Castellaniella gen. nov., to accommodate the phylogenetic lineage of *Alcaligenes defragrans*, and proposal of *Castellaniella defragrans* gen. nov., comb. nov. and *Castellaniella denitrificans* sp. nov.

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Comparative 16S rRNA gene sequence analysis indicates that two distinct sublineages exist within the genus *Alcaligenes*: the *Alcaligenes faecalis* lineage, comprising *Alcaligenes aquatilis* and *A. faecalis* (with the three subspecies *A. faecalis* subsp. *faecalis*, *A. faecalis* subsp. *para-faecalis* and *A. faecalis* subsp. *phenolicus*), and the *Alcaligenes defragrans* lineage, comprising *A. defragrans*. This phylogenetic discrimination is supported by phenotypic and chemotaxonomic differences. It is proposed that the *A. defragrans* lineage constitutes a distinct genus, for which the name *Castellaniella* gen. nov. is proposed. The type strain for *Castellaniella defragrans* gen. nov., comb. nov. is 54Pin^T (= CCUG 39790^T = CIP 105602^T = DSM 12141^T). Finally, on the basis of data from the literature and new DNA–DNA hybridization and phenotypic data, the novel species *Castellaniella denitrificans* sp. nov. (type strain NKNTAU^T = DSM 11046^T = CCUG 39541^T) is proposed for two strains previously identified as strains of *A. defragrans*.

The genus *Alcaligenes* was first described by Castellani & Chalmers (1919). After numerous reclassifications, the authentic genus now encompasses three species: the type species of the genus, *Alcaligenes faecalis*, with the three subspecies *A. faecalis* subsp. *faecalis*, *A. faecalis* subsp. *para-faecalis* (Schroll *et al.*, 2001) and *A. faecalis* subsp. *phenolicus* (Rehfuß & Urban, 2005), *Alcaligenes aquatilis* (Van Trappen *et al.*, 2005) and *Alcaligenes defragrans* (Foss *et al.*, 1998). The species *Alcaligenes latus* has been shown to belong to the family *Comamonadaceae* (Coenye *et al.*, 2003b), and its transfer to the novel genus *Azohydromonas* was proposed recently (Xie & Yokota, 2005). Strains of *A. defragrans* have been isolated from soil and are capable of using alkenoic monoterpenes as sole carbon sources (Foss *et al.*, 1998). Additional isolates (strains TJ4 and NKNTAU^T), presumptively identified as *A. defragrans*, have been shown to

degrade phenol (Baek *et al.*, 2003) and taurine (Denger *et al.*, 1997; Ruff *et al.*, 2003) under denitrifying conditions.

Members of the authentic genus *Alcaligenes* are Gram-negative, strictly aerobic rods or coccobacilli that are motile. They possess oxidase and catalase. They grow well on complex media such as nutrient agar. The predominant fatty acids are C_{16:0}, C_{16:1}ω7c, C_{17:0} cyclo, C_{18:1}ω7c and/or C_{14:0} 3-OH (Foss *et al.*, 1998; Schroll *et al.*, 2001; Coenye *et al.*, 2003b). *A. faecalis* has been reported to possess, among its polar lipids, an ornithine lipid (Yabuuchi *et al.*, 1995). It has been shown in a previous report (Stolz *et al.*, 2005), however, that the fatty acid profiles and polar lipids of *A. defragrans* differ from those of the subspecies of *A. faecalis*. In order to establish the exact taxonomic position of *A. defragrans*, we included the type strain of *A. defragrans* in our polyphasic taxonomic study on strains NKNTAU^T and TJ4.

A. defragrans DSM 12141^T was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany); isolation of strains NKNTAU^T and TJ4 was described previously (Denger *et al.*, 1997; Baek

Abbreviations: pNA, *p*-nitroanilide; pNP, *p*-nitrophenyl.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains NKNTAU^T and TJ4 are U82826 and AF508102, respectively.

et al., 2003). Subculturing was done on nutrient agar (Oxoid) at 30 °C for 24 h. Gram-staining was performed as described by Gerhardt *et al.* (1994). Cell morphology was examined with a Zeiss light microscope at 1000 × magnification, using cells that had been grown for 24 h at 30 °C on nutrient agar. The results regarding cell morphology are given in the formal species descriptions. The 16S rRNA gene was analysed as described previously (Kämpfer *et al.*, 2003). Analysis of sequence data was performed using the software package MEGA, version 2.1 (Kumar *et al.*, 2001) after multiple alignment of the data using CLUSTAL X (Thompson *et al.*, 1997). A distance-matrix method (with distance options according to the Kimura two-parameter model) was employed, using clustering obtained with the neighbour-joining method (Fig. 1) as well as a discrete character-based maximum-parsimony method (data not shown). For each method, bootstrap values were calculated on the basis of 1000 replications. Sequence-similarity calculations indicated that strains NKNTAU^T and TJ4 are closely related (99.9%). The highest level of 16S rRNA gene sequence similarity (98.2%) to a species with a validly published name was found with *A. defragrans* (Foss *et al.*, 1998). The next most similar species were *Pusillimonas noertemannii* (<96.5%) and *A. faecalis* (all subspecies) (<94%).

Chemotaxonomic analyses were performed as follows: respiratory quinones (Tindall, 1990), polar lipids (Tindall, 1990) and fatty acids (Kämpfer & Kroppenstedt, 1996). Quinones, polar lipids and polyamines were extracted from biomass grown for 72 h on PYE medium (0.3% yeast extract, 0.3% peptone from casein, pH 7.2). The quinone system of *A. defragrans* DSM 12141^T, strain NKNTAU^T and strain TJ4 consisted predominantly of ubiquinone Q-8

(98%), with small amounts of Q-7 and Q-9. The three strains exhibited a homogeneous polyamine pattern consisting predominantly of putrescine [48–70 µmol (g dry weight)⁻¹], with moderate amounts of 2-hydroxyputrescine [5–9 µmol (g dry weight)⁻¹] and minor amounts of 1,3-diaminopropane [<0.5 µmol (g dry weight)⁻¹], cadaverine [<0.2 µmol (g dry weight)⁻¹], spermidine [<2.5 µmol (g dry weight)⁻¹], *sym*-homospermidine [<0.1 µmol (g dry weight)⁻¹] and spermine [<0.5 µmol (g dry weight)⁻¹]. Both the quinone systems and polyamine patterns are in excellent agreement with those of members of the *Beta-proteobacteria* (Busse & Auling, 1988; Auling *et al.*, 1991; Hamana & Takeuchi, 1998).

Strains TJ4 and NKNTAU^T exhibited identical polar lipid profiles, containing a predominance of phosphatidylethanolamine, moderate amounts of diphosphatidylglycerol and phosphatidylglycerol and minor amounts of two unknown aminolipids (AL3 and AL1) and an unknown polar lipid (L7) (data not shown). These lipids were also detected in *A. defragrans* DSM 12141^T (Stolz *et al.*, 2005). However, the polar lipid compositions of the two strains were less complex than those of *A. defragrans* DSM 12141^T. Hence, the polar lipid profiles appear to be useful for the differentiation of TJ4 and NKNTAU^T from *A. defragrans* DSM 12141^T.

The fatty acid profiles of strains NKNTAU^T and TJ4 are given in Table 1. The predominant fatty acids are C_{16:0}, C_{16:1ω7c}, C_{17:0} cyclo, C_{18:1ω7c} and/or C_{14:0} 3-OH. The fatty acid C_{12:0} 3-OH, present in all subspecies of *A. faecalis*, could not be detected in strain DSM 12141^T or in strains NKNTAU^T and TJ4.

Fig. 1. Phylogenetic analysis, based on 16S rRNA gene sequences available from the EMBL database (accession numbers are given in parentheses), constructed after multiple alignment of data by CLUSTAL X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura-2 model) and clustering with the neighbour-joining method were obtained by using the software package MEGA, version 2.1 (Kumar *et al.*, 2001). Bootstrap values based on 1000 replications are shown as percentages at the branching points. Bar, 2 substitutions per 100 nucleotide positions.

Table 1. Relative fatty acid compositions and G+C contents of species of the genera *Alcaligenes*, *Castellaniella* gen. nov., *Pusillimonas*, *Achromobacter*, *Pigmentiphaga* and *Kerstersia*

Species/strains: 1, *Alcaligenes faecalis* (data from Coenye *et al.*, 2003b); 2, *C. defragrans* DSM 12141^T; 3, *C. denitrificans* NKNTAU^T; 4, *C. denitrificans* TJ4 (data in columns 2–4 from this study); 5, *Pusillimonas noeremanni* (Stolz *et al.*, 2005); 6, *Achromobacter xylosoxidans*; 7, *Achromobacter denitrificans*; 8, *Achromobacter piechaudii* (data in columns 6–8 from Coenye *et al.*, 2003b); 9, *Achromobacter insolitus* (Coenye *et al.*, 2003a); 10, *Achromobacter spanius* (Coenye *et al.*, 2003a); 11, *Pigmentiphaga kullae* (Blümel *et al.*, 2001); 12, *Kerstersia gyiorum* (Coenye *et al.*, 2003b). C_{10:0} 3-OH, 3-Hydroxydecanoic acid; C_{12:0}, dodecanoic acid (=lauric acid); C_{12:0} 2-OH, 2-hydroxydodecanoic acid; C_{14:0}, tetradecanoic acid (=myristic acid); C_{14:0} 2-OH, 2-hydroxytetradecanoic acid; C_{14:0} 3-OH, 3-hydroxytetradecanoic acid; C_{16:1}ω7c, *cis*-9-hexadecenoic acid (=palmitoleic acid); C_{16:0}, hexadecanoic acid (=palmitic acid); C_{17:0} cyclo, Δ-*cis*-9,10-methylenehexadecanoic acid; C_{16:0} 2-OH, 2-hydroxyhexadecanoic acid; C_{18:1}ω7c, *cis*-11-octadecenoic acid (=vaccenic acid); C_{18:0}, octadecanoic acid (=stearic acid); C_{19:0} cyclo ω8c, Δ-*cis*-11,12-methyleneoctadecanoic acid. —, Not present; tr, trace amount present; ND, not determined. The presence or absence of fatty acids shown in bold is of particular importance for differentiation at the genus level among the listed genera. The relative amounts of all listed fatty acids are also helpful for differentiation. Results from this study were obtained from cells grown on TS agar (Oxoid) at 30 °C for 24 h prior to analysis. *C. defragrans* DSM 12141^T contained small amounts of C_{12:0} aldehyde (2.8%), C_{15:0} (0.4%), C_{17:0} (0.3%) and C_{19:0} iso (0.3%). Strain TJ4 contained small amounts of an unknown fatty acid (1.8%), C_{15:0} (1.4%), C_{17:0} (0.6%) and C_{19:0} iso (0.7%).

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Fatty acids												
Dodecanal	—	0.1	—	—	tr	—	—	—	—	—	—	—
C _{10:0} 3-OH	—	—	—	—	—	—	—	—	—	—	2.9	—
C_{12:0}	2.1	5.6	5.6	6.2	4.0	tr	tr	tr	1.0	tr	—	—
C_{12:0} 2-OH	2.3	—	—	—	3.0	3.5	2.5	3.4	3.8	4.6	—	tr
C _{14:0}	1.4	0.4	0.4	0.7	—	1.1	5.3	5.6	5.6	4.2	—	5.7
C_{14:0} 2-OH	—	—	—	—	—	4.0	—	—	—	—	4.0	4.3
C _{14:0} 3-OH	10.4 ^{a*}	5.0	7.6	6.3	8.0 ^a	10.6 ^a	8.6 ^a	9.6 ^a	12.2 ^a	12.5 ^a	4.6 ^a	12.1 ^a
C _{16:1} ω7c	7.1	27.9	27.4	18.7	tr	9.7	17.4	5.3	10.0	18.1	—	3.9
C _{16:0}	36.8	31.8	33.9	32.5	19.0	38.9	39.7	34.3	35.3	28.8	39.9	33.8
C_{17:0} cyclo	28.8	6.7	6.5	15.7	31.0	24.4	17.3	31.8	22.9	20.0	21.9	23.6
C _{16:0} 2-OH	—	—	—	—	tr	tr	tr	1.3	tr	2.4	4.8	—
C _{18:1} ω7c	3.9	17.7 ^c	15.3 ^c	13.2 ^c	tr ^b	1.8 ^b	3.3	4.3 ^c	3.5	5.2	9.8 ^c	10.7
C_{18:0}	tr	—	—	—	3.0	2.0	1.8	1.8	2.1	2.1	—	2.6
C_{19:0} cyclo ω8c	tr	0.3	0.3	2.1	27.0	tr	tr	tr	tr	—	12.2	2.0
DNA G+C content (mol%)	55.9–59.4	66.9	ND	ND	61.8	66.0–69.8	63.9–68.9	64.0–65.0	64.9–65.5	64.9	68.5	62.9

*Fatty acids listed as the following summed features: *a*, summed feature 3 (C_{16:1} iso I and/or C_{14:0} 3-OH); *b*, C_{18:1}ω7c and/or C_{18:1}ω9t; *c*, summed feature 7 (C_{18:1}ω7c, C_{18:1}ω9t and/or C_{18:1}ω12t).

The results of the physiological and biochemical characterizations (performed using methods described previously; Kämpfer *et al.*, 1991; Schroll *et al.*, 2001) are given in the species description. All of the strains under study were able to utilize a number of organic acids and amino acids. Sugars and sugar alcohols (polyols) were not utilized. Table 2 shows the differentiating characteristics.

DNA–DNA hybridization experiments were performed with both strains (NKNTAU^T and TJ4) and with the type strain of *A. defragrans*, DSM 12141^T, using the method described by Ziemke *et al.* (1998), except that for nick translation, 2 µg DNA was labelled during a 3 h incubation at 15 °C. Strains NKNTAU^T and TJ4 showed DNA–DNA relatedness values of 80 % (reciprocal analysis, 74 %), clearly indicating that the two strains belong to the same species.

Strains NKNTAU^T and TJ4 showed relatively low levels of DNA–DNA relatedness to *A. defragrans* DSM 12141^T (30 %, reciprocal 43 %; 43 %, reciprocal 37 %, respectively). The standard deviation for all of the DNA–DNA hybridizations was 8.5 %. These results clearly showed that NKNTAU^T and TJ4 are members of a single species that is separate from *A. defragrans*.

On the basis of the phylogenetic and chemotaxonomic data, *A. defragrans* is clearly different from strains belonging to the genus *Alcaligenes*. For this reason, a novel genus is proposed for the *A. defragrans* lineage. Moreover, detailed investigations on strains NKNTAU^T and TJ4 show that, on the basis of genotypic and phenotypic results, the two strains represent a novel species of the new genus, for which the name *Castellaniella* gen. nov. is proposed.

Table 2. Physiological characteristics of *Castellaniella* strains

Strains: 1, *C. defragrans* DSM 12141^T; 2, *C. denitrificans* NKNTAU^T; 3, *C. denitrificans* TJ4. +, Positive; −, negative; (+), weakly positive. All strains were positive for the hydrolysis of L-alanine *p*-nitroanilide (pNA) and bis-*p*-nitrophenyl (pNP) phosphate on the basis of the method described by Kämpfer *et al.* (1991). The following compounds are not hydrolysed: pNP β-D-galactopyranoside, pNP β-D-glucuronide, pNP α-D-glucopyranoside, pNP β-D-glucopyranoside, pNP β-D-xylopyranoside, bis-pNP phenylphosphonate, bis-pNP phosphorylcholine, L-aniline pNA, γ-L-glutamate pNA and L-proline pNA. On the basis of the method described by Kämpfer *et al.* (1991), the following compounds are used as sole sources of carbon by all of the strains: acetate, propionate, *cis*-aconitate, 4-aminobutyrate, citrate, fumarate, glutarate, DL-3-hydroxybutyrate, DL-lactate, L-malate, 2-oxoglutarate, pyruvate, L-alanine, β-alanine, L-aspartate, L-leucine, L-proline and L-serine. The following compounds are not assimilated: *N*-acetylgalactosamine, *N*-acetylglucosamine, L-arabinose, L-arbutin, D-cellobiose, D-fructose, D-galactose, D-glucose, D-maltose, D-mannose, α-D-melibiose, L-rhamnose, D-ribose, D-sucrose, salicin, D-trehalose, D-xylose, adonitol, inositol, maltitol, D-mannitol, D-sorbitol, putrescine, adipate, azelate, suberate, L-histidine, L-phenylalanine, L-serine, L-tryptophan, 3-hydroxybenzoate and phenylacetate. No acids are produced from glucose, lactose, sucrose, D-mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, L-arabinose, raffinose, rhamnose, maltose, D-xylose, trehalose, cellobiose, methyl D-glucoside, erythritol, melibiose, D-arabitol or D-mannose.

Characteristic	1	2	3
Assimilation of:			
trans-Aconitate	−	+	+
Itaconate	−	+	+
Mesaconate	−	+	+
D-Gluconate	+	(+)	(+)
Glutarate	(+)	(+)	+
L-Ornithine	+	(+)	(+)

Description of *Castellaniella* gen. nov.

Castellaniella (Cas.tel.la'ni.el.la. N.L. fem. dim. n. *Castellaniella* named after Sir Aldo Castellani, a British–Italian bacteriologist, who first described the bacterial genus *Alcaligenes* in 1919).

Gram-negative, facultatively anaerobic and denitrifying. Cells are motile, short rods, 1.3–2.0 μm long and 0.2–0.8 μm wide. Good growth occurs after 24 h incubation on nutrient agar at 25–30 °C. The polar lipid profile contains the major compound phosphatidylethanolamine, moderate amounts of phosphatidylglycerol and diphosphatidylglycerol and minor amounts of two unknown aminolipids and an unknown polar lipid. The polyamines consist predominantly of putrescine with moderate amounts of 2-hydroxyputrescine; the main component of the quinone system is ubiquinone Q-8. The major fatty acids are C_{16:0}, C_{16:1ω7c}, C_{17:0} cyclo, C_{18:1ω7c} and/or C_{14:0} 3-OH. The DNA G+C content of the type species, *Castellaniella*

defragrans, is 66.9 mol%. As currently circumscribed, the genus contains two species, *Castellaniella defragrans* and *Castellaniella denitrificans*.

Description of *Castellaniella defragrans* (Foss *et al.* 1998) comb. nov.

Basonym: *Alcaligenes defragrans* Foss *et al.* 1998.

The description is essentially that of Foss *et al.* (1998), with the addition of the information obtained in this study. In addition to the characteristics listed in the genus description, the polar lipid profile contains minor amounts of three unknown aminolipids, two unknown phospholipids, two unknown aminophospholipids and four unknown polar lipids (Stolz *et al.*, 2005). The polyamine pattern consists of major amounts of putrescine [69.9 μmol (g dry weight)^{−1}], moderate amounts of 2-hydroxyputrescine [9.2 μmol (g dry weight)^{−1}], minor amounts of spermidine [2.4 μmol (g dry weight)^{−1}] and traces [<0.5 μmol (g dry weight)^{−1}] of 1,3-diaminopropane, cadaverine, *sym*-homospermidine and spermine. The quinone system comprises ubiquinone Q-8. The type strain is strain 54Pin^T (=CCUG 39790^T=CIP 105602^T=DSM 12141^T=LMG 18538^T).

Description of *Castellaniella denitrificans* sp. nov.

Castellaniella denitrificans [de.ni.tri.fi'cans. N.L. part. adj. *denitrificans* (from N.L. v. *denitrifico*) denitrifying].

General characteristics are as described for the genus. On nutrient agar, colonies are beige and circular with an entire margin. Grows at 30, 37 and 42 °C; no growth at 4 °C. On the basis of the method described by Kämpfer *et al.* (1991), L-alanine *p*-nitroanilide (pNA) is hydrolysed. The following compounds are not hydrolysed: *p*-nitrophenyl (pNP) β-D-galactopyranoside, pNP β-D-glucuronide, pNP α-D-glucopyranoside, pNP β-D-glucopyranoside, pNP β-D-xylopyranoside, bis-pNP phosphate, bis-pNP phenylphosphonate, bis-pNP phosphorylcholine, L-aniline pNA, γ-L-glutamate pNA and L-proline pNA. On the basis of the method described by Kämpfer *et al.* (1991), the following compounds are used as sole sources of carbon: D-gluconate (weak), acetate, propionate, *cis*-aconitate, *trans*-aconitate, 4-aminobutyrate, citrate, fumarate, glutarate, DL-3-hydroxybutyrate, itaconate, DL-lactate, L-malate, mesaconate, 2-oxoglutarate, pyruvate, L-alanine, β-alanine, L-aspartate, L-leucine, L-ornithine, L-proline and L-serine. The following compounds are not assimilated: *N*-acetylgalactosamine, *N*-acetylglucosamine, L-arabinose, L-arbutin, D-cellobiose, D-fructose, D-galactose, D-glucose, D-maltose, D-mannose, α-D-melibiose, L-rhamnose, D-ribose, D-sucrose, salicin, D-trehalose, D-xylose, adonitol, i-inositol, maltitol, D-mannitol, D-sorbitol, putrescine, adipate, azelate, suberate, L-histidine, L-phenylalanine, L-serine, L-tryptophan, 3-hydroxybenzoate and phenylacetate. No acids are produced from glucose, lactose, sucrose, D-mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, L-arabinose, raffinose, rhamnose, maltose,

D-xylose, trehalose, cellobiose, methyl D-glucoside, erythritol, melibiose, D-arabitol or D-mannose. The quinone system, the polyamine pattern and the polar lipid profile are identical to those listed in the genus description. The polyamine pattern contains putrescine [$48.1\text{--}53.1\text{ }\mu\text{mol (g dry weight)}^{-1}$], moderate amounts of 2-hydroxyputrescine [$5.3\text{--}5.7\text{ }\mu\text{mol (g dry weight)}^{-1}$], spermidine [$0.7\text{--}1.3\text{ }\mu\text{mol (g dry weight)}^{-1}$] and traces [$<0.5\text{ }\mu\text{mol (g dry weight)}^{-1}$] of 1,3-diaminopropane, cadaverine, *sym*-homospermidine and spermine.

The type strain, NKNTAU^T (=DSM 11046^T=CCUG 39541^T), was isolated from an anaerobic enrichment culture with taurine as sole source of carbon and an inoculum from an anaerobic sludge digester (Denger *et al.*, 1997). Isolate TJ4, which represents a second strain of this species, was isolated from an environmental sample in Korea, enriched under denitrifying conditions with phenol as the sole source of carbon (Baek *et al.*, 2003).

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