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Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.)

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Abstract

Eleven cadmium-tolerant bacterial strains were isolated from the root zone of Indian mustard (*Brassica juncea* L. Czern.) seedlings grown in Cd-supplemented soils as well as sewage sludge and mining waste highly contaminated with Cd. The bacteria also showed increased tolerance to other metals including Zn, Cu, Ni and Co. The isolated strains included *Variovorax paradoxus*, *Rhodococcus* sp. and *Flavobacterium* sp., and were capable of stimulating root elongation of *B. juncea* seedlings either in the presence or absence of toxic Cd concentrations. Some of the strains produced indoles or siderophores, but none possessed C_2H_2-reduction activity. All the strains, except *Flavobacterium* sp. strain 5P-3, contained the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which hydrolyses ACC (the immediate precursor of plant hormone ethylene) to NH_3 and α-ketobutyrate. *V. paradoxus* utilized ACC as a sole source of N or energy. A positive correlation between the in vitro ACC deaminase activity of the bacteria and their stimulating effect on root elongation suggested that utilization of ACC is an important bacterial trait determining root growth promotion. The isolated bacteria offer promise as inoculants to improve growth of the metal accumulating plant *B. juncea* in the presence of toxic Cd concentrations and for the development of plant-inoculant systems useful for phytoremediation of polluted soils.

Keywords: ACC deaminase; *Brassica juncea*; Cadmium; Ethylene; *Flavobacterium* sp.; PGPR; Phytoremediation; Rhizosphere; *Rhodococcus* sp.; *Variovorax paradoxus*

1. Introduction

Among heavy metals, which are widespread pollutants of the surface soil layer, cadmium is one of the most toxic. In plants, Cd inhibits root and shoot growth, affects nutrient uptake and homeostasis, and frequently is accumulated by agriculturally important crops (Sanita di Toppi and Gabrielli, 1999). Thus, Cd is consumed by animals and humans with their diet and can cause diseases. Contamination of soil with Cd also negatively affects biodiversity and the activity of soil microbial communities (McGrath, 1994).

Phytoremediation, an emerging low-cost and ecologically benign technology for decontamination of soils, is defined as the process of utilizing plants to absorb, accumulate and detoxify contaminants in soil through physical, chemical and biological processes (Cunningham and Ow, 1996; Saxena et al., 1999; Wenzel et al., 1999). Phytoremediation helps to prevent landscape destruction and enhances activity and diversity of soil microorganisms to maintain healthy ecosystems. Plants suitable for phytoremediation should have a high biomass production with enhanced metal tolerance and metal uptake potential. Most of the commonly known heavy metal accumulators belong to the Brassicaceae family (Kumar et al., 1995). Although hyperaccumulator plants have exceptionally high metal-accumulating capacity, most of these have a slow growth rate and often produce limited amounts of biomass.

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An alternative is to use species with a lower metal-accumulating capacity but higher growth rates, such as Indian mustard (*Brassica juncea* L. Czern.), which is considered to be one of the most promising species for phytoremediation (Kumar et al., 1995; Saxena et al., 1999).

Along with metal toxicity, there are often additional factors that limit plant growth in contaminated soils including arid conditions, a lack of soil structure, low water supply and nutrient deficiency. Therefore, improvement of plant growth under stressed growth conditions is critical to the optimum performance of phytoremediation of soils using both metal hyperaccumulator plant species and metal accumulating crops like *Brassica juncea*. The bacteria associated with plant roots may have profound effects on plant growth and nutrition through a number of mechanisms such as N_2 fixation, production of phytohormones and siderophores, and transformation of nutrient elements. Improvement of the interactions between plants and beneficial rhizosphere microorganisms can enhance biomass production and tolerance of the plants to heavy metals, and are considered is be an important component of phytoremediation technology (Wenzel et al., 1999; Glick, 2003).

Although many soil bacteria are tolerant to heavy metals and play important roles in mobilization or immobilization of heavy metals (Gadd, 1990), only a few attempts have been made to study the rhizosphere bacteria of metal accumulating and hyperaccumulating plants and their role in the tolerance to and uptake of heavy metals by the plants. A high proportion of metal resistant bacteria persist in the rhizosphere of the hyperaccumulators *Thalaspi caerulescens* (Delorme et al., 2001) and *Alyssum bertolonii* (Mengoni et al., 2001) or *Alyssum murale* (Abou-Shanab et al., 2003a) grown in soil contaminated with Zn and Ni or Ni, respectively. The presence of rhizosphere bacteria increased concentrations of Zn (Whiting et al., 2001), Ni (Abou-Shanab et al., 2003b) and Se (De Souza et al., 1999) in *T. caerulescens*, *A. murale* and *B. juncea*, respectively. Inoculation of Indian mustard and canola (*Brassica campestris*) seeds with the plant growth-promoting rhizobacteria (PGPR) strain *Kluyvera ascorbata* SUD165, which produces siderophores and contains the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, protected the plants against Ni, Pb and Zn toxicity (Burd et al., 1998). Inoculation of rape (canola; *Brassica napus*) with metal-resistant PGPR containing ACC deaminase stimulated growth of plants cultivated in Cd contaminated soil (Belimov et al., 2001). In addition, various N_2-fixing and auxin-producing PGPR immobilized Cd and promoted growth and nutrient uptake by barley plants in the presence of toxic Cd concentrations (Belimov and Dietz, 2000; Pishchik et al., 2002).

Our aim was to isolate and characterize Cd-tolerant bacteria associated with the roots of the metal accumulating plant *Brassica juncea* L. Czern. grown in heavy metal contaminated soils, and to select PGPR strains which might be useful to increase plant biomass production under unfavourable environmental conditions. The creation of such metal tolerant plant-microbe associations is aimed at improving the efficiency of phytoremediation of heavy metal polluted soils.

2. Materials and methods

2.1. Characterisation, sampling and treatment of soils

Characteristics of the soils used are described in Table 1. The soils were classified using the FAO-UNESCO Soil Taxonomic System and soil samples were conventionally labelled 1–5. Sod-podzolic soils (samples 1 and 2) were sampled to a depth of 0–10 cm from agricultural fields in the Saint-Petersburg region (30°37′N, 59°47′E) in June 2001, supplemented with 50 mg Cd kg^{-1}, as $CdCl_2$ dissolved in water, immediately after sampling and incubated in sterile enameled pots in the dark at room temperature (20 °C ± 2 °C) for 3 months before use. Soil moisture was maintained at 60% of water holding capacity via addition of sterile tap water. Sewage sludge pits (samples 3 and 4), which had been left to stand for 13 y, were sampled to a depth of 0–10 cm in the Gatchina sewage treatment works in the Saint-Petersburg region (29°12′N, 58°33′E) in September 2001 and immediately used. Mining waste (sample 5) was sampled to a depth of 0–10 cm at the Campo Pisano mine in the Iglesiente area in southwest Sardinia, Italy (3°54′N, 39°17′E) in March 2001 and stored moist in sterile plastic bags at 4 °C for 6 months before use.

Total C in soil samples 1–4 and 5 was determined as described by Arinushkina (1970) and by the Walkey-Black method (Violante, 2000), respectively. The total N content

Table 1
Characteristics of the soils used for isolation of rhizobacteria

Designation and soil type	pH_{KCl}	Total content of elements (mg kg^{-1})										
		C	N	P	K	Mn	Zn	Cu	Ni	Cr	Cd	Pb
1 Sod-podzolic	4.7	12,300	1240	350	280	240	32	22	24	32	0.1[a]	18
2 Sod-podzolic	5.2	24,800	1830	410	360	340	28	15	23	31	0.2[a]	25
3 Sewage sludge	6.9	36,100	2200	360	1730	714	826	662	63	201	43	84
4 Sewage sludge	6.6	109,000	85,800	640	1890	738	1250	875	87	540	112	106
5 Mining waste	6.9	18,600	700	400	1540	2409	5961	39	30	19	36	2827

[a] The data are given as Cd concentrations at the time of sampling before addition of 50 mg Cd kg^{-1}, as $CdCl_2$ dissolved in water.