Webology (ISSN: 1735-188X)
Volume 18, Number 6, 2021

Structural And Compositional Characterization Of New Strain Of Geobacillus Stearothermophilus Under Heavy Metal-Induced Stress

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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. The efficiency of bioremediation by thermophiles is depended on the presence of biochemical factors present in the cell; like protein/ enzyme, EPS (Extra polymeric substances) or biogenic volatile substances. The first mechanisms by which bioremediation of toxic substances take place in thermophiles is with the help of protein/ enzymes. Structural and compositional characterization of new strain of **Geobacillus stearothermophilus** isolated from hot water spring under stressed and normal conditions were analysed. The band pattern in protein profiling showed the difference in pattern of protein expression in presence and absence of heavy metals. Some proteins were over expressed in presence of heavy metals and some new bands were formed that indicates important role of proteins/enzymes during stress conditions.

Keywords: Thermophiles, protein profiling, Bioremediation, heavy Metals, Heavy metal induced stress

INTRODUCTION

Microbial action catalyzes metal mobilisation and immobilization, which are important processes in environmental biotechnology. Metal mobilization from ores, mining wastes, or solid residues can be used to recover metals and/or clean up polluted surroundings; additionally, immobilization minimizes metal migration; cleans effluents as well as ground- and surface water; and, finally, can help to condense metals.

In most cases, these procedures have advantages over older technologies, such as being more efficient, cost-effective, and ecologically friendly. Because higher temperatures improve chemical kinetics, it stands to reason that replacing mesophiles with thermophiles or hyperthermophiles will improve bioprocesses (Patil 2015).

The key to successful bioremediation is to use bacteria' naturally occurring catabolic capabilities to catalyse environmental contaminant changes. Simulated trials in the lab utilising defined microbial consortia are a fantastic place to start when it comes to getting an early idea of the process (within specific limits). In situ bioremediation, in contrast to bench-scale simulations, is a complicated event involving several contaminants and mediated by distinct strains of bacteria using diverse metabolic pathways across geochemical gradients, geophysical complexity, and hydrological complexities (Burnat 2009).

Modern genomes, transcriptomics, proteomics, metabolomics, phenomics, and lipid omics technologies have recently been used to examine the systems biology of microbial communities in a variety of settings. Bioremediation initiatives that adopt a systems biology approach must include assessment of microbial community composition, cellular and molecular activity, and are complicated by the presence of toxic substances that disrupt the microbial community's typical behaviour (Baptista 2006).

The presence of biochemical components in the cell determines the efficacy of thermophile bioremediation. Proteins, enzymes, EPS (extra polymeric substances), and biogenic volatile compounds are all examples of these substances. To overcome the constraints of their efficient utilisation, research on enzymes and other biogenic volatile chemicals from various thermophilic bacteria involved in the biodegradation of a wide range of contaminants is necessary (Selle 1997).

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

MATERIALS AND METHODS:

The thermophile isolated from hot water springs of Vajreshwari and Ganeshpuri, Thane, Maharashtra, was studied for the effect of heavy metals on the protein and enzyme production. And comparison of protein production from control and treated isolate was studied by SDS PAGE.

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 closed 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 **1**996).
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 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 1979).

3. **Bioremediation:** The Strain isolated during the course of study were investigated for their bioremediation activity. The screening was done by using 1000 ppm 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).

4. **Isolation of Proteins and identification by SDS-PAGE:** The bacterial cell of both the strains were grown at 45oC for 24 hrs. and the cells were harvested by centrifugation at 13,000 rpm for 10 mins. Later the cell pellet was washed with phosphate buffer (pH 7.0) to remove the traces of remaining media and again centrifuged at 10,000 rpm for 10 mins. The supernatant was discarded. The cell pellet obtained was mixed with 1ml of 2X sample buffer (0.5% SDS, 25% beta mercaptoethanol, 0.03% bromophenol blue, 2.5% Glycerol, 15mM tris HCl (pH 6.8)). The samples were vortexed and incubated in a boiling water bath for half an hour. The samples were used directly for SDS-PAGE analysis (Maiti 2009). The same method of isolation was performed with and without the treatment of heavy metals (control and Test). The most probable protein matching according to molecular weightwas carried out by insilico method using by (Patil 2017) http://web.expasy.orgDatabase .

5. **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 gene Ombio, Pune, Maharashtra. The predicted 16S rRNA sequences from this study were compared with 16S rRNA sequences in a BLAST 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.

RESULTS AND DISCUSSION:

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 1	Pin	Irregular	Colour	Butyrous	opaque	Flat	Gram	NM

	point		less				Positive bacilli	

Bioremediation :

The MTC for the isolated strain for bioremediation of heavy metals was found to be above 1000 ppm, Table 2.

Table 2: MTC for heavy Metals

Isolate	Maximum Tolerance Capacity for Heavy metal (concentration in ppm)				
	Cd	Cr	Cu	Fe	Zn
SZP 1	4998	4387	3456	2446	4299

Mechanism involved in Bioremediation:

Protein Isolation:

The gel showed that in presence of heavy metals some of the protein/enzyme for both the strains are over expressed. These protein were identified by insilico method and most probable protein mathching with the observed results are shown in figure 1 and table 3.

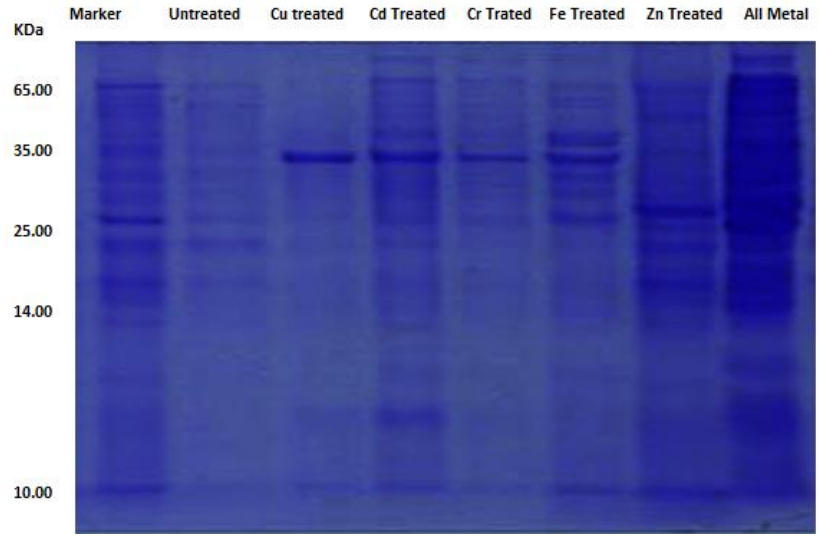

Figure 1 : Protein separation from Geobacillus (SZP 1) by SDS-PAGE

Table 3: The expasy results showed the probable proteins present may be
Geobacillus stearothermophilus BHALPRAVIN (SZP 1). 1. Cadmium accessory protein - 14.01 KD 2. Cadmium transporter - 76.04 KD 3. Metal ion transporter periplasmic protein - 35.67 KD 4. Ferrodoxin - 37.42 KD 5. Zinc Transporter - 28.8 KD

6. Chromate Transporter - 43.17 KD
7. Fe- S Oxidoreductase - 42.42 KD
8. Zn/Fe transporter - 25.75 KD

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 4).

Table 4: NCBI accession number

Thermophile	Strain name	Accession name
Geobacillus stearothermophilus (SZP 1)	BHALPRAVIN	KM527211

>KM527211.1 Geobacillus stearothermophilus strain bhalpravin 16S ribosomal RNA gene, partial sequence

GACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGACCGGATKGGGGCTTGC
YTTGATTCGGT
CAGCGGCGGACGGTAACACGTGGGCAACCTGCCCGCAAGACCGGGATAACTCCGGGAA
ACCGGAGCTAAT
ACCGGATCCGAAGACCGCATGGTCTTCGGTTGAAAGGCGGCCTTTGGGCTGTCACTTGC
GGATGGGCCCG
CGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGGTAGCCGGCCTGAGAGG
GTGCGGCCACA
CTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAA
TGGGCGAAAGC
CTGACGGAGCGACGCCGCGTGAGCGAAGAAGGCCTTCGGGTCGTAAAGCTCTGTTGTGA
GGGACGAAGGA
GCGYCGTTCGAAGAGGGCGGCGCRGTGACGGTACCTCACGAGAAAGCCCCGGCTAACT
ACGTGCCAGCAG
CCGCGGTAATACGTAGGGGGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCA
GGCGGTCTCTT
AAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGGACTT
GAGGGCAGGAG
AGGAGAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAG
TGGCGAAGGCGG
CTCTCTGGCCTGCACCTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGAT
ACCCTGGTAGT
CCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGGTCACACCCTTTAGTGCTGCAGC
TAACGCGATAA
GCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCC
CGCACAAGCGGT
GGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCCCT

GACAACCCAAG
AGATTGGGCGTTCCCCCTTCGGGGGGACAGGGTGACAGGTGGTGCATGGTTGTCGTCAG
CTCGTGTCGTG
AGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCTCTAGTTGCCAGCATTCGGT
TGGGCACTCT
AGAGGGACTGCCGGCGACAAGTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCC
CCTTATGACCTG
GGCTACACACGTGCTACAATGGGCGGTACAAAGGGCTGCGAACCCGCGAGGGGGAGCG
AATCCCAAAAAG
CCGCTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGGAATCGCTAGTA
ATCGCGGATCA
GCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGACTT
GCAACACCCGA
AGTCGGTGAGGTAACCCKYAMGGGAGCCAGCCGCCGAAGGTGGGGCAAGTGATTGGGG
TG

CONCLUSION:

The current research expands the known bioremediation niche in nature to include high (> 60°C) thermal settings. The presence of thermophilic organisms at high temperatures has been linked to bioremediation in the Vajreshwari and Ganeshpuri hot springs. The bacterium was identified using biochemical tests and 16s rRNA analysis. Heavy metal tolerance levels in the isolated strain exceeded 2000 ppm. Protein isolation and characterisation revealed that the required proteins were overexpressed during bioremediation, indicating that many operons are involved in the bioremediation process.

Further studies are required for identification and characterization of the same and for the use of these organisms for bioremediation of other heavy metals. As, the bioremediation of inorganic material is important for health of the people & for monitoring environment, in situ remediation by thermophilic species is one of the best ways for the treatment of industrial effluent containing high levels of heavy metals.

REFERENCES:

1. Baptista, M. S. and Vasconcelos, M. T.(2006). Cyanobacteria metal interactions: requirements, toxicity, and ecological implications. Crit. Rev Microbiol, 32, pp.127–137.
2. Burnat, M., Diestra, E., Esteve, I., Sole, A. (2009). In situ determination of the effects of lead and copper on cyanobacterial populations in microcosms. PLo S ONE 4, e6204.
3. Hungate R. E. (1969). Chapter IV: A roll tube method for cultivation of strict anaerobes. Method. Microbiol. 3, pp. 117–132.

4. Patil S. (2017). Factors responsible for bioremediation in thermophiles. Photon Journal of Microbiology, Photon, 110, pp. 283-286.

5. Patil S. and Unnikrishanan G. (2015). Isolation, characterization and identification of heavy metal tolerating thermophiles from hot water spring. European Journal for Biotechnology and Biosciences, 3(7), pp. 17-22.

6. Postgate, J.R. (1969). Viable Counts and Viability. Methods in Microbiology, 1, pp. 611-628.

7. Selle C, Pohle W., Fritzsche H., (1997). Progress in Fourier Transform Spectroscopy, J. Mink, G. Keresztury, R. Kellner (Eds.), Mikrochim. Acta,14, pp. 449-450.

8. Zeikus, J.G., Hegge, P.W., Anderson, M.A. (1979). Thermoanaerobium brockii gen. nov. and sp. nov., a new chemoorganotrophic, caldo active, anaerobic bacterium. Arch. Microbiol. 122, pp. 41–48.