

***Bacillus vallismortis* EXTN-1-Mediated Growth Promotion and Disease Suppression in Rice**

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(Received on August 4, 2006; Accepted on August 12, 2006)

***Bacillus vallismortis* EXTN-1, a biocontrol agent in cucumber, tomato and potato was tested in rice patho-system against rice fungal pathogens viz. *Magnaporthe grisea*, *Rhizoctonia solani* and *Cochliobolus miyabeanus*. Apart from increasing the yield in the bacterized plants (11.6-12.6% over control), the study showed that EXTN-1 is effective in bringing about disease suppression against all the tested fungal pathogens. EXTN-1 treatment resulted in 52.11% reduction in rice blast, 83.02% reduction in sheath blight and 11.54% decrease in brown spot symptoms. As the strain is proven as an inducer for systemic resistance based on PR gene expression in *Arabidopsis* and tobacco models, it is supposed that a similar mechanism works in rice, bringing about disease suppression. The strain could be used as a potent biocontrol and growth-promoting agent in rice cropping system.**

Keywords : *Bacillus vallismortis*, biological control, induced systemic resistance, rice diseases

Rice is highly vulnerable at all stages of growth to pathogens that affect the quality and quantity of its yield. Rice blast caused by *Magnaporthe grisea* is a problem almost everywhere that rice is grown. This fungal disease is estimated to cause production losses of US \$ 55 million each year in South and Southeast Asia. The losses are even higher in East Asia and other more temperate rice growing regions around the world (Herdt et al., 1991). Sheath blight (caused by *Rhizoctonia solani*) is distributed in 1.2 to 1.4 million ha which is about 32-50% of the world's total rice cropping area. Rice brown spot, caused by *Cochliobolus miyabeanus* has been severely increased recently in Korea.

Sustainable and environmental friendly control measures are a preferred strategy for plant disease control. Beneficial microorganisms form an important component of Integrated Pest Management (IPM). The use of rhizobacteria for controlling soil borne plant diseases has been well documented (Weller and Cook, 1986). The beneficial

effects are brought about by different mechanisms as reported by many researchers (Vessey et al., 2003; Defago et al., 1990). Biocontrol organisms act as antagonists of the targeted pathogen or induce systemic resistance to the host plant (Cook and Baker, 1983), with systemic biochemical and ultra structural changes in the plant system that lead to a greater ability of the host plant to defend itself against pathogens.

The present study dealt with rhizobacteria-mediated plant growth promotion and disease suppression in rice. The rhizobacteria used in the study was *Bacillus vallismortis* strain, EXTN-1. The strain had been demonstrated to induce systemic resistance in many cropping systems leading to significant disease suppression and enhanced plant growth (Park et al., 2001).

Materials and Methods

The microorganisms and the plant source. The rhizobacteria, EXTN-1 that previously showed significant induced systemic resistance (ISR) activity on other crops (Park et al., 1998) was used in the study. For long-term storage, the strain was maintained at -80°C in tryptic soy broth (TSB) with glycerol (20%). The fungal pathogens used in the experiments, *Magnaporthe grisea*, *Rhizoctonia solani* and *Cochliobolus miyabeanus* were maintained on potato dextrose agar (PDA) slants at 15°C . Rice variety cv. Choochung, which is susceptible to the fungal pathogens tested, was used in the experiment.

Treatment of the plants and challenge with the pathogen. The treatment included bacterization of rice seeds with the bacterial strain *B. vallismortis* EXTN-1 (5×10^{10} cells/ml). The inducer of systemic disease resistance, benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH) (100 mM) (Lawton et al., 1996) was included in the treatments as a positive control. Bacterial inoculum was prepared by harvesting cells from Tryptic Soy Agar (TSA) plates (incubated at 28°C for 48 h) and re-suspended in 10 mM MgSO_4 . Seed-bacterization was carried out by soaking the seeds in the bacterial suspension for 4 h. Seeds soaked in 10 mM MgSO_4 served as control. The seeds were sown

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on non-sterile seedbed nursery. Five weeks after seeding, the rice seedlings were transplanted to the field. One set of plants were maintained for measurement of growth parameters and the experiment was carried out in field. The field trial was carried out with a completely randomized block design (CRBD) and repeated in two consecutive years and the yield data obtained. Three other sets of the same treatments were maintained in the green house for challenge inoculation with the three different fungal pathogens.

Plant growth promotion by the bacterial strains. To evaluate the effect of bacterial treatment, the growth of plants was measured in terms of height of the plant and the number of tillers upon a month after transplanting. Also the yield parameters were recorded upon harvest.

Challenge inoculation with the fungal pathogens and scoring disease development. *M. grisea* was plated on rice-bran-agar for spore production. The plates were incubated in diurnal condition at 25°C. The inoculum (2×10^5 conidia/ml) with Tween-20 (0.05% v/v) and gelatin (0.25% w/v) was sprayed until leaves were covered with fine droplets. From the first 20 h in darkness followed by continuous light, inoculated plants were kept at 100% relative humidity (RH) until the end of the experiment. Blast disease was quantified by counting the number of acute lesions with a gray, sporulating center 5 days post inoculation.

For challenge inoculation with *R. solani*, a rice grain:rice hull (1:3) substrate was prepared and sterilized. Mycelial plugs of *R. solani* were introduced into this mixture and allowed to grow for 10 days at 28°C. Before inoculation onto the rice plants, the culm region of the rice tillers of each plant was covered with aluminium foil to form a cone. For artificial inoculation, 40 g of the above inoculum was dropped into the cone at the base of each rice plant. The results on severity of sheath blight development were scored 15 days after inoculation. *C. miyabeanus* was raised in potato dextrose agar (PDA) plates. Conidia were collected in sterile water. Inoculum was adjusted to 10^5 conidia/ml and Tween-20 was added to a final concentration of 0.05% (v/v). The plants were sprayed with the conidial suspension. Plants were kept for 24 h in a dew chamber at 100% RH under fluorescent light, with a 12 h photoperiod, and then maintained in a greenhouse for 7 days. Percent disease (brown spot) suppression obtained in treatments was compared.

Statistical analysis. All data were analyzed with JMP (a PC-version of SAS) (SAS Institute, 1995) software. Significant differences in treatments were determined using Student's T test as LSD at $P = 0.05$.

Results

Plant growth promotion by EXTN-1. Treatment of rice plants with EXTN-1 resulted in growth promotion in the green house and field condition. In the green house, the root proliferation was multifold in the EXTN-1-treated plants when compared with the control (Fig. 1). There was significantly increased growth in the EXTN-1-treated plants in the field in terms of height (10.5% over control) and number of tillers (15.56% over control) (Fig. 2 & 3). Treatment with

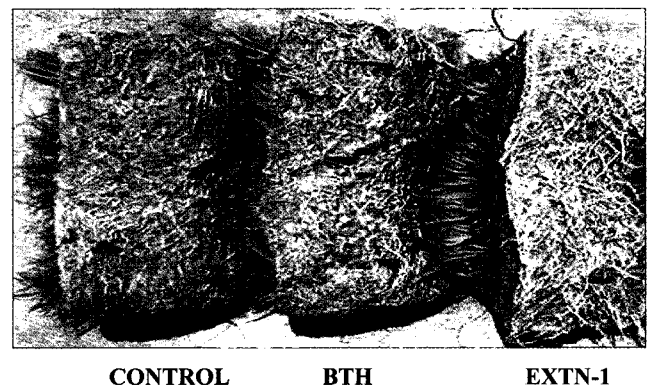


Fig. 1. The root proliferation in EXTN-1-treated rice seedlings in the nursery.

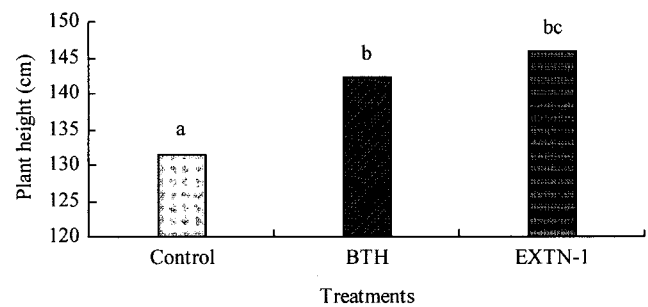


Fig. 2. EXTN-1 mediated growth promotion in paddy field. Different letters indicate significant differences among treatments according to a least significant difference.

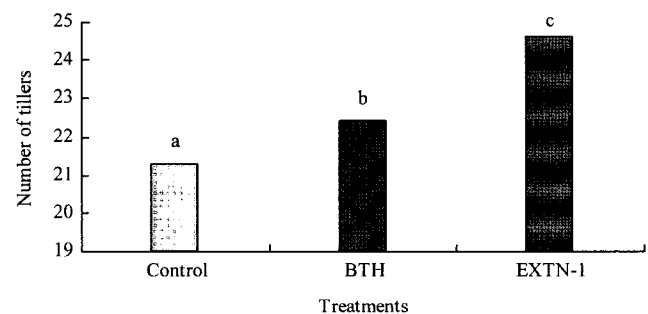


Fig. 3. EXTN-1 treatment increase proliferation of rice-tillers in paddy field. Different letters indicate significant differences among treatments according to a least significant difference.

Table 1. EXTN-1 mediated yield-increase in paddy

Treatments	Yield (kg/10 a)	
	Year 2004	Year 2005
Control	588.4 (0.0) ^a	681.6 (0.0) ^a
BTH	589.1 (0.1) ^a	747.6 (8.0) ^{ab}
EXTN-1	665.8 (11.6) ^b	780.0 (12.6) ^b

The same letters do not differ significantly at $P = 0.05$. The numbers in parenthesis are percent values over control

BTH also resulted in enhanced height (8.23%) and number of tillers (4.70%) in comparison with the control plants. The increased yield in the EXTN-1 treated plants over the untreated control plants were 11.6 and 12.6% in the year 2004 and 2005 respectively (Table 1).

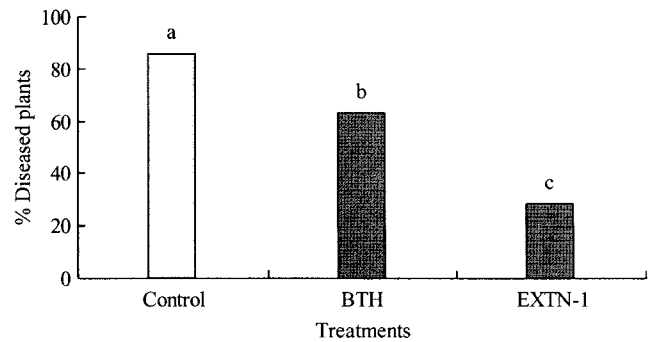
Disease suppression by EXTN-1. The *B. vallismortis* strain, EXTN-1 showed consistent disease protection against tested fungal pathogens. Compared with the control plants, the average reduction in rice blast was 52.11% after treatment with EXTN-1 (Table 2). ISR by treatment with BTH also significantly reduced rice blast (by 78.81%).

The sheath blight symptoms were significantly less in the EXTN-1 treated plants (Fig. 4 and 5). When the untreated

Table 2. Disease (Rice blast and Brown spot) suppression effected in rice plants by treatments with EXTN-1

Treatment	Lesion area	Brown spot
	Rice blast	Lesion number/Leaf
Control	7.1 (0.00) ^a	7.8 (0.00) ^a
BTH (1 mM)	1.5 (78.81) ^b	7.5 (3.85) ^a
EXTN-1	3.4 (52.11) ^b	6.9 (11.50) ^{ab}

The same letters do not differ significantly at $P = 0.05$. The numbers in parenthesis are percent values over control

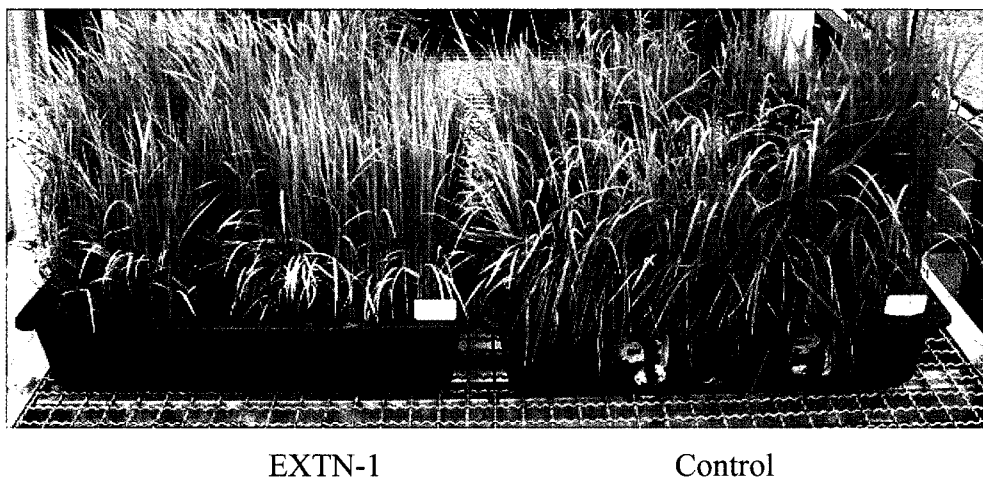
**Fig. 4.** EXTN-1 mediated sheath blight suppression in paddy field against *R. solani*. Bars corresponding to different letters differ significantly at $P = 0.05$.

control had 83.02% diseased plants, the BTH treatment had 63% disease plants while the EXTN-1 treatment had only 28.04% diseased plants.

There was 11.54% reduction in the number of lesions (brown spot) in the EXTN-1 treated plants compared to the untreated control (Fig. 4). Here treatment with BTH also effected significant levels of disease suppression.

Discussion

Rice disease management strategies are mainly through the use of chemical pesticides. The persistent, injudicious use of chemicals has toxic effects on non-target organisms and can cause undesirable changes in the environment. Biological control agents have been used successfully in several pathogen/host systems to enhance plant growth and bring about disease control. The present study revealed the potential of the rhizobacterial strain, EXTN-1 to bring about significant protection against three different fungal pathogens, *M. grisea*, *R. solani* and *C. miyabeanus* and also

**Fig. 5.** EXTN-1 mediated sheath blight suppression by seed soaking with the bacteria.

to enhance plant growth promotion. Plant growth promotion by rhizobacteria could be due to the production of growth hormones, Indole-3-Acetic Acid (IAA) & Gibberellic Acid (GA), suppression of deleterious organisms and promotion of the availability of uptake of mineral nutrients (Kloepper et al., 1980). The exact mechanisms are unclear yet.

EXTN-1 treatment resulted in 52.11% reduction in rice blast, 83.02% reduction in sheath blight and 11.54% decrease in brown spot symptoms in the crop. Reports are few on potential of a strain of biocontrol agent suppressing several diseases of single crop, which is an important strategy to reduce the cost of the technology. The ability of EXTN-1 to suppress multiple diseases proves its multifarious modes of action. It could also be supposed that the bacterium imparts a broad-spectrum systemic resistance to the host plant. As reported by Van loon et al. (1998), if defense mechanisms are triggered by a stimulus prior to infection by a plant pathogen, disease can be reduced. We have reported earlier that pretreatment of tobacco and *Arabidopsis* with EXTN-1 activated the expression of PR-1a and PDF 1.2 defense genes (Park & Kloepper, 2000), which suggested the systemic resistance induced by treatment with EXTN-1 are involved with salicylic acid (SA) mediated and jasmonic acid (JA) mediated systemic resistance pathways. Also demonstrated the hypersensitive reaction (HR), oxidative burst, lignifications and production of cyclo dipeptides and activation of pathogenesis-related genes in cucumber plants upon treatment with EXTN-1 (Park et al., 2001; Jeun et al., 2001; Ahn et al., 2001).

Schweizer (1998) demonstrated a JA-mediated systemic resistance against *M. grisea* in rice. Ahn et al. (2005) suggested that rice employs distinct mechanisms for its defense against *M. grisea* and *C. miyabeanus*. They demonstrated that treatment of BTH and Methyl Jasmonate (MeJA) induced PR gene expression pattern against *M. grisea*, while this defence did not work against *C. miyabeanus* resulting in infection process. These reports reinforced our present observations as EXTN-1 suppressed disease development by both these pathogens and works through a broad-spectrum of systemic resistance via a JA and SA pathway (Park et al., 2001).

In the present study, except for rice blast disease, BTH could not suppress the disease to the level that of EXTN-1. It may be due to the reason that the disease suppression by EXTN-1 is by a multiple mode unlike BTH. In the case of rice blast, systemic-host-resistance-mediated mechanism of disease suppression might be more effective than other modes since BTH brought about 78.81% disease suppression, and EXTN-1 imparted only 52.11% disease suppression. Various researchers have demonstrated different

mechanisms of disease suppression by rhizobacteria (Zehnder et al., 2001; Vessey, 2003), antibiosis being one of the important mechanism (Duffy and Defago, 1999). Homma (1984) observed certain soil bacteria degrade the conidia of *C. miyabeanus*. Also, chitinase-mediated cell wall lysis of *R. solani* has been reported by Lorito et al. (1998). Our recent studies proved that the different fractions of the metabolites produced by EXTN-1 considerably brought down the mycelial development of several fungal plant pathogens (data unpublished).

As EXTN-1 effectively brought about multiple disease suppression in rice via different mechanisms of action, the strain could be effectively explored in large scale for the benefit of rice farming community.

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