Proceedings of the National Academy of Sciences
Vol. 68, No. 1, pp. 136–138, January 1971

Temperature-Sensitive Mutations of the Photosynthetic Apparatus of *Rhodospirillum rubrum*

PAUL WEAVER

Department of Biology, University of California, San Diego, La Jolla, Calif. 92037

Communicated by Martin D. Kamen, October 26, 1970

ABSTRACT **Temperature-sensitive mutants of *Rhodospirillum rubrum* have been isolated by enrichment techniques selecting for conditionally aberrant electron flow in various portions of the electron transport scheme. The temperature sensitivity of a class of these strains is shown to preferentially affect the photosynthetic mode of growth and energy production over the aerobic mode.**

The sequence of electron carriers in the energy producing systems of photosynthetic bacteria remains largely unresolved. Spectroscopic analysis in the presence of inhibitors, which has been of great value in formulating the concept of the mitochondrial electron transport chain, is limited by the paucity of inhibitors affecting specific sites. As an alternative, a broad spectrum of mutant strains would (theoretically) make possible observations on the effects of interrupted electron flow at each particular component of the chain. Since the mutant approach has been so useful in elucidating the sequential events within the metabolic pathways of microorganisms, I attempted to isolate classes of temperature-sensitive (TS) mutants conditionally unable to perform photosynthetic functions associated with electron flow. The feasibility and potential value of such an investigation on photosynthetic bacteria has been indicated by studies on photosynthetically-deficient green algae (1, 2), and the isolation (3) and characterization (4) of a mutant of *Rhodopseudomonas spheroides* lacking reaction-center bacteriochlorophyll.

Rhodospirillum rubrum (strain 1.1.1), a facultative photoheterotroph, was cultured on modified Hutner medium (5) unless otherwise specified. The optimal temperature for growth was 32°C. Approximately 40% of the maximal growth rate was observed at 22 and 35°C; these are termed the "temperature extremes." *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Aldrich) was the mutagenic agent used in all cases. The procedure of Adelberg *et al.* (6) was followed. After treatment with the mutagen, approximately 10% of the cells remained viable. Of these, 0.1–1% showed altered pigment compositions when developed clones were observed on an agar surface. Without enrichment techniques, no TS colonies were detected by replica plating methods between the temperature extremes.

The major selection method used was based on the assumption that the phototactic response of photosynthetic cells requires a properly functioning electron transport chain. A transition from light to dark presumably causes an abrupt change in the energy level of some intermediate in the cell which triggers an immediate reversal of swimming direction, termed the "schreckbewegung" response by Englemann. This interpretation is inferred from the rapidity of the response (~0.1 sec) and relaxation (~1 sec) times (7) and from the action spectra for phototaxis, as determined by Manten (8) and Clayton (9), which follow the *in vivo* absorption spectrum of bacteriochlorophyll and carotenoids. A blue-green mutant (devoid of colored carotenoids) showed dark-induced changes of movement only with light of wavelengths corresponding to the major bacteriochlorophyll absorption bands (unpublished observations).

One edge of an anaerobic Brewer dish filled with a medium containing 0.01% $CaCl_2$, 0.01% $MgCl_2$, 0.1% $(NH_4)_2SO_4$, 15 mM phosphate buffer (pH 7.2), and 3 mM malate was inoculated with 10^{10} mutagenized cells that had been grown at 22°C, centrifuged, and gently suspended in 1 ml of medium. An agar wedge containing 0.02% India ink was made to the dimensions of the top surface of the dish. Light from a 40-W light bulb was directed through the wedge effecting a gradient of light increasing from the inoculation point to the side opposite, 8-cm away. A fan kept the chamber at approximately 22°C. A marked concentration of cells in the small zone of maximal light intensity could be observed after 1.5–2 hr and was completed in 4 hr. At that time the apparatus was incubated at 35°C. After 2 hr. the light gradient was reversed; 6 hr were allowed for the phototactic cells to return to the inoculation point. An aliquot from the zone of maximal phototaxis at 22°C was then taken (Fig. 1). Viable cell density was about 10^4 cells/ml. Cells were plated at 22°C and replica plated to the temperature extremes. The 0.1–0.5% of the clones appearing only on the 22°C plate were isolated as conditionally lethal mutations. Further treatment of the aliquot with 1000 units/ml penicillin G (Sigma) at 35°C for 14 hr decreased the viable cell concentration to 0.2–1.0 $\times$ 10^3 cells/ml, and increased the TS-mutant yield to 1–5%. Conditional loss of the phototactic response in this population usually was not associated with loss of motility. Strains that

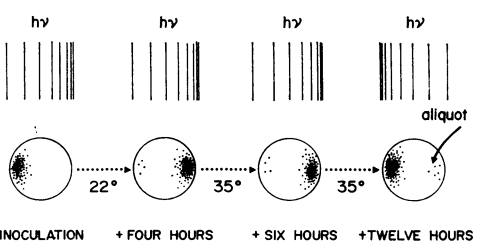
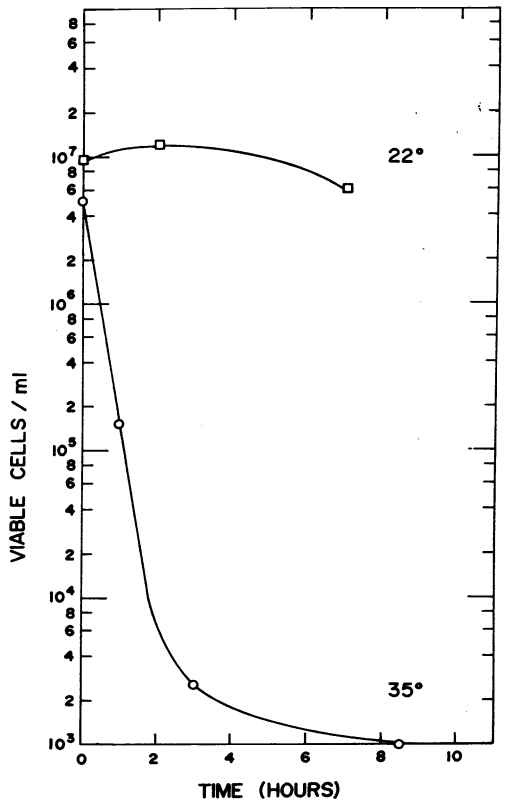
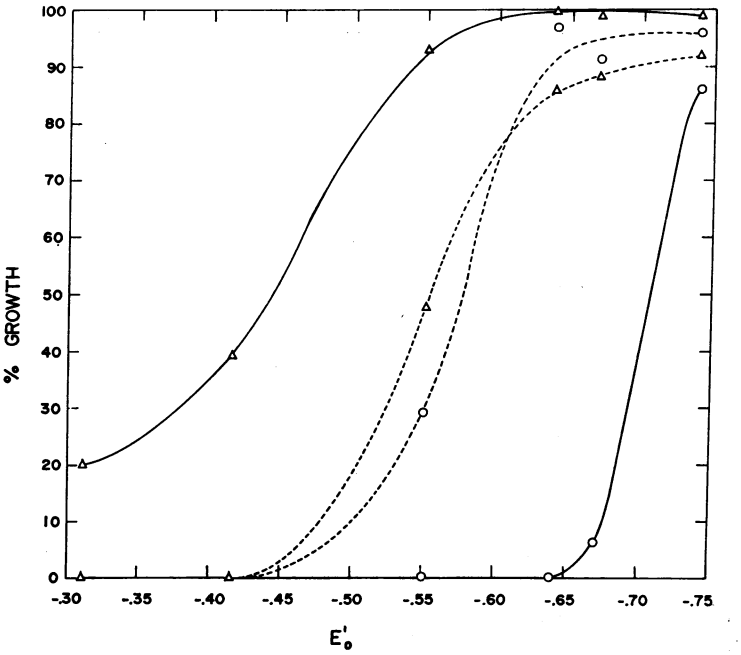


FIG. 1. Scheme for mutant enrichment using phototactic technique.

Abbreviations: TS, temperature-sensitive (mutant).





(*Left*) FIG. 2. Photo-oxidative killing of strain B14. Aliquots of 22 and 35°C cultures were exposed to air and 2×10^5 ergs cm^{-2} sec^{-1} light at 0 time.

(*Right*) FIG. 3. Inhibition of growth in the presence of 0.2 mM viologen dyes of different E'_0. Solid lines: Anaerobic, light growth on modified Hutner (△) and malate–glutamate (○) media. Dashed lines: Aerobic, dark growth on modified Hutner (△) and malate–glutamate (○) media. % growth was determined from the number of generations of growth in a culture containing dye, relative to a control containing 0.2 mM NaCl, at the end of the logarithmic phase of growth. Cultures received 2% inocula; then turbidity doublings were monitored on a Klett-Summerson colorimeter (no. 66 filter). Viologen dyes were obtained through the courtesy of Dr. A. Calderbank, Jealott's Hill Research Station, England.

lost motility and/or their phototactic response, but grew normally, were excluded by replica plating, but could be isolated after microscopic observations of the individual colonies.

B14, a TS carotenoid-producing strain, provided the basis for a second selection method. At 22°C this mutant possesses a normal complement of carotenoids and appears red, but when it is grown at 35°C, no colored carotenoids are synthesized and the cells appear bluish–green. As is true with other blue–green isolates, photosynthetic growth occurs only in the absence of oxygen. The combination of air and light is rapidly lethal to the majority of the cells (Fig. 2). Photo-oxidative injury has been attributed to an interaction of an excited state of bacteriochlorophyll with oxygen (10).

A B14 culture that had been grown at 22°C was mutagenized and anaerobically incubated in growth medium at 35°C. Those cells capable of growth at high temperature replicated, diluting out their previously synthesized carotenoids, and, after 3–5 generations, became susceptible to photo-oxidative killing upon the introduction of air. Theoretically, only those cells incapable of high temperature replication would retain their carotenoids, survive the period of illuminated aerobiosis, and form colonies when plated at 22°C. After 3 hr of aerobic incubation, 0.05% of the cells remained viable. As had been seen with a blue–green strain of *R. spheroides* (11), a large majority of the colonies spread on plates developed very slowly. No attempt was made to segregate them; after replica plating, most were indistinguishable from the original large colonies. About 3% of the total showed TS growth properties. In a comparable enrichment with G9, an *R. rubrum* strain isolated by Dr. Jack Newton (U.S. Dept. of Agriculture, Peoria, Ill.) that never synthesizes colored carotenoids, when the cells were similarly treated with air and light, only 10^{-6} formed normal, large colonies when cultured at 22°C. Of ten such colonies screened for growth at high temperature, five grew aerobically in the dark, but poorly, if at all, photosynthetically. One grew photosynthetically, but not aerobically, and two failed to grow under either condition.

A third enrichment technique was designed to isolate mutations of an as yet ill-defined low potential system in which electron carriers have oxidation–reduction potentials below that of ferredoxin. The activity of this system can be monitored by observing growth rates in the presence of viologen dyes of different redox potentials (Weaver, *et al.*, manuscript in preparation). The oxidized form of the dye apparently is not inhibitory to growth, but when it is reduced by the low potential reductants within the cell bacteriostatic or bacteriocidal effects occur, possibly from the loss of metabolic specificity or from toxic reoxidation products. No indication

TABLE 1. *Growth and phosphorylative capacities of mutant strains at 35°C*

Strain	Parent	Selection method	Growth rate $\left(\frac{\text{generations}}{100\text{ hr}}\right)$ Anaerobic, light	Growth rate $\left(\frac{\text{generations}}{100\text{ hr}}\right)$ Aerobic, dark	ATP formed/A_{650} ($\times 10^{-6}$ M) 10 sec. light	ATP formed/A_{650} ($\times 10^{-6}$ M) 10 sec. air
1.1.1	...	...	14.1	12.5	2.83	2.92
I3	1.1.1	Conditional phototaxis	0.0	9.1	0.89	2.36
I4	1.1.1		0.9	11.1	0.81	2.22
D20	1.1.1		0.7	12.2	1.26	0.82
K1	1.1.1		0.0	4.3	2.34	2.10
B14D	B14	Conditional photo-oxidative	2.1	8.7	2.07	2.26
G9-6	G9		0.0	10.5	0.21	1.84
G9-8	G9	sensitivity	0.0	0.0	<0.005	<0.005
A33*	1·1·1	Conditional resistance to low potential dye	2.4	7.2	1.22	0.78

* Grown on minimal medium.

of a low potential system was observed in photosynthetic cultures grown on complex media. However, in minimal medium with glutamate, as the sole source of nitrogen (12), which is known to induce nitrogen fixation and light-dependent hydrogen evolution (13), marked inhibition of photosynthetic growth was noted in the presence of all viologen dyes more positive than −0.67 V (Fig. 3).

A mutagenized culture was incubated at 35°C for 6 hr in glutamate malata-minimal medium. 1,1′-Tetramethylene-2,2′-bipyridylium diiodide ($E'_0 = -0.64$ V), a viologen dye, was added to a final concentration of 0.5 mM. After 16 hr 0.1% of the cells remained viable. They were plated at 22°C and then replica plated to 35°C. Approximately 0.2% of the colonies grew only at the permissive temperature.

Table 1 shows the growth and phosphorylative capabilities of representative mutants at 35°C. *In vivo* phosphorylation measurements were made on ATP-depleted cells exposed to light or air for 10 sec. The depletion, extraction, and assay conditions of Welsch and Smith (14) were used. A number of strains can be seen to have major deficiencies in their photophosphorylative capacities with little loss of their oxidative phosphorylation abilities.

I thank Prof. M. D. Kamen for helpful discussions and for financial support afforded by grants to him from the National Institutes of Health (HD-01262) and the National Science Foundation (GB-7033X). Also, Dr. R. C. Valentine and Prof. C. B. van Niel generously provided facilities and enthusiasm.

1. Levine, R. P., *Annu. Rev. Plant Physiol.*, **20**, 523 (1969).
2. Powls, R., J. Wong, and N. I. Bishop, *Biochim. Biophys. Acta*, **180** (3), 490 (1969).
3. Sistrom, W. R., B. Ohlsson, and J. Crounce, *Biochim. Biophys. Acta*, **75**, 285 (1963).
4. Clayton, R. K., and W. R. Sistrom, *Biochim. Biophys. Acta*, **88**, 61 (1964).
5. De Klerk, H., R. G. Bartsch, and M. D. Kamen, *Biochim. Biophys. Acta*, **97**, 275 (1965).
6. Adelberg, E. A., M. Mandel, and G. Chen, *Biochem. Biophys. Res. Commun.*, **18**, 788 (1965).
7. Clayton, R. D., *Arch. Mikrobiol.*, **19**, 141 (1953).
8. Manten, A., *Antonie van Leeuwenhoek*, **14**, 65 (1948).
9. Clayton, R. K., *Arch. Mikrobiol.*, **19**, 107 (1953).
10. Griffiths, M., W. R. Sistrom, G. Cohen-Bazire, R. Y. Stanier, and M. Calvin, *Nature*, **176**, 1211 (1955).
11. Dworkin, M., *J. Gen. Physiol.*, **41**, 1099 (1958).
12. Ormerod, J. G., K. S. Ormerod, and H. Gest, *Arch. Biochem. Biophys.*, **94**, 449 (1961).
13. Gest, H., and M. D. Kamen, *Science*, **109**, 558 (1949).
14. Welsch, F., and L. Smith, *Biochemistry*, **8**, 3403 (1969).