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Niabella ginsenosidivorans sp. nov., isolated from compost

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A Gram-reaction negative, strictly aerobic, non-motile, orange colored, and rod-shaped bacterium (designated BS26^T) isolated from compost, was characterized by a polyphasic approach to clarify its taxonomic position. Strain BS26^T was observed to grow optimally at 25–30°C and at pH 7.0 on R2A and nutrient media. Strain BS26^T showed β-glucosidase activity that was responsible for its ability to transform ginsenoside Rb_1 (one of the active components of ginseng) to ginsenoside compound-K (C-K). Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain BS26^T belongs to the genus *Niabella* of family *Chitinophagaceae* and was most closely related to *Niabella soli* DSM 19437^T (94.5% similarity), *N. yanshanensis* CCBAU 05354^T (94.3%), and *N. aurantiaca* DSM 17617^T (93.8%). The G+C content of genomic DNA was 47.3 mol%. Chemotaxonomic data [predominant isoprenoid quinone-MK-7, major fatty acids–iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH, and summed feature 3 (comprising $C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$)] supported the affiliation of strain BS26^T to the genus *Niabella*. However, strain BS26^T could be differentiated genotypically and phenotypically from the recognized species of the genus *Niabella*. The novel isolate therefore represents a novel species, for which the name *Niabella ginsenosidivorans* sp. nov. is proposed, with the type strain BS26^T (=KACC 16620^T =JCM 18199^T).

Keywords: *Niabella ginsenosidivorans*, 16S rRNA gene sequence, polyphasic taxonomy, ginsenoside, decayed feedstuff, compost

Introduction

The genus *Niabella* was proposed by Kim *et al*. (2007). Members of the genus *Niabella* are characterized as Gram-reaction negative, strictly aerobic, non-flagellated, non-spore-forming, and short rods-shaped. When grown on R2A agar plates, colonies are orange and circular. Flexirubin pigment is produced. Chemotaxonomically, they have MK-7 as the predominant isoprenoid quinone and iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH, and summed feature 3 (comprising iso-$C_{15:0}$ 2-OH and/or $C_{16:1}$ $\omega7c$) as major fatty acids. The range of DNA G+C content was described as 42-47.5 mol% (Pham and Kim, 2014). A phylogenetic analysis revealed that the genus *Niabella* was closely related with the genera *Terrimonas* and *Sediminibacterium* within the family *Chitinophagaceae*. At the time of writing, the genus *Niabella* comprises seven recognized species (Euzéby, 1997) isolated from various environments, including the recently described species; *N. hirudinis* and *N. drilacis* (Glaeser *et al*., 2013), *N. terrae* (Ahn *et al*., 2013), *N. thaonhiensis* (Pham and Kim, 2014), and *N. tibetensis* (Dai *et al*., 2011).

In the present study, we conducted a phylogenetic (16S rRNA gene), phenotypic, genotypic and chemotaxonomic analyses to determine the precise taxonomic position of a strain designated BS26^T which was isolated from compost that was made of decayed feedstuff. On the basis of the results obtained in this study, we propose that strain BS26^T should be placed in the genus *Niabella* as the type strain of a novel species, *Niabella ginsenosidivorans* sp. nov.

Materials and Methods

Isolation of bacterial strain and culture condition

During the course of a study on cultivable aerobic bacterial strains using the standard dilution-plating technique on R2A agar (BD) at room temperature from compost that was made of decayed feedstuff, in the Republic of Korea, a large number of novel bacterial strains were isolated. One of them, BS26^T was cultured routinely on nutrient (BD) or R2A agar at 25°C or 30°C and preserved, as a suspension in nutrient with 20% (w/v) glycerol, at -70°C. The strain BS26^T was deposited to the Korean Agricultural Culture Collection (=KACC 16620^T) and Japan Collection of Microorganisms (=JCM 18199^T).

Phenotypic and biochemical characteristics

The Gram reaction was determined using the non-staining method using 3% KOH, as described by Buck (1982). Cell morphology and motility were observed under a Nikon light microscope at × 1,000, with cells grown on R2A agar for 2 days at 30°C. Catalase and oxidase tests were performed as outlined by Cappuccino and Sherman (2002). Anaerobic growth was determined in serum bottles containing R2A

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broth supplemented with thioglycolate (1 g/L), in which the upper air layer had been replaced with nitrogen. Flexirubin pigment was detected by means of a colour shift after exposure to a 20% (w/v) KOH solution (Reichenbach, 1992). Biotransformation tests of ginsenosides were carried out described by Kim *et al.* (2013b) and Du *et al.* (2014). Biochemical phenotypic tests were carried out using API 20NE, API ID 32GN, and API ZYM test kits according to the instructions of the manufacturer (bioMérieux). Degradation for DNA using DNase agar (Scharlau), casein, starch, carboxyl-methyl cellulose (Atlas, 1993), and xylan (Ten *et al.*, 2004) were evaluated after 5 days of incubation. Growth at different temperatures (4, 10, 18, 30, 37, 42, and 45°C) and various pH values (pH 3.5–10.0 at intervals of 0.5 pH units) was assessed after 5 days of incubation. Three different buffers (final concentration, 20 mM) were used to adjust the pH of the nutrient broth. Acetate buffer was used for pH 3.5–5.5, phosphate buffer was used for pH 6.0–8.0 and Tris buffer was used for pH 8.5–10.0. Salt tolerance was tested on a nutrient medium supplemented with 1–10% (w/v at intervals of 1% unit) NaCl after 5 days of incubation. Growth on trypticase soy agar (TSA, Difco) and MacConkey agar (Difco) was also evaluated at 30°C.

PCR amplification, 16S rRNA gene sequencing and phylogenetic analysis

The genomic DNA of strain BS26^T was extracted using a commercial genomic DNA-extraction kit (Solgent). The 16S rRNA gene was amplified from the chromosomal DNA using the universal bacterial primer pair 9F and 1512R and the purified PCR products were sequenced by Solgent Co. Ltd. (Kim *et al.*, 2013a). Full sequences of the 16S rRNA gene were compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank and EzTaxon-e server (Kim *et al.*, 2012). Multiple alignments were performed by Clustal_X program (Thompson *et al.*, 1997) and gaps were edited in the BioEdit

Table 1. Differentiating characteristics of strain BS26^T and the type strains of related *Niabella* species

Strain: 1, *N. ginsenosidivorans* BS26^T; 2, *N. aurantiaca* KACC 11698^T; 3, *N. ginsengisoli* KACC 13021^T; 4, *N. soli* KACC 12604^T; 5, *N. yanshanensis* KACC 14980^T.

All data were obtained from the present study except the DNA G+C contents and tests of temperature, pH, and salt ranges of the reference type strains (Kim *et al.*, 2007; Weon *et al.*, 2008, 2009; Wang *et al.*, 2009). All strains were orange colored and rod-shaped. All strains were negative for hydrolysis of DNA, xylan, and growth on MacConkey agar. In API 20NE and 32GN, all strains were positive for arginine dihydrolase, urease, and assimilation of D-glucose, L-arabinose, D-mannose, *N*-acetyl-D-glucosamine, D-maltose, gluconate, D-mannitol, salicin, L-fucose, D-sorbitol, D-ribose, inositol, and itaconate but negative for nitrate reduction, indole production, glucose acidification, and assimilation of caprate, citrate, propionate, valerate, L-histidine, 4-hydroxybenzoate, L-proline, malonate, L-alanine, glycogen, and 3-hydroxybenzoate. In API ZYM test, all strains were positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, *α*-galactosidase, *β*-galactosidase, α-glucosidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminidase, *α*-mannosidase, and *α*-fucosidase but negative for lipase (C14). +, positive; –, negative.

Characteristic	1	2	3	4	5
Isolation source	Compost	Greenhouse soil	Ginseng cultivating soil	Soil	Soybean rhizosphere
Catalase/oxidase activity	+/+	+/-	+/-	-/+	+/+
Temperature range (°C)	18–42	10–35	5–35	15–35	15–35
pH range	4.5-10.0	5.0–8.0	6.0–8.0	5.0–8.0	6.0–10.0
NaCl range (%)	0–3	0–3	0–2	0–1	0–1
Hydrolysis of:					
Gelatin	-	+	+	+	+
Casein	-	+	-	-	+
Starch	-	-	+	-	+
Carbon utilization of:					
Adipate	-	+	-	+	-
Malate	-	-	-	+	-
Phenyl-acetate	-	-	-	+	-
D-Melibiose	-	+	-	-	-
2-Ketogluconate	-	+	-	-	-
3-Hydroxybutyrate	-	-	+	+	+
L-Rhamnose	-	-	+	+	-
D-Sucrose	-	+	-	+	+
Suberate	-	-	-	+	-
Acetate	-	-	-	+	-
Lactate	-	-	-	-	+
5-Ketogluconate	-	-	-	+	-
L-Serine	-	-	-	+	+
API ZYM test results					
Trypsin	-	-	+	-	+
α-Chymotrypsin	+	+	+	-	+
β-Glucuronidase	-	+	+	+	+
G + C content (mol %)	47.3	45	43	45	42

program (Hall, 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). The phylogenetic trees were constructed by using the neighbor-joining (Saitou and Nei, 1987), maximum-parsimony (Fitch, 1971), and maximum-likelihood methods (Felsenstein, 1981) with the MEGA6 Program (Tamura *et al.*, 2013) with bootstrap values based on 1,000 replications (Felsenstein, 1985).

Isoprenoid quinone and cellular fatty acids analysis

Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under a vacuum and re-extracted in *n*-hexane-water (1:1, v/v). The crude quinone in *n*-hexane solution was purified using Sep-Pak Vac Cartridges Silica (Waters) and subsequently analyzed by HPLC, as described by Hiraishi *et al.* (1996). Cellular fatty acid profiles were determined for strains grown on nutrient agar for 48 h at 30°C. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acid methyl esters were then analyzed by gas chromatography (model 6890; Hewlett Packard) using the Microbial Identification software package (Sasser, 1990).

Determination of DNA G + C content

For measurement of the G+C content of chromosomal DNA, the genomic DNA of strain BS26^T was extracted and purified as described by Moore and Dowhan (1995) and enzymatically degraded into nucleosides. The G+C content was then determined as described by Mesbah *et al.* (1989), using reverse-phase HPLC.

Results and Discussion

Morphological and phenotypic characteristics

Cells of strain BS26^T were Gram-reaction negative, strictly aerobic, non-spore-forming, non-motile, rod shaped, oxidase-positive and catalase-positive. The colonies grown on R2A agar plates for 2 days were smooth, circular, convex, transparent, orange in color, and 2–3 mm in diameter. On R2A agar, BS26^T was able to grow at 18–42°C, but not at 45°C. The isolate grew on nutrient agar and TSA, but not on MacConkey agar. Strain BS26^T had β-glucosidase activity that was responsible for its ability to transform ginsenoside Rb_1 (one of the active components of ginseng) to ginsenoside compound-K (C-K). The morphological, physiological, and biochemical characteristics of strain BS26^T are given in the species description and Table 1, which lists the characteristics that serve to differentiate strain BS26^T from its closest phylogenetic relatives.

Phylogenetic analysis

The 16S rRNA gene sequence of the strain BS26^T determined in this study was continuous stretch of 1,446 bp (base position 28-1491 with respect to the *Escherichia coli* numbering system), which was deposited in a GenBank database (accession numbers JQ349050). A sequence similarity calculation from using the EzTaxon-e server (Kim *et al.*, 2012) indicated that the closest relative of strain BS26^T was *Niabella soli* DSM 19437^T (94.5% similarity), *N. yanshanensis* CCBAU 05354^T (94.3%), *N. drilacis* (93.9%), *N. aurantiaca* DSM 17617^T (93.8%), and *N. ginsengisoli* GR10-1^T (93.2%). This

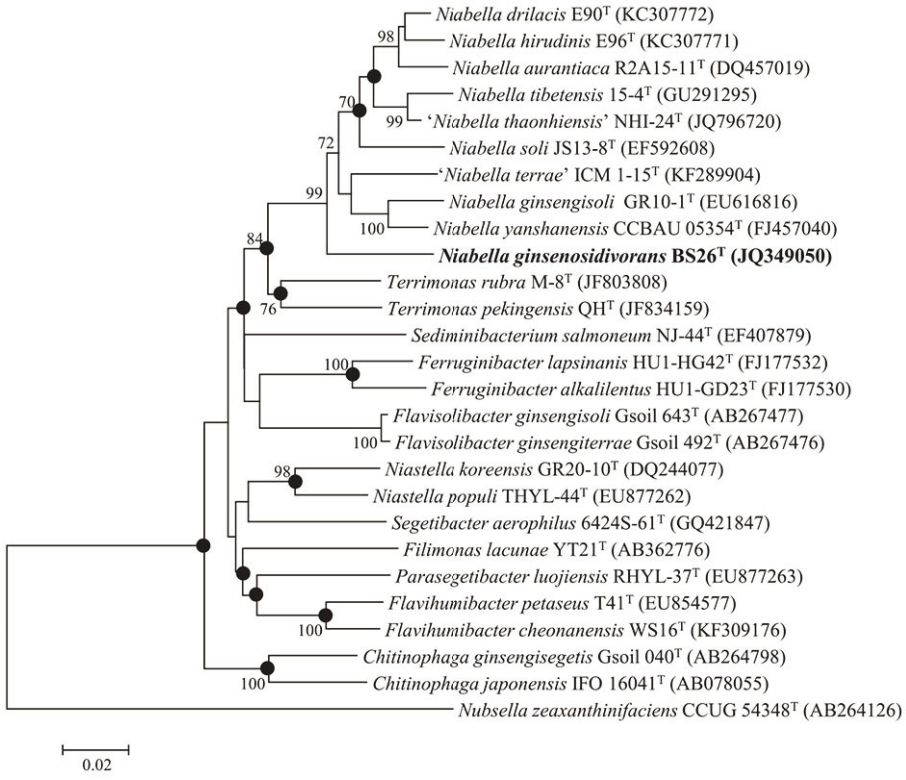

Fig. 1. Phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationships of strain BS26^T with other related species. This tree was made using the neighbor-joining method (Saitou and Nei, 1987) with a Kimura (1983) two-parameter distance matrix and pairwise deletion. Dots indicate generic branches that were also recovered by using maximum-parsimony and maximum-likelihood algorithms. Bootstrap values (expressed as percentages of 1,000 replications) greater than 70% are shown at the branch points. Bar, 0.01 substitutions per 2 nucleotide position. *Nubsella zeaxanthinifaciens* CCUG 54348^T was used as an outgroup.

relationship between strain BS26^T and other members of the genus *Niabella* was also evident in the phylogenetic tree, which used over 1,355 nt (Fig. 1). Strain BS26^T and other members of the genus *Niabella* formed a monophyletic group with a high bootstrap value (84%), which was supported by three kinds of tree making methods used in this study. DNA-NA hybridization tests between strain BS26^T and its nearest phylogenetic neighbor was not attempted since strains differing by >3.0% at the 16S rRNA gene level are unlikely to exhibit >70% relatedness at the whole-genome level (Stackebrandt and Goebel, 1994).

Cellular fatty acids and quinone composition

The predominant isoprenoid quinone of strain BS26^T was menaquinone 7 (MK-7), in line with all other members of the family *Chitinophagaceae*. The cellular fatty acids of strain BS26^T and closely related type strain of genus *Niabella* are listed in the Table 2. The major fatty acids of strain BS26^T were iso-$C_{15:0}$ (42.0%), iso-$C_{17:0}$ 3-OH (16.4%), iso-$C_{15:1}$ G (15.9%), and summed feature 3 (comprising iso-$C_{15:0}$ 2-OH and/or $C_{16:1}$ $\omega7c$, 14.4%), which were typical of those of members of the genus *Niabella*. According to the Table 2, qualitative and quantitative differences in fatty acid content were observed between strain BS26^T and its phylogenetically closest relatives.

DNA G+C content

The DNA G+C content of strain BS26^T was 47.3 mol%, similar to those of *N. soli* DSM 19437^T, *N. aurantiaca* DSM 17617^T, which were in the range of 42–47.5 mol%.

Taxonomic conclusions

The characteristics of strain BS26^T were consistent with the description of the genus *Niabella* with regard to morphological, biochemical and chemotaxonomic properties. However, the phylogenetic distance between strain BS26^T and recognized *Niabella* species and the unique phenotypic characteristics (Table 1) warrant assignment of strain BS26^T to the genus *Niabella* as the type strain of a novel species, for which the name *Niabella ginsenosidivorans* sp. nov. is proposed.

Description of *Niabella ginsenosidivorans* sp. nov.

Niabella ginsenosidivorans (gin.se.no.si.di.vo'rans. N.L. n. *ginsenosidum* ginsenoside; L. part. adj. *vorans* eating, devouring; N.L. part. adj. *ginsenosidivorans*, ginsenoside-devouring).

Cells are Gram-reaction-negative, strictly aerobic, non-motile and rod shaped (measuring 0.3–0.4 μm × 0.9–1.5 μm). Colonies are smooth, translucent, convex, circular with regular margins, orange colored in color and 1–2 mm in diameter. Grows on nutrient and R2A media at 18–42°C and at pH 5.0–10.0, but not at 10°C and 45°C. Optimum growth occurs at 25–30°C and at pH 6–7. Growth occurs up to 3% NaCl supplement. Catalase and oxidase are positive. Does not hydrolyze DNA, skim milk, starch, xylan, and CM-cellulose. Carbon assimilation tests (API ID 32 GN, API 20 NE) and the enzyme activities (API ZYM) are listed in Table 1. MK-7 is the predominant respiratory quinone and iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, iso-$C_{15:1}$ G, and summed feature 3 (comprising iso-$C_{15:0}$ 2-OH and/or $C_{16:1}$ $\omega7c$) are the major cellular fatty acids. The G + C content of the genomic DNA is 47.3 mol%.

The type strain, isolated from the compost, Republic of Korea, is BS26^T (=KACC 16620^T =JCM 18199^T).

Table 2. Cellular fatty acid profiles of strain BS26^T and phylogenetically related species of the genus *Niabella*

Strain: 1, *M. ginsenosidivorans* BS26^T; 2, *N. aurantiaca* KACC 11698^T; 3, *N. ginsengisoli* KACC 13021^T; 4, *N. soli* KACC 12604^T; 5, *N. yanshanensis* KACC 14980^T.

All data from this study. All strains were cultured on R2A agar for 2 days at 30°C. Fatty acids amounting to <0.5% of the total fatty acids in all strains are not listed. -, not detected.

Fatty acid	1	2	3	4	5
Saturated					
$C_{14:0}$	0.8	0.5	0.9	0.5	0.9
$C_{16:0}$	2.1	3.1	1.4	2.7	1.9
Unsaturated					
$C_{16:1}$ $\omega5c$	-	-	-	-	1.7
Branched					
iso-$C_{15:0}$	42.0	42.2	31.1	44.5	37.8
iso-$C_{15:0}$ 3OH	2.9	3.2	8.4	3.1	3.1
iso-$C_{15:1}$ G	15.9	18.2	25.3	19.1	22.9
iso-$C_{16:0}$	0.5	-	-	0.1	0.8
iso-$C_{16:0}$ 3OH	0.7	0.5	-	-	0.6
iso-$C_{17:0}$ 3OH	16.4	17.1	14.9	13.4	15.2
anteiso-$C_{15:0}$	0.7	0.7	0.4	0.7	0.8
Hydroxy fatty acids					
$C_{15:0}$ 2OH	-	0.6	2.9	0.6	-
$C_{16:0}$ 3OH	1.2	2.7	3.2	1.8	1.5
Summed feature*					
3; $C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$	14.4	8.4	9.8	10.8	10.3

*Summed features represent groups of two or three fatty acids that could not be separated by gas chromatography (GLC) with the MIDI system. Summed features consist of: 3, $C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$.

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