Aurantimonas altamirensis sp. nov., a member of the order *Rhizobiales* isolated from Altamira Cave

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A bacterial strain, S21B^T, was isolated from Altamira Cave (Cantabria, Spain). The cells were Gram-negative, short rods growing aerobically. Comparative 16S rRNA gene sequence analysis revealed that strain S21B^T represented a separate subline of descent within the family '*Aurantimonadaceae*' (showing 96 % sequence similarity to *Aurantimonas coralicida*) in the order *Rhizobiales* (*Alphaproteobacteria*). The major fatty acids detected were $C_{16:0}$ and $C_{18:1}\omega7c$. The G+C content of the DNA from strain S21B^T was 71·8 mol%. Oxidase and catalase activities were present. Strain S21B^T utilized a wide range of substrates for growth. On the basis of the results of this polyphasic study, isolate S21B^T represents a novel species of the genus *Aurantimonas*, for which the name *Aurantimonas altamirensis* sp. nov. is proposed. The type strain is S21B^T (=CECT 7138^T=LMG 23375^T).

The genera *Aurantimonas* and *Fulvimarina* constitute the two members of the recently described family '*Aurantimonadaceae*' within the order *Rhizobiales*. Both genera are represented by single species, *Aurantimonas coralicida* (Denner *et al.*, 2003) and *Fulvimarina pelagi* (Cho & Giovannoni, 2003), which were isolated from the marine environment. *A. coralicida* was isolated from a diseased coral (*Dichocoenia stokesi*) and *F. pelagi* was retrieved from bacterioplankton; at present, there are no members of this family from terrestrial environments.

This paper reports on the isolation and characterization of strain S21B^T. This strain was collected from a subterranean environment, Altamira Cave (Cantabria, Spain), being part of a complex microbial community that produces white-coloured colonization on the walls of the cave. This white-coloured microbial growth is threatening the important Palaeolithic paintings of Altamira Cave (Gonzalez *et al.*, 2006).

Strain S21B^T was isolated on 1000-fold-diluted tryptose soy agar (TSA; Oxoid) at 28 °C. *A. coralicida* DSM 14790^T and *F. pelagi* DSM 15513^T were grown on marine agar 2216 (Difco, Becton Dickinson). The methods used in this study were performed as described previously (Jurado *et al.*, 2005a, b), except where indicated otherwise. The cellular fatty acids were determined as described by Gonzalez *et al.* (2004), using the same growth conditions for the tested strains. The growth temperature was tested in the range 4–46 °C. Tolerance of NaCl was studied on TSA and in nutrient broth each supplemented with 0–25 % (w/v) NaCl. Pigment analysis was performed as reported by Denner *et al.* (2003).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain S21B^T is DQ372921.

Analysis of 16S rRNA gene sequences revealed that strain S21B^T belongs to the family '*Aurantimonadaceae*' and is closely related to the members of the genera *Aurantimonas* (96·1 % similarity) and *Fulvimarina* (93·2 % similarity). Within this phylogenetic clade, the members of the genera *Aurantimonas* and *Fulvimarina* showed 93·8 % similarity. Phylogenetic analysis based on 1293 bp was performed as described previously (Jurado *et al.*, 2005b). A consensus tree showed the 16S rRNA gene sequence of strain S21B^T branching off in the proximity of *A. coralicida* strains (not shown). Thus, strain S21B^T represents the first member of this family to have been isolated from a terrestrial environment.

Strain S21B^T grows optimally in nutrient agar without the addition of salts and at salt concentrations below 20 g l^{-1}; these values for salt concentration were lower than the optimum values observed for the type strains of *A. coralicida* (32 g l^{-1}; Denner *et al.*, 2003) and *F. pelagi* (20–25 g l^{-1}; Cho & Giovannoni, 2003). The maximum NaCl concentration tolerated by strain S21B^T was 50 g l^{-1}. Growth of *A. coralicida* and *F. pelagi* occurred at 4–40 °C, while strain S21B^T showed significant growth between 10 and 40 °C, with an optimum at 28 °C. The cells of strain S21B^T were Gram-negative, strictly aerobic, non-spore-forming, non-motile, short rods, whereas *A. coralicida* WP1^T showed motility. No flagella were observed on negatively stained cells of strain S21B^T. The novel strain was oxidase- and catalase-positive. Other morphological and physiological characteristics of strain S21B^T are shown in Table 1. The hydrolysis of adenine, hypoxanthine, tyrosine, xanthine and Tweens 20 and 80 differed among the analysed type strains and S21B^T. *A. coralicida* WP1^T and strain S21B^T could be clearly differentiated on the basis of the production of acid from erythritol and L-xylose; a longer list of substrates utilized

Table 1. Phenotypic characteristics of strain S21B^T and related type strains

Strains: 1, strain S21B^T; 2, *A. coralicida* DSM 14790^T; 3, *F. pelagi* DSM 15513^T. Symbols: −, negative; +, positive; (+), weakly positive. Data were obtained during this study under identical growth conditions, except where indicated otherwise. Strain S21B^T produces acid from D-glucose and D-fucose. It produces alkaline phosphatase, esterase (C1), esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase but not lipase (C14), cystine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase or α-fucosidase. It assimilates mannose, malate, gluconate and glucose but not capric acid, adipic acid or citrate. It is positive for catalase and oxidase. It is positive for urease activity and negative for glucose fermentation and arginine dihydrolase activity. It is negative for the hydrolysis of aesculin, gelatin and starch, for nitrate reduction and in Voges–Proskauer, methyl red and indole tests. It is sensitive to chloramphenicol (30 μg), rifampicin (5 μg), tetracycline (30 μg), norfloxacin (10 μg), novobiocin (30 μg), streptomycin (10 μg), carbenicillin (100 μg), framycetin (50 μg), nalidixic acid (30 μg) and erythromycin (15 μg). Halophilic growth (at 10 % NaCl) is an additional trait that could be useful in discriminating between *Aurantimonas* and *Fulvimarina* species.

Characteristic	1	2	3
Growth with 10 % NaCl (w/v)	−	−	+
Decomposition or hydrolysis of:			
Adenine	+	−	+
Hypoxanthine	+	−	+
Tyrosine	−	−	+
Xanthine	+	−	+
Tween 20	−	+	−
Tween 80	−	+	−
Acid produced from:			
D-Arabinose	(+)	+	−
L-Arabinose	+	+	−
Erythritol	+	−	−
L-Fucose	(+)	−	−
D-Galactose	+	+	−
Gentiobiose	−	(+)	−
D-Mannose	+	+	−
D-Melibiose	(+)	+	−
L-Rhamnose	+	+	−
D-Ribose	+	+	−
D-Xylose	+	+	−
L-Xylose	−	+	−
Assimilation of:			
Arabinose	+	+[a]*	−
Maltose	+	−	+
Mannitol	+	−	−
N-Acetylglucosamine	+	−	+
Phenylacetic acid	−	−	+
Antibiotic susceptibility			
Ampicillin (10 μg)	+	−	−
Doxycycline (30 μg)	+	−	+
Kanamycin (30 μg)	+	+[a]	−[b]
Vancomycin (30 μg)	−	−	+
Gentamicin (10 μg)	+	+[a]	−[b]
Enzymic activities			
β-Glucosidase	−	−	+
Trypsin	+	−	−

*Data from other studies indicated as: *a*, Denner *et al.* (2003); *b*, Cho & Giovannoni (2003).

Table 2. Major fatty acid composition of strain S21B^T and related type strains

Strains: 1, strain S21B^T; 2, *A. coralicida* DSM 14790^T; 3, *F. pelagi* DSM 15513^T. Data were obtained during this study under identical growth conditions. ND, Not detected.

Fatty acid	1	2	3
$C_{16:0}$	11·3±0·1	6·2±0·0	5·2±0·0
iso-$C_{16:0}$	1·4±0·0	ND	ND
$C_{18:0}$	0·7±0·0	2·0±0·0	12·7±0·9
$C_{18:1}$ 2-OH	3·5±0·1	3·2±0·0	1·2±0·0
$C_{18:1}\omega7c$	74·4±0·2	69·4±0·0	77·2±0·2
$C_{19:0}\omega8c$ cyclo	ND	13·2±0·0	2·9±0·3

differently by *F. pelagi* HTCC 2506^T and strain S21B^T is shown in Table 1. Strain S21B^T was able to assimilate maltose, mannitol and *N*-acetylglucosamine, whereas *A. coralicida* WP1^T was unable to utilize these substrates. Strain S21B^T could also be differentiated from the *A. coralicida* and *F. pelagi* type strains on the basis of antibiotic susceptibilities (Table 1). Strain S21B^T produced carotenoid pigments with peaks in the absorption spectra at 447 and 470–471 nm, with a slight inflexion point at 424–427 nm, similar to *A. coralicida* and *F. pelagi*.

The major fatty acids detected in strain S21B^T are shown in Table 2. The predominant fatty acid in strain S21B^T was *cis*-7-octadecenoic acid ($C_{18:1}\omega7c$), as was the case for *A. coralicida* and *F. pelagi*. However, strain S21B^T and these species showed significant differences in the content of $C_{19:0}\omega8c$ cyclo, iso-$C_{16:0}$, $C_{16:0}$, $C_{18:0}$ and $C_{18:1}\omega7c$, as shown in Table 2.

The DNA G+C content of strain S21B^T was 71·8±1·8 mol%, which is slightly higher than the value for *A. coralicida* WP1^T (66·3 mol%; Denner *et al.*, 2003) and much higher than those for *F. pelagi* strains (57·6–59·9 mol%; Cho & Giovannoni, 2003). The degrees of DNA–DNA relatedness between strain S21B^T and the type strains of *A. coralicida* and *F. pelagi* were determined using DNA from strain S21B^T as a probe for DNA–DNA hybridization, according to the method described by Ziemke *et al.* (1998). *A. coralicida* WP1^T and *F. pelagi* HTCC 2506^T showed DNA relatedness values of 56 and 46 %, respectively. The results regarding G+C contents and DNA–DNA relatedness estimates confirmed that strain S21B^T represents a novel genospecies that can be clearly differentiated from *A. coralicida* and *F. pelagi*.

On the basis of the phenotypic and genotypic characteristics described above and their differences with respect to those of previously described related species within the family '*Aurantimonadaceae*', strain S21B^T represents a novel species within the genus *Aurantimonas*, for which the name *Aurantimonas altamirensis* sp. nov. is proposed.

Description of *Aurantimonas altamirensis* sp. nov.

Aurantimonas altamirensis [al.ta.mi.ren′sis. N.L. fem. adj. *altamirensis* referring to Altamira Cave (Cantabria, Spain), where the type strain was isolated].

Gram-negative. Cells are non-motile, short rods, 0·9 μm wide and 1·1 μm long on average. Strictly aerobic. Colonies are about 1 mm in diameter, yellow-coloured, circular, convex and smooth. Growth occurs between 10 and 40 °C, growing optimally at 28 °C. The optimum NaCl concentration for growth is 0–20 g NaCl l^{-1}; concentrations up to 50 g l^{-1} are tolerated. Peaks in absorption spectra are as defined for the genus. Catalase-, oxidase- and urease-positive. Chemotaxonomic characteristics are as reported in Table 1. The predominant fatty acid is $C_{18:1}\omega7c$, with significant proportions of $C_{16:0}$, $C_{18:1}$ 2-OH, iso-$C_{16:0}$ and $C_{18:0}$; $C_{19:0}\omega8c$ cyclo is not detected. The DNA G+C content of the type strain is 71·8 mol%.

The type strain, strain S21B^T (=CECT 7138^T=LMG 23375^T), was isolated from Altamira Cave (Cantabria, Spain).

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