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ORIGINAL PAPER

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Heliorestis convoluta sp. nov., a coiled, alkaliphilic heliobacterium from the Wadi El Natroun, Egypt

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Abstract A morphologically distinct heliobacterium, strain HH, was isolated from Lake El Hamra, a soda lake in the Wadi El Natroun region of northwest Egypt. Strain HH consisted of ring-shaped cells that remained attached after cell division to yield coils of various lengths. Strain HH showed several of the physiological properties of known heliobacteria and grouped in the *Heliorestis* clade by virtue of its phylogeny and alkaliphily. The closest relative of strain HH was the filamentous alkaliphilic heliobacterium *Heliorestis daurensis*. However, genomic DNA:DNA hybridization results clearly indicated that strain HH was a distinct species of *Heliorestis*. Based on its unique phenotypic and genetic properties we describe strain HH here as a new species of the genus *Heliorestis*, *H. convoluta* sp. nov.

Keywords Anoxygenic phototrophic bacteria · Heliobacteria · *Heliorestis* · Akaliphiles · Wadi El Natroun · Soda lakes

Introduction

Heliobacteria are anoxygenic phototrophic bacteria that contain bacteriochlorophyll (Bchl) *g* (Madigan 1992, 2001; Madigan and Ormerod 1995). Phylogenetically heliobacteria are gram-positive bacteria (Woese et al. 1985). Several species of heliobacteria are known including one thermophilic species (Kimble et al. 1995; Madigan 2001). To date, all heliobacteria have been isolated from either soils or microbial mats and most grow best near pH 7 (Stevenson et al. 1997). However, in recent years a new genus of alkaliphilic heliobacteria, *Heliorestis*, has been discovered, with two species now described, *Heliorestis daurensis* and *H. baculata* (Bryantseva et al. 1999, 2000). Cells of *H. daurensis* are filaments of variable length that are bent at one end (Bryantseva et al. 1999). By contrast, cells of *H. baculata* are straight rods, similar in morphology to most species of *Heliobacterium* (Bryantseva et al. 2000; Madigan 2001). Species of *Heliorestis* differ from all other heliobacteria in their alkaliphily and distinct genetic properties (Madigan 2001).

The original enrichment cultures of *H. daurensis* were established using microbial mat and soil from Lake Barun Torey, a southeast Siberian soda lake. In primary enrichments both tightly coiled cells and long rod-shaped to filamentous cells were observed. However, the organism eventually obtained in pure culture was filamentous, some 50–100 μm in length. It was unclear in this work if the coiled organism was a heliobacterium and if so, what its relationship was to *H. daurensis* (Bryantseva et al. 1999). In separate enrichment experiments we have now isolated a truly coiled alkaliphilic heliobacterium that maintains its coiled morphology in pure culture. The isolate, strain HH, was obtained from a soda lake in the Wadi El Natroun, a series of highly alkaline lakes in the Libyan desert of northwest Egypt (Imhoff et al. 1979). Although resembling *H. daurensis* in basic physiology, strain HH differs from this organism in several respects, most notably its morphology and genetic properties. Here we describe the basic properties of the coiled strain HH and propose a new species epithet for it, *H. convoluta* sp. nov.

Materials and methods

Inoculum, enrichment and isolation

Enrichment cultures for anoxygenic phototrophic bacteria were established in an anoxic alkaline (pH 9)

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Dedicated to Prof. Dr. Norbert Pfennig on the occasion of his 80th birthday.

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medium containing 2 mM sulfide and acetate/bicarbonate as carbon sources (Bryantseva et al. 1999). Inocula were sediment and water samples from a red bloom of prokaryotes that had developed near the northeast shore of Lake El Hamra, one of a series of soda lakes in the Wadi El Natroun, Egypt, 35 km west of Sadat City. The salinity of the samples was not determined, but the salinity of Lake El Hamra has been reported to be about 16% (Imhoff et al. 1979). Samples were collected on 5 May 2001, and stored at 4°C until enrichments were established about 2 weeks later. Pure cultures were obtained in agar dilution tubes and maintained in the enrichment medium containing 1 mM sulfide in place of the original 2 mM. Cell suspensions of exponential phase cells could also be stored frozen (−70°C) in growth medium containing 10% DMSO.

Media and growth conditions for pure cultures

Cultures were grown in enrichment medium or the medium designated 1/2 S (described below) in completely filled screw-capped tubes or bottles exposed to 50 $\mu E \cdot m^{-2}\ s^{-1}$ incandescent illumination (33°C). Growth was measured as OD_{700} of cell suspensions. During growth, a few small globules of elemental sulfur were produced from the oxidation of sulfide in the medium. However, this had essentially no effect on growth measurements since cultures grown in media made anoxic with 0.05% ascorbate in place of sulfide showed similar OD readings as sulfide-containing cultures. Since growth was more robust with sulfide than with ascorbate, sulfide-reduced media were used for the routine culture of strain HH.

Physiological tests were performed in medium 1/2 S. This medium contained, per liter of deionized water: EDTA, 5 mg; KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 200 mg; $CaCl_2 \cdot 2H_2O$, 75 mg; NH_4Cl, 0.5 g; sodium acetate, 1 g; yeast extract, 0.1 g; vitamin B_{12}, 20 µg; trace elements (Wahlund et al. 1991), 1 ml; sodium pyruvate, 1 g; BICINE buffer (Sigma, St. Louis, MO), 10 mM; $NaHCO_3$, 3 g; $Na_2S \cdot 9H_2O$, 0.25 g. The pH of the medium was adjusted to 9–9.2 after autoclaving by addition of sterile NaOH solution. NaCl requirements were tested in medium 1/2 S containing varying salt concentrations. Media were prepared by mixing medium 1/2 S containing either 0% NaCl or 20% NaCl in different proportions to yield a medium of a given salinity. For testing pH relationships, medium 1/2 S was supplemented with 10 mM of one of the following buffers (all products of Sigma Chemical Co., St. Louis): pH 7–7.5, MOPS; pH 8–9, BICINE; pH 9.5–10.5, CAPSO.

Microscopy and absorption spectra

All light and electron microscopy was performed as previously described (Kimble et al. 1995; Milford et al. 2000). Phase photomicrographs were taken using the method of Pfennig and Wagener (1986). Absorption spectra of intact cells were performed in anoxic 30% bovine serum albumin (BSA, Sigma). The latter was prepared by adding 0.05% (final concentration) sodium ascorbate to BSA in a 1 ml cuvette, mixing thoroughly, and allowing oxygen removal to occur inside an anoxic chamber for 1 h before performing spectra. Liquid culture was quickly added to the anoxic BSA, the suspension mixed, and absorption spectra performed immediately. Solvent extracts of cells were prepared in methanol. Cell pellets were extracted in ice-cold methanol for 0.5 h in darkness (−20°C). Spectra were performed on a Hitachi U-2000 double-beam spectrophotometer.

Genetic properties

The phylogeny of strain HH was determined as previously described (Bryantseva et al. 1999) and a phylogenetic tree generated using PAUP* 4.0 (Swofford 1998). Genomic hybridization was performed using DNA dot blots. Genomic DNA from *H. daurensis* was obtained using the modified Marmur method described in Kimble et al. (1995). However, this method was unsatisfactory for obtaining genomic DNA from strain HH. For the latter, DNA was isolated using the Puregene® Genomic DNA Purification Kit (Gentra Systems, Minneapolis, MN) following the manufacturer's instruction with a few modifications: (1) the addition of 50 ng of lysozyme to the cell suspension (300 µl), and (2) the addition of 50 µl of DNA hydration solution (instead of 100 µl) to increase DNA yields. For *Rhodobacter capsulatus*, total genomic DNA was isolated from a 1-ml culture using the same kit following the manufacturer's instructions. Dot blot hybridizations were performed as described by Jung et al. (2004) and images were digitally analyzed using a Personal Densitometer SI scanner (Molecular Dynamics, Amersham Biosciences) driven by ImageQuaNT™ software version 4.1.

Results

Enrichment and isolation

When sampled by MTM in May, 2001, the northeast shore of Lake El Hamra, Wadi El Natroun, Egypt, contained an extensive reddish bloom and the pH was 10. Microscopy of the water showed a mixture of prokaryotes, including long rods, square-shaped cells, and occasional spirilla to coiled-shaped cells. A sample of this water including sediment was inoculated into an anoxic alkaline medium lacking NaCl but containing sulfide and incubated phototrophically at 32°C; within one week a greenish cell suspension was obtained. Microscopy revealed cells of a tightly coiled, motile prokaryote that was remarkably free of contaminants. Absorption spectra of cells from the enrichment indi-

cated the presence of bacteriochlorophyll *g* and thus heliobacteria (Madigan 1992, 2001; Madigan and Ormerod 1995). An axenic culture of the coiled heliobacterium was obtained by repeated transfer of single colonies through agar-shake dilution tubes and the pure culture obtained given the designation strain HH.

Morphology of strain HH

The new organism was distinctly and stably coiled in its morphology (Fig. 1). Coils formed from the growth of a single ring-shaped cell that continued to grow and divide without separating (Fig. 1). Coils were tightly wound, especially in early exponential phase cultures, and became more loosely wound in older cultures (Fig. 1a). In scanning electron micrographs it was possible to detect septa between the individual cells making up a coil. This indicates that the coiled morphology of cells of strain HH is the result of incomplete cell division between individual ring-shaped cells rather than coiling of a long filamentous cell (Fig. 1b). The coiled morphology was also evident in thin sections of cells of strain HH observed by TEM. As in other heliobacteria (Madigan 2001), no internal fine structure, such as differentiated photosynthetic membrane systems or chlorosomes, was observed in cells of strain HH (Fig. 1b). Thus, photocomplexes in strain HH likely reside within the cytoplasmic membrane, as is true of other heliobacteria (Madigan 2001; Miller et al. 1986). Exponential phase cells of strain HH were motile although typical flagella were not observed in SEM preparations (Fig. 1b).

Many coils of strain HH were observed by SEM to contain thin connecting structures between cells (Fig. 2a). Although their chemistry is unknown, it is possible that these structures are remnants of a surface polysaccharide that becomes stretched between adjacent cells during the formation of a coil. However, in some preparations the structures appeared to be well affixed on or into cells (Fig. 2b, c), suggesting that they play a role as intercellular connectors. The structures were roughly similar in diameter to flagella; however, it did not appear from the position and connecting points (Fig. 2) of these structures that they were simply flagella wrapped around the coiled cells. Whatever their nature, the fact that some coils contained these intercellular structures (Fig. 2) while others did not (Fig. 1b), indicates that the material is unessential for maintaining the coiled morphology and may be easily lost in preparing cells for electron microscopy.

Heliobacteria are endospore-forming anoxygenic phototrophs (Ormerod et al. 1990, 1996; Starynin and Gorlenko 1993; Stevenson et al. 1997). In enrichment cultures of heliobacteria one often sees sporulating cells and free endospores (Stevenson et al. 1997). In pure cultures, by contrast, endospores are rarely observed (Kimble and Madigan 2001). In the Lake El Hamra enrichments no endospores were observed, and endospores were never observed in either young or old pure cultures of strain HH. However, stationary phase cells of strain HH subjected to a heat treatment (80°C for 15′, a standard test for endospore formation) grew when transferred to fresh medium. This suggests that a few endospores may have been present. But surprisingly, a small proportion of exponential phase cells were observed to retain their motility after the heat shock. This indicates that vegetative cells of strain HH may themselves be unusually heat stable.

Pigments

By in vivo spectral analysis strain HH was a typical heliobacterium. A major absorption maximum was ob-

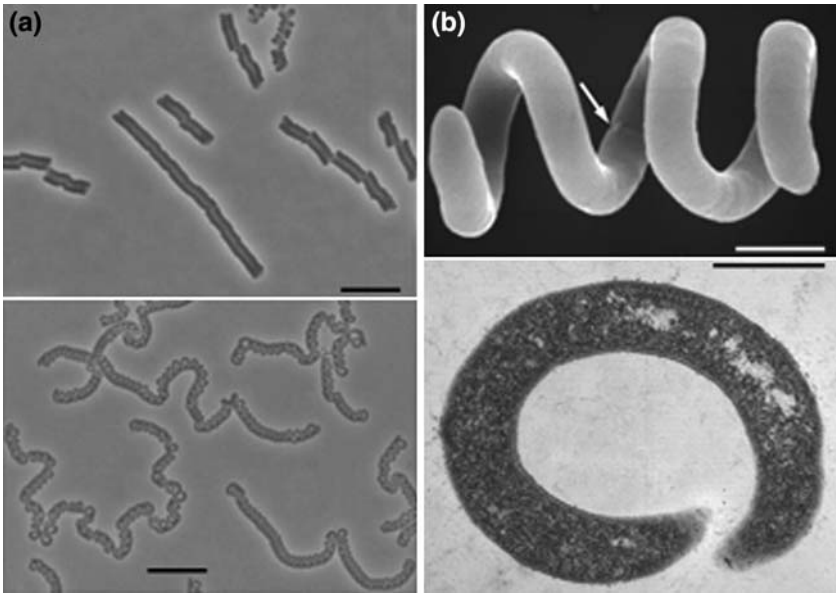

Fig. 1 Morphology of cells of strain HH. **a** Phase photomicrographs: *Upper photo*, tightly coiled cells; *lower photo*, loosely coiled cells (*marker bar* in both micrographs, 10 μm). **b** Electron micrographs: *Upper photo*, scanning electron micrograph; *arrow points* to septum between cells; *lower photo*, transmission electron micrograph of a cross section through a coil (*marker bar* in both micrographs, 1 μm)

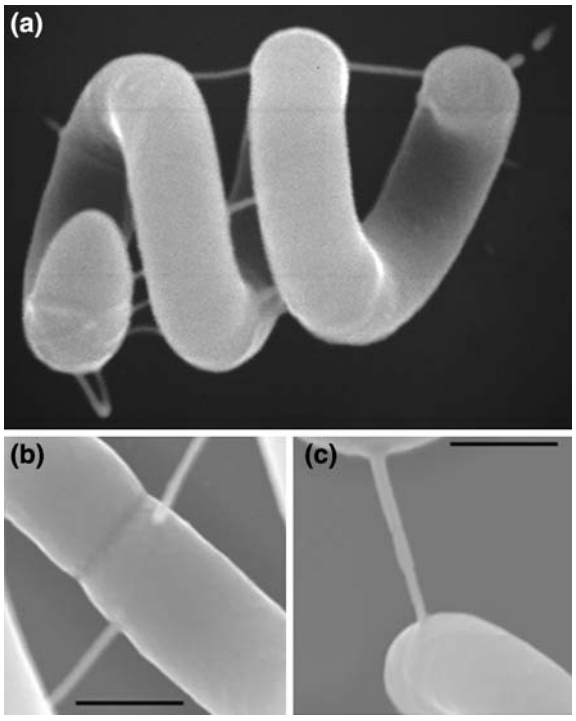

Fig. 2 Scanning electron micrographs of cells of strain HH. **a** An intact coil showing connecting material between cells. **b**, **c** Higher magnification images showing connecting material between cells. *Marker bar* in all three micrographs, 0.5 μm

served at 786 nm in the spectrum of intact cells (Fig. 3); this is very near the absorption maximum of Bchl *g* in intact cells of *Heliobacterium chlorum* (788 nm, Gest and Favinger 1983) and other species of heliobacteria (Madigan 2001). A smaller peak in the in vivo spectrum was observed at 672 nm (Fig. 3); this is due to absorbance by a form of chlorophyll *a*, also found in heliobacteria (Amesz 1995).

In methanol extracts of cells of strain HH peaks were obtained at 746 and 666 nm (Fig. 3). The former is presumably that of Bchl *g* while the latter is clearly due to chlorophyll *a*. Pure Bchl *g* absorbs strongly at 762–776 nm in other organic solvents such as acetone, hexane, and diethyl ether (Brockmann and Lipinski 1983; Kobayashi et al. 1991), but absorption maxima of Bchl *g* in methanol have not been published. To confirm the methanol extract results, cells of strain HH were also extracted with acetone. A major peak at 759 nm was obtained (data not shown), nearly the same as that for acetone extracts of cells of *H. daurensis* (760 nm, Bryantseva et al. 1999). Methanol extracts of cells of *Heliobacterium modesticaldum*, a thermophilic (but not alkaliphilic) heliobacterium that contains authentic Bchl *g* (Kimble et al. 1995), showed maxima in methanol at 750 nm (data not shown). Thus, Bchl *g* in methanol absorbs some 15–30 nm to the blue of solutions made with other solvents.

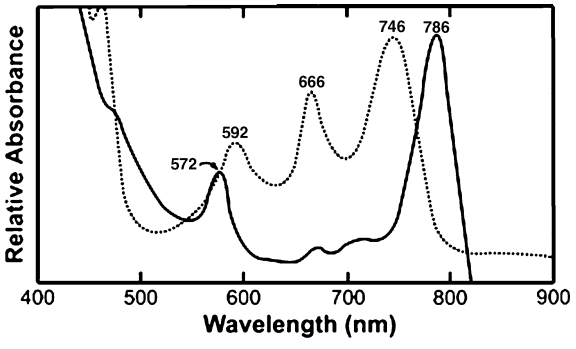

Fig. 3 Absorption spectra of intact cells (*solid line*) and methanol extracts of cells (*dotted line*) of strain HH. The in vivo spectrum is of cells suspended in 30% bovine serum albumin pre-reduced with 0.05% ascorbate

Peaks in the carotenoid region of the in vivo spectrum of strain HH (Fig. 3) initially suggested the presence of 4′,4′-diaponeurosporene, a carotenoid typical of heliobacteria (Takaichi et al. 1997). However, detailed analyses of the carotenoids of strain HH along with established species of the genus *Heliorestis* have shown them to be novel carotenoid glucoside esters, absent from nonalkaliphilic heliobacteria (Takaichi et al. 2003).

Physiology

Strain HH grew photoheterotrophically (anoxic/light) on acetate or pyruvate as carbon sources in the presence of low levels (0.01%) of yeast extract; CO_2 was also present in all media. After growth was achieved in the absence of yeast extract in a biotin-supplemented medium, growth experiments eventually showed that strain HH had no growth factor requirements whatsoever. Reexamination of this issue in *H. daurensis*, previously reported to require biotin (Bryantseva et al. 1999), showed it to be free of growth factor requirements as well. This result differs from that in all other species of heliobacteria, which have an absolute requirement for biotin (Madigan 2001).

Unlike all other heliobacteria, propionate served as a carbon source for photoheterotrophic growth of strain HH. However, reexamination of propionate utilization in *H. daurensis* showed it to grow on propionate as well. But in addition, strain HH but not *H. daurensis* also grew photoheterotrophically on butyrate. Strain HH was quite butyrate tolerant, with growth occurring in media containing up to 20 mM butyrate. Other carbon sources tested included alpha-ketoglutarate (10 mM), arginine (10 mM), ascorbate (0.1%), aspartate (10 mM), benzoate (2, 10 mM), butanol (10 mM), caproate (5, 10 mM), casamino acids (0.1%), citrate (5 mM), ethanol (10 mM), formate (10 mM), fructose (10 mM), fumarate (10 mM), galactose (10 mM), glucose (10 mM), glutamate (5, 10 mM), glycerol (10 mM), lactate (10 mM), malate (10 mM), mannitol (10 mM), methanol (10 mM), propanol (10 mM), ribose (10 mM), sucrose (10 mM), succinate (10 mM), thiosulfate (0.1, 0.2%), valerate (5 mM), and yeast extract (0.1%).

However, none of these substituted for acetate, pyruvate, propionate or butyrate as carbon sources for photoheterotrophic growth of strain HH.

Nitrogen sources supporting growth of strain HH included ammonia, glutamine or asparagine (10 mM), or yeast extract (0.1% w/v). Aspartate, glutamate, or urea did not support growth. Cells grown on limiting (1 mM) ammonia showed nitrogenase activity (acetylene reduction as measured according to Kimble and Madigan 1992, data not shown), indicating that strain HH is also capable of nitrogen fixation.

As was true of *H. daurensis*, strain HH was unable to grow with pyruvate under dark anoxic (fermentative) conditions, a hallmark of nonalkaliphilic heliobacteria (Kimble et al. 1994; Madigan 2001; Pickett et al. 1994). And, as expected, since this is true of all other heliobacteria (Madigan 2001), strain HH was incapable of photoautotrophic growth on $H_2S + CO_2$. Notably, however, strain HH was very sulfide tolerant, growing in media containing up to 10 mM sulfide (in the presence of acetate or pyruvate, data not shown). During growth in sulfide-containing media some sulfide was oxidized to elemental sulfur that appeared as small spheres of presumably amorphous sulfur in the medium. In addition, strain HH grew in ascorbate-reduced defined media containing only sulfate as a sulfur source, indicating that the organism is capable of assimilatory sulfate reduction. This capacity varies among the heliobacteria (Kimble et al. 1995; Stevenson et al. 1997; Madigan 2001).

Strain HH showed similar but not identical growth responses to salt and pH as were originally described for *H. daurensis* (Bryantseva et al. 1999). Optimal growth of strain HH was achieved at 0–1% NaCl and repeated transfers in media devoid of added NaCl showed that the organism lacked an absolute salt requirement. However, unlike for *H. daurensis* (Bryantseva et al. 1999), growth of strain HH occurred up to 3% NaCl (Fig. 4). As regards pH, optimal growth of strain HH occurred at pH 8.5; no growth occurred below pH 7.5 or above pH 10 (Fig. 4). By contrast, *H. daurensis* grew optimally at pH 9 and grew as well at pH 10 as at pH 8.5 (Bryantseva et al. 1999). In strain HH, growth was significantly better at pH 8.5 than at pH 10 (Fig. 4). We therefore conclude that strain HH is slightly less alkaliphilic and slightly more halotolerant than *H. daurensis*. Optimal growth of strain HH occurred at 30–35°C with no growth occurring above 40°C or below 20°C (data not shown).

Genetic analyses

Phylogenetic analyses showed a close yet distinct relationship between strain HH and other *Heliorestis* species (Fig. 5). The sequences of 16S rRNA from the two organisms differed by 2.2%. The genus *Heliorestis* forms a distinct group within the heliobacteria clade (Madigan 2001) and strain HH grouped here, with its closest known relative being *H. daurensis* (Fig. 5). Interestingly, the rod-shaped *Heliorestis* species *H. baculata* (Bryantseva et al. 2000) lies basal on the phylogenetic tree to the filamentous and coiled species, *H. daurensis* and strain HH, respectively (Fig. 5).

To help further genetically distinguish strain HH from *H. daurensis*, dot blot hybridizations of genomic DNA were prepared. As shown in Fig. 6, measurable hybridization was obtained when genomic DNA from either species was probed with that from the other, but the extent of heterologous hybridization was significantly less than in the homologous controls. Compared with *Rhodobacter capsulatus*, which was used as a negative control and showed no detectable hybridization to DNA from either strain HH or *H. daurensis*, reciprocal tests of genomic DNA hybridization of the two alkaliphilic heliobacteria yielded values that varied from 25 to 60% homology; a mean value of 48% was obtained from 22 separate hybridization experiments (Fig. 6). Although some variation was obtained in the genomic hybridization experiments, collectively, these data support the conclusion that strain HH is a member of the genus *Heliorestis* and is a species distinct from that of *H. daurensis*.

Discussion

Soda lakes are excellent habitats for anoxygenic phototrophic bacteria. These highly productive ecosystems typically have anoxic zones and are sulfidic to various extents (Imhoff et al. 1978, 1979; Jones et al. 1998). Through the years several phototrophic purple bacteria have been isolated from soda lakes (Imhoff 2001). These include from the most saline lakes species of *Ectothiorhodospira* and *Halorhodospira* (Imhoff 2001; Imhoff et al. 1978). In less saline soda lakes several purple sulfur and purple nonsulfur bacteria have been isolated (Imhoff 2001; Milford et al. 2000). Like most species of *Ectothiorhodospira* and *Halorhodospira*, these phototrophs are strongly alkaliphilic but differ in that they show little if any NaCl requirement. In recent years it has been recognized that heliobacteria also inhabit alkaline lakes of low salinity, with two species of the genus *Heliorestis*, *H. daurensis* and *H. baculata*, described (Bryantseva et al. 1999, 2000). Now strain HH, a morphologically unique heliobacterium, can be added to this list with its discovery from an Egyptian soda lake.

It is distinctly possible that a strain HH-like organism was present in the original enrichment cultures of *H. daurensis* (Bryantseva et al. 1999). If true, this organism was lost during purification since the axenic culture isolated and described as *H. daurensis* showed no tendency for cells to form stable coils, as occurs in strain HH. Indeed, strain HH has a unique coiled morphology not found in any other anoxygenic phototroph. The final coiled morphology appears to be the result of incomplete cell division of individual curved cells. Then, depending on the number of cells in a chain, the coils can

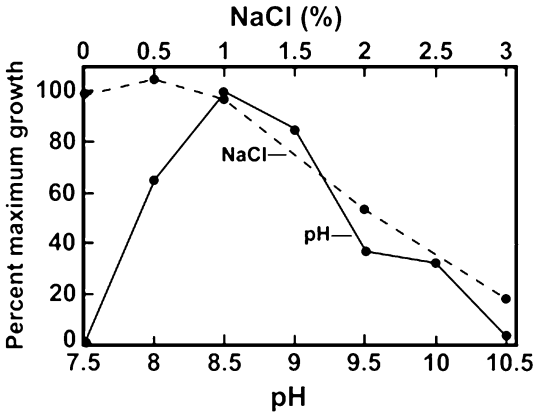

Fig. 4 Growth of strain HH as a function of salt or pH. Cells were grown phototrophically in tubes and cultures scored for growth after incubation for a defined time period, usually 4–5 days. Each data point is the mean of triplicate cultures

be of variable length. Coiled cells are observed in both young and old cultures. Thus, the corkscrew morphology of strain HH appears to be the true morphology of this organism and not an artifact due to any peculiar culture conditions.

No obvious production of endospores was observed in strain HH, even in very old cultures. However, the fact that all heliobacteria tested appear capable of endospore formation (Madigan 2001) suggests that this is also true of strain HH and other *Heliorestis* species. Genes encoding endospore-specific proteins have been found in all heliobacteria tested (Kimble and Madigan 2001), confirming the phylogenetic conclusion (Woese et al. 1985) that they are indeed endospore-forming prokaryotes. Although *Heliorestis* species have not been examined for such genes, we predict that similar endospore-specific genes exist in strain HH and other *Heliorestis* species.

The only physiological surprise with strain HH was its photoheterotrophic growth on propionate and butyrate. Among heliobacteria, propionate is only used by strain HH and *H. daurensis* (Madigan 2001). Butyrate can be used by many phototrophic purple nonsulfur bacteria (Imhoff and Trüper 1989) but is used only by a few species of heliobacteria (Ormerod et al. 1996). No other *Heliorestis* species use butyrate (Bryantseva et al. 1999, 2000). Strain HH grew well on butyrate and did not show any toxic effects of high concentrations typically observed during fatty acid metabolism in purple nonsulfur bacteria (Madigan 1988).

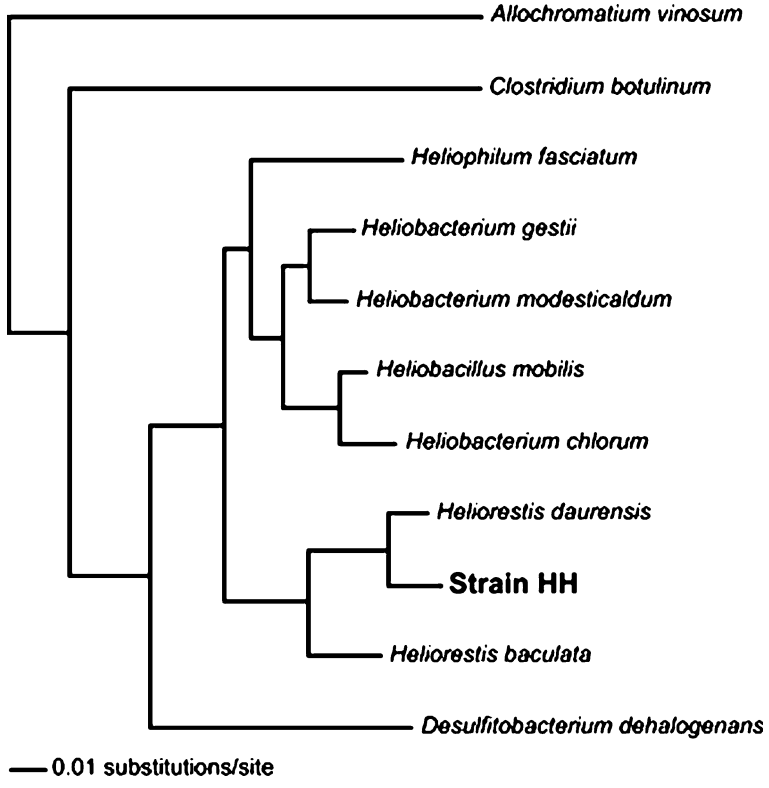

Fig. 5 Phylogenetic tree of strain HH, other heliobacteria, and related organisms based on comparative 16S rRNA gene sequences. A heuristic search using the distance criterion with Jukes–Cantor correction was employed utilizing 1,279 characters. A single tree was obtained with bootstrap values on 100 replicates all exceeding 65%. Sequences were obtained from the following GenBank accession numbers: *Allochromatium vinosum* (M26629), *Clostridium botulinum* (X68187), *Heliophilum fasciatum* (L36197), *Heliobacterium gestii* (L36198), *Heliobacterium modesticaldum* (U14559), *Heliobacillus mobilis* (U14560), *Heliobacterium chlorum* (M11212), *Heliorestis daurensis* (AF079102), *Heliorestis convoluta* (strain HH) (DQ266255), *Heliorestis baculata* (AF249680), *Desulfitobacterium dehalogenans* (L28946). The purple sulfur bacterium *Allochromatium vinosum* was used as an outgroup to the heliobacteria and other gram-positive bacteria

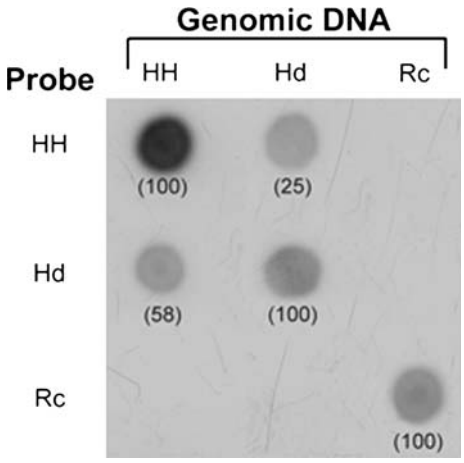

Fig. 6 Dot-blot hybridization of genomic DNAs from strain HH and *Heliorestis daurensis* (Hd); DNA from *Rhodobacter capsulatus* (Rc), an alpha Proteobacterium, was used as a negative control. *Numbers in parentheses* indicate percent hybridization versus homologous controls

Strain HH responded in predictable fashion in its growth requirements for pH and salinity; the organism is alkaliphilic and slightly halotolerant, but not halophilic. This suggests that the geographic distribution of strain HH in lakes of the Wadi El Natroun should be limited to those of relatively low salinity. Surprisingly, however, lakes in this region are all hypersaline to one degree or another. Of the six lakes studied by Imhoff et al. (1979), the lowest salinity reported was in Lake Gabara, which contained about 5% NaCl. Even this is beyond the salinity tolerance of strain HH (Fig. 4). Lake El Hamra, the source of strain HH, contains about 16% NaCl and would thus seem prohibitory as a natural habitat of this organism. However, there are freshwater springs that feed into Lake El Hamra (Imhoff et al. 1979) and thus salinities may vary in different locations of the lake. Other lakes in the Wadi (Lakes Zugm, Grear, and Rizunia) are extremely hypersaline (Imhoff et al. 1979) and would likely not be habitats for strain HH-like organisms.

It is also possible that the major habitats for alkaliphilic heliobacteria are not the soda lakes themselves, but the soils that surround the lakes. Heliobacteria are primarily soil phototrophs (Stevenson et al. 1997). Thus, they may naturally inhabit alkaline low salinity soils adjacent to soda lakes and from there get transmitted to water and sediment only accidentally. For example, the terrain surrounding Lake El Hamra when sampled in May 2001 contained a dense grass cover. Although the salinity of this soil was not determined, it was quite alkaline (about pH 9). Assuming that this soil was not too saline, it may well have contained heliobacteria. In this connection it should be recalled that *H. daurensis*, the close relative of strain HH (Figs. 5, 6), was isolated from soil and microbial mat material growing adjacent to a weakly saline Siberian soda lake (Bryantseva et al. 1999). Whether *Heliorestis* species inhabit moderate salinity soda lakes such as Mono Lake (California, 9% NaCl) or Soap Lake (Washington, 14% NaCl) (Oremland and Miller 1993) is unknown. However, if HH-like organisms can survive in the brines of Lake El Hamra, one would predict that similar organisms would inhabit these USA soda lakes. Like Lake El Hamra, both Mono Lake and Soap Lake are also surrounded by alkaline soils (Oremland and Miller 1993) that could be habitats for alkaliphilic heliobacteria.

Taxonomic conclusions and description of *H. convoluta* sp. nov.

H. daurensis strain BT-H1 and the coiled heliobacterium strain HH described here are related but distinct heliobacteria. The two organisms differ most dramatically in cell morphology and genomic properties (Figs. 1, 2, 5, 6). We therefore describe strain HH as a new species of the genus *Heliorestis*, *H. convoluta* sp. nov.

Heliorestis convoluta; con•vo•lu′ta; L. fem. Adj., coiled; Heliorestis convoluta, the coiled Heliorestis

Cells 0.6 μm wide forming a coil of variable length. Cells stain gram-negatively. Coils grow from ring-shaped cells that elongate and form a septum that fails to completely divide and form individual cells. Individual rings and short coils motile. Obligate phototroph. Cells contain bacteriochlorophyll *g* as major chlorophyll pigment. Grows photoheterotrophically (anoxic/light) in mineral media containing pyruvate, acetate, propionate or butyrate plus CO_2. Neither chemotrophic nor photoautotrophic growth occurs. No growth factors required. Alkaliphilic and mesophilic; optimal growth occurs at pH 8.5–9 and 33°C. Phylogenetic position within the low G+C gram-positive Bacteria. Habitat: shoreline soils and waters of soda lakes. Type strain: HH^T, isolated from shoreline soil and water of Lake El Hamra, Wadi El Natroun, Egypt. Deposited in the American Type Culture Collection as ATCC BAA-1281.

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