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Heavy Metal Tolerance and Antibiotic Profiling of Bacterial Isolates from Hot Springs of Odisha

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Heavy metals released from various industrial effluents have damaging effects on ecosystem and may become a health hazard to man and animals. The present study deals with isolation and characterization of heavy metal and antibiotic resistant thermophilic bacteria isolated from water samples of Atri, Tarabalo and Taptapani hot springs in provinces of Khurda, Nayagard and Ganjam from Odisha, respectively. Endospore forming, aerobic, gram positive, rod-shaped bacteria were isolated from these three hot springs of Odisha. The water temperatures of these hot springs were between 56.8 - 60 °C and pH of water was recorded as 8 - 8.6. These thermophiles showed optimum growth at 55 °C and pH 8. The bacterial isolates exhibit varying degree of resistance to various heavy metals and antibiotics tested. Of the 21 thermophilic bacterial isolates screened, about 33.3%, 38.1%, and 52.38% were found to tolerate high levels of Mercury (200 ppm), Chromium (600ppm) and Lead (800 ppm) respectively. The multiple metal resistances of these isolates were also associated with resistance to antibiotics Erythromycin, Methicillin, Nitrofurantoin, Polymyxin, Sparfloxacin, Streptomycin and Vancomycin. The identified heavy metal resistant thermophilic bacteria could be useful for the bioremediation of heavy metal contaminated sewage and waste water.

Key words: Antibiotic resistance, Metal resistance, Thermophilic bacteria, Hot springs.

Microbes play a large role in the biogeochemical cycling of toxic heavy metals also in cleaning up metal-contaminated sites[1]. Heavy metals are often defined as a group of metals whose atomic density is greater than 5 g cm-3 [2, 3]. Metals play a vital role in the metabolic processes of the biota. Some of the heavy metals are essential and are required by the organisms as micro nutrients (cobalt, chromium, nickel, iron, manganese and zinc etc.) and are known as 'trace elements' [4]. They are involved in redox processes, in order to stabilize molecules through electrostatic interactions, as catalysts in enzymatic reactions, and regulating the osmotic balance [3, 5].

On the other hand some other heavy metals have no biological role and are detrimental to the organisms even at very low concentration (cadmium, mercury, lead etc.). However, at high levels both of the essential and non essential metals become toxic to the organisms. These heavy metals influence the microbial population by affecting their growth, morphology, biochemical activities and ultimately resulting in decreased biomass and diversity [6]. To survive under metal-stressed conditions, bacteria have evolved several types of mechanisms to tolerate the uptake of heavy metal ions. These mechanisms include the efflux of metal ions outside the cell, accumulation

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and complexation of the metal ions inside the cell, and reduction of the heavy metal ions to a less toxic state [3].

Microbes apply various types of resistance mechanisms in response to heavy metals [13]. These mechanisms may be encoded by chromosomal genes, but more usually loci conferring resistance are located on plasmids [12]. Researchers have conducted in the area of mesophilic and certain thermophilic bacteria in the context of reduction, leaching and other forms of mineral extraction [14, 15]. The most important microorganisms which have been successfully used in bioremediation were bacteria such as *Pseudomonas, Bacillus* and several other algae and fungal isolates [16, 17, 18]. Thermophilic bacilli are a group of bacteria that can grow at an optimum growth temperature of 60°C [19]. They have been used in bioremidation of heavy metals associated with different environmental conditions [20, 21].

Hence, the aim of this study is to isolate and characterize heavy metal tolerance and antibiotic resistance bacteria from hot springs of Odisha.

MATERIALS AND METHODS

Isolation of strains

Water samples were collected from three different hot springs (Atri, Tarabalo and Taptapani) and analyzed microbiologically by 10-fold serial dilution method. Each of the samples was spread on pre-sterilized Nutrient Agar (NA-Hi MEDIA Ltd., Mumbai, India) plates and then, incubated at 55 °C for 24–48 h. After incubation, different colonies developed in the media which were selected and purified by subculturing. Isolated and purified bacterial strains were made glycerol stock and stored at -20°C for further studies.

Morphological, physiological and biochemical characterizations of isolates

The temperature ranges for growth were determined by incubating strains from 45 to 80°C. The pH dependence of growth was tested from pH 7 to pH 10.0 (Table 1). Cell morphology of isolates was investigated by light microscopy.

Test for metal resistance

Stock solutions of different metal salts were prepared in distilled water, slightly acidified for mercury (2-6 drops of 6N HCl) and were sterilized. These solutions, in various concentrations according to the metal tested were kept at 4 degree for no more than one month. The glasswares were leached in 2N HNO_3 (nitric acid) and rinsed several times with distilled water before use to avoid metal contamination. The metal salts used were mercuric chloride [$HgCl_2$], potassium dichromate [$K_2Cr_2O_7$], and lead nitrate [$Pb(NO_3)_2$]. Bacterial growth was observed in presence of different concentrations of heavy metals after 24-48 h at 55° ± 1°C. In order to access quantitatively the effect of heavy metal, tube dilution method was used:

Tube dilution method

The range of concentrations of heavy metals was as follows (parts per million):10, 100, 200 and 300 for mercury(Hg), 10, 100, 200, 300, 400, 500, 600 and 700 for chromium(Cr), and 10,100, 200, 300, 400, 500, and 600, 700, 800 and 900 for lead(Pb). Concentrations of appropriate metals were prepared in tubes with final volume of 10 ml of nutrient broth. Three tubes were prepared for each metal concentration, then innoculated with 200µl of 24 hrs old culture of all bacterial isolates. A positive control consisted of a metal -deficient medium innoculated with the micro-organism and a negative one consisted of a metal-supplemented medium without the micro-organism. The tubes were incubated at 56.8°C for 24h and examined for turbidity. Bacterial growth was monitored by measuring the absorbance at 600 nm using spectrophotometer.

Determination of antibiotic resistance

The Antibiotic resistance was checked using Kirby- Bauer disc diffusion method. The isolates were spread on Sterile Mueller Hinton agar plates using sterile cotton swabs. The Antibiotic discs (HiMEDIA Ltd., Mumbai, India) were placed on the plates aseptically and then incubated at 55 ºC for 24 hours. Inhibition zone was noted after 24 h incubation.

RESULTS

A total 21 strains of thermophilic bacilli were isolated from water samples of three different hot springs in odisha. These isolates showed optimum growth at 55 °C and pH 8. The investigation of these strains in terms of morphology, biochemical and physiological

Table 1. Morphological, biochemical and physiological characteristics of thermophilic bacterial isolates three hot springs in odisha

Profiling	Bacterial isolates																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Morphological																					
Gram nature	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Cell morphology	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod
Motility	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve
Endospore forming	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Biochemical																					
Indole	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
MRVP	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
VP	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Citrate	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve
ONPG	+ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	+ve
Catalase	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Oxidase	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Anaerobic Growth	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Starch hydrolysis	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
Urease	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
Caseinase	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
Gelatinase	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
Growth profiling																					
Growth at temperature- 45°C	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
50°C	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
55°C	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
60°C	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
65°C	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
70°C	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
80°C	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
90°C	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Growth at pH - 7	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
8	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
9	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

10	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
11	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Sugar utilisation																					
Fructose	+ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve-
ve																					
Trehalose	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	
Glucose	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Sucrose	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Inulin	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Xylose	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Galactose	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Mannitol	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve

properties were shown in Table.1.

Heavy metal tolerance of bacterial isolates

Out of 21 isolates, examined 33.3% showed tolerance upto 200 ppm mercury, 61.9% isolates to 100ppm and all isolates can tolerate 10ppm mercury. Similarly, 57.14% and 38.1% isolates showed resistance to 500 and 600ppm chromium and all isolates can tolerate 300ppm chromium. On the other hand, 66.6% and 52.38% can tolerate 700, and 800ppm lead and all isolates can tolerate 300ppm lead. The bacterial isolates showed differences in resistance to different metal concentrations as shown in Table 2.

Determination of antibiotic resistance of bacterial isolates

All the thermophilic bacterial isolates were sensitive to Ampicillin(10mcg), Bacitracin(10units), and Chloramphenicol(30mcg) where as some isolates showed resistance to Erythromycin(15mcg), Methicillin(5mcg), Nitrofurantoin(300mcg), Polymyxin-B(300units), Sparfloxacin(5mcg), Streptomycin(10mcg), and Vancomycin(30mcg), respectively as shown in Table 3.

DISCUSSION

Aerobic, endospore forming rods, gram-positive thermophilic bacteria were isolated from water samples of different hot springs of odisha. Several reports corroborates the findings which indicates the presence of *Bacillus spp.* which was isolated and identified based on biochemical and molecular analysis from Ma'in hot spring (Jordan)[22]. More recently, several thermophilic isolates belonging to genus *Bacillus* were isolated and identified[23]. The present study also indicated that all the thermophilic isolates can survive at higher temperature upto 70ºC and pH-10. The extreme conditions present in the hot springs enable these bacteria to change their biochemistry to tolerate higher concentration of heavy metals. Out of 21 isolates screened 33.3%, 38.1% and 52.38% isolates showed tolerance to Mercury (200ppm), Chromium (600ppm) and Lead (800ppm) respectively. Many works have been reported on *Microbacterium* sp. (Pb203) which is able to tolerate Cu (1900 ppm), Ni (580-1100 ppm), Cr (6400 ppm), Cd (300-600 ppm), Pb (660 ppm), U (1500 ppm) and Ag (10 ppm)[24]. De *et al* (2003) isolated

Table 2. Heavy metal resistance pattern of bacterial isolates.

	Growth of bacterial isolates																				
Metal conc(ppm)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Mercury(Hg)10	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
100	+ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve
200	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve
300	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Chromium(Cr)10	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
100	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
200	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
300	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
400	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve
500	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve
600	+ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve
700	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Lead(Pb)10	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
100	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
200	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
300	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
400	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve
500	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve
600	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve
700	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve
800	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
900	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Note: + positive; - negative

Table 3. Antibiotic sensitivity assay of bacterial isolates.

Antibiotics /dose	Bacterial isolates																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Ampicillin(10mcg)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Bacitracin(10units)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Chloramphenicol(30mcg)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Erythromycin(15mcg)	S	S	S	S	S	S	S	S	S	S	S	S	HR	S	S	S	S	S	S	S	S
Methicillin(5mcg)	S	S	S	S	S	S	S	S	S	S	S	S	HR	S	HR	S	S	S	S	S	S
Nitrofurantoin(300mcg)	R	R	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S
Polymyxin-B(300units)	R	S	S	S	R	R	R	S	S	S	S	S	R	S	S	S	S	S	S	S	S
Sparfloxacin(5mcg)	S	S	S	S	S	S	S	S	S	S	S	S	S	HR	S	S	S	S	S	S	S
Streptomycin(10mcg)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	HR	S	S
Vancomycin(30mcg)	S	S	S	S	S	S	S	S	S	S	S	HR	S	S	S	S	S	S	S	S	S

Note: R for resistance, HR for highly resistance and S for sensitive

two strains from an area with intense shipping traffic, which grew on seawater nutrient agar solid medium with 75 ppm mercury[25]. Isolate 13 is showing multiple resistance to antibiotics like Erythromycin, Methicillin and Polymyxin which is also associated with multiple resistance to heavy metals like Hg (200ppm) and Pb(800ppm). The isolates 14, 15 and 19 showing tolerance to Sparfloxacin, Methicillin and Streptomycin are also showing good resistance to Hg(100ppm), Cr(600ppm), Pb(800ppm)., Hg(200ppm), Cr(600ppm), Pb(800ppm) and Hg(200ppm), Cr(600ppm), Pb(800ppm) respectively. The combined expression of metal tolerance and antibiotic resistance is caused by selection resulting from the metals present in the environment[26]. The genes that code for antibiotic resistance traits and genes that code for metal resistance are often carried on the same plasmid or mobile genetic elements [27, 28]. This shows that there is a close association between metal resistance and antibiotic resistance. Singh *et al.* (2010) isolated *Bacillus cereus*, an antibiotic and heavy metal resistant bacterium showing resistance to antibiotics like penicillin, lincomycin, cloxacillin, pefloxacin and heavy metals like arsenic, lead and cesium[29]. Samanta (2012) isolated an organism belonging to the *Bacillus* sp. having the ability to grow in presence of a wide range of metals namely nickel, cadmium, chromium and cobalt in the order Cd2+> Cr6+> Ni2+> Co2+. And also it was observed that the isolate was resistant to a wide range of antibiotics namely Kanamycin (30µg/disc), Ampicillin (25µg/disc) and Methicillin (5µg/disc)[30].

CONCLUSION

The future prospect lies in the application of these microorganisms for purposes like heavy metal remediation and potential use in extracting rare metals from dilute solution or removing toxic metals from industrial effluents.

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