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A Preliminary Study to Evaluate *S. warneri* MF612183 Isolated from Tannery Effluent for Bioremediation of Heavy Metals

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ABSTRACT

Tannery effluent, a major source of water pollution, contains solids, nitrogen, sulphides, neutral salts and heavy metals as major components of pollutant. In the present study Staphylococcus warneri isolated from tannery effluent was evaluated for its ability to reduce the levels of four selected heavy metals; Zinc, Copper, Chromium and Lead. The DNA was isolated from the strain and its 16srRNA have been amplified. The PCR product was sequenced and the amplified product was found to be 1002bp. The isolate was found to be Staphylococcus warneri and the sequence was submitted to genbank and accession number obtained is MF612183. The isolate was grown in nutrient broth along with added heavy metals for about 4 days. The culture was centrifuged and its supernatant was analyzed for heavy metal reduction for every 24hrs. The isolate was found to reduce Zinc, Copper, Chromium and Lead at the end of fourth day by 95%, 86%, 74.6% and 70% respectively at 96 hours.

Key words: *Staphylococcus warneri, Bioremediation, Heavy metals, Tannery effluent.*

INTRODUCTION

Tannery industries are recognized as serious environmental threat, as a variety of chemicals is used in the tanning process along with large quantities of water which are called as effluents. These effluents from tanning industries contain heavy metals and are usually discharged into the soil and local water bodies which are used for irrigation purposes. Heavy metal contamination has been increased since the early 20th century. Presence of heavy metal beyond its tolerance limit makes water unsuitable for crop growth by inhibiting the seed germination and seedling growth. Water contaminated with heavy metals affects not only the plant growth and yield and also their accumulation in plants are biomagnified at different trophic levels through food chain. The various remediation technologies currently used range from *in situ* vitrification and soil incineration to excavation and land filling, soil washing, soil flushing and solidification and stabilization by electrokinetic systems.

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These physicochemical methods of remediation have certain disadvantages associated with it; thus bioremediation- an environmentally friendly approach is used as an alternative to these methods[15]. Bioremediation can be done by using plants, microbes or by the combination of the two. There are a number of microorganisms found to remove the heavy metals from environment such as bacteria, fungi, yeast and algae[14,13]. In the present study *Staphylococcus warneri* isolated from tannery effluent was evaluated for bioremediation of the selected heavy metals- Zinc, Chromium, Lead and Copper.

MATERIAL AND METHODS

Isolation and characterization of heavy metal reducing bacteria

Tannery effluent was collected from the industry and was serially diluted, plated on nutrient agar and incubated for 24hrs at 37°C. A single colony was selected and was grown in Nutrient broth and was found to have 17×10^{-5} CFU/ml. The Culture was gram stained[16], and the biochemical characterization was done.

DNA extraction, 16SrRNA gene amplification and sequencing

DNA was isolated from the 16hrs old bacterial culture using phenol/chloroform method and was amplified for 16s rRNA region using the primers F (5'-GAGTTTGATCATGGCTCAG-3') & R (5'- CTACGGCTACCTTGTTACG-3'). The amplified product was sequenced and the obtained sequence was aligned with the closely related strains in GenBank database using BLAST- N.

Heavy metal reduction studies:

The isolate with 17×10^{-5} CFU/ml was used for the heavy metal reduction studies. The colony was grown in Nutrient broth along with heavy metals- Zinc (1ppm), Lead (30ppm), Chromium (150ppm) and Copper (1ppm). The culture was collected regularly at 0^{th}, 24^{th}, 48^{th}, 72^{nd} and 96^{th} hour. The culture was centrifuged and the supernatant was analyzed for the heavy metals – Zinc[3], Lead[8], Chromium[6], and Copper[4].

Results

Isolation and characterization of heavy metal reducing bacteria

The colony was small sized, yellow in colour in Nutrient agar plates and the isolate was gram stained and was found to be gram positive cocci. The isolate was positive for Methyl red, Voges Proskauer's, citrate utilization, Glucose, mannitol and sucrose fermentation and negative for Indole, adonitol, arabinose, lactose, sorbitol, Rhamnose and raffinose fermentation.

16SrRNA gene amplification and sequencing

The DNA isolated from the bacterial culture using phenol/chloroform method was amplified for its 16sr RNA region. The amplified sequence was found to be 1002bp in size and was also submitted in genbank and the accession no is MF612183 and phylogenetic tree was constructed using Mega 6.0 software for *Staphylococcus warneri* MF612183 and is shown in figure 1

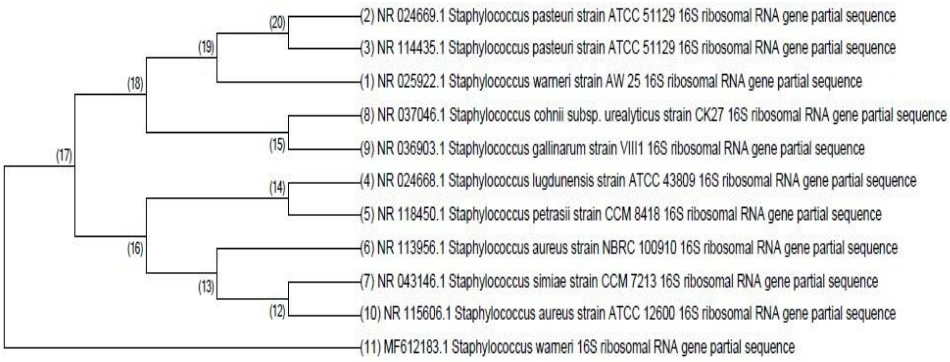

Fig. 1: Phylogenetic tree based on full length 16S rRNA of *Staphylococcus warneri* MF612183

The culture with 17×10^{-5} CFU/ml was evaluated for its ability to bioremediate heavy metals and the heavy metal reduction studies is given in table 1. The percent reduction of heavy metals- Zinc, Copper, Chromium and Lead was calculated and are given in table 2.

Table 1: Zinc, Copper, Lead and Chromium reduction in Nutrient broth for every 24hrs

Time in hours	Zinc (ppm)	Copper (ppm)	Lead (ppm)	Chromium (ppm)
Mean ± S.D.				
0 (Initial value)	1.00± 0.1	1.00± 0.01	30± 1.00	150± 1.00
24	0.6± 0.1	0.66± 0.01	13± 1.00	58± 1.00
48	0.56± 0.01	0.62± 0.01	11± 1.00	58± 1.00
72	0.48± 0.01	0.44± 0.01	11± 1.00	38± 1.00
96	0.05± 0.01	0.14± 0.01	9± 1.00	38± 1.00

Table 2: Percent reduction of heavy metals using *Staphylococcus warneri* MF612183 in Nutrient broth

Heavy metal	Percent reduction at the end of 96th hour
Zinc	95%
Lead	70%
Copper	86%
Chromium	74.6%

DISCUSSION

The isolate *Staphylococcus warneri* MF612183 was found to reduce the selected heavy metals – Zinc, copper, chromium and Lead by 95%, 86%, 74.6% and 70% after 4 days of its growth. *Bacillus Subtilis, Serratia marcescens* and *Pseudomonas fluorescens* were immobilized by Sujitha and Jayanthi[1], and they reported maximum reduction of heavy metals.

Biosorption of lead and cadmium (67-82% and 73-79 %) was observed by Dilna Damodaran[10], when *Saccharomyces cerevisia* was used within 30 days. Krishna *et al*.,[7] showed *Bacillus* sp can be used in reduction of Zinc and also observed a maximum reduction of about 40%. There was reduction in copper level in soil when bacteria was added[9].

Jayanthi *et al.*[11], found that *Staphylococcus aureus* and *Escherichia coli* isolated from tannery sludge can be exploited for bioremediation of Cr (VI) containing wastes, since it seems to have the potential to reduce the toxic hexavalent form of chromium to its nontoxic trivalent form.

Sunitha and Rajkishore[2], found the per cent reduction of chromium in soil ranged from 39 to 79 by *Pseudomonas fluorescens*, 30.8 to 58.4 by *Trichoderma viride* and 50.3 to 83.4 by *Aspergillus niger* after 15 days of incubation.

Seema Sharma and Alok Adholeya[5], isolated 3 *Bacillus sp*. from tannery effluent polluted sites and were found to reduce chromium within 24 hours of incubation in filter sterilized tannery effluent without any nutritional amendment. Emmanuel *et al.*, 2015 found the ability of *R. nigricans* and *A. niger* to reduce chromium ranging from initial concentration 1.517 to 0.067 mg/l.

In the present study *Staphylococcus warneri* was found to reduce all the four heavy metals and found to be promising in Bioremediation of tannery effluent.

CONCLUSION

Staphylococcus warneri MF612183 isolated from tannery effluent was found to reduce the heavy metals (Copper, Lead, Zinc and Chromium) present in the tannery effluent and was found to be promising in bioremediation of tannery effluent.

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