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Effect of Low-Intensity Light on Growth Response and Bacteriochlorophyll Concentration in *Rhodospirillum rubrum* Mutant C[1]

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Photosynthesizing cells of *Rhodospirillum rubrum* mutant C underwent adaptation for growth in low-light conditions. During adaptation there was a transitory increase in the generation time and the bacteriochlorophyll *a* content of the cells.

Rhodospirillum rubrum mutant C grew in strictly anaerobic (1, 4) dark or light conditions (6). Photosynthetically grown cells grew more rapidly when transferred directly into dark conditions than into low-light illumination. This suggested that small amounts of radiant energy repressed growth. The present study describes the growth response of mutant C over a range of light levels (5) to complete darkness.

Cells were grown in culture tubes (16 by 150 mm) containing freshly prepared medium with sodium pyruvate (6, 7). Dark growth conditions were described earlier (7). Growth at different light intensities, measured in lux, was achieved by placing cultures at different distances from the light source. Growth temperature was 30 C, except in saturating light intensity (5,918 lux) where the medium became heated to 32 C. Cell growth was measured turbidimetrically at 660 nm; brief exposure to 660-nm light did not affect growth rates. Growth measurements were begun when cells in cultures reached 0.1 optical density (OD) unit and continued thereafter at 1- or 2-h intervals. To minimize the effect of "self-shading" in light-grown cultures, measurements were stopped when the turbidity of cultures reached 0.45 OD units. This corresponded to approximately 5×10^8 cells per ml (depending upon the growth condition used). OD measurements were converted to equivalent cell numbers by using standard curves derived from direct cell counts (7). Estimates of cell numbers in replicate cultures were subject to a least-squares analysis using a computer. Growth curves were constructed from a minimum of six serial cell number measurements obtained for each culture under different growth conditions used, and the generation times were calculated (3).

Cells were grown in conditions ranging between 0- and 5,918-lux illumination (Fig. 1). As anticipated, generation times increased during growth at light intensities less than 5,918 lux (2) and reached a maximum of 10.3 h in 215.2-lux illumination. Cells exposed to lower light intensities, however, grew faster. In the dark, a generation time of 7.3 h was measured. A series of experiments was performed to determine whether the inhibitory effect of 215.2-lux illumination on cell division persisted during repeated subculture; experimental details are described in the legend to Fig. 2. In this case, a different light source was used. The ratio of bacteriochlo-

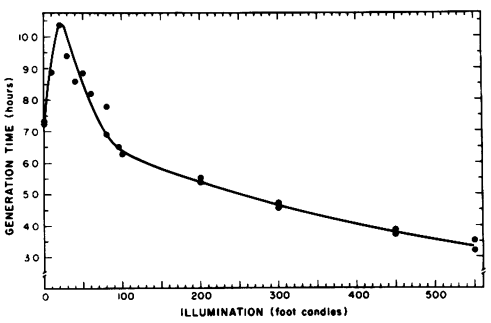

FIG. 1. *Generation times of Rhodospirillum rubrum mutant C in different light conditions. Medium was inoculated with cells serially subcultured twice in 5,918.0-lux intensity; cells grown in high-light intensity were added to produce a final concentration of about 10^8 cells per ml. Mean generation times at specific light intensities represented the average response obtained from two cultures incubated similarly. Light source was 150-W Sylvannia reflector spot lamp; light conditions below 1,076.0 lux were achieved by filtering light through wire mesh screens.*

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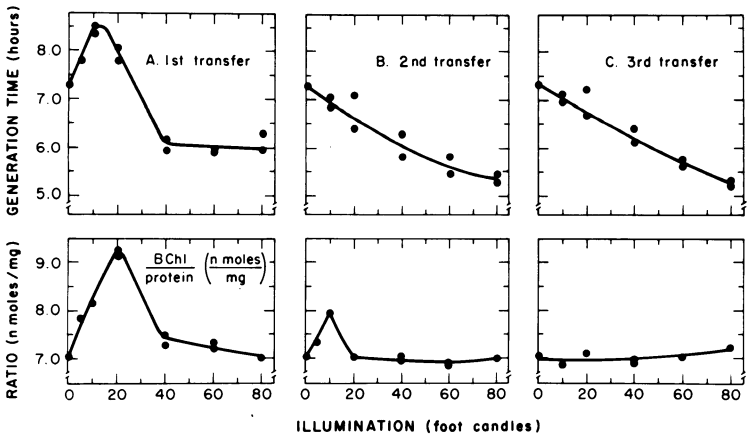

FIG. 2. *Generation times of Rhodospirillum rubrum mutant C during repeated subculture under different light conditions. (A) Medium containing 0.5% (wt/vol) sodium pyruvate was inoculated with cells (subcultured twice in 5,918.0-lux light) to produce a final concentration of 10^8 cells per ml. Two separate cultures were placed into each light condition indicated. After about two generation times, turbidity of cultures was approximately 0.45 OD units. (B) Cells obtained from separate cultures in (A) were inoculated into fresh medium to obtain a final concentration of about 2×10^7 cells per ml. Separate cell samples transferred in this manner were placed back into the previous light-growth condition. After approximately 4.5 generations, separate cultures reached a turbidity of 0.45 OD units. (C) Process described in (B) was repeated, and cells in separate cultures underwent an additional 4.5 generations. From initial transfer of high-light grown cells into low-light conditions (A), to the end of the experiment (C), cells in separate light conditions experienced about 11 generations. Ratio of concentration of bacteriochlorophyll a (BChl) per milligram protein in separate cultures at the end of each growth period are presented below the appropriate growth response curves. Light source was 200-W light bulb (A-23; ITT Lamp Division, Lynn, Mass.).*

rophyll *a* (BChl) to protein (7) was determined in separate cultures at the end of the growth period (i.e., when cultures reached about 0.45 OD units) (Fig. 2).

The growth response of separate cultures of high-light-grown cells placed into different low-light conditions shown in Fig. 1 and Fig. 2A were similar. During approximately two generations of growth after initial transfer from conditions of saturating light intensity (Fig. 2A), *R. rubrum* cells became adapted to low illumination, with the result that generation time decreased and appeared to stabilize at values characteristic of the light intensity (Fig. 2B and C). Cells fully adapted to growth at low-light intensities subsequently grew more rapidly than cells in dark cultures (Fig. 2B and C).

Changes in the concentration of BChl *a* in cells (Fig. 2; below appropriate growth response curves) resembled the growth response of the cultures. Pigment concentration increased in cultures where light energy for photosynthetic growth (2, 5) became limiting (Fig. 2A, below). This increase was transitory and only lasted during the period of adaptation of the cells to growth at low-light intensities (Fig. 2B, below; and 2C, below). After 11 generations of growth in low light (Fig. 2C, below), the amount of BChl *a* in separate cultures placed into dark- or low-light conditions was similar.

These observations suggested that photosynthesizing *R. rubrum* became adapted for growth in low light; a similar response was not observed with dark-grown cells exposed to low amounts of radiant energy (data not shown). The adaptive process in photosynthetically grown cells occurred over a period required for about two cell divisions and resulted in a temporary increase in generation times and changes in BChl *a* concentration. Prolonged division times might have resulted from an inhibitory effect of radiant energy. Certain light-sensitive reactions in the cells may have responded to low-light and "competed" with alteration(s) which were necessary to enable the cells to grow at low levels of illumination. The light-sensitive reactions may be repressed and/or diluted during subsequent low-light growth. Cells placed directly into darkness probably experienced similar change(s). Consequently, study of *R. rubrum* mutant C grown in high-light and then exposed to low-light illumination might be useful in resolving the sequence of events in cells during transition from photosynthetic to dark, fermentative metabolism.

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