Novel heliobacteria of a few semi-arid tropical soils

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Abstract Out of forty rhizosphere soils collected from semi arid tropical regions of Andhra Pradesh, India, 30 gave positive enrichments for heliobacteria. These bacteria were recognized by the presence of bacteriochlorophyll-g together with endospores in the initial enrichments. Using group specific primers of 16S rRNA gene, ten monoheliobacterial cultures were sequenced. They were finally grouped into 3 clusters based on the 16S rRNA gene similarity. Based on a few phenotypic characters, in addition to genetic characterization, we identified them as potential novel species and the 16S rRNA gene sequences were deposited with EMBL.

Keywords Heliobacteria · Endospore · Bacteriochlorophyll g · Semi-arid tropics · 16S rRNA gene sequences.

Introduction

Heliobacteria are a phylogenetic group of anoxygenic phototropic bacteria that contain bacteriochlorophyll g and lack internal cytoplasmic membranes or chlorosomes¹. Heliobacteria belong to the low G+C containing Gram-positive bacteria, specifically closely related to the endospore-forming rod-shaped *Bacillus* and *Clostridium* [1]. All known heliobacteria are strict anaerobes that grow phototrophically on a limited number of organic compounds. These bacteria were reported from garden soils, paddy soils or microbial mats of lake samples [1]. In the present communication we report their presence in the rhizosphere soils of crops grown in the semi-arid tropical regions of Andhra Pradesh, India. The isolates are novel representative members of the family *Heliobacteriaceae*.

Forty rhizosphere soils of crops grown in semi-arid tropical regions of Andhra Pradesh like jowar, maize, tomato, brinjal, sunflower, groundnut, chilly, cucumber were collected during December 2004 and January 2005. One gram of pasteurized soil was used for enrichments in a 20 ml enrichment medium [1] taken in 100 ml narrow mouth reagent bottle, which was sealed with a suba seal and the air was replaced with ultra pure argon using a pair of hypodermic needles. The tubes were incubated at 5000 lux of fluorescent light at 30 ± 2°C, till the enrichments were observed. Whole-cell absorption spectra of the enrichments were recorded in sucrose using Spectronic Genesys-2 spectrophotometer. Microscopic observations were done using phase contrast microscope (Olympus B201). Extraction of genomic DNA, PCR amplification of 16S rRNA gene using heliobacterial specific primers [2] and 16S rRNA gene sequencing was done as described earlier [3].

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Out of forty rhizosphere soil samples used for enrichment, thirty gave positive enrichments with the appearance of green mat on the surface of the soil within 7–10 days of anaerobic phototrophic incubation. Most of the bacteria were either rod- or spiral-shaped, the length ranged between 10–100 μm and the diameter was 1–2 μm . All the bacteria were motile having terminal or sub-terminal endospores. The whole cell absorption spectra of all the positive enrichment samples gave peaks in the region of 785–790 nm, typical of bacteriochlorophyll-g and thus confirm their identity as heliobacteria as this is unique and specifically observed only in this group of bacteria [1].

By using agar shake serial dilutions of the enrichments obtained, we have isolated monoheliobacterial cultures, though they could not be freed of chemotrophs. Viability of many of the cultures was lost during sub-culturing and only ten cultures could be partially purified, which were used for 16S rRNA gene sequence analysis using heliobacterial specific primers. All the 10 cultures were sequenced and based on the sequence similarity, they were grouped into three taxa. The representatives of these taxa are strains, JHB1 (3 isolates), JHB2 (4 isolates) and JHB3 (3 isolates). Their phylogenetic relationships were compared with the so-far reported type species of heliobacteria (Fig. 1), which clearly indicates that all the three could be novel species. However, we are unable to describe them as novel species

because we could not isolate them in pure culture so far, mainly due to the viability problems. Due to which, it was also not possible to determine certain characters like mol % G+C content, utilization of organic carbon sources, nitrogen sources, vitamin requirement etc. Loss of viability during repeated subculturing is commonly observed among the members of Heliobacteria [1].

Some distinguishing characters of strain JHB1

This strain was isolated from the rhizosphere soils of jowar, from Dharmavaram. The color of the enrichment culture is green. Cells are curved rods of $1\text{--}1.5 \times 20\text{--}30 \mu\text{m}$ and have terminal endospores. Cells have peritrichous flagella. Absorption maxima are at 788, 671, 578, 413 and 371, indicating the presence of bacteriochlorophyll-g. 16S rRNA gene sequence (EMBL accession number: AM296477) is most similar to that of *Heliobacterium sulfidophilum* ($\approx 98\%$). However, based on the distinct clustering (Fig. 1), we propose strain JHB1 as a potential novel species of the Genus *Heliobacterium*.

Some distinguishing characters of strain JHB2

Strain JHB2 was isolated from rhizosphere soils of Maize from Godumakunta. Cells are straight long rods of

Fig. 1 Dendrogram depicting the phylogenetic relationships of strain JHB1, JHB2 and JHB3 determined using 16S rRNA sequence analysis. Bar. 1 nucleotide substitution per 100 nucleotides.

Table 1 Comparison of characters of novel isolates of *Helibacteria* with validly described species

Property	Hellobacterium [Hbt]					Hellobacterium [Hbt]		Helophilum [Hpl]	Helioestis [Hrs]			
	Hbt. chlorum	Hbt. gestii	Hbt. Modesticaldum	*Hbt. sulfidophilum	*Hbt. undosum	Strain JHB1	Hbl. mobilis	Strain JHB2	Hbl. fasciatum	Hrs. baculata	Hrs. daurensis	
Habitat	Temperate soil	Tropical paddy soil	Neutral/alkaline hot springs and volcanic soils	Alkaline sulfidic hot springs	Alkaline sulfidic hot springs	Semi-arid tropical soils	Tropical paddy soil	Semi-arid tropical soils	Tropical paddy soil	Semi-arid tropical soils	Alkaline shoreline soils of brackish soda lakes	Microbial mats of brackish soda lakes
Morphology	Rod	Spiral	Rod/curved rod	Rod	Spiral/rod	Rod/curved rod	Rod	Rod	Straight rods with tapered ends grouped in bundles of two to many cells	Rod	Straight or slightly curved rods	Coiled to bent filaments
Dimensions (Diameter x length in μm)	1 × 7–9	1 × 7–10	0.8–1 × 2.5–9	0.6–1 × 4–7	0.8–1.2 × 7–20	1–1.5 × 20–30	1 × 7–10	1–2 × 20–50 (few up to 100)	0.8–1 × 5–8	1–1.5 × 7–10	0.6–1 × 6–10	0.8–1.2 x 10–20
Motility	Gliding	Polar or subpolar flagella	Flagellar or none	Peritrichous flagella	Peritrichous flagella	Peritrichous flagella	Peritrichous flagella	Polar flagella	Polar to subpolar flagella; cell bundles move as a unit	Peritrichous flagella	Peritrichous flagella	Peritrichous flagella
Endospores produced	None observed	Yes (Cylindrical subterminal)	Yes (Cylindrical subterminal)	Yes	Yes	Yes (Terminal spherical)	None observed	Yes (Terminal)	Yes	Yes (Sub-terminal/central)	Yes	Yes
Whole cell absorption spectra	788, 670, 575	788, 480, 415	788, 670, 575	788, 670, 575, 412, 375	790, 720, 671, 576, 412, 370	788, 671, 578, 413, 371	788, 670, 575	785, 671, 578, 416	792, 480, 415	791, 671, 578, 410	788, 671, 575, 413, 370	788, 671, 576, 576,
G+C content (mol%)	52	55.1	54.6–55	51.3	57.2–57.7	ND	50.3	ND	51.8	ND	45	44.9

Source: Madigan [1];

^bBryantseva et al. [5];^aFrom this study

ND = Not Determined

1–2 × 20–50 (few cells are up to 80–100) µm, producing terminal endospores and polar flagella. Absorption maxima of intact cells are at 785, 671, 578 and 416. The 16S rRNA gene sequence (EMBL accession number: AM296478 of strain JHB2) is most similar to that of *Heliobacillus mobilis* (~97%). Phylogenetic relatedness (Fig. 1) and phenotypic properties (Table 1) distinguish the two bacteria and thus we propose strain JHB2 as a potential novel species of the Genus *Heliobacillus*.

Some distinguishing characters of strain JHB3

This strain was isolated from the rhizosphere soils of Brinjal from Godumakunta. The color of enrichment culture is green. Cells are rods of 1–2.5 × 7–10 µm and have subterminal endospores. Cells have peritrichous flagella. Absorption maxima are at 791, 671, 578 and 410 nm. The 16S rRNA gene sequence of strain JHB3 (EMBL accession number: AM296479) is most similar to that of *Heliophilum fasciatum* (~92%) and differed significantly from other heliobacteria. The Phylogenetic relatedness of strain JHB3 is shown in Fig. 1 and some phenotypic differences (Table 1) with other heliobacteria clearly distinguishes strain JHB3 as a potential new Genus of the family Heliobacteriaceae for which a name has to be proposed.

Recalcitrance in microorganisms creates major problems in their cultivation [4]. Heliobacteria represents this recalcitrance due to loss of viability during culturing because of autolysis of the cells [1]. Our study indicated the wide distribution of the members of heliobacteria in semi-arid tropical soils of India, however, we could not isolate them in pure culture so far.

Note added to proof: We release that we missed *Helio-restis convoluta* in the manuscript. this species was isolated from a soda lake [6].

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