Bacterial Polysaccharide and Sorbitol in Fireblight Exudate

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INTRODUCTION

The exudation of gum-like bacterial masses from diseased regions is a frequent feature of fireblight [*Erwinia amylovora* (Burrill) Winslow *et al.*] and other bacterial plant diseases. Exudate usually appears on the plant surface as small globules, a spreading film, or occasionally as fine aerial strands (Eden-Green & Billing, 1972; Billing & Eden-Green, 1973) and may provide a source of inoculum when transported by wind or wind-blown rain. Emergence of exudate follows the development of extensive internal bacterial populations, sometimes before other disease symptoms (wilt or necrosis) appear (Gowda & Goodman, 1970). Since bacteria account for only about 20 % of the dry weight of exudate removed from plant surfaces, analysis of the substantial non-bacterial moiety is of interest in view of the physical properties involved in the formation of aerial strands and its possible role in disease physiology.

METHODS

Potted apple, pear and hawthorn plants were inoculated with *Erwinia amylovora* by needle puncture of the stem below the tip of a growing shoot, or by cutting off the apices of recently unfolded leaves with scissors dipped in inoculum (Eden-Green, 1972). Exudate from a particular host species was removed with a small blade within 2 or 3 days of its emergence, bulked and stored *in vacuo* over P₂O₅. Bacteria were removed from 1 % (w/v) aqueous solutions of exudate by centrifugation (25 000 g) followed by membrane filtration.

Preliminary analyses indicated that the principal constituent of each bacterium-free sample was polysaccharide; this was precipitated from 80 % (v/v) ethanol solution on the addition of a few drops of saturated CaCl₂, and formed the bulk of an '80 % ethanol-insoluble' fraction. The latter was hydrolysed with 0.8 N-H₂SO₄ in an autoclave at 121 °C and neutralized with *N,N*-bis-octylmethylamine (Stoddart, Barrett & Northcote, 1967). Uronic acid present in hydrolysates was identified by paper chromatography (Fischer & Dörfel, 1955) and estimated colorimetrically (Bitter & Muir, 1962; Knee, 1973); neutral sugars were identified and estimated by gas-liquid chromatography of their trimethyl-silyl derivatives (Holligan & Drew, 1971; Knee, 1973). The polysaccharide was further characterized by chromatography on columns of DEAE cellulose in the phosphate form and Sephadex G-200 (Knee, 1973).

Between 20 and 50 % of the exudate matrix was soluble in ethanol. Carbohydrates in this '80 % ethanol-soluble fraction' were analysed by gas chromatography of acetate derivatives (Holligan & Drew, 1971) on a 2 m column of 2 % Silicone Fluid FS 1265 (QF1) on Chromosorb G at 190 °C, and trimethyl-silyl derivatives as above.

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Table 1. *Composition of 80 % ethanol-insoluble fractions of exudate samples from fireblight-infected host plants and from culture filtrates of Erwinia amylovora*

Sample	Constituents (% total accounted for):			
	Galactose	Glucose	Mannose	Uronide
Exudate samples:				
Apple	74.7	6.5	2.5	16.3
Pear	74.1	6.6	2.6	16.7
Hawthorn	74.9	6.3	2.6	16.2
Culture filtrates:*				
YPA + sorbitol	69.0	7.8	0.8	22.4
YPA + glucose	58.3	10.3	7.6	23.8
YPA + galactose	63.5	8.7	5.1	22.7

* Extracted from bacterial growth removed from Oxoid yeast extract + peptone agar (YPA) with carbohydrate added to 1 % (w/v) after autoclaving.

To test the phytotoxic effects of exudate, apple fruit and potato discs were incubated in concentrated (9 mg/ml) aqueous solutions, and apple shoot tip cuttings were placed in dilute (5 µg/ml) aqueous solutions (Hildebrand, 1939; Pierstorff, 1931).

RESULTS AND DISCUSSION

Galactose was the major neutral monomer detected in the 80 % ethanol-insoluble fractions with small amounts of glucose and mannose; the uronic acid had similar mobility to glucuronic acid on a paper chromatograph. The polysaccharides from three host sources had very similar monomeric compositions (Table 1). Polysaccharides with rather similar composition were extracted from the bacterial growth on plates of yeast extract and peptone agar with various carbohydrate supplements (Table 1). This, and the similarity of composition with extracellular polysaccharides produced by other Enterobacteriaceae (Sutherland, 1972) and bacterial plant pathogens (Sutton & Williams, 1970) indicated a bacterial origin for the fireblight-exudate polysaccharide.

The polysaccharide and a small amount of protein in the 80 % ethanol-insoluble fraction were both excluded from a Sephadex G-200 column. Polysaccharide and protein were retained by DEAE cellulose but were separated by elution with a phosphate gradient. The polysaccharide eluted at the higher ionic strength as a single peak containing neutral and acidic residues. This indicated that the 80 % ethanol-insoluble fraction contained a single heteropolymer which was a polysaccharide and not a glycopeptide, of the kind known to have phytotoxic effects when produced in infections of plants by other bacterial species (Strobel, 1972).

Concentrated aqueous solutions of bacterium-free exudate had no toxic effect on apple fruit or potato discs as judged by neutral red staining (Fushtey, 1957), but dilute solutions induced rapid wilting in apple shoot tip cuttings, which recovered in water if lower stem sections were removed. Bacteria are rarely seen in the xylem of diseased stems, however, and demonstration of the direct involvement of exudate polysaccharide in the wilting which often accompanies fireblight infections will depend on further investigation of the presence of this polysaccharide in these tissues.

Sucrose and other sugars could be detected in 80 % ethanol-soluble fractions but the major constituent was a hexitol whose acetate derivative had a similar retention time to that of sorbitol on QF1. This material accounted for 28 % of the dry weight of exudate from the apple sample.

Sorbitol is known to be the principal translocatory carbohydrate in apple (Bieleski, 1969) and other Rosaceae (Zimmerman, 1969), and it seems likely that the large quantities found in the ethanol-soluble fraction were derived directly from the host. Sorbitol is readily metabolized *in vitro* by *Erwinia amylovora*, however, and its predominance in exudate suggests that it is available in excess of demand, as might be the case if the pathogen had access to translocatory material in the phloem. Despite recent reports of bacterial migration in these tissues (Gowda & Goodman, 1970; Lewis & Goodman, 1965) based on the results of ringing experiments, careful histological examinations by many previous workers have shown that bacterial colonization of stem tissues is largely confined to cortical parenchyma, at least in the early stages of disease. Invasion of phloem has rarely been reported. It is not known whether radial movement of sorbitol from phloem to cortical parenchyma could account for the quantities found, or whether sufficient amounts are available in these tissues themselves. An alternative possibility is that sorbitol production is stimulated locally in response to the pathogen.

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Note added in press. R. N. Goodman, J. S. Huang and P. Huang at the Second International Congress for Plant Pathology, Minneapolis, Minnesota, U.S.A. (5 to 12 September 1973) reported the isolation of a polysaccharide from fireblight-infected slices of pear fruits. The preparations contained a small amount of protein and the only monosaccharide detected in hydrolysates was galactose. From observations of the degree of wilting induced by this material in various plant species the authors suggested that it was a host-specific toxin with effects proportional to the field susceptibility of plant species.

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