Structure, function and immunochemistry of bacterial exopolysaccharides

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There has been much written on bacterial exopolysaccharides (EPS) and their role in virulence. Less has been published regarding EPS in free living species. This review focuses on that subject, emphasizing their functions in the environment and the use of antibody probes to study them.

Keywords: polysaccharides; bacterial capsule; biofilm

Composition and structure of bacterial polysaccharides

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Part of the structural diversity of EPS is due to the fact that two identical sugars can bond to form 11 different disaccharides. In contrast two identical amino acids can form only one dipeptide. Additionally, EPS contain a wide variety of sugars as for example glucose, mannose, glucuronic acid, and rhamnose in xanthan gum [32], galactose and glucose (Rhizobium meliloti) [50], xylose (Cryptococcus neoformans) [9], hexosamines, aminouronic acids, aldoses, diaminohexoses [56], 2,3-diamino-2,3-dideoxyxuronic acid, and 5,7-diamino-3,5,7,9-tetradeoxynuloseonic acid [137]. Furthermore, the noncarbohydrate side groups that are found in bacterial EPS add to their heterogeneity that, as a consequence of all of these considerations, far exceeds that of proteins and is reflected in the hundreds of O-antigen serotypes of enterobacteria [49]. Functions for this heterogeneity have been ascribed to pathogens [24] but not environmental strains [106].

The molecular weights of LPS [15,39,43,78,92] and EPS [17,65,99,117] are also extraordinarily heterogeneous. With incomplete stringent control over the number of subunits added to a chain [6], long and short polymers are synthesized, although one molecular weight species predominates. EPS forms higher order structures [49]. Xanthan gum is thought to form double-strand antiparallel helices [84], while the EPS from various Klebsiella spp form left-handed helices [48]. The EPS component of many bacterial films forms thick fibers when examined with electron microscopy [3,8,94]. It has been suggested that these fibers are attached to discrete areas on the outer surface of the cell [8,94]. While EPS and LPS have partial synthetic pathway commonality in some species [58], the remainder of this review shall primarily be concerned with EPS.

Transport attachment and localization of bacterial EPS

A major unanswered question is how the hydrophilic EPS is transported across the hydrophobic interior of the outer membrane to the outer surface of the outer membrane of Gram-negative bacteria. It has been suggested [8], but not documented [12,88], that adhesion sites (Bayer junctions) between the inner and outer membrane are sites of both LPS and EPS transport. Lipid A and O-antigen are synthesized separately on the inner face of the inner membrane and are joined on the periplasmic face of the inner membrane [82]. For E. coli EPS, a 60-kilodalton periplasmic protein is required for translocation to the outer surface of the outer membrane, whereas a protein is not required for LPS translocation [98,103]. Immunochemical analysis of Pseudomonas sp strain S9, suggests that EPS polymerization (or crosslinking) can occur on the outer surface of the outer membrane [135].

The fine structure of the fibrillar structures [3,8,94] radiating out from the cell surface suggests that EPS is bound at a limited number of discrete sites. Once these sites are filled, excess EPS may become the source of slime ([133]; EPS found free in the media). Alternatively, only EPS molecules of the correct length may bind to attachment sites, with larger and smaller molecules forming the slime [112]. Immunoelectron microscopy of a marine pseudomonad suggests that shorter EPS molecules are integrally bound to the outer membrane (integral capsule) while the longer polymers are loosely (peripherally) associated [34]. Without knowledge of export mechanisms it is difficult to theorize just how EPS may be site-specifically deposited. Along with caulobacters, hyphomicrobia are good models to study
mechanisms of EPS deposition. Since export is both polar and temporal, a rare occurrence in procaryotes [93,119,120,122,130], the machinery can be readily correlated with zones of production.

**Regulation of bacterial EPS production**

Many environmental factors can affect the rate of EPS synthesis in bacteria. They include increased oxygen [7], limitation of nitrogen [51,79] and cations (e.g. magnesium, sulfate, phosphate and calcium [27,51,112]), desiccation [87,112,129], low temperature [117], growth on minimal media [117] and growth phase [17,121,129,134]. In most of these cases, enhanced EPS synthesis is a response to environmental stress (e.g. nutrient limitation).

The question of regulation of EPS production has been approached using molecular techniques to analyze the genetic regulation of EPS synthesis in *E. coli* and *Alteromonas atlantica*. The regulatory circuit controlling colanic acid capsule synthesis in *E. coli* includes at least four proteins. RcsA and RcsB are positive trans-acting regulators of capsule synthesis and RcsC is a negative regulator [13,42,89]. RcsA is unstable due to its sensitivity to the Lon protease [116]. RcsC and RcsB are similar to many two-component (sensor-effector) regulatory systems [110]. Even though it is still not clear under what environmental stimulus RcsC (the sensor) activates RcsB (the effector), this is the first genetic evidence linking an environmental stimulus to increased EPS production.

The genetic mechanism for regulation of *A. atlantica* EPS synthesis differs markedly from that of colanic acid [5] and more closely resembles the antigenic phase variation described for *Salmonella*, *E. coli* and *Neisseria* [1,104,114]. These variable systems involve complex reversible genomic rearrangements which can turn EPS synthesis (or antigenic variation) off or on. Under conditions favorable for EPS production, those bacteria which are 'on' will predominate. If the conditions change so that EPS production is no longer favored, those bacteria which were already 'off' will out-compete the 'on' bacteria and predominate [5]. In caulobacters and hyphomonads, EPS regulation is temporal and less influenced by environmental factors [85,124]. In *Pseudomonas aeruginosa*, the intricate regulatory cascade of alginate expression is being worked out [30,35,97,131].

**Functions of EPS**

EPS is produced by the majority of Gram-negative bacteria, some of which invest more than 70% of their energy in its production [45]. Consequently many species grow faster on laboratory media after they mutate and stop producing EPS. This suggests that some environmental factor selects for continuous EPS production. The existence of a genetic switch in the marine bacterium *A. atlantica* [5] to insure the concurrent existence of both EPS-producing and non-producing forms of the organism emphasizes both the importance of EPS and its demand on cellular resources. Polar EPS synthesis, if EPS is an adhesin, may be another resource-saving mechanism.

As discussed in excellent earlier reviews by Decho [29] and Dudman [31a], many functions have been proposed for bacterial EPS (Table 1). They can be divided into four groups, functioning: a) as a physical protective barrier; b) as a response to environmental stress; c) in cell/cell recognition and interaction; or d) in biofilm formation/adhesion. The ability of EPS to act as a physical barrier has been demonstrated with pathogenic bacteria. Encapsulation of *E. coli*, *Klebsiella* sp., and pneumococci renders them resistant to phagocytosis, complement fixation, and antibody [37,102]. In fact, the pathogenicity of bacteria can be artificially increased by coating them with hog gastric mucin, a charged mucopolysaccharide [49]. Even though bacteria may be subjected to phagocytosis-like predation in the natural environment, they are not exposed to antibody or complement. There, EPS may protect against bacteriophage [118], hydrophobic toxins [127] and desiccation [81,87].

In response to stress, when essential cations are required, anionic EPS would sequester them, increasing the gradient across the cell membranes [45]; or, excretion of the charged polymer may provide the driving force for importation of other charged ions [128]. Polymerization of EPS would also produce excess reducing power, used to drive high affinity or high energy uptake systems [45,115]. In symbiotic relationships, the EPS and LPS of some nitrogen-fixing bacteria, most notably *R. meliloti*, but also others [10,68,95], function in the host-specific, bacterial invasion of developing root nodules on leguminous plants [33,64]. The EPS is involved in the initial recognition and attachment of the bacteria, leading directly to morphogenic changes in the plant [10,33,64,95]. Kirchman et al [60] showed that *Pseudomonas marina* EPS is an inductive cue for the metamorphosis of the marine polychaete, *Janua brasiliensis*. The metamorphic trigger might involve the binding of a larval lectin to the EPS [59]. Other marine invertebrates however do not specifically bind with bacterial films prior to larval settlement and metamorphosis [125,126].

Nevertheless, it has long been recognized that there is an ordered sequence of periphytic succession for colonization of clean surfaces immersed in seawater. In the initial phase, after possible coating by organic matter [70], bacteria attach to a surface and begin to grow, forming micro-colonies within several hours [19,25,31,36,77]. Subsequently, diatoms, fungi, protozoans, micro-algae and other microorganisms attach to the surface, adding to the primary slime layer [25,31,36,77,105]. This primary microbial colonization often appears to be a prerequisite for the final stage of succession in which large organisms, viz., invertebrates, attach and grow on the surface [23,26,139].

The biofilm/adhesion functions of EPS are extremely important medically and commercially. The importance of biofilm formation on bacterial growth in dilute nutrient environments has been recognized since Zobell and Anderson's [140] early work on the relationship between bacterial growth and solid surfaces. The involvement of EPS in initial adhesion of the cells [3,20,44], as well as the structural matrix of the biofilm and as an active metabolic component of the biofilm, has received much attention [e.g. 21,22,75,101,113]. Briefly, some EPS may function as an initial adhesion [3], more as a permanent adhesion [44] and many as the biofilm matrix [125].