Oscillochloris trichoides neotype strain DG-6

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Abstract

The new strain of filamentous green bacterium strain DG-6 was isolated in pure culture from the spring of Caucuses. The study of this bacterium allows to suggest that it is a member of the family Chloroflexaceae and may be considered as Oscillochloris trichoides neotype strain. The description of this green bacterium is given.

Introduction

The family Chloroflexaceae includes four genera of filamentous multicellular green bacteria: Chloroflexus, Chloronema, Oscillochloris and Heliothrix (Pfennig 1989). However, only Chloroflexus aurantiacus (Pierson and Castenholz 1974) has been isolated in pure culture.

In this paper a new strain of filamentous green bacteria isolated in pure culture is characterized. The isolate is considered to be Oscillochloris trichoides neotype strain. The earlier described strain SR-1 of this species (Gorlenko and Korotkov 1979) has not been isolated in pure culture and is not maintained in collections.

Materials and methods

The strain DG-6 was isolated from sediment of hydrogen sulfide containing spring of Caucuses (Keppen et al. 1993). Two other green bacteria Chloroflexus aurantiacus strain B-3 and Chlorobium limicola forma thiosulfatophilum strain L used in some experiments, were obtained from the culture collection of the Department of Microbiology, Moscow State University.

The strain DG-6 was maintained on modified medium DGN (Castenholz and Pierson 1981). The medium contained per liter of distilled water: EDTA – 0.02 g; CaSO₄ × 2H₂O – 0.06 g; MgSO₄ × 7H₂O – 0.10 g; NaCL – 0.008 g; Na₂HPO₄ – 0.11 g; NH₄CL – 0.2 g; FeCl₃ solution (100 mg/l) – 3.0 ml; micrunutrient solution (Castenholz and Pierson 1981) – 0.5 ml; glycylglycine 0.8 g; NaHCO₃ – 1.0 g; acetate – 1.0 g; Na₂S × 9 H₂O – 0.5 g; vitamin solution – 1.0 ml; agar-agar – 2.0 g. Vitamin solution contained per 100 ml of distilled water: thiamine HCl – 10.0 mg; biotin – 0.5 mg; riboflavin – 3.0 mg; folic acid – 5.0 mg. For experimental studies the medium (without agar) was modified so that it contained different carbon, nitrogen and sulfur compounds (0.05–0.1%). The cultures were grown under light (1000 lx) anaerobic conditions at 28 °C in completely filled 50-ml to 1-liter vessels or in 100-ml bottles containing 50-ml of medium under atmosphere of H₂, Ar or N₂. In some experiments strain DG-6 was incubated in the dark under anaerobic and aerobic conditions in 250-ml Erlenmeyer flasks containing 100 ml of medium.

The methods of cultivation of Cf. aurantiacus and Cb. limicola forma thiosulfatophilum as well as some analytical methods had been described previously (Keppen and Krasilnikova 1986; Keppen et al. 1989, 1993).

DNA was isolated and purified from the cells by the method of Marmur (1961). DNA base composition was determined by melting point analysis (Marmur and Doty 1962). DNA-DNA homology of isolated strain with other anaerobic bacteria were calculated accord-
ing to De Ley et al. (1970) from the data of optical eassocation kinetics.

For 5S rRNA analysis approximately 1 g of wet cells from the culture was suspended in 0.01 M sodium acetate buffer (pH 5.1) and extracted with hot phenol and 0.5 % dodecyl sodium sulfate (Chumakov 1987). RNA was precipitated and washed with ethanol. Approximately 15 mg RNA was 3' end-labelled in vitro with cytidine 3', 5' - (5'- p)-diphosphate (pCp. 'Isotop', USSR, 1000 Ci mmol, 50 mCi per sample; 1 Ci = 376 GBq) using T4 phage RNA ligase (England and Uhlenbeck 1987). Labelled RNAs were fractionated by high-voltage electrophoresis in 8% (w/v) polyacrylamide gels (Maniatis et al. 1982). 5S rRNA was eluted and sequenced by the slightly modified (Chumakov 1987) chemical method of Peattie (1979). Sequencing gels (Maniatis et al. 1982) were run as 60 x 30 x 0.19 mm slabs at 4500 V.

5S rRNA sequences were aligned as described by Wolters and Erdman (1988) and used to calculate the mutation distance matrix. A tentative phylogenetic tree was constructed using the ‘maximum topological similarity’ method (Chumakov 1988; Yushmanov and Chumakov 1988).

**Results and discussion**

**Morphology and ultrastructure of cells** Single rod-shaped cells (0.8-1.0 x 2.0-5.0 mm) of the isolated green bacterium formed trichomes surrounded by a thin sheath. Slow gliding motility of trichomes was observed. The bacterium multiplied by separation of short sections of filaments or single cells from the mother trichomes.

The structure of the cell wall of isolated organism was typical for Gram-negative bacteria. The cells contained chlorosomes underlying and parallel to the cytoplasmic membrane (Fig. 1). Some cells contained mesosome-like structures as well. Gas vacuoles were found in cells from old cultures. They were located along the cell septa. The electron transparent areas resemble storage areas for poly-β-hydroxybutyrate. Sulfur did not accumulate inside the cells.

**Pigments.** The color of cultures of the isolated strain was green. The in vivo absorption spectra of cell suspensions exhibited maxima at 333, 436, 748 nm (Fig. 2). The maximum at 748 nm indicates the presence of bacteriochlorophyll c. This was confirmed by the absorption spectrum of a methanol extract of the cells, maximum at 668 nm (Fig. 2). The methanol extract also had absorption maximum at 767 nm which indicated the presence in small amount of bacteriochlorophyll a. The major carotenoids were β-carotene and γ-carotene. The same carotenoids has been found in *Cf. aurantiacus* and other filamentous green bacteria (Pfennig 1989).

**Physiology.** Cultures of strain DG-6 were grown in the light under anaerobic conditions only. Optimal growth temperature was 28–30 °C. Growth occurred between pH 6.8 and 9.0; optimal pH 8.0–8.5.

Photolithoautotrophic growth of the new organism occurred in the presence of sulfide and/or molecular hydrogen as electron donors. However it was poor. The optimal concentration of sulfide (Na2S × 9H2O) was 0.5–0.7 g/l although the growth of strain DG-6 was possible at sulfide concentrations up to 1.5 g/l. Sulfide was oxidized to elemental sulfur which was deposited outside the cells.

Cell suspensions fixed more carbon dioxide in the presence of sulfide or molecular hydrogen than on medium containing acetate (Keppen et al. 1993).

The growth of cultures was better on media containing in addition to bicarbonate and sulfide, acetate, pyruvate, casein hydrolysate or yeast extract. Formate, propionate, butyrate, malate, succinate, fumarate, citrate, benzoate did not maintain the growth of cultures. As sources of sulfur strain DG-6 utilized sulfide, cystine, cysteine but not sulfate.

Ammonium salts, urea, glycine, glutamate, glutamine, asparagine and casein hydrolysate were used as nitrogen sources. (Keppen at al. 1989). Poor growth occurred with N2. The capacity of strain DG-6 to fix molecular nitrogen was confirmed by the ability of cell suspensions to reduce acetylene (Keppen et al. 1989). A mixture of such vitamins was thiamine, biotine, riboflavin and folic acid as required for growth.

**Genotypic characteristics.** DNA-base composition in strain DG-6 is not very different from *Cf. aurantiacus* and *Cb. limicola*. The genome sizes of strain DG-6 and *Cf. aurantiacus* are also similar. However, a very low level of DNA-DNA hybridization indicates remote genetic relationship between strain DG-6, *Cf. aurantiacus* and *Cb. limicola* (Table 1).

Nucleotide sequences of 5S rRNA confirm the distant relationship between strain DG-6 and green sulfur bacteria as well as purple bacteria. According to these data strain DG-6 is closer to *Cf. aurantiacus*. However, the level of phylogenetic relationship between