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SII-4

Plant growth promotion and induced systemic resistance by a selected PGPR strain, *Bacillus amyloliquefaciens* EXTN-1

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Various non-pathogenic Rhizobacteria have the ability to induce systemic resistance in plant, which provides protection against a broad spectrum of phytopathogenic microorganisms including fungi, bacteria, and viruses. Researches have demonstrated that induced systemic resistance (ISR) can be a potential mechanism by which plant growth-promoting rhizobacteria (PGPR) reduced diseases (Wei, 1991, Lie *et al.*, 1996). ISR acts through a different signaling pathway to that systemic acquired resistance(SAR), the ISR pathway is induced when the plant is challenged by pathogens. Bacterial determinants that are claimed to produce ISR including sidrophore, O-antigen of lipopolysacharide, pyoveridine and salicylic acid. The purpose of this research was application and elucidation of systemic resistance by *Bacillus amyloliquefaciens* EXTN-1.

Soil drenching or seed priming (10^6 cfu/ml) of *Bacillus amyloliquefaciens* strain EXTN-1 stimulated seed germination and growth of about 20 crops used without any harmful effect. Furthermore, treatment of *B. amyloliquefaciens* strain EXTN-1 showed a broad disease-controlling spectrum to the plant diseases caused by viral, bacterial, and fungal plant pathogens such as cucumber mosaic virus, tobacco mosaic virus, potato virus Y and X, *Pseudomonas syringae* pv. *lacrymans*, *Ralstonia solanacearum*, *Colletotrichum orbiculare*, *Magnaphorte grishia*, and *Fusarium oxysporum* (Park et al.,2001).

Lettuce Plants with EXTN-1 showed great growth promotion compared to that of untreated control (Fig. 1). When *B. amyloliquefaciens* strain EXTN-1 was drenched to lettuce grown in hydroponic system, the population of *B. amyloliquefaciens* strain EXTN-1 was similar or increased in the rhizosphere compared to that of initially treated population, while the population was gradually decreased up to 10 folds in the hydroponic solution 4 weeks after treatment. In another experiment, we found that induced systemic resistance and plant growth promotion activity by EXTN-1 strongly showed in cool season than summer season. In case of cucumber, soil drenching after seed coating with EXTN-1 showed best disease protection and plant growth promotion in soil as well as in hydroponic system as method of application for EXTN-1.

Treatment of EXTN-1 increased H_2O_2 amount in early stage and induced the expression of resistance genes, PR-1a, HMGR, PAL. In the previous reports (Park *et al* 2001, Jeun *et al* 2001, Park *et al.*, 2000, Ahn *et al.*, 2002), *B. amyloliquefaciens* strain EXTN-1 showed various beneficial effects on crops and mode of actions were also proposed for these phenomenon. Among the mechanisms, EXTN-1 provoked the expression of two representative markers, PR-1 and PDF 1.2, in the leaves of Arabidopsis ecotype Col-0 at the same time. This result implied that protection ability induced by EXTN-1 is dependent on salicylic acid and/or jasmonic acid-dependent pathways (Ahn *et al.*, 2002, Fig 2).

Six kinds of Cyclo dipeptide {cyclo(L-tyro-L-pro)} were identified as elicitors inducing systemic resistance, which were purified from butanol extract of EXTN-1 grown on TSA medium. Cyclo dipeptide isolated from EXTN-1 showed induced systemic resistance against cucumber anthracnose fungus as well as PR-1a promoter expression on tobacco plant (Park *et al.*, 2002, Table 1). This result suggests that a bacterial metabolite, cyclo (L-pro-L-tyr) involves in the activation of plant defense reactions, leading to systemic resistance against cucumber anthracnose fungi. Cyclic dipeptide, cyclo (L-pro-L-tyr) was isolated from *Pseudomonas putida* WCS358 (Degrassi, 2002) as a quorum sensing signal compound.

On the other hand, several of cyclic peptides are also produced by the fungal plant pathogen *Alternaria alternate*. Furthermore, cyclo (L-pro-L-tyr) and cyclo (L-pro-L-the) act as host-specific phytotoxins against spotted knapweed (*Centaurea maculosa*; Stierle *et al* 1988). The phygiological or ecological role of these molecules in relation to quorum sensing remains to be investigated. For future work, it may need whether this compound acts as a quorum sensing signal molecule or has other biological functions in our system.

Mechanism involved in induced systemic resistance by EXTN-1 was revealed as simultaneous activation of SA and JA or

ethylene metabolic pathways and pre-treatment of EXTN-1 reduced germination and appressorium formation of conidia of *Colletotrichum orbiculare* on the leaf of cucumber with increase of callus formation. Furthermore, treatment of EXTN-1 promoted growth and quality of paprica grown in cool season with consistency of the effects and inhibited the bacterial wilt on tomato caused by *Ralstonia solanacearum* for 4 weeks after treatment. Treatment of *B. amyloliquefaciens* strain EXTN-1 showed the increased plant height of the three barley varieties and shorten heading stage of two varieties compared with non-treated control.

Table 1. Induce systemic resistance and PR-1a promoter expression in cucumber plant by cyclo (L-tyr-L-pro) from EXTN-1

Treatment	Plant Height (cm)	Stem diameter(mm)	Lesion No / Plant	PR-1aGUS activity (nM MU/10mg F.W/ h)
Water	26.18	5.08	240.33	54.0
BTH 0.1mM	26.94	5.09	58.33*	210,153*
Cyclo dipeptide 1.0ppm	26.27	4.70	153.33*	7,497
Cyclo dipeptide 0.1 ppm	26.68	5.05	157.11*	34,967*
Cyclo dipeptide 0.001 ppm	27.18	4.87	180.22*	58,167*
LSD (p=0.05)	1.07	0.20	48.31	33,267

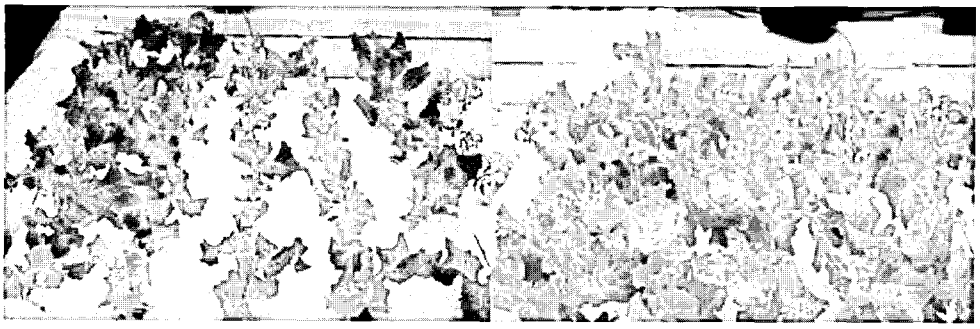

Fig 1. Plant growth promotion by treatment of *B. amyloliquefaciens* EXTN-1 in hydroponic.

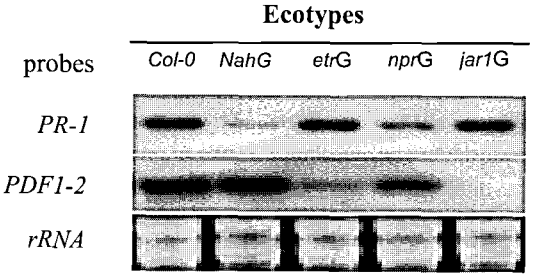

Fig. 2. Activation of Arabidopsis PR-1 and PDF1.2 in response to pretretment with *B. amyloliquefaciens* EXTN-1.