JOURNAL OF PURE & APPLIED MICROBIOLOGY, Nov. 2014. Vol. **8**(Spl. Edn. 2), p. 173-177

Biosorption of Copper using the Fungus *Aspergillus niger* Isolated from Contaminated Soil

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(Received: 10 August 2014; accepted: 20 October 2014)

The effect of copper on the growth of *Aspergillus niger* was studied. In solid media, all the colonies grew faster in the absence of copper heavy metal, *A. niger* growth was luxuriant and the colonies were dark brown in positive control at the 10th day of the experiment. In contrast the growth was decreased gradually at 50, 100, 200, 300 , and 400 ppm respectively. In liquid media, Colonies grown for 10 days with different concentrations of copper heavy metal showed variation in fungal biomass when compared with positive control. An addition no growth was observed in high concentrations of copper heavy metal at 500 ppm.

Key words: Heavy metal, Environmental Pollution, Absorption.

Industrialisation and technical advances have led to an increase in the use of heavy metals and heavy metal pollution. Contrary to organic substances, heavy metals are non-degradable and accumulate in the environment[1].

Heavy metals, a serious form of pollutants greatly affects water supplies and agriculture lands. These toxic heavy metals in air, soil and water are global problems that are a growing threat to humanity[2].

Heavy metal contamination which has increased sharply over the last century due to increasing industrialization imposes stress on organisms, the bioaccumulation of heavy metals being closely connected with their toxicity which causes a restrained metabolism and growth of the microorganisms.

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Contamination of sediments and natural aquatic recaptors with heavy metals is a major environmental problem all over the world[3, 4, 5, 6].

These inorganic micropolluants are released by effluents generated from various industries such as electroplating and metal finishing industries, metallurgy, tannery, and battery manufacturing. The introduction of heavy metal compounds into the environment generally induces morphological and physiological changes in the microbial communities[7], hence exerting a selective pressure on the microbiota[8]. Among microorganisms, filamentous fungi are well recognized for their superior capacities to produce a wide variety of extracellular enzymes, organic acids and other metabolites, and for their capabilities to adapt to severe environmental constraints[9]. For example, members of the Deuteromycetes such as *Aspergillus*, *Penicillium* and *Trichoderma* species are known to produce numerous extracellular enzymes, which are put to good use in biotechnology[9, 10, 11].

Fungus belongs to groups of organisms with very well known heavy metal sorption

capacity. It has been demonstrated that some fungi species are typically associated with heavy metal rich substrata and can be even considered as hyper accumulators of heavy metals[12]. They are a versatile group, as they can adapt and grow under various extreme conditions of pH, temperature and nutrient availability, as well as high metal concentrations[13]. They offer the advantage of having cell wall material which shows excellent metal-binding properties; therefore the fungi are good in the accumulation of heavy metals such as cadmium, copper, mercury, lead and zinc[14].

Aspergillus niger is a member of the genus Aspergillus which includes a set of fungi that are generally considered asexual, although perfect forms (forms that reproduce sexually) have been found. *Aspergillus niger* is a haploid filamentous fungi and is a very essential microorganism in the field of biology. In addition to producing extracellular enzymes and citric acid, *A. niger* is used for waste management and biotransformations[15]. *A. niger* has a high level of metal tolerance and biosorption properties[16].

The addition of copper to soil was reported to significantly decreased the amount of microbial biomass and to have a pronounced toxic effect on the size of the biomass compared to certain metals such as Pb and As. Copper-resistance has been demonstrated in a number of microorganisms, including *Aspergillus niger*, *Penicillium chrysogemum and Rhizopus stolonifer*[17]. In this work we will study the effect of copper on the growth of *Aspirgellus niger*.

MATERIALS AND METHODS

Aspergillus niger provided by Research Lab fungi, Department of Botany and Microbiology, Faculty of Science, King Saud University, was tested for its resistance and growth in presence of an increasing concentrations of Cu. The fungus was stored at 4 °C in Sabouraud agar, to be reactivated; it was subcultured twice on Sabouraud gar. *A. niger* was inoculated on Sabouraud broth liquid media, and allowed to grow for 10 days at 25 °C in an incubator on solid media and for 10 days in liquid media.

Six concentration was prepared using copper sulphate ($Cu_2SO_4 \cdot 5H_2O$) as (50,100, 200,300, 400, and 500 ppm) in 100mL in case of the solid media(five plates replicates for each concentrate) 30petri-plates and 400 mL in case of liquid media(four flasks replicates for each concentrate where each flask containing 100 ml) 24 flasks. Media was prepared according to the manufacturer's instruction. Control group were prepared as positive control (media + fungus) and negative control (only media).

Procedures for measurements

Measuring on solid media

On solid media, results were measured after 10th days by measuring the radius of the colonies (mm) against that of the positive control to determine the index of tolerance. The index was obtained by dividing the radius of the treated colonies on that of the untreated colonies.

Measuring on liquid media

In liquid media, fungus was harvested after 10th days using No. 1 Whatman filter paper, dried at 70 °C for 2 hours and then weighed (g). The net weight of the inoculated replicates was obtained by subtracting the average weight of the negative control replicates. Index of tolerance was calculated by dividing the weight of the treated fungal growth on the weight of the untreated fungal growth.

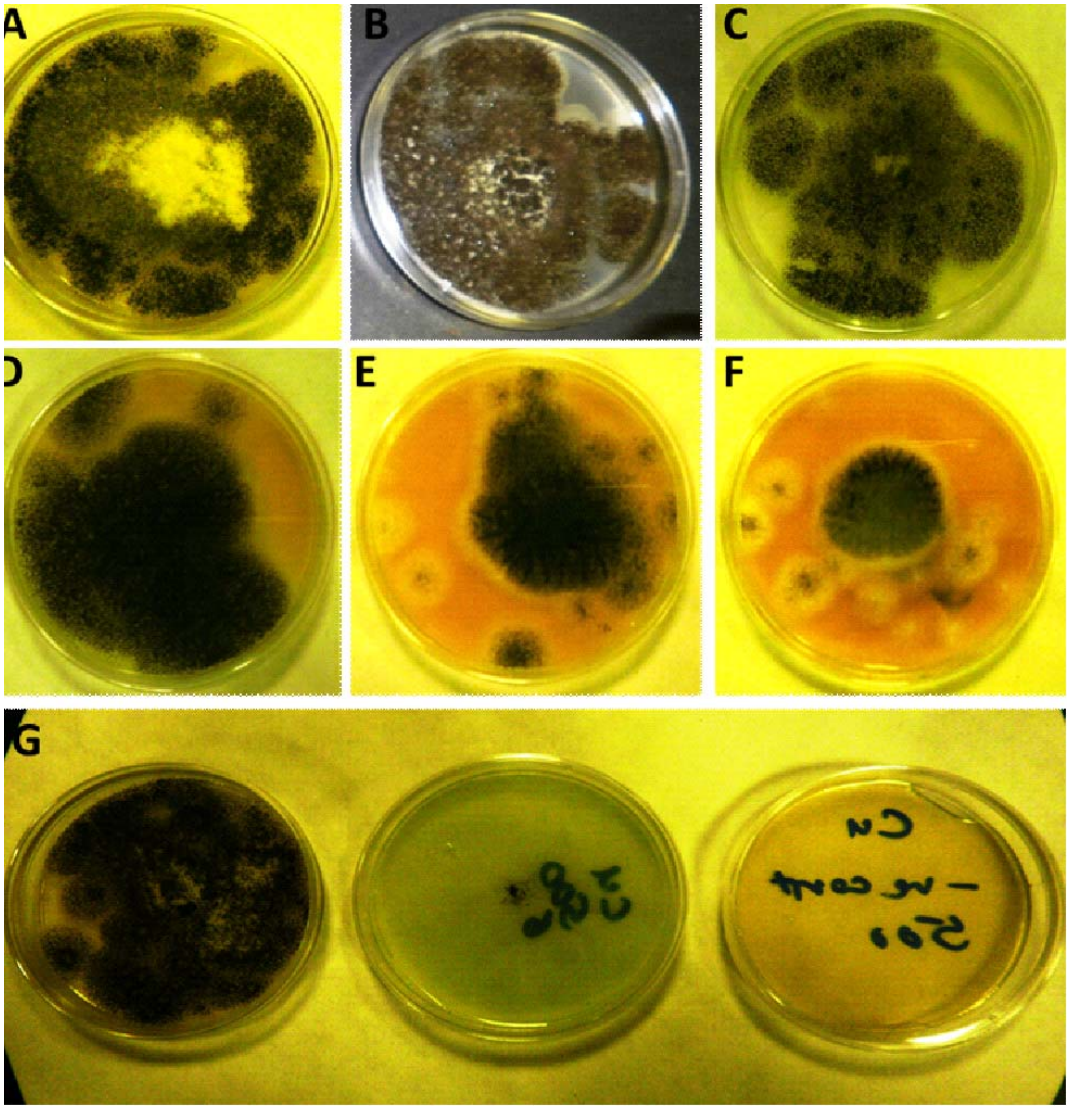
RESULTS AND DISCUSSION

Growth on the solid media

Organism under the stress of the environment develops various mechanisms in order to cope with the adverse conditions. In fungi, severe stresses may be tolerated by inherent physiological characteristics, or by adaptation through a temporary alteration in their developmental pattern (Al-Sohaibani, 2011) The colonies, in the presence of Copper heavy metal, showed striking variations as compared to the positive control which were more pronounced (Figure 1).

(Fig. 1) shows that the mean of the 5 replicates for each copper concentration as well as the results of the positive control at the 10th day of the experiment, and present the radii (mm) of the *A. niger* colonies at different concentrations of copper after 10th days of incubation, the average radius for each concentration and tolerance index.

All the colonies grew faster in the absence of copper heavy metal, *A. niger* growth



A=0 ppm(Positive Control), B=50ppm, C=100 ppm, D=200 ppm, E=300 ppm, F=400 ppm, G= Posit. Cont.,500 ppm, and neg. Cont. respectively.

Fig. 1. Growth of *A. niger* in different concentrations of copper in solid.

Table 1. Effect of different concentrations of copper on *A. niger* dry weight (g)

Cu concentration (ppm)	D.W	SD	SE	Tolerance	Percentage (%)
0	6.90	±0.76	±0.38	1.00	100
50	5.90	±0.67	±0.33	0.86	86
100	4.80	±0.22	±0.11	0.69	69
200	3.75	±0.21	±0.10	0.54	54
300	2.95	±0.13	±0.06	0.42	42
400	1.90	±0.38	±0.19	0.27	27
500	0.00	±0.00	±0.00	0.00	0

ppm=part per million , D.W=Dry weight,SD= Standard deviation , SE= Standard error.

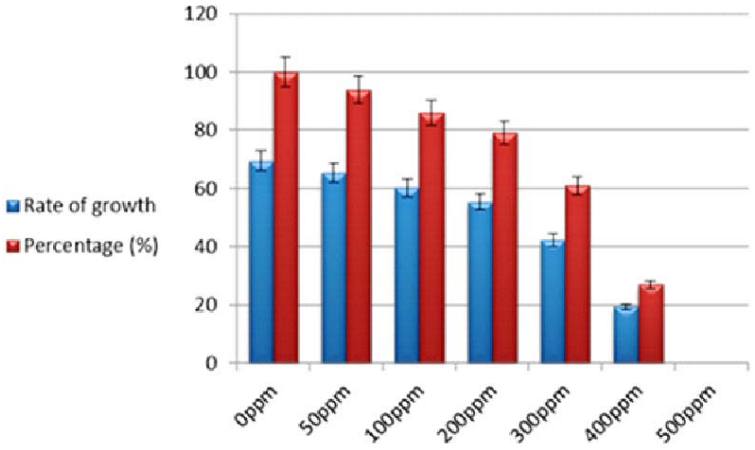


Fig. 1. The values of the five replicates for each copper concentrations and its impact on the growth rate

was luxuriant and the colonies were dark brown in positive control at the 10th day of the experiment. In contrast the growth was decreased gradually at 100, 200, 300 ppm respectively. The decrease in colony diameter of *A. niger* was observed with increase of copper ions. The results were showed the growth of *A. niger* at high concentrations of copper heavy metal (400 ppm and 500 ppm) was inhibited. Al-Kadeeb[17] reported that, the growth of *A. niger* was no affected at lower concentrations of copper ions.

Growth on the liquid media

Colonies grown for 10th days with different concentrations of copper heavy metal showed variation in fungal biomass when compared with positive control. Significant decrease in mycelial mass was observed both in static and aerated incubation. An addition no growth was observed in high concentrations of copper heavy metal at 400 ppm and 500 ppm.

Table (1) shows the weight of the 4 replicates for each copper concentration as well as the results of the positive control at the 10th day of the experiment and present the net weight (g) of the *A. niger* growth at different concentrations of copper after 10th days of incubation, the average net growth for each concentration, and tolerance index.

Number of earlier studies indicated that metal resistant fungi have the ability to grow in an environment containing heavy metals. The relative toxicity of the heavy metals for each strain becomes obvious at the higher concentrations[2].

Metals such as copper and zinc are essential to biological actions, however, all metals, whether essential and inessential will tend to show toxicity at certain levels. Their toxicity may be presented differently, depending on the isolate and its site of isolation[18].

These results demonstrated that, the growth rate of *A. niger* was more sensitive to addition of copper especially at high concentrations whether in solid or liquid media.

ACKNOWLEDGMENTS

This work was supported by King Saud University, Deanship of Scientific Research, College of Science Research Center.

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