Reclassification of *Roseomonas fauriae* Rihs *et al.* 1998 as a later heterotypic synonym of *Azospirillum brasilense* Tarrand *et al.* 1979

Leta O. Helsel, Dannie G. Hollis, Arnold G. Steigerwalt and Paul N. Levett†

Correspondence

Paul N. Levett
plevett@health.gov.sk.ca

Meningitis and Special Pathogens Branch, Division of Bacterial and Mycotic Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA

The relatedness of *Roseomonas fauriae* and *Azospirillum brasilense* was investigated using phenotypic methods and DNA–DNA hybridization. Conventional biochemical tests did not differentiate between the two taxa. DNA–DNA hybridization experiments revealed high values for relatedness between the type strains of these species and suggest that these two taxa constitute a single species. Strains previously identified as *R. fauriae* should be reclassified as *A. brasilense*, with the name *Roseomonas fauriae* as a later heterotypic synonym of *Azospirillum brasilense*.

Roseomonas is a genus of pink-pigmented, oxidative, Gram-negative coccobacilli, first described by Rihs *et al.* (1993, 1998). *Roseomonas* species are opportunistic pathogens and have been isolated from a range of human infections including septicaemia, occurring primarily in patients with underlying medical conditions (De *et al.*, 2004; Struthers *et al.*, 1996). Six genomospecies were defined by DNA relatedness and by phenotypic characterization, of which three were named *Roseomonas gilardii*, *Roseomonas cervicalis* and *Roseomonas fauriae* (Rihs *et al.*, 1993). Five species of *Roseomonas* are currently recognized, including *Roseomonas mucosa* (Han *et al.*, 2003) and *Roseomonas lacus* (Jiang *et al.*, 2006). Recent analyses of 16S rRNA gene sequences and phenotypic characteristics suggested that *R. fauriae* was closely related to *Azospirillum brasilense* (Cohen *et al.*, 2004; Han *et al.*, 2003; Weyant & Whitney, 2005).

There are examples of organisms that have very similar or identical 16S rRNA gene sequences that are nevertheless not classified as members of the same species (Fox *et al.*, 1992; Gee *et al.*, 2004). Such discrepancies are resolved by DNA reassociation studies (Stackebrandt & Goebel, 1994). In order to determine the appropriate position of *R. fauriae*, we characterized strains of *R. fauriae* and *A. brasilense* by using phenotypic methods and DNA–DNA hybridization.

The phenotypic characteristics of five strains of *R. fauriae* and three strains of *A. brasilense* were determined using conventional methods (Weyant *et al.*, 1995). The strains studied were *R. fauriae* strains ATCC 49958^T, C5755, C5596, E5522 and F9190 and *A. brasilense* strains ATCC 29145^T, LOD-4 and RC-1. All strains produced pink, non-haemolytic colonies on rabbit blood agar plates, grew on

MacConkey agar but not on SS agar, cetrimide agar or citrate agar, produced optimum growth at 35 °C and were motile by means of a single polar flagellum; the results of other tests are shown in Table 1.

For preparation of DNA, all strains were cultured on 20–30 blood agar plates and incubated for 24 h at 35 °C. Cells were harvested and lysed and the DNA was isolated and purified according to the method of Brenner *et al.* (1982). DNA from strains *R. fauriae* C610^T (= ATCC 49958^T) and from *A. brasilense* ATCC 29145^T was labelled with [³²P]dCTP using a commercial nick translation kit (BRL) and tested for reassociation with unlabelled DNA from the same strain (homologous reaction), with other strains and also with the type and reference strains studied (heterologous reactions). Relative binding ratios, i.e. 100 × (percentage heterologous DNA bound to hydroxyapatite/percentage homologous DNA bound to hydroxyapatite), and the percentage divergence (the percentage of unpaired bases in related DNA sequences) were calculated as described previously (Brenner *et al.*, 1982). Divergence was calculated to the nearest 0.5 %, with each 1 °C decrease in the thermal stability of a heterologous DNA duplex due to approximately 1 % unpaired bases within related DNA (Bonner *et al.*, 1973). All reactions were performed in duplicate at the optimal temperature (70 °C).

Using the established molecular criteria for species-level relatedness (strains whose DNAs are at least 70 % related under optimal conditions and whose related sequences show ≤5 % divergence; Wayne *et al.*, 1987), the strains of *R. fauriae* and *A. brasilense* formed a single species-level hybridization group.

These data show that strains identified as *R. fauriae* are correctly classified as *A. brasilense*, the name that has priority. Accordingly, we propose that *Roseomonas fauriae*

†Present address: Saskatchewan Health, Provincial Laboratory, 3211 Albert Street, Regina, Saskatchewan S4S 5W6, Canada.

Table 1. Biochemical properties of strains of *R. fauriae* and *A. brasiliense*

Symbols: +, positive; –, negative; W, weakly positive; K, alkaline; NC, no clot; IR, indicator reduced.

Property	<i>R. fauriae</i>					<i>A. brasiliense</i>		
	ATCC 49958 ^T	C5755	C5596	E5522	F9190	ATCC 29145 ^T	LOD-4	RC-1
Catalase/oxidase	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
Acid from (OF base):								
Glucose, lactose, maltose, mannitol, sucrose	–	–	–	–	–	–	–	–
Xylose	W	W	W	W	W	W	W	W
Triple-sugar–iron agar slant	K	K	K	K	K	K	K	K
Triple-sugar–iron agar butt	K	K	K	K	K	K	K	K
Indole production	–	–	–	–	–	–	–	–
H ₂ S (lead acetate paper)	+	+	+	+	+	+	+	+
H ₂ S butt	–	–	–	–	–	–	–	–
Hydrolysis of:								
Aesculin, urea	+	+	+	+	+	+	+	+
Gelatin	–	–	–	–	–	–	–	–
Nitrate reduction	+	+, gas	+	+	+	+	+	+
Litmus milk reaction	NC	K	K	K	K	K, IR	K	K
Growth in/at:								
0 % NaCl	+	+	+	+	+	+	+	+
6 % NaCl	–	–	–	–	–	–	–	–
25 °C	+	+	+	+	+	+	+	+
42 °C	+	+	+	+	+	+	+	+

Rihs *et al.* 1998 be considered as a later heterotypic synonym of *Azospirillum brasiliense* Tarrand *et al.* 1979.

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