UNIVERSITI PUTRA MALAYSIA

TOLERANCE OF *TRICHODERMA ATROVIRIDE* (KARST.) ISOLATED FROM FRESHWATER ECOSYSTEMS TOWARDS COPPER AND ZINC

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TOLERANCE OF *TRICHODERMA ATROVIRIDE* (KARST.) ISOLATED FROM FRESHWATER ECOSYSTEMS TOWARDS COPPER AND ZINC

By

Mazyar Yazdani

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

May 2008

DEDICATION

To:

my dear Siranoosh,
Morad,
Babak
and Shahrzad

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

Tolerance of *Trichoderma atroviride* (karst.) Isolated from Freshwater Ecosystems Towards Copper and Zinc

By

Mazyar Yazdani

May 2008

Chairman: Yap Chee Kong, PhD

Faculty: Science

Isolation of microfungi from sediment samples of Kuyoh River Industrial Area, Kuyoh River Residential Area and Sri Serdang Lake was done on Rose Bengal Agar (RBA). The fungi were exposed to increased concentrations of Cu^{2+} (5 to 600 mg/L) and Zn^{2+} (5 to 6000 mg/L) on Potato Dextrose Agar (PDA) to find the most tolerant isolate. The highest tolerance to both elements was shown by *Trichoderma atroviride.* Further studies on growth rate, uptake capacity and localization of Cu^{2+} and Zn^{2+} were made by using Potato Dextrose Broth (PDB) incorporated with 25 to 300 mg/L of Cu^{2+} and 500 to 1000 mg/L of Zn^{2+}. This study found that the uptake capacity of *T. atroviride* for Cu^{2+} ranged from 0.77 to 11.20 mg/g between 25 to 300 mg/L in the liquid medium while that for Zn^{2+} ranged from 18.1 to 26.7 mg/g between 500 to 1000 mg/L in the liquid medium. The isolate showed that 50.3 to 85.4 % adsorption and 9.6 to 47.1 % absorption for Cu^{2+} and 47.6 to 64% adsorption and 30.4 to 45.1% absorption for Zn^{2+}. Based on the present studies, 2.7 to 5 % of Cu^{2+} and 5.7 to 7.4 % of Zn^{2+}removal was observed due to biomass washing. The mean levels of Cu^{2+} in the surface sediment of the three mentioned sites were 347.64 µg/g, 32.04 µg/g and 21.71 µg/g while the mean levels of Zn^{2+}were 219.75 µg/g, 140.64 µg/g and 85.10 µg/g, respectively. This study suggests that *T. atroviride* is a potential bioremediator of Cu^{2+} and Zn^{2+}. However, further studies are needed to confirm its practical use as a bioremediating agent.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan Ijazah Master Sains

Toleransi *Trichoderma atroviride* (karst.) Yang Diasingkan Dari Pada Ekosistem Air Tawar Terhadap Kuprum dun Zink

Oleh

Mazyar Yazdani

May 2008

Pengerusi: Yap Chee Kong, PhD

Fakulti: Sains

Pengasingan kulat mikro dari sedimen Kawasan Perindustrian Sg Kuyoh, Kawasan Bandar Sg Kuyoh dan Tasik Sri Serdang telah dijalankan di atas media agar Rose Bengal (RBA). Kulat didedahkan kepada kepekatan bertambah ion kuprum (dari 5 ke 600 mg/L) dan ion zink (dari 5 ke 6000 mg/L) di atas media Agar Poato Dextrose (PDA) untuk memperolehi spesies kulat yang mempunyai toleran terbaik. Spesies toleran yang terbaik untuk kedua-dua elemen logam ialah *T. atroviride.*Kajian selanjutnya ke atas kadar pertumbuhan, kapasiti pengumpulan dan lokalisasi ion kuprum dan ion zink telah menggunakan julat kepekatan ion kuprum 25 hingga 300 mg/L dan julat kepekatan ion zink 500 hingga 1000 mg/L di atas media Potato Dextrose Broth (PDB). Kajian mendapati kapasiti pengambilan *T. atroviride* untuk ion kuprum adalah pada julat 0.77 hingga 11.20 mg/g di antara 25 hingga 300 mg/L dalam medium cecair. Sementara itu untuk ion zink adalah pada julat 18.1 ke 26.7 mg/g di antara 500 hingga 1000 mg/L dalam media cecair. Hasil menunjukkan peratus penjerapan ion kuprum spesies ini ialah 50.3 hingga

85.4 %, manakala peratus penjerapan ion zink ialah antara 47.6 hingga 64% dan peratus penyerapan ialah antara 30.4 hingga 45.1%. Berdasarkan pada hasil kajian, didapati peratus penyingkiran ion kuprum ialah antara 2.7 hingga 5% dan ion zink ialah antara 5.7% hingga 7.4% merujuk kepada nilai biojisim. Purata kepekatan ion kuprum yang diperolehi dari permukaan sedimen dari 3 tapak kajian yang telah disebutkan ialah 347.64 µg/g, 32.04 µg/g and 21.71 µg/g sementara purata kepekatan ion zink ialah 219.75 µg/g, 140.64 µg/g and 85.10 µg/g. Kajian ini mencadangkan bahawa *T. atroviride* sebagai spesies yang berpotensi sebagai bioremediator untuk ion kuprum dan ion zink. Walaubagaimanapun, kajian selanjutnya diperlukan untuk memastikan praktikal penggunaan spesies ini sebagai agen bioremediating.

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I certify that an Examination Committee met on 27.May.2008 conduct the final examination of Mazyar Yazdani on his Master of Science thesis entitle "Ecotoxicological studies on the Cu and Zn tolerant fungus *Trichoderma atroviride* (karst.) isolated from freshwater ecosystems" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follow:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follow:

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DECLARATION

I declare that the thesis is my original work except for the quotations and citations which have been duly acknowledged. I also declare that it has not been previously and is not concurrently submitted for any other degree at Universiti Putra Malaysia or at any other institution.

Mazyar Yazdani

Date: 10. July. 2008

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ANOVA	One way analysis of variance
C	Centigrade
CFU	Colony-forms unite
Cu	Copper
DO	Dissolved oxygen
DW	Dry weight
ECM	Ectomycorrhizal fungi
EFLE	Easily and Freely Leachable and Exchangeable
EDTA	Ethylene diamine tetra acetic acid
g	Gram
µg/g	Microgram per gram
mg/g	Miligram per gram
mg/l	Milligram per litre
ppm	Part per million
ppt	Part per thousand
%	Percentage
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
RBA	Rose Bengal Agar
S.E	Standard error
V	Volume
Zn	Zinc

Chapter 1

Introduction

With the rapid urbanization, industrialization and technological innovations in various walks of life, pollution has become an environmental problem (Anand, 2006). Among the different kinds of pollutants, heavy metals have attracted attentions in the past few decades because of their properties such as increasing application, immutable nature and can potentially threat to biolife (Wang and Chen, 2006).

Since majority of contaminations end to the aquatic ecosystems, high concentrations of heavy metals can accumulate in sediments, and especially in fine-grained oozes, which consists high mineral specific surfaces and because of variation in physical and chemical properties they may act as a sink or a source of heavy metals in aquatic environments (Marchand *et al.,* 2006).

Due to mentioned above points there is a need to remove heavy metals before they enter the complex ecosystem (Lacina *et al.,* 2003). Conventional methods for removing dissolved heavy metals from aqueous solution have been studied in detail, such as chemical precipitation and sludge separation, chemical oxidation or reduction, ion exchange, electrochemical treatment, membrane technologies, reverse osmosis, filtration, adsorption on activated carbon and evaporative recovery (Lopez and Vazquez, 2003). However, chemical precipitation and electrochemical treatment are not effective, especially when the concentration of heavy metal ions is as low as 1 to 100 mg/L in aqueous solution, furthermore they produce large amount of sludge to be treated with great difficulties. Other techniques are extremely expensive, especially when treating a large amount of water and wastewater containing heavy metals in low concentrations (Wang and Chen, 2006), and on the other hand, they may not always be feasible and their metal-binding properties are non-specific (Price *et al*., 2001), so they cannot be used at large scale. These are the reasons why alternative processing methods, such as biosorptions (bioremediations), are now being considered more seriously.

Biosorption technology utilizes various natural materials of biological origin, including bacteria, fungi, yeast, algae, etc for the treatment of heavy metals contaminated wastewaters (Bayramoglu *et al.,* 2003). These biosorbents have metal sequestering properties and be able to decrease the concentrations of heavy metal ions in solution from ppt to ppb level. They can effectively sequester dissolved heavy metal ions out of diluting complex solutions with high efficiency and quickly. Hence biosorption is an ideal candidate for the treatment of high volume and low concentration complex in polluted sites (Wang and Chen, 2006).

Heavy metals can disrupt the ecological status of biota (Malik and Jaiswal, 2000) and heavy metal-resistant microorganisms appear in the soil and water of industrial regions (Aleem *et al*, 2003). High level of heavy metals would clearly exert selection pressure for the evolution of metal-resistant organisms (Malik and Jaiswal, 2000) by a range of gene families which are likely to be involved in homeostasis of transition metals (Colpaert *et al.,* 2005). Nevertheless, unpolluted environments may also harbor metal resistant organism or organisms that readily adapt to high concentrations of metals (Malik and Jaiswal, 2000).

Most currently available short-term toxicity assays are according to the bacterial cells. Therefore, there is a need for novel eukaryotic microbial bioassays that will be relevant to higher eukaryotes such as plants and animals (Kozlova *et al.*, 2005), particularly fungi are considered to be best alternatives for those waters purification (Lacina *et al.,* 2003). Fungi 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 (Anand *et al.,* 2006).

Trichoderma atroviride is a potential case in this area of researches with regard of its frequent presence in high polluted area which just a few investigations has been carried out on it and on the other hand, there is no report of bioremediation by this isolate in this region. Thus, there is a need to some experiments be done on *T. atroviride*.

Because of Zn^{2+} and Cu^{2+} are ubiquitous in the environment (Falih1997), these two elements were chosen in this study. Zn and Cu are considered dangerous for organisms at 5 mg/L and 1 mg/L, respectively for organisms.

The objectives of the present study were:

1. To determine the concentrations of Zn and Cu (total, geochemical distribution) in the sediments collected from three sites namely the *Sg*. Kuyoh River industrial area, *Sg*. Kuyoh River Residential area and Sri Serdang Lake.

2. To isolate fungi from sediment of the three sites and screen for the most tolerant strain on Cu^{2+} and Zn^{2+}-incorporated solid media. To determine the tolerance & toxicity of the most tolerant strain to Cu^{2+} and Zn^{2+} concentrations exposed in liquid medium.

3. To determine the adsorption and absorption of Cu^{2+} and Zn^{2+} in the most tolerant strain based on percentages of Cu^{2+} and Zn^{2+} localization in cell walls and intracells.

Chapter 2

Literature Review

2.1 Heavy metals

Heavy metals are a group of metals with a density above 5 g/cm^3. Consequently, the transitional elements from V (but not Sc and Ti) to the half-metal As, from Zr (but not Y) to Sb, from La to Po, including the lanthanides and the actinides, can be referred to as heavy metals. Of the 90 naturally occurring elements, 21 are non-metals, 16 are light metals and the remaining 53 (with As included) are heavy metals (Nies, 1999). In the field of biosorption heavy metals are usually classified as the following three categories: Toxic metals (such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc.), precious metals (such as Pd, Pt, Ag, Au, Ru, etc.), and radionuclides (such as U, Th, Ra, Am, etc.) (Bishop, 2002). Among the metals, Cu and Zn are important and called trace metals (some times described as micronutrients) in the field of biology because of their requirement for the normal growth and maintenance of health in living organisms (Purves, 1985).

The toxicity of heavy metals, can last for a long time in nature, some can even transform low toxic species into more toxic forms within a certain environment, such as the case with mercury, where the bioaccumulation and bioaugmentation in the food chain can damage normal physiological activity and endanger human life eventually (Wang and Chen, 2006). In contrast to organic pollutants, metals can only transform and change in valence and species, but cannot be degraded by any other methods including biotreatment, where heavy metals can occurs toxicity even in low concentrations such as between 1.0 to10 mg/L. Some strong toxic metal ions, such as Hg and Cd, are very toxic in even lower concentrations of 0.001 to 0.1 mg/L (Wang and Chen, 2006).

As a result of various industrial development, heavy metals have been introduced by variety of manufacturing processes, including in mining and smelting of metalliferous, metallurgy, iron and steel, electroplating, electrolysis, electro-osmosis, electric appliance manufacturing, metal surface treating, surface finishing industry, energy and fuel production, fertilizers and pesticide industry, leatherworking, photography, aerospace and atomic energy installation and wastes containing heavy metals directly or indirectly into the environment increasingly, especially in developing countries (Wang and Chen, 2006).

Knowledge of such a metals distribution in marine media, water and sediments, has been traditionally poor primarily because of difficulty in obtaining accurate results. However, due to the perfection of sampling techniques, storage, handling of samples and analytical techniques avoiding sample contamination, and also the availability of highly sensitive analytical instruments, the amount and quality of work published in this field has increased globally (Prego and Cobelo-Garcıa, 2003).

Generally, heavy metals exert an inhibitory action on microorganisms by blocking essential functional groups, displacing essential metal ions, modifying the active conformation of biological molecules and finally react to form toxic compounds in cells (Anand *et al.,* 2006). Though, at low concentration some heavy metals are essential for microorganisms because they provide vital co-factors for metalloproteins and enzymes (Aleem *et al.,* 2003).

2.2 Zn in the environment

The heavy metals concentrations found in the sediment can be both contributed by natural geological processes and man-induced activities. Therefore, it is interesting to know if the metal concentrations in the sediments are contributed to nature or anthropogenic sources. Usually, when the metals concentrations are elevated in the sediment, anthropogenic sources are to be expected (Yap *et al*., 2005a).

Zn is a transition metal that occurs in the centre of the Periodic Table. It is a metal used in galvanizing and alloying. It is also used in the manufacture of electric goods, dying, insecticides and cosmetics (Newton and Baker, 1999). Some properties of Zn have shown in Table 2.1.

Table 2.1. Some properties of Zn (Newton and Baker, 1999)

Name: Zinc
Symbol: Zn
Forms: ZnH_2, $ZnCl_2$, ZnO
number of isotopes: 5

Atomic weight: 65.38	
melting point: 419.5 ºC	
boiling point: 908 ºC	
Heat of vaporization: 115.30 kJ/mol	

Zn abundance in the earth's crust is estimated to be about 0.02 percent which places the element about number 24 on the list of the elements in terms of their abundance (Newton and Baker, 1999).

Zn is found in all natural environments. The bioavailability of Zn is largely determined by its physiochemical state. This metal exists as a soluble, hydrated species in acidic environments. However, it may also exist as a component of insoluble complexes in neutral and alkaline soils and adsorbed to colloids in seawater (Nriagu, 1980). In addition of Zn presence in the natural environmental of the coastal area, the anthropogenic inputs could cause elevated Zn concentration in coastal environment due to high human activities (Yap *et al*., 2005a). It can cause health problem in living organisms such as symptoms of dehydration, electrolyte imbalance, abdominal pain, nausea, vomiting, lethargy, dizziness, acute renal failure, and muscular incoordination in human. Thus, it is listed as one of the hundred and twenty-nine priority pollutants. In aquatic ecosystems, Zn would move to the sediments depending on the pH and the availability of ionic species in water that would react or associate with them (Nriagu, 1980).

Concentration of Zn in tissue of marine plants and animals from throughout the world usually are much higher and more variable than concentration of other metals (Neff, 2002). In comparison with Cu, Zn is readily taken up by plants from Zn–contaminated soils, whereas Cu resemble Pb in being reluctantly taken up by plants from contaminated soils, and nutritionally undesirable level of Cu in plants at the level of soil contamination normally found in urban area do not seem very likely (Purves, 1985).

Zn is considered dangerous for organisms at 5 mg/L for organisms. Knowledge of Zn toxicity in humans is minimal. The most important information reported is its interference with Cu metabolism. The symptoms that an acute oral Zn dose may provoke include: tachycardia, vascular shock, dyspeptic nausea, vomiting, diarrhea, pancreatictis and damage of hepatic parenchyma. Although maximum Zn tolerance for human health has been established for edible parts of crops (20 mg/kg), soil Zn threshold for producing safe vegetables is not available (Ejaz ul *et al*., 2007).

There are a lot of previous studies on Zn concentrations in the marine sediments but only few studies reported on freshwater ecosystem in Malaysian environment (Sarmani 1989; Lim and Kiu 1995; Lau *et al*. 1996; Sahibin *et al.* 2000; Sahibin *et al.* 2000; Yap *et al*. 2004a).

2.3 Cu in the environment

Cu was one of the earliest elements known to man. Cu is a transition metal, one of several elements found in rows 4 through 7 between Groups 2 and 13 in the Periodic Table. Cu and its compounds have many important uses in