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CHARACTERIZATION OF HEAVY METAL-INDUCED METABOLIC CHANGES IN NEW STRAIN OF STREPTOCOCCUS THERMOPHILUS ISOLATED FROM HOT WATER SPRING

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Abstract: Thermophiles can be found in almost any ecological niche from fresh and salt water to terrestrial and extreme environments, including metal-contaminated habitats. Most of the strains are able to produce extracellular polymeric substances (EPS) mainly of polysaccharidic nature which serve as biosorbing agents by accumulating nutrients from the surrounding environment and also play a crucial role in biosorption of heavy metals. Structural and compositional characterization of new spp. of *Streptococcus thermophilus* isolated from hot water spring under stressed and normal conditions were analysed. The structural spectroscopic information was considered together with inductively coupled plasma-mass spectrometric (ICP-MS) analytical data on the content of the heavy metal cations (Co^{2+}, Cu^{2+}, Cr^{2+}, Fe^{2+} Zn^{2+}) in the bacterial cells. As bacteria was able to uptake all the five heavy metals from the culture media in significant amount, the metabolic changes in the bacterium was considerably differentiated by IR spectra. Spectra revealed that chemical compounds of various functional groups were involved during accumulation of these heavy metals.

***Index Terms* - Fourier transform infrared spectroscopy; inductively coupled plasma-mass spectrometry; Heavy metal stress**

I. INTRODUCTION

Use of microbes for bioremediation is not limited to detoxification of organic compounds. In many cases, selected microbes can also reduce the toxic cations of heavy metals (such as selenium) to the much less toxic and much less soluble elemental form. Thus, the study on bioremediation of surface water which has significant contamination of heavy metals should be attempted.

Bioremediation, a process mediated by microorganisms, is a sustainable way to degrade and detoxify environmental contaminants. Though bioremediation has been used to varying degrees for more than 60 years, for example petroleum land farming, it historically has been implemented as a very 'black box' engineering solution where amendments are added and the pollutants are degraded. This approach is often successful but all to often the results are less than desirable, that is, no degradation of the contaminant or even production of more toxic daughter products. The key to successful bioremediation is to harness the naturally occurring catabolic capability of microbes to catalyze transformations of environmental pollutants. Simulated experiments using defined microbial consortia in the laboratory is a great starting point in providing crucial initial indication (within certain con- straints) of the process. However, unlike bench-scale simulations, in situ bioremediation in reality is a complex phenomenon involving more than one contaminant and mediated by different strains of microbes involving differ- ent metabolic pathways, across geochemical gradients, geophysical and hydrological complexities.

Recently, modern tools of genomics, transcriptomics, pro- teomics, metabolomics, phenomics, and lipidomics have been applied to investigate systems biology of microbial communities in a myriad of environments. To use a systems biology approach to bioremediation projects they must involve the characterization of microbial community composition, cellular and molecular activity and are complicated by the presence of toxic chemicals that alters the normal behavior of the microbial community.

The efficiency of bioremediation by thermophiles is depended on the presence of biochemical factors present in the cell. These substances may be present as protein, enzyme, EPS (Extra polymeric substances) or biogenic volatile substance. Thus study on enzymes and other biogenic volatile compounds from various thermophilic microorganisms involved in the biodegradation of wide range of pollutants is required to overcome the limitations of their efficient use.

II. AIMS AND OBJECTIVES OF STUDY

The current study presents the involvement of EPS isolated from selected thermophiles with a view to establish their role as central elements in bioremediation of heavy metals.

III. RESEARCH METHODOLOGY

The thermophile isolated from hot water springs of Vajreshwari and Ganeshpuri, Thane, Maharashtra, was studied for the effect of heavy metals on the EPS production. And comparison of EPS production from control and treated isolate was studied qualitatively and quantitatively by FTIR analysis.

3.1 Sampling for isolation of thermophiles :

Water samples were collected from seven different hot springs (from Vajreshwari & Ganeshpuri, Thane) Mumbai. Surface water samples were taken from the Hot Springs using a grab sampler. A 500-ml plastic cup attached to a 2-m pole was dipped into the water twice to rinse it. The sample was then transferred to a clean, new, polyethylene container with a snap-on lid. The temperature of the sample was taken with a laboratory thermometer and recorded. All samples were taken on the same day to prevent discrepancies due to sample date. Samples were kept cool during transport to the laboratory and processed within 12 h of collection **(**Vieille C**. 1**996).

3.2 Media & growth Conditions :

Bacillus Medium described by Postgate (1969) was used for routine stock maintenance and all enrichment culture studies. Bacillus Medium contained (g/ Lit.: Soluble starch – 30.0 g, Agar – 20.0 g, Peptone – 5.0 g, Yeast Extract – 5.0 g, Distilled Water – 1000 ml, pH 7.5 ± 0.2 (45˚C). Colonies were isolated from anaerobic roll tubes (Hungate*et. al.* 1969) containing Medium and 4% (w/v) purified agar. Stock cultures of strain was prepared from single isolated colonies that proliferated on transfer in Media. The stock cultures were incubated at 50°C. Cultures were routinely checked for contamination (Zeikus*et, al.* 1979).

3.3 Bioremediation:

The Strain isolated during the course of study were investigated for their bioremediation activity. The screening was done by using 100ppm of heavy metals and by calculating MTC (Maximum Tolerance Capacity) for the isolate showing tolerance to 5 specific heavy metals i.e. Cd, Cr, Cu, Fe, Zn (5 heavy metals were chosen as these are common pollutant in industrial wastewater).

3.4 Isolation of EPS:

Six day-culture broths (250ml nutrient broth containing test organisms with and without 100 ppm of heavy metals were incubated at 45˚C at 80rpm) were centrifuged (6000 ×g, 30mins, 4˚C), (Fabienne Francois, *et. al.* 2011). The EPS were isolated either (i) from bacterial pellets or (ii) from supernatants.

(i) The cell pellets from the culture were suspended in 10ml saline (20g NaCl/lit. D/W) and were centrifuged (6000 ×g, 30mins, 4˚C). The resulting pellets were suspended in 10ml saline (10g NaCl /liter D/W) and were centrifuged (6000 ×g, 30mins, 4˚C), and stored at 4˚C.

(ii) The supernatants were filtered through Whatman Filter paper no 40. After the addition of cold ethanol (ethanol/filtrate ratio 2:1), the solutions were kept overnight at 4˚C. The EPS precipitates were recovered by centrifugation (6000 ×g, 30mins, 4˚C). The pellets were suspended in water and stored at 4˚C. The protein contents of EPS and total neutral- carbohydrate content were confirmed by FTIR analysis.

3.5 FTIR Analysis:

FT-IR analysis of EPS from selected isolate in the presence and absence of heavy metals salts was carried out. Strong Bioremediation capacity for the heavy metals is a function of chemical structures present on the biomass. FT-IR analysis of the bacteria is required to know and to confirm the chemical bonds that played a role in the Bioremediation of metal.

3.6 Identification of selected strain by 16s rRNA partial sequencing:

The isolated colonies were sequenced for its conserved sequences and analysed for partial 16s rRNA by geneOmbio, Pune, Maharashtra.The predicted 16S rRNA sequences from this study were compared with 16S rRNA sequences in a BLASTable database constructed from sequences downloaded from the Ribosomal Database Project (release 8.1; http://rdp8.cme.msu.edu).. The obtained sequences were deposited in National Centre for Biotechnology Centre and have got specific accession number with specific strain name.

IV. RESULTS AND DISCUSSION

4.1 Characterization of in situ Bioremediation:

Microbial heavy metal reduction at high temperatures was studied at several sites in Vajreshwari & Ganeshpuri hot springs. Enrichment culture was initiated with Bacillus Medium. After incubation for 6 d at *in situ* temperature, the cultures formed a dense colonies (Table 1).

Table 1: Colony characteristics of selected isolates

Isolate	colony characteristics							
	Size	Shape	Colour	Consistency	Opacity	Elevation	Gram nature	Motility
SZP 18	3 mm	Irregular	Dull white	Butyrous	Trans parent	Convex	Gram Positive Cocci	NM

4.2 Bioremediation :

The MTC for the isolated strain for bioremediation of heavy metals was found to be above 1000 ppm, Table2.

Table 2: MTC for heavy Metals

Isolate	Maximum Tolerance Capacity for Heavy metal (concentration in ppm)				
	Cd	Cr	Cu	Fe	Zn
SZP 18	4778	3900	3889	2787	4209

4.3 Mechanism involved in Bioremediation:

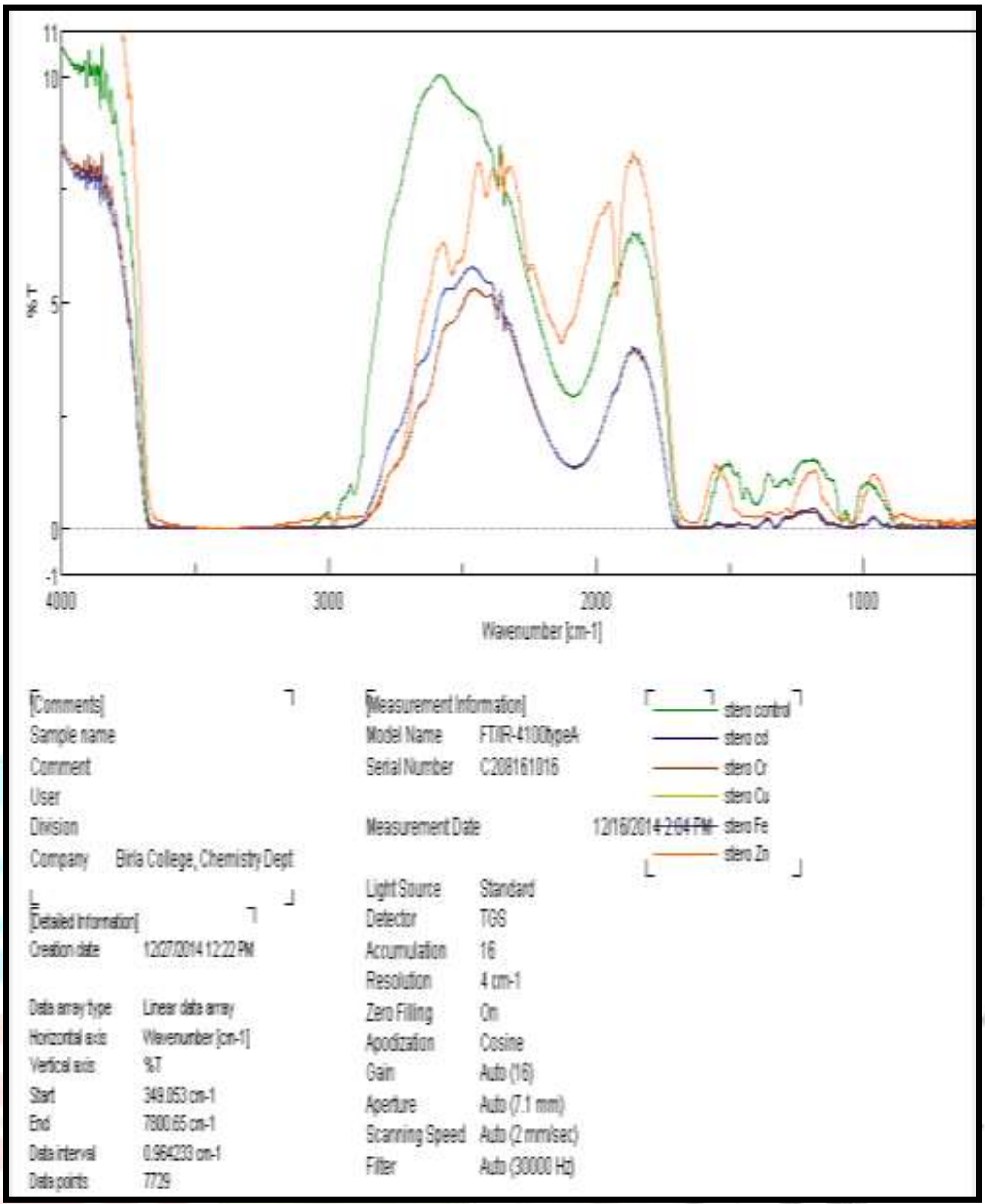
Study of process of EPS- metal binding was carried out to analyze the role of EPS in Bioremediation of heavy metals. Qualitative and quantitative estimation of protein and carbohydrate was carried out to understand amount of EPS produced by the selected isolates, before and after the exposure of heavy metals. FTIR analysis of EPS FT-IR analysis of extracted EPS was carried out and intensity of peaks was compared with that of control spectra.

The most remarkable difference between the control and test spectra was at intensity of 1600-1700 cm^{-1} and 2500 cm^{-1} representing hydroxyl (-OH) and amine (- NH_2) group respectively (Figure 1). This signifies the involvement and changes that occurs during Bioremediation of heavy metals by EPS isolated from the selected isolates.

Similar results were obtained by Sara Pereira (2011), who reported that some cyanobacteria like Gloeothece sp. PCC 6909 and Gloeothece sp. CCY 9612 has ability to remove heavy metals Cu^{2+} and/or Pb^{2+} by producing extracellular polymeric substances (EPS) mainly of polysaccharidic nature. These EPS can remain associated to the cell surface as sheaths, capsules and/or slimes, or be liberated into the surrounding environment as released polysaccharides (RPS).

The isolate SZP 18 showed highest intensity of the peaks for Zn, Cr, Cd and Zn, Fe and Cr. The highest intensity of the peaks indicates that the EPS production during Bioremediation was more (Fig. 1).

Fig. 1: FTIR Spectra for SZP 18



Fabienne François *et al*. (2011), collected and analyzed samples from soil, effluents and river sediments to isolate bacteria having extracellular biosorption capacity for cadmium removal. The seven strains were shown to produce EPS, which were characterized by Fourier transform-infrared (FT-IR) spectroscopy and chemical analysis of neutral-carbohydrate, uronic acid, and protein contents. In the present study, bioremediation of heavy metals by EPS was carried out and it was in accordance with the results shown by Fabienne François *et al.* The results highlight the high potential of selected bacteria for applications in the bioremediation of heavy metals through biosorption onto the biomass surface or secreted EPS.

4.4 Identification of selected thermophiles by 16s rRNA:

The selected strain was sequenced for 16s rRNA. After comparing the sequence using BLAST database, genus and species were confirmed. These sequences were deposited in NCBI database and has been given specific strain name (Table 3).

Table 3: NCBI accession number

Thermophile	Strain name	Accession name
Streptococcus thermophiles (SZP 18)	*ROHANMANALI*	KM527213

>gi|732664276|gb|KM527213.1| *Streptococcus thermophilus* strain *ROHANMANALI* 16S ribosomal RNA gene, partial sequence
TGCAAGTAGAACGCTGAAGAGAGGAGCTTGCTCTTCTTGGATGAGTTGCGAACGGGTGAGTAACGCGTAGGTAACCTG
CCTTGTAGCGGGGGATAACTATTGGAATAGCTAATACCGCATAACAATGGATGACACATGTCATTTATTTGAAAGGGGC
AATTGCTCCACTACAAGATGGACCTGCGTTGTATTAGCTAGTAGGTGAGGTAATGGCTCACCTAGGCGACGATACATAG
CCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCT
TCGGCAATGGGGGCAACCCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTAAG
TCAAGAACGGGTGTGAGAGTGGAAAGTTCACACTGTGACGGTAGCTTACCAGAAAGGGACGGCTAACTACGTGCCAGC
AGCCGCGGTAATACGTAGGTCCCGAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTGATAAGTCTG
AAGTTAAAGGCTGTGGCTCAACCATAGTTCGCTTTGGAAACTGTCAAAGTGCAGAAGGGGAGAGTGGAATTCCATGTG
TAGCGGTGAAATGCGTAGAGGAACACCGGTGGCGAAAGCGGCTCTCTGGTCTGTAACTGACGAGGCTCGAAAGCGTGG
GGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTGGATCCTTTCCGGGATTCA
GTGCCGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGC
CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCCGATGCTATT
TCTAGAGATAGAAAGTTACTTCGGTACATCGGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGG
GTTAAGTCCCGCAACGAGCGCAACCCCTATTGTAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATG
GTTGGTACAACGAGTTGCGAGTCGGTGACGGCGAGCTAATCTCTTAAAGCCAATCTCAGTTCGGATTGTAGGCTGCAAC
T

V. CONCLUSION

The current study on interactions between metals and EPS produced by selected isolate has established the phenomenon of bioremediation of toxic heavy metal by EPS which plays a central role. Study has developed a simple model system which will provide an insight into the basic mechanism(s) of EPS-metal binding, highlight the specific role of each EPS component including proteins and carbohydrates and justify the interaction(s) amongst the components thereof. The study will aid in engineering the extracellular polymeric substances with enhanced characteristics of metal sorption for effective bioremediation of heavy metals of environmental concern.

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