Effect of Light and Organic Acids on Oxygen Uptake by BTAi 1, a Photosynthetic Rhizobium

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A photosynthetic rhizobium, strain BTAi 1, was cultured ex planta to investigate its photosynthetic-respiratory system and the response of this interactive system to light quantity and quality and to the addition of organic acids. Oxygen uptake, as measured with an oxygen electrode, is diminished upon illumination, with the amount of decrease related to light intensity. This oxygen-sparring effect is correlated with the wavelengths of light that are associated with bacteriochlorophyll absorbance. Increasing concentrations of glutamic, succinic, and malic acids enhance the oxygen-sparring effect of light until a threshold concentration is reached, beyond which succinic and malic acids decrease the effect. The photobiology of this unique rhizobium is similar to the photobiology of both anaerobic and aerobic photosynthetic bacteria.

Strain BTAi 1, a photosynthetic rhizobium, is unique because it combines the characteristics of a photosynthetic bacterium with those of nitrogen-fixing symbiotic rhizobia (2). This organism provides the first potential example of energy-self-sufficient nitrogen fixation, in strain BTAi 1 stem nodules on Aeschynomene indica.

Strain BTAi 1 has been characterized as an intermediate type, sharing physiological properties of both fast-growing and slowly growing rhizobia (10). It produces both stem and root nodules. Both types of nodules fix nitrogen, and the stem nodules exhibit light-stimulated CO₂ uptake (6). This organism is closely related to Bradyrhizobium japonicum and Rhodopseudomonas palustris (13). It contains bacteriochlorophyll and photosynthetic reaction centers resembling those of a purple photosynthetic bacterium (3). The amount of bacteriochlorophyll produced is about 1/10th that produced by members of the family Rhodospirillaceae (7). The photosynthetic system in strain BTAi 1 exhibits an oxygen-sparring effect (decreased oxygen uptake during illumination) similar to that found in anaerobic and aerobic photosynthetic bacteria (4, 5). Strain BTAi 1 is an obligate aerobe, as are all rhizobia. It requires a photoperiod for production and accumulation of bacteriochlorophyll (3). In this study we focused on the photobiology of photorhizobium strain BTAi 1 in culture.

MATERIALS AND METHODS

Media and growth. Strain BTAi 1 cells were cultured in liquid medium containing (per liter) 0.5 g of K₂HPO₄, 0.8 g of MgSO₄·7H₂O, 0.2 g of NaCl, 0.01 g of FeCl₃·6H₂O, 9.55 g of glutamic acid, and 1.0 g of yeast extract; the final pH was 6.8.

 Cultures inoculated from slants were grown in a rotary shaker for 7 days; then fresh medium was inoculated with the cell suspension described above (1%, vol/vol), and the resulting cultures were grown in the medium described above at 26°C under incandescent lighting. The light intensity was 34 µmol·s⁻¹·m⁻². The light regime was a cycle consisting of 16 h of light and 8 h of dark. This cycle was necessary for the production and accumulation of bacteriochlorophyll.

Pigment determination. Cell suspensions (10⁷ cells per ml) were centrifuged at 10,000 x g for 20 min. The resulting pellet was resuspended in 1 ml of acetone-methanol (7:2), and the suspension was placed in the dark for 30 min with occasional vortexing. After extraction, the cell debris was removed by centrifugation in a Fisher microcentrifuge, and the supernatant was placed in a Perkin-Elmer dual-beam spectrophotometer and scanned for absorbance between 900 and 300 nm. Bacteriochlorophyll concentration was quantified at 676 nm by using a molar extinction coefficient of 76 mM⁻¹·cm⁻¹.

Oxygen electrode. Cell suspensions (10⁷ cells per ml) were centrifuged at 10,000 x g for 20 min. The resulting pellet was washed with 3% NaCl and centrifuged. This process was repeated twice before final resuspension in a salt buffer containing (per liter) 0.5 g of K₂HPO₄, 0.8 g of MgSO₄·7H₂O, 0.2 g of NaCl, and 0.01 g of FeCl₂·6H₂O (pH 6.8).

Oxygen uptake was measured with a Gilson Oxygraph apparatus. The cuvette was water jacketed, and the temperature was maintained at 27°C. The total volume was 2 ml (salt buffer and bacterial suspension). The initial PO₂ was 275 µM, and measurements were typically recorded at values between 95 and 60% of the initial PO₂. Organic acids used as substrates were added prior to the addition of the bacterial suspension. Each experiment was performed three times, and individual points within each experiment were duplicated.

Light energy was supplied by a halogen lamp which produced a light intensity of 2,500 µmol·s⁻¹·m⁻² at a distance of 5 cm. Bacteriochlorophyll concentration was determined as described above, except that 100 to 200 µl of bacterial suspension was extracted in 800 to 900 µl of acetone-methanol (7:2).

Action spectrum. To determine what effect different bands of light had on the oxygen-sparring effect, the cells were exposed to light passing through the following nine different bandpass filters: filter 1, 850 to 890 nm, 869-nm maximum; filter 2, 800 to 840 nm, 820-nm maximum; filter 3, 780 to 820 nm, 794-nm maximum; filter 4, 760 to 785 nm, 773-nm maximum; filter 5, 640 to 740 nm, 684-nm maximum; filter 6, 520 to 660 nm, 576-nm maximum; filter 7, 430 to 580 nm, 494-nm maximum; filter 8, 370 to 480 nm, 426-nm maximum; and filter 9, 360 to 420 nm, 387-nm maximum. Neutral-
density filters were used to generate saturation curves for each filter in individual experiments. Midpoint values for light intensity and \( \text{O}_2 \) uptake were then determined. The amount of oxygen spared for each wavelength was the difference between the \( \text{O}_2 \) uptake values for the illuminated and non-illuminated samples (in nanomoles of \( \text{O}_2 \) per minute per microgram of bacteriochlorophyll) normalized to the amount of light transmitted by the filters (in micromoles per second per square centimeter). A subsaturating concentration of bacteriochlorophyll, of a constant illumination, was determined and maintained in the cuvette throughout the work.

**Absorption spectra of whole-cell extracts.** Cells suspended in 50 mM Tris (pH 7.5) were broken in a French pressure cell (cell pressure, 10,000 lb/in\(^2\)). The extract was then centrifuged in a Fisher microcentrifuge for 30 min. The supernatant was removed and placed in a Perkin-Elmer dual-beam spectrophotometer and scanned for absorbance between 900 and 300 nm.

**RESULTS**

Figure 1 shows the oxygen-sparing effect as a function of light intensity. An increase in light energy is accompanied by a decrease in oxygen uptake. This response is saturated at 1,000 \( \mu \)mol \( \text{s}^{-1} \text{m}^{-2} \) and is a normal response for a light-absorbing system.

To determine which pigments were contributing to the oxygen-sparing effect, an action spectrum was determined by exposing bacterial suspensions in the oxygen electrode to different wavelengths of light, using a series of bandpass filters (Fig. 2), and then comparing this spectrum with an absorbance spectrum of a cell extract. The major oxygen-sparing effects occur at bacteriochlorophyll absorption maxima of 870, 800, and 420 nm, while there is little effect in the area of carotenoid absorption at 520 nm.

In *Rhodopseudomonas palustris* addition of succinic acid or malic acid stimulates the utilization of \( \text{O}_2 \) in cultures in the dark. Such stimulation is diminished by illumination (4) and was found in *Rhodospirillum rubrum* to be the result of inhibition of \( \text{O}_2 \) uptake instead of \( \text{O}_2 \) production (11). Figure 3 shows the effects of different concentrations of succinic, malic, and glutamic acids in growth medium substrate on \( \text{O}_2 \) uptake in the dark and in the light. Oxygen uptake in the dark and the oxygen-sparing effect were both stimulated by increased concentrations of all three organic acids, until a
concentration was reached beyond which further addition of any of the substrates had no effect on either of the activities. With succinic acid and malic acid, the oxygen-sparing effect decreased at higher concentrations. The other organic substrates which we tested (citric acid, isocitric acid, and alpha-ketoglutaric acid) did not significantly increase oxygen uptake, nor did they enhance the oxygen-sparing effect (data not shown).

**DISCUSSION**

The results of this study, together with previously reported results, enabled us to better characterize photosynthetic rhizobium strain BTAi 1.

Overall, strain BTAi 1 is an organism that is closely related, on the basis of genetics, to the nitrogen-fixing rhizobium *Bradyrhizobium japonicum* and the photosynthetic bacterium *Rhodospseudomonas palustris* (13). It nodulates stems, and the stem nodules exhibit light-stimulated CO₂ uptake (6). Like many photosynthetic bacteria, it exhibits a light-dependent, oxygen-sparing effect (11) which is saturated at high light intensities. This is similar to the saturation effect of light on photosynthesis found by van Neil (12).

The action spectrum for this effect is similar to the phototactic action spectrum determined by Clayton for *Rhodospirillum rubrum* (1). Similarly, the carotenoids in strain BTAi 1, as measured by A_{abs}, do not contribute to this effect. Because of the light scattering in the blue region of the spectrum, the magnitude of the oxygen-sparing effect cannot be directly compared between the red and blue regions of the spectrum. However, on a qualitative basis the oxygen-sparing effect does correlate well with the absorption spectrum of bacteriochlorophyll in cell extracts.

The action spectrum of the oxygen-sparing effect and the increase in the oxygen-sparing effect with increased light intensity both support the conclusion that this organism shares components of its photosynthetic electron chain and respiratory electron chain, as do other photosynthetic bacteria (9). Increased oxygen sparing in the presence of the respiratory substrate succinic acid also provides evidence for shared systems in this organism.

The ability of succinic acid to negate the oxygen-sparing effect at higher concentrations suggests that strain BTAi 1 possesses a bypass system similar to that postulated by Melandri and Zannoni (8) to be present in *Rhodospseudomonas capsulata*.

While strain BTAi 1 possesses the genetic makeup and apparatus essential to a symbiotic rhizobium, the data described above show that its photosynthetic-respiratory system is similar to that found in photosynthetic bacteria. Accordingly, strain BTAi 1 could be a heretofore undiscovered link between rhizobia and photosynthetic bacteria.

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**REFERENCES**