Ureibacillus composti sp. nov. and *Ureibacillus thermophilus* sp. nov., isolated from livestock-manure composts

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Two Gram-negative, rod-shaped, thermophilic bacterial strains, HC145^T and HC148^T, were isolated from a compost sample from a compost facility in Ichon, Korea. Sequencing of the 16S rRNA genes of HC145^T and HC148^T and comparative analyses of the resulting sequences clearly showed that these strains had a phylogenetic affiliation to the genus *Ureibacillus*. The level of 16S rRNA similarity between the two novel strains was 98.4 % and the levels of sequence similarity between them and existing *Ureibacillus* species were 97.8–98.1 (HC145^T) and 97.4–98.7 % (HC148^T). The DNA–DNA reassociation values between the two strains and the type strains of *Ureibacillus* species ranged from 38 to 51 %. The polar lipid profiles for both isolates consisted of phosphatidylglycerol, diphosphatidylglycerol, phospholipids and glycolipids of unknown composition. The major quinones were MK-8, MK-9 and MK-7, the peptidoglycan type was L-Lys←D-Asp and the main cellular fatty acid was iso-C_{16:0}. The DNA G + C contents of strains HC145^T and HC148^T were 42.4 and 38.5 mol%, respectively. On the basis of the data from this polyphasic study, strains HC145^T and HC148^T represent members of the genus *Ureibacillus*, for which the names *Ureibacillus composti* sp. nov. and *Ureibacillus thermophilus* sp. nov., respectively, are proposed. The type strain of *U. composti* is HC145^T (=KACC 11361^T =DSM 17951^T) and the type strain of *U. thermophilus* is HC148^T (=KACC 11362^T =DSM 17952^T).

Members of *Ureibacillus*, a genus comprising thermophilic, aerobic, endospore-forming bacteria, have been isolated from air (*Ureibacillus thermosphaericus*; Andersson *et al.*, 1995), soil (*Ureibacillus terrenus*; Fortina *et al.*, 2001) and composts (*Ureibacillus suwonensis*; Kim *et al.*, 2006). The temperature range for growth for members of this genus is 35–65 °C, the optimum being 50–60 °C (Kim *et al.*, 2006). Composting is a self-heating, aerobic, solid-phase, biodegradative process occurring in organic waste materials (de Bertoldi *et al.*, 1983; Finstein & Morris, 1975). During the thermogenic phase of the composting process, the

temperature usually rises to 65–80 °C for a certain period of time. Thus, hot compost is considered to represent a favourable habitat for thermophilic strains. During the characterization of thermophilic bacteria isolated from livestock-manure composts, strains HC145^T and HC148^T were recovered on trypticase soy agar at pH 7.0 and incubated at 55 °C. Strains HC145^T and HC148^T formed round, brown, convex colonies on both trypticase soy agar and nutrient agar (Oxoid). Gram staining was performed using a Difco Gram-stain kit according to the manufacturer's recommended protocol. A KOH test and an L-alanine aminopeptidase assay were also performed (Gregersen, 1978). Cell morphology was observed under a phase-contrast microscope after 2 days incubation on CASO agar (DSMZ medium no. 220; <http://www.dsmz.de/>

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strain HC145^T and strain HC148^T are DQ348071 and DQ348072, respectively.

media/media.htm) supplemented with MnSO_4 at 10–100 mg l^{-1} . The Voges–Proskauer test and tests for the following physiological traits were carried out according to Gordon *et al.* (1973) and Claus & Berkeley (1986): catalase, anaerobic growth, temperature range for growth (30–70 °C, in increments of 5 °C), growth in the presence of NaCl (0, 2, 5, 7 and 10 %, w/v), growth at pH 4–10, acids from carbohydrates (D-glucose, L-arabinose, D-xylose and D-mannitol), formation of gas from glucose, hydrolysis of starch, nitrate reduction, production of indole, deamination of phenylalanine, decomposition of casein and tyrosine and liquefaction of gelatin. The oxidase test and the determination of aesculin hydrolysis were conducted according to Smibert & Krieg (1994). Motility tests were performed on 1/10-strength CESP agar (1.5 g Casitone, 0.5 g yeast extract, 0.3 g soytone, 0.2 g peptone, 0.015 g MgSO_4 , 0.007 g FeCl_2 and 0.002 g MnCl_2 , made up to 1 l with distilled water; pH 7.2) (Fortina *et al.*, 2001). Strain

HC148^T did not form spores on CASO agar medium supplemented with MnSO_4 at 10–100 mg l^{-1} , unlike other strains within the genus *Ureibacillus*. The physiological and biochemical properties of strains HC145^T and HC148^T and the three existing species within the genus *Ureibacillus* are shown in Table 1.

The 16S rRNA gene sequences were determined by using PCR amplification (Kwon *et al.*, 2003). The sequences were aligned together with those of the type strains of the genus *Ureibacillus* by using the CLUSTAL W program (Thompson *et al.*, 1994). The evolutionary tree for the dataset was inferred with the neighbour-joining and maximum-parsimony methods, using MEGA 3.1 (Kumar *et al.*, 2004). The stability of relationships was assessed by performing bootstrap analyses based on 1000 resamplings. Phylogenetic analyses based on 16S rRNA gene sequences confirmed that strains HC145^T and HC148^T are members

Table 1. Phenotypic characteristics of *Ureibacillus* species

Taxa: 1, strain HC145^T; 2, strain HC148^T; 3, *U. thermosphaericus* (unless indicated, data from Fortina *et al.*, 2001); 4, *U. terrenus* (Fortina *et al.*, 2001); 5, *U. suwonensis* (Kim *et al.*, 2006). For the fatty acid analyses carried out in this study, cells for all strains were harvested after growth on R2A agar at 50 °C for 2 days. All strains were positive for catalase, oxidase and the deamination of phenylalanine. All strains gave negative results in the KOH assay, the L-alanine aminopeptidase assay and for the hydrolysis of casein, gelatin, starch and urease. +, Positive; –, negative or not detected (or <1 % in fatty acid compositions); v, variable; ND, not determined.

Characteristic	1	2	3	4	5
Isolation source	Compost	Compost	Air	Soil	Compost
Spore shape*	s	ND	s	s	s/o
Growth at/with:					
35 °C	–	+	–	–	+
65 °C	–	+	–	+	–
5 % NaCl	+	+	+	v	+
Hydrolysis of:					
Aesculin	+	+	+	+	–
Tyrosine	–	+	–	+	–
Fatty acids (%):†					
iso-C _{14:0}	1.5	2.0	4.7	–	–
iso-C _{15:0}	2.9	4.0	10.2	1.4	–
anteiso-C _{15:0}	–	1.1	–	–	–
C _{16:0}	5.4	3.7	8.1	7.9	6.4
C _{16:1} ω7c alcohol	7.2	8.4	3.0	4.3	8.5
C _{16:1} ω11c	1.0	2.0	1.2	2.9	2.0
iso-C _{16:0}	72.2	60.0	68.4	67.5	77.3
C _{17:0}	–	1.8	–	2.1	–
iso-C _{17:0}	2.3	5.9	2.5	5.5	1.2
anteiso-C _{17:0}	2.7	6.8	–	3.4	–
iso-C _{18:0}	1.0	1.0	–	2.7	1.0
Summed feature 4‡	–	2.6	–	–	–
Major menaquinone(s) (%)	MK-7, MK-8, MK-9, MK-10 (50:40:10:1)	MK-8, MK-9, MK-7, MK-10 (43:35:21:2)	MK-7 (>90)	MK-9, MK-8, MK-10, MK-7, MK-11 (58:22:14:1:1)	MK-9, MK-8, MK-7, MK-10, MK-6 (45:27:18:5:4)
DNA G + C content (mol%)	42.4	38.5	35.7–39.2	39.6–41.5	41.5

*s, Spherical; o, oval.

†Data were obtained from this study, using type strains of *Ureibacillus* species.

‡Comprises iso-C_{17:1} I and/or anteiso-C_{17:1} B.

of the genus *Ureibacillus* (Fig. 1). The level of 16S rRNA gene sequence similarity between strains HC145^T and HC148^T was 98.4% and the levels of similarity between strains HC145^T and HC148^T and the type strains of other *Ureibacillus* species were in the ranges 97.8–98.1% and 97.4–98.7%, respectively. Strains HC145^T and HC148^T and the type strains of the three existing species within the genus *Ureibacillus* were clustered into one clade with a high level of bootstrap support (100%). This was also supported by the tree constructed using the maximum-parsimony method.

DNA–DNA hybridization was carried out by using the filter hybridization method described by Seldin & Dubnau (1985). Probe labelling was conducted using the non-radioactive DIG-High Prime system (Roche) and DNA–DNA relatedness was quantified using a densitometer (Bio-Rad). The DNA relatedness between strains HC145^T and HC148^T was 42%. Strains HC145^T and HC148^T exhibited only 42–51 and 38–45% relatedness, respectively, to the type strains of the three recognized species.

DNA G+C contents were determined by means of HPLC analysis of deoxyribonucleosides, as described by Mesbah *et al.* (1989), using a reversed-phase column (Supelcosil LC-18 S; Supelco). The preparation of cell walls and the determination of peptidoglycan structure was carried out at the DSMZ as described by Schleifer (1985) and Schleifer & Kandler (1972) except that TLC on cellulose was used instead of paper chromatography. Polar lipid analyses were carried out by the Identification Service of the DSMZ (Tindall, 1990). After growth of the cells on R2A for 2 days at 50 °C, fatty acid methyl esters were extracted and prepared using the standard protocol of the Microbial Identification system (MIDI; Microbial ID). The DNA G+C content of strain HC148^T was 38.5 mol%, which lies within the range observed for members of the genus *Ureibacillus*, while the content of HC145^T was 42.4 mol%, which is a little higher than those reported previously for *Ureibacillus* type strains (35.7–41.5 mol%). Fatty acid profiles for members of the genus *Ureibacillus* (Table 1) revealed that all of the strains contained iso-C_{16:0} as the

predominant fatty acid (60.0–77.3%). The minor fatty acid compositions among the strains were found to be very variable. Strain HC148^T was unique in containing summed feature 4 (iso-C_{17:1} I and/or anteiso-C_{17:1} B; 2.6%). The peptidoglycan cross-linkage in the two novel strains was of the L-Lys←D-Asp type (variation A4α). The polar lipids consisted of phosphatidylglycerol, diphosphatidylglycerol, phospholipids and glycolipids of unknown composition. These results were in accordance with those given in the description of the genus *Ureibacillus* (Fortina *et al.*, 2001).

Phenotypic features that serve to distinguish strains HC145^T and HC148^T from recognized *Ureibacillus* species are shown in Table 1. Strains HC145^T and HC148^T could be differentiated from each other on the basis of the following features: temperature range for growth, tyrosine hydrolysis and DNA G+C content. The two novel strains can be distinguished from recognized *Ureibacillus* strains on the basis of several phenotypic properties as well as some chemotaxonomic properties such as fatty acid composition and menaquinone content (Table 1). Furthermore, the DNA–DNA reassociation values (below 70%) and the 16S rRNA gene sequence analysis confirmed the distinct positions of strains HC145^T and HC148^T within the genus *Ureibacillus*.

On the basis of the data from this polyphasic taxonomic study, strains HC145^T and HC148^T represent two novel species of the genus *Ureibacillus*, for which we propose the names *Ureibacillus composti* sp. nov. and *Ureibacillus thermophilus* sp. nov., respectively.

Description of *Ureibacillus composti* sp. nov.

Ureibacillus composti (com.pos'ti. N.L. gen. n. *composti* of compost).

Cells are Gram-negative, motile, rod-shaped bacteria, 0.7–0.9 µm wide and 2.5–4.0 µm long. They bear spherical endospores that lie in subterminal or terminal positions. Round, light-brown, convex colonies are formed. Grows at 37–60 °C and pH 6–8. Tolerates up to 5% NaCl (w/v). Aesculin is hydrolysed. Casein, gelatin, tyrosine, starch and urea are not hydrolysed. Positive for catalase, oxidase and deamination of phenylalanine. Negative for anaerobic growth, glucose fermentation, indole formation, nitrate reduction, acid production from D-glucose, L-arabinose, D-xylose and D-mannitol and in the Voges–Proskauer test. Peptidoglycan cross-linkage is of the L-Lys←D-Asp type (variation A4α). The major cellular fatty acid is iso-C_{16:0}. The major quinones are MK-7, MK-8 and MK-9. Polar lipids consist of phosphatidylglycerol, diphosphatidylglycerol, phospholipids and glycolipids of unknown composition. The DNA G+C content of the type strain is 42.4 mol%.

The type strain, HC145^T (=KACC 11361^T=DSM 17951^T), was isolated from livestock-manure compost from Ichon, Korea.

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of HC145^T and HC148^T and closely related species. Numbers at nodes indicate percentages of bootstrap support, based on a neighbour-joining analysis of 1000 resampled datasets. Bootstrap values below 50% are not indicated. Bar, 0.01 substitutions per nucleotide position.

Description of *Ureibacillus thermophilus* sp. nov.

Ureibacillus thermophilus (ther.mo'phi.us. Gr. adj. *thermos* hot; Gr. adj. *philos* loving; N.L. masc. adj. *thermophilus* heat-loving).

Cells are Gram-negative, motile, rod-shaped bacteria, 0.8–1.2 µm wide and 2.5–3.5 µm long. Round, light-brown, convex colonies are formed. Grows at 30–65 °C and pH 6–8. Tolerates up to 5 % NaCl (w/v). Aesculin and tyrosine are hydrolysed. Casein, gelatin, starch and urea are not hydrolysed. Positive for catalase, oxidase and deamination of phenylalanine. Negative for anaerobic growth, glucose fermentation, indole formation, nitrate reduction, acid production from D-glucose, L-arabinose, D-xylose and D-mannitol and in the Voges–Proskauer test. Peptidoglycan cross-linkage is of the L-Lys←D-Asp type (variation A4α). The major cellular fatty acid is iso-C_{16:0}. The major quinones are MK-8, MK-9 and MK-7. Polar lipids consist of phosphatidylglycerol, diphosphatidylglycerol, phospholipids and glycolipids of unknown composition. The DNA G + C content of the type strain is 38.5 mol%.

The type strain, HC148^T (KACC 11362^T=DSM 17952^T), was isolated from livestock-manure compost from Ichon, Korea.

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