

**INFLUENCE OF DETERGENT ON AMINOACID PRODUCTION AND PROTEOLYTIC ACTIVITY  
OF SOME SPECIES OF FUNGI**

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*Bioproduction of aminoacids by the species of fungi *Aspergillus niger* and *Penicillium verrucosum*, was considerably affected by detergents (Merix<sup>R</sup> Merima, Krusevac) both, concerning relative and total amounts of different aminoacids produced. Stimulation of bioproduction from *Aspergillus niger* by detergent was very expressed for all 15 aminoacids with the exception of leucine and isoleucine which were increased very weakly by detergent. Production of all aminoacids from *Penicillium verrucosum*, was inhibited. Proteolytic activity for the species *Aspergillus niger* was activated by the presence of detergent.*

*Key words: *Aspergillus niger*, *Penicillium verrucosum*, 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 water 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 acrylbensulsulphate tenside is relatively easily decomposed by the microorganisms present in waste waters (Zaric-Horvat and Grbic, 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 biocenotic microorganisms, is also under the influence of toxic matter present. This was found, for instance, for the biomass of *S. cerevisiae* and for the enzymatic activity of *R. chinensis* (Fukamoto 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; Stojanovic, 1987; Stojanovic, 1988; Stojanovic et al., 1994).

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

#### MATERIALS AND METHODS

Monospore cultures of *Aspergillus niger* van Tiegheme and *Penicillium verrucosum* PEYRONEL, taken from the mycothèque of the Faculty of Natural Sciences and Mathematics - Kragujevac, were employed in our experiments. 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 ( $\pm 0,5^{\circ}\text{C}$ ) on potato-dextrose agar slants as the nutrient medium. A monospore culture was developed by the method of exhaustion on a poor agar, in Petri dishes. The fungi were then grown on the nutrient base of Czapek containing (in g/L):

NaNO<sub>3</sub> - 3  
K<sub>2</sub> HPO<sub>4</sub> - 1  
MgSO<sub>4</sub> - 1  
MgSO<sub>4</sub>·7H<sub>2</sub>O - 0,25  
FeSO<sub>4</sub>·7H<sub>2</sub>O - 0,01  
Saccharose - 30  
Distilled water ad 1000

Detergent was added at 1, 0.1 and 0.01 % for observation of the effect of detergent concentration in the nutrient medium of Czapek on the proteolytic activity of fungi. The qualitative and quantitative occurrence of aminoacids, was measured at the detergent concentration of 1%. The sterility of the nutrient bases was tested using mesoseptonic agar.

The Erlenmeyer flasks containing 200ml of nutrient base were placed on an electric shaker enabling uniform and constant mixing. The experiments were carried out at room temperature, under alternate light and dark conditions. Proteolytic activity was determined on the 4 th, 5 th, 6 th, 7 th and 8 th day together with some other parameters, such as pH and rH<sub>2</sub>. At the end of the experiment (on the 8 th day), concentrations of aminoacids were determined. The possible influence of detergent on some other parameters at this point, (amount of nitrogen, protein and biomass, size of colonies, number of spores, amount of glucose, fructose and organic acids) have been partly presented already (Stojanovic, 1988; Stojanovic, 1989) and partly prepared for publishing.

The proteolytic activity of fungi was measured in 1ml of nutrient base by Anson's method (Dudka, 1982; Egorova, 1976; Petrovic and Petrovic, 1971.), on the basis of the amount of tyrosine or tryptophan, 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 aminoacids, was performed with standard methods (Egerova, 1976; Moore et. al. 1958; Petrovic and Petrovic, 1971; Stein and Moore, 1954.).

#### RESULTS AND DISCUSSION

The proteolytic activity of the species of fungi *A. niger* grown on the nutrient medium of Czapek with detergent at 1%, 0,1% and 0,01% concentration is shown on Figure 1.

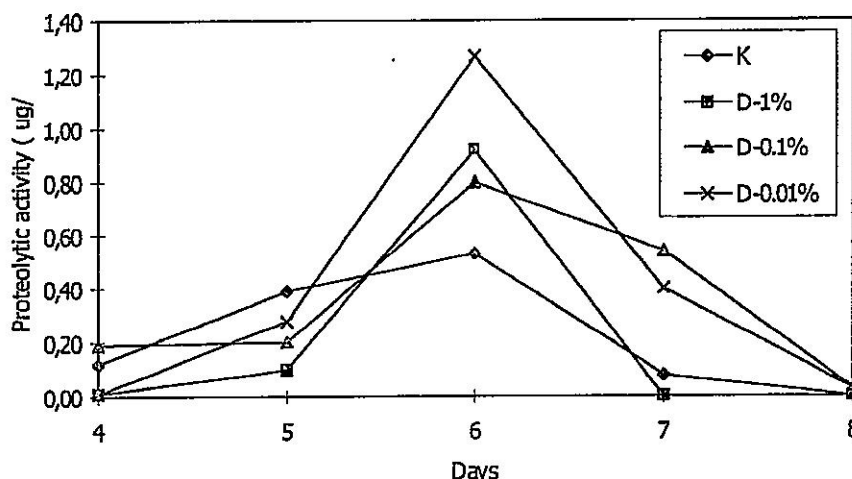


Figure 1. Proteolytic activity of *A. niger* in the nutrient base of Czapek, at various concentrations of detergent

The maximal proteolytic activity occurred in the nutrient medium containing 0,01% of detergent on the 6th day from inoculation. The lowest enzymatic activity of this species was observed in all variants of nutrient medium on the 8th day after the inoculation. With all detergent concentrations from 0,01% to 1% the proteolytic activity of *A. niger* increased up to the 6th day, which agrees with the analyses of enzymatic activity in *Rhizopus chinensis* (Fukamoto et al., 1967). The proteolytic activity of *A. niger* grown in all the variants of nutrient media then significantly decreased (approximately to zero which confirms earlier analyses for many species of fungi (Stojanovic et.al. 1986 Stojanović 1986)).

The proteolytical activity of the species *Penicillium verrucosum*, grown on the medium of Czapek with detergent at 1%, 0,1% and 0,01% concentration is shown in Figure 2.

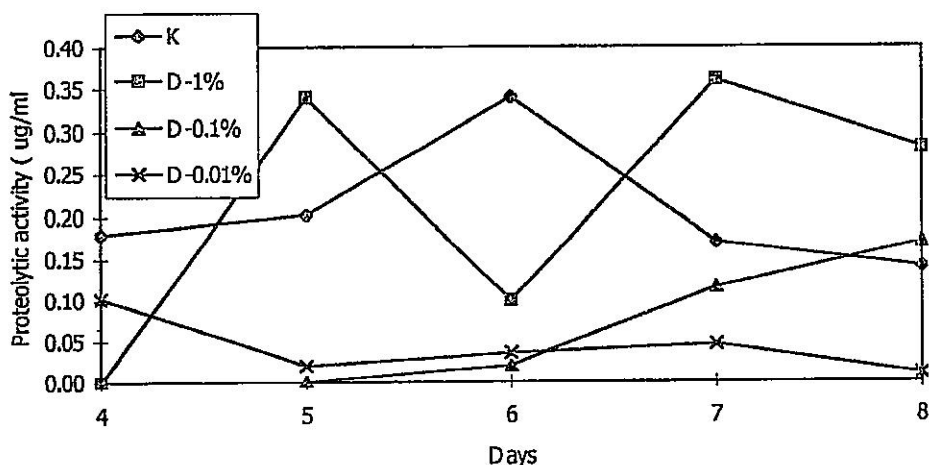


Figure 2. Proteolytic activity of *P. verrucosum* in the nutrient base of Czapek, at various concentrations of detergent

The proteolytic activity of the species *Penicillium verrucosum*, in all the variants of nutrient media was very variable with significant differences in relation to the control during the experimental period. Fluctuation of maximal enzyme activity in the nutrient medium with the maximal concentration of detergent occurred, as found for the species of fungi *Aspergillus saitoi* (Yoshida 1956.), and *Fusarium vasinfectum* (Narayanah 1966.).

The aminoacids produced by the species of fungi *Aspergillus niger* and *Penicillium verrucosum* in the nutrient medium according to Czapek in the presence of 1% detergent is shown in Table 1.

4730 Both species of fungi produced 15 diverse aminoacids. For the species *A. niger* amounts were greater in the nutrient medium with the 1% detergent in relation to the control nutrient medium. The species *Penicillium verrucosum* behaved completely differently producing almost all aminoacids in considerable amounts without detergent. The production of 13 aminoacids was inhibited by 1 % detergent which slightly stimulated the production of leucine and isoleucine in relation to the control nutrient medium. This confirms earlier analyses of aminoacids (Velickovic, 1971 and Dzamic and Velickovic, 1970; Stojanovic, et al., 1994.) and also the effect of detergents and some detergent components on the bioproduction of organic materials and proteolytical activity of fungi (Stojanovic 1989., Stojanovic et al. 1994.).

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

Amino acids	Aspergillus niger		Penicillium verrucosum	
	K	D	K	D
Lysine	0.33	2.37	1.15	0.80
Histidine	0.66	0.55	0.20	0.11
Arginine	0.27	2.49	0.96	0.69
Aspartic acid	0.56	3.40	1.98	1.22
Threonine	0.34	1.89	1.01	0.75
Serine	0.42	1.86	1.14	0.82
Glutamic acid	1.22	5.06	2.41	1.82
Proline	0.34	2.00	0.90	0.60
Glycine	0.36	1.85	0.97	0.69
Alanine	0.54	2.27	1.24	1.06
Valine	0.26	1.92	0.93	0.65
Isoleucine	ND	1.39	0.96	1.17
Leucine	1.30	4.82	1.50	1.56
Tyrosine	0.10	1.44	0.61	0.33
Phenylalanine	0.22	1.77	0.90	0.52

ND - not detected

## CONCLUSIONS

The results of the investigations showed that the fungi *Aspergillus niger* and *Penicillium verrucosum* grown on nutrient medium according to Czapek medium with detergent at 1%, 0.1%, 0.01% and 0 % concentration produced 15 different aminoacids. The detergent behaved as an activator of the bioproduction of these aminoacids and proteolytical activity for *Aspergillus niger* and an inhibitor of production of 13 different aminoacid by *Penicillium verrucosum*

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#### UTICAJ DETERDŽENATA NA PRODUKCIJU AMINOKISELINA I PROTEOLITIČKU AKTIVNOST NEKIH VRSTA GLJIVA

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#### SADRŽAJ

Kod gljivica *Aspergillus niger* i *Penicillium verrucosum* deterdženti utiču na kvalitet i kvantitet bioprodukcije aminokiselina. Stimulacija bioprodukcije deterdžentima bila je veoma izražena kod svih 15 aminokiselina izolovanih iz vrste *A. niger*, a inhibirana kod 13. različitih aminokiselina izolovanih iz vrste *P. verrucosum*. Izuzetak predstavljaju aminokiseline leucin i izoleucin, čija je produkcija kod ove vrste gljivica veoma slabo stimulisana deterdžentom. Proteolitička aktivnost gljivica *A. niger* bila je potencirana prisustvom deterdženata.