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Evaluation of rhizobacteria of some medicinal plants for plant growth promotion and biological control

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Abstract Densities of microbial count in the rhizosphere of eleven medicinal plants viz., *Ocimum basilicum*, *Marrubium vulgare*, *Melissa officinals*, *Origanum syriacum*, *Quisqualis indica*, *Solidago virgaurea*, *Melilotus officinalis*, *Cymbopogon citratus*, *Matricaria chamomilla*, *Thymus vulgaris* and *Majorana hortensis* were determined. The lowest populations were found in the rhizosphere of *M. chamomilla* and *M. hortensis*. A total of 112 bacterial cultures were successfully isolated in pure form from the rhizosphere of the tested medicinal plants. Cultural and morphological characteristics of these isolates showed that they belong to bacilli, azotobacter, fluorescent pseudomonads and actinomycetes. These isolates were screened, in vitro, according to their capacities to produce plant growth promoting substance i.e. IAA, phosphate, potassium solubilization, chitinase activities and hydrogen cyanide. Results indicated that 36 bacterial isolates showed IAA production, 25 HCN production, 57 chitinase activities, 39 phosphate and 105 potassium solubilizers. Eleven bacterial isolates were selected which showed highest plant growth promoting activities and further subjected to estimate siderophores and phenols produced in liquid culture along with antifungal activity against two phytopathogenic fungi. The most potent isolates were identified on the basis of 16S rRNA gene sequence as *Bacillus thuringiensis C110*, *Pseudomonas fluorescens Th98* and *Pseudomonas poae Th75*. Their amounts of soluble phosphate in liquid culture were 2.2, 4.62 and 14.53 ppm in respective order. The indol-3-acetic acid (IAA), gibberellic acid (GA_3) and trans-zeatin riboside (t-zr) produced by the identified strains were determined by HPLC analysis. These strains proved to be effective PGPR inoculants, as they possess a number of traits useful for plant growth.

Introduction

Many reports presented the soil attached to the root system as a hot spot of microbial abundance and activity due to the presence of root exudates and rhizodeposits (Smalla et al., 2006).

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The rhizosphere is a very complex environment in which the effects of plant on soil microorganisms and the effects of microorganisms on plant are interacting and are interdependent (Mukerji et al., 2006). The diversity and composition of bacterial taxa in the rhizosphere can be affected by several factors including plant species (Miller et al., 1989), soil type (Hoitink and Boehm, 1999), soil management practices (Rovira et al., 1990), microbial interactions (Hedges and Messens, 1990) and other environmental variables. Plant growth promoting-rhizobacteria (PGPR) are a group of bacteria that actively colonize plant root and increase plant growth (Deshwal et al., 2010, 2011). Tilak et al. (2005) demonstrated that a number of bacterial species belonging to genera *Azospirillum*, *Alcaligenes*, *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Pseudomonas*, *Rhizobium* and Serratia are associated with the plant rhizosphere and are able to exert a beneficial effect on plant growth. The main mechanisms by which PGPR directly contribute to the plant growth are phytohormone production such as auxins, cytokinins and gibberellins, enhancing plant nutrition by solubilization of minerals such as phosphorus and iron, production of siderophores and enzymes, lowering of ethylene levels and induction of systemic resistance (Bhattacharyya and Jha, 2012). PGPR indirectly benefit the plant growth by the biocontrol of deleterious microorganisms or root pathogens that inhibit plant growth, including antibiotic and hydrogen cyanide production, parasitism, competition for nutrients and niches within the rhizosphere, synthesis of extracellular enzymes to hydrolyze the fungal cell wall and decreasing pollutant toxicity (Bhattacharyya and Jha, 2012; Podile and Kishore, 2006; Zahir et al., 2003). Medicinal plants are known to be rich in secondary metabolites and are potentially useful to produce natural drugs. Medicinal plants support a great diversity of microflora in their rhizosphere including PGPR.

The main objectives of this study were to isolate and characterize the rhizobacteria associated in the rhizosphere of some medicinal plants, to find out high efficient bacterial isolates have the capacities to be used as plant growth promoting and bio-control agents.

Materials and methods

Soil sampling

Soil samples were collected from the rhizosphere of eleven medicinal plants i.e., Basil, Marrubium, Melissa, Origano, Quisqualis, Goldenrod, Melilotus, Lemon grass, German chamomile, Thyme and Margoram grown in loam sandy soil to isolate the rhizospheric bacteria.

Enumeration and isolation the rhizospheric bacteria

Rhizosphere microflora of the eleven medicinal plants were counted and isolated on their selective media; spore forming bacteria and total microbial flora on nutrient agar medium, Pseudomonads on King's B medium (King et al., 1954), actinomycetes on glycerol nitrate agar medium (Waksman, 1961), azotobacters on modified Ashby's medium (Abd El-Malek and Ishac, 1968) and fungi were counted on Potato Dextrose agar medium (ATCC, 1982). Bacterial cultures were maintained on the selective medium. Bacterial colonies were selected according to the cultural and morphological characteristics including pigments; colony form, elevation and margin; texture and opacity (Simbert and Krieg, 1981).

Characterization of rhizobacteria for PGP traits

The collected rhizobacterial isolates were tested for their capacities to produce plant growth promoting substances using standard procedures.

Indole acetic acid (IAA) production

Production of IAA by the collected bacterial isolates was detected according to the method described by Bric et al. (1991). Quantitative measurement of IAA, produced by the selected isolates was determined in liquid culture. The isolates were cultured individually using their liquid selective media (nutrient broth, King's B, Modified Ashby's or Glycerol nitrate broth medium according to the tested microbial group). Each medium was amended with 1 mM tryptophan. After incubation at 28–30 °C for 5–6 days, IAA produced was determined in culture supernatant according to Larsen (1962).

Hydrogen cyanide production

The collected isolates were cultured in liquid media supplemented with 4.4 g/l glycine to detect HCN production according to Bakker and Schippers (1987).

Phosphate solubilization activity

Pikovskaya's agar (Subba-Rao, 1982) plates were inoculated with bacterial cultures and incubated at 30 °C for 4 days. The bacterial colonies forming clear halos were considered as phosphate solubilizers. The screening was conducted based on phosphate solubilization index [The Ratio of the total diameter (colony + halo zone) and the colony diameter] according to Edi-premono et al. (1996). Quantification of soluble phosphate was carried out by using one hundred ml of pikovskaya's broth medium was inoculated individually by standard inoculum of the most potent isolates. After 15 days of incubation period at 28–30 °C under shaking condition (200 rpm), the soluble phosphate was determined in culture filtrate according to Jackson (1958).

Potassium solubilization activity

Fifty milliliters of modified Aleksandrov's medium (Zahra, 1969) was inoculated with collected isolates, then incubated at 28–30 °C under shaking condition (200 rpm) for 15 days. The soluble potassium was determined in culture filtrate as described by Jackson (1958).

Chitinase activity

Chitinase activity of the collected isolates was determined according to Nisa et al. (2010). The isolates were inoculated on colloidal chitin agar medium (Hus and Lockwood, 1975) and incubated for 5–6 days at 28–30 °C. The screening was carried out based on chitinolytic index (ratio of a clear zone and colony diameter).