Taxonomic dissection of *Achromobacter denitrificans* Coenye *et al.* 2003 and proposal of *Achromobacter agilis* sp. nov., nom. rev., *Achromobacter pestifer* sp. nov., nom. rev., *Achromobacter kerstersii* sp. nov. and *Achromobacter deleyi* sp. nov.

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The phenotypic and genotypic characteristics of a historical collection of strains identified as *Achromobacter denitrificans* were examined. Sequence analysis of a 765 bp *nrdA* gene fragment revealed that eight of these strains belonged to the recently described *Achromobacter aegrifaciens, Achromobacter mucicolens,* and *Achromobacter insolitus*, and that one strain belonged to *Achromobacter xylosoxidans.* The analysis also suggested the presence of four novel species of the genus *Achromobacter* among the remaining strains. The latter was confirmed by multilocus sequence analysis of concatenated *nusA, eno, rpoB, gltB, lepA, nuoL* and *nrdA* gene fragments and extensive genotypic and phenotypic characterization. We propose to name these novel species as *Achromobacter agilis* sp. nov., nom. rev. (type strain LMG 3411^T=CCUG 62454^T), *Achromobacter pestifer* sp. nov., nom. rev. (type strain LMG 3431^T=CCUG 61959^T) , *Achromobacter kerstersii* sp. nov. (type strain LMG 3441^T=CCUG 62449^T) and *Achromobacter deleyi* sp. nov. (type strain LMG 3458^T=CCUG 62433^T).

Introduction

Achromobacter denitrificans occurs in soil but can occasionally also be found in human clinical samples (Coenye *et al.*, 2003a). The taxonomy of *Achromobacter* strains has been studied by J. De Ley and K. Kersters (Ghent University, Belgium) since the early 1960s (De Ley *et al.*, 1967, 1970; Kersters, 1978) and led to an extensive characterization of these organisms in the first edition of *Bergey's Manual of Systematic Bacteriology* in 1984. Yet, the taxonomic criteria used in the early 1980s are not the ones we use today as is also apparent in an early DNA–DNA hybridization study where phenotypic consistency rather than high DNA–DNA hybridization level was used to

Abbreviations: MLSA, multilocus sequence analysis; STs, sequence types.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Achromobacter agilis* LMG 3411^T, *Achromobacter pestifer* LMG 3431^T, *Achromobacter kerstersii* LMG 3441^T and *Achromobacter deleyi* LMG 3458^T are HG324050-HG324053, respectively. All MLSA and *nrdA* sequences are available from the *Achromobacter* database at http://pubmlst.org/.

A supplementary figure is available with the online Supplementary Material.

delineate these species (Kiredjian *et al.*, 1981). In the latter study, at least four hybridization groups within strains classified as *Achromobacter denitrificans* were reported (Kiredjian *et al.*, 1981).

We recently developed a multilocus sequence analysis (MLSA) scheme based on *nusA, eno, rpoB, gltB, lepA, nuoL* and *nrdA* sequence analysis and validated 2.1 % concatenated sequence divergence as a threshold value for species delineation in this genus. In the present study, we used this MLSA scheme to reassess the classification of a historical collection of *Achromobacter denitrificans* strains not examined earlier by modern taxonomic methods.

Methods

Isolation, morphological, physiological and biochemical characteristics. The strains studied and their identifications, which resulted from the present study are shown in Table 1. These included 20 strains that were sent to J. De Ley and K. Kersters in the 1960s and 1970s. Data from the present study demonstrate that several of these strains represent novel species and, therefore, additional recent isolates from our own strain collections that represent these same novel species were included as well. Strains were grown aerobically on trypticase soy agar (TSA; BBL) at 28 °C unless otherwise indicated. Conventional phenotypic tests were performed as described previously (Vandamme *et al.*, 1993). API 20NE and API ZYM Microtest systems (bioMérieux) were performed according to the recommendations of the manufacturer. Electron microscopic analysis of strains LMG 3411^T, LMG 3431^T, LMG 3441^T and LMG 3458^T was performed, as described previously (Vandamme *et al.*, 2013a).

Cellular fatty acid analysis. For cellular fatty acid methyl ester analysis, strains were incubated for 24 h at 28 °C. A loopfull of well-grown cells was harvested and fatty acid methyl esters were prepared as described previously (Vandamme *et al.*, 1992), and separated and identified using the Sherlock Microbial Identification System (version 3.1, MIDI Inc.).

16S rRNA gene sequencing, MLSA and DNA G+C content analysis. DNA for performing PCR assays was prepared by heating one or two colonies at 95 °C for 15 min in 20 µl lysis buffer containing 0.25 % (w/v) SDS and 0.05 M NaOH. Following lysis, 180 µl distilled water was added to the lysate. The 16S rRNA gene sequences of strains LMG 3411^T, LMG 3431^T, LMG 3441^T and LMG 3458^T were determined; 16S rRNA gene amplification, purification and sequencing were performed as described previously (Vandamme *et al.*, 2007). Sequence assembly was performed using BioNumerics version 7.5.

PCR amplification and sequencing of *nusA, eno, rpoB, gltB, lepA, nuoL* and *nrdA* gene fragments was performed as described before (Spilker *et al.*, 2012); data for *Achromobacter denitrificans* LMG 1231^T, LMG 1860 and R-50535 (=AU13161) were taken from Spilker *et al.* (2012). Amplification and sequencing of a 765 bp *nrdA* gene fragment was performed as described before (Spilker *et al.*, 2013). Gene number assignments to each unique allele and assignments of sequence types to each unique allelic profile were done using the *Achromobacter* pubMLST database (Jolley & Maiden, 2010); all sequences are publicly available at www.pubmlst.org/achromobacter. A phylogenetic tree of the concatenated sequences (2249 bp) of seven housekeeping gene fragments [*nusA* (355 bp), *eno* (214 bp), *rpoB* (413 bp), *gltB* (241 bp), *lepA* (347 bp), *nuoL* (230 bp) and *nrdA* (449 bp)] was reconstructed using MEGA6 software (Tamura *et al.*, 2013). Between-taxon divergence values were determined as described before (Spilker *et al.*, 2012). Additionally, a phylogenetic tree was also reconstructed based on *nrdA* sequences (765 bp).

High-molecular weight DNA was prepared as described by Pitcher *et al.* (1989). Analysis of the DNA base ratio of strains LMG 3411^T, LMG 3431^T, LMG 3441^T and LMG 3458^T was determined as described by Mesbah *et al.* (1989). DNA was enzymically degraded to nucleosides. The obtained nucleoside mixture was then separated by HPLC using a Waters Symmetry Shield C8 column thermostated at 37 °C. The solvent was 0.02 M $NH_4H_2PO_4$ (pH 4.0) with 1.5 % acetonitrile. Non-methylated Lambda phage (Sigma) was used as the calibration reference.

Results and discussion

Sequence analysis of a 765 bp *nrdA* gene fragment (referred to below as *nrdA*_765) has been proposed as a single-locus-based identification method for species of the genus *Achromobacter* (Spilker *et al.*, 2013). Fig. 1 shows an *nrdA*_765-based phylogenetic tree of all strains examined in the present study and of *Achromobacter* reference strains (data were taken from the PubMLST website). The *nrdA*_765 allele types and multilocus sequence types (STs) of all strains examined in the present study are presented in Table 1. The *nrdA*_765-based phylogenetic analysis assigned none of the historical *Achromobacter denitrificans* strains, other than strains LMG 1231^T and LMG 1860 which were examined previously (Spilker *et al.*, 2012), to *Achromobacter denitrificans sensu stricto*; compared to our previous study (Spilker *et al.*, 2012), only a single isolate (LMG 26680) that was recently obtained from sputum of a cystic fibrosis patient in Belgium was identified as an additional *Achromobacter denitrificans sensu stricto* strain. Four of the historical *Achromobacter denitrificans* strains (i.e. LMG 3342, LMG 3405, LMG 3520 and LMG 5885) were identified as *Achromobacter insolitus* (Coenye *et al.*, 2003b), three (i.e. strains LMG 3396, LMG 3410 and LMG 3467) as *Achromobacter aegrifaciens* (Vandamme *et al.*, 2013b), one (i. e. LMG 3415) as *Achromobacter mucicolens* (Vandamme *et al.*, 2013a), and one (i.e. LMG 3414) as *Achromobacter xylosoxidans*. Two single strains, LMG 3411^T and LMG 3431^T, and two groups of isolates clustered separately from all reference strains (Fig. 1). A first group comprised two of the historical strains of *Achromobacter denitrificans* (i.e. LMG 3441^T and LMG 3442), along with recent soil isolates from Belgium and Peru; a second group comprised five historical strains including LMG 3458^T and a recent isolate from a cystic fibrosis sputum sample from the USA.

MLSA was performed for these thirteen strains that clustered separately from all reference strains (Fig. 1), and for *Achromobacter denitrificans* LMG 26680, and all but two (i.e. LMG 3481 and LMG 3482) represented different sequence types (Table 1). The grouping of the thirteen strains in the concatenated MLSA tree reflected that of the *nrdA*_765-based phylogenetic tree including the distinct clustering of the two unique strains (LMG 3411^T and LMG 3431^T) and the two groups represented by strains LMG 3441^T and LMG 3458^T (Fig. 2). Analysis of concatenated sequence divergence of the two unique strains (LMG 3411^T and LMG 3431^T) and the two groups represented by strains LMG 3441^T and LMG 3458^T towards their respective nearest neighbour species (i.e.

Table 1. Strains included in the present study

Strain	Other strain designations	ST	*nrdA_765*	Depositor	Source
Achromobacter denitrificans					
LMG 1231^{T}*	CCUG 407^{T}, ATCC 15173^{T}	102	2	ATCC	Soil (before 1966)
LMG 1860*	CCUG 61949, CIP 60.83	103	3	CIP	Urine (before 1966, France)
LMG 26680	CCUG 61950, P47052	190	1	O. Denis	Human sputum, cystic fibrosis patient (2004, Belgium)
R-50535*	AU13161	102	1	Own isolate	Human sputum, non-cystic fibrosis patient (2007, USA)
Achromobacter agilis sp. nov., nom. rev.					
LMG 3411^{T}	CCUG 62454^{T}, NCIB 9986^{T}	140	96	Torry Research Station	(Before 1967)
Achromobacter pestifer sp. nov., nom. rev					
LMG 3431^{T}	CCUG 61959^{T}, ATCC 15445^{T}	136	93	M. Popoff	Oil-brine (before 1967)
Achromobacter kerstersii sp. nov.					
LMG 3441^{T}	CCUG 62449^{T}, Holding CS8^{T}	138	94	A. Holding	Soil (before 1968)
LMG 3442	CCUG 62450, Holding CS11	139	95	A. Holding	Soil (before 1968)
LMG 26862	R-47032, CCUG 62451, MP2.5	195	144	T. Coenye	Grass rhizosphere soil (2010, Belgium)
R-41872	P1-12/08	193	94	A. Oswald	Soil (2008, Peru)
R-43761	A3- 51/08 t2	194	145	A. Oswald	Soil (2008, Peru)
Achromobacter deleyi sp. nov.					
LMG 3412	CCUG 62435	132	97	Torry Research Station	(Before 1961)
LMG 3458^{T}	CCUG 62433^{T}	133	98	H. Lautrop	Mouse, lung autopsy sample (before 1966, Denmark)
LMG 3487	CCUG 62434, Lautrop AB104	141	99	H. Lautrop	Culture contaminant (before 1966, Denmark)
LMG 3481	Lautrop AB1214	134	98	H. Lautrop	Used container for rectal thermometer (before 1966, Denmark)
LMG 3482	Lautrop AB1117	134	98	H. Lautrop	Prostate secretion (before 1966, Denmark)
R-50536	AU11459	131	102	Own isolate	Human, deep pharyngeal swab, cystic fibrosis patient (2006,USA)
Achromobacter aegrifaciens					
LMG 3396	API 249-04-76	–	72	J. Gayral	Proctoscopy specimen (Before 1977, USA)
LMG 3410	CIP 73-75	–	100	F. Pichinoty	Soil (before 1979, France)
LMG 3467	ATCC 166	–	63	H. Lautrop	Lung, Guinea pig (Before 1952, USA)
Achromobacter insolitus					
LMG 3342	LMG 3459, CCUG 366, Lautrop AB1416	–	7	H. Lautrop	Pleural fluid (before 1966, Denmark)
LMG 3405	API 011-03-76	–	8	J. Gayral	(Before 1977)
LMG 3520	API 272-04-76	–	8	J. Gayral	(Before 1978)
LMG 5885	API 140-2-84, Gilardi 3982	–	11	D. Monget	Blood (before 1981, USA)
Achromobacter mucicolens					
LMG 3415	CIP 60.81	–	55	CIP	Patient suffering from laryngeal cancer, buccal cavity (before1966, USA)
Achromobacter xylosoxidans					
LMG 3414	CIP 62.30	–	36	CIP	(Before 1953, USA)

Abbreviations: CCUG, Culture Collection University of Gothenburg, Göteborg, Sweden; LMG, BCCM/LMG Bacteria Collection, Laboratorium voor Microbiologie, Gent, Belgium; ATCC, American Type Culture Collection; CIP, Collection of Institut Pasteur France; ST, multilocus sequence type.
*Data from: Spilker *et al.* (2012).

Achromobacter denitrificans, *Achromobacter marplatensis*, *Achromobacter marplatensis* and *Achromobacter insuavis,* respectively) revealed between-taxon divergence values clearly greater than 2.1 % (4.63, 4.04, 4.08 and 4.58, respectively). Our previous comparisons of the latter between-taxon divergence values and DNA–DNA hybridization data between

species of the genus *Achromobacter* validated the use of 2.1 % divergence as a threshold value for species delineation and, hence, the taxa represented by these four strains should be considered distinct species of the genus *Achromobacter* (Spilker *et al.*, 2012; Vandamme *et al.*, 2013a, b).

The 16S rRNA gene sequences of strains LMG 3411^T, LMG 3431^T, LMG 3441^T and LMG 3458^T were more than 99 % similar to those of the current species of the genus *Achromobacter*. The highest percentage similarity values were determined towards the type strains of *Achromobacter dolens* and *Achromobacter anxifer* (both 99.80 %), *Achromobacter spanius* (99.45 %), *Achromobacter spanius* (100 %) and *Achromobacter spanius* (100 %), respectively. The DNA base ratio of strains LMG 3411^T (66.4 mol%), LMG 3431^T (63.8 mol%), LMG 3441^T (63.7 mol%) and LMG 3458^T (66.8 mol%) proved similar to that of other species of the genus *Achromobacter* (Coenye *et al.*, 2003a, b; Vandamme *et al.*, 1996, 2013a, b).

Cellular fatty acid profiles of all 13 *Achromobacter* strains representing novel species are shown in Table 2. Summed features 2 (iso-$C_{16:1}$ I or $C_{14:0}$ 3-OH, or both) and 3 (iso-$C_{15:0}$ 2-OH or $C_{16:1}\omega7c$, or both) most likely correspond to $C_{14:0}$ 3-OH and $C_{16:1}\omega7c$, respectively (Vandamme *et al.*, 2013a). The relative percentages of $C_{12:0}$, $C_{14:0}$ 2-OH, cyclo-$C_{17:0}$ and $C_{18:1}\omega7c$ allow a straightforward differentiation of the taxa represented by strains LMG 3411^T, LMG 3431^T, LMG 3441^T and LMG 3458^T.

The phenotypic characteristics of the 13 *Achromobacter* strains representing novel taxa were compared with those of other species of the genus *Achromobacter* determined earlier (Vandamme *et al.*, 2013a, b). Thirteen characteristics were uniformly present and 55 were uniformly absent in all 13 strains examined. All strains were motile and rod-shaped (Fig. S1, available in the online Supplementary Material) and shared the following characteristics: presence of oxidase, catalase and leucine arylamidase activity; nitrate reduction; growth at 30 and 37 °C on blood agar; growth on Drigalsky agar and in the presence of 0.5 and 1.5 % NaCl; and assimilation of L-malate, citrate and phenylacetate (API 20NE microtest system). The following characteristics were uniformly absent: haemolysis on horse blood agar; tolerance of penicillin (lO-Fg discs); growth at 42 °C, in the presence of 6 % NaCl and in O/F medium with D-glucose, maltose, adonitol, D-xylose or D-fructose; fluorescence on King's B medium; growth in 10 % lactose; hydrolysis of Tween 80; indole production; hydrolysis of aesculin; production of acid or H_2S in triple-sugar-iron agar; activity of amylase, lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, urease, ONPG β-galactosidase and DNase; gelatine liquefaction; and assimilation of glucose, trehalose, L-arginine, DL-norleucine, D-mannitol and sucrose. In addition, when examined by using the API 20NE microtest system, the activities of tryptophanase, arginine dihydrolase, urease, PNPG β-galactosidase; hydrolysis of aesculin; gelatin liquefaction; fermentation of glucose and assimilation of L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine and maltose were all absent, and when examined by using the API ZYM microtest system, the activities of C_8-lipase, C_{14}-lipase, valine arylamidase, cysteine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase were absent as well. Species- or strain-dependent characteristics, along with data for the established type strains of species of the genus *Achromobacter* are shown in Table 3. Only a single test (growth on acetamide) distinguishes the strains grouping with LMG 3458^T from those grouping with LMG 3441^T, yet the two type strains differ in four additional characteristics (Table 3). Similar observations have been reported earlier when distinguishing other pairs of *Achromobacter* species through biochemical characteristics and are related to the biochemical inertness and considerable intraspecies variability of several of these species (Vandamme *et al.*, 2013a, b).

In conclusion, the species represented by strains LMG 3411^T, LMG 3431^T, LMG 3441^T and LMG 3458^T can be distinguished by biochemical characteristics, MLSA, and *nrd*A_765 sequence analysis. Strains LMG 3411^T and LMG 3431^T were sent to K. Kersters and J. De Ley in the early 1970s and were then named '*Achromobacter agile*' and '*Achromobacter pestifer*', respectively. The former organism was first reported as '*Bacillus denitrificans agilis*' in 1896 by Ampola and Garino (Ampola & Garino, 1896) and was isolated from cow manure; the latter organism was first reported as '*Bacillus pestifer*' in 1887 by Frankland and Frankland (Frankland & Frankland, 1887) and was isolated from dust and air samples. Both species were included as species of the genus *Achromobacter* in *Bergey's Manual of Determinative Bacteriology* in 1923 (Bergey *et al.*, 1923). However, it was not until the eighth edition of this manual that strains NCIB 9986^T (=LMG 3411^T) and ATCC 15445^T (=LMG 3431^T), respectively, were listed as reference strains for these species. Both species were then considered species *incertae sedis* (Holding & Shewan, 1974) because they were never included in comparative taxonomic studies (Hendrie *et al.*, 1974). The genera *Achromobacter* and *Alcaligenes* were long considered synonyms (Hendrie *et al.*, 1974) and the genus name *Achromobacter* nor any of the named species of the genus *Achromobacter* were included in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980), so these names temporarily lost standing in bacterial nomenclature. Kersters & De Ley (1984) classified both strains LMG 3411^T and LMG 3431^T as *Alcaligenes denitrificans* to conform to taxonomic practices in the 1970s and 1980s. Below we propose to revive both names to formally classify these strains as *Achromobacter agilis* sp. nov., nom. rev. [with strain LMG 3411^T (=CCUG 62454^T) as the type strain] and *Achromobacter pestifer* sp. nov., nom. rev. [with strain LMG 3431^T (=CCUG 61959^T) as the type strain]. Furthermore we propose to name the species represented by strains LMG 3441^T and LMG 3458^T after K. Kersters and J. De Ley to honour their seminal taxonomic work on

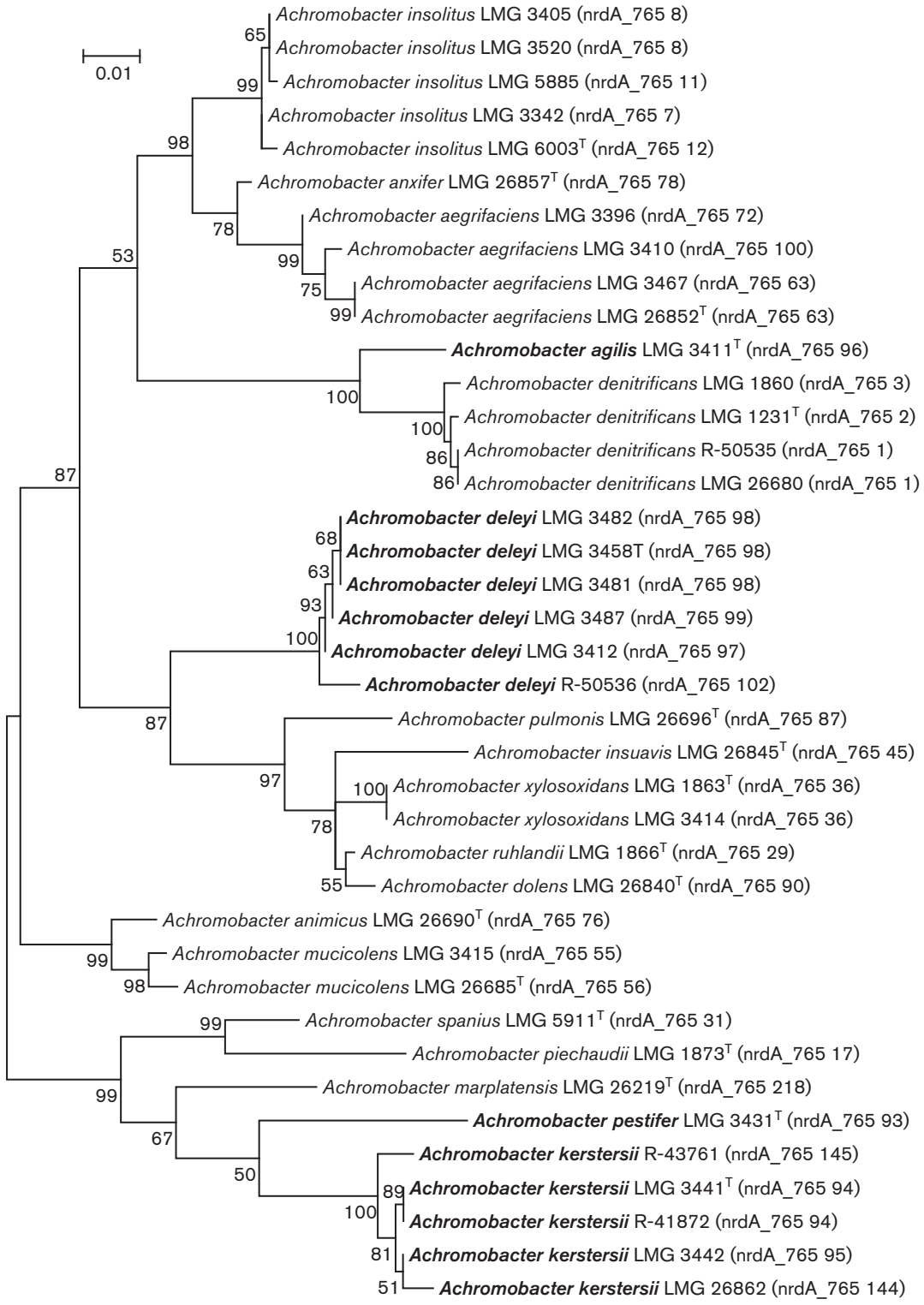

Fig. 1. Maximum-likelihood phylogenetic tree based on *nrdA* sequences (765 bp). Evolutionary history was inferred using the General Time Reversible model (Nei & Kumar, 2000) in MEGA6 (Tamura *et al.*, 2013). The tree with the highest log likelihood (−3014.1616) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter=0.7042)] and allowed for some sites to be evolutionarily invariable [(+I), 62.7394 % sites]. The percentage of trees in which the associated taxa clustered together (1000 bootstrap replicates) is shown next to the branches if >50 %. *nrdA*_765 allele numbers are shown in parentheses. Bar, 0.01 substitutions per site.

Achromobacter, as *Achromobacter kerstersii* sp. nov. [with strain LMG 3441^T (=CCUG 62449^T) as the type strain] and *Achromobacter deleyi* sp. nov. [with strain LMG 3458^T (=CCUG 62433^T) as the type strain].

Description of *Achromobacter agilis* sp. nov., nom. rev.

Achromobacter agilis (a′gi.lis. L. adj. *agilis* agile).

Cells are Gram-stain-negative, motile, small bacilli (about 0.4–0.7 μm wide and 0.9–1.5 μm long). After incubation for 48 h on trypticase soy agar at 28 °C, colonies are low convex, translucent and non-pigmented, with smooth margins and 1–1.5 mm in diameter. Biochemical characteristics are as described above for all *Achromobacter* strains. In addition, *A. agilis* grows on cetrimide agar, on acetamide and in the presence of 3.0 but not 4.5 % NaCl. Nitrite reduction and denitrification is present. Does not assimilate L-arabinose, D-mannose, *N*-acetylglucosamine, maltose, D-gluconate, caprate, adipate, L-malate, citrate, phenylacetate, D-lactate, lactate + methionine (classical tests) or D-glucose, D-gluconate, caprate (API 20NE). Assimilates adipate when tested by the API 20NE microtest system. Activity of C_4-lipase is present but not acid or alkaline phosphatase, phosphoamidase or β-glucosidase. The following fatty acid components were present in major amounts (>30 %): $C_{16:0}$ and summed feature 3 (most likely $C_{16:1}\omega7c$); $C_{18:1}\omega7c$ and summed feature 2 (most likely $C_{14:0}$ 3-OH) were present in moderate amounts (7–10 %); other fatty acids were present in minor amounts (<5 %).

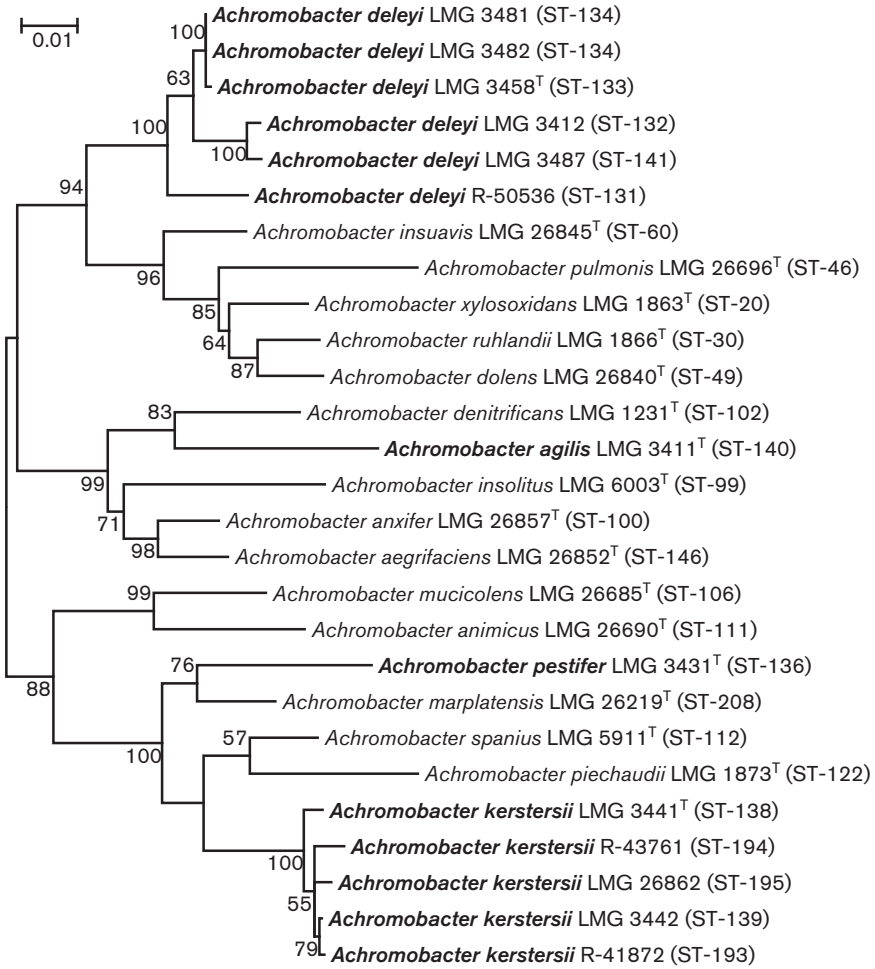

Fig. 2. Maximum-likelihood phylogenetic tree based on concatenated sequences (2249 bp) of seven housekeeping gene fragments [*nusA* (355 bp), *rpoB* (413 bp), *eno* (214 bp), *gltB* (241 bp), *lepA* (347 bp), *nuoL* (230 bp) and *nrdA* (449 bp)]. Evolutionary history was inferred using the General Time Reversible model (Nei & Kumar, 2000) in MEGA6 (Tamura *et al.*, 2013). The tree with the highest log likelihood (−8799.1416) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter=0.1621)] and allowed for some sites to be evolutionarily invariable ([+I], 28.7730 % sites). The percentage of trees in which the associated taxa clustered together (1000 bootstrap replicates) is shown next to the branches if >50 %. Sequence type numbers are shown in parentheses. Bar, 0.01 substitutions per site.

Table 2. Average fatty acid composition of all novel species of the genus *Achromobacter*

Taxa: 1, *Achromobacter agilis* sp. nov., nom. rev. LMG 3411^T; 2, *Achromobacter pestifer* sp. nov., nom. rev. LMG 3431^T; 3, *Achromobacter kerstersii* sp. nov. (data for strain LMG 3441^T and four additional strains); 4, *Achromobacter deleyi* sp. nov. (data for strain LMG 3458^T and five additional strains). Those fatty acids for which the average amount for all taxa was <1 % are not included. Therefore, the percentages may not add up to 100 %. TR, Trace amount (<1 %); ND, not detected.

Fatty acid	1	2	3	4
$C_{12:0}$	1.37	1.81	1.16±0.06	TR
$C_{12:0}$ 2-OH	2.42	3.26	3.05±0.40	4.09±0.97
$C_{14:0}$	4.35	3.56	3.90±0.66	2.53±1.21
$C_{14:0}$ 2-OH	ND	ND	ND	2.76±1.53
$C_{16:0}$	31.66	34.68	28.45±1.18	31.74±2.10
Cyclo-$C_{17:0}$	1.63	5.11	11.32±4.65	12.40±4.52
$C_{18:0}$	1.95	1.00	1.07±0.27	1.48±0.13
$C_{18:1}\omega7c$	9.60	5.93	10.32±0.59	7.33±1.56
Summed feature(s)*				
2	7.85	8.98	9.13±0.76	11.43±2.50
3	37.61	34.87	30.10±5.10	23.97±4.58

*Summed features represent groups of two or more fatty acids that could not be separated using the MIDI system. Summed feature 2 comprises iso-$C_{16:1}$ I or $C_{14:0}$3-OH or both; summed feature 3 comprises iso-$C_{15:0}$ 2-OH or $C_{16:1}\omega7c$ or both.

The type strain is LMG 3411^T (=CCUG 62454^T) and was isolated before 1968 from an unknown source. The DNA G+C content of the type strain is 66.4 mol%.

Description of *Achromobacter pestifer* sp. nov., nom. rev.

Achromobacter pestifer (pes′ti.fer. L. adj. *pestifer* destructive, noxious).

Cells are Gram-stain negative, motile, small bacilli (about 0.4–0.7 μm wide and 0.9–1.5 μm long). After incubation for 48 h on trypticase soy agar at 28 °C, colonies are low convex, translucent and non-pigmented, with smooth margins and 1–1.5 mm in diameter. Biochemical characteristics are as described above for all *Achromobacter* strains. In addition, *A. pestifer* grows on acetamide and in the presence of 3.0 and 4.5 % NaCl, but not on cetrimide agar. Denitrification is present but no reduction of nitrite. Assimilates L-arabinose, D-mannose, *N*-acetylglucosamine, maltose, D-gluconate, caprate, adipate, L-malate, citrate, phenylacetate, D-lactate, lactate + methionine (classical tests) and D-gluconate and adipate (API 20NE), but not D-glucose or caprate (API 20NE). Activity of C_4-lipase and acid phosphatase is present, but not activity of alkaline phosphatase, phosphoamidase or β-glucosidase. The following fatty acid components were present in major amounts (>30 %): $C_{16:0}$ and summed feature 3 (most likely $C_{16:1}\omega7c$); cyclo-$C_{17:0}$, $C_{18:1}\omega7c$ and summed feature 2 (most likely $C_{14:0}$ 3-OH) were present in moderate amounts (5–10 %); other fatty acids were present in minor amounts (<4 %).

The type strain is LMG 3431^T (=CCUG 61959^T) and was isolated from oil-brine before 1967. The DNA G+C content of the type strain is 63.8 mol%.

Description of *Achromobacter kerstersii* sp. nov.

Achromobacter kerstersii (ker.sters′i.i. N.L. masc. gen. n. *kerstersii* of Kersters, named for Karel Kersters, who was one of the pioneers for studying the taxonomy of *Achromobacter*).

Cells are Gram-stain-negative, motile, small bacilli (about 0.4–0.7 μm wide and 0.9–1.5 μm long). After incubation for 48 h on trypticase soy agar at 28 °C, colonies are low convex, translucent and non-pigmented, with smooth margins and 1–1.5 mm in diameter. Biochemical characteristics are as described above for all *Achromobacter* strains. In addition, strains of *A. kerstersii* strains grow in the presence of 3.0 % NaCl but not on acetamide. Nitrite reduction and denitrification are not observed. Assimilates D-gluconate, caprate, adipate, L-malate, citrate, phenylacetate and lactate + methionine, but not L-arabinose, D-mannose, *N*-acetylglucosamine or maltose (classical tests). Assimilate D-gluconate but not caprate when tested by means of the API 20NE microtest system. Activity of acid and alkaline phosphatase and C_4-lipase is present. Growth on cetrimide agar, in the presence of 4.5 % NaCl, assimilation of D-lactate (classical test), D-glucose and adipate (API 20NE), and activity of phosphoamidase and β-glucosidase are strain-dependent characteristics. The following fatty acid components were present in major amounts (about 30 %): $C_{16:0}$ and summed feature 3 (most likely $C_{16:1}\omega7c$); cyclo-$C_{17:0}$, $C_{18:1}\omega7c$ and summed feature 2 (most likely $C_{14:0}$ 3-OH) were present in moderate amounts (9–12 %); other fatty acids are present in minor amounts (less than 4 %).

The type strain is LMG 3441^T (=CCUG 62449^T) and was isolated from soil before 1968. The DNA G+C content of the type strain is 63.7 mol%. More recent isolates were obtained from soil samples in Belgium and Peru (Table 1).

Table 3. Differential characteristics of species of the genus *Achromobacter*

Strains: 1, *A. agilis* sp. nov., nom rev. LMG 3411^T; 2, *A. pestifer* sp. nov., nom. rev. LMG 3431^T; 3, *A. kerstersii* sp. nov. LMG 3441^T; 4, *A. kerstersii* sp nov. LMG 3442; 5, *A. kerstersii* sp. nov. LMG 26862; 6, *A. kerstersii* sp. nov. R-41872; 7, *A. kerstersii* sp. nov. R-43761; 8, *A. deleyi* sp. nov. LMG 3458^T; 9, *A. deleyi* sp. nov. LMG 3487; 10, *A. deleyi* sp. nov. LMG 3412; 11, *A. deleyi* sp. nov. LMG 3481; 12, *A. deleyi* sp. nov. LMG 3482; 13, *A. deleyi* sp. nov. R-50536; 14, *A. animicus* LMG 26690^T; 15, *A. anxifer* LMG 26857^T; 16, *A. denitrificans* LMG 1231^T; 17, *A. dolens* LMG 26840^T; 18, *A. aegrifaciens* LMG 26852^T; 19, *A. insolitus* LMG 6003^T; 20, *A. insuavis* LMG 26845^T; 21, *A. marplatensis* LMG 26219^T; 22, *A. mucicolens* LMG 26685^T; 23, *A. piechaudii* LMG 1873^T; 24, *A. pulmonis* LMG 26696^T; 25, *A. ruhlandii* LMG 1866^T; 26, *A. spanius* LMG 5911^T; 27, *A. xylosoxidans* LMG 1863^T. +, Present; −, Absent.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Growth on cetrimide agar	+	−	+	−	−	+	−	−	−	−	+	+	+	−	+	+	+	−	−	+	+	+	+	+	+	−	+
Nitrite reduction	+	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	+	−	+	+	+	−	−	−	−	+
Denitrification	+	+	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−	+	−	+	−	−	−	−	+
Growth on/in:																											
Acetamide	+	+	−	−	−	−	−	+	+	+	+	+	+	−	+	+	−	+	+	+	−	−	−	−	−	−	+
3.0 % NaCl	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4.5 % NaCl	−	+	+	+	−	+	+	+	+	−	−	−	−	+	−	+	−	+	+	−	−	+	+	−	−	+	+
Assimilation of:																											
L-Arabinose	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
D-Mannose	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
N-Ac-glucosamine	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Maltose	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
D-Gluconate	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
Caprate	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	−
Adipate	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
L-Malate	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Citrate	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenylacetate	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
D-Lactate	−	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	−	+	−	+
D-Lactate + Methionine	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
D-Glucose (API 20NE)	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	+	+	−	+	−	−	−	−	+	+	+
D-Gluconate (API 20NE)	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	+	+
Caprate (API 20NE)	−	−	−	−	−	−	−	+	+	−	+	+	−	−	+	+	−	+	+	+	−	+	−	−	+	−	+
Adipate (API 20NE)	+	+	+	+	+	−	+	+	+	−	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	−	+
Activity of:																											
Alkaline phosphatase	−	−	+	+	+	+	+	+	−	+	+	+	+	−	−	−	+	+	+	+	+	−	−	−	−	−	+
C4-lipase	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	+	+	−	−	+	+	+
Acid phosphatase	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−	+	+	+	+	−	−	−	+	+	+
Phosphoamidase	−	−	−	−	−	+	−	+	−	−	−	+	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−
β-Glucosidase	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

Description of *Achromobacter deleyi* sp. nov.

Achromobacter deleyi (de.ley′i. N.L. masc. gen. n. *deleyi* of De Ley, named for Jozef De Ley, who was one of the pioneers for studying the taxonomy of *Achromobacter*).

Cells are Gram-stain-negative, motile, small bacilli (about 0.4–0.7 μm wide and 0.9–2.0 μm long). After incubation for 48 h on trypticase soy agar at 28 °C, colonies are low convex, translucent and non-pigmented, with smooth margins and 1–1.5 mm in diameter. Biochemical characteristics are as described above for all *Achromobacter* strains. In addition, strains of *A. deleyi* grow on acetamide, do not reduce nitrite or denitrify, and assimilate D-gluconate, adipate, L-malate, citrate, phenylacetate, D-lactate and lactate + methionine, but not L-arabinose, D-mannose, *N*-acetylglucosamine or maltose (classical tests). Assimilates D-gluconate when tested by means of the API 20NE microtest system. Activity of acid phosphatase is present but not β-glucosidase. Growth on cetrimide agar, in the presence of 3.0 % and 4.5 % NaCl, assimilation of caprate (classical test), D-glucose, caprate and adipate (API 20NE), and activity of alkaline phosphatase, C_4-lipase and phosphoamidase are strain-dependent characteristics. The following fatty acid components were present in major amounts (24–32 %): $C_{16:0}$ and summed feature 3 (most likely $C_{16:1}\omega7c$); cyclo-$C_{17:0}$, $C_{18:1}\omega7c$ and summed feature 2 (most likely $C_{14:0}$ 3-OH) were present in moderate amounts (7–13 %); other fatty acids are present in minor amounts (<5 %).

The type strain is LMG 3458^T (=CCUG 62433^T) and was isolated from the lung of a mouse in Denmark before 1966. Other isolates are from human clinical and environmental samples (Table 1). The DNA G+C content of the type strain is 66.8 mol%.

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