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Extracellular Polysaccharides of Phytopathogenic Bacteria *

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Abstract

This article draws attention to the involvement of bacterial exopolysaccharides (EPS) in plant pathology. Different types of EPS from the following phytopathogenic genera *Agrobacterium, Erwinia, Pseudomonas, Xanthomonas* and *Corynebacterium* are described. Reference will also be made to the symbiotic *Rhizobium.*

Although experimental results in this area are limited, the different hypotheses about the possible functions of these EPS are presented; namely the relationship between virulence and the composition of EPS, the protective effects of EPS for the bacterium both in isolation and in contact with the plant and recognition functions in symbiotic or pathogen/plant host interaction.

Exopolysaccharides from phytopathogenic or symbiotic bacteria that are produced commercially and those with likely potential for industrial use are also presented.

Keywords: exopolysaccharides, symbiotic and phytopathogenic bacteria, virulence, recognition

Abbreviations: EPS: exopolysaccharide; HR: hypersensitive reaction; IF: intercellular fluid; LPS: lypopolysaccharide

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1. Introduction

Definition and scope of article

Phytopathogenic and symbiotic bacteria synthesize polysaccharides which lie outside the cell wall or are found secreted into the environment (soil, rhizosphere, phylloplan, plant tissues). Depending on their structural relationship to the bacterial cell, they have been variously termed slime, capsule, microcapsule and glycocalyx. The term exopolysaccharide (EPS) provides a general term for all of these forms of bacterial polysaccharide found outside the cell wall and will be further used in this article and even widened to include the extracellular glycopeptides of corynebacteria, since their sugar residues comprise more than 80% of the molecule. The scope of this article is primarily concerned with the possible role(s) of EPSs in the virulence of phytopathogenic bacteria. Reference will be also given to symbiotic *Rhizobium* as these bacteria are closely related to phytopathogenic *Agrobacterium* and because the role of the EPS in symbiosis is rather well documented and could thus throw some light upon the biological importance of EPS in plant-bacteria interaction. The various functions that have been demonstrated or suggested for EPS will be successively surveyed by drawing examples from amongst the following genera: *Corynebacterium, Pseudomonas, Xanthomonas, Erwinia, Agrobacterium* and *Rhizobium*. Bearing in mind the nature of this conference particular attention will be paid to phytopathogenic and symbiotic bacterial EPSs with a potential for commercialization.

General background

Phytopathogenic bacteria that have the capacity to cause disease are called virulent. Virulence is determined by the outcome of the host-pathogen interaction which can be either compatible or incompatible. Compatibility results when a plant and a pathogenic or symbiotic bacterium are able to coexist at least during the early stage of the interaction, i.e. disease or symbiosis. The inability of a plant and bacteria to coexist results in incompatibility and the absence of disease or symbiosis.

The relationship between virulence and the presence of EPS has long been known in phytopathogenic bacteria (Husain and Kelman, 1979; Ayers et al., 1979; Bradshaw-Rouse et al., 1981). Mucoid virulent strains of phytopathogenic bacteria generally become less virulent or even totally avirulent when they lose their slime and turn rough in appearance. This frequently occurs during subculture *in vitro*.

There are various degrees of specificity in plant-bacteria interaction (Brian, 1976) occurring at the species level (basic compatibility), and also at the race/cultivar level (Heath, 1981). At both of these levels the relationship between EPS and virulence appears complex and far from a simple all-or-none effect due to the presence or absence of EPS. Upon contact, bacteria and plant may recognize each other through interaction of complementary surface molecules, and the events which ensue have profound influences on plant morphogenesis, nutrition and pathogenesis. The increasing awareness of the importance of cellular adhesion as an important determinant of plant-bacteria interactions has been recently reviewed (Schmidt, 1979; Sequeira, 1978; Dazzo, 1980). In studies dealing with the recognition between the host and bacteria, it is important to distinguish two stages: (a) the adsorption, or the immobilization of bacteria on the plant surface. This stage is non specific and depends to a large extent on the nature of the plant surface; (b) the attachment, or the consolidation of the interface between the bacterial and plant surfaces involving the specific formation of polymer bridges between the two surfaces.

2. Roles of Extracellular Polysaccharides

Wilt-inducing bacteria

High molecular weight extracellular glycopeptides and polysaccharides are known to be produced by a number of wilt-inducing phytopathogenic bacteria and the role of slime production in the process of wilting has been known for a long time in the following examples: *Pseudomonas solanacearum* (Husain and Kelman, 1958), *Corynebacterium sepedonicum* (Spencer and Gorin, 1961), *C. insidiosum* (Spencer and Gorin, 1961; Ries and Strobel, 1972), *C. michiganensae* (Rai and Strobel, 1969), *Xanthomonas campestris pv. campestris* (Sutton and Williams, 1970), *X. campestris pv. oryzae* (Angadi, 1978), *Erwinia amylovora* (Ayers et al., 1979), and *E. stewartii* (Bradshaw-Rouse et al., 1981).

Wilting is the result of the disturbance to the water supply throughout the plant. Increased resistance to water flow may result from (a) accumulation of large amounts of high molecular weight EPS in the xylem vessels as the result of active, systemic multiplication of bacteria. Polysaccharides larger than 20,000 daltons in size can cause plants to wilt, as can dextrans (Van Alfen and McMillan, 1982). In addition the viscosity of carbohydrate polymers interferes greatly with water flow. Thus the physical properties of EPS could be responsible for the non specific reduction or even total

blocking of water flow by most of the wilt-inducing bacteria; (b) the accumulation in xylem vessels of substances of plant origin which result from bacterial enzymatic degradation of the plant cell wall; (c) the combination of both mechanisms. There is controversy about the role of bacterial enzymatic degradation of the plant cell wall *in vivo* and unfortunately ultrastructural studies do not distinguish clearly between the possible mechanisms (Wallis et al., 1973; Wallis, 1977; Wallis and Truter, 1978). These wilt-inducing EPSs were previously referred to as toxins. They have been excluded from the toxin definition because of their lack of host specificity and because the loss of membrane permeability could not be sufficient to cause wilting (Daly, 1981; Barzic, 1985).

Compatible/incompatible interactions at the leaf level

Almost all phytopathogenic bacteria enter plant tissues passively through natural openings (stomates, trichomes, hydathodes) or wounds. Having entered the plant tissues, compatible bacteria will multiply quickly and spread from the entry point and in some cases will become systemic by xylem transport. Incompatible bacteria will mostly induce a resistance mechanism, named hypersensitive reaction (HR), that results in the rapid death of a limited number of host cells surrounding the infection site and in the death of the invading bacteria (Kiraly, 1980; Klement and Goodman, 1967). In some cases (Goodman et al., 1977; Sequeira and Graham, 1977; Cason et al., 1978) HR-inducing incompatible or saprophytic bacteria attach to plant cell wall and are readily immobilized within the granular and fibrillar matrix of the plant. In other cases (Daub and Hagedorn, 1980; Hildebrand et al., 1980), immobilization within this matrix is not observed. The hypersensitive reaction is often associated with numerous biochemical changes and accumulation of antibacterial products (terpenoids, salts, enzymes...) that are responsible for the death of incompatible bacteria.

It has been hypothesized that compatible strains of *Pseudomonas solanacearum* prevent their immobilization by their ability to produce soluble EFS which binds to the plant lectins (Sequeira and Graham, 1977). The binding of sufficient amounts of EPS to the host cell may result in the neutralization of all available binding sites and thus in preventing further attachment of the compatible bacteria. The ability of EPS to prevent the binding of lectin has been demonstrated in the cases of: *Erwinia stewartii* (Bradshaw-Rouse et al., 1981) and *Xanthomonas campestris pv. citri* (Takahashi and Doke, 1983; Takahashi and Doke, 1984). However, the above hypothesis does not

explain why many EPS-producing incompatible bacteria are also attached and enveloped by tobacco cell walls (Goodman et al., 1977; Sequeira et al., 1977). It has been shown that the EPS of *Pseudomonas solanacearum* binds quantitatively to the lectin at relatively low ionic strengths *in vitro* (Duvick and Sequeira, 1984). This binding does not occur in the intercellular fluid from tobacco leaf, as obtained by the method of Klement (1965) because of the high ionic strength of the fluid. However because this fluid is artificially generated, this result is not inconsistent with the possible binding of EPS with the lectin *in vivo*.

A typical symptom of many bacterial diseases of leaves is water-soaking, i.e. water congestion in the normally air-filled intercellular spaces of the mesophyll. It has been demonstrated that persistent water-soaking is only induced when purified EPS from *Pseudomonas phaseolicola* is infiltrated into the leaves of susceptible cultivars of bean but not into those of resistant cultivars nor those of other incompatible plant species (El Banoby et al., 1980). *In vitro* studies revealed that the intercellular fluid (IF) from resistant bean cultivars degraded the bacterial EPS whereas no enzymatic degradation occurred in compatible combinations, i.e. in IF from susceptible cultivars (El Banoby et al., 1981). This provides the first indication that at this highly specific level of bacteria/plant interaction, resistant cultivars of bean negate certain bacterial factors which contribute to susceptibility.

It has also been demonstrated that maintenance of free water in the intercellular spaces of leaves by artificial permanent soaking was sufficient to prevent close contact of the bacteria with the host cell wall, a prerequisite for HR induction (Hildebrand et al., 1980; Stall and Cook, 1979). Ultrastructural studies have firmly established that the bacterial EPS appears as a thick, highly-ordered hydrated matrix (Costerton and Irvin, 1981; Politis and Goodman, 1980) whose hydrophilicity and water-holding capacity is likely to maintain water congestion in the mesophyll and prevent bacteria from becoming adsorbed to the plant cell wall, conditions which are most favorable for bacterial multiplication and movement within the plant tissues.

Most *Xanthomonas* species are primarily leaf pathogens that induce water-soaked leaf spots, some of which become systemic and invade xylem vessels. They all produce copious amounts of EPS. Attention has focused on EPSs from *Xanthomonas* sp. because of their commercial potential as rheological agents, i.e. they exhibit unusual and distinctive properties in aqueous solution. These EPSs (Xanthan gums) maintain their highly ordered configuration in solution, and after heating above transition temperature form

strong mixed gels with certain plant polysaccharides (galactomannans, glucomannans, cellulose), even though neither class of these polysacchrides will gel alone, except at high concentrations. No covalent binding occurs between the two polymers and the association is thermally reversible (Morris et al., 1977; Dea et al., 1977). This phenomenon, which appears specific to xanthan gums, occurs at temperature well above physiological and thus relevance xanthan/galactomannan *in planta* is dubious.

EPSs of Rhizobiaceae

Whereas in all phytopathogenic bacteria discussed above firm attachment and engulfing by the plant cell wall are correlated with incompatible reactions, in the case of the Rhizobiaceae firm attachment to the plant cell wall is a prerequisite for a successful compatible reaction (for a review see Vance, 1983).

Agrobacterium tumefaciens initiates crown-gall tumors in a number of dicotyledonous plants only when introduced into wounded tissues. Bacterial attachment to a specific wound site is necessary for the infection process (Lippincott and Lippincott, 1969). The polygalacturonic acid residues of pectin are involved in the adhesion of *A. tumefaciens* on the plant cell wall. Neither cell walls of monocotyldonous plants nor embryos of dicotyledonous plants are susceptible to *A. tumefaciens*. These are high in pectin methyl groups and evidence has been provided that the greater the methylation of pectin residues, the less adhesion and subsequent tumour initiation (Rao et al., 1982). Concerning the nature of bacterial molecules involved in the attachment it has been suggested that there could be a direct polysaccharide-polysaccharide interaction between the O-antigen of lypopolysaccharide (LPS) and polygalacturonic acid residues (Lippincott and Lippincott, 1980). However, *A. tumefaciens* LPS has been found not to inhibit tumorigenesis on potato disks (Pueppke and Benny, 1983). Cellulose fibrils produced by *A. tumefaciens* have been shown to anchor bacteria to the plant cells and to each other during attachment (Matthysse et al., 1981), but do not seem to be implicated in attachment since mutants blocked in cellulose fibril synthesis retain their virulence and attachment ability (Matthysse, 1983). Recent results suggest that beta(1 → 2) linked glucan may be the only component necessary for attachment of *A. tumefaciens* to the plant cell wall (Puvanesarajah et al., 1985).

Rhizobia selectively infect legume root hairs and then form root nodules which fix atmospheric nitrogen. In the establishment of symbiosis several

stages can be schematically distinguished (Vincent, 1980; Hirsch et al., 1982; Patel and Yang, 1981; Vasse and Truchet, 1984), and the involvement of Rhizobia EPSs in the pre-infective stages, namely adhesion to the root hair surface, firm attachment and root hair curling, have long been described (Dazzo, 1980; Dazzo and Hubbell, 1975; Bohlool and Schmidt, 1976) though with some controversy (Law et al., 1982; Sanders et al., 1981). An exhaustive review by Dudman (1983) has recently focused on the structure and possible role(s) of Rhizobia EPSs in the infection process. It now appears that EPSs are complex and that most strains produce different types of polymers. Thus acidic polyssaccharides might be involved in specific adhesion and attachment through a lectin binding mechanism (Dazzo, 1980; Dazzo and Hubbell, 1975; Bohlool and Schmidt, 1976): beta(1 → 2) linked glucans could account for the maintainance of the firm contact and for inducing the formation of the infection thread (Higashi and Abe, 1980; Abe et al., 1982); acidic oligasaccharides of fast-growing Rhizobia bind to lectin and stimulate infection thread formation (Abe et al., 1984). All of these studies are correlative and no decisive conclusion can be drawn until the genes that are involved in EPS production are identified. Recent findings provide the first genetic evidence that EPS of *Rhizobium meliloti* is required for the formation of the effective nodules (Leigh et al., 1985).

3. Composition, Structure and Biosynthesis of EPS

A wealth of literature about composition, structure and biosynthesis has long been devoted to Enterobacteriaceae, and more recently to Rhizobiaceae and Xanthomonas (for review see Tonn and Gander, 1979; Sutheriand, 1982; Sutherland, 1985). In contrast, there is little information, mostly restricted to composition, about the phytopathogenic Pseudomonas, Erwinia and Corynebacteria. In these latter genera it is difficult to establish any relationship between biological properties and structure because of the lack of information.

It appears worthwhile to focus on recent results concerning the structure and biological properties of EPSs. For instance, apart from xanthan gums, other gel forming polysaccharides have been described (Zevenhuizen and Van Neerven, 1983a; Zevenhuizen and Van Neerven, 1983b), whose biological function(s) are still unknown,but which could participate in bacterial polysaccharide/plant polysaccharide interactions. Though no discernible differences have been shown in the glycosyl sequences and the structures of different fast-growing Rhizobia leading to the conclusion that such similar basic

structures could not be responsible for host specificity (McNeil et al., 1986) other workers have reported that the acyl-substitution patterns in polysaccharide side-chains of Rhizobia were different (Kuo and Mort, 1986), as are the acyl and pyruvyl-substitutions in Xanthomonas (Sutherland, 1981). These variations in the degree and nature of the substitution could account for plant host specificity.

Concerning biosynthesis one should draw attention to the glycosyltransferase system. In fact, galactosyltransferases have been isolated from *Erwinia stewartii* (Huang, 1980) and *E. amylovora* (Huang, 1979). These enzymes are known to transfer galactose, a major constituent in most phytopathogenic bacterial EPSs, to side-chains of polysaccharides, and their potential role in adhesion has been proposed (Roseman, 1970; Albersheim and Anderson-Prouty, 1975).

4. EPSs of Practical or Potential Importance

Xanthan gums are produced by different pathovars of *Xanthomonas campestris*, and have received much attention because of their unusual and distinctive properties, namely: remarkable emulsion stabilizing ability, high viscosity even at low concentrations, little variation in viscosity with temperature, and gel formation with usually non gelling polysaccharides (for a review see Sandford, 1979). Industrial procedures developed for production, including continuous fermentation have been published (Weiss and Ollis, 1980; Thomson and Ollis, 1980; Tait et al., 1986).

Besides this well developed example, relatively few phytopathogenic bacterial EPSs have been studied and their use remains at the level of potential. Nevertheless, these EPSs are good candidates since they could be produced under controlled conditions from selected bacterial strains whose genotypes could be selected after transfer of genetic information that controls production of a given type of EPS. Of potential use are the beta-glycans, i.e. succinoglycans and curdlans, that are produced by some strains of *Agrobacterium* (Hisamatsu et al., 1977; Hisamatsu et al., 1978) and *Rhizobium* (Ghai et al., 1981; Harada and Amemura, 1981).

At the present time xanthan gums are the only significant commercial EPS isolated from phytopathogenic bacteria. However polysaccharides from other bacterial sources are of importance. So are the dextrans produced by different *Leuconostoc, Lactobacillus* and *Streptococcus* species (Sidebotham, 1974), and so are new EPS produced by *Pseudomonas elodea* and by some

Alcaligenes strains, and the search of new bacterial polymers is still continuing (Sandford, 1979; Baird et al., 1983; Kang et al., 1983).

5. Concluding Remarks

Little is known about the structure of phytopathogenic bacterial EPSs, with the exception of xanthan gums, and increased knowledge about the type of linkage between adjacent sugar residues, the sequence of oligosaccharides formed from the different monosaccharides, the degree and nature of non-carbohydrate substituents, the degree of polymerization, is needed to throw light upon the biological properties of these molecules. It is important to bear in mind that the term EPS covers an extremely varied range of polymers and that a single bacterium can excrete a different mixture of EPSs under different growth conditions. A better understanding of the role(s) of the EPS of phytopathogenic bacteria can therefore emerge only from concerted genetic, biochemical, cytological and pathological comparative studies.

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