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Plant growth-promoting rhizobacteria mediate induced systemic resistance in rice against bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae*

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ABSTRACT

Seven *Bacillus* plant growth-promoting rhizobacteria spp. were evaluated for growth promotion and induced systemic resistance in rice against *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). The identities of colonies of *X. oryzae* pv. *oryzae* grown on mXOS and PSA medium were confirmed by PCR employing specific primers TXTF and TXT4R. Among the seven strains tested as fresh suspensions, talc and sodium alginate formulations under laboratory and green house conditions, maximum germination of 86% was recorded after seed treatments with fresh suspension of *Bacillus subtilis* GBO3 followed by 85% germination treated with *Bacillus pumilus* SE34 in comparison to only 71% germination in the untreated controls. Similarly, the maximum vigor index of 1374 was obtained by seed treatment with fresh suspensions of *B. subtilis* strain GBO3 followed by treatments with strain SE34 with vigor index of 1323 in contrast to an index of only 834 observed in untreated controls. Among the treatments, seed treatments with fresh suspension of seven strains resulted in better germination and vigor assessments than talc based or sodium alginate formulations. Seed treatments with fresh suspension of strain SE34 gave 71% protection, followed by *B. subtilis* GBO3 and *B. pumilus* T4 with 58% and 52% protection, respectively, compared to the untreated controls. Seed treatments with talc based formulation of SE34 gave 66% protection, while GBO3 and T4 resulted in 52% and 50% protection, respectively, with similar formulation. Seed treatment with talc and sodium alginate formulations of strain SE34 gave 58% protection followed by GBO3 with 40% protection. Seed treatment with fresh suspensions of strains SE34 and GBO3 followed by challenge inoculations with *Xoo* increased accumulation of phenylalanine ammonia lyase, peroxidase and polyphenol oxidase compared to untreated control seedlings. Thus, the results of the present study suggest that the PGPR strains used as fresh suspensions and powdered formulations may have commercial potential in plant growth promotion and in management of rice bacterial leaf blight disease.

1. Introduction

Rice (*Oryza sativa* L.) is the staple food for three fourths of the Indian population. In the global context, India stands first in area with 43.7 million hectares and second in production with 92.24 million metric tonnes (Viraktamath and Rani, 2008). Rice production has intensified to meet consumer demand. The concern to protect the agricultural environment is inevitable because of the harmful effects of excessive use of agrochemicals. Rice diseases have always had a significant impact on rice supply. Considering the large rice production area in the world, even a conservative estimate of 1–5% annual loss would translate into thousands of tonnes of rice and billions of dollars lost. Thus, minimizing the occurrence of disease epidemics and reducing year-to-year losses are central to sustaining rice productivity (Mew et al., 2004).

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a major seed-borne pathogen of rice and is a threat to rice production in both temperate and tropical rice-growing regions, due to its high epidemic potential (Mew, 1987). BLB of rice causes considerable losses in all cultivars of rice in India, particularly in the Punjab state of India (Reddy, 1980).

Currently, the disease is managed through the use of resistant cultivars and systemic bactericides. However, the lack of durable resistance, existence of pathogenic variability, and concerns about chemical resistance has limited the potential of such strategies for managing the disease. Recently, an increasing desire to reduce the pesticides is seen through the attempts to develop integrated pest management approaches, where natural resources are put to

Abbreviations: PAL, phenylalanine ammonia lyase; POX, peroxidase; PPO, polyphenol oxidase; CAT, catalase; PGPR, plant growth promoting rhizobacteria; PR proteins, pathogenesis-related proteins; PSA, peptone sucrose agar; BLB, bacterial leaf blight; ISR, induced systemic resistance; Xoo, *Xanthomonas oryzae* pv. *oryzae*; SA, salicylic acid; JA, jasmonic acid; CMC, carboxymethyl cellulose; cfu, colony forming units.

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maximum use. Hence host resistance is given priority in disease control strategy. Biological control through the use of plant growth-promoting bacteria is high on the list of potential alternative tactics (Nelson, 2004).

PGPR are free living, root colonizing bacteria that have beneficial effects on plants. They reduce disease severity and enhance yield of many crops (Liu et al., 1995; Murphy et al., 2000). In the context of the international concern for food and environmental quality, the use of PGPR has been applied to various crops to enhance growth, seedling emergence and crop yield, and some have been commercialized (Herman et al., 2008; Nayaka et al., 2009; Choong-Min et al., 2007; Saravanakumar et al., 2007; Murphy et al., 2003; Zhang et al., 2004). Rhizobial inoculants have also been reported to improve nutrient uptake, growth, seedling vigor and yield of rice (Biswas et al., 2000).

PGPR bring about disease suppression by various modes of action such as antagonism, competition for space and nutrients, and induction of systemic resistance (ISR) (Kumari and Srivastava, 1999). PGPR may mediate biological control indirectly by eliciting induced systemic resistance against a number of plant diseases (Jetiyanon and Kloepper, 2002). Application of some PGPR strains to seeds or seedlings has also been found to induce ISR in the treated plants (Kloepper et al., 1999). This phenomenon is known as induced systemic resistance and can be triggered by a variety of biotic and abiotic stimuli (Bostock, 2005). In addition, basal resistance responses that act at the site of pathogen infection, plants are also capable of developing a nonspecific resistance that is effective against pathogen attack.

Selected strains of nonpathogenic PGPR can reduce disease in above ground plant parts through the induction of a defense state that is commonly referred to as rhizobacteria-induced systemic resistance (Van Loon et al., 1998). Induced systemic resistance (ISR)-inducing PGPRs have also been demonstrated to enhance the plant's defense capacity by priming for potentiated expression of defense genes (Kim et al., 2004; Tjamos et al., 2005) strongly suggesting that priming is common feature of PGPR-mediated ISR. Ryu and his associates demonstrated that some PGPR can induce priming by the release of volatiles. For instance, *Bacillus subtilis* GBO3 induces a signaling pathway that is independent of salicylic acid (SA), jasmonic acid (JA) and the *Npr1* gene (SA insensitive or nonexpresser of PR genes), yet it requires ethylene (Ryu et al., 2004). Priming offers an energy cost-efficient strategy, enabling the plant to react more effectively to any invader encountered by boosting infection-induced cellular defense responses (Becker and Conrath, 2007; Conrath et al., 2006).

The increased levels of defense related enzymes during ISR are known to play a crucial role in host resistance (Chen et al., 2000; Schneider and Ullrich, 1994; Ramamoorthy et al., 2002). In addition to improvement of plant growth, PGPR are directly involved in increased uptake of nitrogen, synthesis of phytohormones, phosphate solubilization and production of siderophores that chelate iron and make it available to the plant roots (Lalande et al., 1989; Bowen and Rovira, 1999).

Efforts have been made to use bacterial antagonists like *Pseudomonas fluorescens* in the management of bacterial leaf blight of rice (Vidhyasekaran et al., 2001). Babu et al. (2003) reported that treatment with acibenzolar-*S*-methyl (ASM) induced resistance to bacterial leaf blight in rice. *Bacillus* spp. including *Bacillus amyloliquefaciens*, *B. subtilis*, *Bacillus pasteurii*, *Bacillus cereus*, *Bacillus pumilus*, *Bacillus mycoides*, and *Bacillus sphaericus* have been reported to significantly reduce the incidence of disease on a diversity of hosts (Kloepper et al., 2004). Elicitation of ISR by these strains has been demonstrated in greenhouse or field trials on tomato (*Solanum lycopersicum* L.), bell pepper (*Capsicum annuum* L.), muskmelon (*Cucumis melo* L.), watermelon (*Citrulus lanatus* [Thunb.] Matsum. and Nakai), sugar beet (*Beta vulgaris* L.), tobacco (*Nicotiana tabacum* L.), *Arabidopsis* spp., and cucumber (*Cucumis sativus* L.) against leaf-spotting fungal and bacterial pathogens, systemic viruses, a crown-rotting fungal pathogen, root-knot nematodes, and a stem-blight fungal pathogen as well as damping-off, blue mold, and late blight diseases (Kloepper et al., 2004). In most cases, *Bacillus* spp. that elicits ISR also elicits plant growth promotion (Kloepper et al., 2004).

The strains selected in the present study have shown to induce resistance against multiple plant diseases (Raupach and Kloepper, 2000; Jetiyanon and Kloepper, 2002; Niranjan-Raj et al., 2003a,b; Murphy et al., 2003; Choong-Min et al., 2007; Udayashankar et al., 2009). However, very few studies have been made on induced resistance in rice. Hence the present study was conducted to evaluate the effectiveness of seed treatment with fresh suspensions and their powdered formulations of selected PGPR *Bacillus* spp. for management of BLB of rice through ISR.

2. Materials and methods

2.1. PGPR strains and inoculum preparation

Seven PGPR strains (*B. pumilus* INR7, SE34 and T4; *B. amyloliquefaciens* IN937a, *B. subtilis* IN937b and GB03; *Brevibacillus brevis* IPC11) were used in the present study. These strains were obtained from Auburn University, Auburn, AL, USA (Courtesy: Prof. J.W. Kloepper and Prof. M.S. Reddy). The strains were stored in tryptic soy broth amended with glycerol (20%) at −70 °C prior to use.

Bacterial cell suspensions were prepared by streaking the isolates onto tryptic soy agar and incubating at 27 °C for 24 h to check the purity, then transferring single colonies onto tryptic soy agar plates. After 24 h, the bacterial cells were harvested from plates in sterile distilled water and centrifuged at 8000*g* for 5 min (REMI C-24, Bangalore, India). The pellets obtained were resuspended in sterile distilled water and again subjected to centrifugation, and the supernatants were discarded. The pellets were finally collected in sterile distilled water and cell populations were adjusted to 10^8 cfu ml^{-1} as measured spectrophotometrically (Thompson, 1996).

2.2. Host

The seeds of the rice cv., IR-64, susceptible to *X. oryzae* pv. *oryzae* were obtained from National Seeds Corporation, Bangalore, India and were used throughout the study.

2.3. Bacterial pathogen

Rice seed samples and rice plants showing typical bacterial leaf blight symptoms were collected for isolation of *X. oryzae* pv. *oryzae* during a 2006–2007 field survey. The collected rice seed samples and diseased leaves were subjected to liquid assay for detection of *X. oryzae* pv. *oryzae* (Mortensen, 2005). From each rice seed sample collected 400 seeds or leaf materials were ground with autoclaved pestle and mortar, suspended in 200 ml of sterile saline for 2 h. The suspensions were serially diluted 4 × 1:10 in test tubes. Aliquots of 0.05 ml were spread onto mXOS (modified XOS agar; Di et al., 1991; Gnanamanickam et al., 1994) medium and incubated at 28 ± 2 °C for 3–5 days. *X. oryzae* pv. *oryzae* was confirmed by employing specific primers TXTF5′-GTCAAGCCAACTGTGTA-3′ and TXT4R5′-CGTTCGGCACAGTTG-3′ according to Sakthivel et al. (2001). The amplicons generated by PCR were ligated into pTZ57R/T cloning vector using an InsTAclone™ PCR cloning kit (Fermentas Life Sciences) according to the manufacturer's instructions, transformed into competent cells by the heat shock method. Recombinant plasmid DNA was isolated from overnight grown liquid cultures of selected clones using the Plasmid Mini Prep Kit (Sigma–Aldrich) or PCR products were directly sequenced.