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UTILIZATION OF DEGRADED PEACH GUM POLYSACCHARIDE BY *ASPERGILLUS FLAVUS*

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Abstract—D-Galactose, D-mannose, D-xylose, L-arabinose, and D-glucuronic acid and its γ-lactone were examined as carbon sources for the culture of *Aspergillus flavus*. D-Mannose was taken up the most rapidly and D-glucuronic acid and its γ-lactone the least rapidly. A partially degraded polysaccharide from peach tree gum (*Prunus persica* [*L.*] Batsch) containing the above sugars together with D-glucuronic acid and its 4-*O*-methyl ether was used as substrate for another *A. flavus* culture. It was found that D-galactose was the major sugar passing into the culture medium with lower proportions of D-xylose, L-arabinose, 2-*O*-β-D-glucopyranuronosyl-D-mannose, and 6-*O*-β-D-glucopyranuronosyl-D-galactose. This indicates that the fungus produces extracellular exo- and endo-glycanohydrolases which may be useful in structural studies on polysaccharides.

INTRODUCTION

THERE are different opinions on the origin of gum exudates. Some believe that they are normal plant products while others suggest that they arise from pathological infections to seal off the infected region where they are synthesized. The diversity of residues and linkages found in gum polysaccharides may lead to a more effective sealing off of plant wounds since such material may be immune to the degradative enzymes in bacteria or fungi[1]. This diversity of building units and glycosidic linkages indicates it is highly probable that a complex system of enzymes is involved in their biosynthesis and renders structural identification difficult.

Certain gums contain entrapped enzymes capable of effecting their autolysis and this may also be effected by fungi. Studies on such enzymic degradation of gum polysaccharides would be of great value in elucidating their structures since distinctive oligosaccharides should be obtained. This paper describes an examination of the breakdown of a partially degraded polysaccharide from peach tree gum by *Aspergillus flavus*.

RESULTS AND DISCUSSION

The growth of the micro-organism on the various monosaccharides contained in peach gum is shown in Fig. 1. It can be seen that of the various carbon sources, D-mannose was metabolized most rapidly. On the fourth day of culture the surface of the solution was covered by a dense layer of mycelium which sporulated. The reducing power of the medium was by then 0·3 its initial value and by the seventh day this had reduced to zero. Growth in media each containing one of the other three neutral sugars (D-galactose, D-xylose, and L-arabinose) was slower than on mannose but similar to each other; the reducing powers decreased over thirteen days to zero. D-Glucuronic acid and its γ-lactone were the least suitable sources of carbon for the growth of the micro-organism. Even after thirteen days, 69% of D-glucuronic acid and 78% of its γ-lactone were still present in the medium (Fig. 1).

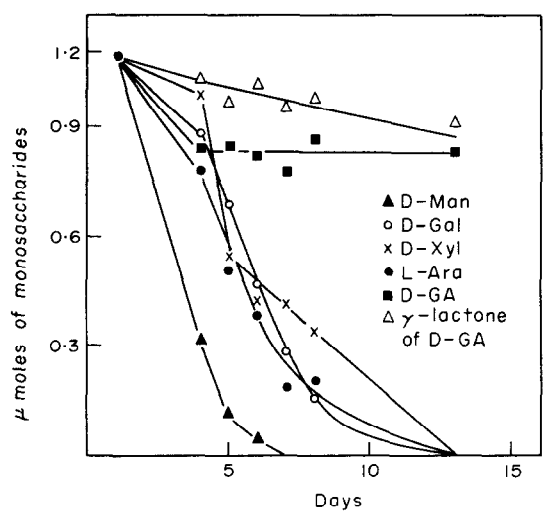

Fig. 1.

Sugars liberated into the solution during growth of *A. flavus* on a partially degraded acidic polysaccharide from peach gum were chromatographically identified. It was shown that the main sugar produced by hydrolysis was D-galactose and that there were smaller amounts of D-xylose and L-arabinose, and trace quantities of two oligosaccharides chromatographically indistinguishable from the standard aldobiouronic acids 2-*O*-*β*-D-glucopyranuronosyl-D-mannose and 6-*O*-*β*-D-glucopyranuronosyl-D-galactose (R_{GlcA} (solvent B) 0·78 and 0·56, respectively). The detection of these degradation products proved that the micro-organism produced exo-glycanohydrolases catalyzing the splitting of D-galactose from non-reducing ends of polysaccharide chains and of pentoses probably present as terminal residues in side-chain [2].

The specific viscosity of the culture medium decreased rapidly during growth. On the fourth day it was 45%, and on the tenth day 20% of the initial value. This indicated the presence of extracellular endo-glycanohydrolases in the medium (Fig. 2).

The degraded polysaccharide used was homogeneous when studied by electrophoresis and by sedimentation on ultracentrifugation. It had a molecular weight of 22,000, an eq. wt of 770, $[\alpha]_D^{20} = +32{\cdot}6°$ (c 1 in H_2O), and –OMe = 0·95%. Its constituent residues, 4-*O*-methyl-D-glucuronic acid: D-glucuronic acid: D-galactose: D-mannose were present in the molar ratio of 0·24:0·76: 2·6:1 and there were traces of L-arabinose and D-xylose. It will be noted that these molar ratios relate to 1 equivalent of the uronic acid residues[3]. In the polysaccharide the D-galactose and the D-mannose units are linked by *β*(1–6) and *β*(1–2) glycosidic linkages, respectively[4]. After the period of culture the polysaccharide isolated from the medium was also homogeneous on electrophoresis. The molecular weight was not determined as the polysaccharide did not sediment on ultracentrifugation at $1{\cdot}8 \times 10^5$G. Its eq. wt was 580, $[\alpha]_D^{20} = +26{\cdot}1°$ (c 1 in H_2O), –OMe = 1·20%. The molar ratios of 4-*O*-methyl-D-glucuronic acid: D-glucuronic acid: D-galactose: D-mannose were 0·22: 0·78: 1·66: 0·83. Pentoses were not present in the hydrolyzate. A comparison of the two sets of molar ratios shows that the ratio of the two uronic acids remains practically unaltered. However, the ratio of D-galactose: D-mannose, 2·6:1, in the initial substrate changed to 2·0:1 in the polysaccharide isolated from the medium after culture. It is not clear whether the D-galactose split off derived from the main chain or from side-chains of the polysaccharide. Further studies dealing with this problem are in progress.

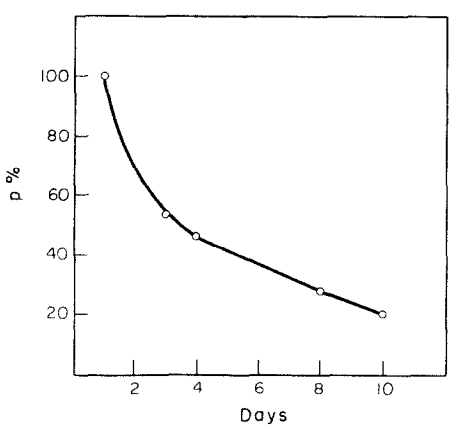

Fig. 2.

EXPERIMENTAL

Material and methods. Aspergillus flavus was isolated from the surface of peach gum and identified in the Institute of Microbiology, Czechoslovak Academy of Sciences, Prague. Paper chromatography solvents: (A) EtOAc pyridine H_2O (8:2:1) and (B) EtOAc HOAc H_2O (18:7:8). Sugars were visualised with aniline hydrogen phthalate[5] and determined densitometrically. Sedimentation constants of polysaccharides were determined using an ultracentrifuge. Partial specific volumes were determined pycnometrically. Reducing groups were determined by Somogyi-Nelson method[6], the absorptions being measured at 530 nm. Polysaccharide degradation was monitored by measuring the loss of viscosity at 20° using an Ubbelohde viscometer. 2-*O*-*β*-D-Glucopyranuronosyl-D-mannose and 6-*O*-*β*-D-glucopyranuronosyl-D-galactose were prepared and characterized in our laboratory[7, 8]. The other sugars were commercial products.

Preparation of the partially degraded polysaccharide. A 2% soln of the polysaccharide from peach gum[7] was hydrolyzed with 0·5 M H_2SO_4 at 100° for 20 min. The mixture was filtered and EtOH added to the filtrate (1:3) giving a precipitate. This was washed (60% EtOH), dissolved in H_2O and freeze-dried.

Hydrolysis of polysaccharides. Samples of each polysaccharide were hydrolysed with 0·5 M H_2SO_4 (1 ml/mg) in sealed glass tubes at 105° for 24 hr. After neutralization ($BaCO_3$) and deionization with Dowex 50W × 4, the acidic sugars were separated from the neutral ones on Dowex 1 × 8 (OAc^- form). Neutral saccharides were eluted from the column by H_2O and the acidic saccharides by 4 M HOAc.

Aspergillus flavus was cultured on synthetic Czapek-Dox media with 1% of each of the following substances as the source of carbon: the partially degraded polysaccharide, D-galactose, D-mannose, D-xylose, L-arabinose, and D-glucuronic acid and

its γ-lactone. The media were initially adjusted to pH 4·5 by the addition of tartaric acid. The conidia of *A. flavus* were suspended in sterile H_2O and used as the inoculum. The inoculated media were incubated without agitation at 28°. Samples withdrawn from the media at different times during growth were used for analysis.

Isolation of the polysaccharide from the culture medium. After 14 days growth on the degraded polysaccharide, the centrifuged filtrate was passed through a column of Sephadex G-25 to remove salts. The aqueous salt-free eluate was reduced in vol. and the polysaccharide precipitated with EtOH. The precipitate was washed with 60% EtOH, dissolved in water, and the solution freeze-dried.

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