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BRIEF COMMUNICATION

Irradiance effects on growth and bacteriochlorophyll content of phototrophic heliobacteria, purple and green photosynthetic bacteria

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Abstract

Two species of heliobacteria along with a purple and green bacterium were tested for their ability to grow phototrophically at irradiances ranging from 0.125 to 50 W m^{-2}. The heliobacteria were incapable of growth below 0.5 W m^{-2}, while both the purple and green bacterium grew at significantly lower irradiances. Specific bacteriochlorophyll contents were higher for the purple and green bacteria than for the heliobacteria at all irradiances tested. Thus in distinct contrast to purple and green bacteria, heliobacteria are "high-irradiance" phototrophs, and this characteristic may influence their distribution in nature.

Additional key words: anoxygenic phototrophs; *Chlorobium;* generation time; *Heliobacillus; Heliobacterium;* photosynthetic pigments; *Rhodobacter*.

Heliobacteria are strictly anaerobic anoxygenic phototrophs that contain bacteriochlorophyll (Bchl) *g* (Brockmann and Lipiniski 1983, Gest and Favinger 1983, Madigan 2001a,b). In contrast to the classical purple and green bacteria, heliobacteria have not been detected in or isolated from typical habitats for anoxygenic phototrophs, such as the depths of stratified lakes. Instead, the eight or so species of heliobacteria known have been isolated only from soils and microbial mats (Kimble *et al.* 1995, Ormerod *et al.* 1996, Bryantseva *et al.* 1999, Madigan 2001a,b). This suggests that for some reason(s), heliobacteria may not be competitive with purple and green bacteria in aquatic habitats.

One variable that could influence the distribution of heliobacteria in nature is irradiance. In this connection, it was noted in early studies of heliobacteria that unlike for purple and green bacteria, enrichment and growth of pure cultures of heliobacteria occurred best at rather high irradiances (Beer-Romero and Gest 1987, Beer-Romero *et al.* 1988). This suggested that heliobacteria may be "high-irradiance-adapted" anoxygenic phototrophs, and as a consequence, possibly have a reduced capacity for growth at low irradiances. We tested this hypothesis and showed that two model species of heliobacteria are indeed incapable of phototrophic growth at low irradiances sufficient to support the growth of purple and green bacteria. We also show that the specific pigment content of heliobacteria is the lowest of all known anoxygenic phototrophs and conclude that this handicap may restrict their distribution in nature to habitats receiving full sunlight.

Heliobacillus (*Hc.*) *mobilis* (ATCC 43427) and *Heliobacterium* (*Hb.*) *gestii* strain HD7 (Ormerod *et al.* 1996) were used as model heliobacteria, *Rhodobacter* (*Rb.*) *capsulatus* strain B10 (ATCC 33303) was used as a model purple bacterium, and *Chlorobium* (*C.*) *vibrioforme* forma *thiosulfatophilum* strain NCIB 8327 (DSM 263) was used as a model green sulfur bacterium. The heliobacteria were grown in medium LYE, a modification of medium PYE (Kimble and Madigan 1992) containing 20 mM sodium lactate in place of pyruvate; dark, fermentative growth of heliobacteria does not occur in medium *LYE* (Kimble *et al.* 1994) and thus growth is strictly phototrophic. *Rb. capsulatus* was grown in medium *RCVB* (Tayeh and Madigan 1987) supplemented with 0.2 % (m/v) yeast extract, and *C. vibrioforme* was grown in medium *Pf-7* (Wahlund *et al.* 1991); in these media, growth of both organisms is also strictly phototrophic.

Heliobacteria were cultured in stoppered culture tubes

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as previously described (Kimble and Madigan 1992), while *Rb. capsulatus* and *C. vibrioforme* were grown in completely filled screw-cap tubes (17 cm^3). All cultures received a 3–5 % (v/v) inoculum and were grown phototrophically in a water bath (35 °C) exposed to tungsten irradiation from a bank of 250 W reflector bulbs; irradiance was controlled by adjusting power to the bulbs with a rheostat. For irradiances under 1 W m^{-2}, the water bath was shrouded with a black cloth to prevent the entrance of stray light. Irradiances were measured with an *INS DX-100* digital light meter. Growth was measured in a *Klett-Summerson* photometer fitted with a no. *66* (red) filter. Cells of all organisms were harvested for pigment analysis before self-shading occurred (*ca.* 200 photometer units for species of heliobacteria and *Rb. capsulatus*, and about 350 photometer units for *C. vibrioforme*).

For bacteriochlorophyll (Bchl) and protein analyses, 5-10 cm^3 aliquots of cultures were transferred to stoppered tubes within an anoxic glove box. Cells were then centrifuged in a bench-top centrifuge for 30 min, placed back in the anoxic hood for removal of the supernatant and addition of extraction solvent. Bchl was extracted from cells under anoxic conditions for 1 h at 0 °C in darkness; Bchl *g* was extracted in 100 % acetone while Bchl *a* or *d* were extracted in 100 % methanol. The resulting suspensions were centrifuged to remove insoluble residue and the absorbance of the supernatant read immediately (Bchl *g*, 762 nm; Bchl *a*, 772 nm; Bchl *d*, 658 nm) in a *Hitachi U-2000* spectrophotometer. Bchl concentrations were calculated using the extinction coefficients of 92.7 m^3 kg^{-1} cm^{-1} for Bchl *g* [recalculated from the extinction coefficient of 76 mM^{-1} cm^{-1} and the molecular mass of Bchl *g* (819.5 g) in van de Meent *et al.* (1991)]; 46.1 m^3 kg^{-1} cm^{-1} for Bchl *a* (Smith and Benitez 1955), and 82.3 m^3 kg^{-1} cm^{-1} for Bchl *d* (Stanier and Smith 1960). Protein was determined by the *BioRad* protein assay (Bulletin 1069; *BioRad Laboratories*, Richmond, CA, USA) using bovine serum albumin as the protein standard. Cell pellets remaining from Bchl extractions were resuspended in 2 cm^3 0.05 M NaOH, boiled for 20 min, and cooled to room temperature before protein analysis. The values reported in Table 1 are the means (5 to 10 determinations for each irradiance).

Table 1. Growth rates and bacteriochlorophyll contents [g kg^{-1}(cell protein)] of various anoxygenic phototrophs as a function of irradiance. Minimum generation time *t* [h] for phototrophic growth at the stated irradiance and pigment contents are means of 5-10 determinations. ND, not determined; NG, no growth, VSG, very slow growth over a 3-week period.

Irradiance	*Heliobacillus mobilis*		*Heliobacterium gestii*		*Rhodobacter capsulatus*		*Chlorobium limicola*	
[W m^{-2}]	*t*	Bchl *g*	*t*	Bchl *g*	*t*	Bchl *a*	*t*	Bchl *d*
50	2.5	16.8	3.3	11.8	2.6	ND	6.6	155.4
25	2.8	16.3	3.8	11.2	3.0	29.9	6.6	213.2
12.5	3.2	18.5	5.9	14.4	ND	ND	ND	ND
5	3.9	19.6	6.8	12.3	3.5	41.7	10.0	238.7
2.5	8.6	12.9	10.4	17.0	7.3	50.6	11.0	300.9
1.25	13.9	11.2	13.5	11.9	12.0	53.5	28.0	308.2
0.875	24.5	15.4	20.5	21.6	29.0	ND	31.2	396.0
0.5	30.1	3.4	NG	NG	29.0	44.6	44.0	347.9
0.25	NG	NG	NG	NG	158.0	35.4	112.0	123.5
0.125	NG	NG	NG	NG	NG	ND	VSG	130.5

At the highest irradiance tested (50 W m^{-2}), the generation times of the heliobacteria and of *Rb. capsulatus* were comparable (2.5-3.3 h) whereas the generation time of *C. vibrioforme* was about twice as long (Table 1). For *Hb. gestii*, generation times increased significantly with reductions in irradiance, with a major reduction in growth rate occurring below 25 W m^{-2}; below 0.875 W m^{-2}, no growth of *Hb. gestii* occurred (Table 1). *Hc. mobilis* showed a similar pattern, however the major reduction in growth rate in this species came only below an intensity of 5 W m^{-2}. Moreover, unlike *Hb. gestii*, *Hc. mobilis* grew at 0.5 W m^{-2}, but at 0.25 W m^{-2} no growth was observed (Table 1). In contrast to the heliobacteria, *Rb. capsulatus* and *C. vibrioforme* continued to grow at irradiances well below 0.5 W m^{-2}. *Rb. capsulatus* grew slowly at 0.25 W m^{-2} but not at 0.125 W m^{-2}, while *C. vibrioforme* grew at 0.125 W m^{-2}, the lowest irradiance tested (Table 1).

From these growth experiments it can be concluded that: (*1*) *Hc. mobilis* is better able than *Hb. gestii* to grow at low irradiances, and (*2*) neither species of heliobacteria grows at irradiances sufficient to support growth of purple and green bacteria. Because the heliobacteria form a very tight phylogenetic clade and seem to be highly similar in terms of their basic physiology (Madigan 2001a,b, Madigan and Ormerod 1995), it is likely that the latter conclusion extends beyond the two species tested here, to include heliobacteria in general. Our data also support previous observations showing that green bacteria can grow at lower irradiances than purple bacteria (Biebl and Pfennig 1978, Guerrero *et al.* 1985, van Gemerden and Mas 1995).

Purple and green bacteria synthesize Bchl and carote-

noids inversely as a function of irradiance (Cohen-Bazire *et al.* 1957, Brock-Due *et al.* 1978). Moreover, the specific pigment content of green bacteria is typically significantly higher than for purple bacteria (van Gemerden and Mas 1995). In our experiments both of these patterns were confirmed (Table 1). However, below an irradiance of about 0.5 W m^{-2}, the specific Bchl content of cells of both *Rb. capsulatus* and *C. vibrioforme* actually decreased rather than increased (Table 1). This suggests that at very low irradiances energy limitation may prevent full adaptation of the photosynthetic apparatus in purple and green bacteria.

The specific contents of Bchl *g* in the two heliobacterial species were far lower than for Bchl *d* (*C. vibrioforme*) or Bchl *a* (*Rb. capsulatus*) at any irradiance tested (Table 1). In fact, our data show that heliobacteria contain the lowest specific Bchl contents of all known anoxygenic phototrophs (Table 1, van Gemerden and Mas 1995). This low specific pigment content is due to the inability of heliobacteria to synthesize intracytoplasmic photosynthetic membranes or chlorosomes (Miller *et al.* 1986). These structures house the antenna pigments of purple bacteria (Drews and Golecki 1995) and green bacteria (Blankenship *et al.* 1995), respectively, and allow cells to accumulate large pigment reservoirs, as shown in this study (Table 1). In heliobacteria, all photocomplexes reside in the cytoplasmic membrane (Miller *et al.* 1986), and this places severe constraints on the total amount of Bchl *g* that can accumulate per cell.

In *Hc. mobilis*, specific Bchl contents were roughly similar at all irradiances from 50 to 5 W m^{-2}, but below 5 W m^{-2} the Bchl content dropped steadily. At 0.5 W m^{-2}, the lower limit for growth of *Hc. mobilis*, Bchl contents were only 17-30 % of that of cells grown at higher irradiances. This suggests that like purple and green bacteria, low irradiance grown *Hc. mobilis* may also be energy starved and thus unable to up-regulate Bchl *g* synthesis. In cells of *Hb. gestii*, a similar pattern to that of *Hc. mobilis* was observed at the higher irradiances but not at the lowest intensity supporting growth (Table 1). At the latter, the specific pigment content of cells of *Hb. gestii* rose significantly (Table 1). Although this suggests some ability to up-regulate pigment synthesis in this species, this trend was short lived, as no growth occurred at the next lowest irradiance tested (Table 1).

The specific Bchl content of cells of *Hc. mobilis* was significantly lower than for *Hb. gestii* in cells grown at 0.875 W m^{-2}, the lower limit for *Hb. gestii*. In cells of *Hc. mobilis* grown at 0.500 W m^{-2}, this value was even lower yet (Table 1). Thus, Bchl content, *per se*, is not the only factor controlling the photon requirements for phototrophic growth of heliobacteria. Although speculative, it is possible that an additional compounding factor involves maintenance energy requirements. Previous studies have shown that the maintenance energy requirements of purple bacteria are significantly higher than for green bacteria and that this advantage for green bacteria contributes to their ability to grow at irradiances insufficient to support the growth of purple bacteria (van Gemerden and Mas 1995). Although the maintenance energy requirements of heliobacteria are currently unknown, maintenance costs of any magnitude coupled with the inherent disadvantage of low specific Bchl contents (Table 1) could be sufficient to prevent growth of heliobacteria at irradiances suitable for purple and green bacteria.

Our results offer an explanation for the apparent absence of heliobacteria from certain aquatic habitats (Stevenson *et al.* 1997). For example, the depths of stratified lakes are ideal habitats for purple and green bacteria, where these organisms can form dense blooms (Pfennig 1978, Guerreo *et al.* 1985, van Gemerden and Mas 1995). Such habitats would be unfavorable ones for heliobacteria, however, because attenuation of radiation through the water columns of the lakes would leave insufficient photons for growth in the lower (anoxic) zones where anoxygenic photosynthesis can occur. The factors discussed in this paper thus likely restrict heliobacteria to habitats in nature that receive full or near full sunlight, such as surface soils, and it is from the latter that enrichment and isolation studies to date have shown them to be most common (Stevenson *et al.* 1997).

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