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ANALYSIS OF THE AMINO ACIDS OF SOME TYPES OF FUNGI CULTIVATED IN THE PRESENCE OF DETERGENT

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The paper presents the results of the influence of anionic-type detergent containing sodium tripolyphosphate and ethoxylated oleyl-cetyl alcohol on the type and quantity of present amino acids in the fermentation broths of the fungi Aspergillus niger, Alternaria tenuis and Fusarium oxysporum cultivated on a medium with the addition of 1% detergent as a function of their application in the process of detergent biodegradation.

In the case of A.niger the stimulation of the biosynthesis of 15 various amino acids was determined. In the case of A.tenuis the production of 14 amino acids was inhibited as compared to the total number of 15 amino acids identified when the fungi were cultivated in the absence of detergent, whereby the explicit inhibition of the synthesis of methionine, isoleucine and leucine was registered.

Detergent (1% concentration) exhibits a stimulating effect on the bioproduction of amino acids in the case of the fungus F.oxysporum when the presence of 14 amino acids was identified.

Key words: amino acids, detergent, ethoxylated oleyl-cetyl alcohol, fungi, phosphates

INTRODUCTION

The increase in the production of synthetic washing and cleaning agents, which originates from the ever-increasing demands and needs of consumers, opens numerous issues regarding their damaging influence on the environment and wildlife (McAvoy *et al.,* 1993; Deshpande *et al.*, 1998).

The chemical composition of detergents depends on their washing capability, as well as degradation in aqueous ecosystems. Omnipresent components of detergents are phosphates in amounts of 45% and surfactants of aliphatic or aromatic structure such as ethoxylated oleyl-cetyl alcohol or alkyl-benzene sulphonates (HERRA, 2002).

By using various test methods for biodegradation on a large number of samples in the USA and Europe, data were obtained that confirmed the high percentage of biodegradation of linear alkyl-benzene sulphates (LAS), as the most abundant active component in detergents (Marcomini *et al.,* 2000a; Rapaport *et al.,* 1992; Moreno *et al.,* 1990). LAS are susceptible to rapid and complete biodegradation in wastewater treatment plants (Sanchez Leal *et al.,* 1994; Kimerle, 1989; Swisher, 1987).

They are also efficiently decomposed on active sludge (about 99%), while filter systems are less efficient (about 77%). In natural waters their biodegradation is extensive (the half-life of LAS is about 0.15 to 0.5 days) (Britton, 1993).

The subject of our investigation was a detergent with an active component of the ethoxylated oleyl-cetyl alcohol type, while previous investigations (Stojanovic *et al.,* 2004; Stojanovic, 1988), confirmed the influence of detergent on the total metabolism of fungi (Jon *et al.,* 2004; Alvarez-Velazquez *et al.,* 2000), their bioproduction, protease activity and amino acid biosynthesis. The investigations should clarify to what extent microorganisms, especially fungi, could be applied to purify wastewaters in natural ecosystems.

MATERIALS AND METHODS

The experiments were performed using fungi of the species *Aspergillus niger* van TIEGHEM, *Alternaria tenuis* NEES ESENBESK*, Fusarium oxysporum* (SCHLECHT) SN. et H., isolated from the Lepenica River basin (location of waste water influx). The fungi determination was performed at the Department of Algae, Fungi and Lichen, Faculty of Biology in Belgrade.

Monosporial fungi cultures were obtained by the exhaustion method on poor agar in Petri dishes. The fungi were cultivated under sterile conditions in a liquid nutritional medium according to Czapek (g/L): $NaNO_3$-3, K_2HPO_4-1, $MgSO_4$-1, $MgSO_4x7H_2O$-0.25, $FeSO_4x7H_2O$-0.01, saccharose-30, distilled water-1000 mL (control medium mark-K) and a variant of the liquid nutritional medium with a 1% concentration of detergent of the following composition: $NaNO_3$-3, K_2HPO_4-1, $MgSO_4$-1, $MgSO_4x7H_2O$-0.25, $FeSO_4x7H_2O$-0.01, saccharose-30, distilled water- 1000 mL and a 1% concentration of detergent (mark-D) for 8 days.

The sterility of the nutritional media was controlled by a mesopeptonic agar (MPA). The volume of the liquid nutritional medium was 200 mL in 250 mL Erlenmeyer bottles. The Erlenmeyer bottles were placed on an electric shaker, enabling constant uniform shaking (fungi aeration). The experiments were performed at room temperature under alternating day-night light conditions.

The fungi *A.niger*, *A.tenuis* and *F.oxysporum* were cultivated in a liquid nutritional medium according to Czapek (control mark-K) and a variant of the liquid nutritional medium according to Czapek with 1% detergent concentration (mark-D). The qualitative and quantitative composition of the amino acids was determined on the eighth day according to the method of Spakman *et al*. (1958) using a BECKMAN model 120 C amino acid analyzer. Measurement conditions: stationary phase: LiChroCART 250-4, mobile phase 0.10 M acetate buffer (pH 4.4)

– acetonitrile in the ratio 70:30, temperature 24°C, mobile phase flow rate 1.0 mL/min, fluorescence detector (Ex 263 nm, Em 313 nm).

RESULTS AND DISCUSSION

In the case of the fungi *A.niger, A.tenuis* and *F.oxysporum* cultivated in a liquid nutritional medium according to Czapek and a variant of the liquid nutritional medium according to Czapek with 1% detergent, the qualitative and quantitative composition of the extracellular amino acids (Dudka, 1982, Egorova, 1976, Petrović and Petrović, 1971, Stein and Moore, 1954) was determined on the eighth day by chromatography using an aminoanalyzer.

The results of the investigation are presented in Figure 1.

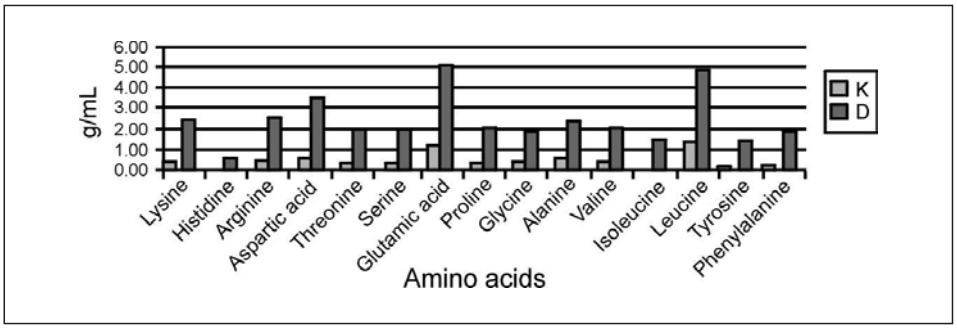

Figure 1. *Aspergillus niger*. Qualitative and quantitative composition of amino acids on 8th day

In the case of *F.oxysporum* 16 amino acids were identified in the standard nutritional medium. The presence of 1% detergent completely inhibited the production of methionine in the case of this fungus. Opposed to these results, in cultures of *A.niger* and *A.tenuis*, methionine was not identified in the standard medium or in the presence of 1% detergent. The synthesis of isoleucine was registered in the presence of detergent in the case of the fungus *A. niger.*

The amounts of the 16 identified amino acids in the case of the fungi *A.niger*, *A.tenuis* and *F.oxysporum* in a standard nutritional medium change in the presence of 1% detergent (Stojanović, 1987; Stojanovic *et al.,* 2004; Stojanović, 1988).

In the case of *A.niger* the applied detergent explicitly stimulated the production of all 15 detected amino acids. The production of most of the analyzed amino acids (lysine, histidine, aginine, asparginic acid, treonine, serine, glutaminic acid, proline, glycine, alanine, valine, leucine, tyrosine and phenylalanine) was stimulated by 4 to 10 times in the presence of 1% detergent.

In the case of the fungus *F.oxysporum*, the presence of 1% detergent stimulated the production of 12 of the 16 identified amino acids, while inhibitory

action was registered in the production of the following amino acids: histidine, methionione, isoleucine and leucine.

The fungus *A.tenuis* is unique compared to *A.niger* and *F.oxysporum.* The detergent (1%) explicitly inhibited the production of 14 of the 15 identified amino acids. Only the production of leucine was stimulated by the detergent. The quantity of this amino acid approximately doubled (0.67 control, 1.30 medium with detergent).

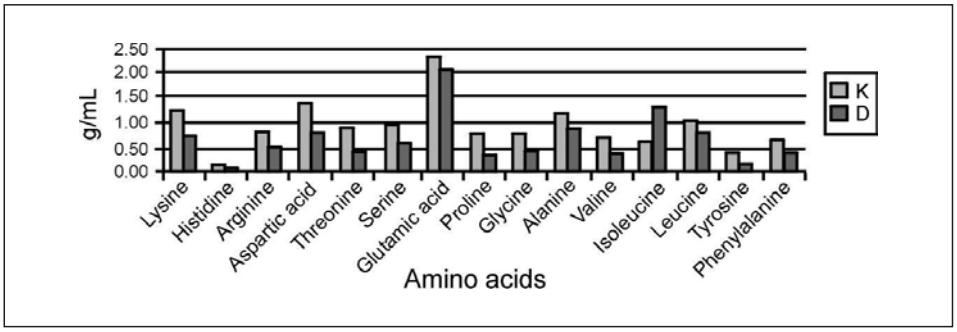

Figure 2. *Alternaria tenius*. Qualitative and quantitative composition of amino acids on 8th day

Analysis of the amounts of the 16 analysed amino acids in cases of the fungi *A.niger, A.tenuis* and *F.oxysporum* indicated considerable differences in the composition of the control nutritional medium and in the nutritional medium with detergent, which indicated relevant differences in the biological characteristics of these fungi, which are reflected in their adaptation and the influence of the detergent as a pollutant in the nutritional medium.

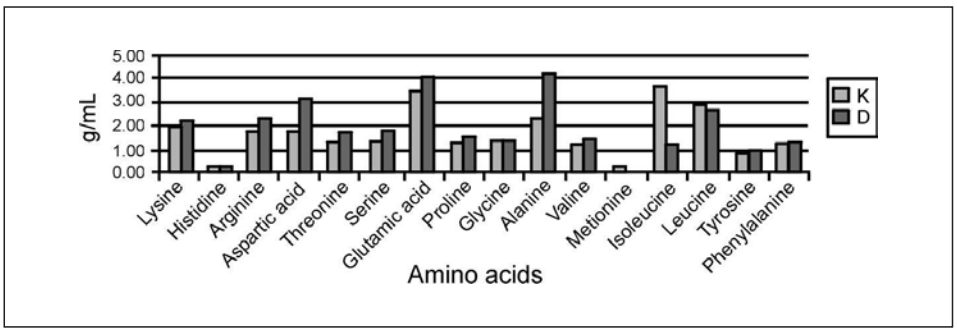

Figure 3. *Fusarium oxysporum*. Qualitative and quantitative composition of amino acids on 8th day

The increased production of some of the identified amino acids in, for example, the case of *A.niger* in the presence of detergent is further evidence of a significant organism that may be utilized for the biodegradation of detergent and detergent components in wastewaters present in natural waterways (Stojanović, 1990; Stojanovic *et al.,* 1994).

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ANALIZA AMINOKISELINA NEKIH VRSTA GLJIVA GAJENIH U PRISUSTVU DETERDŽENTA

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SADRŽAJ

Cilj istraživanja je bio da se ispita uticaj deterdženta na rezistentne mikroorganizme. Među velikim brojem mikroorganizama izdvojene su gljive koje su izolovane iz otpadnih voda. U isto vreme eksperiment je bio postavljen sa ciljem da se ispitaju razlike u bioprodukciji različitih vrsta aminokiselina, kao sto su razlike u kvantitetu identifikovanih aminokiselina ispitivanih vrsta gljiva *Aspergillus niger*, *Alternaria tenuis* i *Fusarium oxysporum* gajenih u prisustvu deterdženta. Rezultati istraživanja ukazuju da je gljiva *A.niger* najotpornija na dejstvo deterdženta.

Gljiva *Aspergillus niger* je u podlozi sa deterdžentom produkovala veliki broj različitih aminokiselina (ukupno 15) i sve one su bile kvantitativno zastupljenije 2 do približno 10 puta u odnosu na kontrolu.