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Cell coatings of surf diatoms

Revestimiento celular de diatomeas de la zona de rompiente de las olas

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ABSTRACT

Many sandy beach surf-zones support characteristic accumulations of diatom cells appearing as dark brown patches at the water surface. These diatoms become buoyant during the day, adhering to air bubbles generated by wave action. This, together with water circulation patterns generated by rip currents results in the accumulation of diatoms. By late afternoon the cells lose buoyancy and the accumulations disappear. Early investigations suggested that flotation and hence accumulation was facilitated by a mucilaginous cell coating. This coating was considered to be a unifying feature of accumulating surf diatoms. However, the degree to which a mucilage coat occurs is extremely variable. For example, *Attheya armatus* (West) Crawford has a thick mucilaginous sheath surrounding the cell, whereas *Aulacodiscus* spp. only have thin mucilaginous strands emerging from the rimoportulae and no surface mucilage. The nature of the mucilage coating also differs between the different species. *Anaulus australis* Drebes *et al.* Schulz has a thin layer of mucilage that continually dissolves into the water, whereas *A. armatus* has a permanent coating. *Asterionellopsis glacialis* (Castracane) Round and *A. armatus* have mucilage coats that are resistant to acid removal techniques, while the mucilage layer of *A. australis* is readily removed. Observations suggest that the flotation mechanism of surf diatoms cannot be simply explained by mucilages associated with surf diatom surfaces.

Key words: *Anaulus, Asterionellopsis, Attheya, Aulacodiscus,* mucilage.

RESUMEN

Muchas de las zonas de rompiente de las olas de playas arenosas presentan acumulaciones características de diatomeas, que aparecen como parches café oscuro en la superficie del agua. Estas diatomeas flotan durante el día adhiriéndose a burbujas de aire generadas por acción del oleaje. Esto, junto a los patrones de circulación del agua generados por corrientes de resaca origina la acumulación de las diatomeas. Al atardecer, las celulas pierden flotabilidad y las acumulaciones desaparecen. Las primeras investigaciones sugirieron que la flotación, y por lo tanto la acumulación, es facilitada por un revestimimiento mucilaginoso de las células. Se consideró que este revestimiento es una característica unificadora de las diatomeas que se acumulan en la zona de rompiente. Sin embargo, el grado de ocurrencia de un revestimiento mucilaginoso es extremadamente variable. Por ejemplo, *Attheya armatus* (West) Crawford tiene una gruesa envoltura mucilaginosa rodeando la célula, mientras que *Aulacodiscus* spp. sólo tiene delgados cordones de mucílago emergiendo de la rimoportula y ningún mucílago superficial. La naturaleza del revestimiento de mucílago es también distinta entre las distintas especies. *Anaulus australis* Drebes et al. Schulz tiene una capa delgada de mucílago que se disuelve continuamente en el agua, mientras que *A. armatus* tiene un revestimiento permanente. *Asterionellopsis glacialis* (Castracane) Round y *A. armatus* tienen revestimientos de mucílago que son resistentes a las técnicas de remoción ácida, mientras que la capa de mucílago de *A. australis* es removida rápidamente. Las observaciones sugieren que el mecanismo de flotación de las diatomeas de zona de rompiente no puede ser explicado simplemente por el mucílago asociado a la superficie de estas diatomeas.

Palabras clave: *Anaulus, Asterionellopsis, Attheya, Aulacodiscus,* mucílago.

INTRODUCTION

Marine habitats require the organisms that inhabit them to develop adaptive features (Mariani et al. 1990). This statement is particularly true of certain marine habitats that are extremely hostile to life. The coastal fringe, where waves break on either rock or sandy beaches, represents the most turbulent habitat in the marine environment. Macrophytic algae have adapted to this habitat by the production of agar and other cell wall constituents, making them pliable and resistant to mechanical damage (Parker & Diboll 1966, Mariani et al. 1990). There are few microalgal species that attain high cell concentrations in this environment. In low energy surf-zones, a high diversity of diatoms is found with some dinoflagellates and silicoflagellates (Campbell & Bate 1991). At several high energy sandy beaches, surf diatoms are the dominant (> 80% of the mi-

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croalgal community) phototrophic organisms coping with this harsh environment. Other non-accumulating diatoms make up most of the remainder of the microalgal community.

Most surf diatoms are confined to surf-zones. In order to remain active they must be retained within the surf-zone where they rise to the water surface by attaching to wave generated bubbles. If they are washed out of the surf-zone they sink to the ocean floor where unfavourable conditions induce them to form resting cells (Du Preez & Bate 1992). Most surf diatoms can only compete with neritic diatoms in the surf-zone.

On the basis of diatom concentration patterns, it is evident that surf diatoms have a mechanism whereby they adhere to bubbles and thereby float to the water surface during the day (Lewin & Hruby 1973, Talbot & Bate 1986). The cells are retained in the beach terrace, at the water surface, by the action of water gyres formed by rip activity and onshore moving waves. In the case of *Anaulus australis* Drebes et Schulz, it has been hypothesised that the cells cease to adhere to bubbles in the late afternoon and relocate into the sand for the night (Talbot & Bate 1988). The appearance of the cell surface changes from smooth in the morning to rough in the afternoon (Talbot & Bate 1988). This selective adherence to bubbles causes the cells to experience a diel periodicity between epipsammic and nektonic modes.

Talbot & Bate (1988) have hypothesised that the cells develop a mucilaginous coating during the day in order to achieve this diel periodicity. In the hypothesis, some unspecified surface features of the cells of *Anaulus australis* enable them to attach to bubbles during the day. In the mid to late afternoon the cells develop a mucilage coating that allows them to attach to sand grains, resulting in their removal from the water column. The cells remain in the sediment until morning when cell expansion causes them to slough off the mucilage and emerge from the sediment to repeat the cycle.

All surf diatoms behave in a similar way and on the basis of observations made of *Attheya armatus* (West) Crawford and *Anaulus australis*, Talbot & Bate (1988) hypothesised that all surf diatom accumulations would be the result of interactions between diatom mucilages and bubbles. If mucilage coats do play a role in controlling accumulation dynamics then there should be similarities between the cell coatings of all surf diatoms.

The mucilaginous secretions of diatoms have long been studied (Mangin 1908, Cholnoky 1928). Early work consisted of non-specific staining of connecting threads of colonial, planktonic diatoms (Mangin 1908) and freshwater benthic diatoms (Cholnoky 1928). More recently, stains of known specificity have been used to identify the type of adhesive involved in fouling benthic diatoms (Daniel et al. 1987). In this case, a mucilaginous material is involved with attachment either by a "sticky" substance cementing the cell surface to the substrate, or as a morphologically distinct structure (Daniel et al. 1987). These structures are pads, envelopes, stalks or tubes (Hendey 1951, Round 1971, Chamberlain 1976). Little histochemical work has been done on mucilaginous coatings of non-colonial, planktonic cells.

In this study, a cytochemical characterization of the surface polysaccharides of surf diatoms was undertaken to compare different species that form accumulations. In addition, the cell coatings of accumulating surf diatoms were compared with those of other species that co-inhabit the surf-zone, but do not become dominant.

MATERIAL AND METHODS

To identify the composition of the adhesive materials of marine fouling diatoms, Daniel et al. (1987) developed a series of cytochemical staining techniques. In this study, not all the methods recommended by Daniel et al. (1987) were employed, but at least one for each group of compounds was used.

Cell collection

Surf diatom cells were collected from Waiuku beach, New Zealand as well as the Sundays River beach, South Africa. The sample from Waiuku beach contained *Attheya armatus*, *Aulacodiscus kittonii*

Arnott, *Anaulus australis*, and *Asterionellopsis glacialis* (Castracane) Round (listed in order of abundance). Because it was difficult to find the latter two species in the Waiuku beach sample, as they were present in low numbers, a second sample where *Asterionellopsis glacialis* was dominant with a co-dominance of about 20% *Anaulus australis* was chosen from the Sundays River beach. The Waiuku beach sample also contained the freshwater diatom *Asterionella formosa* Hassall (third most abundant in the sample). This diatom could have been washed into the surf-zone from a river although the nearest river mouth is almost 23 km away. The cell coating of *A. formosa* is also included in this report (although it is not a surf diatom) because the cells were concentrated into the discoloured foam together with the surf diatoms.

The samples were collected at 14:00 h when a mucilage coat would be expected according to the theory of Talbot & Bate (1988). These two samples provided all but one (*Asterionella socialis* Lewin et Norris) of the surf diatom genera known to form accumulations in surf-zones around the world. A description of the cell coating of *A. socialis* was taken from Lewin & Norris (1970) as no cells were available at the time of this study.

Neutral polysaccharides

Periodic Acid-Schiff method (McCully 1966)

The periodic acid-Schiff method is the best diagnostic histochemical test for carbohydrate material (Chayen et al. 1973). Cells were fixed in neutralised formalin and post-fixed for 5 minutes in picrate formalin with 5% acetic acid. They were washed in 70% ethanol and then immersed for 5 minutes in periodic acid solution made up of 400 mg periodic acid in 15 ml water to which 135 mg sodium acetate dissolved in 35 ml ethanol was added. This solution was made up immediately before use as it does not keep. Cells were washed in 70% ethanol again, after which they were stained for 5 minutes in a solution of 1 g potassium iodide, 1 g sodium thiosulphate in 20 ml water, 30 ml ethanol and 0.5 ml 2N HCl. The cells were again washed in 70% ethanol. They were immersed in Schiff's reagent for 30 minutes, washed in 70% ethanol and viewed.

Charged polysaccharides

Ruthenium Red (Blanquet 1976)

The cells were fixed in neutralised formalin. They were stained for 5 minutes in 0.05% ruthenium red in 0.1 M potassium phosphate buffer at pH 7.4. Before viewing they were washed in potassium phosphate buffer.

Acidic polysaccharides with carboxyl and/or sulphate and/or phosphate groups

Toluidine Blue O, pH 4.4 (Chayen et al. 1973)

Samples were fixed in neutralised formalin and then immersed in 0.05% Toluidine Blue O made up in citrate buffer (pH 4.4) for 15 seconds. Cells were washed in citrate buffer before viewing.

Alcian Blue, pH 2.5 (Sheath & Cole 1990)

Cells were fixed in neutralised formalin. They were stained in 1% Alcian Blue in 3% acetic acid (pH 2.5) for 30 minutes. Before viewing, they were rinsed in distilled water until the supernatant became colourless.

Acidic polysaccharides with sulphate and/or phosphate groups

Alcian Blue pH 1.0 (Sheath & Cole 1990)

The cells were fixed in neutralised formalin. They were stained for 30 minutes in 1% Alcian Blue in 0.1 N HCl (pH 1.0) after which they were rinsed in distilled water until the supernatant became colourless.

Azure A (Chayen et al. 1973)

Cells were fixed in neutralised formalin and postfixed in picrate formalin. They were stained for 30 minutes in 0.1 mg ml^{-1} Azure A in citrate buffer (pH 4). They were washed in citrate buffer before viewing.

Acidic polysaccharides with sulphate

Heath's Aluminium Sulphate (Heath 1961)

The cells were fixed in neutralised formalin and stained in a solution of 1% neutral red and 1.6% aluminium sulphate.

Microscopy

Cells were viewed using the 100x oil immersion objective of a Zeiss Axioplan universal microscope.

RESULTS

Fig. 1 gives line drawings of the surf diatoms *Anaulus australis, Asterionella formosa, Asterionellopsis glacialis, Attheya armatus* and *Aulacodiscus kittonii* showing the structures referred in the text.

Neutral polysaccharides

Periodic Acid-Schiff method

The periodic acid-Schiff method stained neutral polysaccharides pink. Only two species showed staining around the cell body: *Asterionellopsis glacialis* and *Attheya armatus*. Neither *Anaulus australis, Asterionella formosa* nor *Aulacudiscus kittonii* showed any staining. Both *Asterionellopsis glacialis* and *Attheya armatus* had thick coats musilage. The average thickness of the mucilage on *A. armatus* was 2 µm and that on *A. glacialis* was measured at 1 µm thick. Both species had particles embedded in the mucilage.

Charged polysaccharides

Ruthenium Red

Ruthenium Red stained the mucilage of *Asterionellopsis glacialis* and *Attheya armatus* pink. The mucilage thickness' were calculated to be the same as that measured in the periodic acid-Schiff method. In *Aulacodiscus kittonii*, the rim of the frustules stained positively, as did amorphous mucilaginous aggregates found adhering to the frustule. No mucilage was found associated with the rimoportulae. The valvar region of *Anaulus australis* stained positively with the cingular region staining only slightly. No extracellular mucilage was visible. The whole cell stained positively in *Asterionella formosa*, but no extracellular mucilage was detected in this species either.

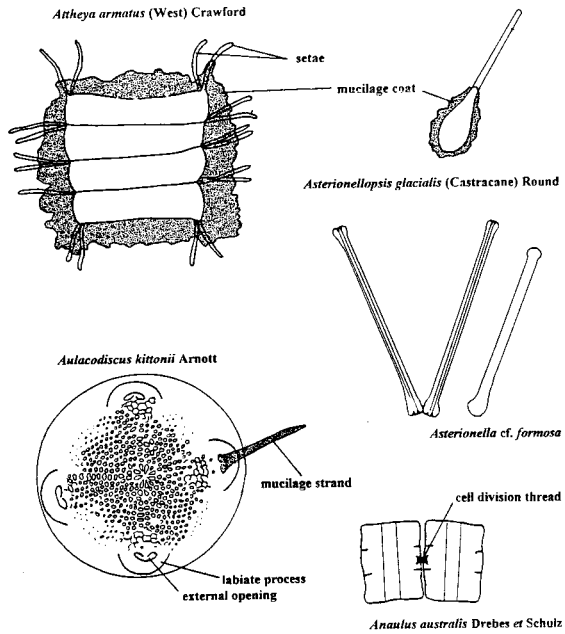

Fig. 1: Line drawings of the surf diatoms *Anaulus australis* Drebes et Schulz, *Asterionella formosa* Hassall, *Asterionellopsis glacialis* (Castracane) Round, *Aulacodiscus kittonii* Arnott and Attheya armatus (West) Crawford showing the structures referred to in the text.

Esquema de las diatomeas de la zona de rompiente de las olas *Anaulus Xaustralis* Drebes et Schulz, *Asterionella formosa* Hassall, *Asterionellopsis glacialis* (Castracane) Round, *Aulacodiscus kittonii* Arnott and Attheya armatus (West) Crawford mostrando las estructuras a las que se refiere en el texto.

Acidic polysaccharides with carboxyl and/or sulphate groups

Toluidine Blue O

Attheya armatus cells stained strongly purple with toluidine blue. The setae of *A. armatus* stained slightly pink. The cell body of *Asterionellopsis glacialis* stained strongly purple, but the spine did not stain at all. In *Aulacodiscus kittoni* the rim of the cells stained purple, but no external mucilage was detected. In several cells, stained mucilage was apparent at the external openings of the rimoportulae. In *Anaulus australis* cells there were a slight staining in the cingular region, but the valvar region did not stain at all. *Asterionella formosa* stained slightly purple.

Alcian blue pH 2.5

Alcian blue (pH 2.5) stained surf diatom cells in a similar fashion to that of toluidine blue. *Attheya armatus* and amorphous mucilaginous aggregates stained strongly, with the setae of *Attheya armatus* staining slightly blue. The cell body of *Asterionellopsis glacialis* stained strongly blue, but unlike with toluidine, the spine stained slightly blue. In *Auladiscus kittonii* the rim of the cells stained blue, but no external mucilage was detected. In several of the cells, strongly stained mucilage was apparent near to, and at the external openings of the rimoportulae. In *Anaulus australis* cells the nuclear region stained strongly blue and there was positive staining of the whole cell, but no external mucilage stained. The nuclear region of *Asterionella formosa* also stained strongly blue with the cell surface also staining positively, but no external mucilage could be found.

Acidic polysaccharides with sulphate and/or phosphate groups

Alcian bue pH 1.0

As with the previous stains, both *Attheya armatus* and *Asterionellopsis glacialis* stained strongly with thick mucilaginous coatings. In both cases, the appendages stained slightly. A few cells of *Aulacodiscus kittonii* were found where strands of weakly stained mucilage emanated from the external opening of the rimoportula. In *Anaulus australis* the thread that holds recently divided cells together stained strongly blue. Several cells were found with a mucilage layer of less than 0.5 um thick, but the majority of the cells had no such coating. The protoplasm of *Asterionella formosa* stained weakly blue. Of the other diatoms present some showed weak blue staining (e.g., *Surirella* sp.) and others showed no staining at all (e.g. *Navicula* sp. and *Biddulphia* sp.).

Azure A

The staining with Azure A showed similar patterns to Alcian Blue (pH 1.0), but stained cells of *Anaulus australis* less strongly.

Sulphated acidic polysaccarides

Heath's Aluminium Sulphate

Heath's technique, using neutral red, stained the mucilage coat of Attheya armatus orange but the coat of *Asterionellopsis glacialis* pink. The whole cell of *Aulacodiscus kittonii* stained positively. Both *Anaulus australis* and *Asterionella formosa* stained very weakly.

DISCUSSION

The mucilage coat of *Asterionellopsis glacialis* and *Attheya armatus* is made up of a mixture of polysaccharides. The mucilage of both species contained neutral polysaccharides, charged polysaccharides and sulphated polysaccharides. All techniques used had a positive result. The polysaccharides associated with the setae, however were acidic phosphated/carboxylated polysaccharides. This staining is probably the diatotepum, i.e. the polysaccharide layer generally produced in diatoms just below the frustule (Schnepf & Drebes 1977). *A. glacialis* has fewer sulphate polysaccharides as indicated by the light staining with Heath's aluminium sulphate.

The mucilage associated with *Aulacodiscus kittoni* rimoportulae was found to be a phosphated acidic polysaccharide. *A. kittonii* also had the acidic phosphated/carboxylated polysaccharide layer below the frustule.

Asterionella formosa showed slight staining of the whole cell, also most likely due to the subfrustular acidic phosphated/carboxylated polysaccharides. No extracellular mucilage was detected nor did the connecting strand joining the cells into colonies stain with any of the methods used.

Anaulus australis had three types of mucilage. A diatotepum of acidic phosphated/carboxylated polysaccharides was evident. Where present, the extracellular mucilage was found to be weakly acidic sulphated polysaccharides. The mucilage thread between recently divided cells was also made up of a sulphated polysaccharide, but a strongly acidic one.

Of the species that form surf diatom patches, three have similar mucilage coats:

Asterionellopsis glacialis, *Asterionella socialis* (Lewin & Norris 1970) and *Attheya armatus*. *Asterionella formosa* appears to have no mucilage coat while *Aulacodiscus kittonii* only has mucilage strands emanating from the rimoportulae. *Anaulus australis* has a very thin mucilage coat recorded in only a few of the cells. The cells are held together as doublets by a mucilage strand until separation, but there was no evidence of a mucilage coat similar to *Asterionellopsis glacialis* and *Attheya armatus*, although Talbot & Bate (1989 have suggested this.

All surf diatom species exhibit a similar pattern of diel periodicity (Lewin & Schaeffer 1983, Kindley 1983, Talbot & Bate 1986). Based on histochemical analysis, the feature causing the diel periodicity cannot be considered to be the mucilage coat as their composition is different. The thickness of the coat varies, as does the temporal variability of the mucilage coat in the different species. If mucilage does play a role in buoyancy regulation of surf diatoms, it is a complex one.

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