Roseomonas aquatica sp. nov., isolated from drinking water

Virginia Gallego, Cristina Sánchez-Porro, Maria Teresa García and Antonio Ventosa

Correspondence
Antonio Ventosa
ventosa@us.es

Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Sevilla, 41012 Sevilla, Spain

Strain TR53^T, a Gram-negative, non-motile, non-spore-forming and strictly aerobic coccobacillus, isolated from the drinking water distribution system of Seville, Spain, was subjected to polyphasic taxonomic analysis using a combination of phenotypic, genotypic and phylogenetic features. Phylogenetic analysis of 16S rRNA gene sequences showed that strain TR53^T had highest similarity to members of the genus *Roseomonas*, with sequence similarity values between 95.7 % (to *Roseomonas* genomospecies 5 strain ATCC 49960) and 94.0 % (to *Roseomonas gilardii* subsp. *rosea* ATCC 49956^T). On the basis of its phenotypic characteristics, 16S rRNA gene sequence data and DNA G + C content (68.6 mol%), strain TR53^T represents a novel species of the genus *Roseomonas*, for which the name *Roseomonas aquatica* sp. nov. is proposed. The type strain of *Roseomonas aquatica* is TR53^T (=CECT 7131^T =JCM 13556^T).

Currently, the genus *Roseomonas* comprises the species *Roseomonas gilardii* (the type species), containing *R. gilardii* subsp. *gilardii* (Rihs *et al.*, 1993) and *R. gilardii* subsp. *rosea* (Han *et al.*, 2003), *R. cervicalis*, *R. fauriae* (Rihs *et al.*, 1993), *R. mucosa* (Han *et al.*, 2003) and *R. lacus* (Jiang *et al.*, 2006). Members of the genus *Roseomonas* belong to the *Alphaproteobacteria* and are pink, Gram-negative coccobacilli with oxidative metabolism that, historically, have been cultured from clinical specimens and are considered to be pathogenic for humans (Rihs *et al.*, 1993; McLean *et al.*, 2006). The recent description of *R. lacus* (Jiang *et al.*, 2006), isolated from freshwater sediment, proves that species of this genus can be isolated from environmental samples. Moreover, other bacterial strains related to this genus have been isolated from very different environments such as drinking water distribution systems (September *et al.*, 2004) or associated with deep-water marine invertebrates (Sfanos *et al.*, 2005), indicating that members of the genus *Roseomonas* are widely distributed in nature (Jiang *et al.*, 2006).

During a broad study carried out in 2003–2004 that focussed on the identification of culturable bacteria occurring in the drinking water distribution system of Seville, Spain, approximately 600 bacterial strains were isolated. Drinking water samples (25 l) were concentrated using a tangential flow filtration system (Filtron), plated on plate count agar (PCA; Difco) and R2A (Difco), and incubated at 28 °C for 7 days. Morphologically different colonies were picked in order to obtain pure cultures. The majority of

the pink-pigmented isolates belonged to the genus *Methylobacterium* (Gallego *et al.*, 2005a, b, c, 2006), but strain TR53^T differed strongly from isolates related to this genus, being unable to grow on one-carbon compounds such as methanol. This isolate was studied phenotypically, phylogenetically and genotypically.

Chromosomal DNA was isolated and purified according to the method described by Marmur (1961). The 16S rRNA gene was amplified using universal primers 16F27 and 16R1488 as described by Mellado *et al.* (1995). The almost-complete nucleotide sequence was determined by NBT-Newbiotechnic (Seville, Spain) using an automated DNA sequencer model 3100 (Applied Biosystems). Subsequent sequence analysis was conducted using the ARB program package (Ludwig *et al.*, 2004). Following the recommendations of Ludwig *et al.* (1998), alternative treeing methods (maximum-parsimony, distance matrix and maximum-likelihood) were used. A comparison of 16S rRNA gene sequences revealed that the sequence of strain TR53^T (1387 bp) displayed the highest level of similarity to those of *Roseomonas* species; highest sequence similarities were to *Roseomonas* genomospecies 5 strain ATCC 49960 (95.7 %) and *R. cervicalis* ATCC 49957^T (94.9 %). The sequence similarity between strain TR53^T and *R. fauriae* ATCC 49958^T was only 85.6 %. The similarity values between *R. fauriae* and *Roseomonas* species with validly published names were lower than 86.3 %, indicating that *R. fauriae* probably represents a member of a different genus, as has been suggested previously (Cohen *et al.*, 2004; Han *et al.*, 2003; Jiang *et al.*, 2006). Similarly, the similarity between *Roseomonas* genomospecies 6 and *R. fauriae* is 96.6 % and that between *Roseomonas* genomospecies 6 and the other

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TR53^T is AM231587.

Roseomonas species is lower than 86.2 %. Overall, the 16S rRNA phylogenetic analysis clearly shows that strain TR53^T constitutes a branch of its own that is separate from the other species of the genus *Roseomonas* (Fig. 1). Our results suggest that the proposal of the genera *Teichococcus* and *Muricoccus* should be investigated in detail since they might represent species of the genus *Roseomonas*. However, this genus is rather heterogeneous and more in-depth studies are necessary. On the other hand, the similarity between strain TR53^T and the type strains of species of the genera *Teichococcus* and *Muricoccus* were 94.9 and 95.5 %, respectively. According to phylogenetic data, strain TR53^T belongs to the genus *Roseomonas*, but as it only shows a relatively low similarity to other species, strain TR53^T represents a novel species of this genus.

The G+C content of genomic DNA was determined from the mid-point value (T_m) of the thermal denaturation profile (Marmur & Doty, 1962) using the equation of Owen & Hill (1979), as described previously (Ventosa *et al.*, 1999). The DNA G+C content of strain TR53^T is 68.6 mol%, a value that is within the range for members of the genus *Roseomonas* (Rihs *et al.*, 1993; Jiang *et al.*, 2006).

The shape and motility of bacterial cells were observed under a phase-contrast microscope ($\times 1000$) from a 48 h liquid culture in R2A medium. Growth at different temperatures (4–40 °C), pH (4–12) and NaCl concentrations (0–5 % NaCl) was tested on R2A agar medium. The isolate was also tested for its ability to grow on nutrient agar medium (NM) and tryptone soy agar (TSA; Difco). H₂S

production and acid and gas production from glucose, lactose and sucrose were determined on Kligler iron agar (Difco). Oxidase activity was detected using a 1 % solution of tetramethyl-*p*-phenylenediamine (Difco) (Kovacs, 1956). Catalase activity was tested by picking up a young colony and smearing it in a drop of H₂O₂. The methyl red and Voges–Proskauer reactions were performed in Clark–Lubs' medium (Scharlau). Indole production was determined with Kovacs' reagent in 1 % tryptone broth. Simmons' citrate test was carried out in Simmons' citrate agar (Sigma). For the determination of acid production from different carbohydrates (1 %), a medium containing 0.5 % peptone, 0.5 % NaCl and 0.001 % phenol red was used (Cowan & Steel, 1974). Nitrate reduction was tested on nitrate broth containing 0.2 % KNO₃ (Skerman, 1967). Urease activity was studied in Christensen's medium (Christensen, 1946). Hydrolysis of gelatin, starch and DNA was tested in the corresponding agar media (Scharlau). Tween 80 hydrolysis was tested in R2A medium containing 1 % Tween 80 and 0.02 % CaCl₂. Casein hydrolysis was tested in R2A medium supplemented with 2 % skimmed milk (Difco). Use of methanol was tested in mineral medium K (Doronina *et al.*, 1998). API 20NE and API ZYM strips (bioMérieux) were inoculated according to the manufacturer's instructions and incubated at 28 °C. API 50CH was inoculated as described by Kersters *et al.* (1984). API ZYM strips were read after 4 and 24 h and API 20NE and API 50CH were read after 7 and 14 days. Antibiotic susceptibility was determined according to the conventional Kirby–Bauer method (Bauer *et al.*, 1966).

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequence comparison showing the relationship between *Roseomonas aquatica* sp. nov. TR53^T, species belonging to the genus *Roseomonas* and related genera. The tree was constructed using the maximum-parsimony method. Bootstrap values >50 % are indicated at branch-points. Bar, 1 % sequence divergence.

Strain TR53^T is a Gram-negative coccobacillus that is strictly aerobic and measures 1.0 µm wide by 1.2–2.0 µm long when grown for 48 h at 28 °C in R2A liquid medium. Cells were non-motile. Colonies of strain TR53^T were irregular, pale-pink-pigmented and mucoid with a diameter of 0.5–1.0 mm on R2A agar after 5 days incubation at 28 °C. On NM, colonies were not mucoid. Strain TR53^T was slow-growing, typically requiring 3–4 days under optimal conditions to give observable turbidity. Growth did not occur in the presence of ≥2 % NaCl. Other phenotypic features are shown in the species description. Differential phenotypic characteristics of strain TR53^T and other *Roseomonas* species are summarized in Table 1.

The whole-cell fatty acid composition was determined using the MIDI system (Microbial Identification System). Cells were cultured for 48 h at 28 °C on R2A medium at pH 7.0 and cellular fatty acids were analysed using GC at the Belgian Co-ordinated Collections of Microorganisms, Laboratory of Microbiology of Gent (BCCM/LMG), Gent, Belgium (Miller, 1982; Kämpfer & Kroppenstedt, 1996). The predominant fatty acids of strain TR53^T were C_{18:1}ω7c, iso-C_{15:0} 2-OH and/or C_{16:1}ω7c, C_{18:1} 2-OH and C_{16:0}. This is similar to the fatty acid profile of *R. gilardii* subsp. *rosea* (Han *et al.*, 2003), except that strain TR53^T has a higher percentage of iso-C_{15:0} 2-OH and/or C_{16:1}ω7c. However, the fatty acid profile of strain TR53^T differed from that reported for *R. lacus* (Jiang *et al.*, 2006). Analysis of quinones was carried out by the Identification Service of the DSMZ (Braunschweig, Germany). As with other member of the genus *Roseomonas*, the major quinone is ubiquinone-10 (Q-10).

Fig. 2. Phase-contrast photomicrograph of cells of *Roseomonas aquatica* sp. nov. TR53^T. Bar, 10 µm.

On the basis of the molecular and physiological characteristics of strain TR53^T, it is proposed that this strain represents a novel species, *Roseomonas aquatica* sp. nov.

Description of *Roseomonas aquatica* sp. nov.

Roseomonas aquatica (a.qua'ti.ca. L. fem. adj. *aquatica* found in water, aquatic).

Gram-negative coccobacillus, 1.0 × 1.2–2.0 µm, occurring singly and in pairs (in liquid R2A medium at 28 °C after 48 h) (Fig. 2). Cells are non-motile, non-spore-forming and

Table 1. Differential characteristics of strain TR53^T and members of the genus *Roseomonas*

Strains: 1, TR53^T; 2, *R. lacus* TH-G33^T; 3, *R. mucosa* ATCC BAA-692^T; 4, *R. gilardii* subsp. *gilardii* ATCC 49956^T; 5, *R. gilardii* subsp. *rosea* ATCC BAA-691^T; 6, *R. cervicalis* ATCC 49957^T; 7, *R. fauriae* ATCC 49958^T. ND, Not determined; v, variable; +, positive; –, negative. Data from Jiang *et al.* (2006), Han *et al.* (2003), Rihs *et al.* (1993) and this study.

Characteristic	1	2	3	4	5	6	7
Motility	–	–	+	v	ND	+	+
Optimum growth temperature (°C)	28	30	35	35	35	35	35
Growth on NaCl (%)	<2	>6.5	ND	≤6	ND	≤6	≤6
Hydrolysis of aesculin	–	–	–	–	–	–	+
Reduction of nitrate	+	+	–	v	–	–	+
Utilization of:							
D-Glucose	–	–	+	v	v	–	v
L-Arabinose	–	+	+	v	+	v	+
Citrate	–	–	+	+	+	v	v
Fructose	–	–	ND	+	ND	+	+
Ribose	–	+	ND	ND	ND	ND	ND
D-Galactose	–	+	ND	v	ND	–	+
D-Xylose	–	+	ND	v	ND	v	+
Glycerol	–	–	ND	+	ND	–	+
Isolation source	Potable water	Freshwater lake sediment	Blood	Potable water	Blood	Cervix	Wound
DNA G+C content (mol%)	68.6	71.9	ND	67.6	ND	70.4	68

strictly aerobic. Colonies are irregular, pale-pink-pigmented, mucoid, with a diameter of 0.5–1.0 mm on R2A agar after 4 days incubation at 28 °C. On NM, colonies are not mucoid and have a diameter $\leq$ 0.5 mm after 4 days incubation. Slow-growing, typically requiring 2–4 days under optimal conditions in order to observe turbidity in liquid media. Does not grow on TSA. Does not grow in the presence of $\geq$ 2% NaCl. Growth occurs at 15–35 °C (optimal temperature 25–28 °C) and pH 5.0–9.0 (optimal pH 7.0). Catalase- and urease-positive. Oxidase-negative. Indole, methyl red and Voges–Proskauer reactions and Simmons' citrate test are negative. Tween 80, starch, gelatin, casein and DNA are not hydrolysed. Hydrogen sulfide is not produced. Nitrate is reduced to nitrite. Acid is not produced oxidatively from D-galactose, D-mannose, D-glucose, D-fructose, D-maltose, glycerol, D-mannitol, D-trehalose, D-xylose or lactose. Does not use any of the carbohydrates or acids present in API 20NE and API 50CH strips as sole carbon sources, i.e. glycerol, erythritol, D- and L-arabinose, D-ribose, D- and L-xylose, D-adonitol, methyl β -D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α -D-mannopyranoside, methyl α -D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, sucrose, D-trehalose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D- and L-fucose, D- and L-arabitol, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Alkaline and acid phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase are present. Negative for arginine dihydrolase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. Cellular fatty acids are C_{12:0} (0.6%), iso-C_{16:0} and/or C_{14:0} 3-OH (2.7%), iso-C_{15:0} 2-OH and/or C_{16:1} ω 7c (27.0%), C_{16:0} (12.5%), C_{16:0} 3-OH (2.9%), C_{18:1} ω 7c (31.3%), C_{18:0} (1.5%) and C_{18:1} 2-OH (21.5%). The major quinone is ubiquinone-10 (Q-10). Resistant to (μ g per disc) tetracycline (30), rifampicin (30), streptomycin (10), neomycin (10), erythromycin (15), kanamycin (30), vancomycin (30), nalidixic acid (30), novobiocin (30), penicillin (10 units), bacitracin (10 units), cephalothin (30), chloramphenicol (30) and trimethoprim/sulfamethoxazole (1.25/23.75).

The type strain is strain TR53^T (=CECT 7131^T=JCM 13556^T), isolated from drinking water. The DNA G+C content of the type strain is 68.6 mol% (T_m).

Acknowledgements

V.G. was supported by a fellowship from the Spanish Ministerio de Educación y Ciencia. This work was supported by grants from the Quality of Life and Management of Living Resources Programme of the

European Commission (QLK3-CT-2002-01972), the Spanish Ministerio de Ciencia y Tecnología (BMC2003-01344) and from the Junta de Andalucía.

References

- Bauer, A. W., Kirby, W. M. M., Sherris, J. C. & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* **45**, 493–496.
- Christensen, W. B. (1946). Urea decomposition as a means of differentiating *Proteus* and paracolon cultures from each other and from *Salmonella* and *Shigella* types. *J Bacteriol* **52**, 461–466.
- Cohen, M. F., Han, X. Y. & Mazzola, M. (2004). Molecular and physiological comparison of *Azospirillum* spp. isolated from *Rhizoctonia solani* mycelia, wheat rhizosphere, and human skin wounds. *Can J Microbiol* **50**, 291–297.
- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- Doronina, N. V., Trotsenko, Y. A., Krausova, V. I., Boulygina, E. S. & Tourova, T. P. (1998). *Methylophilus capsulata* gen. nov., sp. nov., a novel non-pigmented aerobic facultatively methylotrophic bacterium. *Int J Syst Bacteriol* **48**, 1313–1321.
- Gallego, V., Garcia, M. T. & Ventosa, A. (2005a). *Methylobacterium hispanicum* sp. nov. and *Methylobacterium aquaticum* sp. nov., isolated from drinking water. *Int J Syst Evol Microbiol* **55**, 281–287.
- Gallego, V., Garcia, M. T. & Ventosa, A. (2005b). *Methylobacterium variabile* sp. nov., a methylotrophic bacterium isolated from an aquatic environment. *Int J Syst Evol Microbiol* **55**, 1429–1433.
- Gallego, V., Garcia, M. T. & Ventosa, A. (2005c). *Methylobacterium isbiliense* sp. nov., isolated from the drinking water system of Sevilla, Spain. *Int J Syst Evol Microbiol* **55**, 2333–2337.
- Gallego, V., Garcia, M. T. & Ventosa, A. (2006). *Methylobacterium adhaesivum* sp. nov., a methylotrophic bacterium isolated from drinking water. *Int J Syst Evol Microbiol* **56**, 339–342.
- Han, X. Y., Pham, A. S., Tarrand, J. J., Rolston, K. V., Helsel, L. O. & Levett, P. N. (2003). Bacteriologic characterization of 36 strains of *Roseomonas* species and proposal of *Roseomonas mucosa* sp. nov. and *Roseomonas gilardii* subsp. *rosea* subsp. nov. *Am J Clin Pathol* **120**, 256–264.
- Jiang, C.-Y., Dai, X., Wang, B.-J., Zhou, Y.-G. & Liu, S.-J. (2006). *Roseomonas lacus* sp. nov., isolated from freshwater lake sediment. *Int J Syst Evol Microbiol* **56**, 25–28.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Kerstens, K., Hinz, K.-H., Hertle, A., Segers, P., Lievens, A., Siegmund, O. & De Ley, J. (1984). *Bordetella avium* sp. nov., isolated from the respiratory tracts of turkeys and other birds. *Int J Syst Bacteriol* **34**, 56–70.
- Kovacs, N. (1956). Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature* **178**, 703.
- Ludwig, W., Strunk, O., Klugbauer, S., Klugbauer, N., Weizenegger, N., Neumaier, J., Bachleitner, M. & Schleifer, K.-H. (1998). Bacterial phylogeny based on comparative sequence analysis. *Electrophoresis* **19**, 554–568.
- Ludwig, W., Strunk, O., Westram, R. & 29 other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

- Marmur, J. & Doty, P. (1962).** Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **4**, 109–118.
- McLean, T. W., Rouster-Stevens, K., Woods, C. R. & Shetty, A. K. (2006).** Catheter-related bacteremia due to *Roseomonas* species in pediatric hematology/oncology patients. *Pediatr Blood Cancer* **46**, 514–516.
- Mellado, E., Moore, E. R. B., Nieto, J. J. & Ventosa, A. (1995).** Phylogenetic inferences and taxonomic consequences of 16S ribosomal DNA sequence comparison of *Chromohalobacter marismortui*, *Volcaniella eurihalina*, and *Deleya salina* and reclassification of *V. eurihalina* as *Halomonas eurihalina* comb. nov. *Int J Syst Bacteriol* **45**, 712–716.
- Miller, L. T. (1982).** Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* **16**, 584–586.
- Owen, R. J. & Hill, L. R. (1979).** The estimation of base compositions, base pairing and genome sizes of bacterial deoxyribonucleic acids. In *Identification Methods for Microbiologists*, 2nd edn, pp. 217–296. Edited by F. A. Skinner & D. W. Lovelock. London: Academic Press.
- Rihs, J. D., Brenner, D. J., Weaver, R. E., Steigerwalt, A. G., Hollis, D. G. & Yu, V. L. (1993).** *Roseomonas*, a new genus associated with bacteremia and other human infections. *J Clin Microbiol* **31**, 3275–3283.
- September, S. M., Brozel, V. S. & Venter, S. N. (2004).** Diversity of nontuberculous *Mycobacterium* species in biofilms of urban and semiurban drinking water distribution systems. *Appl Environ Microbiol* **70**, 7571–7573.
- Sfanos, K., Harmody, D., Dang, P., Ledger, A., Pomponi, S., McCarthy, P. & Lopez, J. (2005).** A molecular systematic survey of cultured microbial associates of deep-water marine invertebrates. *Syst Appl Microbiol* **28**, 242–264.
- Skerman, V. B. D. (1967).** *A Guide to the Identification of the Genera of Bacteria*, 2nd edn. Baltimore: Williams & Wilkins.
- Ventosa, A., Gutierrez, M. C., Kamekura, M. & Dyal-Smith, M. L. (1999).** Proposal to transfer *Halococcus turkmenicus*, *Halobacterium trapanicum* JCM 9743 and strain GSL-11 to *Haloterrigena turkmenica* gen. nov., comb. nov. *Int J Syst Bacteriol* **49**, 131–136.