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Structural Analysis of Secreted Root Slime from Maize (*Zea mays* L.)

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ABSTRACT

Secreted slime isolated from the incubation medium of *Zea mays* roots maintained axenically contains fucose, arabinose, xylose, galactose, and glucose as the major monosaccharides. The slime preparation contains low levels (3% weight/weight [w/w]) of uronic acids. Methylation analysis reveals an extraordinarily diverse range of glycosyl residues. The fucosyl residues are primarily terminal (60%) and 3-linked (33%) with a relatively small proportion being 2-linked (6%). The methylation data are consistent with, but not proof of, the presence of a range of polymers including arabinogalactan-proteins (AGPs), xyloglucans, arabinoxylans, and glucans in the slime. The specific binding of the β-glucosyl Yariv reagent, a dye which binds and precipitates AGPs, to the slime preparation and to the outer periclinal epidermal cell wall surface in root sections, is further evidence for the presence of AGPs. Low levels of phenolic acids (approximately 0.17% w/w), in particular *trans*-ferulic acid, and protein (approximately 6% w/w) were also detected.

The root surface forms the interface between the plant, the soil and the soil microorganisms. A slime, secreted primarily from the root cap and possibly the epidermal cells, coats the root surface and forms a primary site for colonization of the root by microbial symbionts and pathogens. Colonization requires adhesion of microorganisms to the host root surface and, in some cases, specific interactions involving slime saccharides are apparently involved in the interaction between the root and soil microorganisms (20). In addition, the slime acts as a lubricant aiding the passage of the root through the soil.

In spite of the importance of root slime in rhizosphere colonization and plant root function, our knowledge of the structure of high mol wt components of root slime collected under sterile conditions is poor (26). Monosaccharide analyses of slime collected under axenic conditions (26) are limited to *Zea mays* (7, 19) and *Oryza sativa* (8). Wright and Northcote (32–35) and Wright (31) separated a sterile crude slime preparation from maize roots into neutral and acidic components and performed partial structural analyses.

In this paper we report monosaccharide and methylation analyses of soluble, high mol wt, slime secreted from roots of *Zea mays* under axenic conditions.

MATERIALS AND METHODS

Plant Material. Caryopses of *Zea mays* (cv Flat Red) were supplied by Gippsland and Northern Co. Pty. Ltd., Melbourne, Australia, and Hi-Test Seeds Australia Pty. Ltd., Melbourne, Australia.

Chemicals. Sodium borodeuteride (98 atom % ^{2}H), *myo*-inositol, bis-2-methoxyethyl ether, polygalacturonic acid, gum arabic (from *Acacia*), BSA, chloramphenicol, hog pancreatic α-amylase (Type I-A), 2,7-dichlorofluorescein and potato starch were from Sigma Chemical Co. *Myo*-inositol was prepared as described in Blakeney *et al.* (4). Contaminants in bis-2-methoxyethyl ether were removed by vacuum fractional distillation (140°C, 360 mm Hg) from sodium borohydride. Amberlite IR-120 ion exchange resin was from BDH, Poole, Dorset, U.K. Penicillin and streptomycin were purchased from Flow Laboratories, North Ryde, N.S.W., Australia. 1-Methylimidazole, potassium hydride (approximately 20% in oil), methyl iodide, and 2,2-dimethoxypropane were from Fluka AG, Chemische Fabrik, Buch, Switzerland. *m*-Hydroxydiphenyl and larch arabinogalactan (from *Larix*) were from ICN Pharmaceuticals. Sodium borohydride was obtained from Aldrich Chemical Co. Trifluoroacetic acid was purchased from Pierce Chemical Co. Dialysis membrane (32 mm, mol wt cut-off 6–8000 D) was obtained from Selbys Scientific Ltd., Melbourne, Australia. Diethyl ether (spectroscopic grade, Uvasol) was from Merck. The artificial carbohydrate-binding antigens, β-glucosyl and α-galactosyl Yariv reagents were a kind gift from Dr. M. A. Jermyn, Division of Protein Chemistry, CSIRO, Parkville, Victoria. The monoclonal antibody, PCBC 3, primarily directed toward α-L-arabinofuranosyl residues (1) was a kind gift from Dr. M. A. Anderson, Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Parkville, Victoria, Australia. The mouse myeloma protein J539 was purified from ascities fluid by Mr. A. Gell, Plant Cell Biology Research Center, School of Botany, Universtiy of Melbourne, Parkville, Victoria, Australia. All other chemicals were of analytical grade.

Sterilization and Germination of *Zea mays* Caryopses. Maize caryopses were germinated and grown under axenic conditions. Microbial contamination was excluded by autoclaving (120°C, 106 kPa; 30 min) all solutions and equipment, and carrying out all transfers in a laminar-flow air-cabinet (Gelman Sciences, Australia).

Caryopses were surface sterilized by sequential soaking in 70% (v/v) ethanol (1 min), 0.1% (w/v) mercuric chloride (10 min) and 6% NaOCl (10 min). Each treatment was followed by several washes with sterile distilled H_2O (5–10 min). The caryopses were then imbibed in distilled H_2O containing chloramphenicol (60 μg/ml), streptomycin (100 μg/ml) and penicillin (100 units/ml) with continuous shaking (120 rpm) for 24 h at 24°C prior to a second soaking in 0.1% (w/v) mercuric chloride (10 min). The antimicrobial agents were subsequently removed from the caryopses by washing with sterile distilled H_2O over a period of 15 to 20 min. Surface sterilized caryopses were germinated on moist glass fiber filter discs in culture dishes which were covered with aluminum foil. The caryopses were incubated at 25°C in the dark, until the roots were approximately 1 to 2 cm long (96 h).

Collection of Axenic Secreted Root Slime. Secreted root slime was collected by suspending the roots of germinated seedlings in sterile distilled H_2O, in 10 ml beakers (approximately 10 roots per beaker). The seedlings were supported on plastic grids resting on the top of each beaker. Water loss was minimized by enclosing the beakers in sterile culture dishes covered with foil. The secreted slime was collected for 48 h at 24°C in the dark. The seedlings were then removed and the axenic slime suspension centrifuged (1350*g*, 10 min) to remove the root cap cells and cell debris, concentrated by rotary evaporation to approximately 10 to 20% of the original volume, dialyzed exhaustively at 4°C against distilled H_2O containing 0.02% (w/v) NaN_3 and the nondiffusible material concentrated to approximately 0.5% of the original volume. The resulting high mol wt slime preparation was stored at −20°C, prior to analysis.

General Methods. Microbial contamination of germinated caryopses and collected slime was evaluated by plating out the slime or scraping the root surface onto 1% glucose, 1% peptone agar plates which were incubated for 24 to 72 h at 37°C. Total carbohydrate was determined colorimetrically by the phenol-sulfuric acid method (12), using glucose as a standard. Total protein was determined as BSA by the Bio-rad micro-assay method (6). Sulfate was determined turbidimetrically as described by Dodgson and Price (11). Potassium sulfate was used as a standard.

Amino Acid Analyses. Slime preparations (1-2 mg) were hydrolyzed in 6 N HCl with phenol (1 crystal) at 110°C for 24 h. The amino acids released were identified and quantified on a Beckman 120B Amino Acid Analyser by Dr. A. Ingliss, Division of Protein Chemistry, CSIRO., Parkville, Victoria.

Starch Digestion. Slime preparations (0.1-1 mg) were boiled for 5 min to denature endogenous hydrolases and to gelatinize any granular starch. The solutions were incubated with porcine pancreatic α-amylase at 40°C for 1 h in 10 mM Tris-maleate buffer, pH 6.9, containing 10 mM NaCl and 1 mM $CaCl_2$. The digests were then frozen and lyophilized. Dried samples were resuspended in distilled H_2O (100 μl) by sonication and adjusted to 80% (v/v) ethanol by the addition of four volumes (400 μl) of ethanol. The precipitated high mol wt slime components were then washed exhaustively with 80% ethanol by centrifugation (2800 rpm, 15 min, 3 × 3 ml) to remove the products of α-amylase hydrolysis. The starch-free slime preparations were then dried at 40°C *in vacuo* prior to further analyses.

As a control, potato starch was hydrolyzed under the same conditions described above. The extent of starch digestion was assessed by performing monosaccharide analyses on both the 80% ethanol-soluble and -insoluble fractions using *myo*-inositol hexaacetate as a standard. On average 0.2 U of α-amylase/50μg starch resulted in 95 to 96% hydrolysis of starch to 80% ethanol-soluble products. Increasing the hydrolysis time to 17 h at 40°C, or increasing the concentration of α-amylase did not result in more complete hydrolysis.

Identification and Quantification of Uronic Acids. Total uronic acid content of α-amylase treated slime preparations was estimated colorimetrically and by GC-MS. For colorimetric determination the method of Blumenkrantz and Asboe-Hansen (5) using polygalacturonic acid as a standard was used. For GC-MS analyses, the hexosyluronic acid residues were reduced to their corresponding neutral glycosyl residues and then identified and quantified from the 6,6-dideuterated portion of the corresponding alditol acetate. Hexosyluronic acid residues in α-amylase treated root slime preparation (100 μg) were methyl esterified using a modification of the method of Fazio *et al.* (14) in anhydrous methanolic/HCl (0.08 M, 1 ml, room temperature, 24 h). Samples were dried under a stream of N_2 and washed with methanol (3 × 3 ml). Methyl esterified hexosyluronic acid residues were reduced with sodium borodeuteride (1 ml, 0.25 M, 4°C, 24 h) to their corresponding 6,6-dideutero-labeled neutral sugar residue. The carboxyl-reduced polysaccharides were then hydrolyzed in trifluoroacetic acid (0.5 ml, 2.5 M, 100°C, 2 h) and the released monosaccharides reduced with sodium borohydride and acetylated as described for monosaccharide analysis.

Derivatives eluting from the GC were detected and quantified in the MS using either total ion current (RIC; m/z 100 to m/z 350 in 0.5 s) or selected ion monitoring (SIM; m/z 217 and m/z 219 with 134 ms dwell time for each ion monitored. The ratio of peak heights of the m/z values of 217/217 + 219 was used to determine the proportion of each hexosyluronic acid as described by Fazio *et al.* (14).

Monosaccharide Analysis of Root Slime Polysaccharides. Slime polysaccharides were hydrolyzed in acid and the resultant monosaccharides converted to their alditol acetates and analyzed by GC and combined GC-MS. α-Amylase-treated slime preparations (50–250 μg) were hydrolyzed with trifluoroacetic acid (0.5 ml, 2.5 M, 100°C, 2 h) under argon in a glass tube fitted with a Teflon-lined screw cap. After hydrolysis, TFA was removed by evaporation under a stream of N_2. The resultant monosaccharides were reduced and acetylated by a modification of the method of Blakeney *et al.* (4) in which reduction was carried out using sodium borodeuteride in bis-2-methoxyethyl ether. The acid hydrolysate was dissolved in H_2O (100 μl) and 13 M ammonia (20 μl), then a freshly prepared solution of sodium borodeuteride in bis-2-methoxyethyl ether (1 M, 250 μl) added. Reduction was carried out for 90 min at 40°C. Excess sodium borodeuteride was destroyed by adding 18 M acetic acid (100 μl). Acetylation was carried out by adding 1-methylimidazole (200 μl), followed by acetic anhydride (2 ml). The components were mixed and incubated for 10 min at room temperature, then H_2O (10 ml) was added to destroy excess acetic anhydride. After a further 10 min incubation at room temperature, dichloromethane (1 ml) was added and the solution mixed. After phase separation, the lower phase was back extracted with distilled H_2O (2 × 5 ml) and stored at −20°C for subsequent analysis.

Linkage Analysis of the Root Slime Polysaccharides. Linkage analysis was by methylation using the procedure of Harris *et al.* (18). DMSO (100 μl) was added under argon to dry α-amylase treated slime preparations (0.1–0.5 mg) which were then dissolved by two rapid preliminary methylations. The method was varied in that reduction was carried out using sodium borodeuteride instead of sodium borohydride to provide unambiguous identification of partially methylated alditol acetates by electron impact ionization mass spectrometry (3).

Gas Chromatography-Mass Spectrometry of Alditol Acetates and Partially Methylated Alditol Acetates. Alditol acetates and partially methylated alditol acetates were identified by their mass spectra and their GC retention times relative to *myo*-inositol hexaacetate (2). Combined GC-MS was carried out using a fully automated Finnigan MAT 1020B instrument (Sunnyvale, CA). Alditol acetates and partially methylated alditol acetates were separated on a 25 m × 0.22 mm i.d., BP-75, vitreous-silica, wall-coated open tubular (WCOT) column (SGE Pty. Ltd., Melbourne, Australia) (2). For the separation of alditol acetates an oven temperature program of 170 to 250°C at 5°/min, then held at 250°C for 10 min was used; partially methylated alditol acetates were separated using an oven temperature program of 150 to 250°C at 4°/min, then held at 250°C for 10 min. Partially methylated alditol acetates were also separated on a 15 m × 0.24 mm i.d. SP2100, WCOT column (Supelco Inc., Bellefonte, PA.). An oven temperature program of 120 to 200°C at 2°/min was used.

Compounds that eluted from the GC column were detected using the total ion current by scanning from m/z 100 to 350 in 0.5 s for alditol acetates and in 0.3 s for partially methylated alditol acetates. The amounts of partially methylated alditol

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acetates were determined as molar percentages calculated by [peak area/mol wt of the residue] normalized to 100% (3).

Gas Chromatography of Alditol Acetates. The alditol acetates were separated and quantified on a 28 m × 0.5 mm i.d., Silar 10C support-coated open tubular (SCOT) glass capillary column (SGE Pty. Ltd., Melbourne, Australia) (4) in a Hewlett-Packard 5890A gas chromatograph fitted with a flame ionization detector and a dedicated cool on-column capillary inlet. Ultra-high purity helium was used as the carrier gas at a flow rate of 7 ml/min. The initial oven temperature was 38°C for 30 s followed by a dual ramping program from 38 to 190°C at 70°/min and then from 190 to 230°C at 3°/min. The oven temperature was then held at 230°C for 10 min. The detector temperature was held at 250°C. Peak areas were recorded with a Hewlett-Packard model 3392A integrator. Monosaccharides were quantified from the peak areas and expressed as molar percentages using the weight response factors determined for each of the monosaccharides.

Gel Diffusion of Root Slime Preparations. Gel diffusion was performed in 1% (w/v) agarose containing 1% (w/v) NaCl and 0.02% (w/v) NaN_3 for 24 h at room temperature in a humidity chamber, then examined directly for the presence of precipitation lines and following staining with 0.2% (w/v) Coomassie blue.

Saponification and Extraction of Phenolic Compounds. The method employed was essentially that of Fausch *et al.* (13). α-Amylase treated slime (2.4 mg) was saponified with 0.5 M potassium hydroxide (1 ml) for 90 min at 60°C under N_2. The hydrolysate was acidified with 6 M HCl to pH 2 to 3 and extracted with ether (4 × 1 ml). The ether extracts, containing the liberated phenolic acids, were evaporated to dryness under a stream of N_2 and the residue redissolved in methanol (0.5 ml) for analysis. All manipulations of solutions were carried out in "white" fluorescent light to prevent isomerization of the phenolic acids.

Total extractable phenolic acid was estimated as described by Swain and Hillis (30) using ferulic acid as a standard. The extracted phenolic acids were characterized by their UV absorption spectrum (200–400 nm) and by TLC using cellulose plates and toluene-formic acid-water (8:9:3 [v/v/v], upper phase). Phenolic compounds were detected by uv irradiation and by spraying with 0.1% (w/v) 2,7-dichlorofluorescein.

Microscopy. Bright-field microscopy was carried out using a Zeiss (Oberkochen, West Germany) Cytoscan microscope fitted with a 50 W tungsten lamp. Fluorescence microscopy was performed with a Zeiss Photomicroscope Mk III equipped with a HBO 100 mercury-vapor lamp, epifluorescence optics and an incidence illuminator, with KP 490 and KP 500 excitation filters, FT 510 chromatic beam splitter and a LP 520 barrier filter.

Roots (4 d old) of *Z. mays* were embedded and frozen at −20°C in a gel containing gelatin (15% w/v) and glycerol (1% w/v). Longitudinal sections (8 μm) were cut in a Cryostat (Cryocut, American Optical Co.). For bright-field microscopy the sections were treated with the β-glucosyl Yariv reagent (2 mg/ml in 0.15 M NaCl) for 1 h followed by two washes with distilled H_2O. Control sections were treated with α-galactosyl Yariv reagent (2 mg/ml in 0.15 M NaCl) in a similar manner. For fluorescence microscopy the sections were pretreated with 1% BSA in 0.02 M sodium phosphate buffer, pH 6.8 containing 0.15 M NaCl (PBS) for 15 to 20 min. Sections were then incubated with the α-L-arabinofuranosyl-directed monoclonal antibody PCBC3 (1) (1.9 mg/ml; 1:10 dilution in PBS containing 1% BSA) for 1 h at room temperature, washed (3×) with PBS containing 1% BSA and incubated with FITC-labeled rabbit anti-mouse IgG (1:20 dilution) for 30 min at room temperature. The sections were then washed with PBS (3×) and distilled H_2O (1×) and mounted in Eukitt for examination. Control sections, with monoclonal antibody PCBC3 preincubated with 0.2 M 3-*O*-β-D-galactopyranosyl-D-arabinose were treated in a similar manner.

RESULTS

Composition of the Isolated Root Slime Preparation. The slime preparation is composed primarily of carbohydrate (approximately 94% w/w) and a small amount of protein (approximately 6% w/w) and phenolic acids (approximately 0.17% w/w). No sulfate was detected at the 0.5% (w/w) level. The carbohydrate and protein content are an average of duplicate determinations on three separate batches of slime. Phenolic acid and sulfate analyses were single determinations on one slime preparation.

Monosaccharide Composition of Root Slime Preparations. The monosaccharide composition obtained by acid hydrolysis of the slime preparation is given in Table I. Fucose, arabinose, xylose, galactose and glucose are the major components and mannose is present as a minor component. Treatment of the slime preparation with α-amylase reduced the level of glucose, indicating the presence of some starch in the extracted slime preparation. Analyses of different slime preparations were not identical but this variation was due to the varying amounts of starch present in different preparations, as analyses of different α-amylase-treated samples were similar.

Uronic acids were detected at low levels (3% w/w) in the α-amylase-treated slime preparation by the colorimetric assay of Blumenkrantz and Asboe-Hansen (5). However, no 6,6-dideuterated alditol acetates could be detected by GC-MS using the modified method of Fazio *et al.* (14). Therefore, we are unable to assign an identity to the uronic acids detected by the colorimetric assay.

Methylation Analysis. The linkage composition of the α-amylase-treated root slime preparations determined by methylation analyses is shown in Table II. The following deductions can be made regarding the ring size and the linkage points of the monosaccharide residues in the constituent polysaccharides.

Fucose is in the pyranose form and mainly as terminal and 3-linked fucopyranosyl residues. A small amount of 2-linked fucopyranosyl residues is also present.

Arabinose is present predominantly in the furanose form but a small amount of terminal arabinopyranose was also detected. The arabinofuranosyl residues are mainly terminal and 2-linked, although trace levels of 5-linked arabinofuranosyl residues were consistently detected.

Xylose is present in the pyranose form and is predominantly in a terminal position although there are significant amounts of 2- and 4- and 3,4-linked xylopyranosyl residues. The 2- and 4-linked xylopyranosyl residues cochromatograph on both BP-75 and SP2100 GC capillary columns and hence are quantified as a single component. However, the ratio of 2-:4-linked calculated from the MS by the ratio of the unique ions m/z 117 and 118 respectively is 1:2. Trace amounts of 2,3,4-linked xylopyranosyl residues were also detected.

Mannose is in the pyranose form and is only found as the 2,3-linked mannopyranosyl residue.

Galactose is found only in the pyranose form and is predominantly 2-, 2,3-, and 3,6-linked. There are also significant levels of terminal and 3-linked galactopyranosyl residues with trace

Table I. *Monosaccharide Composition of the Root Slime Preparation*

Slime Preparation	Monosaccharide Composition (mol %)[a]					
	Fuc	Ara	Xyl	Man	Gal	Glc
Untreated	18	20	15	2	26	18
α-Amylase treated	21	18	16	2	31	12

[a] Average of duplicate determinations. Duplicate determinations of three separate preparations of root slime gave Glc values ranging from 18 to 25 mol %. Following α-amylase treatment, the Glc content was reduced and ranged from 12 to 14 mol %.

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Table II. *Methylation Analysis of α-Amylase-Treated Root Slime Preparation*

Monosaccharide	Deduced Glycosidic Linkage[a]	Mol %[b]
Fuc*p*	Terminal	14.5
	2-	1.5
	3-	8
Ara*f*	Terminal	4
	2-	6
	5-	Tr
Ara*p*	Terminal	1
Xyl*p*	Terminal	8
	2- and 4-[c]	3
	3,4-	3
	2,3,4-	Tr[d]
Man*p*	2,3-	3
Gal*p*	Terminal	4
	2-	8
	3-	3
	6-	Tr
	2,3-	6
	2,6-	Tr
	3,6-	7
Glc*p*	Terminal	Tr
	3-	3
	4-	5
	4,6-	7
	2,4,6-	2

[a] 2-Fuc*p* is deduced from 1,2,5-tri-*O*-acetyl-6-deoxy-3,4-di-*O*-methyl-hexitol etc. [b] Average of duplicate determinations. [c] 2- and 4-Xyl*p* coelute on BP-75 and SP2100. [d] Trace amounts (<0.5%).

amounts of 6- and 2,6-linked galactopyranosyl residues.

Glucose is in the pyranose form mainly as 4- and 4,6-linked glucopyranosyl residues. Significant levels of 3- and 2,4,6-linked glucopyranosyl residues are also present together with trace levels of the terminally linked residue.

Amino Acid Composition of the Root Slime Preparation. The amino acid composition of the slime preparation is given in Table III. Glycine and glutamine/glutamate are the major amino acids together with significant levels of asparagine/asparate, proline and alanine. The imino acid hydroxyproline was also present (0.7 mol %). A low level (3.5 mol %) of an unidentified component chromatographing between ammonia and the internal standard, aminoguanidinopropionic acid, was detected. The protein content of root slime calculated from the amino acid analyses is 5% (w/w) and is comparable with that determined colorimetrically by the Bradford (6) procedure. Hexosamines, in particular *N*-acetylglucosamine, were detected at low levels.

Microscopic Observations. The binding of β-glucosyl Yariv reagent to fresh cryostat sections of *Z. mays* roots is shown in Figure 1b. Binding is restricted to a discrete zone at the interface between the thick outer periclinal epidermal cell wall and the secreted root slime and extends from the root cap to the zone of maturation. Occasionally, diffuse binding was observed in the secreted slime. No specific binding of β-glucosyl Yariv reagent was detected in fixed and sectioned roots.

The α-L-arabinofuranosyl-directed monoclonal antibody, PCBC3, binds primarily in the inner and outer regions of the outer periclinal epidermal cell wall in the zone of elongation in fresh cryostat sections (Fig. 1c). Weak binding is also observed in the anticlinal and inner periclinal walls of the epidermal cells, the cortical cell walls, and the root cap cell walls and mucilage.

Gel Diffusion. The α-amylase treated slime preparation gave precipitin bands in double diffusion tests with the α-L-arabinofuranosyl-directed monoclonal antibody, PCBC3, β-glucosyl Yariv reagent and the galactopyranosyl-directed mouse myeloma IgA, J539. The precipitin band given by the β-glucosyl Yariv reagent was weak and diffuse and could only be detected by Coomassie blue staining of the gel at slime concentrations of 5 mg carbohydrate/ml.

Table III. *Amino Acid Composition of the Root Slime Preparation*

Amino Acid	Mol %[a]
Lys	1.8
His	1.5
Arg	2.6
Trp	ND[b]
CysA	6.4
Asx	10.1
Thr	5.6
Ser	6.1
Glx	14.1
Pro	8.2
Gly	13.8
Ala	8.1
Half-Cys	ND
Val	4.6
Met[c]	1.0
Ile	2.7
Leu	4.4
Tyr	1.4
Phe	2.3
Hyp	0.7
Glc NAc	1.1
Gal NAc	Tr[d]
Unknown	3.5

[a] Average of duplicate analysis. [b] Not detected. [c] Met + Met (0). [d] Trace.

Phenolic Constituents Released from Root Slime Preparations by Sodium Hydroxide. *Trans*-ferulic acid was the major constitutent detected by TLC, together with a small amount of an unidentified phenolic component which had an R_F (0.04) similar to *trans-p*-coumaric acid (0.06) and diferulic acid (0.04–0.06) which were poorly resolved in the solvent system used. Insufficient material was available for further characterization.

DISCUSSION

Although many analyses of root cap slime, particularly from *Z. mays*, have been reported (26), few unambiguous conclusions regarding the structure of the components can be drawn. One of the major difficulties in interpreting the data is that, in many cases, the samples analyzed were collected under nonsterile conditions and would probably contain material of both plant and microbial origin. Material collected under such nonsterile conditions is probably equivalent to 'mucigel', a term used to describe gelatinous material at the surface of roots grown in nonsterile soils (27).

In the present study, we isolated water-soluble, high mol wt material secreted by roots of *Z. mays* under axenic conditions. This material is probably a secretory product of the root cap cells (9, 23), although there may be some contribution from epidermal cell secretions. Secreted root slime samples collected axenically are classified as 'crude material' or 'purified secretory product' by Rougier (26). Crude material includes material collected either by wiping root tips onto glass fibre filters or from the root incubation medium (31–35); these samples would contain secreted root cap slime, sloughed root cap cells and debris from lysed cells as well as low mol wt metabolites. Centrifugation and dialysis of this "crude material" gives the 'purified slime' (26). Thus the material we have studied, being collected axenically, then centrifuged and dialyzed, would, in Rougier's (26) classification, be purified slime. In our experience, samples prepared in

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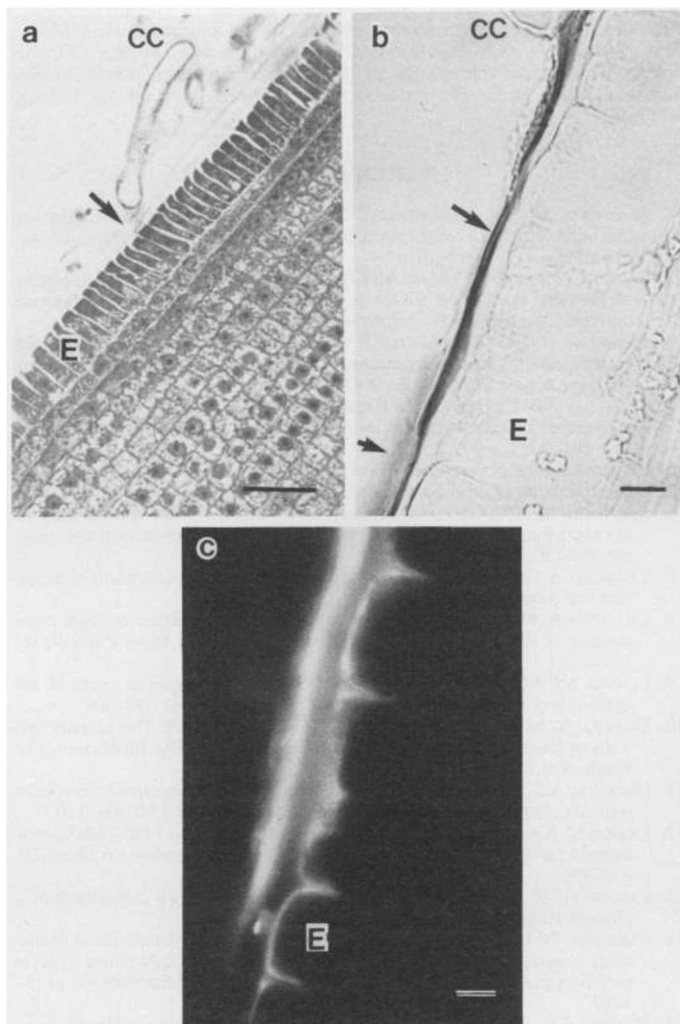

FIG. 1. Micrographs of longitudinal sections from roots of *Zea mays* taken from the zone of elongation near the cap region. a, Light micrograph showing the thick epidermal cell wall (arrow) and sloughed cap cell (cc). Roots were fixed in formaldehyde (4% w/v) and plastic embedded. Bar = 50 μm. b, Light micrograph showing the binding of β-glycosyl Yariv reagent to the outer periclinal face of the epidermal cell wall (long arrow). There is also diffuse binding to the secreted slime (short arrow). Cryostat sections (approximately 10 μm) prepared from fresh (unfixed) material. Bar = 10 μm. c, Fluorescence micrograph showing binding of the antiarabinofuranosyl directed monoclonal antibody, PCBC3, to the epidermis at both the outer periclinal and the anticlinal walls. Cryostat sections (approximately 10 μm) prepared from fresh (unfixed) material. Bar = 10 μm.

this manner contained variable proportions of glucose. Removal of the soluble starch, presumably derived from lysed cap cells, by α-amylase treatment, gave samples with essentially similar monosaccharide compositions. Wright (31) also reported varying proportions of glucose for different slime preparations.

The monosaccharide composition of the purified maize slime is similar to that reported by others (7, 19) and is unusual among higher plant cell walls and secretions in the high fucose content (approximately 20%). The fucose content of root slime preparations from other plants such as rice (8) and wheat (23) (SF Moody, A Bacic, AE Clarke, unpublished data) is less than 8%. The linkage analysis of the fucosyl residues is also remarkable—a high proportion is present as terminal (60%) and 3-linked (33%) fucopyranosyl residues and a smaller amount (6%) as 2-linked fucopyranosyl residues. The 2- and 3-linked fucopyranosyl residues have not been reported previously in higher plant polysaccharides (10) although both are present in fucoidan from brown algae and in heteropolymers isolated from diatoms and blue-green algae (24). Fucosyl residues associated with higher plant polysaccharides are often terminal, for example in xyloglucans, and are also present in the pectic polysaccharides, rhamnogalacturonan I (linkage unknown) and II (as terminal 2-*O*-methyl fucose and 4-linked fucopyranosyl residues) (10). Glycoproteins may also contain terminal fucopyranosyl residues (28). Wright *et al.* (35) reported that an α-L-fucosidase purified from rat epididymides removed only 17% of the fucosyl residues of the slime. However, as the absolute specificity of the enzyme is unknown (35), it is difficult to interpret the data. For example, terminal fucosyl residues present in the core region of glycoproteins (28) may not be accessible to hydrolysis and hence would result in an underestimation of terminal residues by this method. We cannot infer how these fucosyl residues are arranged within the slime components from the methylation data. However, as we detect no branched fucosyl residues, a polymeric fucan accounting for all the residues is not likely. We are currently attempting to isolate the fucose-containing components by lectin affinity chromatography.

Apart from the high fucose content, the other striking feature of the methylation analyses is the extreme complexity of the linkage composition. As well as linkages which are characteristically associated with particular plant polysaccharides, we detect two linkage types: 2,3-linked mannopyranosyl and 2,3-linked galactopyranosyl, not previously reported in higher plants (10). Although the linkage analysis does not give information on the distribution of the residues within or between particular polymers, we can speculate on what types of polymers might be present on the basis of the linkage composition of known plant polysaccharides.

The 3,6- and 3-linked galactopyranosyl residues are probably derived from a Type II arabinogalactan (AG) or AGP[1] (15) as the preparation interacted with β-glucosyl Yariv reagent in gel diffusion tests. This reagent specifically binds and precipitates many AGPs. Diffuse binding of the β-glucosyl Yariv reagent to secreted slime was also observed at the microscopic level (see Fig. 1b). In addition, the low levels of hydroxyproline detected in the amino acid analyses may be derived from AGPs. These polymers are common constituents of plant secretions. Terminal arabinopyranosyl and arabinofuranosyl, 2-linked arabinofuranosyl, terminal galactopyranosyl and 6-linked galactopyranosyl could be side chain constituents on the 3-linked galactopyranosyl backbone. Terminal arabinopyranosyl residues have only been found in AGPs and dicot arabinans (10). The α-L-arabinofuranosyl-directed monoclonal antibody, PCBC3, bound to regions coinciding with the binding of the β-glucosyl Yariv reagent but also bound to the inner region of the outer periclinal wall of root epidermal cells as well as the cortical and root cap cell walls. Polysaccharides other than AGPs containing terminal α-L-arabinofuranosyl residues, for example glucuronoarabinoxylans, also bind this antibody (1) and therefore binding of the antibody to cell wall sites reflecting the presence of polysaccharides containing terminal α-L-arabinofuranosyl residues would be expected.

The xylopyranosyl linkages, terminal, 4-, 3,4-, and 2,3,4-, could be accounted for by the presence of an arabinoxylan. Arabinoxylans are major noncellulosic polysaccharides of cell walls of the Gramineae and comprise a 4-linked xylopyranosyl backbone branched through either (or both) C(O)2 or C(O)3 mainly to terminal arabinofuranosyl residues, although short homo- and hetero-oligosaccharide chains are also found (10). Terminal and 2-linked arabinofuranosyl residues may comprise

[1] Abbreviation: AGP, arabinogalactan-protein.

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side chain constituents on the xylan backbone.

The presence of 4,6-linked glucopyranosyl residues is suggestive of a xyloglucan since this residue is not found in other structural plant polysaccharides (10). Xyloglucans are the major noncellulosic polysaccharides of primary cell walls of dicots, but are only minor components of the primary cell walls of monocots. They have, however, been detected in suspension- cultured monocot cell walls and cell walls of coleoptiles and shoots from maize (Kato and Nevins [21] and references therein). Xyloglucans comprise a 4-linked glucopyranosyl backbone branched through C(O)6 mainly to single terminal xylopyranosyl residues, although short oligosaccharide side chains consisting of 2-linked xylopyranosyl and galactopyranosyl residues are also present. Terminal fucopyranosyl and arabinofuranosyl residues have also been found on xyloglucans (10). All these glycosyl linkages are present in maize root slime. The other glucosyl linkages present are 3- and 4-linked glucopyranosyl linkages which could arise from 3-linked glucan, 4-linked glucan or from 3,4-linked glucan. We can make no prediction about the presence of these polymers from the methylation data.

Low levels (approximately 6% [w/w]) of protein are detected in the purified maize root slime. Chaboud (7) found similar (1-5%) protein levels in purified maize root cap slime. Some of this protein, characterized by the presence of hydroxyproline, is probably associated with the AGPs. The remaining protein may correspond to enzymes either actively secreted (*e.g.* esterases, glycosidases, ATPases, acid phosphatase) or released from root cap cells undergoing lysis (for review see Rougier [26]).

The phenolic acids, ferulic and *p*-coumaric, are widely distributed in cell walls of the Gramineae (17), including wheat root cell walls (29). Ferulic acid is esterified to the cell wall arabinoxylans via the C(O)5 of terminal arabinofuranosyl residues in monocots including *Z. mays* shoot cell walls (Kato and Nevins [22] and references therein). The presence of low levels of ferulic acid (and possibly either *p*-coumaric and/or diferulic acid) in maize root slime confirms earlier cytochemical evidence for the presence of esterified feruloyl residues in maize root slime (17).

A surprising finding of this study are the low levels of uronic acids detected, implying that soluble, high mol wt, secreted root slime contains a low proportion of acidic pectic polysaccharides. Low levels of pectic polysaccharides are also a characteristic of the primary cell walls of the Gramineae (10). Wright and Northcote (34) proposed that the organization of maize root slime is similar to that of mustard seed slime (16), that is, a central cellulosic polymer encased in a hydrophilic uronic acid-containing pectic-like material. Other earlier reports of high levels of uronic acids in "crude slime" preparations could be attributed to material arising from cell wall polymers included in the preparation rather than from secreted slime. Harris and Northcote (29) reported 10% uronic acid in a 90% ethanol-insoluble preparation from maize roots, and we also detect uronic acids (approximately 5%, unpublished data) in cell wall preparations isolated from 1 to 2 mm root tip segments. The pectic polysaccharides are assumed to confer the gel properties on the root slime, but the low levels of pectic polysaccharides in maize root slime does not necessarily preclude the capacity for gel formation. Other polysaccharides such as AGPs form gels, and non-cellulosic polysaccharides can interact to form highly viscous gels (25). As we can find only low levels of these polysaccharides, we infer that the gel properties of secreted slime may be due at least in part to interactions of the other polysaccharides, as described by Rees (25). The root slime does contain an extraordinarily diverse range of glycosyl residues; it is possible that this diversity is of value to the plant not only in providing a protective gel covering for the root, but also in establishing specific relationships with the rhizosphere microflora.

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