Roseisalinus antarcticus gen. nov., sp. nov., a novel aerobic bacteriochlorophyll *a*-producing α-proteobacterium isolated from hypersaline Ekho Lake, Antarctica

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A Gram-negative, aerobic to microaerophilic rod was isolated from 10 m depths of the hypersaline, heliothermal and meromictic Ekho Lake (East Antarctica). The strain was oxidase- and catalase-positive, metabolized a variety of carboxylic acids and sugars and produced lipase. Cells had an absolute requirement for artificial sea water, which could not be replaced by NaCl. A large *in vivo* absorption band at 870 nm indicated production of bacteriochlorophyll *a*. The predominant fatty acids of this organism were 16 : 0 and 18 : 1ω7*c*, with 3-OH 10 : 0, 16 : 1ω7*c* and 18 : 0 in lower amounts. The main polar lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylcholine. Ubiquinone 10 was produced. The DNA G+C content was 67 mol%. 16S rRNA gene sequence comparisons indicated that the isolate represents a member of the *Roseobacter* clade within the α-*Proteobacteria*. The organism showed no particular relationship to any members of this clade but clustered on the periphery of the genera *Jannaschia*, *Octadecabacter* and '*Marinosulfonomonas*' and the species *Ruegeria gelatinovorans*. Distinct morphological, physiological and genotypic differences to these previously described taxa supported the description of a new genus and a novel species, for which the name *Roseisalinus antarcticus* gen. nov., sp. nov. is proposed. The type strain is EL-88^T (=DSM 11466^T=CECT 7023^T).

Aerobic bacteriochlorophyll (bchl) *a*-producing bacteria cover a wide range of micro-organisms from different geographical locations and with different physiological requirements. The spectrum of their ecological niches ranges from oligotrophic picoplankton to microalgal symbioses (Allgaier *et al.*, 2003). Moreover, phototrophy based on bchl *a*-mediated 'aerobic anoxygenic photosynthesis' has been estimated to account for up to 5 % of surface ocean photosynthetic electron transport and 11 % of the total microbial community (Béjà *et al.*, 2002). Ekho Lake, East Antarctica, is a hypersaline, meromictic and heliothermal lake that contains, in its deeper layers, a microflora probably of marine origin (Labrenz & Hirsch, 2001). In an attempt to elucidate the microbial diversity of Ekho Lake, two aerobic bchl *a*-producing genera, *Roseovarius* and *Staleya*, have been described previously by our laboratories (Labrenz *et al.*, 1999, 2000). Here, we present data on a novel aerobic bchl *a*-producing taxon isolated from the lake.

One bacterial isolate was obtained from a 10 m Ekho Lake sample; at this depth, salinity was 70 ‰, temperature was 15·5 °C and pH was 8·01. This isolate is referred to as EL-88^T. Enrichment and isolation of this strain were performed as described by Labrenz *et al.* (1998), and enrichment conditions followed characteristics of the original water samples. Pure cultures were kept as serial transfers on slants, lyophilized or deep-frozen at −72 °C in glycerol. Morphological, physiological and metabolic analyses were performed as described in detail by Labrenz *et al.* (1998, 1999, 2000, 2003) and Lawson *et al.* (2000).

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Abbreviations: ASW, artificial sea water; bchl, bacteriochlorophyll.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain EL-88^T is AJ605747.

A figure showing characteristic absorbance peaks for strain EL-88^T is available as supplementary material in IJSEM Online.

Analysis of fatty acid methyl esters was carried out with 20 mg freeze-dried biomass and using methods that allowed selective hydrolysis of ester- and amide-linked fatty acids as described previously (Labrenz *et al.*, 2000). Respiratory lipoquinones and polar lipids were extracted from 100 mg freeze-dried material using the two-stage method and analysed according to the methods of Tindall (1990a, b). Cell-wall diamino acids were separated by one-dimensional TLC on cellulose plates using the solvent system of Rhuland *et al.* (1955). DNA G+C contents were analysed according to Mesbah *et al.* (1989) as described by us previously (Labrenz *et al.*, 1998).

16S rRNA gene sequence fragments were generated by PCR using universal primers pA (positions 8–28 of the *Escherichia coli* numbering) and pH* (1542–1522). The amplified products were purified using a QIAquick PCR Purification Kit (Qiagen) and sequenced directly using primers to conserved regions of the rRNA gene. Sequencing was performed using a PRISM *Taq* Dyedeoxy Terminator Cycle Sequencing Kit and an automatic DNA sequencer (model 373A, both from Applied Biosystems). To establish the closest relatives to the strain EL-88^T, preliminary searches in the EMBL database were performed with the program FASTA (Pearson & Lipman, 1988). Closely related sequences were retrieved from EMBL and aligned with the newly determined sequences using the program DNATOOLS (Rasmussen, 1995). The resulting multiple sequence alignment had approximately 100 bases at the 5′ end of the molecule omitted from further analysis because of alignment uncertainties resulting from the highly variable region V1, using the program GENEDOC (Nicholas *et al.*, 1997). A phylogenetic tree was constructed according to the neighbour-joining method (Saitou & Nei, 1987) with the programs DNATOOLS and TreeView (Page, 1996), and the stability of the groupings was estimated by bootstrap analysis (1000 replications).

The isolate was a motile Gram-negative rod with one or both cell poles narrower (Fig. 1a–c). However, neither position nor number of flagella could be determined. Holdfast structures were often produced (Fig. 1d) and cells had a strong tendency to form rosettes (Fig. 1). Spores were not produced. Polymers were probably secreted (Fig. 1d), but this was not analysed further. Poly-β-hydroxybutyrate was present. Cell growth appeared to be monopolar because one cell end was usually narrower and shorter, possibly indicating a budding process. Cell size was in the range 0·90–1·02 × 2·18–4·20 μm with a mean size of 0·96 × 3·19 μm.

Aerobic to microaerophilic growth was visible after 3–5 days at 20 °C on peptone/yeast/glucose/vitamin (PYGV) medium (Labrenz *et al.*, 1998, and references herein) plus 25 ‰ artificial sea water (ASW) (Lyman & Fleming, 1940). Colonies were 2 mm in diameter, circular with regular edges, smooth, convex and red. Growth in liquid cultures occurred as small aggregates. The temperature range for growth was < 3–33·5 °C. Optimal growth occurred between 16 and 26 °C at pH 5·5–9·0. Optimal pH for growth was 7·0–7·8. Requirements for Na^+, Cl^-, K^+, Mg^{2+}, Ca^{2+} or SO_4^{2-} were studied in PYGV+ASW, where Na^+ was exchanged with K^+, Mg^{2+} with Ca^{2+}, Cl^- with SO_4^{2-} and vice versa. The isolate had an absolute requirement for Na^+ as well as for Cl^-; K^+, Mg^{2+}, Ca^{2+} and SO_4^{2-} could be replaced by other ions. Tolerance for NaCl could not be detected because growth was not detected without ASW. Osmotolerance ranged from 10 to 130 ‰ ASW, with optimum growth at 50–90 ‰. Growth did not occur on glucose anaerobically in the absence of nitrate. Cells did

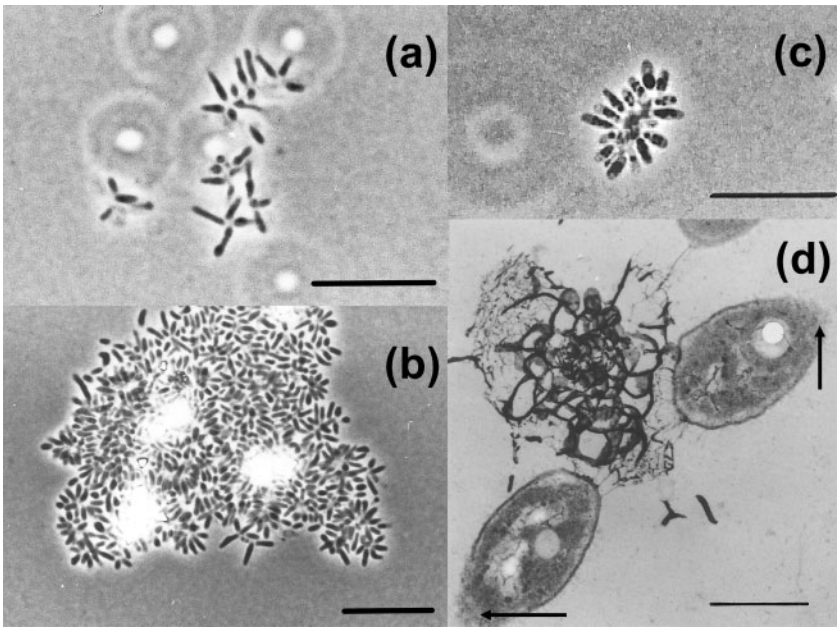

Fig. 1. (a–c) Phase-contrast light micrographs of strain EL-88^T on an agar-coated slide (Pfennig & Wagener, 1986). Bars, 10 μm. (d) Electron micrograph of an ultrathin section of cells of strain EL-88^T stained with uranyl acetate/lead citrate. Arrows indicate holdfast structures. Cells are attached to potentially self-secreted polymeric substances. Bar, 1 μm.

not grow photoautotrophically with H_2/CO_2 (80 : 20) or photo-organotrophically with acetate or glutamate.

Strain EL-88^T exhibited peroxidase, catalase and cytochrome oxidase activity. Cell growth did not depend on vitamins. Weak assimilatory nitrate reduction to nitrite occurred.

Growth of the isolate on different carbon sources as well as further characteristics are given in the species description. The isolate did not grow in a minimal medium (Labrenz *et al.*, 1998) with 0·2 % (w/v) methanol or citric acid. With the API 50CH system the following carbon sources were not metabolized: erythritol, adonitol, methyl β-D-xyloside, D-mannose, L-sorbose, dulcitol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, salicin, lactose, trehalose, inulin, melezitose, D-raffinose, amidone, glycogen, xylitol, D-turanose, D-tagatose, L-arabitol, gluconic acid and 2- as well as 5-ketogluconic acid. In the Biolog system, the isolate did not metabolize α-cyclodextrin, dextrin, glycogen, Tween 80, *N*-acetyl-D-galactosamine, *N*-acetylglucosamine, adonitol, L-arabinose, D-arabitol, cellobiose, *i*-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, *m*-inositol, α-lactose, α-D-lactose-lactulose, maltose, D-mannitol, D-mannose, D-melibiose, methyl β-D-glucoside, psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, xylitol, methylpyruvate, monomethylsuccinate, *cis*-aconitic acid, formic acid, D-galactonic acid, lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, DL-lactic acid, malonic acid, quinic acid, sebacic acid, succinic acid, succinamic acid, glucuronamide, alaninamide, D-alanine, L-alanine, L-alanylglycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl-L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, L-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL-α-glycerophosphate, glucose 1-phosphate or glucose 6-phosphate.

Bchl *a* was present in cell suspensions of strain EL-88^T grown aerobically in sporadic dim light. Characteristic absorbance values were found, with a larger peak at 870 nm and smaller peaks at 800–801 nm and 590–592 nm (data available as supplementary material in IJSEM Online). These differed from the maxima of bchl *a*-containing *Roseobacter denitrificans*, *Roseobacter litoralis*, *Staleya guttiformis* and *Roseovarius tolerans* (Table 1). Other features, such as carotenoids, were not characterized further. Unlike for *Roseobacter denitrificans*, vesicular structures of intracytoplasmic membrane systems (Harashima *et al.*, 1982) were not found in ultra-thin sections of aerobically grown cells (Fig. 1d).

Table 1. *In vivo* absorption band of *Roseisalinus antarcticus* EL-88^T and other aerobic bchl *a*-producing members of the *Roseobacter* group

Taxa: 1, EL-88^T; 2, *Roseobacter*; 3, *Staleya guttiformis* EL-38^T; 4, *Roseovarius tolerans* EL-172^T. Data from Shiba (1991) and Labrenz *et al.* (1999, 2000).

Absorption band (nm)	1	2	3	4
Small				
589–592	+	+	+	+
799–802	+	−	+	+
Large				
805–807	−	+	−	−
861–862	−	−	+	−
868–873	+	+	−	−
877–879	−	−	−	+

The peptidoglycan of this organism contained *meso*-diaminopimelic acid. The strain contained diphosphatidylglycerol, phosphatidylglycerol and phosphatidylcholine, but no phosphatidylmonomethylamine or phosphatidylethanolamine. In addition, cells contained one and three unidentified phospho and amino lipids, respectively. The only respiratory lipoquinone detected was ubiquinone 10. Dominant cellular fatty acids were 16 : 0 and 18 : 1ω7*c*, with 3-OH 10 : 0, 16 : 1ω7*c* and 18 : 0 present in smaller amounts. Fatty acids 16 : 1ω7*c* and 18 : 0 were released by methods which indicated that they were amide-linked. The presence of ubiquinone 10 as the dominant respiratory lipoquinone is characteristic of members of the *α-Proteobacteria*. The predominant fatty acid 18 : 1ω7*c*, accounting for approximately 62 % of the total fatty acids, is a feature characteristic of several major phyletic groups within the *α-Proteobacteria*. The presence of 3-OH 10 : 0 is indicative that the novel isolate belonged within the same phyletic group as members of the genera *Jannaschia*, *Octadecabacter*, *Staleya*, *Sulfitobacter* and *Roseobacter*.

The DNA G+C content of the newly isolated strain was found to be 66·8–66·9 mol%. Characteristics differentiating strain EL-88^T from other related organisms are shown in Table 2.

To establish the phylogenetic affinities of the isolate, the almost-complete 16S rRNA gene sequence was determined. Sequence searches of the EMBL database revealed that the novel organism was related to the *α-Proteobacteria* (data not shown). Pairwise analysis revealed that the novel isolate displayed highest 16S rRNA gene sequence similarity with the members of the *Roseobacter* clade of organisms (90–94 %). Other species belonging to the *α-Proteobacteria* examined showed lower levels of similarity. An unrooted tree constructed using the neighbour-joining method shows the phylogenetic position of strain EL-88^T among the *Proteobacteria*, defined by the *Roseobacter* clade of organisms (Fig. 2). All associations showing bootstrap

Table 2. Differential characteristics of *Roseisalinus antarcticus* EL-88^T and related species

Taxa: 1, EL-88^T; 2, *Jannaschia helgolandensis* DSM 14858^T; 3, *Ruegeria gelatinovorans* ATCC 25655^T; 4, *Octadecabacter arcticus* 238^T; 5, '*Marinosulfonomonas methylotropha*' PSCH4; 6, *Staleya guttiformis* EL-38^T; 7, *Roseobacter litoralis* OCh 149^T; 8, *Roseovarius tolerans* DSM 11457^T; 9, *Sulfitobacter pontiacus* DSM 10014^T; 10, *Ruegeria algicola* ATCC 51440^T. Data from Wagner-Döbler *et al.* (2003), Rüger & Höfle (1992), Uchino *et al.* (1998), Gosink *et al.* (1997), Holmes *et al.* (1997), Labrenz *et al.* (1999, 2000), Shiba (1991), Sorokin (1995) and Lafay *et al.* (1995). V, Variable; W, weak; ND, not determined; +, positive; (+), weakly positive; −, negative.

Characteristic	1	2	3	4	5	6	7	8	9	10
Morphology:										
Rosettes formed	+	−*	−†	−	+	+	−	−	+	−
Budding cell division	+	+	ND	−	ND	+	−	+	+	−
Physiology:										
Oxidase	+	+	+	−	+	+	+	+	+	+
Bchl *a*	+	−	−	−	−	V	+	+	−	−
Growth at 5 °C	+	−	(+)	+	−	+	+	+	+	−
Growth at 37 °C	−	−	−	−	+	−	−	+	−	+
Tween 80 hydrolysis	−	−	ND	ND	ND	+	+	−	−	−
Gelatinase	−	−	+	−	ND	−	+	−	−	+
Vitamin requirement	−	ND	ND	+	ND	+	+	+	ND‡	ND
Susceptibility to tetracycline	−	ND	ND	ND	+	+	+	+	ND	+
Carbon sources:										
Acetate	+	−	+	−	+	+	+	+	+	+
Pyruvate	+	−	+	V	+	+	+	+	+	+
Glutamate	+	−	ND	V	−	+	+	+	+	+
Butyrate	+	−	ND	−	ND	−	−	W	+	−§
Methanesulfonic acid	+	ND	ND	ND	+	−	ND	−	ND	ND
Methanol	−	−	ND	−	+	−	−	−	ND	−
Fatty acids (%):			‖				¶		#**	¶
3-OH 10:0	1·9	4·5		4	ND	5·9	1·9	−	3·6	−
12:1	−	3·4		−	ND	−	−	−	−	−
3-OH 12:1	−	−		−	ND	−	−	3·6	−	−
2-OH 12:0	−	−	−	−	ND	−	−	2·4	−	−
3-OH 12:0	−	−	+	−	ND	−	−	−	−	−
3-OH 14:1	−	2·1††		−	ND	2·1	3·9	−	2·0	−
3-OH 14:0	−	1·4		−	ND	−	−	−	−	−
16:1ω7*c*	2·1	−		8	ND	−	−	0·8	1·2	−
16:0	15·1	−		6	ND	3·9	1·1	6·2	8·1	1·6
17:0	−	1·0		−	ND	−	−	−	−	−
18:2‡‡	−	−		−	ND	5·3	1·4	10·6	−	1·6
Methyl 18:1	−	7·6		−	ND	−	−	−	−	−
18:1ω7*c*	62·0	45·0	+	75§§	ND	79·7	88·8	70·2	79·1	91·5
18:0	2·3	11·7	+	−	ND	0·7	1·3	0·8	−	2·2
Cyclo 19:0	−	22·4		−	ND	−	−	−	−	−
19:1	−	−		−	ND	1·4	−	−	−	−
20:0	−	1·0		−	ND	−	−	−	−	−
Polar lipids:							¶		**	¶
Diphosphatidylglycerol	+	+	ND	ND	ND	−	+	+	+	+
Phosphatidylethanolamine	−	+	ND	ND	ND	+	−	+	+	+
Phosphatidylcholine	+	+	ND	ND	ND	+	−	+	+	+
G+C content (mol%)	67	63·0–63·1	59	57	57	55·0–56·3	56·0–58·8	63·3–63·4	61·7–62·5	64–65

*Cells tend to form chains.

†Sometimes star-shaped aggregates formed.

‡Yeast extract and thiamine stimulate growth.

§Uses butyrate according to Labrenz *et al.* (1999).

‖Major fatty acids are 18:1 and 18:0. 3-OH 12:0 is present, but no 2-hydroxy fatty acids.

¶Data from Labrenz *et al.* (1999).

Table 2. cont.

#Grown on Marine Broth 2216 (Difco).
**Data from Labrenz *et al.* (2000).
††3-OH 14 : 1 and/or 3-oxo 14 : 0.
‡‡Second of two 18 : 2 isomers, which could not be identified further (Labrenz *et al.*, 2000).
§§Unclear: 18 : 1ω7*c*, 18 : 1ω9*t* or 18 : 1ω12*t*.

resampling values of 90 % or more in the neighbour-joining tree were confirmed by parsimony analysis. Analyses demonstrated that strain EL-88^T formed a distinct lineage clustering with the genera *Jannaschia*, *Octadecabacter* and '*Marinosulfonomonas*' and the species *Ruegeria gelatinovorans*. However, EL-88^T did not display a particularly close nor statistically significant association (as shown by bootstrap resampling) with any recognized taxa (Fig. 2). Indeed, from sequence divergence (>6 %) and tree topology considerations, strain EL-88^T appears to be equivalent in rank to the genera of the *Roseobacter* clade of organisms. However, it is also evident from the tree-making analyses that the genus *Ruegeria*, as currently recognized, is interspersed with several other taxa. To fulfil the criteria of being a monophyletic group, the genus *Ruegeria* (Uchino *et al.*, 1998) should be restricted to the species *Ruegeria atlantica* and *Ruegeria algicola*. Note that the phylogenetic distinctiveness of the novel bacterium represented by strain EL-88^T is strongly supported by phenotypic considerations. Strain EL-88^T is distinguishable from its closest relative, *Jannaschia helgolandensis*, by its ability to form rosettes, production of bchl *a*, ability to grow at 5 °C, and utilization of acetate, pyruvate, glutamate and butyrate. Dominant fatty acids of strain EL-88^T are 16 : 0 and 18 : 1ω7*c*, whereas those of *J. helgolandensis* are methyl 18 : 1, 18 : 1ω7*c*, 18 : 0 and cyclo 19 : 0, which in lower amounts are also found in *Sagittula stellata* (Gonzalez *et al.*, 1997) and *Antarctobacter heliothermus* (Labrenz *et al.*, 1998). Interestingly, neither strain EL-88^T nor *J. helgolandensis* is able to grow with NaCl instead of ASW (Wagner-Döbler *et al.*, 2003). However, from the combination of physiological characteristics, chemotaxonomic and biochemical tests, fatty acid profiles, polar lipid data and 16S rRNA gene sequence analyses it is evident that strain EL-88^T represents a hitherto unknown lineage related to, but separate from, the genera *Jannaschia*, *Octadecabacter* and '*Marinosulfonomonas*' and the species *Ruegeria gelatinovorans*. Therefore, based on both phenotypic and genotypic evidence, we propose that the novel strain EL-88^T be classified in a new genus, *Roseisalinus* gen. nov., as *Roseisalinus antarcticus* sp. nov.

Description of *Roseisalinus* gen. nov.

Roseisalinus (Ro.se.i.sal.in′us. L. adj. *roseus* rose-coloured; N.L. adj. *salinus* saline; N.L. masc. n. *Roseisalinus* the rose-coloured bacterium depending on ions).

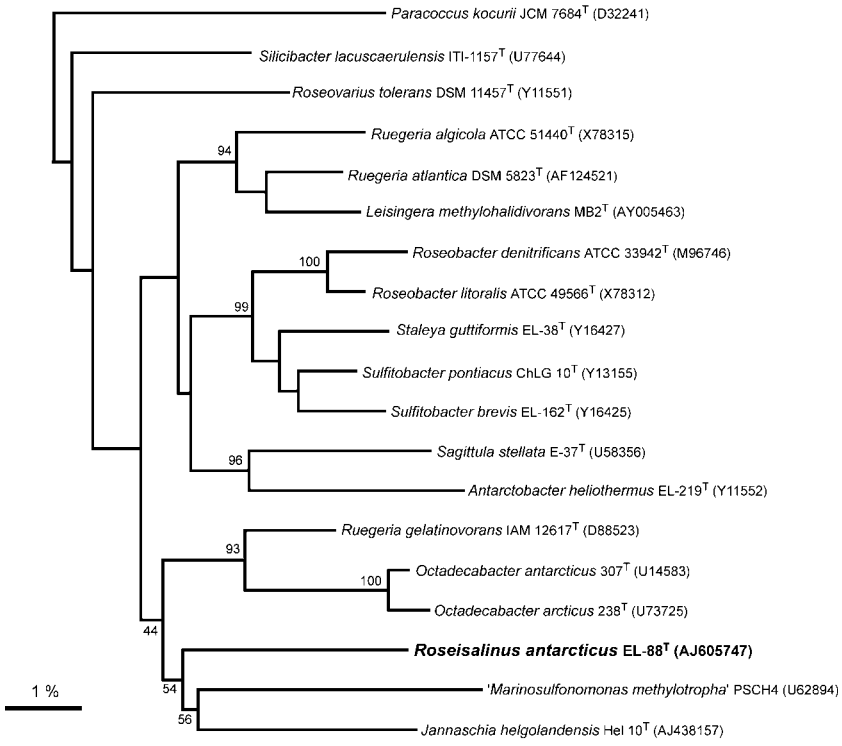

Fig. 2. Unrooted tree showing phylogenetic relationships of strain EL-88^T and closely related *Proteobacteria*. The tree was reconstructed using the neighbour-joining method and was based on a comparison of approximately 1320 nt. Bootstrap values, expressed as a percentage of 1000 replications, are given at branching points. Bar, 1 substitution per 100 nt.

Gram-negative motile rods. Cells contain poly-β-hydroxybutyrate and do not form spores. The temperature range for growth is <3–$33{\cdot}5\,^{\circ}C$. They grow in the presence of 10–130 ‰ ASW. Cells have an absolute requirement for Na^+ and Cl^-, but NaCl cannot replace ASW. pH range for growth is 5·5–9·5. Aerobic to microaerophilic heterotrophs growing on various carboxylic acids and sugars. Cells do not depend on vitamins. No growth on glucose anaerobically in the absence of nitrate. They do not grow photoautotrophically with H_2/CO_2 (80 : 20) or photoorganotrophically with acetate or glutamate. Cells exhibit peroxidase, catalase and cytochrome oxidase activity. The peptidoglycan contains *meso*-diaminopimelic acid. Diphosphatidylglycerol, phosphatidylglycerol and phosphatidylcholine are present, but phosphatidylethanolamine and phosphatidylmonomethylamine are not. Dominant cellular fatty acids are 16 : 0 and 18 : 1ω7*c*, with 3-OH 10 : 0, 16 : 1ω7*c* and 18 : 0 present in smaller amounts. The respiratory quinone is Q-10. Isolated from water samples from Ekho Lake, Vestfold Hills, East Antarctica.

The type species is *Roseisalinus antarcticus*.

Description of *Roseisalinus antarcticus* sp. nov.

Roseisalinus antarcticus (ant.arc′ti.cus. N.L. adj. *antarcticus* pertaining to the Antarctic).

Cell sizes are in the range $0{\cdot}90$–$1{\cdot}02 \times 2{\cdot}18$–$4{\cdot}20$ μm; mean $0{\cdot}96 \times 3{\cdot}19$ μm. One or both cell poles narrower. Cell growth is monopolar, indicating a budding process. Holdfast structures are often produced and cells have a strong tendency to form rosettes. Colonies on PYGV + ASW are 2 mm in diameter, circular with regular edges, smooth, convex and red. Growth in liquid cultures occurs as small aggregates. Bchl *a* is produced. Optimal growth occurs at 16–26 °C at concentrations of 50–90 ‰ ASW. The optimum pH range is 7·0–7·8. Cells are susceptible to chloramphenicol (30 μg), streptomycin (10 μg), polymyxin B (300 U) and penicillin G (10 U), but not to tetracycline (30 μg). Lipase activity, but DNA, gelatin, starch and alginate are not hydrolysed. Growth occurs on acetate, pyruvate, malate, butyrate, succinic acid, methanesulfonic acid, glutamic acid, *cis*-aconitic acid, itaconic acid, propionic acid, D-saccharic acid, bromosuccinic acid, Tween 40 (variably on Tween 80), glycerol, glucose, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, galactose, D-fructose, rhamnose, inositol, mannitol, aesculin, cellobiose, maltose, melibiose, sucrose, β-gentiobiose, D-lyxose, D-fucose, L-fucose, D-arabitol and thymidine. Nitrate is assimilatory slightly reduced to nitrite. Cells do not produce acid or acetoin from glucose. No production of sulfide or indole. The DNA G+C content is 67 mol%. Chemotaxonomic properties and other characteristics are as given for the genus.

The type strain is EL-88^T (= DSM 11466^T = CECT 7023^T).

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