EXTRACELLULAR POLYMERS IN BIOFILMS

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1. Introduction

Many of those who study biofilms view them as a collection of living organisms at an interface but this definition should be expanded to include the products of those organisms. A major product is the matrix in which biofilm cells are found. It is somewhat surprising that there is such an emphasis on the biotic component of the film because this phase occupies only a small fraction of the volume (Characklis & Cooksey, 1983). It is often the biofilm matrix that causes many of the economic problems associated with biofilm formation since it acts as a layer of immobilized water. It is in fact highly hydrated and contains 98-99% water (Christensen and Characklis, 1990). This matrix, which is really a collection of polymers rather than a single material, is made by many organisms in biofilms. The polymers have been referred to collectively as capsules, sheaths, slime and glycocalyces. Costerton _et al_ (1981) proposed the term glycocalyx for use in procaryotic biology. They defined a glycocalyx as "those polysaccharide-containing structures of bacterial origin, lying outside the integral elements of the outer membrane of Gram-negative cells and the peptidoglycan of Gram-positive cells". They further subdivided glycocalyces into (1) glycoprotein subunits at the cell surface and (2) capsules. "Capsules" were further subdivided into (a) those that are rigid and exclude particles such as Indian ink (a classical negative "stain" in bacteriology); (b) those, which in contrast to (a), are flexible and include Indian ink; (c) integral capsules that are closely associated with the cell surface and (d) those capsules that are peripheral to the cell and can be lost to the aqueous phase. In a brief but comprehensive review (Geesey, 1982), Geesey used a less structured term for the high molecular weight material extracellular to cells. He referred simply to extracellular polymeric substances and included all types of cells, not
just bacteria. Although not the first to use it, this review is largely responsible for the general use of the term now universally abbreviated as EPS. Because of its ease of use (e.g. it requires no knowledge of chemical structure), I believe the term EPS has come to be regarded almost as a substance in its own right, rather than a collective term used to describe a poorly-understood group of macromolecules, external to the cell and of differing structure.

In this article I will use the term EPS as described by Geesey rather than by Costerton, since I wish to include all organisms at surfaces. In 1983, Characklis & Cooksey reviewed the role of EPS in biofilm formation and summarized their ideas speculatively as follows: EPS "may (1) provide cohesive forces within the biofilm, (2) adsorb nutrients, (3) protect immobilized cells from environmental changes, including the influence of biocides, (4) adsorb heavy metals from the environment, (5) adsorb particulate and other detritus, (6) serve as a means of intercellular communication within the biofilm, (7) provide short-term energy storage via the cell membrane potential and (8) enhance intercellular transfer of genetic material. Although a great deal of progress has been made since, these thoughts, in some cases, must still be treated as speculation, in spite of the research effort over the intervening years (e.g. Dahlem conferences, 1984, 1989).

Although many organisms in a climax biofilm contribute to the formation of EPS (Marzalek et al, 1979) only those of bacteria and microalgae will be reviewed here.

2. Bacterial EPS

2.1. COMPOSITION

It should be mentioned at the outset that no component of the EPS known to be implicated in the adhesive process has been comprehensively analyzed. Rather we have a series of fragmented studies addressing only part of the problem.

As mentioned in the introduction, many workers have prepared a polysaccharide material from the medium after the growth of bacterial cells. A procedure relying heavily on dialysis to remove low M.W. growth medium components, followed by ethanolic precipitation (80-90% v.v.) is usually used. There have rarely been attempts to discover the number of macromolecules present. Yet in 1973 Fletcher & Floodgate proposed the involvement of two polysaccharides in the adhesive process of Pseudomonas strain NCMB 2021. The first of these was thought to be involved in the initial adhesion and was presumed to exist on the surface of all cells. The second polymer was produced subsequent to the adhesive event and possibly caused a firmer association with the substratum. An alternative explanation is that the secondary polymer is produced only by cells in the "resting phase" - a state sometimes applied to cells adhered in biofilms. Cations, especially calcium, are important in maintaining the structure. Working with the same organism, Christensen et al (1985) have published the most detailed information to date on EPS conformation, but even this does not go as far as a primary structure for the polymers found. These workers found that Pseudomonas strain NCMB 2021