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RESEARCH **P**APER

Isolation and functional characterization of cadmium resistant strains RAN 1 using agronomical parameter of chaulai (*Amaranthus tricolor*)

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Resistance and tolerance are arbitrarily defined terms frequently interchangeable and after based on weather a given isolate can grow in the presence of selected heavy metal concentration in laboratory media. Total bacterial strains were isolated and screened on the basis of their heavy metal tolerance ability for cadmium and their PGPR activity. It was estimated that they have good tolerance power for cadmium the heavy metals and are plant growth promoting rhizobacteria. If we study morphological aspects we can say that Gram negative rods are more efficient in tolerating heavy metals. In case of treatment I, fresh weight was increased 27.5 per cent and dry weight was increased 25 per cent compared to positive control. In case of treatment II, fresh weight was increased 28 per cent and dry weight was increased 14.81 per cent compared to negative control. In case of negative control, fresh weight was decreased 50 per cent and dry weight was decreased 48.07 per cent compared to positive control. By performing experiment it was proved that the cadmium concentration reduced significant percentage of Chaulai fresh weight and dry weight but increased when treated with strain RAN 1.

Key words : Rhizobacteria, Tolerance, Resistance, Functional characterization, Cadmimum

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INTRODUCTION

Heavy metal is natural constituent of the earth crust and is present in varying concentration in all ecosystems. Heavy metal is chemical elements with a specific gravity that is at least 5 times the specific gravity of water. Copper, chromium, cadmium are known to be the most commonly metal used and more widespread contaminants of their environment (Patterson, 1977; Aksu, 1998; Doenmez and Aksu, 2001). Heavy metal could have long term hazardous impact on health of soil micro-organism and adverse influence on soil biological process (Khan *et al*., 2009).

Cadmium is one of the most toxic pollutants of the surface soil layer, released into the environment by mining and smelting activities, atmospheric deposition from metallurgical industries, incineration of plastics and batteries, land application of sewage sludge, and burning of fossil fuels (Tang *et al*., 2006).

Cadmium is heavy metal of no nutrition function, it is a natural element in the earth crust and usually found as a mineral combined with other element such as oxygen (cadmium oxide), chlorine (cadmium chloride), sulphur (cadmium sulfate) and is a toxic agent to micro-organism,

Table A : Samples of soil collected from Rhizosphere

Sr. No.	Soil sample	Source	Plant	Colour	Texture	Arrangement	pH
1.	RAN	Rhizosphere	*Acacia nelotica* (Babul)	Light brown	Fine	Plain	9

but some are microbial strain resistant to the metal. The present investigation was carryout to isolate and functional characterize cadmium resistant strains RAN_1 and its effect on chaulai plant in cadmium contaminated soil.

RESEARCH METHODOLOGY

Collection of soil samples :

Soil sample was collected from the rhizospheric soil of plants from Panki power house. The collected soil samples and strains were stored at 4°C. The details of soil samples collected from rhizosphere are given in Table A.

Isolation of sample :

Soil sample (2 g) was grinded and suspended in 20 ml sterile water. Then isolation of the sample was done by serially diluting it and 0.1 ml of diluted suspension was placed on nutrient agar media and spread evenly on solid plates. The plates were incubated at suitable temperature for different time intervals. The screenings of the strains were done on the basis of their tolerance power with cadmium concentration (Ahmad *et al.*, 2008).

Heavy metal tolerance :

Stock solution of cadmium was made (1 g in 25 ml distilled water). Different concentration of cadmium in media was pipetted and tolerance power of the strains was determined (Mark, 1987).

Identification of bacteria :

Bacterial colonies were identified by Gram staining, starch hydrolysis test, gelatin hydrolysis test, indole test, methyl red test, voges porskauer test, citrate utilization test , triple sugar iron agar test, urease test, catalase test and H_2S production tests (Verma, 2012).

Plant growth promoting activity :

Phosphate solubilization :

The phosphate solubilization by the rhizosperic microbes was detected and estimated by the method of Tank and Saraf, (2010).

HCN production :

HCN production was performed by Bakkar *et al*. (1987)

Siderophore production :

Siderophore production by selected bacterial cultures was detected by Schwyn and Neillands (1987). Both qualitative and quantitative determination of siderophore was done.

Screening of physiological characterization of bacterial strains:

Temperature and pH were determined for screening of physiological characterization of bacterial strains.

RESEARCH FINDINGS AND ANALYSIS

Soil microbial population is known to play a key role in maintaining soil fertility by recycling of plant nutrients and also for removal of metals in contaminated soils. In this context various approaches have been used to study microbial activities in soil. As it is difficult to elucidate soil microbial activity using single monitoring approach, therefore, for a better insight and complete depiction of soil microbial situation, different approaches need to be used. In present investigation total of 5 bacterial isolates were isolated from rhizospheric soil of *Acacia nelotica* (Babul) from Panki power house and tentatively identified on the basis of morphological and biochemical characterization. All bacterial isolates belonged to the

Table 1 : Heavy metal tolerance of soil micro-organism

Sr. No.	Rhizosphere	Strains	Cd (µg/ml)	Incubation time	Incubation temp.	Media
1.	*Acacia nelotica* (Babul)	RAN 1	1150	18-24 h	28°C	Nutrient agar
		RAN 2	510	18-24 h	28°C	
		RAN 3	800	18-24 h	28°C	
		RAN 4	540	18-24 h	28°C	
		RAN 5	580	18-24 h	28°C	

Gram negative groups.

Heavy metal tolerance :

Among all these strains maximum tolerance for cadmium was shown by strain RAN 1 as shown in Table 1.

Characterization of bacteria :

Specific soil strains were isolated from soil sample along with concentration of cadmium on nutrient agar medium. These isolates were tentatively identified on the basis of their staining, behaviour, morphological and cultural characteristics as shown in Table 2.

Biochemical characterization of all isolated strains:

Functional characterization :

The graph showed the comparative analysis between root length in presence and absence of cadmium. In case of treatment I (Media + seeds + strain RAN 1) root length was increased 19.44 per cent compared to positive control (Media + seeds). In case of treatment II (Media + seeds + strain RAN 1 + Cd Conc.) root length was increased 58.82 per cent compared to negative control (Media + seeds + Cd conc.) In case of negative control (Media + seeds + Cd conc.), root length was decreased 5.55 per cent compared to positive control (Media + seeds) as shown in Table 3 and 4, Fig. 1 and 2.

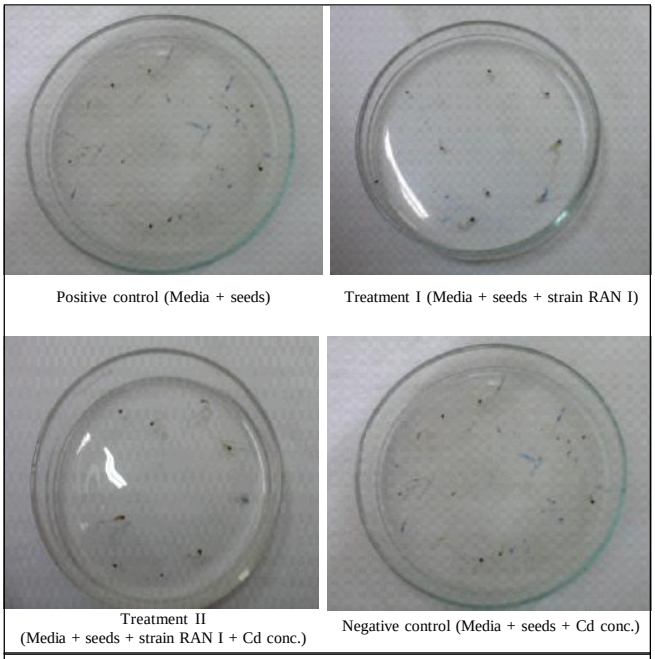

Fig. 1 : Effect of microbial treatment of strain RAN 1 on chaulai seeds germination in presence or absence of cadmium *in vitro* experiment

Table 2 : Morphological characterization of all isolated strains

Strains	Gram staining
RAN 1	Gram negative, rod shaped, fluorescent
RAN 2	Gram negative, rod shaped, fluorescent
RAN 3	Gram negative, rod shaped
RAN 4	Gram negative, rod shaped, fluorescent
RAN 5	Gram negative, rod shaped

Table 3 : Biochemical characterization of all isolated strains

Sr. No.	Srains	Starch hydrolysis	Gelatinase	Indole production	MR	VP	Citrate utilization	Triple sugar iron agar test	Urease	Catalase	H_2S production
1.	RAN 1	+	+	+	+	+	+	+	–	+	–
2.	RAN 2	–	–	–	–	–	–	–	–	+	–
3.	RAN 3	+	–	–	–	+	–	–	–	+	–
4.	RAN 4	–	–	–	+	+	–	–	–	-	–
5.	RAN 5	–	–	–	–	–	–	+	–	+	–

Table 4 : Effect of microbial treatment of strain RAN 1 on chaulai seeds germination in presence or absence of cadmium *in vitro* experiment

Treatments	Root length (in cm)
Positive control (Media + seeds)	1.80
Treatment I (Media + seeds + strain RAN 1)	2.15
Treatment II (Media + seeds + strain RAN 1 + Cd conc.)	2.70
Negative control (Media + seeds + Cd conc.)	1.70

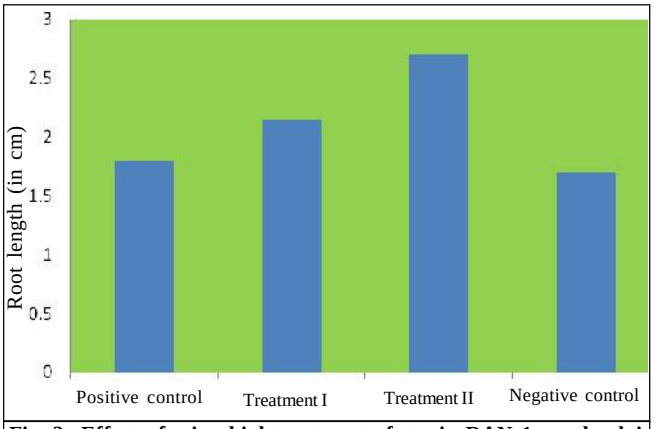

Fig. 2 : Effect of microbial treatment of strain RAN 1 on chaulai seeds germination in presence or absence of cadmium *in vitro* experiment

The graph showed the comparative analysis of root and shoot length in presence or absence of cadmium. In case of treatment I (Soil + seeds + strain RAN 1), root length was increased 40.59 per cent and shoot length was increased 45 per cent compared to positive control (Soil + seeds). In case of treatment II (Soil + seeds + strain RAN 1 + Cd conc.), root length was increased 45.23 per cent and shoot length was increased 38.75 per cent compared to negative control (Soil + seeds + Cd conc.). In case of negative control (Soil + seeds + Cd conc.), root length was decreased 16.83 per cent and shoot length was decreased 14 per cent compared to positive control (Soil + seeds) (Table 5 and Fig. 3). Similar results were also reported by Khan (2005) and Velumurugan (2008).

The graph showed the comparative analysis of fresh and dry weight in presence or absence of cadmium. In case of treatment I (Soil + seeds + strain RAN 1), fresh weight was increased 27.5 per cent and dry weight

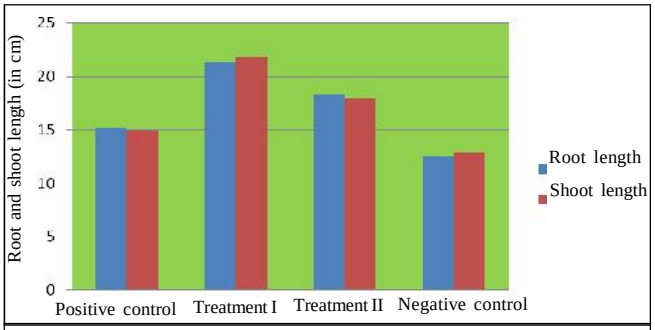

Fig. 3 : Effect of microbial treatment of strain RAN 1 on chaulai seeds in pot experiment in presence and absence of cadmium *in vivo*

was increased 25 per cent compared to positive control (Soil + seeds). In case of treatment II (Soil + seeds + strain RAN 1 + Cd conc.), fresh weight was increased 28 per cent and dry weight was increased 14.81 per cent compared to negative control (Soil + seeds + Cd conc.). In case of negative control (Soil + seeds + Cd conc.), fresh weight was decreased 50 per cent and dry weight was decreased 48.07 per cent compared to positive control (Soil + seeds) (Fig. 4). By performing

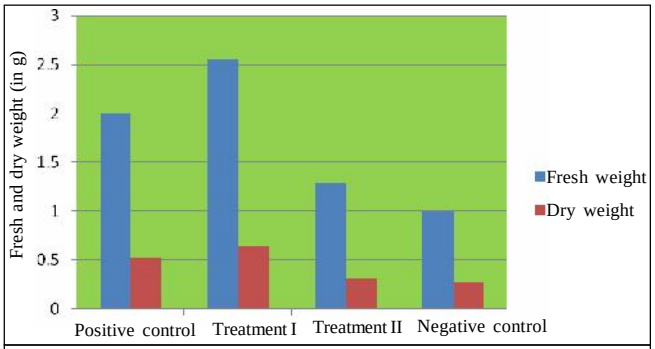

Fig. 4 : Effect of microbial treatment of strain RAN 1 on chaulai seeds in pot experiment in presence and absence of cadmium *in vivo*

Table 5 : Effect of microbial treatment of strain RAN 1 on chaulai seeds in pot experiment in presence and absence of cadmium *in vivo*

	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
Positive control (Soil + seeds)	15.15	15.00	2.00	0.52
Treatment I (Soil+ seeds + strain RAN 1)	21.30	21.75	2.55	0.65
Treatment II (Soil + seeds + strain RAN 1 + Cd conc.)	18.30	17.90	1.28	0.31
Negative control (Soil + seeds + Cd conc.)	12.60	12.90	1.00	0.27

Table 6 : Effect of strain RAN 1 on chlorophyll estimation of chaulai plant

	Chlorophyll A (mg/g)	Chlorophyll B (mg/g)	Total (mg/g)
Positive control (Soil + seeds)	1.2303	0.5361	1.7854
Treatment I (Soil+ seeds + strain RAN 1)	1.3946	0.5345	1.9505
Treatment II (Soil + seeds + strain RAN 1 + Cd Conc.)	1.2231	0.6620	1.9033
Negative control (Soil + seeds + Cd conc.)	1.0134	0.4408	1.5704

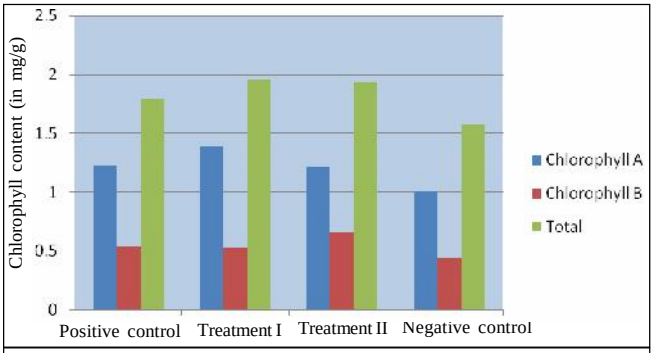

Fig. 5 : Effect of strain RAN 1 on chlorophyll estimation of chaulai plant

where,
1 = Positive control (Soil + seeds)
2 = Treatment I (Soil+ seeds + strain RAN 1)
3 = Negative control (Soil + seeds + Cd conc.)
4 = Treatment II (Soil + seeds + strain RAN 1 + Cd conc.)

Fig. 6 : Effect of microbial treatment of strain RAN 1 on chaulai seeds in pot experiment in presence and absence of cadmium *in vivo* experiment

experiment it was proved that the cadmium concentration reduced significant percentage of chaulai

where,
1 = Positive control (Soil + seeds)
2 = Treatment I (Soil+ seeds + strain RAN 1)
3 = Negative control (Soil + seeds + Cd conc.)
4 = Treatment II (Soil + seeds + strain RAN 1 + Cd conc.)

Fig. 7 : Effect of microbial treatment of strain RAN 1 on chaulai seeds in pot experiment in presence and absence of cadmium : *In vivo* experiment

fresh weight and dry weight but increased when treated with strain RAN 1 Tripathi *et al.* (2004) also reported similar results.

Chlorophyll content increased due to inoculation of strain RAN 1. It provided various function in the plant as nitrogen fixation, synthesizing siderophores, solubilizing minerals as phosphate and other PGPR characteristics. But in presence of heavy metal, the chlorophyll content was decreased.

In the presence of metal a significant reduction in percentage of chlorophyll content was observed (Table 6 and Fig. 5). Similar results were also reported by Stobart *et al*. (1985); Somashekariah *et al*. (1992) and Tripathi *et al*. (2004). When accumulated in plant tissues cadmium causes alteration in the catalytic efficacy of enzymes the change result in inhibition of chlorophyll biosynthesis and photosynthesis when strain RAN 1 incorporated in seeds, this loss over come (Fig. 6 and 7).

LITERATURE CITED

Ahmed, F., Ahmed, I. and Khan, M.S. (2008). Screening of free living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol. Res.*, **163** (2) : 173-181.

Aksu, Z. (1998). Biosorption of heavy metals by microalgae in batch and continuous systems. In: *Algae for waste water treatment*. Springer, Germany, **99** : 37-53.

Bakker, P.A.H.M., Bakker, A.W., Marugg, J.D., Weisbeek, P.J. and Schippers, B. (1987). Bioassay for studying the role of siderophores in potato growth stimulation by *Pseudomonas* spp. in short potato rotations. *Soil Biol. & Biochem.*, **19**(4) : 451-456.

Doenmez, G. and Aksu, Z. (2001). Bioaccumulation of copper (II) and nickel (II) by the non-adapted and adapted growing Candida sp. *Wat. Res.*, **35** (6) : 1425-1434.

Khan, A.G. (2005). Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. *J. Trace Elem. Med. Biol.*, **18** (4) : 355-364.

Khan, S., EI-Latif Heshan, A. and Qiao, M. (2009). Effect of cadmium and lead on soil microbiology community structure and activities. Department of Env Sciences, University of Peshawar.

Mark, R.M. (1987). Heavy metal tolerance in plants: A model evolutionary system. *Trends Ecol. & Evolu.*, **2**(2): 354-359.

Patterson, J.W. (1977). *Waste water treatment technology*. Ann Arbor Science publisher, Ann Arbor, MI, USA.

Schwyn, B. and Neillands, J.B. (1987). Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.*, **160** (1) : 47-56.

Somashekaraiah, B.V., Padmaja, K. and Prasad, A.R.K. (1992). Phytotoxicity of cadmium ions on germinating seedlings of mung bean (*Phaseolus vulgaris*): involvement of lipid peroxides in chlorophyll degradation. *Physiologia Plantarum,* **85** (1) *:* 85-89.

Stobart, A.K., Griffiths, W.T., Bukhari, J.A. and Sherwood, R.P. (1985). The efffect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. *Physiologia Plantarum,* **63** (3) : 293-298.

Tang, X.Y., Zhu, Y.G., Cui, Y.S., Duan, J. and Tang, L. (2006). The effect of ageing on the bioaccessibility and fractionation of cadmium in some typical soils of China. *Environ. Internationa,* **32** (5) : 682-689.

Tank, N. and Saraf, M. (2010). Salinity resistant plant growth promoting rhizobacteria ameliorates sodium chloride stress on tomato plants. *J. Plant Interactions*, **5**(1) : 51-58.

Tripathi, R.D., Srivastava, S., Mishra, S., Singh, N., Tuli, R., Gupta, D.K. and Maathuis, F.J.M. (2004). Arsenic hazards: Strategies for tolerance and remediation by plants. *Trends Biotechnol.*, **25** (4) : 158-165.

Velumurugan, Ganesan (2008). Rhizoremediation of cadmium soil using a cadmium resistant plant growth promoting rhizopseudomonad. *Curr. Microbiol*, **56** (4) : 403-407.

Verma, O.P. (2012). *Practical biotechnology : A methods manual for life sciences*. Parmar Publishers and Distributors, Dhanbad. 14-68.