CHROMIUM HEAVY METAL RESISTANCE ACTIVITY OF MARINE *STREPTOMYCES* VITSVK5 Spp. (GQ848482)

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Summary

Marine actinomycetes, *Streptomyces* spp. VITSVK5 was screened for heavy metal resistance activity. The isolate was also screened for dye decolourization activity. Agar diffusion assay for heavy metal resistance showed that the isolate was resistant to chromium (VI and III). The isolate was less resistant to arsenic. However it was sensitive to lead and nickel nitrate. Based on polyphasic taxonomic characterization the isolate was identified as a novel *Streptomyces* spp. and designated as *Streptomyces* spp. VITSVK5. The 16Sr DNA sequence of the *Streptomyces* spp. VITSVK5 was deposited in GenBank under the accession number GQ 848482. The novelty of this study is that it is the first report that *Streptomyces* spp. VITSVK5 showing resistance against the chromium. Considering the heavy metal resistant activity of the strain, it could be used as potential strain for large scale production and to be used against toxic heavy metal chromium (VI and III).

Keywords*:* Actinomycetes, *Streptomyces* spp. VITSVK5, heavy metal resistance

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Introduction

Heavy metals pollution is caused due to the release of industrial wastewaters and it is an ongoing and serious hazard to livelihood and to the environment. Some heavy metals are toxic, non biodegradable and can accumulate in food chain and in living tissues (1, 2). Toxic metals are being released into the environment mainly untreated effluents from industry and other human activities. Due to their toxic effects on living systems stringent limits have been stipulated for their discharge into the environment. Chromium is a common and very toxic pollutant introduced into natural waters from a variety of sources including industrial wastes. The major sources of contamination are electroplating, metal finishing industries and tanneries (3). Among the several oxidation states of chromium, the main forms present in the environment are trivalent Cr(III) and hexavalent Cr(VI). These two oxidation states have widely contrasting toxicity and transport characteristics. Hexavalent chromium poses a greater risk due to its carcinogenic properties to living organisms, while Cr (III) is generally toxic to plants at very high concentrations and is less toxic or non-toxic to animals (5; 4). Lead is a poisonous metal that can damage nervous connections (especially in young children) and cause blood and brain disorders. Lead poisoning typically results from ingestion of food or water contaminated with lead; but may also occur after accidental ingestion of contaminated soil, dust, or lead based paint (6).Long-term exposure to lead or its salts (especially soluble salts or the strong oxidant PbO_2) can cause nephropathy, and colic-like abdominal pains. Arsenic contamination of groundwater has led to a massive epidemic of arsenic poisoning in Bangladesh (10) and neighboring countries. Presently 42 major incidents around the world have been reported on groundwater arsenic contamination. It is estimated that approximately 57 million people are drinking groundwater with arsenic concentrations greater than the limit (10 parts per billion) prescribed by World Health Organization (WHO).

The impact of heavy metals on the environment and their accretion through the food chain have promoted research aimed at developing alternative, efficient and low cost wastewater purification systems (7). However, these techniques can be expensive, they may not always be feasible and their metal-binding properties are non-specific (8). Today mankind is exposed to the highest levels of these metals. These are the reasons why alternative processing methods, such as those using microbial biomass, are now being considered more seriously (9). Microbial biomass can remove and concentrate a variety of metal ions from aqueous solutions.

Interest in processes involving heavy metal uptake by micro-organisms has increased considerably in recent years, in particular because of the biotechnological potential of micro-organisms in metal removal and/or recovery. For example, biosorption has a possible application as a process for the removal of heavy metals from wastewater.

Marine *Streptomyces* species are Gram-positive bacteria that typically colonize terrestrial and marine soils as free-living saprophytes. In addition, some species also colonize the rhizosphere of plant roots and even plant tissues. They have evolved complex morphological and physiological responses enabling them to adapt to large changes in their environments. Several *Streptomyces* isolated from marine sponge *Fasciospongia cavernosa* has been shown to exhibit resistance against heavy metals, copper, lead, cobalt, nickel, mercury and cadmium (11). Metal resistance in actinomycetes has not been investigated thoroughly despite the fact that actinomycetes are metabolically and biosynthetically versatile. Because of this versatility, actinomycetes are important in biotechnology and have been employed in bioremediation, including metal recovery. The aim of the present work is to evaluate the heavy metal resistance activity of *Streptomyces* spp. VITSVK5.

Materials and methods

Isolation of Actinomycetes

Marine sediment samples collected at the depth upto 0-20 cm in the Marakkanam, (12° 12' 0" North, 79° 57' 0" East) region, Bay of Bengal coast, India was used to isolate actinomycetes using starch casein agar medium (SC agar). The pH was adjusted to 8.0 prior to sterilization and the growth media (SC agar) was supplemented with antibiotics - Cycloheximide (25 mg/ml) and Nalidixic acid (25 mg/ml) (Himedia, Mumbai, India) and incubated for 7 days at 30°C. The colonies were recognized according to their cultural characteristics and then transferred to slant culture maintained at 4°C as well as at 20% (v/v) glycerol stock and stored at -80°C.

Taxonomy

The morphology of the spore bearing hyphae with the entire spore chain with the substrate and aerial mycelium of the strain was examined under light microscope, at 1000X magnification and spore chain surface morphology under scanning electron microscope.

Total DNA preparation from strain was carried out using HiPurA bacterial DNA isolation and purification kit (Himedia, India) and amplified by PCR using a master mix kit, Medoxmix (Medox, India) as per user manual. The primers and the PCR conditions were adapted from Rainey et al. (12). The design of the sequencing primers and the methodology for the sequencing were adapted from previous reports (8-10). The nucleotide sequence of the strain was determined by using Chain termination method (13). The sequence was analyzed for similarity and homology with the existing sequences available in the data bank using BLAST search. The DNA sequences were aligned and phylogenetic tree was constructed by neighbour joining method using ClustalW software (12). Restriction enzyme site analysis and secondary structure prediction for rRNA was carried out using NEB cutter (Version 2.0) and GeneBee softwares respectively and bioinformatics tool available online www.genebee.msu.su/services/rna2_reduced.html.

Preparation of Heavy metal stock solution

The heavy metal standard solutions Cr (III) and Cr (VI), Ni (II), Arsenic and Lead were obtained from the Merck. The concentration of the standard heavy metal solutions was 1000 mg/L, which is then further serially diluted with deionised distilled water to make up the varying concentration to 1000-100 mg/L. The standard solutions were sterilized separately for 15 mins at 110°C.

Screening of heavy metal resistance activity

The isolates from the lawn culture was seeded on the surface of SC agar. Using a sterile well borer wells were made and to each well 500µl of the standard metal solution wth varing concentration was added and incubated at 28°C for 7 days. The area of inhibition (mm) was measured as the distance from the edge of growing colonies to the edge of the well.

Results

The strain showed maximal growth after 7 days when cultivated on SC medium at pH 8.2 when incubated at 30°C with a salt concentration of 8% with glucose and L-cysteine as carbon and nitrogen sources respectively. The arrangement of the spores in the mycelium was found to be spiral under light microscope, 1000X magnification (Figure 2A) by cover slip technique and the surface morphology of spores in the mycelium under scanning electron microscope (Figure

2B) revealed that the spore surface is smooth. Spores are spherical in shape; arranged in long chains and each contains 10-25 spores. The mature spores are 0.5-1.0 mm in diameter and the length is between 0.8 and 1.0 mm (Figure 2 B).

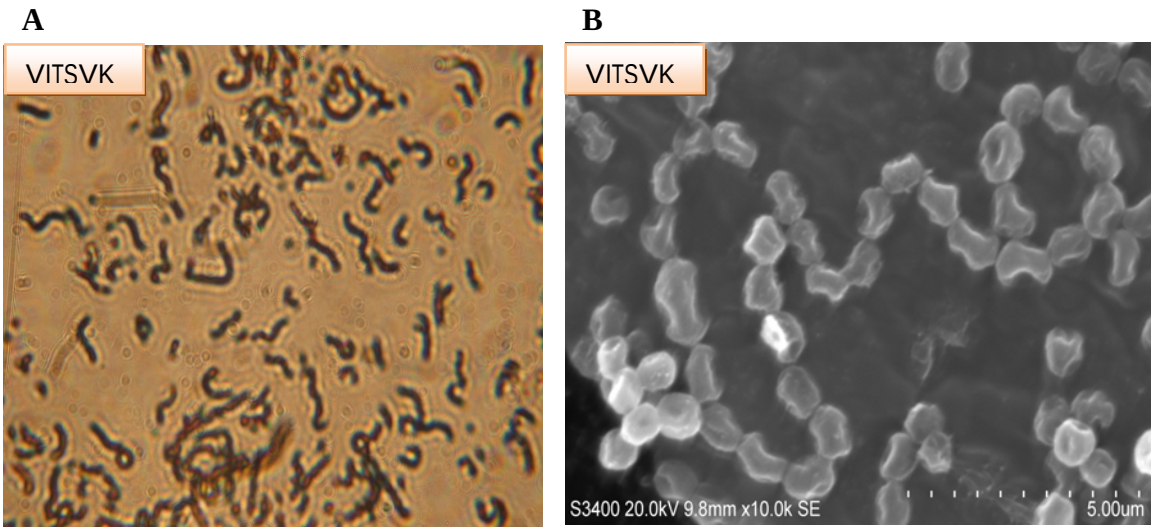

Figure 1. Spiral spore chain morphology **(A)** (arrow) under light microscopy (1000x magnification) and smooth spore surface morphology **(B)** (arrow) under scanning electron microscopy.

The blast search of the 16S rDNA sequence (1424 base pairs) of the isolate showed maximum (93%) similarity with *Streptomyces* MSU2261 (AY232829) and 90 % with bromate-reducing bacterium B7 (AF442523.1). The phylogenetic tree was constructed with bootstrap values (Fig.3). Due to non availability of physiological, biochemical and cultural conditions of the closest phylogenetic neighbours, we are unable to compare the characteristic features of the isolate with others. Based on the molecular taxonomy and phylogeny the strain was identified as *Streptomyces* and designated as *Streptomyces* spp. VITSVK5. The RNA secondary structure (Fig 4) of 16s rRNA gene of *Streptomyces* spp. VITSVK5 showed the free energy of the predicted structure is -320.1kkal/mol. The restriction analysis of the sequence is given in Fig. 5. The restriction sites present on the bacterial 16S rDNA showed 50 restriction sites and GC content was 54 % and AT content was 46 %. The nucleotide sequence of 16s rDNA of 16 S rRNA gene partial sequence was deposited in the GenBank, National Centre for Biotechnological Information, USA under the accession number (GQ 848482).

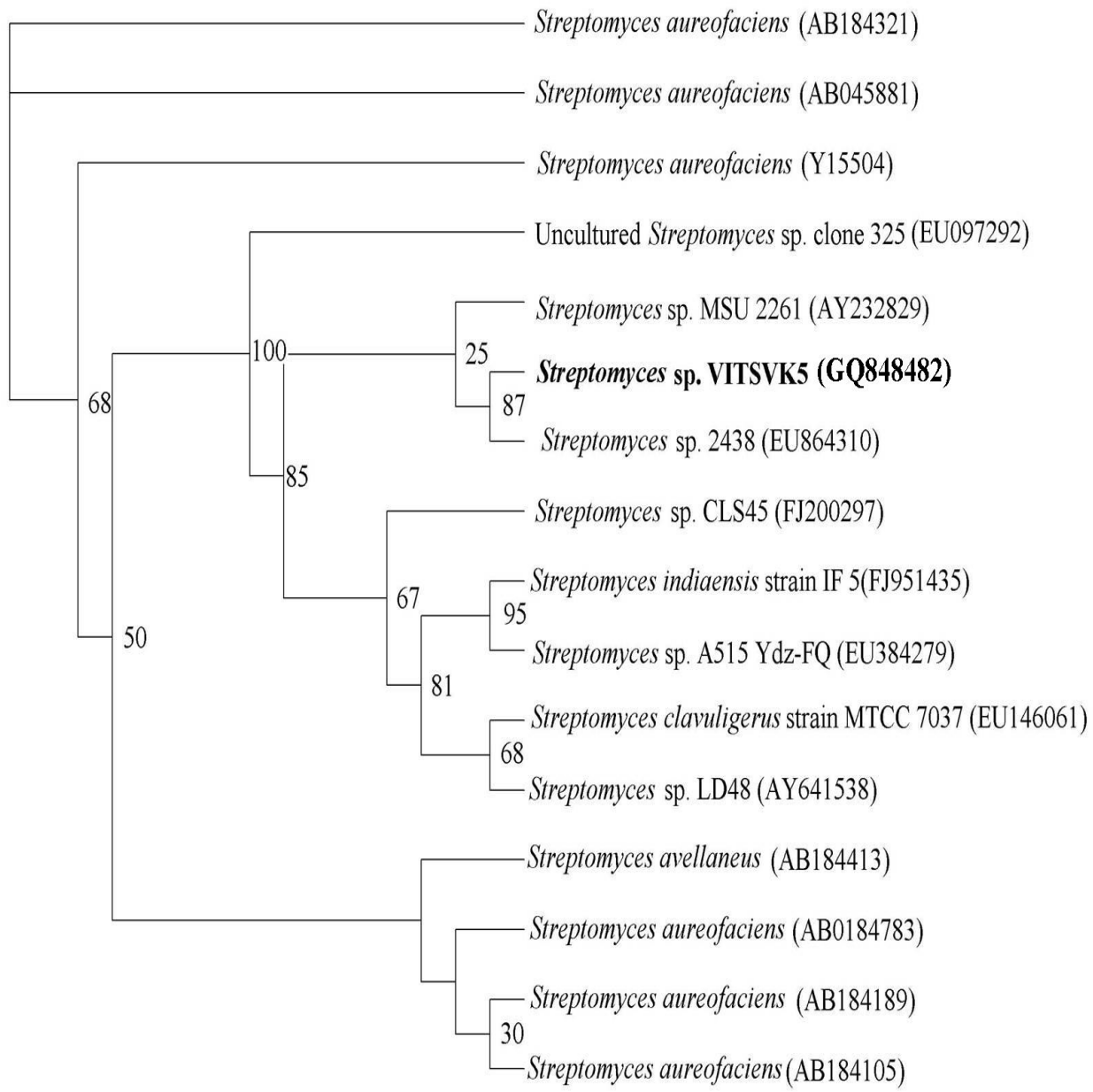

Figure 2. The 16S rDNA sequence of *Streptomyces* spp.VITSVK5 (1424 bp) showed maximum similarity with *Streptomyces* sp. MSU 2261 (94 %) and the phylogenetic analysis by neighbor joining method indicates that the strain belongs to the genus *Streptomyces* and represents as a novel species.

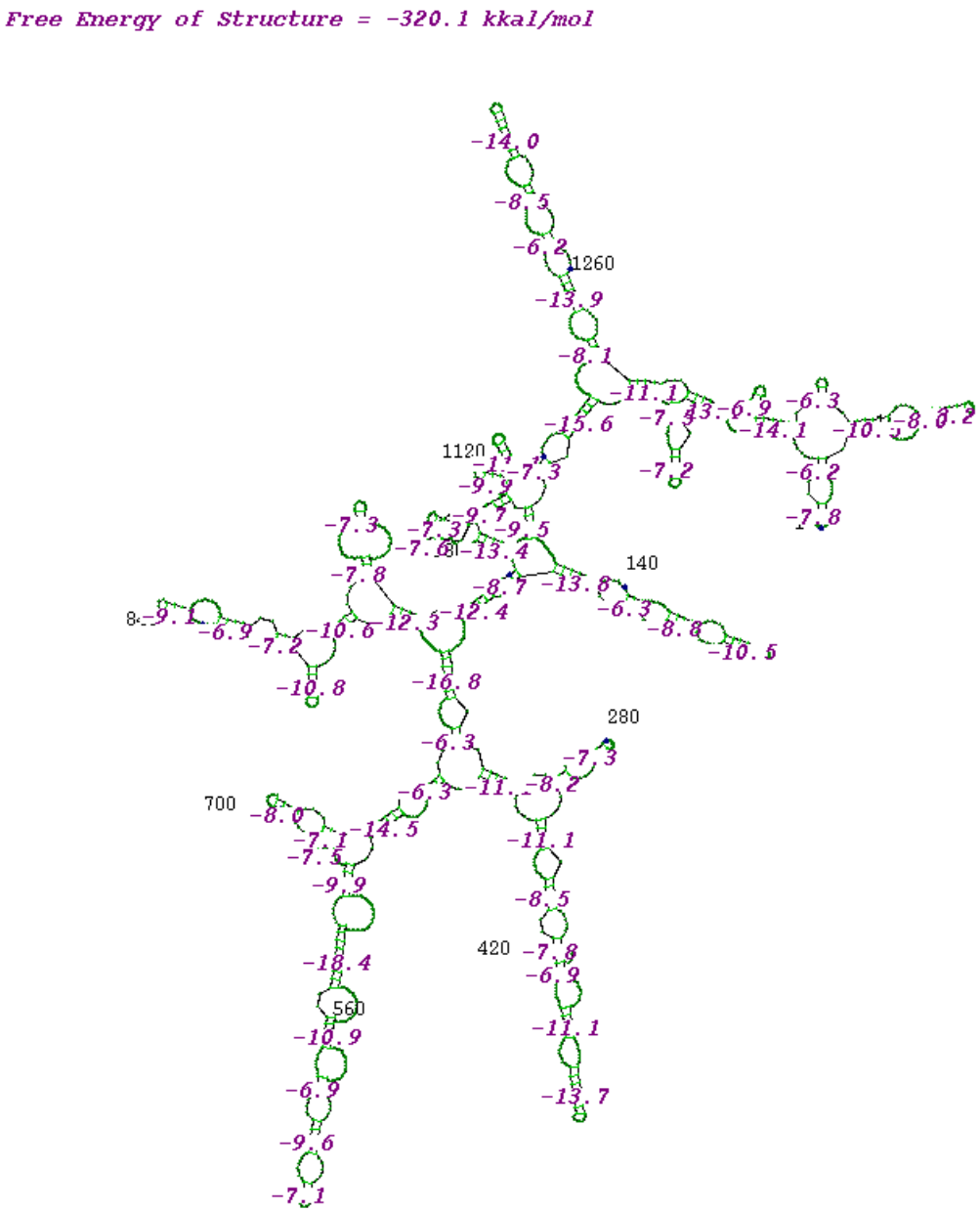

Figure 3. Secondary structure of 16S rDNA of isolate *Streptomyces* spp.VITSVK5 using Genbee software.

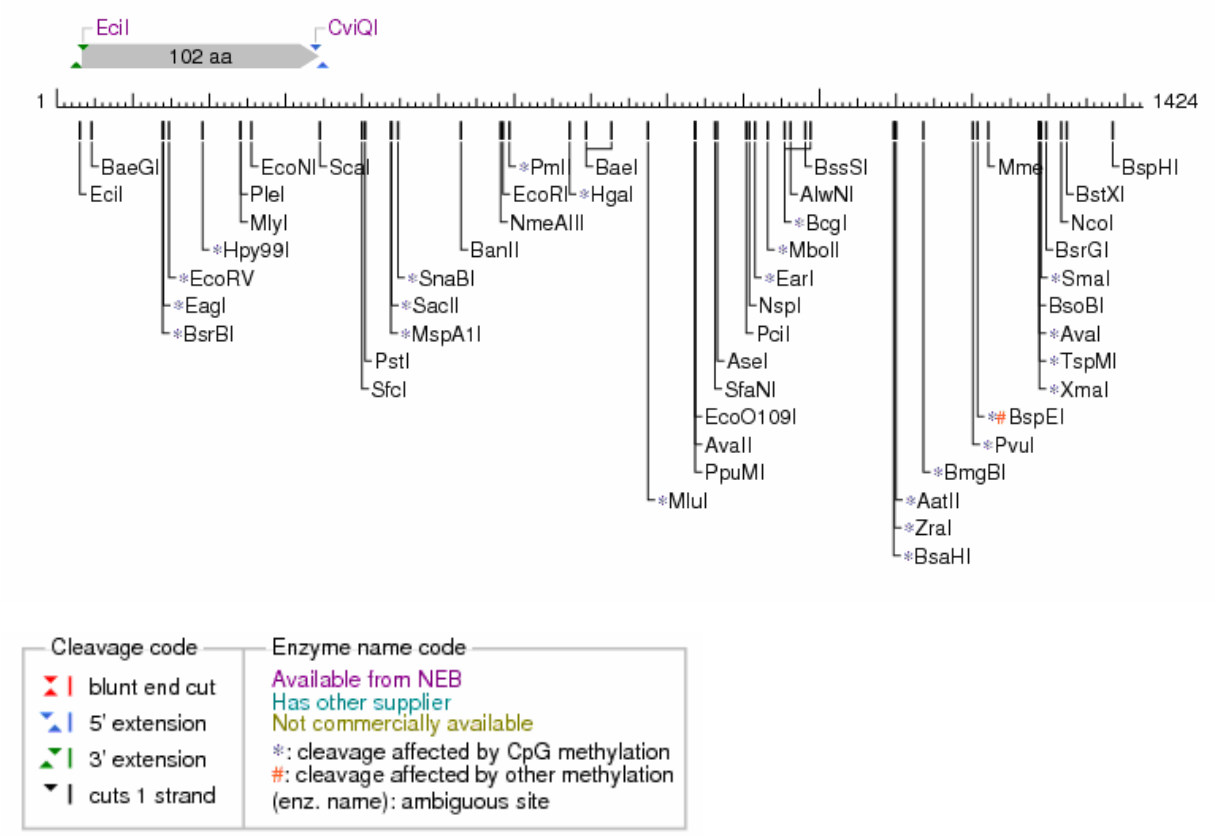

Figure 4. Restriction site analysis of 16S rDNA of the isolate *Streptomyces* spp.VITSVK5 using NEB Cutter program.

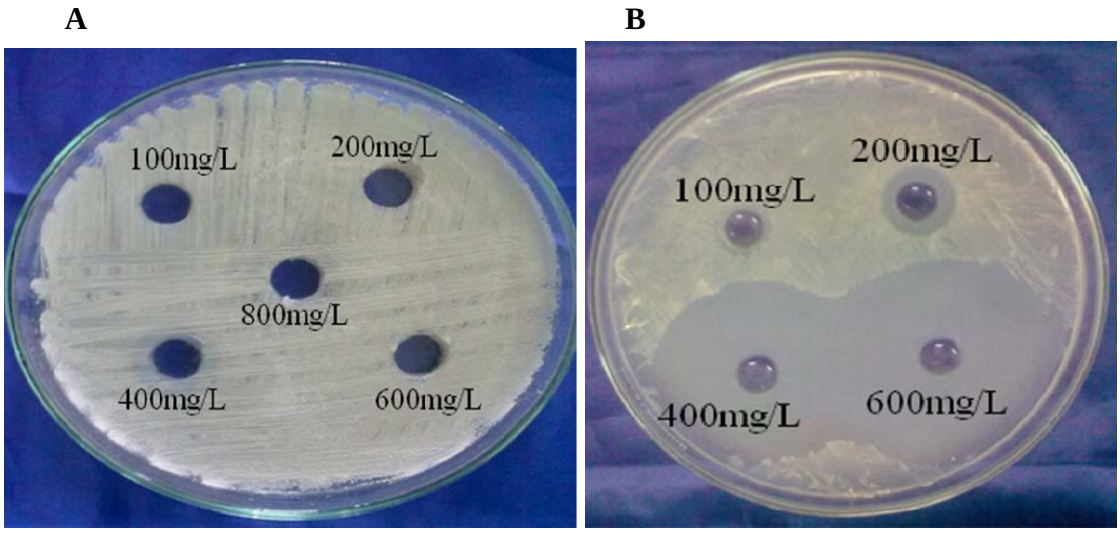

Figure 5. Heavy metal resistance profile exhibited by *Streptomyces* spp. VITSVK5 showing A) Resistance pattern against ChromiumVI. B) Susceptibility pattern against Lead.

Semi quantitative determination of heavy metal resistance by *Streptomyces* spp.VITSVK5 using agar well diffusion method exhibited the extent of resistance for a particular metal. An inhibition zone of 10mm and above on the agar surface was considered as resistant to the particular metal standard. The isolate was found to be resistant to chromiumVI and chromiumIII. *Streptomyces* spp.VITSVK5 showed resistance at low concentration of arsenic and lead (up to 200 mg/L) over and above it was found to be sensitive. *Streptomyces* spp.VITSVK5 was completely sensitive to nickel. *Streptomyces* spp.VITSVK5 showed resistance to boron, molybdenum and silicone (not toxic metals, data not produced).

Table 1. Heavy metals resistance and sensitivity pattern of *Streptomyces* spp. VITSVK5.

Heavy Metals	**Concentration**					
	100mg/L	**200mg/L**	**400mg/L**	**600mg/L**	**800mg/L**	**1000mg/L**
Chromium VI	No zone	No zone	No zone	No zone	No zone	No zone
Chromium III	No zone	No zone	No zone	No zone	No zone	No zone
Arsenic	No zone	No zone	10mm	12mm	15mm	20mm
Lead	5mm*	7mm*	20mm	25mm	-	-

* An inhibition zone of 8mm and above was considered to be sensitive to the metal

No zone- indicates resistance to heavy metals tested with well diffusion technique and inhibition zone indicates sensitivity to metals.

Discussion

Marine actinomycetes, *Streptomyces* spp. VITSVK5 having heavy metal resistance activity was isolated from sediment samples collected at the Marakkanam region, Bay of Bengal coast, India. *Streptomyces* spp. VITSVK5 showed selective resistance up to 1000 mg/L against heavy metals such as chromium VI and chromium III. It showed resistance to arsenic and lead up to 200 mg/L and at higher concentration it was sensitive. It was sensitive to nickel even at lower concentration (100 mg/L) tested. It was reported that several bacteria associated with a marine sponge *Fasciospongia cavernosa*, *Streptomyces* sp. (MSI01), *Salinobacter* sp. (MSI06), *Roseobacter* sp. (MSI09), *Pseudomonas* sp. (MSI016), *Vibrio* sp. (MSI23), *Micromonospora* sp. (MSI28), *Saccharomonospora* sp. (MSI36) and *Alteromonas* sp. (MSI42) showed resistance

against heavy metals, copper, lead, cobalt, nickel, mercury and cadmium. *Streptomyces* sp. C16 (AY741280), M3 (AY741284), M40 **(**AY741285) isolated from sediments has been reported to be resistant to chromiumVI (13). It was reported that two *Streptomyces mirabilis* strains were capable to growing on concentrations higher than100mmol/L nickel. Additional experiments had revealed that both strains were able to withstand up to100mmol/L zinc concentration (14). Recently, van Nostrand et al. (15) have isolated four actinobacterial strains from contaminated riparian sediments; among them two were belongs to *Streptomyces* genus. One of the *Streptomyces* strains was able to grow on 85.2mmol/L nickel which is reported to be the highest nickel resistance detected so far. So far only one heavy metal resistance mechanism has been identified on a molecular basis in *Streptomyces* which is having resistance against mercury. However, mercury resistance is based on the reductive detoxification of Hg(II) to elemental and volatile mercury (16). To the best of our knowledge, ours is the first report that a marine actinomycetes, *Streptomyces* spp. VITSVK5 showing resistance to toxic heavy metal chromium VI and III.

Streptomyces spp. VITSVK5 could be potential actinomycetes capable of utilizing or decomposing the toxic heavy metals. However, further studies are needed to explore its potential and to study the mechanism of resistance against toxic heavy metals. Further, our data supports the role of microorganism in the removal of toxic metals from the contaminated soil to protect the environment.

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