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Isozymes, possible markers for blue-green algae (BGA) identification

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To evaluate the potential of electrophoretically detectable isozymes for identifying BGA strains, especially in soil inoculation experiments, we worked to develop a sample preparation method that maximizes zymograms consistency.

The technique is horizontal starch gel electrophoresis using 14% starch in a tris (0.034 M) histidine (0.018 M) gel buffer with pH 8.0 and a tris (0.400 M) citrate (0.105 M) electrode buffer with pH 8.0. Zymograms were revealed by standard specific histochemical staining.

In a preliminary survey, 4 of 18 enzymes were observed in most of the tested strains (20 species in 9 genera) at different growth stages. They were phosphoglucose isomerase (PGI), isocitrate dehydrogenase (ICD), phosphogluconate dehydrogenase (PGD), and superoxide dismutase (SOD).

We compared zymograms of 63 extracts of an *Anabaena* strain (PCC 7120) for these 4 enzymes: 54 of them were obtained from liquid cultures on BG 11 medium without N. They were

1. grown in 3 environments:
a) *laboratory*: 25 ± 2°C, continuous lighting provided by cold white neon tubes, light intensity about 600 lux;
b) *phytotron:* 28 ± 1°C, continuous lighting provided by neon tubes and incandescent bulbs, light intensity about 5 klux; c) *greenhouse:* 25-38°C, maximum light intensity around 90 klux.

2. harvested 5, 10, 15, 20, 25, and 30 d after inoculation.

3. extracted after deep freezing using three methods: a) simple thawing, b) manual grinding using a glass rod, c) sonication for 5 min, with the culture in an ice bath.

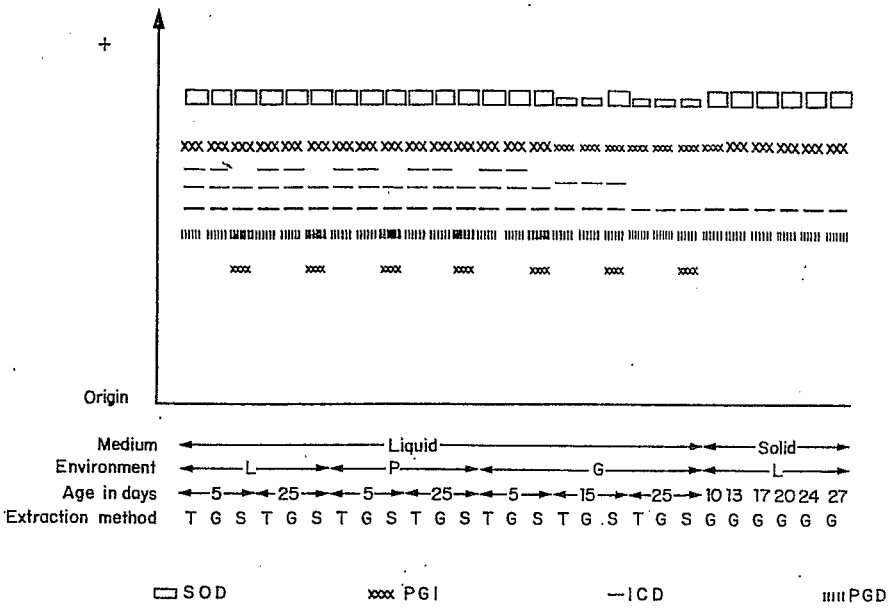
The other 9 extracts were obtained from cultures on solid medium (BG 11 medium without N, 1% bacto agar) grown in the laboratory under continuous lighting (600 lux). Individual colonies were harvested at 10, 13, 15, 17, 20, 22, 24, 27, and 30 d after plating and extracted by manual grinding in cold distilled water.

We observed the following (Fig. 1):

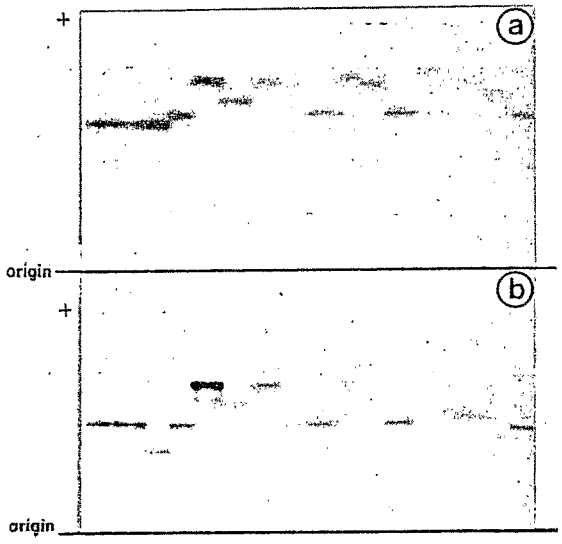
- For a given extraction procedure, there was no difference between laboratory and phytotron grown samples whatever their age. In greenhouse grown samples, the intensity of the bands decreased with the age of the material and a qualitative inconsistency was observed for ICD.
- Manual grinding increased the intensity of the bands as compared with a simple thawing of deep frozen samples. Sonication had a similar effect, but induced development of a new PGI band and the disappearance of an ICD band.
- In material produced on solid medium, a same single band was observed for each of the four enzymes, at all colony ages. This band was the only one common to all the zymograms of the samples grown in liquid medium.

Manual grinding of individual colonies grown on solid medium appears to minimize possible artifacts by selecting bands that consistently appear with the other procedures of extraction and in material grown under different conditions. A major additional advantage is that petri dishes inoculated with suspension dilutions of soil for routine BGA counts can be used directly for isozyme characterization.

This method was tested with nine other *Anabaena* species and nine different genera. In all cases, a single major band was observed for each of the PGI, PGD, and SOD enzymes. Some strains, however, did not produce an ICD band. Variation among strains was large (Fig. 2), which indicates good potential for strain identification. □



1. Synthetic diagram of zymograms of various extracts of Anabaena PCC 7120. L = laboratory, P = phytotron, G = greenhouse, T = thawing, G = thawing + manual grinding, S = thawing + sonication.



2. Zymograms of PGI (a) and PGD (b) from various BGA strains.

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