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Toxicity Assessment of Nickel Using *Aspergillus niger* and Its Removal from an Industrial Effluent

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Abstract

Chemical analysis of electroplating effluent revealed the presence of very high concentrations of nickel (393 ppm) in the effluent. Bioassay was carried out to test the toxicity of nickel chloride to *Aspergillus niger*. In contrast to 50% conidial inhibition at 1.7 m*M* nickel, hyphal extension was affected even at a lower concentration (0.4 m*M*), suggesting that hyphae are more sensitive than conidia to nickel. An increase in nickel concentration resulted in a proportionate decrease in the hyphal extension. Nickel (II)-resistant mutants of *A. niger* M1, M2, and M3, were obtained using direct selection, stepwise adaptation, and ultraviolet mutation techniques. Biosorption of Ni (II) by the mutant M3 was 50% more than that of its parent strain.

Index Entries: *Aspergillus niger*; nickel toxicity; bioremediation; biosorption.

Introduction

Heavy metals are widespread pollutants of great environmental concern, because they are nondegradable and thus persistent *(1)*. Toxic metal species are discharged from industrial activities and are eventually accumulated through the food chain, leading to serious ecologic and health problems *(2)*. Microorganisms can affect heavy metal concentrations in the environment because they exhibit a strong ability for metal removal from solution through enzymatic or nonenzymatic mechanisms *(3)*. Biosorption is a process that utilizes inexpensive dead biomass to sequester toxic heavy metals and is particularly useful for removing contaminants from industrial effluents *(4)*. The removal of heavy metals can occur through several mechanisms such as simple adsorption, enzymatic synthesis, or the

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production of extracellular polymers *(5)*. Fungal biomass offers the advantage of having a high concentration of cell-wall material that shows excellent binding *(6)*.

Nickel is a toxic metal found in the environment as a result of various natural and industrial activities *(7)*. In the biosphere, nickel is ubiquitous and circulated through the system by the chemical and physical processes and through the biologic transport mechanism of living organisms *(8)*. In some organisms, nickel acts as a cofactor of certain metallozymes *(9)*. At higher concentrations, Ni (II) ions interact with many components, such as nucleotides *(10)*, phospholipids *(11)*, and amino acids *(12)*. Nickel is toxic even at low concentrations *(13)* and is an important source of contamination in industrial societies *(2)*.

Nickel exhibits mutagenic, teratogenic, and carcinogenic effects *(14)* having affinity for bioaccumulation *(15)*. Toxic and carcinogenic effects of nickel compounds are associated with nickel-mediated oxidative damage to DNA and proteins and to inhibition of cellular antitoxicant defenses. Nickel-dependent formation of an activated oxygen species is a primary molecular event in acute nickel toxicity and carcinogenicity *(16)*. Inside the mammalian cell, nickel is accumulated in the nucleus and nucleolus *(17)*. It disturbs DNA metabolism and causes crosslinks and strand breaks *(18)*. Therefore, the development of suitable and less expensive methods for determination of nickel present in the environment and its removal is important.

Materials and Methods

Culture and Growth Conditions

Aspergillus niger NRRL 330 was obtained from Northern Regional Research Laboratory Peoria, IL. *A. niger* was propagated on potato dextrose agar (PDA) medium at 35°C and maintained at 4°C. The medium was also supplemented with a solution of nickel chloride hexahydrate ($NiCl_26H_2O$).

Inhibition of Germination of Conidia and Hyphal Growth

Nickel concentrations for bioassay studies were selected based on the nickel content in a local electroplating industry effluent. Conidia of *A. niger* were plated on PDA with different concentrations of nickel (0.4–2.1 m*M*) and also without it. After 4 d of incubation at 37°C, the colonies were counted and expressed as the percentage of germination of conidia.

Three equidistant inocula with mycelial disks were made in Petri plates containing medium with 0.4–2.1 m*M* nickel and the plates were incubated at 37°C. The diameter of the colonies was measured daily for 4 d. The growth rate of the colony was expressed as the increase in the square diameter of the colony per day.

Isolation of Ni (II)–Resistant Mutants

Three different methods described by Kung and Lee *(19)* were employed for selection of Ni (II)–resistant mutant of *A. niger*. Mutants were

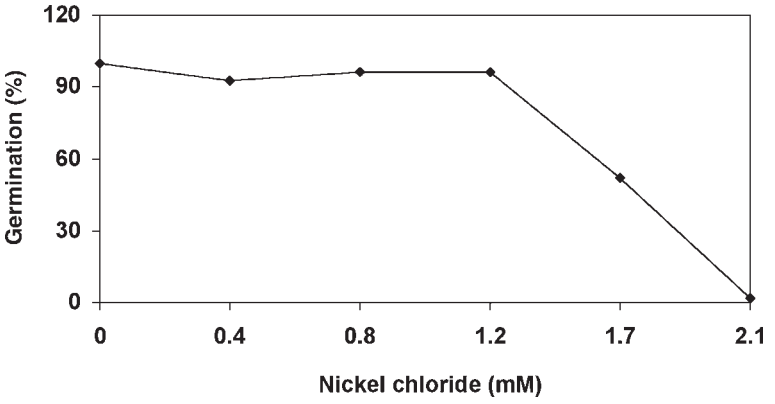

Fig. 1. Effect of nickel concentration on germination of conidiospores of *A. niger*. The conidiospore suspensions (1×10^5 spores/mL) were diluted and plated on PDA agar plates containing varying concentrations of $NiCl_2$. The number of colony-forming units was determined after 72 h of incubation at 37°C.

isolated by direct selection on medium containing 1.7 m*M* nickel, which was highly inhibitory to the parent strain. In the second method, the cells were repeatedly subcultured onto PDA medium containing increasing concentrations of nickel from 0.4 to 2.1 m*M*.

In the third method, a suspension of conidia (1×10^5 spores/mL) was irradiated with ultraviolet (UV) light (2 μJ/[$mm^2 \cdot s^{-1}$]) from a distance of 30 cm for 5 min. The irradiated conidia were plated on PDA incubated at 37°C for 4 d and mutants resistant to Ni (II) were selected.

Estimation of Nickel

Nickel analysis was done by gravimetric method with dimethylglyoxime and complexometric titration using murexide as indicator *(20)*.

Results and Discussion

Chemical analysis of the electroplating effluent showed a relatively high level of nickel (393 ppm). In addition, the effluent contained other heavy metals such as copper (3 ppm) and lead (0.5 ppm) in lower concentrations. Williams and Edyvean *(21)* suggested that, usually, only the toxic metal with the high concentration is targeted for removal.

Effect of Nickel on Conidiospore Germination and Hyphal Growth of A. niger

The effect of nickel on the germination of conidiospores of *A. niger* was studied (Fig. 1). The germination was not inhibited up to a concentration of 1.2 m*M* Ni (II). A further increase in Ni (II) concentration affected conidial germination. A 50% inhibition of conidiospore germination was observed at a concentration of 1.7 m*M* Ni (II). A concentration of 2.1 m*M* Ni (II)

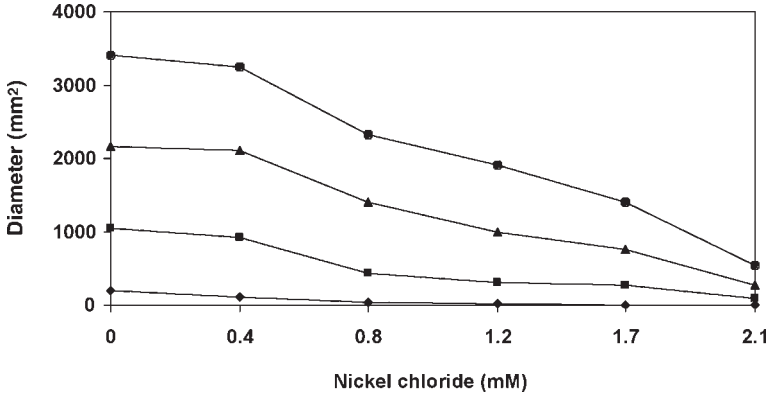

Fig. 2. Effect of nickel concentration on hyphal growth of *A. niger*. The hyphal disks were plated on the PDA agar plates containing different concentrations of $NiCl_2$. The diameter of the colony was measured at 24-h intervals. (—◆—) 24 h; (—■—) 48 h; (—▲—) 72 h; (—●—) 96 h.

completely inhibited the germination. Similarly, the germination of conidiospores of *Aspergillus nidulans* was inhibited at 175 µ*M* $CuCl_2$ *(22)*.

Inhibition of *A. niger* hyhae extension was also demonstrated with varying concentrations of $NiCl_2$. In contrast to the inhibition of germination of conidiospores, the hyphal growth was not affected up to 0.4 m*M* nickel. However, the hyphal growth inhibition occurred at higher concentrations of nickel. Inhibition was 64, 53, and 34% at 2.1, 1.7, and 0.8 m*M* $NiCl_2$, respectively (Fig. 2). These results suggested that hyphae were highly sensitive to nickel. However, the hyphae continued to grow at a slower rate at lower concentrations Ni (II). Similarly, the hyphal growth of *A. nidulans* was found to be highly sensitive to Co concentrations. Mutants resistant to Co showed increased hyphal growth in the presence of Co *(23)*.

Nickel-Resistant Mutants of A. niger *and Their Growth Characteristics*

Ni (II)–resistant mutants of *A. niger* M1, M2, and M3 were obtained by direct selection, stepwise adaptation, and UV radiation techniques respectively. The mutants showed improved hyphal growth rates and sporulation at 0.4–2.1 m*M* Ni (II) (Fig. 3). Phelan et al. *(22)* isolated copper-resistant mutants of *A. nidulans* using a similar technique. Brown and Hall *(24)* demonstrated that fungi can accumulate heavy metals, which are toxic, and that the fungal biomass can be used for detoxification or recovery of valuable metals. Therefore, we studied the Ni (II)–resistant mutants for bioremediation of electroplating effluents.

Accumulation of Nickel by A. niger

At a concentration of 1.7 m*M* Ni (II), the resistant *A. niger* mutant M3 showed twofold biosorption of Ni (II) (88.5 mg/g) compared to the wild

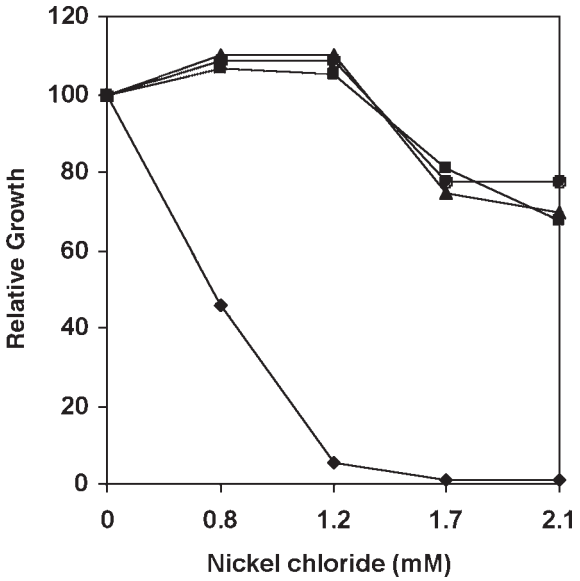

Fig. 3. Effect of nickel concentration on growth of *A. niger* mutants. The spores of nickel-resistant mutants M1, M2, and M3 and the wild type of *A. niger* were spot inoculated on PDA medium containing varying concentrations of $NiCl_2$ and incubated for 96 h at 37°C. The relative colony diameters were compared with their growth in the plate without $NiCl_2$. (—■—) Mutant M1; (—▲—) mutant M2; (—●—) mutant M3; (—◆—) parent strain.

type (45.25 mg/g). The metal-binding ability may be owing to adsorption and inorganic precipitation, complexation, ion exchange, or active transport *(25)*. High tolerance toward Ni (II) ion concentration rendered *Candida* BG55 more significant for its use as a potential biosorbent for the recovery of nickel from industrial effluents *(26)*. We suggest that the mutants of *A. niger* are also good candidates for removal of Ni (II) from the electroplating industry effluent.

Conclusion

Chemical analysis of the electroplating industry effluent showed a higher concentration of nickel (383 ppm) and lower concentration of other metals such as copper (3 ppm) and lead (0.5 ppm). Bioassay demonstrated the inhibition of conidial germination and hyphal extension by nickel present in the effluent. Conidial germination was inhibited by nickel concentrations >1.2 m*M*. However, hyphal extension was affected even at lower concentrations (0.8 m*M*), suggesting that hyphae are more sensitive to nickel. Among the nickel-resistant mutants M1, M2, and M3, obtained by different techniques, the mutant M3 showed a higher growth rate compared to the wild type and showed a twofold increase in absorption at a concentration of 1.7 m*M* Ni (II).

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