Reclassification of *Acinetobacter grimontii* Carr *et al.* 2003 as a later synonym of *Acinetobacter junii* Bouvet and Grimont 1986

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Using tDNA-PCR, the type strain CCM 7198^T (←CIP 107470^T ←17A04^T) of *Acinetobacter grimontii* was found to be indistinguishable from *Acinetobacter junii* strains. Therefore, the phenotypic properties, amplified fragment length polymorphism (AFLP) patterns and 16S rRNA and *rpoB* gene sequences of the type strain of *A. grimontii* (CCM 7198^T) were determined. We found that the strain used L-arginine and L-glutamate, in contrast to the original description and in accordance with the phenotypic properties of *A. junii*. By AFLP analysis, *A. grimontii* CCM 7198^T clustered at 50.2 % with a set of *A. junii* strains previously identified by DNA–DNA hybridization, which is in accordance with the previously established intraspecies values of this technique. Sequence similarity of the 16S rRNA gene between the type strains of the two species was found to be 99.9 %. Finally, DNA–DNA relatedness between the type strains of *A. junii* and *A. grimontii* was redetermined and was found to be 85 %. These findings were corroborated for a second representative of the *A. grimontii* type strain, DSM 14968^T. These data confirm that *Acinetobacter grimontii* is a later heterotypic synonym of *Acinetobacter junii*.

Carr *et al.* (2003) described *Acinetobacter grimontii* as one of seven novel species from activated sludge from Australia on the basis of 16S rRNA gene sequence analysis, phenotypic characterization and DNA–DNA hybridization. The type strain of *A. grimontii* (17A04^T) was deposited at two public culture collections, the Collection Institut Pasteur, Paris, France (CIP 107470^T) and the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany (DSMZ; DSM 14968^T). A subculture of CIP 108470^T was subsequently obtained by the Czech Collection of Microorganisms, Prague, Czech Republic, and designated CCM 7198^T.

When comparing the tDNA-PCR pattern (Baele *et al.*, 2000, 2001; Vaneechoutte *et al.*, 1998) of *A. grimontii* CCM 7198^T with those of other *Acinetobacter* strains, we found the strain to be indistinguishable from *Acinetobacter junii* strains (data not shown). Later, we also found *A. grimontii*

DSM 14968^T to be indistinguishable from *A. junii* by tDNA-PCR (not shown).

To reassess the taxonomic relationships of *A. grimontii* and *A. junii*, phenotypic analysis and amplified fragment length polymorphism (AFLP) fingerprinting were carried out for a selection of *A. junii* strains and both the CCM and DSMZ representatives of the *A. grimontii* type strain, the 16S rRNA gene sequence was determined for both representatives of the type strain, the *rpoB* gene sequence was determined for *A. grimontii* CCM 7198^T and DNA–DNA hybridization was performed between *A. grimontii* CCM 7198^T and *A. junii* LMG 998^T.

Phenotypic analysis for *A. grimontii* CCM 7198^T and DSM 14968^T and four *A. junii* strains which had previously been assigned to the species by DNA–DNA hybridization (CCM 2376^T, RUH 204, RUH 2229, RUH 2230) was carried out as described by Nemec *et al.* (2001). None of the six strains produced acid from D-glucose or hydrolysed gelatinase. Two strains (RUH 2229, RUH 2230) showed haemolytic activity on sheep-blood agar at 30 or 37 °C, while the others were non-haemolytic. When inoculated onto brain heart infusion broth (Oxoid), all strains showed clear

Abbreviation: AFLP, amplified fragment length polymorphism.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *A. junii* ATCC 17908^T, *A. junii* SH215.SHVI (=ACI289) and *A. grimontii* CCM 7198^T are respectively AM410704–AM410706.

growth after 1 day at 37 °C, while showing weak or doubtful growth at 41 °C and no growth at 44 °C within 2 days of incubation. In assimilation tests, modified from the method of Bouvet & Grimont (1986), all strains utilized DL-lactate, L-arginine, L-glutamate and acetate with clearly positive results after 2 days of incubation, whereas none of the strains grew on *trans*-aconitate, glutarate, azelate, β -alanine, malonate, histamine, L-phenylalanine, phenylacetate, laevulinate, citraconate, 4-hydroxybenzoate, L-tartrate, L-ornithine, 2,3-butanediol, L-arabinose, D-glucose, D-gluconate, gentisate, tryptamine, trigonelline, tricarbaldehyde, putrescine or adipate within 10 days. With the exceptions indicated in parentheses, all strains utilized Simmons' citrate (RUH 2229), L-histidine (RUH 2230), ethanol (RUH 2230) and 4-aminobutyrate (RUH 2229, RUH 2230), while only one strain (CCM 2376^T) grew on L-aspartate. There was no difference in the phenotypic properties of *A. grimontii* CCM 7198^T and *A. grimontii* DSM 14968^T. Whereas Carr *et al.* (2003) reported that *A. grimontii* could be differentiated from *A. junii* by its inability to assimilate L-arginine or L-glutamate, we found that *A. grimontii* strains CCM 7198^T and DSM 14968^T clearly grew on both L-arginine and L-glutamate within 2 days of incubation. Thus, the phenotypic properties of *A. grimontii* reported here are fully consistent with those of *A. junii*.

AFLP analysis was carried out as described previously (Nemec *et al.*, 2001). Briefly, DNA was digested simultaneously with *Eco*RI and *Mse*I and with simultaneous adaptor ligation. PCR was carried out with a Cy5-labelled *Eco*RI+A primer and an *Mse*I+C primer. Amplified fragments were separated with the ALF Express system (Amersham Biosciences). Cluster analysis of profiles was done with the BioNumerics software release 4.5 (Applied Maths) with Pearson's product moment coefficient (*r*) as a measure of similarity and the unweighted pair group average linkage method for clustering. Profiles were compared to a library of reference strains of all described species. Generally, the grouping of strains according to their AFLP similarity at ~50 % corresponds to the grouping obtained by employing a DNA–DNA hybridization cut-off of 70 % (Nemec *et al.*, 2001). Cluster analysis of AFLP profiles of *A. grimontii* CCM 7198^T with those of reference strains of all described named and unnamed *Acinetobacter* species, all identified by DNA–DNA hybridization, showed that *A. grimontii* falls within the *A. junii* cluster (Fig. 1). This cluster, which is delineated at a clustering level of 50.2 %, was well separated from all other species, with *Acinetobacter haemolyticus* being closest, at 19.9 %. AFLP analysis showed a same-run similarity of 96.5 % for the two representatives of the *A. grimontii* type strain (CCM 7198^T and DSM 14968^T), which is above the strain delineation level of 90 % determined previously (van den Broek *et al.*, 2006) (data not shown).

From the publication of Carr *et al.* (2003), it could be observed that the 16S rRNA gene sequence of *A. grimontii* 17A04^T as published in their study showed highest

Fig. 1. Dendrogram of AFLP fingerprints obtained from the type strains of *A. junii* and *A. grimontii* and from *A. junii* and *A. haemolyticus* strains already present in the Leiden University Medical Centre (LUMC) database. All strains were previously identified by DNA–DNA hybridization. Numbers on branches indicate similarity values. LUH and RUH are designations of strains of the LUMC strain collection.

similarity to that of the type strain of *A. junii*, i.e. 99 %, with 7 mismatches and 20 gaps over the compared length of 1310 bp. Here, we redetermined the 16S rRNA gene sequence of the *A. grimontii* type strain, as obtained from both the CCM and DSMZ. The 16S rRNA gene sequence of strain CCM 7198^T was 100 % identical to that of strain DSM 14968^T. The *A. grimontii* type strain sequence was found to be even more similar (99.9 %) to that of the type strain of *A. junii* (Fig. 2) than was reported by Carr *et al.* (2003). The only differences between the two sequences are an ambiguity in the sequence of the *A. junii* type strain, which was determined to be adenine (A) in our sequence, and the presence of two different gaps, one in each sequence.

Finally, the synonymy of the two species names is corroborated by an independent source. La Scola *et al.* (2006), reporting on *rpoB* gene sequencing as an approach with high discriminatory power, found that the complete *rpoB* gene sequences (4089 bp) of the type strains of *A. junii* and *A. grimontii* showed 99 % similarity whereas, for all other species pairs except for *A. baylyi* and *Acinetobacter* genomic species 11, less than 95 % similarity was observed in pairwise comparisons. In addition, *rpoB* sequences were determined in this study for *A. junii* CCM 2376^T and *A. grimontii* CCM 7198^T for both zones 1 and 2 (according to La Scola *et al.*, 2006). Firstly, our sequencing results, obtained for the CCM type strains for both species, were 100 % identical to those published by La Scola *et al.* (2006), obtained for the CIP type strains, for both strains and zones, confirming that the *A. grimontii* type strain held by the CCM was indeed obtained from the CIP. Secondly, we established 99 % identity between *A. junii* CCM 2376^T and *A. grimontii* CCM 7198^T for *rpoB* zone 1 and 97 % identity

Fig. 2. Rooted tree showing phylogenetic relationships within the genus *Acinetobacter* on the basis of a region of the 16S rRNA gene sequence corresponding to nucleotides 110–1443 of the gene from *Escherichia coli* ATCC 11775^T (GenBank accession no. X80725). Cluster analysis was performed using Genebase (Applied Maths) and the tree was constructed on the basis of the neighbour-joining method using members of the closest related genus *Alkanindiges* as an outgroup. Bar, 1 % sequence diversity. Numbers at nodes indicate bootstrap values. NIPH, National Institute of Public Health, Prague, Czech Republic. *Acinetobacter* sequences not determined in this study were reported by Rainey *et al.* (1994), Ibrahim *et al.* (1997), Vanechoutte *et al.* (1999), Nemec *et al.* (2001, 2003) and Carr *et al.* (2003).

for *rpoB* zone 2, corroborating the synonymy of *A. grimontii* and *A. junii*.

Carr *et al.* (2003) reported that the DNA–DNA relatedness between the type strains of *A. grimontii* and *A. junii* was 63 %, which was the highest of all interspecies values reported in their study. In our study, DNA–DNA hybridization between *A. junii* LMG 998^T and *A. grimontii* CCM 7198^T was carried out by the Culture Collection of the LMG (Gent, Belgium). Total DNA was prepared according to a modification of the method described by Wilson (1987), while hybridizations were performed at 37 °C according to a modification of the method described by Ezaki *et al.* (1989). A DNA–DNA relatedness value of 85 % (mean of at least four hybridizations) was obtained, with a maximum difference of 21 units between the reciprocal values. With this technique, the mean standard deviation is 14 units and deviations up to 20–25 units are

still acceptable (Goris *et al.*, 1998). In conclusion, the value of 85 % is clearly above 70 %, the generally accepted limit for species delineation (Wayne *et al.*, 1987), and indicates that the two strains belong to the same species.

All data presented here, as well as results published independently by La Scola *et al.* (2006), clearly indicate the synonymy of *A. grimontii* and *A. junii*. The reclassification of *Acinetobacter grimontii* Carr *et al.* 2003 as a later heterotypic synonym of *Acinetobacter junii* Bouvet and Grimont 1986 is therefore proposed in accordance with Rule 24a of the Bacteriological Code (Lapage *et al.*, 1992).

Acknowledgements

T.D.B. is indebted to the FWO (Fonds voor Wetenschappelijk Onderzoek – Vlaanderen) for a post-doctoral fellowship.

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