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NOTE

Proposal of *Craurococcus roseus* gen. nov., sp. nov. and *Paracraurococcus ruber* gen. nov., sp. nov., novel aerobic bacteriochlorophyll *a*-containing bacteria from soil

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Sequences of the 16S rRNA gene were determined for three strains of aerobic bacteriochlorophyll *a*-containing bacteria isolated from soil. The sequences of two strains (NS89^T and NS102) were identical for approximately 1500 nucleotides. Phylogenetic analysis revealed that the three strains belonged to the α-1 subclass of the *Proteobacteria*, constituting one line of descent. The three strains are comparatively related to *Roseococcus thiosulfatophilus*, which is an aerobic bacteriochlorophyll *a*-containing bacterium. The 16S rRNA gene sequence similarity and the DNA–DNA relatedness allow the proposal of two new genera, *Craurococcus* gen. nov. and *Paracraurococcus* gen. nov. The type species are *Craurococcus roseus* sp. nov. and *Paracraurococcus ruber* sp. nov., and their type strains are NS130^T (=JCM 9933^T) and NS89^T (=JCM 9931^T), respectively.

Keywords: *Craurococcus roseus* gen. nov., sp. nov., *Paracraurococcus ruber* gen. nov., sp. nov., bacteriochlorophyll *a*, soil bacteria, *Proteobacteria*

Since formation of bacteriochlorophyll (Bchl) *a* was found in aerobic methanol-utilizing bacteria, aerobic Bchl *a*-containing bacteria have been isolated from a variety of habitats (2, 4, 5, 8, 12–15, 17–19, 21–23). However, there is no report of the isolation of such bacteria from soil, except our previous paper (9), which described the isolation of three strains (NS89^T, NS102 and NS130^T) from soil as novel aerobic Bchl *a*-containing bacteria. Strains NS89^T and NS102 were significantly different from strain NS130^T in the absorption spectra of membrane fractions in the near-infrared region, and in several physiological properties. In this study the phylogenetic positions of the three isolated strains were investigated on the basis of 16S rRNA gene sequence analysis and DNA–DNA hybridization.

Strains NS89^T (T = type strain), NS102 and NS130^T were isolated from soils in Noda, Osaka and Tokyo, Japan, respectively (9). The strains were deposited in the Japan Collection of Microorganisms (JCM), Institute of Physical and Chemical Research (RIKEN), Wako, Japan, as JCM 9931^T (=NS89^T), JCM 9932 (=NS102) and JCM 9933^T (=NS130^T). Strains NS89^T and NS102 were cultivated with NPG liquid medium, which was a twofold dilution of a nutrient broth plus extract from 100 g potato and 10 g glucose. Strain NS130^T was cultivated on the NPG agar medium.

Cells harvested by centrifugation or by scraping from agar slants were washed and suspended in 25 mM Tris/HCl buffer (pH 7·5) containing 25 mM $CaCl_2.2H_2O$. Then the cells were treated with lysozyme (final concn, 1·0 mg ml^{-1}) and SDS (final concn, 5·0 mg ml^{-1}) for thorough lysis. Chromosomal DNA was purified by standard procedures (11). Amplification of the 16S rRNA gene and sequencing of the amplified DNA fragments were performed as described previously (16). The sequences determined in this study were aligned with the sequences of members of the α subclass of the *Proteobacteria*, taking account of the secondary structure. Evolutionary distances (K_{nuc} values) were calculated, and a phylogenetic tree was constructed by applying the algorithm of the neighbour-joining method (10) to K_{nuc} values.

Purified chromosomal DNA was labelled with $[^3H]$deoxy-(1′,2′,5′-^{3}H) cytidine 5′-triphosphate (ammonium salt) by DNA-labelling techniques (3), using a

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Abbreviation: Bchl, bacteriochlorophyll.

The DDBJ accession numbers for the 16S rRNA sequences of strains NS89^T, NS102 and NS130^T are D85827, AB009013 and D85828, respectively.

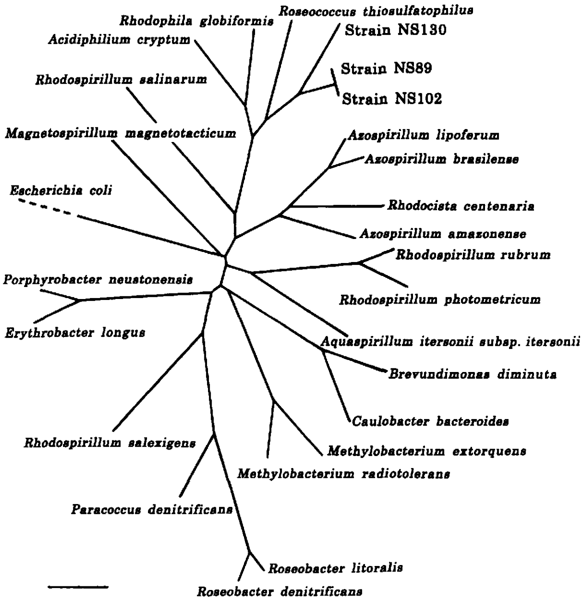

Fig. 1. Unrooted distance matrix tree based on 16S rRNA sequences, showing phylogenetic relationships among strains NS89^T, NS102 and NS130^T, and representatives of the α subclass of the *Proteobacteria*. Bar, 0·02 K_{nuc}.

DNA-labelling kit (Nippon Gene). Reassociation of labelled and unlabelled DNAs was performed on membrane filters at 73 °C for 48 h. Otherwise, the procedures were carried out as described previously (6, 7).

The 16S rRNA gene sequences of strains NS89^T, NS102 and NS130^T determined in this study were 1439, 1439 and 1443 nucleotides long, respectively. Strains NS89^T and NS102 had identical sequences. Signature sequences and secondary structural features at the stems from positions 180–220 and 455–480 [*Escherichia coli* numbering system (1)] suggested that the strains are members of the α subclass of the *Proteobacteria* (4, 20). The phylogenetic tree reconstructed from 1072 nucleotide sequences that were unambiguously alignable regions, showed that the isolated strains are members of the α-1 subclass of the *Proteobacteria*, forming a separate line of descent in the subclass (Fig. 1). In the phylogenetic tree the strains formed a cluster with *Roseococcus thiosulfatophilus*, *Rhodophila globiformis* and *Acidiphilium cryptum*, although the strains were not closely related to any bacteria in the cluster. *Roseococcus thiosulfatophilus*, which is an aerobic Bchl *a*-containing bacterium isolated from a freshwater cyanobacterial mat (22, 23), was comparatively related to the isolated strains in the cluster. The similarity values of the 16S rRNA gene sequences of strains NS89^T and NS130^T to *Roseococcus thiosulfatophilus* were 92·9 and 89·9%, respectively. The similarity of the 16S rRNA gene sequences between NS89^T and NS130^T was 94·2%. The homology of chromosomal DNAs between strains NS89^T and NS102 was 86%, while strain NS130^T exhibited low homologies (less than 21%) with the other strains (Table 1).

Table 2 shows the distinguishing characteristics in the isolates and *Roseococcus thiosulfatophilus*. The 16S rRNA gene sequence analysis and several taxonomic properties indicate that the isolated strains differ from *Roseococcus thiosulfatophilus* at the genus level. Strains NS89^T and NS102 had identical 16S rRNA gene sequences, almost the same physiological properties and a high level of DNA homology. On the other hand, the similarity of the 16S rRNA gene sequences and the DNA homologies between these two strains and strain NS130^T were low. Strain NS130^T also differed from the other two strains in many phenotypic properties (9). Therefore, the isolated strains should be divided into two genera. We propose strain NS130^T to belong to *Craurococcus roseus* gen. nov., sp. nov., and strains NS89^T and NS102 to belong to *Paracraurococcus ruber* gen. nov., sp. nov. The descriptions of the genera and the species are given below.

Description of *Craurococcus* gen. nov.

Craurococcus (cra.u.ro.coc′cus. Gr. adj. *crauros* fragile; Gr. n. *coccus* a grain, berry; M.L. masc. n. *Craurococcus* fragile coccus, referring to lysis in aqueous solution to which divalent cations are not added).

The description of the genus is based on the original description of Saitoh & Nishimura (9) and the results in this study. Gram-negative. Coccus. Non-motile. Cells divide by binary fission. Catalase and oxidase tests are positive. Forms pink irregular colonies on agar media. Grows heterotrophically under aerobic condition, but not under anaerobic conditions even in the light. Bacteriochlorophyll *a* is present, as well as spirilloxanthin and carotenoic acids. Mesophilic. No

Table 1. Homology values of the DNA preparations from the isolated strains

Source of unlabelled DNA	Homology (%) with labelled DNA from:		
	NS89^T	NS102	NS130^T
NS89^T	100	92	21
NS102	86	100	19
NS130^T	20	17	100

Table 2. Distinguishing characteristics among strains NS89^T, NS102 and NS130^T, and *Roseococcus thiosulfatophilus* strain RB3^T

+, Positive; –, negative; ND, not determined.

Characteristic	NS89^T, NS102*	NS130*	RB3^T†
Cell shape, cocci	+	+	–
Cell size (μm)	0·8–1·5	0·8–2·0	0·9–1·3 × 1·3–1·6
Motility	–	–	+
Growth at 42 °C	+	–	ND
Hydrolysis of:			
Casein	–	–	+
Tween 80	–	+	–
Utilization of as sole carbon and energy source:			
D-Arabinose	+	+	–
D-Ribose	+	+	–
D-Fructose	+	+	–
Acetate	–	–	+
Citrate	–	–	+
Fumarate	+	+	–
Succinate	–	–	+
Near-IR peak for Bchl *a* (nm)‡	856	872	855

* Data from reference 9.

† Data from reference 23.

‡ Peaks were measured for the membrane fractions.

growth occurs at 42 °C. Nitrate is reduced to nitrite. Denitrification is not observed. Nicotinic acid and pantothenate are required for growth. Sodium ion is not required for growth, and no growth is observed in the presence of more than 4·0 g NaCl l^{-1}. Divalent cations such as Ca^{2+} or Mg^{2+} are required for growth in natural and complex liquid media. Tween 80 is hydrolysed. Gelatin and casein are not hydrolysed. Methanol and ethanol are not utilized as sole carbon and energy sources. Sulfate is not utilized as sole sulfur source. Accumulates poly-β-hydroxybutyrate. The major cellular fatty acid is C18:1. Both 2-hydroxy and 3-hydroxy fatty acids are present, and the major hydroxy fatty acid is 2-OH C18:1. Quinone system is Q-10. The G+C content of the DNA is 70·5 mol% (as determined by HPLC). The phylogenetic position is the α-1 subclass of the *Proteobacteria*. The type species is *Craurococcus roseus*.

Description of *Craurococcus roseus* sp. nov.

Craurococcus roseus (ro.se.us. L. adj. *roseus* rose-coloured, pink, pink bacterium).

Colony colour is pink. Cell size is 0·8–2·0 μm. Optimum growth condition is pH 7·5 and 28 to 32 °C. It can not grow at pH 6·8. Hydrolyses Tweens 20, 40, 60 and 80. Acids are produced from D-arabinose, D-fructose, D-fucose, D-galactose, D-glucose, D-ribose, D-mannose, D-fructose, L-rhamnose and D-xylose. No acid is produced from lactose, maltose, sucrose, inositol, D-mannitol, D-sorbitol and starch. Good carbon sources for growth are D-xylose, fumarate and L-glutamate. Moderate growth occurs with glycerol, D-fructose, D-galactose, D-glucose, D-arabinose, L-rhamnose, D-ribose, D-fucose, DL-lactate, malonate, pyruvate, gluconate and L-malate. D-Mannitol, D-sorbitol, methanol, ethanol, 1,2-propanediol, kerosene, acetate, butyrate, citrate, formate, glycolate, propionate, succinate, phthalate, *p*-hydroxybenzoate and benzoate are not utilized. Cells are susceptible to chloramphenicol, penicillin, streptomycin and tetracycline, but are resistant to polymyxin B. The absorption spectrum of bacteriochlorophyll *a* in the intact cells has maxima at 800 and 872 nm. The habitat is soil. The G+C content of the DNA is 70·5 mol%. The type strain is NS130^T (=JCM 9933^T).

Description of *Paracraurococcus* gen. nov.

Paracraurococcus (pa.ra.cra.u.ro.coc′cus. Gr. prep. *para* like, along side of; Gr. adj. *crauros* fragile; Gr. n. *coccus* a grain, berry; M.L. masc. n. *Paracraurococcus* coccus like *Craurococcus*).

The description of the genus is based on the original description of Saitoh & Nishimura (9) and the results in this study. Gram-negative. Coccus. Non-motile. Cells divide by binary fission. Catalase and oxidase tests are positive. Forms red irregular colonies on agar media. Grow heterotrophically under aerobic conditions, but not under anaerobic conditions even in the light. Facultatively photoheterotrophic. Bacteriochlorophyll *a* is present, as well as spirilloxanthin and carotenoic acids. Mesophilic. Capable of growth at 42 °C. Nitrate is reduced to nitrite. Denitrification is not observed. Nicotinic acid and pantothenate are required for growth. Sodium ion is not required for growth, and no growth is observed in the presence of more than 4·0 g NaCl l^{-1}. Tween 80, gelatin and casein are not hydrolysed. Methanol and ethanol are not utilized as sole carbon and energy sources. Sulfate is not utilized as sole sulfur source. Accumulates poly-β-hydroxybutyrate. The major cellular fatty acid is C18:1. Both 2-hydroxy and 3-hydroxy fatty acids are present, and the major hydroxy fatty acid is 2-OH C18:1. Quinone system is Q-10. The G+C content of the DNA is 70·3–71·0 mol % (as determined by HPLC). The phylogenetic position is the α-1 subclass of the *Proteobacteria*. The type species is *Paracraurococcus ruber*.

Description of *Paracraurococcus ruber* sp. nov.

Paracraurococcus ruber (ru.ber. L. adj. *ruber* red-coloured, red bacterium).

Colony colour is red. Cell size is 0·8–1·5 μm. Optimum growth condition is pH 6·6–6·8 and 30–34 °C. Hydrolyse Tweens 20, 40 and 60. Acids are produced from D-arabinose, D-fucose, D-glucose, D-ribose, D-mannose, L-rhamnose and D-xylose. No acid is produced from D-fructose, D-galactose, lactose, maltose, sucrose, inositol, D-mannitol, D-sorbitol and starch. Good carbon source of growth are D-xylose, fumarate and L-glutamate. Moderate growth occurs with glycerol, D-fructose, D-galactose, D-glucose, D-arabinose, L-rhamnose, D-ribose, D-fucose, DL-lactate, malonate, pyruvate, gluconate and L-malate. D-Mannitol, D-sorbitol, methanol, ethanol, 1,2-propanediol, kerosene, acetate, butyrate, citrate, formate, glycolate, propionate, succinate, phthalate, *p*-hydroxybenzoate and benzoate are not utilized. Cells are susceptible to chloramphenicol, streptomycin and tetracycline, but are resistant to penicillin and polymyxin B. Utilizes light energy as an auxiliary source for growth. The absorption spectrum of bacteriochlorophyll *a* in the intact cells has maxima at 802 and 856 nm. The habitat is soil. The G+C content of the DNA is 70·3–71·0 mol%. The type strain is NS89^T (=JCM 9931^T).

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