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Development and commercialization of a biocontrol agent, TORY using *Trichoderma harzianum* YC459 for controlling gray mold rot

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Introduction

In the decades since the concepts of biological control were first articulated, there have been dramatic advances in both the theory and practice of biological control of plant diseases. Due to increases in fungicide resistance and loss of fungicide registrations, there is a worldwide growing consensus that sole reliance on chemically based disease management is not desirable. Biological control is defined as "the use of natural or modified organisms, genes, or gene products to reduce the effects of undesirable organisms (pests) and to favor desirable organisms such as crops, trees, animals and beneficial insects and microorganisms". For the biological control of plant diseases, antagonistic microorganisms and microbial products, so called 'Biofungicides' are used in the sustainable farming system and many products are now commercially available. Among these, Trichoderma-based biofungicides are known to be most successful in the world markets due to their good controlling efficacies and plant growth promoting effects. A venture company, JGreen Inc. was established to develop a strain of T. harzianum YC459 as commercial products (TORY, 土利), which are now sold in the greenhouse, field crops, and turf industries. This presentation will deal my experiences with Trichoderma in both academic and commercial aspects of biological control.

Background for the development

Since the start of my research career by working on the biological control of ginseng root rots, I have tried to develop biofungicides that are used in commercial agriculture. Most of my research and development effort has focused then on the biological control of soil-born plant diseases by antagonistic bacteria and *Trichoderma* species. I initially sought to develop biofungicides for controlling fusarium root rots and wilts, rhizoctonia damping-off. These efforts were somewhat successful as academic projects. However, soil-born diseases seemed not to be proper target diseases for developing a commercial product in Korea, because it takes long time for farmers to recognize the efficacy of biofungicides comparing to aerial diseases. I, therefore, decided a decade ago that a priority for my research efforts would be to develop biofungicides for aerial plant diseases such as gray mold rot and powdery mildews. Especially, gray mold rot is a good target disease for the biofungicide because of three reasons: (i) there are no adequate chemical replacements due to fungicide resistance; (ii) the effect of biological control can be observed within a few days like chemicals; (iii) the possibility of success may be high under controlled conditions, or plastic film houses.

Strategies for the isolation of *Trichoderma*

In developing a screening system for antagonistic microorganisms, in vivo tests using cucumber seedlings were always utilized in addition to agar plate tests. Among hundreds of tested bacterial and fungal isolates, several Trichoderma spp. were most effective in the control of gray mold rot in repeated growth chamber and greenhouse tests. In the selection of Trichoderma sp. for developing a commercial product, two key strategies were considered: (i) fast growth on cheap nutrients; (ii) good sporulation on high cellulose materials such as saw dust or rice straw. Especially, the YC459 strain grows fast and sporulates very well on saw dust media, which might indicate the good activity of colonization and degradation of senescent or dead plant tissues. The removing activity of the strain of senescent flower and leaf tissues can be an important asset of biological control agent for gray mold rot.

Mass production

For mass production of *T. harzianum* YC459, a semisolid fermentation technology was developed. The technology consists of four steps: preparation of seed inoculum; mycelial growth; production of conidia; collection and concentration of conidia. All steps are processed under aseptic conditions. However, contamination of the culture media is often serious problem in the mass production. It takes one week for a cycle of production and 10^{12} conidia can be obtained per gram of concentrated conidia. The conidial formulation is stable for at least 18 months at ambient temperature.

Formulations and control efficacy

Two kinds of formulations, wettable powders and granules, were developed and the control efficacy of wettable powders in field tests is described in a table below.

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.

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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-