Description of *Comamonas aquatica* comb. nov. and *Comamonas kerstersii* sp. nov. for two subgroups of *Comamonas terrigena* and emended description of *Comamonas terrigena*

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Three clusters of isolates have previously been defined within the species *Comamonas terrigena*, on the basis of DNA–rRNA and DNA–DNA hybridization data, and of protein electrophoretic patterns and immunotyping. More detailed characterization in the current study shows that representatives of these three groups can also be differentiated phenotypically from each other. Strains of *C. terrigena sensu stricto* (*C. terrigena* DNA group 1) are pyrrolidone aminopeptidase-positive, do not grow at 40 °C, are L-alanine-positive and are always negative for 4-hydroxybenzoate. Strains of *C. terrigena* DNA groups 2 and 3 are pyrrolidone aminopeptidase-negative; the former is the only group that is tyrosine-negative, and only the latter can grow at 42 °C (with an optimal growth temperature of 40 °C). These findings are corroborated by differences in 16S rDNA sequence and tRNA intergenic spacer lengths. Therefore, it is proposed to rename *C. terrigena* DNA group 2 [containing former *Aquaspirillum aquaticum* and E. Falsen (EF) group 10 strains] as *Comamonas aquatica* sp. nov., and *C. terrigena* DNA group 3 (containing former EF group 10 strains) as *Comamonas kerstersii* sp. nov.

Previously published data indicate that the species *Comamonas terrigena* actually comprises three genotypically separate groups. Willems *et al.* (1991) reported that three DNA groups could be recognized within *C. terrigena*; these three groups could also be recognized by immunotyping and whole-cell protein patterns, but could not be differentiated phenotypically. They were therefore not named as separate species, but maintained as genospecies within *C. terrigena*. Vaneechoutte *et al.* (1992) showed that these three DNA groups gave clearly different rDNA restriction digestion patterns, based on amplified 16S, spacer and the 5' part of 23S rDNA.

In a comparative study of SDS-PAGE whole-cell protein

patterns of *Comamonas* strains (Willems *et al.*, 1991), *Comamonas testosteroni* and *Comamonas acidovorans* (now *Delftia acidovorans*) were found to have distinct and characteristic protein patterns. For 46 *C. terrigena* strains, including E. Falsen (EF) group 10 strains and the monotype strain of *Aquaspirillum aquaticum*, more variable protein patterns were observed. By numerical analysis, they were grouped into four electropherovars, 1a and 1b, 2 and 3, which correspond to *C. terrigena* DNA groups 1, 2 and 3, respectively.

In immunodiffusion analysis with antisera against one or two representative strains of each of the DNA groups of *C. terrigena*, three groups were again easily distinguishable. Strains reacted strongly with the antiserum of their own DNA group, and not at all, or weakly to moderately, with antisera of other groups (Willems *et al.*, 1991).

Data obtained in the present study enable us to describe these three DNA groups as separate species. We propose to

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences reported in this paper are AJ430342–AJ430348.

Tables containing details of the strains used and 16S rDNA sequence similarity values are available as supplementary data in IJSEM Online.

Fig. 1. Rooted 16S rDNA sequence-based tree showing the relationship of the different *Comamonas* species. Cluster analysis, performed using GeneBase (Applied Maths), was based on the neighbour-joining method and *Delftia acidovorans* IAM 12409^T was used as the outgroup. The region used corresponds to *Escherichia coli* positions 45–1393. Bootstrap values are shown at the branching points (proportion of 100 resamplings). Sequences in bold were obtained during this study. Bar, 1% estimated sequence divergence.

rename *C. terrigena* DNA group 2 as *Comamonas aquatica* comb. nov., and *C. terrigena* DNA group 3 as *Comamonas kerstersii* sp. nov. The strains used in this study and the characteristics tested for each strain are available as supplementary data in IJSEM Online.

Sequencing of the 16S rRNA gene and DNA similarity calculations and cluster analysis of the sequences obtained together with sequences from GenBank were carried out as described previously (Vanechoutte *et al.*, 2000; Nemec *et al.*, 2001). Fig. 1 presents the 16S rDNA similarity tree, with *Delftia acidovorans* IAM 12409^T as the outgroup. The distance matrix is available as supplementary data in IJSEM Online. From these data, it is clear that the three DNA groups cluster separately and cannot be confused with *C. testosteroni* or the recently described species *Comamonas denitrificans* (Gumaelius *et al.*, 2001), *Comamonas nitrivorans* (Etchebehere *et al.*, 2001) or *Comamonas koreensis* (Chang *et al.*, 2002).

Biochemical characterization was carried out according to Gilligan & Whittier (1999); the API20NE and API ID 32 GN systems (bioMérieux) and susceptibility testing to desferrioxamine (Desferal) (Lindsay & Riley, 1991; Laffineur *et al.*, 2002) were also used. It was possible to differentiate the three groups from *C. testosteroni* and from each other using these methods (Table 1).

An important differential characteristic between the newly described species *C. denitrificans* (Gumaelius *et al.*, 2001) and *C. nitrivorans* (Etchebehere *et al.*, 2001) and the other *Comamonas* species is that both of the former species reduce nitrate to nitrogen. *C. koreensis* (Chang *et al.*, 2002) is the only non-motile *Comamonas* species described so far.

All three species described in the present study are Gram-negative rods that are motile by multitrichous polar flagella, often with bipolar tufts. They are aerobic and non-fermentative, do not acidify or assimilate sugars, are urease-negative, positive for nitrate reduction and negative for nitrite reduction. They are negative for the production

of indole and H₂S, do not hydrolyse gelatin and produce no lysine decarboxylase, ornithine decarboxylase or arginine dihydrolase. Aesculin is not hydrolysed. Phosphatase, trypsin and acidification of ethylene glycol (Laffineur *et al.*, 2002) give negative reactions. Citrate is not assimilated at 30 °C when tested with the ID 32 GN system (AUX medium; bioMérieux), but assimilation–alkalinization is variable on Simmons' citrate agar.

The major cellular fatty acids of the three species are C_{16:1}, C_{16:0} and C_{18:1ω9c}, but C_{10:0} 3-OH, C_{12:0} and C_{14:0} are also present, as well as small amounts of C_{15:0} and C_{17:0} cyclo. In contrast to *C. testosteroni*, C_{16:0} 2-OH is either not detected or is only present in trace amounts.

Table 1. Differential biochemical characteristics, as determined in this study, for *C. testosteroni*, *C. terrigena*, *C. aquatica* and *C. kerstersii*

Species: 1, *C. testosteroni* (n=14); 2, *C. terrigena* (n=8); 3, *C. aquatica* (n=11); 4, *C. kerstersii* (n=15). R, Resistant; S, susceptible; +, 100% positive; (+), positive (weak or delayed); –, 0% positive; v (variable), 72% positive.

Characteristic	1	2	3	4
Desferrioxamine	R	S	S	S
Testosterone utilization	+	–	–	–
Alkalinization on Simmons' base of glycine	+	–	–	–
Growth at:				
40 °C	–	–	(+)	+
42 °C	–	–	–	+
44 °C	–	–	–	+
Pyrrolidone aminopeptidase	+	+	–	–
Tyrosine hydrolysis	+	+	–	+
Assimilation on ID 32 GN of:				
3-Hydroxybenzoate	+	–	–	–
4-Hydroxybenzoate	+	–	v	+
L-Alanine	+	+	–	–

Amplification of tRNA intergenic spacers (Welsh & McClelland, 1991) and separation of the amplified DNA fragments by capillary electrophoresis (Vanechoutte *et al.*, 1998; Baele *et al.*, 2000, 2001) also makes it possible to differentiate between isolates of these three species and *C. testosteroni* and *Delftia acidovorans* (Table 2). A full database with all established tRNA PCR fingerprints is freely available upon request.

Given the previously published results, indicating the existence of three DNA hybridization groups within *C. terrigena* that are supported by serological and protein electrophoretic data (Willems *et al.*, 1991), and given the clear biochemical and genotypic (16S rDNA sequence and tRNA-PCR profile) differences between the three DNA groups as established in this study, we propose that *C. terrigena* actually comprises three separate species and that *C. terrigena* DNA group 2 should be renamed as *C. aquatica*, and *C. terrigena* DNA group 3 as *C. kerstersii*.

Description of *Comamonas aquatica* (Hylemon *et al.* 1973) comb. nov.

Comamonas aquatica (a.qua'ti.ca. L. fem. adj. *aquatica* living in water).

Basonym: *Aquaspirillum aquaticum* Hylemon *et al.* 1973.

Table 2. Summary of the lengths of the amplified tRNA intergenic spacer DNA fragments for the different species studied

Species: 1, *Delftia acidovorans* ($n=8$); 2, *C. terrigena* ($n=6$); 3, *C. aquatica* ($n=3$); 4, *C. kerstersii* ($n=20$); 5, *C. testosteroni* ($n=5$). Values are the number of isolates for which a tRNA intergenic spacer with this length was observed. Fragment lengths are means $\pm$ SD.

Fragment length (bp)	1	2	3	4	5
74.2 $\pm$ 0.3				14	
85.5 $\pm$ 0.3				20	
94.3 $\pm$ 0.5	7				
98.2 $\pm$ 0.3				13	
106.7 $\pm$ 0.2	8				
112.1 $\pm$ 0.3					5
113.2 $\pm$ 0.1				18	
128.6 $\pm$ 0.1			3		
129.2 $\pm$ 0.2				15	
138.7 $\pm$ 0.1		6			
190.9 $\pm$ 1.5					5
202.9 $\pm$ 0.2					3
213.5 $\pm$ 0.3				18	
255.4 $\pm$ 0.3	8				
273.5 $\pm$ 0.5					5
311.7 $\pm$ 0.7	8				
313.9 $\pm$ 0.8					3
374.8 $\pm$ 0.2	5				

Gram-negative rods, motile by multitrichous polar flagella, often with bipolar tufts. Grows aerobically on tryptic soy agar and broth at 30 and 35 °C. Moderate and delayed growth in broth may be observed at 40 °C; there is no growth at 42 °C. After 24 h incubation at 30 °C, colonies reach a diameter of 1–1.5 mm on blood agar. Oxidase and catalase activities are positive. Carbohydrates are neither acidified nor assimilated. Ethylene glycol is not acidified. Urease, indole and H₂S are not produced; negative for lysine decarboxylase, ornithine decarboxylase and arginine dihydrolase. Nitrates are reduced, but nitrites are not. Tyrosine and gelatin are not hydrolysed. Simmons' citrate agar test is positive. On Simmons' minimal agar base, L-alanine and glycine are neither assimilated nor alkalinized. Using ID 32 GN strips, propionate, valerate, 3-hydroxybutyrate, L-proline, itaconate, suberate, acetate and lactate are assimilated. Utilization of caprate and 4-hydroxybenzoate is variable. 3-Hydroxybenzoate and L-alanine are not utilized. Alkaline phosphatase, trypsin and pyrrolidone arylamidase are negative. Strains are susceptible to desferrioxamine and colistin. The main cellular fatty acids are C_{16:1}, C_{16:0}, C_{18:1}ω9c, C_{10:0} 3-OH, C_{12:0} and C_{14:0}. The DNA G + C content is 62.8–66.7 mol%.

The type strain is LMG 2370^T (=CCUG 15845^T = ATCC 11330^T). Its DNA G + C content is 64.0 mol%. The sequence of its 16S rDNA is available under EMBL/GenBank accession number AJ430344.

Description of *Comamonas kerstersii* sp. nov.

Comamonas kerstersii (ker.ster'si.i. N.L. masc. gen. n. *kerstersii* of Kersters, in honour of Karel Kersters, a Belgian microbiologist, for his contribution to bacterial taxonomy).

Gram-negative rods, motile by multitrichous polar flagella, often with bipolar tufts. Grows aerobically on tryptic soy agar and broth at 30, 35, 42 and 44 °C. Optimal growth occurs at 35–40 °C. After 24 h incubation at 30 °C, colonies reach a diameter of 1.5 mm on blood agar, and a diameter of more than 2 mm may be reached at 40 °C. Oxidase and catalase activities are positive. Carbohydrates are neither acidified nor assimilated. Ethylene glycol is not acidified. Urease, indole and H₂S are not produced; negative for lysine decarboxylase, ornithine decarboxylase and arginine dihydrolase. Nitrates are reduced but nitrites are not. Simmons' citrate agar test may be positive, but irregular and delayed. Tyrosine hydrolysis is positive but gelatin hydrolysis is negative. On Simmons' minimal agar base, L-alanine and glycine are neither assimilated nor alkalinized. Using ID 32 GN strips, valerate, 3-hydroxybutyrate, L-proline, itaconate, suberate, acetate, lactate and 4-hydroxybenzoate are assimilated. Utilization of caprate is variable. L-Alanine and 3-hydroxybenzoate are negative. Alkaline phosphatase, benzylarginine arylamidase and pyrrolidone arylamidase are negative. Strains are susceptible to desferrioxamine and colistin. The main cellular fatty acids are C_{16:1}, C_{16:0}, C_{18:1}ω9c, C_{10:0} 3-OH, C_{12:0} and C_{14:0}. DNA G + C content is 59.7–63.3 mol%.

The type strain is LMG 3475^T (=CCUG 15333^T=AF61^T). Its DNA G+C content is 61.0 mol%. The sequence of its 16S rDNA is available under EMBL/GenBank accession number AJ430347.

Emended description of *Comamonas terrigena sensu stricto* (ex Hugh 1962) De Vos et al. 1985

Gram-negative rods, motile by multitrichous polar flagella, often with bipolar tufts. Grows aerobically on tryptic soy agar and broth at 30 and 35 °C, but not at 40 °C. After 24 h incubation at 30 °C, colonies reach a diameter of 1 mm on blood agar. Oxidase and catalase activities are positive. Carbohydrates are neither acidified nor assimilated. Ethylene glycol is not acidified. Urease, indole and H₂S are not produced; negative for lysine decarboxylase, ornithine decarboxylase and arginine dihydrolase. Nitrates are reduced but nitrites are not. Simmons' citrate agar test is positive, but may be delayed. Tyrosine hydrolysis is positive but gelatin hydrolysis is negative. On Simmons' minimal agar base, L-alanine is assimilated and alkalinized but glycine is not. Using ID 32 GN strips, propionate, valerate, 3-hydroxybutyrate, L-proline, suberate, acetate, lactate and L-alanine are assimilated. Utilization of itaconate and caprate is variable. 3-Hydroxybenzoate and 4-hydroxybenzoate are always negative. Alkaline phosphatase and benzylarginine arylamidase activities are negative but pyrrolidone arylamidase is positive. Strains are susceptible to desferrioxamine and colistin. The main cellular fatty acids are C_{16:1}, C_{16:0}, C_{18:1}ω9c, C_{10:0} 3-OH, C_{12:0} and C_{14:0}. DNA G+C content is 64.0–66.6 mol%.

The type strain is LMG 1253^T (=CCUG 15327^T=NCIB 8193^T). Its DNA G+C content is 64.0 mol%. The sequence of its 16S rDNA is available under EMBL/GenBank accession number AJ430342.

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