

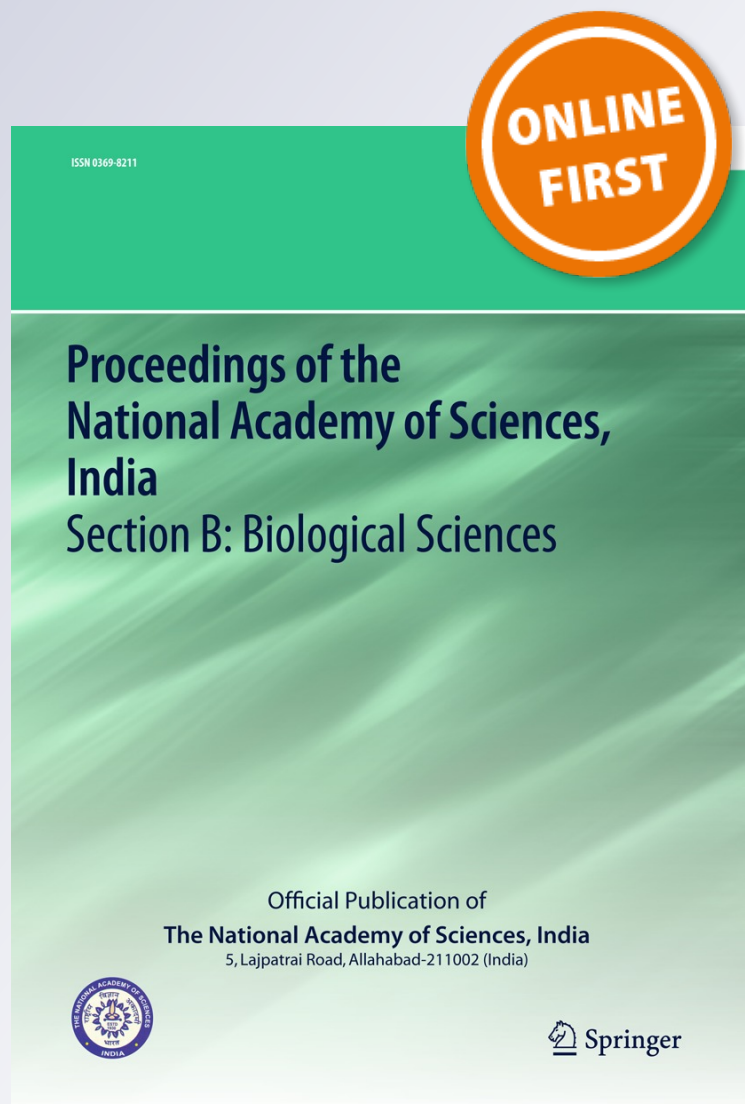
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**Barnali Sarma, Celin Acharya & Santa
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Plant Growth Promoting and Metal Bioadsorption Activity of Metal Tolerant *Pseudomonas aeruginosa* Isolate Characterized from Uranium Ore Deposit

Barnali Sarma · Celin Acharya · Santa Ram Joshi

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Abstract In the present study, a metal-tolerant fluorescent pseudomonad isolated from uranium ore rich deposit of Domiasiat in North-East India was identified as *Pseudomonas aeruginosa* DPs-13 based on morphological, biochemical and molecular analysis. The isolate showed higher tolerance to uranium and other metals like copper, cadmium, zinc and lead when compared with the reference strain *P. aeruginosa* MTCC2474. When checked for uranyl bioadsorption potential, the isolate showed 94 % (22.5 mg/L) and 72 % (342.7 mg/L) removal of uranium (VI) when challenged with 100 μ M (23.8 mg/L) and 2 mM (476 mg/L) uranyl nitrate solutions within 1 h of incubation as compared to 68 % (16.18 mg/L) and 25 % (119 mg/L) when challenged with above concentrations respectively of uranyl nitrate by the reference strain. The isolate was resistant to most of the commonly used antibiotics like Ampicillin, Kanamycin, Chloramphenicol, Erythromycin, Aztreonam, Tetracycline, Ciprofloxin and Streptomycin. The isolate had no phytotoxic effect, produced siderophores, possessed phosphate solubilising ability as well as two antibiotic producing genes, and had antagonistic activity against plant pathogens. Plasmid occurrence was also noticed in the isolate. The isolate from the uranium ore rich site besides

being a promising metal tolerant bacterium had potent plant growth promoting activity and can be used to promote plant growth in bioremediation approaches in metal contaminated sites.

Keywords *Pseudomonas aeruginosa* DPs-13 · Metals tolerance · Bioadsorption · Bioremediation

Introduction

Metals are essential part of the environment but elevated concentration of metals due to different natural or anthropogenic activities like volcano, leaching, metal smelting, mining, metallurgical and industrial/municipal waste disposals cause adverse effects in environmental health including its plant biota [1, 2]. Chemical remediation processes are not applicable to cost-effective remediation of large-scale sub-surface contamination in situ. On the other hand, biological approaches or bioremediation processes possess immense potentiality for the highly selective removal of toxic metals coupled with considerable operational flexibility both in in situ or ex situ approaches. Many such processes utilise microorganisms that have key roles in the biogeochemical cycling of toxic metals [3]. Resistance/tolerance to metal and antibiotic contaminants is an adaptive capacity of an organism developed due to constant pressure exerted on it by its environment [4]. Microbes in heavy metal rich environments show activities for bioadsorption, bioprecipitation, extracellular sequestration, transport mechanisms, and/or chelation. Such resistance mechanisms are the basis for the use of microorganisms in bioremediation approaches [5]. A properly selected bacterial isolate with metal bioremediation efficiency, plant

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B. Sarma · S. R. Joshi (✉)
Microbiology Laboratory, Department of Biotechnology
and Bioinformatics, North-Eastern Hill University, Umshing,
Shillong 793022, Meghalaya, India
e-mail: srjoshi2006@yahoo.co.in

C. Acharya
Molecular Biology Division, Bhabha Atomic Research Centre,
Trombay, Mumbai 400085, Maharashtra, India

growth promoting (PGP) properties and agricultural biocontrol potentialities may provide multiple advantages [6].

Pseudomonas aeruginosa is a metabolically versatile, highly adaptive, ubiquitous, gram negative rod shaped bacterium having metal bioabsorption efficiency, widely used as plant growth promoter and biocontrol agent in agricultural soil [7–9]. The indirect plant growth promotion is mostly due to the synthesis of antibiotics or siderophores by the bacteria that decrease or prevent deleterious effect of pathogenic microorganisms and direct growth promotion can be through the synthesis of phytohormones, N₂ fixation, reduction of membrane potential of the roots, as well as solubilization of inorganic phosphate and mineralization of organic phosphate [10]. Siderophores have ability to bind metals like cadmium, lead, nickel, arsenic (III, V), aluminium, magnesium, zinc, copper, cobalt and strontium [11, 12]. This makes siderophore producing, metal tolerant pseudomonads of great interest in bioremediation, biological control as well as plant growth promotion.

In the present study, a multi-metal tolerant fluorescent pseudomonad was selected that was isolated from uranium ore rich area of Domiasiat in North-East India and characterized for metal bioadsorption, PGP and biocontrol potentials to assess if the isolate can be a consortial candidate to supplement phytoremediation technology.

Material and Methods

Description of the Study Site and Isolation of the Test Isolate

Soil, water and sediment samples were collected from uranium ore rich sites of Domiasiat in North-East India and transported in ice to laboratory. The global positioning system coordinates were noted down and pH was measured using pH meter. Uranium was analysed using inductively coupled plasma spectrometer (ICP-MS, Perkin Elmer Elan DRC) and the trace metals like Cu, Cd, Zn and Pb were analysed using atomic absorption spectrophotometer (AAS, Perkin Elmer 3110) from the acid digested samples. The chemical yield and digestion process were repeated several times to extract the elements of interest and standardized for consistency. Metal tolerant pseudomonads were isolated using *Pseudomonas* agar (HiMedia, India) media, supplemented with 1 mM U [UO₂(NO₃)₂·6H₂O]. Purity of the isolates was confirmed by streak plate method and were preserved using 15 % glycerol stocks.

Type Cultures

The reference strain *P. aeruginosa* MTCC2474, the fungal pathogens *Fusarium oxysporum* MTCC284 and *Botrytis*

cinerea MTCC359 were obtained from microbial type culture collection (MTCC) and Gene Bank, IMTECH, India.

Morphological and Biochemical Identification of the Isolate

The selected isolate DPs-13 was presumptively identified as *Pseudomonas* based on its sticky nature in growth media as well as presence of fluorescence and greenish pigment. Biochemical characterization was done using API 20NE kit (Biomérieux, France). The API stripe was incubated for 48 h at 30 °C under ambient air. The results from the stripes along with the oxidase test were interpreted with the apiweb™ online software (<https://apiweb.biomerieux.com>).

Molecular Identification of the Isolate

Molecular characterization was done by sequencing 16S rRNA gene as well as 16–23S rRNA gene spacer region. Total genomic DNA was isolated using DNA isolation kit-HiPurA™ Bacterial and Yeast Genomic DNA Kit (HiMedia, India).

16S rRNA Gene Sequencing

The 16S rDNA region was amplified using the universal 16S rRNA gene forward primer 7–26 (5'-AGAGTTTGTAT CCTGGCTCAG-3') [13] and reverse primer 1,513–1,494 of the *Escherichia coli* 16S gene (5'-GGCTACCTTGTTA CGACTT-3') [14]. The PCR amplification involved an initial cycle of 94 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min, with a final extension at 72 °C for 5 min carried out in a Gene AMP PCR system 9700 (Applied Biosystems, USA). The amplified products were analysed on 1.2 % agarose gel. Amplified products were purified using QIAquick Gel Extraction Spin Kit (QIAGEN, Germany) and were bi-directionally sequenced using the same forward and reverse primers by Genetic Analyzer ABI 3130XL (Applied Biosystems, USA) with Big Dye (3.1) terminator protocol. Basic Local Alignment Search Tool (BLAST) was used to determine the existing phylogenetic neighbours from the nucleotide database of NCBI [15] and Ez-Taxon [16]. The obtained sequences of existing neighbors along with the sequence of DPs-13 were aligned using Clustal W inbuilt with Molecular Evolutionary Genetics Analysis software (MEGA 4) [17]. Neighbor-Joining, Maximum parsimony and Maximum evolutionary methods were employed to construct the phylogenetic tree with 1,000 bootstrap replications to assess nodal support in the tree. *Deinococcus radiodurans* M21413 was taken as an outgroup. The 16S rRNA gene partial sequence was submitted to GenBank and accessions obtained.

16–23S rRNA Gene Spacer Region Sequencing

16S–23S rRNA gene spacer (ITS1) region of the isolate DPs-13 was amplified using the primers A1 (5'-GCCCG TCACACCATGGGAG-3') and A2 (5'-TCGCCT(G/C)TG (A/G)(A/G)GCCA-3'). Forward A1 was from 16S rRNA gene, and reverse A2 was from 23S rRNA gene and PCR mixture of 50 µL reaction volume was prepared according to Tyler et al. [18] with minor modifications. Approximately 700 nucleotides were amplified and the amplified products were purified and bi-directionally sequenced using the primers A1 and A2 as described for 16S rRNA gene. BLAST was used to determine the existing phylogenetic neighbours from the nucleotide database of NCBI [15]. The sequences of existing neighbors were aligned with the sequence of the selected isolate using Clustal W inbuilt with MEGA 4. Neighbor-joining, Maximum parsimony and Maximum evolutionary methods were employed to construct the phylogenetic tree with 1,000 bootstrap replications to assess nodal support in the tree. The ITS-1 sequence with embedded tRNA-Ile and tRNA-Ala genes along with flanking 16S and 23S rRNA gene partial sequence was submitted to GenBank and accession number obtained.

Determination of Minimum Inhibitory Concentration (MIC) of Metals

To study MICs for the isolate DPs-13, five different metals namely U, Cu, Zn, Cd and Pb were taken in two different culture conditions. Analytical grade salts of uranyl nitrate [$\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$], copper sulphate [$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$], cadmium nitrate [$\text{Cd}(\text{NO}_3)_2 \cdot 5\text{H}_2\text{O}$], zinc sulphate [$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$] and lead nitrate [$\text{Pb}(\text{NO}_3)_2$] were used to prepare stock solutions. The stock solutions were filter sterilized through a 0.22 µm nitrocellulose membrane filter (Millipore, India). The isolate was grown to mid-exponential phase in low phosphate broth and studied for its tolerance in two different agar media, Mueller–Hinton Agar (MHA) (HiMedia, India) and low phosphate agar (LPA) media with increasing concentration of heavy metals. Streak plate technique was used to inoculate the mid-exponential phase culture of DPs-13 and the reference strain *P. aeruginosa* MTCC2474 on metal enriched agar plates. The minimum concentration of metal that completely prevented growth on agar plates after 72 h incubation at 32 °C was taken to be the minimal inhibitory concentration (MIC) of the metal.

Antibiotic Resistance

The isolate DPs-13 was screened for intrinsic antibiotics resistance using antibiotic discs (HiMedia, India) on MHA

plates along with the reference strain *P. aeruginosa* MTCC2474. Eleven antibiotics viz., Ampicillin (10 µg)-A, Kanamycin (30 µg)-K, Chloramphenicol (30 µg)-C, Erythromycin (10 µg)-E, Gentamicin (10 µg)-G, Aztreonam (30 µg)-Ao, Tetracycline (30 µg)-T, Imipenem (10 µg)-I, Ciprofloxacin (5 µg)-Cf, Streptomycin (25 µg) and Amikacin (30 µg)-Ak were taken for this study. The results were recorded as Resistant (R), Intermediate (I) and Susceptible (S) on the basis of the diameter of the inhibition zone in reference to the Zone Size Interpretative Chart supplied by the manufacturer.

Uranyl Bioadsorption Capacity and Viability Test of the Isolate

For uranyl bioadsorption study, the wild isolate along with the reference strain were grown in M9 minimal medium. Mid-exponential phase cells obtained after centrifugation ($5,600 \times g$ for 5 min) were washed with sterile deionised water. 10 mL of sterile uranyl nitrate solutions containing four different concentrations viz., 100 µM, 500 µM, 1 mM, and 2 mM U were allowed to equilibrate for 30 min under aerobic conditions. Both the wild isolate and the reference strain at $\text{OD}_{600} 2$, were resuspended in the uranium solutions separately and incubated at 32 °C under continuous shaking for 24 h. 100 µL from this solution was withdrawn and centrifuged at $9,500 \times g$ for 3 min. The supernatants containing residual uranium were acidified with 0.01 N HCl to prevent precipitation. The uranium-loaded cell pellets were washed with distilled water to remove loosely bound uranium and digested at room temperature with 0.2 % HCl. The mineralised samples were then estimated for uranium by using the arsenazo III method [19]. Spontaneous chemical precipitation of uranium was checked in the absence of the test organism. Each treatment consisted of 3 replicates.

The viability test to evaluate the tolerance of strains to acidic condition (pH 3.5) and uranium (100 µM and 2 mM) were done as described by Kumar et al. [20] with minor modifications. Three aliquots of 1 mL mid-log phase cells of equivalent OD ($\text{OD}_{600} 2$) were washed with MilliQ water (pH 7.0) and each aliquot was assayed for viability as follows: (a) serial 10-fold dilutions were made and plated on LB agar, (b) transferred to MilliQ water (pH 3.5) without uranium, incubated for 1 h at 32 °C and plated (serial 10-fold dilutions) on LB agar and (c) inoculated in uranyl nitrate solutions (100 µM and 2 mM separately) at pH 3.5 and incubated for 1 h at 32 °C. Viability test was done to ensure minimum loss in cell density due to nutrient limitation for 1 h as the present study showed that the uranyl adsorption reached equilibrium by 1 h, and serial 10-fold dilutions were plated on LB agar. All the plates were incubated for 24 h and enumeration was done for 3 replicates.

Occurrence of Plasmid

Occurrence of plasmid in both the wild isolate and reference strain was checked using plasmid isolation kit—Hi-Per™ HP Plasmid DNA Mini Kit (HiMedia, India). *E. coli* DH5 α was taken as negative control. To visualize its presence, plasmid DNA was run in 1 % agarose gel along with supercoiled plasmid DNA ladder (Merck, India) stained with ethidium bromide.

PGP Factors and Biocontrol Potentialities

Siderophore Production Activity

Siderophore production ability was screened by inoculating the isolate into sterile succinic acid medium (SAM) containing (g l⁻¹ in distilled water): K₂HPO₄–6.0; KH₂PO₄–3.0; (NH₄)₂SO₄–1.0; MgSO₄·7H₂O–0.2; C₄H₄Na₂O₄·6H₂O–4.0 and pH 7, at 28 ± 2 °C at 120 rpm for 48 h. Change in the colour of SAM from colorless to fluorescent green after 48 h of incubation indicated siderophore production.

Phosphate Solubilising Activity

Pikovskaya's agar (HiMedia, India) medium was used to test phosphate solubilising ability of the test isolate and the reference strain MTCC2474. A clear zone around the isolate confirms the phosphate solubilising ability.

Antagonistic Activities

Antagonistic activity of the isolate was studied against the fungal plant pathogens *F. oxysporum* MTCC284 and *B. cinerea* MTCC359 using secondary metabolite of the culture broth. 100 μ L of 72 h old culture supernatant was used to study antimicrobial activity by agar well diffusion method [21]. Zone of inhibition was recorded after 5 days with three replications.

Antibiotic Coding Genes

The isolate was screened for two antibiotic coding genes, viz., 2, 4-diacetyl-phloro-glucinol (DAPG) and pyoluteorin (PLT) as described by Naik et al. [22]. For the amplification of DAPG (745 bp), the two primers, viz., Phl2a (5'-GAG GACGTCGAAGACCACCA-3') and Phl2b (5'-ACCGCA GCATCGTGTATGAG-3') were used. PLT (779 bp) region was amplified by the primers PltBf (5'-CGGAGCATGGAC CCCAGC-3') and PltBr (5'-GTGCCCGATATTG GTCTT GACCGAG-3').

Phytotoxicity Test

To check for any phytotoxic effect of the isolate DPs-13, chickpea (*Cicer arietinum*) seeds were soaked overnight with 72 h old culture supernatant after centrifugation. Uninoculated media was taken as control. Overnight soaked seeds were sandwiched with wet filter paper and kept for 3 days in the petri plates. Both the control seeds and test seeds were moisturized by spraying sterilized water. After 5 days, the seeds in both the plates were checked for toxicity effect.

Results and Discussion

Sample Analysis

The pseudomonad considered for this study was isolated from sediment sample of Kylleng mining site (zero point area) (25°19'14.8'' N and 91°12'46.6'' E, altitude 811 m above sea level) having a pH value of 5.6. This acidic nature of the sediment may be due to the presence of pyrite (FeS₂) and humic substance (coal) in the prevailing sandstone rocks [23]. Uranium concentration of the sample was recorded as 480 ppm while concentrations of other metals like Cu, Pb, Zn and Cd were 28.8, 23, 377 and 0.9 ppm respectively. U concentration was found to be high whereas concentrations of other metals tested were found to be within the range of background concentration of trace elements as observed in non-anthropogenic soils [24].

Identification of the Isolate

The results of API 20NE kit (20 + 1) test for the wild isolate and the reference strain were interpreted using apiweb™ software and identified as *P. aeruginosa* in highest level of confidence i.e., “Excellent Identification” (Table 1).

BLAST results of 16S rRNA gene sequence of the isolate showed more than 99 % pairwise similarity with *P. aeruginosa* LMG 1242T in EzTaxon database [16]. The isolate DPs-13 produced a single band of approximately 700 bp of the 16–23S rRNA spacer region. BLAST results of the obtained sequences matched the ITS1 region of *P. aeruginosa*. Neighbor joining tree of 16S rRNA gene and ITS1 sequences of the isolate DPs-13 with its nearest homologs were constructed using MEGA 4 (Figs. 1, 2). Accession numbers for 16S rRNA gene and ITS1 sequences are JN247764 and JN418879 respectively.

Table 1 Identification result of API 20NE and the aptweb™ online software

	NO ₃	TRP	GLU	GLU	ADH	URE	URE	ESC	ESC	GEL	PNG	GLU	ARA	MNE	MAN	NAG	NAG	MAL	GNT	CAP	ADI	MLT	CIT	PAC	OX	Identification
Type strain MTCC24742	+	-	-	-	+	+	+	+	+	+	-	+	-	-	+	+	+	-	+	+	+	+	+	+	+	<i>P. aeruginosa</i>
Isolate DPs-13	+	-	-	-	+	+	+	+	+	+	-	+	-	-	+	+	+	ND	+	+	+	+	+	+	+	<i>P. aeruginosa</i>

ND not detected. (The abbreviations denote: Oxidation of nitrate (NO₃), indole production (TRyptoPhane), fermentation of GLUcose, Arginine DiHydrolyase, UREase; hydrolysis of (β-glucosidase) (ESCulin), (protease) (GELatin) and β-galactosidase (Para-NitroPhenyl-8DGalactopyranosidase), utilization of GLUcose, ARAbinose, MaNnosE, MANnitro, N-Acetyl-Gluco-samine, MALtose, GlucoNaTe, CAPrate, ADIpate, MaLaTe, CITrate, PhenylACetate, OXidase)

Determination of MICs of Metals

The MIC of U, Cu, Cd, Zn and Pb for the growth of the wild isolate DPs-13 was compared with the reference strain *P. aeruginosa* MTCC2474. The metal tolerance ability of the test isolate was found to be higher than the reference strain. The toxicity of the metals was in the order of Cd > U > Zn > Cu > Pb in MHA, while in LPA it was Pb > Cd > U = Zn > Cu (Figs. 3, 4). In natural environments, the availability of metals to the bacteria may vary due to the presence of other agents which can bind with metals [25]. The differences between MIC on rich medium and LPM could be explained as mentioned previously by Mergeay et al. [26]. They demonstrated that the interference of high phosphate content in high phosphate minimal medium with metal effect, can lead to overestimation of the MIC [26]. Considering that, in this study DPs-13 was tested for its multi-metal resistance ability using both rich and low phosphate minimal media, to mimic nearest natural conditions of metal availability.

Antibiotic Resistance

The wild isolate DPs-13 could resist six out of eleven commonly used antibiotics taken for this study namely, Ampicillin, Kanamycin, Chloramphenicol, Aztreonam, Tetracycline and Amikacin as compared to three antibiotics by the reference strain MTCC2474 (Table 2). Some studies suggested that the presence of metals could increase resistance to antibiotics in *P. aeruginosa* because of possible coregulation [27, 28].

Uranyl Bioadsorption Potential and Viability of the Isolate

Uranyl adsorption potential of the wild isolate DPs-13 was evaluated with respect to incubation time (5 min to 24 h) as well as initial uranium concentrations (100 μM to 2 mM) (23.8–476 mg/L). Uranyl binding by the wild isolate was found to be very high, showing 94 % uranium removal from the uranium solutions (100–500 μM) at pH 3.5 within 1 h and the activity was retained even at 24 h of incubation (Fig. 5). The reference strain showed 68 % removal from 100 μM solution and only 25 % uranium removal was observed from 2 mM test solutions at pH 3.5. Uranium remained stable under the experimental conditions and did not precipitate as seen in the abiotic controls. Uranyl adsorption results beyond 24 h are not shown due to nutrient limitation and an increase in cell density owing to replication. Both the wild isolate and the reference strain could not remove uranium from test solutions at pH >7. The viability study revealed that *P. aeruginosa* isolate showed tolerance to acidic pH (pH 3.5) and toxic

Fig. 1 Neighbor-joining tree based on 16S rRNA gene sequences depicting the phylogenetic relationship of DPs-13 with its nearest homologs and *D. radiodurans* M20539T as the outgroup organism

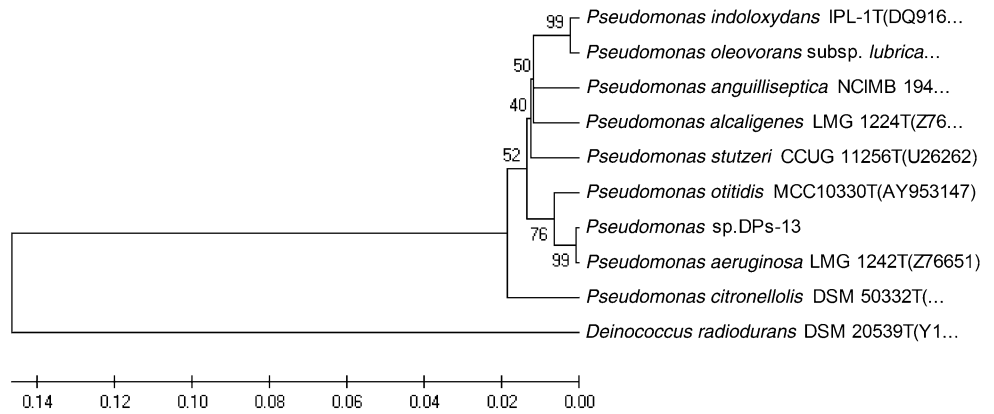


Fig. 2 Phylogenetic tree constructed from 16–23S rRNA gene spacer (ITS1) region sequences of DPs-13 and its nearest homologs

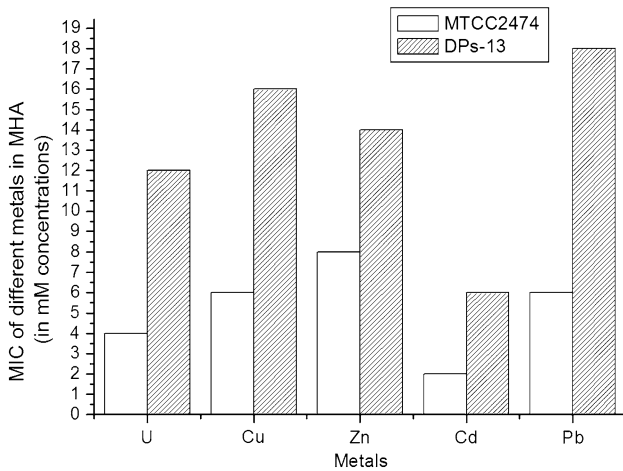
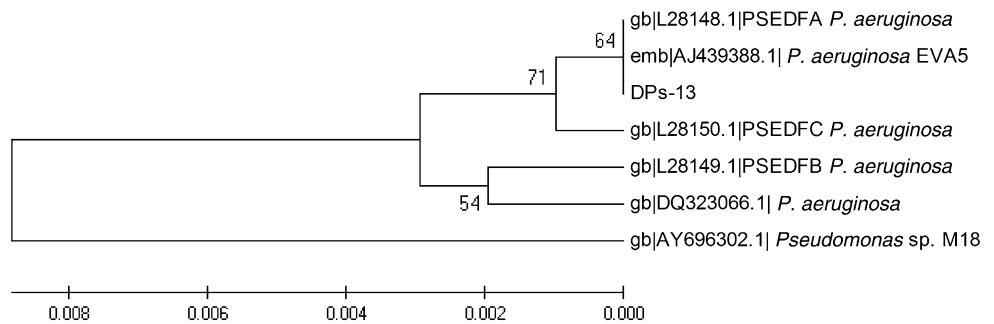


Fig. 3 MIC of five different metals in milliMolar (mM) concentrations in MHA plates

concentrations of uranium U(VI) (100 μ M to 2 mM) (Fig. 6). The characteristics of uranyl binding at acidic pH and tolerance to toxic concentrations of heavy metals including uranium exhibited by the wild isolate correlates with the in situ conditions prevalent at the studied sites.

Occurrence of Plasmid

The isolate DPs-13 carried plasmid under metal stressed conditions while the reference strain did not reveal the

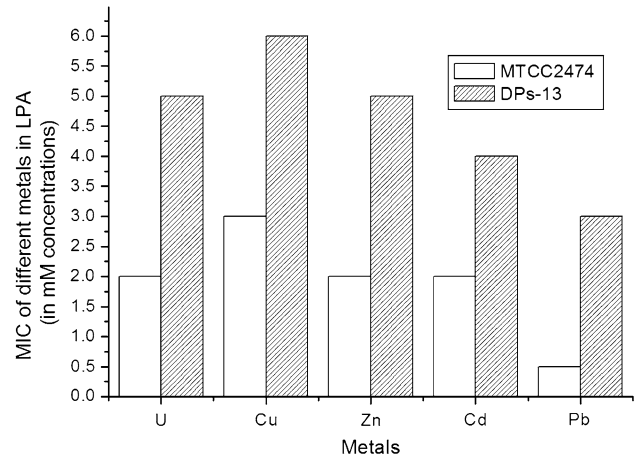


Fig. 4 MIC of five different metals in milliMolar (mM) concentrations in LPA plates

presence of the same. Several studies revealed plasmid mediated metal tolerance in bacteria [29–31].

PGP Factors and Biocontrol Potentialities

The culture was found to produce siderophore after 24 h in SAM. A clear zone around the isolate on Pikovskaya's agar (HiMedia, India) plate confirmed the phosphate solubilising ability of the isolate whereas the reference strain lacked this ability (Online resource 1). The wild isolate had a

Table 2 Antibiotic sensitivity of the wild isolate and the type strain of *P. aeruginosa*

Antibiotics and their concentrations	<i>P. aeruginosa</i> MTCC2474	<i>P. aeruginosa</i> DPs-13
Ciprofloxin (5 µg)	Susceptible	Intermediate
Chloramphenicol (30 µg)	Intermediate	Resistant
Tetracycline (30 µg)	Intermediate	Resistant
Erythromycin (10 µg)	Intermediate	Intermediate
Gentamicin (10 µg)	Susceptible	Susceptible
Amikacin (30 µg)	Susceptible	Resistant
Imipenem (10 µg)	Susceptible	Susceptible
Ampicillin (10 µg)	Resistant	Resistant
Kanamycin (30 µg)	Resistant	Resistant
Aztreonam (30 µg)	Resistant	Resistant
Streptomycin (25 µg)	Susceptible	Resistant

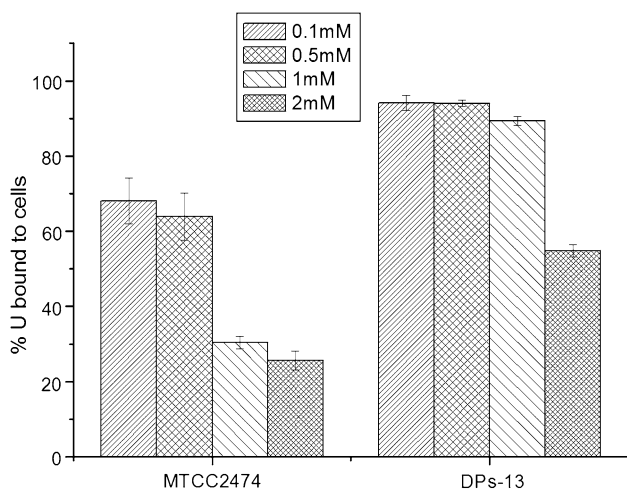


Fig. 5 Uranium binding by wild isolate DPs-13 and reference strain *P. aeruginosa* MTCC2474. [Equivalent cells (OD_{600} 2) of both the organisms were exposed to four different concentrations (100 µM, 500 µM, 1 mM and 2 mM) of uranyl nitrate solutions and checked for their U binding potentiality for 24 h. Error bars denote standard deviation of triplicate experiments]

strong antagonistic nature as it showed inhibition zone against both the fungal plant pathogens tested at culture supernatant screening level (Table 3, Online resource 2 and 3). Productions of DAPG and PLT have been shown to be important mechanism of biological control of a wide variety of plant pathogens by fluorescent *Pseudomonas* spp. [32]. Both the tested antibiotic coding genes i.e., DAPG and PLT were found to be present in the isolate DPs-13 (Online resource 4). Secondary compound of the isolate DPs-13 did not possess any phytotoxic effect as the root and shoot development in the culture filtrate treated seedlings of chickpea was normal (Online resource 5).

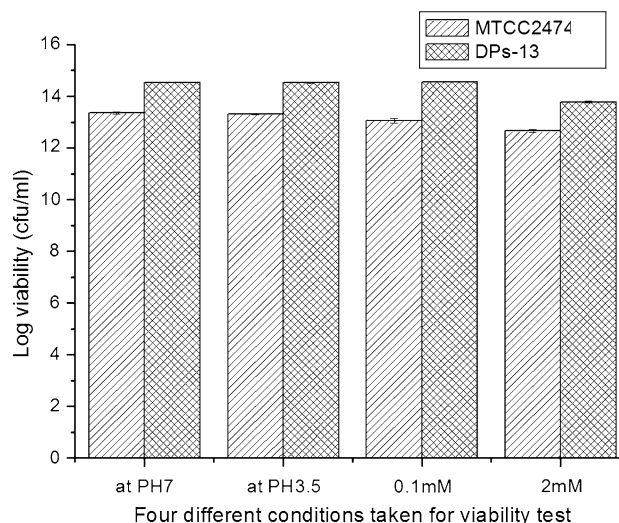


Fig. 6 Viable cell counts (log CFU/mL) determined after washing and incubating for 1 h at pH 7.0, at pH 3.5, at pH 3.5 in 100 µM uranyl nitrate solution and at pH 3.5 in 2 mM uranyl nitrate solution. (Error bars denote standard deviation of triplicate experiments)

Table 3 Antagonistic activity as zone of growth inhibition shown by the wild isolate and the type strain of *P. aeruginosa*

	<i>F. oxysporum</i> (MTCC284)	<i>B. cinerea</i> (MTCC359)
DPs-13	++	+
MTCC2474	-	-

- = negative, + = 10–15 mm, ++ =>15 mm

Conclusion

This study was aimed to isolate potential pseudomonad from uranium ore rich area for metal/uranium tolerance and bioremediation as well as soil quality enhancement. The isolate DPs-13 showed potentials in metal bioadsorption as well as PGP and biocontrol activity. It showed higher tolerance to multiple metals and antibiotics when compared to reference strain *P. aeruginosa* MTCC2474. In this study, DPs-13 was tested for its multi-metal tolerance ability using both rich and minimal media to mimic nearest natural conditions of metal availability and found to have higher MIC values for each metal in both the media. Uranium bioadsorption study of the isolate revealed up to 94 and 72 % removal of U(VI) when challenged with 100 µM and 2 mM uranyl nitrate solutions within 1 h of incubation, whereas the reference strain could remove 68 and 25 % uranium when challenged with the above concentrations respectively at pH 3.5. The siderophore producing isolate DPs-13 was found to carry two important antibiotic producing genes. The isolate showed phosphate solubilisation and strong antagonistic activity against the fungal plant pathogens without any phytotoxic affect. The

soil quality enhancement through PGP potential of the isolate from uranium rich site can be an added advantage for it to be considered as a potential candidate for concerted approach in bioremediation along with phytoremediation technology to convert metal contaminated sites to productive land.

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References

- Moore JW, Ramamoorthy A (1984) Heavy metals in Natural waters: applied monitoring and impact assessment. Springer, New York
- Malik A, Ahmad M (1995) Genotoxicity of some waste waters in India. *Environ Toxicol Water Qual* 10:287–293. doi:10.1002/tox.2530100409
- Lloyd J, Lovley D (2001) Microbial detoxification of metals and radionuclides. *Curr Opin Biotechnol* 12:248–253
- Sarma B, Acharya C, Joshi SR (2010) Pseudomonads: a versatile bacterial group exhibiting dual resistance to metals and antibiotics. *Afr J Microbiol Res* 4:2828–2835
- Haferburg G, Kothe E (2007) Microbes and metals: interactions in the environment. *J Basic Microbiol* 47:453–467
- Tripathi P, Srivastava S (2007) Development and characterization of nickel accumulating mutants of *Aspergillus nidulans*. *Indian J Microbiol* 47:241–250
- Cho Y, Kim JS, Crowley DE, Cho B (2003) Growth promotion of the edible fungus *Pleurotus ostreatus* by fluorescent pseudomonads. *FEMS Microbiol Lett* 218:271–276
- Preston GM (2004) Plant perceptions of plant growth-promoting Pseudomonas. *Philos Trans R Soc Lond B Biol Sci* 359:907–918
- Sayed RZ, Patel PR (2011) Biocontrol potential of siderophore producing heavy metal resistant *Alcaligenes* sp. and *Pseudomonas aeruginosa* RZS3 vis-a-vis organophosphorus fungicide. *Indian J Microbiol* 51:266–272. doi:10.1007/s00284-008-9264-z
- Rodríguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol Adv* 17:319–339. doi:10.1016/S0734-9750(99)00014-2
- Nair A, Juwarkar AA, Singh SK (2006) Production and characterization of siderophores and application in arsenic removal from contaminated soil. *Water Air Soil Pollut* 180:199–212. doi:10.1007/s11270-006-9263-2
- Sayed RZ, Chincholkar SB (2010) Growth and siderophore production *Alcaligenes faecalis* is influenced by heavy metals. *Indian J Microbiol* 50(2):179–182
- Brosius J, Dull TJ, Sleeter DD, Noller HF (1981) Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. *J Mol Biol* 148:107–127
- Weisburg WG, Barns SM, DA Pelletier Z, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 173:697–703
- Altschul SF, Madden TL, Schaeffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25:3389–3402
- Chun J, Lee JH, Jung Y, Kim M, Kim S, Kim BK, Lim YW (2007) EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* 57:2259–2261
- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* 24:1596–1599
- Tyler SD, Strathdee CA, Rozee KR, Johnson WM (1995) Oligonucleotide primers designed to differentiate pathogenic pseudomonads on the basis of the sequencing of genes coding for 16S–23S rRNA internal transcribed spacers. *Clin Diagn Lab Immunol* 2:448–453
- Savvin SB (1961) Analytical use of arsenazo III: determination of thorium, zirconium, uranium and rare earth elements. *Talanta* 8:673–685
- Kumar R, Acharya C, Joshi SR (2011) Isolation and analyses of uranium tolerant *Serratia marcescens* strains and their utilization for aerobic uranium U(VI) bioadsorption. *J Microbiol* 49:568–574. doi:10.1007/s12275-011-0366-0
- Schillinger U, Lucke FK (1989) Antibacterial activity of Lactobacillus sake isolated from meat. *Appl Environ Microbiol* 55:1901–1906
- Naik PR, Sahoo N, Goswami D, Ayyadurai N, Sakthivel N (2008) Genetic and functional diversity among fluorescent pseudomonads isolated from the rhizosphere of banana. *Microbiol Ecol* 56:492–504
- Lenka P, Jha SK, Gothankar S, Tripathi RM, Puranik VD (2009) Suitable gamma energy for gamma-spectrometric determination of ²³⁸U in surface soil samples of a high rainfall area in India. *J Environ Radioact* 100:509–514
- Burt R, Wilson MA, Mays MD, Lee CW (2003) Major and trace elements of selected pedons in the USA. *J Environ Qual* 32:2109–2121
- Hassen A, Saidi N, Cherif M, Boudabous A (1998) Resistance of environmental bacteria to heavy metals. *Bioresour Technol* 64:7–15
- Mergeay M, Nies D, Schiegel HG, gerits J, Charles P, Van Gijsegem F (1985) *Alcaligenes eutrophus* CH34 is a facultative chemolithotroph with plasmid-bound resistance to heavy metals. *J Bacteriol* 162:238–334
- Perron K, Caille O, Rossier C, Delden CV, Dumas J, Köhler T (2004) CzcR-CzcS, a two-component system involved in heavy metal and carbapenem resistance in *Pseudomonas aeruginosa*. *J Biol Chem* 279:8761–8768
- Caille O, Rossier C, Perron K (2007) A copper-activated two-component system interacts with zinc and imipenem resistance in *Pseudomonas aeruginosa*. *J Bacteriol* 189:4561–4568
- Lim C, Cooksey DA (1993) Characterization of chromosomal homologs of the plasmid-borne copper resistance operon of *Pseudomonas syringae*. *J Bacteriol* 175:4492–4498
- El-Deeb B (2009) Plasmid mediated tolerance and removal of heavy metals by enterobacter sp. *Am J Biochem Biotechnol* 5:47–53
- Raja CE, Selvam GS (2009) Plasmid profile and curing analysis of *Pseudomonas aeruginosa* as metal resistant. *Int J Environ Sci Technol* 6:259–266
- Schnider-Keel U, Seematter A, Maurhofer M, Blumer C, Duffy B, Gigot-Bonnefoy C, Reimann C, Notz R, De'fago G, Haas D, Keel C (2000) Autoinduction of 2,4-diacetylphloroglucinol biosynthesis in the biocontrol agent *Pseudomonas fluorescens* CHA0 and repression by the bacterial metabolites salicylate and pyoluteorin. *J Bacteriol* 182:1215–1220