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Tricalcium phosphate solubilization and nitrogen fixation by newly isolated *Aneurinibacillus aneurinilyticus* CKMV1 from rhizosphere of *Valeriana jatamansi* and its growth promotional effect

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ABSTRACT

Aneurinibacillus aneurinilyticus strain CKMV1 was isolated from rhizosphere of *Valeriana jatamansi* and possessed multiple plant growth promoting traits like production of phosphate solubilization (260 mg/L), nitrogen fixation (202.91 nmol ethylene mL^{-1} h^{-1}), indole-3-acetic acid (IAA) (8.1 μg/mL), siderophores (61.60%), HCN (hydrogen cyanide) production and antifungal activity. We investigated the ability of isolate CKMV1 to solubilize insoluble P via mechanism of organic acid production. HPLC study showed that isolate CKMV1 produced mainly gluconic (1.34%) and oxalic acids. However, genetic evidences for nitrogen fixation and phosphate solubilization by organic acid production have been reported first time for *A. aneurinilyticus* strain CKMV1. A unique combination of glucose dehydrogenase (*gdh*) gene and pyrroloquinoline quinone synthase (*pqq*) gene, a cofactor of *gdh* involved in phosphate solubilization has been elucidated. Nitrogenase (*nif* H) gene for nitrogen fixation was reported from *A. aneurinilyticus*. It was notable that isolate CKMV1 exhibited highest antifungal against *Sclerotium rolfsii* (93.58%) followed by *Fusarium oxysporum* (64.3%), *Dematophora necatrix* (52.71%), *Rhizoctonia solani* (91.58%), *Alternaria* sp. (71.08%) and *Phytophthora* sp. (71.37%). Remarkable increase was observed in seed germination (27.07%), shoot length (42.33%), root length (52.6%), shoot dry weight (62.01%) and root dry weight (45.7%) along with NPK (0.74, 0.36, 1.82%) content of tomato under net house condition. Isolate CKMV1 possessed

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traits related to plant growth promotion, therefore, could be a potential candidate for the development of biofertiliser or biocontrol agent and this is the first study to include the *Aneurinibacillus* as PGPR.

Introduction

Soil is the reservoir for a variety of living organisms comprising microorganisms, plants, and animals, which do not live in isolation, but form a complex interactive network. A very special ecological niche, the rhizosphere is present around the roots of the plants that support a group of metabolically versatile microorganisms.[1] Some of these rhizospheric bacteria that are beneficial to plants are often referred as plant growth promoting rhizobacteria (PGPR). Microbial inoculants or biofertilizers are used to hasten biological activity to improve availability of plant nutrients by fixing atmospheric nitrogen, making insoluble phosphate soluble and decomposing farm wastes, which result in the release of nutrients and antagonize various pathogenic fungi by producing siderophore, HCN (hydrogen cyanide), Chitinase, β-1,3-glucanase and a variety of different antibiotics.[2]

Phosphorus (P) is one of the major essential macronutrients for biological growth and development but it is commonly deficient in most natural soils since it is fixed as insoluble iron and aluminium phosphates in acidic soils or calcium phosphates in alkaline soils. It is generally accepted that the major mechanism of mineral phosphate solubilization is by the action of organic acids synthesized by soil microorganisms yet the exact biochemical basis of these transformations are not completely understood but can be explored. The concentration of soluble P in soil is usually very low, normally at levels of 1 ppm or less (10 M $H_2PO_4^-$). The cell might take up several P forms but the greatest part is absorbed in the forms of HPO_4^{2-} $H_2PO_4^-$.[3,4]

The genetic basis of mineral phosphate solubilization phenotype of bacteria is not well understood. It is believed that organic acids produced by the action of glucose dehydrogenase, which require pyrroloquinoline quinone (PQQ) as cofactor and it is a primary mechanism behind phosphate solubilization; this assumption has been confirmed by the cloning of gene involved in gluconic acid production viz. *gdh* and PQQ. PQQ-dependent glucose dehydrogenase (GDH) is capable of oxidizing glucose to gluconate. Gluconate is further oxidized to 2-ketogluconic acid by gluconate dehydrogenase (GADH). Glucose dehydrogenase (GDH) is a member of quinoproteins, catalysing the oxidation of glucose to gluconic acid, requiring PQQ and also metal ions such as Ca^{2+} (or Mg^{2+} *in vitro*) for its activity. Glucose, gluconate, manitol, and glycerol are among the possible inducers of the halo enzyme activity, but the information regarding the synthesis of this holoenzyme (GDH-PQQ) is not available.[5]

Microbial communities are a main component of ecosystems that play critical roles in the biochemical transformations of elements including nitrogen fixation.[6] Therefore, nitrogen that is available to plants grown for many years without N fertilizers is considered to be due to biological fixation.[7] This process catalysed by nitrogenase enzymes is essential for maintaining fertility in many ecosystems.[8] The ability to fix nitrogen is widely distributed among diverse groups of Bacteria and Archae in different ecosystems. A substantial molecular diversity of N_2 fixing bacteria has been detected in field grown rice and maize based on retrieval of *nif* H or *nif* D gene fragments from root DNA.[9,10]

Therefore, the present study aims to understand the possible mechanism and to characterize the genes involved in the phosphate solubilization and nitrogen fixation by *Aneurinibacillus aneurinilyticus* isolated from the rhizosphere of medicinal plant *Valeriana jatamansi*. Information about genus *A. aneurinilyticus* with plant growth promoting attributes from any medicinal plants is limited. Hence, the results could be useful for better understanding of mechanism of plant growth promoting traits, which can help us to engineer this PGPR for agronomic interest.

Material and methods

Isolation and characterization of PGPR from Valeriana jatamansi

Sixty rhizobacteria with multiple PGP traits were obtained by using serial dilution technique from the rhizospheric soils of *V. jatamansi*, located in Chamba, Himachal Pradesh, India. A potential isolates were screened and selected on the basis of halo zone produced in Pikovskaya's (PVK) agar for phosphate-solubilization and chrome azurol S medium for siderophore production and growth on nitrogen free medium (NFb) for N_2-fixing ability. Among sixty isolated strains, CKMV1 showed maximum effectiveness of multifarious plant growth-promoting attributes, *i.e.* phosphate-solubilization, IAA production, nitrogen fixing ability, HCN production, siderophore production and broad spectrum of antagonistic activity against common phytopathogenic fungi, *i.e. Sclerotium rolfsii*, *Rhizoctonia solani*, *Phytopthora* sp., *Alternaria* sp., *Fusarium oxysporum* and *Dematophora necatrix*.

Phenotypic characterization of the bacterial isolate was done based on their colony morphology, microscopic observations, and biochemical tests.[11] The isolate was tested for the utilization of different carbon sources using KB009 Hi carbohydrate™ kit (Himedia, Mumbai). The isolate was identified based on whole cell fatty acids derivatized to methyl esters and analysed by gas chromatography at IMTECH, Chandigarh and by 16S rDNA sequence analysis.

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