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# Antifungal Effects of Fermented *Sophora flavescens* and *Eleutherococcus sessiliflorus* Extract

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**Abstract:** In this study, a microbial strain was isolated from humus soil to ferment *Sophora flavescens* and *Eleutherococcus sessiliflorus* extracts. The isolated microbial was identified as the *Bacillus* genus by 16S rRNA sequence analysis. The fermented plant extracts exhibited antifungal effects against four types plant pathogen, *P. carotorum*, *B. cinerea*, *C. fructicola* Sau-3, and *C. gloeosporioides*, according to incubation time. In particular, the fermented plant extracts showed the most activity for *Colletotrichum* genus in inhibiting mycelium growth. Metabolite changes in fermented *S. flavescens* and *E. sessiliflorus* extracts were confirmed through LC-Q-TOF/MS. Flavonoid and peptide derivatives were improved in fermented *S. flavescens* and *E. sessiliflorus* extracts compared to their unfermented counterparts. This study suggested that isolated *Bacillus* microbial fermentation could be a valuable tool in improving the bioactivity of *S. flavescens* and *E. sessiliflorus* extracts, with the potential to form more environmentally friendly antifungal agents.

**Keywords:** antifungal effects; *Sophora flavescens*; *Eleutherococcus sessiliflorus*; fermented plant extracts; LC-Q-TOF/MS analysis



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## 1. Introduction

Plant extracts are known for promising potential antimicrobial activity against microorganisms and fungi. Many studies have reported the antimicrobial properties. One such study investigated the antimicrobial properties of water and ethanolic extracts from plants such as roselle, rosemary, clove, and thyme, against a range of foodborne pathogens and spoilage microorganisms [1–3]. Microbial activities occur in characterized metabolites such as terpenes, flavones, flavonols, alkaloids, and phenylpropanoids [4,5]. In recent years, there has been growing interest in improving antibacterial activity through efficient metabolite changes [6–8]. Several research studies have been presented, showcasing innovative methods of enhancing the antioxidant and antibacterial properties of natural substances. For instance, the fermentation of *Salvia miltiorrhiza* roots by *Aspergillus oryzae* has been shown to significantly increase its antioxidant and antibacterial activity [9]. Additionally, the development of a colorimetric sensor with high selectivity for antibacterial agents using green synthesis of gold nanocubes (utilizing sunlight and horsetail leaf extract) has shown promising results [10]. Furthermore, the antibacterial activity of olive fruit extract has been improved through the use of silver nanoparticles [11]. These advancements in antibacterial research hold great potential for the development of more effective and eco-friendly solutions.

*Sophora flavescens* exhibits a variety of pharmacological properties, including anti-inflammatory, antioxidant, and anticancer activities [12,13]. The root contains a range of secondary metabolites, including isoprenoid flavonoids such as kushenol, kurarinone, and maackiain, as well as alkaloids and other flavonoids [14]. The key to these beneficial effects lies in the diverse array of compounds found within *S. flavescens*, with flavonoids and alkaloids being the major components. Through extensive research, over 200 compounds have been isolated from the plant, and at least 50 of these have been found to possess potent antifungal properties [15]. Notably, alkaloids such as oxymatrine and matrine, extracted from *S. flavescens*, have been identified as having significant pharmacological effects and are commonly used in the treatment of inflammatory conditions [16].

*Eleutherococcus sessiliflorus*, also known as *Acanthopanax sessiliflorus*, is a shrub or small tree native to Eastern Asia [17]. It has been used in traditional medicine for its various properties [18]. Some of the properties associated with *E. sessiliflorus* include immune-stimulating, anti-inflammatory, and anticancer activities [19]. It has been found to inhibit receptor activator of nuclear factor kappa-B ligand (RANKL)-induced osteoclast differentiation and has been evaluated for its potential to prevent osteoporosis [20,21]. The chemical constituents of *E. sessiliflorus* include triterpenoids, phenolic compounds, alkaloids, flavonoids, and other bioactive compounds. These constituents have been found in various parts of the plant, including the leaves, roots, and fruits [22,23]. Some specific compounds that have been identified include chiisanoside, chiisanogenin, eleutherosides, 3,4-seco-lupanetype triterpenoids, elesesterpenes AK, and lignans [24].

*Bacillus* fermentation is a process in which bacteria from the *Bacillus* genus are used to produce a variety of products through the breakdown of organic materials. *Bacillus* bacteria are aerobic, Gram-positive soil bacteria that have been used for decades in the production of foods such as fermented soybean products, cheese, and yogurt [25]. During fermentation, the bacteria utilize the nutrients in the organic material to produce various substances, including enzymes, organic acids, and other metabolites. These substances can have beneficial effects on health and are used in various applications, such as food, agriculture, and industry [26]. Examples of substances produced through *Bacillus* fermentation include nattokinase, phytase, amylase, protease, and biosurfactants [27,28]. The process is generally carried out in liquid or solid-state fermentation systems under controlled conditions to maximize product yield and quality.

In this study, *Phytophthora cactorum*, *Botrytis cinerea*, *Colletotrichum fructicola* Sau-3, and *Colletotrichum gloeosporioides* were used to confirm the antifungal activities of *bacillus*-fermented *S. flavescens* and *E. sessiliflorus* extracts. *P. cactorum* is a well-known and significant pathogen in the field of agriculture; it is known particularly for its detrimental effects on strawberries and other fruit crops [29]. This pathogen is often transported through plant material as a latent infection and can also survive in soil and plant debris [30]. In recent years, *P. cactorum* has caused significant losses in the strawberry industry in Finland and has also been responsible for an increase in culling of silver birch seedlings in forest nurseries due to stem lesions [31]. The impact of this pathogen on the agricultural sector cannot be overlooked and requires immediate attention for effective management and control.

*B. cinerea* is a fungal pathogen that is known to cause a soft decay of plant tissues, accompanied by the growth of fuzzy gray–brown mold [32]. It can have a significant impact on many plants, particularly those grown in humid conditions. The pathogen typically enters the plant's tissue through injuries, where it forms gray, velvety mats of sporulating tissues [33]. This can result in the death of infected twigs, causing them to die back several inches. Additionally, infected blossoms can lead to increased fruit drop and injuries to the developing fruit.

*C. gloeosporioides* is a well-known fungal plant pathogen that is responsible for causing bitter rot in a variety of crops worldwide, with a particular impact on perennials in tropical regions [34]. This pathogen is known to infect a range of important host plants, including citrus, yam, papaya, avocado, coffee, eggplant, sweet pepper, and tomato. In fruits, it

commonly causes black or brown lesions, while in inflorescence, blight, necrosis, and lesions with flecks and streaks can occur. The symptoms produced by this fungus vary depending on the host species and the infected tissue. It is considered the most widespread and serious post-harvest disease of several tropical fruits, such as mango, papaya, pitaya, and avocado [35]. The most damaging phase of this disease is when it remains dormant and undetected, particularly during the preclimacteric phase of fruit development.

*C. fructicola*, commonly known as Sau-3, is a well-known and extensively studied fungus belonging to the *C. gloeosporioides* complex [36]. This polyphagous fungus has been reported from all five continents, causing anthracnose, bitter rot, and leaf spotting diseases on over 90 cultivated and non-cultivated woody or herbaceous plant species [37]. Its wide distribution and ability to infect a diverse range of plants make it a significant pathogen, causing significant economic losses to important crops such as apple, pear, strawberry, and other Rosaceae and non-Rosaceae species [38]. This fungus has been reported to cause anthracnose on various plants in different regions, including white jute in China [39], cassava in Brazil [40], tea in Taiwan [41], and shine muscat in Korea [42]. In southern Brazil and Uruguay, it is the primary cause of apple bitter rot and Glomerella leaf spot [43,44]. Furthermore, *C. fructicola* has been identified as a major pathogen of strawberries worldwide, exhibiting strong pathogenicity to both leaves and petioles [45]. Its broad host range and ability to cause severe diseases make it a significant concern for farmers and researchers alike.

This study aimed to verify the antifungal activities of *S. flavescens* and *E. sessiliflorus* through fermentation by *Bacillus* species. A single microbial strain was isolated from the humus soil to ferment *S. flavescens* and *E. sessiliflorus*. The fermented extract showed efficacy compared with unfermented plant extracts by exerting antifungal effects on *Phytophthora cactorum*, *Botrytis cinerea*, *Colletotrichum gloeosporioides*, and *Colletotrichum fructicola* Sau-3. The plant extracts and their fermented extracts were analyzed using LC-Q-TOF/MS to identify metabolite changes.

## 2. Materials and Methods

### 2.1. Isolation of Microbial Strains

The fermentation strain used in this study was isolated from the humus from filed soil in Gyeongsang National University (35°10′50.3″ N 128°05′38.1″ E, Jinju-si, Gyeongsang-nam-do, Republic of Korea). Sterilized water (9 mL) was added to the collected humus soil (1 g) using a sonicator. Then, 1 mL of the solution was diluted with 9 mL of sterilized water. The diluted humus solution (200 µL) was spread on starch plate medium, which was incubated for 18 h at 37 °C. The colonies observed on the plate were segregated based on their type and then cultured. After the culturing, the amylase, CMCase, and protease activities were measured based on the size of the clear zone of the separated colonies, which were sprayed with 0.05% KI-I solution. Single strains with highly effective enzymes were isolated to use for fermentation of the plant sample.

### 2.2. 16S rRNA Sequence Analysis

Genomic DNA of the isolated microorganisms was extracted using a genomic DNA extraction kit (Bioneer, Daejeon, Republic of Korea). The reaction mixture consisted of genomic DNA (1 µL), universal primers (27F/1492R, 5 µM), DNA polymerase, dNTPs, and PreMix (20 µL, AccuPower PCR PreMix, Bioneer), and PCR was performed. The PCR conditions were as follows; initial denaturation (95 °C, 2 min), denaturation (95 °C, 30 min), annealing (55 °C, 30 s), and extension (72 °C, 30 s) for a total of 30 cycles. After the cycles, extension was carried out at 72 °C for 5 min. An Accuprep TM PCR purification Kit (Bioneer) was used to remove and purified the remaining primers, nucleotides, polymerase, and salts. Then, 30 µL of the elution buffer (10 mM Tris-HCl, pH 8.5) was added. The base sequences of purified PCR products were analyzed by Macrogen Co., Ltd. (Seoul, Republic of Korea).

### 2.3. Fermentation and Cultivation of Plant Extracts

The dried roots of *Sophora flavescens* and *Eleutherococcus sessiliflorus* were purchased from a local market in Haman-gun, Gyeongsangnam-do, Republic of Korea. Each 200 g of the dried roots of *S. flavescens* and *E. sessiliflorus* was extracted with 1 L of distilled water using a sonicator for 7 h at 80 °C to make *S. flavescens* and *E. sessiliflorus* extract samples. The LB broth (0.5 g) was added to 20 mL of the plant extracts (*S. flavescens* and *E. sessiliflorus*). The pre-cultured strain was inoculated with 1 mL based on the optical density (O.D.) at 0.7. The mixture was incubated for 48 h at 37 °C. Then, it was obtained via filtration through filter paper (No. 2, Whatman, Maidstone, UK).

### 2.4. Antifungal Activity

The plant extract, fermented plant extract, and pre-cultured strain broth were measured for antifungal activity against *Phytophthora cactorum*, *Botrytis cinerea*, *Colletotrichum gloeosporioides*, and *Colletotrichum fructicola* Sau-3 by monitoring the inhibition zone. *Phytophthora cactorum* (Lebert & Cohn) Schroeter (KACC 40166), *Botrytis cinerea* Pers. (KACC 40573), and *Colletotrichum gloeosporioides* (Penzig) Penzig & Saccardo (KACC 40003) were distributed from Korean Agricultural Culture Collection (KACC, Wanju-gun, Jeollabuk-do, Republic of Korea). *Colletotrichum fructicola* Sau-3 was provided by Gyeongsangnam-do Agricultural Research & Extension Services at Jinju-si, Gyeongsangnam-do, Republic of Korea. Cork borer (5 mm) holes were drilled in the center and edges of the high-pressure sterilized PDA (potato dextrose agar) plate. Then, 180 µL of fungal strain was dispensed into the center hole. The plant extracts, fermented plant extracts, and pre-cultured strain medium were centrifuged at 12,000 rpm for 3 min to obtain the supernatant, which was filtered through a 0.45 µm membrane filter. Then, the filtered samples (160 µL) were loaded into three edge holes on the fungal inoculated plate. The plate was incubated for 48 h at 25 °C to observe the inhibition zone as antifungal effects of the samples.

### 2.5. LC-Q-TOF/MS Analysis

Identification of the metabolites from the plant extract was performed by LC-Q-TOF/MS equipped with high-performance liquid chromatography (HPLC, Shimadzu, Kyoto, Japan) and quadrupole time-of-flight mass spectrometry (Q-TOF/MS, X500R, AB Sciex, Framingham, MA, USA). The mobile phases A and B were water containing 0.1% acetic acid and acetonitrile containing 0.1% acetic acid, respectively. An Infinity Lab Poroshell 120 C18 column (2.1 × 100 mm, 2.7 µm, Agilent Technology, Santa Clara, CA, USA) was used as the analytical column. The mass ionization method used was electrospray ionization (ESI) in a positive mode, with the following source parameters: ion spray voltage, 5500 V; ion source temperature, 450 °C; curtain gas pressure, 30 psi; nebulizer gas pressure, 50 psi; heating gas pressure, 50 psi; and declustering potential, 50 V. The mass detection range was configured from $m/z$ 50 to 1000. The resulting MS data were acquired by utilizing SCIEX OS 3.1.6 software.

### 2.6. Statistical Analysis

All experiments were conducted in triplicate to ensure accuracy and reproducibility. The antifungal activity data (the degree of inhibition of fungi growth) was collected using Sigma Plot (Version 10.0), which allowed for reliable statistical analysis of the results obtained from the experiments. The experimental results were then compared using a t-test, with a $p$-value less than 0.05 considered statistically significant enough to determine any differences between samples or treatments used in the experiment.

## 3. Results and Discussion

### 3.1. Identification of the Isolated Microbial Strains

The products amplified by 16S ribosomal RNA PCR were determined by DNA sequencing data identification of the GenBank database (http://www.ncbi.nlm.nih.gov) (accessed on 1 March 2023). As shown in Figure 1, a phylogenetic tree was observed with

the *Bacillus*, *Lactobacillus*, and *Streptomyces* genus. The dominance of *Bacillus*, *Lactobacillus*, and *Streptomyces* was 93%, 98%, and 89%, respectively. Furthermore, the isolated bacteria (SM38b) was determined to be a similar strain to *Bacillus amyloliquefaciens* and *Bacillus velezensis*. The *Bacillus* genus is a diverse group of bacteria that play important roles in various environments. *Bacillus subtilis* and *Bacillus cereus* have been found in grass, soil, and the gastrointestinal tracts of ruminants, humans, and sponges, respectively. In this study, *Bacillus amyloliquefaciens* and *Bacillus velezensis* were isolated from humus, a nutrient-rich layer of soil. These bacteria are frequently found in soil environments and have been found to have beneficial effects on plant growth. *Bacillus amyloliquefaciens,* as a Gram-positive bacterium, is known for its biocontrol abilities. It has been shown to effectively combat plant root pathogens in various agricultural settings. Similarly, *Bacillus velezensis* has the ability to inhibit the growth of plant pathogens by competing with other bacteria. These bacteria can also produce bacterial metabolic active substances that help strengthen the plant's immune system. Thus, *Bacillus velezensis* can contribute to promoting plant growth, suppressing pathogens, and maintaining the balance of the soil ecosystem.

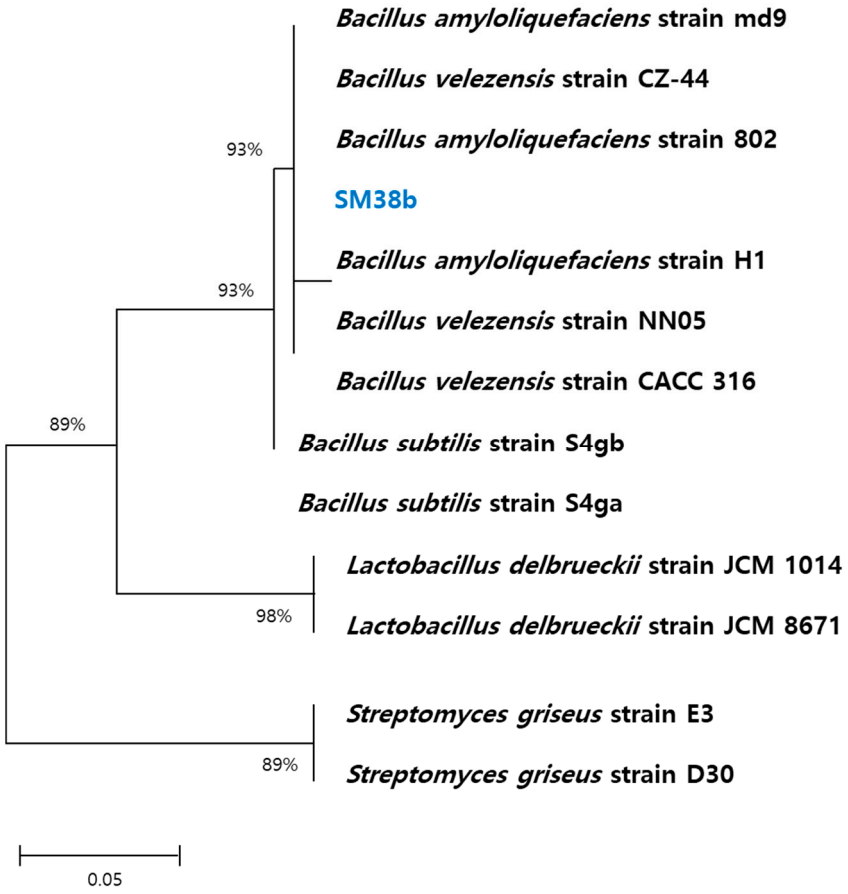



**Figure 1.** Classification and 16S rRNA sequences of bacteria isolated from humus soil.

### *3.2. Antifungal Effects of the Fermented and Unfermented Plant Extracts*

*S. flavescens* and *E. sessiliflorus* extracts were inoculated with the isolated microbial strain to prepare the fermented plant extracts. The fermented and unfermented *S. flavescens* and *E. sessiliflorus* extracts were tested for their antifungal effects against four plant pathogens including *Phytophthora cactorum*, *Botrytis cinerea*, *Colletotrichum fructicola* Sau-3, and *Colletotrichum gloeosporioides* according to incubation times of 24, 48, 72, 96, and 120 h, respectively.

*P. cactorum* is a soilborne pathogen that can infect many plant species. It causes various types of damage, including leaf spots, shoot blight, root rots, and crown rots [29,46]. *B. cinerea* is a destructive fungal pathogen that affects numerous plant hosts. It causes fuzzy

gray-brown mold and soft decay of plant tissues [47,48]. *C. fructicola* is known to cause anthracnose, bitter rot, and leaf spotting diseases on over 90 cultivated and non-cultivated woody or herbaceous plant species. The pathogen has been reported from all five continents and affects a wide range of crops, including apple, pear, strawberry, mango, avocado, and coffee [36,49]. *C. gloeosporioides* is a fungal pathogen that causes anthracnose and fruit rotting diseases in a wide range of host plants [34].

As shown in Figure 2 and Table 1, the fermented *S. flavescens* and *E. sessiliflorus* extracts showed more active antifungal activities against four fungi than the unfermented extracts. The growth inhibitory potential of *P. cactorum* was slightly exhibited by the effects of fermented *S. flavescens* extract (FSE) and fermented *E. sessiliflorus* extract (FEE). FSE and FEE exhibited the same inhibition patterns against *P. cactorum*, as follows: no inhibition at 24 h, mycelial growth inhibition with less than 2 mm at 48 h, and more than 2 mm from 96 h. Similarly, *B. cinerea* growth was inhibited by less than 2 mm up to 72 h and more than 2 mm thereafter by FSE and FEE treatments. The most inhibition of FSE and FEE was observed against the *Colletotrichum* genus (*C. fructicola* Sau-3 and *C. gloeosporioides*). For the inhibition of *C. fructicola* Sau-3, we observed that mycelial growth was hindered at a level of more than 2 mm by 96 h; moreover, it was inhibited by more than 3 mm when cultured for up to 120 h. The growth of *C. gloeosporioides* was inhibited by approximately 2 mm for up to 72 h, but FSE inhibited its growth by over 3 mm. The effects of FEE confirmed that *C. gloeosporioides* was suppressed at the 2 mm level throughout the entire culture period. These findings suggested that the fermented extracts of *S. flavescens* and *E. sessiliflorus* have potential as antifungal agents against these plant pathogenic fungi.

**Table 1.** Inhibitory effects of targeted fungi by the fermented and unfermented *Sophora flavescens* and *Eleutherococcus sessiliflorus* extract.

| Target Fungi | Incubation Time (h) | Antifungal Effects | | | |
|---|---|---|---|---|---|
| | | SE [a] | FSE [b] | EE [c] | FEE [d] |
| *P. cactorum* | 24 | - [e] | - | - | - |
| *B. cinerea* | | - | + | - | + |
| *C. fructicola* Sau-3 | | - | ++ | - | ++ |
| *C. gloeoporioides* | | - | ++ | - | ++ |
| *P. cactorum* | 48 | + | + | - | + |
| *B. cinerea* | | - | + | - | + |
| *C. fructicola* Sau-3 | | - | ++ | - | ++ |
| *C. gloeoporioides* | | - | ++ | - | ++ |
| *P. cactorum* | 72 | + | + | - | + |
| *B. cinerea* | | + | ++ | - | ++ |
| *C. fructicola* Sau-3 | | - | ++ | - | ++ |
| *C. gloeoporioides* | | - | ++ | - | ++ |
| *P. cactorum* | 96 | + | ++ | - | ++ |
| *B. cinerea* | | + | ++ | - | ++ |
| *C. fructicola* Sau-3 | | - | ++ | - | ++ |
| *C. gloeoporioides* | | - | +++ | - | ++ |
| *P. cactorum* | 120 | + | ++ | - | ++ |
| *B. cinerea* | | + | ++ | - | ++ |
| *C. fructicola* Sau-3 | | - | +++ | - | +++ |
| *C. gloeoporioides* | | - | +++ | - | ++ |

[a] SE means *Sophora flavescens* extract, [b] FSE means fermented *Sophora flavescens* extract, [c] EE means *Eleutherococcus sessiliflorus* extract, and [d] FEE means fermented *Eleutherococcus sessiliflorus* extract. [e] The effectiveness of antifungal activities is expressed depending on the degree of inhibition of fungi growth. The inhibition is displayed as +, ++, and +++, referring to less than 2 mm, less than 3 mm, and more than 3 mm in comparison to no inhibition, respectively.

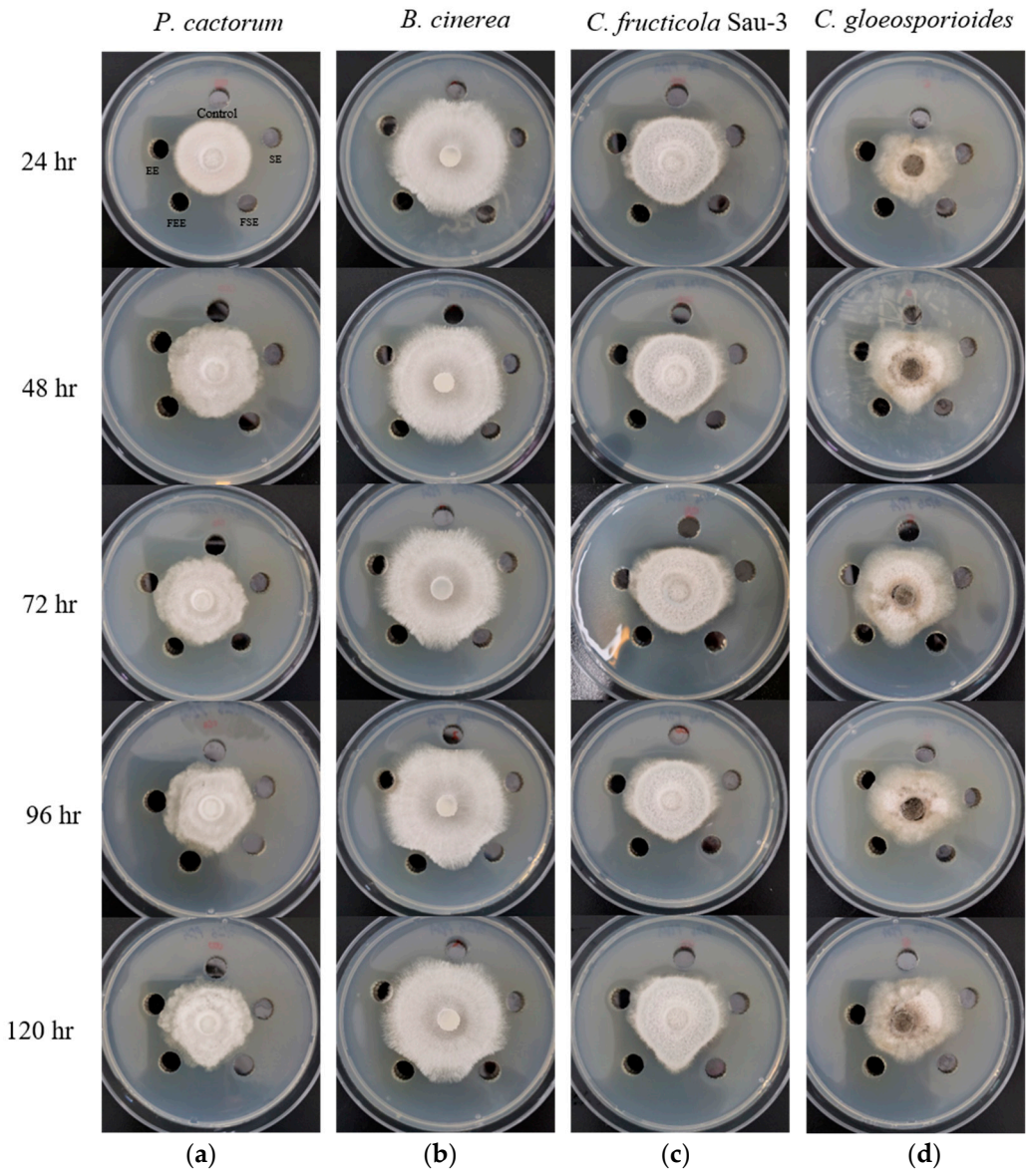



**Figure 2.** Antifungal effects of the fermented and unfermented Sophora flavescens and Eleutherococcus sessiliflorus extract against (**a**) *Phytophthora cactorum*, (**b**) *Botrytis cinerea*, (**c**) Colletotrichum fructicolo Sau-3, and (**d**) *Colletotrichum gloeosporioides*.

### *3.3. LC-Q-TOF/MS Analysis*

Figure 3 shows that the peaks from the BPI gram were well separated in the unfermented and fermented *S. flavescens* and *E. sessiliflorus* extracts, according to a positive mode of LC-Q-TOF/MS. The different patterns of the peaks represented the metabolite changes of the fermented extracts compared to the control.

The BPI gram of FSE displayed (Figure 3c) predominant seven peaks (peaks 1–7), but only five peaks (peaks 1, 2, 5–7) were identified by comparing observed and theoretical mass in the individual mass gram (Table 2 and Figure 4a). Peak 1 of FSE ($t_R$ = 1.79 min) had an observed ion mass at $m/z$ 249.1944 compared with the theoretical mass ($m/z$ 249.1967) used to annotate matrine. Peak 2 of FSE ($t_R$ = 3.46 min) possessed a reasonable error value of −4.87 ppm, which was presented as tryptophan. Peaks 5–7 of FSE were confirmed as flavonoids, named kushenol F, G, and L, respectively. Peak 5 ($t_R$ = 14.39 min) had an error value of −4.7 ppm between the observed $m/z$ 425.1944 and the theoretical mass at $m/z$ 425.1964, referred to as kushenol F. Kushenol G, with the chemical formula $C_{25}H_{28}O_8$, confirmed the molecular ion peaks at $m/z$ 457.1891 $[M + H]^+$ in the mass gram of peak 6

($t_R$ = 15.21 min). Peak 7 ($t_R$ = 17.58 min) showed an observed mass at $m/z$ 441.1921 with a +1.81 ppm of error value compared with the theoretical mass used to annotate kushenol L. Based on the identification of five peaks, FSE was found to contain one alkaloid, one amino acid, and three flavonoids.

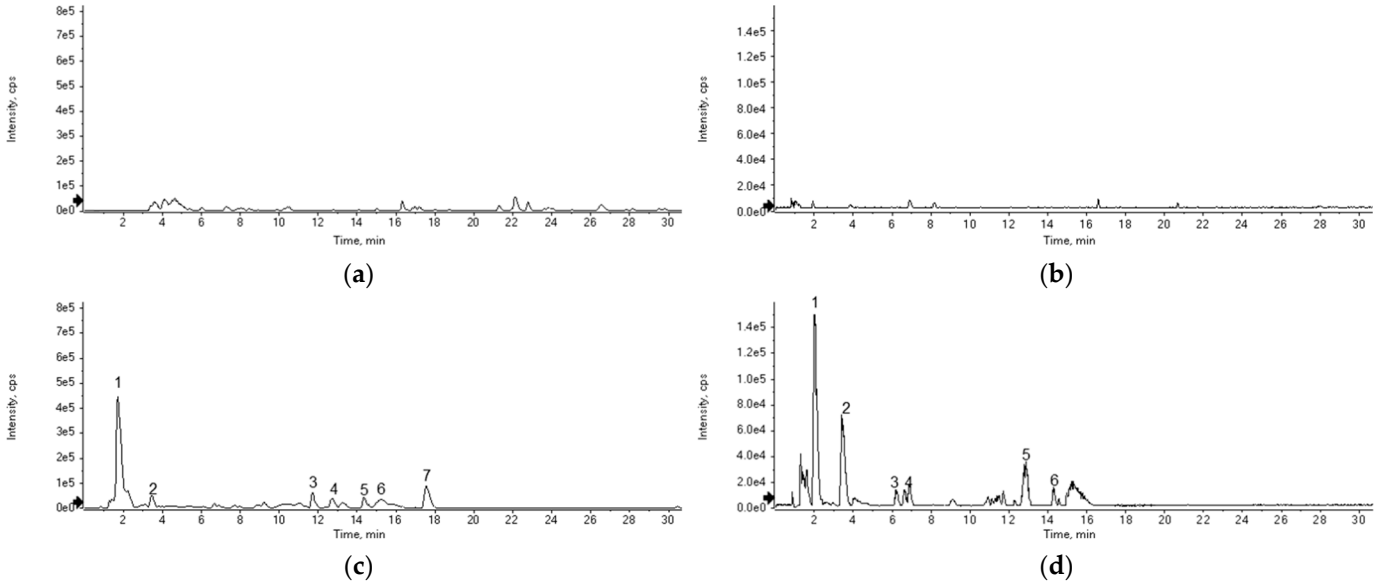


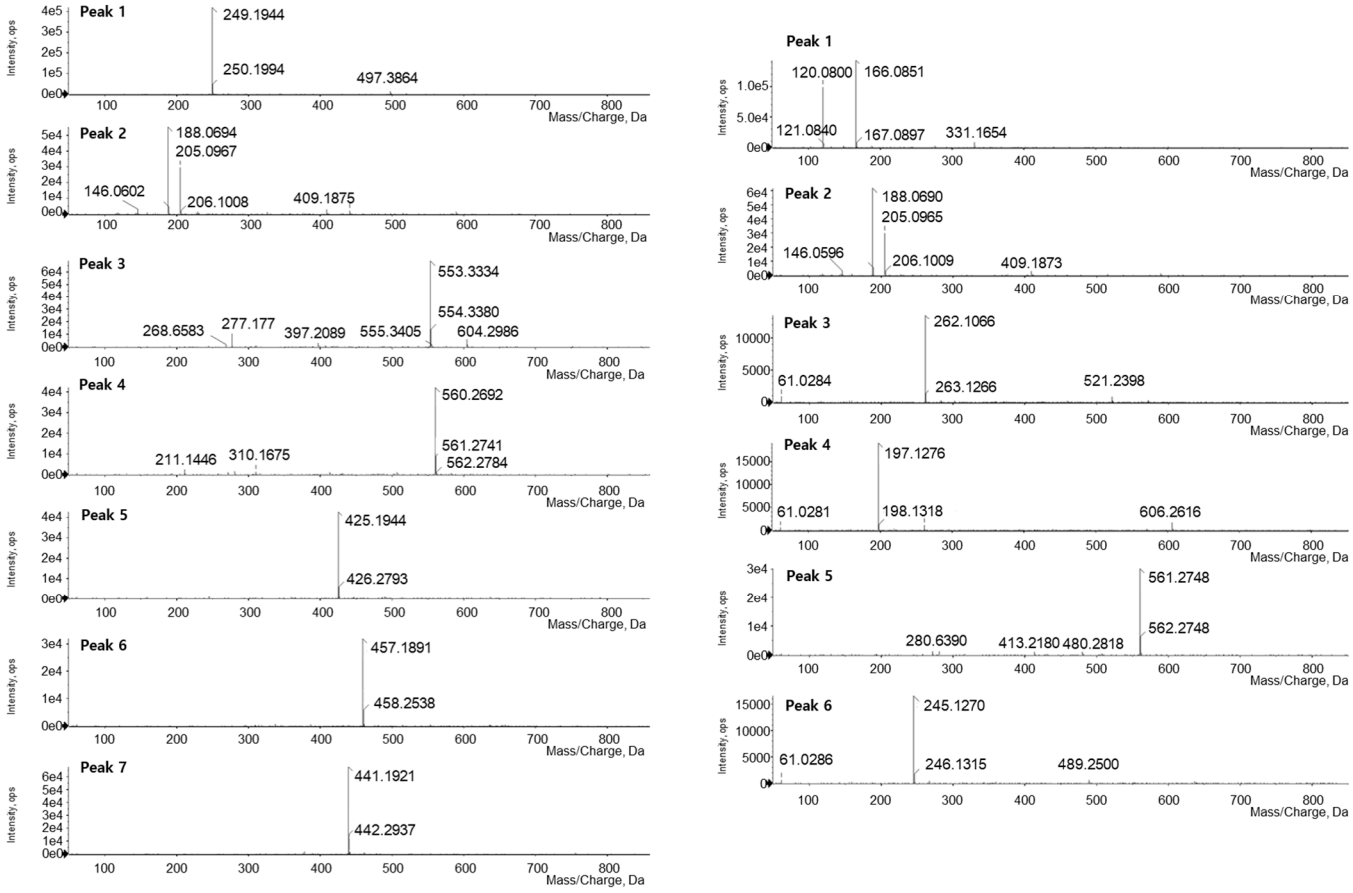

**Figure 3.** LC-Q-TOF/MS analysis of the fermented and unfermented *Sophora flavescens* and *Eleutherococcus sessiliflorus* extract. Base peak intensity (BPI) of (**a**) unfermented *Sophora flavescens* extract, (**b**) fermented *Sophora flavescent* extracts, (**c**) unfermented *Eleutherococcus sessiliflorus* extract, and (**d**) fermented *Eleutherococcus sessiliflorus* extract.

**Table 2.** Identification of metabolites from the fermented plant extracts using LC-TOF/MS.

| No. | $t_R$ (min) | Observed Ion ($m/z$) | Theoretical Ion ($m/z$) | Error (ppm) | Adducts | Formula | Identification |
|---|---|---|---|---|---|---|---|
| Fermented *Sophora flavescens* Extract | | | | | | | |
| 1 | 1.79 | 249.1944 | 249.1967 | −9.23 | M + H | $C_{15}H_{24}N_2O$ | Matrine |
| 2 | 3.46 | 205.0967 | 205.0977 | −4.87 | M + H | $C_{11}H_{12}N_2O_2$ | Tryptophan |
| 3 | 11.72 | 553.3334 | - | - | - | - | Unknown |
| 4 | 12.72 | 560.2692 | - | - | - | - | Unknown |
| 5 | 14.39 | 425.1944 | 425.1964 | −4.70 | M + H | $C_{25}H_{28}O_6$ | Kushenol F |
| 6 | 15.21 | 457.1891 | 457.1862 | +6.34 | M + H | $C_{25}H_{28}O_8$ | Kushenol G |
| 7 | 17.58 | 441.1921 | 441.1913 | +1.81 | M + H | $C_{25}H_{28}O_7$ | Kushenol L |
| Fermented *Eleutherococcus sessiliflorus* extract | | | | | | | |
| 1 | 2.06 | 166.0851 | 166.0868 | −10.24 | M + H | $C_9H_{11}NO_2$ | Phenylalanine |
| 2 | 3.48 | 205.0965 | 205.0977 | −5.85 | M + H | $C_{11}H_{12}N_2O_2$ | Tryptophan |
| 3 | 6.24 | 262.1066 | 262.1039 | +10.30 | M + H | $C_9H_{15}N_3O_6$ | Asn-Glu |
| 4 | 6.87 | 197.1276 | 197.1290 | −7.10 | M + H | $C_{10}H_{16}N_2O_2$ | Cyclo(-Pro-Val) |
| 5 | 12.87 | 561.2748 | - | - | - | - | Unknown |
| 6 | 14.31 | 245.1270 | 245.1290 | −8.16 | M + H | $C_{14}H_{16}N_2O_2$ | Cyclo(-Phe-Pro) |

FEE had six peaks (peaks 1–6) detected in the BPI gram within 30 min (Figure 3d). Among the peaks, five peaks were annotated with molecular ion peaks using individual mass grams (Table 2 and Figure 4b). Peak 1 ($t_R$ = 2.06 min) and peak 2 ($t_R$ = 3.48 min) were determined as phenylalanine and tryptophan, respectively, and the theoretical masses were found by examining the molecular ion peaks in on a mass gram. Based on the compatibility between their observed and theoretical masses, peaks 3, 4, and 6 were thought to be peptide derivatives. Peak 3 ($t_R$ = 6.24 min) displayed molecular ion peaks at $m/z$ 262.1066

$[M + H]^+$, indicating the presence of Asn-Glu with a theoretical mass of $m/z$ 262.1039. Peak 4 ($t_R$ = 6.87 min) was thought to be cyclo(-Pro-Val) due to its error value of −7.10 ppm, which was determined by comparing the theoretical mass with the chemical formula ($C_{10}H_{16}N_2O_2$) with the observed one. Similarly, cyclo(-Phe-Pro) also displayed a molecular ion peak at $m/z$ 245.1270 $[M + H]^+$, which was consistent with its theoretical mass of $m/z$ 245.1290. Thus, it was confirmed that peptide derivatives were more enhanced in FEE than in unfermented samples.




(**a**) (**b**)

**Figure 4.** Mass gram of individual peaks from (**a**) fermented *S. flavescens* extract (FSE) and (**b**) fermented *E. sessiliflorus* extract (FEE) from BPI chromatograms.

## 4. Conclusions

The isolated microorganism from humus soil was identified as belonging to the *Bacillus* genus (including species such as *Bacillus amyloliquefaciens* and *Bacillus velezensis*) due to its high dominance, which itself was based on a phylogenetic tree formed through 16S rRNA sequence analysis. The isolated *Bacillus* was inoculated into *S. flavescens* and *E. sessiliflorus* extracts for fermentation. The fermented plant extracts showed antifungal effects against *P. carotorum*, *B. cinerea*, *C. fructicola* Sau-3, and *C. gloeosporioides*, as plant pathogens, depending on incubation time (24, 48, 72, 96, and 120 h). The fermented plant extracts showed more active anti-fungal effects than unfermented extracts and were especially effective in inhibiting the growth of the *Colletotrichum* genus. Furthermore, the metabolites in the fermented plant extracts were identified by LC-Q-TOF/MS to determine the causality associated with improved biological effects. It was confirmed that the fermented *S. flavescens* and *E. sessiliflorus* extracts improved the flavonoids and peptide derivatives, respectively, based on BPI and individual mass grams. Overall, this study suggests that the fermentation-

enhanced anti-fungal effects of *S. flavescens* and *E. sessiliflorus* extracts may result in the production of specific metabolites.

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