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Effect of Cadmium Levels on the Growth Curve of *Candida albicans*

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ABSTRACT

Environmental pollution by toxic heavy metals is one of the most pressing problems. Metals are released in the environment in industrial effluent. Cadmium is one of the heavy metal toxic to microorganisms; however, there are yeast strains resistant to this metal. One of yeast species *Candida albicans* is a diploid fungus (a form of yeast) that is a causal agent of opportunistic oral and genital infections in humans. In the present study, effect of Cadmium on the growth curve of *Candida albican*s was demonstrated. *Candida albicans* was grown both in the presence and absence of Cadmium in YEPD media. Growth curves of the *Candida albicans* were plotted to study the growth pattern of the yeast isolate. It was found that *Candida albicans* grow well at 50 µg/ml concentration of cadmium. The organism was found to be resistant to the used heavy metal. Growing metal resistant cells is very important as it can ensure better removal through the process of biosorption. Such approaches may help in the removal of toxic metals from the environmental thus reducing environmental pollution.

Keywords: Biosorption, *Candida albicans*, Cadmium, Detoxification, Heavy metals.

INTRODUCTION

Rapid industrialization and urbanization have enhanced the levels of organic and inorganic contaminants in the environment (Chaudhari *et al*., 2009). The inorganic minerals like sodium, potassium, calcium, magnesium and heavy metals like iron, manganese, lead, mercury, chromium, cadmium, nickel, cobalt, beryllium copper etc., when present above the permissible limit are harmful. Agricultural water pollution is caused by fertilizers, insecticides, pesticides, farm animal wastes and sediments (Begum *et al*., 2009). Industrial activities have led to large-scale contamination of the environment with toxic heavy metals and radio nuclides (Lloyd and lovely, 2001). Heavy metals are chemical elements with a specific gravity that is at least 5 times the specific gravity of water. Some well-known toxic metallic elements with a specific gravity that is 5 or more times that of water are arsenic, 5.7; cadmium, 8.65; iron, 7.9; lead, 11.34; and mercury, 13.546. Toxic heavy metals in air, soil, and water are global problems that are a growing threat to humanity. There are hundreds of sources of heavy metal pollution, including the coal, natural gas, paper, and chore-alkali industries. In response to the growing problems, federal and state governments have instituted environmental regulations to protect the quality of surface and ground water from heavy metal pollutants, such as Cd, Cu, Pb, Hg, Cr, and Fe. Interactions between microorganisms and metals can be conveniently divided into three distinct processes, a) intracellular interactions, b) cell-surface interactions, and extracellular interactions (Gaylarde and Videla, 1995). Microbes have evolved to deal with toxic metals using several mechanisms. Heavy metals such as mercury, lead, copper, and arsenic are metabolic poisons in that they inhibit the activity of certain enzymes. Heavy metal ions often damage viable cell severly if they play essential role in many metabolic processes at low concentrations. *Candida albicans* is yeast that showed resistance to certain

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heavy metals. It lives in the mouth, throat, intestines and genitourinary tract of most humans and is usually considered to be a normal part of the bowel flora (the organisms that coexist with us in our lower digestive tract). It is actually a member of a broader classification of organisms known as fungi. *Candida albicans* is a diploid organism which has eight sets of chromosome pairs. Interestingly, Candida is one of the few microorganisms that have a diploid gene controlling the same protein – this means that is capable of pleomorphic activity being able to mutate forms from the budding form to the mycelial, pathogenic form (Bruins *et al*., 2009).

Cadmium is an important toxic metal whose in vivo metabolism and cellular mechanisms of toxicity appear to be highly complex (Fowler, 1978). Cadmium is a lustrous, silver-white, ductile, very malleable metal. Its surface has a bluish tinge and the metal is soft enough to be cut with a knife, but it tarnishes in air. It is soluble in acids but not in alkalis. It is similar in many respects to zinc but it forms more complex compounds. Cadmium metal exhibits excellent resistance to corrosion, particularly in alkaline and seawater environments, possesses a low melting temperature and rapid electrical exchange activity, and has both high electrical and thermal conductivity (Lenntech, 2010). In mammals, Cadmium exerts multiple toxic effects and has been classified as a human carcinogen by the International Agency for Research on Cancer. Cadmium affects cell proliferation, differentiation, apoptosis and other cellular activities. The inhibition of DNA repair processes by cadmium represents a mechanism by which cadmium enhances the genotoxicity of other agents and may contribute to the tumor initiation by this metal. Cadmium modulates also gene expression and signal transduction, reduces activities of proteins involved in antioxidant defenses (Waisberg *et al*., 2003). Cd depletes many essential metal antioxidants including selenium in the body. Oxidative stress occurs as a result of an increase in Cd-induced peroxidation of membrane lipids in the organs when it accumulates. (Chen *et al*., 1995). Fungal sporulation was more sensitive to Cd than was mycelia growth, as spore formation was inhibited at Cd concentrations that were no inhibitory to mycelia proliferation. In *C. utilis* there is a sharp decline in cell growth even at very low concentrations of cadmium (up to 0.0625mM) followed by a slow decrease in cell growth from 0.125 to 1mM cadmium (Bertin and Averbeck, 2006). Cadmium-binding proteins have an important role in moderating cadmium toxicity in some fungi. In a previous study, Candida was exposed to cadmium via soil or food. They demonstrated that the effect of cadmium on juvenile production of Candida decreased with time (Smit *et al*., 2004). *C. tropicalis* was found to be resistant to Cd up to a concentration of 2,800 mg L-1. *C. albicans* and *C. tropicalis* are known for high levels of resistance to the water-soluble ions Hg2 ,Pb2+, Cd2+, arsenate ,and selenite (Rehman and Anjum, 2010).

In this study effect of cadmium levels on the activity of *Candida albicans* was observed by plotting the growth curve.

MATERIALS AND METHODS

Culture: Candida albicans.

Chemical: Cadmium chloride solution ($CdCl_2$)

Media: Yeast Extract Peptone Dextrose media (YEPD)

Glass wares and instruments: Petri plates, justers, tips, conical flasks, hot air oven, spectrophotometer, incubator, autoclave.

Procedure: Inoculate 100ml of YEPD media with 16 hour old culture of *Candida albicans* and mark as control. Incubate at 37^{o}C for 24 hours. Prepare stock solution of cadmium chloride in 100ml of distilled water. 50ug of cadmium chloride is dissolved in 100ml of distilled water and autoclave. Inoculate another 100ml YEPD media with 16 hour old culture of *Candida albicans* and solution of cadmium. Incubate at 30^{o}C for 48 hours. After 48 hours, take the optical density of both control and test at 600 nm

after interval of 2 hours.

RESULTS AND DISCUSSION

The use of yeast as biosorbent is particularly important because of the ease of genetic manipulation. Cadmium is one of the important heavy metal present in industrial effluent, so there is need to grow metal resistant cells for the removal of cadmium. Normally at higher concentration the metal can form certain complex compounds inside the microbial cell and may lead to toxic effects. But certain micro organisms are the potent agents for bioremediations such as yeast isolates accumulate toxic metals in significant values. The yeast can transform the absorbed metal into complex polymeric compounds non toxic for the cells. In yeast, several resistance mechanism can activated on exposure to toxic metal and identify certain genes involved in the metal detoxification. The resistance of yeast cell by cadmium is increase by the metallotheinoine and gluthione. Yeast cells containing cad 2 exhibit a resistance to cadmium intracellular level through enhanced cadmium efflux system. In the present study, *Candida albicans* was used to observe the effect of cadmium on its growth curve. Microscopic examination showed that the growth of yeast isolates and the size of cell become smaller in the presence of heavy metal i.e. Cd but the growth of the organism was not inhibited by the cadmium and the organism continues to grow in the presence of 50 ug/ml of cadmium (Table I).

Table I. Optical Density of *Candida albicans* in control and in 50µg/ml cadmium concentration at 600 nm.

S. No.	Time (hrs)	Control	Cd (50) ug/ml
1	1	0.37	0.131
2	2	0.587	0.188
3	3	0.615	0.203
4	4	0.623	0.228
5	5	0.861	0.375
6	6	0.904	0.499

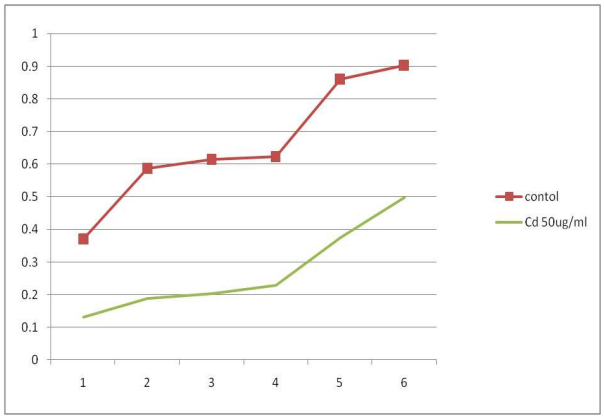

Figure 1. Growth curve to show the effect of Cadmium on *Candida albicans.*

The organism removed about 80% of Cadmium from the YEPD after 96 hours of incubation. In previous study it was observed that prolonged exposure of certain *Candida albicans* strains to inhibitory concentrations of cadmium resulted in the appearance of resistant colonies (Malavasic and Cihlar, 1992). Previous studies revealed that certain strains of yeast can show high resistance to cadmium in the YEPD medium. At 50ug/ml concentration of cadmium in the YEPD medium, there was a decline and a number of colonies start decreasing after 48 hours of incubation (Muneer *et al*., 2007). While in the present study the growth of *Candida albicans* in the 50ug/ml concentration of cadmium appears to be slower as compared to the control in which no cadmium was present but the growth was not inhibited by the heavy metal and the organism continues to grow at this concentration of cadmium. Clinical isolates of *C. albicans* and *Candida glabrata* exhibit high levels of resistance to both copper and cadmium salts, although the molecular basis of this resistance is not known (Oh *et al*., 1999). Whether the yeast isolates studied in this investigation, possess reduction abilities or they simply uptake the metal from the medium and accumulate with resultant lowering of the metal concentration in the medium has to be determined. These yeast isolates can be exploited for bioremediation of cadmium containing wastes, since they seem to have the potential to accumulate the toxic metal from the environment.

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