Massilia jejuensis sp. nov. and *Naxibacter suwonensis* sp. nov., isolated from air samples

Hang-Yeon Weon,¹ Seung-Hee Yoo,¹ Soo-Jin Kim,¹ Yi-Seul Kim,¹
Rangasamy Anandham² and Soon-Wo Kwon¹

Correspondence
Soon-Wo Kwon
swkwon@rda.go.kr

¹Korean Agricultural Culture Collection (KACC), National Agrobiodiversity Center, National Academy of Agricultural Science, Rural Development Administration, Suwon 441-707, Republic of Korea

²Agricultural Microbiology, Department of Agricultural Microbiology, Agricultural College and Research Institute, Madurai, India

Two Gram-negative, motile, rod-shaped bacteria (strains 5317J-18^T and 5414S-25^T) were isolated from air samples collected in the Jeju Island and Suwon region of Korea, respectively. Phylogenetically, strain 5317J-18^T was grouped with the genus *Massilia* with *Massilia brevitalea* byr23-80^T as the closest relative (98.8 % sequence similarity). Strain 5414S-25^T was affiliated with the genus *Naxibacter* with *Naxibacter haematophilus* CCUG 38318^T as the closest relative (98.8 % sequence similarity). The mean DNA–DNA relatedness values between strain 5317J-18^T and *M. brevitalea* DSM 18925^T and *Massilia aurea* DSM 18055^T were 43 and 36 %, respectively. The mean DNA–DNA hybridization values between strain 5414S-25^T and *N. haematophilus* KACC 13771^T, *M. brevitalea* DSM 18925^T, *Massilia timonae* DSM 16850^T, *Naxibacter varians* KACC 13770^T, *M. aurea* DSM 18055^T, *Massilia lutea* DSM 17473^T and *Massilia albidiflava* DSM 17472^T ranged from 33 to 42 %. Both novel strains had ubiquinone Q-8 as the predominant isoprenoid quinone and summed feature 3 (comprising iso-C_{15:0} 2-OH and/or C_{16:1} ω7c) and C_{16:0} as the major fatty acids. Both strains also showed similar polar lipid profiles with phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol as the major polar lipids. The DNA G + C contents of strains 5317J-18^T and 5414S-25^T were 66.1 and 67.8 %, respectively. On the basis of their phenotypic, chemotaxonomic and genotypic characteristics, the new strains represent novel species in the genera *Massilia* and *Naxibacter*. Strain 5317J-18^T (=KACC 12634^T=DSM 21309^T) is proposed as the type strain of *Massilia jejuensis* sp. nov. and strain 5414S-25^T (=KACC 12635^T=DSM 21311^T) is proposed as the type strain of *Naxibacter suwonensis* sp. nov.

La Scola *et al.* (1998) proposed the genus *Massilia* for an isolate from the blood of an immunocompromised patient with cerebellar lesions. Members of the genus are aerobic, Gram-negative, motile, non-spore-forming rods to short-rods and are catalase-positive. Chemotaxonomically, members of this genus contain summed feature 3 (comprising iso-C_{15:0} 2-OH and/or C_{16:1} ω7c) and C_{16:0} as the major fatty acids and Q-8 as the predominant isoprenoid quinone

and show relatively high G + C contents (62–68.9 mol%). To date, this genus comprises ten species with validly published names, including the type species *Massilia timonae* (La Scola *et al.*, 1998; Gallego *et al.*, 2006; Zhang *et al.*, 2006; Weon *et al.*, 2008, 2009; Zul *et al.*, 2008). The genus *Naxibacter* was originally erected to represent one soil isolate, *Naxibacter alkalitolerans* (Xu *et al.*, 2005). Later, two additional species, *Naxibacter varians* and *Naxibacter haematophilus*, were described and the genus description was emended (Kämpfer *et al.*, 2008). The genus *Naxibacter* is closely related to the genus *Massilia*. This paper describes the isolation and characterization of two novel strains collected from air samples.

Strains 5317J-18^T and 5414S-25^T were isolated from air samples collected from Jeju Island and the Suwon region, Korea, respectively. The air samples were collected by using an MAS-100 air sampler (single-stage multiple-hole impactor; Merck) that contained Petri dishes with R2A

Abbreviations: NJ, neighbour-joining; ML, maximum-likelihood; MP, maximum-parsimony.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains 5317J-18^T and 5414S-25^T are FJ969486 and FJ969487, respectively.

Additional phylogenetic trees based on analysis of 16S rRNA gene sequences using the maximum-likelihood and maximum-parsimony algorithms and the polar lipid profile are available as supplementary figures with the online version of this paper.

medium (BBL) supplemented with cycloheximide (Sigma) at 200 µg ml⁻¹.

Tests to determine Gram staining, catalase and oxidase activities, and hydrolysis of casein, DNA, hypoxanthine, starch, Tween 80 and xanthine were performed according to the methods of Smibert & Krieg (1994). Cellular morphology was determined by light microscopy (Axio; Zeiss) and transmission electron microscopy (TEM) using 1-day-old cells. For TEM observations, the cells were negatively stained with 0.5 % (w/v) uranyl acetate and examined with a model 912AB (LEO) microscope. The temperature range (4–50 °C) and pH range (4–10 at intervals of 1 pH unit) for growth and the requirement for NaCl [0, 1, 2, 3, 5 and 7 % (w/v)] were determined using R2A medium. Hydrolysis of carboxymethylcellulose (0.1 %, w/v), chitin from crab shells (1 %, w/v), pectin (1 %, w/v) and tyrosine (0.5 %, w/v) were also tested. The commercially available API 20NE and API ID 32 GN test kits (bioMérieux) were used to determine the biochemical properties, utilization of carbohydrates and enzymic activities of the novel strains according to the manufacturer's instructions. The API ZYM tests were read after 4 h incubation at 37 °C, the other API tests were read after 72 h at 28 °C. Strains 5317J-18^T and 5414S-25^T could be differentiated from members of the genera *Massilia* and *Naxibacter* on the basis of the results of assimilation tests and other physiological properties. Some of these results are given in the species description; Table 1 shows a comparison of the characteristics of strains 5317J-18^T and 5414S-25^T and closely related strains.

Chromosomal DNA was extracted and purified according to the method of Ausubel *et al.* (1987), except that the lysates were extracted twice with chloroform to remove residual phenol. The 16S rRNA genes were amplified by PCR from chromosomal DNA using two universal primers for bacteria (Weisburg *et al.*, 1991). The PCR products were purified and sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) according to the manufacturer's instructions. The 16S rRNA gene sequences from the novel isolates and related taxa (obtained from the NCBI database) were aligned using the CLUSTAL W multiple sequence alignment program (Thompson *et al.*, 1994). Sequence similarities were calculated using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Phylogenetic trees were constructed with the neighbour-joining (NJ; Saitou & Nei, 1987), maximum-parsimony (MP; Kluge & Farris, 1969) and maximum-likelihood (ML; Felsenstein, 1981) methods using the MEGA3 program (Kumar *et al.*, 2004), with bootstrap values based on 1000 replications (Felsenstein, 1985). Phylogenetic trees (Fig. 1 and Supplementary Figs S1 and S2 in IJSEM Online) indicated that strain 5317J-18^T was phylogenetically affiliated with *Massilia brevitalea* byr23-80^T with high bootstrap values (99, 98 and 97 % by the NJ, ML and MP methods, respectively) and strain 5414S-25^T grouped with the members of the genus *Naxibacter* (77, 66 and 65 % by the NJ, ML and MP

methods, respectively). Strain 5317J-18^T showed the highest sequence similarities with *M. brevitalea* byr23-80^T (98.8 %) and *Massilia aurea* AP13^T (97.9 %). Strain 5414S-25^T revealed high gene sequence similarities with *N. haematophilus* CCUG 38318^T (98.8 %), *M. brevitalea* byr23-80^T (98.2 %), *M. timonae* CIP 105350^T (97.6 %), *N. varians* CCUG 35299^T (97.5 %), *M. aurea* AP13^T (97.3 %), *Massilia lutea* 101^T (97.2 %) and *Massilia albidiflava* 45^T (97.0 %).

For the assessment of DNA–DNA relatedness values, DNA–DNA hybridization was carried out according to the method of Seldin & Dubnau (1985). The mean DNA–DNA relatedness values between strain 5317J-18^T and *M. brevitalea* DSM 18925^T and *M. aurea* DSM 18055^T were 43 and 36 %, respectively. The mean DNA–DNA hybridization values between strain 5414S-25^T and *N. haematophilus* KACC 13771^T, *M. brevitalea* DSM 18925^T, *M. timonae* DSM 16850^T, *N. varians* KACC 13770^T, *M. aurea* DSM 18055^T, *M. lutea* DSM 17473^T and *M. albidiflava* DSM 17472^T were 42, 42, 39, 38, 34, 33 and 33 %, respectively. These DNA–DNA relatedness values of less than 70 % (Wayne *et al.*, 1987) indicated that the two novel isolates represent two distinct genospecies.

Cell biomass for the analysis of cell-wall and isoprenoid quinones, polar lipids and fatty acids was obtained from cells grown in R2A medium at 28 °C for 2 days. Isoprenoid quinones and polar lipids were obtained using the methods of Minnikin *et al.* (1984). The fatty acids were analysed according to the standard protocol of the Sherlock Microbial Identification System (Sasser, 1990). The DNA G+C content was determined using an HPLC method (Mesbah *et al.*, 1989). The predominant isoprenoid quinone for strains 5317J-18^T and 5414S-25^T was ubiquinone Q-8 with a trace amount of Q-7. Strains 5317J-18^T, 5414S-25^T and the two reference strains, *M. brevitalea* DSM 18925^T and *N. alkalitolerans* KACC 12188^T, had similar polar lipid patterns with the predominant polar lipids of phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol (see Supplementary Fig. S3 in IJSEM Online). The fatty acid contents of strains 5317J-18^T and 5414S-25^T both contained summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 49 and 45.8 %, respectively) and C_{16:0} (22.5 and 28.8 %, respectively) as the major components and moderate amounts of C_{18:1}ω7c (12.1 and 7.8 %, respectively), C_{10:0} 3-OH (6.1 and 5.4 %, respectively) and C_{12:0} (5.9 and 4.4 %, respectively) (Table 2). The same fatty acid patterns were also found for other species of the genera *Massilia* and *Naxibacter*. The DNA G+C contents of strains 5317J-18^T and 5414S-25^T were 66.1 and 67.8 %, respectively.

The genus *Naxibacter* was originally reported by Xu *et al.* (2005) on the basis of several phenotypic properties that were different from those of closely related genera. In particular, the genera *Massilia* and *Naxibacter* could be distinguished on the basis of starch hydrolysis, urease production, oxidase production, utilization of sole carbon

Table 1. Differential properties of species of the genera *Massilia* and *Naxibacter*

Strains: 1, 5317J-18^T (data from this study); 2, 5414S-25^T (data from this study); 3, *M. aerilata* KACC 12505^T (Weon *et al.*, 2008); 4, *M. albidiflava* DSM 17472^T (Weon *et al.*, 2008); 5, *M. aurea* DSM 18055^T (Weon *et al.*, 2008); 6, *M. brevitalea* DSM 18925^T (Zul *et al.*, 2008; Weon *et al.*, 2009); 7, *M. dura* DSM 17513^T (Weon *et al.*, 2008); 8, *M. lutea* DSM 17473^T (Weon *et al.*, 2008); 9, *M. niabensis* KACC 12632^T (Weon *et al.*, 2009); 10, *M. niastensis* KACC 12599^T (Weon *et al.*, 2009); 11, *M. plicata* DSM 17505^T (Weon *et al.*, 2008); 12, *M. timonae* DSM 16850^T (Weon *et al.*, 2008); 13, *N. alkalitolerans* KACC 12188^T (data from this study); 14, *N. haematophilus* KACC 13771^T (data from this study); 15, *N. varians* KACC 13770^T (data from this study). +, Positive; (+), weakly positive; –, negative.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Oxidase	+	+	+	+ ^{ax}	(+) ^b	– ^c	+ ^a	+ ^a	+	+	– ^a	+ ^e	– ^f	+ ^g	+ ^g
Nitrate reduction	–	–	+	+	–	+ ^c	–	–	+	–	–	–	– ^f	–	–
Urease	–	–	+	–	–	– ^d	–	–	–	–	–	–	+ ^f	–	–
Hydrolysis of aesculin	+	–	+	+	+	+ ^d	+	+	–	+	+	+	+ ^f	–	(+)
Assimilation of:															
Adipic acid	–	+	–	+	+	+ ^d	–	+	–	–	–	–	–	+	–
L-Alanine	–	+	+	+	+	–	+	–	–	+	+	+	+	–	+
L-Arabinose	–	–	+	+	–	– ^d	+	+	–	+	+	+	+	–	+
D-Glucose	–	–	+	+	+	+ ^d	+	–	–	+	+	+	+	–	–
Glycogen	+	+	+	+	+	–	+	–	–	–	+	+	–	+	+
L-Histidine	–	+	+	–	–	–	–	–	–	–	–	+	+	–	–
3-Hydroxy-benzoic acid	–	–	–	–	–	–	–	–	–	+	–	–	+	+	–
4-Hydroxy-benzoic acid	–	–	–	+	–	–	+	–	–	+	–	–	–	+	–
3-Hydroxy-butyric acid	–	+	+	+	+	+	+	–	–	+	+	+	+	+	+
Lactic acid	–	–	–	+	–	–	–	–	–	–	–	+	–	–	–
Potassium gluconate	–	–	–	+	–	– ^d	+	+	–	+	+	+	–	–	–
L-Proline	–	+	+	+	+	+	+	+	–	+	+	+	+	–	+
Propionic acid	–	+	+	–	(+)	+	–	–	–	–	–	–	+	+	+
L-Rhamnose	–	–	–	+	+	–	+	–	–	+	+	+	+	–	+
L-Serine	–	+	+	+	+	–	–	–	–	–	+	+	+	–	+
Suberic acid	–	+	–	–	+	+	–	–	–	–	–	–	–	+	–
Trisodium citrate	–	–	–	–	+	– ^d	–	–	–	–	–	+	+	–	–
Valeric acid	–	+	–	–	–	+	–	–	–	–	–	–	–	+	–
DNA G + C content (mol%)	66.1	67.8	68.9	65.3 ^a	66.0 ^b	65.3	65.9 ^a	63.3 ^a	67.8	66.6	65.1 ^a	62–67 ^e	62.4 ± 0.3 ^f	ND	ND

*Data from: *a*, Zhang *et al.* (2006); *b*, Gallego *et al.* (2006); *c*, Zul *et al.* (2008); *d*, Weon *et al.* (2009); *e*, Lindquist *et al.* (2003); *f*, Xu *et al.* (2005); *g*, Kämpfer *et al.* (2008).

sources and the sources of isolation. According to phenotypic comparisons between many recently described members of the genera *Naxibacter* and *Massilia*, the above-mentioned phenotypic properties are not genus-specific but can be variable among species of the two genera. Furthermore, phosphatidylinositol mannosides originally described as being present in members of the genus *Naxibacter* (Xu *et al.*, 2005) were absent and phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol were detected as the major polar lipids (Kämpfer *et al.*, 2008). According to previously reported data, there were no unique genus-specific phenotypic characteristics that distinguished the genera *Massilia* and *Naxibacter*. In

addition, the polar lipid profiles and fatty acids of both genera have not been found to show any significant differences. In the future, some genera, including the genera *Massilia* and *Naxibacter* and another closely related phylogenetic relative, the genus *Telluria*, will need further reclassification as more taxonomic data become available. At present, based on the polyphasic taxonomic approach described here, particularly on the basis of the phylogenetic evaluation, it is suggested that strains 5317J-18^T and 5414S-25^T should be classified as novel species of the genera *Massilia* and *Naxibacter*, for which the names *Massilia jejuensis* sp. nov. and *Naxibacter suwonensis* sp. nov. are proposed, respectively.

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences. The positions of strains 5317J-18^T and 5414S-25^T are shown with respect to other closely related species. The tree was generated using the NJ method. Numbers at the nodes indicate bootstrap values, expressed as percentages of 1000 replications; only values >50% are shown. Bar, 0.005 accumulated changes per nucleotide.

Description of *Massilia jejuensis* sp. nov.

Massilia jejuensis (je.ju.en'sis. N.L. masc. adj. *jejuensis* referring to Jeju Island, Republic of Korea, where the type strain was found).

Cells are aerobic, Gram-negative, motile with single flagellum, rod-shaped (1.1–1.5 × 1.3–3.0 µm). Catalase- and oxidase-positive. Colonies are light orange, round and convex with clear margins. Grows at 5–35 °C and pH 6.0–9.0; optimum

growth occurs at 30 °C and pH 7.0. Does not require NaCl for growth, but can tolerate 1% (w/v) NaCl. Hydrolyses hypoxanthine and Tween 80 and weakly hydrolyses starch and tyrosine. Does not hydrolyse carboxymethylcellulose, casein, chitin, DNA, pectin or xanthine. Grows on R2A and nutrient agar (Difco), but not on tryptic soy agar (Difco) or MacConkey agar (Difco). According to API 20NE test strips, positive for aesculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation,

Table 2. Cellular fatty acid contents (%) of species of the genera *Massilia* and *Naxibacter*

Strains: 1, 5317J-18^T; 2, 5414S-25^T; 3, *M. aerilata* KACC 12505^T; 4, *M. albidiflava* DSM 17472^T; 5, *M. aurea* DSM 18055^T; 6, *M. brevitalea* DSM 18925^T; 7, *M. dura* DSM 17513^T; 8, *M. lutea* DSM 17473^T; 9, *M. niabensis* KACC 12632^T; 10, *M. niastensis* KACC 12599^T; 11, *M. plicata* DSM 17505^T (Zhang *et al.*, 2006); 12, *M. timonae* DSM 16850^T; 13, *N. alkalitolerans* KACC 12188^T; 14, *N. haematophilus* KACC 13771^T; 15, *N. varians* KACC 13770^T. Data for taxa 1–2 and 13–15 are from this study; data for taxa 3–10 and 12 are from Weon *et al.* (2009). –, <1% or not detected.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
C _{10:0} 3-OH	6.1	5.4	4.7	7.0	6.0	4.9	5.5	5.7	6.6	5.5	10.1	4.6	3.2	3.0	3.3
C _{12:0}	5.9	4.4	3.4	5.3	4.4	5.0	3.9	4.0	8.9	4.7	7.1	3.3	3.3	3.3	3.7
C _{12:0} 2-OH	1.7	2.4	—	—	—	2.0	—	—	—	2.4	1.6	2.2	2.2	1.5	2.0
C _{14:0}	—	1.2	1.9	2.6	2.0	—	1.1	1.2	—	—	—	—	—	—	—
C _{14:0} 2-OH	—	—	2.5	2.4	2.5	—	2.6	2.9	—	—	6.1	—	—	—	—
anteiso-C _{15:0}	—	—	—	—	—	—	—	—	—	—	—	—	—	1.5	—
C _{16:0}	22.5	28.8	30.6	23.4	27.2	23.0	27.5	26.6	23.6	26.9	25.1	30.5	26.6	26.2	28.5
iso-C _{16:0}	—	—	—	—	—	—	—	—	—	—	—	—	—	2.1	—
C _{17:0} cyclo	1.0	2.9	6.1	—	—	—	—	—	—	3.4	—	3.7	—	2.7	—
iso-C _{17:0}	—	—	—	—	—	—	—	—	—	—	—	—	—	1.6	—
iso-C _{17:1} ω9c	—	—	—	—	—	—	—	—	—	—	—	—	—	1.4	—
C _{18:1} ω7c	12.1	7.8	11.7	7.4	7.9	9.0	7.03	7.8	7.8	12.3	11.7	7.9	6.6	8.7	8.1
C _{20:0}	—	—	—	—	—	—	—	—	—	2.6	—	—	—	—	—
Summed feature 3*	49.0	45.8	35.2	46.0	48.6	54.2	52.0	51.1	46.0	38.1	36.9	47.0	55.2	44.7	51.7

*Summed feature 3 included C_{16:1} ω7c and/or iso-C_{15:0} 2-OH.

arginine hydrolysis, urease, gelatin hydrolysis and β -galactosidase. Assimilates glycogen, but does not assimilate any other substrates embedded in the API 20NE and API ID 32GN strips. Produces alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase and α -glucosidase, but a weak reaction for valine arylamidase and acid phosphatase. Does not produce lipase (C14), cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase or α -fucosidase (API ZYM test strip). The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. The major fatty acids are summed feature 3 (C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH) and C_{16:0}. Q-8 is the predominant respiratory quinone.

The type strain, 5317J-18^T (=KACC 12634^T=DSM 21309^T), was isolated from an air sample from Jeju island, Republic of Korea. The DNA G+C content of the type strain is 66.1 mol%.

Description of *Naxibacter suwonensis* sp. nov.

Naxibacter suwonensis (su.won.en'sis. N.L. masc. adj. *suwonensis* referring to Suwon region, Republic of Korea, where the type strain was found).

Cells are aerobic, Gram-negative, motile with a single flagellum and rod-shaped (0.6–0.8 $\times$ 1.2–3.0 μ m). Catalase- and oxidase-positive. Colonies are ivory coloured, round and convex with clear margins. Grows at 5–40 °C and pH 6.0–9.0; optimum growth occurs at 30 °C and pH 7.0. Does not require NaCl for growth, but can tolerate 1% (w/v) NaCl. Hydrolyses casein, hypoxanthine, Tween 80 and tyrosine, hydrolyses xanthine weakly, but does not hydrolyse carboxymethylcellulose, chitin, DNA, pectin or starch. Grows on R2A, nutrient agar (Difco) and tryptic soy agar (Difco), but not on MacConkey agar (Difco). According to API 20NE test strips, positive reaction for gelatin hydrolysis, but negative result for nitrate reduction, indole production, glucose fermentation, arginine hydrolysis, urease, aesculin hydrolysis and β -galactosidase. Assimilates maltose, adipic acid, malic acid, phenylacetic acid, suberic acid, L-alanine, glycogen, L-serine, propionic acid, valeric acid, L-histidine, 3-hydroxybutyric acid and L-proline, but does not assimilate D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, potassium gluconate, capric acid, trisodium citrate, L-rhamnose, D-ribose, inositol, sucrose, itaconic acid, sodium malonate, sodium acetate, lactic acid, potassium 5-ketogluconate, 3-hydroxybenzoic acid, salicin, melibiose, L-fucose, D-sorbitol, potassium 2-ketogluconate or 4-hydroxybenzoic acid. Produces alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -glucosidase. Weak reaction for esterase (C4) and cystine arylamidase, but negative reaction for lipase (C14), trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -man-

nosidase and α -fucosidase. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. The major fatty acids are summed feature 3 (C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH) and C_{16:0}. Q-8 is the predominant respiratory quinone.

The type strain, 5414S-25^T (=KACC 12635^T=DSM 21311^T), was isolated from an air sample from the Suwon region, Republic of Korea. The DNA G+C content of the type strain is 67.8 mol%.

Acknowledgements

This work was supported by National Academy of Agricultural Science (NAAS), Rural Development Administration, Republic of Korea.

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