

Vol. 6, Issue 12, December 2017

ISSN: 2319-9490



IJCRLS

Impact Factor 2017:5.379

International Journal of Current Research in Life Sciences

A Peer-Reviewed International
Monthly Research Journal

Journal Indexed in:



VIBUS INNOVATIVE PUBLISHING SOLUTIONS



RESEARCH ARTICLE

DEVELOPMENT OF HEAVY METAL BIOREMOVAL SYSTEM UTILIZING MRB *PSEUDOMONAS STUTZERI*

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Received xxxxxxxx, 2017; Accepted xxxxxxxx, 2018; Published xxxxxxxx, 2018

ABSTRACT

As compared to toxic organics and inorganics, that in many cases can be degraded, the metallic species that are released into the environment tend to persist indefinitely, accumulating in living tissues throughout the food chain. Heavy metal pollution in aquatic ecosystems has become a global phenomenon because these metals are indestructible and most of them have toxic effects on each component of the ecosystem. Most of the fresh water bodies all over the world are getting contaminated thus declining their suitability. In this scenario, monitoring and assessment of such freshwater systems has become an environmental concern. In the state of Gujarat particularly Central Gujarat, South Gujarat and some parts of Western Gujarat many pharmaceuticals industries, dye manufacturing units, electroplating industries, pesticides/ insecticides manufacturing industries are situated. Effluents of these industries are constantly adding heavy metals either in the running water or are discharged in an open land which percolates down and contaminate the ground water and thus pollute the water making it unusable for drinking purpose. This effluent contaminates ground water as well as running water. Removal of heavy metals from the environment is of the immediate importance of the present time. Adsorption or accumulation of heavy metals by microorganisms has received much attention recently due to its potential use in waste treatment processes involving removal of heavy metal pollutants from a contaminated environment. Heavy metal like mercury has threatened the sustainability aquatic biodiversity as well as the quality of human life too. Mercury complexes with organic and inorganic ligands and, is easily adsorbed to surfaces of particulates owing to its high reactivity and affinity to thiol groups. In present research, the effect of thiol group containing compound (Sodium Thiosulphate and Sodium Thioglycolate) was checked on the growth of Bacteria. Some pharmaceutical effluents contain organic compounds such as Phenol, Toluene, along with heavy metals like mercury and cadmium. In the present work MRB (Mercury Resistant Bacteria) were studied for the degradation of aromatic organic compounds especially phenol and Toluene. The isolated MRB have displayed mercury tolerance upto 100 ppm concentrations. The results reveal that MRB have ability to degrade toluene and heavy metal bioremoval capacities can be enhanced by treating them with thiol compounds like sodium thioglycolate. The concentration of Sodium Thioglycolate which is most suitable for bioremoval efficiency of MRB is around 0.1 Gm/L. Heavy metal tolerant bacteria also demonstrate resistant to various traditional antibiotics too. Ecotoxicological studies on selected plant, bacterial and yeast species reveal that the effluent treated with MRB has shown decreased toxicity thereby promoting the better growth of respective species as compared to the growth in presence of untreated effluent.

Key words: Ecosystem, Effluents, Electroplating industries, Ecotoxicological studies, Food chain, Gujarat, Heavy metal pollution, Mercury, Pharmaceuticals industries, Sodium Thiosulphate, Sodium Thioglycolate, Phenol, Toluene.

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Citation: Dr. Mehul P Dave, 2018. "Development of heavy metal bioremoval system utilizing mrb pseudomonas stutzeri" *International Journal of Current Research in Life Sciences*, 7, (03), xxxxxxxx.

INTRODUCTION

Heavy metal pollution in aquatic ecosystems has become a global phenomenon because these metals are indestructible and most of them have toxic effects on each component of the ecosystem. Most of the fresh water bodies all over the world are getting contaminated thus declining their suitability. In this scenario, monitoring and assessment of such freshwater systems has become an environmental concern.

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Mercury complexes with organic and inorganic ligands and, is easily adsorbed to surfaces of particulates owing to its high reactivity and affinity to thiol groups. In present research, the effect of thiol group containing compound (Sodium Thiosulphate and Sodium Thioglycolate) was checked on the growth of Bacteria. Some pharmaceutical effluents contain organic compounds such as Phenol, Toluene, along with heavy metals like mercury and cadmium. In the present work MRB (Mercury Resistant Bacteria) were studied for the degradation of aromatic organic compounds especially phenol and Toluene. The isolated MRB have displayed mercury tolerance upto 100 ppm concentrations.

MATERIALS AND METHODS

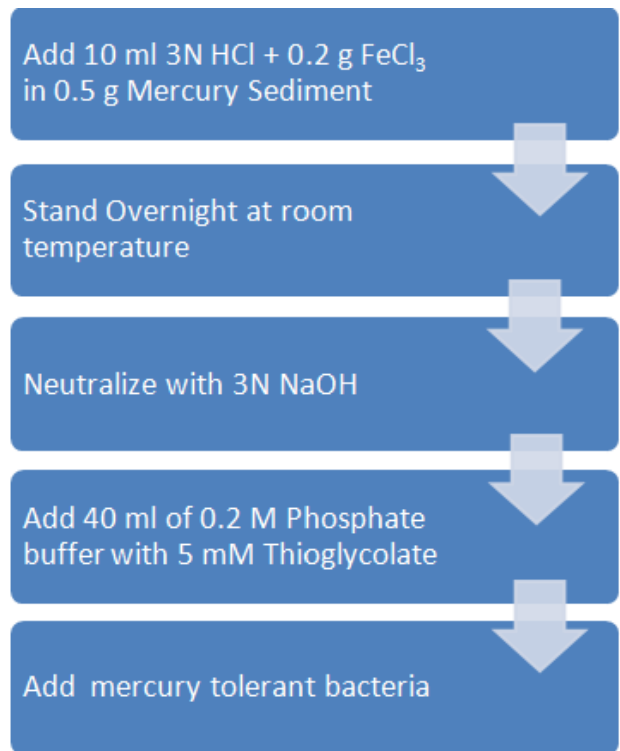
Isolation of MRB from polluted water samples



Identification based on Molecular characterization

- DNA isolation
- 16s rDNA Sequencing
- Amplification of conserved region
- Sequences Generated
- Assembly of sequences generated from 3 Forward Primers
- Assembly alignment
- Consensus generated
- Nucleotide BLAST analysis

Study of effect of thiol compounds like Sodium Thioglycolate, Sodium Thiosulfate and 2-Mercapto Ethanol on heavy metal bio removal. Assessment of the effect of toluene on heavy metal bio removal. Ecotoxicological analysis on plants, bacteria and yeast



Protocol for chemical leaching and determination of bio removal

RESULTS

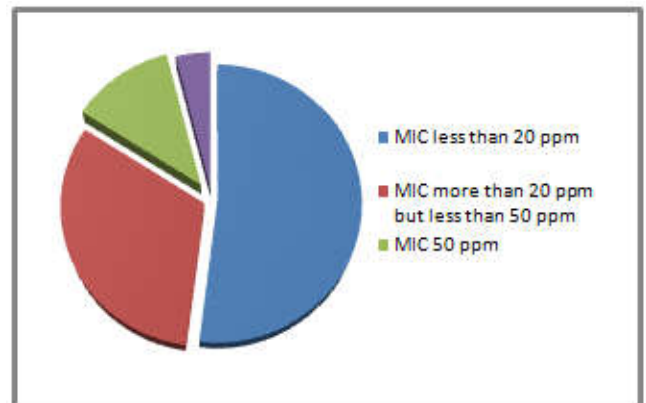


Fig 1. Proportion of various MRB in the effluent sample

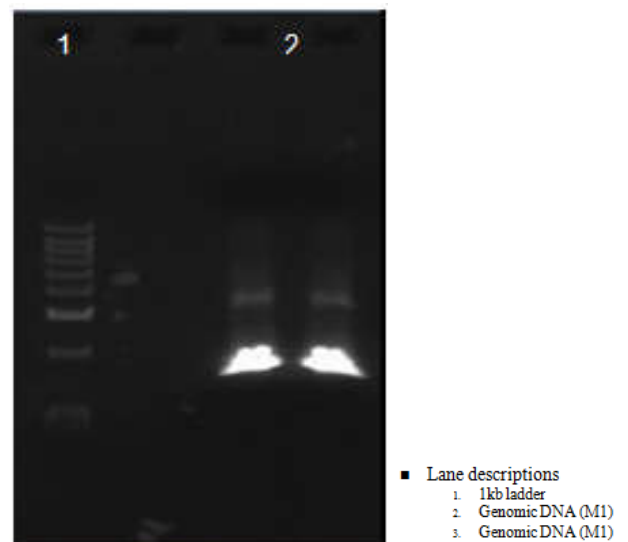


Fig 2. Isolation of Genomic DNA for molecular identification

Table title missing

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
NR_041715.1	<i>Pseudomonas stutzeri</i> ATCC 17588 = LMG 11199 strain ATCC 17588 16S ribosomal RNA, partial sequence	2615	2615	72%	0.0	99%

BLASTN Results

- Identification using FASTA Sequences generated from PCR Product
- (sequences generated using 27F, 530F, 1114F Primers)
- Nucleotide Sequence (1978 letters)
- Molecule Type : Nucleic Acid
- Query Length : 1978 letters
- Database Name TL/16S_ribosomal_RNA_Bacteria_and_Archaea
- Description 16S ribosomal RNA sequences (Bacteria and Archaea)
- Program BLASTN 2.2.26+



Fig 6. Effect of Treated and Untreated effluent on *Trifolium aestivum*

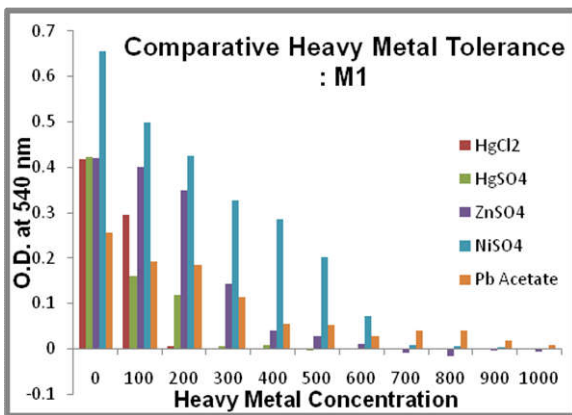


Fig 3. Comparative Heavy Metal Tolerance of isolate M1

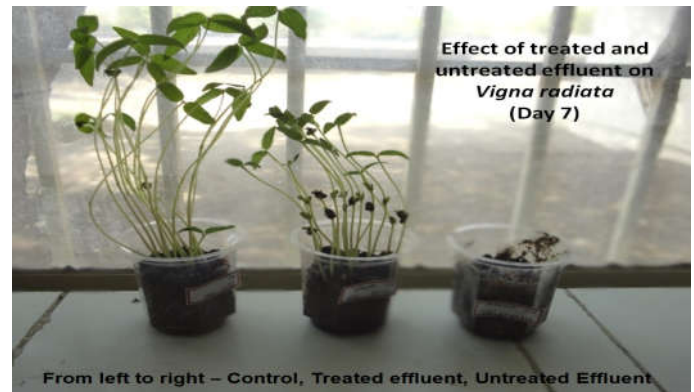


Fig 7. Effect of Treated and Untreated effluent on *Vigna radiata*

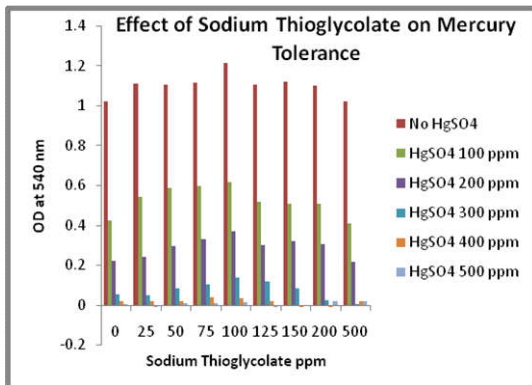


Fig 4. Effect of Sodium Thioglycolate on Mercury Tolerance

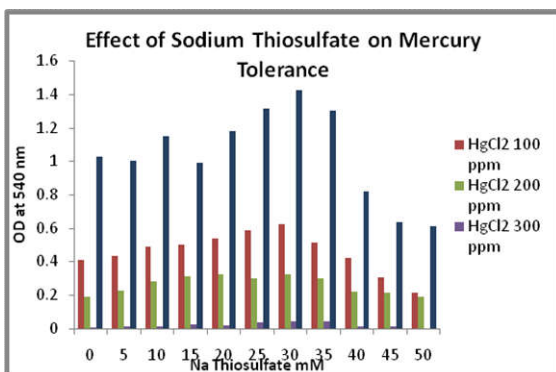


Fig 5. Effect of Sodium Thiosulfate on Mercury Tolerance

Conclusion

- MRB displays tolerance to 100 ppm HgCl₂.
- The MRB is identified as *Pseudomonas stutzeri* by molecular characterization.
- Mercury complexes with organic and inorganic ligands and, is easily adsorbed to surfaces of particulates owing to its high reactivity and affinity to thiol groups. Sodium thiosulphate enhances the mercury tolerance and bioremoval efficiency of MRB.
- 100 ppm (0.1 g/L) Sodium Thioglycolate (C₂H₃O₂SNa, Molecular Weight: 114.1) is the most suitable concentration for the bio removal of mercury from polluted water.
- The organism has tremendous potential for detoxification of polluted industrial effluents. The polluted effluent treated by this organism, could support the growth of plants, yeast as well as bacteria as compared to untreated polluted effluent.

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