UDC: 631.453:631.465
original scientific paper

Acta Agriculturaae Serbica, Vol. VI, 12 (2001) 37-42

Acta Agriculturae Serbica FACULTY OF AGRONOMY ČAČAK

Effects of Copper on the Growth and Glucoamylase Activity of *Aspergillus Niger D-IV-8*

K.Tsekova and D. Dentchev

Department of Microbial Ecology,
Bulgarian Academy of Sciences, Sofia, Bulgaria

Abstract: The effects of copper ions on the growth and glucoamylase activity of *Aspergillus niger D-IV-8* were investigated. The enzyme activity was more strongly inhibited than the growth process. The high copper ion concentrations provoking inhibition show a high resistance of this fungus towards these ions. The morphological and physiological changes provoked in the presence of heavy metal reveal the possibility for application of this microorganisms as an indication for copper pollution.

Key words: coper effect, growth, glucoamylase activity, *Aspergillus niger.*

Introduction

Heavy metals are one of the major pollution sources in soil environment and a number of soil characteristics, including biological properties, are influenced by heavy metals. The strong inhibition of the activities of a variety of enzymes have been reported in metal polluted soil over the past years (Huang and Shindo, 2000, Geiger et el., 1998, Marzadori et al.,1996. Some investigators have emplyed the LD_{50} (i.e. the heavy metal concntration at which enzyme activity is half of the uninhibited level) to describe metal toxicity to enzymes. Deng and Tabalabai (1995) recorded higher inhibition of 25 trace elements on cellulase activity in field-moist than in air-dried soils. In view of the sensitivity of soil enzymes to heavy metals the use of enzyme activity as a bioindicator to evaluate the degree of soil contamination by heavy metals was proposed (Nannipieri,1995).

Glucoamylase is one of numerous amylases functioning in soil and is largely responsible for the hydrolysis of starch-containing substances in soils to glucoes. Copper is a nutritional element for plants and it plays an essential role in fungal metabolism, but its overabundance may lead to excessive cellular accumulation and consequent toxicity (Morby and Gadd, 1995).

Material and Methods

Microorganisms. The fungal strain *Aspergillus niger D-IV-8*, isolated from the soil and selected at the Institute of Microbiology, was used as a model microorganism. The strain was maintained on the potato-glucose agar slants.

Media and Cultivation Petri-dishes with Czapek-Dox agar ssupplemented with 3% (w/v) soluble as a carbon source were used for the isolation of colonies.

The basal fermentation medium for shaking flasks contained ($g \cdot l^{-1}$), maltodextrine 45 and corn steep liqour 40. The pH of the medium was adjusted to 4.8-5.0 with sodium hydroxide.

Spore suspension ($1.10^6 \cdot ml^{-1}$) was cultivated on agar plates and in 500 ml Erenmeyer flasks containing 100 ml of basal medium at 30°C for 72 hours.

The copper ions as a CuSO45H2O were added to the media in various concentrations indicated in the illustrations in the text.

Analytical methods. The cell growth on the solid medium was determined by the number of the emerged single colonies and expressed as percentage compared to the control (without heavy metal). The cell growth in submerged cultures was measured by drying of washed mycelia at 105°C to constant mass.

The glucoamylase [EC: 3.2.1.3.] was measured by enzymatic method of Muller (1971) and expressed in μmoles of glucose released per minute by hydrolysis of 1% (w/v) starch solution at 30°C and pH 4.7.

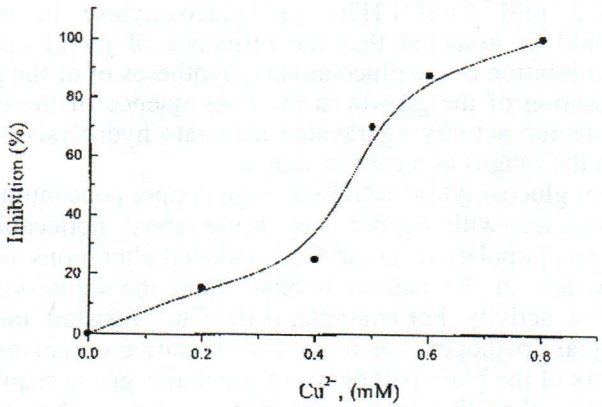
Results and Discussion

Toxic effect of copper ions on growth of Aspergillus niger soild-cultures.

Copper ions were highly toxic to the growth of *Aspergillus niger* on the agar plates and growth was progressively inhibited as the metal concentrations increased (Fig.1.) The half-maximal growth inhibition was observed at 0.45 mM copper ions in the medium. No change in the pigmentation of the mycelium occurred in the presence of copper but the concentration 0.8 mM was lethal for the culture.

Based on the macroscopic observations, a general correlation could be established.: an increase in the copper ions concentrations caused a visible decrease in the site of colonies, the air-mycelium became more loose, the sporulation was delayed and weaker, whereas connidia became shorter.

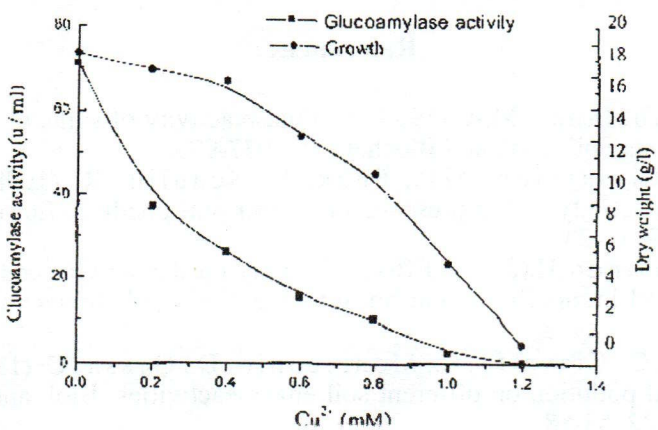
Fig.1. Inhibition of growth by copper in solid-cultures of *Aspergillus niger.*
Inhibition was represented as a percentage of growth after 72 h in copper-free medium. The values serve as the means for three experiments.



Effect of copper ions on the growth and glucoamylase activity in liquid cultures of Aspergillus niger.

Experimental results are shown in Fig.2. The biomass quantity was strongly decreased at copper concentrations higher than 1 mM. The survival ability of the strain dropped to 6-7% at the concentration of 1.2 mM. The concentrations higher than 1.2 mM were noticed to completely break off the development of *Aspergillus niger* spores in the medium.

Fig.2. Growth and glucoamylase activity in liquid cultures of *Aspergillus niger* at different copper concentrations after 72 hours of cultivation. The values concerned serve as the means of the three experiments.



The results shown Fig.2 also indicated that copper ions were an inhibitor og glucoamylase activity of *Aspergillus niger*. The enzyme levels were strongly decreased even at 0.2 mM copper ions in the medium an dropped to about 50%. The concentrations higher than 0.8 mM provoked a complete loss of the enzyme activityand at 1.2 mM CuSO4·H2O the glucoamylase in not presented practically. It could be assumed that the influence of metal ions is directly connected to the inhibition of the glucoamylase syntheses of or the glucoamylase activity. The inhibition of the growth is the consequence of these effects. The absence of the enzyme activity aggravated substrate hydrolysis, as well as its assimilation from the fungus as a carbon source.

The loss of glucoamylase activity at high copper concentrations suggests that soil contamination with copper may bring about noticeable qualitative alterations in the soil population. These Cu^{2+} induced alterations are likely to be of greater importance in the natural habitats than the inhibition of enzyme synthesis or enzyme activity. For example, if the Cu^{2+} resistant members of the population were plant pathogens, or if the Cu^{2+} sensitive organisms were either normal competitors of the plant pathogens or the major group required for some degrading function other than starch hydrolysis, major changes in disease incidence or substrate degradation would occur. This aspect of Cu^{2+} pollution will be examined in the future.

Conclusion

The results on the influence of copper ions on the growth and the glycoamylase activity of *Aspergillus niger* D-IV-8 suggest the following:

-the growth process is less inhibited than the product formation process. The high copper ion concentrations provoking inhibition also show high resistance of *Aspergillus niger* towards these ions. The morphological and physiological changes of the strain tested provoked in the presence of copper ions reveal the possibility for application of this microorganism as an indicator of copper pollution.

References

Deng, S., Tabalabai, M.A. (1995): Cellulase activity of soils: effect of trace elements Soil. Biol. and Biochem. 27, 977-979.

Geiger,G., Livingston, M.P., Funk, F., Schulin, R. (1998):β-Glucosidase activity in the presence of copper and goethite. Europ.J. of Soil Sci. 49, 17-23.

Huang,Q., Shindo,H.(2000): Effect of copper on the activity and kinetics of free and immobilized acid phosphatase. Soil Biol. Biochem. 32, 1885-1892.

Marzadori, C., Ciavatta, C., Montecohio, D., Gessa, C. (1996): Effect of lead pollution on different soil enzymeactivities. Biol. and Fertil. Of Soils. 22, 53-58.

Morley, G.F. and Gadd, G.M. (1995): Sorption of toxic metals by fubgi and clay minerals. Mycol. Res. 99, 1429-1438.

Muller, H., (1971): Enzymatsche Bestimmung von D-Glucose mit Glucose-oxydase. Ersatz des o-Dianisidin durch Kaliumhexacyanoferrat (II). Mechanisierunf der Methode. Starke. 23, 314-319.

Nannipieri, P. (1995): The potential use of soil enzymes as indicators of productivity, sustainability and pollution. In: Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R., Grace, East Melbourne, Victoria, Australia, pp. 238-244.

EFEKTI BAKRA NA RAZVOJ I AKTIVNOST GLIKOAMILAZE *Aspergillus niger* D-IV-8

-originalni naučni rad-

K. Tsekova i D. Dentchev

Mikrobiološki Institut, Bugarska Akademija Nauka, Sofija

Rezime

Ispitivani su uticaji jona bakra na razvoj i aktivnost glikoamilaze *Aspergillus niger* D-IV-8. Enzimska aktivnost je jače inhibirana nego sam proces razvoja ove vrste plesni, što se odnosi i na visoke koncentracije jona bakra. Morfološke i fiziološke promene izazvane u prisustvu ovog teškog metala ukazuju na mogućnost primene ovih mikroorganizama, kao indikacija zagađenosti zemljišta bakrom.