Mycobiology 36(4) : 266-269 (2008)

In Vitro Antagonistic Characteristics of Bacilli Isolates against *Trichoderma* spp. and Three Species of Mushrooms

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(Received November 26, 2008. Accepted December 16, 2008)

Twenty isolates of *Bacillus* species obtained from livestock manure composts and cotton-waste composts were tested for their antagonistic effects *in vitro* against three green mold pathogens of mushrooms (*Trichoderma harzianum*, *T. koningii*, and *T. viridescens*). However, there exists a possibility *Bacillus* species may have antagonistic effects against mushrooms themselves, and thus the same 20 isolates were tested *in vitro* against three species of mushrooms (*Flammulina velutipes*, *Lentinus edodes*, and *Pleurotus ostreatus*). Of the 20 *Bacillus* species isolates tested, two inhibited mycelial growth of *T. harzianum*, seven that of *T. koningii*, and eight that of *T. viridescens*. Importantly, the bacterial isolates M27 and RM29 strongly inhibited mycelial growth of all the *Trichoderma* spp. isolates tested. The isolate M27 was subsequently identified as the most effective in inhibiting mycelial growth of all the *Trichoderma* species. Interesting results of the effect *Bacillus* isolates had upon the mushroom species followed. It was found that most *Bacillus* isolates except 5T33 at least somewhat inhibited mycelial growth of the three mushroom species or some of the mushrooms. Furhermore, the antagonistic effects of the bacterial isolates against the three species of mushrooms varied depending on the mushroom species, suggesting a role for mushroom type in the mechanism of inhibition. The bacterial isolates M27 and RM29 were identified as having the most antagonistic activity, inhibiting mycelial growth of all the *Trichoderma* spp. as well as mycelial growth of the three species of mushrooms. These results suggest that the bacterial isolates and their antagonistic effects on green mold pathogens should be further studied for their practical use for biological control of green mold in the growing room of the mushrooms.

KEYWORDS : Antagonistic effect, *Bacillus* species, Inhibition zone, Mushrooms, Mycelial growth, *Trichoderma* spp.

Edible mushrooms are a widely cultivated food source throughout the world and are integral in the cuisines of numerous cultures. The genera of commonly cultivated mushrooms for edibility include *Agaricus*, *Auricularia*, *Flammulina*, *Lentinus*, *Pleurotus*, and *Volvariella* (Chang *et al*., 1993; Chang and Miles, 1989). In Korea, oyster mushroom [*Pleurotus ostreatus* (Jacq.:Fr.) Kummer], winter mushroom [*Flammulina velutipes* (Curt.:Fr.) Sing.], oak mushroom [*Lentinus edodes* (Berk.) Sing.], and button mushroom [*Agaricus bisporus* (Lange) Imbach] are the most widely cultivated mushroom species. Mushrooms comprise the spore-forming, fruiting body of a fungus and therefore are commonly found on compost in wooden trays, in bags, in plastic bottles, on shelves, or on beds in growing rooms at low temperatures with high humidity (Hall *et al*., 2003). However, an emerging problem particularly relevant to agriculture is the development of green mold that frequently occurs on composts or the beds of mushroom populations. It has been reported that several *Trichoderma* spp. are responsible for the green mold therefore can potentially cause substantial economic losses in mushroom production worldwide (Jaklitsch *et al*., 2006; Samuels *et al*., 2002; Seaby, 1998).

Both mushrooms and the green mold made of *Trichoderma* spp. that grows on them belong to the Kingdom Fungi and thus it is reasonable to assume their growth conditions may be similar. This implies preventing the growth of green mold while simultaneously promoting, or at least maintaining, mushroom growth could be difficult. For example, the growth of mushroom green mold is very difficult to control using chemicals because growth of the mushrooms themselves is affected by chemicals as well. This is even excluding that food safety considerations for cultivated mushrooms demand alternatives to chemical control of diseases, including that of green mold.

Biological control using antagonistic bacteria has been applied as one of alternative methods to chemical control of plant diseases (Baker and Cook, 1974; Cook and Baker, 1983; Hornby, 1990; Gnanamanickam, 2002; Kheten, 2001; Parker *et al*., 1985). Although Kim *et al*. (2008) recently tested prospective antagonistic bacteria against four soilborne phytopathogenic fungi, there have been no reports on using microorganisms for the biological control of mushroom green mold. It is considered that effective biological control of green mold relies both on the selection of antagonists that act specifically against the

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pathogens which cause the green mold, and that these same antagonists have no inhibition on the mycelial growth of mushrooms. This condition considered, this study was thus conducted to select prospective antagonists for the biological control of green mold of mushrooms caused by *Trichoderma* spp.

Materials and Methods

Isolates of *Bacillus* species. Twenty isolates of *Bacillus* species obtained from livestock manure composts and cotton-waste composts (Table 1) were used for antagonistic tests *in vitro* against three species of *Trichoderma*, which cause green mold of mushrooms. The bacterial isolates used in this study were identified in the previous report (Kim *et al.*, 2008).

Isolates of *Trichoderma* species and mushrooms. Isolates of *Trichoderma harzianum* Rifai, *T. koningii* Oudem. and *T. viridescens* (A.S. Horne & H.S. Williamson) Jaklitsch & Samuels were obtained from samples of green mold found on the mycelia and beds of oyster mushrooms and button mushrooms. After these isolates were confirmed as causing green mold on three species of mushrooms (*F. velutipes*, *L. edodes*, and *P. ostreatus*), subsequent antagonistic tests were performed with the selected *Bacillus* species isolates. Additional isolates of *F. velutipes*, *L. edodes*, and *P. ostreatus* obtained from the National Agrobiodiversity Center, National Academy of Agricultural Science were also used for antagonistic tests with the bacterial isolates.

Table 1. List of *Bacillus* species isolates from livestock manure composts and cotton-waste composts used in this study

Isolate No.	Source isolated	Location	Year isolated
M15	Cotton-waste compost	Gimpo	2000
M16	Cotton compost	Gimpo	2000
M18	Cotton compost	Suwon	1999
M27	Cotton compost	Gimpo	2000
M34	Cotton compost	Suwon	1999
M47	Cotton compost	Incheon	1999
M49	Cotton compost	Wonju	2000
M75	Manure compost	Icheon	2000
M78	Manure compost	Icheon	1999
M79	Manure compost	Icheon	1999
RM29	Cotton compost	Suwon	1999
RM43	Cotton compost	Suwon	1999
RT03	Cotton compost	Suwon	1999
4T01	Cotton compost	Suwon	1999
4T47	Cotton compost	Suwon	1999
4T49	Cotton compost	Suwon	1999
5M35	Cotton compost	Suwon	1999
5M50	Cotton compost	Suwon	1999
5T32	Cotton compost	Suwon	1999
5T33	Cotton compost	Suwon	1999

Test of antagonistic effects. Twenty isolates of the *Bacillus* species were tested *in vitro* for antagonistic effects against the three species of *Trichoderma* previously confirmed to cause green mold on mushrooms. A 5 mm-mycelial mat taken from the isolate of each *Trichoderma* species was placed on one side of potato dextrose agar (PDA). All 20 bacterial isolates were streaked on the other side of the medium, each individually paired to the three *Trichoderma* species. The paired PDA plate was cultured at 25°C for 6 to 12 days and the dual culture test was performed in triplicate. During cultivation, the plates were examined for any possible antagonistic effect between the bacterial isolates and the *Trichoderma* isolates by measuring the inhibition zone formed between them to the nearest width. The bacterial isolates were also tested with the same method *in vitro* for possible antagonistic effects against the three species of mushrooms.

Results and Discussion

Formation of inhibition zones. Of the twenty *Bacillus* species isolates analyzed in the dual culture experiments, several isolates demonstrated antagonistic effects against the mycelial growth of *Trichoderma* species and mushrooms. The width of the inhibition zone produced in dual culture tests shows the degree of antagonism bacterial isolates have against target organisms, and has been used extensively as an *in vitro* test for preliminary screening of biological control agents (Desai *et al.*, 2002). Therefore, the width of the inhibition zones between the *Bacillus* isolates and the *Trichoderma* isolates was a reliable measure and was used to rate the antagonistic effects of the *Bacillus* species (Fig. 1). The mode of antagonism generally observed with *Bacillus* spp. is antibiosis (Edwards *et al.*, 1994). This is supported by reports that most *Bacillus* spp. produce many antibiotics such as bacillomycin, fengycin, mycosubtilin and zwittermicin, which are all effective at suppressing growth of target pathogens *in vitro* and/or *in situ* (Pal and Gardener, 2006). This evidence allows the assumption that antibiotics are related to the formation of inhibition zones between the bacterial and the fungal isolates shown in this study.

Antagonistic effects. Of the 20 *Bacillus* species isolates tested, two inhibited mycelial growth of *T. harzianum*, seven that of *T. koningii*, and eight that of *T. viridescens* (Table 2). The bacterial isolates M27 and RM29 provided the most noteworthy result as both strongly inhibited mycelial growth of all *Trichoderma* spp. isolates tested. The isolate M27 was the most effective in the formation of inhibition zones against the *Trichoderma* spp. isolates.

Bacillus species have previously been reported as effective biological control agents against plant diseases (Boland and Kuykendall, 1998; Gnanamanickam, 2002;

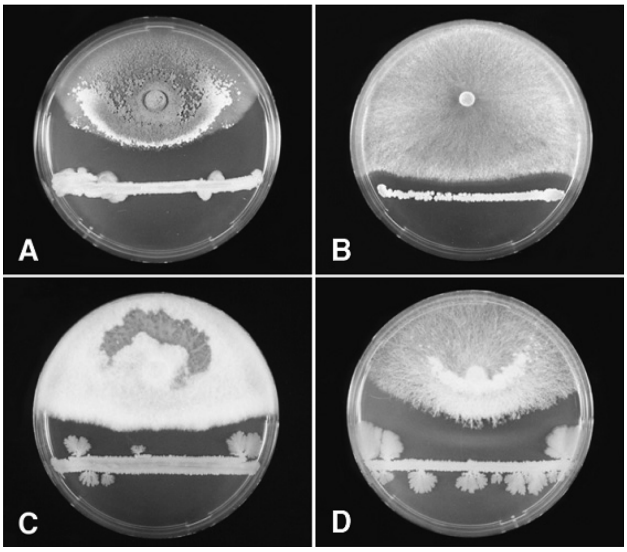

Fig. 1. Dual culture tests for the antagonistic evaluation of *Bacillus* species isolates against mycelial growth of *Trichoderma* spp. (A and B) and mushrooms (C and D) on PDA medium. A, an inhibition zone of mycelial growth of *T. harzianum* by *Bacillus* sp. M27; B, an inhibition zone of mycelial growth of *T. viridescens* by *Bacillus* sp. RM29; C, an inhibition zone of mycelial growth of *Flammulina velutipes* by *Bacillus* sp. M27; D, an inhibition zone of mycelial growth of *Lentinus edodes* by *Bacillus* sp. M27.

Table 2. Antagonistic effect of *Bacillus* species isolates against three species of *Trichoderma*

Isolate No. of *Bacillus* species	Inhibition zone (mm)[a] of mycelial growth of *Trichoderma* spp. isolates		
	T. harzianum	*T. koningii*	*T. viridescens*
M15	–	7.0	–
M16	–	6.7	3.7
M18	–	1.3	4.7
M27	8.7	9.3	5.7
M34	–	2.3	2.3
M47	–	12.3	1.0
M49	–	4.0	–
M75	–	2.7	2.7
M78	–	7.0	2.0
M79	–	7.7	–
RM29	1.3	6.7	4.0
RM43	–	3.7	–
RT03	–	–	–
4T01	–	3.0	–
4T47	–	4.7	–
4T49	–	2.3	–
5M35	–	1.0	–
5M50	–	–	–
5T32	–	1.3	–
5T33	–	–	–

[a]Measurement was made after six days of cultivation. The data represents the average of three replicates. –, no inhibition.

Jacobsen *et al*., 2004; Tjamos *et al*., 1991). Supporting this claim is a study where Kim *et al*. (2008) identified several prospective antagonistic isolates for the biological control of diseases caused by soilborne phytopathogenic fungi by screening 20 *Bacillus* species isolates for antagonistic effects *in vitro*. It has further been reported that *T. harzianum*, *T. koningii*, *T. viridescens*, etc. cause green mold of mushrooms (Jaklitsch *et al*., 2006; Samuels *et al*., 2002; Seaby, 1998). Our study therefore attempted to examine the antagonistic effects of *Bacillus* on *Trichoderma* spp. and indeed two *Bacillus* species isolates were identified as prospective antagonists against *Trichoderma* spp. Accordingly, these selected antagonists could be used for control of green mold caused by the *Trichoderma* spp.

Except 5T33, most isolates of *Bacillus* species inhibited mycelial growth of the three experimental mushroom species, *Flammulina velutipes*, *Lentinus edodes*, and *Pleurotus ostreatus* (Table 3). The antagonistic effects of the bacterial isolates against the three species of mushrooms varied depending on the mushroom species. The bacterial isolates M27 and RM29, which had antagonistic effects against all the *Trichoderma* spp., also inhibited mycelial growth of the three species of mushrooms. Therefore, although the *Bacillus* isolates may be effective antagonists against green mold pathogens, *Trichoderma* spp., it must be recognized that the additional inhibitive effect on

Table 3. Antagonistic effect of *Bacillus* species isolates against three species of mushrooms

Isolate No. of *Bacillus* species	Inhibition zone (mm)[a] of mycelial growth of mushroom isolates		
	Flammulina velutipes	*Lentinus edodes*	*Pleurotus ostreatus*
M15	5.0	9.3	3.0
M16	6.3	7.0	9.7
M18	2.7	–	1.3
M27	8.0	11.3	0.7
M34	9.7	1.0	2.0
M47	8.7	11.0	8.7
M49	1.7	7.0	1.7
M75	6.7	13.3	5.0
M78	7.7	2.0	5.0
M79	0.7	7.7	–
RM29	9.3	5.7	10.3
RM43	2.7	3.0	–
RT03	–	3.3	–
4T01	2.3	2.3	3.0
4T47	1.3	18.0	–
4T49	9.3	–	3.7
5M35	–	4.0	5.7
5M50	–	9.3	–
5T32	2.0	–	5.3
5T33	–	–	–

[a]Measurement was made after 12 days of cultivation. The data represents the average of three replicates. –, no inhibition.

the mycelial growth of mushrooms is not useful as prospective antagonists for the biological control of green mold in the growing room of the mushrooms. Further study is needed in order to explore any possible practical use of the antagonistic bacterial isolates for the biological control of green mold in the growing room of the mushrooms.

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