

INDUCED SYNTHESIS OF PHOSPHATASES
IN *ANACYSTIS NIDULANS*
BY *p*-NO₂-PHENYLSERINAL*

M. L. MUÑOZ-CALVO, M. RODRIGUEZ-LOPEZ
and M. VILLARROYA-SANCHEZ

Instituto "Jaime Ferrán" de Microbiología, C.S.I.C.
Joaquín Costa, 32, Madrid-6, Spain

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Antibiotic inhibitors of protein synthesis induce the synthesis of phosphatases in different genera of microalgae with prokaryotic or eukaryotic organization¹⁻⁵). Among the antibiotics used, chloramphenicol induced acid phosphatase synthesis significantly⁵).

It has been demonstrated recently that chloramphenicol and *p*-NO₂-phenylserinal (lacking the dichloroacetamide side chain of the antibiotic) stimulate acid phosphatase synthesis in the eukaryotic phytoflagellate *Ochromonas danica*⁶) more strongly than chloramphenicol. However, since chloramphenicol is not an inhibitor of protein synthesis in eukaryotes, we have considered it of interest to compare the effect on a prokaryote of chloramphenicol and *p*-NO₂-phenylserinal to establish any possible relationship between the inhibitory effects of the compounds in protein synthesis and their action in inducing synthesis of phosphatases. *p*-NO₂-Phenylserinal has only about 2% of chloramphenicol activity as an inhibitor of protein synthesis in prokaryotes⁷).

In this paper we describe the results obtained on the blue-green alga *Anacystis nidulans* using essentially the same techniques and methods as those in our previous work⁵). The micro-alga was obtained from the Culture Collection of Algae and Protozoa (The Botany School, University of Cambridge, England). It was grown in medium D⁸) autotrophically at 24°C and aerated with air containing 2% of CO₂. The antibiotics used were *p*-NO₂-phenylserinal and chloramphenicol at a concentration of 20 µg/ml. In all assays, a culture in a complete medium without the antibiotic was used as the control.

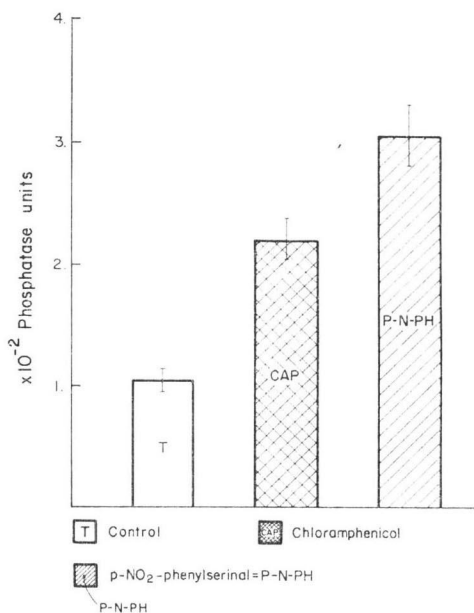
After 24 hours of incubation the cells were harvested by centrifugation and washed several times by buffer solution, acetate 0.1 M pH 3.65. The cells were resuspended in the buffer at the

rate of 1 g (wet weight) per 10 ml of the buffer and were broken in the Ribbi Cell Fractionator at 30,000 psi at 0°C. This suspension of broken cells in the acid medium was centrifuged at 15,000 rpm for 20 minutes. Phosphatases were present in the supernatants. The phosphatase activity of the extract was estimated with *p*-nitrophenylphosphate following the SCHURR and YAGIL method⁹); the results were expressed in phosphatase units contained in 500 mg dry weight of the alga. To detect the phosphatase cytochemically, the GOMORI method with β-glycerophosphate as substrate was used¹⁰).

Analysing the results (Fig. 1) we observed that chloramphenicol increased acid phosphatase synthesis in *Anacystis nidulans* two times over the control, whereas *p*-NO₂-phenylserinal increased it three times. Fig. 2 shows a normal control cell of *Anacystis nidulans*. In cells treated with chloramphenicol and *p*-NO₂-phenylserinal, the phosphatases are evident within the vacuoles (Figs. 3 and 4, respectively). These results clearly show that the effect of chloramphenicol in inducing synthesis of phosphatases in prokaryotes is unrelated to its inhibitory effect in protein synthesis⁵).

Fig. 1. Acid phosphatases induced by antibiotics in *Anacystis nidulans*.

Results expressed per 500 mg of dry weight of the alga.



* *p*-NO₂-phenylserinal = Chloramphenicol base.

Fig. 2. Normal cell of *Anacystis nidulans* showing several polyhedral bodies. Pb. ($\times 62,400$)

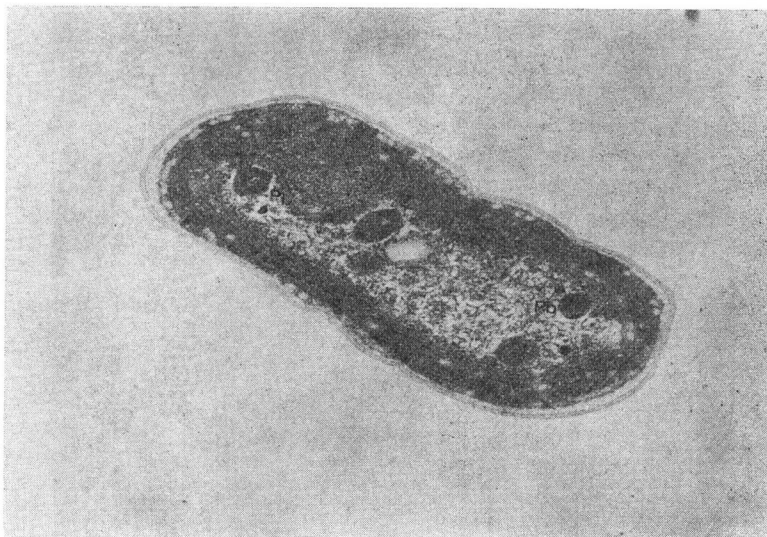
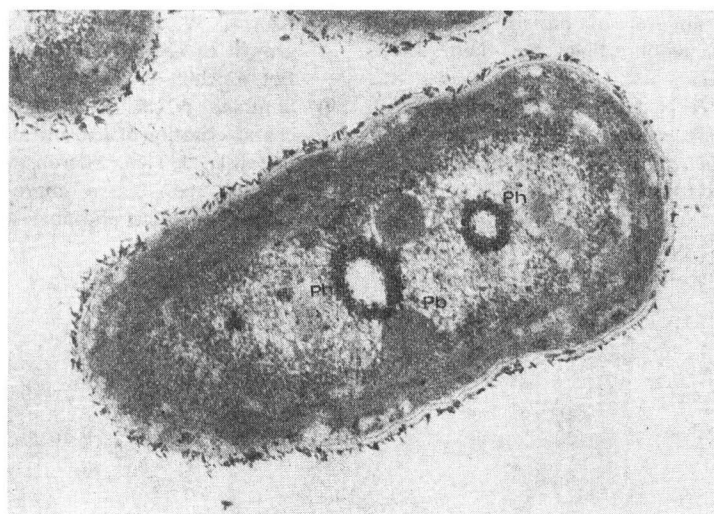


Fig. 3. Cell of *Anacystis nidulans* incubated in the presence of *p*-NO₂-phenylserinal with acid phosphatase. Ph. ($\times 78,400$)



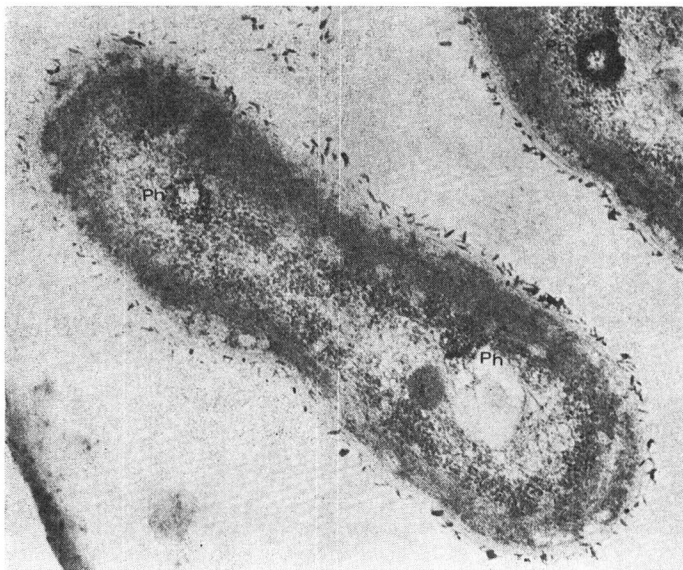
Acknowledgement

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Fig. 4. Cells of *Anacystis nidulans* incubated in the presence of chloramphenicol. ($\times 75,600$)



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