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A natural terrestrial biofilm

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Material recovered from an extensive and viscous biofilm found on areas of rough hill pasture in Southern Scotland proved to consist of a thick mucilaginous deposit of polysaccharide in which species of green algae, with *Gleocystis* spp as the dominant microorganism and lesser numbers of *Oocystis* spp. Cyanobacteria were also entrapped. On laboratory culture other green algal and cyanobacterial species were detected. Analysis of the native polysaccharide and of the exopolysaccharide from a mixed culture of the dominant algal species derived from the original material, revealed the major components as glucose, galactose, mannose and rhamnose. The content of uronic acids was very low. The viscosity of the polysaccharide preparations was determined and compared with bacterial biofilm material; viscosity was lost following phenol extraction indicating that the original material was probably closely associated with proteins.

Keywords: green algae; cyanobacteria; polysaccharides

Introduction

In any natural aqueous environment the biofilm mode of growth is frequently the main source of biological productivity and such complex biofilms have been the subject of many studies. In all cases, the biofilms comprise complex mixtures of microbial cells together with exopolysaccharides (EPS) which play a major role in microbial adhesion and in maintaining the structure of the biofilm [10,11]. Algal crusts are of widespread occurrence and have been implicated in erosion. In one example, such crusts were initially composed predominantly of cyanobacteria with subsequent development of green algae [16]. Similarly, the algal mats found on or near marine shores and in other environments contain large amounts of polysaccharide [14] some of which may be rich in uronic acids [4]. These algal polymers are presumably derived from the sheaths and slime material which are found surrounding the surface of many cyanobacteria and green algae [6]. The exopolysaccharides of cyanobacteria have been the subject of a number of studies and contain a range of neutral monosaccharides together with uronic acids and, occasionally, pentoses or O-methyl pentoses (reviewed by Bertocchi *et al* [3]). Less is known about the sheath material and exopolysaccharides of green algae, although polymer from *Botryococcus braunii* was found to contain galactose as its major monosaccharide, together with 3-O-methyl-fucose and 3-O-methyl-rhamnose [1].

Most studies on biofilms relate to material obtained from solid–liquid interfaces in freshwater or saline environments. Few reports relate to material observed on solid/air interfaces. In November 1994, extensive tracts of coarse grass (*Festuca* spp) in areas of hill pasture at a height of *c* 400–700 m in a tributary valley of the River Tweed to the south of Edinburgh were observed to be covered with thick mucilaginous material; it was absent from the heather (*Calluna vulgaris*) which is also present on such hills on tracts adjacent to the grass. Similar material was observed in the following year at about the same time. In both years, the production of this material was observed during a prolonged period of relatively calm and sunny weather without either high rainfall or low nocturnal temperatures, although there was heavy dew. The following report describes our investigation of the microorganisms and polymers present in this mucilaginous material.

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Materials and methods

Harvesting and culture of biofilm material

The material from the biofilm after passage through muslin was cultured onto slopes of BG11 medium [9] and incubated in the same medium in static culture in Roux bottles or Erlenmeyer flasks with illumination at room temperature, 20°C and 25°C. Cultures were also maintained on slopes of the same medium containing 1.5% agar.

Purification of the biofilm material

The crude material was passed through muslin to remove plant material and yield a viscous green solution. On centrifugation at 50 000 × *g* for 45–60 min this yielded a yellow-green clear supernatant fluid (Supernate 1) and a large volume of gelatinous material associated with a deposit of green cells. The supernatant fluid was dialysed exhaustively against tap water then distilled water and aliquots were either lyophilised or retained for viscosity measurements. After addition of a few drops of formalin, the gelatinous deposit was mixed with several volumes of distilled water and treated in a domestic blender prior to re-centrifugation as above. A second supernate fraction (Supernate 2) was obtained together with a compact deposit of green cell material. The supernatant fluid was treated in the same way as before. Some of the lyophilised material from each supernate fraction was redissolved in 100 ml water and treated with an equal volume of 90% w/v aqueous phenol at 60°C for 10 min to remove protein. The upper, aqueous supernatants from the phenol treatment were again dialysed

and lyophilised. These preparations were designated Supernates 1P and 2P respectively.

Analytical procedures

Viscosity of the different samples was measured at 30°C using a Brookfield LVTD instrument (Brookfield Engineering, Stoughton, MA, USA). Monosaccharides were identified in acid hydrolysates of the polysaccharides (0.5 N H_2SO_4 for 16 h at 100°C) by descending paper chromatography using butan-1-ol/pyridine/water (6/4/3,v/v/v) as mobile phase and development with alkaline silver nitrate or saturated aniline oxalate, then quantified by HPLC on strong cation exchange columns (SCX) in the Pb^{2+} form as indicated by Kennedy and Sutherland [7].

Results

Examination of the mucilaginous material

The major species present in the material were *Gleocystis* spp and smaller numbers of *Oocystis* spp. A small number of filamentous cyanobacteria were also present as were other green algae such as *Ankinistrodesmus* spp. On culturing the material from the field on BG11 medium under illumination, green algae were still the dominant microorganisms present with greatly increased numbers of the *Ankinistrodesmus* spp and also types such as *Ecballocystis* spp which were not seen in the field material. More cyanobacteria were also visible including *Oscillatoria* and *Phormidium* spp.

Analysis of the mucilage

When hydrolysates of the crude viscous mucilage were examined by paper chromatography, the major sugars identified were fucose, mannose, glucose and galactose. The same monosaccharides were also present in the supernate fractions prepared (Supernates 1 and 2) and in the material partially purified by phenol treatment. However the molar ratio of the sugars differed in the different preparations (Table 1). The material from the laboratory culture contained rhamnose, mannose and galactose as the major neutral sugar components along with smaller amounts of fucose but lacked significant amounts of glucose.

Table 1 Composition of biofilm and algal material

	Molar ratio in:				
	Natural biofilm material				Algal material
	Supernate 1	Supernate 1P	Supernate 2	Supernate 2P	
Monosaccharide					
Rhamnose	—	—	—	—	2.19
Fucose			1.00	1.00	1.00
		1.00	1.00		
Mannose	2.05	1.34	2.13	2.21	3.25
Glucose	1.11	3.05	1.05	1.16	0.28
Galactose	4.21	2.25	3.45	3.29	3.16
Uronic acid[a]	4.25%	10.9%	0.5%	4.42%	5.63%

Molar ratios were calculated relative to fucose which was present in all preparations at approximately the same level.
[a]Uronic acid values were calculated as % dry weight of polysaccharide.

Mucilage viscosity

Comparison of the response of mucilage viscosity to shear indicated that while it showed shear thinning, it differed from a typical commercial polymer such as xanthan or from a typical viscoelastic polysaccharide derived from a bacterium previously found by us in an aqueous biofilm (Figure 1) (J Clark and IW Sutherland, unpublished results). Viscosity was totally lost following treatment of the initial supernate with phenol. This possibly indicates that the viscosity is due either to the complex of polysaccharides with protein rather than the polysaccharide mixture *per se* or that phenol treatment has dissociated the polysaccharides in such a way that they cannot reassemble on cooling to form the previous interactive system. It is also possible that ions responsible for the previous physical state had been lost during the extraction process, although addition of various monovalent and divalent cations to solutions of the purified material failed to reproduce the original viscosity nor did they stimulate any gel formation in either the original or phenol-treated polymers. The polysaccharide recovered from the laboratory culture appeared to show similar properties to the original mucilage despite its differing monosaccharide composition.

Discussion

The current report describes a biofilm found on a solid surface exposed to air rather than the commoner surface/liquid interface. As such, it clearly developed under the climatic conditions pertaining during a particular time period and probably only occurs rarely at the site described. It did however persist for several weeks as repeated visits to the same site recovered similar material over a period of

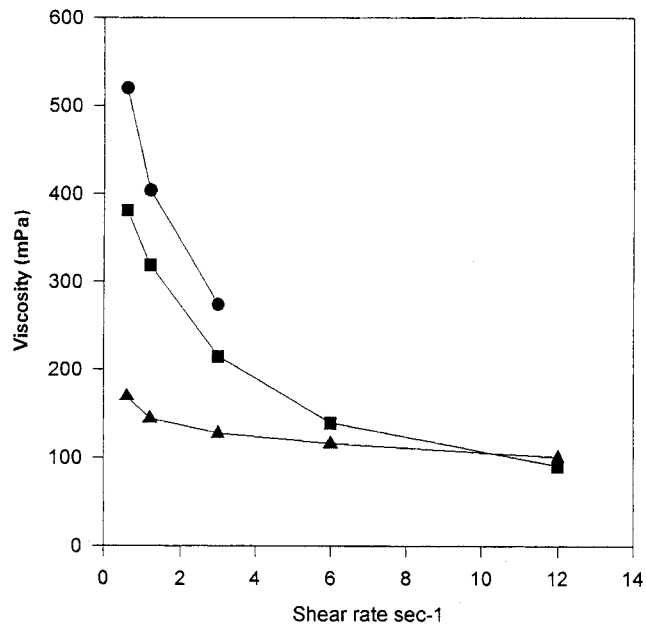

Figure 1 Viscosity of biofilm material. Viscosities were measured at 25°C using the Brookfield LVTD instrument.

approximately 3 weeks. Similar biofilms have been reported on grass in golf courses and in gardens at lower altitudes, where they may eventually dry out to form a thick, relatively water-resistant film affecting the plant growth and detracting from the appearance of the grass beneath. In gross composition they may resemble the algal mats which have been described in marine and other locations where they play a role in the stabilization of sediments and sand particles [8]. However, both the original material and the exopolysaccharide recovered from the algal cultures were extremely low in uronic acid content and would thus not be expected to play a major role in ion sequestration. This might also account for the unchanged viscosity of lack of gelation noted when multivalent ions were added to various preparations. One of their functions may instead be to provide a hydrophilic surface or oxygen- and light-permeable film permitting the survival and multiplication of the microbial cell community on the surface of the grass on which they were found. The known capacity for many microbial polysaccharides to absorb water may also provide a mechanism for ensuring some degree of hydration of the cell environment.

Although structural studies carried out on many green algae and on cyanobacteria, have revealed details of ultrastructure such as the fibrillar nature of some of the sheaths and slime surrounding the wall material [6], relatively few reports indicate the monosaccharide composition of such excreted polysaccharides and structural details are even more sparse. Unlike bacterial exopolysaccharides which are essentially free of covalently bound protein, the polysaccharide material found with the cells of green algae is closely associated with protein from which it can be separated by phenol extraction. In examinations of the sheath material from *Gleothece* spp, rhamnose, mannose, glucose and galactose were observed to be the major sugars [13,15]. These polymers also contained 2-O-methyl-D-xylose and both glucuronic and galacturonic acids. Thus while the major sugars are similar to those detected in the present study, and might indicate that the cyanobacteria contributed much of the biofilm material, no methylxylose was detectable under the assay conditions used. The exopolysaccharide from strain J-1 of *Phormidium* proved to contain rhamnose, mannose and galactose in the molar ratio 1 : 2 : 0.5 together with a smaller amount of uronic acid and sulphate [2]. Uronic acids are major components of polysaccharides from *Chlamydomonas* spp. An exopolysaccharide from *C. humicola* contained xylose, glucose and glucuronic acid in the molar ratio 3 : 1 : 1. The polymers from *C. peterfi* and *C. sajao* contained galactose and glucuronic acid in the molar ratio 2 : 1 and that of *Chlorella stigmatophora* comprised 30% uronic acid. However, the EPS from *Chlorella salina* more closely resembled the material in the present study as it contained only 6% uronic acid [5,12]. The polysaccharide from *Botryococcus* was a macromolecule of M_r 1.4–2 m in which galactose (80%) was the major component along with minor amounts of rhamnose (5%) [1]. Several of these green algal polymers also contained sulphate but this was not assayed in the present study.

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