NOTES & TIPS

In Situ Analysis of Carboxyl and Sulfhydryl Groups of Extracellular Polymeric Secretions by Confocal Laser Scanning Microscopy

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Extracellular polymeric secretions (EPSs)[2] are a complex matrix of molecules secreted by bacteria and cyanobacteria in the construction of microbial biofilms. Biofilms are important in microbial disease processes, binding of antibiotics and metals, environmental contaminant cycling, and water purification processes (1). The EPS matrix of a biofilm consists mainly of polysaccharides, proteins, and lipids (2). Confocal scanning laser microscopy (CSLM) coupled with various probes has been used for localization of specific components of EPSs (3). Lectin-specific binding has made it possible to probe for the presence of specific sugar residues including mannose, glucose, galactose, glucosammine, and fucose. However, labeling of important functional groups such as carboxyl (—COOH) and sulfhydryl (—SH) groups in EPSs has been difficult.

Here we report a new method to visualize fluorescently labeled carboxyl and sulfhydryl groups through biotinylation of those functional groups followed by streptavidin-conjugated fluorescein and CSLM. We used marine stromatolites, Highborne Cay, Bahamas, containing filamentous cyanobacteria, *Schizothrix* sp., as a model. *Schizothrix* sp. is coated with sheath and EPSs containing uronic acids and acid polysaccharides. Carboxyl and sulfhydryl groups of acid polysaccharides in EPSs are believed to play important role in binding Ca^{2+} in calcification of marine stromatolites (4).

Freshly collected marine stromatolite samples, Highborne Cay, Bahamas, were sectioned using sterile razor blades. Immediately after collection, the sections of stromatolite were preserved in 3% formaldehyde in seawater.

For biotinylation of carboxyl groups of EPSs secreted, a piece of stromatolite section was placed in a small vial containing 1 ml of 0.1 M *N*-morpholinoethanesulfonic acid (MES) buffer, pH 5.5. Then, 50 μl of 50 mM EZ-Link 5-(biotinamido) pentylamine (Pierce, Rockford, IL) solution was added. Immediately before use, a water-soluble carbodiimide (EDC) was dissolved in 0.1 M MES buffer, pH 5.5, at a concentration of 100 mg/ml. Twelve and one-half microliters of EDC in 0.1 M MES buffer was added to a same vial. The stromatolite section was incubated in a small vial at room temperature for 2 h using a rotary shaker. The unreacted biotinylation reagent and EDC by-product were removed by washing three times with phosphate-buffered saline (PBS), pH 7.4.

For biotinylation of sulfhydryl groups of EPSs secreted by *Schizothrix* sp., a piece of stromatolite section was placed in a small vial containing 2.5 ml of 50 mM mercaptoethylamine-HCl (MEA) in 100 mM sodium phosphate, 5 mM EDTA, pH 6.0 buffer. Then the sample was incubated at 37°C for 90 min. One hundred microliters of EZ-Link polyethylene oxide (PEO)–maleimide-activated biotin (Pierce, Rockford, IL) solution was added; then the sample was incubated at room temperature overnight and washed three times in PBS.

After biotinylation of both samples, samples were placed on a slide glass and ImmunoPure streptavidin–fluorescein conjugate (Pierce) in PBS (1:100 dilution) was added to this slide. Samples were incubated at room temperature for 1 h in the dark. Then, samples were washed three times in PBS.

Sample embedding was carried out according to the method of Decho and Kawaguchi (5). Stromatolite sections were trimmed (3 × 8 × 5 mm) using a sterile razor blade (2.5 × 4 cm), then placed in BEEM embedding molds (5 × 10 × 5 mm). Resin and catalyst were thoroughly mixed on site and then added to molds containing the stromatolite samples. The molds were placed in a temperature-controlled heat block at 25°C for 48–60 h to allow slow penetration and complete mixing of the Nanoplast resin (Ted Pella, Redding, CA) with the hydrated sample. After penetration of the medium, the temperature was raised to 40°C for 48 h to dry and then 60°C for 48 h to harden the medium

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[2] Abbreviations used: EPS, extracellular polymeric secretion; MES, 0.1 M *N*-morpholinoethanesulfonic acid; PBS, phosphate-buffered saline; MEA, mercaptoethylamine-HCl.

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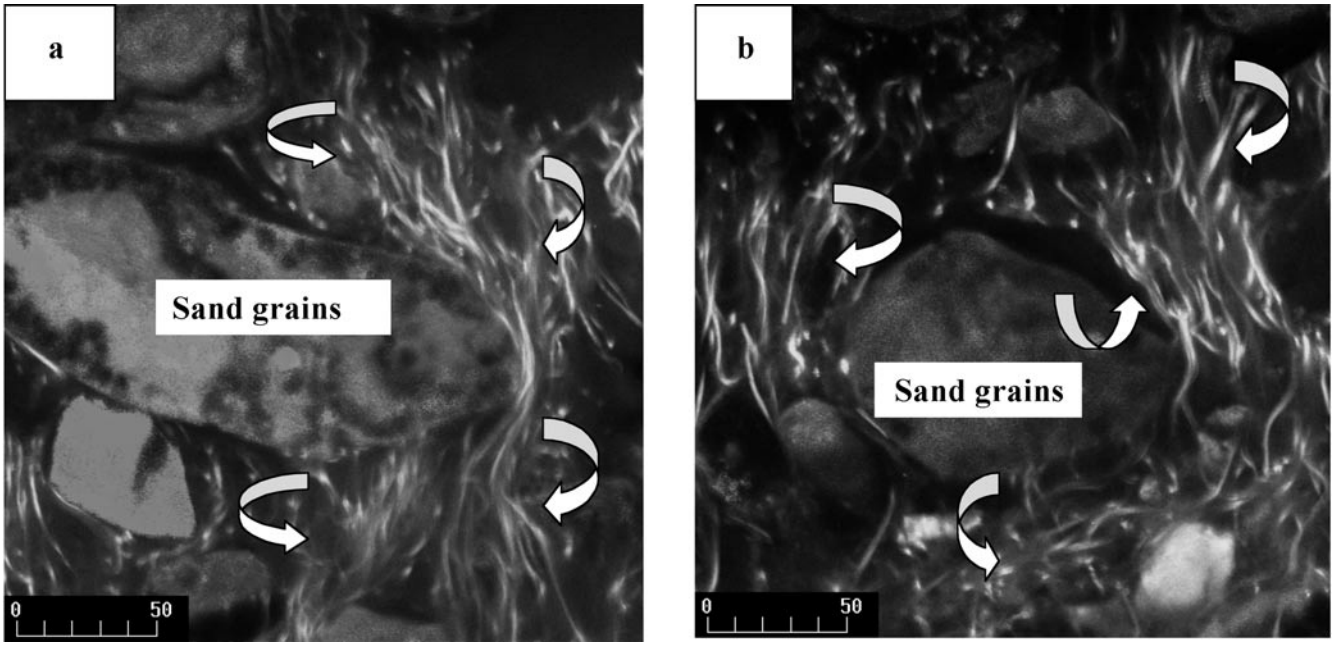

FIG. 1. Fluorescein-labeled carboxyl (a, arrows) and sulfhydryl (b, arrows) groups of cyanobacterial (*Schizothrix* sp.) sheath surrounding carbonate sand grains (autofluorescence) in marine stromatolite by confocal laser scanning microscopy.

into blocks. The resulting blocks were thick sectioned (approximately 2 mm), mounted on glass microscope slides using Epon 812, and then observed using confocal laser scanning microscopy.

Sheaths of filamentous cyanobacteria, *Schizothrix* sp., among carbonate sand grains in marine stromatolite sections were clearly labeled by fluorescein. Figures 1a and 1b show the presence of carboxyl and sulfhydryl groups on the sheath of *Schizothrix* sp. In conclusion, this method could be applicable to labeling polymers such as alginate-containing uronic acids and could serve as a great tool in studying a complex structure of EPSs containing carboxyl and sulfhydryl groups.

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REFERENCES

1. Costerton, J. W., Lewandowski, Z., Caldwell, D. E., Korber, D., and Lappin-Scott, H. M. (1995) Microbial biofilms. *Annu. Rev. Microbiol.* **49,** 711–745.
2. Decho, A. W. (1990) Microbial exopolymer secretions in ocean environments: Their role(s) in food webs and marine processes. *Oceanogr. Mar. Biol. Annu. Rev.* **28,** 73–154.
3. Neu, R. T., and Lawrence, J. R. (1999) In situ characterization of extracellular polymeric substances (EPS) in biofilm systems. *In* Microbial Extracellular Polymeric Substances (Wingender, J., Neu, T. R., and Flemming, H.-C., Eds.), pp. 21–47. Springer-Verlag, New York.
4. Reid, R. P., Visscher, P. T., Decho, A. W., Stolz, J. F., Bebout, B. M., Dupraz, C., Macintyre, I. G., Pinckney, J., Paerl, H. W., Prufert-Bebout, L., Steppe, T. F., and Des Marais, D. J. (2000) The role of microbes in accretion, lamination, and lithification in modern marine stromatolites. *Nature* **406,** 989–992.
5. Decho, A. W., and Kawaguchi, T. (1999) Confocal imaging of in-situ natural microbial communities and their extracellular polymeric secretions using nanoplast resin. *BioTechniques* **27,** 1246–1252.

One-Tube Protocol for Single-Cell Reverse Transcriptase-Polymerase Chain Reaction

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Reverse transcriptase-polymerase chain reaction (RT-PCR) has been widely used to assess the expression of specific genes in cells and tissues. RT-PCR involves several steps including RNA preparation, complementary DNA (cDNA) synthesis by reverse transcription, and finally cDNA amplification by PCR. While RT-PCR is usually performed on RNA isolated from a population of cells, it has also been performed

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