Copper Tolerance of a New Strain of *Penicillium ochro-chloron*

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Copper is essential to most organisms and is known as a constituent of many metalloenzymes. At the same time, however, it is very toxic to most microorganisms so that copper salts have been used extensively as fungicides. In the course of our studying the role of copper in microorganisms, we have recently isolated a new strain of *Penicillium ochro-chloron* from our laboratory air which grows in the medium saturated with copper sulfate (40% CuSO₄·5H₂O solution) and is remarkably tolerant to other heavy metal ions, too.

The high tolerance of *Penicillium ochro-chloron* towards copper has been reported by various workers and reviewed by Raper and Thom.3) Bedford2) has observed earlier that a *Penicillium* species, subsequently identified as *P. ochro-chloron*, was also tolerant to zinc, manganese, iron and cadmium. Kendrick3) has isolated *P. ochro-chloron* from the soil with high copper content.

The mechanism of copper tolerance has scarcely been investigated and it is virtually unknown. In this paper we report, therefore, the effect of the external copper concentration on the growth and uptake of copper by the fungus to elucidate the cause of the nature of the tolerance, comparing it with those by other ordinary species, *Aspergillus niger* ATCC 6275 and *Penicillium chrysogenum* IAM 7114.

*P. ochro-chloron* was grown in a 500 ml Erlenmeyer flask with 200 ml of the synthetic medium at 30°C, placed on a rotary shaker. The one liter basal medium, pH 4, contained: Glucose 40 g, (NH₄)₂SO₄ 3.3 g, KH₂PO₄ 2.5 g, MgSO₄·7H₂O 1 g, Ca(NO₃)₂·4H₂O 0.5 g, 5 mg Fe as FeSO₄, 5 mg Zn as ZnSO₄, 1 mg Mn as MnSO₄, 0.5 mg Mo as Na₂MoO₄, 0.1 mg Co as CoSO₄ and 0.01 mg V as NaVO₃. In order to examine the copper tolerance, various amounts of copper sulfate, none to the saturated, were added to the upper medium, 0.1 mg Cu being the basal. After 4 days growth the mycelium was collected on a filter paper, washed with deionized water, dried at 105°C to a constant weight and ashed at 450°C overnight in a muffle furnace. The mycelial ash was dissolved in 6 N HCl and made up to a volume with deionized water for metal analysis. Copper was determined by HITACHI 207 atomic absorption spectrophotometer at 3248 Å.

The effect of the external copper concentration on the growth and copper uptake is illustrated in Fig. 1. As is shown clearly, the growth of *P. ochro-chloron* is little affected by the copper ions of whole range of concentrations investigated and the yield at even saturated concentration after 30 days is almost equal to those at the lower levels of copper. Whereas *A. niger* and *P. chrysogenum* do not grow in the medium of more than 200 and 100 μg/ml of copper, respectively.

The uptake of copper into mycelium expressed in log scale proportionally increases with the external copper concentration for both *A. niger* and *P. chrysogenum*. In case of *P. ochro-chloron*, however, copper uptake is proportional only in the lower concentration.

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but is leveled off above 1000 µg/ml of copper in the medium. In this range, the copper content of this fungus is found approximately 1000 µg/g dry wt., rather independent of the copper concentration of the medium. Bedford has observed that the copper content of this fungus was found to depend on added copper in the lower concentration but above 1600 to 2000 µg/ml of copper the amount of copper in the mycelial ash was almost constant, similar to our finding. Our observation agrees, except that the constant uptake occurs in much wider range. This independency of copper content at the extreme condition appears to be most important for the elucidation of the mechanism of copper tolerance.

Permeability of the cell wall to metals has been considered. For instance, from the results of the low copper content of mycelium, Pulst has concluded that the cell wall of copper tolerant fungus *Penicillium glaucum* was less permeable to copper. In case of *P. ochro-chloron*, however, there is no difference of copper uptake with other fungi in the lower concentration. The constant copper content above 1000 µg Cu/ml seems to be a particular characteristic of this fungus, suggesting a different mechanism other than a simple permeability.

It has been well-known that small quantity of copper is essential to the growth of *A. niger*. Nicholas has found the growth of *A. niger* to be proportional, within limits, to the copper concentration of the medium. In this experiment a slight decrease of the growth is observed, as is seen in Fig. 1, by the omission of copper from the medium, coincident with his finding. Whereas in *P. ochro-chloron* and *P. chrysogenum* the effect of copper deficiency is not observed.

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