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System. Appl. Microbiol. 6, 105–106 (1985)

A Modified DAPI Fluorescence Staining Procedure Suitable for the Visualization of Lithotrophic Bacteria

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Received December 7, 1984

Summary

A DAPI fluorescence staining method, modified by a combination with low concentrations of SDS, is described which is suitable for the microscopic visualization of lithotrophic organisms growing on ores.

Key words: Ore – Leaching – Metal – Stain – Lithotrophy – Fluorescence microscopy – Geomicrobiology – *Thiobacillus* – *Sulfolobus*

In geomicrobiology, fluorescence microscopy after staining with suitable dyes is a simple and sensitive method for the detection of bacteria within their natural environments (Doetsch, 1981). The various fluorescence dyes that have been employed (Doetsch, 1981; Paton and Jones, 1973; Conn and Holmes, 1926) are either autofluorescent by themselves (e. g. acridine orange) or become fluorescent in the inciting light after binding to special cell components (e. g. 4'-6'-Diamidino-2-phenylindole = DAPI; Dann et al., 1979; Schweizer, 1976). In the case of autolithotrophic bacteria which are fixed to solid surfaces like ore particles, two problems arise during application of these dyes: (a) with autofluorescent dyes, the residual staining solution must be removed in order to improve the contrast of the stained cells against the background. During the necessary washing steps, most of the cells become detached from their solid surfaces. Furthermore, this washing is very tedious, especially for routine inspections; (b) dyes which become fluorescent only after binding to specific elements in the interior of the cell, like DNA, are normally not been taken up by these organisms, even after fixation. Therefore, fluorescence staining could not be successfully applied to most bacteria of this group, e. g. metal mobilizers.

Here we report a rapid and reliable modified DAPI staining method in which the cells are made permeable by low amounts of detergent allowing the stain to form its fluorescing complex with the DNA. To prepare the stock staining solution, 2 mg of 4'-6'-Diamidino-2-phenylindole (No. 18860 Serva, Heidelberg) and 100 mg of sodium

dodecylsulfate (SDS No. 20760 Serva, Heidelberg) are dissolved in 100 ml of buffer, containing 0.05 molar ethylene-diaminetetraacetic acid (EDTA, Na₂-salt; No. 11280 Serva, Heidelberg) and 0.7 molar sodium acetate, pH 4.7. For extended periods, this solution can be stored at +4°C. In order to stain the cells, 1 µl of the stock staining solution is mixed with 20 µl of the bacterial suspension on a microscopic slide. Under the UV fluorescence light microscope (e. g. Zeiss Standard 16, fluorescence equipment IV FL; filter set 487702, consisting of BP 365, FT 410 and LP 420), a bright blue fluorescence of the bacteria against a dark background becomes visible after about 30 seconds.

With this method a variety of ore-colonizing bacteria becomes visible on the particles without further treatment, e. g. *Thiobacillus ferrooxidans* (Fig. 1a) or *Sulfolobus brierleyi*-like archaeobacteria (not shown). By phase contrast microscopy, however, these bacteria are invisible (Fig. 1b). This procedure is also well suited for investigation of eubacteria and archaeobacteria in particle-containing environments such as soils or volcanic muds.

Acknowledgements. The excellent technical assistance of H. Nowarra is highly appreciated. Thanks are also due to T. Langworthy for critically reading the manuscript. This work was supported by a grant of the Bundesministerium für Forschung und Technologie (BMFT) to K. O. S.

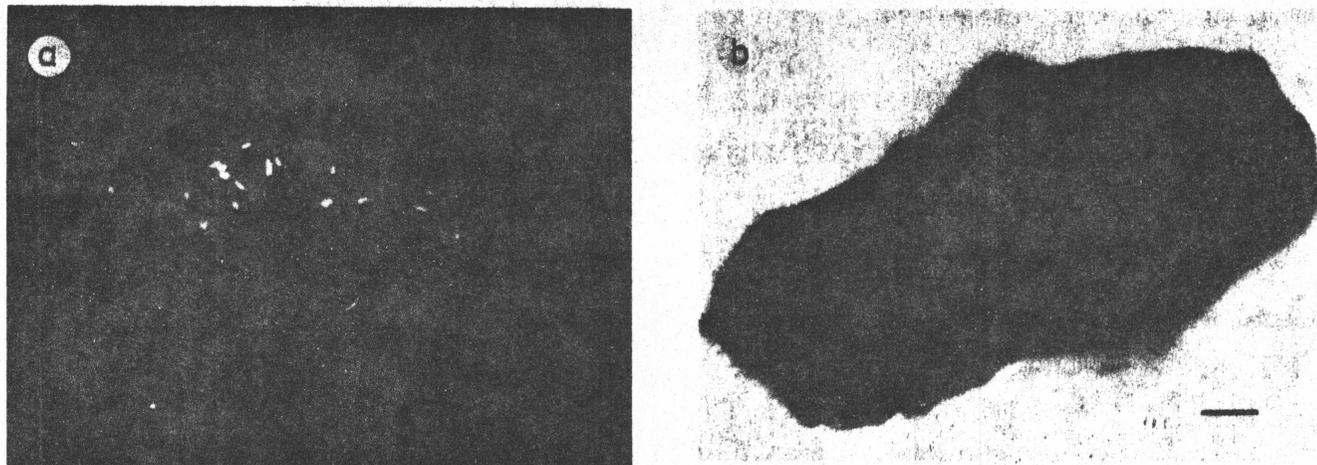


Fig. 1. Pyrite fragment colonized by *Thiobacillus ferrooxidans*. Bar, 5 μm .

(a) Fluorescence microscopy after the modified DAPI staining procedure. The cells on the ore surface are visible.

(b) Phase contrast microscopy of the same particle.

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