ASPERGILLUS PIPERIS A/5 FROM PLUM-DISTILLING WASTE COMPOST PRODUCES A COMPLEX OF ANTIFUNGAL METABOLITES ACTIVE AGAINST THE PHYTOPATHOGEN PYTHIUM APHANIDERMATUM

Jelena Jovičić-Petrović1,*, Sanja Jeremić2, Ivan Vučković1, Sandra Vojnović2, Aleksandra Bulajić1, Vera Raičević1 and Jasmina Nikodinović-Runić2

1 Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Zemun, Belgrade, Serbia
2 Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvode Stepe 444a, 11000 Belgrade, Serbia
3 Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Serbia

*Corresponding author: jelenap@agrif.bg.ac.rs

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Abstract: Adding compost to soil can result in plant disease suppression through the mechanisms of antagonistic action of compost microflora against plant pathogens. The aim of the study was to select effective antagonists of Pythium aphanidermatum from compost, to assess the effect of its extracellular metabolites on the plant pathogen, and to characterize antifungal metabolites. The fungal isolate selected by a confrontation test was identified as Aspergillus piperis A/5 on the basis of morphological features and the internal transcribed spacer (ITS) region, β-tubulin and calmodulin partial sequences. Liquid chromatography-mass spectroscopy (LC-MS) analysis showed that gluconic and citric acid were the most abundant in the organic culture extract. However, the main antifungal activity was contained in the aqueous phase remaining after the organic solvent extraction. The presence of considerable amounts of proteins in both the crude culture extract as well as the aqueous phase remaining after solvent extraction was confirmed by SDS-PAGE. Isolated Aspergillus piperis A/5 exhibits strong antifungal activity against the phytopathogen Pythium aphanidermatum. It secretes a complex mixture of metabolites consisting of small molecules, including gluconic acid, citric acid and itaconic acid derivatives, but the most potent antifungal activity was associated with proteins resistant to heat and organic solvents. Our findings about the activity and characterization of antagonistic strain metabolites contribute to the understanding of the mechanism of interaction of antifungal metabolites as well as fungal-fungal interaction. The obtained results provide a basis for further application development in agriculture and food processing.

Key words: antifungal activity; antifungal proteins; Aspergillus piperis; organic acids; Pythium aphanidermatum; compost.

INTRODUCTION

Growing concerns regarding the sustainability requirements, environmental quality, human health and plant pathogen resistance to conventional pesticides have resulted in the need to find new approaches to plant protection [1,2]. Among others, an integrated approach in plant protection involves the use of residue management and compost as a soil organic amendment [3-5].

Many filamentous fungi are destructive pathogens of plants and are thus responsible for enormous crop losses worldwide. Pythium aphanidermatum is a widespread soil-borne plant pathogen and a member of the class Oomycetes [6]. It causes diseases in multiple plant species, leading to significant losses in agriculture [7]. New hosts and diseases of this pathogen are continuously reported and it has recently been also associated with human invasive wound infection [8]. P. aphanidermatum is susceptible to different biotic and abiotic factors during its saprophytic phase in soil [9]. Biotic factors include interactions with other microorganisms in soil, and P. aphanidermatum sensitivity to competition and antagonism may be the key potential for its biological control [4,10].
Soil amendment with compost is an agricultural practice commonly used to improve soil quality and manage organic wastes [11]. Recycling composted organic residues in agriculture can reduce the need for mineral fertilizers and improve the physicochemical and biological properties of cultivated soils [2,12]. The microbial community contributes to the soil and compost suppressiveness, but disease suppression may be based on the activity of a small proportion of the total biomass [13,14]. There are numerous examples of soil-borne pathogens controlled effectively by the application of compost as an organic amendment including suppression of *Pythium* species [15-17]. On the other hand, filamentous fungi are known as important inhabitants of compost and a rich source of bioactive compounds, with many of them identified as plant pathogen antagonists [18]. Compost produced in the open air (field compost), has been known for its high biocontrol potential for a long time [19].

In this study, we explored field compost from agro-industrial waste as a source of efficient phytopathogen fungal antagonists. Our aim was to select effective antagonists of *P. aphanidermatum* and to study and characterize their bioactive metabolites against this harmful plant pathogen.

**MATERIALS AND METHODS**

**Strains and media**

*P. aphanidermatum* and *Botrytis cinerea* cultures were from the collection of the Institute for Pesticides and Environmental Protection, Belgrade, Serbia, and *Fusarium oxysporum* was from the Culture Collection of the Institute for Phytomedicine, Faculty of Agriculture, Belgrade, Serbia. *P. aphanidermatum* culture was maintained on potato dextrose agar (PDA; Sigma Aldrich®, Saint Louis, USA) at 25°C and transferred to fresh media every week. *B. cinerea* and *F. oxysporum* were maintained on PDA at 4°C and subcultured every month. Potential fungal antagonists of *P. aphanidermatum* were isolated from mature compost made of stillage derived from plum-brandy production. Fungi were isolated by serial dilution technique on selective media, Rose Bengal Agar with streptomycin (30 µg mL⁻¹) [20]. The obtained monosporic cultures were maintained at 4°C on PDA. Selected isolates were cultivated in potato dextrose broth (PDB; Sigma Aldrich®, Saint Louise, USA) for metabolite production and characterization.

**Selection of antagonistic fungi by confrontation assay**

Selection of plant pathogen antagonists was performed by confrontation test as previously described [21]. Briefly, disks (5-mm diameter) obtained from the edge of actively growing colonies of plant pathogens and a specific isolate from compost were placed 3 cm apart on a PDA plate. The interaction between the two colonies was observed after incubation at 25°C in the dark, and the appearance of the colony of pathogens was also monitored and recorded. Incubation time was 3, 5 and 7 days for *P. aphanidermatum*, *B. cinerea* and *F. oxysporum*, respectively. The colony diameter of the plant pathogen was measured and compared to that of the control, which was the diameter of plant pathogen grown in pure culture on PDA plates. The test was conducted in three replications in two independent experiments. Inhibition percentage was determined as: (colony diameter of control – colony diameter after treatment)/colony diameter of control × 100.

**Taxonomic identification of the fungal isolate**

Morphological identification of the antagonistic fungal isolate was performed on the basis of colony appearance and microscopic examination (Leica DMLS, Leica Microsystems GmbH, Wetzlar, Germany) on 7-day-old culture grown on PDA at 25°C in darkness.

Molecular identification was carried out by amplification and sequencing of three genes (18S ITS region, β-tubulin and calmodulin). Fungal DNA was extracted by DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and amplifications were conducted in 25 µL reaction volumes containing 12.5 µL of Master Mix (Fermentas, Vilnius, Lithuania), 10 pmol of each primer, 100 ng of DNA and 6.5 µL of RNase-free wa-
The ITS region was amplified using an ITS1/ITS4 primer pair [22], the β-tubulin region was amplified using a Bt2a/Bt2b primer pair [23], and the calmodulin region was amplified using CL1/CL2A primer pair according to the previously reported protocol [24].

Amplified products were visualized by 1% (w/v) agarose gel electrophoresis. Polymerase chain reaction (PCR) products were sequenced on an ABI 3730XL Sequencer (Macrogen, Inc., Seoul, Korea) in both directions using the same respective primer pairs, and the BLASTN program (NCBI, http://www.ncbi.nlm.nih.gov) [25] was used for sequence similarity searches. Alignment of the obtained ITS sequence (586 bp), partial β-tubulin (524 bp) and calmodulin (653 bp) sequences and homologous sequences taken from GenBank was performed by Clustal W 2.0 algorithm [26]. A phylogenetic tree was constructed by the maximum-likelihood algorithm using Tamura-Nei distance correction and Bootstrap resampling method, all included in the MEGA6 package [27]. The phylogenetic tree was rooted using ITS, partial β-tubulin and calmodulin sequences of *Aspergillus robustus* NRRL 6362T as an outgroup. Sequences obtained within this research were deposited in the GenBank database with accession numbers: KF991390 (ITS sequence), KJ469441 (β-tubulin), and KP019623 (calmodulin A).

*Aspergillus piperis* A/5 maintenance, growth, crude culture filtrate and extract preparation

The A/5 isolate was precultured on PDA in darkness at 25°C for 7 days. For crude culture filtrate (CCF) preparation, A/5 was grown in PDB. Flasks containing 200 mL of sterile PDB were inoculated with five 5-mm-diameter mycelial disks obtained from the edge of the colony and incubated in darkness at 25°C for 7 days on a rotary shaker (Environmental Shaker Incubator ES-20, BIOSAN, Riga, Latvia) at 160 rpm. Liquid culture was filtered through sterile cheese cloth and then centrifuged at 25°C at 13000 x g (Centrifuge U-320, BOECO, Hamburg, Germany). The supernatant was carefully collected and sterilized by filtering through a 0.45-μm Millipore membrane (Merck KGaA, Darmstadt, Germany) to obtain CCF. In some cases, the CCF was autoclaved at 121°C for 15 min.

The CCF was subjected to extraction with an equal volume of ethyl acetate by vigorous shaking at 25°C, 150 rpm for 12 h. Ethyl acetate extract was separated from the remaining aqueous phase (AP) using a separating funnel, evaporated under reduced pressure and weighed. This crude culture ethyl acetate extract (CCE) was dissolved in dimethyl sulfoxide (DMSO) and used in antimicrobial screening. Any remaining ethyl acetate was removed from the AP under reduced pressure and was further used in antimicrobial screening.

A qualitative enzyme assay of the CCF and AP was conducted by API ZYM (Biomerieux, Marcy l’Etoile, France) according to the manufacturer’s instructions.

**Treatment of CCF and AP with proteinase K**

The pH values of the CCF and AP were measured electrochemically (CyberScan Ion 510, Eutech Instruments, Singapore), and then adjusted to 4.5 by addition of NaOH to 0.1 mol L⁻¹. Subsequently, CCF and AP were treated with 0.1 mg mL⁻¹ of proteinase K (Sigma, Saint Lois, USA) for 1 h at 37°C. The enzyme was inactivated by boiling for 15 min. Inhibition of *P. aphanidermatum* was tested in the presence of CCF and AP in following treatments: original CCF and AP, CCF and AP with pH adjusted to 4.5 and CCF and AP treated with proteinase K.

**Antifungal activity screening**

Inhibition of *P. aphanidermatum* by the CCF and fractions obtained after ethyl acetate extraction (CCE and AP) was assessed by dissolving appropriate amounts in PDA medium. PDA medium melted at 45°C was amended with sterile CCF, AP and CCE in a comparable range of concentrations of 2-10% (v/v). The 5-mm-diameter disks cut from the edge of the 2-day-old colony of *P. aphanidermatum* were plated centrally on media containing filtrate or extract. Plant pathogen growth on PDA without CCF was used as a control. The experiment was conducted in three independent
replicates. After two days of incubation at 25°C in the dark, the colony diameter was measured.

Protein separation by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

Proteins in CCF (10 mL) were precipitated by trichloroacetic acid (TCA) according to the standard protocol [29]. This procedure was also used for the precipitation of proteins from the AP after the ethyl acetate extraction of the supernatant of A. piperis A/5 culture, but the starting volume was doubled for adequate comparison (20 mL). In both cases, pelleted proteins were resuspended in 0.5 mL of 20 mM phosphate buffer pH 6.5 and additionally concentrated (5x) using Microcon Centrifugal Filters-cut off 10 kDa (Millipore, Merck KGaA, Darmstadt, Germany). Protein concentration was determined using the standard Bradford assay [30]. Prepared samples (25 µL) were analyzed by SDS-PAGE [31]. The polyacrylamide concentration in the separating gel was 12% (w/v). The Amersham Low Molecular Weight Calibration Kit for SDS Electrophoresis (GE Healthcare Buckinghamshire, UK) was used as a protein molecular size standard. Proteins were stained with Coomassie Brilliant Blue R 250 (Sigma, Saint Lois, USA).

Chemical analysis of A. piperis A/5 extracellular metabolites

Citric acid, gluconic acid, succinic acid and other chemicals were purchased from Sigma-Aldrich (Saint Lois, USA). LC-MS analysis was performed on the CCF, AP and CCE using the HPLC instrument Agilent 1200 Series (Agilent Technologies, Santa Clara, USA) with a Zorbax Extend C18 column (RR HT 50 × 4.6 mm, 1.8 µm) and a diode-array detector (DAD), coupled with a 6210 Time-Of-Flight LC/MS system (Agilent Technologies, Santa Clara, USA). The column temperature was 40°C with a constant flow rate of 0.5 mL min⁻¹. The mobile phase was a gradient prepared from 0.2% formic acid in water (A) and acetonitrile (B), according to the following program: 0-0.24 min, 5% B; 0.24-10 min 5-95% B; 10-15 min 95% B; 15-15.5 min 95-5% B; 15.5-18.5 min 5% B. High resolution electrospray ionisation mass spectrometry (ESI-MS) spectra were recorded in the range of 100-2500 m/z in both positive and negative ion mode, with 4000 V ion source potential and 70 V fragmentor potential.

Statistical analysis

Collected data were subjected to analysis of variance (ANOVA) by the software Statistica (StatSoft, Tulsa, OK, USA). Mean values of data were compared by Fisher’s LSD test at a significance level of P=0.05.

RESULTS

Selection of antagonistic fungal isolates from the compost heap sample

Pure cultures of 14 morphologically different isolates representing dominant compost fungi were obtained and tested to select for efficient antagonists of P. aphanidermatum. Results of the confrontation test showed that the isolate A/5 produced the highest inhibition percentage of P. aphanidermatum (81%), and was chosen for further study (Fig. 1a). The same isolate inhibited B. cinerea growth and completely overgrew its colony, while in a paired culture plate assay using F. oxysporum it induced colony diameter reduction on average by 33%. Based on the most efficient antagonistic activity against P. aphanidermatum, as well as exhibited activity against other phytopathogens, the isolate A/5 was selected for further study.

Isolate A/5 identification and characterization

The morphological features of the isolate A/5 were consistent with common features of Aspergillus sp., a member of section Nigri. The isolate was characterized by creamy white substrate mycelia with sparsely produced black conidial areas, transparent conidiophores, oval vesicles bearing transparent one-row phialides with conidia 3-4 µm in diameter (Fig. 1a and 1b). Upon 7 days’ incubation in liquid medium, the culture broth from pH 6.8 reached pH 2. Its maximal growth temperature was 45°C, indicating that it could
survive the composting conditions in the outer layers of a compost heap. On the basis of these features, the A/5 isolate was assigned as a member of the Aspergillus niger ‘aggregate’, which consists of morphologically indistinguishable taxa: A. niger, A. tubingensis, A. acidus, A. brasilienis, A. costaricaensis, A. lactocofeatus, A. piperis and A. vadensis [32]. Section Nigri consists of 26 closely related species that are hardly distinguishable by morphological properties [33].

Based on the ITS sequences, A/5 strain was also placed in the genus Aspergillus, and grouped with A. tubingensis and A. piperis strains. In the case of β-tubulin and calmodulin partial sequences, strain A/5 was unequivocally clustered with A. piperis NRRL 62631, based on 99% sequence identity. These results are in accordance with the search results of the BLAST NCBI nucleotide collection (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Strain A/5 was identified as A. piperis based on molecular identification and morphological features that are consistent with the description of A. piperis given previously [34].

Inhibition of P. aphanidermatum by A. piperis A/5 extracellular metabolites

Microbial extracts represent a good source of biotechnologically important new molecules. Aspergillus and Penicillium species are known as producers of a large number of useful metabolites, especially those with antimicrobial activity [35]. Indeed, the CCF of A. piperis A/5 exhibited significant activity against P. aphanidermatum. CCF in a concentration of 10% (v/v) completely inhibited the growth of the plant pathogen, while at lower concentrations of 2% (v/v) it stimulated P. aphanidermatum growth (Table 1). Surprisingly, the AP exhibited much higher activity in comparison to the CCF with 2% (v/v) inhibiting P. aphanidermatum by 92% (Table 1). Both the CCF and AP completely
retained their activities after autoclaving. The difference in CCF and AP activity could be attributed to the presence of small organic molecules, predominantly organic acids in the CCF that were removed during solvent extraction. From 100 mL of A. piperis A/5 CCF, on average 12.0 mg of ethyl acetate extract was obtained. This fraction contained gluconic and citric acid amounting to 90% (w/w) and some other organic acids, including succinic, muconic and itaconic acids (results not shown). Species of the genus Aspergillus section Nigri are known as organic acid producers in biotechnology. They are mainly used in the production of gluconic and citric acid [36]. Nevertheless, CCE, which predominantly consisted of organic acids, had no antifungal activity. Thus, from these results it could be concluded that the main bioactive metabolites in A. piperis were proteins or peptides, and that the presence of organic acids affected their activity.

Proteins were detected in CCF and AP (Fig. 2). The 7-day-old CCF of A. piperis A/5 usually contained 6.8-10.2 mg mL⁻¹ of proteins, while after ethyl acetate precipitation, the amount of protein remaining was between 1.8-2.5 mg mL⁻¹ (Fig. 2). On the other hand, the pH values of both CCF and AP were 2.0. To further assess the activity of CCF and AP, we raised their pH to 4.5 and treated both with proteinase K. The AP completely retained its antifungal activity after pH elevation to 4.5, while the effect of the CCF was reduced by 30%. Treatment of the AP treatment with protease K decreased its inhibitory effect on P. aphanidermatum growth almost two-fold; despite this, addition of this AP still led to significant inhibition of the phytopathogen. Protease K treatment reduced the inhibitory effect of the CCF, but only by 5.5% in comparison to the effect of CCF with adjusted pH (Fig. 3).

The qualitative enzyme activity test of the CCF showed strong activity of alkaline and acid phosphatases, phosphohydrolase and α-galactosidase, moderate activity of N-acetyl-β-glucosaminidase
Chemical characterization of extracellular metabolites

The crude culture filtrate and the aqueous phase remaining after the extraction of small organic molecules still retained a pH of 2 and were subjected to chromatographic separation and analysis (Fig. 4). The metabolic profiles of CCF and AP were comparable, with several organic acids identified in both (Fig. 2). The identification was based on high-resolution ESI-MS spectra recorded in positive and negative ion mode. The major components in CCF were gluconic acid and citric acid, and glucose acid, tartaric acid, succinic acid and muconic acid were also present (Fig. 2a). In addition, two compounds corresponding to the molecular formulae C11H18O5 (RT 4.62 min) and C11H16O6 (RT 4.70 min) were detected. They could be attributed to itaconic acid derivatives, the same or similar to those previously isolated from a seawater isolate of *A. aculeatus*, namely 9-hydroxylitaconic acid and 9-hydroxylitaconic acid-4-methyl ester [42].

Apart from citric acid, the AP contained considerably lower amounts of all acids in comparison to the CCF (Fig. 4b). In both fractions, the presence of polypeptide component was detected, based on the characteristic ESI-MS spectrum recorded in positive ion mode [results not shown].

### DISCUSSION

We have shown that field compost from agricultural waste is a good source of new organisms that can possibly be used as biocontrol agents against important phytopathogens. *A. piperis* A/5 from composted plum-brandy production stillage displayed significant antagonistic activity against the important plant pathogen *P. aphanidermatum*, causing between 40-90% inhibition. According to the achieved inhibition, the A/5 isolate was comparable to some known *P. aphanidermatum* antagonists, such as *Trichoderma viride* 1433, which caused 72% inhibition in the confrontation test and 50.8% inhibition in glasshouse conditions [43]. Arunachalam and Sharma [21] reported that *P. aphanidermatum* was efficiently inhibited by 12

**Table 2.** Qualitative enzyme screening of *A. piperis* A/5 crude culture filtrate (CCF) and aqueous phase (AP) after ethyl acetate extraction.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>CCF</th>
<th>AP</th>
</tr>
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<tbody>
<tr>
<td>Alkaline phosphatase</td>
<td>+++a</td>
<td>++</td>
</tr>
<tr>
<td>Esterase[C4]</td>
<td>-b</td>
<td>-</td>
</tr>
<tr>
<td>Esterase lipase [C8]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lipase [C14]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Leucine arylamidase</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Valine arylamidase</td>
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</tr>
<tr>
<td>Cystine arylamidase</td>
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<td>Trypsin</td>
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<tr>
<td>α-chymotrypsin</td>
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<td>-</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Naphthol-AS-phosphohydrolase</td>
<td>+++</td>
<td>-</td>
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<tr>
<td>α-galactosidase</td>
<td>+++</td>
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<td>β-galactosidase</td>
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<td>β-glucuronidase</td>
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<td>α-glucosidase</td>
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<td>β-glucosidase</td>
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</tr>
<tr>
<td>N-acetyl-β glucosaminidase</td>
<td>++</td>
<td>-</td>
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<tr>
<td>α-mannosidase</td>
<td>-</td>
<td>-</td>
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<td>α-fucosidase</td>
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a+, weak positive activity; ++, positive activity; ++++, strong positive activity; b-, absence of activity

and weak activity of β-galactosidase (Table 2). The only activity detected in the AP was that of alkaline phosphatase (Table 2). N-acetyl-β-glucosaminidase is a lytic enzyme produced by numerous fungi and has been associated with plant pathogen inhibition [37]. Considering that no N-acetyl-β-glucosaminidase activity was detected in the AP (Table 2), the enhanced AP activity was most likely due to some other polypeptidaceous compound with a lower molecular weight.

The predominant protein bands in the CCF were 20 kDa and 38 kDa, while the most abundant protein in the AP was about 20 kDa (Fig. 2). This protein was solvent-tolerant and possibly involved in the antifungal activity of the AP. Representatives of genus *Aspergillus*, including *A. niger*, were previously reported to produce bioactive proteins [38-41]. However, these peptides are about 10-15 kDa, and we did not detect this particular fraction amongst precipitated proteins from *A. piperis* A/5 CCF or AP (Fig. 2).
different isolates of Trichoderma sp. in the confrontation test in a range from 56.5% to 86.4%.

Aspergillus spp. that are members of section Nigri are important fungi in biotechnology, providing enzyme activities (amylase, lipase) and as organic acid (citric, gluconic) producers [33]. There are a few studies describing Aspergillus from section Nigri as antagonists to plant pathogens. In a similar study, 10 fungal isolates from compost were antagonistic to F. oxysporum f. sp. melonis, with the two exhibiting the highest biological control activity being Aspergillus spp., members of section Nigri [17]. A. niger was efficient in the suppression of rice sheath blight caused by Rhizoctonia solani [44].

To date, this is the first report of A. piperis as an antagonist of P. aphanidermatum. Some of the antagonists of P. aphanidermatum described so far include Gliocladium virens, T. harzianum, T. viride, P. fluorescens, Agrobacterium radiobacter K-84 and Bacillus sp. [7,43]. Biocontrol in terms of introducing a living organism to the environment has certain limitations associated with safety [45]. These limitations require particular caution, and research into some naturally occurred antagonistic interactions are often directed at the isolation and characterization of bioactive metabolites. Thus, we have examined the effect of the A. piperis A/5 CCF, its organic extract and the aqueous filtrate remaining after organic extraction (AP) on P. aphanidermatum growth.

We established that A. piperis A/5 was capable of producing a mixture of organic acids in the liquid medium. There are different findings about P. aphanidermatum tolerance to low pH in the environment. Pythium species are found in soils with a pH ranging from 3.6 to 7.2 [9]. The presented results suggest that the main potential of A. piperis for the biological control of P. aphanidermatum is not in the organic acid production.

We have also shown the antifungal activity as well as the presence of proteins and protein activities in the crude culture filtrate and in the aqueous phase remaining after solvent extraction (Table 2, Fig. 2 and Fig. 3). N-acetyl-β-glucosaminidase is related to chitin hydrolysis whereby it hydrolyzes N-acetyl- β-D-glucosamine residues from the terminal ends of chitooligosaccharides [46]. Purified β-glucosaminidase from T. harzianum demonstrated antifungal activity against several plant fungal pathogens, including B. cinerea [47]. It was purified and biochemically characterized as the 149 kDa protein from A. niger [48]. Furthermore, similar to our study,
N-acetyl-β-glucosaminidase isolated from A. niger 419 was characterized by some degree of thermostability and resistance to different organic solvents [49]. On the other hand, it was reported that fungal N-acetyl-β-glucosaminidase (72 kDa) [47] is larger than the proteins detected in this study. Considering that no N-acetyl-β-glucosaminidase activity was detected in the AP (Table 2), the enhanced activity of the AP was most likely due to some other proteinaceous compound with a lower molecular weight.

Peptides and small proteins exhibiting antimicrobial activity have been described as a promising tool for the development of new antifungal therapies [50, 51]. They have been shown to be effective in plant disease control as well [52]. Antifungal peptides derived from fungi are more active than those isolated from bacteria and plants [53]. Besides their activity, a significant advantage of antifungal peptides derived from filamentous fungi is their stability in various environmental conditions, such as pH, temperature, enzyme treatment [54]. Some of the isolated antifungal proteins are PAF from Penicillium chrysogenum, AnAFP from A. niger, AFP from A. giganteus and AcAFP from A. clavatus [38, 41, 55, 56]. However, they do not usually show antibacterial properties [41].

Taking into account our bioactivity results, it can be concluded that the presence of organic acids and derivatives inhibited proteins with antifungal activity. Microbial interactions within a complex compost community are important during the composting process and also after compost addition to soil [57]. Compost used in agriculture becomes part of the food chain and it is very important to prevent its contamination with human pathogens [58]. Plant disease suppression with compost is related to the microbial diversity that develops during compost maturation [18]. Therefore, the isolated A. piperis A/5 can be considered as a valuable multitasking compost community member, acting as a plant pathogen antagonist and thereby contributing to compost suppressiveness. In addition to the clarification of its role in agroecosystems, the presented results also indicate its potential in biotechnology, particularly in the production of organic acids and antifungal compounds.

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Authors’ contributions: Jelena Jovičić-Petrović and Aleksandra Bulajić isolated and characterized fungal strains, Sanja Jeremić carried out bioinformatic analysis, Sandra Vojnović and Ivan Vučković characterized bioactive components of the fermentation broth. Vera Raičević and Jasmina Nikodinovic-Runic designed the study and prepared the manuscript.

Conflict of interest disclosure: The authors state that there is no conflict of interest.

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