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POLYSACCHARIDES OF THE CELL WALLS OF FUNGI PATHOGENIC FOR THE COTTON PLANT

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The dynamics of the secretion of polysaccharides by fungi pathogenic for the cotton plant — Verticillium dahliae *and* Fusarium oxysporum — *have been studied. An alkaline hydrolysate of the fungal cell walls has been obtained. Total fractions of the polysaccharides have been separated on Sephadex G-50 and Acrilex P-4, and their monosaccharide compositions have been determined.*

According to the concept of the immune protection of plants that has been best substantiated experimentally at the present time, the switching on of protective mechanisms on fungal invasion takes place when plant cell receptors interact with the pathogen.

It is known from the literature that immunomodulatory properties are possessed by, among others, 1,3-, 3,6-, and 1,6-β-*D*-bound glucans [1, 2], which may not only be structural components of the cell walls of the parasite but may also be found among its metabolites. The hydrolytic breaking down of the plant cell by the enzymes of the cellulolytic complex of the fungus is accompanied by the cleavage of β-bound glycosides — endogenous elicitors of the protective reactions of plants [3, 4], in view of which there is obvious interest in the isolation, purification, and characterization of the carbohydrate components participating in the host–pathogen inteaction.

We have studied the nature of the secretion of polysaccharides into the culture medium in the dynamics of the growth of the fungi *V. dahliae* and *F. oxysporum*. Experiments have enabled us to show that, beginning from the sixth day of cultivation, the rate of secretion of the polysaccharides gradually rises, reaching a maximum by the 16-17th day in the case of *V. dahliae* and by the 11-12th day in the case of *F. oxysporum* (Fig. 1). Similar results were obtained by Kin and Long, who investigated the dynamics of the accumulation of polysaccharides in a culture medium of the fungus *V. dahliae* and found the maximum polysaccharide content for a two-week culture [5].

It must be mentioned that, in the light of their origin from the cell wall of the pathogen, the change in the amount of polysaccharides in the culture liquid indicates a change in the qualitative composition of the cell wall. We studied the composition of an alkaline hydrolysate of it.

To isolate the cell walls we used a 15-day culture of *V. dahliae* and a 10-day culture of *F. oxysporum*. The alkaline hydrolysate obtained was subjected to chromatographic separation on two supports: Sephadex G-50 (fine) and Acrilex P-4. The gel filtration of the total fraction on Sephadex G-50 (fine) showed that the polysaccharide mixture contained several components, one of which had a high molecular weight and issued with the free volume, and also poly- and oligosaccharides as minor components (Fig. 2). Chromatography of the same hydrolysate on Acrilex P-4 enabled us to convince ourselves of the presence of two fractions, one of which likewise issued with the free volume (Fig. 3).

It must be mentioned that similar results have been obtained by R. G. Ovodova et al. [6], who subjected bioglycans from marine invertebrates to gel filtration on Sephadex G-75. As a result, it was established that the total fraction consisted of two components — high-molecular, issuing with the free volume, and low-molecular.

For a further study of the structures of the polysaccharides isolated, a total preparation of the cell walls of the fungus *V. dahliae* was subjected to specific hydrolysis by its own β-1,3-glucanase. As a result of the cleavage of the polysaccharide mixture with the β-1,3-glucanase isolated from the culture liquid of *V. dahliae*, the presence of β-1,3-glycosidic bonds in the

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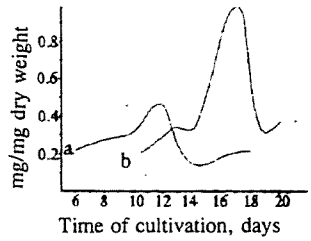

Fig. 1. Amount of exopolysaccharides in the dynamics of the growth of the fungi *F. oxysporum* (*a*) and *V. dahliae* (*b*).

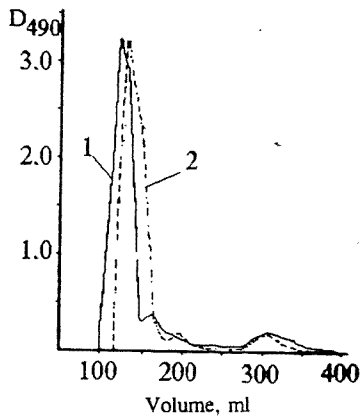

Fig. 2

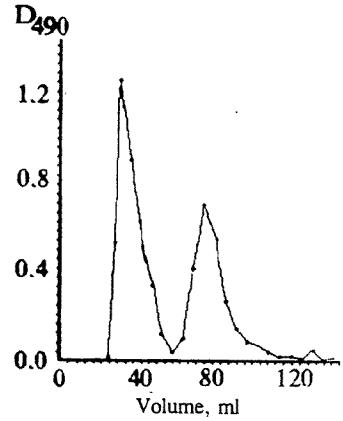

Fig. 3

Fig. 2. Gel chromatography of alkaline hydrolysates of the cell walls of the fungi *V. dahliae* (**1**) and *F. oxysporum* (**2**) on Sephadex G-50.

Fig. 3. Chromatography of an alkaline hydrolysate of the cell walls of the fungus *V. dahliae* on Acrilex P-4.

polysaccharide under study was established. On analyzing the monosaccharide composition of the extract by paper chromatography we were able to convince ourselves of the presence in the polysaccharide under investigation of glucose (70%), galactose (15%), and glucosamine (15%).

Thus, the experimental material obtained permits the conclusion that the polysaccharide isolated was a glucan containing β-1,3-bonds.

EXPERIMENTAL

Materials and Methods. Monospore cultures of the fungi *V. dahliae* and *F. oxysporum* were grown at 28 and 26°C, respectively, in Czapek-Dox medium in liter flasks each containing 200 ml of medium.

The amounts of sugars in the solutions analyzed were determined by the phenol—sulfuric acid method [7]. Spectral analysis and acid hydrolysis of the polysaccharides were performed under the conditions described in [8]. Monosaccharide compositions were determined by a standard procedure [9].

Determination of the Amounts of Exopolysaccharides. The fungal mycelium was separated from the culture liquid by filtration, and the filtrate was centrifuged at 10,000 rpm for 15 min. After this, the mycelium was washed twice with Czapek-Dox medium without the carbohydrate component and was incubated at the optimum temperature in the same medium for 3 h. Then the samples were centrifuged under the same conditions, and their polysaccharide contents were determined by the phenol—sulfuric acid method.

Alkaline Extraction of the Polysaccharides. The fungal mycelium was separated from the culture liquid by filtration, and to disrupt the cells it was ground in a mortar with quartz glass to a uniform mass. This was then washed repeatedly with distilled water (15 min at 15,000 rpm), after which it was freed from lipids by extraction with chloroform—alcohol (1:1) twice over a period of 12 h. The samples with the cell walls were covered with water and after 2 h were placed in a water bath for 2.5 h. The mixture was left overnight in the cold and was then centrifuged for 15 min at 15,000 rpm and was covered with 1 N NaOH after the flask with the sample had been placed on a stirrer in a refrigerator. The mixture was centrifuged at 15,000 rpm for 30 min and was subjected to dialysis, first against distilled water and then against 0.01 M ammonium acetate buffer, pH 5.2. It was freed from proteins by extraction with chloroform—butanol (5:1) with vigorous stirring for 30-40 min.

Fractionation of the Polysaccharides. Solutions of 40 mg of the alkaline extract of the polysaccharides (3 ml) were deposited on a column (2.3 × 76 cm) filled with Sephadex G-50 (fine). Elution was conducted with 0.01 M ammonium acetate buffer, pH 5.2, at the rate of 54.6 ml/h. The fraction volume was 8.2 ml. Aliquots of each fraction were analyzed for their sugars content.

For gel filtration on Acrilex P-4, 25 ml of the alkaline extract was dissolved in 2 ml of the same buffer, deposited on a column (1.6 × 65 cm), and eluted at the rate of 14.4 ml/h. The fraction volume was 2.7 ml.

Specific Hydrolysis. A preparation of β-1,3-glucanase was obtained from a 15-day culture liquid of the fungus *V. dahliae* by concentration, precipitation with alcohol, and gel chromatography on Sephadex G-50 [10]. To 40 ml of a solution of the polysaccharides with a concentration of 0.5 mg/ml was added 8 ml of a solution of the enzyme having a concentration of 0.125 mg/ml. A 0.01 M ammonium acetate buffer, pH 5.2, was used to dissolve the substances. The reaction mixture was incubated at 37°C for 1 h. The efficacy of hydrolysis was determined from the increase in the amount of reducing sugars by the Somogyi–Nelson method [7], using glucose as standard.

REFERENCES

1. P. Albersheim and A. G. Darvill, V Mire Nauki, No. 11, 16 (1985).
2. J. R. Sharp, V. Barbara, and P. Albersheim, J. Biol. Chem., **259**, No 18, 11312 (1984).
3. L. A. Rivas and L. R. Pont, Eur. J. Biochem., **163**, 129 (1987).
4. M. Kopp, J. Rouster, B. Tritig, A. Darvill, and P. Albersheim, Plant Physiol., **90**, 208 (1989).
5. V. V. Filippov, L. N. Andreev, and R. V. Bazilinskaya, Phytopathogenic Fungi of the Genus *Verticillium* [in Russian], Nauka, Moscow (1980), p. 258.
6. R. G. Ovodov, V. I. Molchanovsa, L. V. Mikheiskaya, and Yu. S. Ovodov, Khim. Prir. Soedin., 739 (1990).
7. S. D. Barfolomeev (ed.), Itogi Nauki i Tekhniki, Ser. Biotekhnol., VINITI, Moscow, Vol. 25 (1990), p. 33.
8. S. A. Avilov, A. I. Kalinovskii, and V. A. Stonik, Khim. Prir. Soedin., 53 (1990).
9. O. A. Semikhatov, Procedure for the Quantitative Paper Chromatography of Sugars, Organic Acids and Amino Acids in Plants, Izd-vo Akad. Nauk SSSR, Moscow (1962), p. 5.
10. R. S. Sattarova, Isolation, Physicochemical Properties and Some Structural Characteristics of a β-1,3-Glucanase from the Fungus *Verticillium dahliae*, Author's Abstract of Dissertation, Candidate of Biological Sciences [in Russian], Tashkent (1993), p. 97.