Roseibaca ekhonensis gen. nov., sp. nov., an alkalitolerant and aerobic bacteriochlorophyll a-producing alphaproteobacterium from hypersaline Ekho Lake

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A Gram-negative, aerobic rod was isolated from the hypersaline, heliothermal and meromictic Ekho Lake (East Antarctica) at a depth of 6 m. The novel strain (designated EL-50T) was oxidase-positive and weakly catalase-positive and metabolized a variety of carboxylic acids, alcohols, sugars and lipids. Cells of strain EL-50T had an absolute requirement for artificial seawater or NaCl. Optimum growth occurred at 16 °C and at pH values ranging from 7.0 to 9.5. A large in vivo absorption band at 865–866 nm indicated the production of bacteriochlorophyll (bchl) a. The predominant cellular fatty acid of strain EL-50T was 18:1ω7c, with 3-OH 14:1, 16:1ω9c, 16:0 and 18:1ω9c present in lower amounts. Fatty acids 16:0 and 18:1ω9c were probably amide-linked. The main polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylcholine. Ubiquinone 10 was produced. The cell-wall diamino acid was meso-diaminopimelic acid. The DNA G+C content of strain EL-50T was 61 mol%. 16S rRNA gene sequence comparisons indicated that the novel isolate was phylogenetically most closely related to alkaliphilic Rhodobaca and Roseinatronobacter species (approximately 96 % 16S rRNA gene similarity). The organism had no particular relationship to any other cultivated members within the Alphaproteobacteria. The distinct morphological, physiological and genotypic differences from the previously described taxa studied supported the description of a new genus and novel species, for which the name Roseibaca ekhonensis gen. nov., sp. nov. is proposed. The type strain is EL-50T (=DSM 11469T = CECT 7235T).

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), often accounting for a significant fraction of the microbial community in the upper ocean (Béja et al., 2002, Masín et al., 2006). However, only two alkaliphilic aerobic bchl a-producing species, Roseinatronobacter thiooxidans and Roseinatronobacter monicus, have been described to date (Sorokin et al., 2000; Boldareva et al., 2007), whereas their phylogenetically close relative Rhodobaca bogoriensis is an alkaliphilic, phototrophic, purple non-sulfur bacterium, producing bchl a anaerobically (Milford et al., 2000).

In an attempt to elucidate the cultivated microbial diversity of the hypersaline, meromictic and heliothermal Ekho Lake (Vestfold Hills, East Antarctica), three aerobic bchl a-producing taxa belonging to the Roseobacter clade of organisms were described previously (Labrenz et al., 1999, 2000, 2005). Here, we present data on a novel alkalitolerant, aerobic, bchl a-producing taxon isolated from Ekho Lake, which is phylogenetically related to alkaliphilic species of the Rhodobaca/Roseinatronobacter group.

Enrichment and isolation of strain EL-50T were performed according to Labrenz et al. (1998). Enrichment conditions followed the characteristics of original water samples. Strain EL-50T was isolated at a depth of 6 m; at this depth the salinity was 65.0 %, the temperature was 14.8 °C and...
the pH was 8.22. 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). For these analyses, strain EL-50T was usually cultivated at 20 °C on peptone-yeast-glucose-vitamin (PYGV) medium (Labrenz et al., 1998, and references herein) plus 40 % artificial seawater (ASW; Lyman & Fleming, 1940) at pH 8.0.

Strain EL-50T was a non-motile, Gram-negative rod (Fig. 1a, b). Stem-like structures were often produced (Fig. 1b) and cells had a tendency to form rosettes. Endospores were not produced. Poly-β-hydroxybutyrate was accumulated. Cell growth appeared to be monopolar as one cell end was usually narrower and shorter, possibly indicating a budding process. The temperature range for growth was 10–30 °C. Optimum growth occurred at 16 °C and the optimum pH was 7.0–9.5, but strain EL-50T was able to grow at all pH values tested (5.5–9.5). Requirements for Na+, Cl−, K+, Mg2+, Ca2+ or SO42− were studied in PYGV + ASW, where Na+ was replaced with K+, Mg2+ with Ca2+, Cl− with SO42− and vice versa. The isolate had an absolute requirement for Na+; K+, Mg2+, Ca2+ or SO42− could be replaced by other ions. Tolerance of NaCl ranged from <1.0 to <4.0 %, with an optimum of 2.5 %. The osmotolerance ranged from 0–10 to 90–100 % ASW, with an optimum between 30 and 80 %. Growth did not occur on glucose anaerobically in the absence of nitrate. Cells of strain EL-50T did not grow photoautotrophically with H2/CO2 (80:20, by vol.) or photo-organotrophically with acetate or glutamate. Neither dissimilatory nor assimilatory nitrate reduction occurred.

Bchl a was present in cell suspensions of strain EL-50T grown aerobically in sporadic dim light. Characteristic absorbance values were found, with a large peak at 865–866 nm and smaller peaks at 800–801 nm and 590–592 nm. These differed from the larger peak of bchl a observed in Roseinatronobacter species or Rhodobaca bogoriensis (Table 1). Other features, such as carotenoids, were not characterized further. Unlike Rhodobaca bogoriensis, vesicular structures of intracytoplasmatic membrane systems (Milford et al., 2000) were not found in ultrathin sections of aerobically grown cells and cells of strain EL-50T were not able to produce bchl a or even grow anaerobically. Other physiological data are given in the genus and species descriptions.

Analysis of fatty acid methyl esters was carried out with 20 mg freeze-dried biomass, and employed 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 were analysed according to Tindall (1990a, b). Cell-wall diamino acids were separated by using one-dimensional thin-layer chromatography on cellulose plates with the solvent system of Rhuland et al. (1955). The DNA guanine-plus-cytosine (G+C) contents were analysed by HPLC according to Mesbah et al. (1989), and as described previously (Labrenz et al., 1998).

The peptidoglycan of strain EL-50T contained meso-diaminopimelic acid. The strain contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine, but not phosphatidylmonomethylamine. In addition, cells contained three unidentified phospho- and aminolipids. The only respiratory lipoquinone detected was ubiquinone 10. The predominant cellular fatty acid was 18:1o7c (80.5 %); fatty acids 3-OH 14:1 (7.2 %), 16:1o9c (5.9 %), 16:0 (1.3 %) and 18:1o9c (1.5 %) were present in lower amounts. Fatty acids 16:0 and 18:1o9c were released by using methods that indicated they were amide linked. The presence of ubiquinone 10 as the dominant respiratory lipoquinone is characteristic of members of the Alphaproteobacteria. The predominant fatty acid, 18:1o7c, is a feature characteristic of several major phyletic groups within the Alphaproteobacteria (Labrenz et al., 2005). The DNA G+C content of strain EL-50T was found to be 61 mol %.

Fig. 1. (a) Phase-contrast light micrograph of strain EL-50T on an agar-coated slide (Pfennig & Wagener, 1986). Bar, 10 μm. (b) Electron micrograph of isolate EL-50T negatively stained with phosphotungstic acid. Bar, 1 μm.
16S rRNA gene fragments were generated by PCR using universal primers pA (positions 8–28, *Escherichia coli* numbering, AGAGTTTGATCCTGGCTCAG) and pH* (1542–1522, AAGGAGGTGATCCAGCCGA). The amplified products were purified by 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 (Applied Biosystems) and an automatic DNA sequencer (model 373A; Applied Biosystems). To establish the closest relatives of strain EL-50T, preliminary searches in the EMBL data library were performed with the program FASTA (Pearson & Lipman, 1988). Closely related sequences were retrieved from GenBank and aligned and analysed with the newly determined sequence within the program ARB (Ludwig et al., 2004). Phylogenetic analysis was performed on 1320 nucleotides using the neighbour-joining, maximum-par-simony and maximum-likelihood algorithms. Sequence searches of the EMBL database revealed that the novel organism was related to the *Alphaproteobacteria* (data not shown). Pairwise analysis revealed that the novel isolate displayed the highest 16S rRNA gene sequence similarity (95–96%) with alkaliphilic *Roseinatronobacter thiooxidans*, *Roseinatronobacter monicus* or 'Natronohydrobacter thiooxidans' AHO 1, as well as *Rhodobaca bogoriensis*, although this organism lacks standing in the nomenclature (Euzéby & Tindall, 2004). Other species belonging to the *Alphaproteobacteria* examined showed lower levels of relatedness (94.0% or lower sequence similarity). An unrooted tree reconstructed using the neighbour-joining method shows the phylogenetic position of strain EL-50T amongst the *Proteobacteria* (Fig. 2). Treeing analyses demonstrated that strain EL-50T clustered with 'N. thiooxidans', an organism isolated from an alkaline environment (Sorokin, D. Y. and Kuenen, G. J.; http://www.ncbi.nlm.nih.gov/nuccore/
18076041), within the clade of alkaline Roseinatronobacter and Rhodobaca species (Fig. 2). Strain EL-50\(^T\) did not display a particularly close nor statistically significant association with any other recognized taxa. There was no precise correlation between the percentage 16S rRNA gene sequence divergence and species delineation, but it is generally recognized that divergence values of 3 % or more are significant (Stackebrandt & Goebel, 1994). However, it is pertinent to note that the phylogenetic separateness of strain EL-50\(^T\) was strongly supported by phenotypic considerations. Strain EL-50\(^T\) is distinguishable from the whole alkaliphilic group by its ability to grow at pH values below 7. Because of this physiological feature it could be speculated that cells of strain EL-50\(^T\) derived originally from more alkaline environments and adapted to the neutral to just slightly alkaline Ekho Lake conditions (Labrenz & Hirsch, 2001). In particular, strain EL-50\(^T\) can be distinguished from R. bogoriensis by its strictly aerobic growth and aerobic chl\(_a\) production and from Roseinatronobacter species by their fatty acid profiles, where nearly all fatty acids present in lower amounts are different (Table 1). Additional characteristics useful for differentiating the antarctic isolate (strain EL-50\(^T\)) from related organisms are given in Table 1.

Based on both the phenotypic and genetic evidence, strain EL-50\(^T\) should be classified as representing a new genus and novel species, for which the name Roseibaca ekhonensis gen. nov., sp. nov. is proposed.

**Description of Roseibaca gen. nov.**

Roseibaca (Ro.se.i`baca: L. adj. roseus rose-coloured; L. fem. n. baca berry; N.L. fem. n. Roseibaca the rose-coloured berry, recognizing the close relationship to the anaerobic chl\(_a\)-producing genus Rhodobaca).

Gram-negative non-motile rods. One or both cell poles are narrower. Cell growth is monopolar indicating a budding process. Stem-like structures are often produced. Cells have a tendency to form rosettes and contain numerous fimbriae on their surface. Cells contain poly-β-hydroxy butyrate and do not form endospores. Alkalitolerant; cells have an absolute requirement for Na\(^+\). Aerobic heterotrophs. Do not grow photoautotrophically with H\(_2\)/CO\(_2\) (80 : 20, by vol.) or photo-organotrophically with acetate or glutamate. Peptidoglycan contains meso-diaminopimelic acid. Diphosphatidyglycerol, phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine are present, but phosphatidylmonomethylamine is not. The dominant cellular fatty acid is 18 : 1, with 3-OH 14 : 1, 16 : 1, 18 : 1 and Molar volume. Temperature range for growth is 10–30 °C. Optimum growth occurs at 16 °C and pH 7.0–9.5. NaCl tolerance ranges from 0–1.0 to 3.5–4.0 %, with an optimum of 2.5 %. Positive for peroxidase and cytochrome oxidase, and weakly-positive for catalase activity. Cells depend on thiamine and vitamin B\(_12\), but not on biotin, nicotinic acid or sodium pantothenate. Susceptible to chloramphenicol (30 μg), penicillin G (10 μg) and streptomycin (10 μg), and resistant to polymyxin B (300 μg) and tetracycline (30 μg). Does not grow on α-D-glucose anaerobically in the absence of nitrate. DNA, gelatin, lipase (Tweek 80), starch and alginate are not hydrolysed. Does not grow in minimal medium (Labrenz et al., 1998) with 0.2 % (w/v) methanol,
methanesulfonic acid or butyrate. With the API 50 CH system the following carbon compounds are not metabolized: erythritol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, adonitol, methyl β-D-xylidine, L-sorbose, dulcitol, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, arbutin, melibiose, inulin, melezitose, raffinose, amideone, glycogen, xyitol, β-gentiobiose, D-tagatose, D-fucose, L-fucose, L-arabitol, gluconic acid, 2-ketogluconic and 5-ketogluconic acid. In the Biolog system, does not metabolize α-cyclodextrin, dextrin, glycerin, methyl β-D-glucose, pyruvic acid methyl ester, succinic acid monomethyl ester, formic acid, D-galactonolactone, lactate, β-hydroxybutyric acid, γ-hydroxybutyric acid, DL-lactic acid, sebacic acid, bromosuccinic acid, succinic acid, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, DL-carnitine, γ-aminoobutyric acid, urocanic acid, phenylethylamine, putrescine, 2-aminoethanol or 2,3-butanediol. Growth occurs on acetate, pyruvate, malate, citrate, succinic acid, glutamic acid, Tween 40, N-acetyl-D-galactosamine, N-acetylglucosamine, cellobiose, D-fructose, D-galactose, α-D-glucose, α-D-lactose, lactulose, maltose, D-mannitol, D-mannose, D-psicose, rhamnose, D-sorbitol, sucrose, trehalose, turanose, cis-aconitic acid, D-galacturonic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, p-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, glucuronamide, alaninamide, D-alanine, L-alanine, L-alanylglycine, L-asparagine, L-aspartic acid, glycol-L-aspartic acid, glycol-L-glutamic acid, L-histidine, L-pyrrolglutamic acid, D-serine, L-serine, L-threonine,inosine, uridine, thymidine, DL-α-glycerol phosphate, glucose 1-phosphate, glucose 6-phosphate, glycerol, inositol, aesculin, salicin, D-lyxose and D-arabitol. Does not produce acid or acetoin from α-D-glucose. Sulfide and indole are not produced. The DNA G+C content of the type strain is 61 mol%.

The type strain, EL-50T (=DSM 11469T=CECT 7235T), was isolated from Ekho Lake, Antarctica (Vestfold Hills).

Note added in Proof

After this work was submitted for publication, the name Rhodobaca barguzinensis was validly published [Boldareva, E. N., Akimov, V. M., Boychenko, V. A., Stadnichuk, I. N., Moskalenko, A. A., Makhneva, Z. K. & Gorlenko, V. M. (2009). Rhodobaca barguzinensis sp. nov. In List of New Names and Combinations Previously Effectively, but not Validly, Published, Validation List no. 127. Int J Syst Evol Microbiol 59, 923–925].

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