Protective Activity of the Fungal Polysaccharides against Fusariosis

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Abstract

Most of the experiments carried out in the area of plant protection have used chitin and chitosan obtained from the crustacean chitin which production is rather expensive. In our study we have applied the chitin-glucan complex prepared from the waste mycelia of filamentous fungi, from baker's yeast. Five different polysaccharides have been used for the preparation of water-soluble compounds and the assay of their antifungal activity against plant pathogen *Fusarium oxysporum*. In the field experiments, application of the polysaccharides led to the diminished infestation as well as to significantly increased productivity of fresh weight of the plants (tomato). The results demonstrated that application of the polysaccharides led to increased production of cell wall and some outher and intermembrane-bound proteins. Although the nature of the observed proteins has not been yet established, it can be speculated that they represent some enzymes involved in the antiinfective defense mechanisms in plants.

Keywords: fungal polysaccharides; chitin-glucan; *Fusarium oxysporum*; tomato; membrane-bound proteins

INTRODUCTION

It is known that like many other fungal cell walls, those of *Aspergillus niger* contain as a skeletal component a covalently-linked complex of chitin with (1→3)-β-glucan – SIETSMA & WESSELS (1981) and others. Since it is known, that fungal (1→3)-β-glucans also possess immunomodulating properties and reveal among other plant-protective activity, serving as elicitors of phytoalexins and other protective substances (ALBERSHEIM & VALENT 1978; ROUHIER *et al*. 1995). In the previously published results obtained in our Laboratory of Microbila Polymers, we have prepared several water-souble carboxymethylated derivates of the chitin-glucan complex and demonstrated their antimutagenic activity agains cyclophosphamide (CHORVATOVIČOVÁ *et al*. 1996) and antimycotic effect against *Candida albicans*, as well as plant-protective effect against *Tobacco necrosis virus* using bean and cucumber (KOGAN *et al*. 1998). The results obtained and known literature data on the antifungal action of crustacean-derived chitosan inspired us to use fungal chitin-glucan in experiments with fungal pathogen.

MATERIAL AND METHODS

Five polysaccharides or their derivates from waste material have been:

1. *Carboxymethyl-glucan* prepared from yeast *Saccharomyces cerevisiae* – MW 350 kDa
2. *Carboxymethyl chitin-glucan* prepared from *Aspergillus niger* – fraction with MW 20 kDa
3. *Carboxymethyl chitin-glucan* prepared from molecular weight fraction with MW 600 kDa
4. *Sulfoethyl chitin-glucan* prepared from *Aspergillus niger* – MW 600 kDa
5. *Mannan* from *Candida albicans* serotype A – MW 87 kDa.

The procedure originally developed for polysaccharides isolation and sulfoethylation were used (CHORVATOVIČOVÁ *et al*. 1996; SLOVÁKOVÁ *et al*. 1993).

Work was done during bilateral cooperation SAS/CNR and partly supported by Grant Agency VEGA, Grant No. 2/3051/23.

Tomato seedlings infection. Tomato seeds were pretreated with polysaccharide solution and infected with *Fusarium oxysporum* f.sp. *lycopersici* after 5 weeks of growing. The infection and evaluation has been done according the KROON and ELGERSMA (1993). Drops of 20 μl a conidial suspension (10^5/ml) were placed 1 cm above the cotyledons, one of each of the main vascular bundles of the plants. The plants were sprayed with polysaccharides solution in 0.01% concentration twice a day. The productivity was compared with the control plants (non-infected with and without polysaccharide spraying, and infected without polysaccharide spraying).

Evaluation of proteins assay was done on the tomato leaves infected with *Fusarium oxysporum*. Collected leaves were frozen in liquid nitrogen and ground to a fine powder in a cooled mortar. The powder was homogenized in extraction buffer (50 mmol/l Tris, pH 8.0; 1 mmol/l EDTA (ethylenediamine tetraacetate acid); 1 mmol/l DTT (dithiothreitol); 3% PVP (polyvinylpyrrolidone) using homogenizator. The homogenate was centrifuged at 1500 g for 10 min and subsequently at 14 000 g for 10 min. The microsomal membrane fraction was isolated from the supernatant by ultracentrifugation at 150 000 g for 30 min. The pellet was washed with 10 mmol/l Tris-maleate buffer, pH 7.3 that contained 0.15 mol/l NaCl, 1 mmol/l EDTA, and 10 μ/ml BHT (butylhydroxytoluene) or 1 mol/l NaCl or 0.1 mol/l Na_2CO_3 and centrifuged at 150 000 g to obtain peripheral membrane proteins were isolated from the supernatant obtained upon ultracentrifugation at 150 000 g. After passing through Sephadex G-25 column and concentration, proteins were fractionated on a anion-exchange column Bio-Scale Q2 in 25 mmol/l Tris buffer, pH 8.0. Fractions were eluted with a linear 0–1.0 mol/l NaCl gradient in the same buffer. The proteins were precipitated overnight at –20°C with two volumes of ice-cold acetone. Proteins were solubilized and separated under denaturing conditions on 15–20% gradient polyacrylamide gel using discontinous buffer system (LAEMMLI 1970). Protein concentration were determined according to LOWRY *et al*. (1951) with BSA as the standard.

Table 1. Antifungal and growth stimulating activity of polysaccharides on tomatoes infected with *Fusarium oxysporum* after 10 days of treatment

Model tested	Fresh weight		Fresh weight	
	degree	%	infection	without infection
PS 1	2.0	90	32.90	33.90
PS 2	1.4	40	36.20	34.23
PS 3	2.5	100	33.60	33.68
PS 4	1.0	80	35.95	29.22
PS 5	3.5	100	26.73	36.15
Control				
not infected	0.0	0	25.24	without
infected	1.6	80	22.99	spraying

RESULTS AND DISCUSSION

Productivity parameters. After 10 days the infection spread is from 40 to 100% and is accompanied by the higher average degree of infestation (Table 1). Effect of polysaccharides on non-infected plants was also observed. All polysaccharides caused significant increase of the fresh weight. Polysaccharides 2 and 4 revealed the highest antifungal activity as in case of antiviral reaction (SLOVÁKOVÁ *et al*. 1993). However, PS 1, 3 and 5 seemed to increase a degree of infestation but only in case of P5 accompanied by loss of weight compared to the notinfected samples (but sprayed with polysaccharides).

Protein composition of tomato plants. In the fraction of extracellular proteins cell wall (CW) proteins between 45 and 67 kDa in polysaccharide-treated samples were accumulated and their quantity is higher then in the infected and control samples. Proteins between 60–80 kDa may be involved in the export and assembly of semitrial subunit across the outer membrane (KORONAKIS *et al*. 2000) or organic solventy tolerance protein precursor involved in OM (outer membrane) tolerance (SAMPSON *et al*. 1989) to toxins as in yeast model system was identified. Another mode of polysaccharide action is enhancing of cell wall resistance of plants to pathogen (POSPIESZNY & ZIELINSKA 1997; GHAOUTH 1994). Six cytoplasmatic proteins (CP) have been evaluated in comparison to control, but no differences in quantity have been observed, except for the samples treated with PS 4 and PS 5 (45 and 60 kDa), CP_1 band with mass of around 35 kDa is induced in association with PS 2, PS 3 and CP_1 (55 kDa) with PS_5. Two intermembrane-bound proteins (IMP) have been accumulated that were associated with polysaccharide application. That IMP, between 50–60 kDa, namely around 55 kDa may be as hypothetical fusaric acid resistance protein Fus A (LINK *et al*. 1997) and may be in interaction with efflux (JENKINSON 1996) which is caused by it. The used strains of *F. oxysporum* produced fusaric acid *in vitro* and *in vivo*, too. No important differences in protein accumulation were observed for different

polysaccharides, but there are quantitative changes between samples treated and non treated.

Two PMP (peripheral membrane – proteins) have been detected as well 67 and 55 kDa as a reaction to the PS_3 and PS_5 treatment. There are less visible differences between samples pretreated with different PS.

In general, it can be said that the applied polysaccharides induced of proteins which can enhance, accelerate or contribute to recovery of the plant cell membranes damaged as the result of the metabolic products of pathogenic fungi.

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