Heteropolysaccharide produced by the red alga *Dixoniella grisea* affects the host–parasite relationship in the system *Scenedesmus incrassatulus–Phlyctidium scenedesmi*

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Dedicated to Assoc. Prof. Dr Stefan Draganov in honour of his 75th anniversary

Abstract. Treatment of the *Scenedesmus incrassatulus* (*Chlorophyta*) culture with the heteropolysaccharide produced by *Dixoniella grisea* (*Rhodophyta*) before inoculation with the obligate unicellular fungal parasite *Phlyctidium scenedesmi* (*Chytridiomycota*) has stimulated both host growth and parasite invasion. It has been cytochemically established that this treatment enhances the activities of α-esterase, α- and β-galactosidase, α- and β-glucosidase, and glutamate dehydrogenase in the non-infected and infected algal cells, as wells in the parasite cysts. The possible mechanism involved in the stimulation of the infection process by the heteropolysaccharide produced by *Dixoniella* is discussed.

Key vords: *Dixoniella grisea*, heteropolysaccharide, hydrolytic enzymes, pathosystem *Scenedesmus–Phlyctidium*

Introduction

There is accumulating evidence that sugars take part in the stress response of plants as biotic and abiotic stress-related stimuli. Sugars have important hormone-like functions as central signalling molecules (Rolland & al. 2002). Moreover, a number of investigations have established the existence of a complex signalling network that interconnects transduction pathways from sugars and other hormone signals (Roitsch 1999; Sheen & al. 1999). A number of defence genes are modulated by carbohydrates and the products of these genes mediate the plant–pathogen interaction (Koch 1996).

Investigations on higher plants show that oligosaccharide and glucopeptide elicitors are essential for the signal exchange between plant hosts and microbial pathogens that leads to activation of host defences (Hahn1996). Bouarab & al. (2001) reported that in the host–pathogen association of red algal host and green algal pathogen (*Chondrus crispus–Acrochaete operculata*) the virulence of the pathogen is mediated by oligosaccharides released from the host. Along with this, it has been proved that the cell wall sulfated polysaccharide of the red alga *Porphyridium* sp. has impressive antiviral activity (Huleihel & al. 2001).

The aim of the present investigation was to elucidate the effect of the polysaccharide isolated from the red alga *Dixoniella grisea* (Geitler) Scott on the host–parasite pathosystem of green microalga–fungal parasite.

Materials and methods

The pathosystem *Scenedesmus incrassatulus* Bohlin (*Chlorophyta*)–*Phlyctidium scenedesmi* Fott *(Chytridiomycota)* was cultivated under optimally intensive conditions described by Pouneva (2006). From the culture of the red alga *D. grisea* sulfur containing the heteropolysaccharide was isolated in the stationary phase, according to the method of Simon & al. (1992). Material from the intensively cultivated alga was centrifuged at 6000 G for 30 min to pellet the cells. The medium was collected and the polysaccharide was harvested from the supernatant suspended in water. Ethanol (2 volumes to the original solutions volumes) was added to precipitate the polysaccharide. After dialysis for 48 h against deionized water, the polysaccharide was used in the experiment. It was established that it contains about 50 % of carbohydrates: 39.6 % xylose; 21.5 % 3-0-methylpentose; 12.9 % rhamnose; 7.8 % 4-0-methylgalactose; 7 % galactose; 5 % glucuronic acid; 3.2 % glucose; 3 % protein, and 10 % sulfate (Evans & al. 1974).

Asynchronous culture of *S. incrassatulus* was inoculated with 0.32 mg/ml polysaccharide and its effect on the algal growth was determined gravimetrically 24 h, 48 h, 72 h, and 96 h after treatment. Influence of the polysaccharide on the infection process was investigated after inoculation of the host culture and treatment with 0.32, 0.032 and 0.0032 mg/ml polysaccharide. Furthermore, the infection degree was studied after inoculation of *S. incrassatulus* cultures pretreated with the same concentrations of the polysaccharide. For this purpose host cultures were incubated for 7 days in nutrient mediums with the same concentrations of the polysaccharide. After that the polysaccharide was removed, the algal cells were washed with the nutrient medium and infected with the parasite.

The percentage of invaded *S. incrassatulus* cells was determined by a Bürker camera 48 h from the beginning of the infection. After 48 h treatment with 0.32 mg/ml polysaccharide, the activities of the enzymes α-esterase, α- and β-galactosidase, α- and β-glucosidase, and glutamate dehydrogenase (GDH) in algal host cells and parasite cysts were investigated cytochemically (Lojda & al. 1979). This is the only suitable approach for the examination of enzyme activities of host and parasite in the pathosystem, because of the high obligation of *Phlyctidium* and the impossibility to have it isolated from the host alga in monoculture for biochemical assay.

The experiments were carried out with four replications. The standard errors were determined according to Student & Fisher (Diem & Lentner 1970).

Results

Cultivation of *S. incrassatulus* in nutrient medium with 0.32 mg/ml polysaccharide led to significant stimulation of the algal biomass production. Seventy-two hours after the beginning of cultivation the growth of polysaccharide–treated algal cultures exceeded the controls by 17 mg/ml (Fig. 1).

Addition of polysaccharide into the nutrient medium enhanced the infection degree from the beginning of the infection process until the stage of polyinfection. The results show that all polysaccharide concentrations under study have stimulated the development of *P. scenedesmi* and, simultaneously with the increase of concentration of the carbohydrate, the number of infected cells has risen. The highest stimulation was achieved by treatment with 0.32 mg/ml polysaccharide. After this treatment, at the end of the infection process (48 h) the infected algal cells exceeded by 18 % the non-treated control (Fig. 2).

Pretreatment of algal cells with the polysaccharide led to higher susceptibility to *P. scenedesmi*. It was established that the percentage of infected *S. incrassatulus* cells was highest when they were pretreated with the highest concentration under study (Fig. 3).

Cytochemical investigation of the enzymes α-esterase, α- and β-galactosidase, α- and β-glucosidase, and glutamate dehydrogenase has shown that treatment of the pathosystem *Scenedesmus–Phlyctidium* with 0.32 mg/ml heteropolysaccharide increases the activities of all investigated enzymes. This enhancement was observed in parasite cysts (Fig. 4), as well as in non-infected (Fig. 5) and parasite-infected (Fig. 6) cells of *S. incrassatulus*.

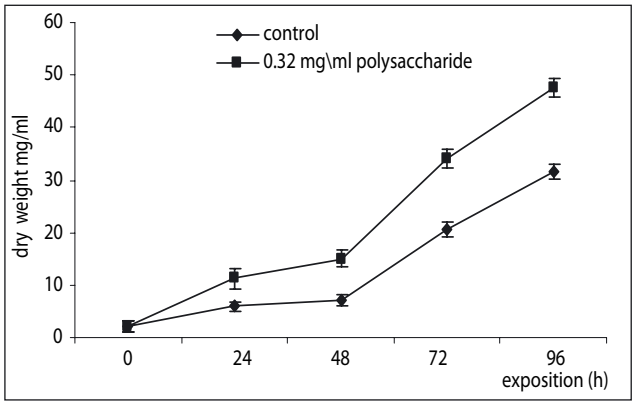

Fig. 1. Influence of the polysaccharide on the growth of *S. incrassatulus.*

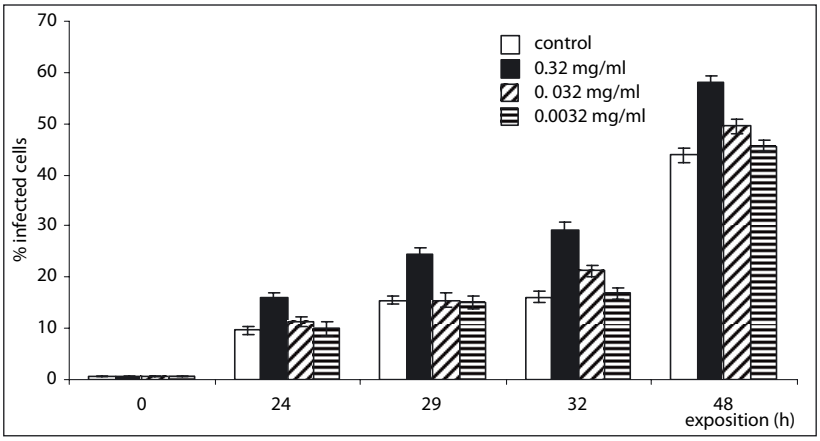

Fig. 2. Influence of different concentrations of the polysaccharide of *D. grisea* on the development of *P. scenedesmi.*

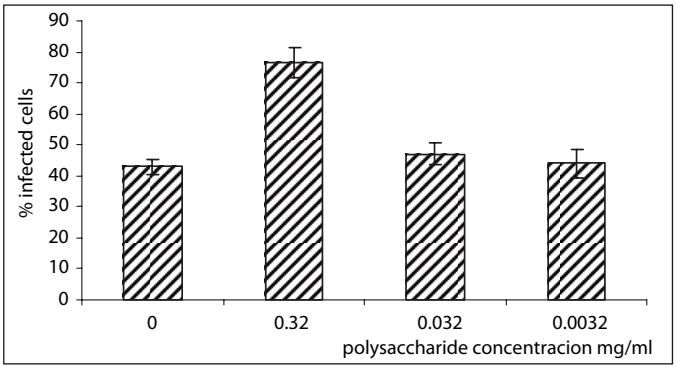

Fig. 3. Rate of infection in *Scenedesmus* cultures pretreated with different concentrations of the polysaccharide.

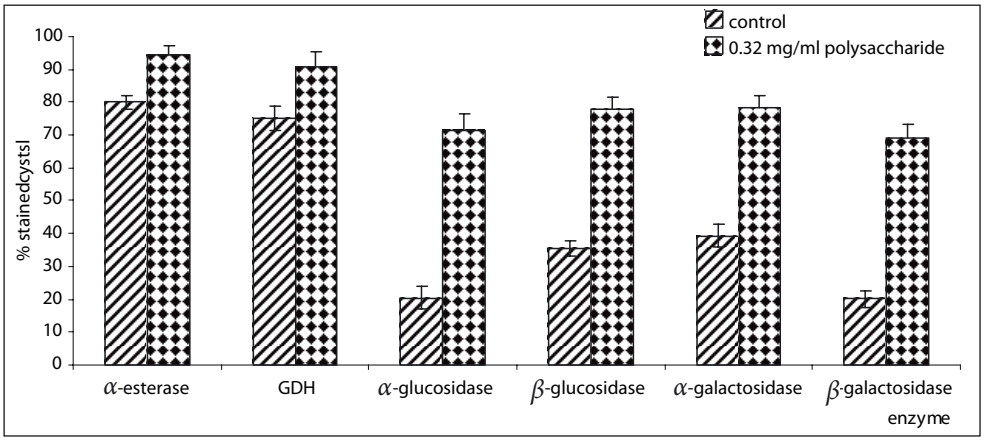

Fig. 4. Influence of the polysaccharide of *D. grisea* on the enzyme activity of hydrolases and dehydrogenases in *Phlyctidium* cysts.

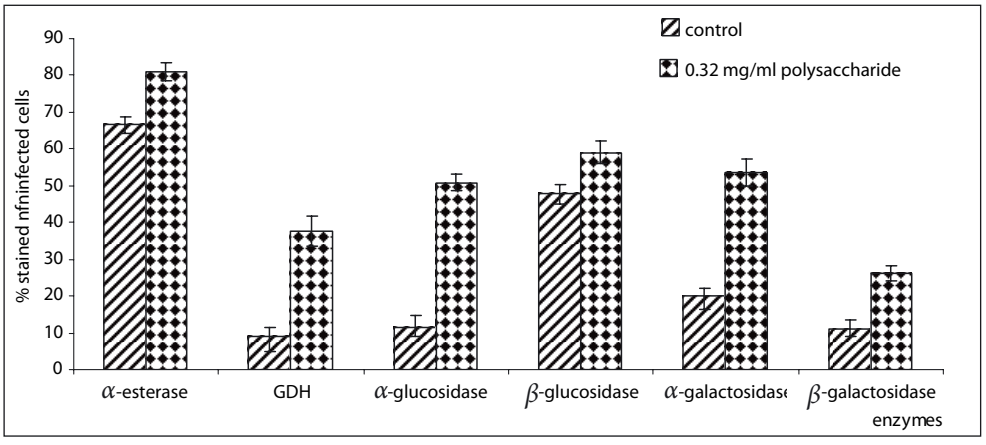

Fig. 5. Influence of *D. grisea* polysaccharide on the enzyme activity of hydrolases and dehydrogenases in non-infected cells of *S. incrassatulus.*

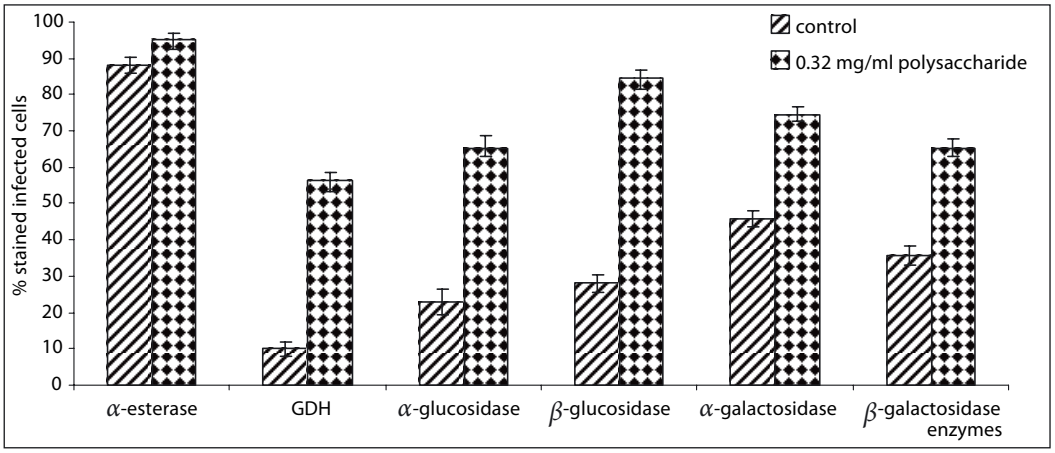

Fig. 6. Influence of the polysaccharide of *D. grisea* on the enzyme activity of hydrolases and dehydrogenases of *S. incrassatulus* infected with *P. scenedesmi*.

Discussion

Our early papers have proved that bacteria which naturally accompany the non-axenic algal cultures of genus *Scenedesmus* have high hydrolytic activities (Pouneva & Toncheva–Panova 1981). Their growth on medium prepared with cells and polysaccharide of the red microalga (Toncheva–Panova & al. 1991) has confirmed their ability to degrade the heteropolysaccharide to low molecular fragments (oligosaccharides and monosaccharides).

It was also established that in infected tissues of higher plants the intracellular concentration of monosaccharides has enhanced. The sucrose may be utilized as energy supply to the appoplast for ready use by endophytic pathogenic microbes (Blee & Anderson 1998). Similarly could be explained the activation of the infection process after pretreatment of algal cells with the polysaccharide from the red alga established in the present study. In all probability, after bacterial degradation of the polysaccharide, oligo-, di- and monosaccharides penetrate into the host cells and entail the observed stimulation of the pathogenesis. Treatment of the pathosystem *Scenedesmus–Phlyctidium* with the heteropolysaccharide produced by *D. grisea* simultaneously increases both host growth and parasite development.

A number of authors have reported activation of the infection process in different pathoassociations of red alga and bacterial or algal parasites as an effect of treatment with oligosaccharides. They have established that certain forms of oligosaccharides have been identified as endogenous and exogenous elicitors. These elicitors are recognized by the host that responds hypersensitively to the pathogen (Weinberger & Friedlander 2000). On the other hand, virulence of the pathogen is associated with identification of the oligosaccharides released by the host. Presumably, recognition of host and parasite is connected to the oligosaccharide signal (Bouarab & al. 2001), and oligosaccharide enhances the pathogenicity of endophytic green alga *Acrochaete operculata* as an induced release of H_2O_2 and synthesis of specific polypeptides in the pathogen. It was also reported that in higher plants oligosaccharides released from the damaged cell walls elicit an oxidative burst (Leon & al. 2001) and regulate the expression of genes coding the pathogenesis-related proteins.

The enhanced activities of hydrolases observed in the cysts of *P. scenedesmi* can explain the mechanism of its increased virulence. It is known that the enzymes α-esterase, α- and β-galactosidase, and α- and β-glucosidase take part in degradation of the host cell walls during parasite penetration and in the trophic process during pathogenesis. Furthermore, Bishop & al. (2002) have established that carbohydrate signalling has defined roles in the induction of genes encoding the hydrolytic enzymes.

As to the growth stimulation of host cells after treatment with the heteropolysaccharide, it can be associated with the observed increase of glutamate dehydrogenase activity, since it is a key enzyme connecting the carbohydrate metabolism with the metabolism of amino acids.

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