BULG. J. PLANT PHYSIOL., 1999, 25(1–2), 76-82

EFFECT OF *FUSARIUM OXYSPORUM* EXUDATE ON THE GROWTH AND VIABILITY OF *SCENEDESMUS ACUTUS*

Irina Puneva*, Tonka Toncheva-Panova, Milka Bozhkova

Acad. M. Popov Institute of Plant Physiology, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria

Received 22 August 1999

Summary. The influence of exudate of *F. oxysporum* on the viability, productivity and the content of some ingredients of *S. acutus* cells was studied. It was established that the fungal exudate stimulates cell growth (6.6%) and enhances the content of proteins (13%), lipids (7.6%) and pigments (4.8%). Furthermore, it was found that treatment with fungal exudate resulted in enhanced activities of both esterase and glutamatedehydrogenase in algal cells as well as increased percentage of viable *S. acutus* cells upon prolonged storage. The effect of dimethylamine, present in the fungal exudate, is discussed.

Key words: dimethylamine, fungus, green alga

Abbreviations: DMA – dimethylamine, GDH – glutamatdehydrogenase, TTC – triphenyltetrazolium chloride, DW – dry weight

Introduction

It has been suggested, that algae might have similar biochemical systems as higher plants, their growth and development is regulated by endogenously produced growth substances, and the role of plant growth regulators controlling algal development has been discussed (Bradley, 1991; Evans and Trewavs, 1991).

It is known that amines are involved in the process of plant growth and regulation. Diamine putrescine and polyamine spermidine interact with nucleic acids and play an important role in plant growth and development (Daoudi and Biondi, 1995). They influence considerably the activities of enzymes and are implicated in protein synthe-

* Corresponding author, E-mail: ipuneva @bio.bas.bg

sis (Smith, 1980). Kordy and Soeder (1984) suggest that changes in polyamine concentration in unicellular alga *Chlorella emersonii* are in fact related to cell development.

Dimethylamine (DMA) is a lower aliphatic monoamine widely distributed in higher plants, some fungi and algae. Data about the influence of DMA on different organisms are contradictory. It was shown, that DMA is toxic to some organisms (Wotzka, 1975) and humans (Hoffmann et al., 1976; Evelo et al., 1998). It was also supposed, that DMA itself exerts no toxic effect, but the dangerous substance is rather ammonia, released after DMA decomposition (Wotzka, 1975).

Our previous investigations revealed the presence of DMA in the exudate of *Fusarium oxysporum* (Toncheva-Panova et al., 1995). Besides, it was found that some microorganisms possess enzymes that oxidize secondary amines and especially DMA (Meiberg and Harder, 1979; Wassenaar et al., 1998) thus reducing its content in the culture medium. Furthermore, it was suggested that algal cells decompose DMA more efficiently than bacterial cells (Chromek et al., 1983).

To gain further insight into the influence of DMA containing exudate from *F. oxysporum* on the unicellular alga *Scenedesmus acutus*, cell growth and protein, carbohydrate, lipid and pigment production were estimated. Moreover, the activities of esterase involved in the basic metabolism of the cells and glutamate dehydrogenase (GDH), connected with carbohydrate and protein metabolism were also determined. The effect of the fungal exudate on the number of contaminating bacteria was assessed as well.

Materials and Methods

Fusarium oxysporum strain F 2 was cultivated under standard conditions (Puneva and Toncheva-Panova, 1995). Fungal mycellium was removed from the medium by filtration. The exudate was added in ratio at 1:4 v/v to the non axenic cultures of *Scenedesmus acutus* M Tomaselli 8 cultivated both intensively and extensively in the medium of Georgiev et al. (1978). Control samples were diluted with equal volumes of distiled water.

The cells of *S. acutus* were cultivated intensively during 3 days at standard conditions of illumination and mixing with CO_2 enriched air (Dilov, 1985). The extensive cultivation was conducted for 6 months on solid medium.

The effect of fungal exudate on algal growth was registered by daily measurement of cell DW. The growth coefficient was computed as a 24 h standing crop versus inoculum ratio. Protein content was measured after Lowry et al. (1951), carbohydrates – by the antron method (Jaska, 1964), lipids – after extraction with chloroform:ethanol 2:1 (Popov et al., 1970), pigments – after methanol extraction. The activities of both esterase and glutamate dehydrogenase (GDH) were determined cytochemically, esterase – by the method of simultaneous azocopulation, GDH – by the

method of tetrazolium reductases (Lojda et al., 1979). The effect of the treatment was registered as the percentage of algal cells with positive cytochemical reactions using Burker camera. The viability of the algal cells was tested by the Triphenyltetrazolium test (TTC) (Jones, 1987).

The total number of contaminating bacteria in algal suspension was determined on meat peptone agar and the predominating bacterial strain *Pseudomonas* sp. was isolated. From each sample the statistical significance of the results was computed by the Student's criterion.

Results

Treatment with *Fusarium* exudate of algo-bacterial culture of *Scenedesmus acutus* for 24 h had a negligible effect on algal biomass, production rate, the differences on growth coefficients of treated and non treated cultures. However, 72 h after cell inoculation the growth coefficient of the experimental cultures rose (Table 1). The higher growth rate was accompanied by a rise in protein (with 13%), lipid (with 7.6%) and pigment (with 4.8%) contents and a slightly lower carbohydrate content (Fig. 1).

Significant enhancement of the percentage of algal cells with positive cytochemical reaction for esterase and GDH after addition of *Fusarium* exudate was observed. This effect concerning GDH was better expressed in the intensively as compared to the extensively grown cultures (Fig. 2).

Table 1. Influence of *Fusarium oxysporum* exudate on the dry weight and growth coefficient of *Scenedesmus acutus.* DW of the inoculum – 0.2 mg/ml. Data are means from three independent experiments

	Growth coefficient	
Hours after treatment	control cultures	treated cultures
24	3.05	3.25
48	13.95	15.00
72	22.85	24.35

The results from TTC-test showed that *Fusarium* metabolites affected the viability of *Scenedesmus* cells both in intensive and in extensive cultures. In the intensive cultures the percentage of viable cells exceeded that of controls by 11%, whereas in the extensive cultures this percentage was twice as high (25%) (Fig. 3).

Fusarium exudate caused increasing in the number of contaminating bacteria from 40–90%. Among the bacteria prevailence of representatives of *Pseudomonas* with 7.10^7 cells/ml at 72 h was demonstrated.

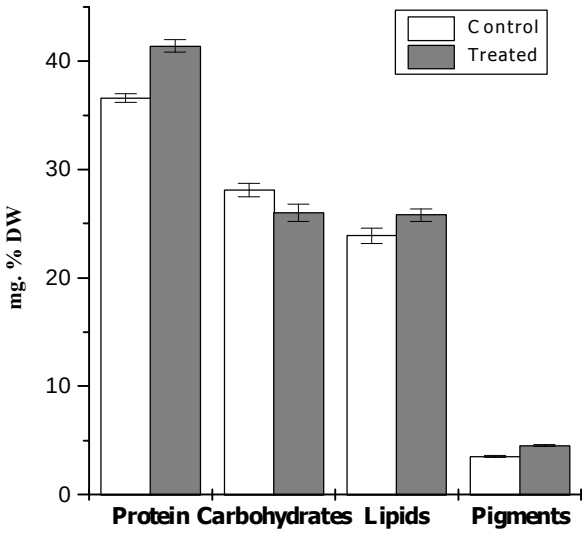

Fig. 1. Influence of exudate of *Fusarium oxysporum* on the content of protein, carbohydrates, lipids and pigments (mg % cell DW) in *Scenedesmus acutus* cells after 72 h cultivation.

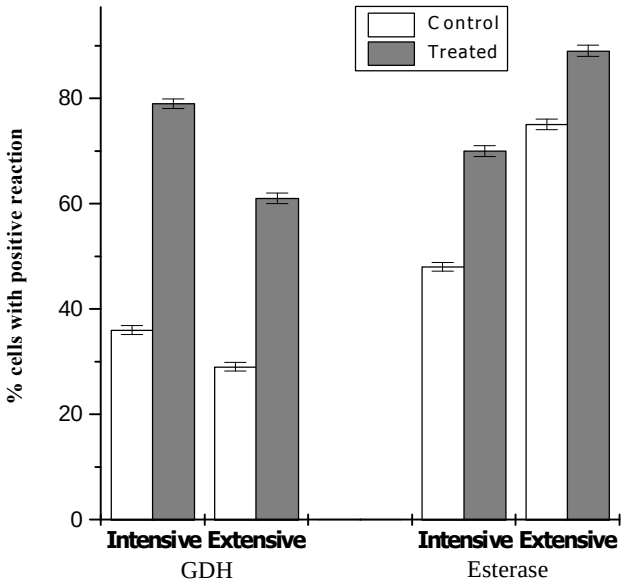

Fig. 2. Influence of *Fusarium oxysporum* exudate on the activity of esterase and GDH in *Scenedesmus acutus,* expressed as percentage of cells with positive cytochemical reaction.

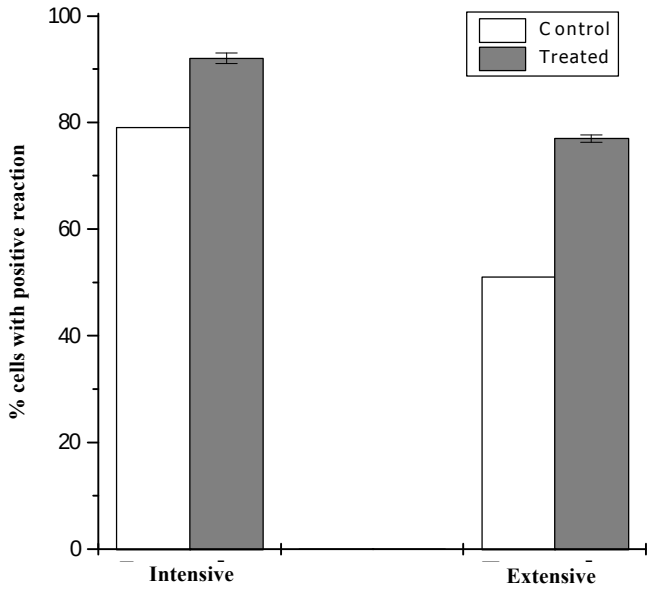

Fig. 3. Influence of *Fusarium oxysporum* exudate on the viability of *Scenedesmus acutus* after intensive and extensive cultivation expressed as percentage of cells with positive TTC reaction.

Discussion

The present data showed that some ingredients in the exudate of *F. oxysporum* enhanced cell growth and increased protein, lipid and pigment content of *S. acutus* cells.

The rise of *S. acutus* cells percentage with positive GDH activity after treatment is probably linked with the stimulatory effect of *Fusarium* exudate on the enhanced protein content and productivity of the algal culture.

It was proven that the enhanced NH_4^+ concentration in the culture medium was associated with stimulation of *de novo* synthesis of GDH (Molin et al., 1981; Ahmad and Hollebust, 1984). The higher percentage of *S. acutus* cells with a GDH activity after treatment with *Fusarium* exudate might be attributed to increased ammonium content in the culture medium after DMA decomposition by the algal cells (Chromek et al., 1983).

Increased percentage of algal cells with active esterase is probably related with stimulation of biosynthetic processes, since this enzyme is involved not only in catabolic but in anabolic processes as well.

We established previously that the exudate of *Fusarium oxysporum* contained 1–1.2 mg/l DMA (Toncheva-Panova et al., 1995). Interestingly it has been reported that a related fungal species *Fusarium (Fusarium moniliforme)* produces a high amount

of gibberellins, exclusively GA_3, GA_4 and GA_7 (Rachev et al., 1997). So production of gibberellins by *F. oxysporum* in the present experiment can not be excluded.

The possibility of a combined stimulatory effect of DMA and gibberellins on the exudate of *F. oxysporum* on the growth and development of *S. acutus* cells can not be excluded.

It was reported that *S. acutus* cultures decompose DMA (Chromek et al., 1983) to secondary products which are taken up by algal cells and utilized as a source of nitrogen and carbon. Besides, it was found that some aerobic microorganisms from the *Pseudomonas* group possesses a multicomponent enzyme system for DMA oxidation thus reducing DMA via a monooxygenase reaction (Eady and Large, 1969). In our opinion some bacterial contaminants of *S. acutus* cultures and especially *Pseudomonas* sp. are also involved in DMA decomposition thus supplying algal cells with additional nitrogen and carbon sources. These data are in agreement with the enhanced activities of GDH and esterase and with the increased viability of algal cells in the exudate-treated algal cultures.

The observed favourable effect of DMA on the growth and viability of *S. acutus* is in concert with the data which showed that mono- , di- and polyamines play important role in the regulation of plant metabolism (Sheviakova, 1981) thus stimulating similarly to hormones many macromolecular biosyntheses.

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