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A simple method for the preparation of permanent mounts of freshwater cyanobacteria for light microscopy

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Abstract

Taxonomic studies of microalgae in many laboratories use drawings, photographs, and type-specimens because, continuous maintenance of live specimens is mostly impractical. The most common and easy-to-handle type-specimens for microalgae are permanent slide preparations. Although there are established methods for preparing permanent slides for diatoms and green algae, no such methods have been developed specifically for cyanobacteria. Therefore, the objective of the present study was to develop a simple method for the preparation of permanent microscopic slides for freshwater cyanobacteria. Six species were selected to represent three forms of cyanobacteria, filamentous, unicellular colony-forming and unicellular non-colony-forming for the preparation of slides. Two acidic dyes, Fast Green and Brilliant Blue were used along with Mayer's egg albumin adhesive agent, fixative, dehydration and clearing agents. In the procedure with Fast Green, we used acetic acid: 96% ethanol (1:3) as the fixative in combination with gentle heat fixation. The specimens were dehydrated using a series of acetic acid and 96% ethanol solutions with different retention times. Finally, cleared with Xylene and mount on a drop of Canada balsam. This method successfully stained filamentous cyanobacteria. However, it poorly stained colony forming *Microcytis*. Apparently, the mucilage of the colony does not allow penetration of the dye to reach cells that are embedded within the mucilage. When this procedure was used for *Synechococcus,* which consists of single cells, those cells were loosely adhered to the glass slide and easily washed out. Therefore, a modified method was developed with Brilliant Blue including steps to overcome poor staining of colony forming cyanobacteria and loose adherence of unicellular non-colony forming cyanobacteria. Specimens were fixed to the glass slide using gentle heat and dehydrated using a series of 30-70% ethanol with various retention times. This modified method was able to stain *Microcytis*, *Synechococcus* and filamentous cyanobacteria. Slide preparations were found well preserved in terms of color and cellular structures when observed after 10 months of storage at room temperature. We recommend the method developed with Fast Green particularly for filamentous cyanobacteria while the method developed with Brilliant Blue for all three forms of filamentous, unicellular, and colony-forming cyanobacterial species for the preparation of permanent slides.

Keywords: Brilliant Blue, Cyanobacteria, Fast Green, Permanent slides

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