Water Extract from Spent Mushroom Substrate of *Hericium erinaceus* Suppresses Bacterial Wilt Disease of Tomato

A Min Kwak¹, Kyeong Jin Min¹, Sang Yeop Lee² and Hee Wan Kang¹,³*¹

¹Graduate School of Future Convergence Technology, Hankyong National University, Anseong 17579, Korea
²Agricultural Microbiology Division, National Academy of Agricultural Science (NAAS), Rural Development Administration (RDA), Wanju 55365, Korea
³Institute of Genetic Engineering, Hankyong National University, Anseong 17579, Korea

**Abstract**

Culture filtrates of six different edible mushroom species were screened for antimicrobial activity against tomato wilt bacteria *Ralstonia solanacearum* B3. *Hericium erinaceus*, *Lentinula edodes* (Sanjo 701), *Grifola frondosa*, and *Hypsizygus marmoreus* showed antibacterial activity against the bacteria. Water, n-butanol, and ethyl acetate extracts of spent mushroom substrate (SMS) of *H. erinaceus* exhibited high antibacterial activity against different phytopathogenic bacteria: *Pectobacterium carotovorum* subsp. *carotovorum*, *Agrobacterium tumefaciens*, *R. solanacearum*, *Xanthomonas oryzae* pv. *oryzae*, *X. campestris* pv. *campestris*, *X. axonopodis* pv. *vesicatoria*, *X. axonopodis* pv. *citri*, and *X. axonopodis* pv. *glycine*. Quantitative real-time PCR revealed that water extracts of SMS (WESMS) of *H. erinaceus* induced expressions of plant defense genes encoding β-1,3-glucanase (*GluA*) and pathogenesis-related protein-1a (*PR-1a*), associated with systemic acquired resistance. Furthermore, WESMS also suppressed tomato wilt disease caused by *R. solanacearum* by 85% in seedlings and promoted growth (height, leaf number, and fresh weight of the root and shoot) of tomato plants. These findings suggest the WESMS of *H. erinaceus* has the potential to suppress bacterial wilt disease of tomato through multiple effects including antibacterial activity, plant growth promotion, and defense gene induction.

**Keywords**

Antibacterial activity, Bacterial wilt disease of tomato, Defense genes, Spent mushroom substrate, Suppression

The global cultivation of edible mushrooms is attributed to their nutritional, medicinal, ecological, and economical value. In Korea, the production of edible mushrooms was estimated to be 614,224 tons a year [1]. Mushrooms belong to class Basidiomycetes and produce a large number of biologically active compounds that exhibit antibacterial, antifungal and antiviral, cytotoxic, or hallucinogenic activity [2-4]. Previous screening studies on 317 isolates representing 204 species of basidiomycetes evaluated their antimicrobial activity against a range of human pathogens including bacterial and fungal species [3]. Several bioactive compounds such as antibiotics, pigments, antioxidants, antihypertensive agents, antitumor agents, and biosurfactants have been extracted from mycelial leachate and fruiting bodies of mushrooms and have been characterized. The culture types of fungi have been classified into solid-state fermentation (SSF) and submerged fermentation (SmF) based primarily on the type of substrate they use [5, 6]. SSF utilizes solid substrates such as bran, bagasse, and paper pulp and is best suited for fermentation of fungi to obtain high yield of certain bioactive compounds. Recent advances have also suggested that SSF produces more stable and greater quantity of antibiotics than that obtained by SmF [6]. Spent mushroom substrate (SMS) is the post-harvest substrate and retains a variety of bioactive substances such as extracellular enzymes, antibiotics, secondary metabolites, and carbohydrates produced during mycelium and fruiting-body formation [7]. Approximately 5 kg of substrate is estimated to produce 1 kg of mushroom, and consequently about 2.5 million tons of SMS is annually generated as agricultural waste in Korea. Different SMSs have been used for production of value-added products...
such as decolorization [1] and lignocellulolytic enzymes [8], bioconversion into organic fertilizer [9], and for use as animal feed supplements [7]. Mushrooms are known to naturally produce low- and high molecular weight, compounds against gram-negative and -positive bacteria [4].

Current researches have focused mainly on fungal therapeutics for human pathogens rather than control of plant diseases. Phytopathogenic bacteria have resulted in tremendous economic loss of different crops. One such soil borne bacterium is Ralstonia solanacearum, which causes bacterial wilt in tomato, resulting in severe yield and quality reductions (about 20–25% yearly) in Korea. The bacterium enters plant roots through wounds and colonizes the vascular tissues of the stem, leading to wilt and decay of the infected tissues [10, 11]. Agrochemicals, copper, and antibiotics are being used as pesticides to control these bacterial disease, but are expensive and detrimental to the environment because of their effect on groundwater, air pollution, human health, and the ecosystem. Hence, nonchemical (biological) control of plant pathogens has received much attention worldwide. SMS derived from edible mushrooms are eco-friendly and can be used for suppression of plant pathogenic bacteria as it not harmful to humans. Water extract of SMS from mushroom species have been used to suppress plant diseases such as Pythium dampening-off, apple scab, cucumber anthracnose caused by fungal pathogens [12, 13].

Mycelial leachate of Lentinula edodes (Shiitake) was applied as biological control of plant disease, to suppress the bacterial wilt of tomato [14]. SMS is believed to possess multiple beneficial functions. Recently, it was reported that autoclaved water extract from SMS of the edible mushroom, Lyophyllum decastes (hatakeshimeji) could induce expression of systemic acquired resistance (SAR) related defense genes in plants [15]. H. erinaceus has been known to have anti-microbial activity on bacteria infecting humans [16]; however, there has been no report of its activity for bacterial disease control in plants.

Our study aims to investigate antibacterial activity of H. erinaceus SMS against phytopathogenic bacteria and evaluate the role of this extract in improving plant defense and growth. Further, the water extracts from SMS of H. erinaceus were also tested for their ability to suppress tomato bacterial wilt disease caused by R. solanacearum.

### MATERIALS AND METHODS

#### Mushroom species and bacterial strains.

Edible mushroom species Hericium erinaceus Noru 2, Pleurotus eryngii, Lentinula edodes, Flammulina velutipes, Grifola frondosa, Daedaleopsis tricolor, Pleurotus ostreatus, Pycnoporus coccineus, and Hypsizygus marmoreus were obtained from the Mushroom Research Institute (Gwangju, Korea). These edible mushrooms were maintained on potato dextrose agar (PDA; Dièco, Sparks, MD, USA). The plant pathogenic bacteria used in this study, namely, Xanthomonas campestris pv. campestris (black rot on Chinese cabbage), Ralstonia solanacearum (bacterial wilt on tomato), Agrobacterium tumefaciens 208 (crown gall), Pectobacterium carotovorum subsp. carotovorum (soft rot), Xanthomonas oryzae pv. oryzae KACC10859 (bacterial blight on rice), X. axonopodis pv. citri KACC 10443, Pseudomonas tauri KACC10365, and X. axonopodis pv. glycines KACC 11144 were obtained from Korean Agricultural Type Collection (KACC) and National Academy of Agricultural Science (NAAS), Rural Development Administration (RDA), Wanju. Escherichia coli DH5 was obtained from a laboratory collection.

#### Preparation of culture filtrates of edible mushrooms.

Myccelia of edible mushrooms (listed in Table 1) were grown on PDA (Dièco). Mycelial blocks (5 mm × 5 mm) were cut out from the media, inoculated in 100 mL of sterile potato dextrose broth (PDB) in a 500-mL Erlenmeyer flask, and incubated at 24°C for 28 days with shaking at 120 rpm. The culture fluid was harvested by filtration through a Whatman No. 1 filter paper and a 0.22-μm filter (Millipore, Billerica, MA, USA).

#### Water extract of SMS (WESMS).

Post-harvest substrates from bag-cultivated mushroom species were provided by MushArt farm in the Gyeonggi-do region, Republic of Korea. The SMS (300 g) was mixed with 900 mL distilled water by shaking at 150 rpm for 2 hr. The mixture was then filtered through two layers of Miracloth (Calbiochem, Billerica, MA, USA). The filtrate was centrifuged for 10 min at 10,000 rpm and the supernatant was used as the water extract of SMS.

#### Antibacterial assay.

Each aliquot (50 μL) of SMS extracts or culture filtrate from each edible mushroom species was

---

**Table 1.** Primer used for the quantitative real-time RT-PCR analysis of defense related tomato genes

| Genes | GenBank accession No. | Sequences (5'-3') | References |
|-------|-----------------------|-------------------|------------|
| GlaA  | M80604                | F-TCAGCGAGGTTGCAAAATCA  
R-CCTGACTGTTGGAATTGTTGATAA | [17] |
| PR-1a | M69247                | F-GAGGGGAGCGGTGCAA  
R-CAATTGTTGGCCAACACACATTG  
F-TCAAGCACTGGGATGATAG | [17] |
| Actin | BT013524              | F-TCAGCGAGGTTGCAAAATCA  
R-TTAGGGTTGAGAGGTGCTTC | [17] |

RT-PCR, reverse transcriptase polymerase chain reaction.
Antibacterial solvent extraction from SMS of H. erinaceus. Methanol solution (70%) was used to extract SMS (500 g) of H. erinaceus, homogenized, and filtered through two layers of Miracloth (Calbiochem) and the filtrate was centrifuged for 10 min at 10,000 rpm. The supernatant was evaporated under reduced pressure to remove methanol, and the resulting residue was extracted with n-hexane. Subsequently, the resulting water layer residue was serially extracted with chloroform, ethyl acetate, and n-butanol to separate different antibacterial compounds. Each solvent extract was concentrated in a rotary evaporator, dried at room temperature, dissolved in 70% methanol and adjusted to a final concentration of 150 mg/L.

Bacterial wilt disease assay on tomato. Seedlings (25 days old) of tomato cultivar, Superdoterang with four to five fully expanded leaves grown in pot (9 cm) were used for this experiment. The tomato seedlings were irrigated with WESMS (50 mL) of H. erinaceus. Three days later, marginal roots of the seedlings were wounded with scissors and drenched in the bacterial suspension (5 × 10^5 cells) of R. solanacearum B3. After 6–14 days of inoculation, disease index was evaluated on a scale of 0–5; with 0, no visible disease symptoms; 1–4, 1–25%, 26–50%, 51–70% disease symptoms; 4, 71–90% of plant wilt; and 5, complete plant wilt.

Gene expression analysis using quantitative real-time PCR (qRT-PCR). Expression patterns of the defense response genes encoding β-1,3-glucanase (GluA) and pathogenesis-related protein-1a (PR-1a) in tomato [17] were analyzed using qRT-PCR. Each primer set used in this study to amplify GluA and PR-1a are listed in Table 1. For RNA extraction, tomato seedlings grown in greenhouse for four weeks were treated with (50 mL) and roots and leaves were collected at 0, 24, 48, and 72 hr post-treatment. Samples were snap frozen in liquid nitrogen and stored at −80°C until required. Total RNA was isolated using TRIzol- Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions, and treated with DNase (Qiagen, Valencia, CA, USA). One microgram of DNA-free RNA was used for first-strand cDNA synthesis using the SuperScript III First-strand Synthesis system for RT-PCR (Invitrogen) according to the manufacturer's instructions. High-throughput qRT-PCR reactions were performed in a LightCycler 96 System (Roche, Mannheim, Germany) using SYBR Green 1 (Roche). Reaction parameters were set as follows: 10 min for polymerase enzyme activation, followed by 40 cycles of 95°C for 15 sec and 57°C for 1 min. Actin was used as a reference gene and tomato plants were used as the no treatment controls (NTC). The fold increase in the expression of each gene was calculated after normalization using the formula:

\[ \text{Fold gene expression} = \frac{\text{Quantity of target gene}}{\text{Quantity of reference gene}} \]

### RESULTS AND DISCUSSION

**Antibacterial activity of mushroom species.** The paper disk assay was performed to analyze the antibacterial activity of SMS from mushroom species. Culture filtrates and WESMS from different edible mushroom species was concentrated thrice for use in the assay. Culture filtrates of *Hericium erinaceus*, *Lentimina edodes* (Sanjo 701), *Grifola frondosa*, and *Hypsizygus marmoreus* showed antibacterial activity against *R. solanacearum* B3 forming clear zones of inhibition (Table 2). However, culture filtrate of *Pleurotus eryngii*, *Flammulina velutipes*, and *Pleurotus ostreatus* did not exhibit this property. The water extracts of different edible mushrooms showed different degrees of growth inhibition of tomato bacterial wilt pathogen, *R. solanacearum* B3. WESMS of *H. erinaceus* formed a larger inhibition zone (12.5 mm) than that of its culture filtrate (3.2 mm) of its culture. Studies on edible and wild type mushrooms, such as *Agaricus bisporus*, *Lentimina edodes*, and *Auricularia auricula*, and many *Pleurotus* species have focused primarily on the antagonistic effects against human pathogenic bacteria and fungi [3, 4]. Fruiting bodies of edible mushrooms and

| Mushroom species | Inhibition zone (mm) |
|------------------|----------------------|
| *Hericium erinaceus* Noru 2, CF | 3.2 ± 0.09 e' |
| *Hericium erinaceus* Noru 2, WESMS | 12.5 ± 1.02 a |
| *Hericium erinaceus* 843, CF | 1.2 ± 0.15 f |
| *Pleurotus eryngii*, CF | 0.0 |
| *Lentimina edodes* (Sanjo 701), CF | 7.5 ± 0.39 b |
| *Flammulina velutipes*, CF | 0.0 |
| *Grifola frondosa*, CF | 6.2 ± 0.16 c |
| *Duxaleopsis tricolor*, CF | 0.0 |
| *Pleurotus ostreatus*, CF | 1.5 ± 0.19 f |
| *Pycnoporus coccineus*, CF | 3.3 ± 0.18 e |
| *Hypsizygus marmoreus*, CF | 4.5 ± 0.22 f |
| Distilled water | 0.0 |

All data is abstained from four replicates. CF, cultural filtrate; WESMS, water extract spent mushroom substrate.

The different letters are significantly different (p < 0.05) according to Duncan's multiple range test.
mycelia have been the main source of clinical effects against these pathogens. Recently, mycelial cultures of certain mushroom species have been implicated in the suppression of phytopathogenic fungi and bacteria [18]. In practice, however, the use of mushroom culture filtrates in the field is limited owing to the need for expensive equipment for mass culturing and labor-intensive aseptic culturing processes. Though SMS from edible mushrooms such as *Lentinus edodes*, *Lyophyllum decastes*, *P. eryngii*, and *P. columbines* have been used to suppress fungal and bacterial diseases [12, 13], most previous investigations using SMS focused on the biological control of fungal plant diseases than bacterial. Interestingly, the mycelial leachate of *L. edode* suppressed the growth of several plant pathogenic bacteria [14]. Although WESMS of *H. erinaceus* have been shown to exhibit antibacterial activity against human bacterial pathogens [16], this is the first report of its growth inhibition effect on a plant pathogenic bacterium.

The mushroom substrate uses lignocellulosic wastes such as corn cobs, sugarcane wastes, cottonseed hull, cotton and beet pulp, sawdust supplement and rice bran similar to that used for SSF. SSF is the best fermentation technique for fungi [5], since the substrates are utilized very slowly and steadily through long fermentation periods and bioactive compounds with high quantity are released in the substrate. It is known that SSF produces higher quantities of certain bioactive compounds and more stable antibiotics compared to SmF. Therefore, higher antibacterial activity in SMS of *H. marmoreus* than that of its culture filtrate in this study may be attributed to the analogous characteristics of SMS and SSF.

**Antibacterial spectrum of solvent extracts from SMS of *H. erinaceus***. In order to know antibacterial property in *H. erinaceus* SMS, 70% methanol extraction solution from the SMS was used for extracting antibacterial fractions with the procedure depicted in Fig. 1. To start with, the solution was extracted with hexane; however, antibacterial activity exhibited in water fraction and not in hexane fraction. The extraction from the water layer was done with chloroform, ethyl acetate and *n*-butanol. As shown in Fig. 1, out of different solvent extracts, ethyl acetate and *n*-butanol extracts showed antibacterial activity against the bacterial cells, forming inhibition zone; whereas the remnant water fractions did not show antibacterial activity. Generally, it has been known hexane is used to extract lipophilic compounds, while ethyl acetate and *n*-butanol is useful for extracting non-polar compounds [19]. Actually, the dried ethyl acetate and *n*-butanol fractions were dissolved in absolute methanol, but not in water. Thus, it is assumed that the nonpolar compounds in the WESMS of *H. erinaceus* play critical role in antibacterial function. Furthermore, antibacterial spectrum of WESMS, ethyl acetate and butanol extract was tested on nine plant pathogenic bacteria that caused major bacterial diseases on different crops in Korea (Table 3, Fig. 2). The extracts exhibited growth inhibition zones ranging from 2.4 to 26.5 mm on the bacterial cells, especially, strongly inhibiting the growth of *Agrobacterium tumefaciens* 208 and *Xanthomonas campestris* pv. *campestris*, *Xanthomonas axonopodis* pv. *vesicatoria*, *X. axonopodis* pv. *citr*., and *X. axonopodis* pv. *glycines* forming inhibition zones greater than 14 mm.

In previous studies, culture filtrates of 27 edible mushrooms were screened for antimicrobial activity against plant pathogens [18], and *Agrocybe cylindracea*, *Grifola frondosa*, and *Lentinula edodes* showed varying zones of inhibition against different bacterial species. In addition, the mycelial leachate of *L. edodes* has also been reported to contain substances that suppressed the growth of plant pathogens, such

---

**Fig. 1.** Antibacterial activity on *Ralstonia solanacearum* B3 cells of layers of spent mushroom substrate (SMS) extracted by different solvents. Numbers on extraction layers of scheme are in accordance with those of image.
Suppression of Bacterial Wilt Disease of Tomato by SMS of *H. erinaceus* as *Pseudomonas syringae* pv. *glycinea*, *P. syringae* pv. *tabaci*, *Xanthomonas campestris* pv. *glycines*, *Erwinia amylovora*, and *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* [14].

**Effect of WESMS on expression of defense genes in *H. erinaceus***. Gene expression analysis using real-time PCR showed that the expressions of the *PR-1a* and *GluA* genes were significantly enhanced in WESMS-treated tomato plants compared to water-treated control plants at 72 hr (Fig. 3). Both these genes are related to SAR, which can be induced by a variety of agents, including necrotizing pathogens and certain chemicals and are regulated by a salicylic acid-dependent process [20]. In contrast, induced systemic resistance (ISR) is caused because of colonization of plant roots by certain strains of plant growth-promoting rhizobacteria and is mediated by a jasmonate- and ethylene-sensitive pathway. Treatment of plants with agents such as cell wall fragments, plant extracts, and synthetic chemicals, can induce resistance to subsequent pathogen attack both locally and systemically [21]. In tomato, defense genes related to SAR and ISR are activated by acibenzolar-S-methyl [22], β-amino butyric acid [23], and extracellular polysaccharide of *R. solanacearum* [17].

Several studies have reported the use of elicitors from the cell walls of pathogens and non-pathogens to induce resistance in plants [17]. Chitins and glucans are key skeletal polysaccharides of fungal cell walls, and in general, have been recognized as elicitors of a plant defense response for many years [24, 25]. In mushrooms, elicitors from fruiting body of Shiitake (*Lentinula edodes*) have been purified to control cucumber anthracnose [26]. In addition, it was also demonstrated that water extract from SMS of *Hatakeshimeji* (*Lyophyllum decastes*) could control cucumber anthracnose disease and enhance the state of SAR inducing defense genes such as chitinase and β-1,3-glucanase [15].

In the present study, water extract from SMS of *H. erinaceus* activated defense genes related to SAR. SAR is widely known to suppress a broad spectrum of plant pathogens, including fungi, bacteria, and viruses [21]. Carbohydrate and protein elicitors that induce defense mechanism in plants are released from the mycelia of fungal pathogens. Therefore, SMS of *H. erinaceus* that diffuses into the mushroom mycelia could be an abundant source of elicitors for SAR. However, future investigations are needed to identify the elicitors associated with SAR induction in tomato plants treated with WESMS of *H. erinaceus*.

**Table 3. Antibiotic activity of different extracts from spent mushroom substrate of *Hericium erinaceus***

| Bacterial species | WESMS (100 mg/L) | Butanol extract (150 mg/L) | Ethyl acetate extract (150 mg/L) | Tetracycline (30 mg/mL) |
|-------------------|-----------------|---------------------------|---------------------------------|------------------------|
| *Xanthomonas campestris* pv. *campestris* | 9.0 ± 0.10 c’ | 10.3 ± 0.19 e | 14.2 ± 0.07 e | - |
| *Ralstonia solanacearum* B3 | 6.0 ± 0.42 e | 4.3 ± 0.03 f | 9.5 ± 0.08 f | 10.3 ± 0.21 a |
| *Agrobacterium tumefaciens* 208 | 14.3 ± 0.36 a | 12.2 ± 0.29 d | 18.1 ± 0.06 d | - |
| *Xanthomonas axonopodis* pv. *vesicatoria* | 12.0 ± 0.28 b | 13.4 ± 0.30 c | 20.2 ± 0.12 c | - |
| *Xanthomonas oryzae* pv. oryzae KACC10859 | 2.5 ± 0.06 f | 3.5 ± 0.17 g | 7.7 ± 0.07 h | - |
| *X. axonopodis* pv. *citri* KACC 10443 | 12.0 ± 0.25 b | 18.4 ± 0.22 b | 21.2 ± 0.07 b | - |
| *Pseudomonas tolaasi* KACC10365 | 6.4 ± 0.27 d | 5.3 ± 0.05 f | 5.4 ± 0.04 g | - |
| *Pectobacterium carotovorum* subsp. *carotovorum* | 2.4 ± 0.13 f | 2.3 ± 0.05 a | 5.1 ± 0.13 i | - |
| *X. axonopodis* pv. *glycines* KACC11144 | 12.5 ± 0.56 b | 21.6 ± 0.13 h | 26.5 ± 0.18 a | - |

All data was abstained from four replicates.
WESMS, water extract spent mushroom substrate.
*The different letters are significantly different (p < 0.05) according to Duncan’s multiple range test.*

Fig. 2. Antibacterial activity of different extracts from spent mushroom substrate of *Hericium erinaceus* on *Ralstonia solanacearum* Biovar 3 (A), *Agrobacterium tumefaciens* A208 (B), and *X. campestris* pv. *glycines* KACC 10491 (C), with water extract (1); ethyl acetate extract (2) (150 mg/L); butanol extract (3) (150 mg/L); and negative control treated with water (c).
Protective effect of *H. erinaceus* WESMS against bacterial wilt disease of tomato. To investigate if the protective effect of WESMS is derived from SMS of *H. erinaceus*, tomato plants were treated with WESMS before bacterial inoculation of PIS. Plants incubated for 14 days after WESMS, water extract of pre-inoculated substrate substrate (WEPIS), and water treatment showed 15%, 68%, and 98% disease incident, showing highly protective effect of 85% against tomato wilt disease (Fig. 4). As shown in Fig. 5, tomato plants treated with WESMS were healthy; however, all the water-treated tomato plants exhibited wilted symptoms. It is speculated that the antibacterial compounds and elicitors in WESMS of *H. erinaceus* may be play critical role in suppressing the tomato wilt disease by inhibiting cell density of the pathogen and inducing defense genes in tomato.

The focus of research has mainly been on the role of WESMS in suppression of diseases caused by fungal plant pathogens. However, in a previous study that investigated the suppression effect of SMS of the edible mushrooms...
Lyophyllum decastes and Pleurotus eryngii against fungal and bacterial diseases in cucumber plants [13], Lyophyllum decastes was found to significantly reduce powdery mildew by Podosphaera xanthii and, angular leaf spot by Pseudomonas syringae pv. lachrymans. It was demonstrated that the bacterial suppression event may be due to induction of SAR-related defense genes such as β-1,3-glucanase [17].

In addition, we investigated if WESMS of H. erinaceus promotes tomato growth, by measuring plant height, leaf number, and fresh weights of root and shoot. Interestingly, WESMS-treatment dramatically promoted the growth of tomato plants compared to water- and WEPIS-treatment (Table 4). Particularly, the highest mean number of 48 leaves in WESMS-treated plants; this is significant since leaves are vital sites of photosynthesis in green plants. Moreover, WESMS treatment also increased the fresh weight of root and shoot from 2 to 20 folds. These results demonstrate that WESMS of H. erinaceus are beneficial to tomato plants with respect to growth promotion as well as disease suppression. Recently, it was reported that SMS of oyster mushroom and button mushroom promoted growth of pepper plant [27]. SMS is considered to be a good source of organic matter and rich in macro- and microelements, thus promoting plant growth, disease suppression and help to increase the soil biological activity [7, 28].

To conclude, this study reveals that WESMS from H. erinaceus has multiple effects including antibacterial activity, defense gene induction, plant growth promotion and in suppressing the bacterial wilt disease of tomato. Hence, SMS of the edible mushroom H. erinaceus can be used as a eco-friendly control of this disease. Further, this is a new way of recycling the waste from mushroom cultivation, which helps alleviate the waste disposal problem in the mushroom industry. However, identification of the key antibacterial compound and main elicitor of SAR response in WESMS of H. erinaceus requires further investigation.

**ACKNOWLEDGEMENTS**

This research is supported by research grant (Agenda project No. PJ009969) from Rural Development Administration, Wanju, Korea.

**REFERENCES**

1. Lim SH, Lee YH, Kang HW. Efficient recovery of lignocellulolytic enzymes of spent mushroom compost from oyster mushrooms, Pleurotus spp., and potential use in dye decolorization. Mycobiology 2013;41:214-20.
2. Benedict RG, Brady LR. Antimicrobial activity of mushroom metabolites. J Pharm Sci 1972;61:1820-2.
3. Suay I, Arenal F, Asensio FJ, Basilio A, Cabello MA, Diez MT, García JB, del Val AG, Gorrochategui J, Hernández P, et al. Screening of basidiomycetes for antimicrobial activities. Antonie Van Leeuwenhoek 2000;78:129-39.
4. Alves MJ, Ferreira IC, Dias J, Teixeira V, Martins A, Pintado M. A review on antimicrobial activity of mushroom (Basidiomycetes) extracts and isolated compounds. Planta Med 2012;78:1707-18.
5. Subramaniyam R, Vimala R. Solid state and submerged fermentation for the production of bioactive substances: a comparative study. Int J Sci Nat 2012;3:3480-6.
6. Vinkneswary S, Kumanan KS, Ling SK, Dinesh N, Shim YL. Solid substrate fermentation of agroresidues for value added products: the Malaysian experience. In: Wise DL, editor. Global environmental biotechnology. Dordrecht: Springer; 1997. p. 301-5.
7. Suess A, Curtis J. Report: value-added strategies for spent mushroom substrate in BC. British Columbia Mushroom Industry; 2006.
8. Lim SH, Lee YH, Kang HW. Optimal extraction and characteristics of lignocellulolytic enzymes from various spent mushroom composites. Kor J Mycol 2013;41:160-6.
9. Sochtig H, Grabbe K. The production and utilization of organic-mineral fertilizer from spent mushroom compost. Mushroom Sci 1995;14:907-15.
10. Hayward AC. Biology and epidemiology of bacterial wilt caused by Pseudomonas solanacearum. Annu Rev Phytopathol 1991;29:65-87.
11. Denny TP. Ralstonia solanacearum: a plant pathogen in touch with its host. Trends Microbiol 2000;8:486-9.
12. Inagaki R, Yamaguchi A. Spent substrate of shiitake (Pleurotus eryngii) inhibits symptoms of anthracnose in cucumber. Mushroom Sci Biotechnol 2009:17:301-5.
13. Parada RY, Murakami S, Shimomura N, Otani H. Suppression of fungal and bacterial diseases of cucumber plants by using the spent mushroom substrate of Lyophyllum decastes and Pleurotus eryngii. J Phytopathol 2012;160:390-6.
14. Pacumbaba RP, Beü CA, Pacumbaba RO Jr. Shiitake mycelial leachate suppresses growth of some bacterial species and symptoms of bacterial wilt of tomato and lima bean in vitro. Plant Dis 1999;83:20-3.
15. Parada RY, Murakami S, Shimomura N, Egusa M, Otani H. Autoclaved spent substrate of hatake-shimeji mushroom

### Table 4. Effect on tomato growth promotion of WESMS of *Hericium erinaceus*

| Treated     | Plant height (cm) | Leaf length (cm) | Leaf width (cm) | Leaf number | Fresh weight (g) |
|-------------|-------------------|------------------|-----------------|-------------|------------------|
|             |                   |                  |                 |             | Shoot            | Root             |
| WESMS       | 28.95 ± 2.26       | 4.54 ± 0.31      | 2.32 ± 0.37     | 46 ± 2.33 b | 0.95 ± 0.13 a    | 0.22 ± 0.03 a    |
| WEPIS       | 25.29 ± 2.17       | 4.51 ± 0.24      | 2.16 ± 0.30     | 30 ± 2.08 b | 0.82 ± 0.23 b    | 0.11 ± 0.03 b    |
| Water       | 17.30 ± 1.85       | 3.69 ± 0.33      | 1.73 ± 0.24     | 28 ± 1.65 c | 0.30 ± 0.11 c    | 0.05 ± 0.02 c    |

WESMS, water extract spent mushroom substrate; WEPIS, water extract of pre-inoculated substrate.

*The different letters are significantly different (p < 0.05) according to Duncan’s multiple range test.
(Lyophyllum decastes Sing.) and its water extract protect cucumber from anthracnose. Crop Prot 2011;30:443-50.

16. Wong KH, Sabaratnam V, Abdullah N, Kuppusamy UR, Naidu M. Effects of cultivation techniques and processing on antimicrobial and antioxidant activities of Hericium erinaceus (Bull.:Fr.) Pers. extracts. Food Technol Biotechnol 2009;47:47-55.

17. Milling A, Babujee L, Allen C. Ralstonia solanacearum extracellular polysaccharide is a specific elicitor of defense responses in wilt-resistant tomato plants. PLoS One 2011;6:e15853.

18. Chen JT, Huang JW. Antimicrobial activity of edible mushroom culture filtrates on plant pathogens. Plant Pathol Bull 2010;19:261-70.

19. Rydberg J, Cox M, Musikas C, Choppin GR. Principles and practice of solvent extraction. 2nd ed. New York: Marcel Dekker, Inc.; 1992.

20. Walters DR, Fountaine JM. Practical application of induced resistance to plant diseases: an appraisal of effectiveness under field conditions. J Agric Sci 2009;147:523-35.

21. Walters DR, Ratsep J, Havis ND. Controlling crop diseases using induced resistance: challenges for the future. J Exp Bot 2013;64:1263-80.

22. Herman MA, Restrepo S, Smart CD. Defense gene expression patterns of three SAR-induced tomato cultivars in the field. Physiol Mol Plant Pathol 2007;71:192-200.

23. Hassan MA, Abo-Elyour KA. Activation of tomato plant defence responses against bacterial wilt caused by Ralstonia solanacearum using DL-3-aminobutyric acid (BABA). Eur J Plant Pathol 2013;136:145-57.

24. Shibuya N, Minami E. Oligosaccharide signalling for defense responses in plant. Physiol Mol Plant Pathol 2001;59:223-33.

25. Walters D, Walsh D, Newton A, Lyon G. Induced resistance for plant disease control: maximizing the efficacy of resistance elicitors. Phytopathology 2005;95:1368-73.

26. Di Piero RM, Wulff NA, Pascholati SF. Partial purification of elicitors from Lentinula edodes basidiocarps protecting cucumber seedlings against Colletotrichum lagenarium. Braz J Microbiol 2006;37:175-80.

27. Roy S, Barman S, Chakraborty U, Chakraborty B. Evaluation of spent mushroom substrate as biofertilizer for growth improvement of Capsicum annuum L. J Appl Biol Biotechnol 2015;3:02-027.

28. Ahlawat OP, Manikandan K, Sagar MP, Rai D, Gupta P, Vijay B. Effect of composted button mushroom spent substrate on yield, quality and disease incidence of Pea (Pisum sativum). Mushroom Res 2011;20:87-94.