

# Structure and function of marine microbial aggregates

Bacterial extracellular processes  
Detritus  
Macroaggregates  
Processus bactériens  
extra-cellulaires  
Détritus  
Macroagrégats

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## ABSTRACT

A transmission electron microscope study of laboratory-produced marine aggregates showed bacterial extracellular polysaccharide processes to be responsible for aggregate formation. Adjacent bacteria appear to be attached to one another by these processes. It also appears that similar extracellular material is responsible for attachment of a bacterium to particles of decomposing seaweed. The functional significance of this structure, in carbon and energy flow (in the shape of bacterial macroaggregates and detritus) in aquatic environments is briefly discussed.

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## RÉSUMÉ

Structure et fonction des agrégats microbiens marins

Une étude au microscope électronique par transmission des agrégats marins produits en laboratoire montre que les processus bactériens extra-cellulaires polysaccharides sont responsables de la formation d'agrégats. Les bactéries contiguës paraissent s'être jointes au moyen de ces processus. Il semble qu'un matériel extra-cellulaire du même type soit aussi responsable de l'attache d'une bactérie aux grosses molécules des algues en voie de décomposition. L'importance fonctionnelle de cette structure vis-à-vis des flux carbonique et énergétique dans l'environnement aquatique est brièvement discutée.

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## INTRODUCTION

Attempts by microbial ecologists to correlate microorganism structure with function have continued to evoke interest in the fine structure of bacteria (Fletcher, Marshall, 1982; Paerl, 1973; Pedros-Alio, Brock, 1983; Sieburth, 1979; Wiebe, Chapman, 1968).

The question of the origin and fate of aggregates in the sea is closely tied to that of the interaction between micro and macro food webs, since aggregations of bacteria and non-living particulate matter are more accessible to macroconsumers than free-living bacteria (Pomeroy, 1984). Also, physico-chemical processes promoting exchange between dissolved and particulate organic pools of carbon in the ocean are often used by ecologists to explain the calculated shortfall in the amount of particulate food required by macro food chains and that available to them in the form of phytoplankton cells and detritus (Riley, 1963). In the past, particle aggregation leading to production of marine detritus has been attributed to physico-chemical flocculation (Krank, Milligan, 1980). However, a duplication of these experiments with bacteria-killed controls indicated that presence of active bacteria is necessary for

aggregate formation (Biddanda, 1985). Microbial aggregation is a surface phenomenon. In this study, therefore, I have examined the fine structure of bacterial aggregates and bacterio-seaweed particle aggregates in sea water, to reevaluate the role of microorganisms in the ecology of detritus and aggregate formation.

## MATERIALS AND METHODS

Bacterial aggregates and bacterio-seaweed particle aggregates were produced in laboratory microcosms as described in Biddanda (1985). Briefly, the method consisted of suspending, by agitation, an organic inoculum (prepared by grinding seaweeds — *Ulva*, *Gracillaria* and *Sargassum* — in a mortar and pestle and extracting in sea water), in sea water, either as dissolved organic matter (extract filtered through 0.45 µm Millipore filter), or as dissolved organic matter + particulate organic matter (extract screened through 100 µm Nitex screen). Visible bacterial aggregates and bacterio-seaweed particle aggregates, respectively, formed in a 2-4 day period (Biddanda, 1985). About 50 ml subsamples were centrifuged gently to concentrate the aggregates for transmis-

sion electron microscopy. Fixation was done in 2% Glutaraldehyde in 1M Cacodylate buffer with 5.5% sucrose (the sucrose concentration was raised by 0.5% to match the osmolarity of marine bacterial cytoplasm) for 2 hr. and centrifuged gently to form pellets. 2% noble agar was melted at 55°C and drops were put on a dental plate. The pellet was transferred very carefully (taking care not to admit air bubbles) by means of a pasteur pipette into the drops of agar and gently mixed. After three minutes, the solidified agar drops with the aggregates were minced into 1 mm cubes, treated in 2% osmium tetroxide, then 4% osmium tetroxide + 0.2 M Cacodylate buffer for 1 hr. The specimen was dehydrated in graded concentrations of Ethanol, infiltrated in propylene oxide and embedded in Spurr media (Spurr, 1969). Blocks were polymerized at 60°C for 48 hr, sectioned in Reichert OMU2, and stained in Uranyl acetate followed by Lead citrate. A separate set of samples was stained in Ruthenium red to ascertain the nature of the extracellular adhesive material (Fletcher, Floodgate, 1973). The Philips 200 model was used for electron microscopy and photography.

## RESULTS

The high magnification electron micrograph of the interface between two bacteria in a bacterial aggregate (Fig. 1) details the bacterial extracellular processes that constitute inter-bacterial bridging, a feature that enables the bacteria to attach to one another resulting in the formation of macroaggregates. The electron micrograph of the thin section of the bacterio-seaweed particle aggregate (Fig. 2), illustrates the attachment of a bacterium to a decomposing seaweed particle. My observations (morphological and staining characteristics with Ruthenium red), indicate that the same bacterial extracellular polysaccharide processes are responsible for attachment in both cases. The classification of the polysaccharide region into primary and secondary types is based on Ruthenium red staining (Fletcher, Floodgate, 1973; 1976). In addition, the electron opaque and fibrillar appearance of the extracellular material resembles that observed by Fletcher and Floodgate (1973), Leppard *et al.* (1977), and is similar to the capsular polymers observed by Marshall *et al.* (1971).

## GENERAL DISCUSSION AND CONCLUSIONS

Adhesion of bacteria to surfaces and particles with the aid of extracellular processes has been suggested (Busch, Stumm, 1968; Goldman, 1984, Fletcher, Floodgate, 1973; Harris, Mitchel, 1973; Imam *et al.*, 1984; Leppard *et al.*, 1977; Marshall, Cruickshank, 1973; Paerl, 1973; 1978; Sieburth, 1983). Results of this study (the first detailed description of aggregates produced under defined conditions), confirm the direct involvement of bacterial extracellular processes (biological glue) in attachment and aggregate formation. Such aggregate formation may explain the occurrence of deep-sea marine snow and other organic-inorganic complexes. In fact, these laboratory-produced aggregates

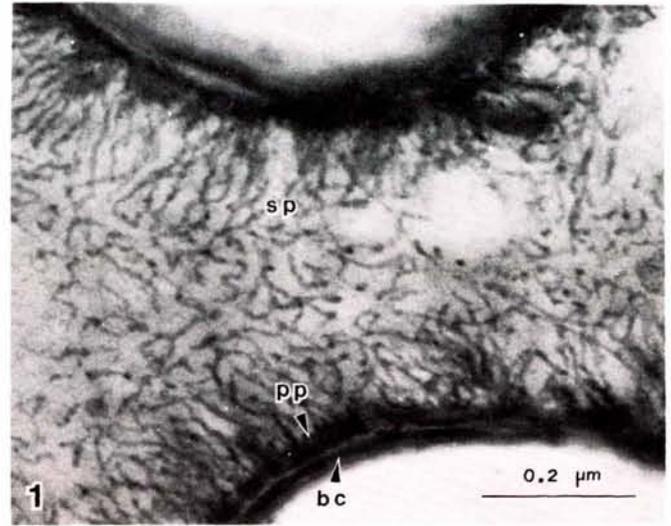


Figure 1

Transmission electron micrograph showing the details of the interface between two adjacent bacteria in a marine bacterial aggregate. Bacterial cell (bc), primary polysaccharide (pp), and secondary polysaccharide (sp).  $\times 144000$ .

resemble closely in both their morphology and microbiology the aggregates produced from the later stages of degrading *Trichodesmium* blooms at sea (Pomeroy, pers. comm; Biddanda, pers. observations-manuscript in prep.). Hobbie and Lee (1979), have emphasized the quantitative significance of microbially-produced extracellular material as a carbon and energy source for consumer organisms. The present study, suggests the need for its qualitative appreciation as well, viz., its role in the ecology of detritus (Paerl, 1975) and in macroaggregate synthesis (Biddanda, 1985).

It is obvious that microorganisms invest considerable energy into the production and maintenance of extracellular structures that aid them in attachment. This may well be an adaptation to exploit nutrients that accumulate at the solid-liquid interface (Marshall, 1976). Also, since attached bacteria experience a microenvironment that is quite different from the surrounding bulk phase, their activity is different from that of free-living bacteria (Kirchman, Mitchell, 1982; Hoppe, 1984). In this context, Pedros-Alio and Brock (1983) and Kefford *et al.* (1982) have investigated reversible and irreversible attachment by microorganisms at interfaces and considered its ecological importance. From a consideration of these findings, I propose that the presence or absence of microbial aggregation and attachment may be of significant causal importance, so as to shift the role of the "microbial loop" (Azam *et al.*, 1983; Pomeroy, 1974; Williams, 1981) from that of a "link" to a "sink" (for carbon and energy; Pomeroy, 1974), or *vice versa* in aquatic ecosystems.

Aggregation of bacteria and their attachment to particles enhances the efficiency of bacteria consumption by consumer organisms (Caron *et al.*, 1982; Seki, 1972), and both selective as well as non-selective feeders may derive significant amounts of energy from bacterial aggregates and bacteria in detritus (Pomeroy, 1984). In addition, transfer of microbial macroaggregates directly to the macro food chains without the mediation of the multi-step microbial loop, may be a major pathway for

the flow of carbon and energy to the higher trophic levels in aquatic systems. This consideration may account for the "shortfall" in mass balance, discussed earlier.

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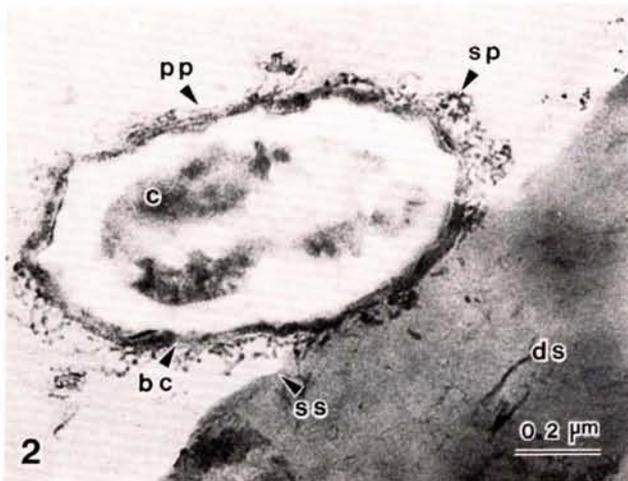


Figure 2

Transmission electron micrograph of a marine bacterio-seaweed particle aggregate showing details of the interface between a bacterium and a decomposing seaweed particle. Bacterial cell (bc), seaweed particle surface (ss), decomposing seaweed particle (ds), primary polysaccharide (pp), secondary polysaccharide (sp), and bacterial cytoplasm (c).  $\times 68000$

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