Roseibacterium elongatum gen. nov., sp. nov., an aerobic, bacteriochlorophyll-containing bacterium isolated from the west coast of Australia

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A novel aerobic, chemoheterotrophic, bacteriochlorophyll-containing bacterium, strain OCh 323^T, was isolated from sand at Monkey Mia, Shark Bay, located on the west coast of Australia. The cells were Gram-negative, non-motile rods of variable length; one or both cell poles was narrower. Bacteriochlorophyll *a* was synthesized under aerobic conditions. Catalase, oxidase and urease were produced. The ONPG reaction was positive. The major component of the cellular fatty acid was octadecenoic acid (18:1). The DNA G + C content was 68.1 mol%. The results of 16S rRNA gene sequence comparisons revealed that strain OCh 323^T formed a novel, separate line of descent within the α -3 group of the *Alphaproteobacteria*. The similarity value for the 16S rRNA gene sequence of strain OCh 323^T and that of the most closely related species, *Rhodovulum sulfidophilum*, was 91.4%. It is concluded that strain OCh 323^T (= JCM 11220^T = CIP 107377^T) should be placed in a novel genus, *Roseibacterium* gen. nov., as the type strain of a novel species *Roseibacterium elongatum* sp. nov.

Since Sato (1978) reported on aerobic methylotrophs containing bacteriochlorophyll *a*, a variety of aerobic, anoxygenic, phototrophic bacteria have been described (Yurkov & Beatty, 1998). Recently, many novel genera of aerobic bacteriochlorophyll-containing bacteria, including *Craurococcus* (Saitoh *et al.*, 1998), *Paracraurococcus* (Saitoh *et al.*, 1998), *Roseovarius* (Labrenz *et al.*, 1999), *Rubrimonas* (Suzuki *et al.*, 1999a), *Roseateles* (Suyama *et al.*, 1999), *Roseivivax* (Suzuki *et al.*, 1999b), 'Citromicrobium' (Yurkov *et al.*, 1999), *Roseinatronobacter* (Sorokin *et al.*, 2000), *Staleyia* (Labrenz *et al.*, 2000), *Acidisphaera* (Hiraishi *et al.*, 2000) and *Roseibium* (Suzuki *et al.*, 2000), have been described.

Aerobic and chemoheterotrophic bacteriochlorophyll-containing bacteria have been isolated from specimens from a variety of marine environments on the east and west coasts of Australia (Shiba *et al.*, 1991). Most of these isolates have been divided into several groups on the basis of phenotypic characteristics and DNA–DNA relatedness (Nishimura *et al.*, 1994). On the basis of phenotypic characteristics and phylogenetic considerations, we have previously proposed three novel genera, *Rubrimonas*, *Roseivivax* and *Roseibium*, for three groups of those isolates

(Suzuki *et al.*, 1999a, b, 2000). The genera *Rubrimonas* and *Roseivivax* belong to the α -3 group, while the genus *Roseibium* belongs to the α -2 group of the *Proteobacteria*.

In the present study, the phenotypic characteristics and the phylogeny of strain OCh 323^T, which has not previously been included in any group of these isolates, were investigated. On the basis of the results of these investigations, a novel genus and novel species are proposed.

Strain OCh 323^T was previously isolated from sand at Monkey Mia, Shark Bay, located on the west coast of Australia, by Shiba *et al.* (1991). The strain was cultivated on PPES-II medium (Taga, 1968) at 27 °C. The pH was adjusted to 7.8 with 10% NaOH. Colonies of strain OCh 323^T were circular, smooth, convex, entire, glistening, opaque and pink. Cells were stained with 1% (w/v) aqueous uranyl acetate and examined under a JEOL model JEM-1200 EX electron microscope at an accelerating voltage of 80 kV. Cells were Gram-negative, non-motile rods (with one or both cell poles narrower) and of variable length (Fig. 1). Cell growth appeared to be monopolar, since one cell end was usually narrower and shorter than the other, possibly indicating a budding process (Hirsch, 1974). Cells of strain OCh 323^T were 0.5–0.8 × 1.6–10.0 µm.

Physiological and biochemical characteristics were examined according to the methods of Shiba & Simidu (1982). Acid production from D-fructose, D-glucose and lactose was

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Fig. 1. Electron micrograph of negatively stained cells of strain OCh 323^T. Bar, 500 nm.

tested on ZOF medium (Lemos *et al.*, 1985) supplemented with a 1 % (w/v) carbohydrate source.

Strain OCh 323^T grew chemoheterotrophically under aerobic conditions but could not grow phototrophically under anaerobic conditions in the light. Optimum growth occurred at pH 7.5–8.0 and at 27–30 °C. The strain required NaCl, with growth occurring in 0.5–7.5 % NaCl. The biochemical and physiological characteristics of strain OCh 323^T are given in Table 1.

Cells were washed and sonicated for 3 min in 30 mM Tris/HCl (pH 7.5). The absorption spectrum of cell extracts was recorded with a Shimadzu UV-VIS recording spectrophotometer (model UV-160). Ultrasonically disrupted cells in buffer had absorption peaks in the near-infrared region at 879 and 800 nm (Fig. 2), indicating the presence of bacteriochlorophyll *a* in light-harvesting complex I and the photochemical reaction centre.

Strain OCh 323^T was grown in PPES-II broth at 27 °C with shaking. Cells were washed with distilled water and freeze-dried. A 100 mg portion of lyophilized cells was methylated with 3 ml 5 % (v/v) HCl/methanol at 100 °C for 3 h. Fatty acid methyl esters were extracted with hexane three times. The hexane fraction was washed with distilled water, dehydrated with anhydrous Na₂SO₄ and then concentrated by using a rotary evaporator. Hydroxylated fatty acid methyl esters were separated from non-polar fatty acid methyl esters by TLC (silica gel 60 F₂₅₄; Merck) using hexane/diethylether (85:15, v/v) as the developing solvent. The spots were visualized by spraying with 0.02 % (w/v)

Table 1. Comparative physiological and biochemical characteristics of aerobic, chemoheterotrophic, bacteriochlorophyll-containing bacteria

Strains: 1, strain OCh 323^T; 2, *Roseivivax halodurans* OCh 239^T; 3, *Roseivivax halotolerans* OCh 210^T; 4, *Rubrimonas cliftonensis* OCh 317^T; 5, *Roseobacter litoralis* OCh 149^T; 6, *Roseobacter denitrificans* OCh 114^T. +, Positive; –, negative; w, weakly positive. All strains are positive for catalase and oxidase and for growth in the presence of 0.5 % NaCl. All strains are negative in the Voges–Proskauer test, for H₂S production and for the hydrolysis of alginate and starch.

Characteristic	1	2	3	4	5	6
Nitrate reductase	–	+	–	w	–	+
Phosphatase	–	+	–	+	–	+
Urease	+	–	+	+	–	–
Indole production	–	+	+	+	+	+
ONPG reaction	+	+	+	–	+	+
Hydrolysis of:						
Gelatin	+	–	+	–	+	+
Tween 80	–	–	–	–	+	+
Utilization of:						
D-Glucose	–	+	+	+	+	+
Acetate	–	+	+	–	+	w
Citrate	–	+	+	+	+	+
DL-Malate	–	+	+	–	+	+
Pyruvate	–	+	+	+	+	+
Succinate	–	+	+	–	+	+
Ethanol	–	–	–	+	–	–
Acid production from:						
D-Fructose	–	+	w	+	–	w
D-Glucose	–	+	w	+	–	–
Lactose	–	+	–	–	–	–
Growth in the presence of NaCl at:						
0 %	–	w	–	–	–	–
7.5 %	+	+	+	+	–	–
10.0 %	–	+	+	–	–	–

2',7'-dichlorofluorescein ethanol solution and then scraped off the TLC plate under UV light. Hydroxylated fatty acid methyl esters were extracted with diethylether. After removal of the diethylether from the extracted solution, the sample was dissolved in a small volume of hexane. To verify the presence of cyclopropanic and unsaturated acids, they were saturated with hydrogen. The analysis was performed in a GC-8A gas chromatograph (Shimadzu) equipped with an FFS ULBON HR-1 capillary column (0.25 mm × 50 m; Shinwa Chemical Industries), at 170–230 °C (rate: 4 °C min^{–1}). The detector used was flame-ionized. The chromatogram was processed by using Chromatopac C-R3A (Shimadzu).

The cellular fatty acids of strain OCh 323^T are described in Table 2; the major cellular fatty acid is octadecenoic acid (18:1).

The cells were suspended in saline EDTA (0.15 M NaCl, 0.1 M EDTA, pH 8.5) and then lysed at 60 °C for 10 min

Fig. 2. Absorption spectrum of ultrasonically disrupted membrane of strain OCh 323^T, showing the presence of the photosynthetic reaction centre (minor peak at 800 nm) and light-harvesting complex I (major peak at 879 nm).

with 0.5 % SDS (final concentration). Chromosomal DNA was purified according to standard procedures (Sambrook *et al.*, 1989).

The DNA G+C content was determined by the HPLC method (Katayama-Fujimura *et al.*, 1984). Chromosomal DNA was denatured to the single stranded form by means of heating at 95 °C for 5 min. The single-stranded DNA was hydrolysed to nucleotides with P1 nuclease (DNA-GC kit; Seikagaku Kogyo) in 40 mM sodium acetate and 2 mM ZnSO₄ (pH 5.3) at 50 °C for 2 h. The nucleotide composition was analysed by HPLC, which was performed in a Shimadzu high-performance liquid chromatograph (model LC-6A) equipped with an octadecyl-silica column (YMC pack AQ-312, 6 mm × 150 mm), and detected at 260 nm. The mobile phase was 10 mM phosphate buffer (pH 3.5). The DNA G+C content was corrected by dividing the value of the peak area of the sample nucleotide by the peak area of the standard nucleotide. The G+C content of the DNA from strain OCh 323^T was 68.1 mol%.

Amplification of the 16S rRNA gene was performed on a Quick Thermo Personal QTP-1 thermocycler (Nippon Genetics) in a 100 µl reaction volume, as described previously (Suzuki *et al.*, 1999a, b). The amplified DNA fragments were purified by gel electrophoresis on 1 % agarose S (Nippon Gene) and recovered with glass powder, using Prep-A-Gene DNA purification systems (Bio-Rad Laboratories). Sequencing was carried out as described previously (Suzuki & Yamasato, 1994).

The sequences of strain OCh 323^T and of reference bacterial species were aligned with the CLUSTAL X program, version 1.64b (Thompson *et al.*, 1997); the alignment was checked manually. The phylogenetic analysis was performed with PHYLIP, version 3.57c (Felsenstein, 1995). A distance matrix was calculated with DNADIST, using the Kimura

Table 2. Fatty acid compositions (%) of whole-cell hydrolysates of aerobic, chemoheterotrophic, bacteriochlorophyll-containing bacteria

Strains: 1, strain OCh 323^T; 2, *Roseivivax halodurans* OCh 239^T; 3, *Roseivivax halotolerans* OCh 210^T; 4, *Rubrimonas cliftonensis* OCh 317^T; 5, *Roseobacter litoralis* OCh 149^T; 6, *Roseobacter denitrificans* OCh 114^T. —, Not detected; tr, trace.

Fatty acid	1	2	3	4	5	6
14:0	tr	2	—	tr	tr	tr
15:0	1	tr	tr	—	tr	—
16:0	12	13	5	16	1	2
17:0	1	2	1	tr	1	1
18:0	8	7	4	2	1	1
19:0	—	15	10	—	tr	tr
16:1	1	tr	tr	2	tr	1
18:1	68	44	69	69	94	92
cyclo 19:0	4	—	—	—	—	—
18:2	tr	—	—	—	2	1
2-OH 16:0	2	—	—	—	—	—
3-OH 14:0	—	—	—	2	—	—
Unknown	2	16	10	8	1	2

two-parameter method, and a phylogenetic tree was reconstructed using NEIGHBOR. The stability of the clusters was ascertained by performing bootstrap analyses (1000 replications) with SEQBOOT, DNADIST, NEIGHBOR and CONSENSE.

The 16S rRNA gene sequence of strain OCh 323^T was determined and aligned with other available 16S rRNA gene sequences. A comparison of the 16S rRNA gene sequences, for which a phylogenetic tree was reconstructed (Fig. 3), revealed that strain OCh 323^T belonged to the α-3 group of the *Proteobacteria*, forming a separate line of descent. The 16S rRNA gene sequence similarity values between strain OCh 323^T and other strains belonging to this group were low: 91.4 % for *Rhodovulum sulfidophilum*, 90.7 % for *Ruegeria algicola*, 90.5 % for *Rhodovulum adriaticum*, 90.2 % for *Rhodobacter sphaeroides* and 90.0 % for *Rhodovulum iodolum*.

Aerobic bacteriochlorophyll-containing, budding bacteria are known, e.g. *Porphyrobacter neustonensis* (Fuerst *et al.*, 1993), *Roseovarius tolerans* (Labrenz *et al.*, 1999) and *Staleyia guttiformis* (Labrenz *et al.*, 2000). Phylogenetically, *Porphyrobacter neustonensis* is located in the α-4 group, whereas *Roseovarius tolerans* and *S. guttiformis* belong to the α-3 group of the *Proteobacteria*. Strain OCh 323^T is distantly related to *Roseovarius tolerans* and *S. guttiformis*. It has been demonstrated previously that phenotypic characteristics are not necessarily reflected in phylogenetic relationships; this inconsistency was also apparent in the present study.

The phylogenetic analysis supports the need for a novel genus, *Roseibacterium* gen. nov., to be created for strain OCh

Fig. 3. Neighbour-joining phylogenetic tree derived from analysis of the 16S rRNA gene sequences of *Roseibacterium elongatum* OCh 323^T and other members of the α -3 group of the *Proteobacteria*. Numbers at nodes indicate levels of bootstrap support based on 1000 resamplings; only values above 500 are considered as significant and are indicated.

323^T, and we propose that strain OCh 323^T should be described as the type strain of *Roseibacterium elongatum* gen. nov., sp. nov.

Description of *Roseibacterium* gen. nov.

Roseibacterium (Ro.sei.bac.te'ri.um. L. adj. *roseus* rose, pink; Gr. neut. n. *bakterion* rod; N.L. neut. n. *Roseibacterium* pink, rod-shaped bacterium).

Cells are Gram-negative, non-motile rods that multiply by monopolar growth, i.e. by an apparent budding process. Aerobic chemoheterotrophs. Cells exhibit catalase and oxidase activities. Bacteriochlorophyll *a* is synthesized under aerobic conditions. The major cellular fatty acid is octadecenoic acid (18:1). The genus *Roseibacterium* belongs to the α -3 group of the *Proteobacteria*. The type species is *Roseibacterium elongatum*.

Description of *Roseibacterium elongatum* sp. nov.

Roseibacterium elongatum (e.lon.ga'tum. L. part. neut. adj. *elongatum* elongated, stretched out).

Colonies are circular, smooth, convex, entire, glistening, opaque and pink. Cells are 0.5–0.8 × 1.6–10.0 μ m. Optimum growth occurs at pH 7.5–8.0 and at 27–30 °C. Growth occurs in the presence of 0.5–7.5 % NaCl, but not in the absence of NaCl. Cells exhibit urease activity, but not

nitrate reductase or phosphatase activities. Negative in the Voges–Proskauer test. The ONPG reaction is positive. Cells do not produce indole or H₂S. Gelatin is hydrolysed, but alginate, starch and Tween 80 are not. Cells do not utilize D-glucose, acetate, citrate, DL-malate, pyruvate, succinate or ethanol. Acid is not produced from D-fructose, D-glucose or lactose. The absorption spectrum of ultrasonically disrupted cells in the near-infrared region has maxima at 879 nm and 800 nm. The G + C content of the DNA is 68.1 mol%.

The type strain, strain OCh 323^T (=JCM 11220^T=CIP 107377^T), was isolated from sand at Monkey Mia, Shark Bay, located on the west coast of Australia.

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References

- Felsenstein, J. (1995). PHYLIP (phylogeny inference package), version 3.57c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Fuerst, J. A., Hawkins, J. A., Holmes, A., Sly, L. I., Moore, C. J. & Stackebrandt, E. (1993). *Porphyrobacter neustonensis* gen. nov., sp. nov., an aerobic bacteriochlorophyll-synthesizing budding bacterium from fresh water. *Int J Syst Bacteriol* **43**, 125–134.
- Hiraishi, A., Matsuzawa, Y., Kanbe, T. & Wakao, N. (2000). *Acidisphaera rubrifaciens* gen. nov., sp. nov., an aerobic bacteriochlorophyll-containing bacterium isolated from acidic environments. *Int J Syst Evol Microbiol* **50**, 1539–1546.
- Hirsch, P. (1974). Budding bacteria. *Annu Rev Microbiol* **28**, 391–444.
- Katayama-Fujimura, Y., Komatsu, Y., Kuraishi, H. & Kaneko, T. (1984). Estimation of DNA base composition by high performance liquid chromatography of its nuclease P1 hydrolysate. *Agric Biol Chem* **48**, 3169–3172.
- Labrenz, M., Collins, M. D., Lawson, P. A., Tindall, B. J., Schumann, P. & Hirsch, P. (1999). *Roseovarius tolerans* gen. nov., sp. nov., a budding bacterium with variable bacteriochlorophyll *a* production from hypersaline Ekho Lake. *Int J Syst Bacteriol* **49**, 137–147.
- Labrenz, M., Tindall, B. J., Lawson, P. A., Collins, M. D., Schumann, P. & Hirsch, P. (2000). *Staleyia guttiformis* gen. nov., sp. nov. and *Sulfitobacter brevis* sp. nov., α -3-Proteobacteria from hypersaline, heliothermal and meromictic antarctic Ekho Lake. *Int J Syst Evol Microbiol* **50**, 303–313.
- Lemos, M. L., Toranzo, A. E. & Barja, J. L. (1985). Modified medium for the oxidation-fermentation test in the identification of marine bacteria. *Appl Environ Microbiol* **49**, 1541–1543.
- Nishimura, Y., Muroga, Y., Saito, S., Shiba, T., Takamiya, K. & Shioi, Y. (1994). DNA relatedness and chemotaxonomic feature of aerobic bacteriochlorophyll-containing bacteria isolated from coasts of Australia. *J Gen Appl Microbiol* **40**, 287–296.
- Saitoh, S., Suzuki, T. & Nishimura, Y. (1998). Proposal of *Craurococcus roseus* gen. nov., sp. nov. and *Paracraurococcus ruber* gen. nov., sp. nov., novel aerobic bacteriochlorophyll *a*-containing bacteria from soil. *Int J Syst Bacteriol* **48**, 1043–1047.

- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989).** *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Sato, K. (1978).** Bacteriochlorophyll formation by facultative methylotrophs, *Protaminobacter ruber* and *Pseudomonas* AM 1. *FEBS Lett* **85**, 207–210.
- Shiba, T. & Simidu, U. (1982).** *Erythrobacter longus* gen. nov., sp. nov., an aerobic bacterium which contains bacteriochlorophyll *a*. *Int J Syst Bacteriol* **32**, 211–217.
- Shiba, T., Shioi, Y., Takamiya, K., Sutton, D. C. & Wilkinson, C. R. (1991).** Distribution and physiology of aerobic bacteria containing bacteriochlorophyll *a* on the east and west coasts of Australia. *Appl Environ Microbiol* **57**, 295–300.
- Sorokin, D. Y., Tourova, T. P., Kuznetsov, B. B., Bryantseva, I. A. & Gorlenko, V. M. (2000).** *Roseinatronobacter thiooxidans* gen. nov., sp. nov., a new alkaliphilic aerobic bacteriochlorophyll *a*-containing bacterium isolated from a soda lake. *Microbiology* (English translation of *Mikrobiologiya*) **69**, 75–82.
- Suyama, T., Shigematsu, T., Takaichi, S., Nodasaka, Y., Fujikawa, S., Hosoya, H., Tokiwa, Y., Kanagawa, T. & Hanada, S. (1999).** *Roseateles depolymerans* gen. nov., sp. nov., a new bacteriochlorophyll *a*-containing obligate aerobe belonging to the β -subclass of the *Proteobacteria*. *Int J Syst Bacteriol* **49**, 449–457.
- Suzuki, T. & Yamasato, K. (1994).** Phylogeny of spore-forming lactic acid bacteria based on 16S rRNA gene sequences. *FEMS Microbiol Lett* **115**, 13–18.
- Suzuki, T., Muroga, Y., Takahama, M., Shiba, T. & Nishimura, Y. (1999a).** *Rubrimonas cliftonensis* gen. nov., sp. nov., an aerobic bacteriochlorophyll-containing bacterium isolated from a saline lake. *Int J Syst Bacteriol* **49**, 201–205.
- Suzuki, T., Muroga, Y., Takahama, M. & Nishimura, Y. (1999b).** *Roseivivax halodurans* gen. nov., sp. nov. and *Roseivivax halotolerans* sp. nov., aerobic bacteriochlorophyll-containing bacteria isolated from a saline lake. *Int J Syst Bacteriol* **49**, 629–634.
- Suzuki, T., Muroga, Y., Takahama, M. & Nishimura, Y. (2000).** *Roseibium denhamense* gen. nov., sp. nov. and *Roseibium hamelinense* sp. nov., aerobic bacteriochlorophyll-containing bacteria isolated from the east and west coasts of Australia. *Int J Syst Evol Microbiol* **50**, 2151–2156.
- Taga, N. (1968).** Some ecological aspects of marine bacteria in the Kuroshio current. *Bull Misaki Mar Biol Inst Kyoto Univ* **12**, 56–76.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997).** The CLUSTAL_X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Yurkov, V. V. & Beatty, J. T. (1998).** Aerobic anoxygenic phototrophic bacteria. *Microbiol Mol Biol Rev* **62**, 695–724.
- Yurkov, V. V., Krieger, S., Stackebrandt, E. & Beatty, J. T. (1999).** *Citromicrobium bathyomarinum*, a novel aerobic bacterium isolated from deep-sea hydrothermal vent plume waters that contains photosynthetic pigment-protein complexes. *J Bacteriol* **181**, 4517–4525.