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Characterize heavy metal tolerant rhizospheric sewage bacteria isolated from Tolly nullah (with special emphasis on strain *Microbacterium radiodurans* K12016)

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Abstract

Because of rapid industrialization and urbanization, heavy metals are deposited in the environment in frightening quantity. Literature survey manifested, that the heavy metal contaminated soil born microbes can be a potent mode of environmental heavy metal degradation. In the present study, to reveal microbial heavy metal tolerant activity rhizosperic microbes were isolated from sewage sludge of circular canal (Tolly Nullah) and characterize. Microbial heavy metal resistance pattern was checked against four heavy metal salts (Cr_2O_3, CrO_3, $CdCl_2$ and $CoCl_2$) individually and in consortium. Plant growth promoting rhizobacterial (PGPR) traits of isolated microbes were evaluated. One interesting isolated strain (*Microbacterium radiodurans* strain K12016), identified by 16s rDNA, shown total tolerance against 100 mM Cr_2O_3 (Cr^{3+}) and also mentionable Cobalt and hexavalent Chromium (Cr^{6+}) tolerance, but didn't produce a significant amount of plant growth promoting IAA whereas the most of the rest isolates produced adequate amount of IAA. 16s rDNA of that particular isolate was analyzed and phylogenic tree was constructed using the neighbor-joining method to ensure its taxonomic position. Furthermore experimentation and gene study should require to determining heavy metal resistance mechanism of *Microbacterium radiodurans* K12016.

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Keywords:

Heavy Metal tolerance;
Biochemical analysis;
Antibiotic resistance;
Indole Acetic Acid (IAA);
16s rDNA.

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1. Introduction

As a result of different unrestricted anthropogenic activities, different concerning heavy metals like Chromium (Cr), Lead (Pb), Cadmium (Cd), Cobalt (Co), Nickel (Ni), Iron (Fe) are alarmingly increased in the environment, particularly the areas where waste materials from different industrial sectors, tannery, household, medical sectors are coming from. The presence of trace amount of heavy metal in soil, water and even in the atmosphere is a huge threat for different living organisms including human being [17]. Some

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microbes which grow in hot and humid natural habitat of the urbanized sewage site developed certain biological mechanisms to overcome the heavy metal stress like efflux, accumulation or reduction of heavy metals or they can also use metal ions as terminal electron acceptors of respiration [27]. But all of the microbes do not withstand a certain level of heavy metal at their natural habitat and they gradually change their community structure and lowering their total biomass and diversity [18]. Bacteria resistant to heavy metals by developing such mechanisms are potent to grow to in high concentration of heavy metals and good candidate for heavy metal bioremediation. So, investigate the microbial habitat containing increasing levels of heavy metals have a vital role in microbial heavy metal tolerance because the solubility and toxicity of different heavy metals depends on their oxidation state.

The bioremediating bacteria might be a good candidate for plant growth promotion and might be able to help in cultivation at poorly cultivated heavy metal containing soil [30]. Some plant friendly soil rhizobacteria which are present in plant rhizosphere regions are called plant growth-promoting rhizobacteria [PGPR] [20] help in plant growth promotion by producing plant growth hormones or exhibiting other different plant growth inducing mechanisms. The soil is the natural habitat for a large number of bacteria (10^8 to 10^9 bacterial cells per gram of soil) but among them only about 1% of total cell are cultivable [36], and at environmentally stressed soil the average number of bacterial cells might remain 10^4 cells per gram of soil [41] but the plant root adjacent regions (rhizosphere) are rich in microbes because the presence of different amino acids, organic acids, sugars, and other molecules secreted from plant root for the which may help in plant growth by accounting upto a third carbon which is fixed by plant [3]-[5],[46] Rhizosperic microbe-plant interaction may be utilized in bioremediation and biomass improvement at a time, which is good for human beings [45]. Tryptophan secreted by plant roots enhanced bacterial IAA production [26]. Interestingly, when IAA remains present in soil, it helps in phytoremediation by increasing plant root heavy metal uptake [32]. Phosphate solubulizing Microorganisms [PSM] are the most accurate environment friendly Phosphorous source for crop plants because they increase the available soil Phosphorus level for plant [33].

This present work describes the isolation and characterization of some rhizospheric soil bacteria, isolated from the sewage water fed soil of Circular Canal (Tolly Nullah; 22° 33"N 88° 30"E) which is an important tide influenced drainage canal of south Kolkata, India. The warm, humid atmosphere of this particular area acts as a natural incubator and helps the microbes to grow. That's why this site has chosen to isolate heavy metal resistance rhizospheric soil microbes. Most of the economically backward people live on the bank of canal cultivate and consume vegetables grow in that heavy metal contaminated region which may be life threatening for them. Saha el al. (2005) [34] already reported that the region is polluted with heavy metal. Out of the 12 selected microbial strain, strain C12 (*Microbacterium radiodurans* strain K12016) which was isolated from rhizosphere of *Pteris vitatta*, shown high resistance against trivalent Chromium and also considerable resistance against hexavalent Chromium and Cobalt also. So, it can be predicted from the result that this strain can be a potent candidate for heavy metal bioremediation. But interestingly, this rhizospheric bacterial strain doesn't show any considerable plant growth promoting features like IAA production, phosphate solubilization capability, whereas the most of the other isolated strain produces a considerable amount of IAA.

2. Research Method

2.1. Isolation of soil Microbes:

The rhizosperic soil sample was collected randomly from 10 cm depth and then dissolved 10 g of wet soil in 90 ml of 0.85% salt solution and incubated at 90 RPM for 2 hours in sterile condition and further experimentation was carried out with the collected bacterial suspension [16]. The bacterial suspension was serially diluted (10^{-1} to 10^{-9}) and each dilution plated in triplicate in Luria Bertani (LB) medium at 37ºC for 24 to 48 hours to get single microbial colonies. Among the isolated microbes only twelve are randomly chosen for the present study on the basis of their colony morphology. Strains are cultivated and maintained by Luria Bertani broth and agar medium as per requirement and stored for future use at -80ºC in 70% glycerol stalk.

2.2. Morphological characterization of bacterial isolates:

Colony morphology of different isolated soil microbes was checked by measuring growth specifications like colony form, elevation, surface, edge, color etc. when single colonies were found after spreading serially diluted bacterial suspension on LB agar plates after 48 to 72 hours incubation. Cellular morphology of the Gram stained preparations and endospore producing capacity of each of the individual isolates were determined at 100X magnification using bright field microscope by using standard protocols.

2.3. Standardization of bacterial growth conditions:

The individual pure colonies are isolated and maintained in different pH (6.0, 6.4, 6.8, 7.0, 7.2 and 7.4); different temperature (27ºC, 37ºC and 47ºC) and by applying 5%, 10% and 20% inoculums at 160 RPM

and optimized the growth condition. The growth efficiency was measured by measuring the optical density at 595 nm in UV-VIS spectrophotometer.

2.4. Biochemical characterization of bacterial isolates:

Production of different extracellular substances and enzymes like Gelatinase, Catalase, Amylase, Urease etc. by bacterial isolates examined using different supplementary and commercially available media. To detect diffusible pigment production ready-made King's B media was used and mixed acid fermentation capability was checked using MRVP (Methyl Red and Voges-Proskauer) broth tubes whereas to check Gelatinase, Urease and Amylase production Gelatin, Urea and Starch supplemented media was prepared respectively.

2.5. Antibiotic sensitivity test of bacterial isolates:

Antibiotic discs were applied to the Muller Hinton Agar plate after spreading 10^{-2} times diluted log phase bacterial suspension culture. The antibiotics were used to characterize microbial isolates are ampicillin (10 µg/ml), norfloxacin (10 µg/ml), doxycycline (30 µg/ml), tetracycline (30 µg/ml). To measure bacterial resistance or sensitivity, the zone of inhibition against different antibiotics was standardized by the commercially available National Committee for Clinical Laboratory Standard's (NCCLS) chart.

2.6. Determine heavy metal resistance pattern:

Four heavy metal salts [Cr_2O_3, CrO_3, $CdCl_2$, and $CoCl_2$] were used in different concentrations for this experiment. Different gradations of salt solutions were prepared from one Molar stalk solutions. Bacterial heavy metal resistance pattern was checked by agar cup method [12]. In this method, Luria Bertani (LB) medium was inoculated with 10% of overnight grown single colony broth culture when it reaches a sustainable temperature and after mixing properly it was poured into sterile petri plate. After setting the LB agar medium, on each plate five wells of equal width and equal depth were made on equal distance using cork and borer aseptically. Then 80µl of each salt solution of each Molarity were put in the wells and plates are incubated at 37ºC for 24 hours and after the incubation tenure measure the zone of inhibition in mm. The same process was repeated in triplicate. The heavy metal resistance pattern of microbial isolates was checked individually and also in the consortium.

Figure1. Formation of bacterial clear zone of inhibitions against heavy metals in agar cup-disc diffusion method

Minimum Inhibitory Concentration (MIC) was assessed by checking growth pattern after 24 and 48 hours of inoculation using turbidimetry method. In this method until growth ceased, the inoculums were added to elevated concentration heavy metal solution and the procedure was repeated thrice [47].

2.7. Bacterial growth profiling in the presence and absence of heavy metals:

To check bacterial growth pattern 10% inoculums were transferred from fresh confluent overnight culture to pure LB broth and LB broth supplemented with different heavy metals in Minimal Inhibitory Concentrations for each individual bacterium and measure optical density values after each hour of time span from the time of inoculation. Then get the growth curves and compare the presence and absence of heavy metals.

2.8. Molecular characterization of bacterial isolates:

Soil bacterial Genomic DNA was isolated with microbial DNA isolation kit (GCC Biotech) as per manufacturer's protocol. The 16s rDNA region was amplified from the genomic DNA using standard primer 16sF and 16sR. The PCR product was then analyzed by agarose gel electrophoresis and purified by MOBIO gel extraction kit. The purified PCR product was then sequenced by the Sanger sequencing methodology (using ABI 3730Xl machine).

2.9. In silico analysis of 16s rDNA sequence to Identify and analyze the bacterial isolate:

Obtained 16s rDNA sequence was annotated using Chromas Lite software (version 2.01) and then find its near relatives and taxonomic position using A BLAST search [2],[48] at the NCBI server(http://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple alignment of the 16s sequence was carried out using clustal omega server [25] and after aligning the sequence phylogenetic and molecular evolutionary analyses were conducted using *MEGA* version 6 [40] with neighbor joining method [35] and evolutionary distances

were computed using Kimura 2 parameter model [19]. The acquired partial16s rDNA sequence was submitted to the NCBI GenBank database [7] and getting the accession number. Then search close relatives of our identified soil bacteria through 16s rDNA based BLAST, which construct different environmental heavy metal processing proteins.

2.10. Indole Acetic Acid (IAA) Production test:

Bacterial Indole Acetic Acid production was checked with the help of Spectro nanometer using ferric Chloride-perchloric acid as colorimetric reagent. Using this method perceived bacteria generating L-Tryptophan, the precursor of Indole compound.

To govern the impact of isolated microbes on Indole production, they were grown in 20 ml volumes of Tryptophan supplemented Luria Bertani Broth media for overnight at 150 RPM. The medium was prepared by adding 1mg/ml filter sterilized, ether extracted and reduced L-tryptophan solution. After 24 hour incubation with 1 ml volume of each culture was withdrawn, centrifuged and to the cell free supernatant, ferric Chloride-perchloric reagent, $FeCl_3$-$HClO_4$ [15] was added in 1:1 ratio and then incubated for 1 hour and when the colour intensity become maximum, the colour intensity was measured at spectrophotometer at 530 mm absorbance using a standard curve which indicate the IAA production amount. The procedure was repeated in triplicate.

2.11. Phosphate Solubilization test:

A quantitative phosphate solubilization test was carried out by inoculating bacterial isolates on Pikovskaya agar medium, and checks the presence of clear zone surrounding bacterial colonies after five days of incubation as a result of phosphate solubilization.

3. Results and Analysis

3.1. Soil study:

To measure the bioremediation efficiency of isolated bacteria, their heavy metal resistance pattern should be assessed. It can be anticipated that the microbes present at the harsh polluted Tolly Nullah area may resist harmful heavy metal to some extent. Our results support this conviction and among different microbial isolates which were considered in the present study five (C5, C6, C7, C8 and C12) shown ample heavy metal tolerance level. The basic soil physiology is the best indicator for soil microbial community and its diversity [42],[23]. The soil microbes present in the Tolly Nullah area have developed in a slightly alkaline environment. The soil collected from Tolly Nullah at Kalighat area of Kolkata was grayish black with the smooth clayey texture. The soil pH varied from 7.5 to 8.1.

3.2. Morphological, biochemical characters and antibiotic sensitivity of bacterial isolates:

The characterization of the isolates was shown that most of the microbes are Gram positive endospore forming. Formation of endospore indicates that the microbes can withstand environmental adverse conditions. Biochemical investigation manifested, that most of the isolated strain produced diffusible pigment after 6 days of incubation. Most of the isolated strains, including strain C12 produced Catalase but didn't produce Gelatin hydrolyzing enzyme.

Table 1: Morphological characters of isolated soil bacteria

Strain No.	**Colony Character**		**Cell Character**		
	Colour	**Nature**	**Gram Nature**	**Shape**	**Endospore**
C1	Straw yellow	Rapid, entire, circular, smooth, flat	Gram +Ve	Cocci	Present
C2	Whish orange	Rapid, entire, circular, smooth, concave	Gram +Ve	Cocci	Present
C3	Orange	Less rapid, entire, circular, rough, flat	Gram +Ve	Cocci	Present
C4	Yellow	Rapid, entire, circular, smooth,	Gram – Ve	Cocci	Present

		flat			
C5	Whitish yellow	Less rapid, entire, elongated, smooth, flat	Gram +Ve	Rod	Present
C6	White	Rapid, entire, elongated, smooth, flat	Gram +Ve	Rod	Present
C7	Whitish yellow	Rapid, slightly wavy, circular, smooth, flat	Gram +Ve	Rod	Present
C8	Translucent	Rapid, entire, circular, smooth, concave	Gram – Ve	Rod	Present
C9	White	Rapid, entire, circular, smooth, flat	Gram +Ve	Cocci	Present
C10	White	Rapid, entire, elongated, smooth, flat	Gram +Ve	Long rod	Present
C11	Yellow	Rapid, entire, circular, smooth, flat	Gram +Ve	Rod	Present
C12 (*Microbacterium radiodurans* K12016)	Grayish white	Rapid, wavy, circular, rough, flat	Gram + Ve	Short Rod	Present

Previously Zhang et al.(2010)[49] also reported that strain *Microbacterium radiodurans* sp. nov. GIMN 1.002^{T} produces Catalase but not Gelatinase. All of the isolated strain excluding strain C3 produce extracellular α-amylase enzyme which was previously described [21] from cold stressed *Microbacterium foliorum* GA2 and *Bacillus cereus* GA6. A small number of the isolated bacterial strains, including strain C12 formed Urea degrading extracellular enzyme, but most of the soil microbes perform mixed acid fermentation which supports the formerly accounted data [22] which says that most of the *Microbacterium* species doesn't produce Urease but *Microbacterium esteraromaticum* LMG 4020T produce it and majority of considered *Microbacterium* species have taken part in the mixed acid fermentation.

Table 2: Biochemical characters of isolated soil bacteria (+ denotes the enzyme producing property of the isolate and – denotes the absence of enzyme producing property, **Microbacterium radiodurans* K12016**)**

Biochemical Tests	**Bacterial strains**											
	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	**C11**	**C12***
1.Gelatinase Test	-	+	+	-	-	-	+	-	-	-	+	-
2. Catalase	+	+	-	+	+	+	+	+	-	+	+	+

Test												
3. Amylase Test	+	+	-	+	+	++	+	+	+	+	+++	+
4. Fluorescence Test	-	-	-	-	-	-	-	-	-	-	-	-
5. Methyl red Test	++	+++	+	+	+	+	+++	+	+++	+	+	+
7. Urease Test	+	+	-	+	+	-	+	-	-	-	-	-
8. Diffusible pigmentation Test												
After 1 days	-	+	-	-	+	+	-	-	-	-	-	-
After 6 days	+++	+++	++	+++	+++	+++	+++	+++	++	+++	+++	+++

Different prior reports indicate that there is a correlation between heavy metal and antibiotic resistance [39]. Chen et al (2015) [11] declared that the presence of heavy metal in considerably lower, even MIC concentration induce bacterial antibiotic resistance. Heavy metal resistance pathway might be contributing in microbial antibiotic tolerance.

Table 3: Antibiotic sensitivity (µg/mL) of the twelve isolates using Muller Hinton Agar (The concentration of the antibiotics in µg/disc. R denotes resistant, I denotes the intermediate response and S denotes sensitive.)

Bacterial Strain	**Antibiotics**			
	Ampicillin (10 µg/ml)	**Tetracycline (30 µg/ml)**	**Deoxycyclin (30 µg/ml)**	**Norfloxacin (10 µg/ml)**
C1	S	R	I	R
C2	I	R	R	R
C3	I	R	S	I
C4	S	I	R	R
C5	S	R	I	R
C6	I	I	S	I
C7	S	I	I	R
C8	I	S	I	S
C9	S	I	S	I
C10	S	R	R	R
C11	R	R	R	I
C12 (*Microbacterium radiodurans* K12016)	S	R	I	R

The reason behind it is heavy metal and antibiotic resistance genes may cluster in same plasmid. The present study indicates most of the isolated heavy metal tolerant microbes had shown considerable resistance against different considered antibiotics.

3.3. Study heavy metal tolerance pattern of isolated soil bacteria:

Though metals are important for growth and development of microbes, they can also cause harm to living organisms [37] because of their tendency to form complexes with body protein and inactivate those. The heavy metal tolerance capability of soil microbes was estimated by measuring zone of inhibition against different salt concentrations. Soil microbe can accumulate metals within cells in 50 times higher than their habitat [31] Bacteria also can detoxify heavy metals using techniques like metal removal, immobilization or acid production [43]. As in present heavy metal tolerance become the most important trend to analyze the functional diversity of indigenous bacteria and their bioremediation potentiality.

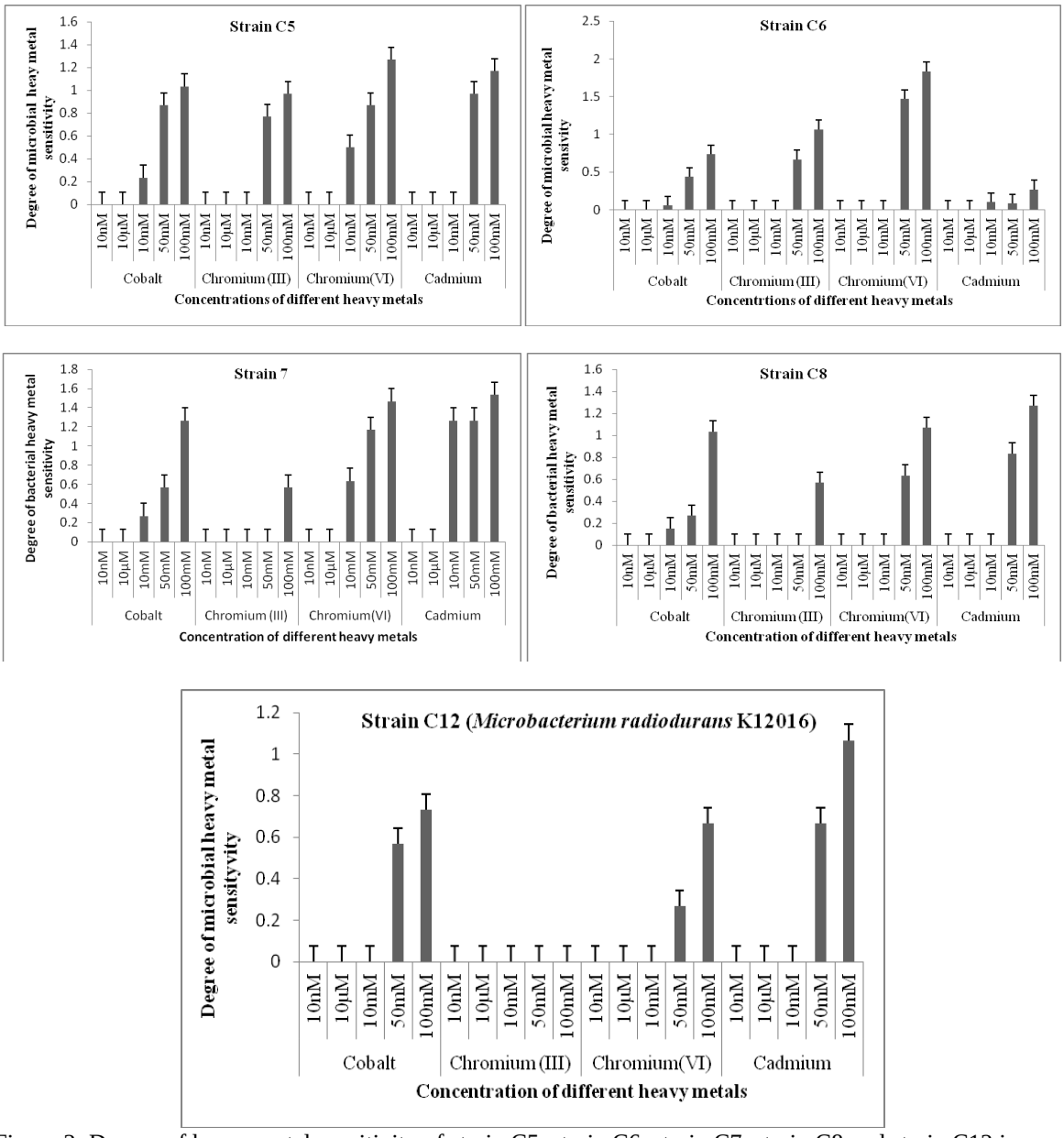

Figure 2: Degree of heavy metal sensitivity of strain C5, strain C6, strain C7, strain C8 and strain C12 in agar cup-disc diffusion method (all values were taken by calculating bacterial zone of inhibition in triplicate)

The bacterial isolates retrieved from the Tolly Nullah region showed a variegated susceptibility limit against different heavy metals. Isolated strain C7 had shown high susceptibility against 10 mM $CdCl_2$ but most of the other isolates demonstrated resistance against that particular concentration of different heavy metal salts. As the metal concentration increases microbial resistance against heavy metal decreases and at 100 mM metal concentration, the maximum salt concentration used all of the microbes shown maximum

susceptibility except strain C12 which manifested total resistance against Cr_2O_3 till 100 mM concentration. Basu et al. (2014) [6] reported about the hexavalent Chromium removal by *Bacillus subtilis*. Strain C7 and strain C8 had also showed a high degree of resistance against trivalent Chromium salt (Cr_2O_3). Strain C7 had shown maximum sensitivity against Cadmium. Strain C12 had shown absolute tolerance against 10 mM Cobalt Chloride whereas other strains performed a certain degree of susceptibility. Hence the present study show that the isolated microbial strains shown their metal resistance capability, but most of the isolated strains showed high level of susceptibility against the highest considered metal concentration (100 mM). Among the bacterial isolates, strain C12 shown absolute resistance upto 100 mM of trivalent Chromium, 50 mM of hexavalent Chromium and 10 mM of Cobalt and 10mM Cadmium which is quite inflated with respect to other isolates. Interestingly, strain C12 had also shown total tolerance against Cr_2O_3 till 100 mM concentration, the maximum salt concentration used, in consortium with strain C5, strain C6 and strain C7 each of which do not show that level of resistance in separate culture. Recently, Nesheli et al (2018) [29] had reported that in radio resistance bacterium *Kocuria* sp ASB107, live bacterial cells are more potent in Chromium(VI) removal at acidic pH and higher concentration of hexavalent Chromium affect bacterial growth curve by lengthening lag phase, in our study strain C12 also shown extended preparatory phase in the presence of hexavalent Chromium but in the presence of trivalent Chromium the bacterium reaches to exponential phase at more or less the same time in comparison with heavy metal free environment. Chromium tolerant C12 strain shown satisfactory growth at neutral to highly alkaline pH and Gupta et al (2012) [16] also previously reported that the maximum Chromium removal occurred in highly alkaline condition.

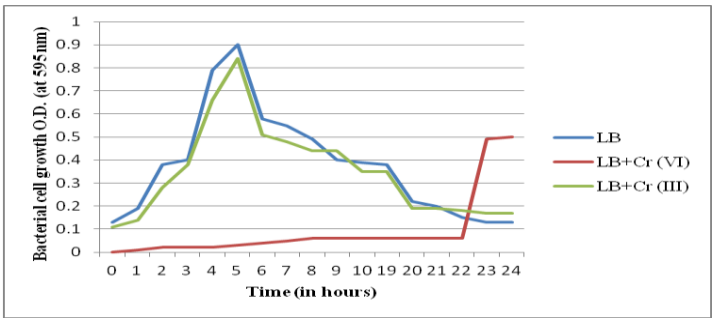

Figure 3: The growth curve of strain C12 (*Microbacterium radiodurans* strain K12016) shown extended preparatory phase in the presence of hexavalent Chromium but in the presence of trivalent Chromium the bacterium attains exponential phase at more or less the same time in comparison with heavy metal free environment.

Table 4: Minimal Inhibitory concentrations (in mM) of 12 heavy metal tolerant strains isolated from Tolly Nullah sewage sludge

Strain	**Metal**			
	Cr^{3+}	**Cr^{6+}**	**Co**	**Cd**
C1	26	8	9	22
C2	60	9	11	15
C3	45	32	15	11
C4	20	15	7	13
C5	35	7	8	26
C6	30	8	10	9
C7	72	10	7	7
C8	75	40	9	32
C9	55	42	7	28
C10	48	35	9	22
C11	75	7	8	35
C12 (*Microbacterium radiodurans* K12016)	Didn't find till 100	42	40	45

3.4. Characterize the metal resistance bacterium on the basis of its 16s rDNA:

Five of the twelve isolated microbial strains (viz C5, C6, C7, C8 and C12) had shown considerable heavy metal resistance. Amongst the selected microbial isolates, C12 showed considerably higher tolerance against Chromium and Cobalt. That's why 16s rDNA of this particular strain was sequenced for identification. Its taxonomic position was also seen with the aid of the standard identification hierarchy. After getting the 16s rDNA sequence it was concerned to mega BLAST to find its near relatives. The strain exhibited 88% sequence identity with a strain of *Microbacterium radiodurans.* Preliminary morphological, physiological characterization and taxonomic key analysis also confirmed this report. The newly identified strain named as *Microbacterium radiodurans* K12016 was submitted to the NCBI database with the GenBank accession number MF600628. The 16s rDNA sequence of strain K12016 considered in this present study is a 708 bp continuous linear stretch partial sequence. Zhang et al.(2010) [49] previously reported UV radiation resistance *Microbacterium radiodurans* from the soil. As per my knowledge this is the first report about heavy metal tolerant *Microbacterium radiodurans.*

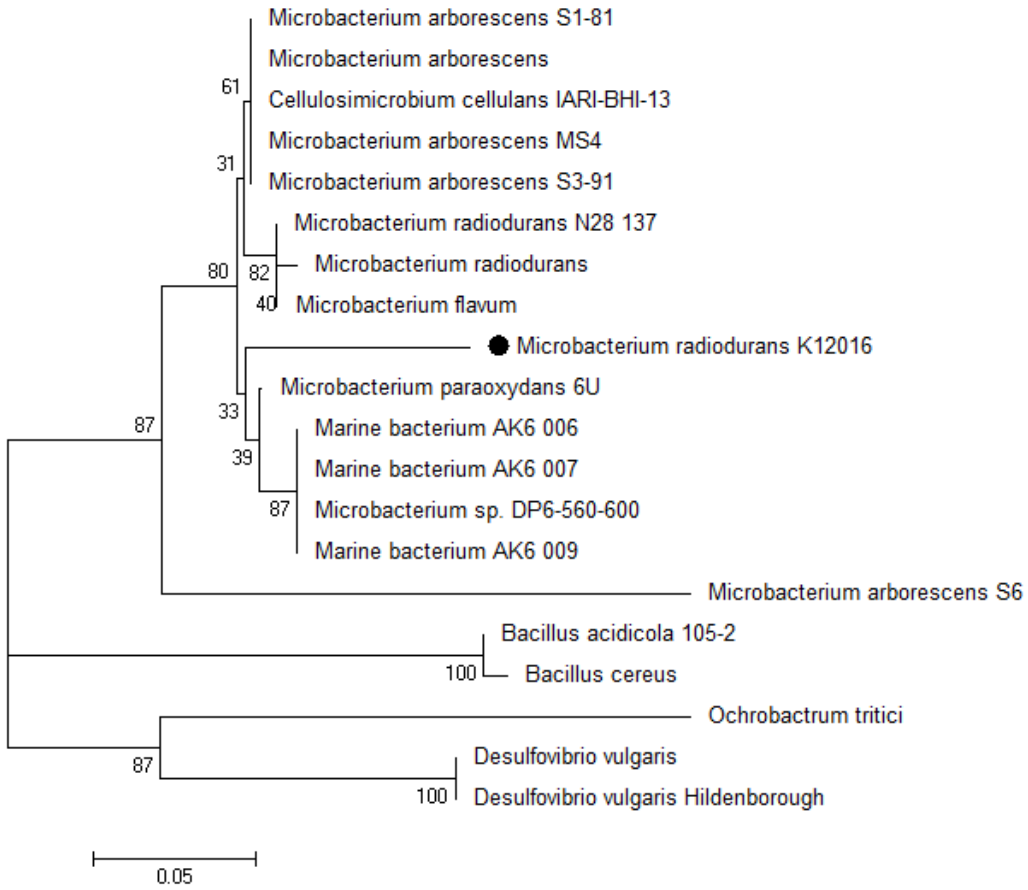

Figure 4: Phylogenetic tree constructed with MEGA 6 software using the neighbor-joining method based on 16S rDNA sequences in which the associated taxa clustered together in the bootstrap test (600 replicates) are shown next to the branches. The evolutionary distances were computed using the Kimura 2-parameter method and the sum of the optimum branch length is 0.79039173. In the analysis 20 nucleotide sequences involved and all gaps and missing data were eliminated.

Phylogenetic tree was constructed using the neighbor joining method with the newly identified strain manifested maximum 16s rDNA based similarity with *Microbacterium* paraoxydans and marine bacterium but not with other *Microbacterium radiodurans* strains. Other Chromium tolerant strains like *Ochrobactrum tritici, Bacillus cereous, Desulphovibrio vulgaris* used as out group in the phylogenetic tree.

Microbacterium radiodurans K12016 shown 16s rDNA based 67.58% identity with Chromium resistance chr gene containing *Ochrobactrum tritici* (EF469735). *Ochrobactrum tritici* gene translates Chromate Transporter (ABO70325) and Transposase (ABO70322) protein, which convert hexavalent to trivalent Chromium and transport Chromium [10],[9]. Chromium reducing *Bacillus cereous* satrin S612 (AN402) showing the genomic relatedness with *Microbacterium radiodurans* K12016, translate different proteins like ATP dependent chaperon, ABC transporter, GTP binding protein, amino acid permease family protein, Inner spore coat family protein, siderophore biosynthesis protein, copper resistance protein etc. [44].

Desulphovibrio vulgaris strain Hildenborough has shown almost 50% 16s rDNA sequence based similarity with *Microbacterium radiodurans* K12016, translates a chemoreceptor protein dcrA [13]. Further genetic study with the *Microbacterium radiodurans* K12016 whole genome sequence is required to find Chromium resistance mechanisms.

3.5. PGPR like trait analysis:

Metal resisting bacteria may play important role in plant survival by formation of different plant growth promoting factors like IAA (Indole Acetic Acid). The present study indicates that rhizospheric bacterial strain C12 (*Microbacterium radiodurans* K12016) produced an insignificant concentration of IAA, whereas strain C5 and C6 build considerably higher.

Table 5: Concentration of rhizospheric soil bacteria formed IAA get through standard curve. This result obtained from three separate experiments and the values were calculated with the help of standard solutions (± standard deviation)

Bacterial Strain Number	**IAA Production (µg/ml)**
C1	37.5±0.2
C2	25±0.28
C3	37.5±0.11
C4	43.75±0.16
C5	43.75±0.1
C6	37.5±0.2
C7	37.5±0.2
C8	31.25±0.3
C9	37.5±0.05
C10	24.5±0.11
C11	25±0.5
C12(*Microbacterium radiodurans* K12016)	25±0.36

Another PGPR trait, the precipitated phosphate solubilization ability was also tested, but none of the bacterial isolates showed any significant result. PGPR like traits and metal absorption capability was reported in phytoremediating bacteria [1]. Plant rhizosperic bacteria play important role in the changing environment by adapting different plant cellular metabolic reactions [8]. Indigenous bacteria, present at heavy metal contaminated area exhibiting heavy metal resistance and plant growth promoting activity may be used in heavy metal remediation in the near future. Studying bacterial heavy metal resistance and their PGPR like trait is the preliminary work to categorize soil bacteria present in heavy metal contaminated soil.

4. Conclusion

All of the examined strains showed heavy metal resistance till certain considerable metal concentration and most of them also able to produce plant growth promoting hormone IAA which indicates that isolated soil microbes might convenient candidate for heavy metal bioremediation and plant growth induction at a time. Enthrallingly, at 100 mM metal concentration, the maximum salt concentration used all of the microbes shown susceptibility except strain C12 (*Microbacterium radiodurans* strain K12016) which manifested total resistance against Cr_2O_3 till 100 mM concentration individually and in consortium of some other strain. This strain also showed significant tolerance against Cadmium also. Further experiments like observing the bacterial heavy metal absorption and removal efficiency, detecting bacterial heavy metal accumulation site and genetic study should required to understand heavy metal resistance mechanisms of *Microbacterium radiodurans* strain K12016.

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