

## THE INFLUENCE OF DETERGENTS ON THE BIOPRODUCTION OF ORGANIC MATTER AND THE ENZYMATIC ACTIVITY OF TWO FUNGAL SPECIES

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*The effects of a detergent product (Merix, Merima, Krusevac) on the production of aminoacids and monosaccharides and the proteolytic enzyme activity of the fungi *Alternaria tenuis* and *Trichotecium roseum* were examined. After incubation for 8 days the concentrations of all aminoacids, except for isoleucine in the case of *A. tenuis* and alanine in the case of *T. roseum*, were found to be lower in media with 1 % detergent than in the control media without added detergent. However, progressively increasing concentrations of detergent from 0.01 to 1 % did not appear to affect consistently the proteolytic activity of either fungus in comparison with controls after 4 to 8 days incubation. After 4 days incubation the production of glucose by *A. tenuis* was greater than the production of fructose and concentrations of both monosaccharides were greater than with *T. roseum* with or without detergent in the medium. It may be concluded that the metabolic activity of both fungal species is generally maintained in the presence of small quantities of the examined detergent.*

*Key words: bioproduction, aminoacid, fungi, detergent*

### INTRODUCTION

Recently, a large number of authors have shown that microorganisms, including fungi, are potential degraders of detergents. This applies particularly to surface active material of heterogenous structure with linear or branched hydrophobic unipolar chains and different polar groups (Bull, 1980). Thus, the tenside linear acryl-benzosulphate is dissolved relatively easily by the action of microorganisms from polluted water and in that way is eliminated (Žarić-Horvat and Grbić, 1977). It has been established that more than one hundred species of microorganisms, classified into sixty orders of fungi and bacteria, have the ability to oxidise detergents at one or more carbon atoms (Dzokić, 1985; Gilmore *et al.*, 1994).

Due to the ever greater concentration of detergents in the environment, there is an increasing need for purification i.e. removal of detergents from water, soil etc. Microorganisms, including fungi, play an important role in this process.

The influence of a detergent product on fungal morphological properties including colony size, number of spores and biomass have already been reported (Stojanovic, 1988, 1989). Moreover, Stojanović and coworkers (1994) found that detergent concentrations up to 1 % did not appear to affect the proteolytic activity of the fungus *Trichotecium roseum*, although concentrations of all aminoacids, except alanine, in the medium were reduced after 8 days incubation in comparison with detergent free medium. In this report we extend this study to include an examination of the effect of the same detergent on some aspects the metabolic activity of the fungus *Alternariae tenuis*. The results are compared with those obtained earlier for *T. roseum*.

#### MATERIALS AND METHODS

Monospore cultures of the fungi *Alternariae tenuis* NEES ESENBECK and *Trichotecium roseum* LINK, taken from the mycotheque of the Faculty of Natural Sciences and Mathematics, Kragujevac, were maintained in a chamber at  $4 \pm 0.5$  °C on potato-dextrose agar slant medium. Monospore cultures were obtained by culturing to exhaustion on nutrient poor agar in Petri dishes.

During the experiments the fungi were grown on the nutrient base of Czapek containing (g/L):  $\text{NaNO}_3$ -3;  $\text{K}_2\text{HPO}_4$ -1;  $\text{MgSO}_4$ -1;  $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ -0.25;  $\text{FeSO}_4 \times 7\text{H}_2\text{O}$ -0.01; sucrose -30; distilled water -1000. The sterility of the nutrient base was tested using mesopeptone agar.

Detergent was added to the medium at the concentrations of 1, 0.1, 0.01 or 0% for examination of fungal proteolytic activity, while aminoacids were determined at the detergent concentrations of 1 and 0% only. Erlenmayer flasks containing 200ml medium were uniformly and constantly shaken on an electric mixer at room temperature for 8 days with alternate light/dark cycles. Proteolytic activity, pH and rH2-redox potential were monitored after 4, 5, 6, 7 and 8 days incubation and aminoacid concentration after 8 days only.

Proteolytic activity was measured in 1 ml medium by Anson's colorimetric method (Dudka, 1982; Egorova 1976; Petrovic and Petrovic, 1971), which determines the amount of tyrosine or tryptophan produced during hydrolysis of casein in relation to a standard curve. Aminoacids were determined by standard methods (Egerova, 1976; Moore et al., 1958; Petrovic and Petrovic 1971; Stein and Moore 1954). The monosaccharides, glucose and fructose, were determined after cation-exchange chromatography on a column of AmberliteIR-120 followed by descending chromatography on Whatman No 1 paper. After reaction with suitable reagents to produce a green-blue complex, the amounts of glucose and fructose were determined spectrophotometrically using a red filter in comparison with the appropriate standard curves (Velicković, 1971).

#### RESULTS AND DISCUSSION

The effect of the examined detergent on the proteolytic activity found in the media, expressed as the concentration of tyrosine ( $\mu\text{g/ml}$ ) produced per unit time, during growth of the fungi *A. tenuis* and *T. roseum* are shown in Figures 1 and 2.

In both cases the presence of detergent in the medium did not appear to affect proteolytic activity consistently at each time interval, although high activity

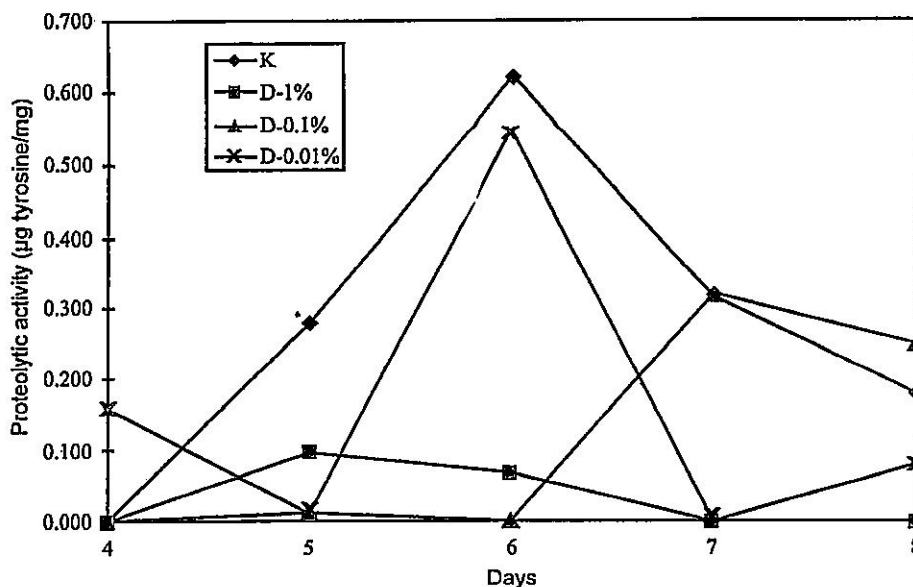


Figure 1. Proteolytic activity of *A. tenuis* in the nutrient base of Czapek, at various concentrations of detergent (control - K, D - 1%, D - 0.1%, D - 0.01%)

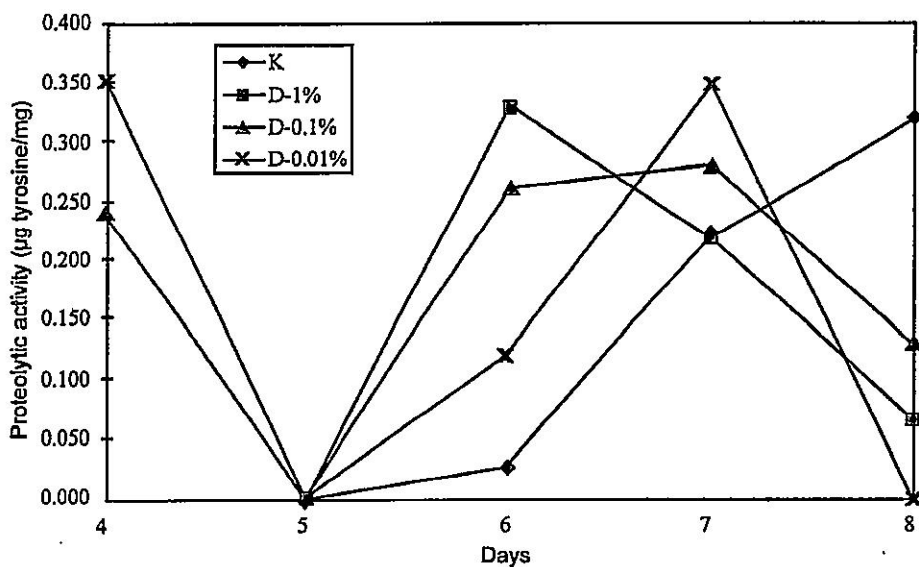


Figure 2. Proteolytic activity of *T. roseum* in the nutrient base of Czapek, at various concentrations of detergent (control - K, D - 1%, D - 0.1%, D - 0.01%)

with *A. tenuis* was not detected at any time interval in the presence of 1 % detergent. *A. tenuis* showed higher proteolytic activity in the examined medium

Table 1. Glucose and fructose concentrations (g/100 ml) in the liquid medium of Czapek after 4 and 8 days incubation of *A. tenuis* and *T. roseum*, without (K) and with different concentrations of detergent (D)

Culture	Glucose		Fructose	
	Days		Days	
	4	8	4	8
<i>A. niger</i> (K)	0.000	0.298	0.000	0.400
<i>A. niger</i> (AOC-1%)	0.000	0.285	0.000	0.200
<i>A. niger</i> (AOC-0.1%)	0.000	0.160	0.000	0.300
<i>A. niger</i> (AOC-0.01%)	0.000	1.100	0.000	0.700
<i>A. tenuis</i> (K)	0.980	0.330	0.490	0.330
<i>A. tenuis</i> (AOC-1%)	0.020	0.710	0.030	0.420
<i>A. tenuis</i> (AOC-0.1%)	1.340	0.520	0.645	0.520
<i>A. tenuis</i> (AOC-0.01%)	1.320	0.280	0.770	0.230
<i>T. roseum</i> (K)	0.000	0.050	0.000	0.025
<i>T. roseum</i> (AOC-1%)	0.680	0.085	0.070	0.060
<i>T. roseum</i> (AOC-0.1%)	0.010	0.145	0.010	0.125
<i>T. roseum</i> (AOC-0.01%)	0.310	0.625	1.090	0.660

than *T. roseum* especially at 6 days incubation in the media with no or 0.01 % detergent. After 8 days incubation of *A. tenuis*, the proteolytic activity in the media with no or 0.1 % detergent remained higher than the very low value recorded for the medium containing 1 % detergent.

The amounts of glucose and fructose found after 4 and 8 days incubation of the fungi in media with or without detergent are shown in Table 1. At 4 days the concentrations of both monosaccharides in the media with *A. tenuis* were markedly higher than with *T. roseum* without and at all concentrations of detergent examined. Moreover, the concentration of glucose at this time interval was always higher than that of fructose in *A. tenuis* culture media. The concentrations found at 8 days indicated a decline in sugar production by *A. tenuis*. There were no consistent effects of fungal species and detergent concentration on sugar levels found at 8 days.

The qualitative and quantitative amino acid content of the media found after 8 days incubation of each fungal species without or with 1 % detergent is shown in Table 2. Fifteen amino acids were detected in each case. The concentrations were higher in the control media without detergent for all the amino acids except for isoleucine produced by *A. tenuis* and alanine produced by *T. roseum* in 1 % detergent.

Table 2. The aminoacids detected and their amount (g/L) in the liquid medium of Czapek after 8 days incubation of *A. tenuis* and *T. roseum* without (K) and with 1% detergent (D)

Amino acids	<i>Alternariae tenuis</i>		<i>Trichotecium roseum</i>	
	K	D	K	D
Lysine	1.25	0.73	1.51	0.75
Histidine	0.15	0.09	0.29	0.08
Arginine	0.76	0.45	1.29	0.71
Aspartic acid	1.39	0.76	2.40	1.19
Threonine	0.87	0.41	1.20	0.73
Serine	0.98	0.56	1.28	0.88
Glutamic acid	2.31	2.04	3.38	1.59
Proline	0.80	0.33	1.09	0.57
Glycine	0.81	0.42	1.24	0.56
Alanine	1.14	0.89	1.61	1.64
Valine	0.69	0.39	1.14	0.66
Isoleucine	0.67	1.30	2.85	0.45
Leucine	1.05	0.81	2.76	1.08
Tyrosine	0.39	0.15	0.65	0.33
Phenylalanine	0.60	0.38	0.97	0.67

In an earlier study Stojanović and coworkers (1994) showed that the same detergent appeared to stimulate proteolytic activity and aminoacid production by *Aspergillus niger*. Thus, a particular product may have different effects on the metabolic activity of different species of fungi, varying from predominantly inhibitory as in the cases examined here (*A. tenuis* and *T. roseum*) to stimulatory, as found previously for *A. niger*. The enzymatic activity of *Rhizopus chinensis* was also found to be affected by toxic matter (Fukamoto *et al.*, 1967), while fungal vitality can be affected by sodium tripolyphosphate (Stojanović, 1987). Various other factors have also been found to influence the composition of aminoacids produced by microorganisms (Džamić and Veličković, 1970).

However, it may be concluded that the metabolic activity of both fungal species examined here is generally maintained in the presence of small quantities of the examined detergent.

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UTICAJ DETERDŽENATA NA BIOPRODUKCIJU ORGANSKIH MATERIJA I ENZIMSKU  
AKTIVNOST NEKIH VRSTA GLJIVA

STOJANOVIĆ JELICA, VELIČKOVIĆ D I VUČETIĆ J

SADRŽAJ

Rezultati ispitivanja izneti u ovom radu ukazuju na inhibitorni ili izrazito inhibitorni uticaj deterdženta (MERIX, "Merima", Kruševac) na produkciju 14 različitih aminokiselina u ispitivanim kulturama gljiva *Alternaria tenuis* i *Trichothecium roseum*. Bioprodukcija većine analiziranih aminokiselina je inhibirana deterdžentom u koncentraciji od 1% sa izuzetkom alanina i izoleucina čija je produkcija stimulirana. Proteolitička aktivnost ispitivanih vrsta gljiva je različita i zavisi od koncentracije deterdženta kao i karakteristikama samih gljiva. Produkcija ugljenih hidrata (glikoze i fruktoze) je izraženija kod vrste *A. tenuis* u odnosu na *T. roseum*.