**Roseivivax halodurans** gen. nov., sp. nov. and **Roseivivax halotolerans** sp. nov., aerobic bacteriochlorophyll-containing bacteria isolated from a saline lake

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Phenotypic and phylogenetic studies were performed with two strains (OCh 239\(^T\) and OCh 210\(^T\), \(T = \) type strain) of aerobic bacteriochlorophyll-containing bacteria isolated from the charophytes and the epiphytes on the stromatolites, respectively, of a saline lake located on the west coast of Australia. Both strains were chemoheterotrophic, Gram-negative and motile rods with subpolar flagella. Catalase and oxidase were produced. ONPG reaction was positive. Cells utilized D-glucose, acetate, butyrate, citrate, DL-lactate, DL-malate, pyruvate, succinate, L-aspartate and L-glutamate. Acids were produced from D-fructose and D-glucose. Bacteriochlorophyll \(a\) was synthesized under aerobic conditions. Strain OCh 239\(^T\) had nitrate reductase and phosphatase. Acids were produced from L-arabinose, D-galactose, lactose, maltose, D-ribose and sucrose. The strain could grow in 0-200\% (w/v) NaCl. Strain OCh 210\(^T\) had urease. Hydrolysis of gelatin was positive. Acids were produced from D-xylene. The strain could grow in 0.5-20.0\% (w/v) NaCl. The results of 16S rRNA sequence comparisons revealed that strains OCh 239\(^T\) and OCh 210\(^T\) formed a new cluster within the \(\alpha\)-3 group of the \(\alpha\) subclass of the class Proteobacteria. The similarity value of the 16S rRNA sequences between strains OCh 239\(^T\) and OCh 210\(^T\) was 95.8\%. Therefore, it was concluded that these two strains should be placed in a new genus, Roseivivax gen. nov., as the new species **Roseivivax halodurans** sp. nov. and **Roseivivax halotolerans** sp. nov. The type species of the genus is **Roseivivax halodurans**. The type strains of **Roseivivax halodurans** and **Roseivivax halotolerans** are OCh 239\(^T\) (= JCM 10272\(^T\)) and OCh 210\(^T\) (= JCM 10271\(^T\)), respectively.

**Keywords:** Roseivivax halodurans, Roseivivax halotolerans, aerobic bacteriochlorophyll-containing bacteria, saline lake, 16S rRNA

**INTRODUCTION**

The first paper on aerobic bacteriochlorophyll-containing bacteria was published by Sato in 1978. Afterwards, aerobic bacteriochlorophyll-containing bacteria were isolated from various sources (Shiba et al., 1979) and identified (Nishimura et al., 1981). Recently, new aerobic photosynthetic bacteria have been isolated from a hot spring (Hanada et al., 1997) and deep-ocean hydrothermal vents (Yurkov & Beatty, 1998). Based on the phylogenetic analysis, it has been shown that these and newly isolated organisms belong to the \(\alpha\) subclass of the class Proteobacteria. Acidiphilium (Wakao et al., 1993), Roseococcus (Yurkov et al., 1994), Craurococcus (Saito et al., 1998) and Paracrurococcus (Saito et al., 1998) are located in the \(\alpha\)-1 group. Methylobacterium (Green & Bousfield, 1983) and Rhizobium sp. BT Ai l (Evans et al., 1990) are located in the \(\alpha\)-2 group. Roseobacter (Shiba, 1991) is a member of the \(\alpha\)-3 group. Erythrobacter (Shiba & Simidu, 1982), Erythromicrobium (Yurkov et al., 1994), Erythromonas (Yurkov et al., 1994), the GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of strains OCh 239\(^T\) and OCh 210\(^T\) are D85829 and D85831, respectively.

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1997), Porphyrobacter (Fuerst et al., 1993) and Sandaracinobacter (Yurkov et al., 1997) comprise the α-4 group.

Shiba et al. (1991) isolated aerobic bacteriochlorophyll-containing bacteria from the east and west coasts of Australia. These organisms have been divided into four groups (Group I–IV) on the basis of colony colour, absorption spectrum type of bacteriochlorophyll and cell morphology (Nishimura et al., 1994). Based on the results of the DNA–DNA hybridization (Nishimura et al., 1994), each group (except Group III) has been divided into several genotype groups. Group II (19 strains) is divided into four genotype groups and the others (strains OCh 215, 231, 210, 239 and 263). In the present study, we investigated the phenotypic characteristics and the phylogenetic situation of strains OCh 239T (T = type strain) and OCh 210T in Group II. Based on the results of this study, we propose the new genus Roseivivax with two new species, Roseivivax halodurans and Roseivivax halotolerans.

METHODS

Bacterial strains. Strains OCh 239T and OCh 210T were previously isolated from the charophytes and the epiphytes on the stromatolites, respectively, at Lake Clifton (isolated saline lake located in the west coast of Australia) by Shiba et al. (1991). The strains were cultivated on PPES-II medium (Taga, 1968) containing 2·0 g Bacto Peptone, 1·0 g Proteose Peptone No. 3, 1·0 g Bacto Soytone, 1·0 g Bacto Yeast extract, 0·1 g Fe(III)-EDTA, 1000 ml artificial sea water and 15–0 g agar (if needed). The artificial sea water contained (per litre) 30·0 g NaCl, 0·7 g KCl, 10·8 g MgCl2·6H2O, 54·g MgSO4·7H2O and 1·0 g CaCl2·2H2O. The pH was adjusted to 7–8 with 10% (w/v) NaOH. Roseobacter littoralis OCh 149T and Roseobacter denitrificans OCh 114T were used as references for physiological and biochemical characterization.

Electron microscopy. 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.

Physiological and biochemical characteristics. Physiological and biochemical characteristics were examined according to the methods of Shiba & Simidu (1982).

Preparation of chromosomal DNA. Strains OCh 239T and OCh 210T were grown in PPES-II broth at 27°C with shaking. The cells were suspended in 0·1 M saline EDTA (0·15 M NaCl, 0·1 M EDTA; pH 8·5), and then lysed at 60°C for 10 min with 0·5% SDS as a final concentration. Chromosomal DNA was purified according to standard procedures (Sambrook et al., 1989).

Amplification of 16S rRNA gene. Amplification of 16S rRNA gene was performed on a Quick Thermo Personal QTP-1 (Nippon Genetics) in 100 μl reaction volume containing 100 ng chromosomal DNA, 10 μl 10 × Ex Taq buffer (Takara Shuzo), 200 μM each dNTP, 1 μM each primer and 2·5 U Takara Ex Taq (Takara Shuzo). The primers were 5′ AGTGGATCCTGGCCTC 3′ [Escherichia coli numbering system (Brosius et al., 1978): positions 10–25] and 5′ AAGGAGGTGATCCAGCC 3′ (positions 1525–1541). Amplification conditions were described previously (Suzuki & Yamamoto, 1994). The amplified DNA fragments were purified by gel electrophoresis on 1% Agarose S (Nippon Gene), and recovered with glass powder using the Prep-A-Gene DNA Purification System (Bio-Rad).

Sequencing and analysis of sequence data. Sequencing was carried out as described previously (Suzuki & Yamamoto, 1994). The sequences that were determined and the sequences of reference bacterial species were aligned using the program CLUSTAL W version 1.7 (Thompson et al., 1994). The alignment was checked manually. Phylogenetic analysis was performed using the PHYLIP (phylogeny inference package) version 3.57c (Felsenstein, 1995). A distance matrix was calculated with DNADIST using the Kimura 2-parameter model, and a phylogenetic tree was reconstructed using NEIGHBOR. The stability of the clusters was ascertained by performing a bootstrap analysis (1000 replications) with DNABOOT, DNADIST, NEIGHBOR and CONSENSE.

**Fig. 1.** Electron micrographs of negatively stained cells of strains OCh 239T (top) and OCh 210T (bottom). Bars, 1 μm.
RESULTS

Colony and cell morphology

Colonies of strains OCh 239T and OCh 210T were circular, smooth, slightly convex, entire, glistening, opaque and pink. Electron micrographs of negatively stained cells showed that they were rods with subpolar flagella (Fig. 1). Cells of strain OCh 239T were 0.5–1.0 x 1.0–5.0 μm, and cells of strain OCh 210T were 0.5–1.0 x 1.0–3.0 μm.

Physiological and biochemical characteristics

Strains OCh 239T and OCh 210T grew chemoheterotrophically under aerobic conditions, but could not grow phototrophically under anaerobic conditions in the light. They synthesized bacteriochlorophyll a under aerobic conditions. Optimum growth occurred at pH 7.5–8.0 and at 27–30 °C. The physiological and biochemical properties of strains OCh 239T and OCh 210T are shown in Table 1. These two strains had catalase and oxidase. Voges–Proskauer test was negative. ONPG reaction was positive. Both strains produced indole, but did not generate H₂S. Starch, Tween 80 or alginate were not hydrolysed. Both strains utilized D-glucose, acetate, butyrate, citrate, DL-lactate, DL-malate, pyruvate, succinate, L-aspartate and L-glutamate, but did not utilize fumarate, glycolate, ethanol or methanol. Acids were produced from D-fructose and D-glucose. Both strains were resistant to penicillin and tetracycline, but were sensitive to chloramphenicol and streptomycin.

Strain OCh 239T had nitrate reductase and phosphatase, but did not have urease. Gelatin was not hydrolysed. Acids were produced from L-arabinose, D-galactose, lactose, maltose, D-ribose and sucrose, but were not produced from D-xylose. The strain could grow in media supplemented with 0–20.0 % (w/v) NaCl.

Strain OCh 210T had urease, but did not have nitrate reductase or phosphatase. Gelatin was hydrolysed. Acids were produced from D-xylose, but were not produced from L-arabinose, D-galactose, lactose, maltose, D-ribose or sucrose. The strain required NaCl for growth, and could grow in 0.5–20.0 % (w/v) NaCl.

Phylogenetic analysis

The 16S rRNA gene sequences of strains OCh 239T and OCh 210T were determined, and aligned with the other available 16S rRNA sequences of the strains that belong to the α-3 group of the α subclass of the class Proteobacteria. A comparison of the 16S rRNA

Table 1. Differential characteristics of isolates and reference strains Roseobacter denitrificans OChl14T and Roseobacter litoralis OCh149T

<table>
<thead>
<tr>
<th>Character</th>
<th>Och239T</th>
<th>Och210T</th>
<th>OChl14T</th>
<th>OCh149T</th>
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<tbody>
<tr>
<td>Nitrate reductase</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<td>Phosphatase</td>
<td>+</td>
<td>–</td>
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<td>–</td>
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<td>Urease</td>
<td>–</td>
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<td>Hydrolysis of:</td>
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<tr>
<td>Gelatin</td>
<td>–</td>
<td>+</td>
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<td>Tween 80</td>
<td>–</td>
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<td>+</td>
<td>+</td>
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<td>Utilization of:</td>
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<tr>
<td>Butyrate</td>
<td>+</td>
<td>+</td>
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<td>Fumarate</td>
<td>–</td>
<td>–</td>
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<td>Glycolate</td>
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<td>–</td>
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<td>L-Aspartate</td>
<td>+</td>
<td>+</td>
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<td>Acid production from:</td>
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<td>L-Arabinose</td>
<td>+</td>
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<td>D-Fructose</td>
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<td>w</td>
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<td>D-Galactose</td>
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<td>D-Glucose</td>
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<td>–</td>
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<td>Lactose</td>
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<td>Maltose</td>
<td>+</td>
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<td>D-Ribose</td>
<td>+</td>
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<td>Sucrose</td>
<td>+</td>
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<td>–</td>
<td>–</td>
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<tr>
<td>D-Xylose</td>
<td>–</td>
<td>w</td>
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<td>Growth in the presence of:</td>
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<tr>
<td>0% NaCl</td>
<td>w</td>
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<td>–</td>
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<tr>
<td>20% NaCl</td>
<td>w</td>
<td>+</td>
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</table>
sequences revealed that strains OCh 239T and OCh 210T belonged to the α-3 group of the α subclass of the class Proteobacteria, forming a distinct cluster (Fig. 2). The cluster neighboured on Roseobacter algicola and Roseobacter gallaeciensis. The similarity values of the 16S rRNA sequences of strains OCh 239T and OCh 210T to Roseobacter algicola and Roseobacter gallaeciensis were 91.8–93.3%. The similarity value of the 16S rRNA sequences between strains OCh 239T and OCh 210T was 95.8%.

**DISCUSSION**

Aerobic bacteriochlorophyll-containing bacteria isolated from the east and west coasts of Australia (Shiba et al., 1991) have been divided into four groups on the basis of colony colour, absorption spectrum type of bacteriochlorophyll and cell morphology (Nishimura et al., 1994). These phenotypic groups have been investigated for their chemotaxonomic characteristics and by DNA–DNA hybridization. Strains OCh 239T and OCh 210T have been included in Group II, but did not belong to any of the genotype groups. To define the phylogenetic positions of these strains, we performed comparative 16S rRNA sequence analysis. In consequence, it was revealed that strains OCh 239T and OCh 210T formed a new distinct cluster moderately related to Roseobacter algicola, Roseobacter gallaeciensis, Sagittula stellata, Octadecabacter arcticus and Sulfitobacter ponticus. The cluster was distantly related to other taxa at the generic level (93.3% 16S rRNA sequence similarity or less). The phylogenetic analysis supports the proposal of the new genus Roseivivax for strains OCh 239T and OCh 210T. Strain OCh 239T was phylogenetically distant from strain OCh 210T (95.8% 16S rRNA sequence similarity). Phenotypic characteristics of strain OCh 239T were very different from those of strain OCh 210T, i.e. nitrate reduction, urease, phosphatase, hydrolysis of gelatin and acid production from carbon sources. These results indicate that strains OCh 239T and OCh 210T are different species.

In conclusion, we propose that strains OCh 239T and OCh 210T should be described as Roseivivax halodurans gen. nov., sp.nov. and Roseivivax halotolerans sp. nov., respectively.

**Description of Roseivivax gen. nov.**

Roseivivax (Ro.se.i.vii’vax. M.L. adj. roseus rose-coloured, pink; L. adj. vivax living; M.L. masc. n. Roseivivax pink living organism).

Cells are aerobic and Gram-negative rods that are motile by means of subpolar flagella. Catalase and oxidase are produced. Chemoheterotrophic. Bacteriochlorophyll a is synthesized under aerobic conditions. The ubiquinone system is Q-10 (Nishimura et al., 1994). The major cellular fatty acids are C18:1.

**Description of Roseivivax halodurans sp. nov.**

Roseivivax halodurans (ha.lo.du’rans. Gr. n. hals salt; L. pres. part. durans enduring; M.L. part. adj. halodurans salt-enduring).

Colonies are circular, smooth, slightly convex, entire, glistening, opaque and pink. Cells are 0.5–1.0 x 1.0–5.0 μm. Optimum growth occurs at pH 7.5–8.0 and at 27–30 °C. Growth occurs in the presence of 0–20.0% (w/v) NaCl. Cells have nitrate reductase and phosphatase, but do not have urease. Voges–Proskauer test is negative. ONPG reaction is positive. Cells produce indole, but do not generate H2S. Hydrolysis of alginate, gelatin, starch and Tween 80 is negative. Cells utilize D-glucose, acetate, butyrate, citrate, DL-lactate, DL-malate, pyruvate, succinate, L-aspartate and L-glutamate, but do not utilize fumarate, glycolate, ethanol or methanol. Acids are produced from L-arabinose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-ribose and sucrose, but are not produced from D-xylose. Cells are resistant to penicillin and
tetraacycline, but are sensitive to chloramphenicol and streptomycin. The absorption spectrum of the membrane fraction in the near-IR region has maxima at 803 and 873 nm (Nishimura et al., 1994). The source of the strain is the charophytes of the saline lake. The G+C content of the DNA is 64.4 mol% (Nishimura et al., 1994). The type strain is strain OCh 239^T (= JCM 10272^T).

**Description of Roseivivax halotolerans sp. nov.**

*Roseivivax halotolerans* (ha.lo.to’le.rans. Gr. n. hals salt; L. pres. part. tolerans tolerating; M. L. part. adj. halotolerans salt-tolerating).

Colonies are circular, smooth, slightly convex, entire, glistening, opaque and pink. Cells are 0·5–1·0 x 1·0–5·0 μm. Optimum growth occurs at pH 7·5–8·0 and at 27–30 °C. Growth occurs in the presence of 0·5–20·0 % (w/v) NaCl. No growth occurs in the absence of NaCl. Cells have urease, but do not have nitrate reductase or phosphatase. Voges–Proskauer test is negative. ONPG reaction is positive. Cells produce indole, but do not generate H₂S. Hydrolysis of gelatin is positive, but hydrolysis of alginate, starch and TWEEN 80 is negative. Cells utilize d-glucose, acetate, butyrate, citrate, DL-lactate, DL-malate, pyruvate, succinate, L-aspartate and L-glutamate, but do not utilize fumarate, glycolate, ethanol or methanol. Acids are produced from d-fructose, d-glucose and d-xylene, but are not produced from L-arabinose, D-galactose, lactose, maltose, D-ribose or sucrose. Cells are resistant to penicillin and tetracycline, but are sensitive to chloramphenicol and streptomycin. The absorption spectrum of the membrane fraction in the near-IR region has maxima at 803 and 871 nm (Nishimura et al., 1994). The source of the strain is the epiphytes on the stromatolites of the saline lake. The G+C content of the DNA is 59·7 mol% (Nishimura et al., 1994). The type strain is strain OCh 239^T (= JCM 10271^T).

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