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Development and commercialization of *Trichoderma asperellum* SKT-1 (Ecohope®), a microbial pesticide*

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Trichoderma asperellum (=*T. atroviride* Karsten) SKT-1, a biocontrol agent (BCA) that is highly effective against rice seedborne diseases, was discovered and developed by Kumiai Chemical Industry Co., Ltd. and Shizuoka Prefecture Agricultural Experiment Station. To commercialize this fungus as a microbial pesticide, we studied the economical fermentation methods, stable formulation, and identification of this fungus. As a result, we successfully developed "Ecohope®" and "Ecohope-Dry®", microbial pesticides that are comparable in price to chemical pesticides, retain high biological effects in distribution, and show excellent stability. "Ecohope®" and "Ecohope-Dry®" were registered as agricultural pesticides in 2003 and 2004, respectively. © Pesticide Science Society of Japan

Keywords: biocontrol, *Trichoderma*, rice seedborne diseases, Bakanae disease.

Introduction

Chemically synthesized pesticides have been used for the efficient production of crops. In recent years, whereas environmental pollution and load due to synthetic chemicals including chemical pesticides have been taken up as a social problem, efficient agricultural production to obtain crops from limited cultivation areas is an important issue to maintain the current population. With such a background, microbial pesticides for biological control using microorganisms present in nature are noted as a means to reduce environmental pollution and for efficient agricultural production.

However, microbial pesticides in general are considered unsuitable for commercialization by business enterprises because of the following problems: (1) they have slow effect manifestation, (2) they are effective only for a limited number of pests, (3) because they are made of living organisms, they are unstable and unsuitable for distribution, and (4) they are more expensive than chemical pesticides.

Kumiai Chemical Industry Co., Ltd. and Shizuoka Prefecture Agricultural Experiment Station have found that SKT-1, a fungal strain of genus *Trichoderma*, was highly effective against four diseases of rice caused by seedborne pathogens, Bakanae disease caused by *Gibberella fujikuroi*, bacterial seedling blight caused by *Burkholderia plantarii*, bacterial grain rot caused by *Burkholderia glumae* and bacterial brown stripe caused by *Acidovorax avenae* subsp. *avenae*. The suppressive effects of *T. asperellum* SKT-1 were nearly equivalent to those of chemicals such as ipconazol-copper for Bakanae disease and oxolinic acid for bacterial rice seedling diseases. We gave priority to the submerged spores of *T. asperellum* SKT-1 obtained by deep-tank fermentations because of their economical production, and marketed under the brand name of Ecohope in Japan (Registration No. 21009) in 2003. In 2005, we released an aerial spore (=conidia) of *T. asperellum* SKT-1 obtained by koji fermentation with the brand name Ecohope-Dry wettable powder in Japan (Registration No. 21434). This paper describes the biological control activity, fermentation, formulation and mode of action of SKT-1.

* See Part II for full Japanese article.

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1. Biological Control Activity

Trichoderma asperellum SKT-1 (FERM P-16510; International Patent Organism Depository, Ibaraki, Japan) was isolated from the rhizosphere of Japanese lawngrass (*Zoysia japonica*). *T. asperellum* SKT-1, when rice seeds were soaked in a suspension of its conidia at $4\times10^4-1\times10^6$ conidia/ml, gave high control of Bakanae disease, which was nearly equivalent to that of seed disinfection with ipconazol-l-copper flowable. At $2\times10^5-1\times10^6$, it also gave high control of bacterial seedling blight causing bacterial grain rot and bacterial brown stripe, nearly equivalent to seed infection with oxolinic acid wettable powder. SKT-1 conidia with those washed by centrifugation gave clear control against Bakanae disease and bacterial seedling blight, but neither SKT-1 conidia killed by autoclaving nor supernatant solution from centrifugation controlled disease. SKT-1 strain gave high control of Bakanae disease and bacterial seedling blight in treatments during soaking and germination. In post-seeding treatments of conidia at low concentrations, however, control of Bakanae disease decreased and bacterial seedling blight was not controlled by any SKT-1 concentration tested. Tests performed on the loss of disease control in combination with benomyl revealed that SKT-1 had lost its inhibitory effects against Bakanae disease by one day after seeding and against bacterial seedling blight by the time of seeding. In subsequent combined treatments, however, SKT-1 controlled both diseases.

2. Fermentation and Formulation

T. asperellum SKT-1 forms submerged spores by liquid culture. These submerged spores had some common properties with aerial spores, but differed in surface topology and internal organization. Aerial spores, so called conidia, maintained high viability under dry storage conditions, whereas the viability of submerged spores was reduced. The cell wall of submerged spores was thinner than that of aerial spores, and the surface was smooth in contrast to the irregular pyramidal warts of aerial spores. Both submerged and aerial spores were highly effective against Bakanae disease by seed-treatment therefore, these two-types of spores were suitable for different applications. Although the viability of submerged spores with a thin cell wall was reduced under dry storage conditions, submerged spores stored in water at 4°C maintained high viability and biological control activity for 4 months. The wettable powder, a formulation for hydrophobic aerial spores, could be stored at room temperature, and maintained high viability and biological control activity for 6 months.

3. Mode of Action

T. asperellum SKT-1 and *Gibberella fujikuroi*, known as the causal agents of Bakanae disease, were both transformed with genes encoding green fluorescent protein (GFP) and hygromycin B (hygB) by restriction enzyme-mediated integration (REMI). Rice seeds inoculated with GFP-tagged *G. fujikuroi* showed Bakanae symptoms. GFP-tagged SKT-1 maintained biocontrol activity against the pathogen by soaking seeds in SKT-1 spore suspension. Then, we monitored *in situ* interactions between SKT-1 and *G. fujikuroi* on rice seeds using GFP-tagged transformations under confocal scanning laser stereomicroscopy. *G. fujikuroi* disappeared from embryos of rice seeds after treatment with SKT-1, whereas SKT-1 was observed on embryos 24 hr after quickening of germination. In addition, hyphae of *G. fujikuroi* was penetrated by hyphae of SKT-1, and degradation of the cell walls of *G. fujikuroi* was observed under the scanning electron microscope (SEM) in their co-culture. The cell wall of *G. fujikuroi* on embryos of rice seeds was lysed, suggesting that mycoparasitism is the mode of action of *T. asperellum* SKT-1.

4. Conclusion

The active ingredient counts in the application of Ecohope® and Ecohope-Dry® are "zero" because they are microbial pesticides. Thus, they are useful for farmers who run reduced-pesticide cultivation and meet the needs of producers. We were able to commercialize a "microbial pesticide" that had been considered unsuitable for commercialization, because it is a useful microorganism, and because innovative technologies for fermentation and storage stability of the microorganism were developed.