Paracoccus haeundaensis sp. nov., a Gram-negative, halophilic, astaxanthin-producing bacterium

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An aerobic, non-motile, Gram-negative, orange-pigmented, rod-shaped, astaxanthin-producing marine bacterium was isolated from the Haeundae Coast, Korea. This strain, BC74171T, produced carotenoids, mainly astaxanthin. All the type strains of the genus Paracoccus were compared with strain BC74171T using 16S rRNA gene sequence analysis, fatty acid patterns and physiological reaction profiles. Based on the results of these analyses, it is proposed that strain BC74171T represents a novel species, Paracoccus haeundaensis sp. nov. The type strain is BC74171T (= KCCM 10460T = LMG P-21903T).

An orange-pigmented, astaxanthin-producing bacterial strain, BC74171T, was isolated from sea water collected on the Haeundae Coast, Korea. This strain was isolated on nutrient agar medium (Difco) and maintained on PPES-II medium (0·2 % polypeptone, 0·1 % Bacto-yeast extract, 0·1 % Bacto-soytone, 0·1 % Bacto-tryptone, 10 p.p.m. ferric citrate and 3 % NaCl) by serial inoculation. Strain BC74171T was Gram-negative and rod-shaped. The cells ranged from 0·3 to 0·7 μm in diameter and 0·8 to 2·5 μm in length. Cells were non-motile and non-sporoform. Colonies on agar were smooth, flat and bright orange in colour.

Cultured cells were suspended in 0·1 M phosphate buffer (pH 7·2). Cells were fixed with 2 % glutaraldehyde, washed with 0·05 M cacodylate buffer and post-fixed with 1 % osmium tetroxide. Fixed cells were dehydrated in ethanol and the ethanol was replaced with propylene oxide before embedding in Epon resin and sectioning with an ultramicrotome. Sections were examined with a JEM 1200EX-II transmission electron microscope (Fig. 1; see also the transmission electron micrograph available as supplementary material in IJSEM Online).

Strain BC74171T was cultivated for 2 days at 25 °C on modified tryptcase soy agar (TSA, 2 % NaCl added) adjusted to pH 8·0. Harvesting, saponification, methylation and extraction of the fatty acid methyl esters were performed according to Sasser (1990). Fatty acid methyl esters were identified by GC-MS, as described previously (Lipski & Altendorf, 1997).

The fatty acid profile (C18:1, 84·32 %; C18:0, 7·79 %; C10:0 3-OH, 2·06 %; C12:1 cis8, 2·0 %; C14:0, 1·47 %; C17:0, 0·80 %; C16:0, 0·78 %; and unknown peaks, 0·78 %) is characteristic of the α-Proteobacteria. The major cellular non-hydroxyl fatty acid is unsaturated C18:1. The major

Astaxanthin (3,3’-dihydroxy-β,β-carotene-4,4’-dione) is a carotenoid that is widely distributed in nature and is present in marine animal tissues, including those of red sea bream, salmon and lobster (Fujita et al., 1983; Johnson & An, 1991; Nels & De Leenheer, 1991). Organisms that produce astaxanthin include the basidiomycetous yeast Phaffia rhodozyma (Miller et al., 1976), the green alga Haematococcus pluvialis (Bubrick, 1991), and the Gram-negative bacteria ‘Agrobacterium aurantiacum’ (Yokoyama et al., 1994), Paracoccus marcusii (Altendorf, 1997).

The genus Paracoccus consists of Gram-negative, oxidase- and catalase-positive bacteria that show substantial metabolic versatility. At present, the genus Paracoccus includes 17 recognized species. Phylogenetically, the genus belongs to the α-3 subclass of the Proteobacteria. In this study, results of a taxonomic study and a phylogenetic analysis based on 16S rRNA gene sequence comparisons are described. Data show that a newly isolated marine strain, BC74171T, should be classified as a representative of a novel species within the genus Paracoccus. The name Paracoccus haeundaensis sp. nov. is proposed; the type strain is BC74171T (= KCCM 10460T = LMG P-21903T) and its characteristics are described.
BC74171T was, in most cases, cultivated on TSA or in broth (TSB) at concentrations of 0–10 % (w/v). Motility and 11

Strain BC74171T, an authentic astaxanthin. Coloration of the bacterium was shown to result from biosynthesis of carotenoids within the cells. Pigments were extracted with acetone, analysed by HPLC and monitored by measuring $A_{470}$. One of the major pigments that accumulated in the cell wall was astaxanthin, which was identified by spectroscopic analysis using a standard solution of authentic astaxanthin.

Strain BC74171T, P. marcusii DSM 11574T and Paracoccus sp. strain MBIC 01143 were grown on PPES-II medium and subjected to physiological characterization. The temperature range for growth was determined by incubating cells for 10 days on plates of nutrient agar at the following temperatures: 4, 10, 20, 25, 28, 30, 37, 40 and 50 °C. The pH range for growth was determined by incubating cells in PPES-II broth medium at 25 °C for 10 days at the following pH: 3-0, 4-0, 5-0, 6-0, 6-5, 7-0, 8-0, 9-0, 10-0, 10-5 and 11-0. NaCl tolerance was measured in trypticase soy broth (TSB) at concentrations of 0–10 % (w/v). Motility was determined with an optical microscope using the hanging-drop technique (Skerman, 1967). To investigate its morphological and physiological characteristics, strain BC74171T was, in most cases, cultivated on TSA or in broth with 2 % NaCl added, at 25 °C. Acid production from some carbohydrates was detected using the method of Hugh & Leifson (1953). Growth was monitored by measuring turbidity after 10 days incubation at 25 °C with shaking. Starch hydrolysis was determined as described by Cowan & Steel (1965). Denitrification was determined by growth and gas formation in a stab culture of nutrient agar containing 0-1 % (w/v) agar. Catalase activity was determined by the presence of bubbles in a 3 % H$_2$O$_2$ solution. Oxidase activity was determined by oxidation of 1 % p-aminodimethylaniline oxalate. Urease production was determined as described by Lanyi (1987).

Strain BC74171T grew at 20–37 °C, but not at 4, 10, 40 or 50 °C; the optimal growth temperature was 25–30 °C. Slow growth was observed in TSB in the absence of NaCl. The optimum NaCl concentration for growth was 1–6 %. When the NaCl concentration in the medium was increased to 7 %, growth was slow. No growth occurred in the presence of more than 8 % (w/v) NaCl. Growth was very slow or inhibited below pH 6-0 and above pH 10-5. Glucose was not fermented. Metabolism was aerobic.

Extraction of genomic DNA and amplification of the 16S rRNA gene were carried out as described by Rainey et al. (1992). The PCR product was ligated into a pGEM-T vector (Promega), sequenced using a Termination Sequencing Ready Reaction kit (Perkin Elmer) and electrophoresed using an ABI 377 genetic analyser (Perkin Elmer). The resulting sequence data were analysed with programs BLASTN and BLASTX of GenBank. Sequences were aligned using the program CLUSTAL W. Gaps at the 5′ and 3′ ends of the alignment were omitted from further analyses. To indicate confidence in the branching order, bootstrap analysis (1000 replications) was completed for both the distance and parsimony methods. A phylogenetic dendrogram was obtained using the program TREEVIEW (Fig. 2). The full-length 16S rRNA gene from strain BC74171T was sequenced. The sequence of strain BC74171T shared the greatest similarity with the sequences of P. marcusii and P. carotinifaciens (99-8 and 99-6 % similarity, respectively).

The G + C content of the genomic DNA was determined by the method of Tamaoka & Komagata (1984). The DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. DNA–DNA hybridization was performed fluorometrically by the method of Ezaki et al. (1989) using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. Of the values obtained, the highest and lowest values in each sample were excluded; the DNA–DNA relatedness values are expressed as the mean of the remaining three values.

The G + C content of the DNA of strain BC74171T was 66-9 mol % (by HPLC analysis). DNA–DNA hybridization was performed to determine the genomic relatedness between strain BC74171T and some Paracoccus species. Strain BC74171T exhibited mean levels of DNA–DNA relatedness to the type strains of Paracoccus denitrificans, Paracoccus thiocyanatus, Paracoccus versutus and P. marcusii,

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**Fig. 1.** Transmission electron micrograph of cells of *Paracoccus haeundaensis* strain BC74171T during the exponential stage of growth. Bar, 200 nm.
and Paracoccus sp. strain MBIC 01143 of 8, 7, 6, 47 and 11 %, respectively, when each of their DNAs was used separately as a labelled probe.

The genus Paracoccus comprises a group of aerobic, Gram-negative bacteria that are catalase- and oxidase-positive and reduce nitrate. Seventeen recognized species of this genus have been identified. Details of physiological characteristics of P. haeundaensis and several type strains belonging to the genus Paracoccus are available as supplementary material in IJSEM Online.

The 16S rRNA gene sequence of P. haeundaensis shared high similarity with the sequences of P. marcusii and P. carotinifaciens and all three are astaxanthin-producing bacteria. The type strain of P. carotinifaciens is deposited only in IFO (=IFO 16121T) as a patented strain and is not publicly available. In a case like this, P. carotinifaciens contravenes the revised Rule 27(3) and Rule 30 of the Bacteriological Code (Euzeby & Tindall, 2004). Therefore, direct biochemical comparison and DNA–DNA hybridization studies between P. haeundaensis and P. carotinifaciens could not be performed. The biochemical characteristics of P. carotinifaciens were taken from a published report (Tsubokura et al., 1999). P. marcusii occurs as a coccus or short rod and forms short chains, whereas both P. haeundaensis and P. carotinifaciens occur as rods, but do not form chains. P. carotinifaciens has flagella, whereas P. haeundaensis does not. Moreover, the species have different phenotypic characteristics. P. haeundaensis does not utilize mannitol, maltose or mannose, whereas both P. marcusii and P. carotinifaciens utilize these compounds. In addition, P. haeundaensis hydrolyses starch, whereas neither P. marcusii nor P. carotinifaciens does.

Description of Paracoccus haeundaensis sp. nov.

Paracoccus haeundaensis (hae.un’dae.n.sis. N.L. adj. haeundaensis referring to Haeundae beach in Busan, Korea, where the type strain was isolated).

Gram-negative, non-motile, rod-shaped and non-spor-forming. Cells are 0·3–0·7 µm in diameter and 0·8–2·5 µm in length. Colonies are orange to red in colour. Optimal growth temperature is 25 °C; no growth occurs below 10 °C or above 40 °C. The optimum NaCl concentration for growth is 1–6 % (w/v). No growth occurs in the presence of more than 8 % (w/v) NaCl. Optimal pH for growth is 8. Capable of producing astaxanthin. The major cellular non-hydroxyl fatty acid is unsaturated C18 : 1. The major hydroxyl fatty acid is C 10 : 0 3-OH. The following carbon and energy sources can be used for growth: D-arabinose and galactose. No growth occurs on D-glucose, lactose, maltose, sucrose, trehalose, D-mannitol, D-sorbitol, inositol, D-raffinose, D-fructose, D-mannose, dimethylformamide, L-glutamic acid, acetone, L-leucine, L-asparagine, L-rhamnose, salicin, D-cellobiose, adonitol, dulcitol, D-xylene or glycerol. L-Ornithine and L-lysine decarboxylases, and arginine dihydrolase are not detected. Citrate utilization test is

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positive. Starch is hydrolysed. No indole is produced from tryptophan. Cytochrome oxidase and catalase reactions are positive. Urease-negative. Nitrate is reduced. Denitrification does not occur. Glucose is not fermented. Metabolism is aerobic. DNA G+C content is 66.9 mol%.

The type strain is BC74171T (=KCCM 10460T =LMG P-21903T).

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References


