Mutation of the Blue-Green Alga, Anacystis nidulans

Abstract. *Cells of* Anacystis nidulans *in the logarithmic phase of growth were briefly treated with the mutagen,* N-*methyl*-N′-*nitro*-N-*nitrosoguanidine, and plated under conditions suitable for single-cell growth. Selection of aberrant colonies and examination of the cultural characteristics of these clones suggest that mutants of at least several types are easily derivable in* Anacystis.

Most of the accumulated information on blue-green algae, aside from comparative studies on photosynthesis, pertains to their growth and nutritional characteristics (*1, 2*). Little is known of their biochemistry, and even less of their genetics. Kumar (*3*) attempted recombination experiments using penicillin- and streptomycin-resistant strains of *Anacystis nidulans*, but resistant clones were not isolated and the data are therefore only tentative.

Development of methodology for producing mutants is essential for investigation of blue-green algal genetics. Auxotrophic and pigment mutants can be expected to aid in investigating biochemical problems and details of reaction mechanisms in photosynthesis.

I now report data on the effect of the chemical mutagen, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (NTG) on *Anacystis nidulans*; these data suggest that mutants of blue-green algae can be obtained.

Anacystis nidulans used for inoculum was routinely grown in batches on Medium C (*1*) at 39°C, 8 cm from two fluorescent lamps; carbon dioxide (1 percent in air) was bubbled through the cultures. Single cells were plated on a modified Medium C, manganese chloride was omitted from the A-5 microelements, and A-5 was used at one-half the usual concentration (*1*). The medium was solidified with 1 percent Difco agar (Difco No. 0140), and the plates were sealed with Scotch tape. They were incubated at 32° to 35°C, 26 cm from a linear bank of 60-watt tungsten lamps. Under these conditions single cells quantitatively gave rise to pinpoint colonies after 3 days (*4*).

With *Anacystis* and several marine coccoids as test organisms, previous attempts to produce mutants with standard methods such as ultraviolet irradiation (2537 Å), heat, or nitrous acid have not been successful. However, clearly recognizable variant colonies were obtained in the following way. Cells of *Anacystis* in the logarithmic phase of growth were exposed to NTG (200 μg/ml) in Medium C for 2 to 10 minutes; the cells were separated by centrifugation and resuspended in fresh Medium C and placed for 3 hours under optimum conditions for growth. Samples were then removed and plated on modified Medium C appropriately enriched (Table 1).

Mutants 330y, 2a, and 2b represent commonly occurring types blocked at different stages in nitrate reduction. Mutant Pt6i was somewhat impaired in growth rate and the pigment ratio was permanently altered. The ratio OD_{678}/OD_{620} calculated from whole cell spectra, determined according to the method of Shibata (*5*), was 0.39. The ratio approaches that reported for wild-type *Anacystis* grown under predominately red illumination (*6*). So far no mutants have been found in which either chlorophyll or phycocyanin was entirely absent or in which a change in structure of the phycobilin was indicated. Mutant Hpur represents another common type in which cell division was apparently impaired. The colony morphology was altered from the normal circular, convex form to forms with an irregular or filamentous appearance. Microscopically the cells were normal in width but 12 to 15 times as long as normal, and the filaments were distorted and twisted. Similar mutant types are found in *Escherichia coli* (*7*). Only occasional cross walls were found. This aberrant morphology was not altered by the presence of vitamins, purines, or amino acids during growth. Mutant Bpur is apparently a temperature mutant. It did not grow at 39°C, and even at 30°C growth was slow. The growth rate at 30°C was stimulated slightly by vitamin B_{12} and thiamine, but not by exogenous purines or amino acids.

From the foregoing results there should be no great hindrance to eventual development of specific mutants as experimental tools for photosynthetic studies and studies on problems in blue-green algae.

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Table 1. Specific growth-rate constant (k) for mutants of *Anacystis nidulans*. Medium C prepared according to method of Kratz and Myers (*1*) with no modifications. C + 50 and C + 100 indicate Medium C plus 50 mg and 100 mg of NH_4Cl per liter, respectively. Illumination provided by two 20-watt fluorescent lamps 8 cm from the growth tubes; CO_2 (1 percent in air) bubbled through the tubes. Each of the mutants has been serially transferred in liquid culture at least three times with no change in characteristics.

Stock designation of mutant	Growth temperature (°C)	k ($\log_{10}$ unit/day)		
		Med. C	Med. C + 50	Med. C + 100
Wild-type	39	1.92	1.92	
Wild-type	30	1.58		
330y	39	0.00	1.68	1.68
2a	39	0.52	1.96	2.00
2b	39	0.26	1.50	1.40
Pt6i	39	1.15		
Hpur	39	1.50		
Bpur	39	0.00		
Bpur	30	0.80		

References and Notes

1. W. A. Kratz and J. Myers, *Amer. J. Bot.* **42**, 282 (1955). A-5 microelements stock solution is composed of (per liter): H_3BO_3, 2.86 g; $MnCl_2 \cdot 4H_2O$, 1.81 g; MoO_3, 0.0177 g; $CuSO_4 \cdot 5H_2O$, 0.079 g. One milliliter of stock solution is used per liter of Medium C.
2. C. Van Baalen, *Bot. Marina* **4**, 129 (1962).
3. H. D. Kumar, *Nature* **196**, 1121 (1962).
4. C. Van Baalen, *J. Phycol.* **1**, 19 (1965).
5. K. Shibata, *J. Biochem.* **45**, 599 (1958).
6. L. Jones and J. Myers, *J. Phycol.* **1**, 7 (1965).
7. J. Curry and J. Greenberg, *J. Bacteriol.* **83**, 38 (1962).
8. Supported by NSF grant GB 2006. The technical assistance of Rita O'Donnell is gratefully acknowledged.

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Nature of the Excitatory Sarcoplasmic Reticular Junction

Abstract. *The appositional regions between the surface membrane and sarcoplasmic reticulum in insects, decapod crustaceans, and barnacles are largely diadic and show a four-layered structure which is roughly circular in surface view. Each consists of the 56-angstrom double-membrane of the intermediary (here called excitatory) element, and the 75-Å double-membrane of the cisternal element of the reticulum, separated by a space of about 100 Å. A sheet of electron-dense material is found between the two elements, giving the superficial appearance of an additional membrane. The orbits of thin filaments around the thick filaments adjacent to both excitatory and reticular elements are incomplete on the contact side. Regularly spaced bridges connect the thick filaments with both the excitatory elements and cisternal elements and hold the diads in place during stretch and contraction.*

One of the most important unresolved problems in muscle physiology is the nature of the coupling processes between the electrical changes at the