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Attenuated Effect of Oxygen on Photopigment Synthesis in *Rhodospirillum centenum*

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Rhodospirillum centenum **resembles typical nonsulfur photosynthetic bacteria in a number of respects, including its ability to grow either anaerobically as a phototroph or aerobically as a heterotroph. We demonstrate, however, that *R. centenum* is unusual in its ability to synthesize a functional photosynthetic apparatus regardless of the presence of molecular oxygen. Aerobically expressed photopigments were shown to be functionally active, as demonstrated by the ability of heterotrophically grown cells to grow photosynthetically, without a lag, when suddenly placed under anaerobic conditions. An *R. centenum* mutant that has acquired the ability to repress synthesis of photopigments in the presence of oxygen was also characterized. Both the wild type and the oxygen-repressed mutant of *R. centenum* were found to exhibit high light intensity repression of photopigment biosynthesis. The latter result suggests that *R. centenum* contains separate regulatory circuits for controlling synthesis of its photochemical apparatus by light intensity and oxygen.**

The recently discovered nonsulfur purple bacterium *Rhodospirillum centenum* exhibits an in vivo photopigment spectrum virtually identical with that of *Rhodospirillum rubrum* (6, 13), to which it is closely related (on the basis of 16S rRNA sequence analysis [16]). *R. centenum*, however, differs from *R. rubrum* and from other more frequently studied photosynthetic bacteria in a number of respects. Thus, *R. centenum* (i) is able to grow at temperatures as high as 47°C, (ii) forms desiccation- and heat-resistant cysts, (iii) produces intracellular R bodies typically observed in endosymbionts of paramecia, (iv) forms motile colonies that migrate towards light (19), and (v) shows an atypical absence of oxygen repression of photopigment biosynthesis. In this study, we undertook an analysis of the last trait and provide evidence that the wild-type *R. centenum* isolate synthesizes a functional photosynthetic apparatus when grown aerobically. Mutants of *R. centenum* that have acquired enhanced repression of pigment biosynthesis by oxygen have also recently been described (18). Phenotypically, the oxygen-repressed mutants are shown in this study to behave more like typical representatives of nonsulfur purple bacteria than does the wild-type isolate during growth shift experiments. The results of this study are discussed in terms of our current understanding of oxygen regulation of photosynthesis in *Rhodobacter* species.

MATERIALS AND METHODS

Bacterial strains and growth media. The wild-type strain of *R. centenum* used in this study (ATCC 43720) is a thermotolerant nonsulfur purple bacterium isolated from Thermopolis Hot Springs, Wyo. (6). *R. centenum* FY040, that fails to synthesize a reaction center complex, and FY013, which exhibits oxygen repression of pigment biosynthesis, have been described previously (18). *R. centenum* strains were routinely grown at 42°C in CENMED, a synthetic minimal medium containing pyruvate as the carbon source (6), or in CENS medium, which consists of CENMED supplemented with 0.4% Difco Soytone (13).

The wild-type strain of *Rhodobacter capsulatus* used in this study, strain SB1003, has been described previously (17). It was grown at 35°C either anaerobically under photosynthetic conditions or aerobically in darkness as a heterotroph in synthetic medium RCV (15) or in RCV supplemented with peptone and yeast extract (RCV 2/3 PY; described in reference 20).

Growth conditions. For photosynthetic growth, unless otherwise noted, cells were cultivated in 17-ml screw-cap tubes completely filled with medium. In some experiments, cultures were grown photosynthetically in sealed sidearm flasks with large headspace that was sparged with an anaerobic gas mixture composed of 95% N_2 and 5% CO_2. Cultures were routinely illuminated by banks of incandescent Lumiline 60-W lamps at a light intensity of 5,380 lx. In experiments requiring high light intensities, cultures were incubated in a glass-sided water bath and illuminated with two 150-W reflector flood lamps. For experiments requiring low light intensities, cultures were shaded by a neutral density filter. Light intensities were determined with a Weston Illumination Meter model 755 (Weston Inst. Inc.).

For aerobic (heterotrophic) growth, 20-ml cultures were incubated in 250-ml sidearm flasks that were shaken at 200 to 250 rpm in a gyrotory water bath shaker. Defined O_2 tensions were obtained by continually sparging shaking flasks at a rate of 500 ml/min with O_2-N_2-CO_2 gas mixtures which were generated with a Linde Mass Flowmeter (FM4575). Dissolved-O_2 tension in the culture medium was determined prior to cell harvesting by using an oxygen electrode (4004 Clark Oxygen Probe; Yellow Springs Instruments). In order to avoid secondary effects known to occur at high cell densities (such as O_2 depletion and self shading), cultures were harvested at the relatively low cell density of 50 Klett-Summerson photometer units (filter no. 66), which corresponds to a cell density of ca. 1.5×10^8 cells per ml.

Extraction of pigments. The total bacteriochlorophyll (Bchl) *a* content of cells was measured by extracting pigments from cell pellets with cold acetone-methanol (7:2) as described previously (3). For quantitative determination of carotenoids, cells pellets were extracted with ethyl ether-methanol (3:5) and then saponified as described in reference

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1. Carotenoids were subsequently dried under nitrogen, suspended in benzene, and measured for carotenoid content using $E^{1\%}_{1cm}$ 2.470 at 510 nm. The protein content of the cells was determined with a Bradford Protein Assay Kit (Bio-Rad Inc).

Other procedures. In vivo absorption spectra of photosynthetic pigments were determined by suspending intact cells in 30% (wt/vol) bovine serum albumin (Sigma Chemical Co.), followed by scanning such cell suspensions from 400 to 900 nm with a Beckman DU-50 recording spectrophotometer (12, 14). For electron microscopy, cells were grown to mid-log phase in CENMED medium, fixed by treatment with glutaraldehyde, dehydrated in an ethanol series, embedded, and sectioned as in previous studies (8).

RESULTS AND DISCUSSION

***R. centenum* exhibits attenuated oxygen regulation of photopigment biosynthesis.** In typical representatives of nonsulfur purple bacteria, the synthesis of photopigments is known to be repressed by molecular oxygen (2, 5). We previously reported (13) that *R. centenum* appears to lack oxygen repression of photopigment biosynthesis, as evidenced by the observation that cells grown aerobically form pigmented colonies that do not have a halo of unpigmented growth. The marked attenuation of oxygen regulation of photopigments in *R. centenum* is more clearly seen by comparing in vivo spectra of *Rhodobacter capsulatus* and *R. centenum* cells grown photosynthetically in the absence of oxygen and aerobically in darkness. As demonstrated by spectral analysis (Fig. 1) and analytical measurements of photopigments (see Table 2), the presence of 20% oxygen during growth of *Rhodobacter capsulatus* results in a 28-fold reduction in Bchl *a* levels whereas in *R. centenum*, photopigment absorbancies are reduced to a relatively minor extent (<2-fold). Thin-section electron micrographs confirm aerobic synthesis of characteristic photosynthetic membranes in the cell periphery (in the form of concentric lamellae) in aerobically grown *R. centenum* (Fig. 2B). These observations indicate that *R. centenum* lacks stringent oxygen regulation of photopigment synthesis.

Evidence that intracytoplasmic membranes produced during dark aerobic growth of *R. centenum* contain a functional photosynthetic apparatus was obtained from growth experiments. With L-glutamate as the sole carbon and energy source, the aerobic doubling time of *R. centenum* is quite long, namely, ca. 25 h (Table 1). However, illumination of aerobically growing cultures with saturating light decreased the doubling time to 15 h, which is equivalent to that observed with cells growing photosynthetically in the same medium. The decrease in aerobic generation time as a result of illumination most likely results from the utilization of light as an energy source, as evidenced by the absence of an effect of light on the generation time of a strain of *R. centenum* that lacks photosynthetic reaction centers (FY040). Additional evidence for aerobic synthesis of a functional photosynthetic apparatus is presented below.

Mutants of *R. centenum* with acquired oxygen repression of photopigment biosynthesis. Mutants of *R. centenum* were obtained by adapting a procedure described by Madigan et al. (10) for isolation of spontaneous mutants of *Rhodobacter capsulatus* incapable of photosynthetic growth (PS^-). Nonpigmented mutants of *Rhodobacter capsulatus* arise frequently after a number of serial subcultures of cells growing under dark anaerobic growth conditions in a medium containing glucose as the carbon and energy source and dimethyl sulfoxide as an electron acceptor. Although *R. centenum* does not show appreciable growth under the conditions noted, after 48 h of incubation under similar conditions, PS^- mutants are found at frequencies as high as 10^{-3} (18). Also, among the cells surviving the anaerobic dark incubation period are mutants, such as strain FY013, which form colonies that exhibit halos of unpigmented growth when grown in the presence of oxygen. Such colonies represent mutants that appear to have acquired repression of photopigment synthesis in response to oxygen. Analytical measurement of pigment levels in this strain demonstrates that under anaerobic growth conditions FY013 exhibits a 1.6-fold reduction in Bchl *a* and a 2.6-fold reduction in carotenoid levels relative to those in wild-type *R. centenum* (Table 2). The factor(s) that leads to a reduction in photopigment levels under anaerobic conditions remains to be determined. In contrast, under aerobic growth conditions FY013 exhibits an 11-fold-lower level of Bchl *a* and a 26-fold reduction of carotenoid content compared with levels in aerobically grown wild-type *R. centenum*. Thus, FY013 behaves more like the wild-type strain of *Rhodobacter capsulatus* in its response to changes in oxygen tension.

A functional test of the effects of oxygen on the growth characteristics of the wild type and the oxygen-repressed strain of *R. centenum* was also undertaken by performing a growth shift experiment (dark aerobic to photosynthetic). It has long been observed, with species of photosynthetic bacteria that exhibit oxygen repression, that shifting exponentially growing cells from dark aerobic to photosynthetic growth conditions causes a lag period of no growth (4). Presumably, this lag represents an adaptation period during which cells must induce anaerobic synthesis of the photochemical apparatus before the cells can utilize light as an energy source. Synthesis of a functional photosynthetic apparatus in wild-type *R. centenum* grown aerobically is evidenced by the absence of a lag of growth when cells undergo such a shift (Fig. 3). With FY013, however, growth stops as soon as the shift is made and does not resume until after a 3-h lag period. This phenotype resembles that observed for *Rhodobacter capsulatus*, which in a parallel experiment exhibited a 5-h lag (19).

Molecular oxygen and light intensity appear to control photopigment biosynthesis by independent mechanisms. Synthesis of photopigments in photosynthetically growing nonsulfur purple bacteria is, within limits, inversely proportional to light intensity (4). Cohen-Bazire et al. (4) proposed that a regulatory signal, related to the redox state of an unspecified regulatory electron carrier, is directly involved in the control of photopigment biosynthesis by both molecular oxygen and light intensity. The availability of FY013 provides the opportunity to test this model by measuring the response of the mutant to changes in light intensity relative to that shown by wild-type *R. centenum*. The data of Table 2 show that the extent of repression of Bchl synthesis caused by an increase of light intensity is approximately the same in FY013 and the wild-type strain, namely, three to fivefold. The analytical data are further supported by electron micrographs which show an increased amount of intracytoplasmic membrane present in cells grown under dim light conditions for both wild-type and FY013 cells (19). Accordingly, the data at hand suggest that the light intensity control mechanism remains intact in FY013.

Conclusions. Oxygen repression of bacterial photosynthesis is thought to be one of the most conserved physiological traits in photosynthetic bacteria. It has been reported for representative isolates of the nonsulfur purple genera *Rho-*

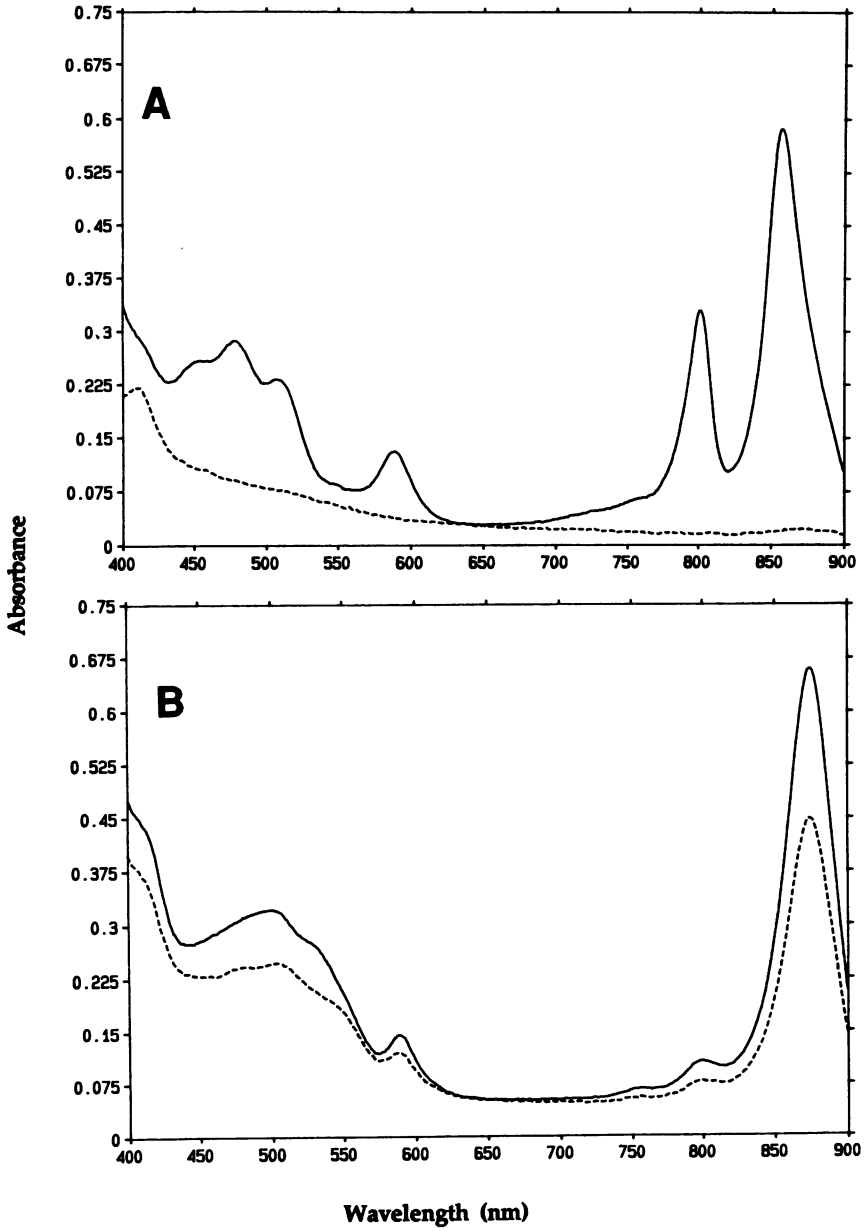

FIG. 1. In vivo absorption spectra of aerobically and anaerobically grown cells. Equal numbers of cells were scanned for their in vivo spectra as indicated in Materials and Methods. Dashed lines denote aerobically grown cells, whereas solid lines indicate anaerobically grown cells. (A) *Rhodobacter capsulatus* cells grown in PY; (B) *R. centenum* cells grown in CENS.

dopseudomonas, *Rhodospirillum*, and *Rhodobacter* (reviewed in reference 5) as well as for the evolutionary distant filamentous green bacterium *Chloroflexus aurantiacus* (7). The ability to synthesize photopigments under aerobic growth conditions is not unique to *R. centenum*, since certain obligate aerobes, such as *Erythrobacter* spp. (11), have been reported to synthesize Bchl *a* as well as a functional photosynthetic apparatus under aerobic conditions. However, such obligate aerobes differ from *R. centenum* in that they have not been demonstrated to utilize light as the only source of energy for growth. To our knowledge, *R. centenum* is the first bacterium described which has the ability to both utilize light as an anaerobic energy source for growth and also synthesize functional photopigments under aerobic growth conditions. Collectively, the results cited suggest that there are a number of species of bacteria not yet isolated or characterized which have very different responses to environmental signals such as molecular oxygen.

From the present studies with *R. centenum*, it is obvious that the oxygen control mechanism is subject to modification by mutational events. Lascelles and Wertlieb (9) described the isolation of an *Rhodobacter sphaeroides* mutant (TA-R) which synthesized photopigments aerobically. Their mutant exhibited behavior similar to that of wild-type *R. centenum*, including an absence of a growth lag in cultures suddenly shifted from dark aerobic to light anaerobic conditions. They also concluded that TA-R synthesized a functional photosynthetic apparatus during aerobic growth. Although the

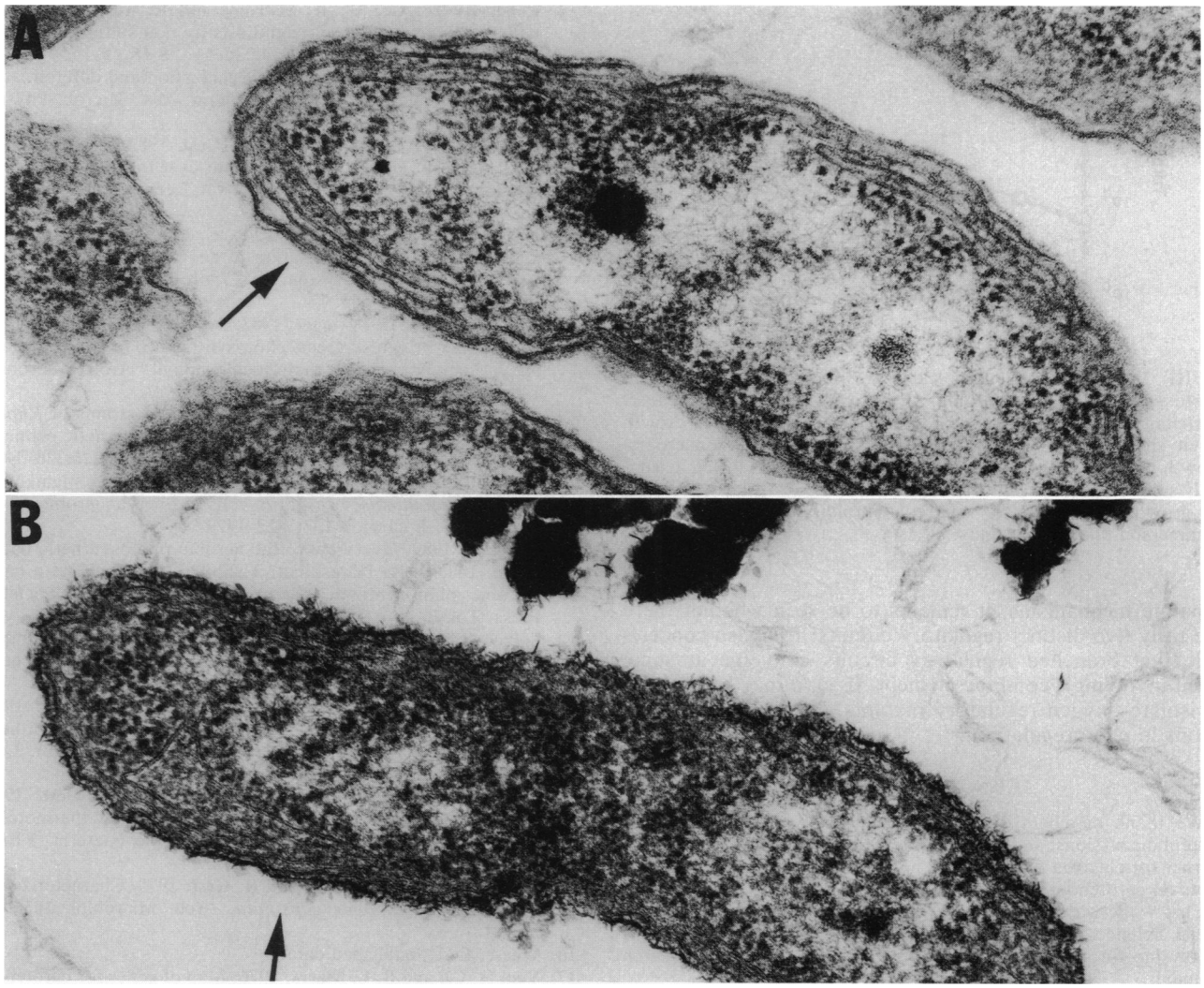

FIG. 2. Ultrathin-section electron micrographs of *R. centenum* cells. Lamellar-type photosynthetic membranes (arrows) are visible in cells grown anaerobically in the light (A) or aerobically in the dark (B). Magnification, ×93,000.

molecular mechanism of the mutational events that allow FY013 to control pigment biosynthesis by oxygen and the constitutive production of photopigments by *Rhodobacter sphaeroides* TA-R are unknown, these mutants should be valuable in future investigations.

In regard to our conclusion that control of photopigment synthesis by oxygen and high light intensity occurs through

TABLE 1. Effect of light on aerobic growth rate of *R. centenum*

Growth conditions[a]	Time (h) required for doubling optical density	
	Wild type	FY040 (RC^{-}[b])
Aerobic dark	25	24.5
Aerobic light	15	24.5
Anaerobic light	14	—[c]

[a] Aerobic and anaerobic growth conditions are as described in the text with the light intensity set at 5,380 lx.
[b] RC^{-}, does not form reaction center complexes.
[c] —, no growth.

TABLE 2. Effects of oxygen and light intensity on photopigment contents of cells

Growth condition	Bchl (μg/mg of protein) in:			Carotenoid (μg/mg of protein) in:	
	R. centenum Favinger/Gest	*R. centenum* FY013	*Rhodobacter capsulatus* SB1003	*R. centenum* Favinger/Gest	*R. centenum* FY103
% Dissolved oxygen					
0	12.8	8.0	19.9	5.0	1.9
5	12.1	2.8	2.3	—[a]	—
10	10.5	1.3	1.3	—	—
20	9.0	0.8	0.7	3.2	0.1
Light intensity[b]					
Low	62.9	40.0	74.8	—	—
High	20.2	8.2	27.4	—	—

[a] —, not determined.
[b] Low, 215 lx for *R. centenum* and 323 lx for *Rhodobacter capsulatus*; high, 5,380 lx for *R. centenum* and 21,520 lx for *Rhodobacter capsulatus*.

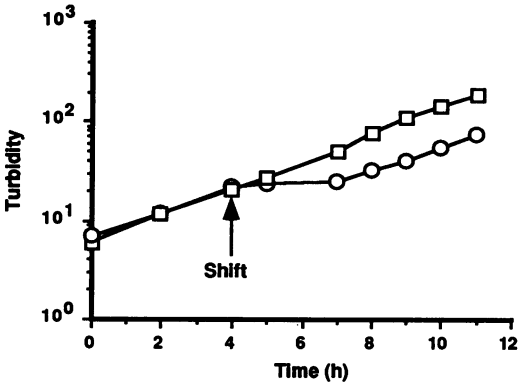

FIG. 3. Response of aerobically growing cells to a shift to photosynthetic growth conditions. Aerobically growing wild-type *R. centenum* cells (open squares) exhibit no significant lag in growth when shifted from darkness to photosynthetic (light anaerobic) growth conditions. In contrast, *R. centenum* FY013, which aerobically suppresses photopigment synthesis, exhibits a 3-h lag period of no growth when similarly shifted. Turbidity is given in Klett-Summerson photometer units.

separate mechanisms, it remains to be seen whether there are truly two distinct regulatory circuits. It is also conceivable that branched regulatory circuits converge at some point involving a common element. If so, it may be possible to isolate oxygen regulatory mutants that also show alterations in photoregulation.

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REFERENCES

1. **Britton, G., and T. W. Goodwin.** 1971. Biosynthesis of carotenoids. Methods Enzymol. **18**:672–693.
2. **Chory, J., T. J. Donohue, A. R. Varga, L. A. Staehelin, and S. Kaplan.** 1984. Induction of the photosynthetic membranes of *Rhodopseudomonas sphaeroides*: biochemical and morphological studies. J. Bacteriol. **159**:540–554.
3. **Clayton, R. K.** 1966. Spectroscopic analysis of bacteriochlorophylls *in-vitro* and *in-vivo*. Photochem. Photobiol. **5**:669–677.
4. **Cohen-Bazire, G., W. R. Sistrom, and R. Y. Stanier.** 1957. Kinetic studies of pigment synthesis by non-sulfur purple bacteria. J. Cell. Comp. Physiol. **49**:25–51, 58–68.
5. **Drews, G., and J. Oelze.** 1981. Organization and differentiation of membranes of phototrophic bacteria. Adv. Microb. Physiol. **22**:1–81.
6. **Favinger, J., R. Stadtwald, and H. Gest.** 1989. *Rhodospirillum centenum, sp. nov.*, a thermotolerant cyst forming anoxygenic photosynthetic bacterium. Antonie van Leeuwenhoek J. Microbiol. **31**:317–322.
7. **Foster, J. M., T. E. Redlinger, R. E. Blankenship, and R. C. Fuller.** 1986. Oxygen regulation of development of the photosynthetic membrane system in *Chloroflexus aurantiacus*. J. Bacteriol. **167**:655–659.
8. **Gest, H., and J. L. Favinger.** 1983. *Heliobacterium chlorum*, an anoxygenic brownish-green photosynthetic bacterium containing a "new" form of bacteriochlorophyll. Arch. Microbiol. **136**:11–16.
9. **Lascelles, J., and D. Wertlieb.** 1971. Mutant strains of *Rhodopseudomonas sphaeroides* which form photosynthetic pigments aerobically in the dark. Biochim. Biophys. Acta **226**:328–340.
10. **Madigan, M. T., J. C. Cox, and H. Gest.** 1982. Photopigments in *Rhodopseudomonas capsulata* cells grown anaerobically in darkness. J. Bacteriol. **137**:1422–1429.
11. **Shiba, T.** 1989. Overview of the aerobic photosynthetic bacteria, p. 1–8. *In* K. Harashima, T. Shiba, and N. Murata (ed.), Aerobic photosynthetic bacteria. Japan Scientific Societies Press, Tokyo.
12. **Sojka, G. A., H. H. Freeze, and H. Gest.** 1970. Quantitative estimation of bacteriochlorophyll in situ. Arch. Biochem. Biophys. **136**:578–580.
13. **Stadtwald-Demchick, R., F. R. Turner, and H. Gest.** 1990. Physiological properties of the thermotolerant photosynthetic bacterium, *Rhodospirillum centenum*. FEMS Microbiol. Lett. **67**:139–144.
14. **Stadtwald-Demchick, R., F. R. Turner, and H. Gest.** 1990. *Rhodopseudomonas cryptolactis, sp. nov.*, a new thermotolerant species of budding phototrophic purple bacteria. FEMS Microbiol. Lett. **71**:117–122.
15. **Weaver, P. F., J. D. Wall, and H. Gest.** 1975. Characterization of *Rhodopseudomonas capsulata*. Arch. Microbiol. **105**:207–216.
16. **Woese, C.** Unpublished data.
17. **Yen, H. C., and B. L. Marrs.** 1976. Map of genes for carotenoid and bacteriochlorophyll biosynthesis in *Rhodopseudomonas capsulata*. J. Bacteriol. **126**:619–629.
18. **Yildiz, F. H., H. Gest, and C. Bauer.** 1991. Genetic analysis of photosynthesis in *Rhodospirillum centenum*. J. Bacteriol. **173**: 4163–4170.
19. **Yildiz, F. H., H. Gest, and C. Bauer.** Unpublished data.
20. **Young, D. A., C. E. Bauer, J. C. Williams, and B. L. Marrs.** 1989. Genetic evidence for superoperonal organization of genes for photosynthetic pigments and pigment binding proteins in *Rhodobacter capsulatus*. Mol. Gen. Genet. **218**:1–12.