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Studies on isolation and characterization of Zn bioaccumulating bacteria from their natural habitat

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ABSTRACT

Many industry such as the electroplating & mining companies produced large amount of waste water that contain hazardous amount of mercury, lead, cadmium, silver, copper & zinc ions. The search for new technology involving the removal of toxic metal from waste water has directed attention to biosorption which can be defined as the ability of biological material to accumulate heavy metal from physico-chemical pathway of uptake. The topic under interest is Bioaccumulation of heavy metal by using bacterial isolates obtain from Zn containing waste. Study gives focus on Isolation of Zinc Bioaccumulating bacteria from Zinc containing waste from Electroplating industry. Total four Zn accumulating bacteria were isolated and studied for their Morphological, Cultural and enzymatic Characters. The isolated bacterial species are from Genus Plesiomonas, Psedomona and Staphylococci .Analysis of Bioaccumulation with Atomic Absorption Spectroscopy showed that among all isolates Psedomonas has maximum Bioaccumulation capacity and has highest MTC against Zn as Compared to other genera. This study leads to the conclusion that, Psedomonas is an efficient organism for Zn bioaccumulation.

Key words: Biosorption, AAS, *Pseudomonas.*

INTRODUCTION

Many industry such as the electroplating &mining companies produced large amount of waste water that contain hazardous amount of mercury, lead, cadmium, silver, copper & zinc ions. They are require by law to reduce the concentration of these toxic metal in their waste water before it is discarded into sewer lakes & stream. The search for new technology involving the removal of toxic metal from waste water has directed attention to biosorption can be defined as the ability of biological material to accumulate heavy metal from physico-chemical pathway of uptake. [1] The industrial activities led to substantial release of toxic metal into the environment. Heavy metals constitute a major hazard for the human health & ecosystem.[2]The heavy metal are defined as ions with partially or completely filled d-orbital some including ions, zinc, copper & manganese are micro-nutrient used in the redox processes regulation of osmotic pressure, enzyme cofactor and are also important in the maintenance of the protein stricture. However even essential metals such as zinc and copper are toxic at high concentration. [3]

The presence of heavy metal in aquatic environment is known to cause severe damage to aquatic life beside the fact that these metals kill micro-organism during biological treatment of waste water with a consequent delay of the process of water purification.[4] Many aquatic micro- organisms such as bacteria yeast & algae can take up

dissolved metal from their surrounding into their bodies and can be used for removing heavy ions successfully.[5] Micro-organism uptake metal either activity (bioaccumulation) and or passively (biosorption).[6,7]

The chemical method such as precipitation, oxidation or reduction has been widely used to remove metal ions from industrial waste water. These methods are ineffective or expensive.[8] The activity of micro- organism is extended to environmental management and microbes have superseded the conventional techniques of remediation.[9] Biological method such as biosorption & bioaccumulation provide promising alternative to chemical methods.[10]

The mechanism by which micro- organism remove heavy metals can be divided into three categories. The first mechanism is the biosorption of metal ions the cell surface. Second intracellular uptake of metal ion and third chemical transformation of metal ion by microorganism.[11] Among the different technique employed for metals removal from multi elemental system, biosorption has been found to be highly selectively.[12] Furthermore metal accumulating bacteria can be used to remove concentration & recover metals from industrial effluents.[13, 14]

The biosorption of heavy metals by microbial cells has been recognizes as a potential alternative to existing technology for recovery of heavy metals from industrial waste stream. [15, 16, 17] Feasibility studies for large scale application demonstration that , biosorption process are more applicable than the bio accumulation process, because living system often required the addition of nutrients and hence increased biological oxygen demand or chemical oxygen demand in the effluent. In addition, maintenance of healthy microbial population is difficult due to toxicity & other unsuitable environment factors. [18, 19]

MATERIALS AND METHODS

Sample Collection and Study of Physicochemical Properties of Sample

Waste water sample were collected from industrial area nearby SANGLI and analyzed for presence of Zinc by performing AAS of sample. Collection of sample was done in sterile bottle and preserved at low temperature till further analysis to be done physicochemical properties like $P^{H,}$ total solids (TS), total dissolved solid (TDS), total suspended solid (TSS) & total alkalinity of sample was measured. The COD & BOD were also measured by the titration method.

Bioaccumulation of heavy metals

Serially diluted waste water sample were plate inoculated on Nutrient agar containing 0.4 gm of ZnO and plates were incubated at R.T. for 48 hrs. After proper incubation period, four well defined colonies were isolated and purified by sub culturing. Purified biomass of four bacterial isolates was develop and analyzed for Zn accumulation by AAS (NIKHIL Laboratory SANGALI.).

Identification of isolate

The identification and characterization of isolate has been done up to genus level by performing Gram Character, colony Character, biochemical character and enzymatic activity.

Determination of MTC (maximum tolerance concentration)

Maximum Tolerable Concentration of Zn^{+2} in the form of ZnO in varying concentration from 1gm-10gm against each bacterial Isolate was determined.

RESULTS AND DISCUSSION

Physicochemical Properties of Sample: - The physical properties of waste water sample studied and given in table No. 1

Table No. 1 **Physicochemical Properties of Sample:**

Sr. No.	Name of Property	Quantity
1	Total solid	8.8g/lit
2	Total dissolved solid	6.4g/lit
3	Total suspended solid	2.4g/lit
4	Total alkalinity	20mg/lit
5	BOD	43mg/lit
6	COD	300mg/lit

Isolation of zinc accumulating bacteria:-
From collected waste water sample, total 4 bacterial isolates were obtained on Nutrient agar having 0.4 gm concentration of Zn, coded and their Zn accumulation was measured by Atomic Absorption Spectroscopy result of Zn accumulation was cited in table No.2

Table No.2 - Isolate from Waste Sample and their Zn accumulation by AAS

Bacterial Isolates	Isolate Code	Zinc Accumulations (%) per 0.1gm of dry biomass
1	P_1	0.15
2	P_2	0.17
3	P_3	0.55
4	P_4	0.20
	E.coli (Reference)	0.17

Bioaccumulation of Zn: From above table it can be seen that isolate P_3 was found to be accumulate maximum zinc **(0.55%)** & P_1 was found to be accumulate minimum zinc **(0.15%)**.

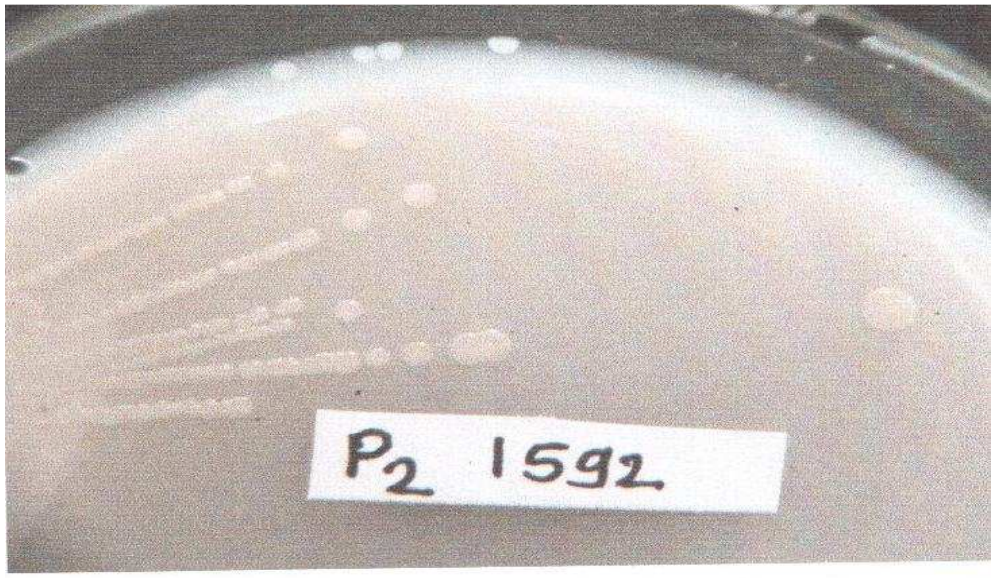
Identification of isolate: The colony characters of all isolates were studied and result are recorded in table No.2 Morphological character of isolates were studied by gram staining (Hucker's modification of gram staining 1884) & motility (Hanging drop technique). The result are cited in table No.3

Table No.3 – Colony Characters, Gram nature and Motility of Isolates

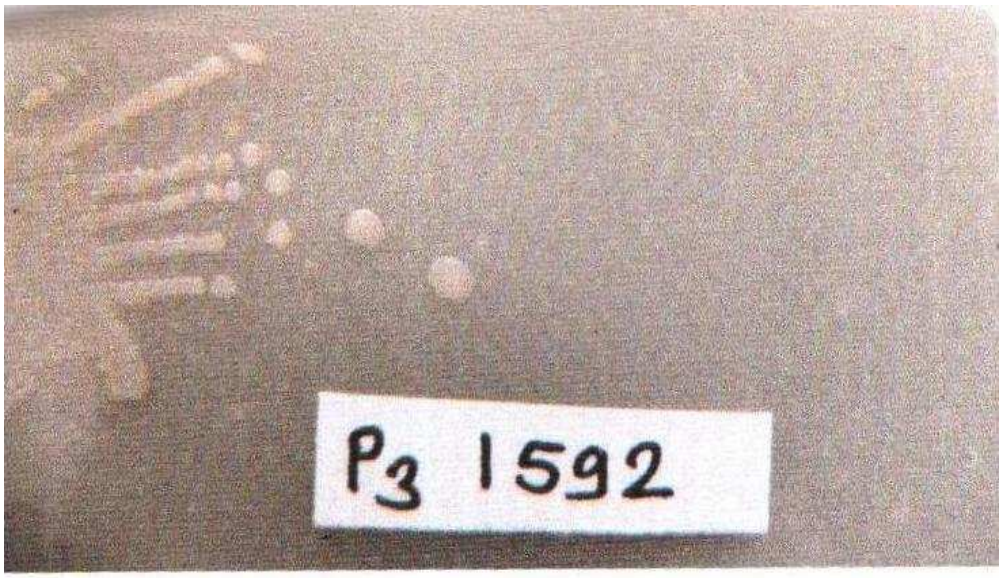
Isolate	Size	Shape	Margin	Color	Elevation	Opacity	Consistency	Gram Nature	Motility
P_1	3mm	circular	entire	white	convex	opaque	moist	Gram negative short rod	non motile
P_2	2mm	circular	entire	white	convex	opaque	moist	Gram positive long rod	non motile
P_3	2mm	circular	entire	white	convex	opaque	moist	Gram negative rod	Motile
P_4	1mm	circular	entire	golden yellow	convex	opaque	moist	Gram positive cocci	non motile

From above table it can be seen that colonies of all isolates were found to be 1-3 mm in size & circular, where as P_4 shows golden yellow color & other colonies isolated were white in color.

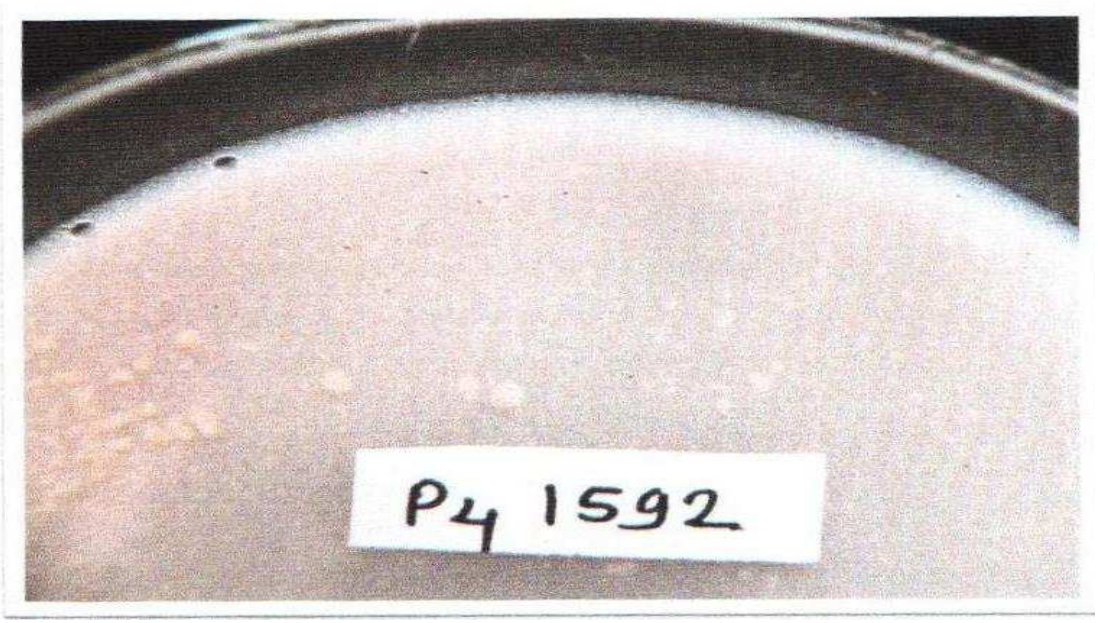
POTOGRAPH NO. -1 –ISOLATION OF P_1



POTOGRAPH NO. -2 –ISOLATION OF P_2



POTOGRAPH NO. - 3 –ISOLATION OF P_3



POTOGRAPH NO. 4- ISOLATION OF P_4

Table 4–Result of Biochemical and Enzymatic Activity

Sr.No.	Isolate	Result HL test		Oxidase	Catalase
		Aerobic Tube	Anaerobic Tube		
1	P_1	+	-	+	+
2	P_2	-	+	-	+
3	P_3	+	-	-	+
4	P_4	+	-	+	+

+ = positive test, - = negative test.

From above table it is seen that, isolates P_1 P_3 P_4 were aerobic & P_2 was anaerobic in nature. The enzymatic studies of all isolates were studied & results are recorded in table no.4. As shown in above table it can be seen that isolates P_1, P_3, P_4 were aerobic & P_1 P_2 was anaerobic. Isolate $P_{1\&}$ P_4 were oxidase positive & P_2, P_3 were negative. All isolates catalase positive. All 4 isolates tentatively identified up to genus level as

Isolate P_1 = *Plesiomonas species*
Isolate P_2 = not identified
Isolate P_3 = *Psedomonas species*
Isolate P_4 = *Staphylococci species*

Determination of MTC. - The maximum tolerance concentration of each isolates was studied & results are cited in table No.5 & as in photograph.

Table 5 – MTC of 4 isolates in gm on nutrient agar

Isolate Coded	MTC of ZnO (gm)
Plesiomonas	3
P2 (Not Indentified)	5
Psedomonas	8
Staphylococci	5

From above table it can be seen that Isolates P_3 has highest MTC for ZnO it is about 8 gm per 100 ml of medium. P1, P2, P4 have moderate MTC.

CONCLUSION

This study gives focus on Isolation of Zinc Bioaccumulating bacteria from Zinc containing waste from Electroplating industry. Total four Zn accumulating bacteria were isolated and studied for their Morphological, Cultural and enzymatic Characteristics. The isolated bacterial species are from Genus *Plesiomonas, Psedomonas, Staphylococci* .After studies on Bioaccumulation with Atomic Absorption Spectroscopy it is observed that among all isolate *Psedomonas* has maximum Bioaccumulation capacity and has highest MTC against Zn as Compared to other genera.

This study lead to the conclusion that, *Psedomonas* is an efficient organism for Zn Accumulation This high Zinc accumulation capacity made such bacterial species well suited for removal of heavy metal from contaminated water, bioleaching, bioremediation of polluted sites and effluent treatment. These types of waste treatment for removal of heavy metal are a cost effective and are Ecofriendly.

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