**In situ** cell and glycoconjugate distribution in river snow studied by confocal laser scanning microscopy*

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ABSTRACT: Aggregates from the river Elbe (Germany) were collected and examined by confocal laser scanning microscopy. The river snow was directly transferred into coverslip chambers and observed without any embedding and fixation procedure. River snow samples were examined in the reflection and fluorescence mode to record mineral content and autofluorescence signals. Spatial distribution of microbial cells within the aggregates was shown with general nucleic acid specific stains (SYTO 9, SYTO 64). The polymeric matrix of the river snow was demonstrated by using a panel of fluorescently labelled lectins. Staining with FITC and TRITC labelled lectins and simultaneous dual channel imaging showed the distribution of different types of glycoconjugates. The binding pattern observed included bacterial cell surface labelling and cloud-type labelling of extracellular polymeric substances (EPS). In addition, dual staining combined with triple-channel recording was employed to clearly separate the signal in the red channel originating from TRITC-lectin or SYTO 64 nucleic acid stain from the far red autofluorescence originating from chlorophyll-containing organisms. With this approach the fully hydrated, living, 3-dimensional architecture of aquatic aggregates was investigated. Application of lectins demonstrated the heterogeneity of the EPS matrix in between the cellular constituents of the river snow community. The chemical heterogeneity of river snow may be significant for sorption and transport of nutrients and contaminants in lotic aquatic environments. This **in situ** technique may be also useful to examine interfacial microbial consortia in other habitats. Finally, it is suggested to employ **in situ** lectin staining to probe for the glycoconjugate distribution at the polymer level similar to **in situ** hybridisation with rRNA targeted oligonucleotides at the cellular level.

KEY WORDS: River/stream - Aggregates - Suspended particulate matter - River/lake/marine snow - Biofilm - Polysaccharide - Lectin - Fluorescence - **In situ** analysis - Confocal laser scanning microscopy

INTRODUCTION

By analogy to other aquatic habitats, the lotic aggregates in this paper were termed 'river snow'. In addition to natural aggregates, artificial aggregates, e.g., activated sludge flocs, have been the subject of intensive research due to their significance in waste water treatment (e.g., Wagner et al. 1994a, b, Amann et al. 1995, Manz et al. 1998).

Most microscopic techniques applied to microbial biofilms and aquatic aggregates require fixation and dehydration in order to microscopically examine the 3-dimensional structure by scanning electron microscopy (SEM) (Richard & Turner 1984, Costerton et al. 1986, Ladd & Costerton 1990) or transmission electron microscopy (TEM) (Leppard et al. 1996). Fixation and dehydration, however, may result in artifacts which create difficulties in interpretation of previously hydrated biological structures. For this reason, there is a shift in preparative procedures from dehydration of samples to techniques where partly dehydrated samples can be examined. Partly dehydrated samples may be employed for environmental SEM and for atomic force microscopy (Bremer et al. 1992, Kasas et al. 1994, Sutton et al. 1994, Lavoie et al. 1995, Surman et al. 1996). Further, there has been the development and application of techniques allowing the observation of fully hydrated interfacial microbial consortia using confocal laser scanning microscopy (CLSM) (Caldwell et al. 1992, Lawrence et al. 1996a, Lawrence & Neu 1999).

The purpose of this study was to assess a direct procedure to investigate the 3-dimensional structure of living, lotic aggregates. The emphasis was to develop a method that does not require embedding or fixation. By employing nucleic-acid-specific and carbohydrate-specific probes in conjunction with multi-channel CLSM, the fully hydrated architecture of river snow with respect to the distribution of microbial cells, glycoconjugates and autofluorescence was demonstrated.

### Table 1. Characteristics of lectins employed in this study. Lectins which are presented in the figures are given in bold. Data are taken from the material data sheet of the supplier

<table>
<thead>
<tr>
<th>Lectin source</th>
<th>Common name [abbreviation]</th>
<th>Molecular weight [×10³]</th>
<th>Sugar specificity (major only)</th>
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</thead>
<tbody>
<tr>
<td>Abris precatorius</td>
<td>Abrin</td>
<td>134</td>
<td>D (+) galactose</td>
</tr>
<tr>
<td>Arachis hypogaea</td>
<td>PNA</td>
<td>120</td>
<td>D (+) galactose t lactose</td>
</tr>
<tr>
<td>Bandeiraea simplicifolia</td>
<td>BS I</td>
<td>114</td>
<td>D (+) galactose 1-O-methyl α-D-galactopyranoside</td>
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<tr>
<td>Canavalia ensiformis</td>
<td>ConA</td>
<td>102</td>
<td>Methyl α-D-mannopyranoside D (+) mannose</td>
</tr>
<tr>
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<td>Lentil</td>
<td>49</td>
<td>α-methyl-D-mannopyranoside D (+) mannose</td>
</tr>
<tr>
<td>Limulus polyphemus</td>
<td>Limulin</td>
<td>400</td>
<td>N-acetylneuraminic acid D-glucuronic acid</td>
</tr>
<tr>
<td>Ricinus communis</td>
<td>RCA</td>
<td>60/120</td>
<td>D (+) galactose N-acetylglactosamine</td>
</tr>
<tr>
<td>Tetraxonolobus purpureas</td>
<td>Lotus</td>
<td>120</td>
<td>L (-) fucose N-acetyl-D-glucosamine</td>
</tr>
<tr>
<td>Triticum vulgareus</td>
<td>WGA</td>
<td>36</td>
<td>NN'-diacetylchitobiose NN'-N'-triacyctiyctobiose</td>
</tr>
<tr>
<td>Ulex europaeus</td>
<td>UEA I</td>
<td>68</td>
<td>L (-) fucose</td>
</tr>
</tbody>
</table>

### MATERIALS AND METHODS

The river Elbe (Germany) is one of the largest rivers in middle Europe having a discharge of 537 m³ s⁻¹, a particulate load of 24.2 mg l⁻¹, TOC (total organic carbon) 7.8 mg l⁻¹, TIN (total inorganic nitrogen) 5.19 mg l⁻¹, total P 0.26 mg l⁻¹, COD (Chemical oxygen demand) 20.8 mg l⁻¹ and a saprobic index of 2.5 (all mean values) (Landesamt für Umweltschutz Sachsen-Anhalt 1997). Water samples were taken from the river Elbe in Mudgeburg at 322 km on the right side of the river. Initially, the samples were transported in 1 l glass bottles within 30 min to the laboratory. After this time the first aggregates were already sedimented at the bottom of the flasks. These aggregates were carefully transferred with an inverted 10 ml glass pipette to an 8-well coverslip chamber (Nunc, Roskilde, Denmark). Later, the aggregates were collected directly into coverslip chambers. For reproducibility, 4 wells were used for staining and observation. Optical sectioning of the river snow aggregates by CLSM was performed in these chambers.

The nucleic acid stains SYTO 9 and SYTO 64 (Molecular Probes, Eugene, Oregon) and a panel of fluorescently labelled lectins (Sigma, St. Louis, Missouri) were used to stain the aggregates. The characteristics of the lectins are described in Table 1. The lectins were custom labelled with fluorescein isothiocyanate (FITC) and tetramethyl rhodamine isothiocyanate (TRITC). Staining of aggregates was carried out with the nucleic acid stains only, nucleic acid and lectin stain as well as lectin double stain.

Each of the nucleic-acid-specific stains SYTO 9 and SYTO 64 (7.5 µl) were diluted in 5 ml filtered (0.2 µm) demineralized water. The samples (200 µl) were covered with the staining solution (200 µl) and immediately examined by CLSM.

For lectin staining, the freeze-dried lectins were diluted to a concentration of 0.1 mg ml⁻¹ protein. This solution (200 µl) was added to the samples (200 µl) in
In the coverslip chambers and incubated for 20 min. The samples were then carefully washed 4 times with filtered river water to remove unbound lectins, by using a standard filter paper to draw off excessive liquid (Neu & Lawrence 1997). Subsequently, the samples were carefully covered again with filtered river water and examined by CLSM.

Controls for lectin inhibition were performed using Canavalia ensiformis FITC-lectin and methyl-α-D-mannopyranoside (Sigma). The lectin was used at the same concentration as for staining (0.1 mg ml⁻¹ protein). From the inhibiting carbohydrate a stock solution was prepared (100 mg ml⁻¹ in 30 mM phosphate buffered saline, pH 7.2). This concentration as well as a dilution series from 1:10 to 1:100 000 was used for the inhibition experiments. The procedure employed for inhibition experiments was identical to the lectin staining described above.

CLSM was performed with a TCS 4D (Leica, Heidelberg, Germany) attached to an inverted microscope and equipped with an argon-krypton laser. The aggregates were observed with 20 × 0.6 NA, 63 × 0.7 NA and 63 × 1.2 NA W CORR lenses. Reflection images were taken with RT 30/70 and polarisation filters. Specific settings were used for FITC (excitation = 488 nm, emission filter = BP FITC) in the green channel and TRITC (excitation = 568 nm, emission filter = BP 600) in the red channel. The settings intended for CY5 (excitation = 647 nm, emission filter = LP 665) in the far red channel were used to record the autofluorescence of chlorophyll-containing organisms.

For presentation of micrographs the standard software ScanWare Ver. 5.1A (Leica) was used. The optical sections were presented as maximum intensity projections in Photoshop 5.0 (Adobe, Edinburgh, UK).

RESULTS

Initially, the CLSM was run in the 1- or 2-channel mode only. This sometimes resulted in the overlay of 2 clearly different signals in 1 channel. For example, in the 2-channel mode, the chlorophyll signal was recorded in the same channel as the TRITC signal from the lectins (e.g., Fig. 1f). To separate these 2 signals, the instrument was run in the 3-channel mode with simultaneous recording. The filter settings of the CLSM in the 3-channel mode allowed the separation of the TRITC signal into the red channel and the chlorophyll signal into the far red channel.

Control images of unstained samples were scanned in the reflection and fluorescent mode. The reflection images were used to examine the river snow samples for the presence of geogenic or biogenic mineral particles which are typical for aquatic aggregates. The reflection image is shown as a maximum intensity projection (Fig. 1a). As a further control, images of the same aggregate were taken in simultaneous 3-channel fluorescent mode, to record autofluorescence. At equivalent settings, the images showed little signal in the green and red channels. However, in the far red channel the chlorophyll a signal was recorded (Fig. 1b). It clearly shows the signals originating from a variety of green algae.

The direct nucleic acid staining with SYTO 9 gave a bright signal in the green channel without any background staining. An example of nucleic acid staining of microbial cells associated with river snow is presented as a maximum intensity projection (Fig. 1c). The aggregates were colonized by a variety of morphological bacterial cell types and microcolonies.

Lectin staining of the river snow resulted in 2 types of information. Usually the images showed, as opposed to the nucleic acid stain, a cloud-type distribution of glycoconjugates. Two examples with single Lens culinaris FITC (Fig. 1d) or single Bandeiraea simpilifolia TRITC (Fig. 1e) labelled lectins are presented. The staining was clearly different from possible background fluorescence or non-specific binding. Sometimes, the glycoconjugates were located at the microbial cell surface, thereby staining single microbial cell surfaces or cell surfaces within microcolonies.

Double staining with FITC and TRITC labelled lectins showed the single glycoconjugates in green and red as well as the overlay of both glycoconjugates in yellow. This is demonstrated by combining the lectins from Lens culinaris and Bandeiraea simpilifolia (Fig. 1f). The images collected in the 2-channel mode showed the cloud-type distribution of the FITC-lectin recorded in the green channel. The binding reveals the size and outline of the whole aggregate. The red signal of the TRITC-lectin could only be found in a small area of the aggregate (large arrow, Fig. 1f). The remaining information in the red channel originates from the autofluorescence of chlorophyll-containing organisms, e.g., sessile or motile algae (small arrow, Fig. 1f). The same sample showed inside the red cloud a double labelling with both lectins at the cell surfaces of bacteria in yellow (large arrow, Fig. 1f). It is suggested that the microcolony within the aggregate produced its own micro-habitat by the production and release of a certain specific type of extracellular polysaccharide.

Other images were collected in the 3-channel mode by combining nucleic acid and lectin staining. This is shown for the green nucleic acid stain SYTO 9 and Ulex europaeus TRITC-lectin (Fig. 2a) and for the reversal stain with red nucleic acid stain SYTO 64 and Limulus polyphemus FITC-lectin (Fig. 2b). Both examples show, with respect to glycoconjugates, the chemical heterogeneity within river snow samples.
Fig. 1. Confocal laser scanning micrographs of river snow aggregates. Scale bars: 20 μm. (a) Single-channel 3-dimensional maximum intensity projection of the reflection signal from a river snow aggregate. (b) Three-channel (green, red, lar red = blue) maximum intensity projection of the autofluorescence signals of the river snow aggregate in (a). (c) Dual-channel maximum intensity projection of river snow aggregate. Nucleic acid staining is shown in green (SYTO 9); far red autofluorescence is shown in blue. (d) Single-channel maximum intensity projection of a single FITC-lectin (L. culinaris)-stained river snow aggregate. (e) Single-channel maximum intensity projection of a single TRITC-lectin (Bandeiraea simplicifolia)-stained river snow aggregate. (f) Two-channel maximum intensity projection of a double-stained river snow aggregate. The aggregate was stained with 2 different lectins, FITC-lectin (L. culinaris) = green signal and TRITC-lectin (B. simplicifolia) = red signal. Large arrow: a yellow microcolony surrounded by a cloud-type polysaccharide microhabitat; small arrow: autofluorescence signal from green algae

In another example, the double staining of an aggregate with 2 different lectins labelled with *Tetragonolobus purpureus* FITC and *Triticum vulgaris* TRITC is shown (Fig. 2c). The images were collected in the 2-channel mode. The nearly identical signal of the 2 channels resulted in an overlay of the green and red lectin signal, which is presented in yellow. This example shows an aggregate with a more homogeneous distribution of glycoconjugates (Fig. 2c). The same approach with 3-channel recording allowed in addition the collection of the signal from phototrophic organisms (Fig. 2d). This example shows the very specific binding of the *Limulus polyphemus* FITC-lectin to several microcolonies (small arrow), and in addition the specific binding of *Bandeiraea simplicifolia* TRITC-lectin to the polysaccharide cloud of another microcolony (large arrow) (Fig. 2d).

Finally, another 3-channel combination is presented as color stereo pair (Fig. 2e). The signals recorded included *Triticum vulgaris* FITC-lectin, *Tetragono lobus purpureas* TRITC-lectin and autofluorescence of chlorophyll a-containing organisms. This color stereo pair may be ideally examined by using stereo glasses to pick up the 3-dimensional color impression (Fig. 2e).

Controls for lectin specificity were done using *Canavalia ensiformis* FITC-lectin and methyl-α-D-mannopyranoside. The rising carbohydrate concentration clearly showed the inhibition of the lectin (2 μg/200 μl) in the range of 10,000–100 μg/200 μl (Fig. 3).

**DISCUSSION**

The challenge of analyzing EPS in interfacial microbial communities is well known in the literature and several solutions have been discussed (Neu 1994). One of the most promising tools for probing the EPS matrix in biofilm systems may be the use of lectins (Neu & Lawrence 1999).

Lectins are carbohydrate-recognizing proteins which may have bi-functional properties (Sharon & Lis 1989). Due to their specificity for carbohydrates they have been used as probes for microbial cell surface carbohydrates in pure culture. Examination of cells with colloidal gold labelled lectins was done by electron microscopy (Vasse et al. 1984, Monoka et al. 1987, Sanford et al. 1995, Hood & Schmidt 1996) whereas bacteria stained with fluorescently labelled lectins were observed by epifluorescence microscopy or measured by flow cytometry (Jones et al. 1986, Yagoda-Shagam et al. 1988, Quintero & Weiner 1995). Lectins were employed in studies of adhesive polymers such as *Cal uobacter* holdfasts (Merker & Smit 1988, Ong et al. 1990), bacterial 'footprints' (Neu & Marshall 1991) and *Hyphomonas* cell surface structures (Quintero et al. 1998). Fluorescent lectins have been suggested to distinguish between Gram-positive and Gram-negative bacteria (Sizemore et al. 1990) and between different cell surface mutants of *Rhodospiridium* (Buck & Andrews 1999). Furthermore, lectins were used as a measure for biofilm matrix material in *Staphylococcus* biofilms (Thomas et al. 1997).

Natural marine biofilm systems on inert and living surfaces were also the subject of lectin analyses (Michael & Smith 1995). It was found that the marine films showed a spatial and chemical heterogeneity of glycoconjugates similar to the results of the present study. In a pure culture study with *Staphylococcus epidermis* cellular autofluorescence and the lectin of *Triticum vulgaris* was used for visualization of bacteria and extracellular matrix material (Sanford et al. 1996). A CLSM study of environmental biofilms employed double lectin labelling in combination with a nucleic acid stain to examine the architecture of river biofilms (Neu & Lawrence 1997). The non-uniform distribution of glycoconjugates and charged and hydrophobic regions within a defined 9-member biofilm community was very recently described (Wolfaardt et al. 1998).

The application of fluorescently labelled lectins to stain aggregates is only limited by 2 factors. These are the need to remove unbound lectin which may move the aggregates and the presence of motile eukaryotic organisms which can interfere with imaging aggregates. The first disadvantage may be overcome by reducing the lectin concentration and increasing the incubation time. Movement of the lotic aggregates may not be as critical as for the very fragile marine and
lake snow samples. Lotic aggregates are exposed to a permanent shear force created by turbulent flow and thereby may have a stabilized structure. Some trouble may be caused by motile organisms creating a track through an image (Fig. 1f, top right) or moving aggregates, thereby degrading image quality. However, they do provide evidence of imaging fully hydrated, living systems.

A further area of development will have to be the quantification of the 3-dimensional image data. During CLSM the 3-dimensional sample is optically sectioned; consequently, a series of 2-dimensional sections is collected. For digital image analysis these sections are usually quantified 2-dimensionally and the result is presented in relation to the third dimension. True 3-dimensional quantification is difficult and requires sophisticated hardware and software. Nevertheless, the first approaches for pseudo-3-dimensional quantification of CLSM data have been presented (Kuehn et al. 1998, Lawrence et al. 1998b).

With respect to the structure of aquatic aggregates and activated sludge flocs the following points have to be discussed:

Firstly, there are reports on the structure of activated sludge flocs. These may be divided into those discussing the general architecture revealed after fixation, embedding, dehydration and sectioning followed by light microscopic (Li & Ganczarczyk 1990) or electron microscopic (Zartarian et al. 1994) examination and those applying rRNA targeted oligonucleotide probes to label certain microbial groups within the activated sludge floc. These fixed samples were examined by employing epifluorescence microscopy or CLSM (e.g., Wagner et al. 1994a, b, Amann et al. 1995, Manz et al. 1998). The CLSM images show the location and clustering of distinct bacteria within the activated

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Fig. 2. Confocal laser scanning micrographs of river snow aggregates. Scale bars: 20 μm. (a) Three-channel maximum intensity projection of a double-stained river snow aggregate. The aggregate was nucleic acid stained (SYTO 9) = green signal, TRITC-lectin stained (Ulex europaeus) = red signal and recorded together with the autofluorescence of green algae in the far red channel = blue signal. (b) Three-channel maximum intensity projection of a double-stained river snow aggregate. The aggregate was FITC-lectin stained (Limulus polyphemus) = green signal, nucleic acid stained (SYTO 64) = red signal, and recorded together with the autofluorescence of green algae in the far red channel = blue signal. (c) Two-channel maximum intensity projection of double-stained river snow aggregate. The aggregate was stained with FITC-lectin (Tetragonolobus purpureas) = green signal and TRITC-lectin (Triticum vulgare) = red signal. The overlay of both stainings at the same location resulted in the yellow signal. (d) Three-channel maximum intensity projection of double-stained river snow aggregate. The aggregate was stained with FITC-lectin (L. polyphemus) = green signal and TRITC-lectin (Bandeiraea simplicifolia) = red signal. In the third far red channel autofluorescence was recorded = blue signal. Small arrow: FITC-lectin (L. polyphemus)-stained microcolonies; large arrow: a microcolony surrounded by a TRITC-lectin (B. simplicifolia)-stained glycoconjugate cloud. (e) Three-channel maximum intensity projection of double-stained river snow aggregate shown as a stereo pair. The aggregate was stained with FITC-lectin (T. vulgare) = green signal and TRITC-lectin (T. purpureas) = red signal. In the third far red channel autofluorescence was recorded = blue signal. Stereo glasses will reveal the distribution of the 2 glycoconjugates in different layers, an interior pocket labelled with T. vulgare FITC-lectin and the channel structure of the aggregate.

Fig. 3. Control experiment with Canavalia ensiformis FITC-lectin and its target carbohydrate, methyl-α-D-mannopyranoside. (a) Control with lectin binding; (b) control with added carbohydrate but without lectin; (c–h) lectin inhibition starting at a high carbohydrate concentration to a dilution of 1:100 000.
sludge floc. However, due to the necessity of fixation, the floc may be condensed to about 25% of its original size (W. Manz pers. comm.). It is evident that probing the EPS with lectins would add additional information with respect to the chemical composition of the polymer matrix.

Secondly, there are several studies in which the ultrastructure of marine aggregates was investigated by TEM. The presence of the capsular envelope in more than 95% of marine-snow-associated bacteria was shown (Heissenberger et al. 1996a). Different approaches for preparation of aggregates to resolve structural details at 1 nm were evaluated. The best preservation of polymeric substances was achieved after fixation and embedding of the aggregates in a hydrophilic resin (Leppard et al. 1996). It was concluded that the polymeric substances found in marine snow may originate from the capsules of bacteria associated with the aggregates (Heissenberger et al. 1996b).

Thirdly, one may find in the literature the first attempts to use CLSM to study aquatic aggregates (Droppo et al. 1996, 1997, Liss et al. 1996, Holloway & Cowan 1997). The FITC-stained aggregates showed the cell distribution within the floc. However, all the CLSM samples were examined after embedding in agarose, which may interfere with the multiple application of lectins (Droppo et al. 1996, 1997, Liss et al. 1996). Very recently, the development of a CLSM technique for examination of formaldehyde-fixed marine snow samples has been described. The stains applied allowed the sequential imaging of selected polysaccharides, proteins, free DNA and chlorophyll (Holloway & Cowan 1997).

Fourthly, in addition to the structure-related publications, there are some studies on aggregates in the river Elbe estuary with emphasis on the microbial community (Zimmermann & Kausch 1996, Zimmermann 1997) and the significance of ammonia-oxidizing bacteria (Stehr et al. 1995). Furthermore, there are several German publications discussing the significance of lotic aggregates in the river Elbe or its tributaries (Greiser et al. 1996a,b) as well as their structure examined by TEM and micro element analysis (EDX) (Bähr et al. 1997).

This study showed that the biological constituents of the river Elbe aggregates were EPS, microbial cells and algae. When nucleic-acid-specific stains were used, they revealed the distribution of single microorganisms and microcolonies immobilized in the non-visible polymeric matrix. The space in between the individual microorganisms can be demonstrated by using a panel of fluorescently labelled lectins. This 'empty' space may be represented by EPS of microbial and algal origin as well as humic substances. Control experiments clearly showed the inhibiting effect of methyl-α-D-mannopyranoside on the binding of Canavalia ensiformis FITC-lectin to river snow. This is in agreement with another report where the identical lectin and carbohydrate combination was employed in control experiments with environmental biofilms (Michael & Smith 1995).

The polymeric matrix of river snow may have a crucial role in the interaction and transport of nutrients and contaminants in lotic systems. For extraction of 3 types of information the instrument was run in the 3-channel mode. This approach allowed the simultaneous collection of 3 signals in the green (FITC), red (TRITC) and far red (autofluorescence) channels (Lawrence et al. 1998b). Thus multi-channel CLSM of living, fully hydrated aquatic aggregates in conjunction with a variety of stains for nucleic acids and for probing the extracellular matrix with lectins proved to be a powerful tool for the assessment of chemical heterogeneity and of glycoconjugate distribution.

In nature many microorganisms are associated with interfaces. These so-called biofilm systems have been defined as microorganisms and their EPS associated with interfaces (Marshall 1984). So far general nucleic acid stains such as acridine orange (AO), 4',6-diamidino-2-phenylindole (DAPI) or the SYTO series have been used to stain cellular constituents in biofilm systems. In the meantime in situ hybridisation allows the determination of specific bacterial groups in environmental communities. Similarly, general EPS stains such as alcian blue, calcofluor white, and congo red have been employed to stain polymeric compounds in microbial communities. With the lectin approach described in this paper it is possible to detect specific glycoconjugates and their relation to microbial communities. Thus the technique allows, on the level of EPS, a more spe-

<table>
<thead>
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<th>Table 2. Strategy and comparison of general and specific in situ stains at the level of bacterial cells and extracellular polysaccharides as suggested</th>
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<tr>
<td><strong>Bacterial cells</strong></td>
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<tr>
<td><strong>General stains</strong></td>
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<tr>
<td>Acridine orange (AO)</td>
</tr>
<tr>
<td>4',6-diamidino-2-phenylindole (DAPI)</td>
</tr>
<tr>
<td>SYTO series</td>
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<td><strong>Challenge</strong></td>
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<td><strong>Specific in situ technique</strong></td>
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<tr>
<td>Oligonucleotide probes</td>
</tr>
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pecific staining. Consequently, there is the potential to use the lectin approach at the EPS level in a way similar to the in situ hybridisation approach with rRNA targeted oligonucleotide probes at the cellular level. It may be suggested to employ lectin staining of EPS as an additional technique to in situ hybridisation to specifically characterize microbial communities on the basis of their extracellular polymers (see Table 2).

In conclusion, confocal laser scanning microscopy is the key instrument to elucidate the hydrated, 3-dimensional architecture of interfacial microbial consortia (Lawrence et al. 1991). The same is true for aquatic aggregates in the form of river snow. Therefore, it is suggested that this technique may be added to the microbial ecologist's toolbox suggested by Paerl & Pinckney (1996).

Acknowledgements. The author would like to thank D. Spott for support in collecting water samples. The excellent technical assistance by U. Kuhlicke is gratefully acknowledged.

LITERATURE CITED


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Submitted: July 25, 1999, Accepted: November 30, 1999
Proofs received from author(s): February 7, 2000