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Regulation of formation of the photosynthetic system in a photosynthetic rhizobium

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Introduction

Bradyrhizobium strains that form nitrogen-fixing stem and root nodules on some species of the legume *Aeschynomene* possess photosynthetic systems (Fleischman and Kramer, 1998). Such stem-nodulated legumes have been of interest to rice farmers in developing countries, since they can grow and fix nitrogen in flooded rice fields, and so provide biologically produced nitrogen fertilizer. Photosynthetic rhizobia have been found in the vascular systems of wild rice plants in Africa, where they apparently can fix nitrogen and stimulate plant growth significantly (Chaintreuil *et al*., 2000). The photosynthetic rhizobia that have been identified thus far are aerobic photosynthetic bacteria (aerobic anoxygenic photorophs). The latter are a phylogenetically diverse group of bacteria that form the photosynthetic system and grow only in the presence of oxygen or an alternative electron acceptor (Shimada, 1995). Recently aerobic photosynthetic bacteria have been found to contribute substantially to the carbon cycle in the ocean (Kolber et al., 2001).

The ability of *Bradyrhizobium* strain BTAi 1 to form a photosynthetic system was discovered when W. R. Evans noticed that cultures turned pink when grown under cyclic illumination, but not when grown in constant light or darkness, and that pigment accumulation was dependent on the carbon source employed (Evans *et al*., 1990). In a study of the regulation of pigment accumulation in BTAi 1,Wettlaufer and Hardy (1995) found that bacteriochlorophyll forms in darkness after cultures have been illuminated, They also reported that blue light is effective in triggering pigment synthesis (Wettlaufer and Hardy, 1993). Thus light is necessary to initiate pigment synthesis, but the pigment can accumulate only in subsequent darkness. Formation of the photosynthetic system is triggered by light in some other aerobic photosynthetic bacteria (including other *Bradyrhizobium* isolates), but not in others (reviewed in Shimada, 1995 and Fleischman and Kramer, 1998). In this study we further examine the effects of light and carbon source on bacteriochlorophyll accumulation in BTAi 1.

Methods

For study of the dependence of pigment accumulation on light quality, cultures were grown under continuous tungsten illumination in crystallization dishes covered by aluminum foil except for a window covered by a sharp-cutoff long pass filter. A mineral salts medium containing glutamate (0.15%) was employed (Evans *et al*., 1990). Cells were collected nine days after inoculation, pigment was extracted with acetone/methanol (7/2, v/v) and the bacteriochlorophyll content of the extract was determined from the absorbance at 770 nm. The dry weight of the extracted cells was determined. For growth experiments, 100 ml cultures containing the appropriate carbon source (0.15 %) were inoculated with 1.0 ml of a late-log phase BTAi 1 culture and grown under cyclic tungsten illumination (16 h light-8 h dark). Each day, a culture was harvested and the bacteriochlorophyll content and dry weight of the cells determined.

Results

Cells were grown in continuous light under a series of sharp-cutoff long-pass filters. Little bacteriochlorophyll was found in cells exposed only to light of wavelength greater than 830 nm (Fig. 1).

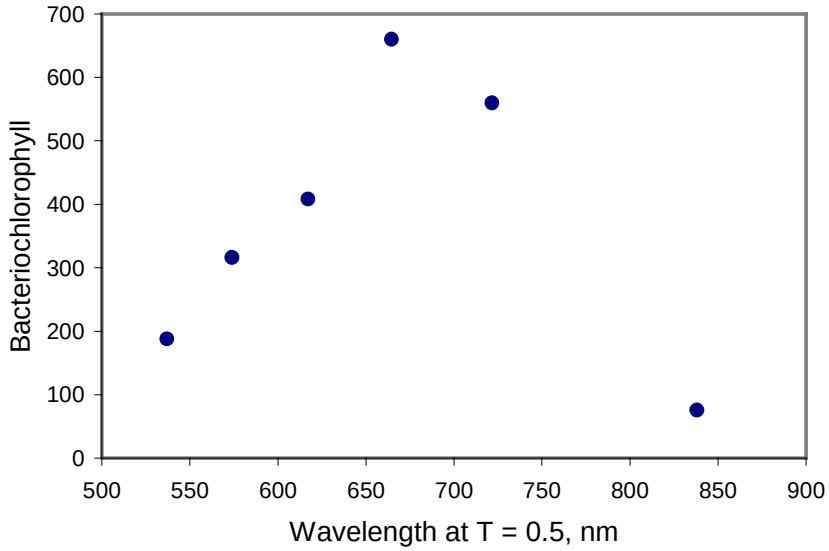

Figure 1. The effect of wavelength of incident light on bacteriochlorophyll accumulation in BTAi 1. Cells were grown for nine days under continuous illumination by tungsten light passed through sharp-cutoff long pass filters whose transmittance was half-maximal at the indicated wavelength.

Since LH1, the light-harvesting bacteriochlorophyll protein of BTAi 1 absorbs strongly near 870 nm, the photosynthetic light-harvesting system must not be the photoreceptor responsible for triggering bacteriochlorophyll accumulation. As shorter wavelengths were added, bacteriochlorophyll began to accumulate. But as wavelengths below 650 nm were added, bacteriochlorophyll accumulation decreased. It appears that a photoreceptor absorbing at wavelengths less than 750 nm

triggers pigment accumulation, while a photoreceptor absorbing at wavelengths below 650 nm inhibits it.

When cells are grown in a medium containing glutamate as the carbon source, bacteriochlorophyll content parallels cell growth (data not shown). However, when the carbon source is arabinose (Fig. 2) or malate (Fleischman *et al*. 1995), bacteriochlorophyll begins to accumulate only when the cultures reach the end of exponential growth.

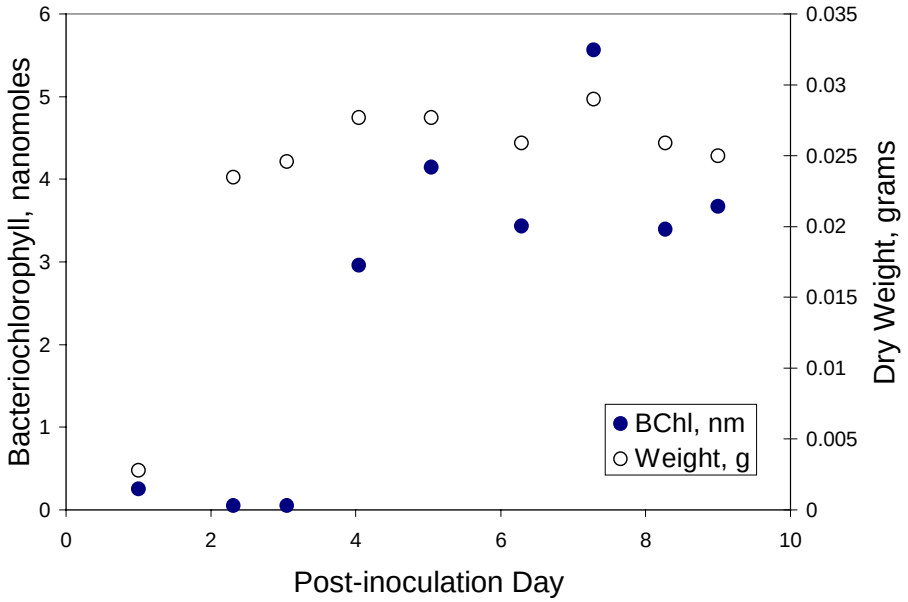

Figure 2. Growth and bacteriochlorophyll accumulation in BTAi 1 cultures grown in arabinose medium.

We have noticed that the amount of bacteriochlorophyll that cultures accumulate when they are grown on different carbon sources is inversely related to their growth rate and respiration rate. Thus bacteriochlorophyll content increases in the order: glutamate > malate > succinate > arabinose (W. R. Evans, unpublished data). Growth rate increases in the order: arabinose > malate > glutamate, while the respiration rate increases in the order succinate > malate > glutamate (Wettlaufer and Hardy, 1992).

Discussion

The wavelength dependence of bacteriochlorophyll accumulation presented above is clearly not a true action spectrum, since broad spectral bands were used and fluence through the various filters was not constant. Nevertheless it indicates that bacteriochloophyll accumulation can be triggered by light of wavelength between 550 and 750 nm, and suppressed by light of wavelength below about 650 nm. Visible light suppresses bacteriochlorophyll synthesis in many photosynthetic bacteria, and the mechanism by which it does so may be similar in aerobic

photosynthetic bacteria such as BTAi 1. But what is the photoreceptor responsible for triggering bacteriochlorophyll accumulation? Few biological pigments absorb in the 750 nm region. We have suggested that the receptor might be a tetrapyrrole, some of which do absorb here (Fleischman and Kramer, 1998). This possibility would be consistent with the observation that the *puf* operon in ORS278, a *Bradyrhizobium* closely related to BTAi 1, includes what appears to be a heme oxidase gene (Giraud *et al*., 2000). Heme oxidase is involved in the synthesis of linear tetrapyrroles.

In sum, the data presented above suggest that BTAi 1 forms the photosynthetic system only when it is needed to provide free energy - when good carbon sources are absent or become depleted, and light is available (bacteriochlorophyll is not present in BTAi 1 bacteroids in subterranean root nodules). Formation of the photosynthetic system in some marine aerobic photosynthetic bacteria is similarly suppressed in rich media (Zbigniew *et al*., 2001).

Oh and Kaplan (2000) have suggested, on the basis of studies with electron transport mutants, that the expression of photosynthesis genes in *Rhodobacter sphaeroides* is regulated by the rate of electron flow through the cbb_3 cytochrome c oxidase. Higher electron flow rates correlate with reduced gene expression. Photosynthesis genes in BTAi 1 might be regulated in a similar fashion, since accumulation of bacteriochlorophyll is inversely correlated with electron flow to oxygen when the carbon source is varied. Some bradyrhizobia possess cbb3.

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