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INFLUENCE OF DETERGENG ON THE BIOCHEMICAL PROCESSES OF SOME FUNGI UNDER IN VITRO CONDITIONS

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In this paper, the results of an investigation on the influence of various concentrations of detergent in microbial medium on the ecophysiological characteristics of Aspergillus niger and Trichotecium roseum are presented. The proteolytic activity of these fungi varied, as a function of detergent concentration, from an inhibitory to a stimulative effect. Parallel to this, tests for the occurrence of amino acids in the liquid medium were performed after addition of 1% detergent. In the case of Aspergillus niger, detergent had a stimulative effect on the production of amino acids, with an opposite effect for Trichotecium roseum.

Key words: Aspergillus niger, Trichotecium roseum, proteolytic activity, amino acids

INTRODUCTION

Detergents and other washing substances with their harmful components (surface active substances, tensides, sodium polyphosphate), are significant pollutants of the environment, despite a tendency towards the introduction of "ecological detergents". In combination with other pollutants (only rarely, alone), they directly change the physicochemical characteristics of the receiver, i. e. indirectly affect the qualitative and quantitative composition of living populations.

Autochthonous flora, including fungi, naturally purify waters through degradation and transformation of toxic matter, to an extent that depends on their nature and concentration, either directly, or as a component of the subsequently formed active sludge. For instance, the linear acrylbenzensulphate tenzide is relatively easily decomposed by the microorganisms present in waste waters (Žarić-Horvat, and Grbić, 1977). Every change of the ecosystem caused by abiotic factors, influences the qualitative and quantitative composition of the organisms present (fungi, in our case) and the dynamics of biocenosis, as a whole. These changes are accompanied by individual changes in the species

and individuals and their ability to adapt to the altered conditions, expressed through changes in their biochemical activity. The dynamics of development, for most biocenosic microorganisms, is also under the influence of toxic matter present. This was found, for instance, for the biomass of *Saccharomyces cerevisiae* and for the enzymatic activity of *Rhizopus chinensis* (Fukomoto, et al., 1967). These changes may either inhibit or activate their proteolytic activity, as reflected in changes of: the pH value of the nutrient base, morphology and reproduction, population biomass. Contemporary taxonomy is also based on an understanding of the biochemical characteristics of fungi (Gorlenko and Sokolov, 1976; Stein and Moore, 1954; Stojanović et al. 1986; Stojanović, 1987; Stojanović, 1988).

A study of the process of biodegradation of detergents and change of biochemical activity of the fungi in relation to the presence of various concentrations of detergents in nutrient media and determination of the mechanisms by which the fungi resist the xenobiotic compounds, is best attained by extrapolation of the environmental conditions and biomodelling, using an "in vitro" method. In this paper, part of some complex investigations, performed over a number of years, on the influence of a specific detergent in a nutrient base on proteolytic activity and production of amino acids by *Aspergillus niger* and *Trichotecium roseum,* is presented.

MATERIALS AND METHODS

In our experiments, monospore cultures of *Aspergillus niger* van Tiegheme and *Trichotecium roseum* Link, taken from the mycotheque of the Faculty of Natural Sciences and Mathematics – Kragujevac, were employed. When deciding on the species of fungi to be used we took into consideration their taxonomic order and ecology. The fungi were maintained in a chamber, at 4°C (± 0.5°C), on a potato-dextrose agar slant as the nutrient medium. A monospore culture was developed by the method of exhaustion on a poor agar, in Petri dishes. In our experiment, the fungi were grown on the nutrient basis of Czapek (in g/L): $NaNO_3$ - 3, K_2HPO_4 - 1, $MgSO_4$ -1, $MgSO_4$. $7H_2O$ - 0.25, $FeSO_4$. $7H_2O$ - 0.01, saccharose - 30, distilled water - 1000. Detergent was added in the concentrations of 1, 0.1 and 0.01% for observation of the effect of concentration of detergent in the nutrient medium of Czapek on the proteolytic activity of fungi. The qualiative and quantitative occurrence of amino acids, was measured at the detergent concentration of 1%. The sterility of the nutrient bases was tested using a mesopeptonic agar.

The influence of detergent on the biochemical processes of the fungi was investigated in 250 mL Erlenmeyer flasks, containing 200 mL of nutrient base. The flasks were placed on an electric shaker, thus enabling uniform and constant mixing. The experiments were carried out at room temperature, under alternate light and dark. During incubation, the proteolytic activities on the 4th, 5th, 6th, 7th and 8th day were measured, together with some other parameters, such as pH and rH2. In the end of the experiment (on the 8 th day), the concentration of amino acids was determined. Also, at this point, the possible influence of

detergent on some other parameters (change of the amount of nitrogen, protein and biomass, the size of colonies, the number of spores, the amount of glucose, fructose and organic acids) of biochemical activity of the fungi was observed, thus completing the picture of activity of the fungi. These results have been partly presented already (Stojanović, 1988; Stojanović, 1989) and partly prepared for publishing.

The proteolytic activity of fungi was measured in 1 mL of nutrient base, by Anson's method (Dudka, 1982; Egorova, 1976; Petrović and Petrović, 1971.), on the basis of the amount of tyrosine or tryptophane, produced by hydrolysis of casein. From the curve obtained, the intensity of the activity was colorimetrically determined. Proteolytic activity of the fermentation liquids was determined by standard methods.

The qualitative and quantitative determination of amino acids, presented in Table 1, was performed by standard methods (Egerova, 1976; Moore et al. 1958; Petrović and Petrović, 1971; Stein and Moore, 1954.).

RESULTS AND DISCUSSION

The proteolytic activity of *Aspergillus niger,* depending on the concentration of detergent in the liquid nutrition medium of Czapek and in the control, is presented in Figure 1.

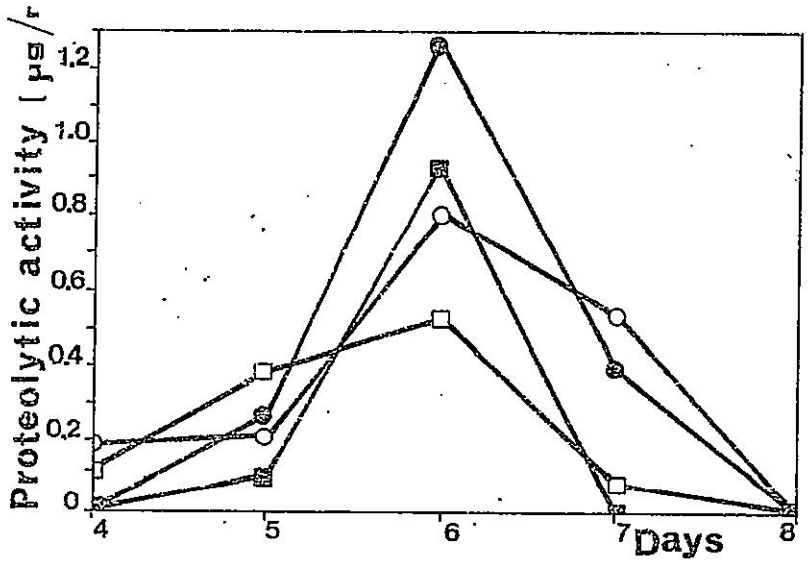

Figure 1. Proteolytic activity of A. niger in the nutrient base of Czapek, at various concentrations of detergent (□ - control, □ - 1%, O - 0.1%, ⊙ - 0.01%)

The maximum proteolytic activyty of this fungus, shown both in the presence of detergent and in the control, was reached on the sixth day following the innoculation of spores, while it completely stopped on the eighth day. The most intense activity was achieved in the presence of 0.01% of detergent (curve 4). At the concentrations of 0.1% and 1%, the activity was somewhat lower and approximately the same at both concentrations. The influence of detergent on

the course of enzymatic reaction was strongest and regular at the highest concentration of detergent, which can be explained by adaptation of fungi to the changed environmental conditions. A positive contribution of toxic substances to the enzymatic activity of *Rhizopus chinensis* species was also registered by other authors (Fukomoto et al., 1967).

The proteolytic activity in the liquid nutrition base caused by the activity of *Trichothecium roseum,* under the same laboratory conditions, as those applied for *Aspergillus niger,* is presented in Figure 2. The maximum proteolytic activity in the control variant (curve 1) was expressed on the eighth day after the inoculation of spores; while on the fourth and fifth day, activity could not be registered. Further on, the activity in the control showed a rising tendency, while in the medium with detergent it was changeable, reaching a maximum after 6-7 days, followed by an abrupt decrease. According to the literature, very changeable proteolytic activity was also detected for *Aspergillus saitoi* species (Yoshida and Hagasawa, 1956).

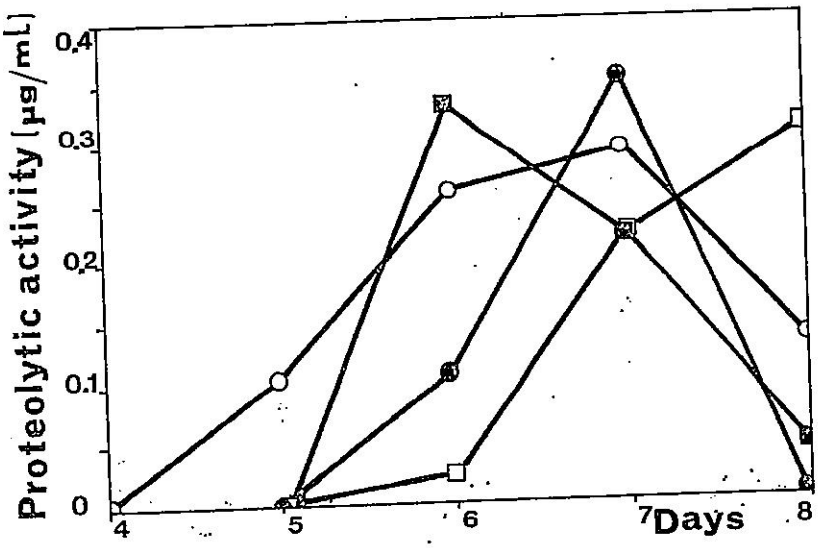

Figure 2. Proteolytic activity of T. roseum in the nutrient base of Czapek, at various concentrations of detergent (□ - control, □ - 1%, O - 0.1%, ● - 0.01%)

Since the same experiment has been carried out with a number of species or genera of fungi, *(Alternaria tenuis, Fusarrium oxysporum, Penicillium verrucosum),* it can be generally concluded that proteolytic activity of fungi varies as a function of time and concentration of detergent in the liquid nutrient medium. For all investigated species of fungi, detergent present at the concentration of 1 % did not prevent proteolytic activity, i. e. at this concentration it still did not have a lethal effect. The relatively intense activity of the fungi in the presence of detergents, which occasionally produced even a stimulative effect at, increased concentrations should be utilized in the process of purification of waste waters containing detergents, where these fungi should constitute part of an active sludge. The change of activity of proteinases was also investigated for the *Fusarium vasinfectum* species (Narayanan and Shanmugsundaram, 1966).

The qualitative and quantitative occurence of amino acids (g/mL) for in *A. niger* and *T. roseum,* at in the concentration of detergent of 1 % (D), are presented in Table 1.

Table 1. The amino acids detected and their amount (g/mL) for different fungi, on the eighth day, in the liquid medium of Czapek without (K) and with 1 % of detergent (D)

Amino acids	Aspergillus niger		Trichotecium roseum	
	K	D	K	D
Lysine	0.33	2.37	1.51	0.75
Histidine	0.06	0.55	0.29	0.08
Arginine	0.27	2.49	1.29	0.71
Aspartic acid	0.56	3.40	2.40	1.19
Threonine	0.34	1.89	1.20	0.73
Serine	0.42	1.86	1.28	0.88
Glutamic acid	1.22	5.06	3.38	1.59
Proline	0.34	2.00	1.09	0.57
Glycine	0.36	1.85	1.24	0.56
Alanine	0.54	2.27	1.61	1.64
Valine	0.26	1.92	1.14	0.66
Isoleucine		1.39	2.85	0.45
Leuc8ine	1.30	4.82	2.76	1.08
Tyrosine	0.10	1.44	0.65	0.33
Phenylalanine	0.22	1.77	0.97	0.67

In the case of *A. niger,* all the investigated amino acids (15 of them) were produced more intensely when detergent was present in the medium, than in the control. The ratio varied from 1:14 (for tyrosine) to1:3.7 (for leucine). The greatest amount, both in the medium with detergent and in the control, was registered for aspartic and glutamic acids and leucine. The composition of amino acids is not only influenced by the composition of the biological system, but also by changes in the dynamics of development of the biological system (Veličković, 1971).

The production of amino acids in the nutrient base in the presence of *T. roseum* was quite different. With the exception of alanine, their production was much more intense in the control, i. e. in the absence of detergent. The ratio of amino acid production between the test and the control was between 1:6 (for isoleucine) and 1:1.5 (for serine and phenylalanine). For this species, detergent in the concentation of 1% had an inhibitory effect on the production of amino acids. The greatest overall amount was measured for the same amino acids as for *A. niger.* Neither of the species of fungi, examined grown on the ordinary nutrient base and with the addition of detergent, produced methionine. Various factors have been shown to have on influence on the qualitative and quantitative composition of amino acids produced by the microoorganisms (Džamić and Veličković, 1970).

In order to have a more complete insight into the acivity of the fungi at various concentrations of detergent, other parameters, such as pH and Eh

values, the change of biomass density, the size of colonies, the amount of glucose and fructose, free and total organic acids and the number of spores during the tests, were also observed. Beside an influence of the increased concentrations of detergent described in this paper, the listed parameters were also monitored in the presence of inorganic concentrations of sodium polyphosphate and enthoxylized oleilacetyl alcohol. The obtained results were partly reported (Stojanović, 1989.).

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UTICAJ DETERDŽENTA NA BIOHEMIJSKE PROCESE NEKIH GLJIVA POD USLOVIMA "IN VITRO"

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SADRŽAJ

U radu se iznose rezultati istraživanja uticaja različitih koncentracija deterdženata uneti u mikrobiološku podlogu na ekofiziološke karakteristike gljiva *Aspergillus niger* i *Trichotecium roseum.* Proteolitička aktivnost ovih gljiva je različita u funkciji koncentracije deterdženta, kreće se od inhibitornog do stimulativnog efekta. Uporedo je rađen test na zastupljenost aminokiselina u tečnoj podlozi sa dodatkom deterdženata u koncentraciji od 1%. Kod *A. niger* deterdžent u navedenoj koncentraciji deluje stimulativno na produkciju aminokiselina, dok je ovaj efekat obrnut kod vrste *T. roseum.*