An improved DAPI staining procedure for visualization of polyphosphate granules in cyanobacterial and microlagal cells

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Method Article

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Abstract

DAPI staining method is broadly used for the detection of polyphosphate granules in microbial cells. The present protocol modifies the existing methods for better detection of the granules with higher resolution on confocal microscopy, especially for cyanobacteria and microalgae. The approximate time required to prepare one slide for microscopic detection is around two and a half hours and to visualize it is around fifteen to thirty minutes.

Introduction

A widely accepted method for _in situ_ polyphosphate \(polyP) detection is fluorescence microscopy using a fluorochrome, 4', 6 - diamino-2-phenylindole 2HCl \(DAPI) at high concentrations ranging from 3-50 µg/ ml[1]. Interaction of polyP with DAPI shifts the emission maxima of the dye from 456 nm emitting blue fluorescence to 526 nm emitting bright yellow- green fluorescence[2]. The present protocol modifies the existing method of cell preparation by using McIlvaine's buffer[3] instead of PBS buffer[4, 5] for more efficient microscopic detection of the polyP granules in cyanobacteria and microalgae _in vivo_. The use of PBS buffer greatly reduces the contrast and resolution during confocal microscopy as compared to McIlvaine's buffer. It was also observed that the application of the dye to the specimen at concentrations greater than 25 µg/ ml resulted in only blue light emission making the detection of polyP impossible.

Reagents

• Microscopic glass slides \(Blue Star) • Microscopic cover glasses \(Blue Star, 22x50 mm) • McIlvaine's buffer preparation I. Stock solution A: 0.1 M citric acid solution. Dissolve 4.2 g of citric acid \(Merck, Cat. No. 61799705001730) in 200 ml double distilled water II. Stock solution B: 0.2 M Na_2HPO_4 solution. Dissolve 22.7 g of Na_2HPO_4 \(Merck, Cat. No. 1.06559.0500) in 800 ml double distilled water III. Mix 2 parts of stock solution A and 8 parts of stock solution B to make pH 7.0 buffer • Formaldehyde \(Merck, Cat No. 8.18708.1000) • Triton-X-100 \(HiMedia, Cat. No. MB031-100ML) • 4', 6 - diamidino-2-phenylindoledihydrochloride \(DAPI) solution preparation I. Dissolve 2 mg DAPI \(Sigma-Aldrich, Cat. No. D9542) in 1 ml McIlvaine's buffer to prepare a main stock solution of concentration of 2 mg/ ml. Store it in dark at - 20°C II. Prepare working stock solution of the concentration of 20 µg/ ml by diluting 10 µl of the main stock solution with 990 µl of McIlvaine's buffer. Store it in dark at - 20°C

Equipment

• Confocal microscope \(OLYMPUS 1X81 with FV 1000 software)

Procedure

1. Take 1 ml of microalgal and cyanobacterial culture grown for 25 days at 28°C in BG-11 \(non-N_2 fixer medium) and BG-0 \(N_2 fixer medium). 2. Centrifuge the samples at 2350 g for 5 minutes. Discard the

supernatant. 3. Wash the cell pellet with 200 µl McIlvaine's buffer. 4. Fix the pellet with 200 µl of 4% formaldehyde \(prepared in McIlvaine's buffer) and incubate for 30 minutes. 5. Wash again with the buffer. 6. Make the cells permeable by treating them with 200 µl of 0.3% \(v/v) Triton-X-100 solution \ (prepared in McIlvaine's buffer) for 5 minutes. Repeat washing with the buffer. 7. Mix the cells on the slide with 200 µl of DAPI solution and incubate for 30 minutes in the dark. CRITICAL STEP: All the further steps including the present one should be strictly carried out in the dark. 8. Re-wash it gently with the buffer. CRITICAL STEP: Proper and gentle washing should be done to minimize the background noise due to the fluorescence of extra DAPI stain present on the slide during microscopy. 9. Dry the slides at room temperature in the dark. 10. Observe the slides under confocal microscope by setting the excitation filter at 370 nm and emission filter at 526 nm.

Timing

Approximately two and a half hours will be required to prepare one slide for confocal microscopic observation. Visualization of one sample specimen may take around fifteen to thirty minutes.

Troubleshooting

1. The pH of the McIlvaine's buffer should be 7.0 as the DAPI stain does not react with the polyphosphate granules even at the slight variation of pH.

Anticipated Results

DAPI-stained polyphosphate granules present in the cyanobacterial and microalgal cells show bright yellow- green fluorescence at 526 nm upon observation under confocal microscope \(Figure 1) whereas the cells devoid of the granules emit a light blue fluorescence at 456 nm.

References

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Figures

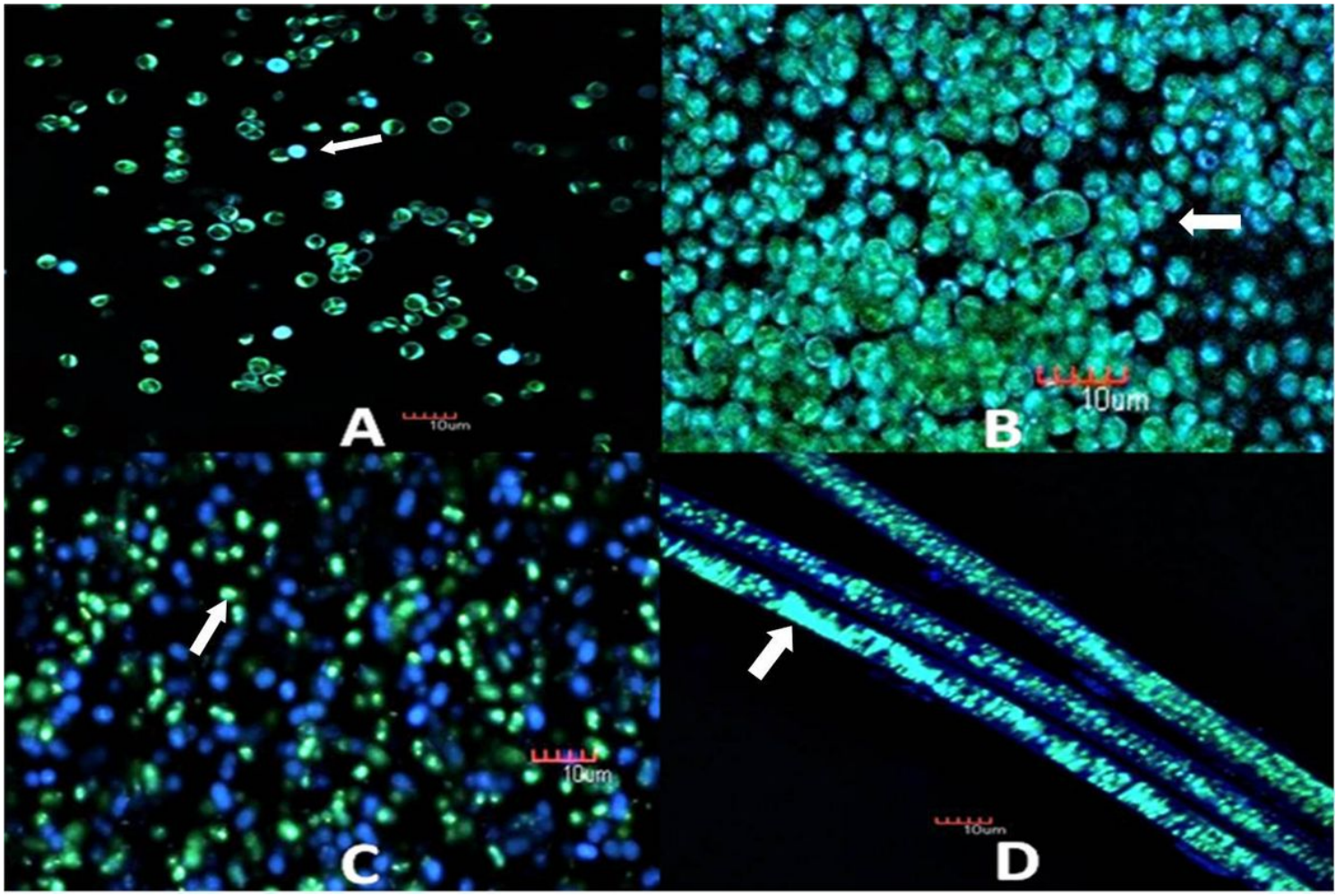

Figure 1

DAPI staining of polyphosphate granules present in the cyanobacterial and microalgal cells observed under confocal microscope White arrows indicate the presence of polyphosphate granules in the cells of: A) _Chlorella_ sp. isolate 10.2; B) _Chlorella_ sp. isolate H36; C) _Cyanobacterium_ sp. isolate Fardillapur; D) _Lyngbya_ sp. isolate 2.1.