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Isolation, Identification and Pathogenic Effects of Trichoderma spp. from Auricularia auricula

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Abstract

Auricularia auricula, one of the most important edible mushrooms, is affected heavily by *Trichoderma*. We collected the diseased samples from the main *A.auricula* cultivation regions to characterize the pathogen and study the effect of *Trichoderma* spp. on *A.auricula* species. We identified one *Trichoderma* species, *T.pleuroticola*, based on the internal transcribed spacer and morphology characteristics and two types of *A.auricula* strains, Heiwei 15 (HW 15) and Hei 29 (H 29) were tested in this work. The growth rate of *T.pleuroticola* was 3.26–3.52 times higher than that of *A.auricula* and advantageously competed for living space and nutrients. In confrontation culture, *T.pleuroticola* completely inhibited the mycelium growth of *A.auricula* and grew on it, resulting in the diverse impact on HW 15 and H 29. In addition, *T.pleuroticola* can produce metabolites with antibacterial activity. The inhibition rate of volatile metabolites to H-29 and HW 15 was 13.46% and 10.44%, and the inhibition rate of nonvolatile metabolites to H-29 and HW 15 was 36.04% and 31.49%, respectively. These antifungal activities of inhibiting the growth of *A.auricula* were abbtributed to the organic compounds from *T.pleuroticola*, nonanal, tyrosine, beta-sitosterol, and wortmannin, which were identified by gas chromatography-ion mobility spectroscopy (GC-IMS) and liquid chromatography-mass spectrometry (LC-MS/MS). In short, *T. pleuroticola* was a highly pathogenic fungi in the production of *A.auricula.*

1 Introduction

Trichoderma is widely distributed and diverse. Hundreds of *Trichoderma* species have been identified, such as *T.atroviride, T.longibrachiatum, T.aggressivum*, and *T.harzianum*[1]. *Trichoderma* species have produced peptide chain, terpene, laccase, cellulase, and other substances. They also have anti-tumor, antifungal, antibacterial, and antiviral biological activities. Additionally, the *Trichoderma* species are widely used in agriculture, papermaking and chemical industries[2, 3, 4].

However, recent studies have shown that some *Trichoderma* strains can cause green mold disease of edible fungi, which is the most devastating disease. Massive attacks of the disease have been reported in South Korea, Sri Lanka, China, and Europe countries, and pollute a variety of edible fungi, such as *Agaricus bisporus, Lentinus edodes, Flammulina velutioes* and *Auricularia auricula. Trichoderma* spp. has caused serious losses in the production of edible fungi all over the world[5, 6, 7, 8]. Studies have shown that *Trichoderma* can contaminate edible fungi strains, fruiting bodies, and compost. The contaminated edible fungus mycelium was brown, the fruiting body no longer grew, and there were green spores on the compost surface, which could not reproduce the fruiting body[9, 10]. The antagonistic mechanism of *Trichoderma* includes indirect and direct mechanisms. The indirect mechanism is to compete for space and nutrientswhile the direct mechanism is to parasitize fungi, produce active metabolites, and decompose enzymes[11]. The mycelium growth rate of *Trichoderma* is 1.25–5.35 times that of *L.edodes* and *P.ostreatus*, and can quickly occupy living space and compete for nutrients[12, 13]. *Trichoderma* can parasitize in the mycelium of edible fungi. Studies have shown that *T.harzianum* and *T.longibrachiatum* cause the mycelium of edible fungi to swell, distort, dissolve, brown, wither and die[14, 15]. *Trichoderma* can secrete secondary toxic metabolites, extracellular enzymes and volatile organic compounds to inhibit the edible fungi's growth, significantly reducing or even completely hindering commercial production[16]. Studies have shown that *T.harzianum* can produce extracellular chitinase to act on the cytoderm of *P.ostreatus*, leading to the disintegration of the cytoplasm[17]. There are many studies on the enzyme system of *Trichoderma* spp., and few on active metabolites. Only a few active substances have been identified, but the in-depth action mechanism is unclear.

Most of the studies on *Trichoderma* diseases of edible fungi are related to *L.edodes, P.ostreatus* and *A.bisporus*, with less *Auricularia auricula*. This study collected a wealth of green mold disease logs from main *A.auricula* cultivation

areas in Zhashui, Shaanxi, China. Based on the morphology and internal transcribed spacer (ITS) sequence, we analyzed the species of *Trichoderma* spp. To elaborate the mechanism of the *Trichoderma* spp., we undertook the *Trichoderma* spp. on the mycelial growth of *A.auricula* via scanning electron microscope. In addition, we studied the volatile and nonvolatile metabolites of *T.pleuroticola* on the growth of *A.auricula* and speculated on the properties of antibacterial substances based on detecting the specific metabolites. This study provides an effective way to reduce or release the inhibition of *T.pleuroticola* metabolites on the growth of *A.auricula*, and also provides a reliable strategy for the safe production of *A.auricula*.

2 Materials And Methods

2.1 Sample Collection and Disease Symptoms

Green mold disease occurred continuously in *A.auricula* production in Zhashui, Shaanxi, leading to a significant negative effect on the development of *A.auricula*. The diseased logs of *A.auricula* were collected from six main cultivation areas (see Table 1 in Supporting materials). The surface of the cultivation substrate was completely covered with *Trichoderma* spores, which became dark green, rotten and soft, with the obvious musty smell.

2.2 *A.auricula* Strains

Hei 29 (H 29) and Heiwei 15 (HW 15) were sourced from the Institute of Microbiology (Heilongjiang, China).

2.3 Chemical Materials

GC-grade methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). GC-grade formic acid was obtained from Aladdin (Shanghai, China). AR-grade isoamyl acetate, ethanol absolute, glutaraldehyde, and potato dextrose agar (PDA) were obtained from 'Xi'an Jingbo Biotechnology Co., Ltd (Xi'an, China), *A.auricula* complete media (ACM; 200 g potato, 30 g wheat bran, 20 g glucose, 3 g peptone, 2 g KH_2PO_4, 1 g $MgSO_4 \cdot 7H_2O$, 100 mg VB_1, 15 g agar, and 1 L water). Extraction of fungal genomic DNA kit and PCR purification kit (Dynabeads) were obtained from Beijing Genomics Institution (Beijing, China).

2.4 Isolation and Identification of *Trichoderma* Strains

Trichoderma strains were isolated from green mold-affected logs of *A.auricula*. About 1 g of infected tissue of each sample was placed in a 250 mL flask containing 99 mL sterile distilled water and shaken for 20 min at 150 rpm. Samples were then serially diluted in sterile distilled water and transferred onto PDA. The plates were incubated at 28 °C. Pure subcultures were obtained from growing colonies on PDA.

The isolated species were incubated on PDA at 28°C in darkness, during which colony shape and pigment were documented. Simultaneously, the cover glasses were inserted slantingly into the ACM medium to observe the conidia and conidiophores via the SEM (Quanta 200 Environmental Scanning Electron Microscope, FEI Company) when the mycelia spread on the cover glass. The strains were identified by Trichoderma Classification and Identification[18].

To identify *Trichoderma* spp, the growing mycelium on PDA plates was taken and DNA was extracted using the fungal genomic DNA kit. The amplification was made using the universal primer pairs ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The reaction mixture for PCR amplification was template DNA (1 µL), forward and reverse primers (1 µL), super mix (15 µL), ddH_2O (12 µL). The conditions of the thermocycler were: 96 °C for 5 min; followed by 35 cycles of 96 °C for 20 s, 56 °C for 20 s and 72 °C for 30 s, and finally, 72 °C for 10 min. The amplicons obtained were confirmed in 1% agarose gel. PCR products

were tested and sequenced by Beijing Genomics Institution. The obtained ITS sequences were submitted to the NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and blasted in GenBank. Additionally, the phylogenetic trees were constructed with a neighbor-joining method by MEGA 7.0[19].

2.5 Comparison of the Mycelium Growth Rate of *Trichoderma* and *A.auricula*

T.pleuroticola was recovered from the zhashui. *T.pleuroticola* and *A.auricula* (H 29, HW 15) were selected as the test strains. The 5 mm diameter mycelial plugswere separately inoculated in the center of ACM, and cultured at 28 ℃ until the mycelium had overgrown the petri plate. Each treatment was replicated three times. The growth rate (cm/d) was calculated with the formula (1).

$$S = \frac{D - 0.5}{T} \quad (1)$$

where D is colony diameter (cm); 0.5 is plug diameter (cm); and T is cultivation days (d).

2.6 Effect of *T.pleuroticola* on *A.auricula* Mycelium in Petri Plates

To verify the interaction between *T.pleuroticola* and *A.auricula* (H29, HW15), dual culture was used based on the Owaid method with slight modifications[20]. Mycelial agar plugs (5 mm in diameter) were cut from the growing front of 7-day-old colonies of *A.auricula* species. They were separately inoculated in ACM at 1 cm from the edge in Petri plates of 9 cm in diameter, and 5 mm mycelial plugs of *T.pleuroticola* were inoculated 6 cm apart on the same plates 7 d later. All combinations of *T.pleuroticola* × H 29 and *T.pleuroticola* × HW 15 were performed in triplicate. All plates were maintained at 28°C in the dark, and the radial growth of *A.auricula* was measured every 12 hours. The control group was plated with only the *A.auricula*. The confrontation inhibition of *T.pleuroticola* against *A.auricula* was calculated with Formula (2).

When the mycelium of *T.pleuroticola* and *A.auricula* were about to come into contact, a cover glass was placed on the medium to promote the mycelium to climb and observe mycelium interaction. After 24 h of incubation, the cover glass was removed, and then the changes of *A.auricula* mycelium treated by *T.pleuroticola* were observed via SEM.

$$P = \frac{R - R_1}{R} \times 100 \quad (2)$$

where R is the radial growth distance of the control plate (mm); R_1 is the radial growth distance of the test plate (mm); and P is the percent of inhibition (%).

2.7 Effect of *T.pleuroticola* Volatile Substances on *A.auricula* Mycelium

2.7.1 Inhibition of *T.pleuroticola* Volatile Substances on *A.auricula* Mycelium

The inhibition ability of *T.pleuroticola* volatile substances on *A.auricula* Mycelium was evaluated with two partition plate confrontation assay methods based on the method of Ebadzadsahrai with slight modifications[21]. Firstly, a 5

mm diameter plug of each *A.auricula* strain was inoculated on one side of the ACM plate and cultured for 3 d. Then, a 5 mm diameter plug of *T.pleuroticola* was inoculated on the opposite side. All plates were sealed with parafilm film and incubated at 28 ℃. Radial growth of *A.auricula* was measured after 3 d. The control group was plated with only the *A.auricula*. Each treatment was replicated three times. The inhibition of *T.pleuroticola* volatile substances against *A.auricula* was calculated with the formula (2).

2.7.2 Analysis of Volatile Organic Compounds (VOCs) of *T.pleuroticola*

2 mL sterile ACM placed in a 20 mL headspace bottle and inoculated 5 mm diameter plug of *T.pleuroticola* after condensation. All headspace bottles were incubated at 28 ℃ for 3 d, and the headspace bottle only contained 2 mL ACM as the blank control group (KB).

VOCs were identified with gas chromatography-ion mobility spectroscopy (GC-IMS, FlavourSpec® Gas Phase Ion Mobility Spectrometer, GAS Company, Germany). The test conditions were as follows: the headspace bath temperature was performed at 40 ℃ for 15 min; the temperature of headspace injection was performed at 85 ℃, the injection volume was set at 500 μL, and the incubation speed was 50 rpm. The GC-IMS system was equipped with an MXT-5 (15 m × 0.53 mm and 1.0 μm) chromatographic column (RESTEK, USA). The chromatographic column was programmed to 40 ℃ and worked for 20 min. Nitrogen (99.999%) was used as the carrier gas and drift gas, and the flow rate of drift gas was 150 mL/min. The carrier gas was programmed to start at 2 mL/min (held for 2 min) and to linearly ramp up to 10 mL/min within 8 min, and then it was linearly increased to 100 mL/min within 10 min. The temperature of IMS was 45°C. The analysis was carried out in triplicate.

2.8 Effect of *T.pleuroticola* Culture Filtrate on *A.auricula* Mycelium

2.8.1 Inhibition of *T.pleuroticola* Culture Filtrate on *A.auricula* Mycelium

3 mL of *T.pleuroticola* spore suspension (5×10^6 CFU/mL) was inoculated in 100 mL of sterile potato dextrose broth (PDB). The liquid cultures were respectively incubated for 2, 4, 6, 8 and 10 d at 28°C under stirring (150 rpm). The cultures were filtered through sterile gauze and a 0.22 μm filter in turn, and the filtrates in different culture periods were stored at -80°C.

Based on the method of Stracquadanio with slight modifications[22], *T.pleuroticola* filtrates in different culture periods were added to sterile ACM at 20% (v/v). A 5 mm diameter plug of each *A.auricula* was inoculated in the center of the plate. The *A.auricula* on a fresh ACM plate as control. All dishes were incubated at 28 ℃ for 10 d, and then, the radial growth of the *A.auricula* colonies was determined. The inhibitory activity of *T.pleuroticola* nonvolatile organic compounds against *A.auricula* was calculated with the formula (2). Three replicates for each treatment were considered.

2.8.2 Analysis of Nonvolatile Organic Compounds (nVOCs) of *T.pleuroticola*

For the identification of nVOCs, the *T.pleuroticola* filtrates were taken out from the − 80°C refrigerator and thawed on ice, vortex for 10 s. Mix 50 μL of *T.pleuroticola* filtrates and 150 μL of 20% acetonitrile methanol internal standard extractant using vortex the mixture for 3 min and centrifuge (12000 rpm, 4°C) for 10 min. Then transfer 150 μL of the

supernatant and stand still at -20°C for 30 min. Finally, centrifugation (12000 rpm, 4°C) for 3 min followed by analysis of the supernatant

T3 UPLC Conditions: The *T.pleuroticola* filtrate extracts were analyzed using an LC-ESI-MS/MS system (UPLC, ExionLC AD https://sciex.com.cn/; MS/MS, QTRAP® System, https://sciex.com/). The analytical conditions were as follows, UPLC: column, Waters ACQUITY UPLC HSS T3 C18 (1.8 μm, 2.1 mm*100 mm); column temperature, 40°C; flow rate, 0.35 mL/min; injection volume, 2 μL; solvent system, water (0.1% formic acid), acetonitrile (0.1% formic acid); gradient program, 95:5 V/V at 0 min, 10:90 V/V at 11.0 min, 10:90 V/V at 12.0 min, 95:5 V/V at 12.1 min, 95:5 V/V at 14.0 min.

LIT and triple quadrupole (QQQ) scans were acquired on a triple quadrupole-linear ion trap mass spectrometer (QTRAP), QTRAP® LC-MS/MS System, equipped with an ESI Turbo Ion-Spray interface, operating in positive and negative ion mode and controlled by Analyst 1.6.3 software (Sciex). ESI source conditions were set as follows: source temperature 500°C; ion spray voltage (IS) 5500 V (positive), -4500 V (negative); ion source gas I (GSI), gas II (GSII), curtain gas (CUR) was set at 50, 50, and 25 psi, respectively; the collision gas (CAD) was high. The analysis was carried out six times.

3 Results And Discussion

3.1 Morphology identification of the isolates

One *Trichoderma* specie, KQQ-3, was isolated based on the colony shape, conidia, conidiophore, and pigment (Table 2 in Supporting materials). The color of the colony firstly is white round, with cotton-like aerial mycelium. The colony becomes dark green after 4 d because conidia clusters are generating. The mycelium grew all over the plate after 5 d, and the growth rate was 1.56 cm/d. The spores entirely covered the dish and arranged 2 ~ 4 concentric rings after 7 d. The color of the back of the colony is uneven, the center is yellow and the edge is yellow-green.

The microscopic characteristics of KQQ-3 are seen in Fig. 1. The conidia were smooth oval with a concave center, (0.45 ~ 1.82) μm × (1.36 ~ 2.73) μm in diameter. The branch of the conidiophore was dendritic, (2.67 ~ 40) μm × (1.33 ~ 4) μm in (length × width). The base of the primary branch was thick, and the peak became slender, with single or opposite secondary branches extending at vertical angles. The spores were attached to the top of secondary branches.

3.2 Molecular identification of the isolates

For further information, we applied the ITS sequences to identify the KQQ-3 accurately. The results suggested that the ITS sequence sizes of KQQ-3 were 606 bp (Fig. 2). The phylogenetic trees were constructed by neighbor-joining method (Fig. 3), and demonstrated that the ITS sequence of KQQ-3 showed 99% identity with *T.pleuroticola* (JQ040377.1). Combined with microscopic characteristics, the agents causing green mold in the cultivation of *A.auricula* were identified as *T.pleuroticola*.

3.3 Comparison of Mycelium Growth Rate of *T.pleuroticola* and *A.auricula*

As shown in Tables 3, 4 and 5 in Supporting materials, the colony of H 29 and HW 15 were white rounds with short aerial mycelium, and the growth rate was slow, respectively 0.56 cm/d and 0.52 cm/d, with no significant difference

($P < 0.05$). However, the growth rate of *T.pleuroticola* on ACM was 1.83 cm/d, which was 2.05 ~ 3.85 times that of *A.auricula*. It could achieve superiority in the occupation of the living space and in its competition for nutrients.

3.4 Effect of *T.pleuroticola* on *A.auricula* Mycelium in Petri Plates

In dual culture, we observed the confrontation colony of two *A.auricula* species with *T.pleuroticola* and measured the inhibition rate for *A.auricula* mycelium (see Table 6 in Supporting materials, Fig. 4). *T.pleuroticola* inhibited heavily *A.auricula* mycelium growth, the inhibition is observed only on the 12th hour. With the prolongation of confrontation, the inhibition ratio gradually increased, up to 100% after 60 h. From the perspective of mycelium morphology, *T.pleuroticola* mycelium could overgrow and spread on *A.auricula* mycelium, forming conidial clusters, resulting in the gradual withering of *A.auricula* mycelium. Besides, brown pigment appeared on the back of *A.auricula.* The phenomenon of the test was consistent with what Wang's group reported[7].

By analyzing the dark brown on the back of the mushroom colony, Li believed that the pigment produced by the *L.edodes* mycelium infiltrated the medium. It may inhibit the growth of *Trichoderma* or as an indicator of the inhibition of the growth of *L.edodes*[23]. Wang found that *Trichoderma* and *L.edodes* could produce dark substances during confrontation through microscopic observation, and the mycelium of *L.edodes* infected by *Trichoderma* could produce dark brown vesicles rupture later. The contents infiltrated the medium, resulting in the dark brownness of the medium. At the same time, the top of *Trichoderma* mycelium in contact with *L.edodes* was dark yellow and secreted dark antibacterial substances to act on *L.edodes* mycelium, resulting in roughness and depression[24]. Marik showed that the dark substance peptaibols secreted by *T.pleuroticola*, which inhibited the mycelium growth of *A.bisporus*, may be related to the dark brown color of the edible fungus colony[25]. It is speculated that T.pleuroticola and A.auricula may cause the formation dark brown color of the edible fungus colony in this test. However, a few studies on the dark brown edible fungi are present in the literature, and many more studies should be performed to understand the production of dark substances.

From the perspective of the mycelium interaction between *T.pleuroticola* on *A.auricula*, we observed that the mycelium untreated by *T.pleuroticola* was smooth and straight (Fig. 5A and E). In contrast, the mycelium morphology of *A.auricula* from the interaction zone was abnormal. After getting contact with *T.pleuroticola* mycelium, H 29 mycelium became shrunken, broken, and dissolved (Fig. 5B, C, D). While HW 15 mycelium became swelled, broken, and dissolved (Fig. 5F, G, H), it may be attributed to *Trichoderma* β-1,3-glucanase, chitinase, protease and other cell wall degrading enzymes. Additionally, there was no significant difference in the morphology of the different *A.auricula* strains treated by *T.pleuroticola*.

The mycelium interaction results were consistent with those Wu and Yan reported. However, the winding effect of *Trichoderma* was not found in this test. Wu thought that the mycelium twine only occurred in the *L.edodes*, which is very rare and was not the main reason *Trichoderma* infected edible fungi[15]. However, not all *Trichoderma* strains are able to inhibit the edible fungi by mycelium interaction. Innocenti reported that *T.pleuroticola* and *P.ostreatus* mycelium grew in parallel during the confrontation, and the former inhibited the latter only by competing for space and nutrition[26].

3.5 Effect of *T.pleuroticola* Volatile Substances on *A.auricula* Mycelium

3.5.1 Inhibition of *T.pleuroticola* Volatile Substances on *A.auricula* Mycelium

We evaluated the inhibition of *T.pleuroticola* volatile substances. The results showed that the inhibition rate of *T.pleuroticola* to H 29 and HW 15 was 13.46% and 10.44%, respectively, with a significant difference ($p < 0.05$), and HW 15 had relatively strong resistance (Table 1). Only weak inhibition was observed for the three days the test was conducted. This may suppose that the inhibition of *T.pleuroticola* is not mainly due to the secretion of volatile substances or that volatile antibiotic substances are not generally produced if it is not approaching the *A.auricula*.

Table 1 Influence of *T.pleuroticola* volatile metabolites on *A.auricula* colony

A.auricula	Control Group	Test Group	Inhibition(%)
H 29			13.46±0.16 a
HW 15			10.44±0.12 b

Different letters mean a significant difference at the 5% level

3.5.2 Analysis of Volatile Organic Compounds (VOCs) of *T.pleuroticola*

To study the volatile substances of *T.pleuroticola*, GC-IMS was performed to analyze the samples. The results presented the information of each sample in the form of three-dimensional topographic maps depicted in Fig. 6. The volatile substances of *T.pleuroticola* differed from KB, with a variety of volatile organic compounds in different concentrations.

The qualitative and quantitative results of the volatile compounds in *T.pleuroticola* are shown in Fig. 7 and Table 2. GC-IMS detected 52 signal peaks and 40 typical compounds were identified, but there were still 12 compounds with no qualitative results due to the limited data of the library database. Since monomer ions and neutral molecules might form adjunct substances in the drift region, several single compounds might produce multiple signals so that the same substance could detect monomers or dimers. Based on the identified compounds, the volatile compounds in *T.pleuroticola* were alcohols (9.05%), aldehydes (14.33%), ketones (15.25%), furans (1.26%), and esters (45.20%). Among the compounds most identified in *T.pleuroticola* culture filtrate were ethyl acetate (31.52%), Isopentyl alcohol (9.05%), acetone (8.03%), and Butanal (4.93%).

The bioactive compounds detected in the culture of *T.pleuroticola* are members of the following compound classes: ethyl acetate could attract nematodes and collembolans, which nibble off fungus mycelium[27]; 2-heptanone has a penetrating fruity odor and is proven as attractants for the bacterium *Bacillus nematocidal* lures nematodes to their death by a Trojan horse mechanism[28]; nonanol has various biological activities such as antimicrobial and antifungal activities, inhibiting sclerotia and ascospore germination, and mycelial growth of *Sclerotinia sclerotiorum*[29]; isoamyl acetate and isoamyl alcohol, the main Ginjo-flavour components of sake, had broad antimicrobial activity against filamentous fungi, bacteria, yeast, and *Escherichia coli* and *Acetobacter aceti* were markedly sensitive to them. Isoamyl acetate was hydrolyzed to acetic acid and isoamyl alcohol. Acetic acid and isoamyl alcohol had a high affinity for the cell membrane and abolished respiration in *E.coli* by damaging the cell membrane[30, 31].

These volatile compounds may play a role in inhibiting the growth of *A.auricula*. However, we still do not know the mechanism of volatiles compounds inhibition. Furthermore, as the VOCs are complex mixtures and the environmental conditions induce their production, it is difficult to attribute the effects to individual volatile compounds or their mechanisms[32].

Table 2
GC–IMS integration parameters of *T.pleuroticola* volatile compounds

Compounds	CAS	Formula	MW	RI	Rt	Dt	Comment	Relative Content (%)
Ester								
Isoamyl acetate monomer	C123922	$C_7H_{14}O_2$	130.2	878.1	355.44	1.30765	monomer	1.2407 ± 0.0361
Isoamyl acetate dimer	C123922	$C_7H_{14}O_2$	130.2	876.8	353.99	1.7474	dimer	0.7316 ± 0.2272
Propyl acetate monomer	C109604	$C_5H_{10}O_2$	102.1	714.9	195.596	1.16412	monomer	0.8708 ± 0.0984
Propyl acetate dimer	C109604	$C_5H_{10}O_2$	102.1	714.6	195.343	1.47299	dimer	4.3263 ± 0.0329
Ethyl 2-methylpropanoate monomer	C97621	$C_6H_{12}O_2$	116.2	757.2	230.414	1.19724	monomer	0.6430 ± 0.0157
Ethyl 2-methylpropanoate dimer	C97621	$C_6H_{12}O_2$	116.2	753.6	227.426	1.56341	dimer	0.8481 ± 0.4103
Ethyl acetate	C141786	$C_4H_8O_2$	88.1	616.9	143.462	1.33714		31.5195 ± 0.8717
Isobutyl acetate monomer	C110190	$C_6H_{12}O_2$	116.2	769.8	240.786	1.23088	monomer	0.8981 ± 0.1338
Isobutyl acetate dimer	C110190	$C_6H_{12}O_2$	116.2	769.8	240.786	1.61141	dimer	3.9043 ± 0.6590
Diethyl malonate	C105533	$C_7H_{12}O_4$	160.2	1062.9	687.646	1.25485		0.2183 ± 0.0108
Aldehyde								
2-Methylbutanal monomer	C96173	$C_5H_{10}O$	86.1	684.8	172.279	1.16113	monomer	0.5863 ± 0.0292
2-Methylbutanal dimer	C96173	$C_5H_{10}O$	86.1	677.1	169.007	1.40015	dimer	0.9777 ± 0.3872
3-Methylbutanal monomer	C590863	$C_5H_{10}O$	86.1	655.7	159.946	1.17028	monomer	0.7931 ± 0.0970
3-Methylbutanal dimer	C590863	$C_5H_{10}O$	86.1	658.7	161.204	1.40524	dimer	2.0547 ± 0.2817
Butanal monomer	C123728	C_4H_8O	72.1	574.6	125.464	1.11231	monomer	3.6632 ± 0.1765
Butanal dimer	C123728	C_4H_8O	72.1	580.5	127.981	1.28216	dimer	1.2675 ± 0.0206
Pentanal monomer	C110623	$C_5H_{10}O$	86.1	702.8	185.619	1.18249	monomer	0.9319 ± 0.0954

Compounds	CAS	Formula	MW	RI	Rt	Dt	Comment	Relative Content (%)
Pentanal dimer	C110623	$C_5H_{10}O$	86.1	702.5	185.367	1.42049	dimer	0.2510 ± 0.1031
Hexanal monomer	C66251	$C_6H_{12}O$	100.2	796.7	266.283	1.25357	monomer	0.8031 ± 0.0586
Hexanal dimer	C66251	$C_6H_{12}O$	100.2	797.4	267.027	1.56103	dimer	0.6157 ± 0.1641
Heptanal monomer	C111717	$C_7H_{14}O$	114.2	905.5	394.08	1.33111	monomer	0.2829 ± 0.0044
Heptanal dimer	C111717	$C_7H_{14}O$	114.2	906.1	395.13	1.69563	dimer	0.0367 ± 0.0037
(E)-2-Heptenal monomer	C18829555	$C_7H_{12}O$	112.2	963	493.936	1.25281	monomer	0.2463 ± 0.0394
(E)-2-Heptenal dimer	C18829555	$C_7H_{12}O$	112.2	963.5	494.854	1.66498	dimer	0.0567 ± 0.0044
Furfurol monomer	C98011	$C_5H_4O_2$	96.1	861.6	337.428	1.08786	monomer	1.4107 ± 0.0775
Furfurol dimer	C98011	$C_5H_4O_2$	96.1	859.2	334.698	1.33061	dimer	0.2005 ± 0.0385
N-Nonanal	C124196	$C_9H_{18}O$	142.2	1103.7	770.912	1.47259		0.1556 ± 0.0104
Ketone								
2-Butanone monomer	C78933	C_4H_8O	72.1	602.4	137.294	1.05942	monomer	0.5660 ± 0.0700
2-Butanone dimer	C78933	C_4H_8O	72.1	599.5	136.035	1.24657	dimer	2.1852 ± 0.4211
Acetone	C67641	C_3H_6O	58.1	543.2	112.124	1.11434		8.0291 ± 0.4864
2-Heptanone monomer	C110430	$C_7H_{14}O$	114.2	895.9	377.29	1.25881	monomer	0.6277 ± 0.0286
2-Heptanone dimer	C110430	$C_7H_{14}O$	114.2	893.5	373.092	1.62935	dimer	0.4306 ± 0.0347
3-octanone monomer	C106683	$C_8H_{16}O$	128.2	995.8	550.865	1.30605	monomer	0.2896 ± 0.0483
3-octanone dimer	C106683	$C_8H_{16}O$	128.2	996.3	551.783	1.71231	dimer	0.0917 ± 0.0163
Acetoin	C513860	$C_4H_8O_2$	88.1	728.6	206.816	1.33554		3.0264 ± 0.4430
Alcohol								
Isopentyl alcohol monomer	C123513	$C_5H_{12}O$	88.1	742	217.892	1.2382	monomer	1.3668 ± 0.1422

Compounds	CAS	Formula	MW	RI	Rt	Dt	Comment	Relative Content (%)
Isopentyl alcohol dimer	C123513	$C_5H_{12}O$	88.1	742.6	218.399	1.48836	dimer	7.0629 ± 0.7864
Isopentyl alcohol polymer	C123513	$C_5H_{12}O$	88.1	740.5	216.625	1.79023	multimer	0.6222 ± 0.0796
Furan								
2-Butylfuran	C4466244	$C_8H_{12}O$	124.2	895.9	377.29	1.18048		0.1335 ± 0.0208
2-Pentylfuran	C3777693	$C_9H_{14}O$	138.2	1000.9	561.114	1.24912		1.1310 ± 0.2106

MW represents the molecular mass; RI represents the relative retention index; Rt represents the retention time; Dt represents the relative drift time (normalization); Relative Content was obtained by dividing the area of each peak by the total area of the chromatogram peaks ± standard deviation.

3.6 Effect of *T.pleuroticola* Culture Filtrate on *A.auricula* Mycelium

3.6.1 Inhibition of *T.pleuroticola* Culture Filtrate on *A.auricula* Mycelium

The results of the inhibition of nonvolatile obtained from the *T.pleuroticola* showed that the extracted compounds inhibited the growth of *A.auricula*, as shown in Table 3 and Fig. 8. The nonvolatile metabolites in *T.pleuroticola* culture liquid accumulated day by day, and the inhibition rate on *A.auricula* was gradually enhanced. The inhibition rate of *T.pleuroticola* culture filtrate cultured for 10 d to H 29 and HW 15 was 36.04% and 31.49%, respectively, with a significant difference ($p < 0.05$), and HW 15 had relatively strong resistance. In addition, the inhibition of nonvolatile metabolites on *A.auricula* was stronger than that of volatile metabolites.

Table 3 Colony of *A.auricula* on the medium c*ontaining T.pleuroticola* culture filtrate

A.auricula	Control group	Incubation time of *T.pleuroticola* culture filtrate (d)				
		2	4	6	8	10
H 29						
HW 15						

3.6.2 Analysis of Nonvolatile Organic Compounds (nVOCs) of *T.pleuroticola*

In order to predict the *T.pleuroticola* metabolites with inhibiting *A.auricula*, the nVOCs of *T.pleuroticola* were used for metabolic profiling based on the widely targeted metabolomics approach. One thousand nine hundred seventy-three metabolites were detected and grouped into 15 known classes based on the structure of the metabolites (Table 4). *T.pleuroticola* was rich in metabolites belonging to the classes of amino acids and metabolites, organic acids and derivatives, coenzyme and vitamins, and nucleotide and metabolomics.

Table 4
Classification of the detected metabolites in *T.pleuroticola*

Type	Number	Percentage (%)
Amino acids and metabolomics	194	5.72
Organic acids and derivatives	109	4.62
CoEnzyme and vitamins	17	2.83
Nucleotide and metabolomics	75	1.85
FA	36	1.05
Carboxylic acids and derivatives	47	0.91
Benzene and substituted derivatives	62	0.60
Heterocyclic compounds	50	0.60
GP	30	0.31
Alcohol and amines	35	0.19
Others	10	0.17
GL	4	0.15
SL	2	0.13
Aldehyde, Ketones, Esters	12	0.09
Tryptamines, Cholines, Pigments	4	0.02

The analysis of nVOCs of the culture filtrates of *T.pleuroticola* tested led to the identification of 10 molecules in the literature (Table 5). The results showed that tyrosine inhibited the growth of *A.auricula*. At the same time, threonine, glutamine, glycine, glutamic acid, phenylalanine, arginine, leucine, lysine and methionine promoted the growth of *A.auricula* at low concentrations and inhibited the growth of *A.auricula* at high concentration[33]. In addition, wortmannin is an antibiotic and has remarkably specific antifungal properties, inhibiting the spore germination of *Botrytis allii*, *Cladosporium herbarum* and *Rhizopus stolonifera* at the concentrations of 0.4–3.2 µg/ml[34]. Beta-sitosterol isolated from *T.asperellum* and *T.harzianum* showed inhibitory activity against *Rhizoctonia solani*, *Sclerotinia rolfsii*, and *Fusarium oxysporum*[35]. The secondary metabolites of *Trichoderma* sp. YM 311505 Daidzein exhibited antibacterial activity against *E. coli* with a MIC value of 64 g/mL[36].

However, a few studies on the identification of nVOCs produced by *T.pleuroticola* are present in the literature. To understand the antibacterial of the secondary metabolites produced, more studies should be performed.

Table 5
Nonvolatile compounds of *T.pleuroticola* identified by LC-MS/MS

Compound	MW	Formula	m/z	Ion	RT
L-Tyrosine	181.074	$C_9H_{11}NO_3$	-	[M + H]+	-
L-Glutamine	146.069	$C_5H_{10}N_2O_3$	-	[M + H]+	-
L-Glutamic Acid	147.053	$C_5H_9NO_4$	-	[M + H]+	-
L-Phenylalanine	165.079	$C_9H_{11}NO_2$	-	[M + H]+	-
DL-Leucine	131.095	$C_6H_{13}NO_2$	-	[M + H]+	-
L-Lysine	146.106	$C_6H_{14}N_2O_2$	-	[M + H]+	-
L-Methionine	149.051	$C_5H_{11}NO_2S$		[M + H]+	
Beta-sitosterol	414.386	$C_{29}H_{50}O$	-	[M + H-H2O]+	10.467
Wortmannin	428.147	$C_{23}H_{24}O_8$	-	[M-H]-	2.283
Daidzein	254.058	$C_{15}H_{10}O_4$	-	[M-]-	4.800

4 Conclusions

T.pleuroticola is a seriously pathogenic fungus in the production of *A.auricula*. It is not only a competitive disease but also an infectious disease, and the pathogenic mechanism results from multiple synergistic effects. Moreover, it was demonstrated that *T.pleuroticola* produced compounds with antifungal activity and might be used for inhibiting the growth of *A. auricula*.

Declarations

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Author Contributions Statement

B.Z. and Z.W. have designed this project and contributed to the main manuscript text. H.D. and Q.K. conducted experiments and wrote the main manuscript text. X.W., Y.L., H.Z., Y.Z., X.L., W. W. and B. B. X. have contributed to conducting the experiments, preparing figures, and writing. All authors reviewed the manuscript.

Conflict of interest The authors declare no competing interests.

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Figures

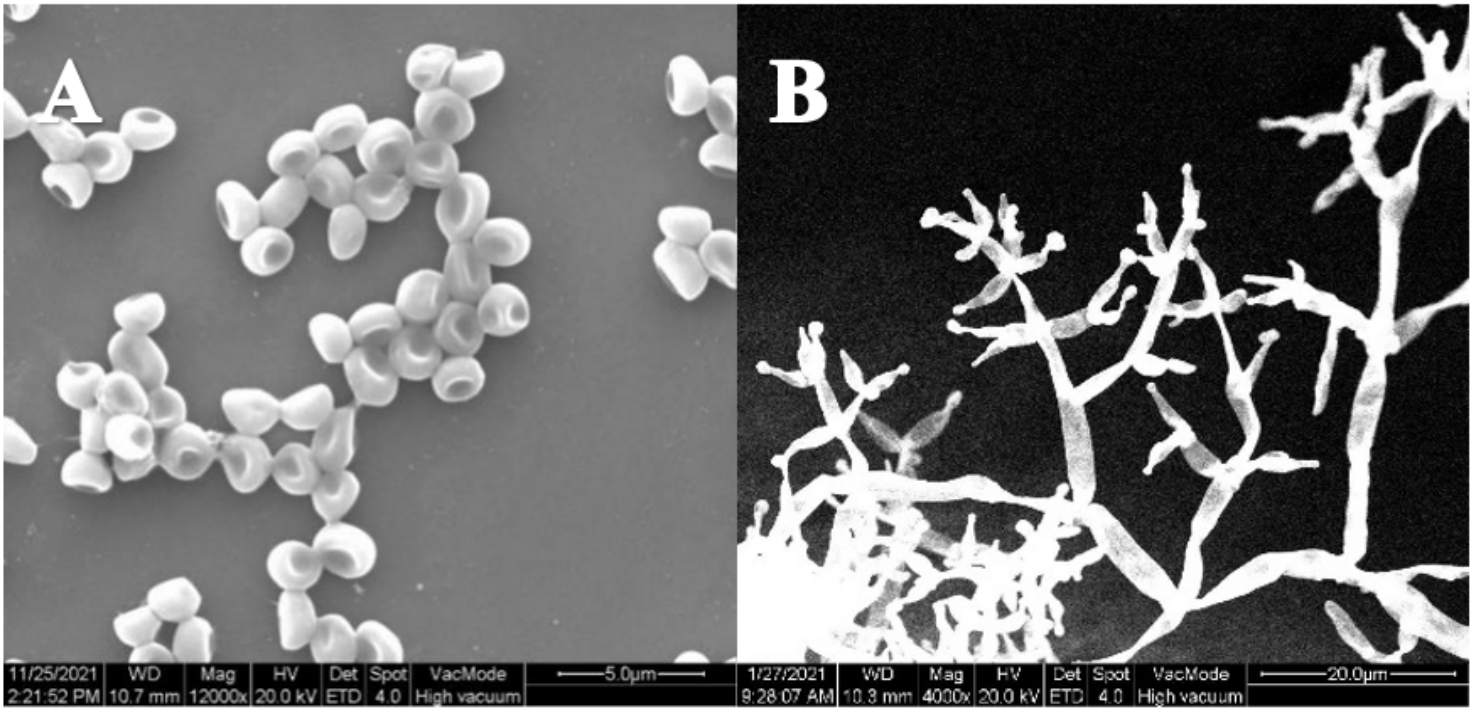

Figure 1

Conidia and conidiophore of KQQ-3. A: Conidia B: Conidiophore

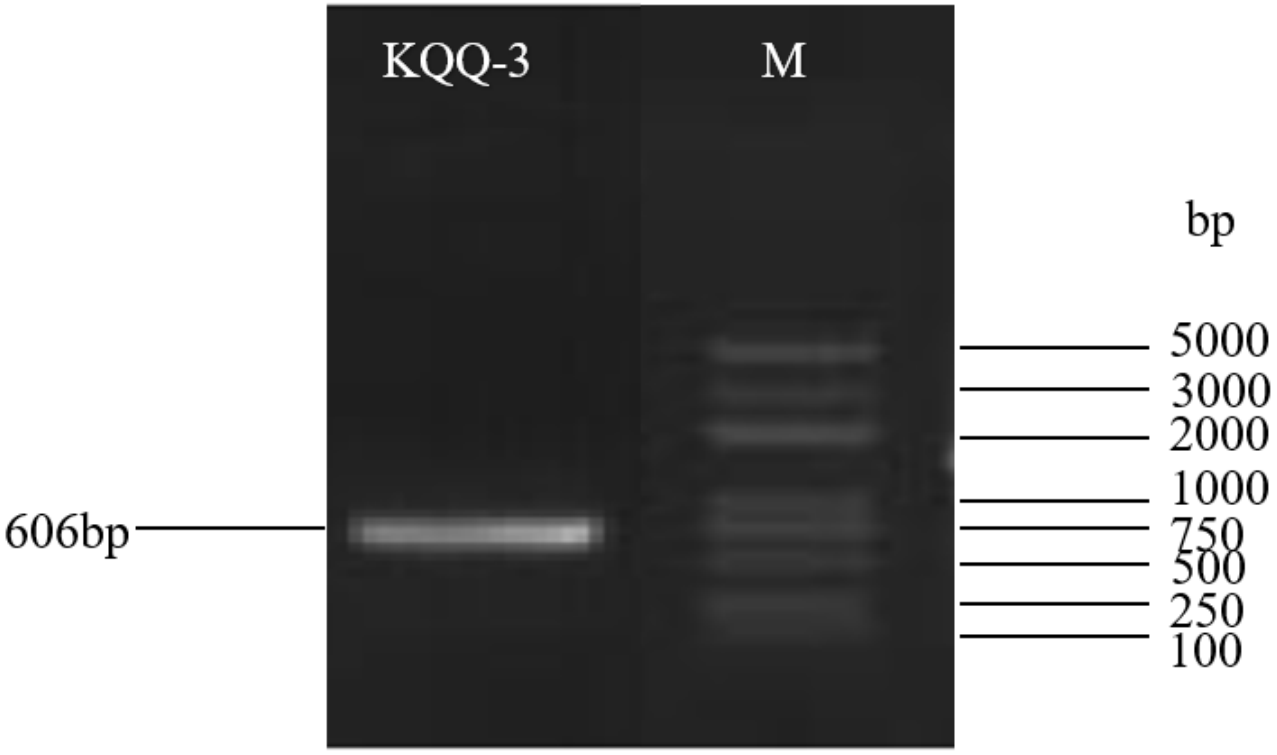

Figure 2

Agarose gel electrophoresis of KQQ-3

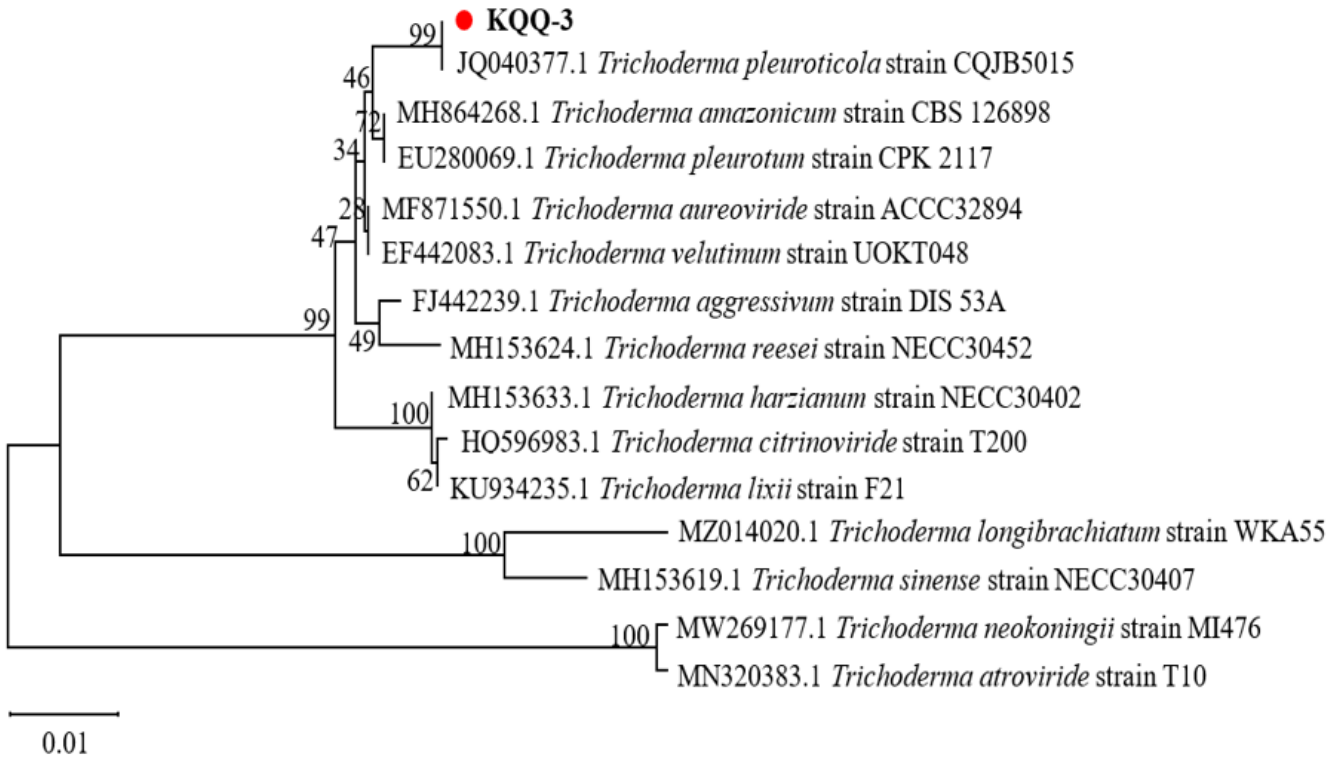

Figure 3

Phylogenetic tree of KQQ-3

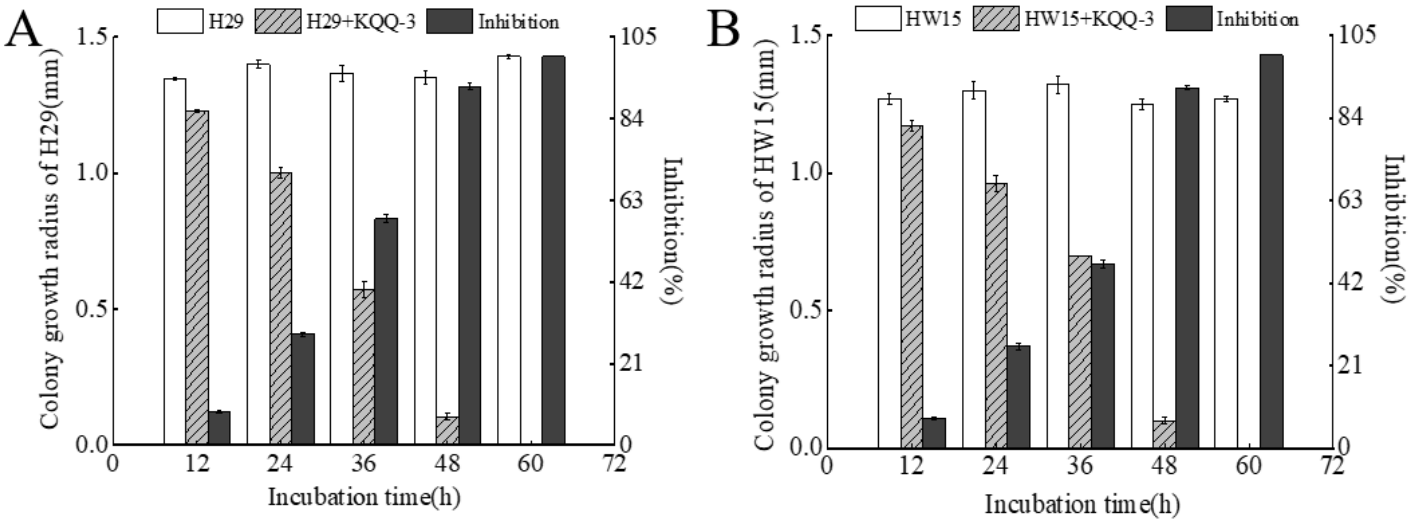

Figure 4

Inhibition rate of *T.pleuroticola* on *A.auricula* during confrontation culture

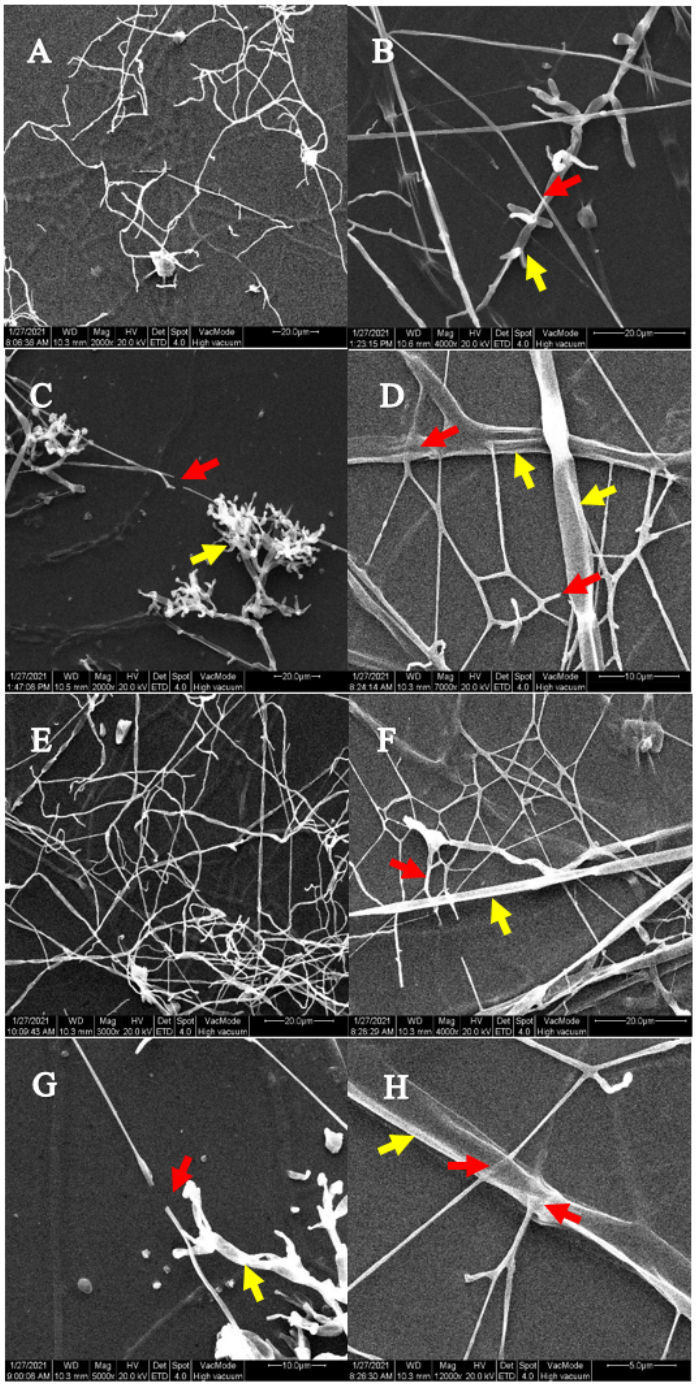

Figure 5

Mycelium interaction between *T.pleuroticola* and *A.auricula*

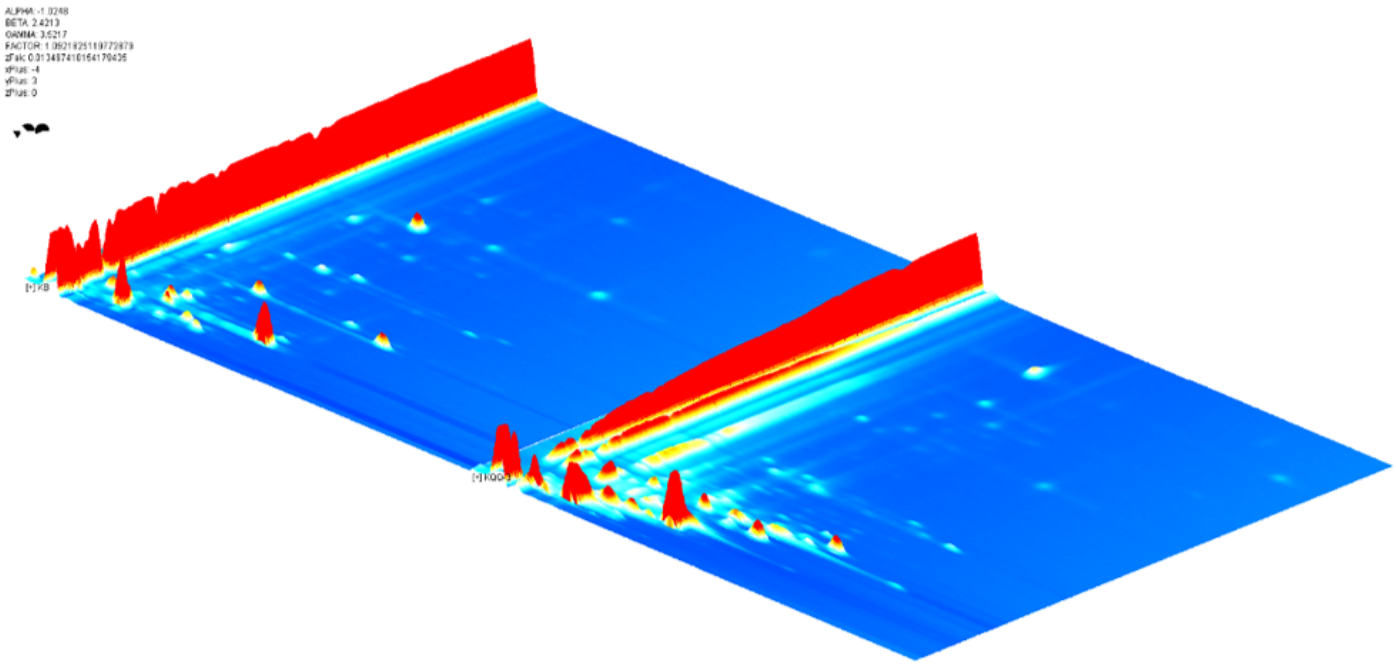

Figure 6

3D-topographic view of the volatile components of KB and *T.pleuroticola*

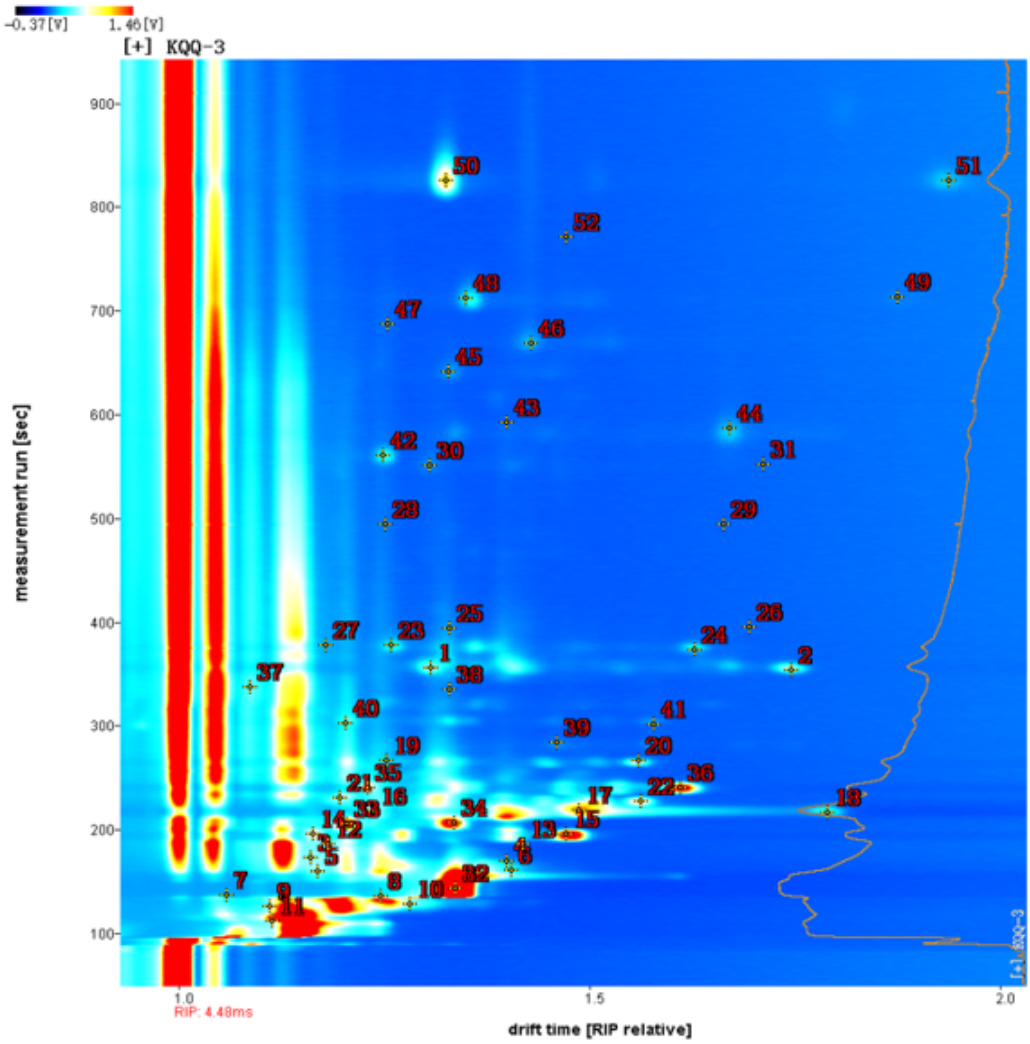

Figure 7

Topographic plots of GC-IMS spectra with the selected markers obtained from the *T.pleuroticola*

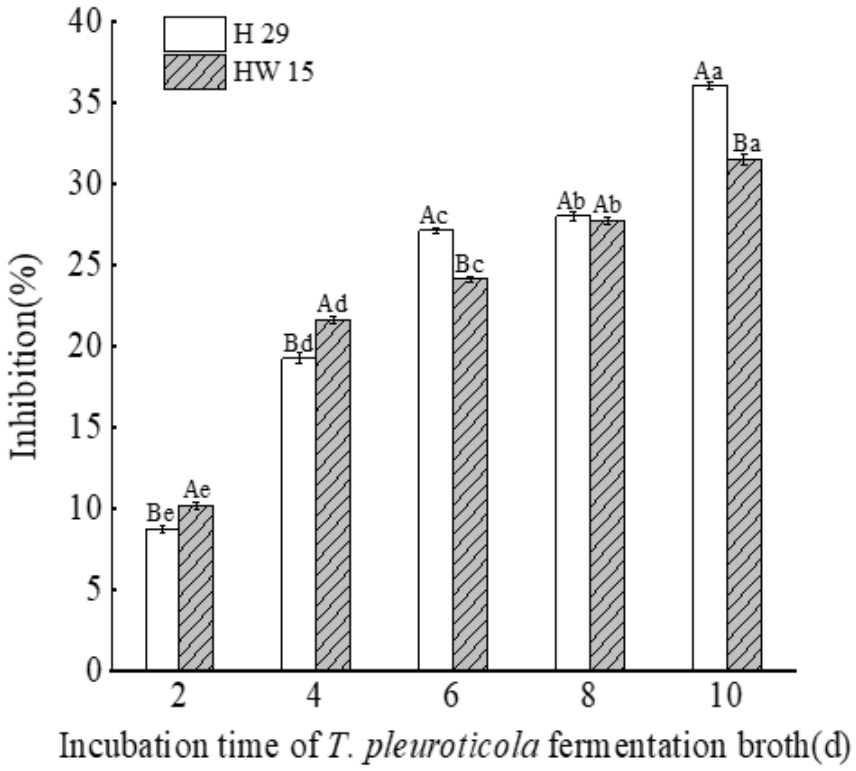

Figure 8

Inhibition of *T.pleuroticola* culture filtrate on *A.auricula* mycelium

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