Achromobacter marplatensis sp. nov., isolated from a pentachlorophenol-contaminated soil

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A polyphasic taxonomic approach was applied to the study of a Gram-negative bacterium (B2^T) isolated from soil by selective enrichment with pentachlorophenol. 16S rRNA gene sequence analysis of strain B2^T showed that the strain belongs to the genus *Achromobacter* within the *Betaproteobacteria*. The 16S rRNA gene sequence displayed more than 99 % similarity to the sequences of the type strains of all species of *Achromobacter*, with the highest sequence similarity to those of *Achromobacter spanius* CCM 7183^T and *A. piechaudii* CCM 2986^T (99.8 %). On the basis of phylogenetic analysis, genomic DNA–DNA relatedness and phenotypic characteristics, including chemotaxonomic (cellular fatty acid profile) analysis, a novel species is proposed, *Achromobacter marplatensis* sp. nov., with the type strain B2^T (=CCM 7608^T =CCUG 56371^T =CECT 7342^T).

Pentachlorophenol (PCP) was generally believed to be resistant to environmental degradation until numerous investigators reported the isolation of fungi and bacteria able to degrade it (Litchfield & Rao, 1998; Tiirola *et al.*, 2002). Although many bacteria are able to metabolize organic pollutants, very few strains have the capability to mineralize high concentrations of PCP in contaminated wastewaters (Lee *et al.*, 1998). With the objective of finding novel strains better able to degrade PCP, strain B2^T was isolated from a PCP-contaminated site in Argentina (Murialdo *et al.*, 2003). Initial analyses indicated that the isolate could be a strain of *Alcaligenes* or *Bordetella*, but further taxonomic characterization of strain B2^T led to its recognition as a novel member of the genus *Achromobacter*.

The genera *Alcaligenes* and *Achromobacter* belong to the class *Betaproteobacteria*, grouped together in the family *Alcaligenaceae* (Busse & Auling, 2005). The taxonomy of the genera *Alcaligenes* and *Achromobacter* is closely

Abbreviations: CFA, cellular fatty acid; FAME, fatty acid methyl ester; PCP, pentachlorophenol.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain B2^T is EU150134. The accession numbers for the other 16S rRNA gene sequences determined in this study are FM999731–FM999735.

Four supplementary tables and a supplementary figure are available with the online version of this paper.

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intertwined; several *Alcaligenes* species were reclassified in *Achromobacter* by Yabuuchi *et al.* (1998). The genus *Achromobacter* now contains six species: *Achromobacter denitrificans*, *A. insolitus*, *A. piechaudii*, *A. ruhlandii*, *A. spanius* and *A. xylosoxidans* (the type species). These species have been isolated from a range of different sources, mainly from water and soil, but also from clinical samples. *A. xylosoxidans* is widespread in oligotrophic aquatic niches and is an opportunistic human pathogen, able to cause a variety of infections (Busse & Auling, 2005). *A. denitrificans* strains are found in soil but are also occasionally found in human clinical samples (Busse & Auling, 2005; Coenye *et al.*, 2003b). *A. piechaudii* has been isolated from soil and human clinical samples, including blood (Kiredjian *et al.*, 1986). *A. ruhlandii* is considered to be a soil inhabitant and is not known to be associated with human clinical conditions (Busse & Auling, 2005) and *A. insolitus* and *A. spanius* were isolated from a leg wound and blood samples, respectively (Coenye *et al.*, 2003a).

We performed a polyphasic taxonomic study to elucidate the taxonomic position of strain B2^T, isolated from soil by selective enrichment with PCP. On the basis of comparative 16S rRNA gene sequence analysis, it was observed to cluster most closely with species of the genus *Achromobacter*. Phenotypic data, including chemotaxonomic characteristics, ribotyping and DNA–DNA hybridization suggest that strain B2^T represents a novel species in the genus *Achromobacter*.

Strain B2^T was isolated in mineral salts base (MS) medium supplemented with PCP as sole carbon source by enrichment of a sample of soil containing PCP collected near a wastewater discharge site. The initial PCP concentration was 5 mg l^{-1} and the concentration was increased in subsequent enrichment subcultures to 100 mg l^{-1}, as described previously (Murialdo *et al.*, 2003). Strain B2^T was able to metabolize PCP alone or in the presence of glucose as co-substrate (Murialdo *et al.*, 2003).

Type strains of all species of the genus *Achromobacter* were included in this study: *A. denitrificans* CCM 3427^T and CCUG 407^T, *A. insolitus* CCM 7182^T and CCUG 47057^T, *A. piechaudii* CCM 2986^T and CCUG 724^T, *A. ruhlandii* CCM 7494^T and CCUG 57103^T, *A. spanius* CCM 7183^T and CCUG 47062^T and *A. xylosoxidans* CCM 2741^T and CCUG 56438^T. These strains were cultivated on nutrient agar (Merck) unless stated otherwise and incubated for 2–3 days at 37 °C, except for strains of *A. denitrificans* and *A. ruhlandii*, which were incubated at 30 °C.

The 16S rRNA genes of all strains employed in this study were amplified by PCR using primers 16F27 and 16R1492 and sequenced as described previously (Gomila *et al.*, 2005). PCR products were purified with Microcon centrifugal filter devices (Microcon-Millipore) according to the manufacturer's instructions. Sequencing reactions were carried out using the ABI Prism Big Dye Terminator version 3.1 cycle sequencing kit and the sequences were read with an automatic sequence analyser (ABI Prism 3730 DNA sequencer; Applied Biosystems). Nearly full-length gene sequences (1302 positions) were aligned with reference sequences of the closest relatives, retrieved using the BLAST analysis tool from the NCBI nucleotide sequence database (Altschul *et al.*, 1990). Sequences were aligned using a hierarchical method for multiple alignments implemented in the CLUSTAL_X program (Thompson *et al.*, 1997). Automatically aligned sequences were edited manually. Similarities and evolutionary distances were calculated with programs contained in PHYLIP (Felsenstein, 1989). Gene distances were calculated from nucleotide sequences by the Jukes–Cantor method (Jukes & Cantor, 1969) and dendrograms were generated by the neighbour-joining method. Alternative analyses of the sequence data were carried out using different algorithms (maximum-likelihood, parsimony and Fitch–Margoliash) and all analyses supported the derived phylogenetic position of strain B2^T within the genus *Achromobacter*. Bootstrap analysis of 1000 replications was performed in order to assess the reliability of the dendrogram branching order. Topologies of the trees were visualized with the TreeView program (Page, 1996). We found that the published sequence data for *A. denitrificans* LMG 1231^T (M22509), *A. piechaudii* ATCC 45552^T (AB01041) and *A. xylosoxidans* LMG 1863^T (D88005) included a number of undetermined positions and possible erroneous gaps. Since the 16S rRNA gene sequences of *Achromobacter* species have high levels of similarity to one another, exclusion of these undetermined or ambiguous positions could affect the elucidation of interspecies relationships. Therefore, the 16S rRNA gene sequences for the type strains of the other *Achromobacter* species were determined, including those species whose sequences were already available. We used the new versions of the sequences, containing no ambiguities, for the phylogenetic analyses. The 16S rRNA gene sequence similarity between B2^T and the type strains of all other *Achromobacter* species was greater than 99 %. The highest similarity observed was 99.8 %, with *A. piechaudii* CCM 2986^T and *A. spanius* CCM 7183^T (*A. piechaudii* CCM 2986^T and *A. spanius* CCM 7183^T showed 100 % similarity in their 16S rRNA gene sequences). The neighbour-joining tree and the distance matrix are given in Fig. 1 and in Supplementary Table S1 (available in IJSEM Online), respectively.

Genomic DNA–DNA hybridizations were performed, in duplicate, using a non-radioactive method as described by Ziemke *et al.* (1998). Genomic DNA was isolated according to the method of Marmur (1961). Reference DNAs of strain B2^T, *A. piechaudii* CCUG 724^T, *A. spanius* CCM 7183^T and *A. xylosoxidans* CCUG 56438^T were double-labelled with DIG-11-dUTP and biotin-16-dUTP using a nick translation kit (Roche). Labelled DNA was hybridized separately with DNAs from strain B2^T, *A. denitrificans* CCM 3427^T, *A. insolitus* CCM 7182^T, *A. piechaudii* CCM 2986^T, *A. spanius* CCM 7183^T, *A. ruhlandii* CCUG 57103^T and *A. xylosoxidans* CCM 2741^T. The levels of DNA–DNA relatedness of strain B2^T with the other strains were, in all

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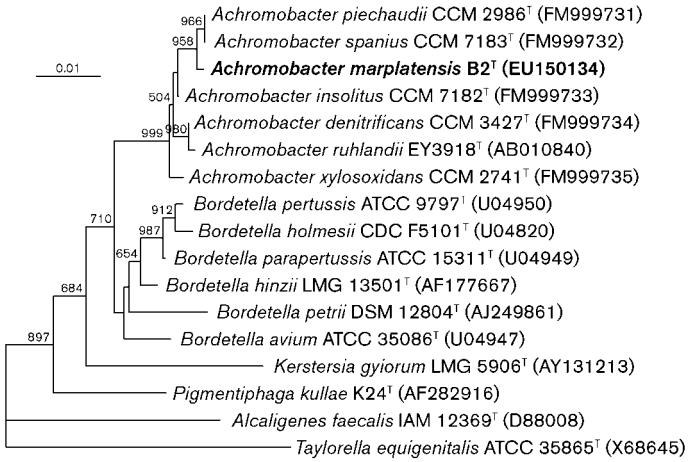

Fig. 1. Neighbour-joining tree showing derived phylogenetic relationships among strain B2^T and type strains of the genus *Achromobacter*, based on 16S rRNA gene sequence comparisons. Numbers at branch nodes are bootstrap values from 1000 replicates. Bar, 1 substitution per 100 nucleotide positions.

cases, lower than 56 % (Supplementary Table S2), confirming that strain B2^T was genomically distinct from the type strains of all species within the genus.

Strain B2^T is a motile, rod-shaped (1 μm long), Gram-negative bacterium (Supplementary Fig. S1). Phenotypic analyses (API 20 NE, API ZYM and Biotype 100) were carried out to determine the characteristic profile for assimilation of organic compounds as sole carbon sources, according to the manufacturer's recommendations (bioMérieux). Inocula were taken from cultures grown for 16–20 h at 35 °C on nutrient agar. Conventional phenotypic tests were done according to Barrow & Feltham (1993). The results showed that strain B2^T was able to assimilate phenylacetate, citrate and malate and showed weak growth with gluconate. It was also capable of nitrate and nitrite reduction, but showed a negative reaction in the Voges–Proskauer test. It was not capable of hydrolysis of aesculin or production of acid and H_2S from triple-sugar-iron agar. The inabilities to use D-glucose, adipate, caprate and D-gluconate as carbon sources for growth were physiological characteristics that differentiate B2^T from the other *Achromobacter* species (Table 1). The strain demonstrated oxidase, leucine arylamidase, alkaline and acid phosphatase, phosphoamidase and catalase activities, with few differences being observed between *Achromobacter* species for these tests (Supplementary Table S3).

Antibiotic resistance to kanamycin, amoxicillin, ampicillin and carbenicillin was tested by two different tests. The first test employed Luria–Bertani plates supplemented with 30 or 50 μg antibiotic ml^{-1}. For the second test, antibiotic susceptibility discs (500 μg) were placed on Mueller–Hinton agar. Strains were inoculated onto the medium and grown for 20–48 h at 35 °C. The presence of growth in the first test and the absence of an inhibition halo in the second test were assessed. Strain B2^T was observed to be resistant to 30 μg kanamycin ml^{-1} and sensitive to amoxicillin, ampicillin and carbenicillin at this concentration.

Growth was tested on nutrient agar plates for 24–48 h at 4, 15, 20, 30, 37, 40 and 42 °C. Strain B2^T was able to grow at 15, 20 and 40 °C but not at 4 or at 42 °C, nor in the presence of 6.5 % NaCl (Table 1). Growth was observed on MacConkey agar.

The relative amounts of cellular fatty acids (CFAs) can be useful for the chemotaxonomic characterization of Gram-negative, non-fermenting taxa. Cellular fatty acid methyl ester (FAME) profiles were determined using GC and a standardized protocol similar to that of the MIDI Sherlock MIS system (http://www.ccug.se/pages/cfanew.pdf). Prior to CFA extraction, strains were grown and harvested under the same conditions, using blood agar as the cultivation medium. The relative amounts of each CFA were expressed as percentages of the total fatty acids. Only CFAs with chain lengths of 9 to 18 carbon atoms were analysed. Predominant fatty acids observed in strain B2^T were $C_{16:0}$, $C_{16:1}\omega7c$ and $C_{17:0}$ cyclo. Detailed fatty acid compositions are indicated in Table 2. The CFA profile of strain B2^T conformed to the general profile of *Achromobacter* species; only slight differences could be established in comparison with other type strains of the genus (e.g. in $C_{14:0}$ 2-OH).

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used for additional differentiation and characterization of strain B2^T with respect to the type strains of other species of the genus *Achromobacter*. Cells for MALDI-TOF MS were harvested from 24 h cultures, grown as a second subculture at 30 °C on tryptone soy agar (Oxoid). The cells were suspended in 0.5 ml acetonitrile/water (1 : 1). Bacterial suspensions were mixed with MALDI matrix solution in a ratio of 1 : 4 (v/v) and 0.6 μl aliquots of the mixture were pipetted onto three individual spots of a stainless-steel MALDI target. As a MALDI matrix, sDHB (90 % 2,5-dihydroxybenzoic acid and 10 % 2-hydroxy-5-methoxy-benzoic acid, 20 mg ml^{-1} in 20 % acetonitrile and 1 % trifluoroacetic acid) was used (Tvrzová *et al.*, 2006). MALDI-TOF MS measurements were carried out using a Reflex IV instrument (Bruker Daltonik) operated in linear positive mode with 20 kV acceleration voltage. Mass spectra were accumulated in the mass range 4–20 kDa

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Table 1. Phenotypic characteristics of strain B2^T and type strains of *Achromobacter* species

Strains: 1, strain B2^T; 2, *A. denitrificans* CCM 3427^T; 3, *A. insolitus* CCM 7182^T; 4, *A. piechaudii* CCM 2986^T; 5, *A. ruhlandii* CCM 7494^T; 6, *A. spanius* CCM 7183^T; 7, *A. xylosoxidans* CCM 2741^T. +, Positive; −, negative; w, weak; ND, not determined. These data were obtained in this study. Data in parentheses give information on intraspecific variability as follows and were taken from Busse & Auling (2005) and Coenye *et al.* (2003b): +, ⩾90 % strains positive; [+], ⩾80 % strains positive; d, 11–79 % of strains positive; −, ⩽10 % of strains positive. No information on strain variability has been reported for *A. insolitus* or *A. spanius*. All strains are negative for aesculin hydrolysis, DNase, urease, lysine decarboxylase, ornithine decarboxylase and β-galactosidase and positive for nitrate reduction. All strains grow with L-malate, but not with L-arabinose, D-mannitol, *N*-acetylglucosamine or maltose.

Characteristic	1	2	3	4	5	6	7
Growth at:							
22 °C	+	ND	−	+	+	−	ND
42 °C	−	w	+	w	+	−	+
Hydrolysis of:							
Gelatin, starch	−	−	−	−	−	−	−*
Tween 80	−	−	−	−	−	−	−*
Use of carbon sources for growth							
D-Glucose	−	− (−)	−	−	+ (+)	−	+ (+)
D-Gluconate	w	− ([+])	+	+ (+)	+ (+)	+	+ (+)
Caprate	−	w (d)	w	−	+ (+)	−	+ (+)
Adipate	−	+ (+)	+	+ (+)	+ (+)	−	+ (+)
Citrate	+	+ (+)	+	+†	+ (+)	+	+ (+)
Phenylacetate	+	+ (d)	+	+ (+)	+ (+)	+	+ (+)
D-Mannose	−	− (−)	−	−	− (−)	−	− (d)
Tube tests							
Acid from glucose in OF medium	−	− (−)	−	− (−)	+ (+)	−	+ (+)
Nitrite reduction	+	+ ([+])	−	− (−)	− (−)	−	+ (+)
Malonate utilization	w	+	+	+	−	+	+
Acetamide utilization	+	−	+	−	−	−	+
Acid from xylose	−	− (−)	−	− (−)	− (−)	−	+ (+)

*Reported as positive by Busse & Auling (2005).
†According to Yabuuchi *et al.* (1998), *A. piechaudii* is not able to grow with citrate.

using a 10 Hz nitrogen laser operating at 337 nm. At least five spectrum accumulations involving 100 laser shots were acquired from each sample spot. External mass spectrum calibration was carried out applying the $[M+H]^+$ and $[M+2H]^{2+}$ molecular peaks of lysozyme. Software FlexControl 1.1 and Xtof 5.1.5 was used for spectrum acquisition and evaluation, respectively. The MALDI-TOF MS mass profile of intact cells was useful for differentiating strain B2^T from the type strains of other *Achromobacter* species (see Fig. 2 and Supplementary Table S4). The presence of single peaks at *m/z* 6210, 7086, 8928, 9972 and 12 626 allowed good discrimination of B2^T from the other *Achromobacter* strains examined.

Automated ribotyping was performed using cells of *Achromobacter* type strains cultivated for 24 h at 30 °C on Columbia agar base (Oxoid) supplemented with 5 % sheep blood, *Eco*RI restriction enzyme and a RiboPrinter microbial characterization system (DuPont Qualicon), in accordance with the protocol provided by the manufacturer. The ribopatterns obtained were normalized, automatically categorized into ribogroups and analysed according to Švec & Sedláček (2008). Automated ribotyping with *Eco*RI generated bands ranging from 1.2 to 50 kbp and separated all seven analysed strains into different ribogroups. Identification using the RiboExplorer software assigned only the strains of *A. piechaudii*, *A. xylosoxidans* and *A. denitrificans* correctly to the species level. Strain B2^T and the remaining three type strains were not identified. *Achromobacter* species revealed great heterogeneity in ribopatterns (Fig. 3), and strain B2^T was distinguished from the other *Achromobacter* type strains.

Although 16S rRNA gene sequence analysis did not provide the resolution necessary to differentiate strain B2^T clearly, distinction of strain B2^T from the type strains of other *Achromobacter* species could be achieved definitively by DNA–DNA hybridization, MALDI-TOF MS and ribotyping analyses. Its capabilities for acetamide utilization, nitrite reduction and assimilation of gluconate (weakly) as a carbon source for growth together with its inability to assimilate adipate or caprate and to produce acid from xylose allowed the differentiation of strain B2^T from the type strains of other *Achromobacter* species. Based on genotypic, phylogenetic, phenotypic and chemotaxonomic analyses, we conclude that strain B2^T represents a novel

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Table 2. CFA profiles of strain B2^T and type strains of *Achromobacter* species

Strains: 1, strain B2^T; 2, *A. denitrificans* CCUG 407^T; 3, *A. insolitus* CCUG 47057^T; 4, *A. piechaudii* CCUG 724^T; 5, *A. spanius* CCUG 47062^T; 6, *A. ruhlandii* CCUG 57103^T; 7, *A. xylosoxidans* CCUG 56438^T. −, Not detected. Data are percentages of total fatty acids and were obtained in this study.

Fatty acid	1	2	3	4	5	6	7
$C_{9:0}$ 3-OH	−	0.6	−	−	−	−	−
$C_{12:0}$ aldehyde	0.5	−	−	−	−	−	0.2
$C_{12:0}$	1.0	1.6	1.3	0.9	1.3	0.6	0.5
$C_{12:0}$ 2-OH	2.2	2.7	2.9	2.3	1.9	2.5	2.8
$C_{12:0}$ 3-OH	−	−	−	0.3	0.4	−	−
$C_{14:0}$	4.9	5.9	5.2	5.7	3.0	1.7	1.2
$C_{15:1}\omega6c$	−	0.5	−	0.3	0.5	−	−
$C_{15:0}$	−	0.7	1.3	0.6	0.7	−	−
$C_{14:0}$ 2-OH	−	−	−	−	−	2.8	3.1
$C_{14:0}$ 3-OH/iso-$C_{16:1}$ I	5.5	7.7	6.7	7.0	3.7	6.6	6.5
$C_{16:1}\omega7c$	25.3	21.9	26.9	12.8	35.8	22.2	14.8
$C_{16:0}$	33.6	30.7	31.5	34.3	33.2	37.2	41.0
$C_{17:0}$ cyclo	16.9	19.9	14.7	29.8	5.9	19.3	24.1
$C_{17:0}$	0.6	0.7	0.7	0.6	1.2	0.6	0.5
$C_{16:0}$ 2-OH	−	−	−	−	0.4	0.6	0.6
$C_{16:0}$ 3-OH	−	0.3	0.3	−	−	−	−
$C_{18:2}\omega6,9c$/anteiso-$C_{18:0}$	−	−	−	0.8	0.6	−	−
$C_{18:1}\omega7c/\omega12t/\omega9t$	8.6	5.5	7.1	2.5	8.1	4.5	2.8
$C_{18:0}$	1.1	0.7	1.1	0.9	1.5	1.5	1.5
iso-$C_{19:0}$	−	−	−	0.2	−	−	−
Unidentified	−	−	0.4	0.5	2.0	−	−

species in the genus *Achromobacter*, for which the name *Achromobacter marplatensis* sp. nov. is proposed.

Description of *Achromobacter marplatensis* sp. nov.

Achromobacter marplatensis (mar.pla.ten′sis. N.L. masc. adj. *marplatensis* pertaining to Mar del Plata, the Argentinian city where the type strain was isolated).

Motile, small rod-shaped, Gram-negative bacterium, 0.5–1 μm long, able to grow at 15–40 °C. Unable to grow at 6.5 % NaCl. Asaccharolytic. Resistant to kanamycin and sensitive to amoxicillin, ampicillin and carbenicillin (at 30 μg ml^{-1}). Grows on MacConkey agar. Positive for oxidase, leucine arylamidase, alkaline and acid phosphatase, phosphoamidase and catalase activities and tyrosine hydrolysis. Negative for activities of amylase, arginine dihydrolase, lysine and ornithine decarboxylases, C4 esterase, C8 esterase lipase, C14 lipase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, urease, tryptophan deaminase and α-fucosidase. Able to assimilate malate, citrate and phenylacetate and to assimilate gluconate weakly. Capable of nitrate and nitrite reduction. Tests for the Voges–Proskauer reaction and indole production are negative. Unable to grow on inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin, glucose, *p*-nitrophenyl β-D-galactopyranoside, arabinose, mannose, mannitol, *N*-acetylglucosamine, maltose, caprate, arginine, King's media A and B, α-(+)-D-glucose, β-(+)-D-fructose, (+)-D-galactose, (+)-trehalose, (+)-D-mannose, (+)-L-sorbose, (+)-melibiose, (+)-raffinose, maltotriose, lactose, lactulose,

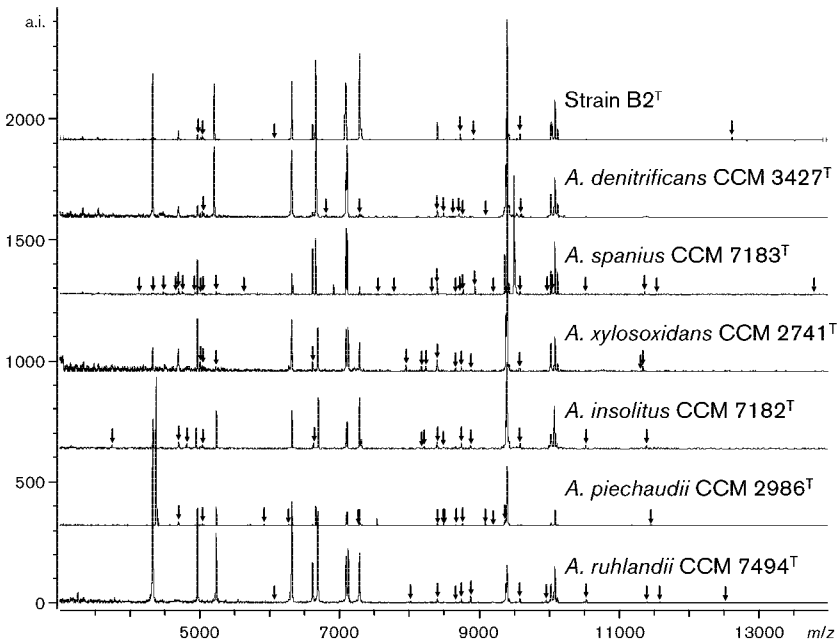

Fig. 2. Profiles of strain B2^T and the type strains of *Achromobacter* species based on characteristic peaks obtained by intact cell MALDI-TOF MS. Peaks with relatively low intensities are highlighted with arrows. More details are given in Supplementary Table S4.

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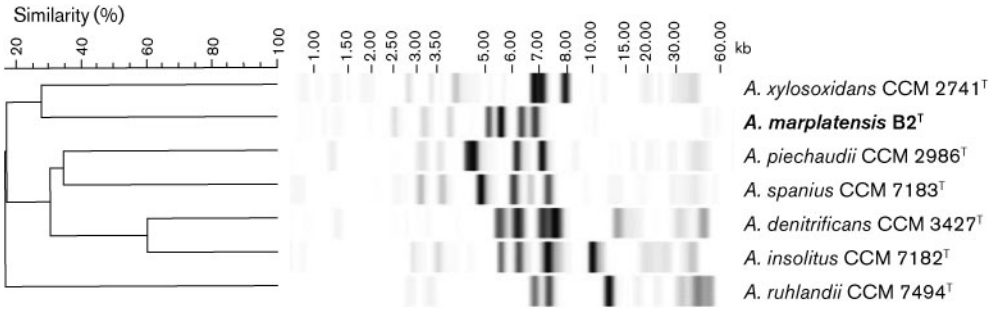

Fig. 3. Dendrogram based on cluster analysis of *Eco*RI ribotype patterns obtained by automated ribotyping with the RiboPrinter microbial characterization system. The dendrogram was calculated with Pearson's correlation coefficient, using the UPGMA clustering method (*r* is expressed for convenience as percentage similarity).

1-*O*-methyl β-galactopyranoside, 1-*O*-methyl α-galactopyranoside, (+)-cellobiose and gentiobiose. Does not utilize 1-*O*-methyl β-D-glucopyranoside, (−)-D-ribose, (+)-L-arabinose, (+)-D-xylose, palatinose, α-L-rhamnose, α-(−)-L-fucose, (+)-melezitose, (+)-D-arabitol, xylitol, dulcitol, D-tagatose, glycerol, *myo*-inositol, D-mannitol, maltitol, (+)-turanose, D-sorbitol, adonitol, hydroxyquinoline β-glucuronide, D-lyxose, i-erythritol, 1-*O*-methyl α-D-glucopyranoside, 3-*O*-methyl D-glucopyranose, D-saccharate, mucate, (+)-L-, (−)-D- and *meso*-tartrate, (+)-D- and (−)-L-malate, *cis*- and *trans*-aconitate, tricarballylate, D-glucuronate, D-galacturonate, 2-keto-D-gluconate, 5-keto-D-gluconate, L-tryptophan, *N*-acetyl-D-glucosamine, D-gluconate, phenylacetate, protocatechuate, *p*-hydroxybenzoate, quinate, gentisate, *m*-hydroxybenzoate, benzoate, 3-phenylpropionate, *m*-coumarate, trigonelline, betaine, putrescine, DL-α-amino-n-butyrate, histamine, DL-lactate, caprylate, L-histidine, succinate, fumarate, glutarate, DL-glycerate, DL-α-amino-n-valerate, ethanolamine, tryptamine, D-glucosamine, itaconate, DL-β-hydroxybutyrate, L-aspartate, L-proline, D- and L-alanine, L-serine, malonate, propionate, L-tyrosine or α-ketoglutarate. No hydrolysis of aesculin, Tween 80, gelatin, starch, casein, lecithin, elastin or DNA or production of acid or H_2S from triple-sugar-iron agar.

The type strain is B2^T (=CCM 7608^T =CECT 7342^T =CCUG 56371^T), isolated in 2001 from soil in Mar del Plata, Argentina, by selective enrichment with PCP.

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