| Disease | Host | Pathogen | Control Value (%) | |
|---|---|---|---|---|
| | | | TORY (*T. harzianum*) | Chemical Fungicide |
| Gray mold rot | Tomato, Strawberry, Egg-plant, etc | *Botrytis cinerea* | 69-82* | 40 (Sumilex) |
| Damping-off | Onion | *Rhizoctonia solani* | 81.6* | 72 (Benomyl) |
| Rust | Garlic | *Puccinia allii* | 69.7* | 3.0 (Mycob+Mancoz) |
| Sheath blight | Rice | *Rhizoctonia solani* | 50.9 | 78.9 (Monceren) |
| Brown patch | Turfgrass | *Rhizoctonia solani* | 60.0* | n.t. |
| Early blight | Potato | *Alternaria solani* | 57.6 | 57.6 (Otiva) |
| Sprout rot | Potato | *Fusarium oxysporum* | 45.0* | n.t. |
| Gummy stem rot | Water melon | *Didymella bryoniae* | 58.2* | 79.9 (Dipheconazole) |
| Anthracnose | Chilli pepper | *Colletotrichum gloeosporioides* | 82.0* | 78.4 (Propi WS) |

**Reaction mechanisms**

Reaction mechanisms of T. harzianum YC459 have not been elucidated exactly yet, but mycoparasitism, induced resistance and enhanced root and plant development appear to be involved in the mechanisms of action based on many observations in greenhouse and field experiments. It is likely that other mechanisms, such as antibiosis, competition for nutrients or space, solubilization of inorganic nutrients also exist but have not yet been studied. In addition, it has been recently found that YC459 has particularly good activity of removing old flower and leaf tissues, an important habitat for many fungal pathogens by producing high cellulase and polygalacturonase.

**References**

Chung, Y. R. and Hoitink, H. A. J. 1990. Interactions between thermophilic fungi and *Trichoderma hamatum* in suppression of *Rhizoctonia* damping-off in a bark compost-amended container medium. Phytopathology 80:73-77.

Elad, Y. 1996. Mechanisms involved in the biological control of *Botrytis cinerea* incited diseases. Europ. J. Pl. Pathol. 102:719-732.

Elad, Y. 2000. Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. Crop Protection 19:709-714.

Harman, G. E. 2000. Myths and dogmas of biocontrol: changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Disease 84:377-393.

Kim, G. G. 2004. Biological control of gray mold rot by *Trichoderma harzianum* YC459 and its interaction with host plants. MS Thesis, Gyeongsang National University, Jinju, Korea.

**SII-3**

# Biological activity of Ethaboxam: the first Korean fungicide

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**Abstract**

Ethaboxam is a new fungicidal active ingredient that inhibits growth of plant pathogens specifically belonging to Oomycetes with protective, curative, systemic and translaminar activity in plants. Modes of action studies revealed that ethaboxam simultaneously inhibits cytoskeleton formation and mitochondrial respiration of *Phytophthora infestans* at low concentrations. There have been no indications of resistance development when tested for baseline resistance monitoring to 261 isolates of *P. infestans* in Korea and Europe and 150 populations of *Plasmopara viticola* populations in Europe for 3 years since 2000. In a selective study with vine trees artificially inoculated with *P. viticola* repeatedly for 10 generations in greenhouse, there have been no changes in sensitivity to ethaboxam among four natural populations of *P. viticola*. Furthermore, ethaboxam has not shown any cross resistance with azoxystrobin, mefenoxam, dimethomorph and cymoxanil. Based on the study results from modes of action and resistance development, ethaboxam appears to be unlikely to develop resistance in field applications.

**Introduction**

Ethaboxam {IUPAC name: *(RS)-N-*(□-cyano-2-thenyl)-4-ethyl-2-(ethylamino)-1,3-thiazole-5-carboxamide; CAS name: *N*-(cyano-2-thienylmethyl)-4-ethyl-2-(ethylamino)-5-

thiazolecarboxamide; CAS registration number 162650-77-3; code name: LGC-30473} is a new aminothiazole carboxamide fungicide and grouped into U5 by the Fungicide Resistance Action Committee (FRAC) mainly due to its unique molecular structure. Ethaboxam is first discovered in 1993, developed and registered in Korea by LG Life Sciences in 1998 and now submitted with full data packages for global registration in more than 50 countries of Asia, Europe, Africa, Middle East and America. Ethaboxam mainly targets diseases caused by pathogenic Oomycetes, such as grape downy mildew caused by *Plasmopara viticola*, potato and tomato late blight caused by *Phytophthora infestans*, cucumber downy mildew caused by *Pseudoperonospora cubensis* and pepper Phytophthora blight caused by *P. capsici* (Kim *et al.*, 1999, 2002, 2004). Many additional plants or crops are considered as potential application targets such as sesame, cabbage, lettuce, melon, pineapple, onion, pea and roses throughout the world. In this symposium, ethaboxam is addressed mainly for its fungicidal activity, modes of action and resistance.

## Fungicidal activity

Minimum inhibitory concentrations (MICs) of ethaboxam were 0.1-0.5 mg/liter when tested with nine isolates of P. infestans and 1.0-5.0 mg/liter when tested with eight isolates of P. capsici (Kim et al., 2002). In planta bioassay, ethaboxam suppresses tomato late blight by preventive and curative activity when applied onto foliage (Kim et al., 2004). Ethaboxam also systemically suppresses tomato late blight and pepper Phytophthora blight when drenched onto soil surface at 24 h before inoculation with each pathogen (Kim et al., 2004). Furthermore, ethaboxam is fairly persistent when examined to tomato late blight for 14 days in glasshouse. These characteristics in planta bioassay indicate that ethaboxam has a high potential as an effective fungicide against Oomycetes diseases. In natural field conditions throughout the world, various formulated products of ethaboxam have resulted in effective control to most diseases, whereas rather weak control to potato late blight (Kim et al., 1999, 2002).

## Modes of action

Ethaboxam has dual modes of fungicidal action, i.e. inhibition of cytoskeleton formation and mitochondrial respiration. Ethaboxam disrupts microtubules of P. infestans when tested using standard immunofluorescence and laser scanning confocal microscopy (Uchida et al., 2004). Microtubules are disrupted even after exposure to ethaboxam for 30 min at 0.01 mg/liter. However, microtubules integrity in the model fungus Aspergillus nidulans (Ascomycota) and in mammalian mouse 17cl1 cells is not affected by the ethaboxam. These results indicate that ethaboxam is specific for the targeting of microtubules disruption in P. infestans. In addition, ethaboxam also exerts a rapid and pronounced effect on mitochondrial respiration in P. infestans strains. Based on inhibition dynamics and magnitude, ethaboxam was equal to known respiratory chain inhibitors, cyanide, azoxystrobin and propyl gallate. However, ethaboxam activity is not an uncoupler, and mitochondrial membrane potentials are dissipated following addition of ethaboxam to whole cells of *P. infestans*.

## Resistance and cross resistance

Baseline resistance has been extensively monitored for P. infestans in Korea and Europe and P. viticola in Europe. In Korea, 261 isolates of P. infestans collected for 3 years (2000-2002) have been tested and MICs were determined as 5.0 mg/liter (Kim et al., 2003). However, only four isolates were capable of growing at 5.0 mg/liter with severe retardation and the rest 257 isolates were not able to grow at all at 1.0 mg/liter. In Europe, 64 populations of P. infestans from potato were collected from five countries (UK, Netherlands, Belgium, France and Spain) and their baseline sensitivity was 0.2 mg/liter. For P. viticola with 150 populations collected from five European countries (France, Italy, Germany, Spain and Portugal) for 3 years, the MICs of all populations were below 10.0 mg/liter when determined by leaf disc assay and approximately 96% of the populations 1.0-3.0 mg/liter. Further studies have been conducted to examine resistance dynamics of P. viticola to ethaboxam by a selective study. Four sets of young vines (cultivar Cabernet Sauvignon) were applied with ethaboxam at 200 g a.i./ha at 12 days before inoculation with each of four natural populations of P. viticola collected in 2003 for the baseline resistance monitoring study. This application and inoculation has been repeated 10 times and then checked any sensitivity change of P. viticola to ethaboxam after 10 cycles. There was no change in sensitivity after 10 cycles, indicating that it is unlikely to select resistance to ethaboxam after repeated applications. Cross resistance has been tested by leaf disc bioassay with 21 strains of P. viticola showing differential sensitivity to azoxystrobin, mefenoxam (isomer of metalaxyl), dimethomorph and cymoxanil. The range of variation was lower and narrower for ethaboxam than the other standards. No cross resistance has been detected between ethaboxam and azoxystrobin, mefenoxam, dimethomorph or cymoxanil, respectively.

## Closing remarks

Ethaboxam has a high potential as a fungicide to control Oomycetes diseases not only in efficacy but also in resistance management. Furthermore, development of ethaboxam has a symbolic meaning because it is the first active ingredient of fungicide discovered in Korea and currently in the process of global registration in more than 50 countries after completing all data generation by the GLP standards. However, there have been so many trials and errors during development (nearly 10 years) mainly due to lack of knowledge, experience and system for development of new active ingredients. Since KSPP members have capability of playing important roles in developing new fungicides that are industrially very significant in the Korean agriculture, we expect more attention to disease management and fungicide development in the future.

## References

Kim, B. S., Xhang, X. Z., Chung, E. K., Kim, D. S., Chun, S. J. and Choi, W. B. 2003. Sensitivity of *Phytophthora infestans* isolates to fungicides metalaxyl and ethaboxam in Korea.

*Plant Pathol. J.* 19:143-147.

Kim, D. S., Chun, S. J., Jeon, J. J., Lee, S. W. and Joe, G. H. 2004. Synthesis and fungicidal activity of ethaboxam against Oomycetes. *Pest Management Sci.* 60: 1007-1012.

Kim, D.S., Lee, Y. S., Chun, S. J., Choi, W. B., Lee, S. W., Kim, G. T., Kang, K. G., Joe, G. H. and Cho J. H. 2002. Ethaboxam: a new oomycetes fungicide. *Proc. British Crop Protect Conf.*-Pest and Disease BCPC, Farnham, Surrey, UK, pp 377-382.

Kim, D. S., Park, H. C., Chun, S. J., Yu, S. H., Choi, K. J., Oh, J. H., Shin, K. H., Koh, Y. J., Kim, B. S., Hahm, Y. I. and Chung, B. K. 1999. Field performance of a new fungicide ethaboxam against cucumber downy mildew, potato late blight and pepper phytophthora blight in Korea. *Plant Pathol. J.* 15:48-52.

Uchida, M., Roberson, R. W., Chun, S. J. and Kim, D. S. 2004. *In vivo* effects of the fungicide ethaboxam on microtubules integrity in *Phytophthora infestans. Pest Management Sci.* (submitted).

**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