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THE EFFECTS OF DETERGENT, SODIUM TRIPOLY-PHOSPHATE AND ETHOXYLED OLEYL-CETYL ALCOHOL ON METABOLIC PARAMETERS OF THE FUNGUS *TRICHOTHECIUM ROSEUM* LINK

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Abstract - The degradation of detergents that are dispersed in water and soil partially depends on the metabolic activities of fungi. Among the fungi that have this ability, *Deuteromycetes* are particularly noted for their biochemical characteristics. Taking this into account, it was of interest to analyze the influence of detergent and its main compounds, ethoxyled oleyl-cetyl alcohol (AOC) and sodium tripoly-phosphate (TTP), on the metabolism of the fungus *Trichothecium roseum*. Our results revealed that both detergent and AOC had an inhibitory effect on the bioproduction of free organic acids, while TTP stimulated their production. Also, detergent inhibited the bioproduction of basic amino acids, with the exception of alanine. In addition, detergent applied at 1% concentration inhibited the bioproduction of proteins and the total biomass of the fungus, while AOC and TTP inhibited the production of proteins, but stimulatedl the production of *Trichothecium*.

Key words: Trichothecium roseum, detergents, bioproduction, free organic acids, amino acids, proteins, biomass

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INTRODUCTION

Fungi are recognized for their ability to produce a large variety of extracellular proteins, organic acids and other metabolites, and for their adaptability to severe environmental constraints (Cochrane, 1958). Beyond the production of relevant metabolites, fungi have been attracting a growing interest for the biodegradation of organic compounds which come to the living environment by the activity of man (Coulibaly and Aghatos, 2003).

Among the fungi that have this ability, the filamentous fungi from the group *Deuteromycetes* or *Imperfect fungi* are especially notable for their physiological, morphological and biochemical characteristics (Raimbault, 1981). The apical growth of the hypha enables penetration of the fungus into substrates and the secretion of extracellular enzymes from the vesiculae on the tip of the hypha into the environment. Under the effects of these enzymes, complex organic compounds are decomposed into simpler ones which can be used by the fungus for its growth and the development of the mycelium, and thus they are built into a protein rich biomass (Raimbault, 1998; Saucedo-Castaneda et al., 1992a; Saucedo-Castaneda et al., 1992b).

Trichothecium roseum link is a fungus that grows rapidly and produces a pinkish colony within ten days when incubated at 25°C. It has worldwide distribution but is commonly isolated from air, decay-

ing plant material, compost, sewage sludge, water and rarely from soil. *Trichothecium roseum* link produces simultaneously trichothecin and proteolytic enzymes possessing fibrinolytic, thrombolytic and esterase activity. In addition to the function of splitting and consuming the substrate, the proteolytic enzymes of *Trichothecium roseum* may be considered as a protective mechanism for the detoxication of metabolites toxic to the organism.

A genuinely new dimension to microbiology concerns the fate of synthetic compounds dispersed throughout the biosphere whose degradation depends on the use of the metabolic activity of microorganisms. Among various pollutants found in water and soil, detergents play an important role in regard to the extent of their production and usage. They can be divided into two groups: phosphate detergents and detergents with active surface agents. Phosphate detergents are highly caustic since they modify pH media, induce eutrophication and toxin release. On the other hand, if present in significant quantities, detergents with active surface agents may have toxic effects on all aquatic species (Moreno et al., 1990). Linear alkyl benzene sulfonate (LAS) is used worldwide as a surfactant in detergent production and it is present in domestic and industrial wastewaters where it may result in the inhibition of microorganisms that degrade organic matter. Degradation of LAS is significant in natural waters and very efficient in activated sludge (around 99%) (McAvoy et al., 1993, Rapaport et al., 1992, Britton, 1993). A great number of different fungi species that are capable of degrading detergents have been identified in aerobic and anaerobic conditions (Sanz et al., 2003).

The results from our previous studies concerning the degradation of MERIX detergent ("Merima", Kruševac, Srbija) and its main components (ethoxyled oleyl-cetyl alcohol and sodium tripoly-phosphate) by different water microorganisms (Stojanović et al., 1994; Stojanović et al., 2010) prompted us to investigate whether this particular detergent affects the metabolism of the fungus *Trichothecium roseum* LINK by affecting its bioproduction, enzyme activity and biosynthesis of amino acids.

MATERIALS AND METHODS

Isolation of Trichothecium roseum LINK and treatment with detergents

A monosporic culture of the fungi species *Trichothecium roseum* LINK was isolated from the river basin of Lepenica at the place of wastewater flood (sewage). A quantity of the particular fungi species, in a precisely determined volume of the water sample, was taken into consideration when deciding which species of fungi to use.

The isolated fungi were maintained in a chamber at a constant temperature of 4°C ± 0.5°C, on a potato-dextrose agar slant under sterile conditions. A monospore culture was developed by the method of exhaustion on a poor agar in Petri dishes under sterile conditions. Mesopeptonic agar was used for a sterility control.

During the experiment, the fungi were grown on a sterile growth base of the following composition: $NaNO_3$-3 g, K_2HPO_4-1 g, $MgSO_4$ x7H_2O-0.25 g, $FeSO_4$x7H_2O-0.01 g, sucrose-30 g, distilled water up to 1000 ml, and on variation of the liquid growth base, according to Czapek, with MERIX detergent ("Merima", Kruševac), sodium tripoly-phosphate (TTP) and ethoxyled oleyl-cetyl alcohol (AOC) in concentrations of 1%.

The variants of the liquid growth base were stored in Erlenmeyer bottles (200 mL of base in 250 mL bottles). The Erlenmeyer bottles were placed on an electric shaker, thus enabling uniform and constant mixing (aeration of the fungi). All the experiments were carried out at room temperature under alternating light and dark cycles.

Analysis of the metabolism of the fungus Trichothecium roseum link

The analysis of the metabolism of *Trichothecium roseum* which was grown on a liquid growth base with or without the detergent, TTP and AOC, included an analysis of the amount of free organic acids on

the 4th and 8th days, the amount of 15 different basic amino acids, the amount of proteins on the 4th, 5th, 6th, 7th and 8th days, and the quantity of the total biomass on the 8th day.

The amount of free organic acids was determined by proportion and by acidimetric titration with 0,1 mol/dm^3 NaOH with an added phenolphthalein indicator (Stojanović et al., 2010).

The qualitative and quantitative determination of amino acids produced by the *Trichothecium roseum* was performed on the 8th day from the day of inoculation by the method of Sparkman et al. (1958) using an amino analyzer (Beckman Model 120C). The conditions of the measurement were as follows - stationary phase: LiChro CART 250-4; mobile phase: 0,10 mol/dm^3 acetate buffer (pH 4,4), acetonitrile in a proportion 70:30, temperature 24°C; speed of the mobile phase 1,0 mL/min; fluorescent detector: Ex 263 nm, Em 313 nm.

The amount of the protein from the fungi species was calculated according to the amount of nitrogen present in fungal tissue and determined by the method of Kjeldahl (Stojanović et al., 2010) according to following formula:
amount of proteins = 6.25 x amount of nitrogen (in mg)

The quantity of the biomass of the fungi *Trichothecium roseum* was determined on the basis of the mass difference between dry filter paper and the total mass with fungus mycelium. The quantity of the biomass was expressed in grams (g).

RESULTS

The effects of detergent and its main components at a concentration of 1% on the metabolism of the fungus *Trichothecium roseum* are presented in Figs. 1 to 4. These effects were assessed by the following biochemical parameters: the amount of free organic acids, qualitative and quantitative composition of amino acids, the total amount of proteins and total biomass.

As shown in Fig. 1, the amount of free amino acids on the 4th day of the experiment was not significantly elevated in the growth bases with added detergent (0.17%) and AOC (0.6%), but was considerably higher with TTP (6.83%) with respect to the control. On the 8th day from the day of inoculation, production of free organic acids was 7.16% higher with TTP than the production of free organic acids with AOC.

The fungus *Trichothecium roseum* grown in a liquid growth base with and without detergent produced 15 structurally different basic amino acids on the 8th day after inoculation (Fig. 2). The production of 14 amino acids was significantly (*lysine, histidine, arginine, glutamic acid, proline, glycine, isoleucine, leucine, tyrosine*) or partly (*threonine, serine, valine and phenylalanine*) higher in the control than in the detergent-treated sample. The production of the amino acid *alanine* was not decreased by the presence of 1% of detergent in the growth.

As shown in Fig. 3, the bioproduction of proteins in all types of growth media had the tendency of growth from 4th to 8th day, but a significantly larger amount of protein was produced in the control medium than in the media containing detergent or its components. On the 8th day, the amount of protein was significantly decreased in the control growth medium and partly decreased in the growth medium with AOC and TTP. In the medium with detergent, the bioproduction of proteins was increased.

The biomass of the fungus grown in the AOC or TTP media was higher compared with the control medium, as is shown in Fig. 4. Conversely, the biomass of the fungus grown in the medium with detergent was considerably lower than in the control medium (Fig. 4).

DISCUSSION

The degradation of synthetic compounds dispersed throughout the biosphere and found in water and soil depends on the metabolic activity of microorganisms, especially fungi (Ojo and Oso, 2009).

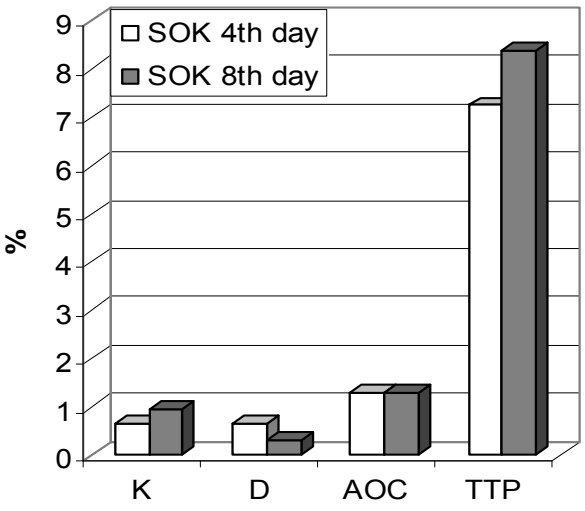

Fig. 1. The quantity of free organic acids (SOK) (in percentage) produced by *Trichothecium roseum* grown on the growth base according to Czapek (control-K) and the variants of the growth medium with detergent (D), ethoxyled oleyl-cetyl alcohol (AOC) and sodium tripoly-phosphate (TTP) in concentration of 1% on the 4th and the 8th days.

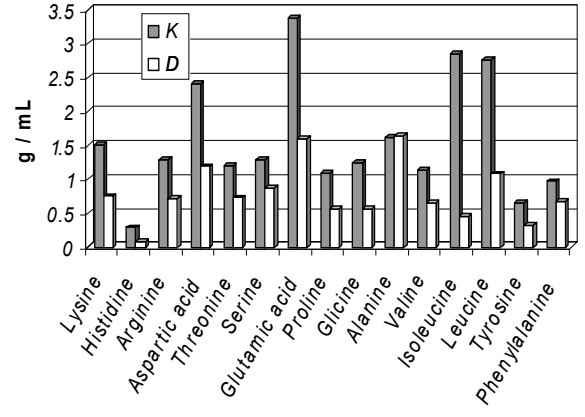

Fig. 2. The qualitative and quantitative composition of amino acids (g/mL) of *Trichothecium roseum* grown on the growth medium according to Czapek (control-K) and on the growth base with 1% detergent (D) on the 8th day.

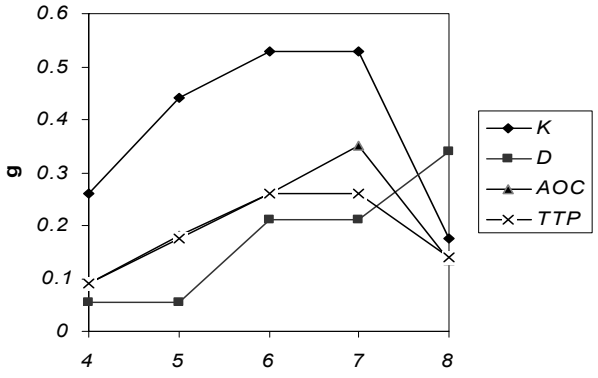

Fig. 3. The quantity of proteins (in grams) produced by *Trichothecium roseum* grown on the control growth medium (K) and the growth medium with detergent (D), 1% sodium tripoly-phosphate (TTP) and 1% ethoxyled oleyl-cetyl alcohol (AOC) from the 4th to the 8th day.

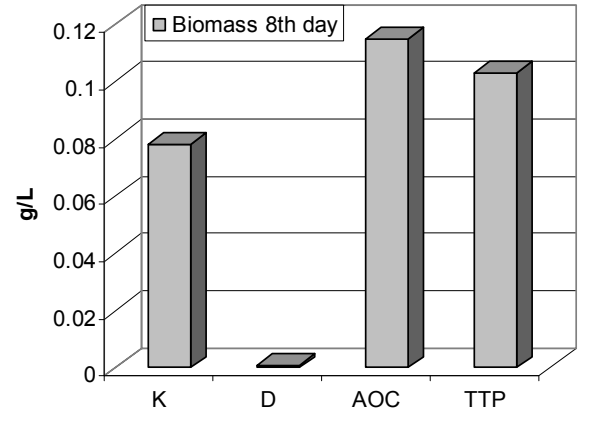

Fig. 4. The quantity of biomass (g/L) of *Trichothecium roseum* grown on the control growth medium (K) and the growth medium with detergent (D), 1% ethoxyled oleyl-cetyl alcohol (AOC) and 1% sodium tripoly phosphate (TTP) on the 8th day.

Among the various pollutants, detergents play an important role in regard to the extent of their production and usage. Linear alkyl benzene sulfonate (LAS) is a surfactant used in detergent production present in wastewaters where it may result in the inhibition of microorganisms that degrade organic matter (Kimerle, 1989). Degradation of LAS is very efficient in activated sludge and the different fungi species degrading it were identified in aerobic and anaerobic conditions (Sanz et al., 2003). Taking this into consideration, it was of interest to analyze whether the LAS type detergent, or two of its main compounds, affects the metabolism of the fungus *Trichothecium roseum* link at the level of its bioproduction, enzyme activity and biosynthesis of amino acids. In order to achieve this, the fungi were grown

in a liquid nutrition base, according to Czapek, and also on variants of nutrition base with detergent, ethoxyled oleyl-cetyl alcohol (AOC) and sodium tripoly-phosphate (TTP).

The treatment with detergent, AOC and TTP led to the production of different amounts of free organic acids on the 4th and 8th days from the day of inoculation of the fungus. With aging of the fungus, the intensity of production of the free organic acids was considerably reduced in the detergent base and significantly higher in the base with AOC of the same concentration. Interestingly, on the 8th day from the day of inoculation the detergent was still behaving as an inhibitor of the bioproduction of organic acids, while AOC, and especially TTP, even stimulated the production of amino acids. The observed effects of AOC and TTP could possibly represent a protective mechanism for the detoxication of the metabolites toxic to the organism. Taken together, the maximum production of free organic acids during the whole experimental period was found in the base with TTP on the 8th day and minimum production was also detected on the 8th day, albeit in the growth medium with detergent.

Furthermore, the fungus *Trichothecium roseum* produced 15 different basic amino acids on the 8th day from the day of inoculation. The production of 14 amino acids was higher in the control nutritious-growth medium then in the medium with the detergent, with the exception of the amino acid *alanine* whose production was not changed by the presence of detergent in the growth medium. This result was not unexpected since our previous studies, on other fungi species, showed a similar effect of inhibition of amino acid production by the detergent which is a known pollutant (Džamić and Veličković, 1970; Veličković, 1971; Stojanović et al., 1994) .

The presence of detergent, AOC and TTP in the liquid growth medium affected the production of proteins and variable amounts of proteins were found on 4th, 5th, 6th, 7th and 8th days from day of inoculation. The obtained results revealed that with the aging of the mentioned fungus, bioproduction of proteins had the tendency of significantly improved growth in the control medium than in the medium with added detergent or its main components. These findings were in accordance with the results of other authors and our previous studies (Raimbault, 1981; Stojanović, 1994), confirming the inhibitory effect of pollution by LAS type detergents. On the 6th and 7th days of the experiment, the stagnation in bioproduction of the proteins was observed for the fungus grown in the control and TTP media, while growth of the fungus continued in the medium with AOC. On the 8th day, the amount of proteins was decreased in the control medium, as well as in the AOC and TTP smedia. The exception was the bioproduction of proteins in the medium with detergent, which was rapidly increased.

The total biomass which the fungus *Trichothecium roseum* produced during the growth period was measured on 8th day. The total amount of biomass of the fungus grown in bases with AOC and TTP was higher compared to the control, which means that these compounds acted as stimulators of bioproduction. On the contrary, the detergent behaved like an inhibitor, judging by the measured value of the total biomass amount grown in growth medium with added pollutant. The results revealed that highest biomass production was obtained in the medium with 1% AOC and the lowest in the medium with 1% of added detergent.

In summary, based on the obtained results it can be concluded that detergent and its active component ethoxyled oleyl-cetyl alcohol had an inhibitory effect on the bioproduction of free organic acids, while sodium tripoly-phosphates stimulated their production. The detergent had an inhibitory effect on the bioproduction of basic amino acids, except on the amino acid alanine. In addition, detergent applied in 1% concentration also had an inhibitory effect on the bioproduction of proteins and on the total production of biomass, while its components inhibited the production of proteins but stimulatedively the production of *Trichothecium roseum* biomass.

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