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Bioremediation of Zinc by *Streptomyces aureofacienes*

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Abstract: Heavy metals are generally toxic to microorganisms, especially if they exist at high concentrations. Environmental pollution particularly in soil with heavy metals can stem from industrial activities or sewage discharges. In this study, twelve *Streptomyces* species isolated from Egyptian soil and identified by using physiological tests as Bergey's manual was assessed quantitatively to the effects of zinc using plate diffusion method. Biosorption of zinc by biomass microorganisms is an innovative and alternative technology for removal of these pollutants due to their good performance, low cost and large available quantities. Experiments in liquid culture were used to determine concentration ranges of the metals at which the most tolerant species could grow showed that *Streptomyces aureofacienes* had maximum uptake of zinc (734.8 μg g^{-1} biomass).

Key words: Heavy metals, zinc, *Streptomyces*, identification, bioremediation

INTRODUCTION

Metals are found in the environment in varying concentrations in soil, water, air and in all biological matter. Through they have a well known concentration range characteristic for live organisms.

Growing attention is being given to health hazards presented by the existence of heavy metals. These metals are redistributed in the biosphere and dispersed in the air, soil and water and through their accumulation in living tissues throughout the food chain, human may have serious health problems. Exposure to heavy metals has been linked with development retardation. Various cancers, kidney damage and even death in some instances of exposure to very high concentrations of heavy metals which has also been associated with the development of autoimmunity too (Brooks *et al*., 2010).

Zinc constitutes about 0.02% of the earth's crust (Fisher, 1992). It also present in waste waters from plating and metal processing industry and can be removed by precipitation as zinc hydroxide (Eckenfelder and Wesley, 1989).

Zinc is an essential metal and its deficiency results in severe nutritionally related health problem (Sharma and Dugyala, 1996) and it is required for functioning of many metallo-enzymes which participate in variety of cellular metabolic process, protein synthesis and association of nuclear proteins with DNA (MacDonald, 2000).

Actinomycetes are Gram positive bacteria characterized by the formation of aerial mycelium on solid media, presence of spores and high G+C content of DNA (60-70 mol%). They are important class of bacteria since they produce numerous natural products such as antibiotics and enzymes (De-Schrijver and De Mot, 1999).

Streptomycetes is an actinomycetes with cell wall type I, belonging to the family Streptomycetaceae (Suutari *et al*., 2002). This member of the order Actinomycetales with complex life cycle involving three stages of differentiation. It is thought that the morphological and physiological differentiation and onset of secondary metabolites production result from common elements of regulation (Brown *et al*., 1998; Krishna Kumari *et al*., 2006). Recently, streptomycetes play important role in bioremediation of zinc specially which have high resistance to zinc (Chergui *et al*., 2007; Lin *et al*., 2010; Li *et al*., 2010; Deepika; Kannabiran, 2010).

The aim of the present study is to isolate streptomycetes from dfferent Egyptian soil. Also a rapid method for assessing the tolerance of streptomycete isolates to zinc. Identification of active tolerant streptomycete isolates and studying the bioremediation of zinc by *Streptomyces* species selected.

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MATERIALS AND METHODS

Sampling: Soil samples were collected at the last of 2007 in polyethylene bags by two ways. The first one which considered to be polluted with some heavy metals from Alexandria, Behera, Dakahlia, Ismaelia, Helwan and Shubra El Kheima. The second is two concentrations of Zinc sulphate solution (200 and 2000 μg g^{-1}) were added to another samples collected from Dakahlia which is far from any sources of heavy metals. The water content of the soil was adjusted to 28% (w/v) to permit good aeration. Treatments were setup in duplicates in addition to the control. Bags were incubated at 28±2°C and samples were taken after 1, 4 and 12 weeks (Hemida *et al*., 1997).

Isolation of streptomycetes: Using the serial dilution method of Hayakawa and Nonomura (1987) four different media were used for the isolation of streptomycetes namely: Starch nitrate agar (Waksman, 1961), Malt extract-yeast extract agar (Pridham *et al*., 1957), inorganic salt starch agar (Kuster, 1959) and Glycerol-L-asparagine agar (Pridham and Lyons, 1961).

Assessment of zinc toxicity: In order to assess quantitatively the effects of zinc, the plate diffusion method was used. To each plate of starch nitrate agar, 0.5 mL of the appropriate zinc solution was added in a central well of 1 cm in diameter and 4 mm in depth. Plates were then incubated at (37±2°C) for 24 h to allow diffusion of metal into the agar. On each plate, six strains were inoculated in radial streaks and in duplicate. Plates were then incubated at (28±2°C) for one week. The area of growth inhibition (in cm) was measured as that from the edge of central well to the leading edge of the growing streak. The percentage of streptomycetes tolerance was calculated in terms of the ratio: length of the growth in cm vs length of total inoculated streak. The range of concentrations for zinc in millimols were 25, 50, 100, 150, 200 and 250 (Hassen *et al*., 1998).

Identification of active biosorption streptomycete isolates: The isolated streptomycetes which showed high resistance to zinc were subjected to identification using keys proposed by Szabo *et al*. (1975) and Holt *et al*. (1994).

Uptake of zinc by selected Streptomyces species: In order to assess quantitatively biosorption of zinc by streptomycete species. It is grown in a liquid salts medium and the appropriate metal was added to the desired concentration after autoclaving. Liquid media were inoculated with 0.1 mL of spore suspension and incubated at 30°C with shaking at 200 rpm for 7 days. Culture samples of known volumes were centrifuged at 1500 rpm for 10 min. The pellets were washed twice, dried for 24 h at 105°C and digested by nitric acid. The digested cells were used for the determination of zinc concentrations (Abbas and Edwards, 1989).

Determination of zinc concentrations: The slightly acid solution (pH 2-3) containing not more than 10 μg of Zn and not larger than 25 mL in volume was placed in a separating funnel, 5 mL of acetate buffer and 5 mL of thiosulphate solution was added and shaked with portions of the dithizone solution in CCl_4 (1 mL of 0.002% H_2Dz solution corresponds to 2.6 μg of Zn) until the green CCl_4 layer showing no longer changes in colour. Each shaking should last not more than 2 min. The combined extracts were shaken with 5 mL portions of wash solution. Free dithizone was washed out from the CCl_4 layer with dilute ammonia (1drop of conc. NH_3 solution in 25 mL of water). The pink solution of Zn $(H_2Dz)_2$ with CCl_4 was diluted in a 25 mL or smaller flask (according to colour intensity) and mixed well. If the solution was turbid (owing to formation of an emulsion), it was filtered through a filter paper previously washed with dilute dithizone solution. The absorbance of the clear solution was measured at 536 nm and the solvent used as reference (Marczenko, 1986).

RESULTS AND DISCUSSION

Conventional methods for removing metals from aqueous solution include chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, membrane technologies and evaporation recovery. These processes may be ineffective and extremely expensive especially when the metals in solution are in the range of 1-100 mg L^{-1} (Nourbakhsh *et al*., 1994). Also when the metals are one of the components of the soil. Another major disadvantage with conventional treatment technologies is the production of toxic chemical sludge and its disposal/treatment become a costly affair and is not eco-friendly. Therefore, removal of toxic heavy metals to an environmentally safe level in a cost effective and environment friendly manner assumes great importance.

Rather than searching of microbial species for particular metal sequestering features, it is beneficial to look for biomasses that are readily available in large quantities to support potential demand. Different biomass types have been used to adsorb heavy metal ions from environment (De Rome and Gadd, 1991;

Tiemann *et al*., 1999). Sea-weed, mold, bacteria, crab shell (Rana *et al*., 2009) and yeasts are among the different kinds of biomass that have been tested for metal biosorption or removal (Volesky and Holan, 1995).

Isolation of streptomycetes: Streptomycetes were isolated according to their special colony morphological characteristics which are usually round, convex, shaped colonies, with deeply rooting growth into the medium. Colonies are usually covered with spore masses, they are dry and powdery. Randomly 237 streptomycete isolates were isolated from polluted agricultural soils subjected to some sources of heavy metals. The highest number of streptomycete isolates was recovered from Ismaelia followed by Behera, Dakahlia and Alexandria, while the lowest number from Shubra El Kheima and Helwan (Table 1).

Streptomycetes were also isolated from soils after addition of zinc with specific concentrations of 200 and 2000 μg g^{-1} after 1, 4 and 12 weeks. The total number of isolated streptomycetes was 148 isolates as shown in (Table 2).

Many media formulations have been recommended for isolation of streptomycetes. The most widely used include starch casein and malt extract-yeast extract agar. Kuster and Williams (1964), who examined several carbon and nitrogen sources, found "starch-casein-nitrate" to be the most selective for isolation of streptomycetes. The use of L-arginine as selective nitrogen source favouring streptomycetes over bacteria was recommended by El-Nakeeb and Lechevalier (1963).

Resistance of streptomycete isolates to zinc: By using a simple plate diffusion assay system, we have been able to estimated the effects of zinc on a number of streptomycete species The obtained results indicated that zinc yielded a range of sensitivities and tolerance on *Streptomyces* species (Table 3). These results are almost the same to those described by Abbas and Edwards (1989) studying the effect of metals on rang of *Streptomyces* species. As pointed out by Trevors *et al*. (1985), Simeonova *et al*. (2008) and Lakshmipathy *et al*. (2010), there is a problem in defining exactly what is mean by resistance to heavy metals. Duxbury (1981) proposed an equation where by resistance could be defined more precisely.

Identification of streptomycete isolates: Several tests have been used for phenotypical characteristics of streptomycetes isolated from soils subjected to some sources of zinc or after addition of zinc with variable concentrations. In case of numerical analysis of the phenotypical data, similarity calculations were made with the simple matching coefficient (S_{SM}). Trees were generated by using the UPGMA technique to enable the comparison of results.

Table 1: Total number of streptomycetes isolated from polluted agricultural soils with zinc

Locality	No. of isolates
Alexandria	40
Behera	44
Dakahlia	43
Ismaelia	46
Shubra El Kheima	30
Helwan	34
Total	237

Table 2: Total number of streptomycetes isolated from agricultural soils after the addition of zinc

Heavy metals	1 week	4 weeks	12 weeks
200 μg g^{-1} Zn	35	28	21
2000 μg g^{-1} Zn	28	27	22
Total	63	55	43

Table 3: Tolerance of streptomycetes to $ZnSO_4$ and Bioremediation of zinc from aqueous solution Streptomycetes biomass isolates

	Concentration of $ZnSCO_4$ (nM)						
Sample	25	50	100	150	200	250	Zn removed (μg g^{-1} biomass)
Str. aureofacienes	1	1	1	1	1	1	734.8
Str. badius	1	1	1	1	1	1	684.0
Str. tanashiensis	1	1	1	1	1	1	542.6
Str. halstedii	1	1	1	1	1	1	419.8
Str. diastaticus	1	1	1	1	1	0.9	663.2
Str. acrimycini	1	1	1	1	1	0.9	644.5
Str. viridodiastaticus	1	1	1	1	1	0.8	696.5
Str. cyaneus	1	1	1	1	1	0.8	588.9
Str. anulatus	1	1	1	1	0.9	0.8	572.9
Str. misakiensis	1	1	1	1	0.8	0.8	640.1
Str. griseoflavus	1	1	1	0.6	0.5	0.5	452.3
Str. rochei	1	0.9	0.8	0.7	0.6	0.6	507.9

Uptake of zinc by selected *Streptomyces* species: Some selected *Streptomyces* species that showed tolerance to the heavy metals were examined for their ability to remediate the zinc from aqueous solution (Table 3). *Streptomyces aureofacienes* showed the maximum uptake (734.8 Zn μg g^{-1} biomass) followed by *Streptomyces viridodiastaticus*, *Streptomyces badius* and *Streptomyces diastaticus* 696.5, 684.0 and 663.2 (μg g^{-1} biomass) respectively. The other *Streptomyces* species showed different pattern of uptake, while the lowest uptake (419.8 Zn μg g^{-1} biomass) was detected with *Streptomyces halstedii*. The obtained results were in accordance with this reported by many authors examined the uptake of zinc by different *Streptomyces* species (Abbas and Edwards, 1989; Mameri *et al*., 1999; Schmidt *et al*., 2009; Lin *et al*., 2010).

CONCLUSION

Experimental results showed that among all studied species of Streptomyces, *Streptomyces aureofacienes* has maximum uptake of zinc.

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