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The fluorescent sediment thin section technique: spatial distribution of microorganisms in North Sea microbial mat systems

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Abstract

A new technique to process sediment thin sections with fluorescent dyes is presented. It enables to examine the distribution of bacteria on and between sediment particles in the um-scale, using epifluorescent microscopy and/or simultaneous transmittent light and epifluorescence microscopy using a laser scanning microscope. It was possible to assign biomass data to the structures of sediment and organic matter of surface layers of young microbial mats, mature mats and stages of early (microbial) diagenesis. The gradual transformation of cyanobacterial mats after drastic events of sedimentation can be analyzed and demonstrated this way.

Introduction

According to WATLING (1988) the understanding of sedimentary ecosystems requires the assignement of bulk parameters to optical informations about the biological and mineralogical structures, for example the spatial distribution of microorganisms in interstices. For such micro-scale investigations it is of great importance to avoid preparation-induced shrinking and contraction of the mucilagenous matrix and other artefacts. WATLING (1988) developed a suitable technique combining histological protocols for fixation and dehydration with petrological protocols for embedding and thin sectioning, opening the possibility to process undisturbed samples of siliciclastic sediments, maintaining microorganisms and organic matrices *in situ*. This technique has been improved and applied to study microbial mat systems. In contrast to FRANKEL and MEAD (1973) who embedded pre-stained sediment samples, we used the DNA-specific fluorescent dye DAPI to stain the thin sections after embedding and polishing.

The microbial mats of Mellum Island (North Sea) have been subject of several investigations (STAL et al. 1985, GERDES et al. 1985, 1986 and 1987), more or less limited to the surface mat layer. Only GERDES et al. (1985 and 1986) included the buried ancient mat layers studying thin sections under sedimentological and paleontological aspects.

Applying the fluorescent thin section technique three layers of a microbial mat system of Mellum Island - surface mat (SM), ancient mat 1 (ca. 10 mm depth) and ancient mat 2 (ca. 20 mm depth) - have been compared. Thus we received informations about the increasing decomposition of the mat building cyanobacteria and about the microbial community of mature and diagenetic mats. Concerning the significance of recent microbial mats as an example of potential stromatolites (KRUMBEIN 1983), persisting fragments of these organisms have to be expected in ancient mat layers.

Material and methods

Sediment samples were taken from microbial mats of Mellum Island with small aluminium dishes (3,8 cm diameter); additionally sediment cores for the analysis of biological parameters were taken by using plastic tubes.

The thin sections were prepared according to WATLING (1988) using SPURR's Resin-Mixture for embedding. The sediment thin sections were treated with the DNA-specific fluorescent dye DAPI: They were covered with DAPI-solution (5 µg/ml a. dest) for 5 min., then several times rinsed with a. dest and carefully dried. For the microscopic examination a ZEISS AXIOPHOT equipped with epifluorescence (excitation filters: autofluorescence: BP 400-440, FT 460, LP 470; DAPI: G 365, FT 395, LP 420) and a ZEISS LSM 44 laser scanning microscope (Ar-laser: 488 nm; Ne-laser: 633 nm) was used.

Total organic carbon, proteins and carbohydrates were chosen as corresponding bulk parameters. The sediment cores were cut in 2 mm-slices. Total organic matter was determined from dried samples (24 h, 60 °C) homogenized in a mortar. Total organic carbon (TOC) was calculated after combustion (2 h, 475 °C). Carbohydrates were measured according to LIU et al. (1973) with glucose (Merck) as standard. The protein analysis with coomassie-blue (Sigma) was based on the method of SETCHELL (1981) using albumin (Sigma) as standard.

Results and discussion

The microbial mat system shows several mat generations as dark, reduced layers alternating with bright sediment insertions caused by (drastic) events of sedimentation. The distinct peaks in the distribution of total organic carbon, carbohydrates and proteins (Fig. 1) mark both, surface mat as well as ancient mat 1 and 2.

Characterization of the mats

1. Surface mat (SM)

The quartz crystals in the surface mat are embedded in a tight mucilagenous matrix produced by cyanobacteria. The ensheated bundles of *Microcoleus chthonoplastes* (Fig. 2) as main mat-building organism are dominant. *Phormidium spec., Oscillatoria spec.* and different diatoms are frequent too (Fig. 2).

The laser scanning microscope (LSM) enabled us to detect a small filamentous cyanobacterium (*Nodularia spec.?*) between quartz-grains: Transmittent light has been superimposed upon epifluorescence (Fig. 3). This way we were able to identify this thin filamentous bacterium as photoautotrophic by its autofluorescence.

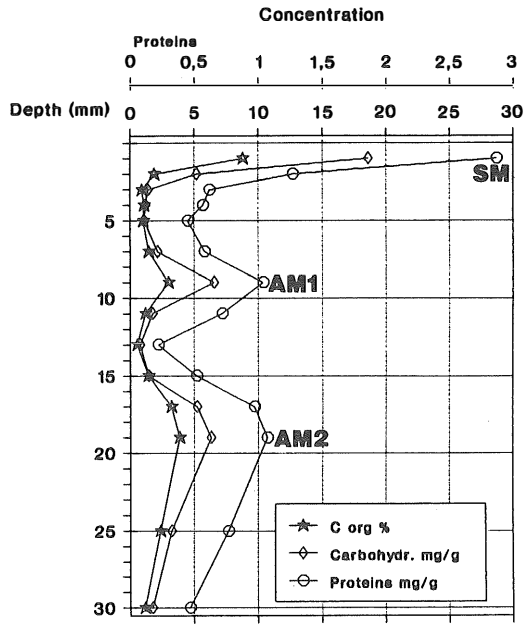

Fig. 1. Profiles of total organic carbon (TOC), carbohydrate- and protein-concentration (per g sediment dry weight); SM = surface mat, AM1 = ancient mat 1, AM2 = ancient mat 2.

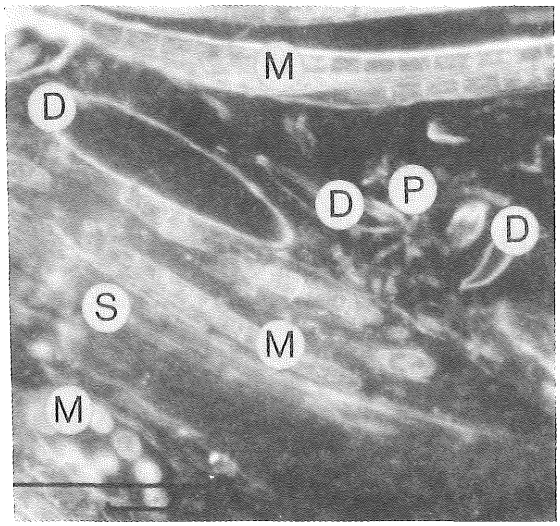

Fig. 2. Cyanobacteria of the surface mat (SM) *in situ*. DAPI-stained thin section. M = *Microcoleus chthonoplastes*, P = *Phormidium* spec., D = diatom, S = sheath, bar = 10 µm.

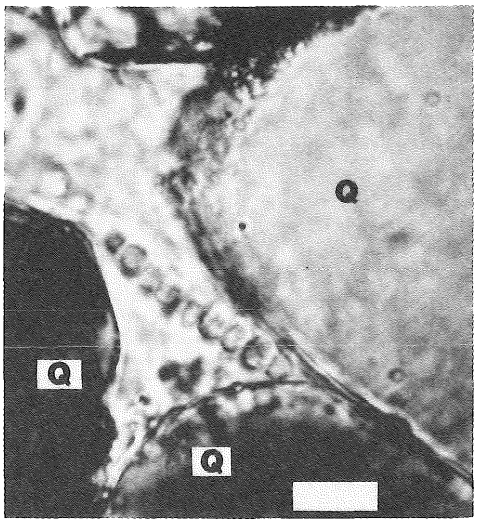

Fig. 3. Autofluorescing filamentous cyanobacterium between quartz-grains. Laser scanning microscope - simultaneous transmittent light and epifluorescence. Q = quartz-grain, bar = 10 µm.

2. Ancient mat 1 (AM1; Fig. 4)

This layer is characterized by the empty, partially decomposed sheaths of *Microcoleus chthonoplastes* and by numerous coccoid, rodshaped and colony-building bacteria.

3. Ancient mat 2 (AM2; Fig. 5)

Only remnants of *Microcoleus*-sheaths resisted to decomposition at this depth. In comparison to AM1 the mucilagenous matrix occurs in a very fragmented shape in AM2. Numerous large filamentous bacteria, but only few cocci and rods are abundant.

Methodological considerations

The evaluation of sediment thin sections based on the autofluorescence of photoautotrophs (Fig. 3) and on processing the sections with the fluorescent dye DAPI (Fig. 2, 4 and 5) is a promising and fruitful approach to the differentiation of mat microbiota.

Using an epifluorescence microscope, autofluorescence is ideal to investigate the distribution of microorganisms up to magnifications of 200 x. At higher magnifications the intensity of fluorescence causes scattered light effects decreasing considerably contrast and resolution. The laser scanning microscope (LSM) allows to reduce such effects by electronical contrast enhancement and electronical zooming (WILKE 1985). Furthermore the superimposition technique (Fig. 3) improves the images of microorganisms in thin sections retaining clear shaped mineral compounds. In this method the information is processed by computer and

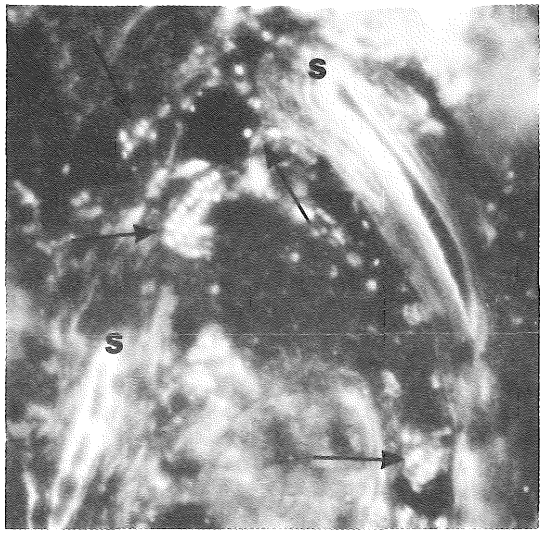

Fig. 4. *Microcoleus*-sheaths and different bacteria in ancient mat 1 (DAPI-stained thin section). Arrow: colony-building bacteria, cocci and rodshaped bacteria, s = sheath, bar = 10 µm.

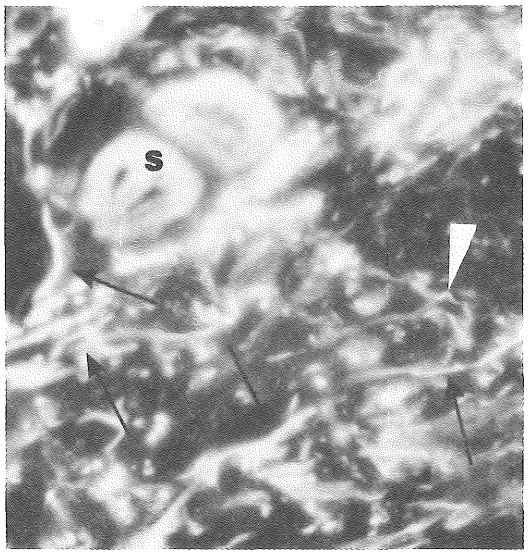

Fig. 5. *Microcoleus*-sheats and bacterial community of ancient mat 2 (DAPI-stained thin section). Arrows: large bacterial filaments, arrowhead: spirilloidal bacterium, s = sheat, bar = 10 µm.

options to choose different ratios of reflected laser light and transmitted light for imaging exists.

The use of DAPI provides images comparable to the TEM and light microscopical investigations of microbial mats by STOLZ (1983) and D'ANTONI D'AMELIO (1989), although limited in magnification. STOLZ (1983) and D'ANTONI D'AMELIO (1989), however, used only mat parts void of any associated sedimentary clastic particles, thus disregarding the interrelationsship between sediment and organic matrix. Microorganisms of various size are distinct and well contrasted (Fig. 2, 4 and 5). The elements of the concentric sheath of *Microcoleus chthonoplastes* - usually shrinked even after careful REM-preparations (STAL et al. 1985, GERDES et al. 1985, COHEN 1984) - are recognizable in their original shape.

The Mat Layers

The profiles of TOC, carbohydrates and proteins corresponding to the microscopical analysis show maximal concentrations in the surface mat SM (Fig. 1). The nearly identical data of the biomass parameters in the ancient mats were quite astonishing in view of the high degree of decomposition witnessed by our histological data. In Solar Lake KRUMBEIN et al. (1977) found comparable peaks in ancient mat layers.

The dominance of *Microcoleus chthonoplastes* as main mat building cyanobacterium (Fig. 2) was also found by STAL et al. (1985) and GERDES et al. (1985). The mat community produces large amounts of sediment fixing exopolymers. Only the sheaths of *Microcoleus chthonoplastes* are persisting even down to AM 2 (Fig. 5). At low sedimentation rates the gliding filaments of the latter leave their sheaths behind by positive phototactic movements (GERDES and KRUMBEIN 1986). But the compact mat layers in 10 and 20 mm (AM1 and AM2) have obviously been buried by drastic sedimentation events. In this case empty sheaths result from decomposition of the filaments according to observations in subrecent mats at Laguna Figueroa, Baja California (STOLZ and MARGULIS 1984).

However, the organic matrix has been reduced gradually with increasing depth by chemoorganotrophic bacteria. Based on reports of the morphological diversity of sulfate-reducers (PFENNIG et al. 1981) and methanogenic bacteria (MAH et al. 1981) the cocci and rods in both ancient mats and even the large filamentous and spirilloidal microorganisms in AM2 probably could be assigned to these physiological groups. Furthermore the high numbers of other chemoorganotrophs, for example aerobic heterotrophic bacteria (we found more then 10^8 colony-forming units per g dry weight), should be considered as parts of this microbial community.

The fluorescent sediment thin section technique opens new possibilities in the interdisciplinary analysis of siliciclastic marine microbial mat sediments. This method enables working on the same large-scaled sample under (micro)-biological and geo-/mineralogical aspects, for example including investigations referring to the fractal-porous geometries of pore structures.

The extension of bulk measurements by exact optical informations, favourable digitalized by means of computer aided image analysis (PLOEM 1986), fulfils the demand of WATLING (1988).

Applications of other fluorescent dyes and histochemical methods are of great importance for future research. First experiences with immunofluorescent dyes are promising.

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