NOTE

Comamonas koreensis sp. nov., a non-motile species from wetland in Woopo, Korea

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A bacterial strain, designated YH12^T, was isolated from a wetland sample collected from Woopo, Republic of Korea, and characterized using a polyphasic approach. Analysis of 16S rDNA indicated that the isolate formed a monophyletic clade with the members of the genus *Comamonas*. The closest phylogenetic relative among the valid species was *Comamonas testosteroni*, with 96·6% 16S rDNA similarity. The chemotaxonomic properties of the wetland isolate supported its membership of the genus *Comamonas*, as it contained ubiquinone Q-8 as a major respiratory quinone and hexadecanoic, methylene-hexadecanoic and octadecenoic acids as major cellular fatty acids. The G+C content of the DNA was 66 mol%. The isolate is a Gram-negative, non-pigmented, rod-shaped, oxidase- and catalase-positive, non-motile, non-endospore-forming and non-fermentative bacterium. The phenotypic properties of the isolate were compared with those of the type strains of *Comamonas terrigena*, *C. testosteroni* and *Delftia acidovorans*. A number of tests, including motility, can differentiate our isolate from related taxa. On the basis of the 16S rDNA phylogenetic, chemotaxonomic and phenotypic evidence given in this study, it is proposed that strain YH12^T (= KCTC 12005^T = IMSNU 11158^T) be assigned as the type strain of a novel species of the genus *Comamonas*, *Comamonas koreensis* sp. nov.

Keywords: polyphasic taxonomy, 16S rDNA, taxonomy, *Comamonas koreensis* sp. nov.

Woopo, one of the oldest freshwater wetlands in the Republic of Korea, represents a unique ecological niche for many animals and plants, covering 8·54 km^2. Geological investigations have indicated that it was created some 140 million years ago. In recent years, it has received great attention from the public, due to its potential for biodiversity and biological conservation. There have been several investigations to survey the biodiversity of animals and plants, but none has been done on micro-organisms (KyongNam Development Institute, 1997). Therefore, we initiated a systematic screening programme to catalogue the microbial composition of Woopo. In this study, we report the isolation and identification of a previously unknown bacterium from Woopo, for which the name *Comamonas koreensis* sp. nov. is proposed.

Abbreviation: NA, nutrient agar.

The GenBank accession number for 16S rDNA sequence of *Comamonas koreensis* YH12^T (= KCTC 12005^T) is AF275377.

A sediment sample was collected from a depth of 30 cm from the Woopo wetland. The sample was serially diluted with 0·85% (w/v) saline solution, spread onto plates of peptone/yeast extract/glucose/vitamin agar (Staley, 1968) and incubated at 30 °C under anaerobic conditions. The gas phase was $H_2/CO_2/N_2$ (7:5:88 by vol.) in a Forma anaerobic chamber. A bacterial strain, designated YH12^T, was isolated and subcultured several times to obtain a pure culture. The reference strains used were *Comamonas terrigena* KCTC 2989^T, *Comamonas testosteroni* KCTC 2990^T and *Delftia acidovorans* KCTC 2991^T. Test strains were routinely grown using nutrient agar (NA, Difco) at 30 °C and maintained as glycerol suspensions (10%, w/v) at −80 °C.

For the determination of cell shape and size and the detection of flagella, cells were negatively stained with 1% (w/v) phosphotungstic acid according to the method of Cole & Popkin (1981) and observed with a CM-20 Philips transmission electron microscope at a

voltage of 100 kV. Motility was checked using a hanging-drop method (Schaal, 1986). An additional test was performed by the modified Craigie's technique (Craigie, 1931), in which the organism was inoculated into a central tube of semi-solid NA and diffusion of growth from the line of inoculum was observed. Catalase was detected by placing drops of 3 % (w/v) H_2O_2 on cultures growing on NA and observing for oxygen bubbles. The presence of oxidase was determined using Bactident Oxidase test paper (Merck). Biochemical characteristics of test strains were determined using the API 20 E, 20 NE and 50 CH systems (bioMérieux) following the manufacturer's protocols. Screening for carbon substrates utilized was performed with the BIOLOG GN2 microplate system release 4.0. For temperature characterizations, cultures were grown on NA at 5–45 °C for 7 d. NA containing 3–6·5 % (w/v) NaCl was inoculated and incubated at 30 °C for 7 d to test for salt tolerance. The pH range for growth was checked using a 500 ml flask containing 250 ml NA broth with a pH of 6–10 at 30 °C; 100 mM Na_2HPO_4/NaH_2PO_4 (pH 6–8) or 100 mM $Na_2CO_3/NaHCO_3$ (pH 9–10) were used as buffers. Growth was checked by monitoring optical density at 650 nm.

Cellular fatty acids of test strains were analysed as methyl esters by GC according to the instructions of the Microbial Identification System (MIDI). Respiratory quinones were extracted using the small-scale method of Komagata & Suzuki (1987) and examined by TLC and HPLC. DNA was prepared according to Chun & Goodfellow (1995) and DNA G+C contents were determined by using the thermal-denaturation method (Mandel & Marmur, 1968).

A nearly complete 16S rDNA of strain YH12^{T} was determined according to Chun & Goodfellow (1995). The resultant sequence (1464 bp) was aligned manually with sequences of representatives of the genus *Comamonas* and related taxa using known 16S rRNA secondary structure information. Ambiguous bases were omitted from the final dataset. Phylogenetic trees were inferred by using the neighbour-joining (Saitou & Nei, 1987), Fitch–Margoliash (Fitch & Margoliash, 1967) and maximum-parsimony (Fitch, 1972) methods. Evolutionary distance matrices for the neighbour-joining and Fitch–Margoliash methods were generated according to the model of Jukes & Cantor (1969). The sequence of *Burkholderia cepacia* ATCC 25416^{T} (GenBank accession no. M22518) was used as an outgroup. The PHYLIP package (Felsenstein, 1993) was used for all analyses. The resultant tree topology was evaluated by bootstrap analysis (Felsenstein, 1985) of the neighbour-joining tree based on 1000 resamplings.

A preliminary comparison of the nucleotide sequence with sequences in the GenBank and Ribosomal Database Project (Maidak *et al.*, 1997) databases indicated that the isolate was closely related to members of the genus *Comamonas*. The 16S rDNA sequence of the test

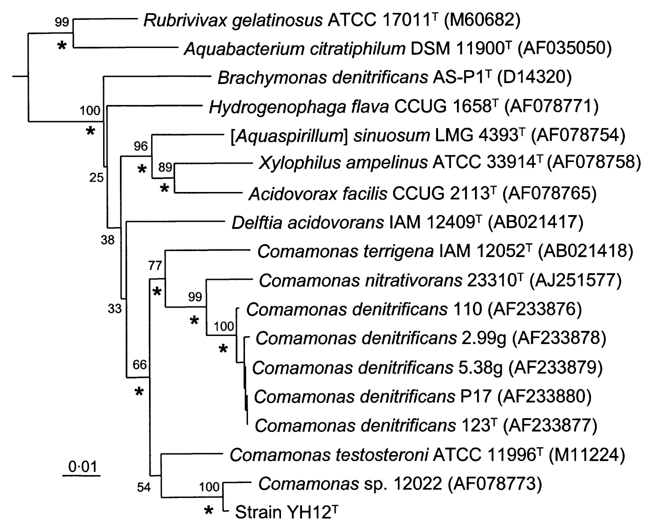

Fig. 1. Rooted neighbour-joining tree based on 1449 nucleotide positions of 16S rDNA sequences showing relationships between strain YH12^{T} and some closely related taxa. *Burkholderia cepacia* was used as an outgroup. Percentages at the nodes indicate levels of bootstrap support based on neighbour-joining analyses of 1000 resampled datasets. Asterisks indicate clades that were also recovered in the Fitch–Margoliash and maximum-parsimony trees. Bar, 0·01 substitutions per nucleotide position.

strain was aligned manually with representatives of the genus *Comamonas* and related taxa. A rooted phylogenetic tree showing the relationships between strain YH12^{T} and representatives of the family *Comamonadaceae* is given in Fig. 1. The test strain formed a moderately significant monophyletic clade with the members of the genus *Comamonas*, which at the time of writing had two validly described species (Wen *et al.*, 1999), *C. terrigena* and *C. testosteroni*. The *Comamonas* clade was also confirmed in other treeing algorithms, but was supported by a moderate bootstrap value of 66 %. Strain YH12^{T} formed a significant monophyletic clade with *Comamonas* sp. 12022, a bacteriocin-producing bacterium isolated from the Baltic Sea (Hantula *et al.*, 1996). On the basis of pairwise 16S rDNA similarities, the closest phylogenetic relative of strain YH12^{T} was *Comamonas* sp. 12022 (98·8 %), followed by *C. testosteroni* ATCC 11996^{T} (96·7 % similarity), *D. acidovorans* IAM 12409^{T} (95·7 %) and *Comamonas denitrificans* 110 (95·5 %). It is evident from 16S rDNA analysis that isolate YH12^{T} represents a novel genomic species in the genus *Comamonas*, as none of the validly described species showed more than 97 % 16S rDNA similarity (Stackebrandt & Goebel, 1994). A previously unidentified strain, *Comamonas* sp. 12022, showed 16S rDNA similarity of 98·8 % (i.e. over 97 %). At this stage, it cannot be concluded whether our isolate and strain 12022 belong to the same species.

Our isolate contained the characteristic chemical markers of the genera *Comamonas* and *Delftia*, that is ubiquinone Q-8 and 16 : 0, $16:1\omega7c$, 17 : 0 cyclo and 18 :

Table 1. Cellular fatty acid composition of strain YH12^T and related bacteria

Values are percentages of total fatty acid methyl esters. tr, Trace (< 0·5%).

Fatty acid	YH12^T	*C. terrigena* KCTC 2989^T	*C. testosteroni* KCTC 2990^T	*D. acidovorans* KCTC 2991^T
12:0	2·3	2·8	2·4	2·3
14:0	1·0	3·3	1·0	1·4
15:1	–	–	–	0·7
15:0	9·4	3·7	1·0	4·0
16:1$\omega 7c$	26·1	38·4	33·1	35·2
16:0	29·9	27·5	30·4	28·3
17:1	0·7	tr	–	tr
17:0	2·6	1·5	0·8	1·6
18:1	9·6	14·9	17·9	15·3
18:0	tr	tr	tr	tr
17:0 cyclo	12·3	2·4	3·8	8·1
19:0 cyclo	–	–	0·9	–
20:0 iso	–	–	1·1	–
8:0 3OH	–	–	–	tr
10:0 3OH	3·5	5·3	4·8	3·2
15:0 2OH	0·6	–	tr	–
16:0 2OH	2·2	–	2·0	–
16:1 2OH	–	–	0·6	–

1 fatty acids (Table 1), as reported in previous studies (Tamaoka *et al.*, 1987; Willems *et al.*, 1989). However, significant differences were found in the relative amounts of 15:0, 18:1 and 17:0 cyclo fatty acids, which can be employed to differentiate our isolate from these related taxa. 3-Hydroxy fatty acids were found in all of the test strains, whereas only strain YH12^T and *C. testosteroni* KCTC 2990^T contained 2-hydroxy fatty acids. The G+C content of the DNA was 66 mol%, which is slightly higher than values reported for *C. terrigena* (65 mol%) and *C. testosteroni* (61–62 mol%; Tamaoka *et al.*, 1987). However, the value still lies within the range expected for members of the same genus.

The following characters gave positive results for strain YH12^T: assimilation of cellobiose, malate, maltose, D-psicose, D-raffinose and L-rhamnose; hydrolysis of acetic acid, D-galactonic acid lactone, D-gluconic acid, itaconic acid, methyl pyruvate, monomethyl succinate, quinic acid, D-saccharic acid, succinamic acid, succinic acid, bromosuccinic acid, L-leucyl 2-naphthylamide, 2-naphthyl caprylate, 2-naphthyl phosphate (pH 5·4 and 8·5) and naphthol-AS-BI phosphate. The following characters gave negative results for strain YH12^T: production of acetoin, H_2S and indole; β-galactosidase and tryptophan deaminase activities; assimilation of *N*-acetyl D-galactosamine, *N*-acetylglucosamine, adonitol, aesculin, amygdalin, DL-arabinose, DL-arabitol, arbutin, dulcitol, erythritol, DL-fucose, galactose, α-gentiobiose, glycogen, inulin, 2-ketogluconate, 5-ketogluconate, lactose, lactulose, D-lyxose, D-mannose, melibiose, melezitose, methyl α-D-glucoside, methyl β-D-glucoside, methyl α-D-mannoside, methyl β-D-xyloside, ribose, salicin, sorbitol, sorbose, starch, sucrose, trehalose, D-turanose, DL-xylose and xylitol; hydrolysis of alaninamide, L-alanyl glycine, γ-aminobutyric acid, 2-aminoethanol, arginine, L-asparagine, DL-carnitine, glucuronamide, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, hydroxy-L-proline, phenylethylamine, L-proline, putrescine, L-pyroglutamic acid, DL-serine, citric acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, formic acid, D-galacturonic acid, D-glucosaminic acid, D-glucuronic acid, inosine, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, DL-lactic acid, malonic acid, thymidine, Tween 40, urocanic acid, uridine, 2,3-butanediol, α-cyclodextrin, dextrin, gelatin, DL-α-glycerol phosphate, glucose 1-phosphate, glucose 6-phosphate, *N*-benzoyl-DL-arginine 2-naphthylamide, 6-bromo-2-naphthyl α-D-galactopyranoside, 6-bromo-2-naphthyl β-D-glucopyranoside, 6-bromo-2-naphthyl α-D-mannopyranoside, L-cystyl 2-naphthylamide, *N*-glutarylphenylalanine 2-naphthylamide, 2-naphthyl butyrate, 2-naphthyl myristate, 2-naphthyl β-D-galactopyranoside, naphthol-AS-BI β-D-glucuronate, 2-naphthyl α-D-glucopyranoside, 1-naphthyl-*N*-acetyl β-D-glucosaminide, 2-naphthyl α-L-fucopyranoside and L-valyl 2-naphthylamide.

All *Comamonas* species have been reported to be motile by means of polar or bipolar tufts of flagella (De Vos *et al.*, 1985; Tamaoka *et al.*, 1987; Wen *et al.*, 1999; Willems *et al.*, 1991). However, our isolate neither showed motility nor contained flagella, as revealed by light microscopy and negatively stained TEM analysis. Motility can be a simple and excellent

Table 2. Differential phenotypic characteristics of strain YH12T and related bacteria

Data were taken from this and earlier studies (De Vos *et al*., 1985; Tamaoka *et al*., 1987; Wen *et al*., 1999; Willems *et al*., 1991).

Characteristic	YH12T	*C. terrigena* KCTC 2989T	*C. testosteroni* KCTC 2990T	*D. acidovorans* KCTC 2991T
Motility/flagella	−	+	+	+
Hydrolysis of:				
Tween 80	+	−	+	+
2-Naphthyl butyrate	−	+	+	+
2-Naphthyl phosphate (pH 8·5)	+	−	−	+
Naphthol-AS-BI phosphate	+	+	+	−
Used of carbon source for growth:				
Adipate, D-gluconate	−	+	+	+
Aconitate	+	+	−	+
Caprate	−	−	+	−
Citrate	−	−	+	+
D-Fructose	−	−	−	+
Glycerol, L-threonine	−	+	+	+
D-Glucose	+	−	−	−
D-Alanine, L-alanine	−	+	+	+
L-Aspartate, L-leucine	−	+	+	+
L-Histidine, L-phenylalanine	−	−	+	+
m-Inositol	+	−	−	−
D-Mannitol	+	−	−	+
Phenylacetate	−	−	−	+
Propionate	−	+	+	+
Sebacate	−	+	+	+
D-Tagatose	+	−	−	+

marker to differentiate strain YH12T from its phylogenetic relatives, as *D. acidovorans* and other *Comamonas* species have been shown to be motile. In comparison with the description of the genus *Comamonas*, strain YH12T showed several phenotypic characteristics that imply an intermediate position of the isolate between *Comamonas* and *Delftia* and which can be used to differentiate the test strain other members of these genera (Table 2). Wen *et al.* (1999) summarized the characters that are diagnostic between the members of the genus *Comamonas* and *D. acidovorans*. It is noteworthy that the ability to utilize D-mannitol as a sole carbon source is no longer useful in differentiating *Comamonas* from *D. acidovorans*, as strain YH12T was able to utilize D-mannitol, in common with *D. acidovorans* strains. On the basis of the molecular, chemical and phenotypic evidence presented in this study, we propose strain YH12T, isolated from the Woopo wetland, as the type strain of a novel species in the genus *Comamonas*, *Comamonas koreensis* sp. nov.

Description of *Comamonas koreensis* sp. nov.

Comamonas koreensis (ko.re.en′sis. N.L. fem. adj. *koreensis* of Korea, the geographical origin of isolation).

Cells are straight to slightly curved rods, 0·4–0·6 × 1–1·5 μm, which occur singly or in pairs. Gram-negative, catalase- and oxidase-positive and non-fermentative. Grows at 10, 25 and 37 °C but not at 5 or 42 °C. Grows optimally at pH 7·0 at 30 °C. Grows on NA containing 3% (w/v) NaCl but not with 4·5 or 6·5% NaCl. Aerobic, but able to grow under anaerobic conditions (gas phase $H_2/CO_2/N_2$; 7:5:88 by vol.) on NA. No pigment production on NA. Neither endospores nor flagella are produced. Nitrate is reduced to nitrite, but the species is unable to denitrify. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and urease are not produced. Assimilates D-glucose and D-mannitol, but not D-fructose or starch. Hydrolyses Tween 80 but not gelatin. Hydrolyses naphthol-AS-BI phosphate but not 2-naphthyl butyrate, 2-naphthyl phosphate (pH 8·5), adipate, D-gluconate, glycerol, propionate, sebacate, L-threonine, D- or L-alanine, L-aspartate or L-leucine. Major fatty acids are hexadecanoic acid (16:0), *cis*-9-hexadecenoic acid (16:1*ω*7*c*), methylene-hexadecanoic acid (17:0 cyclo) and octadecenoic acid (18:1). 2-Hydroxy fatty acids (2-OH 15:0 and 16:0) and 3-hydroxy fatty acids (3-OH 10:0) are present. Ubiquinone Q-8 is the major quinone. The G+C content of the DNA is 66 mol%. The species was isolated from a wetland sample collected from Woopo, Republic of Korea.

The type and only strain is strain YH12^T [= KCTC 12005^T = IMSNU (Institute of Microbiology, Seoul National University) 11158^T].

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