

Copper Uptake by *Penicillium brevicompactum*

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The copper binding properties of *Penicillium brevicompactum* biomass were influenced by growth phase of mycelium and concentration of copper in reaction mixtures. The efficiency of copper uptake increased with growth time and was largest at the mid-logarithmic growth phase. The time course of copper uptake was biphasic. Double reciprocal plots of absorption velocity of copper vs. copper concentration gave straight lines at concentration between 0.5 to 4 mM. The apparent affinity of copper to the biomass of the stationary growth phase was the same as that of logarithmic growth phase and K_m values were about 1.4 mM. Pretreatment of the mycelium with glucose increased the amount of metal uptake about five times in comparison with the controls.

Introduction

The use of microorganisms in treating waste water containing toxic heavy metals is becoming an attractive technique (Kreamer and Meisch, 1999; Van Causyen *et al.*, 1999; Koronelli *et al.*, 1999). There are many reports concerning the bio-sorption of heavy metals by the passive metal uptake of different forms dead biomass (Kappor and Viraraghavan, 1997; Sag *et al.*, 1998; Sad *et al.*, 2000). However, there are few reports concerning the incorporation of heavy metals in living cells of filamentous fungi (Sanna *et al.*, 1997; Mogollon *et al.*, 1998).

This paper describes the relationships between copper uptake and growth phase of *Penicillium brevicompactum*.

Materials and Methods

Microorganisms

The fungus used in this study, *Penicillium brevicompactum*, is deposited at the Collection of the Institute of Microbiology at the Bulgarian Academy of Sciences. Spores of 6–7 days old culture incubated on potato-glucose agar slants at 30 °C were used for inoculation (concentration of spore suspension 1×10^6 /ml).

Culture medium and growth conditions

The fungus was grown in 500 ml Erlenmeyer flasks with 100 ml Chapek-Dox medium at 30 °C

for 3 days, shaking at 220 rpm. Every 6 h (from the beginning of the cultivation) the mycelium was centrifuged, washed with bidistilled water and used as a biosorbent.

Copper uptake

Biomass was incubated in the copper solution (0.5–4 mM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) at 30 °C under stirring. The pH of the mixture was 5.0 in all experiments. At given times after addition of copper II ion, an aliquot of the mixture was centrifuged at $5000 \times g$ for 20 min. The contents of copper in supernatant were determined, using a Perkin Elmer model 403 atomic absorption spectrophotometer.

The final concentration of biomass in incubation mixture was about 1 g dry mycelium per liter.

Effects of glucose, glycerol, ethanol and Na_2HAsO_4 on Cu^{2+} -uptake were determined as follows: Glucose, glycerol and ethanol at concentrations from 1 to 20 mM were added directly to the suspension (mycelium – Cu^{2+} solution) at the beginning of the uptake (To). In another experiment the mycelium was preincubated with glucose 60 min prior to add the Cu^{2+} -solution (To-60). The metabolic inhibitor (1 mM Na_2HAsO_4) was added to the suspension (mycelium – Cu^{2+} solution) at the beginning of the process (To) or 20 min later (To+20).

Aliquots of wet biomass, followed by 2-d over drying at 105 °C, was considered as dry biomass to calculate the uptake.

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Uptake of metal ions was calculated from a metal mass balance yielding (Volesky, 1990): $q = V(C_i - C_f) / m$, where q is mmol metal ions / g dry biomass, V is the sample volume (l), C_i and C_f are the initial and residual metal concentrations (mg / l) respectively, m is the amount of dry biomass (g) and M is the relative molecular mass of the metal. Control samples with no added biomass were used as blanks.

Chemicals

All chemicals were commercial preparations of analytical grade.

Results and Discussion

Relationships between copper uptake and growth phase

The efficiency of copper uptake was represented as copper content of fungal biomass on each growth phase. The efficiency of uptake increased with growth time and was largest at the mid-logarithmic growth phase, while at the stationary growth phase it decreased about two third of the maximum and then became approximately constant as shown in Fig. 1.

The time courses of copper uptake was biphasic as shown in Fig. 2. Mycelium of the logarithmic phase (20 h) absorbed copper more than that of the stationary growth phase (40 h). Both the rate and quantity of accumulated Cu^{2+} in the second phase were controlled by the glucose concentration. For example, to obtain a linear uptake at the

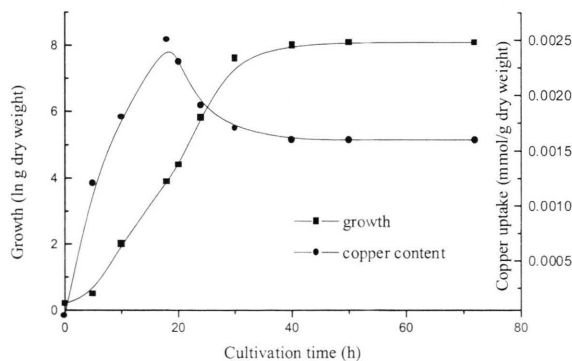


Fig. 1. Culture growth and copper uptake by *Penicillium brevicompactum* biomass. Data (three determinations from each of two separate experiments) were pooled to give a mean \pm S. D. of 10–15%.

maximum rate, at least 10 mM glucose was required. At concentrations higher than 10 mM, copper uptake was the same. Similar results were received when glycerol or ethanol at equivalent carbon concentrations were added at the same time as glucose (data not shown). Moreover, before energy-dependent Cu^{2+} -uptake could begin, the cells had to be provided with glucose for least 60 min. Therefore, in this experiment, 10 mM glucose was added 60 min before the addition of Cu^{2+} , i. e. at time T_0-60 . In these cases the values of uptake increased about five times in compared to the controls without glucose. However, the direct addition of glucose to the suspension (fungal cells – Cu^{2+} solution) had little effect on the amount of metal accumulation.

To test the hypothesis that glucose transport and subsequent catabolism yield the energy for Cu^{2+} -uptake, we added the metabolic inhibitor (Na_2HAsO_4) at various times during the experimental period. When the poison was added after the energy-dependent Cu^{2+} -uptake had been initiated (T_0+20), Na_2HAsO_4 inhibited the rate of Cu^{2+} -transport (Fig. 2). Similarly energy-dependent transport was inhibited when this poison was added at the same time either as glucose (T_0-60) or Cu^{2+} (T_0).

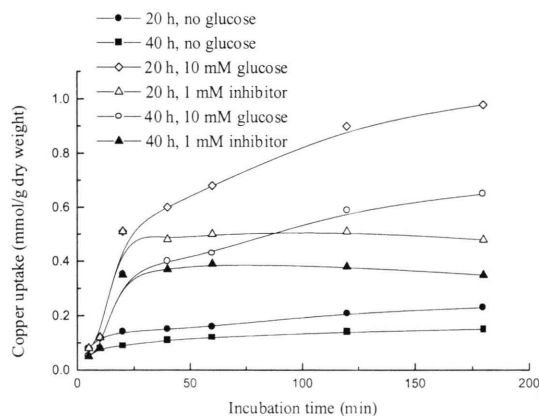


Fig. 2. Time courses of copper uptake by *Penicillium brevicompactum* biomass. Mycelia from the different age (1 g/l dry weight) were incubated for 3 h in the solution containing 1 mM CuSO_4 at 30 °C. Glucose was added at $T_0 - 60$; Cu^{2+} was added at T_0 , inhibitor (1 mM Na_2HAsO_4) was added at T_0+20 as described in Materials and Methods. The data shown are mean values of three experiments. Individual values deviated less than $\pm 10\%$ of the mean values.

Kinetics of the copper absorption

Both the absorption velocity and the Cu^{2+} -absorption during 180 min increased with copper concentration. Double reciprocal plots of absorption velocity of copper vs. copper concentration gave straight lines at concentration between 0.5–4 mM. The straight lines were extrapolated to find the intercept on the abscissa ($-1/K_m$) and the intercept on the ordinate ($1/V_{\max}$). The kinetics parameters for copper absorption by *Penicillium brevicompactum* are given in Table I.

The apparent affinity of copper to the cells of stationary growth phase was the same as that of logarithmic growth phase and the K_m values were

Table I. Summary of kinetics parameters of copper uptake by *Penicillium brevicompactum* biomass. Mycelia from different age were incubated in solutions containing 0.5–4 mM CuSO_4 , as described in Materials and Methods. All data points are means of four experiments.

Age of mycelia [h]	K_m [mM]	V_{\max} [nmol×mg ⁻¹ min ⁻¹]
18	1.04	49.6
24	1.04	30.2
48	1.04	20.4
72	1.04	15.8

about 1.04 mM. On the other hand, the differences in the absorption velocities values, may depend on the efficiency to form stable copper complexes on the cell surface. Furthermore, it may depend on the number of copper binding sites, which could be changed by the age of the mycelium.

In this study, abundant and common fungal biomass of *Penicillium brevicompactum* has been examined for its capacity to uptake copper ions from aqueous solution. The copper binding properties were influenced by the growth phase of the mycelium (Fig. 1) and the concentration of copper in the reaction mixture (Table I). The levels of metal accumulation were depended on glucose concentration as well as metabolite inhibitor (Na_2HAsO_4) added in reaction mixture during the experiments (Fig. 2). These results confirm the partial energy-dependent nature of Cu^{2+} -uptake by this fungus, because cells supplied with an energy source prior to metal uptake are capable to accumulate enhance levels of metal ions. However, the failure of the direct addition of glucose to the suspension, to elicit a similar response, suggests the inability of the fungal cells to internalize the glucose in the presence of high metal ion concentrations. It is possible, that, heavy metal may interfere with the uptake system of glucose into the cells as described by Stoll and Duncan (1996).

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