ISSN 0975 - 6299 Vol 2 / Issue 4 / Oct - Dec 2011
International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

PHARMACOLOGY

COMPARATIVE STUDIES OF BACTERIA AND FUNGI FOR THE REMOVAL OF CU^{2+} METAL.

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ABSTRACT

The aim of this research work is to evaluate the efficiency of fungus and bacteria for the Cu^{2+} removal capacity .The bacteria and fungi was spread on Cu^{2+}(50mg/l) and monitored after 24hr and fungus after 96hrs, purified & characterized based on morphological characteristics. Finally the result indicated that fungal strain A. lentulus absorb maximum amount of copper and have a high absorbing capacity.

KEY WORDS

Copper metal, morphological characteristic, A. lentulus

INTRODUCTION

Removal of heavy metals in wastewater has been a major concern due to their non-biodegradability and toxicity (Gadd 1998).Conventional wastewater treatment technologies are less effective. Moreover, due to lack of effluent treatment plants in industries, the untreated effluents are discharged directly into the local water bodies and they become a major source of groundwater pollution. At present, growing interest in the application of fungi for bioremediation of industrial effluents.

Filamentous fungi are common and important residents of soil biota, and many of them have been used for bioremediation of heavy metal contaminated wastes (Gadd, 2001; Gadd, 1997). They are represented by a wide range of morphological types and the availability of large amounts of fungal biomass and products derived from industrial processes and fermentations favor their use as biosorbents. Fungus has proved to be a suitable organism for the treatment of industrial effluents because fungal mycelia have an additional advantage over single cell organisms due to an increased cell to surface ratio and a greater physical and enzymatic contact with the environment. Further, the versatility of fungal strains to utilize different carbon sources is another advantage in the favor of fungal strains. However, little information about the capability of growing filamentous fungi to accumulate copper in their cells or to reduce the Cu^{2+} concentration is found in the available literature (Dursen et al., 2003) .

Copper, a widely used metal in industry, is an essential trace element for human health and plays an important role in carbohydrate and lipidmetabolism and in the maintenance of heart and blood vessel activity. The adult human body contains 100- 150 mg of Cu (II), but excess amount in the body can be toxic (Gupta et al., 2006) . In aqueous environments, the specification of the metal is dependent both on ligant concentration and pH. While the cupric ion (Cu(II)) in the metallic form is more toxic to flora and fauna, it is also a nutrient necessary for algae growth (Murphy et al., 2007). If allowed to enter the human body, the excessive amounts of Cu(II) can cause serious potential health issues such as nausea, headache dizziness, respiratory difficulties, hemolytic anemia, massive gastrointestinal bleeding , liver and kidney failure, and death (Gong et al., 2008; Ayhan and Ozacar, 2008; Siao et al., 2007; Yazici et al., 2008, Anirudhan and Radhokrishman, 2008; Chan et al., 2008) . The World Health Organization recommended a maximum acceptable concentration of Cu(II) in drinking water of 1.5 mgL- 1 (Ayhan and Ozacar, 2008).In recent years , increasing concern about the effect of toxic metals in the environment has resulted in more strict environmental regulations for industries that discharge metal bearing effluents (Papageorgiov et al., 2008).

MATERIALS AND METHODS

Material and Isolation of strain

Industrial effluents contaminated with Cu^{2+} were collected from tannery industry and textile industry from the industrial area, Banmore, located in Gwalior, India. Both the effluents were kept at 4°C during storage and characterized. The pH, COD and Cu^{2+} concentrations in tannery effluent were 8.0, 520 mg/l 102 mg/l, whereas in textile effluent the values obtained were 8.0, 540 mg/l and 137 mg/l, respectively.

To isolate the microorganism, serially diluted effluent was spread on Cu^{2+} amended (50 mg/l) nutrient agar plates for bacteria and potato dextrose agar plates for fungus and these plates were incubated at 30°C. For the bacterial growth, plates were monitored after 24h and for fungus, monitoring was done after 96 h. The microbial colonies (bacterial as well fungus) that appeared on Cu^{2+} amended plates were isolated, purified and characterized based on their morphological characteristics such as colour, texture, size. Further, microscopic characterization was also done. Four bacterial (B1,B2,B3, B4) and one fungal strains (AML05) were obtained. The preliminary characterization of the bacterial isolate is shown in Table 1.1. The fungal strain was identified as Aspergillus sp (Dursun et al 2006., Kapoor et al 1999), based on the microscopic characteristics as shown in Table 1.2.

Table 1.1
Characteristics of bacterial isolate

Parameter	**Bacterial strain**			
	B1`	**B2**	**B3**	**B4**
Gram strain	+	+	+	-
Cell shape	Rod shaped	Circular shaped	Rod shaped	Round shaped
Colony colour	Light yellow	Creamish	Lemon	Lemon
Motility	+	+	+	-
Indole test	+	-	+	+
Catalase test	+	+	-	+
Oxidase	+	-	+	-

Table 1.2
Morphological and microscopic characteristics of the fungal isolate

Parameter	**Remarks**
Growth	Fast Growth
Colonial reverse	Uncolored to flesh coloured
Conidial color	Blue green
Conidial shape	Subgllobose
Vesicle shape	Flask shape
Conidiophore shape	Short and smooth
Sterigmata shape	Fertile, uniseriate

Biomass production and growth kinetics of A. lentulus

Isolated A.lentulus was grown in 250 ml Erlenmeyer flask containing 100 ml of growth media containing 0.5g/l K_2HPO_4 ; 5g/l Yeast extract; 0.1g/l $MgSO_4$;0.5g/l NH_4NO_3 and 5g/l glucose , pH =6 at 35± ^oC using 180 rpm . Then growing A.lentulus was used for finding the growth kinetics.

RESULT

The results of MIC determination are shown in Table 1.3. It was found that fungal strain AML05 was able to tolerate Cu^{2+} concentration up to 500mg/l. On the other hand the bacterial strains B1, B2, B3, and B4 showed resistance up to 150mg/l. Although bacterial strains were also isolated from metal contaminated effluents, they did not display

Table 1.3
MIC of Cu^{2+} for isolated microbial strains

S.No.	MIC of Cu^{2+} (mg/l)	Number of isolates
1	20-50	5
2	50-100	4
3	100-150	3
4	150-300	1
5	300-500	1

tolerance to a higher concentration of Cu^{2+}. Many microbial strains (bacterial and fungal) have been reported (Dursun et al., 2003) to be resistant to toxic Cu^{2+} but the tolerance for high metal concentration may be observed in all the cases. The fungal isolate (AML05) could tolerate upto 550mg/l in solidified composite media (Basu and Paul (1999)) . Hence AML05 seems to be a better candidate for remedial measures as it could withstand Cu (II) levels even in both media. The A. niger raw biomass contains chitin - chitosan units and a reasonable amount of protein and amino acids which serves as a matrix of COOH,-NH_2 and –OH group (Mukhopadhyay et al 2006a) which in turn takes part in binding of metal ion (Tsezos ,1983).

It is beneficial to work further with more resistant strains, since the toxicity of Cu (II) can inhibit the survival and also the removal capacity of the strain (Kaushik et al., 2008)

Growth kinetics of A. lentulus

Fig 1.2 and 1.3 shows the change in glucose concentration and biomass production during the growth of A.lentulus in absence and in presence of 100 mg/l Cu^{2+} using the optimized composite media. No lag phase was observed in the absence of Cu^{2+} as compared to 12h of lag phase in its presence. In absence of the metal, very fast growth and glucose consumption were observed during the first 18 hr which was coupled with a decrease in pH (initial pH -6) and it was dropped to 5.0 at 24 hr. Subsequently, pH values displayed a rise as6.5 in next 48 h. In the presence of Cu(II), the fall in pH initiated at 12 hand a maximum pH (5.0) was recorded at 48 h, beyond which it started rising. This could be attributed to the delayed and slow rate of glucose consumption in presence of Cu(II), which led to substantial reduction in the rate of fungal growth. Alentulus displayed toxicity response and its growth was delayed in presence of Cu (II). However the organism could eventually acclimatize to produce almost equal quantity of biomass(in absence of Cu (II)) after 120 h Dursun et al.(2003).

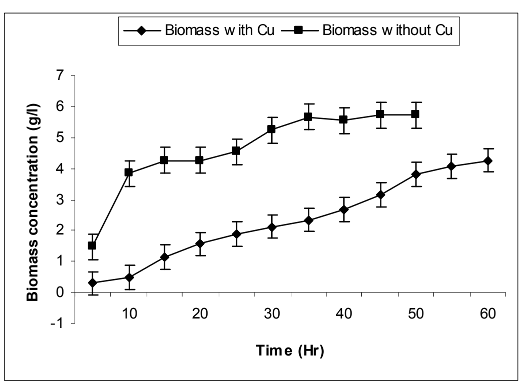


Fig 1.2

ISSN 0975 - 6299 Vol 2 / Issue 4 / Oct - Dec 2011

Variation in biomass during growth of A. lentulus in presence (100mg/l) and absence of Cu(II).

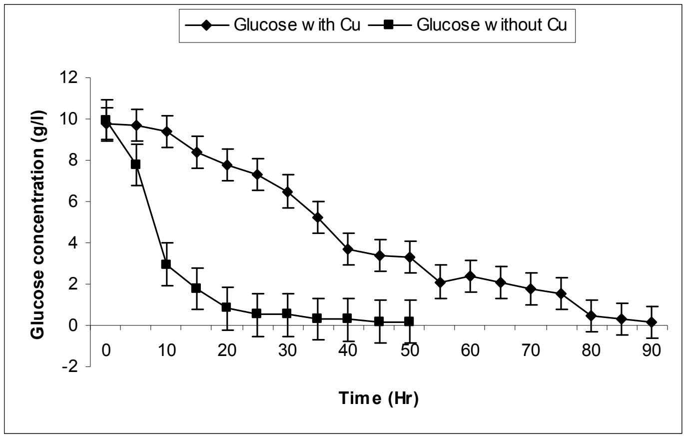


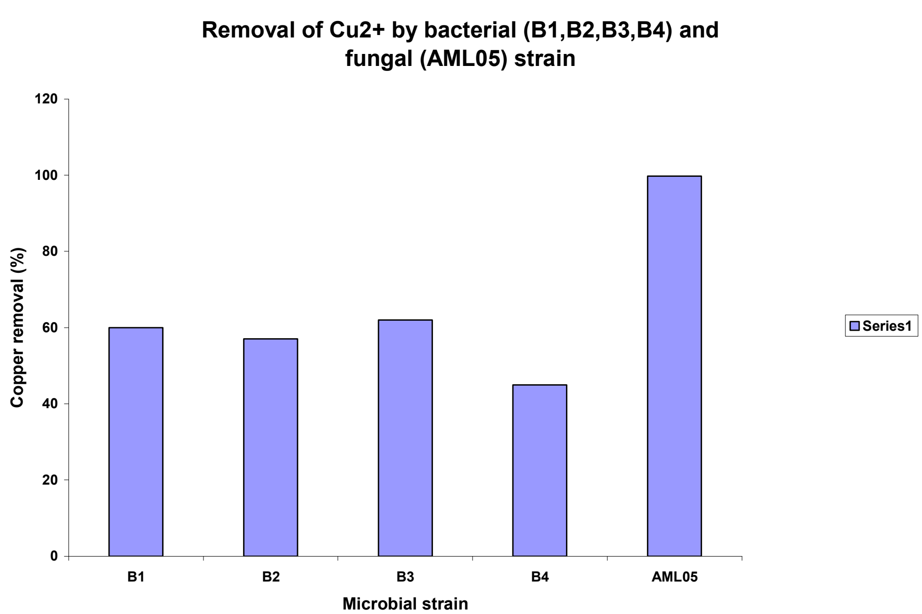
Fig 1.3

Variation in glucose concentration during growth of A. lentulus in presence (100mg/l) and absence of Cu(II).

CONCLUSION

Comparison of Cu^{2+} removal during growth of these microbial strains is shown in Fig. 1.1. It is evident from this figure that fungal strain was not only tolerant to higher concentrations of Cu^{2+} but also caused higher removal as compared to the bacterial strains at all the concentrations tested. Although these concentrations were sub- lethal to all the bacterial and fungal strains, higher removal in case of fungal strain indicates that the latter has better ability to uptake Cu^{2+} during its growth (Hauang and Huang 1996). The metal uptake by living fungal cells involves the cell across the cell membrane is dependent on cellmetabolism and is referred to as intracellularuptake, active uptake.For living cells metal uptake is also done by the production of metal- binding proteins.

Fig. 1.1



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