A Gram-negative halophilic carotenoid-producing bacterium, *Paracoccus* sp.

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Abstract

A new species of Gram-negative halophilic cartenoid producing bacterium was isolated from the Haeundae Coast, Korea. This strain is non-motile, aerobic, orange-pigmented, rod-shaped, and produced carotenoids, mainly astaxanthin. All the type strains of the genus *Paracoccus* were compared with this strain using 16S rDNA sequence analysis, fatty acid patterns, and physiological reaction profiles. From the results obtained, this strain is classified as a new species, *Paracoccus* sp..

Key words: *Paracoccus* sp., astaxanthin, 16S rDNA analysis, carotenoid

Introduction

Astaxanthin (3,3'-dihydroxy-,-carotene-4,4'-dione) is a carotenoid that is widely distributed in nature and is present in marine animal tissues, including those of the red seabream, salmon, and lobster.[1] Organisms that produce astaxanthin include the basidiomycetous yeast *Phaffia rhodozyma*[2], the green alga *Haematococcus pluvialis*[3], the Gram-positive bacterium *Brevibacterium* 103[4], the Gram-negative bacteria *Agrobacterium aurantiacum*[5]. The genus *Paracoccus* consists of Gram-negative, oxidase and catalase positive bacteria that show substantial metabolic versatility. At present, the genus *Paracoccus* includes 14 validated species. In this study, we describe the results of a taxonomic and phylogenic analysis based on 16S rDNA sequence comparisons, which show that the bacterium should be classified as a new species within the genus *Paracoccus*.

Methods

Phenotype characteristics. The strain was grown on PPES-II medium and subjected to morphological and physiological characterization. Motility was determined with an optical

microscope, using the hanging-drop technique.

Fatty acid analysis. The strain was cultivated for 2 d at 25 ℃ on modified Trypticase Soy agar (TS agar 2% NaCl added) adjusted to pH 8.0. The FAMEs were identified by GC-MS, as described previously.[6]

DNA base composition. The G + C content of the genomic DNA was determined by the method of Tamaoka & Komagata.[7] The DNA was hydrolyzed and the resultant nucleotides were analyzed by reverse-phase HPLC.

Determination of the 16S rDNA sequence and phylogenetic analysis. Extraction of genomic DNA and amplification of 16S rDNA were carried out as described by Rainey *et al.*.[8]

Results and discussion

Morphological features of *Paracoccus* sp. strain

Paracoccus sp. strain was Gram-negative and rod-shaped. The cells ranged from 0.3 to 0.7 mm in diameter and 0.8 to 2.5 mm in length. The cells were non-motile and non-spore forming. The colonies on agar were smooth, flat, and bright orange in color.

Physiological characteristics

The optimal growth was at 25-30 ℃. The optimum NaCl concentration for growth was 1-6%. When the NaCl concentration in the medium was increased to 7%, growth was slow. The optimal pH for growth was 8. Growth was very slow or inhibited below pH 6 and above pH 10.5. The following carbon and energy sources could be used for growth: darabinose and galactose. No indole was produced from tryptophan. The citrate utilization test was positive. The cytochrome oxidase and catalase reactions were positive. Nitrate was reduced. Metabolism is aerobic.

Chemotaxonomic characteristics

The fatty acid profile ($C_{18:1}$, 84.32%; $C_{18:0}$, 7.79%; $C_{10:0}$ (3-OH), 2.06%; $C_{12:1}$cis5, 2.0%; $C_{14:0}$ (3-OH), 1.47%; $C_{17:0}$, 0.80%; $C_{16:0}$, 0.78%; and unknown peaks, 0.78%) is characteristic of the alpha-subclass of the *Proteobacteria*. 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. The G + C content of the DNA of strain BC74171^T was 66.9 mol% (by HPLC analysis).

Summary

Colonies are orange to red. The optimal growth temperature is 25 ℃. The optimum NaCl concentration for growth is 1-6%. No growth occurs in thepresence of more than 8% (w/v) NaCl. The optimal pH for growth is 8. Capable of producing astaxanthin. The major cellular non-hydroxyl fatty acid is unsaturated $C_{18:1}$. 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. The citrate utilization test is positive. Starch is hydrolyzed. The cytochrome oxidase and catalase reactions are positive. Nitrate is reduced. Denitrification does not occur. Metabolism is aerobic. DNA G + C composition is 66.9 mol%.

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