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Roseibium denhamense gen. nov., sp. nov. and *Roseibium hamelinense* sp. nov., aerobic bacteriochlorophyll-containing bacteria isolated from the east and west coasts of Australia

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Phenotypic and phylogenetic studies were performed with 10 strains of bacteriochlorophyll-containing bacteria isolated from a variety of marine environments (surface of Rhodophyta, sand and algal sand mat) on the east and west coasts of Australia. The strains were aerobic, chemoheterotrophic, Gram-negative, motile rods with peritrichous flagella. Bacteriochlorophyll *a* was synthesized under aerobic conditions. Catalase, nitrate reductase, oxidase and phosphatase were produced. ONPG reaction was positive. The strains have been divided into genotype group 1 (seven strains) and genotype group 2 (three strains) according to previously described DNA–DNA hybridization data. Strains OCh 254^T and OCh 368^T have been included in genotype groups 1 and 2, respectively. The results of 16S rRNA gene sequence comparisons revealed that strains OCh 254^T and OCh 368^T formed a new cluster within the α-2 group of the α subclass of the *Proteobacteria*. The similarity value of the 16S rRNA gene sequences between strain OCh 254^T and the most closely related species, *Stappia aggregata*, was 95·6%. The sequence similarity value between strains OCh 254^T and OCh 368^T was 97·1%. It was concluded that these two strains should be placed into a new genus, *Roseibium* gen. nov., as *Roseibium denhamense* sp. nov. and *Roseibium hamelinense* sp. nov. The type species of the genus is *Roseibium denhamense*. The type strains of *Roseibium denhamense* and *Roseibium hamelinense* are OCh 254^T (= JCM 10543^T) and OCh 368^T (= JCM 10544^T), respectively.

Keywords: *Roseibium denhamense*, *Roseibium hamelinense*, aerobic bacteriochlorophyll-containing bacteria, 16S rRNA

INTRODUCTION

Phototrophic bacteria generally synthesize bacteriochlorophylls under anaerobic conditions. However, Sato (1978) reported on aerobic methylotrophs containing bacteriochlorophyll *a*. To date, a lot of aerobic anoxygenic phototrophic bacteria have been described (Yurkov & Beatty, 1998).

Aerobic and chemoheterotrophic bacteriochlorophyll-containing bacteria have been isolated from a variety of marine environments (surface of Rhodophyta, sand and algal sand mat) on the east and west coasts of Australia (Shiba *et al*., 1991). These isolates have been divided into four phenotype groups (Group I–IV) based on colony colour, bacteriochlorophyll absorption spectrum and cell morphology. Each phenotype group (except Group III) has been divided into several genotype groups according to DNA–DNA hybridization data (Nishimura *et al*., 1994). Based on phenotypic characteristics and the phylogenetic considerations, we have recently proposed two new genera, *Rubrimonas* and *Roseivivax*, for Group III strains and

The GenBank accession numbers for the 16S rRNA gene sequences of strains OCh 254^T and OCh 368^T are D85832 and D85836, respectively.

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two strains that do not belong to any genotype groups in Group II, respectively (Suzuki *et al.*, 1999a, b). The genus *Rubrimonas* consists of one species, *Rubrimonas cliftonensis*, which is a short rod with polar flagella. *Roseivivax* possesses two species, *Roseivivax halodurans* and *Roseivivax halotolerans*, which are rods with subpolar flagella and can grow in up to 20·0% (w/v) NaCl. These two genera belong to the α-3 subclass of the *Proteobacteria*.

In the present study, the phenotypic characteristics of strains OCh 254^T, 281, 282, 326, 374, 375 and 445 of genotype group 1 and strains OCh 368^T, 334 and 355 of genotype group 2 in Group II were investigated and the 16S rRNA genes of strains OCh 254^T and OCh 368^T were analysed. Based on the results of these investigations, a new genus, *Roseibium* gen. nov., with two new species, *Roseibium denhamense* sp. nov. and *Roseibium hamelinense* sp. nov., is proposed.

METHODS

Bacterial strains. Strains OCh 254^T, 281, 282, 326, 374, 375, 445, 368^T, 334 and 355 were previously isolated from specimens from a variety of marine environments on the east and west coasts of Australia by Shiba *et al.* (1991). Strains OCh 254^T and OCh 326 were isolated from *Botryocladia* sp. at Denham, Shark Bay. Strain OCh 281 was isolated from *Botryocladia* sp. at Little Lagoon, Shark Bay. Strains OCh 374 and OCh 375 were isolated from *Laurencia* sp. at Little Lagoon, Shark Bay. Strain OCh 282 was isolated from sands at Monkey Mia, Shark Bay. Strains OCh 368^T and OCh 355 were isolated from sands at Hamelin Pool, Shark Bay. Strain OCh 334 was isolated from *Botryocladia* sp. at Shell Beach, Shark Bay. Strain OCh 445 was isolated from an algal sand mat at Arcadia, Magnetic Island. The strains were cultivated on PPES-II medium (Taga, 1968). The pH was adjusted to 7·8 with 10% NaOH.

Electron microscopy. Cells of strains OCh 254^T and OCh 368^T 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 254^T and OCh 368^T were grown in PPES-II broth at 27 °C with shaking. The cells were resuspended 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 (final concentration). Chromosomal DNA was purified according to standard procedures (Sambrook *et al.*, 1989).

Amplification of the 16S rRNA gene and sequence analysis. Amplification of the 16S rRNA gene was performed on a Quick Thermo Personal QTP-1 (Nippon Genetics) in 100 µl reaction volume as described previously (Suzuki *et al.*, 1999a, b). The amplified DNA fragments were purified by gel electrophoresis on 1% (w/v) Agarose S (Nippon Gene) and recovered with glass powder using Prep-A-Gene DNA Purification Systems (Bio-Rad). Sequencing was carried out according to the previous paper (Suzuki & Yamasato, 1994).

Phylogenetic analysis. The determined sequence and the sequences of reference bacterial species were aligned with the program CLUSTAL X version 1·64b (Thompson *et al.*, 1997). The alignment was checked manually. Phylogenetic analysis was performed with PHYLIP version 3·57c (Felsenstein, 1995). A distance matrix was calculated with DNADIST using the Kimura two-parameter correction 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.

RESULTS

Colony and cell morphology

Colonies of all the strains were circular, smooth, slightly convex, entire, glistening, opaque and pink. Electron micrographs of negatively stained cells (OCh 254^T and OCh 368^T) showed that they were rods with peritrichous flagella (Fig. 1). Cells of strains OCh 254^T, 281, 326, 374, 375, 445, 368^T, 334 and 355 were 0·5–0·8 × 1·0–4·0 µm and cells of strain OCh 282 were 0·5 × 3·0–6·0 µm.

Physiological and biochemical characteristics

All strains 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. Some of the physiological and biochemical properties of strains OCh 254^T and OCh 368^T are shown in Table 1. All strains were positive for catalase, nitrate reductase, oxidase and phosphatase activity. Voges–Proskauer test was negative. ONPG reaction was positive. The strains produced indole, but not H_2S. They hydrolysed gelatin, but not alginate, starch or Tween 80. All strains utilized butyrate, L-glutamate, pyruvate and L-aspartate, but did not utilize glycolate, ethanol and methanol. Acids were produced from D-fructose, D-glucose, D-ribose and maltose, but were not produced from L-arabinose or lactose. They were resistant to penicillin and tetracycline, but were sensitive to chloramphenicol and streptomycin.

Genotype group 1 strains possessed urease activity. The strains hydrolysed gelatin (except OCh 445), but did not hydrolyse Tween 80. They utilized D-glucose (except OCh 281), acetate, citrate (except OCh 282, 326 and 445), fumarate, glycolate (except OCh 254, 281 and 282), DL-lactate (except OCh 254, 281 and 282), DL-malate (except OCh 282), pyruvate (except OCh 281), succinate (except OCh 282 and OCh 326) and L-aspartate (except OCh 281 and OCh 282). Acids were produced from D-galactose, D-xylose, maltose (except OCh 281 and OCh 326) and sucrose. All genotype group 1 strains required NaCl for growth and could grow in 0·5–7·5% NaCl (strain OCh 254^T could grow up to 10·0% NaCl).

Genotype group 2 strains also possessed urease activity (except OCh 368^T). The strains hydrolysed gelatin and Tween 80 (except OCh 368^T). They utilized D-glucose,

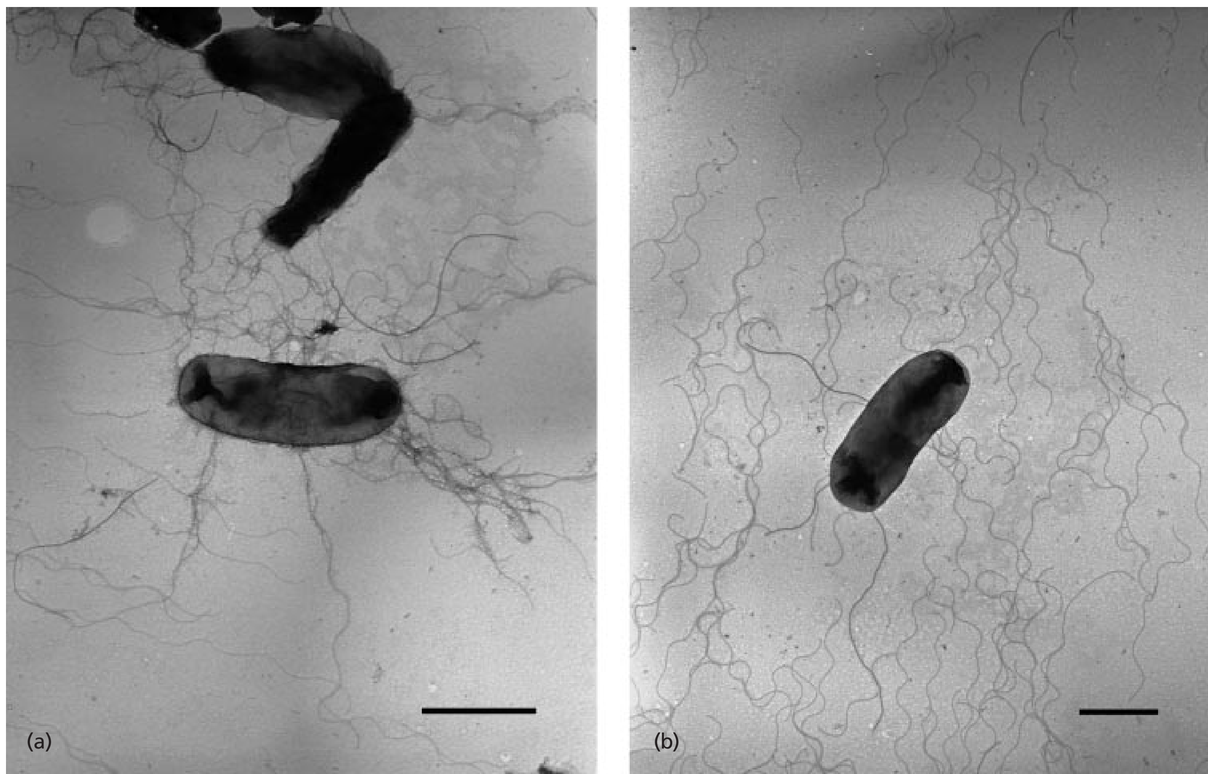

Fig. 1. Electron micrographs of negatively stained cells of strains OCh 254^T (a) and OCh 368^T (b). Bars, 1 μm.

Table 1. Physiological and biochemical characteristics of *Roseibium denhamense* OCh 254^T and *Roseibium hamelinense* OCh 368^T

+, Positive; −, negative; w, weakly positive.

Character	OCh 254^T	OCh 368^T
Urease	+	−
Utilization of:		
D-Glucose	w	+
Acetate	+	−
Citrate	w	−
Fumarate	+	−
DL-Lactate	−	+
DL-Malate	+	−
Succinate	w	−
Acid production from:		
D-Galactose	+	−
D-Xylose	+	−
Sucrose	+	w
Growth in the presence of 0% NaCl	−	+

DL-lactate, pyruvate, succinate (except OCh 368^T) and L-aspartate, but did not utilize acetate (except OCh 334), citrate, fumarate (except OCh 334), glycolate or DL-malate (except OCh 355). Acids were produced from maltose and sucrose (except OCh 334), but were not produced from D-galactose or D-xylose. The strains could grow in 0–10·0% NaCl.

Phylogenetic analysis

The 16S rRNA gene sequences of strains OCh 254^T and OCh 368^T were determined and aligned with the other available 16S rRNA gene sequences of strains belonging to the α subclass of the *Proteobacteria*. A comparison of the 16S rRNA gene sequences in which a phylogenetic tree was reconstructed (Fig. 2) revealed that strains OCh 254^T and OCh 368^T belonged to the α-2 subclass of the *Proteobacteria*. The 16S rRNA gene sequence similarity values of strain OCh 254^T to *Stappia aggregata* and *Stappia stellulata* were 95·6 and 92·0%, respectively. The 16S rRNA gene sequence similarity values of strain OCh 368^T to *S. aggregata* and *S. stellulata* were 96·7 and 93·4%, respectively. The similarity value of the 16S rRNA gene sequences between strains OCh 254^T and OCh 368^T was 97·1%.

DISCUSSION

Shiba *et al.* (1991) isolated aerobic and chemoheterotrophic bacteriochlorophyll-containing bacteria from specimens from a variety of marine environments on the east and west coasts of Australia. Nishimura *et al.*

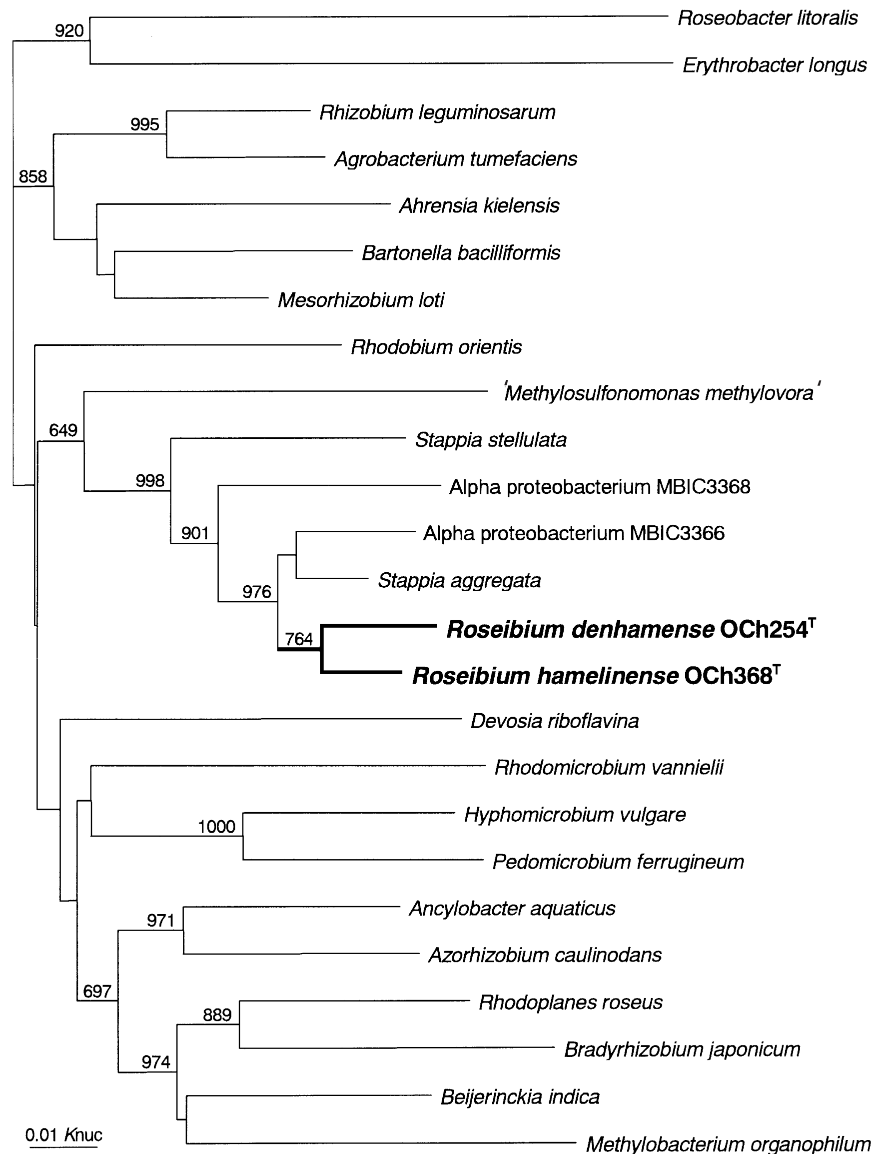

Fig. 2. Unrooted phylogenetic tree derived from the analysis of the 16S rRNA gene sequences of *Roseibium denhamense* OCh 254^T, *Roseibium hamelinense* OCh 368^T and other members of the α subclass of the *Proteobacteria*. The numbers at the nodes indicate the levels of bootstrap support based on 1000 resamplings. Only significant values (above 600) are indicated.

(1994) divided these isolates into four groups (I–IV) on the basis of colony colour, bacteriochlorophyll absorption spectrum and cell morphology. Strains of Group II have been divided into four genotype groups according to DNA–DNA hybridization data. Strains OCh 254^T and OCh 368^T have been included into genotype groups 1 and 2, respectively.

To date, the taxonomic positions of these genotype groups has not been clear. To solve this problem, a phylogenetic analysis based on 16S rRNA gene sequences was carried out. In consequence, it was revealed that strains OCh 254^T and OCh 368^T formed a new cluster within the α-2 subclass of the *Proteobacteria*. The sequence similarity values of the cluster to the related organisms *Stappia aggregata* and *Stappia stellulata* were 92·0–96·7 %. The genus *Stappia* was proposed by Uchino *et al.* (1998) to accommodate two marine '*Agrobacterium*' species. The phylogenetic analysis supports the creation of a new genus, *Roseibium*, for strains of genotype groups 1 and 2 in Group II.

The 16S rRNA gene sequence similarity value between strains OCh 254^T and OCh 368^T was 97·1 %. The chromosomal DNA homology values between these strains were 12–17 % and the DNA homology values between strains of genotype groups 1 and 2 were

75–100% and 98–99%, respectively (Nishimura *et al*., 1994). These results indicate that the strains of genotype groups 1 and 2 are different species.

In conclusion, we propose that strains OCh 254^T, 281, 282, 326, 374, 375 and 445 of genotype group 1 should be described as *Roseibium denhamense* gen. nov., sp. nov., and strains OCh 368^T, 334 and 355 of genotype group 2 as *Roseibium hamelinense* sp. nov.

Description of *Roseibium* gen. nov.

Roseibium (ro.sei′bi.um. M. L. adj. *roseus* rose/pink; Gr. n. *bios* life; M. L. neut. n. *Roseibium* pink life).

Cells are Gram-negative rods that are motile by means of peritrichous flagella. Growth is aerobic and chemoheterotrophic. Bacteriochlorophyll *a* is synthesized under aerobic conditions. Cultures do not grow anaerobically in the light. Catalase, nitrate reductase, oxidase and phosphatase are produced. The ubiquinone system is Q-10 and the major cellular fatty acid is C18:1 (Nishimura *et al*., 1994). The genus *Roseibium* belongs to the α-2 subclass of the *Proteobacteria*. The type species is *Roseibium denhamense*.

Description of *Roseibium denhamense* sp. nov.

Roseibium denhamense (den.ha.men′se. M. L. adj. *denhamense* referring to Denham, Australia, the source of the type strain).

Colonies are circular, smooth, slightly convex, entire, glistening, opaque and pink. Cells are 0·5–0·8 × 1·0–4·0 µm, except strain OCh 282 which is 0·5 × 3·0–6·0 µm. Voges–Proskauer test is negative. ONPG reaction is positive. Cells produce indole, but do not produce H_2S. Alginate, starch and Tween 80 are not hydrolysed. Cells utilize acetate, butyrate, fumarate and L-glutamate, but do not utilize ethanol or methanol. Acids are produced from D-fructose, D-galactose, D-glucose, D-ribose, D-xylose and sucrose, but are not produced from L-arabinose or lactose. Cells are resistant to penicillin and tetracycline, but are sensitive to chloramphenicol and streptomycin. Optimum growth occurs at pH 7·5–8·0 and at 27–30 °C. Growth occurs in the presence of 0·5–7·5% (w/v) NaCl, except strain OCh 254^T which can grow in the presence of 0·5–10·0% (w/v) NaCl. No growth occurs in the absence of NaCl. The absorption spectrum of the membrane fraction in the near-IR region has maxima at 803–805 and 863–864 nm (Nishimura *et al*., 1994). The DNA G+C content is 57·6–60·4 mol% (Nishimura *et al*., 1994). The type strain is OCh 254^T (= JCM 10543^T), which was isolated from *Botryocladia* sp. at Denham, Shark Bay, Australia.

Description of *Roseibium hamelinense* sp. nov.

Roseibium hamelinense (ha.me.li.nen′se. M. L. adj. *hamelinense* referring to Hamelin Pool, Australia, the source of the type strain).

Colonies are circular, smooth, slightly convex, entire, glistening, opaque and pink. Cells are 0·5–0·8 × 1·0–4·0 µm. Voges–Proskauer test is negative. ONPG reaction is positive. Cells produce indole, but do not produce H_2S. Gelatin is hydrolysed, but alginate and starch are not. Cells utilize D-glucose, butyrate, DL-lactate, pyruvate, L-aspartate and L-glutamate, but do not utilize citrate, glycolate, ethanol or methanol. Acids are produced from D-fructose, D-glucose, D-ribose and maltose, but are not produced from L-arabinose, D-galactose, D-xylose or lactose. Cells are resistant to penicillin and tetracycline, but are sensitive to chloramphenicol and streptomycin. Optimum growth occurs at pH 7·5–8·0 and at 27–30 °C. Growth occurs in the presence of 0–10·0% (w/v) NaCl. The absorption spectrum of the membrane fraction in the near-IR region has maxima at 803–805 and 872–873 nm (Nishimura *et al*., 1994). The DNA G+C content is 59·2–63·4 mol% (Nishimura *et al*., 1994). The type strain is OCh 368^T (= JCM 10544^T), which was isolated from sands at Hamelin Pool, Shark Bay, Australia.

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REFERENCES

Felsenstein, J. (1995). PHYLIP (phylogeny inference package), version 3.57c. Seattle: University of Washington.

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.

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* AM1, *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.

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 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.

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* **24**, 4876–4882.

Uchino, Y., Hirata, A., Yokota, A. & Sugiyama, J. (1998). Reclassification of marine *Agrobacterium* species: proposals of *Stappia stellulata* gen. nov., comb. nov., *Stappia aggregata* sp. nov., nom. rev., *Ruegeria atlantica* gen. nov., comb. nov., *Ruegeria gelatinovora* comb. nov., *Ruegeria algicola* comb. nov., and *Ahrensia kieliense* gen. nov., sp. nov., nom. rev, *J Gen Appl Microbiol* **44**, 201–210.

Yurkov, V. & Beatty, J. T. (1998). Aerobic anoxygenic phototrophic bacteria, *Microbiol Mol Biol Rev* **62**, 695–724.