WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

SJIF Impact Factor 6.647

Volume 7, Issue 1, 592-599 Research Article ISSN 2278 – 4357

FOURIER TRANSFORM INFRARED SPECTROSCOPIC CHARACTERIZATION OF HEAVY METAL-INDUCED METABOLIC CHANGES IN THE THERMOPHILES

Sonali Patil* and Geetha Unnikrishnan

Birla College of Arts, Science and Commerce, Kalyan.

Article Received on
30 October 2017,

Revised on 20 Nov. 2017,
Accepted on 10 Dec. 2017

DOI: 10.20959/wjpps20181-10668

***Corresponding Author**
Dr. Sonali Patil
Birla College of Arts,
Science and Commerce,
Kalyan.

ABSTRACT

Structural and compositional features of cells of thermophiles under stressed and normal conditions were analysed using Fourier transform infrared (FTIR) spectroscopy. The structural spectroscopic information is 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 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 group were involved during accumulation of these heavy metals.

KEYWORDS: Fourier transform infrared spectroscopy; inductively coupled plasma-mass spectrometry; Heavy metal stress.

INTRODUCTION

Thermophiles can be found in almost any ecological niche from fresh and salt water to terrestrial and extreme environments, including metal-contaminated habitats (Burnat *et al*., 2009). Most of the strains are able to produce extracellular polymeric substances (EPS) mainly of polysaccharidic nature comprising polysaccharides, proteins, nucleic acids, uronic acids, humic substances, lipids, etc (De Philippis & Vincenzini, 2003; Pereira *et al*., 2009). Bacterial secretions, shedding of cell surface materials, cell lysates and adsorption of organic constituents from the environment result in EPS formation in a wide variety of free-living bacteria as well as microbial aggregates like biofilms, bioflocs and biogranules. Irrespective

of origin, EPS may be loosely attached to the cell surface or bacteria may be embedded in EPS. Compositional variation exists amongst EPS extracted from pure bacterial cultures and heterogeneous microbial communities which are regulated by the organic and inorganic constituents of the microenvironment. Functionally, EPS aid in cell-to-cell aggregation, adhesion to substratum, formation of flocs, protection from dessication and resistance to harmful exogenous materials. In addition, exopolymers serve as biosorbing agents by accumulating nutrients from the surrounding environment and also play a crucial role in biosorption of heavy metals. Being polyanionic in nature, EPS forms complexes with metal cations resulting in metal immobilization within the exopolymeric matrix. These complexes generally result from electrostatic interactions between the metal ligands and negatively charged components of biopolymers. Moreover, enzymatic activities in EPS also assist detoxification of heavy metals by transformation and subsequent precipitation in the polymeric mass. Although the core mechanism for metal binding and / or transformation using microbial exopolymer remains identical, the existence and complexity of EPS from pure bacterial cultures, biofilms, biogranules and activated sludge systems differ significantly, which in turn affects the EPS - metal interactions.

On the other hand, information about the effects of the metals on the physiology of thermophiles is still scarce particularly for situations of simultaneous exposure to more than one metal, which frequently occur in polluted environments (Baptista & Vasconcelos, 2006; Burnat *et al.*, 2009; Fiore & Trevors, 1994; Heng *et al*., 2004). Thermophilic bacteria can decrease metal uptake by sequestering the metals ions in their extracellular surfaces and/or by releasing ligands to the surrounding environment. Once the metals ions enter the cell, their toxicity can be avoided by their reduction to a less toxic oxidation state, intracellular complexing (e.g. metallothioneins) or metal efflux by export systems, e.g. P-type ATPases (Baptista & Vasconcelos, 2006; Nies, 1999; Roy *et al.*, 2008). When these protection mechanisms fail, the cell experiences the toxic effects of high metal concentration that can cause the disintegration and disorganization of thylakoid membranes, large intra-thylakoidal spaces, increase of polyphosphate bodies and even cell death (Baptista & Vasconcelos, 2006; Nies, 1999).

Therefore, it is urged to develop a simple model system which will provide an insight into the basic mechanism(s) of EPS-metal binding, highlight the functional group of each EPS component and justify the interaction(s) amongst the components thereof. Such an

understanding will aid in engineering the extracellular polymeric substances with enhanced characteristics of metal sorption for effective bioremediation of heavy metals of environmental concern.

Hence, the aim of this work was to evaluate the effects of different heavy metals in the growth/survival of the selected isolates, to extract the EPS, examine EPS qualitatively and quantitatively and also to study their functional group by using FTIR.

Hence, the aim of this work was to evaluate the effects of different heavy metals in the growth/survival of the selected isolates, to extract the EPS, examine EPS qualitatively and quantitatively and also to study their functional group by using FTIR.

MATERIALS AND METHODS

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

1. 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.

2. FTIR Analysis

FT-IR analysis of EPS from all the selected isolates 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. Identification of selected strain by 16s rRNA partial sequencing and evolutionary relationship.

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). Comparisons were made using the program BLAST (ftp://ftp.ncbi.nih.gov/BLAST/executables/LATEST/) and a FASTA-formatted file containing the predicted 16S rRNA sequences.

The obtained sequences were deposited in National Centre for Biotechnology Centre and have got specific accession number with specific strain name. The genotypic relationship between the isolates were studied by constructing a phylogenetic tree by using CLUSTAL W (NCBI service).

RESULTS AND DISCUSSION

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.

Richard *et. al*. (2002) reported that Cu^{+2} and Pb^{+2} seemed to bind to certain groups present on the cell surface. Lead is precipitated in an insoluble form that is localized to the cell membrane or cell surface (Aiking *et al*., 1985: Levinson *et al*., 1996). This could be generally explained by the fact that the negatively charged groups (carboxyl, hydroxyl and phosporyl) of the bacterial cell wall adsorb metal cations through various mechanisms (Chojnacka *et al*., 2005).

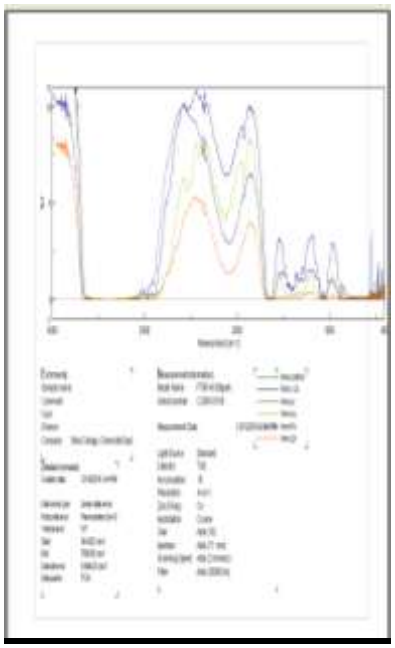
The isolates SZP 4, SZP 8, SZP 12, SZP 16 and SZP 18 showed highest intensity of the peaks for Zn, Cr, Cd and Zn, Fe and Cr, respectively. The highest intensity of the peaks indicates that the EPS production during Bioremediation was more. SZP 4 did not show any change in efficiency of EPS production in case of Cr and Zn. More EPS production was observed for Cd, Cr and Fe by SZP8 and SZP 12 strains showed EPS production in case of Cd, Cu and Zn. While SZP 16 and SZP 18 showed various in intensity of peaks for all the five heavy metals proving that EPS has been over expressed while uptake of heavy metals (Table 1).

Table 1: Summary of involvement of EPS in Bioremediaition of heavy metals.

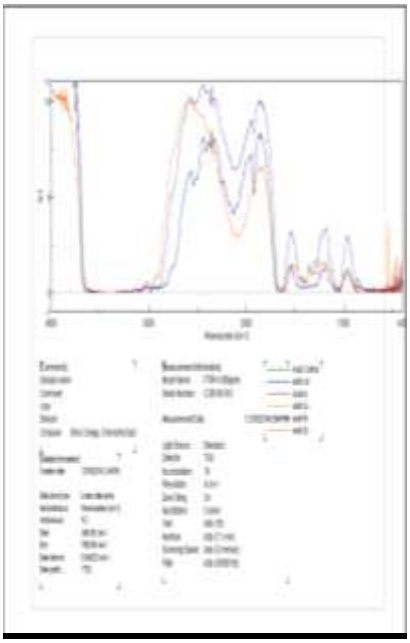
	Cd	Cr	Cu	Fe	Zn
SZP 4	Yes	No	Yes	No	Yes
SZP 8	Yes	Yes	No	Yes	No
SZP 12	Yes	No	Yes	No	Yes
SZP 16	Yes	Yes	Yes	Yes	Yes
SZP 18	Yes	Yes	Yes	Yes	Yes

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.

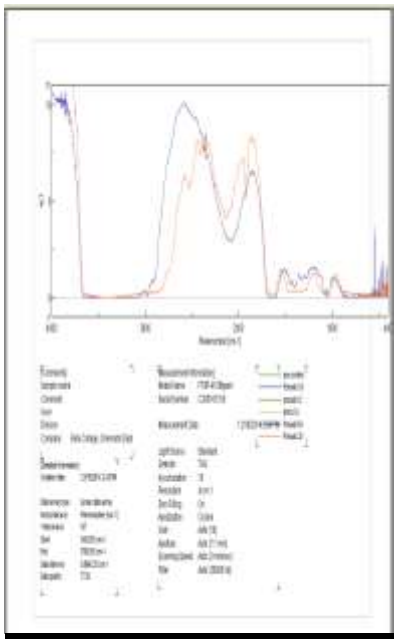
In the present study, bioremediation of heavy metals by EPS was carried out and it was in accordance with the results shown by Fabienne François *et al*. The results highlight the high potential of selected bacteria for applications in the bioremediation of heavy metals through biosorption onto the biomass surface or secreted EPS.



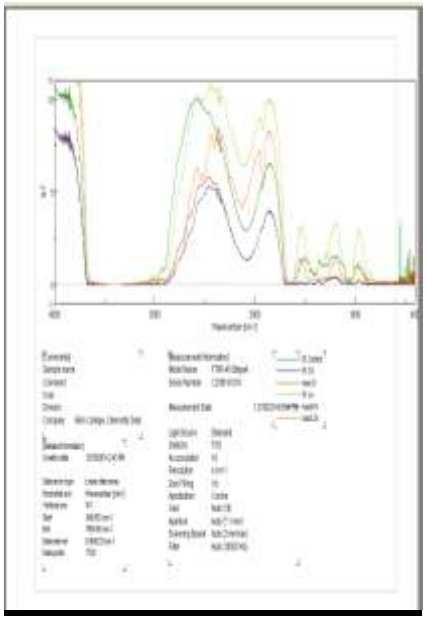
SZP 4



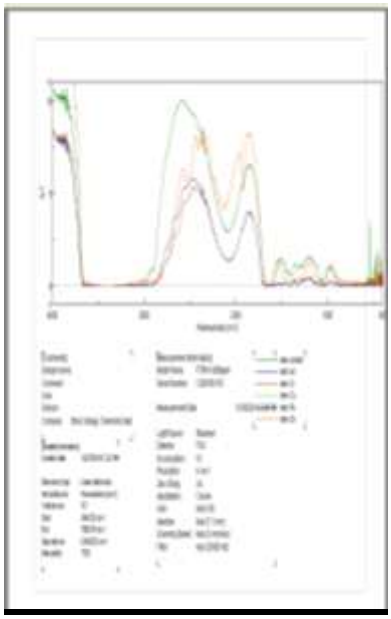
SZP 8



SZP 12



SZP 16



SZP 18

Figure 1: FTIR analysis of EPS extracted from SZP4, SZP8, SZP12, SZP 16 and SZP 18.

Identification of selected thermophiles by 16s rRNA

The selected five strains were 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 2). Phylogenetic and evolutionary relationship were studied using CLUSTAL W (Figure 2).

Table 2: NCBI accession number.

Thermophile	Strain name	Accession name
Acidithiobacillus ferrooxidans (SZP 4)	*ABHAY*	KM527215
Acidiphilium acidophilum (SZP 8)	*POPATNP*	KM527210
Pseudomonas fluorescens (SZP 12)	*SONALIZANKAR*	KM527212
Geobacillus stearothermophilus (SZP 16)	*BHALPRAVIN*	KM527211
Streptococcus thermophiles (SZP 18)	*ROHANMANALI*	KM527213

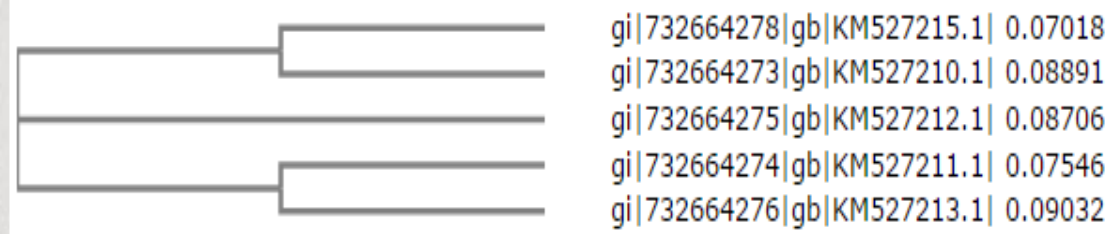


Figure 2: Clustal w of selected five isolates.

CONCLUSION

Above studies on interactions between metals and EPS produced by selected isolates have 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.

REFERENCES

1. Baptista, M. S., Vasconcelos, M. T. Cyanobacteria metal interactions: requirements, toxicity, and ecological implications. *Crit. Rev Microbiol*, 2006; 32: 127–137.
2. Burnat, M., Diestra, E., Esteve, I., Sole, A. In situ determination of the effects of lead and copper on cyanobacterial populations in microcosms. *PLoS ONE 4*, 2009; e6204.
3. C. Selle, W. Pohle, H. Fritzsche, Progress in Fourier Transform Spectroscopy, J. Mink, G. Keresztury, R. Kellner (Eds.), Mikrochim. Acta, 1997; 14 449-450 Supplimentry issue.
4. De Philippis, R., Paperi, R., Sili, C., Vincenzini, M. Assessment of the metal removal capability of two capsulated cyanobacteria, *Cyanospira capsulata* and *Nostoc* PCC7936. *J. Appl. Phycol*, 2003; 15: 155–161.

5. Fabienne, F., Carine, L., Jean-Michel, G., Paul, S., Florence Brian-Jaisson, Grégory M., Manon, Daniel G., Anne-Laure M., David, P., Jean, P., Séverine, Z., Sylvie, R. Isolation and Characterization of Environmental Bacteria Capable of Extracellular Bisorption of Mercury. *Applied and Environmental Microbiology*, 2011; 1097–1106.
6. Fiore, M. F., Trevors, J. T. Cell composition and metal tolerance in cyanobacteria. Biometals, 1994; 7: 83–103.
7. Heng, L. Y., Jusoh, K., Ling, C. H. M., Idris, M. Toxicity of single and combinations of lead and cadmium to the cyanobacteria Anabaena flos-aquae. *Bull Environ Contam Toxicol*, 2004; 72: 373–379.
8. Nies, D. H. c. Microbial heavy-metal resistance in prokaryotes. *FEMS Microbiologcal Reviews*, 1999; 27: 313-339.
9. Patil Sonali, Geetha Unnikrishanan. "Isolation, characterization and identification of heavy metal tolerating thermophiles from hot water spring", European *Journal for Biotechnology and Biosciences*, 2015; 3(7): 17-22.
10. Patil Sonali. "Factors responsible for bioremediation in thermophiles". *Photon Journal of Microbiology*, 2017; Photon, 110: 283-286.
11. Pereira, S., Ernesto, M., Andrea, Z., Arlete, S., Pedro, M., Paula T., De Philippis R. Using extracellular polymeric substances (EPS) producing cyanobacteria for the bioremediation of heavy metals: do cations compete for the EPS functional groups and also accumulate inside the cell? *Microbiology*, 2011; 157: 451–458.
12. Roy, S., Ghosh, A. N., Thakur, A. R. Uptake of Pb2+ by a cyanobacterium belonging to the genus Synechocystis, isolated from East Kolkata Wetlands. *Biometals*, 2008; 21: 515–524.
13. W. Babel, A. Steinbuochel (Eds.), Advances in Biochemical Engineering/Biotechnology Biopolyesters, 2001. Vol. 71, Springer, Berlin, Heidelberg, pp. 51-79 See also pages 81-123, 125-135.