensis* sp. nov. is therefore proposed for strain 5G38^T (KACC 13938^T =NBRC 107692^T) as the type strain. Furthermore, the reclassification of *Nubsella zeaxanthinifaciens* as *Pedobacter zeaxanthinifaciens* comb. nov. is proposed.

Keywords: *Pedobacter namyangjuensis* sp. nov., Chinese cabbage soil, taxonomy

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Introduction

The genus *Pedobacter*, which includes 4 species, was proposed by Steyn *et al.* (1998) to be classified as heparinolytic bacteria, with the type species, *Pedobacter heparinus*. Asker *et al.* (2008) proposed that the genus *Nubsella* be used to accommodate a zeaxanthin-producing bacterium. At the time that this paper was written, the genus *Pedobacter* consisted of 32 species with valid names and the genus *Nubsella* was comprised of only 1 species: *Nubsella zeaxanthinifaciens.* These two genera were characterized as Gram-negative, obligately aerobic, and non-spore forming rod bacteria. Their colonies exhibit various colours from yellow to white or pink (Gallego *et al.*, 2006; Asker *et al.*, 2008), but the pigment does not exhibit the typical flexirubin reaction. Chemotaxonomically, the members of these genera contain menaquinone-7 (MK-7) as the predominant menaquinone and iso-$C_{17:0}$ 3-OH, iso-$C_{15:0}$ and summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}$ $\omega7c$) as the primary fatty acids. The G+C contents of the genomic DNA are 33.8% to 44.2% and 38.6% for the *Pedobacter* and *Nubsella*, respectively. In this study, we studied the taxonomic characterization of a bacterial strain, 5G38^T, that is closely related phylogenetically to the genus *Pedobacter*, and we also studied the taxonomic reassignment of *Nubsella zeaxanthinifaciens* as a member of the genus *Pedobacter*.

Materials and Methods

Bacterial strains

Bacterial strain 5G38^T was isolated during a study of the culturable bacterial community in soil cultivated with Chinese cabbage (*Brassica campestris L.*). Soil samples were taken from field plots in Namyangju (37° 35′ 03″ N 127° 14′ 00″ E), South Korea, during the early vegetative growth stage of Chinese cabbage. The strain designated as 5G38^T was then isolated by a standard dilution plating technique on tryptic soy agar (TSA; Difco) at 28°C. The *N. zeaxanthinifaciens* KACC 14260^T and six reference strains of the *Pedobacter* species were obtained from the Korean Agricultural Culture Collection (KACC) in order to compare their phenotypic and chemotaxonomic characteristics. These strains included the *P. alluvionis* KACC 14286^T, *P. borealis* KACC 14287^T, *P. roseus* KACC 11594^T, *P. africanus* KACC 11492^T, *P. suwonensis* KACC 11317^T, and *P. terrae* KACC 13760^T.

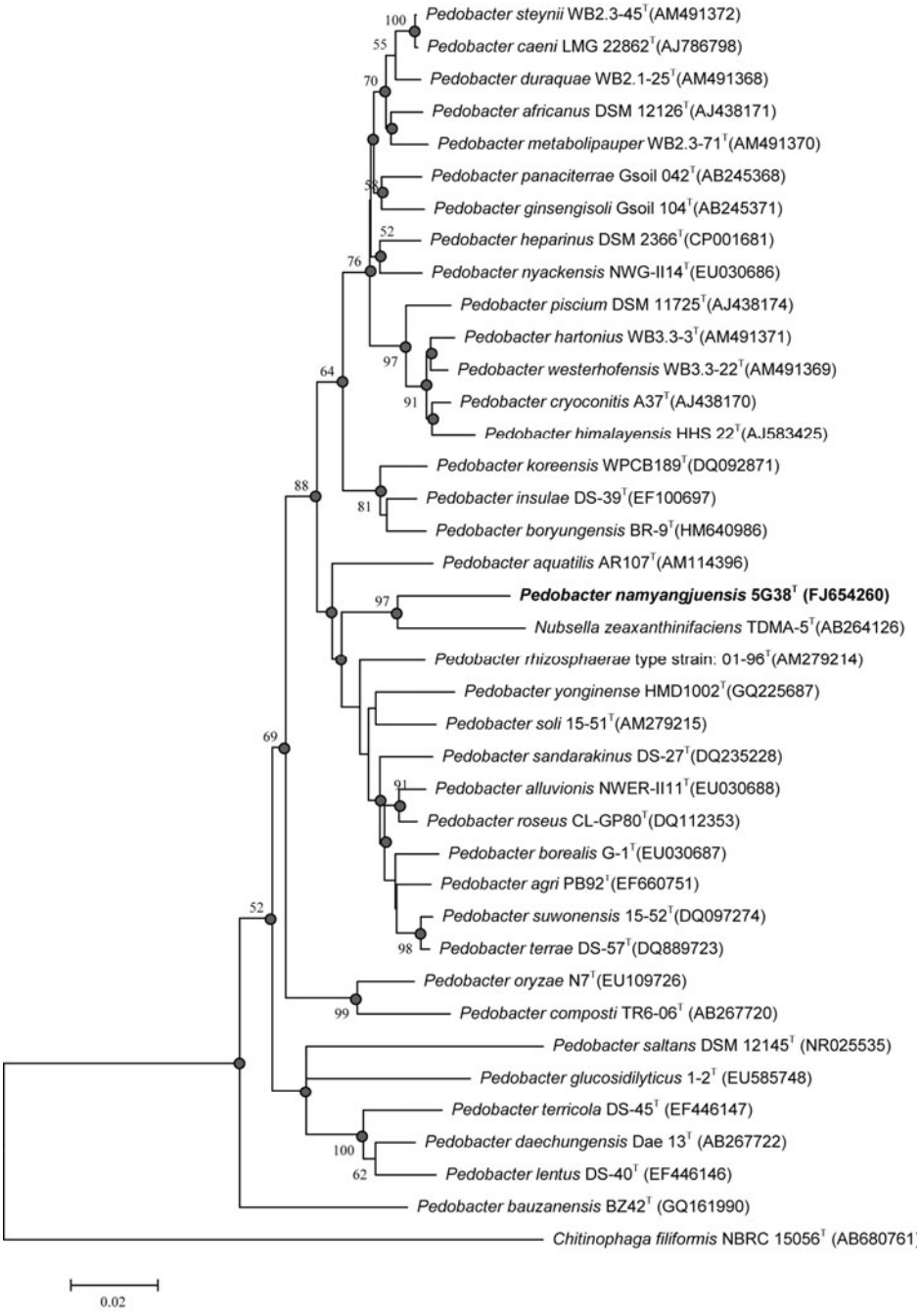

Fig. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain 5G38^T in relation to the type strains of 36 *Pedobacter* species and *Nubsella zeaxanthinifaciens* KACC 14260^T. *Chitinophaga filiformis* NBRC 15056^T was used as an outgroup. Bootstrap values (expressed as percentages of 1,000 replications) >50% are shown at branch points. Filled circles indicate nodes that were also recovered using maximum-parsiomony algorithms. Bar, 2% sequence divergence.

Morphology and physiological characteristics

The pH range for growth on the R2A agar (Difco) was determined and then adjusted to a pH level between 4 and 10 at 1 pH unit intervals by the addition of HCl and NaOH. Tolerance to NaCl was tested on 0, 2, 3, 5, and 7% NaCl (w/v) R2A agar prepared according to the formula for the Difco medium. The temperature range for growth was tested between 5 and 45°C (at 5°C intervals). Growth was also tested on Luria-Bertani agar (LB; Difco), nutrient agar (NA; Difco), tryptic soy agar (TSA; Difco), and MacConkey's agar (Difco). Growth under anaerobic condition was assessed in a GasPak (BBL) jar at 30°C for 15 days on a R2A agar. The cellular morphology and motility of cells grown for 2 days at 28°C on a R2A agar supplemented with 1% agar were studied using transmission electron (model 912AB; LEO) and phase-contrast (AXIO; Zeiss) microscopy, respectively. Cells of the strain 5G38^T grown on the R2A agar at 28°C for 2 to 3 days were used for the physiological and biochemical tests. The catalase and oxidase activities as well as the hydrolysis of casein, tyrosine, DNA, chitin, pectin, cellulose, hypoxanthine, xanthine, starch, tyrosine, Tween 80, and carboxymethyl-cellulose were performed as previously described (Groth *et al.*, 1996). In addition, aesculin hydrolysis was assessed using an Enterococcosel agar (BBL, USA)

Table 1. Differential characteristics of strain 5G38^T, *Nubsella zeaxanthinifaciens*, and several *Pedobacter* species. Strains: 1, *Pedobacter namyangjuensis* sp. nov. 5G38^T; 2, *Nubsella zeaxanthinifaciens* TDMA-5^T (data in column 1-2 from this study); 3, *Pedobacter alluvionis* KACC 14286 ^{T} (Gordon *et al*., 2009); 4, *Pedobacter borealis* KACC 14287 ^{T} (Gordon *et al*., 2009); 5, *Pedobacter roseus* KACC 11594^T (Gordon *et al*., 2009); 6. *Pedobacter suwonensis* KACC 11317^T (Gordon *et al*., 2009); 7, *Pedobacter terrae* KACC 13760^T (Kwon *et al*., 2007); 8, *Pedobacter africanus* KACC 11492^T (Gordon *et al*., 2009). +, Positive; -, negative; v, weak; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8
Source	Soil	Fresh water	Flood-plain sediment	Soil	Hypertrophic fresh water	Cabbage rhizosphere	Soil	Soil, activate sludge
Temperature range (°C)	5–40	18–40	4–30	4–30	5–33	1–37	4–35	ND
Hydrolysis of:								
Gelatin hydrolysis	+	+	+	+	+	+	+	v
Arginine dihydrolase	+	-	+	+	-	-	ND	-
Starch	+	+	+	+	ND	-	+	v
Assimilation of :								
L-Arabinose	+	-	+	+	+	+	+	v
D-Galactose	+	-	+	+	+	+	+	+
L-Rhamnose	-	-	+	+	+	+	-	+
D-Mannitol	+	-	-	-	-	-	ND	-
D-Sorbitol	-	-	-	-	-	-	ND	-
Maltose	+	+	+	+	+	+	+	v
Gluconate	-	-	-	-	-	ND	-	-
DNA G+C content (mol%)	37.0	38.6	39.3	39.7	41.3	44.2	39.7	43-45

at 37°C. Other physiological and biochemical properties were tested by using commercial systems including API ZYM, API 20NE, and API 50CH (bioMérieux). All of the kits were used according to the manufacturer's instructions. The API ZYM tests were read after 4 h of incubation at 37°C and the other API tests were read after 48 h at 28°C.

Determination of 16S rRNA gene sequencing, phylogenetic analysis, and isoprenoid quinone analysis

Chromosomal DNA was isolated from the 5G38^T strain with the Wizard Genomic DNA Purification Kit (Promega, USA). The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using the universal primers 27f and 1492r (Baker *et al*., 2003). Sequencing was performed with an ABI Prism BigDye Terminator Cycle Sequencing Ready Kit (Applied Biosystems, USA) according to the manufacturer's instructions with the sequencing primers 519r, 926f (Lane, 1991), and 1055r (Lee *et al*., 1993). Partial 16S rRNA gene sequences were assembled using SEQMAN software (DNASTAR). The almost full-length similarity was determined using the EzTaxon server (Chun *et al*., 2007) and sequences from the 5G38^T strain and related taxa (retrieved from the NCBI database) were aligned using the multiple sequence alignment program CLUSTAL W (Thompson *et al*., 1994). Phylogenetic trees were inferred using neighbor-joining (Saitou and Nei, 1987) in the MEGA4 software program (Tamura *et al*., 2007) with a bootstrap value based on 1,000 replications (Felsenstein, 1985). The isoprenoid quinones of the 5G38^T strain were analyzed by HPLC as described previously (Groth *et al*., 1996).

Determination of G+C content and cellular fatty acid analysis

The DNA G+C content (mol%) was determined by HPLC analysis of the deoxyribonucleosides using a reverse-phase column (Supelcosil LC-18-S; Supelco) (Mesbah *et al*., 1989). Polar lipids were analyzed according to Minnikin *et al*. (1984). The cellular fatty acids of the 5G38^T strain, *N. zeaxanthinifaciens* TDMA-5^T, and the *Pedobacter* species were grown on a LB agar for 3 days at 30°C. The cellular fatty acids were extracted, methylated, and separated by gas chromatography (model 6890; Hewlett Packard) according to the protocol of the Sherlock Microbial Identification System (MIDI, 1999). The fatty acid methyl esters were identified and quantified using the TSBA 6 database (version 6.10) of the Sherlock Microbial Identification System (MIDI).

Nucleotide sequence accession number

The GenBank accession number for the 16S rRNA gene sequence of the 5G38^T strain is FJ654260.

Results and Discussion

Morphological and physiological characteristics

The 5G38^T strain grew on the R2A, LB, TSA, and NA agars, but not on the MacConkey agar. On the R2A agar, the 5G38T strain was able to grow at 5 to 40°C (optimum was 28°C), but not at 45°C. The 5G38^T strain showed positive results for oxidase and catalase activities, as well as casein, starch, tyrosine, and Tween 80 hydrolysis. However, aesculin, DNA, xanthine, hypoxanthine, chitin, pectin, and cellulose were not hydrolyzed. Strain 5G38^T could be differentiated from the related *Pedobacter* species and *N. zeaxanthinifaciens* TDMA-5^T by means of some phenotypic characteristics, such as assimilation of D-mannitol and DNA G+C content. The detailed results of the morphological, physiological, and biochemical characteristics of the 5G38^T strain are noted in the species description and compared with those of *N. zeaxanthinifaciens* TDMA-5^T in Table 1.

Table 2. Fatty acid composition (%) of strain 5G38^T and related species. Strains: 1, *Pedobacter namyangjuensis* sp. nov. 5G38^T; 2, *Nubsella zeaxanthinifaciens* KACC 14260^T; 3, *Pedobacter alluvionis* KACC 14286^T; 4, *Pedobacter borealis* KACC 14287^T; 5, *Pedobacter roseus* KACC 11594^T; 6. *Pedobacter suwonensis* KACC 11317^T; 7, *Pedobacter terrae* KACC 13760^T; 8, *Pedobacter africanus* KACC 11492^T. Data were obtained in this study; values are percentages of total fatty acids. Fatty acids that represent less than 1% of total fatty acids in the strains are not shown. tr, Trace; -, not detected; ECL, equivalent chain length.

	1	2	3	4	5	6	7	8
Straight-chain saturated								
$C_{14:0}$	1.2	tr	tr	1.0	tr	tr	tr	1.0
$C_{16:0}$	3.7	5.2	2.2	2.0	3.2	2.8	1.8	3.9
$C_{16:0}$ 3-OH	2.1	Tr	2.0	2.2	1.7	1.6	1.1	1.4
$C_{17:0}$ 2-OH	1.4	tr	tr	tr	1.0	-	tr	tr
Branched saturated								
iso-$C_{15:0}$	27.8	36.1	28.9	25.4	32.0	27.7	31.4	26.9
iso-$C_{15:0}$ 3-OH	2.5	2.4	3.5	3.3	2.5	2.5	2.9	2.1
iso-$C_{16:0}$ 3-OH	1.0	tr	tr	tr	tr	-	tr	tr
iso-$C_{17:0}$ 3-OH	11.3	10.3	14.6	14.6	16.4	18.6	17.0	15.5
anteiso-$C_{15:0}$	tr	2.0	tr	1.1	1.9	tr	tr	1.1
Summed feature*								
3	34.2	23.5	30.3	30.6	26.8	27.5	29.8	27.7
4	tr	2.2	3.5	2.3	tr	2.7	1.2	2.2
Monounsaturated								
$C_{16:1}\omega5c$	3.5	1.4	2.0	2.7	1.4	2.2	1.5	1.4
iso-$C_{17:1}\omega9c$	3.3	7.4	4.3	6.6	5.9	8.7	7.1	9.9
Unknown								
ECL 13.565	1.0	2.0	tr	tr	tr	tr	tr	tr
ECL 16.582	tr	tr	1.1	1.3	1.3	1.6	1.0	1.1

* Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contained $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH. Sumnmed feature 4 contained anteiso-$C_{17:1}$ B and/or iso-$C_{17:1}$ I.

Phylogenetic analysis and isoprenoid quinone analysis

The 5G38^T strain exhibited 16S rRNA gene sequence similarity values ranging from 95.2% (*P. koreensis* WPCB189^T) to 89.5% (*P. saltans* DSM12145^T) with respect to strains of the *Pedobacter* species and 94.3% with respect to *N. zeaxanthinifaciens* TDMA-5^T. The 16S rRNA gene similarity between the *N. zeaxanthinifaciens* TDMA-5^T and *P. koreensis* WPCB189^T strains was 93.9%. The neighbor-joining

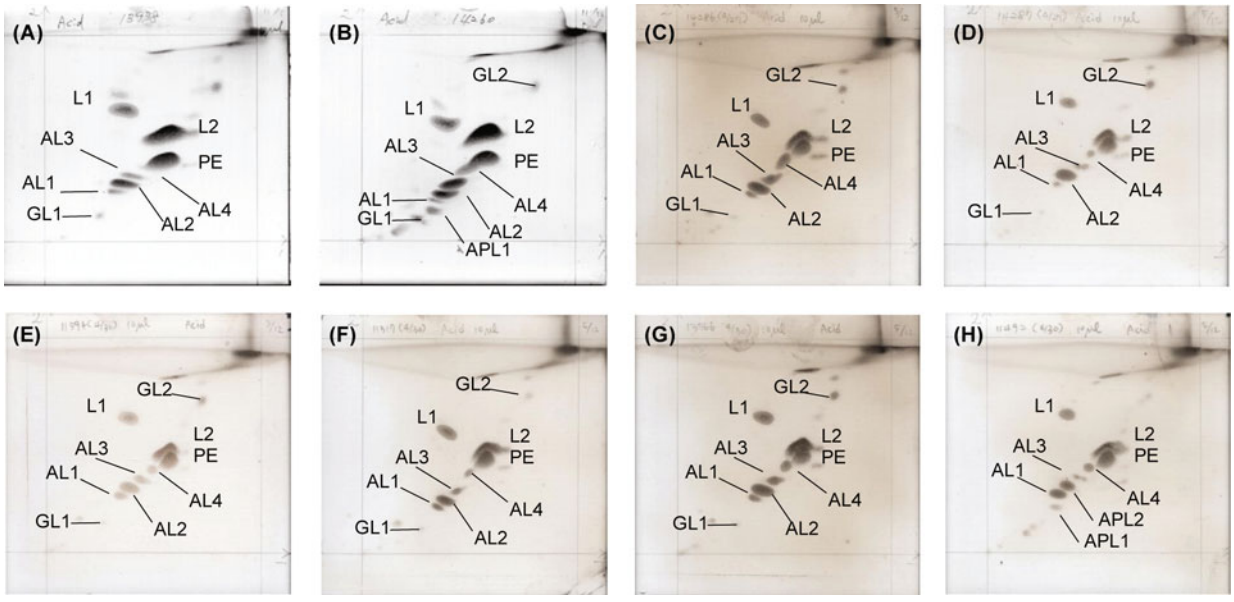

Fig. 2. Two-dimensional thin-layer chromatography of polar lipids extracts obtained from strain 5G38^T (A), *N. zeaxanthinifaciens* KACC 14260^T (B), *P. alluvionis* KACC 14286^T (C), *P. borealis* KACC 14287^T (D), *P. roseus* KACC 11594^T (E), *P. suwonensis* KACC 11317^T (F), *P. terrae* KACC 13760^T (G), *P. africanus* KACC 11492^T (H). L1–L2, unidentified polar lipids; GL1–GL2, unidentified glycolipid; AL1–AL4, unknown aminolipids; APL, unidentified aminophospholipids; PE, phosphatidylethanolamine.

tree based on the 16S rRNA gene sequences clearly showed that the 5G38^T strain formed a distinct phylogenetic lineage with the *N. zeaxanthinifaciens* TDMA-5^T (97% bootstrap support) (Fig. 1). The major respiratory quinone of the 5G38^T strain was menaquinone-7 (MK-7) in line with all of the other members of the family *Sphingobacteriaceae*.

G+C content and analysis of cellular fatty acid

The DNA G+C content of the 5G38^T strain (37.0 mol%) was similar to that of *N. zeaxanthinifaciens* TDMA-5^T (38.6 mol%). The fatty acid profile of the novel strains was similar to those of *N. zeaxanthinifaciens* KACC 14260^T and the six recognized species of the genus *Pedobacter* (*P. alluvionis* KACC 14286^T, *P. borealis* KACC 14287^T, *P. roseus* KACC 11594^T, *P. africanus* KACC 11492^T, *P. suwonensis* KACC 11317^T, and *P. terrae* KACC 13760^T), which possess large amounts of iso-$C_{15:0}$, summed feature 3 (comprising $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH), and iso-$C_{17:0}$ 3-OH. However, the fatty acids of *P. nayangjuensis* are slightly different from the others with different respective proportions of several fatty acids (Table 2). The polar lipid profile of the 5G38^T strain was composed of a phosphatidylethanolamine, two unknown lipid components (L1 and L2), and four unknown aminophospholipids (APL1, APL2, AL3, and APL4), and this pattern was in agreement with those of the *N. zeaxanthinifaciens* TDMA-5^T and the six *Pedobacter* species (Fig. 2).

In their previous study, Asker *et al.* (2008) proposed that TDMA-5^T should be classified as a novel genus, *Nubsella*, which has different minor fatty acids, is more mesophilic (less psychrotolerant), and is able to assimilate malate better than the members of *Pedobacter*. However, the difference in the minor fatty acids could be found among different species within the same genus. The temperature range of *Nubsella* (18–40°C) is within the range of the *Pedobacter* (1–45°C) and the assimilation of malate varied within intraspecies. In the neighbour-joining phylogenetic tree, a cluster of *N. zeaxanthinifaciens* TDMA-5^T and the novel 5G38^T strain are positioned within the radiation of the genus *Pedobacter*. Similar results were also obtained from the maximum-parsimony algorithm. In addition, we could not find any convincing evidence in this study that the genus *Nubsella* has phenotypic characteristics different from those of the *Pedobacter* with respect to the fatty acid compositions and the polar lipid patterns. Therefore, we propose to reclassify *N. zeaxanthinifaciens* to the genus *Pedobacter* as *P. zeaxanthinifaciens* comb. nov. Based on the the polyphasic taxonomic data obtained from this study, the 5G38^T strains represent a novel member of the genus *Pedobacter* for which the name *Pedobacter namyangjuensis* sp. nov. is proposed.

Description of *Pedobacter namyangjuensis* sp. nov.

Pedobacter namyangjuensis (nam.yang.ju.en'sis. N.L. fem. adj. *namyangjuensis* referring to Namyangju region, where the bacterium was first found).

The cells of this strain are Gram-negative, non-spore forming, non-motile, yellow pigmented, strictly aerobic rods, and they are between 0.7 and 0.9 μm in diameter and between 1.0 and 1.7 μm in length after cultivation for 2 days on a R2A agar. Growth occurs on the R2A, LB, TSA, and NA agars, but not on the MacConkey's agar. Growth occurs at a pH level between 6 and 8 (the optimum pH is 8) and between 5 and 40°C (the optimum temperature is 28°C). This strain is positive for oxidase, catalase, and arginine dihydrolase activities and for glucose fermentation. It does not produce H_2S and indole. Casein, starch, and tyrosine are hydrolysed by this strain, but aesculin, DNA, xanthine, urea, hypoxanthine, chitin, pectin, Tween 20, and cellulose are not. Acid is produced from D-galactose, D-glucose, D-mannose, D-cellobiose, D-maltose, D-lactose, amidon, glycogen, and gentiobiose by this strain, but acid is not produced from glycerol, erythritol, D-arabinose, D-ribose, L-xylose, D-adonitol, methyl-*β*-D-xyloppyranoside, D-fructose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-*α*-D-glucopyanoside, *N*-acetylglucosamine, amygdalin, arbutin, salicin, D-melibiose, D-saccharose, D-trehalose, inulin, D-melezitose, D-raffinose, xylitol, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate, and potassium 5-ketogluconate. Arabinose, mannose, mannitol, maltose, capric acid, adipic acid, and malic acid are assimilated by this strain, but glucose, potassium gluconate, *N*-acetyl-glucosamine, and phenylacetic acid are not. In the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, *α*-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, *β*-galactosidase, *α*-glucosidase, *β*-glucosidase, and *N*-acetyl-*β*-glucosaminidase activities are present, but lipase (C14), trypsin, *α*-galactosidase, *β*-glucuronidase, acid phospatase, and *α*-fucosidase activities are absent. The major fatty acids are iso-$C_{15:0}$, summed feature 3 (comprising $C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH), and iso-$C_{17:0}$ 3-OH. Menaquinone-7 is the predominant quinone. The DNA G+C content is 37.0 mol%.

The 5G38^T strain (KACC 13938^T =NBRC 107692^T) was isolated from rhizosphere soil cultivated with Chinese cabbage in Namyangju, Korea.

Description of *Pedobacter zeaxanthinifaciens* comb. nov.

Basonym: *Nubsella zeaxanthinifaciens* Asker *et al.* 2008.
The description is based on that given for *Nubsella zeaxanthinifaciens* by Asker *et al.* (2008). The TDMA-5^T strain was (=NBRC 102579^T =CCUG 54348^T =KACC 14260^T) isolated from a freshwater sample collected at Misasa (Tottori, Japan).

Acknowledgements

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