Songklanakarin J. Sci. Technol.
46 (6), 499–506, Nov. – Dec. 2024

http://www.sjst.psu.ac.th

Original Article

The efficacy of bioproduct of *Bacillus* spp. on mycelium growth and controlling green mold disease of *Ganoderma lucidum*

Wiparat Noochaichaw, Patcharaporn Vanichpakorn, and Pornsil Seephueak*

Division of Plant Science, Faculty of Agriculture,
Rajamangala University of Technology Srivijaya, Thung Yai, Nakhon Si Thammarat, 80240 Thailand

Received: 25 February 2024; Revised: 14 August 2024; Accepted: 12 September 2024

Abstract

This research aimed to study the potential of bioproduct of *Bacillus* spp. on promoting mycelial growth and controlling green mold disease caused by *Trichoderma velutinum* in *Ganoderma lucidum.* The antagonistic native *Bacillus* three isolates *via Bacillus subtilis* subsp. *subtilis* RUTs001, *Bacillus subtilis* RUTs002 and *Bacillus subtilis* RUTs003 were used to test interaction with *G. lucidum* mushroom mycelium and control green mold disease in the laboratory by dual culture and in a mushroom house. The results showed that all *Bacillus* spp. had a positive interaction with *G. lucidum* on PDA plates, with mushroom mycelium growth of 79.29 - 100.00%. Additionally, there were no harmful effects found on the mycelium of *G. lucidum*. The growth inhibition of *T. velutinum* by *Bacillus* spp. was 22.28 - 73.93%. Biological products of *Bacillus* spp. were significantly different in their effectiveness in controlling green mold disease in mushroom houses ($P<0.01$). The combination of *B. subtilis* subsp. *subtilis* RUTs001, *B. subtilis* RUTs002 and *B. subtilis* RUTs003 showed the lowest disease incidence and disease severity index of 12.50 and 2.08%, respectively. Yield was 158.24 g/bag and biological efficiency (B.E.) was 40.26%, There was no significant difference form control checked (normal spawn) disease incidence, disease severity index, yield and biological efficiency obtained 4.17%, 0.69%, 162.96 g/bag and 41.47%, respectively.

Keywords: *Bacillus*, *Ganoderma* mushroom, biocontrol, green mold disease, inhibition

1. Introduction

Ganodermataceae is a large family of polypores with seven accepted genera: *Amauroderma*, *Foraminispora*, *Furtadoa*, *Ganoderma*, *Haddowia*, *Humphreya* and *Polyporopsis* (Hapuarachchi *et al*., 2018). *Ganoderma* species have a worldwide distribution on land, in tropical and temperate forests. The genus *Ganoderma* was established by Karsten (1881). *Ganoderma lucidum* is a highly sought-after medicinal mushroom and is distributed worldwide but was actually frequently mistaken for *G. lingzhi* (Cao, Wu, & Dai, 2012). In Thailand, *G. lucidum* was discovered engaging in parasitism of a living root of a species of tree found in the rainforests, *Milletia leucantha* Kurz. (Fabaceae) (Hawkeswood, Sommung, & Sommung, 2020). The scale of cultivation of lingzhi mushroom in the mushroom farm industry has increased significantly in southern Thailand, with the rapid expansion of *G. lucidum* (G2), and usually the cultivation takes place in a plastic bag. *Ganoderma* is a food supplement used for health maintenance or as an allegedly therapeutic “drug” for medical purposes.

Disease has become a serious threat to the production of these mushrooms. The virulent disease in *G. lucidum* is caused by green mold, specifically *Trichoderma* spp. It infects the spawn and substrates and causes harm to the fruiting body, and it could be seen to contaminate parts of the basidiocarps. *Trichoderma* is an aggressive green mold, and the cause of epidemics that sometimes cause yield losses of 100% (Santric *et al*., 2018).

Biological control comes to the forefront with its features, such as being able to specifically target disease microorganisms, being cost-effective, and being environmentally friendly. Biocontrol agents, antagonistic bacteria, can reduce the deleterious effects of pathogens and have the ability to promote plant growth and mushroom formation, exerting beneficial effects on mushrooms (Braat,

*Corresponding author
Email address: pornsil.s@rmutsv.ac.th

Koster, & Wosten, 2022). *Bacillus* species have antagonistic *in vitro* and *in vivo* activity against green mold disease (Milijasevic-Marcid *et al*., 2017; Nagy *et al*., 2012; Stanojevic *et al*., 2016; Stanojevic *et al*., 2019; Sivasakthi, Usharani, & Saranraj, 2014; Torres *et al*., 2020). Moreover, previous studies reported not only that bacterial samples were found involved in antifungal activity against green mold but also that they can promote the growth of mushrooms, for example in the cases of *Bacillus velezensis* QST713 (Pandin, Le Coq, Deschamps, & Vediet, 2018), *B. subtilis* B-233 (Stanojevic *et al*., 2019), *B. subtilis* isolate MSG-5 (Aydogdu, Sulu, Krubetli, & Sulu, 2021), *B. pumilus* B-138 and *B. amyloliquefaciens* B-241 (Stanojevic *et al*., 2019). Therefore, the aim of this research was to study the efficacy of bioproduct of *Bacillus* biocontrol agents against green mold and for mycelial growth of *G. lucidum*.

2. Materials and Methods

2.1 Mushroom culture

G. lucidum strain G2 was used in the experimental procedure, using the culture received from the Biotechnology Research and Development office, Department of Agriculture, Thailand. A culture of *G. lucidum* was prepared by using a tissue-transplanting technique in the laboratory. The culture was multiplied on potato dextrose agar (PDA) and maintained in glass tubes at 10 ºC for further studies.

2.2 Bacterial isolates

The three species of *Bacillus subtilis* subsp. *subtilis* RUTs001, *B. subtilis* RUTs002 and *B. subtilis* RUTs003 were isolated from spent mushroom compost (SMC) of *Pleurotus* spp., from a mushroom farm in the southern region of Thailand. The antagonistic *Bacillus* spp. had been identified previously, based on the partial sequence of their 16S rDNA gene at the National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand (Zulfikar, Nur Layla, Preecha, & Seephueak, 2018). Pure cultures of bacterial isolates were maintained on nutrient agar (NA) slant at 4 ºC, at the Plant Disease Laboratory Room, Rajamangala University of Technology, Thung-Yai, Nakhon Si Thammarat Campus, Thailand.

2.3 Mushroom green mold disease

The *Trichoderma* was isolated from contaminated samples of spawn substrates of *G. lucidum* in a mushroom farm at the Faculty of Agriculture, Rajamangala University of Technology Srivijaya, Thung Yai, Nakhon Si Thammarat province. The *Trichoderma* identified based on partial ITS rDNA gene sequence at Biotech Thailand was *Trichoderma velutinum* (Accession MN533734*)*. Culturing of *T. velutinum* was performed on PDA at room temperature and maintained on slant at 10 ºC.

2.4 Effect of *Bacillus* spp. on mycelial growth of *Ganoderma lucidum*

Before testing the efficacy of *Bacillus* spp. for suppression of *T. velutinum* in mushroom growing house, the effects of antagonistic *B. subtilis* subsp. *subtilis* RUTs001, *B. subtilis* RUTs002 and *B. subtilis* RUTs003 on the growth of *G. lucidum* were examined using dual culture. *Bacillus* spp. and *G. lucidum* were co-cultivated on Petri dishes (9 cm diameter) on PDA. Mycelial plugs (0.5 cm) of the colonies of *G. lucidum* were taken from the 10 - days - old growing on PDA and placed nearly 2 cm away from the edge of Petri dish containing PDA. After 5 days of the initial incubation, a loopful of each bacterial isolate was streaked on the opposite side and incubated at room temperature (28-32 ºC). The experimental procedure was performed using a Completely Randomized Design (CRD) consisting of 4 treatments and 4 replicates (4 plates/replicates). In the control, only mycelial plugs of the *G. lucidum* were placed on PDA. The relative growth (RG) of the mycelia co-cultivated with bacteria compared to mycelia without bacteria as a control were calculated according to the following formula: RG = (radius sample – radius control) / radius control x 100 (Aydogdu *et al*., 2021; Dygico *et al*., 2019; Orban *et al*., 2023).

2.5 Effect of *Bacillus* spp. on the growth of *Trichoderma velutinum*

Dual culture tests were used to examine the effects of the bacterial isolates on mycelial growth of *T. velutinum*. Mycelial plugs (0.5 cm) of the colonies of *T. velutinum* were taken from the 7 - days - old growing on PDA and placed nearly 2 cm away from the edge of Petri dish containing PDA. After 24 h of the initial incubation, a loopful of each bacterial isolated was streaked to the opposite and incubated at room temperature. The experimental procedure was performed using CRD consisting of 4 treatments and 4 replicates (4 plates/replicate). The data were collected on percentage of inhibition by bacteria inhibiting the mycelium growth of *T. velutinum* for 20 days. The percent inhibition of mycelial growth was calculated according to the formula by Aydogdu *et al*. (2021).

2.6 The effect of bioproduct of *Bacillus* spp. on green mold diseases in the mushroom house

The bioproducts of *B. subtilis* subsp. *subtilis* RUTs001, *B. subtilis* RUTs002 and *B. subtilis* RUTs003 were tested for green mold disease control in a *Ganoderma* mushroom house. The formula for the *Bacillus* spp. bioproduct consists of 99 g of talcum, 1 g of sodium carboxymethyl cellulose (sCMC), and 40 ml of endospore suspension of *Bacillus* spp. The formulation of *Bacillus* spp. was prepared in the laboratory at Rajamangala University of Technology Srivijaya, Nakhon Si Thammarat, Thailand. The concentration of the *Bacillus* spp. bioproduct was 50 g WP/ 20 L (10^9 CFU/g WP), confirmed by plate count technique. The commercial bio-fungicide based on *B. subtilis* (Bio-Censer®), Thailand, was tested at the standard dose of 50 g WP/ 20 L (10^9 CFU/g WP) compared with spore suspensions of *T. velutinum* (control) and control checked (distilled water). Each experimental setup involved composted mushroom spawn at 800 g/bag.

The biological product of *Bacillus* sp., concentration 10^9 CFU/g WP, was sprayed on the spawning surface of *G. lucidum*, approximately 3 ml/bag. After 48 hours of inoculation, the spore suspension of *T. velutinum* at

concentration 10^5 spores/ml was sprayed, approximately 1 ml/bag. The fruiting bodies were hand-picked in two flushes from thirty-day-old basidiocarps. The experiments were set up by using CRD with ten treatments and four replicates. In analyses the level of significance was set at P<0.01. Statistical data analysis was performed using the software Statistica for Windows.

The potential of different treatments for suppression of *G. lucidum* fungal pathogens was determined based on disease incidence (%DI), meaning the number of infected spawn mushroom / total number of spawn mushroom x 100. The disease severity index is (%DSI) = [Σ(ab)]/[(NK)] x 100, where a = class frequency, b = score of rating class, N = total number of plants, K = maximal disease index (Chiang, Liu, & Bock, 2017). The disease severity scores (DSS) are based on the infected mushroom spawn, scale 0 to 5: Level 0 = no disease, Level 1 = mild disease (1-20%), Level 2 = moderate disease (21-30%), Level 3 = severe disease (31-40%), Level 4 = very severe disease (more than 41%), and Level 5 = disease affecting the entire mushroom spawn. The effect of bioproduct of *Bacillus* sp. on mushroom productivity was evaluated as biological efficacy (%B.E.), calculated as the ratio of the fresh weight of total fruiting body yield and the weight of dry spawned substrate mass x 100 (Potocnik *et al*., 2018).

3. Results and Discussion

3.1 Interaction between *Bacillus* spp. and *Ganoderma* mycelium

Interaction between *Bacillus* spp. and *Ganoderma* mycelium was evaluated by dual culture tests, compared with control, with all *Bacillus* treatments having statistical significance (P<0.01) (Table 1). In this experiment, the interactions between bacterial *B. subtilis* subsp. *subtilis* RUTs001 showed the highest promotion of mycelial growth, as high as 100%, and mushroom primordia showed on PDA media at 30 days of tests (Figure 1). There was no statistically significant difference when compared with the mycelium of *G. lucidum* in control. On the other hand, the use of *B. subtilis* RUTs002 and *B. subtilis* RUTs003 showed interactions with growth by 81.86 and 79.29%, respectively. However, there were no negative effects on the mycelial growth of *Ganoderma* mushroom.

Suarez *et al.* (2019) reported that bacteria play an important role in several aspects of the life cycle of fungi, such as mycelial growth, fruiting body induction, spore formation, enzyme activity, and gene expression. Several bacteria have been tested as agents to be used to control plant disease and some of the corresponding studies have focused on the effect of bacterial volatile organic compounds (VOCs) on fungal growth (Wang *et al*., 2021). As a result, bacteria affect the mushroom by influencing positively or negatively mycelial growth. In particular, *B. subtilis* subsp. *subtilis* RUTs001 showed significant promotion of vegetative growth and mushroom formation on Petri dishes. On the other hand, *B. subtilis* RUTs002 and *B. subtilis* RUTs003 slightly inhibited the growth of the mycelium of *G. lucidum* but they can grow, and the mushroom's mycelium does not sustain damage. Stanojevic *et al.* (2019) reported that some types of *Bacillus* when used as a mushroom substrate decrease the incidence of disease with no negative effects on growth, in the case of white button mushrooms.

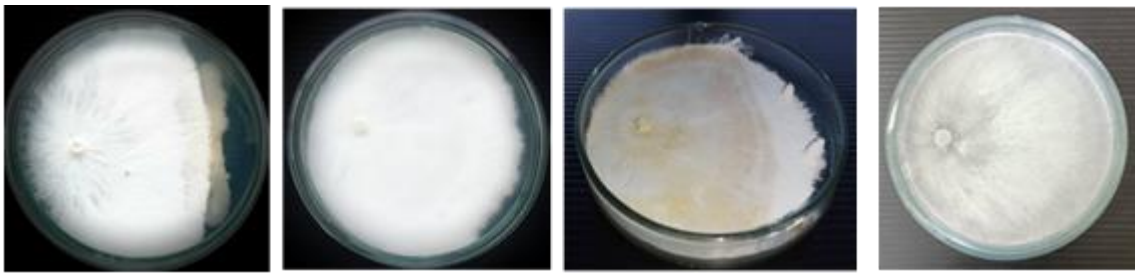
In previous research that studied this topic, the researchers reported that not only were bacterial materials found to have antifungal activity against green mold, but also, they can promote mushroom production, for example, *B. velezensis* QST713 (Pandin *et al*., 2018), *B. subtilis* B-233 (Stanojevic *et al*., 2019), *B. subtilis* MSG-5 (Aydogdu *et al*., 2021), *B. pumilus* B-138 and *B. amyloliquefaciens* B-241 (Stanojevic *et al*., 2019) and *B. megaterium* (Braat *et al*., 2022). Culture filtrates of bacteria gave results indicating that metabolites of these microbes induce mushroom formation, these including biotin, gibberellic acid (GA) or indole acetic acid (IAA) that also had an inducing effect on mushroom formation (Park & Agnihotri, 1969). However, the research on *Bacillus* in stimulation of mushroom formation of *G. lucidum* has not been reported, this is the first report on that aspect.

Orban *et al.* (2023) reported that bacteria isolated from *Pleurotus ostreatus* or cultures significantly enhanced mycelial growth only for fungi of the genus *Pleurotus*. However, this experiment shows that all of the bacteria were isolated from the spent *Pleurotus* compost, and they are growth co-cultivated with *G. lucidum* especially *B. subtilis* subsp. *subtilis* RUTs001 which were used to stimulate the growth and produce the primordia on PDA. This research showed positive interaction between *Bacillus* spp. and *G. lucidum*. Braat *et al.* (2022) reported on the bacterial diversity at different stages of mushroom cultivation and that some bacterial species are supportive of vegetative growth and mushroom production, as well as tend to protect mushrooms against pathogens (Stanojevic *et al*., 2019).

Table 1. Effects of *Bacillus* spp. on the mycelial growth of *Ganoderma lucidum* when co-cultivated on a Petri dish

Treatments	Mycelium growth (%)					
	3 days	5 days	7 days	10 days	20 days	30 days
Control	100.00±0.00$^{a1/}$	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
B. subtilis subsp. RUTs001	92.99±2.54^{b}	78.57±0.00^{b}	85.72±0.00^{b}	88.92±1.55^{b}	96.79±2.22^{b}	100.00±0.00^{a}
B. subtilis RUTs002	87.07±0.91^{c}	78.80±0.29^{b}	78.40±0.70^{c}	78.95±0.70^{c}	81.86±0.69^{c}	81.86±0.69^{b}
B. subtilis RUTs003	79.69±2.88^{d}	75.43±0.60^{c}	78.24±1.84^{c}	78.24±1.84^{c}	79.29±0.75^{d}	79.29±0.75^{c}
F-test	**	**	**	**	**	**
C.V. (%)	2.19	0.40	1.15	1.45	1.37	0.57

$^{1/}$ Means of percent mycelial growth significantly different (P< 0.01) were indicated by different lower cases.



(A) (B) (C) (D)

Figure 1. Interaction between mycelium of *Ganoderma lucidum* and *Bacillus subtilis* subsp. *subtilis* RUTs001 on PDA at 10 days (A), 20 days (B), 30 days and primordia on media (C) compared with control (D)

3.2 Inhibitory effect on mycelium growth of *Trichoderma velutinum*

The results showed that all *Bacillus* spp. were potentially antagonistic bacteria owing to the inhibitory effect seen in the dual test (Table 2 and Figure 2). *B. subtilis* RUTs003 showed the highest inhibition of the mycelium of *T. velutinum*, at 20 days after a co-inoculation test were 73.93%, followed by *B. subtilis* RUTs002 by which the inhibition was 49.48%. On the other hand, use of *B. subtilis* subsp. *subtilis* RUTs001 gave 22.28% at 10 days after test.

In this study, green mold disease of *G. lucidum* caused by *T. velutinum* identified based on ITS rDNA was investigated. There are various strains of *Trichoderma* that infect *G. lucidum* cultivation such as *T. atroviride* (Li, Dong, Wen, & Liu, 2016; Ubolsuk & Pornsuriya, 2022; Wang, Zeng, Wu, & Jiang, 2022; Yan, Zhang, Moodley, Zhang, & Xu, 2019; *T. hengshanicum* (Cai, Idress, Zhou, Zhang, & Xu, 2020), *T. harzianum* (Lu *et al*., 2016; Ubolsuk & Pornsuriya, 2022; Wang *et al*., 2022), *T. guizhouense*, *T. hamatum*, *T. koningiopsis*, *T. pleuroticola*, *T. irale*, and *T. virens* (An *et al*., 2022; Wang *et al*., 2022), *T. ganodermatigerum* sp. nov. (An *et al*., 2022). In Thailand, Ubolsuk and Pornsuriya (2022) reported that *Trichoderma* species identified from *Ganoderma* include *T. harzianum*, *T. pleuroticola* and *T. reesei*. This is the first report of *T. velutinum* causing the green mold disease of *G. lucidum* in Thailand. Whereas, Sharma, Singh, and Verma (2018) reported that *T. velutinum* is effective against the important plant pathogens, such as *Fusarium oxysporum*, *Verticillium dahliae*, *Alternaria alternata,* and *Colletotrichum capsici* which was demonstrated by *in vitro* dual culturing experiments.

Green mold disease has significantly damaged production of *G. lucidum* and other mushrooms and various growth stages of edible fungi. The competitive activity is based on antifungal metabolites and/or enzyme production, mycoparasitism, or ecological competition (Mukherjee, Horwitz, & Kenerley, 2012). The direct effects of *Trichoderma* spp. were expressed as the inhibition of *G. lucidum* growth by non-volatile and volatile metabolites. *Trichoderma* produced secondary metabolites with antifungal activity, including terpenes, pyrones, gliotoxin and peptaibols (Khan, Najeeb, Hussain, Xie, & Li, 2020). The antagonistic function may contribute to the accumulation of their population and threaten the growth and development of *G. lucidum*, in contrast, microbial competition and antagonism of *Trichoderma* makes it a preventive that controls plant pathogens (Zin & Badaluddin, 2020).

3.3 Efficacy of the bioproduct of *Bacillus* spp. For controlling green mold disease in the mushroom house

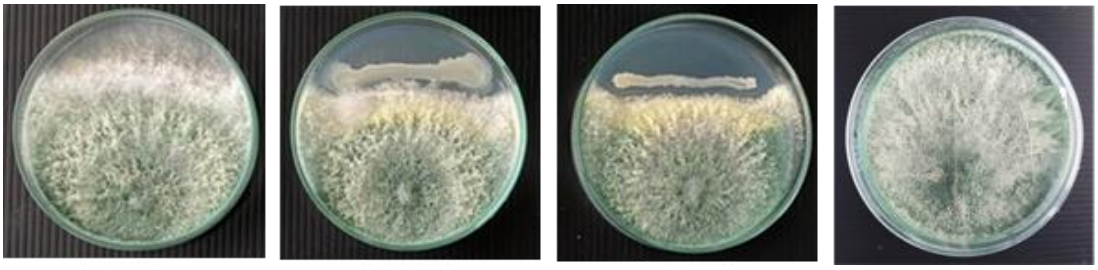
The results showed that all the bioproducts of *Bacillus* effectively control the green mold disease of *G. lucidum* and the results were significant at P<0.01. Disease incidence (%DI) in treatments inoculated with bioproduct of *Bacillus* spp. ranged within 12.50-47.92% and disease severity index (%DSI) was 2.08-12.15%. The combination of *B. subtilis* subsp. *subtilis* RUTs001+ *B. subtilis* RUTs002+ *B. subtilis* RUTs003 showed the lowest disease incidence at 12.50% and disease severity index at 2.08%. This was followed by the combination *B. subtilis* subsp. *subtilis* RUTs 001 + *B. subtilis* RUTs 002, with disease incidence and disease severity index 18.75 and 3.13%; and *B. subtills* subsp. *subtilis* RUTs001 + *B. subtilis* RUTs003 gave disease incidence and disease severity index 22.92 and 3.82%, being non-significant when compared with uninoculated control (control checked) that had disease incidence and disease severity index 4.17 and 0.69%. On the other hand, the inoculated control showed the highest disease incidence and disease severity index at 83.33 and 21.18% (Table 3).

Regarding the yields of *G. lucidum*, the results showed that all antagonistic *Bacillus* do not inhibit the mycelial growth of *G. lucidum*. The average yields in treatments inoculated with bioproduction of *Bacillus* spp. were 137.27-158.24 g/bag (B.E.=34.93-40.26%). The combination *B. subtilis* subsp. *subtilis* RUTs001+ *B. subtilis* RUTs002+ *B. subtilis* RUTs003 tended to give a high yield of 158.24 g/bag (B.E.=40.26%) followed by use of *B. subtilis* subsp. *subtilis* RUTs001, *B. subtilis* RUTs003, *B. subtilis*, *B. subtilis* RUTs002, combination of *B. subtilis* subsp. *subtilis* RUTs001+ *B. subtilis* RUTs002, *B. subtilis* RUTs002, combination of *B. subtilis* subsp. *subtilis* RUTs001+ *B. subtilis* RUTs003 and combination of *B. subtilis* RUTs 002+ *B. subtilis* RUTs003 giving 154.83 g/bag (B.E.=39.39%), 150.20 g/bag (B.E.=38.22%), 148.10 g/bag (B.E.=37.68%), 138.53 g/bag (B.E.=35.23%), 141.86 g/bag (B.E.=36.10%), 138.53 g/bag (B.E.=35.23%), 137.72 g/bag (B.E.=35.04%) and 137.27 g/bag (B.E=34.93%), respectively. On the other hand, uninoculated control (control checked) was 162.96 (B.E.=41.47%) and inoculated control was the lowest at 130.42 g/bag (B.E.=33.19%) (Table 3).

Table 2. Percent growth inhibition of *Trichoderma velutinum* by antagonistic *Bacillus* spp. *in vitro*

Treatment	Inhibition of mycelium growth (%)					
	3 days	5 days	7 days	10 days	15 days	20 days
Control	0.00±0.00$^{c1/}$	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{c}
B. subtilis subsp. *subtilis* RUTs001	59.97±1.60^{b}	49.48±0.70^{c}	25.28±0.50^{c}	22.28±0.05^{c}	0.00±0.00^{c}	0.00±0.00^{c}
B. subtilis RUTs002	60.04±0.91^{b}	56.13±2.71^{b}	49.95±1.19^{b}	47.95±1.19^{b}	49.47±0.00^{b}	49.48±0.58^{b}
B. subtilis RUTs003	70.06±0.00^{a}	62.27±0.00^{a}	51.00±0.00^{a}	51.00±0.00^{a}	73.93±0.00^{a}	73.93±0.75^{a}
F-test	**	**	**	**	**	**
C.V. (%)	1.98	2.64	1.42	1.45	1.32	1.32

1/ Means of percent inhibition significantly different (P< 0.01) were indicated by different lower cases.



(A) (B) (C) (D)

Figure 2. The inhibition of *Trichoderma velutinum* by *Bacillus subtilis* subsp. *subtilis* RUTs001 (A), *Bacillus subtilis* RUTs002 (B), *Bacillus subtilis* RUTs003 (C), compared with control (D) at 20 days after test

Table 3. The efficacy of bioproduct of *Bacillus* spp. on disease incidence (%DI), disease severity index (%DSI), diameter of basidiocarp, yield and biological efficacy (% B.E.) of *Ganoderma lucidum* inoculated with *Trichoderma velutinum*

Treatment	Disease incidence (%)	Disease severity index (%)	Diameter of basidiocarp (mm)	Yield (g/bag)	% B.E.
B. subtilis subsp. *subtilis* RUTs001	35.42±10.49$^{bc1/}$	6.60±2.37^{bc}	71.70±4.12	154.83±2.12^{ab}	39.39^{a}
B. subtilis RUTs002	37.50±20.97^{bc}	7.64±4.74^{bc}	66.64±3.94	138.53±0.66^{ab}	35.23^{ab}
B. subtilis RUTs003	33.33±20.41^{bc}	7.99±5.12^{bc}	69.11±6.06	150.20±2.61^{ab}	38.22^{ab}
B. subtilis subsp. *subtilis* RUTs001 + *B. subtilis* 002	18.75±12.50^{cd}	3.13±2.08^{bc}	66.31±8.26	141.86±2.74^{ab}	36.10^{ab}
B. subtilis subsp. *subtilis* RUTs001 + *B. subtilis* 003	22.92±17.18^{bcd}	3.82±2.86^{bc}	67.32±6.76	137.72±1.81^{ab}	35.04^{ab}
B. subtilis RUTs002 + *B. subtilis* 003	47.92±27.53^{b}	10.42±6.16^{bc}	67.61±3.21	137.27±2.02^{ab}	34.93^{ab}
B. subtilis subsp. *subtilis* RUTs001 + *B. subtilis* RUTs002 + *B. subtilis* RUTs003	12.50±8.33^{cd}	2.08±1.39^{c}	73.37±5.17	158.24±0.50^{ab}	40.26^{a}
B. subtilis	35.42±21.91^{bc}	12.15±7.29^{b}	69.08±8.76	148.10±2.99^{ab}	37.68^{ab}
T. velutinum (control)	83.33±13.61^{a}	21.18±5.71^{a}	64.10±11.39	130.42±2.24^{b}	33.19^{b}
Control checked	4.17±8.34^{d}	0.69±1.39^{c}	70.57±2.03	162.96±0.72^{a}	41.47^{a}
F-test	*	**	ns	**	**
C.V. (%)	25.11	9.68	9.57	11.96	9.68

1/ Means of percent inhibition significantly different (P< 0.01) were indicated by different lower cases

Some beneficial bacteria, such as *Bacillus* and *Pseudomonas* have been used in the disease management to reduce *Trichoderma* infected mushroom (Tong *et al*., 2020). Liu *et al*. (2007) reported that several species of *Bacillus* were very effective for the control of green mold disease as they can secrete several antimicrobial lipopeptide substances, such as iturin which can cause pore formation in the cell membrane i.e. create holes in the cell wall, reducing the cell's ability to reproduce or survive, thereby suppressing the growth of green mold.

The results showed that *B. subtilis* RUTs003 has a high effectiveness for the control of *T. velutinum* in the laboratory, determined by using a dual culture test giving 73.93% for 20 days after test, and this did not inhibit the growth of *G. lucidum* (mushroom growth was 79.29%). Moreover, the mushroom mycelial growth tendency is to have fast growth after making contact with the bacteria. However, the efficacy of bioproduct of *Bacillus* spp. for the control of green mold disease in the mushroom house showed that bioproduct combination *B. subtilis* subsp. *subtilis* RUTs001+ *B. subtilis* RUTs002 + *B. subtilis* RUTs003 gave very good results for control of green mold disease. The disease incidence, disease severity index, diameter of basidiocarp and yields obtained were 12.50%, 2.08%, 73.37 mm and 158.24 g/bag (B.E.=40.26%). *Bacillus* not only does not inhibit the growth of *G. lucidum* but also controls green mold disease,

which is consistent with the research conducted by Potocnik *et al*. (2018); Milijasevic-Marcic *et al.* (2017) in *Agaricus bisporus* and *P. ostreatus* (Nagy *et al*., 2012).

According to Pandin *et al.* (2018), *B. subtilis* QST713 has a good interaction on yield and efficacy against *T. aggressive* f. *europaeum*, with no significant difference when compared to the use of prochloraz manganese and showing impact on *A. bisporus* yield of biological efficiency (B.E.) obtained 91.95%. Potocnik *et al.* (2018, 2019) reported that *B. subtilis* B-358 was a good effective native strain against *T. pleuroti and T. pleuroticala* green mold of *Pleurotus* spp. with growth inhibition ranging in 54.44-62.22 and 55.56-69.62%, respectively. However, some research reported that there was slightly inhibited mycelial growth in *Flamulina velutipes*, *Lentinus edodes* and *A. bisporus* (Kim *et al*., 2008; Kosanovic *et al*., 2013).

In this study, combinations of *Bacillus* spp. reduced the disease incidence and the disease severity index of *Ganoderm*a mushroom. According to Dombrowski *et al.* (2018) combined bacterial inoculum has higher environmental adaptability, biological viability, and synergistic metabolism levels than single strains and has a greater impact. However, the effect of plant growth-promoting rhizobacteria (PGPR) *via Bacillus* on the fungal community, especially pathogenic fungi remains unclear (Ji *et al*., 2022). The findings may provide a theoretical foundation and obtain support for constructing efficient PGPR-community and carrying out research to clarify its mechanisms of pathogenic fungi inhibition. Therefore, this research also confirmed *Bacillus* spp. as having a good potential for use in biological control of green mold in *G. lucidum* mushroom production *in vivo* under growing conditions, especially a combination of bioproduct *Bacillus* spp. having good potential for biological control agents.

4. Conclusions

B. subtilis subsp. *subtilis* RUTs001, *B. subtilis* RUTs002 and *B. subtilis* RUTs003 showed the ability to suppress green mold disease caused by *T. velutinum* in *G. lucidum*. Especially effective was the bioproduct of combined *B. subtilis* subsp. *subtilis* RUTs001, *B. subtilis* RUTs002 and *B. subtilis* RUTs003 which tended to give the best results for the control (i.e. suppression of) green mold disease. Moreover, there were no harmful effects on the mycelium of the *G. lucidum*. Therefore, there are good possibilities to use the bioproduct of *Bacillus* spp. for disease incidence reduction and increase and improve mushroom production. However, the application of bioproduct of *Bacillus* is considered to have varying efficiency under different conditions such as the various stages of mushroom spawn and the various types of mushrooms.

Acknowledgements

This work was supported by (i) Rajamangala University of Technology Srivijaya, (ii) Thailand Science Research and Innovation (TSRI), and (iii) National Science, Research and Innovation Fund (NSRF). (Fundamental Fund: Grant no.180992/2566).

References

An X. Y., Cheng, G. H., Gao, H. X., Li, X. F., Yang, Li, U., & Li, Y. (2022). Phylogenetic analysis of Trichoderma species associated with green mold disease on mushrooms and two new pathogens on *Ganoderma sichuanense*. *Journal Fungi*, *8*, 704. doi:10.3390/jof8070704

Aydogdu, M., Sulu, S. M., Kurbetli, L., & Sulu, G. (2021). *In vitro* and *in vivo* biological control of the green mold using different bacteria in button mushroom cultivation. *Egyptian Journal of Biological Pest Control*, *31*(70). doi:10. 1186/s41938-021-00401-w

Braat, N., Koster, M. C., & Wosten, H. A. B. (2022). Beneficial interactions between bacteria and edible mushrooms. *Fungal Biology Reviews*, *39*, 60-70. doi:10.1016/j.fbr.2021.12.001

Borriss, R., Wu, H., & Gao, X. (2019). Secondary metabolites of the plant growth promoting model rhizobacterium *Bacillus velezensis* FZB42 are involved in direct suppression of plant pathogens and in stimulation of plant-induced systemic resistance. In H. B. Singh, C. Keswani, M. S. Reddy, E. Sansinenea, & C. Garcia-Estrada (Eds.), *Secondary Metabolites of Plant Growth Promoting Rhizomicroorganisms* (pp. 147-168). Singapore: Springer Nature

Cai, M., Idrees, M., Zhou, Y., Zhang, C., & Xu, J. (2020). First report of green mold disease caused by *Trichoderma hengshanicum* on *Ganoderma lingzhi*. *Mycobiology*, *48*(5), 427-430. doi:10.1080/12298093.2020.1794230

Cao, Y., Wu, S. H., & Dai, Y. C. (2012). Species clarification of the prize medicinal *Ganoderma* mushroom "Lingzhi". *Fungal Diversity*, *56*, 49-62.

Chiang, K. S., Liu, H. I., & Bock, C. (2017). A discussion on disease severity index values. Part I: warning on inherent errors and suggestions to maximise accuracy: warning on disease severity index values. *Annals of Applied Biology*, *171*, 134-154. doi:10.1111/aab.12362

Dombrowski, N., Teske, A., & Baker, B. (2018). Expansive microbial metabolic versatility and biodiversity in dynamic guaymas basin hydrothermal sediments. *Nature Communications*, *9*, 1-13. doi:10.1038/s41467-018-07418-0

Dygico, L. K., O'Connor, P. M., Hayes, M., Gahan, C. G. M., Grogan, H., & Burgess, C. M. (2019). *Lactococcus lactis* subsp. *lactic* as a natural anti-listerial agent in the mushroom industry. *Food Microbiology*, *82*, 30-35. doi:10.7717/peerj.5741

Hapuarachchi, K. K., Elkhateeb, W. A., Karunarathna, S. C., Cheng, C. R., Bandara, A. R., Kakumyan, P., . . . Wen, T.C. (2018). Current status of global *Ganoderma* cultivation, products, industry and market. *Mycosphere*, *9*(5), 1025-1052. doi:10.5943/mycosphere/9/5/6

Hawkeswood, T. J., Sommung, B., & Sommung, A. (2020). First record of the bracket fungus, *Ganoderma lucidum* (Crutis) P. Karsten (1881) (Basidiomycota: Ganodermataceae) from Sisaket Province, Thailand. *Caloderma*, *714*, 1-5.

Ji, C., Chen, Z., Kong, X., Xin, Z., Sun, F., Xing, J., . . . Cao, H. (2022). Biocontrol and plant growth promotion by combined *Bacillus* spp. inoculation affecting pathogen and AMF communities in the wheat rhizosphere at low salt stress conditions. *Frontiers Plant Science, 13*. doi:10.3389/ fpls.2022.1043171

Karsten, P. A. (1881). Enumeralio boletinearum et polypore arum fennicarum, systemate novo dispositarum. *Revue Mycologie, 3*, 16-19.

Khan, R. A. A., Najeeb, S., Hussain, S., Xie, B., & Li, Y. (2020). Bioactive secondary metabolites from *Trichoderma* spp. against phytopathogenic fungi. *Microorganisms, 8*, 817. doi:10.3390/micro organisms8060817

Kim, M. K., Math, R. K., Cho, K. M., Shin, K. J., Kim, J. O., Ryu, J. S., . . .Yun, H. D. (2008). Effect of *Pseudomonas* sp. P7014 on the growth of edible mushroom *Pleurotus eryngii* in bottle culture for commercial production. *Bioresource Technology, 99*, 3306-3308. doi:10.1016/j.biortech. 2007.06.039

Kosanovic, D., Potocnik, I., Duduk, B., Vukojevic, J., Stajic, M., Rekanovic, E., & Milijasevic-Marcic, S. (2013). *Trichoderma* species on *Agaricus bisporus* farms in Serbia and their biocontrol. *Annals of Applied Biology, 163*(2), 218-230. doi:10.1111/aab.12048

Li, S., Dong, C., Wen, H., & Liu, X. (2016). Development of ling-zhi industry in China – emanated from the artificial cultivation in the Institute of Microbiology, Chinese Academy of Sciences (IMCAS). *Mycology, 7*(2),74-80.

Liu, Y., Chen, Z., Ng, T. B., Zhang, J., Zhou, M., Song, F., . . . Liu, Y. (2007). Basizubin, an antifungal protein with ribonuclease and hemagglutinating activities from *Bacillus subtilis* strains B-916. *Peptides, 28*(3), 553-559.

Lu, B. H., Zuo, B., Liu, X. L., Feng, J., Wang, Z. M., & Gao, J. (2016). *Trichoderma harzianum* causing green mold disease on cultivated lingzhi in Jilin Province, China. *Plant Disease, 100*, 2524-2525. doi:10.1094/ PDIS-04-16-0422-PDN

Milijasevic-Marcic, S., Sttepanovic, M., Todorovic, B., Duduk, B., Stepanovic, J., Rekanovic, E., & Potocnik, I. (2017). Biological control of green mould on *Agaricus bisporus* by a native *Bacillus subtilis* strain from mushroom compost. *European Journal of Plant Pathology, 148*(3), 509-519.

Mukherjee, P. K., Horwitz, B. A., & Kenerley, C. M. (2012). Secondary metabolism in *Trichoderma* - a genomic perspective. *Microbiology, 158*, 35-45. doi:10.1099/ mic. 0.053629-0

Nagy, A., Manczinger, L., Tombacz, D., Hatani, L., Gyorfi, J., Antal, Z., & Kerdics, L. (2012). Biological control of oyster mushroom green mould disease by antagonistic *Bacillus* species. *International Organisation for Biological and Integrated Control Bulletin, 78*, 289-293.

Orban, A. M., Jerschow, J. J., Birk, F., Ruhl, M., Suarez, C., & Schnell, S. (2023). Effect of bacterial volatiles on the mycelial growth of mushrooms. *Microbiological Research, 266*, 127250. doi:10.1016/j.micres.2022. 127250

Pandin, C., Le Coq, D., Deschamps, J., & Vediet, R. (2018). Complete genome sequence of *Bacillus velezensis* QST713: A biocontrol agent that protects *Agaricus bisporus* crops against the green mould disease. *Journal of Biotechnology, 278*, 10-19.

Park, J. Y., & Agnihotri, V. P. (1969). Sporophore production of Agaricus bisporus in aseptic environments. *Antonie van Leeuwenhoek, 35*, 523-528.

Potocnik, I., Milijasevic-Marcic, S., Stanojevic, O., Beric, T., Stankovic, S., Kredics, L., & Hatvani, L. (2019). The activity of native *Bacillus subtilis* strains in control of green mould disease of oyster mushroom (*Pluerotus* spp.). *Journal Pesticides and Phytomedicine, 34*(2), 97-102.

Potocnik, I., Todorovic, B., Rekanovic, E., Lukovic, J., Paunovic, D., & Milijasevic-Marcic, S. (2018). Impact of *Bacillus subtilis* QST713 mushroom grain spawn treatment on yield and green mould control. *Journal Pesticides and Phytomedicine, 33*(3-4), 205-211.

Santric, L., Potocnik, I., Radivojevic, L., Umiljendic, J. G., Rekanovic, E., Duduk, B., & Milijasevic-Marcic, S. (2018). Impact of a native *Streptomyces flavovirens* from mushroom compost on green mold control and yield of *Agaricus bisporus*. *Journal of Environmental Science and Public Health, 53*(10), 677-684. doi:10.1080/03601234.2018.1474559

Sharma, R., Singh, J., & Verma, N. (2018). Production, characterization and environmental applications of biosurfactants from *Bacillus amyloliquefaciens* and *Bacillus subtilis. Biocatalysis and Agricultural Biotechnology, 16*, 132-139. doi:10.1016/j.bcab. 2018.07.028

Sivasakthi, S., Usharani, G., & Saranraj, P. (2014). Biocontrol potentiality of plant growth promoting bacteria (PGPR)- *Pseudomonas fluorescens* and *Bacillus subtilis. African Journal of Agricultural Research, 9*(16), 1265-1277.

Stanojevic, O., Beric, T., Potocnik, I., Rekanovic, E., Stankovic, S., & Milijasevic-Marcic, S. (2019). Biological control of green mould and dry bubble diseases of cultivated mushroom (*Agaricus bisporus* L.) by *Bacillus* spp. *Crop Protection, 126*(5), 104944. doi:10.1016/j.cropro.2019.104944

Stanojevic, O., Milijasevic-Marcic, S., Potocnik, I., Stepanovic, M., Dimkic, I., Stankovic, S., & Beric, T. (2016). Isolation and identification of *Bacillus* spp. form compost material, compost and mushroom casing soil active against *Trichoderma* spp. *Archives of Biological Sciences, 68*(4), 845-852. doi:10. 2298/abs151104073s

Suarez, C., Ratering, S., Weigel, V., Sacharow, J., Bienhaus, J., Ebert, J., . . . Schnell, S. (2019). Isolation of bacteria at different points of *Pleurotus ostreatus* cultivation and their influence in mycelium growth. *Microbiological Research, 234*, 126393. doi:10. 1016/j.micres.2019.126393

Tong, X., Jiang, H., Liang, Y., Rao, Y., Mei, L., & Wang, Y. (2020). Waterlogging reduce soil colonization by antagonistic fungi and restores production in *Ganoderma lucidum* continuous cultivation. *Crop*

Protection, 137, 105314. doi:10.1016/j.cropro.2020.105314

Torres, M., Llamas, I., Torres, B., Toral, L., Sampedro, I., & Béjar, V. (2020). Growth promotion on horticultural crops and antifungal activity of *Bacillus velezensis* XT1. *Applied Soil Ecology, 150*, 103453. doi:10.1016/j.apsoil. 2019.103453

Ubolsuk, C., & Pornsuriya, C. (2022). *Trichoderma* species associated with green mold disease of *Ganoderma lingzhi* in Thailand. *Songklanakarin Journal Science and Technology, 44*(1), 1-5.

Wang, Z., Zhong, T., Chen, K., Du, M., Chen, G., Chen, X., . . . Kan, J. (2021). Antifungal activity of volatile organic compounds produced by *Pseudomonas fluorescens* ZX and potential biocontrol of blue mold decay on postharvest citrus. *Food Control, 120*, 107499. doi: 10.1016/j. foodcont.2020.107499

Wang, Y., Zeng, L., Wu, J., & Jiang, H. (2022). Diversity and effects of competitive *Trichoderma* species in *Ganoderma lucidum*–cultivated soils. *Frontiers Microbiology, 13*. doi:10.3389/fmicb.2022.1067822

Yan, Y. H., Zhang, C. L., Moodley, O., Zhang, L., & Xu, J. Z. (2019). Green mold caused by *Trichoderma atroviride* on the lingzhi medicinal mushroom, *Ganoderma lingzhi* (Agaricomycetes). *International Journal of Medicinal Mushrooms, 21*, 515-521. doi:10.1615/IntJMedMushrooms.2019030352

Zin, N. A., & Badaluddin, N. A. (2020). Biological functions of *Trichoderma* spp. for agriculture applications. *Annals of Agricultural Sciences*, *65*, 168-178. doi:10.1016/j.aoas.2020.09.003

Zulfikar, A., Nur Layla, I., Preecha, C., Seephueak, W., & Seephueak, P. (2018). Use of antagonistic bacteria from spent mushroom compost for controlling damping-off cause by *Fusarium solani* in tomato. *Proceedings of the 6th Asian Academic Society International Conference (AASIC): A Trans formative Community: Asia in Dynamism, Innovation, and Globalization 6*, 630-638.