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Acidotolerant *Streptomyces* sp. MBRL 10 from limestone quarry site showing antagonism against fungal pathogens and growth promotion in rice plants

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Abstract Acidotolerant *Streptomyces* sp. MBRL 10 isolated from limestone deposit site on Gauze's medium No. 1 (pH 5.3) showed significant antagonism against the tested fungal pathogens. It exhibited the highest mycelial growth inhibition by diffusible and volatile compound(s) production against *Rhizoctonia solani*. Culture filtrates also exhibited significant inhibition zone but the inhibition activities vanished when sterilized. The strain produced chitinase, β-1,3-glucanase, lipase, protease and ammonia but not β-1,4-glucanase. It could produce 25 μg/ml of indole acetic acid, solubilize up to 140 μg/ml of phosphate with a concomitant decrease in pH of the medium. The bioactive actinomycete strain produced hydroxamate type of siderophore. Casamino acid was found to be the best medium for siderophore production (87% siderophore units).

MBRL 10 showed the highest rice seedlings vigor index corresponding to an inoculum size of 0.3×10^8 cfu/ml. Strain treated rice seeds at an inoculum size of 0.3×10^8 cfu/ml showed higher germination percentage and significantly enhanced ($P \leqslant 0.05$) the growth of seedlings. Strain treated rice seedlings challenged with pathogens also exhibited higher germination percentages and

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significantly enhanced ($P \leqslant 0.05$) growth over seedlings challenged with pathogen alone in the absence of the bioinoculant. Rice plants treated with the strain significantly promote ($P \leqslant 0.05$) the growth under nethouse conditions.

1. Introduction

Rice (*Oryza sativa*) is one of the most important staple foods for more than three billion people i.e. over half the world's population (IRRI, 2006) and this cereal crop influences the livelihoods and economies of several billion people across the world. It provides 27% of dietary energy and 20% of dietary protein in the developing world (Redoña, 2004). The majority of the global rice production (88%) is done in Asian countries, with China and India being the major producers (IRRI, 2008). However, since 2000, the world's average growth rate in rice production has not kept up with population increases and demand for rice has outstripped its production (FAO, 2000). Intensive research on plant growth promoting bacteria (PGPB) is underway worldwide for developing biofertilizers and biocontrol agents as better alternative to agrochemicals, as the latter harm the environment and human health besides demanding high costs (Ningthoujam et al., 2009).

Actinomycetes are prolific producers of several agriculturally important secondary metabolites and several members have been considered as plant growth promoting agents (Goodfellow and Williams, 1983; Nimaichand et al., 2013). Until the investigations of Corke and Chase (1964), and Khan and Williams (1975) had been published, all soil actinomycetes were believed to be neutrophilic. Acidophilic isolates grow in the pH range 3.5–6.5, with optimum growth between pH 4.5 and 5.5 (Khan and Williams, 1975). The most frequently encountered acidophilic/acidotolerant actinomycetes belong to the genus *Streptomyces* (Hagedorn, 1976; Poomthongdee et al., 2015). Soil pH can drop below 5.0 after prolonged use of ammonia-based fertilizers or acid rain and this can cause considerable reduction in bacteria and actinomycetes population and increase the relative abundance of fungi in soil (Ventura, 2000; Haney et al., 2000). Acidophilic actinomycetes may be a potential source of new effective agents for controlling fungal plant diseases and plant growth promotion for sustainable agricultural product where soil has been contaminated with the excessive use of agrochemicals and environmental factors.

Streptomyces sp. has played an important role in chitin decomposition in acidic soil and litter, where fungi are important colonizers (Williams and Robinson, 1981). Release of ammonia by the deacetylation and deamination of N-acetylglucosamine residues may raise the pH of the soil (Williams and Robinson, 1981) making the way for the other neutrophilic PGPB to colonize and compete with the pathogens. In an acidic soil environment, they probably involved in competition with fungi, and therefore, it is logical that acidophilic actinomycetes possess an antifungal activity (Zakalyukina and Zenova, 2007). Acidophilic/acidotolerant actinomycetes that can inhibit the growth of different *Fusarium* sp. have been reported (Zakalyukina and Zenova, 2007). But there is limited report for their potential as biocontrol and plant growth promoting bacteria especially for rice. Acidophilic/acidotolerant *Streptomyces* sp. has been reported to show antagonistic activity against rice fungal pathogens such as *Fusarium moniliforme*, *Helminthosporium oryzae* and *Rhizoctonia solani* (Poomthongdee et al., 2015).

Hundung limestone deposit site is a unique, non-rhizospheric habitat in Ukhrul, Manipur, India falling under the Indo-Burma Biodiversity hotspot. The present investigation aimed to study the native acidotolerant actinomycetes, *Streptomyces* sp. MBRL 10 showing in vitro antagonistic activity against important rice fungal pathogens as well as plant growth promoting activity. It also aimed to study rice plant growth promotion under nethouse conditions.

2. Materials and methods

2.1. Isolation

Soil samples were collected from Hundung limestone deposit, Ukhrul District, Manipur. Isolation was performed on Gauze's Medium No. 1 (GM 1, pH 5.3) by serial dilution technique (10^{-3} to 10^{-6}) as described earlier (Nimaichand et al., 2012). After incubation at 30 °C for 1 week, colonies obtained with distinct morphologies were selected and subcultured in the same medium to get pure cultures. The purified cultures were preserved as agar slants (4 °C) and glycerol stocks (20% v/v, −20 °C) for further use.

2.2. Preliminary antagonistic bioassays

The isolates obtained were subjected to preliminary antagonistic assays by dual culture method (described in the following section) against fungal pathogens. Of the isolates showing antagonistic activity, the best isolate MBRL 10 was characterized and further screened for other antagonistic and plant growth promoting traits.

2.3. Genomic DNA isolation and strain characterization

Genomic DNA extraction and PCR amplification of the 16S rRNA gene was performed as described by Li et al. (2007). The almost complete 16S rRNA gene sequence of the strain was identified using the EzTaxon-e server database (Kim et al., 2012) and aligned with the 16S rRNA gene sequences of related species using CLUSTAL X version 2.1 (Larkin et al., 2007). Phylogenetic analyses were performed using the software package MEGA version 5 (Tamura et al., 2011). Phylogenetic distances were calculated with the Kimura two-parameter model (Kimura, 1983) and tree topologies were inferred using the neighbor-joining method (Saitou and Nei, 1987). To determine the support of each clade, bootstrap analysis was performed with 1000 resamplings (Felsenstein, 1985).

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