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Bioremediation of Heavy Metal (Copper) Using Indigenous Bacteria (*Staphylococcus sps*.) Isolated from Mithi River.

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ABSTRACT

Mithi River is one of the polluted rivers in Mumbai. It is most vulnerable to pollution from discharge of sewage through municipal outlets as well as improper outlets through slums and further by untreated discharge of industrial effluents, containing heavy metals. The isolation and narrowing down of copper resistant bacteria were carried out from samples collected from different locations on the river. It was screened for resistance at different concentrations of Copper, i.e. 50ppm, 100ppm, 500 ppm and 1000ppm. The resistant bacteria revealed that it belonged to the *staphylococcus* genus. Bioremediation studies were carried for a period of 120 hours in the medium. The decrease in metal was analysed at 24 hours and 120 hours. There was considerable removal of copper from the medium, proving the capacity of *Staphylococcus. sps*. to bioremediate.

Introduction

Heavy metal pollution has been a common consequence of industrialization. Heavy metals are usually a part of the untreated effluents discharged from industries, small scale factories, illegal processing units etc. into the nearest waterbody. These heavy metals interfere with the natural processes occurring in the waterbody, often leading to accumulation of it in the organisms and aquatic environment. Metals when consumed can have toxic effects. Some of the metals like arsenic, copper, iron, nickel, etc. are useful to the body in low concentration but are toxic at high concentration (Suranjana *et.al*., 2009). Many methods have been incorporated to reduce the toxicity of metals. Some conventional methods to remediate sites contaminated with heavy metals are excavation and solidification/ stabilization which are suitable in controlling contamination but not permanently remove heavy metals (Bahi *et. al.,* 2012). Unconventional methods like using microorganisms to study their natural mechanisms which help in reducing the toxicity of harmful effluents has been explored. Bioremediation is the "use of living organisms (primarily microorganisms) for removal of a pollutant from the biosphere" (Samal and Kotiyal, 2013). Bioremediation is an effective process to reduce environmental pollution due to heavy metals (Ghosh and Saha, 2012). Microbial communities respond to heavy metals depending upon the concentration and availability of heavy metals and is also a complex process which is controlled by factors such as, the type of metal, the nature of the medium, and microbial species (Goblenz *et.al.*,1994). Copper is commonly present in various industrial effluents (Ho *et. al.,* 2002). Many studies revealed that marine life is damaged due to high copper concentration in water (Saha *et.al*., 2008) including humans (Dursun, 2006).

In the light of the adverse effects of copper, the objective of the study was taken up, to check for the bioremediation capacity of active *staphylococcus sps*. indigenous to the river in bringing down the level of copper in an aqueous medium and understand that life exists in the river and is in a continual process of remediation.

Materials and Methods

Study Area

Mithi River, emerges from the pristine hilly areas, strengthened by discharges from Tulsi, Vihar and Powai lakes as it flows down. It meanders through Filterpada, Bamandayapada, Marol, Sakinaka, L&T Junction, JVLR, Bail Bazaar, Chattrapati Shivaji International Airport, BKC and finally the Mahim bay completing its journey into the Arabian Sea. On its course, it traverses through highly populated industrial and residential areas.

Preparation of bacterial culture

Three sets of 100 ml nutrient broth medium devoid of any metals was prepared. Sterilization was carried out by autoclaving the medium and necessary glasswares. The medium was inoculated with 24-48-hour pure culture previously isolated and identified.

Screening for metal tolerance

The experimental bacteria were exposed to varying concentrations of copper using the chemical salt - Copper sulphate. The concentrations ranged from 50ppm-1000ppm. Two percent nutrient agar plates were prepared in aseptic conditions and were allowed to solidify was overnight. Approximately 0.1 ml of bacterial culture was smeared with the help of a sterile spreader. A sterile cork borer (8mm bore size), was used to create a cavity in the agar plate which was divided into four quadrants. Using 1ml pipetting tips approximately 100 ul of the metal solution of different concentration were added in four different quadrants. It was allowed to diffuse for 1 hour and then incubated at 37^0C for 24hours-48hours. After 24hours all the plates were observed for the presence and absence of zones of inhibition.

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Metal removal analysis

The bioremediation of copper was evaluated by allowing a known bacterial biomass to interact with metal ions in the aqueous medium. The *Staphylococcus* pre-cultures were prepared by inoculating a loopful of it into 50mL of nutrient broth and were incubated for 24- hours. Approximately 1000ul of the 24-hour culture was inoculated into three flasks containing 100 ml of nutrient broth each. Bacterial cells were harvested from the flasks after 24 -hours by centrifugation at 10,000 rpm for 10 min at 4° C. The wet biomass was weighed using an analytical balance. Approximately,1 gram of wet biomass was added into three flasks containing 100 mL of 10ppm primary solution of copper prepared in nutrient medium. Primary solution was prepared by dissolving Copper sulphate ($CuSO_4$) in distilled water. The solutions were adjusted to pH 7. The flasks containing the culture was subjected to an orbital shaker at ambient temperature overnight. Centrifugation was carried out after 24 hours and 120 hours of contact time. The supernatants were filtered and digested and thereafter it was analysed using ICP-OES.

Percentage metal removal and specific metal uptake (Q) were calculated (Volesky and May-Phillips, 1995) as:

a. Percentage metal removal: % Biosorption = [(Ci – Cf) ÷ Ci] × 100

b. Specific metal uptake (mg metal/biomass weight):

Q = [V × (Ci – Cf)] ÷ [1000 × M]

Where:

Q = specific metal uptake (mg metal/g biosorbent)

V = volume of metal solution (mL)

Ci = initial concentration of metal in solution (mg/L)

Cf = final concentration of metal in solution (mg/L)

M = mass of biosorbent

Results

Screening for metal tolerance

The bacteria showed no inhibition zone at 50 ppm implying its tolerance, but it showed inhibition zones of 12mm, 17mm and 19mm at 100ppm, 500 ppm and 1000 ppm respectively.

Metal removal analysis

The removal capacity of copper in the aqueous medium by the *Staphylococcus sps*. was observed to be 32% and 84% in 24 and 120 hours respectively.

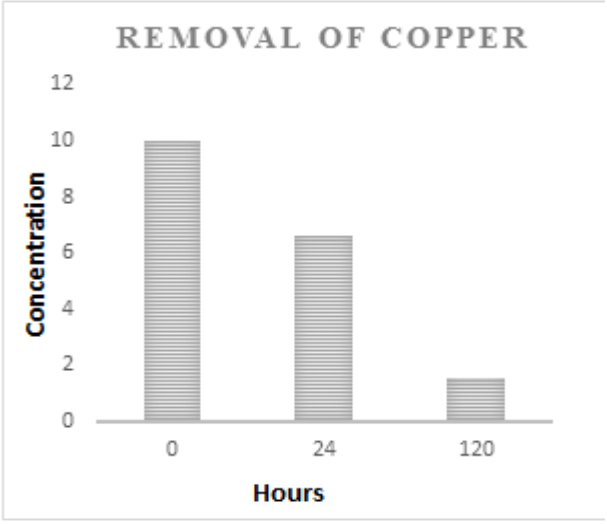

Figure 1. Reduction of copper by *Staphylococcus sps*.

Discussion and Conclusion

Most species of staphylococcus are associated with diseases production in animals. They are also found in waters which have sewage outlets emptying into them. Exposure to copper has been linked to gastrointestinal, hepatic, and renal effects (Parungao *et.al.*, 2007). Excessive amounts of copper in fresh water resources and aquatic ecosystem damage the osmo-regulatory mechanism of fresh water animals (Lee *et. al.*, 2010).

They are efficient in bioremediation and have been studied for their metal uptake characteristics by many researchers. Hakeem and Bhatnagar, (2010), observed 82% copper was reduced by the indigenous *Staphylococcus sp*s.in their work area. Rajaram *et.al.*, (2013), observed that the species in the environment they studied had a copper removal rate percentage of 83.3 and 87.25 at 24 hours and 120 hours respectively. In this study, the *Staphylococcus* species isolated from Mithi river were explored for their bioremediation capabilities and prove that they have potential in bringing down the intensity of heavy metals in water, in this case, Copper. The Central Pollution Control Board (CPCB) standards for discharge of environmental pollutants: Effluents, (copper) into the inland surface water is 3mg/l. The study conducted further shows that if the pollutant is present up to a concentration of 10ppm, it certainly possesses the capacity of reducing the pollutant to a concentration that is not toxic to the environment.

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