Niabella aurantiaca gen. nov., sp. nov., isolated from a greenhouse soil in Korea

Byung-Yong Kim,¹ Hang-Yeon Weon,² Seung-Hee Yoo,¹
Seung-Beom Hong,¹ Soon-Wo Kwon,¹ Erko Stackebrandt³
and Seung-Joo Go¹

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

¹Korean Agricultural Culture Collection (KACC), Microbial Genetics Division, National Institute of Agricultural Biotechnology, Rural Development Administration, Suwon 441-707, Korea

²Applied Microbiology Division, National Institute of Agricultural Science and Technology, Rural Development Administration, Suwon 441-707, Korea

³Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig, Germany

An orange-coloured bacterial strain, designated R2A15-11^T, was isolated from greenhouse soil. The strain was found to be strictly aerobic, Gram-negative, non-spore-forming and non-flagellated. The cells were short rods (0.7–0.9 × 1.0–1.5 µm) and produced flexirubin. Growth of the strain was observed at 10–35 °C, pH 5.0–8.0 and 0–3 % (w/v) NaCl. The predominant isoprenoid quinone was MK-7. The major fatty acids were 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} ω7c). The genomic DNA G+C content was 45.0 mol%. Phylogenetic analysis of the 16S rRNA gene sequence of strain R2A15-11^T revealed a clear affiliation with the phylum *Bacteroidetes*, and the highest levels of sequence similarity were found with respect to *Terrimonas ferruginea* ATCC 13524^T (91.5 %), *Terrimonas lutea* DY^T (90.2 %), *Niastella yeongjuensis* GR20-13^T (89.9 %) and *Niastella koreensis* GR20-10^T (89.7 %). On the basis of the polyphasic evidence from this study, strain R2A15-11^T represents a novel genus and species, for which the name *Niabella aurantiaca* gen. nov., sp. nov. is proposed. The type strain of *Niabella aurantiaca* is R2A15-11^T (=KACC 11698^T=DSM 17617^T).

Although the taxonomic relationships among species within the genera *Flavobacterium*, *Flexibacter* and *Cytophaga* have been largely resolved through phylogenetic analysis (Sly *et al.*, 1999; Nakagawa & Yamasato, 1993; Nakagawa *et al.*, 2002), these genera remain taxonomically heterogeneous. The genus *Terrimonas* (Xie & Yokota, 2006) was recently proposed for the misclassified species *Flavobacterium ferrugineum* and a novel garden-soil isolate. A second recently described genus, *Niastella*, was created for isolates from Korean ginseng fields (Weon *et al.*, 2006).

We isolated a bacterial strain, designated R2A15-11^T, from greenhouse soil, and found it to be related to members of the genera *Terrimonas* and *Niastella*. The soil sample was obtained from a greenhouse cultivated with lettuce (*Lactuca sativa* L.) in the Yeosu region of Korea. The sample was serially diluted with 0.85 % NaCl (w/v) and the dilutions

plated onto R2A agar (Difco). The strain was isolated after incubation for 4 days at 28 °C.

The cell morphology of cells grown on R2A for 2 days was examined by transmission electron microscopy (Fig. 1), using cells negatively stained with 0.5 % uranyl acetate. Gliding motility was investigated by using oil-immersion phase-contrast microscopy of the edges of colonies when the cells were in the exponential phase of growth. Growth at different temperatures and pHs was tested at 5–45 °C (in increments of 5 °C) and pH 4–10 (in increments of 1 pH unit), respectively. Salt tolerance was tested on R2A broth supplemented with 0, 1, 2, 3, 5 and 7 % (w/v) NaCl. Gram staining, catalase activity, cytochrome oxidase activity and the hydrolysis of alginate acid, carboxymethylcellulose, casein, chitin (from crab shells), DNA, starch and tyrosine were investigated according to the methods of Smibert & Krieg (1994). Flexirubin pigment was detected by means of a colour shift after exposure to a 20 % (w/v) KOH solution (Reichenbach, 1992). Other biochemical tests were carried out by using API 20NE, API ID 32 GN and API ZYM test kits according to the instructions of the manufacturer (bioMérieux).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain R2A15-11^T is DQ457019.

The cellular fatty acid compositions of strain R2A15-11^T and related strains are available in a supplementary table in IJSEM Online.

Fig. 1. Transmission electron micrograph of cells of strain R2A15-11^T. Bar, 2 μ m.

After cultivation of R2A15-11^T on R2A at 28 °C for 48 h, the whole-cell fatty acids were analysed according to Sasser (1990). Isoprenoid quinones were examined according to Groth *et al.* (1996). The G+C content of the DNA was determined as described by Mesbah *et al.* (1989), using a reversed-phase column (Supelcosil LC-18-S; Supelco).

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and sequencing of PCR products were carried out as described by Kwon *et al.* (2003). The 16S

rRNA gene sequences were aligned using the CLUSTAL W program (Thompson *et al.*, 1994). A phylogenetic analysis was performed using the software package MEGA (version 3.1) (Kumar *et al.*, 2004). Distances (using distance options according to the Kimura two-parameter model) and clustering (using the neighbour-joining method) were determined by using bootstrap values based on 1000 replicates.

The cultural and metabolic properties of strain R2A15-11^T are indicated in Table 1 and in the genus and species descriptions. Analysis of quinone compounds revealed MK-7 to be the major isoprenoid quinone. The cellular fatty acids of R2A15-11^T were iso-C_{15:0} (33.7 %), iso-C_{15:1} G (22.3 %), iso-C_{17:0} 3-OH (15.5 %), summed feature 3 (10.6 %; comprising iso-C_{15:0} 2-OH and/or C_{16:1} ω 7c), C_{16:0} (3.5 %), iso-C_{15:0} 3-OH (2.9 %), C_{16:0} 3-OH (2.4 %), anteiso-C_{15:0} (1.6 %) and an unknown fatty acid (1.2 %; equivalent chain-length 16.582). The DNA G+C content of strain R2A15-11^T was 45.0 mol%.

The predominance of MK-7 and the large amounts of fatty acids 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} ω 7c) in strain R2A15-11^T are features similar to those of members of the genera *Terrimonas* (Xie & Yokota, 2006; Weon *et al.*, 2006) and *Niastella* (Weon *et al.*, 2006) (see Supplementary Table S1, available in IJSEM Online). The DNA G+C content (45.0 mol%) falls within the range (44.3–48.9 mol%) encompassed by the members of the genera *Terrimonas* and *Niastella*.

Table 1. Phenotypic comparisons among R2A15-11^T and related strains

Strains: 1, R2A15-11^T; 2, *T. ferruginea* IAM 15098^T (data from Xie & Yokota, 2006; Weon *et al.*, 2006); 3, *T. lutea* IAM 15284^T (Xie & Yokota, 2006); 4, *Niastella koreensis* KACC 11465^T (Weon *et al.*, 2006); 5, *Niastella yeongjuensis* KACC 11466^T (Weon *et al.*, 2006). +, Positive; –, negative; w, weakly positive; ND, not determined. All strains are positive for gelatin hydrolysis, negative for urea hydrolysis and possess MK-7 as the major menaquinone.

Characteristic	1	2	3	4	5
Cell size (μ m)	0.7–0.9 $\times$ 1.0–1.5	0.3–0.5 $\times$ 1.0–3.0	0.3–0.5 $\times$ 1.0–3.0	0.4–0.5 $\times$ 10–50	0.4–0.6 $\times$ 10–40
Catalase/oxidase	+ / –	w / +	w / +	– / –	– / +
Flexirubin reaction	+	–*	ND	–	–
Gliding motility	–	–	–	+	+
Colony colour	Orange	Salmon red	Yellow	Light yellow	Milky
Nitrate reduction	–	+	+	–	–
Indole production	+	–	–	–	–
Highest NaCl concentration tolerated (%)	3.0	1.0	1.0	ND	ND
Hydrolysis of:					
Starch	–	+*	ND	–	–
Chitin	–	–	–	+	+
Carboxymethylcellulose	–	–*	ND	+	+
Casein	+	–*	ND	+	+
DNA G+C content (mol%)	45.0	48.9	47.2	45.8	44.3

*Data from Weon *et al.* (2006).

The sequence analyses confirmed the affiliation of the novel isolate with the members of the phylum *Bacteroidetes* (Fig. 2). Strain R2A15-11^T is related to members of the genera *Terrimonas* and *Niastella*. The 16S rRNA gene sequence similarity values for strain R2A15-11^T with respect to *Terrimonas ferruginea* ATCC 13524^T, *Terrimonas lutea* DY^T, *Niastella yeongjuensis* GR20-13^T and *Niastella koreensis* GR20-10^T were 91.5, 90.2, 89.9 and 89.7 %, respectively. The highest level of sequence similarity (97.6 %) for strain R2A15-11^T was obtained with respect to a cloned 16S rRNA gene sequence derived from an activated sludge with an enhanced biological phosphate removal capacity (clone Ebpr2; Liu *et al.*, 2001). All of the other species (with validly published names) showed sequence similarity levels below 89 %.

In terms of physiological and biochemical properties, strain R2A15-11^T differed from members of the genera *Terrimonas* and *Niastella* in being able to produce catalase, flexirubin and indole. Furthermore, strain R2A15-11^T can be differentiated from members of the genus *Terrimonas* on the basis of the tolerance of higher NaCl concentrations and the absence of oxidase and nitrate reduction. In addition, strain R2A15-11^T can be distinguished from *Niastella* species by the absence of gliding motility, by differences in cellular morphology and by the inability to hydrolyse chitin and cellulose. The characteristics that differentiate strain R2A15-11^T from other closely related species are listed in Table 1.

On the basis of the results of this polyphasic approach, strain R2A15-11^T represents a novel genus and species, for which the name *Niabella aurantiaca* gen. nov., sp. nov. is proposed.

Description of *Niabella* gen. nov.

Niabella (Ni.a.bel'la. L.L. dim. suff. -ella; N.L. fem. n. *Niabella* arbitrary name, after NIAB, National Institute of Agricultural Biotechnology, where taxonomic studies of this taxon were conducted).

Cells are Gram-negative, strictly aerobic, non-flagellated, non-spore-forming, short rods. When grown on R2A plates, colonies are orange and circular. Catalase-positive and oxidase-negative. Gliding motility is not observed.

Flexirubin pigment is produced. The predominant isoprenoid quinone is MK-7. The major fatty acids are 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}ω7c). Phylogenetically, the genus is a member of the phylum *Bacteroidetes*. The type species is *Niabella aurantiaca*.

Description of *Niabella aurantiaca* sp. nov.

Niabella aurantiaca (au.ran.ti.a'ca. N.L. fem. adj. *aurantiaca* orange-coloured).

In addition to the properties described above for the genus, the following properties are observed. Cells are approximately 0.7–0.9 µm wide and 1.0–1.5 µm long. Growth occurs with NaCl concentrations in the range 0–3 % (w/v) (optimum, 1–2 %), at temperatures between 10 and 35 °C (optimum, 25–30 °C) and at pH 5.0–8.0 (optimum, pH 6–7). Casein and tyrosine are hydrolysed. Alginate, chitin, carboxymethylcellulose, DNA and starch are not hydrolysed. Positive for indole production, aesculin hydrolysis and β-galactosidase, but negative for nitrate reduction, glucose fermentation, arginine dihydrolase, urease and gelatin hydrolysis (API 20NE). Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities, but negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin and β-glucuronidase activities (API ZYM). Assimilates D-glucose, L-arabinose, D-mannose, N-acetylglucosamine, D-maltose, L-rhamnose, sucrose, salicin and D-melibiose (API 20NE and API ID 32 GN). Does not assimilate D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, D-ribose, inositol, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, L-fucose, D-sorbitol, propionic acid, valeric acid, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid or L-proline (API 20NE and API ID 32 GN). The DNA G + C content of the type strain is 45.0 mol% (HPLC).

Fig. 2. Neighbour-joining phylogenetic tree for strain R2A15-11^T and related strains, based on almost-complete 16S rRNA gene sequences. Numbers at nodes indicate percentages of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets. Bar, 2 substitutions per 100 nt.

The type strain, R2A15-11^T (=KACC 11698^T=DSM 17617^T), was isolated from greenhouse soil in Yeosu Region, Republic of Korea.

Acknowledgements

This study was supported by a programme of international collaborative research between the Rural Development Administration (Suwon, Republic of Korea) and the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany).

References

- Groth, I., Schumann, P., Weiss, N., Martin, K. & Rainey, F. A. (1996). *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. *Int J Syst Bacteriol* **46**, 234–239.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Kwon, S. W., Kim, J. S., Park, I. C., Yoon, S. H., Park, D. H., Lim, C. K. & Go, S. J. (2003). *Pseudomonas koreensis* sp. nov., *Pseudomonas umsongensis* sp. nov. and *Pseudomonas jinjuensis* sp. nov., novel species from farm soils in Korea. *Int J Syst Evol Microbiol* **53**, 21–27.
- Liu, W. T., Nielsen, A. T., Wu, J. H., Tsai, C. S., Matsuo, Y. & Molin, S. (2001). In situ identification of polyphosphate- and polyhydroxyalkanoate-accumulating traits for microbial populations in a biological phosphorus removal process. *Environ Microbiol* **3**, 110–122.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Nakagawa, Y. & Yamasato, K. (1993). Phylogenetic diversity of the genus *Cytophaga* revealed by 16S rRNA sequencing and menaquinone analysis. *J Gen Microbiol* **139**, 1155–1161.
- Nakagawa, Y., Sakane, T., Suzuki, M. & Hatano, K. (2002). Phylogenetic structure of the genera *Flexibacter*, *Flexithrix*, and *Microscilla* deduced from 16S rRNA sequence analysis. *J Gen Appl Microbiol* **48**, 155–165.
- Reichenbach, H. (1992). The order Cytophagales. In *The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications*, 2nd edn, pp. 3631–3675. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.
- Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC Newsl* **20**, 1–6.
- Sly, L. I., Taghavi, M. & Fegan, M. (1999). Phylogenetic position of *Chitinophaga pinensis* in the *Flexibacter*–*Bacteroides*–*Cytophaga* phylum. *Int J Syst Bacteriol* **49**, 479–481.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Weon, H. Y., Kim, B.-Y., Yoo, S.-H., Lee, S.-Y., Kwon, S.-W., Go, S.-J. & Stackebrandt, E. (2006). *Niastella koreensis* gen. nov., sp. nov. and *Niastella yeongjuensis* sp. nov., novel members of the phylum *Bacteroidetes*, isolated from soil cultivated with Korean ginseng. *Int J Syst Evol Microbiol* **56**, 1777–1782.
- Xie, C. H. & Yokota, A. (2006). Reclassification of [*Flavobacterium*] *ferrugineum* as *Terrimonas ferruginea* gen. nov., comb. nov., and description of *Terrimonas lutea* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **56**, 1117–1121.