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NOTE

Antifungal Activity of *Streptomyces padanus* isolate TH04 against *Monilinia fructicola*, Brown rot Fungus on Stone-fruits

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Abstract

The *Streptomyces padanus* isolate TH04, isolated from mummified peaches, showed strong antifungal activity to *Monilinia fructicola*. The inhibition activity of the isolate TH04 to mycelial growth and spore germination at 1% concentration of sub-antifungal powder made from culture suspension (CS) was ranged from 79.8% to 81.0% and from 73.9% to 75.8% to *M. fructicola* four strains, respectively. In the test of antifungal activity in mixed culture of the isolate and *M. fructicola*, inhibition rate was 7.5%, 86.8% and 94.0% in 0.01, 0.1, and 1% concentration of CS containing bacterial cell of the isolate, respectively. On apples (cultivar; Fuji), the control values of the isolate TH04 crude filtrates (0.1 and 1%) were 85.9% and 100%, respectively. The results suggest that the isolate TH04 indicate development possibility as biocontrol agent of brown rot caused by *M. fructicola* with the study on delivery method and fermentation condition to produce an antifungal compound.

Key words *Streptomyces padanus*, *Monilinia fructicola*, biocontrol agent

Brown rot by *Monilinia* spp. is a major global disease of all commercially grown *Prunus* species in the world (Adaskaveg *et al.*, 2000). Under suitable environments, the brown rot fungi can infect all Drupaceous and Pomaceous species and many other members of the rosaceae which also serve as hosts to these fungi (Landgraf and Zehr, 1982; Sutton and Clayton, 1972; Wittig *et al.*, 1997).

Introduction of the benzimidazole, dicarboximide, and EBIs (ergosterol biosynthesis inhibitors) since 1970's have dramatically improved to control brown rot in the field and in packing sheds (Ogawa *et al.*, 1985). Fungicides were used to control plant pathogenic fungi for a long time repeatedly, but arose issues on environmental impact, fungicide resistance, and safety of crop products (Ames, 1979; Bus *et al.*, 1991; Delp, 1988; Elmer and Gaunt, 1994; Lim *et al.*, 1998).

To overcome the dilemmas of pesticides mentioned above, *Streptomyces* spp., *Trichoderma* spp., *Bacillus* spp., *Agrobacterium* spp., and *Pseudomonas* spp. have frequently been used for biocontrol of plant pathogenic fungi recently (Baker *et al.*, 1983, Becker, 1993; Katz and Demain, 1977; Powel and Fox, 1993; Sivan and Chet, 1989). Wittig *et al.* (1997) reported that *Aureobasidium pullulans*, *Epicoccum purpurascens*, and *Gliocladium roseum* from cherry blossom infected by *M. fructicola* were isolated and showed antifungal activity against *M. fructicola* under mist chamber and field condition. Hong *et al.* (2000) also reported that mycoflora of stone fruit mummies was

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diverse and contained a number of antifungal microorganism such as *Aureobasidium*, *Penicillium*, *Trichoderma*, and yeast.

The objective of this study was investigation on potential as biocontrol agent of the isolate TH04 obtained from mummified peach caused by *M. fructicola*. The potential was tested with inhibition mycelial growth and spore germination and antifungal activity on apple of substance from cultural suspension, mixed culture with pathogen.

The antifungal microorganisms were isolated from mummified peaches. To isolation of microorganisms, Collected 120 samples were heated for 30 min at 60°C to reduce undesirable microorganism and then used for isolation sources. Actinomyces colonies were isolated after 2-4 weeks incubation on starch casein agar (starch 10 g, casein 0.3 g, KNO_3 2 g, K_2HPO_4 2 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $CaCO_3$ 0.02 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, agar 0.2 g, and dH_2O 1 L) and they were freeze-dried and stored at -40°C until use.

For identification of isolate TH04, showed inhibition activity of 80% to mycelial growth among *Streptomyces* spp. (120 isolates) on dual culture method (Lim *et al.*, 2000), 16S rDNA sequences were analyzed. Target DNA was amplified with a PCR kit using universal primers (27F 5'-AGA GTT TGA TCA TGG CTC AG-3', 1492R 5'-GGA TAC CTT ACG ACT T-3'). After purification of PCR product, it was sequenced with DNA analyzer (ABI PRISM 3700). The determined sequences were analyzed by a BLASTN program. The isolate TH04 has a 99.16% homology with *Streptomyces padanus* strain MITKK (Fig. 1). The culture and morphological characteristics of isolate TH04 was reported by Lim *et al.* (2000).

The culture broth (Yeast extract 3 g, Malt extract 3 g, Peptone 5 g and dH_2O 1 L) of isolate TH04 was dried with vacuum freeze dryer (Samwon, SFDSM24L, Korea). The dried powder containing bacteria was treated with MeOH to eliminate bacteria and make sub-antifungal powder (SAP). After concentration with vacuum rotary evaporator (EYELA, N-1000-W, Japan), the activity was tested on PDA added with 1% of SAP dissolved in MeOH. The inhibitory effect was evaluated in percentage using the following formula: [1-(fungal growth or germination rate of treatment/fungal growth or germination rate of control)] ×100. Against fungicide-resistant isolates, the inhibitory activity to mycelial growth ranged from 79.8 to 81.0%, depending on fungicide-response of the isolates (Table 1). The activity to CD97, a double-resistance isolate to carbendazim and iprodione, was the highest, even though

CCTTCGGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCNCTCTGGGACAAGCC
CTGGAAACGGGGTCTAATACCGGATATGACCATCTTGGGCATCCTTGATGGTGTAAAGCTCCGGCGGTGCAN
GATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTG
AGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCANACTCCTACGGGAGGCAGCAGTGGGGAATATT
GCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTC
AGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGT
AATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGATT
GTGAAAGCTCGGGGCTTAACCCCGAGTCTGCAGTCGATACGGGCTAGCTAGAGTGTGGTAGGGGAGATCGG
AATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCA
TTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACG
GTGGGAACTAGGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACGCATTAAGTTCCCCGCCTGGGG
AGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAA
TTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCATTAGAGATAGTGCCCCCCTTGT
GGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG
CGCAACCCTTGTCCCGTGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAA
CTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGG
CCGGTACAATGAGCTGCGATACCGTGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTC
TGCAACTCGACCCCATGAAGTCGGAGTCNCTAGTAATCGCAGATCAGCATTGCTTGCGGTGAATACNTTTTCC
CCGGGCCCTTGTACA

Fig. 1. 16S rDNA sequences of *Streptomyces padanus* isolate TH04. The sequences were analyzed with DNA analyzer (ABI PRISM 3700) and a BLASTN program.

there was no significant difference among tested isolates. The activity against spore germination was recorded from 73.9 to 75.8%. But, no significant difference was found among tested isolates (Table 1).

To test antifungal activity of isolate TH04 on the growth of *M. fructicola* in liquid culture, *M. fructicola* (1×10^8 spores/mL) was seeded first to the medium which contains same volume of PDB and YMB. Together with inoculation of pathogen, 0.01, 0.1, and 1% of isolates TH04 culture suspension (1% means 1×10^6 TH04 cells) were added to the medium respectively. After incubation for 7 days at 27°C, mycelial mass was collected by filtering the medium through cheesecloth and air-dried to measure the dried mycelial weight. Inhibition rates on mycelial growth were 7.5, 86.8 and 94.0%, according to the treatment (Fig. 2).

For assay on apple, which was selected with pathogenicity and host (Byrde and Willetts, 1997), *M. fructicola* was incubated for 7 days to take agar plugs for inoculation. Apples (cultivar: Fuji) were washed with distilled water and disinfected with 70% ethanol. Wounds (width: 5 mm, depth: 5 mm) were made on the surface of apples using cork borer. The wounds were pre-treated with 0.5 mL of 0.1 and 1% sub-antifungal powder (SAP) of isolate TH04. After one hour, the agar plugs were inoculated to the wounds and incubated for 7 days to measure lesion size. The control values were 85.9% and 100%, respectively (Fig. 3).

Antifungal activity of isolates TH04 to *M. fructicola* increased by treatment of isolate TH04 cells instead of culture filtrate (Fig. 2). It was thought that the increase in the activity could be caused by synergistic effect of antifungal substances and chitinase (Lim *et al.*, 2000; 2007).

The results suggest that the isolate TH04 is a possible agent for biocontrol of brown rot caused by *M. fructicola*. It is necessary that the study on delivery method and fermentation condition to produce an antifungal compound as chitinase and valinomycin and fungichromin which has bioactivity such as antifungal, insecticidal, nematocidial, and anticancer (Lim *et al.*, 2000; 2007; O'Neil *et al.*, 2001; Shih *et al.*, 2003).

Table 1. Activity of crude culture filtrates of *Streptomyces padanus* isolate TH-04 to fungicide resistant isolates of *Monilinia fructicolaa*

Isolates	Response to fungicides[b]	Inhibition activity (%)[c]	
		Mycelial growth	Spore germination
CH06	C^SI^S	80.5d	75.8d
KY95	C^RI^S	80.7d	74.5d
CH12	C^SI^R	79.8d	73.9d
CD97	C^RI^R	81.0d	75.8d

[a]Test media were adjusted with 1% of sub-antifungal powder (SAP).
[b]C^SI^S: sensitivity to carbendazim and iprodione, C^RI^S: resistance to carbendazim and sensitivity to iprodione, C^SI^R: sensitivity to carbendazim and resistance to iprodione, C^RI^R: resistance to carbendazim and resistance to iprodione.
[c]Means with the same letter in the columns are not significantly different at P=0.05 in Duncan's multiple range test.

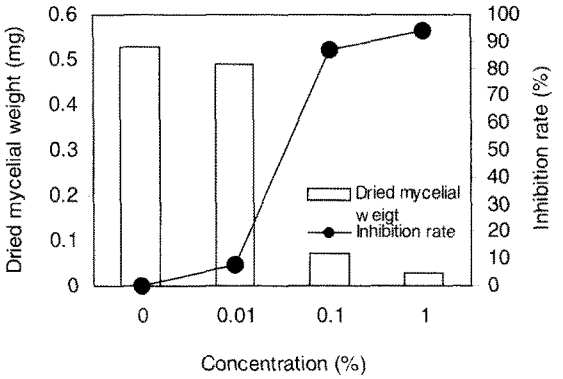

Fig. 2. Antifungal activity of isolate TH04 in co-incubation with *Monilinia fructicola* for 7 days. Inhibition activity (%) = 1-(dried mycelial weight on medium with isolate TH04/ dried mycelial weight on medium without isolate TH04)×100. Concentration represents density of isolate TH04 culture suspension with bacterial cells.

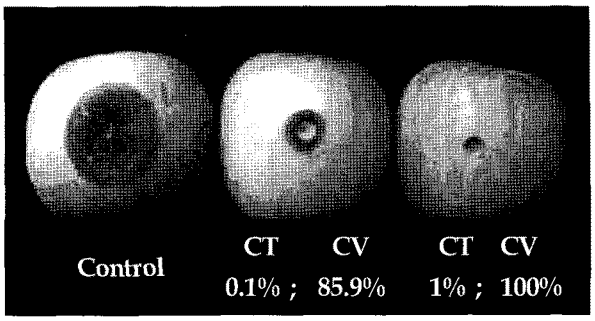

Fig. 3. Antifungal activity of substances from *Streptomyces padanus* isolate TH04 to *Monilinia fructicola*. Photo was taken after 5 days from inoculation. CT and CV represent treated-concentration and control values, respectively. There were 15 apples in each treatment. They were selected according to pathogenicity and host (Byrde and Willetts, 1997).

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잿빛무늬병균에 대한 *Streptomyces padanus* isolate TH04의 항균활성

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요 약 복숭아 미이라 과일로부터 복숭아 잿빛무늬병 *Monilinia fructicola*에 대하여 강한 항균활성을 보이는 방선균 Streptomyces padanus TH04를 분리하였다. TH04 균주의 배양 추출물 1%를 함유한 배지에서의 균사생육 및 포자발아는 시험한 *M. fructicola*의 strain에 따라 각각 79.8~81%와 73.9~75.8% 억제되었다. 병원균과 TH04 균주 초기접종 밀도를 0.01%, 0.1% 및 1%로 달리하여 동시배양 한 결과, 항균활성은 선발 방선균의 밀도에 따라 7.5%~94%로 나타났다. 사과(품종; 후지)를 이용한 조추출물의 항균활성은 0.1% 처리구 85.9%, 1%처리구 100%로 나타났다. 항균활성 물질 생산, 안정성 및 제형화에 관한 연구가 이루어질 경우 선발한 *Streptomyces padanus* TH04는 생물학적 방제제로의 개발 가능성 있을 것으로 생각된다.

색인어 *Streptomyces padanus*, *Monilinia fructicola*, 생물적 방제