



Isolation And Identification Of Heavy Metal-Tolerant Bacteria From Soil Polluted By Industrial Activities In Kanjikode

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Abstract: A fascinating class of microorganisms and plants known as heavy metal resistant organisms have developed special adaptations to survive in environments where high concentrations of heavy metals are present. The goal of the current study was to identify heavy metal-tolerant bacteria from Kanjikode soil samples that had been industrially contaminated and to evaluate the heavy metal resistance of the chosen bacterial isolates. A quantitative analysis of heavy metal-tolerant organisms was also conducted using morphological, biochemical, molecular identification, and assessment of heavy metal-tolerance in order to isolate and characterize the total heterotrophic population. *Bacillus flexus* and *Bacillus aryabhattai* were identified as the two chosen bacterial isolates following 16S RNA sequencing. Because of their resistance to and potential for degrading heavy metals, the identified bacteria suggest potential for bioremediation efforts. As per previous studies, the potential of *Bacillus flexus* was maintained in arsenic-contaminated environments, whereas *Bacillus aryabhattai* promotes plant development and has a high resistance to heavy metals. The present investigation presents light on heavy metal resistant soil bacteria in industrial pollution scenarios, revealing possible uses in bioremediation methods in the future.

Index Terms - Heavy metal resistance, *Bacillus flexus*, *Bacillus aryabhattai*, Industrial pollution.

I. INTRODUCTION

Steel factories are notable sources of soil pollution, releasing dust and gases containing metals like iron into the environment. While iron is crucial for many living systems as a cofactor for enzymes, its high concentrations can hinder microorganisms due to the production of free radicals. In polluted soil from steel factories, various elements affect the microbiological aspect differently. Iron, abundant and essential, can hinder microbial growth due to high concentrations catalysing free radicals. Steel production emits dust particles that alter soil pH and accumulate metals like iron, impacting microbial composition and processes [1, 2]. Additionally, solid and liquid wastes contain heavy metals such as cadmium, zinc, lead, manganese, posing health risks and further disturbing microbial ecosystems. As a result, the microbiological aspect of polluted soil experiences disruptions, necessitating comprehensive assessments to understand and mitigate ecological risks.

Heavy metal pollution of soil has been recognized as major factor impeding soil microbial processes. The existing regulatory values regarding soil pollution with heavy metals are mainly design to protect the food chain and to control the risk of excessive accumulation of metals in plants. Recently, there has been a growing interest in protecting soil habitat function, which requires a quantitative assessment of toxicity factor influencing soil processes mediated by microorganisms. Soil contamination by heavy metals is of most important apprehension throughout the industrialized world. Heavy metal pollution not only result in adverse effects on various parameters relating to plant quality and yield but also cause changes in the size, composition and activity of the microbial community. Therefore, heavy metals are considered as one of the major sources

of soil pollution. Heavy metal pollution of the soil is caused by various metals especially Cu, Ni, Cd, Zn, Cr, and Pb. [3]

The adverse effects of heavy metals on soil biological and biochemical properties are well documented. The soil properties i.e. organic matter, clay contents and pH have major influences on the extent of the effects of metals on biological and biochemical properties. In response to heavy metal presence, certain microorganisms, particularly bacteria, have developed mechanisms to resist and tolerate these toxic substances. These metal-resistant bacteria are essential in bioremediation, detoxifying contaminated environments. Understanding their significance is crucial for developing effective strategies to mitigate heavy metal pollution and its adverse effects. Metal toxicity to humans is a growing environmental concern, with human activities mobilizing a total toxicity of metals surpassing that of radioactive and organic wastes combined. [3]

Heavy metal resistant organisms employ various mechanisms to combat the toxic effects of heavy metals. One common strategy is metal sequestration, where the organism binds the metals intracellularly, preventing them from interfering with essential cellular processes. Another mechanism involves the production of metal-chelating molecules such as phytochelatins and metallothioneins, which bind to heavy metals and facilitate their detoxification or removal from the cell. Additionally, some organisms possess efflux pumps that actively pump heavy metals out of the cell, reducing their intracellular concentration. Furthermore, certain microbes can enzymatically transform toxic heavy metals into less harmful forms through processes like reduction or oxidation. These diverse mechanisms collectively enable heavy metal resistant organisms to thrive in contaminated environments and play crucial roles in biogeochemical cycles. [4] The present study aimed to isolate and characterization of heavy metal resistance bacteria from Kanjikode industrial area by morphological, biochemical, molecular identification and assessment of heavy metal tolerance.

II. MATERIALS AND METHODS

2.1 Sample collection

20g soil sample each were collected from five different locations of Kanjikode steel industrial area, Palakkad district Kerala by appropriate collection procedure. The samples were designated as IFS1-5 respectively.

2.2 Enumeration of Total Heterotrophic population

The total heterotrophic population of soil refers to the number of organisms in the soil ecosystem that use organic carbon as an energy source. The method of estimation begins with small amounts of soil that is serially diluted in water. Then, a portion of the diluted soil suspension is distributed on the surface of an agar plate. The agar acts as a substrate for microbial development, enabling for the identification of microorganism in the soil sample. [5]

2.3 Morphological and biochemical characterization

Gram's staining was performed for the colonies obtained. IMViC, Catalase and Oxidase tests were performed for the selected isolates. [6]

2.4 Analysis of heavy metals in soil

The collected industrially polluted soil sample were analysed for the presence of heavy metals by atomic absorption spectrophotometer (acid digestion method).

2.5 Screening of heavy metal resistant isolates

The antimicrobial assay was determined using the well diffusion method. Petri plates were then incubated at 37°C for 24 hours, after which zone formed by the bacterial strains doesn't have the ability to resist that heavy metal. If there is no zone, which meant the organism had heavy metal resistant capability. [7]

2.6 16S rRNA sequencing for bacterial identification

The PCR amplification of the 16S rRNA gene is a fundamental technique for bacterial identification and phylogenetic studies. The process begins with the extraction of DNA from a pure bacterial culture. For more detailed protocols and procedures, researchers refer to specialized literature and databases in the field.

III. RESULTS AND DISCUSSION

Soil Contamination in Steel industrial areas such as Heavy metals released during industrial activity, particularly in steel factories, can cause soil contamination. Copper, lead, zinc, cadmium, chromium, nickel, arsenic, and mercury are all common contaminants found around steel mills, according to studies. These metals can offer serious health problems because they can enter the food chain, affecting both soil quality and food safety. [8]

In the present study, five soil samples of 20g each were collected in sterile zip lock bags from the

industrial area of Kanjikode, Palakkad, Kerala. The soil texture was clay-type and black in colour. After enumeration of industrial polluted soil samples in IFS5- Five different types bacterial colonies were observed (Fig. 1).



Fig.1. THP of IFS5 sample

From IFS5 sample five different bacterial colonies were observed, designated as IFS5A-E and subcultured on Nutrient agar plates (Fig.2a-e). IFS5B, IFS5C and IFS5D were observed as Gram positive rod shape. IFS5A was observed as Gram positive cocci and IFS5E was observed as gram negative (Table. 1) IFS5 B and IFS5 D were selected for further studies as they were predominantly seen in mother culture plate.

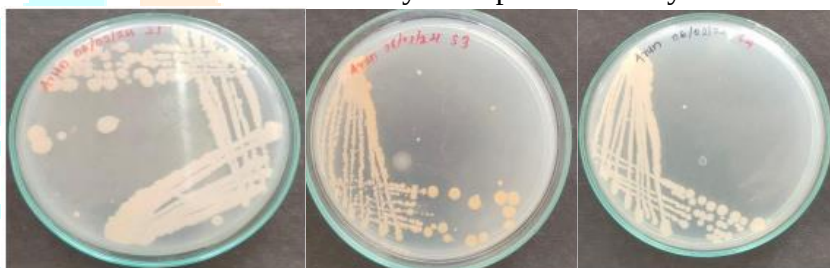


Fig.2a

Fig.2b

Fig.2c

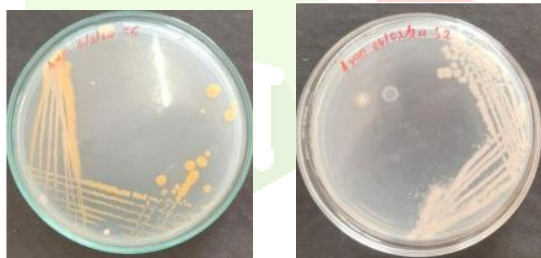


Fig.2d

Fig.2e

Table 1. Morphological Identification of selected isolates

Bacterial Isolate	IFS5 A	IFS5 B	IFS5C	IFS5 D	IFS5 E
Colony morphology	Whitish colony	Opaque, creamish raised margin colonies	yellowish	Non pigmented	whitish appearance
Gram staining	Gram positive	Gram positive	Gram positive	Gram positive	Gram negative
Shape	Cocci in chain	Rod shape	Rod shape	Rod shape	Rod shape

Analysis of heavy metals in IFS5 soil sample

The collected soil samples were analyzed for the presence of heavy metals by atomic absorption spectrophotometer (acid digestion method) The test report showed that the soil contains heavy metals such as zinc, lead, manganese (Table 2)

Table 2. Soil Analysis

Sl. no.	Parameters	Test Method	Unit	Test result	Limit of Quantification(<LOQ)
1	Zinc (Zn)	By Atomic absorption spectrophotometer (Acid digestion method)	mg/kg	137	0.10
2	Lead (Pb)	By Atomic absorption spectrophotometer (Acid digestion method)	mg/kg	17.6	0.50
3	Manganese (Mn)	By Atomic absorption spectrophotometer (Acid digestion method)	mg/kg	1731	0.50
4	Mercury (Hg)	By Atomic absorption spectrophotometer (Acid digestion method)	mg/kg	<LOQ	0.10
5	Arsenic(As)	By Atomic absorption spectrophotometer (Acid digestion method)	mg/kg	<LOQ	0.10

Screening of heavy metal-resistant bacteria for zinc, lead, manganese

After incubation period no zone formation was observed i.e., IFS5B and IFS5D were resistant against zinc, manganese and lead. (Fig 3a,b,c and Fig. 4a,b,c).



Fig 3a



Fig 3b

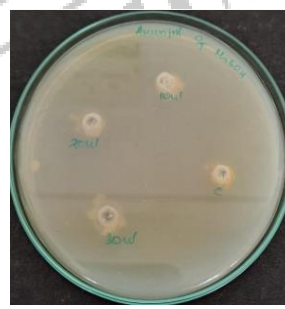


Fig 3c



Fig 4a



Fig 4b

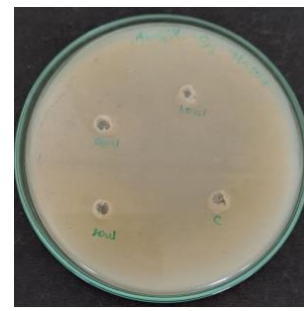


Fig 4c

16S rRNA Sequence of the Isolate IFS5 B

>Contig - S2

CTCACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGAC
ACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAGTCTGACG
GAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACA
AGTACAAGAGTAACTGCTTGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTG
CCAGCAGCCGCGGTAATCGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCG
CAGGCGGTTTCTTAAGTCTGATGTGAAAGCCACGGCTCAACCGTGGAGGGTCATTGGAAACT
GGGGAAGTTGAGTGCAGAAGAGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGA
TGTGGAGGAACACCAGTGGCGAAGGCGGCTTTTTGGTCTGTAAGTACGCTGAGGCGCGAAAG
CGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTG
TTAGAGGTTTCCGCCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGT
CGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCCGACAAAGCGGTGGAGCATGTGGTTTA
ATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACTCTAGAGATAGAG
CGTTCCTTCCGGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCTGTGAGA
TGTTGGGTAAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTTAGTTGGGCAC
TCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCC
TTATGACCGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTGCAAGACCGCGAGGTCA
AGCCAATCCCATAAAACCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTG
GAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTAATACGTTCCCGGGCCTTGTACACACCG
CCCGTCAC

Result: *Bacillus flexus*

Phylogeny tree

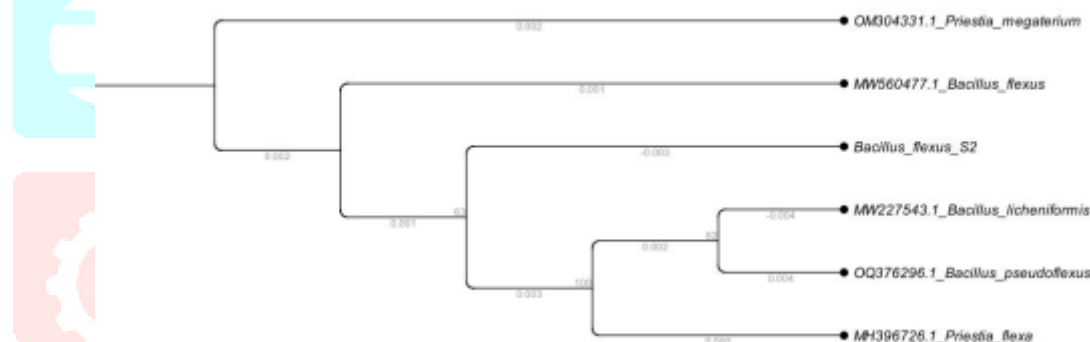


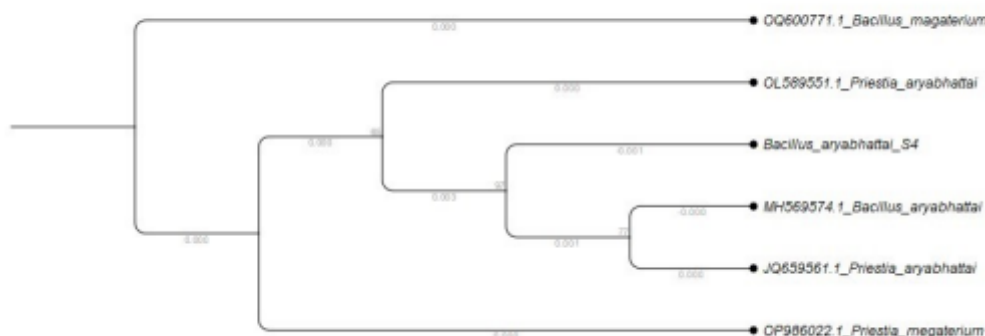
Fig. 5 Phylogeny tree of *Bacillus flexus*

16S rRNA Sequence of the Isolate IFS5 D

>Contig - S4

GAATCCTGGCTCAGGATGAACGCTGGCGCGTGCTAATACATGCAAGTCGAGCGAACTGATTA
GAAGCTTGCTTCTATGACGTTAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCTGTAA
GACTGGGATAACTTCGGGAAACCGAAGCTAATACCGGATAGGATCTTCTCCTTCATGGGAGAT
GATTGAAAGATGGTTTCGGCTATCACTTACAGATGGGCCCCGCGGTGCATTAGCTAGTTGGTGA
GGTAACGGCTCACCAAGGCAACGATGCATAGCCGACCTGAGAGGTGATCGGCCACACTGGGA
CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAA
GTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGG
GAAGAACAAGTACGAGAGTAACTGCTCGTACCTTGACGGTACCTAACCAGAAAGCCACGGCT
AACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGT
AAAGCGCGCCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCACGGCTCAACCGTGGAGGGTCA
TTGGAAACTGGGGAAGTTGAGTGCAGAAGAGAAAAGCGGAATTCCACGTGTAGCGGTGAAAT
GCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTTTTGGTCTGTAAGTACGCTGAG
GCGCGAAAGCGTGGGGAGCAAACAGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAG
TGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCTAACGCATTAAGCAC

Phylogeny tree



After 16 S rRNA sequencing the IFS5 B and IFS5 D was found to be *Bacillus flexus* and *Bacillus aryabhattai* respectively. (Fig 5 and 6).

Heavy metal-resistant organisms exhibit remarkable adaptations to thrive in contaminated environments, offering insights into resilience mechanisms and potential applications in bioremediation. These organisms employ diverse strategies such as metal sequestration, metal-chelating molecule production, and efflux pumps to combat metal toxicity. *Bacillus flexus* has been examined for its ability to withstand and degrade heavy metals in the environment. For example, a strain of *Bacillus flexus* known as SSAI demonstrated remarkable resistance to arsenite and was discovered to include genes responsible for the oxidation of highly poisonous arsenite to less deadly arsenate. This demonstrates *Bacillus flexus* potential in bioremediation attempts to clear up arsenite contaminated areas. [9]

Bacillus aryabhatai is known for its plant growth-promoting properties and capacity to flourish under heavy metal stress. It produces siderophores and exopolysaccharides, which can block the flow of harmful ions and regulate ionic balance and water transport in plant tissues. Furthermore, a specific strain, *Bacillus aryabhatai* PM34, demonstrated high tolerance to heavy metals such as nickel. [10] *Bacillus flexus* and *Bacillus aryabhatai* exemplify the remarkable resilience of microorganisms in heavy metal-contaminated environments. Their unique adaptations not only enable them to survive under metal stress but also position them as promising candidates for bioremediation applications. With their ability to degrade harmful metals and support plant growth, these bacteria offer valuable solutions for addressing industrial pollution and improving environmental health.

IV. CONCLUSION

Bacillus flexus and *Bacillus aryabhatai*, two heavy metal-resistant bacteria that were isolated from industrially polluted soils, are highlighted in this study for their potential. These bacteria may be useful in bioremediation because of their special adaptations that allow them to survive high concentrations of heavy metals. In environments contaminated with arsenic, *Bacillus flexus* exhibits promise, whereas *Bacillus aryabhatai* may promote plant growth and provide resistance to a variety of heavy metals. This study emphasizes how important soil bacteria are to reducing industrial pollution and developing bioremediation techniques.

V. ACKNOWLEDGMENT

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