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POLYSACCHARIDES OF ROOT-CAP SLIME FROM FIVE MAIZE VARIETIES

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Abstract—Radioactive root-cap slime from five maize varieties consisted of a mixture of neutral, weakly-acidic and acidic polymers. The distribution of radioactivity between monosaccharides of these components showed that the slimes were essentially the same but that preparations, even those made at different times from the same variety, contained variable proportions of glucose. Results obtained with different maize varieties can therefore be considered together.

INTRODUCTION

The plant root-cap has been studied intensively from the point of view of its role in geotropism [1–4] and the cytology of the formation of its protective slime [5–8] but less attention has been directed to the biochemistry of the slime itself. Maize slime contains a polymer rich in fucose [9] but no such polymer is present in the rest of the maize seedling [10]. Further, fucose is only a minor component of root-tip material isolated from sycamore, pea and wheat roots [10]. Hence the occurrence of large amounts of fucose in maize slime represents the end-product of a differentiation process specific to the maize outer root-cap cell. In this report radioactive slime from five maize varieties has been prepared and separated into component polymers. The sugar labelling patterns of the polymers have been measured and some differences suggest a way in which the polymers may be related to one another, and a possible reason for variations between published data. Overall however, the slimes were essentially the same and therefore results obtained using different varieties can be combined to build up a picture of the biochemistry of maize slime.

RESULTS

Sterilization of seeds

The seeds supplied coated with a fungicide (Caldera 535, WF-9 × 38) were easier to sterilize than the others and either sterilization procedure was satisfactory for these. Variety WF-9 × M-14 could also be sterilized by either method but Kelvedon 33 and Golden Bantam were more frequently successfully sterilised by method 2; in these cases, as an extra precaution, the seeds were also soaked in 0·1% $HgCl_2$ for 20 min before washing in chloramphenicol solution and planting.

Composition of bacteria

The composition of the whole freeze-dried bacteria isolated from the slime was—glucose 24%, galactose 41%, mannose 8%, xylose 7%, arabinose 4%, ribose 4%, fucose 6%, rhamnose 6%. When bacteria from a different isolate were fed with D-glucose [U-^{14}C] for 2·5 hr over 50% of the label was found in hexose and the remainder was in rhamnose and material which did not run in the solvent system used to separate the neutral sugars; no label was detectable in arabinose, xylose or fucose.

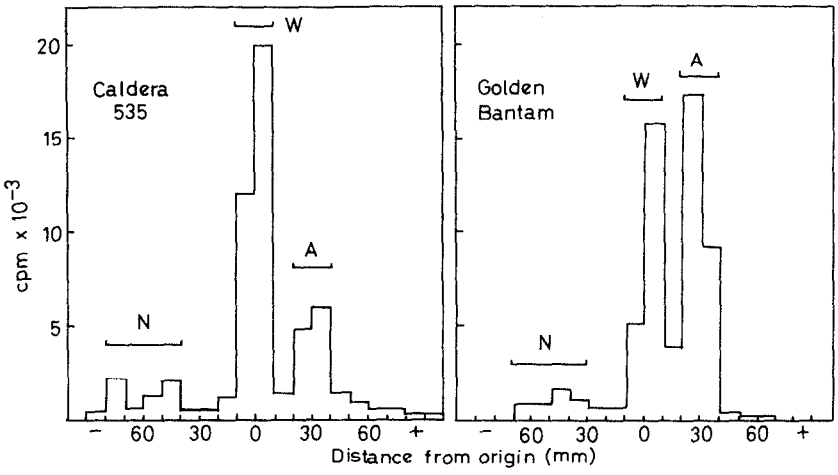

Fig. 1. Separation of slime components by glass-fibre paper electrophoresis. The procedure used is described in Experimental. N = neutral; W = weakly-acidic; A = acidic; + and − indicate anode and cathode.

Separation of slime polymers

Two examples of the separation obtained by glass-fibre paper electrophoresis are given in Fig. 1. Equivalent peaks of the different varieties ran in exactly similar positions in the same electrophoresis run and the peaks obtained were designated "neutral", "weakly-acidic" and "acidic" (Fig. 1). The acidic component is to be distinguished from the strongly-acidic one found in some preparations of Caldera 535 slime [10]. In some cases there was a suggestion of two peaks in the neutral region but these were analysed together. The distribution of total radioactivity between the components is given in Table 1. The whole material of each peak was analysed to provide the radioactive sugar compositions of the polymers.

Compositions of the polymers

The distribution of radioactivity between the individual monosaccharides of the neutral, weakly-acidic and acidic polymers is shown in Tables 2–4 respectively, and data for the whole slime are given in Table 5. Material remaining at the origin when the sugars were separated chromatographically was eluted and electrophoresed at pH 3·5 [9]. The

Table 1. Distribution of radioactivity between the polymeric components of maize slime isolated from different varieties*

Component	Variety radioactivity (cpm × 10^{-2}) Kelvedon 33	Caldera 535	WF-9 × M-14	Golden bantam	WF-9 × 38
Neutral	59 (1)	49 (1)	81 (1)	44 (1)	45 (1)
Weakly-acidic	607 (10)	355 (7)	1017 (13)	208 (5)	380 (8)
Acidic	122 (2)	107 (2)	239 (3)	264 (6)	225 (5)

* Figures in parentheses indicate the relative amounts of each component in the material from each variety.

Table 2. The distribution of radioactivity between component monosaccharides in the neutral polysaccharide of maize slimes

Monomer	Variety radioactivity (%) Kelvedon 33	Caldera 535	WF-9 × M-14	Golden bantam	WF-9 × 38
Uronic acid	0·8	1·2	3·0	2·1	0
Galactose	32·1	36·3	33·1	28·9	27·8
Glucose	25·3	62·4	25·1	47·1	13·7
Mannose	0·6	0	0	0·4	0·2
Arabinose	23·2	0	24·1	14·0	26·7
Xylose	16·2	0	12·4	4·7	22·5
Fucose	1·8	0	2·2	3·1	3·6

Table 3. The distribution of radioactivity between component monosaccharides in the weakly acidic polysaccharide of maize slimes

Monomer	Variety radioactivity (%) Kelvedon 33	Caldera 535	WF-9 × M-14	Golden bantam	WF-9 × 38
Uronic acid	14·1	17·4	16·2	13·0	15·4
Galactose	43·0	36·0	30·0	26·1	28·1
Glucose	3·2	19·1	4·5	8·7	4·5
Mannose	1·3	2·4	1·4	0	0
Arabinose	9·4	6·5	13·6	17·8	16·8
Xylose	10·0	10·4	14·8	22·0	19·4
Fucose	18·8	8·1	19·6	12·4	15·9

Table 4. The distribution of radioactivity between component monosaccharides in the acidic polysaccharide of maize slimes

Monomer	Variety radioactivity (%) Kelvedon 33	Caldera 535	WF-9 × M-14	Golden bantam	WF-9 × 38
Uronic acid	22·1	21·1	21·0	20·0	21·5
Galactose	33·5	33·7	34·4	36·4	35·1
Glucose	0	0	0	0	0
Mannose	1·3	1·6	1·9	1·2	1·4
Arabinose	8·3	8·4	8·8	9·1	9·8
Xylose	4·2	5·6	5·4	5·0	4·8
Fucose	30·6	29·6	28·5	28·3	27·4

small amount of radioactivity which did not move under these conditions was taken to be an unhydrolysed representative sample of the polysaccharide taken for hydrolysis and the percentages shown in Tables 2–4 have been adjusted accordingly. All the material which was negatively-charged at pH 3·5 is grouped together as uronic acid.

The relative amounts of radioactivity in the three peaks were not constant between preparations. For instance, preparations of slime have been made from varieties Golden Bantam and WF-9 × M-14 in which the ratios of the neutral, weakly-acidic and acidic peaks were respectively 1:1·3:1·3 and 1:4:1·4 (cf. values in Table 1). Acid hydrolysates of the peak material were neutralised with $BaCO_3$ and this precludes valid comparison of the uronic acids. However, in the case of Golden Bantam slime the ratio of radioactivities in the neutral sugars of the 3 polymers was virtually the same as in the results given in Tables 2–4. The WF-9 × M-14 slime possessed a considerably greater neutral peak compared with the acidic components, and this was largely accounted for by an increase in the proportion of label in glucose. There was also more label in glucose than in galactose in the weakly-acidic material, and, unusually, radioactivity in both galactose and glucose in the acidic peak. Otherwise the neutral sugars were labelled to relatively the same extent as shown in the Tables.

Table 5. Distribution of radioactivity between the component monosaccharides of whole slime from maize varieties*

Monomer	Variety radioactivity (%) Kelvedon 33	Caldera 535	WF-9 × M-14	Golden bantam	WF-9 × 38
Uronic acid	14	16	17	16	16
Galactose	40	36	31	31	30
Glucose	4	20	5	8	4
Mannose	1	2	1	1	1
Arabinose	10	6	14	13	15
Xylose	10	8	13	12	14
Fucose	19	12	19	19	19

* These data are calculated from those given in Tables 1–4.

DISCUSSION

Seeds germinated without special precautions to ensure their sterility showed a high degree of contamination and this could also be observed electron-microscopically using a negative staining method [11]. Since fucose is not uncommon in bacteria [12] it was thought that this might have accounted for the different content of this sugar reported in the literature. No evidence was found for any incorporation of radioactivity into fucose polymers by the cultured bacteria and a different isolate contained only a small proportion of fucose by bulk analysis. The presence of bacteria which were not rich in fucose in a slime preparation would artificially depress the apparent fucose content of the slime and this argument predicts that the higher estimates of the fucose content might be the more accurate. However, many bacteria of different polysaccharide compositions may be able to use slime as a food source and so sterilization of starting material is the only certain way to avoid this problem.

The overall labelling patterns of slimes from four of the varieties were extremely similar, although the fifth, Caldera 535, had a much higher proportion of its label in glucose. An explanation for this difference is suggested by the labelling patterns of the polymers after separation by glass-fibre paper electrophoresis.

Precisely analogous peaks were identified in material from the different varieties although the relative amounts varied. However, the variation found was no greater than has been noticed between different preparations of material from the same variety; e.g. compare the results presented here for Caldera 535 slime with those shown in Fig. 3 of Wright and Northcote [10]. Also, separate preparations of slime from varieties WF-9 × M-14 and Golden Bantam gave relative incorporations (neutral:weakly-acidic:acidic) of 1:4:1·4 (cf. 1:13:3, Table 1) and 1:1·3:1·3 (cf.

1:5:6, Table 1) respectively. Since slime is produced with a 3-hr periodicity [13] this may have been due to conducting the experiments with groups of roots at different stages within this cycle.

The labelling patterns of the acidic fractions were almost identical and therefore the higher proportion of label in glucose in Caldera 535 slime stemmed from differences in the other two fractions. It is known that, in pectin, material which is present as a separate netural polymer can later combine with acidic material to yield a product which is weakly-acidic [14]. If this metabolic relationship also exists in slime it follows that, for instance, an unusually high proportion of label in glucose in the netural component will lead to the weakly-acidic component containing more label in glucose than would otherwise have been the case. This does not imply that all the neutral polymers need be combined in this way, and nor does it exclude the likely possibility that some sugars are incorporated into the weakly acidic material as single units. Without further evidence it cannot be excluded that the fractions examined are breakdown products of a single molecular slime species.

If the pulse incorporation patterns for Caldera 535 slime given in Table 5 had shown only 5% label in glucose, the rest of the values would have been similar to those found with the other varieties. In a separate preparation the pulse incorporation patterns of WF-9 × M-14 slime showed label in glucose in all three components. Also, Jones and Morré [15] found 22% glucose in a bulk analysis of a slime preparation from this variety whereas a previous analysis [16] had yielded 37%. Table 5 shows that, at the time of this experiment, only 5% of the label being incorporated into polysaccharide by WF-9 × M-14 root-caps was contained in glucose. These results support the idea that the glucose-containing polymer is a variable component of the slime and that the variation resides between preparations rather than between varieties.

The glucose polymer apart, the overall labelling patterns of the slimes were very alike and no varietal differences could be demonstrated. Data obtained with different varieties of maize can therefore be taken together to provide an overall picture of the biochemistry of the slime. It is synthesized by the dictyosomes of the outer root-cap cells [5–8, 17] and may be passed across the whole area of the plasmalemma before subsequent migration to the outer tangential cell wall [18]. If the water supply is inadequate, it will accumulate here rather than form a droplet at the root tip [8, 13]. Under conditions which do permit droplet formation its diameter varies with a periodicity of about 3 hr [13]. A depletion of the starch reserves takes place at the same time as the slime formation [8] and exogenously applied sugars can in some way regulate the amount of slime which is produced [15]. The experiments described here do not measure the total composition of the slime but instead show the biosynthetic activity of the roots at the time of the experiment [10]. However, similar radioactivity incorporation patterns are unlikely to be produced by batches of roots synthesising slimes of radically different compositions. If the pulse incorporation patterns are accepted as an approximation to the overall composition of the slime then the fucose content, about which there has been some discussion [8, 9, 15, 16, 18–20] was about 20% in these experiments.

The monomeric constituents of the uronic acid fractions have not been considered here. The major acidic product of hydrolysis ran close to, but not coincidentally with, galacturonic acid on electrophoresis at pH 3·5 [10] and this will be the subject of a further report. Galacturonic acid has been chromatographically identified as a component of the slime [16] and it is generally assumed to be of a pectic nature. The results presented here and earlier [10] confirm similarities with pectin in respect of the polymers which the slime contained. However there must also be differences since the EtOH-insoluble material contains only *ca* 12% uronic acid, [15] a figure which would be low for a pectin [21]. The slime also contains protein [8, 16]. It is not yet known whether this is actively synthesized as a component of the slime or whether it is released from dying cells of the outer root-cap.

EXPERIMENTAL

Plant material. Zea mays L cv. Caldera 535, Golden Bantam and Kelvedon 33 were obtained from commercial sources. Samples of varieties WF-9 × M-14 and WF-9 × 38 were gifts. They were sterilized and germinated by 2 methods. (1) Seeds were washed with numerous changes of tap H_2O which largely removed fungicide where it was present. They were soaked overnight in 5× their own vol. of sterile H_2O in a closed vessel. The H_2O was decanted and replaced by a similar vol of 10% Milton soln [9] for 1–2 hr. This was washed away with

several changes of a 10 mg/l chloramphenicol (CAP) soln and the seeds were germinated bathed in this soln in a sterile crystallizing dish covered with aluminium foil and lined with chromatography paper. (2) Seeds with fungicide were allowed to stand overnight in 5× their vol. of CAP soln without prior washing. Seeds not supplied with fungicide were soaked in a soln prepared in this way from Caldera 535 seeds; this was sometimes followed by soaking for 20 min in 0·1% $HgCl_2$ with a trace of Triton X-100 as a wetting agent. Seeds were then washed in CAP soln and treated as above. Seedlings were used after 2–3 days when primary roots were 30–40 mm long. Sterility checks were made by wiping the roots over the surface of a nutrient agar medium [9] and incubating at 26°.

The contaminating bacteria were also isolated in this way and they were grown in liquid medium of the same composition, harvested and washed by centrifugation (17 000 *g*, 40 min), and freeze-dried.

Production and isolation of radioactive slime. Seedlings were placed in circular groups so that the tips of the longest roots of each were in contact. 10–20 Roots per variety were used. A soln of D-glucose-[U-^{14}C] (sp. act. 268 mCi/mmol) was placed at the point of contact at the rate of 2 μCi (20 μl) per root. They were maintained in high-humidity conditions, in the dark, for 2·5 hr. Roots were then excised and transferred to a small vial with the washings from the glass plate on which the incubations were carried out. A few drops of toluene were added and the soln was gently agitated occasionally over a period of 18 hr. It was dialysed for 24 hr against 5 changes of 3 l. H_2O. Solutions were decreased to small vol. under reduced pressure and freeze-dried.

Analysis of slime. Slime was dissolved in buffer at pH 6·5 (100 ml pyridine, 3 ml HOAc made up to 1 l. with H_2O) and applied to a wetted piece of Whatman GF81 paper to minimize sticking. Electrophoresis at pH 6·5, 2 kV was carried out for 30 min in an apparatus described by Harris and Northcote [9]. The counting procedure has been described [9]. Strips corresponding to peaks of radioactivity were washed in toluene and C_6H_6 [10], dried and submerged in 4% (w/v) H_2SO_4 prior to hydrolysis and separation of component monomers [9]. During this procedure any amino-acids and peptides formed from protein hydrolysis were removed by electrophoresis at pH 2.

Analysis of bacteria. Freeze-dried preparations were dissolved in 72% (w/w) H_2SO_4 and this was diluted to 4% with 28 vol. of H_2O before hydrolysis [9]. The composition was measured by GLC [10]. Their radioactive incorporation pattern was measured by resuspending a pellet of bacteria in a small volume (*ca* 5 ml) of nutrient medium for 2 days and then pulsing for 2·5 h with 10 μCi (100 μl) D-glucose-[U-^{14}C]. Bacteria were isolated by centrifugation, freeze-dried and analysed as above.

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