Phylum BXIII. Firmicutes

Gibbons and Murray 1978, 5[AL] emend. Garrity and Holt

Class I. *"Clostridia"*

Order I. **"Clostridiales"**

Family VI. **"Heliobacteriaceae"** Beer-Romero and Gest 1987, 113

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He.li.o.bac.te.ri.a'ce.ae. M.L. neut. n. *Heliobacterium* type genus of the family; *-aceae* ending to denote a family; M.L. fem pl. n. *Heliobacteriaceae* the *Heliobacterium family.*

Rod-shaped to short filaments or spirillum-shaped cells; multiplication by binary fission. **Anoxygenic phototrophs.** Rod-shaped cells are frequently curved slightly and in one genus (*Heliophilum*), cells are straight and tapered and group together to form bundles that are motile as a unit. **Cells stain Gram-negative, although the phylogenetic position of the group is Gram-positive** (Woese et al., 1985a; Madigan and Ormerod, 1995; Ormerod et al., 1996a). Motility is by gliding or flagellar means; if the latter then by polar, subpolar, or peritrichous flagella. **Contain bacteriochlorophyll *g* as major bacteriochlorophyll** (Brockman and Lipinski, 1983; Michalski et al., 1987) and diaponeurosporene (Taikaichi et al., 1997) as the major carotenoid. Small amounts of 8′-OH-chlorophyll *a* is also present (Amesz, 1995). Bacteriochlorophyll *g* and 8′-OH-chlorophyll *a* are esterified with the C-15 alcohol farnesol rather than the C-20 alcohol phytol. **Color of phototrophically grown cultures is brownish green** although cultures turn a more emerald green in color in stationary phase. Extensively developed **intracytoplasmic membranes, of the kind observed in phototrophic purple bacteria or chlorosomes characteristic of the green sulfur or green nonsulfur bacteria, are not observed**. Gas vesicles are not present. Metabolism is strictly anaerobic; cells grow under anoxic conditions in the light as photoheterotrophs on a limited number of organic carbon sources or chemoorganotrophically (in darkness) by pyruvate fermentation (pyruvate fermentation has not been observed in *Heliorestis*) (Kimble et al., 1994). Aerobic or microaerobic dark growth does not occur. **Photoautotrophic growth on CO_2/H_2 or CO_2/H_2S has not been observed**, although if sulfide is added to culture media, it is frequently oxidized to S^0. Optimal pH is ~7 with a pH growth range of 5.5–8, depending on the species; *Heliorestis* is alkaliphilic, having a pH optimum of 9. Optimal temperature is 37– 42°C (52°C for *Heliobacterium modesticaldum* and 30°C for *Heliorestis daurensis*). Ammonium salts and glutamine are used as nitrogen sources and certain species can use other amino acids as well. **Nitrogen fixation is a property of all heliobacteria. Biotin is required as a growth factor by all heliobacteria** and some species also require a reduced sulfur compound (sulfide, thiosulfate, or cysteine) as a sulfur source for biosynthesis. Poly-β-hydroxybutyrate has not been observed as a storage material. True endospores containing dipicolinic acid and elevated levels of Ca^{2+} have been observed in some species and, considering the phylogenetic position of all members of the family, this property may well be universal among the group; the loss of ability to form endospores in laboratory culture has been a common observation with several isolates. ***Heliobacteriaceae* occur in nature primarily in soil and in this regard differ significantly in their ecology from purple and green anoxygenic phototrophs.**

The mol% G + C of the DNA is: 44.9–55.

Type genus: **Heliobacterium** Gest and Favinger 1985, 223 (Effective publication: Gest and Favinger 1983, 15.)

FURTHER DESCRIPTIVE INFORMATION

The family *Heliobacteriaceae* contains all of the anoxygenic phototrophic bacteria that produce bacteriochlorophyll *g*. This structurally and spectrally unique bacteriochlorophyll distinguishes the heliobacteria from the purple bacteria, which contain bacteriochlorophyll *a* or *b*, and the green sulfur and green nonsulfur (*Chloroflexus*) bacteria, which contain bacteriochlorophylls *c*, *d*, or *e*, or bacteriochlorophyll c_s, respectively. *In vivo* absorption spectra (performed anoxically) of heliobacteria show a major peak at 786–792 nm (depending on the species) due to absorption by bacteriochlorophyll *g*, and comparatively small peaks at about 575 and 670 nm; the latter is due to a small amount of 8′-OH-chlorophyll *a* present in the photosynthetic reaction center. Exposure of cultures of heliobacteria to air causes the apparently irreversible oxidation of bacteriochlorophyll *g* to chlo-

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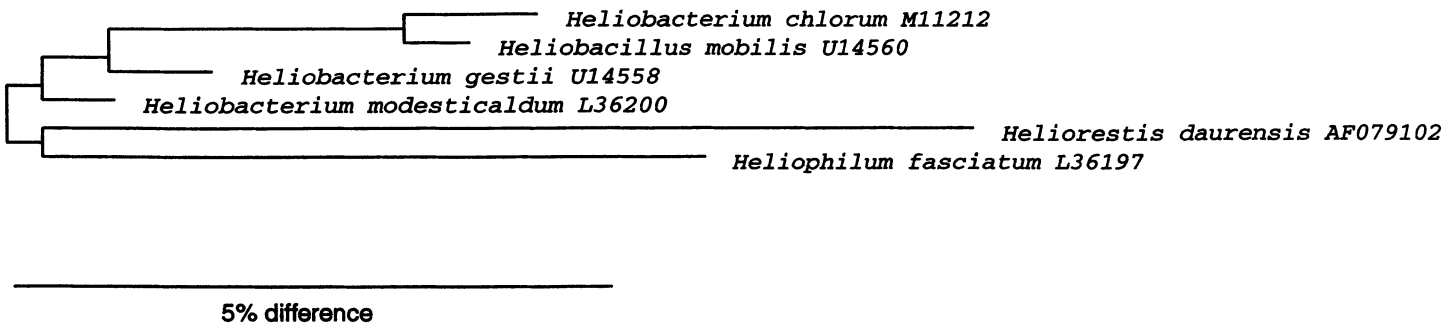

FIGURE 1. Evolutionary distance tree of the family *"Heliobacteriaceae"* based on comparative 16S rRNA sequencing. The tree shown was computed from evolutionary distances by the algorithm of De Soete using the Jukes and Cantor correction as detailed in Wahlund et al. (1991). Organisms and their GenBank accession numbers include: *Heliobacterium chlorum* (M11212); *Heliobacterium gestii* (strain Chainat, U14558); *Heliobacterium modesticaldum* (strain Icel, L36200); *Heliobacillus mobilis* (U14560); *Heliophilum fasciatum* (strain Tanzania, L36197); *Heliorestis daurensis* (strain BT-H1, AF079102).

rophyll *a*, a major increase in 670 nm absorbance coupled to a corresponding loss of 788 nm absorbance, and loss of cell viability. Fermentatively (dark)-grown cells of heliobacteria also produce photopigments.

The cell wall of heliobacteria species is rather fragile and stationary phase cells of some species tend to form spheroplasts. The peptidoglycan, which is present in small amounts, contains L,L-diaminopimelic acid instead of *meso*-diaminopimelic acid as muramic acid crosslinks. Cell walls also contain a considerable amount of lipid, although this lipid is not in the form of lipopolysaccharide (Beck et al., 1990). Heliobacteria are unusually sensitive to penicillin—growth of *Heliobacterium chlorum* is inhibited by 2 ng/ml of penicillin G, a level 1000-fold lower than the inhibitory level for *E. coli* (Beer-Romero et al., 1988).

From a phylogenetic standpoint, heliobacteria belong to the low mol% G + C Gram-positive phylum (*Firmicutes*) (Fig. 1), that includes chemotrophic endospore-forming bacteria. Currently, four genera with a total of six species are recognized.

Enrichment, Isolation, and Ecology Enrichment of heliobacteria takes advantage of their ability to form endospores and their presence in soils; rice soils are particular good sources of heliobacteria (Stevenson et al., 1997). A soil sample is pasteurized (80°C for 10 minutes) in a stoppered tube of anoxically prepared growth media (for enrichment, a 0.25% [w/v] yeast extract medium at pH 7 works well), sealed under an atmosphere of N_2/CO_2 (95:5 v/v), and incubated in incandescent light (40 $\mu E \cdot m^{-2} \cdot s^{-1}$) at 38–40°C. It should be emphasized that all heliobacteria isolated to date are strict anaerobes, such that extreme care should be taken to ensure that media and growth conditions for the isolation of heliobacteria are strictly anoxic. Successful enrichments usually show a green film of cells atop the soil in the tube. This material can be removed with a sterile Pasteur pipette and plated onto enrichment medium (or other media; see Kimble and Madigan, 1992; Kimble et al., 1995; Stevenson et al., 1997; Bryantseva et al., 1999a). Plating should be done inside an anoxic chamber. Streaked plates are incubated inside anoxic jars (e.g., Gas-Pak® jars) placed in the light and green colonies with irregular edges form within 2–3 d. Pure cultures can usually be obtained by repeated restreaking. Alternatively, isolated colonies of heliobacteria can be obtained by using the agar "shake culture" technique (Trüper and Pfennig, 1982). If the latter is used without benefit of an anoxic chamber, it is advisable to add 1–2 mM sulfide to the medium to ensure reducing conditions.

In a survey of soils for the presence of heliobacteria it was found that these anoxyphototrophs are common in rice (paddy) soils in both traditional rice-growing regions such as Southeast Asia and also in modern cultivated rice fields such as those in the southern United States (Stevenson et al., 1997). Other agricultural or garden soils were not reliable sources of heliobacteria, although occasional isolates from these soils were obtained. Aquatic habitats, with the exception of neutral to alkaline hot springs ≤60°C, did not yield heliobacteria (Stevenson et al., 1997). The close connection between heliobacteria and rice plants suggest that a specific plant-bacterium association may exist, with the photoheterotrophic heliobacteria assimilating organic compounds excreted by rice plant roots and the plants benefiting from fixed nitrogen excreted by the nitrogen-fixing heliobacteria; all species of heliobacteria have been shown to be strong nitrogen fixers (Kimble and Madigan, 1992; Madigan, 1995).

Genus I. **Heliobacterium** *Gest and Favinger 1985, 223[VP] (Effective publication: Gest and Favinger 1983, 15)*

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He.li.o.bac.ter'i.um. Gr. n. *helios* sun; Gr. neut. n. *bakterion,* a small rod; M.L. neut. n. *Heliobacterium* sun bacterium.

Rod-shaped cells, slightly curved, 0.8–1 × 2.5–9 μm (Fig. 2), **or spirilla,** 1–1.2 μm in width and of similar length to rod-shaped cells (Fig. 3). From an ultrastructural standpoint, cells of heliobacteria lack intracytoplasmic membranes or chlorosomes, and thus electron micrographs of the cytoplasm give no indication that the cells are phototrophic (Fig. 4). Colonies on a yeast extract/salts medium (medium PYE, see Kimble et al., 1995) frequently have irregular margins because of wavy protrusions