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Influence of plant growth promoting bacteria and Cr^{6+} on the growth of Indian mustard

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Abstract

The Cr^{6+} resistant plant growth promoting bacteria (PGPB), *Pseudomonas* sp. PsA4 and *Bacillus* sp. Ba32 were isolated from heavy metal contaminated soils and their plant growth promoting activity on the Indian mustard (*Brassica juncea*) were assessed with different concentrations of Cr^{6+} in soil. Production of siderophores and the solubilization of phosphate were observed in both strains, PsA4 and Ba32. Production of IAA was only observed in strain PsA4. Inoculation of PsA4 or Ba32 promoted the growth of plants at 95.3 and 198.3 μg of $Cr^{6+}g^{-1}$ soil. The maximum growth was observed in the plants inoculated with strain PsA4. Both strains, PsA4 and Ba32 did not influence the quantity of accumulation of chromium in root and shoot system. The present observations showed that the strains PsA4 and Ba32 protect the plants against the inhibitory effects of chromium, probably due to the production of IAA, siderophores and solubilization of phosphate.
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Keywords: *Brassica juncea*; Chromium; Siderophore; Rhizosphere bacteria

1. Introduction

Chromium is one of the common heavy metals affecting the soil quality, especially Cr^{6+}, which is introduced into the environment from electroplating, pigment production and leather tanning industries (Bartlett and James, 1988). Unlike organic pollutants, the heavy metals cannot be degraded to harmless products and hence persist in the environment indefinitely. In order to remediate the soil contaminated with toxic heavy metals, it should be concentrated and extracted by conventional methods for reuse or for proper disposal. A promising option to achieve this is through phytoremediation—the use of plants to remove, destroy or sequester hazardous substances from the environment (Cunningham et al., 1995). Currently, there are a number of reports available on metal accumulating plants that are used in removing toxic metals from the soil (Zayad et al., 1998; Burd et al., 2000). Indian mustard (*Brassica juncea*) is one of such plant species, which has attracted considerable attention because of its ability to grow in

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heavily polluted soil together with its capacity for metal ion accumulation (Blaylock and Huang, 2000). As reported (Terry, 1981) the heavy metals at elevated levels in the environment lead to impair the metabolic activities and result in reduced plant growth. Hence the alternate ways to reduce the toxicity of heavy metals to the plants is using the rhizosphere microbes (Burd et al., 2000). The rhizosphere microorganisms have exceptional ability to promote the growth of host plant by various mechanisms such as the production of phytohormones (Gaudin et al., 1994), siderophore (Burd et al., 2000) and solubilization of minerals (Gupta et al., 2002).

As far as the role of rhizosphere microbes on plant growth in polluted soil is concerned, no general conclusions has been made. In some reports it has been claimed that the rhizosphere microbes can increase the tolerance of their host plants to heavy metals when these are present at toxic levels (Hasnain et al., 1993; Hoflich and Metz, 1997). Further, the rhizosphere microbes can protect the plants by facilitating the uptake of Fe^{3+} against the toxic effects of Nickel (Burd et al., 1998). However, no published evidence showing the role of chromate resistant bacteria on plants growth and tolerance to Cr^{6+} pollution under controlled conditions is available. The present study was therefore designated to investigate the effects of Cr^{6+} resistant plant growth promoting bacteria (PGPB) on the growth of Indian mustard under three different concentrations of Cr^{6+} in soil.

2. Materials and methods

2.1. Isolation of Cr^{6+} resistant PGPB

The soil samples were collected from the metal contaminated region near Chennai, India an area which had been exposed to heavy metal contamination from tannery effluents. About 1 g of wet soil samples were serially diluted using 25 mM phosphate buffer and spread over on nutrient agar (Difco) amended with 25 mg of Cr^{6+} l^{-1} ($K_2Cr_2O_7$). The plates were incubated at 37 °C for 24 h. From the Cr^{6+} resistant colonies, different strains were picked and purified on the nutrient agar medium containing 25 mg l^{-1} of Cr^{6+} according to the procedure of Hasnain et al. (1993). Purified colonies were gradually taken to higher concentration of Cr^{6+} (25–500 mg l^{-1}) and the same procedure was continued to isolate Cr^{6+} resistant strains.

In order to isolate the PGPB, the Cr^{6+} resistant strains were grown on DF salt minimal medium (Dworkin and Foster, 1958) supplemented with 3 mM 1-aminocyclopropane-1-carboxylic acid (ACC) to provide a nitrogen source at 30 °C for 120 h at 175 rpm. The bacterial growth was measured once in every 24 h by dilution plate method. The diluted culture was plated on to solid DF salt minimal medium with ACC and incubated at 30 °C for 48 h. Further, the ACC utilizing strains were assessed for the plant growth promoting activity by roll towel method. Indian mustard seeds were procured from the Tamil Nadu Agricultural College and Research Institute, Madurai, India. The seeds were surface sterilized in 1% sodium hypochlorite for 30 s and rinsed several times with sterile water. The seeds were inoculated by soaking in a bacterial suspension containing 10^8 cell ml^{-1} for 1 h then placed in wet blotters and incubated in a growth chamber for 15 d. The germination percentage of seeds was recorded and the vigour index was calculated using the formula described by Abdul Baki and Anderson (1973). Vigour index = (mean root length + mean shoot length) × germination (%).

2.2. IAA, siderophore production and phosphate solubilization

2.2.1. IAA

IAA production was determined as described by Brandle and Lindow (1996). The bacterial strains were grown in minimal medium (50 mM KH_2PO_4, 50 mM K_2HPO_4, 5 mM $MgSO_4$, 25 mM $(NH_4)_2SO_4$, 1% glucose) amended with 0.05% of L-tryptophane at 30 °C for 36 h at 175 rpm. The culture supernatants were collected by centrifugation at 10000 rpm for 15 min and adjusted to pH 2 with HCl. The acidified supernatants were extracted twice with equal volumes of ethyl acetate and then dried by using a rotary evaporator. The residues were dissolved in methanol, filtered, and analyzed on a Knauer Ministart-501 A4040 high pressure liquid chromatograph (HPLC) equipped with a Microsorb C18 reverse phase column. The solvent system was 30% methanol in water. Eluates were detected at 254 nm with a variable-wavelength spectrophotometer. The retention time for peaks were compared with those of authentic standards added to the medium and extracted by the same procedure used for bacteria cultures.

2.2.2. Siderophore

Siderophore production was determined in Fe-deficient mineral salt medium. Mineral salt medium contained: 0.36 g KH_2PO_4, 1.4 g K_2HPO_4, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.2 g NaCl, 0.02 g $CaCl_2 \cdot 2H_2O$, 15 mg EDTA, 0.16 mg $ZnSO_4$, 0.25 mg H_3BO_3, 0.2 mg Na_2MoO_4, 0.2 mg $MnSO_4 \cdot 4H_2O$, 0.02 mg $CuSO_4$ $5H_2O$ l^{-1} of water with 20 mM of mannitol and 10 mM of NH_4Cl_2. The isolates were inoculated in mineral salt medium and incubated on a rotary shaker at 30 °C for 48 h at 175 rpm. The culture supernatants were collected by centrifugation at 10000 rpm for 15 min. The supernatant was assayed for siderophore production by using the Chrome Azurol S (CAS) assay described by Schwyn and Neilands (1987): 0.5 ml of blue CAS solution was added to 0.5 ml of culture supernatant. A reference

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