Chitinimonas koreensis sp. nov., isolated from greenhouse soil in Korea

Byung-Yong Kim,¹ Hang-Yeon Weon,² Seung-Hee Yoo,¹
Wen-Ming Chen,³ Soon-Wo Kwon,¹ Seung-Joo Go¹
and Erko Stackebrandt⁴

Correspondence

Soon-Wo Kwon
swkwon@rda.go.kr

^{1,2}Korean Agricultural Culture Collection (KACC), Microbial Genetics Division, National Institute of Agricultural Biotechnology¹ and Applied Microbiology Division, National Institute of Agricultural Science and Technology², Rural Development Administration, Suwon, Republic of Korea

³Department of Seafood Science, National Kaohsiung Marine University, Kaohsiung, Taiwan

⁴Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig, Germany

Strain R2A43-10^T was isolated from a greenhouse soil in Korea. Cells were Gram-negative rods, motile by means of a single flagellum. Growth occurred at 10–40 °C and at pH 5–8. Ubiquinone-8 (Q-8) was the only respiratory lipoquinone. Major fatty acids were summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) and C_{16:0}. 16S rRNA gene sequence analysis revealed that strain R2A43-10^T was closely related to *Chitinimonas taiwanensis* cf^T (sequence similarity of 94.8%), but it exhibited low sequence similarities (<92%) to other members of the *Betaproteobacteria*. The G + C content of the genomic DNA of strain R2A43-10^T was 65.0 mol%. The novel isolate could be differentiated from *C. taiwanensis* cf^T by several physiological properties. On the basis of genomic and phenotypic data, it is concluded that R2A43-10^T (=KACC 11467^T=DSM 17726^T) is the type strain of a novel species of the genus *Chitinimonas*, for which the name *Chitinimonas koreensis* sp. nov. is proposed.

The genus *Chitinimonas* (Chang *et al.*, 2004) was classified as a distinct subline within the *Betaproteobacteria*, showing a distant relatedness to members of the genera *Propionivibrio*, *Burkholderia*, *Pandoraea* and others. At the time of writing, the genus comprised only one species, *Chitinimonas taiwanensis*, which was characterized as a Gram-negative, flagellated, chitinolytic, rod-shaped bacterium.

A single strain, designated R2A43-10^T, was isolated from greenhouse soil in Korea. The strain was purified by streaking the soil sample on R2A medium (Reasoner & Geldreich, 1985). The morphological, physiological and biochemical characteristics of strain R2A43-10^T were investigated using routine cultivation on R2A medium at 28 °C. Cell morphology was examined by light microscopy (AXIO; Zeiss) and transmission electron microscopy. Chitinolytic activity was characterized by using the method of Chang *et al.* (2004). Gram staining, catalase and oxidase activities, production of poly-β-hydroxybutyrate and hydrolysis of agar, casein, DNA, gelatin and starch were determined as described by Smibert & Krieg

(1994). Growth was assessed at 5, 10, 20, 25, 30, 37, 40 and 45 °C, at pH 4, 5, 6, 7, 8, 9 and 10 and at 0, 1, 3, 5 and 7 % NaCl. Hydrolysis of carboxymethylcellulose (Sigma) (0.1 %) and tyrosine (0.5 %, w/v) was also tested. The strain was additionally characterized by using the whole test spectrum of the API 20NE, API ID 32 GN and API ZYM systems (bioMérieux) according to the manufacturer's instructions.

Cells were harvested after 24 h growth on R2A medium and the identification of fatty acids was performed by use of the standard protocol of the Microbial Identification System (MIDI; Microbial ID). Isoprenoid quinones were analysed by HPLC as described by Groth *et al.* (1996). The DNA G + C content was determined by using an HPLC-based method as described by Mesbah *et al.* (1989).

The 16S rRNA gene sequence of strain R2A43-10^T was analysed as described by Kwon *et al.* (2003). The sequence was aligned together with those of representative members of selected genera by using the CLUSTAL W program (Thompson *et al.*, 1994). A phylogenetic analysis was performed using the software package MEGA (Molecular Evolutionary Genetics Analysis) version 3.1 (Kumar *et al.*, 2004). Distances (using distance options according to the Kimura two-parameter model) and clustering using the

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain R2A43-10^T is DQ256728.

An HPLC profile of chitin-digested products of strain R2A43-10^T is available as a supplementary figure in IJSEM Online.

Fig. 1. Transmission electron micrograph of cells of strain R2A43-10^T, each with a single flagellum. Bar, 500 nm.

neighbour-joining and maximum-parsimony (Kumar *et al.*, 2004) methods were determined by using bootstrap values based on 1000 replicates.

Colonies of strain R2A43-10^T were irregular and milky coloured on R2A medium. The strain grew well on chitin basal medium (Chang *et al.*, 2004), R2A and nutrient agar (NA; Difco), but did not grow on trypticase soy agar (TSA; Difco) or MacConkey agar (Difco). Cells were Gram-negative, rod-shaped (0.7–0.8 by 1.6–3.8 µm) and motile by means of a single polar flagellum (Fig. 1).

Phylogenetic analysis of the 16S rRNA gene sequence of strain R2A43-10^T (Fig. 2) revealed that it was related most closely to *C. taiwanensis* cf^T (94.8 % sequence similarity). Sequence similarities were below 92 % to the type strains of other members of the *Betaproteobacteria*. The neighbour-joining (Fig. 2) and maximum-parsimony methods placed strains R2A43-10^T and *C. taiwanensis* cf^T as phylogenetic neighbours within the *Betaproteobacteria*. The branching pattern was strongly supported by bootstrap analysis. This result was supported by other trees which included additional type strains of a diverse range of genera of the *Betaproteobacteria* (data not shown).

Analysis of the respiratory quinones of strain R2A43-10^T gave only one characteristic peak, corresponding to ubiquinone Q-8. Cellular fatty acid analysis revealed that

summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) was the most abundant component (39.5 %), followed by C_{16:0} (33.8 %) and C_{18:1}ω7c (4.6 %). The fatty acid profile of R2A43-10^T was similar to that of *C. taiwanensis* cf^T except that the latter strain contained C_{12:0} (3.7 %; absent from strain R2A43-10^T) while strain R2A43-10^T contained C_{16:0} 2-OH (3.0 %; absent from *C. taiwanensis* cf^T) (Table 1). The G + C content of its DNA was 65.0 mol%.

The physiological and biochemical characteristics of strain R2A43-10^T and *C. taiwanensis* cf^T are compared in Table 1. Strain R2A43-10^T differs clearly from *C. taiwanensis* cf^T on the basis of a positive reaction for gelatin hydrolysis, negative reactions for urea and aesculin hydrolysis, and the presence of C_{16:0} 2-OH in the fatty acid profile. In the chitinolytic activity test, *C. taiwanensis* cf^T produced chitotriose [(GlcNAc)₃] as the major product of chitin cleavage. Isolate R2A43-10^T, by contrast, produced mono-(GlcNAc) and disaccharides [(GlcNAc)₂] as the major products and only a small amount of chitotriose (see Supplementary Fig. S1 in IJSEM Online).

On the basis of the polyphasic data presented, strain R2A43-10^T is considered to represent a novel species of the genus *Chitinimonas*, for which the name *Chitinimonas koreensis* sp. nov. is proposed.

Emended description of the genus *Chitinimonas* Chang *et al.* 2004

The description of the genus *Chitinimonas* is as given by Chang *et al.* (2004) except that members of the genus are negative for indole production and glucose fermentation and may or may not hydrolyse aesculin, gelatin and urea.

Description of *Chitinimonas koreensis* sp. nov.

Chitinimonas koreensis (ko.re.en'sis. N.L. fem. adj. *koreensis* of Korea, where the type strain was isolated).

Cells are Gram-negative rods, motile by a single flagellum. Colonies are irregular, and no diffusible pigments are produced. Growth occurs at 10–40 °C, 0–1 % NaCl and pH 5–8. Poly-β-hydroxybutyrate is produced. Positive for catalase and oxidase, and hydrolysis of casein, chitin, gelatin, tyrosine and starch. Negative for hydrolysis of DNA, carboxymethylcellulose and urea. Other phenotypic properties are listed in Table 1. Ubiquinone-8 (Q-8) is the only respiratory lipoquinone. Major fatty acids are summed

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences (*Escherichia coli* positions 50–1483) of strain R2A43-10^T and its closest relatives. A sequence from *E. coli* (strain unknown) was used as the outgroup to root the tree. Numbers at nodes indicate levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets. Bar, 2 substitutions per 100 nt.

Table 1. Phenotypic comparison between strain R2A43-10^T and *C. taiwanensis* cf^T

Data for *C. taiwanensis* cf^T were taken from Chang *et al.* (2004). According to API 20NE and API ZYM test strips, both strains are positive for nitrate reduction, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α -galactosidase and negative for indole production, glucose fermentation, arginine dihydrolase, β -glucuronidase, α -glucosidase, β -glucosidase, α -mannosidase and α -fucosidase. According to API 20NE and API ID 32 GN test strips, both strains are positive for D-glucose, D-maltose, glycogen, valeric acid, 3-hydroxybutyric acid and L-proline and negative for assimilation of L-arabinose, potassium gluconate, capric acid, adipic acid, trisodium citrate, phenylacetic acid, L-rhamnose, D-ribose, inositol, itaconic acid, suberic acid, sodium malonate, sodium acetate, L-alanine, potassium 5-ketogluconate, 3-hydroxybenzoic acid, salicin, D-melibiose, L-fucose, propionic acid, potassium 2-ketogluconate and 4-hydroxybenzoic acid. +, Positive; –, negative; ND, not detected.

Characteristic	R2A43-10 ^T	<i>C. taiwanensis</i> cf ^T
Major products of chitin cleavage	GlcNAc, (GlcNAc) ₂	(GlcNAc) ₃
API 20NE and API ZYM strip results		
Urease	–	+
Aesculin hydrolysis	–	+
Gelatin hydrolysis	+	–
β -Galactosidase	–	+
Alkaline phosphatase	+	–
Lipase (C14)	–	+
β -Galactosidase	–	+
Alkaline phosphatase	+	–
Valine arylamidase	–	+
Cystine arylamidase	–	+
Trypsin	–	+
α -Chymotrypsin	–	+
N-Acetyl- β -glucosaminidase	–	+
Assimilation according to API 20NE and API ID32 GN strips		
D-Mannose	+	–
D-Mannitol	–	+
N-Acetylglucosamine	–	+
Malic acid	+	–
Sucrose	+	–
Lactic acid	–	+
L-Serine	+	–
D-Sorbitol	–	+
L-Histidine	+	–
Fatty acids (% of total)*		
C _{12:0}	ND	3.7
C _{12:0} 2-OH	3.3	1.8
C _{14:0}	1.7	1.3
C _{16:0}	33.8	29.2
C _{16:0} 2-OH	3.0	ND
C _{16:1} ω 5c	ND	0.6
C _{18:1} ω 7c	4.6	11.6
C _{18:0}	1.9	0.6
Summed feature 3†	39.5	49.4
DNA G+C content (mol%)	65.0	62.8

*Fatty acids that account for <0.5 % of the total are not shown.

†Summed feature 3 consisted of C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH.

feature 3 (C_{16:1ω7c} and/or iso-C_{15:0} 2-OH) and C_{16:0}. The G+C content of the genomic DNA is 65 mol%.

The type strain, R2A43-10^T (=KACC 11467^T=DSM 17726^T), was isolated from greenhouse soil cultivated with cucumber in Sangju Province, Korea.

References

- Chang, S. C., Wang, J. T., Vandamme, P., Hwang, J. H., Chang, P. S. & Chen, W. M. (2004). *Chitinimonas taiwanensis* gen. nov., sp. nov., a novel chitinolytic bacterium isolated from a freshwater pond for shrimp culture. *Syst Appl Microbiol* **27**, 43–49.
- 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.
- 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.
- Reasoner, D. J. & Geldreich, E. E. (1985). A new medium for the enumeration and subculture of bacteria from potable water. *Appl Environ Microbiol* **49**, 1–7.
- 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.