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Comamonas granuli sp. nov., Isolated from Granules Used in a Wastewater Treatment Plant

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A Gram-negative, motile, rod-shaped, non-spore-forming bacterial strain, designated as Ko03^T, was isolated from microbial granules, and was characterized, using a polyphasic approach, in order to determine its taxonomic position. The isolate was positive for catalase and oxidase, but negative for gelatinase and β-galactosidase. Phylogenetic analyses using the 16S rRNA gene sequence showed that the strain formed a monophyletic branch towards the periphery of the evolutionary radiation occupied by the genus *Comamonas*, its closest neighbors being *Comamonas koreensis* KCTC 12005^T (95.9% sequence similarity), *Comamonas nitrativorans* DSM 13191^T (95.7%), and *Comamonas odontotermitis* LMG 23579^T (95.7%). Strain Ko03^T had a genomic DNA G+C content of 68.4 mol% and the predominant respiratory quinone was Q-8. The major fatty acids were $C_{16:1}$ *ω7c* (44.7%), $C_{16:0}$ (28.1%), $C_{18:1}$ (16.1%), and $C_{10:0}$ 3-OH (3.5%). These chemotaxonomic results supported the affiliation of strain Ko03^T to the genus *Comamonas*. However, low 16S rRNA gene sequence similarity values and distinguishing phenotypic characteristics allowed genotypic and phenotypic differentiation of strain Ko03^T from recognized *Comamonas* species. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain Ko03^T represents a novel species of the genus *Comamonas*, for which the name *Comamonas granuli* sp. nov. is proposed. The type strain is Ko03^T (= KCTC 12199^T= NBRC 101663^T).

***Keywords*:** betaproteobacteria, polyphasic taxonomy, *Comamonas granuli*, 16S rRNA gene

The genus *Comamonas* was proposed by Davis and Park (1962), but was not validated until De Vos *et al.* (1985) revived the genus and type species *Comamonas terrigena*. Tamaoka *et al*. (1987) transferred [*Pseudomonas*] *acidovorans* and [*Pseudomonas*] *testosteroni* to this genus *Comamonas* as [*Comamonas*] *acidovorans* and *Comamonas testosteroni*, respectively. Later [*Comamonas*] *acidovorans* was transferred to the genus *Delftia acidovorans* and excluded from the member of the genus *Comamonas* (Wen *et al*., 1999). Two denitrifying species, *Comamonas denitrificans* (Gumaelius *et al*., 2001) and *Comamonas nitrativorans* (Etchebehere *et al*., 2001), and the only non-motile species, *Comamonas koreensis* (Chang *et al*., 2002) were reported subsequently. Wauters *et al.* (2003) proposed to separate *Comamonas terrigena* into three species such as *Comamonas terrigena*, *Comamonas aquatica*, and *Comamonas kerstersii* based on their biochemical and genotypic differences. Recently, a floc-forming species, *Comamonas badia* (Tago and Yokota, 2004) and *Comamonas odontotermitis* (Chou *et al.,* 2007) were reported as eighth and ninth members of the genus *Comamonas.* Representatives of this genus were isolated from various environments including soil, freshwater, wetland, activated sludge, and termite gut (Lim *et al.*, 2005; Chou *et al.,* 2007).

In this study, we describe a Gram-negative, motile, rod-shaped, non-spore-forming strain Ko03^T isolated from microbial granules treating wastewater. Based on polyphasic taxonomic approach combining the phenotypic, genotypic and chemotaxonomic characterization, this isolate appears to represent a distinct novel species of genus *Comamonas*, for which the name *Comamonas granuli* has been proposed.

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Materials and Methods

Isolation of bacterial strain and culture condition

Strain Ko03^T was isolated from microbial granule, collected from the bioreactor treating industrial wastewater in Kongju using direct plating onto R2A agar (Difco, USA). Single colony on these plates was purified by transferring them onto fresh plates of R2A agar and incubating again for 3 days at 30°C. One isolate, Ko03^T, was cultured routinely on R2A agar at 30°C and maintained as a glycerol suspension (20%, w/v) at -70°C. This organism was then submitted to the Korean Collection for Type Cultures (= KCTC 12199^T) and the National Institute of Technology and Evaluation Biological Resource Center, Japan (= NBRC 101663^T).

Phenotypic and biochemical characteristics

The Gram reaction was performed using the non-staining method, as described by Buck (1982). Cell morphology was observed under a Nikon light microscope at ×1,000, with cells grown on R2A agar for 3 days at 30°C. Catalase and oxidase tests were performed as outlined by Cappuccino

and Sherman (2002). Substrate utilization as sole carbon source and some physiological characteristics were determined with API 32GN and API 20NE galleries according to the instructions of the manufacturer (bioMérieux, France). Anaerobic growth was determined in serum bottles containing R2A broth supplemented with thioglycolate (1 g/L), and in which the upper air layer had been replaced with nitrogen. Reduction of nitrate and nitrite was determined using serum bottles containing R2A broth supplemented with KNO_3 (10 mM) and $NaNO_2$ (10 mM), respectively. The reduction of nitrate and nitrite was monitored by ion chromatography on a model 790 personal IC (Metrohm, Swiss) equipped with a conductivity detector and an anion exchange column (Metrosep Anion Supp 4; Metrohm). Growth at a variety of temperatures (4, 20, 30, 37, and 42°C) was assessed on R2A agar and at pH 5.0~10.0 (in increments of 0.5 pH units) was assessed in R2A broth. Salt tolerance was tested on R2A medium supplemented with 1~6% (w/v) NaCl after incubation for up to 5 days.

PCR amplification, 16S rRNA gene sequencing, and phylogenetic analysis

For phylogenetic analysis of strain Ko03^T, genomic DNA was extracted using a commercial Genomic DNA Extraction Kit (Core Biosystem, Korea) and PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Kim *et al.* (2005). Full sequences of the 16S rRNA gene were compiled using SeqMan software (DNASTAR, USA). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed using the CLUSTAL X program (Thompson *et al.*, 1997) and gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were constructed by using the neighbour-joining (Saitou and Nei, 1987) and max-

Table 1. Differential phenotypic characteristics of Ko03^T and related type species of the genus *Comamonas*

Characteristic	1	2	3	4	5	6	7	8	9	10
Motility	+	-	+	+	+	+	+	+	+	+
Growth at 42°C	+	-	NR	-	-	NR	-	-	+	-
Nitrite reduction to nitrogen	-	-	+	-	-	-	-	+	-	-
Arginine dihydrolase	+	-	NR	-	-	-	-	NR	-	NR
Urease	+	-	NR	-	-	v	-	v	-	NR
Aesculin hydrolysis	-	-	NR	-	-	+	-	v	-	NR
Assimilation of										
Acetate	+	+	+	NR	+	+	+	NR	+	NR
Adipate	-	+	+	-	+	+	+	-	+	+
L-Alanine	+	-	+	NR	-	+	+	+	-	NR
Caprate	-	-	-	-	-	+	-	-	-	-
Citrate	-	+	-	+	-	+	-	+	-	+
DL-3-Hydroxybutyrate	+	-	NR	NR	+	+	+	+	+	NR
D-Glucose	-	+	-	-	-	-	-	-	-	-
Inositol	-	+	-	NR	-	-	-	-	-	-
D-Maltose	-	+	-	-	-	-	-	-	-	-
D-Mannitol	-	+	-	NR	-	-	-	-	-	-
Itaconate	-	+	NR	NR	+	+	v	NR	+	NR
D-Gluconate	-	+	-	+	+	+	+	-	-	+
Propionate	+	-	+	NR	+	+	+	NR	NR	NR
Malate	+	+	+	+	+	+	-	-	+	+
L-Proline	+	-	NR	NR	+	+	+	NR	+	NR
Phenylacetate	-	-	+	+	-	-	-	-	-	+
L-Rhamnose	-	+	-	NR	-	-	-	-	-	-
DL-Lactate	+	-	+	NR	+	+	+	+	+	NR
L-Serine	-	-	NR	NR		-	-	+	NR	NR
DNA G+C content (mol%)	68.4	66.0	ND	61.6	64.0	62.5-64.5	64.0	60.8	61.0	66.3

Strains: 1, *Comamonas granuli* Ko03^T; 2, *Comamonas koreensis* KCTC 12005^T; 3, *Comamonas nitrativorans* DSM 13191^T; 4, *Comamonas odontotermitis* LMG 23579^T; 5, *Comamonas aquatica* LMG 2370^T; 6, *Comamonas testosteroni* ATCC 11996^T; 7, *Comamonas terrigena* LMG 1253^T; 8, *Comamonas denitrificans* ATCC 700936^T; 9, *Comamonas kerstersii* LMG 3475^T; 10, *Comamonas badia* IAM 14839^T. Data from this and earlier studies (Willems *et al*., 1991; Etchebehere *et al*., 2001; Gumaelius *et al*., 2001; Chang *et al*., 2002; Wauters *et al*., 2003; Tago and Yokota, 2004; Chou *et al.,* 2007). +, positive; -, negative; v, variable; NR, not reported.

imum parsimony (Fitch, 1972) methods with the MEGA3 program (Kumar *et al*., 2004) with bootstrap values based on 1,000 replications (Felsenstein, 1985).

Determination of DNA G+C content

For measurement of the G+C content of chromosomal DNA, the genomic DNA of strain Ko03^T was extracted and purified as described by Moore and Dowhan (1995) and enzymically degraded into nucleosides. The G+C content was then determined as described by Mesbah *et al.* (1989), using reversed-phase HPLC.

Isoprenoid quinones and cellular fatty acids

Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under vacuum conditions and re-extracted in n-hexane/water (1:1, v/v). The crude quinone in n-hexane was purified using Sep-Pak Vac Cartridges Silica (Waters) and subsequently analyzed by HPLC, as described previously (Collins and Jones, 1981). Cellular fatty acid profiles were determined for strains grown on TSA for 3 days 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 acids were then analyzed by gas chromatography (model 6890; Hewlett Packard) using the Microbial Identification software package (Sasser, 1990). Duplicate experiments were performed.

Results and Discussion

Morphological and phenotypic characteristics

Cells of strain Ko03^T were Gram-negative, motile, and rod shaped. Fast movements were observed by phase contrast microscopy. The colonies grown on R2A agar for 3 days were smooth, circular, non-glossy, creamy color, and 0.5∼2.0 mm in diameter. The strain was able to grow at 20∼42°C, but not at 4°C on R2A agar. Growth occurred in the absence of NaCl and in the presence of 1% (w/v) NaCl, but not in the presence of 2% (w/v) NaCl. The biochemical characteristics of strain Ko03^T were similar to those reported for members of the genus *Comamonas* (Wauters *et al*., 2003; Chou *et al.*, 2007), i.e. positive for catalase, oxidase and nitrate reduction to nitrite, negative for assimilation of L-arabinose, mannose and *N*-acetyl-glucosamine. Phenotypic and chemotaxonomic characteristics that differentiate strain Ko03^T from other members of the genus *Comamonas* are listed in Table 1.

Cellular fatty acid and quinone compositions

Strain Ko03^T contained the characteristic chemical markers of the genus *Comamonas,* that is ubiquinone Q-8 and $C_{16:0}$, $C_{16:1}$ *ω7c*, $C_{18:1}$, and $C_{10:0}$ 3-OH fatty acids (Table 2), as reported in previous studies (Chang *et al*., 2002; Tago and Yokota, 2004; Chou *et al*., 2007). However, some qualitative and quantitative differences in fatty acid content could be observed between strain Ko03^T and the phylogenetically closest relatives. It could be clearly distinguished from the type strains of its closest phylogenetic neighbours *Comamonas koreensis*, *Comamonas nitrativorans*, and *Comamonas odontotermitis* by the proportions of $C_{17:0}$ cyclo, $C_{14:0}$, $C_{15:0}$, $C_{17:0}$ and 2-hydroxy fatty acids.

DNA G+C content

The G-C content of strain Ko03^T was 68.4 mol%, which is slightly higher than values reported for *Comamonas koreensis* (66.0 mol%) (Chang *et al*., 2002) and *Comamonas badia* (66.3 mol%) (Tago and Yokota, 2004). However, the

Table 2. Cellular fatty acid profile of strain Ko03^T and related type species of the genus *Comamonas*

Fatty acid	1	2	3	4	5	6	7	8	9	10
$C_{12:0}$	2.8	2.3	2.9	2.7	3.0	2.4	2.8	3.0	2.6	2.9
$C_{14:0}$	1.7	1.0	3.4	-	3.9	1.0	3.3	3.2	2.9	1.5
$C_{15:0}$	1.6	9.4	-	-	-	1.0	3.7	-	-	-
$C_{16:0}$	28.1	29.9	21.2	33.6	25.2	30.4	27.5	17.8	23.4	33.3
$C_{17:0}$	1.5	2.6	-	-	-	0.8	1.5	-	0.6	-
$C_{18:0}$	-	tr	-	-		tr	tr	-		-
$C_{17:0}$ cyclo	-	12.3	-	5.9	-	3.8	2.4	-	0.7	-
$C_{19:0}$ cyclo	-	-	-	-	-	0.9	-	-	-	-
$C_{20:0}$ iso	-	-	-	-	-	1.1	-	-	-	-
$C_{10:0}$ 3-OH	3.5	3.5	5.0	3.8	5.0	4.8	5.3	4.2	4.5	2.4
$C_{15:0}$ 2-OH	-	0.6	-	-	-	tr	-	-	-	-
$C_{16:0}$ 2-OH	-	2.2	-	2.5	-	2.0	-	-	-	2.3
$C_{16:1}$ 2-OH	-	-	-	-	-	0.6	-	-	-	-
$C_{16:1}$ *ω7c*/iso-$C_{15:0}$ 2-OH	44.7	26.1	42.9	33.9	42.4	33.1	38.4	48.6	28.2	41.9
$C_{17:1}$	-	0.7	-	-	-	-	tr	-	-	-
$C_{18:1}$/$C_{18:1}$ *ω7c*	16.1	9.6	23.8	16.2	19.0	17.9	14.9	22.4	36.1	13.1

Strains: 1, *Comamonas granuli* Ko03^T; 2, *Comamonas koreensis* KCTC 12005^T; 3, *Comamonas nitrativorans* DSM 13191^T; 4, *Comamonas odontotermitis* LMG 23579^T; 5, *Comamonas aquatica* LMG 2370^T; 6, *Comamonas testosteroni* ATCC 11996^T; 7, *Comamonas terrigena* LMG 1253^T; 8, *Comamonas denitrificans* ATCC 700936^T; 9, *Comamonas kerstersii* LMG 3475^T; 10, *Comamonas badia* IAM 14839^T. Data in columns 2-10 were obtained from Chang *et al*. (2002); Chou *et al.* (2007) and Tago and Yokota (2004). Values are percentages of total fatty acids. -, not detected; tr, fatty acids representing less than 0.5%.

value still lies within the range expected for members of the same genus and the G+C content range of genus *Comamonas* should be extended taking into account our result.

Phylogenetic analysis

1,443 bp nucleotide positions of 16S rRNA gene sequence of strain Ko03^T were obtained and deposited in GenBank database under the accession numbers AB187586. A comparison of the sequence with those of representatives of genera classified in the family *Comamonadaceae* of the class *Betaproteobacteria* showed that the organism fell within the evolutionary radiation occupied by the genus *Comamonas* (Fig. 1). According to sequence similarity calculations, the organism was most closely related to *Comamonas koreensis* KCTC 12005^T (95.9% similarity), *Comamonas nitrativorans*

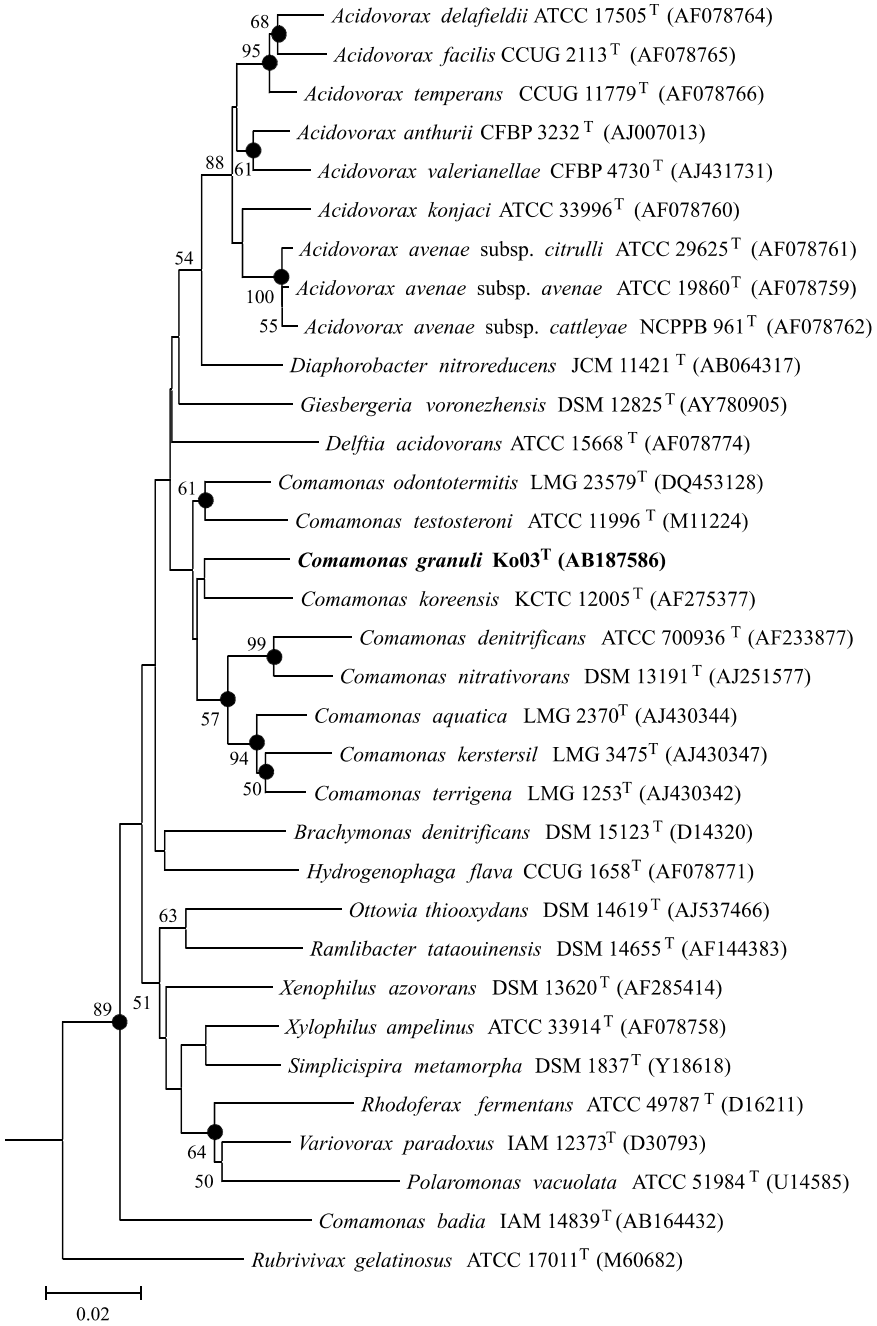

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strain Ko03^T among neighboring species selected from the class *Betaproteobacteria* on the basis of 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1,000 replications) greater than 50% are shown at the branch points. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum parsimony algorithm. *Paracoccus versutus* ATCC 25364^T was used as an outgroup (not shown). A bar represents 0.02 substitutions per nucleotide position.

DSM 13191^T (95.7%), and *Comamonas odontotermitis* LMG 23579^T (95.7%). The 16S rRNA gene sequence similarity of strain Ko03^T with other species within the class *Betaproteobacteria* was less than 95.6%. Strain Ko03^T formed a monophyletic clade with the other nine members of the genus *Comamonas*, which was supported by the neighbour-joining and maximum-parsimony methods employed. The generally accepted criteria for delineating bacterial species state that strains with a DNA-DNA relatedness value of below 70% (as measured by hybridization), or strains with 16S rRNA gene sequence dissimilarity above 3%, are considered as belonging to separate species (Wayne *et al.,* 1987; Stackebrandt and Goebel, 1994). Taking into account this definition, our data indicate that strain Ko03^T represents a novel species of the genus *Comamonas.*

Taxonomic conclusions

All characteristics determined for strain Ko03^T are in accordance with those of the genus *Comamonas.* On the basis of phylogenetic distance from established *Comamonas* species, indicated by relatively low 16S rRNA gene sequence similarities (<95.9%) and a specific combination of phenotypic characteristics, it is demonstrable that Ko03^T is not affiliated to any species of this genus. Therefore, on the basis of the data presented, strain Ko03^T should be placed in the genus *Comamonas* as a novel species, for which the name *Comamonas granuli* sp. nov. is proposed.

Description of *Comamonas granuli* sp. nov.

Comamonas granuli (gra.nu'li. L. gen. n. *Granuli* of a small grain, pertaining to a granule, from which the type strain was isolated).

Cells are Gram-negative, rod-shaped (0.5~0.7 μm wide and 1.2~4.0 μm long), non-spore-forming, and motile. After 3 days incubation at 30°C on R2A, colonies are smooth, circular, non-glossy, creamy color, and 0.5~2.0 mm in diameter. Anaerobic growth does not occur. The isolate grows on R2A agar at 20~42°C but not at 4°C. Tolerates NaCl at 1% (w/v) but not 2%. The pH range for growth is 6.0~8.5. Catalase- and oxidase-positive. Does not hydrolyze esculine and gelatine. Negative for D-glucose fermentation. Produces arginine dihydrolase and urease but not tryptophan deaminase and β-galactosidase. The following substrates are utilized for growth: malate, propionate, valerate, L-histidine, DL-3-hydroxybutyrate, L-proline, acetate, DL-lactate, and L-alanine. The following substrates are not utilized for growth: D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, D-gluconate, caprate, adipate, citrate, phenylacetate, salicin, D-melibiose, L-fucose, D-sorbitol, 2-ketogluconate, 4-hydroxybenzoate, L-rhamnose, D-ribose, inositol, D-sucrose, itaconate, suberate, malonate, 5-ketogluconate, 3-hydroxybenzoate, L-serine, and glycogen. Predominant lipoquinone is Q-8. Major fatty acids are $C_{16:1}$ $\omega7c$, $C_{16:0}$, $C_{18:1}$, and $C_{10:0}$ 3-OH. The G+C content of the genomic DNA of the type strain is 68.4 mol%.

The type strain, Ko03^T (= KCTC 12199^T= NBRC 101663^T), was isolated from microbial granules obtained from industrial wastewater treatment plant in Kongju, Republic of Korea.

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References

Buck, J.D. 1982. Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl. Environ. Microbiol.* 44, 992-993.

Cappuccino, J.G. and N. Sherman. 2002. Microbiology: a laboratory manual, 6th ed. Pearson Education, Inc., California, USA.

Chang, Y.H., J.I. Han, J. Chun, K.C. Lee, M.S. Rhee, Y.B. Kim, and K.S. Bae. 2002. *Comamonas koreensis* sp. nov., a non-motile species from wetland in Woopo, Korea. *Int. J. Syst. Evol. Microbiol.* 52, 377-381.

Chou, J.H., S.Y. Sheu, K.Y. Lin, W.M. Chen, A.B. Arun, and C.C. Young. 2007. *Comamonas odontotermitis* sp. nov., isolated from the gut of the termite *Odontotermes formosanus*. *Int. J. Syst. Evol. Microbiol.* 57, 887-891.

Collins, M.D. and D. Jones. 1981. A note on the separation of natural mixtures of bacterial ubiquinones using reverse-phase partition thin-layer chromatography and high performance liquid chromatography. *J. Appl. Bacteriol.* 51, 129-134.

Davis, G.H. and R.W. Park. 1962. A taxonomic study of certain bacteria currently classified as *Vibrio* species. *J. Gen. Microbiol.* 27, 101-119.

De Vos, P., K. Kersters, E. Falsen, B. Pot, M. Gillis, P. Segers, and J. De Ley. 1985. *Comamonas* Davis and Park 1962 gen. nov., nom. rev. emend., and *Comamonas terrigena* Hugh 1962 sp. nov., nom. rev.. *Int. J. Syst. Bacteriol.* 35, 443-453.

Etchebehere, C., M.I. Errazquin, P. Dabert, R. Moletta, and L. Muxi. 2001. *Comamonas nitrativorans* sp. nov., a novel denitrifier isolated from a denitrifying reactor treating landfill leachate. *Int. J. Syst. Evol. Microbiol.* 51, 977-983.

Felsenstein, J. 1985. Confidence limit on phylogenies: an approach using the bootstrap. *Evolution* 39, 783-791.

Fitch, W.M. 1972. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst. Zool.* 20, 406-416.

Gumaelius, L., G. Magnusson, B. Pettersson, and G. Dalhammar. 2001. *Comamonas denitrificans* sp. nov., an efficient denitrifying bacterium isolated from activated sludge. *Int. J. Syst. Evol. Microbiol.* 51, 999-1006.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41, 95-98.

Kim, M.K., W.T. Im, H. Ohta, M. Lee, and S.T. Lee. 2005. *Sphingopyxis granuli* sp. nov., a β-glucosidase producing bacterium in the family *Sphingomonadaceae* in α-4 subclass of the *Proteobacteria*. *J. Microbiol.* 43, 152-157.

Kimura, M. 1983. The neutral theory of molecular evolution. Cambridge: Cambridge University Press, Cambridge, New York, N.Y., USA.

Kumar, S., K. Tamura, and M. Nei. 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform.* 5, 150-163.

Lim, Y.W., S.A. Lee, S.B. Kim, H.Y. Yong, S.H. Yeon, Y.K. Park, D.W. Jeong, and J.S. Park. 2005. Diversity of denitrifying bacteria isolated from Daejeon sewage treatment plant. *J. Microbiol.* 45, 383-390.

Mesbah, M., U. Premachandran, and W. Whitman. 1989. Precise measurement of the G+C content of deoxyribonucleic acid by

high performance liquid chromatography. *Int. J. Syst. Bacteriol.* 39, 159-167.

Moore, D.D. and D. Dowhan. 1995. Preparation and analysis of DNA, p. 2-11. *In* F.W. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl (eds.), Current protocols in molecular biology. Wiley, New York, N.Y., USA.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol*. 4, 406-425.

Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Technical Note 101. MIDI Inc., Newark, DE, USA.

Stackebrandt, E. and B.M. Goebel. 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.* 44, 846-849.

Tago, Y. and A. Yokota. 2004. *Comamonas badia* sp. nov., a floc-forming bacterium isolated from activated sludge. *J. Gen. Appl. Microbiol.* 50, 243-248.

Tamaoka, J., D.M. Ha, and K. Komagata. 1987. Reclassification of *Pseudomonas acidovorans* den Dooren de Jong 1926 and *Pseudomonas testosteroni* Marcus and Talalay 1956 as *Comamonas acidovorans* comb. nov. and *Comamonas testosteroni* comb. nov., with an emended description of the genus *Comamonas*. *Int. J. Syst. Bacteriol*. 37, 52-59.

Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin, and D.G. Higgins. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 24, 4876-4882.

Wauters, G., T. De Baere, A. Willems, E. Falsen, and M. Vaneechoutte. 2003. Description of *Comamonas aquatica* comb. nov. and *Comamonas kerstersii* sp. nov. for two subgroups of *Comamonas terrigena* and emended description of *Comamonas terrigena*. *Int. J. Syst. Evol. Microbiol.* 53, 859-862.

Wayne, L.G., D.J. Brenner, R.R. Colwell, P.A.D. Grimont, O. Kandler, M.I. Krichevsky, L.H. Moore, W.E.C. Moore, R.G.E. Murray, E. Stackebrandt, M.P. Starr, and H.G. Truper. 1987. International committee on systematic bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Bacteriol.* 37, 463-464.

Wen, A., M. Fegan, C. Hayward, S. Chakraborty, and L.I. Sly. 1999. Phylogenetic relationships among members of the *Comamonadaceae*, and description of *Delftia acidovorans* (den Dooren de Jong 1926 and Tamaoka *et al.* 1987) gen. nov., comb. nov. *Int. J. Syst. Bacteriol.* 49, 567-576.