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Effect of culture filtrates of *Trichoderma* sp. isolated from wild mushrooms on the infectious behavior of plant pathogenic fungi

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Abstract *Trichoderma* sp. (strain H921) was isolated from the fruiting bodies of wild mushrooms and evaluated for fungicidal activity against plant pathogenic fungi. When spores of the plant pathogenic fungi *Alternaria alternata* Japanese pear and tomato pathotypes), *Botrytis cinerea, Colletotrichum orbiculare, Corynespora cassiicola*, and *Fusarium oxysporum* f. sp. *spinaciae* were incubated in culture filtrates of strain H921 and incubated onto glass slides, spore germination of these fungi was significantly inhibited.

Keywords: Culture filtrate, Antifungal compound, Plant pathogenic fungi, Mushrooms, *Trichoderma*

Introduction

Plant diseases caused by fungi are a major limiting factor in agricultural production. Resistant cultivars and chemical fungicides play important roles in plant disease control. However, the durability of genetic resistance in improved rice cultivars is often short lived in the field because of the pathogen's ability to rapidly evolve and overcome resistance (Ahn 1994). The key to the chemical control of plant diseases is to overcome fungicide–resistant strains of the pathogen (So et al., 2002; Yamaguchi et al., 2002). Therefore, a search for antifungal compounds is required to develop new fungicides. Antifungal compounds of microbial origin play an important role in the biological and chemical control of plant diseases (Fravel 1998; Shimizu et al., 2000; Uddin and Viji 2002).

Some phylloplane and endophytic fungi are involved in protecting plants from pathogens and produce antimicrobial compounds (Aremu et al., 2003; Evueh et al., 2008; Koitabashi et al., 2004). The number of mushroom species in the world is estimated to be approximately 140, 000 but only around 10% of these species have been identified (Wasser 2002). Interestingly, studies on the existence of symbiotic and parasitic fungi in wild mushrooms and their potential for plant disease control are not get reported.

Recently, we isolated *Trichoderma* strain H921 from the fruiting bodies of wild mushrooms. Ethyle acetate (EtOAc) extracts of H921 culture filtrates inhibited spore germination and appressorium formation of *Magnaporthe oryzae*. Furthermore, blast lesion formation was significantly inhibited by EtOAc extracts of H921 culture filtrates. The potential of the H921 culture filtrates isolated from the fruiting bodies of wild mushrooms to control other fungal disease remains to be elucidated. The objective of this study is to investigate the antifungal activity of culture filtrates of H 921 isolated from the fruiting bodies of wild mushroom against various plant pathogenic fungi. We reported that these culture filtrates can inhibit the infectious behavior of plant pathogenic fungi.

Materials and Methods

Microorganisms and culture conditions

In July 2010, we isolated the *Trichoderma* sp. (strain H 921) from fresh fruiting bodies of wild mushrooms from a paddy field in Shobara (Hiroshima prefecture). Strain H921 was grown on potato sucrose ager (PSA) medium, and a mycelial disk (8 mm I diameter) from the growing colony was transferred in a test tube containing 20 mL of

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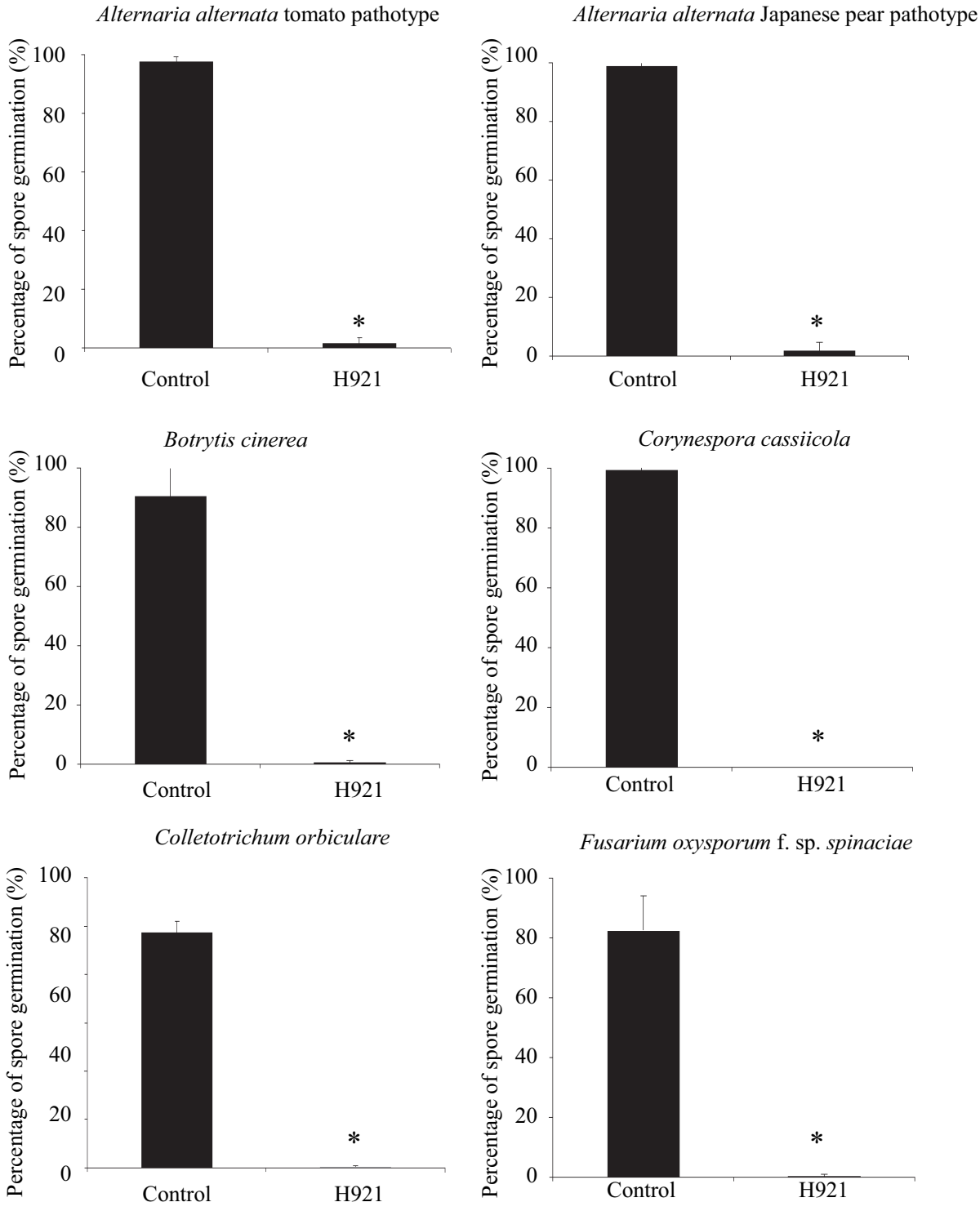

Fig. 1 Effect of ethyl acetate extract of culture filtrates of strain H921 isolated from wild mushrooms on spore germination of plant pathogens. A spore suspension of each pathogen was incubated onto glass slides in the absence (Control) or presence (H 921) of the ethyl acetate extract of culture filtrates of strain H921 and incubated in a moist chamber at 26°C. After 24 h, the percentage of spore germination was determined under light microscopy. Experiments were repeated 3 times. Bars represent mean ± SD. Asterisk indicate the significant difference compared with the result of the control (Fisher's exact test, $P<0.05$).

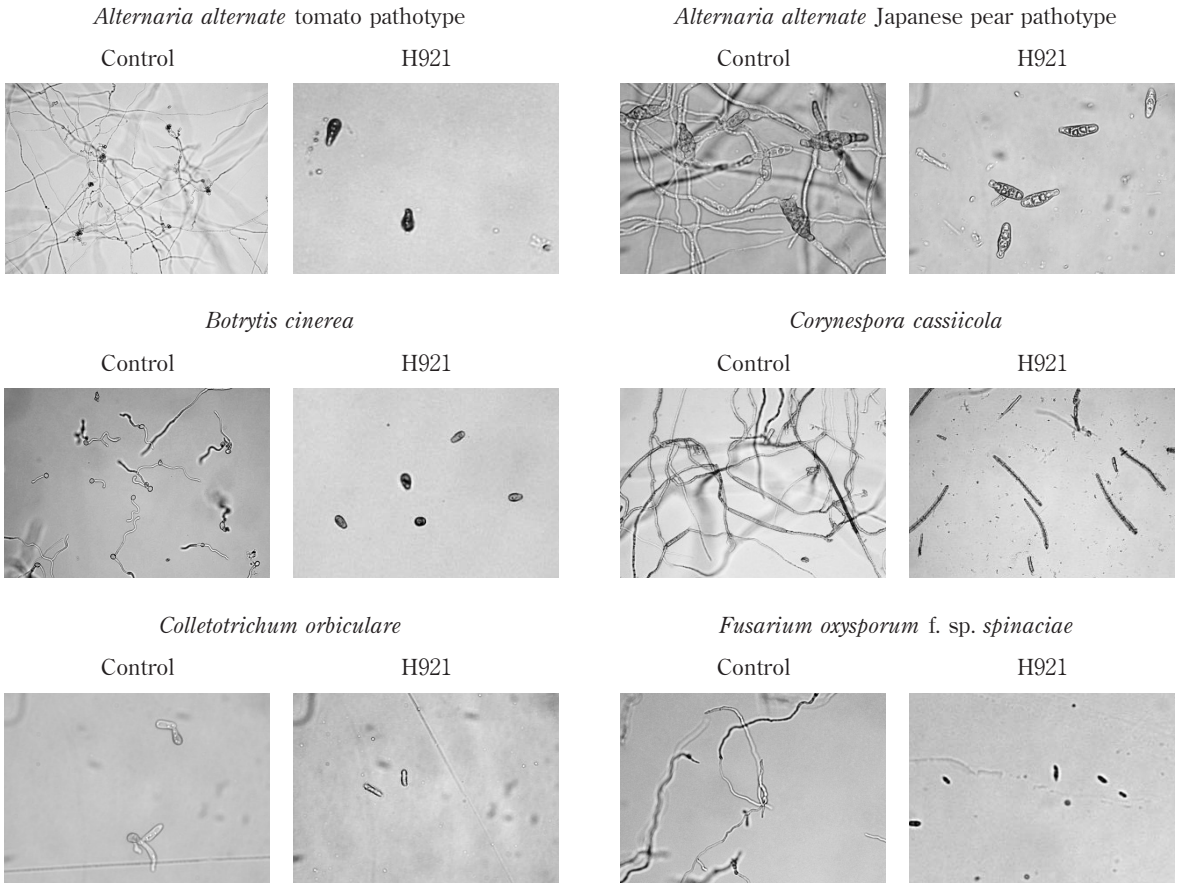

Fig. 2 Suppression of spore germination of plant pathogens by ethyl acetate extract of culture filtrates of strain H 921 on a glass slide. A spore suspension of each pathogen was incubated onto slides in the absence (Control) or presence (H921) of the ethyl acetate extract of culture filtrates of strain H921 in a moist chamber at 26° C. After 24 h, spore germination was observed under light microscopy.

potato sucrose broth (PSB). The culture was incubated at 27° C in the dark for 5 days with shaking. Crude culture filtrate (20ml) was extracted with EtOAc, followed by the addition of distilled water to the EtOAc fraction. This mixture was rotary-evaporated at 40° C under reduced pressure until only water remained, and the resulting aqueous volume was adjusted to 2 mL.

The *Alternaria alternata* Japanese pear and tomato pathotypes, *Colletotrichum orbiculare* and *Corynespora cassiicola* were grown on either rice bran agar or PSA medium for 7 days at 27° C and then for approximately 2 days at 27° C under near-ultraviolet radiation provided by fluorescent lamps (FL20s B-B; Panasonic, Osaka, Japan) to induce abundant sporulation. *Botrytis cinerea* and *Fusarium oxysporum* f. sp. *spinaciae* were grown on PSA medium for 7 days at 27° C.

Influence of strain H921 on the spore germination of plant pathogenic fungi

The spores of plant pathogenic fungi and EtOAc extracts of H921 culture filtrates were prepared as described above. To investigate the inhibitory effects of H921 culture filtrates on the spore germination of *A. alternata* Japanese pear and tomato pathotypes, *B. cinerea, C. orbiculare, C. cassiicola*, and *F. oxysporum* f. sp. *spinaciae*, the fungal spores (5×10^4 spores/mL) were incubated in the presence of H 921 culture filtrates in the dark at 26° C. After 24 h, the percentage of spore germination of fungal spores was determined by light microscopy.

Statistical analysis

Data have been showed in terms of the mean ± standard deviation (SD) values. Statistically significant differences were determined using the Fisher's exact test.

Results and Discussion

To determine the effects of H921 culture filtrates on the spore germination of the *A. alternate, B. cinerea, C. orbiculare, C. cassiicola*, and *F. oxysporum* f. sp. *spinaciae*, spore suspensions (5×10^4 spores/mL) were placed onto glass slides in the presence of EtOAc extracts of H921 culture filtrates and incubated in a moist chamber at 26°C. After 24 h, spore germination was observed by light microscopy. The percentages of spore germination of *A. alternata* (Japanese pear and tomato pathotypes), *B. cinerea, C. orbiculare, C. cassiicola* and *F. oxysporum* f. sp. *spinaciae* were 1.7 ± 2.8%, 1.3 ± 1.9%, 0.3 ± 0.8%, 0.2 ± 0.6%, 0 %, and 0.2 ± 0.6%, respectively (Fig. 1). In the control, the percentages of spore germination of the *A. alternata* (Japanese pear and tomato pathotypes), *B. cinerea, C. orbiculare, C. cassiicola*, and *F. oxysporum* f. sp. *spinaciae* were 99.0 ± 1.7%, 97.6 ± 1.9%, 96.9 ± 3.2%, 97.3 ± 4.7%, 99.5 ± 0.8%, and 82.4 ± 11.8%, respectively (Fig. 1). Thus, spore germination of *A. alternata, B. cinerea, C. orbiculare, C. cassiicola* and *F. oxysporum* f. sp. *spinaciae* was significantly inhibited by EtOAc extracts of the H921 culture filtrates but was not inhibited in the control (Fig. 2).

These results clearly show that spore germination of air-borne and soil-borne plant pathogens is inhibited by EtOAc extracts of H921 culture filtrates.

We have previously reported that EtOAc extracts of H 921 culture filtrates contribute to blast control in rice and barley (Nguyen et al., 2011). In the current study, we observed that EtOAc extracts of H921 culture filtrates exhibited antifungal activity against the *A. alternate, B. cinerea, C. orbiculare, C. cassiicola*, and *F. oxysporum* f. sp. *spinaciae*. Interestingly, some *Trichoderma* spp. have been reported to produce 6-pentyl-α-pyrone, which inhibits the growth of plant pathogens such as *Rhizoctonia solani, F. oxysporum, Botrytis* sp., and *Athelia rolfsii*. However, the Rf value on the TLC plate of the antifungal compound in H921 culture filtrates differed from that of 6-pentyl-α-pyrone (data not shown). Additional studies are required to identify the active antifungal compound in H921 culture filtrates.

Studies on H921 culture filtrates from mushrooms may provide further insights into the protection of plants from pathogens.

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References

Ahn, W.W. (1994) International collaboration on breeding for resistance to rice blast. In: Rice blast disease. RS Zeigler, SA Leong, PS Teng, eds. CAB International, Wallingford, UK, pp. 137-153.

Aremu, E.A., Furumai, T., Igarashi, Y., Sato, Y., Akamatsu, H., Kodama, M. and Otani, H. (2003) Specific inhibition of spore germination of *Alternaria brassicicola* by fistupyrone from *Streptomyces* sp. TP-A0569. Journal of General Plant Pathology, 69: 211-217.

Evueh, G.A. and Ogbebor, N.O. (2008) Use of phylloplane fungi as biocontrol agent against Colletotrichum leaf disease of rubber (Hevea brasiliensis Muell. Arg.). African journal of biotechnology, 15: 2569-2572.

Fravel, D.R. (1988) Role of antibiotics in the biocontrol of plant diseases. Annual Review of Phytopathology. 26: 75-92.

Koitabashi, M., Kajitani, Y. and Hirashima, K. (2004) Antifungal substances produced by fungal strain Kyu-W 63 from wheat leaf and its taxonomic position. Journal of General Plant Pathology, 70: 124-130.

Nguyen, T.Q., Ueda, K., Kihara, J., Arase, S., Oshima, A., Akihiro T., Furuta, K., Yoshikiyo, K. and Ueno, M. (2011) Control of rice blast disease by Trichoderma sp. isolated from a mushroom. Japanese Journal of Phytopathology, 78: 52 (Abstract, in Japanese).

Shimizu, M., Nakagawa, Y., Sato, Y., Furuma, T., Igaroshi, Y., Onaka, H., Yoshida, R. and Kunoh, H. (2000) Studies on endophytic actinomycetes and Streptomyces sp. isolated from Rododendron and its antifungal activity. Journal of General Plant Pathology, 66：360-366.

So, K., Fuji, M., Iwabuchi, H., Kanayama, M. and Yamaguchi, J. (2002) Effects of various fungicides against less carpropamid-sensitiverice blast fungus isolated from the northwest area in Saga prefecture. Japanese Journal of Phytopathology, 68: 262 (Abstract, in Japanese).

Uddin, W. and Viji, G. (2002) Biological control of turfgrass disease. In: Gnanamanickam, SS eds.Biological control of crop disease. Marcel Dekker, New York, pp. 313-337.

Wasser, S.P. (2002) Medicinal mushroom as a source of antitumor and immunomodulating polysaccharides. Applied Microbiology and Biotechnology, 60: 258-274.

Yamaguchi, J., Kuchiki, F., Hirayae, K. and So, K. (2002) Decreased effect of carpropamid for rice blast control in the west north area of Saga prefecture in 2001. Japanese Journal of Phytopathology, 68: 261 (Abstract, in Japanese).