Actibacterium mucosum gen. nov., sp. nov., a marine alphaproteobacterium from Mediterranean seawater

Teresa Lucena, María A. Ruvira, Esperanza Garay, M. Carmen Macián, David R. Arahal and María J. Pujalte

Correspondence

María J. Pujalte
maria.j.pujalte@uv.es

Departamento de Microbiología y Ecología and Colección Española de Cultivos Tipo (CECT), Universidad de Valencia, Valencia, Spain

Strain R46^T, a marine alphaproteobacterium, was isolated from Mediterranean seawater at Malvarrosa beach, Valencia, Spain. It is an aerobic chemo-organotrophic, mesophilic and slightly halophilic organism, with complex ionic requirements. Phylogenetic analyses based on the 16S rRNA and *gyrB* gene sequences showed that strain R46^T formed a separate branch within the family *Rhodobacteraceae*, bearing similarities below 94.7 and 80.3 %, respectively, to any other recognized species. It contained Q10 as the predominant isoprenoid quinone and C_{18:1}ω7c/C_{18:1}ω6c as the major cellular fatty acid. Phosphatidylglycerol was the only identified polar lipid, although other lipids were also detected. The DNA G+C content of the genomic DNA was 61.3 mol%. On the basis of extensive phenotypic and phylogenetic comparative analysis, it is concluded that the strain represents a novel genus and species, for which the name *Actibacterium mucosum* gen. nov., sp. nov. is proposed. The type strain of the type species is *Actibacterium mucosum* R46^T (=CECT 7668^T=KCTC 23349^T).

The family *Rhodobacteraceae* in the class *Alphaproteobacteria* of the phylum *Proteobacteria* mainly comprises bacteria of aquatic origin, many of them requiring NaCl for growth. Within this family, the so-called *Roseobacter* group is one of the most abundant and ubiquitous. Since the description of the genus *Roseobacter* over two decades ago (Shiba, 1991), this group has expanded to include more than 54 genera, as can be seen in the release (LTPs106_SSU) of the Living Tree Project database (Yarza *et al.*, 2010).

Strain R46^T was isolated from surface seawater collected at Malvarrosa Beach (Valencia, Spain, 39° 28' 29" N 0° 19' 23" W; temperature 25 °C; pH 8.0) in July 2008. Seawater was plated directly on Marine R2A agar, and incubated at 26 °C for 8 days, after which isolated colonies were randomly selected and restreaked on the same medium

Abbreviations: ASW, artificial sea water; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PHB, polyhydroxybutyrate; MALDI-TOF, matrix-assisted desorption/ionization time of flight; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; PG, phosphatidylglycerol; PE, phosphatidylethanolamine.

The GenBank/EMBL/DBJ accession numbers for the *gyrB* gene sequences of *Palleronia marisminoris* CECT 7066^T, strain R46^T, *Jannaschia seosinensis* CECT 7799^T, *Maribius salinus* CECT 7800^T, *Jannaschia donghaensis* CECT 7802^T, *Oceanicola nanhaiensis* CECT 7831^T and *Oceanicola pacificus* CECT 7832^T are HE590587–HE590593, and that of the 16S rRNA gene of strain R46^T is HE590855.

Six supplementary figures are available with the online version of this paper.

until pure cultures were obtained. Marine R2A agar was prepared with R2A agar (Difco) using aged, filtered seawater as the suspending fluid instead of distilled water. Strain R46^T showed good growth on marine broth (MB; Difco) and marine agar (MA, MB plus 1.2 % bacteriological agar; Oxoid). Thus, for routine use, the strain was subsequently cultured on MA or in MB at 28 °C for 2–4 days. It was stored at –80 °C in MB supplemented with 20 % (v/v) glycerol. Strain R46^T was deposited in CECT and KCTC as CECT 7668^T and KCTC 23349^T, respectively.

A 1000 bp region of the 16S rRNA gene (Arahal *et al.*, 2008) of strain R46^T was obtained and a BLAST search in public databases (Zhang *et al.*, 2000) suggested that this strain represented a new taxon within the alphaproteobacteria branch containing members of the genus *Roseobacter* and related marine bacteria. Therefore, strain R46^T was submitted to a taxonomic study using a polyphasic approach, including classical biochemical and physiological characterization, chemotaxonomic and phylogenetic analyses, using almost complete (1412 bp) 16S rRNA and partial (1000 bp) *gyrB* gene sequences. The following type strains were included in the study and tested under the same conditions: *Pseudoruegeria aquimaris* CECT 7680^T, *Jannaschia rubra* CECT 5088^T, *Jannaschia helgolandensis* DSM 14858^T, *Jannaschia donghaensis* CECT 7802^T, *Jannaschia seosinensis* CECT 7799^T, *Palleronia marisminoris* CECT 7066^T, *Maribius salinus* CECT 7800^T and *Thalassobacter stenotrophicus* CECT 5294^T.

The cell morphology and motility of strain R46^T were examined on wet mounts under phase-contrast microscopy (DMRB fluorescence microscope; Leica). Half-strength artificial sea water (ASW; 400 mM NaCl, 100 mM MgSO₄·7H₂O, 20 mM KCl and 20 mM CaCl₂·2H₂O) was used as the suspension medium. Cells grown on MA plates for 48 h were rod-shaped and non-motile. Inclusion bodies or granules were not seen. The absence of polyhydroxybutyrate (PHB) accumulation was also noted in cells grown on MA and basal medium agar [BMA; 50 mM Tris/HCl, pH 7.5; 19 mM NH₄Cl; 0.33 mM K₂HPO₄·3 H₂O; 0.1 mM FeSO₄·7H₂O and 1.3 % w/v, purified agar (Oxoid)] on half-strength ASW (Baumann & Baumann, 1981)] plus pyruvate for 7 days at 25 °C that were stained with Nile Blue A (Smibert & Krieg, 1994). *T. stenotrophicus* CECT 5294^T was used as a positive control. The cell size and morphology of strain R46^T were also determined by using scanning electron microscopy at the Servicio Central de Soporte a la Investigación Experimental (University of Valencia, Spain). Samples were examined in an Hitachi S-4100 field emission scanning microscope with a 7–15 mm working distance and at an acceleration voltage of 10 kV (Fig. S1, available in IJSEM Online). Cells were ovoid to rod-/club-shaped, with moderate tendencies to aggregate. The size of the cells was 0.2–0.5 × 0.6–1.4 µm. Cells lysed readily when suspended in 3 % (w/v) KOH solution, indicating that the strain was Gram-negative. Strain R46^T grows on MA as regular, mucous, non-pigmented, non-luminescent colonies.

The biochemical and physiological profile of the strain along with reference type strains was determined, in duplicate, in two independent assays. The ability to grow at different temperatures was tested on MA incubated at 4 °C (15 days), 15 °C (15 days), 28, 37, 40 and 45 °C (48 h). The strain grew from 15 to 37 °C, while no growth was ever observed at 4 or 40 °C. Ionic requirements were tested on salt tolerance agar (STA), containing 1 % peptone, 0.3 % yeast extract and 1.2 % purified agar. This medium was supplemented with the following salt combinations (in w/v): (i) with and (ii) without 2 % NaCl, (iii) with 2 % KCl, (iv) with 2 % NaCl and 0.9 % MgCl₂·6H₂O, (v) with 2 % NaCl and 0.2 % CaCl₂·2H₂O, (vi) with 2 % NaCl and 0.06 % KCl, (vii) with 2 % NaCl, 0.9 % MgCl₂·6H₂O and 0.2 % CaCl₂·2H₂O, (viii) with 2 % NaCl, 0.9 % MgCl₂·6H₂O, 0.2 % CaCl₂·2H₂O and 0.06 % KCl, (ix) with 2 % NaCl, 0.9 % MgSO₄·7H₂O, 0.2 % CaCl₂·2H₂O and 0.06 % KCl. The ionic requirements were complex, as the strain did not grow on media with only NaCl or KCl added, or with the various combinations of NaCl and Mg, Ca and K chlorides. Good growth was obtained only when STA was supplemented with NaCl, CaCl₂, KCl and MgSO₄ or when the full combination of marine salts was present (as in MA or seawater-based media). The salinity range for growth was tested on MA as reported previously (Macián *et al.*, 2005a) and it was 1–5 % (w/v) total salinity.

The strain was positive in oxidase and catalase tests. It was unable to ferment glucose in O/F medium supplemented

with ASW. Acid from glucose was not produced aerobically. Nitrate reduction to nitrite or further products was tested in marine nitrate broth, with an inverted Durham tube for the detection of gas production, and also in API 20NE strips; negative results were always obtained. Other enzymic activities also tested for by two methods (classical and API 20NE) were: arginine dihydrolase (Baumann & Baumann, 1981), indole production from tryptophan (in MB) and glucose fermentation; however, all these tests were negative. Hydrolysis of casein, alginate, starch and lecithin was tested on MA as basal medium and after 6 days of incubation (as recommended by Baumann & Baumann, 1981). Tween-80 hydrolysis was tested on medium containing 1 % peptone, 0.01 % CaCl₂, 1 % Tween-80 and 1.2 % agar powder, supplemented with marine cation supplement (MCS; Farmer & Hickman-Brenner, 2006), while DNase activity was tested on DNase agar (Oxoid), also prepared with MCS. Gelatin hydrolysis was assayed in API 20NE and was positive after 48 h of incubation. Sole carbon and energy sources used for growth were investigated on BMA supplemented with carbohydrates, organic acids or amino acids and incubated for up to 14 days. API 20NE and API ZYM profiles were obtained for strain R46^T and reference strains by following the manufacturer's instructions, except for the inclusion of MCS in the suspension medium. In API 20NE, conventional tests were read after 24 h incubation but assimilation and gelatin tests were reincubated for an additional 24 h after the first reading. The results of strain R46^T were negative for all tests, except for gelatin and aesculin hydrolysis (48 h). In API ZYM, strain R46^T was positive only for leucine arylamidase and gave a weak result in the alkaline phosphatase reaction. Results of Biolog GN2 microplates (inoculated by using ASW-based suspension fluid) were erratic. Consistent positive results were only obtained for the oxidation of the following substrates: cellobiose, L-rhamnose, D-sorbitol, turanose, citric acid, α-ketoglutarate, propionic acid, quinic acid, succinic and bromo-succinic acids, alaninamide, D-serine, glucose 1-phosphate and glucose-6-phosphate.

Fatty acid methyl esters were extracted from biomass grown for 48 h on MA at 28 °C and prepared according to standard protocols as described for the MIDI Microbial Identification System (Sasser, 1990) at the CECT. Cellular fatty acid content was analysed by GC with an Agilent 6850 chromatographic unit, with the MIDI Microbial Identification System using the RTSBA6 method (MIDI, 2008) and identified using the Microbial Identification Sherlock software package. Twelve different cellular fatty acids were detected in strain R46^T, two hydroxylated, six saturated and four unsaturated. The main component was C_{18:1}, which accounted for 75.3 % of the total, as occurs in all species so far analysed in the *Roseobacter* group. Other components are shown in Table 1, along with the results obtained in parallel with some reference type strains. The presence of C_{12:0} 3-OH in appreciable amounts (4.6 %) is a distinctive feature of the fatty acid profile of strain R46^T.

Analysis of respiratory quinones and polar lipids was carried out by the Identification Service of the DSMZ and

Table 1. Comparison of cellular fatty acid composition of R46^T and related species

Taxa: 1, strain R46^T; 2, *Pseudoruegeria aquimaris* CECT 7680^T; 3, *J. donghaensis* CECT 7802^T; 4, *J. helgolandensis* DSM 14858^T; 5, *Jannaschia rubra* CECT 5088^T; 6, *J. seosinensis* CECT 7799^T; 7, *Palleronia marisminoris* CECT 7066^T; 8, *Maribius salinus* CECT 7800^T; 9, *Thalassobacter stenotrophicus* CECT 5294^T. All data are from this study. –, Not detectable; tr, trace amount (<1 %). Cellular fatty acid composition carried out according to the Microbial Identification System (Microbial ID) using cultures grown on MA for 48 h at 28 °C. Fatty acids giving only trace amounts or not detected for all strains are not represented.

Cellular fatty acid (%)	1	2	3	4	5	6	7	8	9
Hydroxy									
C _{10:0} 3-OH	8.1	4.5	4.0	7.2	3.5	3.4	3.8	6.1	5.0
C _{12:0} 3-OH	4.6	–	–	–	–	–	–	–	–
Saturated									
C _{9:0}	–	–	–	1.1	tr	–	–	–	–
C _{12:0}	1.0	tr	–	tr	tr	–	–	tr	tr
C _{14:0}	tr	tr	–	1.7	tr	–	1.4	–	1.0
C _{16:0}	8.3	tr	tr	3.2	2.5	1.0	2.7	4.3	1.7
C _{17:0}	tr	tr	tr	tr	tr	tr	tr	1.6	2.2
C _{18:0}	1.4	4.1	5.4	9.6	7.9	6.0	3.1	2.3	2.8
C _{18:0} iso	–	–	tr	–	tr	–	1.9	–	19.6
C _{19:0}	–	–	2.6	1.0	–	tr	–	–	–
C _{19:0} cyclo ω8c	–	1.4	–	15.1	–	–	14.7	5.4	–
Unsaturated									
C _{17:1} iso ω9c	–	–	–	–	–	–	–	–	1.2
C _{18:1} ω7c 11-methyl	1.3	3.0	3.9	4.9	tr	–	–	4.0	2.5
C _{18:1} iso H	–	–	–	–	8.8	–	–	–	–
C _{18:1} ω9c	–	–	–	–	–	–	–	–	2.7
C _{20:1} ω7c	tr	tr	2.8	tr	tr	tr	–	–	–
C _{20:2} ω6,9c	–	–	–	1.2	tr	–	1.0	–	–
Summed feature									
2 (C _{14:0} 3-OH/C _{16:1} iso I)	–	–	–	2.5	tr	1.6	–	–	–
3 (C _{16:1} ω7c/C _{16:1} ω6c)	tr	tr	–	–	tr	tr	–	tr	1.0
7 (C _{19:1} ω7c/C _{19:1} ω6c)	–	–	1.4	1.3	–	–	–	1.6	tr
8 (C _{18:1} ω7c/C _{18:1} ω6c)	73.1	81.9	77.1	49.2	72.1	85.5	70.7	73.4	59.0

Dr B. J. Tindall, DSMZ, Braunschweig, Germany. Strain R46^T had a ubiquinone (Q10) system, in agreement with reported data for other members of the *Roseobacter* clade, and the following polar lipids: phosphatidylglycerol (PG), two aminolipids, two phospholipids, three lipids and one glycolipid (Fig. S2).

Sample preparation for matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS protein analysis was carried out at the CECT, according to the ethanol/formic acid extraction protocol recommended by Bruker Daltonics (<http://www.bdal.de>). MALDI-TOF MS was conducted using a Reflex IV mass spectrometer (Bruker Daltonics) equipped with an N₂ laser. All spectra were recorded in linear positive ion mode. The acceleration voltage was 20 kV. Spectra were collected as a sum of 240 shots across a spot, and between 12–16 spots per strain. A mass range of 2–20 kDa was used for analysis. Peak recognition and comparison of the spectra were done with the BioTyper software 1.1 as described by Maier *et al.* (2006). The dendrogram obtained with MALDI-TOF MS illustrates that strain R46^T can be well separated from the type strains of the following species: *J. helgolandensis* DSM 14858^T, *J.*

rubra CECT 5088^T, *J. seosinensis* CECT 7799^T, *J. donghaensis* CECT 7802^T, *M. salinus* CECT 7800^T, *Palleronia marisminoris* CECT 7066^T, *Phaeobacter inhibens* CECT 7251^T, *Phaeobacter gallaeciensis* CECT 7277^T, *Phaeobacter daepo-nensis* CECT 7309^T, *Roseobacter litoralis* CECT 5395^T, *Roseobacter denitrificans* CECT 5011^T, *Roseovarius crassostreae* CECT 7676^T, *Roseovarius aestuarii* CECT 7745^T, *Roseovarius tolerans* CECT 7738^T, *Roseovarius nubinihibens* CECT 7750^T, *Pseudoruegeria aquimaris* CECT 7680^T and *T. stenotrophicus* CECT 5294^T (Fig. S3).

The DNA G+C content, determined by the Identification Service of the DSMZ using HPLC according to the method described by Tamaoka & Komagata (1984) and Mesbah *et al.* (1989), was 61.3 mol%.

Cell pellets were collected and DNA was extracted using a microbial DNA isolation kit (Ultra Clean, Mo Bio Laboratories). Amplification of 16S rRNA and *gyrB* genes was performed as described previously (Lucena *et al.*, 2010; Pascual *et al.*, 2010). PCR amplicons were purified using an UltraClean PCR clean-up kit (Mo Bio Laboratories). Subsequent sequencing reactions were carried out on an ABI PRISM 3730 automated sequencer using the Big Dye

Terminator v3.1 cycle sequencing kit, premixed format. The sequencing primers were the same ones used in the amplification reaction but diluted tenfold (5 pmol). The new sequences were compared with public sequences in the EMBL gene databases using the BLAST program (National Center for Biotechnology Information; <http://www.ncbi.nlm.nih.gov/>). Related sequences were further analysed using the ARB program package (Ludwig *et al.*, 2004) (<http://www.arb-home.de>). Automated sequence alignments were corrected manually using the ARB_EDIT sequence editor. 16S rRNA gene sequence alignments retrieved from SILVA and LTP latest updates were also used as references (Pruesse *et al.*, 2007; Yarza *et al.*, 2010). Phylogenetic analysis using alternative treeing methods (maximum-parsimony, MP, maximum-likelihood, ML, and distance matrix) and data subsets were performed using the appropriate ARB tools (Ludwig *et al.*, 2004).

The 16S rRNA gene sequence analysis showed that even the highest similarities were below 95%. *Oceanicola nitrator-reducens* JLT1210^T, *Poseidonocella sedimentorum* KMM 9025^T, *Shimia isopora* SW-6^T and *Vadicella arenosi* KMM 9024^T displayed 94.7% similarity to strain R46^T, whereas *Pseudoruegeria aquimaris* SW-255^T had 94.4%, *Roseibacterium elongatum* JCM 11220^T 94.3% and *Shimia marina* CL-TA03^T and *J. donghaensis* DSW-17^T exhibited 94.2%. All other members of the family *Rhodobacteraceae* shared less than 94.0% similarity to strain R46^T. Given the low similarity values, tree topology considering alternative treeing methods was taken into account as a better indicator of relatedness. The *gyrB* gene sequence retrieved from strain R46^T showed similarities lower than 80.0% when compared with the available *gyrB* sequences from *Rhodobacteraceae*, with the single exception of *Roseovarius nubinihibens* (80.3% similarity).

Phylogenetic analyses performed using all three methods (neighbour-joining, NJ, MP and ML) and both 16S rRNA and *gyrB* genes always placed strain R46^T in a separate lineage, only distantly related to neighbour taxa within the *Roseobacter* clade (Figs 1, S4, S5 and S6). In the case of the trees based on the 16S rRNA gene, the genera *Jannaschia*, *Maribius*, *Palleronia* and *Hwanghaeicola* appear as the closest relatives of strain R46^T (supported by resemblance in topology and high resampling values in the connecting nodes). Although *gyrB* trees showed differences in the phylogenetic inference with respect to the 16S rRNA trees, it has to be noted that it is based on a smaller set of species and that possible recombination events cannot be accurately evaluated with the present data. In any case, it gives further support to the recognition of strain R46^T as a new taxon within the *Roseobacter* clade.

All together, the information gathered for strain R46^T in this study indicates that it belongs to a yet unrecognized genus of marine alphaproteobacteria, that can be differentiated from members of the *Roseobacter* group, and for which the name *Actibacterium mucosum* gen. nov., sp. nov. is proposed.

The new genus can be phenotypically differentiated from the nearest genera on the basis of polar lipid composition, DNA

G+C molar content, maximum halotolerance, PHB production, oxidase activity and motility behaviour. Thus, the genera *Jannaschia*, *Roseovarius*, *Litoreaibacter*, *Celeribacter* and *Vadicella* differ in the presence of phosphatidylethanolamine (PE) and phosphatidylcholine (PC) (plus diphosphatidylglycerol, DPG, in some instances) in their cells, while these polar lipids are absent in strain R46^T. In addition, members of the genus *Celeribacter* are motile and oxidase-negative, those of the genus *Vadicella* are able to reduce nitrates, and most species of the genus *Roseovarius* have a high halotolerance (up to 15% salinity). Differences with the genus *Thalassobacter* include the presence in this genus of PC and DPG and accumulation of PHB. One of the two members of the genus *Pseudoruegeria* (*P. aquimaris*) could be differentiated by the polar lipids PC, PE and DPG, while the other (*P. lutimaris*) is readily differentiated by the presence of fatty acid C_{19:0} cyclo and a much higher G+C mol%. Members of the genus *Maribius* show a different halotolerance pattern, being able to grow with up to 10–15% salinity (maximum salinity allowing growth of strain R46^T is 5%), accumulate PHB and have a DNA G+C content from 67 to 70 mol%. Members of the genus *Palleronia* are negative for oxidase, accumulate PHB and grow at 15% salinity, unlike those of the genus *Actibacterium*. Finally, members of the genus *Hwanghaeicola* are motile cocci differing from species of the genus *Actibacterium* in traits such as pigmentation, indole production, β -galactosidase activity, Voges–Proskauer reaction and a different cellular fatty acid composition that includes C_{19:0} cyclo but not C_{12:0} 3-OH. Additional differentiation of strain R46^T from the type strains of several species of the *Roseobacter* clade is shown in Table 2.

Description of *Actibacterium* gen. nov.

Actibacterium (Ac.ti.bac.te'ri.um. L. n. *acta*, *ae* seaside, shore; *-i-* connecting vowel; L. neut. n. *bacterium* a small rod; N.L. neut. n. *Actibacterium* a rod from the seaside).

Aerobic chemo-organotroph with complex ionic requirements. Positive for oxidase and catalase. Does not accumulate PHB. The major polar lipids are PG, an unidentified aminolipid and an unidentified lipid. The major cellular fatty acids are C_{18:1} ω 7c/C_{18:1} ω 6c. The predominant isoprenoid quinone is Q10. The DNA G+C content of the type species is 61.3 mol%. The type species is *Actibacterium mucosum*.

Description of *Actibacterium mucosum* sp. nov.

Actibacterium mucosum (mu.co'sum. L. neut. adj. *mucosum* slimy, mucous, a property of the colonies).

In addition to the features included in the genus description above, strain R46^T shows the following traits: cells are non-motile and oval- to rod-shaped (0.6–1.4 μ m long and 0.2–0.5 μ m wide). It grows on MA as regular, mucous and unpigmented colonies, measuring 2 mm diameter after 4 days of incubation at 28 °C. Growth occurs at 15–37 °C, but not at 4 or 40 °C. Optimal growth

Fig. 1. MP phylogenetic trees based on almost complete 16S rRNA (a) and partial *gyrB* gene (b) sequences of strain R46^T (=CECT 7668^T) and closely related species. The 16S rRNA tree is a subtree of the one shown in Fig. S6. GenBank accession numbers are given in parentheses. Bootstrap values $\geq 70\%$ confidence are shown at branching points (percentage of 1000 resamplings). Nodes coincident in the ML and NJ trees are indicated by a filled circle and an empty circle, respectively. Bar, number of substitutions per position.

requires the presence of Na^+ , K^+ , Ca^{2+} , Mg^{2+} and SO_4^{2-} ions or the addition of seawater to the medium. The salinity range for growth on MA is 1.7–5‰; no growth is produced at 6‰ or more, or below 1‰ salinity. It does not ferment carbohydrates and is not able to reduce nitrate to nitrite or further. It is also negative for the following activities: arginine dihydrolase, indole production from tryptophan, PNPG, urease, aesculin, casein, starch, alginate, lecithin, DNA hydrolysis, esterase (C4), esterase lipase (C8), lipase (C14), cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. Gelatin is hydrolysed after 2 days. Alkaline phosphatase and leucine arylamidase are positive or weakly positive. The strain grows on defined medium (BMA) using the following sole carbon and energy sources:

D-ribose, D-xylose, D-glucose, D-fructose, D-galactose, D-mannose, cellobiose, glycerol, D-glycerate, *myo*-inositol, acetate, pyruvate, propionate (slow), butyrate, citrate, 2-oxoglutarate, succinate, fumarate, malate, lactate, 3-hydroxybutyrate and L-tyrosine. The ability to grow with sucrose, lactose, melibiose, D-sorbitol, L-alanine and L-glutamate is variable. No growth is obtained with the following carbon sources: L-arabinose, L-rhamnose, salicin, amygdalin, *N*-acetyl-D-glucosamine, D-gluconate, D-glucuronate, D-galacturonate, D-mannitol, saccharate, aconitate, glycine, L-leucine, L-serine, L-threonine, L-arginine, L-ornithine, L-citrulline, L-4-aminobutyrate, L-aspartate, L-lysine, L-histidine, L-sarcosine or putrescine. Polar lipid composition includes phosphatidyl glycerol, two unidentified aminolipids, three unidentified lipids, two unidentified phospholipids and one unidentified glycolipid. Major cellular fatty acids are $\text{C}_{18:1}\omega 7\text{cl}\omega 6\text{c}$, $\text{C}_{16:0}$, $\text{C}_{10:0}$ 3-OH and $\text{C}_{12:0}$ 3-OH.

Table 2. Differential characteristics between strain R46^T and some related species

Taxa: 1, strain R46^T; 2, *Pseudoruegeria aquimaris* CECT 7680^T; 3, *J. donghaensis* CECT 7802^T; 4, *J. helgolandensis* DSM 14858^T; 5, *J. rubra* CECT 5088^T; 6, *J. seosinensis* CECT 7799^T; 7, *Palleronia marisminoris* CECT 7066^T; 8, *M. salinus* CECT 7800^T. All data from this study unless otherwise indicated. +, Positive; –, negative; tr, trace amount.

	1	2	3	4	5	6	7	8
Colony colour	Beige	Greyish yellow	Deep orange	Beige orange	Red	Deep red	Pink	Beige
Motility	–	–	–	–	+	+	–	–
Growth at								
37 °C	+	+	–	–	+	+	+	+
0.25 % salinity	–	+	–	+	+	+	+	+
6 % salinity	–	+	–	–	+	+	+	+
Assimilation (API 20NE) of								
D-Glucose	–	+	–	–	+	–	–	–
D-Mannose	–	+	–	–	+	–	–	–
D-Mannitol	–	+	–	+	+	–	+	–*
Maltose	–	+	–	–	–	–	+	–
Adipate	–	+	–	–	+	–	+	–
Malate	–	+	–	+	–	–	+	–
Citrate	–	+	–	+	–	–	+	–*
Hydrolysis of								
DNA	–	–	–	+	–	+	–	–
Starch	–	–	–	+	–	+	–	–*
Gelatin	+	–	–	–	–	–	–	–
Enzymatic activity								
Alkaline phosphatase	+	+	+	+	+	+	+	–
Acid phosphatase	–	+	+	+	+	–	+	–
α-Galactosidase	–	–	–	–	+	–	–	–
N-Acetyl-β-glucosaminidase	–	+	–	–	–	–	–	–
α-Glucosidase	–	–	–	–	+	–*	+	–
Fatty acids (%)								
C _{12:0} 3-OH	4.6	–	–	–	–	–	–	–
C _{16:0}	8.3	tr	tr	3.2	2.5	1.0	2.7	4.3
C _{18:0}	1.4	4.1	5.4	9.6	7.9	6.0	3.1	2.3
C _{18:1} ω7c 11-methyl	1.3	3.0	3.9	4.9	tr	–	–	4.0
DNA G + C content (mol%)†	61.3	67 ^a	65.2 ^b	63.1 ^c	64.6 ^d	63.0 ^e	64.2 ^f	66.7 ^g

*Results that differ from the original description.

†Data from: a, Yoon *et al.* (2007a); b, Yoon *et al.* (2007b); c, Wagner-Döbler *et al.* (2003); d, Macián *et al.* (2005b); e, Choi *et al.* (2006); f, Martínez-Checa *et al.* (2005); g, Choi *et al.* (2007).

In addition, C_{12:0}, C_{18:0} and C_{18:1}ω7c 11-methyl are also detected in a lower proportion.

The type strain, R46^T (=CECT 7668^T=KCTC 23349^T), was isolated from seawater from the western Mediterranean Sea. The DNA G + C content of the type strain is 61.3 mol%.

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