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Identification and characterization of heavy metal-resistant *Pseudomonas aeruginosa* and its potential for bioremediation

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ABSTRACT

The present study deals with isolation, identification and characterization of heavy metal resistant bacteria isolated from sewage water collected in and around Trichy district, South India. Initially, among 26 of the total isolates screened from sewage water, one isolate was selected for study based on high level of heavy metal resistances. On the basis of morphological, biochemical, 16S rDNA gene sequencing and phylogeny analysis revealed that, the isolate was authentically identified as *Pseudomonas aeruginosa*. The sewage isolate exhibited resistance to Silver (Ag), Copper (Cu) and Zinc (Zn). The maximum tolerance concentration (MTC) of the isolate against Ag, Cu and Zn was determined in solid media. The uptake of heavy metals, present in sewage and detoxification of metal ions by bacteria provide an additional mechanism of environmental bioremediation. The identified heavy metal resistant bacteria could be useful for the bioremediation of heavy metal contaminated sewage and waste water.

Key words: Heavy metals; copper; silver; zinc; metal tolerance

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INTRODUCTION

Heavy metal pollution of soil and wastewater is an important environmental problem that has gained significance[1]. Many metal ions are essential as trace elements, but at higher concentrations, they become toxic. Such heavy metals are not easily removable from the environment and are also indestructible, unlike many other pollutants that can be chemically or biologically degraded[2]. Hence, heavy metals constitute a global environmental hazard[3]. Bacteria develop heavy-metal resistance mostly for their survivals, especially a significant portion of the resistant phenomena was found in the environmental strains (with or without the presence of heavy metals). These bacteria possessing plasmids carry the genes for metal resistance, and since such traits are plasmid borne, they can be easily transmitted from one bacterium to an entire population. Such metal tolerable bacteria are currently favored for their capacity to depollute the environment and hence regarded as instruments for bioremediation. This has made the study of metal resistant significant. Among the several bacteria studied, *Pseudomonas sp* is well known for the capacity to exhibit resistance due to efflux mechanism, hence this study was undertaken to screen for the resistance of the bacteria for the three metals chosen.

MATERIAL AND METHODS

Isolation of bacteria

The sewage water samples were collected in and around Trichy district, South India. The samples were collected in sterile glass container and transported to laboratory for bacteriological analysis. The bacterial isolates were screened on Luria Bertani (LB) agar plates supplemented with 5 mg/l concentration of each metal one time by the standard pour plate method. Plates were incubated at 30^{o}C for 5 days and colonies differing in morphological characteristics were selected and used for further studies.

Identification and characterization of the sewage bacteria

Selected sewage isolates were grown on MacConkey agar media (HiMedia, India). The shape and colours of the colonies were examined under the microscope after Gram staining. Isolates were biochemically analyzed for various biochemical tests. The tests were used to identify the isolates according to Bergey's Manual of Systematic Bacteriology[4].

Determination of the Effect of Metals on Bacterial Growth

Toxicity of the selected metal to the bacterial isolates was determined using 10µg/ml concentration of the metal. 48 well sterile polystyrene microplates was used in this study as growth vessels. Sterile NB was amended with heavy metal and inoculated with exponentially

growing cultures (24 h old, optical density of 0.090 at 600 nm) of bacterial isolates prepared in the same medium. Medium without metal but the bacterial inoculum (bacterial growth control) and medium with metal but without bacteria (abiotic control) served as controls. All the experiments were conducted in triplicates. Bacterial growth was measured in terms of optical density at 600 nm at 0hr, 24 hrs and 48 hrs respectively.

Maximum tolerable concentrations

MTC of heavy metals

To determine the Maximum Tolerable Concentration (MTC) of the metal, several dilutions of the metal salt was prepared based on preliminary screening (1µg, 5 µ, 10 µg , 25 µg , 50 µg , 75 µg , 100 µg). To each set, a bacterial culture was inoculated and plates were incubated and observed for growth by streaks on Nutrient agar plates. Strains that could not grow on 1.0 µg were termed as sensitive to the metal, while that which grew in 50 µg were further tested for higher concentration.

16S rDNA gene amplification

Genomic DNA was isolated and analyzed from sewage bacteria by the method of Chen and Kuo.[5] Bacterial 16S rDNA was amplified by using the universal bacterial 16S rDNA primers, F (5 '- AGA GTT TGA TCC TGG CTC AG – 3') and R (5'- GGT GTT TGA TTG TTA CGA CTT - 3'). PCR was performed with a 50-µl reaction mixture containing 1-µl(10ng) of DNA extract as a template, each primer at a concentration of 5 mM, 25 mM MgCl2 and dNTPs at a concentration of 2 mM, as well as 1.5 U of *Taq* polymerase and buffer used as recommended by the manufacturer (Fermentas, Hanover, Germany). PCR products were analyzed by 1.5% (w/v) agarose gel electrophoresis in 1x TAE buffer with ethidium bromide (0.5 µg/ml).

Sequence of 16s rDNA

TGGCCAGTTTCGAGCGGATGAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGACTA
ATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCA
TACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGT
CGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAG
AGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTG
GGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTC
TTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTT
TGACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAA
GGGTGCAAGCGTTAATCGGAATTACTGGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGT
TGGATGTGAAATATCCCGGGCTCGGCCTGGGTAACTGCATCCAAACCTACTGACTTAGAG

TTCGTCAGAGGGTGGNGGAATTTCCTGTGTAGGGGTGAAATGCGAGATTTAGGTAGGAAC
CACCGTTGGCAAAGGCGACCCCCTGGACTGATCTTGAC

Nucleotide sequencing

Sequences were matched with previously published bacterial 16S rDNA sequences in the NCBI databases using ADVANCED BLAST[6]. Based on the scoring index the most similar sequences were aligned with the sequences of other representative bacterial 16S rDNA regions by using ClustalX software version 1.83.[7]

RESULTS AND DISCUSSION

In the present study we identified and characterized heavy metal resistant bacteria isolated from sewage water. Among the several isolates, only one exhibited remarkable activity and was hence chosen for the study.

Comparative analysis of the 16s rDNA sequences with already available database showed that the strain was *Pseudomonas aeruginosa* (98% similarity to *Pseudomonas aeruginosa* PD100, accession number AY 025034). Phylogeny based on ClustalX clearly indicates that S1 strain is *Pseudomonas aeruginosa.*

To find out whether metal tolerance mechanism was inducible or not, growth with respect to OD600 were obtained and the presence of copper sulphate was stimulating the growth of *Pseudomonas sp*, while the other two compounds showed an initial increase followed by a dip in the growth.

The chosen isolate, (*Pseudomonas aeruginosa*) was seen to grow upto 1200µg of Zinc sulphate, while 1500µg of Silver nitrate could be tolerated. It was surprising to note that even 2100µg of copper sulphate could not inhibit the growth of *Pseudomonas sp* (Table 1& 2).

Table 1: MTC of heavy metals by *Pseudomonas aeruginosa*

Conc. of metal	AgNO3	Zinc SO4	CuSO4
300µg	+	+	+
600µg	+	+	+
900µg	+	+	+
1200µg	+	+	+
1500µg	+	-	+
1800µg	-	-	+
2100µg	-	-	+
2400µg	-	-	-
2700µg	-	-	-
3000µg	-	-	-

Table: 2 Growth of bacteria treated with silver, copper and zinc salts at 100μg/ml

Organism	OD at 600nm		
	0hr	24hr	48hr
Silver nitrate	0.276	0.728	0.176
Zinc Sulphate	0.03	0.17	0.25
Copper sulphate	0.16	0.36	0.37

Heavy metals are found in natural environments and are stable and persist as environmental contaminants since they are nondegradable.[8] Few metals are required in trace amounts as nutrients, also as coenzymes, many inhibit for microbial growth at relatively low concentrations[9,10]. Microorganisms and microbial products can be highly efficient bio-accumulators of soluble and particulate forms of metals especially dilute external solutions. The ability of microbial stains to grow in the presence of heavy metals would be helpful in the biological treatment where microorganisms are directly involved in the decomposition of organic matter[11].The present results show *Pseudomonas* species to be highly resistant to all the three metals tested and hence such bacteria could be utilized for detoxification and removal of heavy metals from polluted environment[12]. Thus this pilot study suggests the use of *Pseudomonas sp* as a tool for bioremediation (Figure 1).

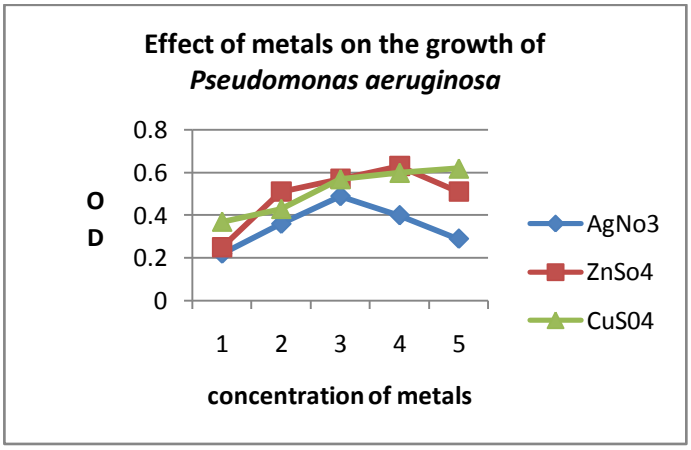

Figure 1: Effect of metals on growth of *Pseudomonas aeruginosa*

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