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Ability of soil microorganisms to degrade aminopyralid and its effect on their growth

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Abstract: The study aimed at the isolation of microorganisms from aminopyralid (AP)-contaminated soil, evaluation of their ability to degrade AP, and examination of the effects of AP on microbial growth. *Geotrichum candidum*, *Cladosporium herbarum*, *Candida subhashii*, and *Paenarthrobacter nicotinovorans* were isolated and identified using enrichment. None of those strains were able to degrade 100 ppm AP within 2–3 weeks. In contrast, collection strains *Pleurotus ostreatus* 'Spoppo' and *Bacillus velezensis* FZB42 reduced AP concentration by 35.1% and 47.8%, respectively. Low sensitivity of growth to AP (400 ppm) on the malt-extract-agar medium was observed; inhibition values for *C. herbarum* and *G. candidum* were 52.4% and 22.8%, respectively, compared to 33.7% inhibition found with *P. ostreatus* 'Spoppo'. Promotion of fungal growth was observed at low AP concentrations in the Czapek-Dox medium, the highest effect being in *G. candidum*. The growth promotion effect was confirmed with *P. ostreatus* 'Spoppo'-growing on wheat straw contaminated with Mustang Forte and Corello herbicides; total fruiting body mass yield increased 1.25- and 1.37-fold, respectively. The study offers insight into future strategies for mitigating the environmental impact of synthetic auxin herbicides.

Keywords: soil microbiota; contamination; weed control; taxonomical identification; biodegradation capacity; liquid-chromatography mass-spectrometry analysis

The pyridine carboxylic acid family of synthetic auxin herbicides that includes aminopyralid (AP) has been widely used for the control of broadleaf weeds in agriculture. AP is highly soluble and exhibits low sorption to soil. It is stable for hydrolysis, and its toxicity for humans and animals is low, but the long-term effect on human health is unknown (Kashuba et al. 2005, US EPA 2020). Synthetic auxin

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herbicides mimic the plant auxin hormone indole-3-acetic acid (IAA) that controls the growth process and development of plant organs (Zhang et al. 2022). Auxins are also synthesised by bacteria and fungi, where they affect cell morphology, stimulate fungal growth and influence spore germination. They are also involved in symbiotic interactions between plants and microorganisms (Chanclud and Morel 2016, Keswani et al. 2020).

After field application, AP persists in the environment for a long time. When exposed to aerobic metabolism of soil microbiome, AP is degraded to CO_2 and non-extractable residues, which indicate mineralisation of the phenyl ring (Kashuba et al. 2005, Daehli et al. 2010). The half-life values in soil vary from 31.5 to 533.2 days (Kashuba et al. 2005). Aerobic soil degradation efficiency of AP indicated a 90% removal after 88–488 days (Daehli et al. 2010). The above studies worked with whole soil microbiomes, and the biodegradation capacities of individual soil microbes are not known. Our aim was to characterise the biodegradation capacity of individual soil microorganisms. Strains of fungi and bacteria were isolated from AP-contaminated soil, and their potential for biodegradation of the herbicide was measured. Their biodegradation capacity was compared with strains of fungi and bacteria renowned for their capacity to degrade various recalcitrant organopollutants but whose capacity to degrade AP was unknown. Those efficient biodegrades were represented by the bacterium *B. velezensis* FZB42 and the fungus *P. ostreatus* 'Spoppo' that were obtained from acknowledged collections. *B. velezensis* FZB42 is a ubiquitous bacterium whose broad capacity to degrade various herbicides, polyaromatic hydrocarbons and other persistent pollutants was proven (Jakinala et al. 2019, Sultana et al. 2021, etc.). *P. ostreatus* belongs to the group of wood-rotting fungi capable of degradation of recalcitrant compounds such as lignin, herbicides, polychlorobiphenyles, and phthalates (Gadd 2008, Chun et al. 2019, etc.).

As significant crop injuries by AP in several plants such as bell pepper, eggplant, tomato, strawberry and watermelon were documented (Fast et al. 2011, Koudela et al. 2023), we focused on research of biological tools reducing soil contamination by AP. Our study aimed at isolating and taxonomically characterising fungal and bacterial strains from AP-contaminated soil using MALDI-TOF MS and 16S rRNA and ITS region sequencing. This result represents a novel finding. The ability of the isolated strains to degrade AP was measured and compared with the biodegradation efficiency of *Bacillus velezensis* FZB42 and *Pleurotus ostreatus* 'Spoppo'. The degradation of AP was measured by the LC-MS/MS method. Further, the effect of AP on the growth of the isolated strains was studied in solid and liquid media to bring information on how AP affects the growth of microorganisms, which is unknown and would permit insight into the environmental effects at the level of the soil microbiome. The results documented a low efficiency of the isolated microorganisms in degrading AP and a relatively low sensitivity of various fungal and bacterial species to the inhibition of their growth by AP.

MATERIAL AND METHODS

Microorganisms and materials. The soil from which microorganisms were isolated was obtained from an experimental strawberry plantation in the Demonstration and Research Station in Prague Troja, Czech Republic (50.1217261N, 14.3991272E). The climate characteristics of the locality are moderately warm and dry area, 195 m a.s.l., average air temperature of 8.2 °C, and long-term average rainfall of 590 mm. The soil was manifold sprayed with Mustang Forte solution containing AP before it was used for the isolation of microorganisms. The soil samples were removed and used in experiments in 2021. The sandy loam soil was pedologically characterised as Eutric Fluvisol. The pH_{H_2O} value of the soil was neutral (range 6.76), total N (Primacs) 0.12% and C_{ox} 1.19%. Carbonates were present in small to trace amounts. Sorption capacity was moderate, and the sorption complex was mainly saturated. C:N ratio characterising the soil quality was 10:1. The contents of mineral nutrients in the soil were (mg/kg): NO_3^--N 9.77, NH_4^+-N 2.27 (extracted by 1% KCl in deionised water), Ca 2 783, K 300, Mg 164, P 372 (Mehlich 1984).

The following microorganisms were isolated from the soil at the Department of Biochemistry and Microbiology (DBM) of the University of Chemistry and Technology Prague: the fungi *Cladosporium herbarum* strain F-968, *Geotrich*um *candidum* strain F-966, *Candida subhashii* strain F-967, the bacterium *Paenarthrobacter nicotinovorans* strain DBM 3799. The fungal strains were deposited in the culture collection of microorganisms of Crop Research Institute, Prague, Czech Republic, and the bacterium in the culture collection of the Department

https://doi.org/10.17221/586/2024-PSE

of Biochemistry and Microbiology, University of Chemistry and Technology, Prague.

Other microorganisms studied were strains obtained from the following collections: *Bacillus velezensis* FZB42 (DSM 23117, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany), *Pleurotus ostreatus* 'Spoppo' (Sylvan Nederland, Horst, the Netherlands), *Pleurotus ostreatus* 'Ivory' (Sylvan Nederland, Horst, the Netherlands), and *Irpex lacteus* [617/93, Culture Collection of Basidiomycetes (CCBAS), Inst. Microbiol, Prague], *Trametes versicolor* (167/93, CCBAS), and *Ganoderma lucidum* (530/93, CCBAS).

Synthetic auxin herbicide Mustang Forte (AP 10 g/L, florasulam 5 g/L, 2,4-D 180 g/L) (Dow AgroSciences s.r.o., Prague, Czech Republic) was applied on the soil (2 L/ha) from which the microorganisms were isolated.

Enrichment isolation method. The enrichment cultivation method is used to obtain a culture capable of efficient biodegradation of organopollutants. After several passages, a population with the highest specific growth rate is expected to predominate in the consortium. In our case, the initial inoculum was prepared according to Strejcek et al. (2018). The soil suspension was incubated overnight under shaking with glass beads (100 rpm at 12, 20, or 37 °C) and inoculated (ratio 1:9) into minimal medium (Strejcek et al. 2018) amended with sterile sea sand (Lachner, Neratovice, Czech Republic) and AP (Shanghai Tianfu Chemical Ltd., Zhengzhou, China) as a sole carbon source that was used at final concentrations of 100, 1 000, 10 000 ppm. For each AP concentration, three parallels were used, each of them cultivated under a different temperature (12, 20 or 37 °C). The isolation was carried out using a modified method by Uhlík et al. (2016). The whole enrichment experiment represented 15 passages; starting from the 12th passage, the cultures were supplemented with yeast extract 0.25 g/L and a solution of minimum essential medium-vitamins (both Merck, Darmstadt, Germany). Controls without AP and controls without inocula were incubated under the same conditions as the enrichment consortia. The growth of enriched populations was checked periodically, since 11th passage using minimal medium plates with AP (1 000 ppm) as the sole carbon source. Microorganisms obtained from both types of solid media were inoculated on Plate Count Agar (PCA) medium and subjected to identification.

Identification of isolates. The obtained microorganisms were characterised by MALDI-TOF mass spectrometry (MS) using a direct transfer protocol according to Smrhova et al. (2022), and the protein spectra measured were compared with spectral library and identified using MALDI Biotyper® 3.1 (Bruker Daltonics, GmbH & Co. KG, Bremen, Germany). In the case that the identification by MS was not sufficient, the microorganisms were identified by taxonomical characterisation of the 16S rRNA gene or internal transcribed spacer (ITS) region sequence. The genetic analysis of bacterial genomic DNA was carried out using a procedure described by Smrhova et al. (2022). The sequences were processed in MEGA X and the EzBio Cloud Identify service was used for further classification (Smrhova et al. 2022). The fungal genomic DNA was extracted using Phire Plant Direct PCR Kit (Thermo Fisher Scientific, Waltham, USA). Internal transcribed spacer (ITS) strains' fragments were amplified with primers ITS1F and ITS4. The obtained sequences were checked and compared with Chromas and BioEdit programs. Both in the case of prokaryotes and eukaryotes, the species identity was determined by comparing the DNA sequence to the NCBI database using BLAST.

Long-term biodegradation experiment. Biodegradation experiments were carried out aseptically using a modified method of Novotny et al. (2001) in liquid Czapek-Dox medium pH 7.3 (Himedia, Bombay, India), aerobically (rotary shaker, 120 rpm) at 24 °C for 2–3 weeks. The initial concentration of AP was 100 ppm. Bacterial and fungal cultures were pre-incubated at 24 °C on a rotary shaker (120 rpm) for one week to grow up before the biodegradation experiment was started by adding AP. Three replicates for each culture were used in the experiment. 5 % (*v*/*v*) inoculum was used for both bacterial and fungal cultures. Bacterial inocula were grown overnight in LB medium pH 7.5 (VWR International, Stříbrná Skalice, Czech Republic) at 24 °C on a rotary shaker (120 rpm). Fungal inocula were grown in liquid Czapek-Dox medium at 24 °C for 1 week after inoculation with mycelium-covered, 1-cm agar cubes. Before being used for inoculation of the biodegradation cultures, the fungal inocula were aseptically homogenised in a Waring blender (3 times, low speed, 20 s). After removal of samples, bacterial and fungal biomasses were separated by filtration (pore size 0.22 μm) or centrifugation (4 000 rpm, 4 °C; Rotanta 460 R, Hettich, Germany), respectively. An aliquot of the separated medium was then diluted 10 000-fold with MiliQ® water and used for the analysis.

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The AP concentrations were measured in the supernatants by LC-MS. The analysis searched for metabolites originating from the biodegradation of AP. Samples were analysed using tandem mass spectrometry with a liquid chromatography system (LC-MS/MS) consisting of UHPLC chromatograph Exion (Shimadzu, Kyoto, Japan) coupled with mass spectrometer Q-Trap 4600+ (AB Sciex, Framingham, USA). Chromatography was carried out on Ace® Excel 2 Super C18 column (100 × 2.1 mm, 3 µm, 90 Å, Avantor, Radnor, USA) by gradient elution using Mili-Q water (A) and acetonitrile (B), both supplemented with 0.2% formic acid (*v*/*v*) as mobile phases. The gradient started with 3% of B (0–3 min), then ramped to 58% at the 5th min, where it was kept until the 7th min, then it returned to starting conditions (3% of B) at the 8th min and was equilibrated until the 12th min. The flow rate was 0.3 m/min, and the column was tempered at 35 °C. AP was ionised using ESI in positive mode, and the signal was collected by multiple reaction monitoring (MRM) of transitions 207/134 m/z (quantifier) and 207/161 m/z (qualifier). Samples were cooled at 15 °C during the analysis, and the injection volume was 5 uL. The acquisition and data processing was carried out using Analyst software 1.7.1 (AB Sciex, Framingham, USA). The amount of AP was calculated from an 8-point matrix-matched calibration curve based on the cultivation medium (concentration range 1 to 200 ng/mL) with a commercial analytical standard of AP (PESTANAL®, purity ≥ 98.0%, Supelco-Merck, Mainz, Germany). Acetonitrile for chromatography was purchased from Honeywell (Riedel-de Haën, Chromasolv^{TM}, Seelze, Germany).

Effect of AP on fungal and bacterial growth. Agar plates containing malt extract agar (MEA) (20 g/L malt extract, 24 g/L agar, VWR International, Stříbrná Skalice, Czech Republic) were used. They were inoculated with a 1 cm agar cube covered with fungal mycelium inserted in the centre of a plate and incubated at 24 °C. The fungal colony diameter was measured in time to determine the effect of AP on fungal growth. The effect of AP on growth was also measured in submerged cultures (Czapek-Dox medium pH 7.3, Himedia, India) at 24 °C (rotary shaker, 120 rpm). The difference between the maximal biomass yield obtained in the presence of AP and a control without AP was determined.

Statistical evaluation. Statistical analysis was carried out using the analysis of variance (ANOVA) and Fisher's *LSD* (least significant difference) test ($P \leq 0.05$) in TIBCO Statistica Ultimate Academic software (version 13.5; StatSoft Europe) GmbH, Hamburg, Germany). Error bars in column diagrams showing growth inhibition (Figures 2 and 3) express standard error.

RESULTS AND DISCUSSION

Isolation of microorganisms. The genetic potential of the indigenous microbial community adapted to a particular herbicide pollution is one of the key factors ensuring contaminant biodegradation in contaminated soil. In our study, soil exposed to repeated treatment with Mustang Forte herbicide containing AP was the environment from which microorganisms expected to have a potential for degradation of AP were isolated. To cover a broad range of microorganisms, including bacteria and fungi, different growth temperatures (12, 20, 37 °C) and various AP concentrations (0.5, 5, 50 mmol/L) were used as selective factors. In the first 11 subsequent passages, AP was used as the sole carbon source for the growth of the isolates. In the last four passages, yeast extract and a mixture of vitamins were used to support the growth of the isolated strains in the presence of 1 000 ppm of AP. The isolates were kept only in the presence of AP as the carbon source was spread on plates with a solid minimal medium with 1 000 ppm of AP as the sole carbon source and then reinoculated on PCA plates. All growing microorganisms were subjected to MALDI-TOF analysis for identification. The microorganisms were isolated under the following conditions: *G. candidum* 20 °C, 10 000 ppm AP; *C. subhashii* 12 °C, 10 000 ppm AP; *C. herbarum* 20 °C, 100 ppm AP; *P. histidinovorans* 20 °C, 1 000 ppm AP. Later, the bacterium *P. histidinovorans* was reclassified after sequencing to be *P. nicotinovorans*.

The capability of microorganisms to degrade AP. Fungi *C. subhashii*, *C. herbarum* and *G. candidum* and the bacterium *P. nicotinovorans* that were isolated from AP-contaminated soil were tested for the ability to degrade AP together with two other microorganisms, *B. velezensis* FZB42 and *P. ostreatus* 'Spoppo'. The two latter microorganisms were included in the experiment since they were shown to be able to efficiently degrade various recalcitrant organopollutants (Jakinala et al. 2019, Chun et al. 2019) and their ability to degrade AP was not known. The conditions of biodegradation were: liquid Czapek-Dox medium pH 7.3, shaken cultures (120 rpm), 24 °C. The initial concentration of AP was 100 ppm, and the removal of AP was measured over 2–3 weeks (Figure 1). The

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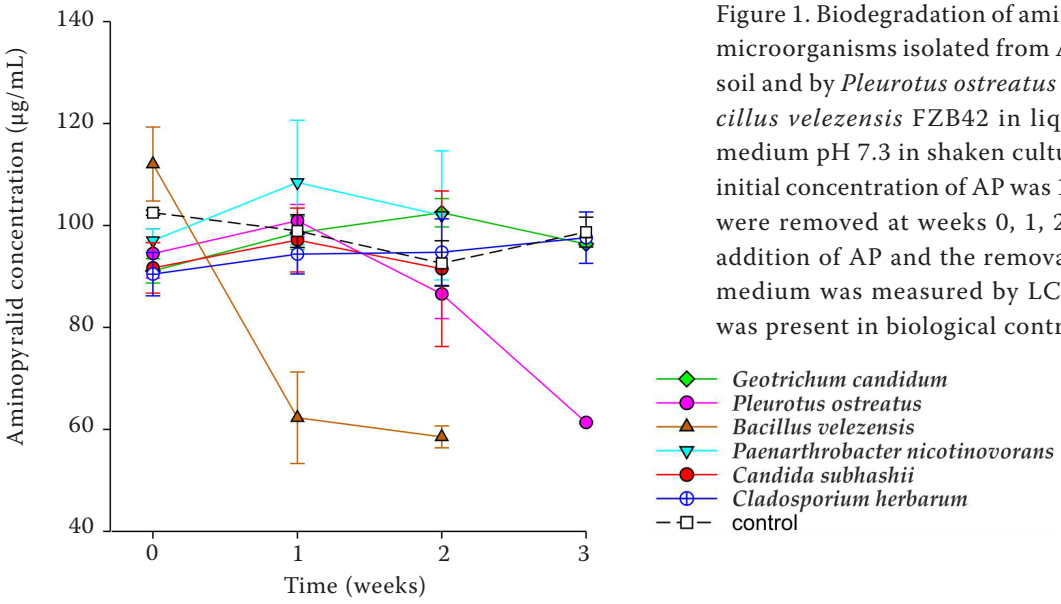

Figure 1. Biodegradation of aminopyralid (AP) by microorganisms isolated from AP-contaminated soil and by *Pleurotus ostreatus* 'Spoppo' and *Bacillus velezensis* FZB42 in liquid Czapek-Dox medium pH 7.3 in shaken cultures at 24 °C. The initial concentration of AP was 100 ppm. Samples were removed at weeks 0, 1, 2, and 3 after the addition of AP and the removal of AP from the medium was measured by LC-MS/MS. No AP was present in biological control cultures

degradation of AP by *B. velezensis* FZB42 was followed only for two weeks as the degradation process stopped after one week (Figure 1). The purpose was to obtain an insight into the potential of various soil fungi and bacteria to degrade AP.

C. subhashii, *C. herbarum*, *G. candidum*, and *P. nicotinovorans* did not decrease the AP amount after 3 weeks, whereas *P. ostreatus* 'Spoppo' and *B. velezensis* FZB42 reduced the concentration of AP by 35.1% and 47.8% (Figure 1). The analysis did not indicate a significant sorption of AP to microbial biomass, nor were any metabolites attributable to AP biodegradation measured in *P. ostreatus* 'Spoppo' and *B. velezensis* FZB42 cultures. To compare with the reduction rates reported for herbicides by fungi: *P. ostreatus* INCQS 40310 degraded 39–90% of atrazine (10 g/L) within 10–15 days, *Rigidoporus* sp. FDM21, *Fusarium* sp. T1-BH.1, and *Verticillium* sp. T1-BH.2 reduced 2,4-D and 2,4,5-T amounts by 20–30, 10–20 and 25–30% within two weeks (Pereira et al. 2013, Nguyen et al. 2022). *B. velezensis* MHNK1 decreased the amount of atrazine used at 200 mg/L by 69.4% after four days, whereas the degradation rates reported for *Rhodococcus* sp. BCH2 and *Pseudomonas* sp. EGD-AKNS were 75% after 7 days and 98.3% after 3.6 days (Kolekar et al. 2014, Bhardwaj et al. 2015, Jakinala et al. 2019).

During the experiment, a slight increase in AP concentration was observed with *C. herbarum*, *G. candidum* and *P. ostreatus* (Figure 1). The reason is unclear. Bacteria and fungi are able to synthesise auxins, namely IAA derivatives, that affect mycelium growth, morphogenesis and sporulation (Chanclud and Morel 2016), but the specificity of the LC-MS analysis does not support a view that the increase could be caused by those compounds.

The half-life of AP described in various soils spans from several weeks to as much as 1.5 years and indicates the persistence of AP in the environment after its application, but the members of the microbiota active in AP degradation have not been specified (Daehli et al. 2010, Tomco et al. 2016). In this context, our finding that the isolated soil microorganisms, even after long previous exposure to AP in the soil, were not able to degrade the herbicide may indicate that rather consortia of microorganisms than individual strains are responsible for the removal of the herbicide in the environment (Zhang and Zhang 2022).

Inhibition of growth of microorganisms by AP. The residual concentrations of AP in soil were documented to cause significant crop injuries in a number of plants such as bell pepper, eggplant, tomato, strawberry and watermelon (Fast et al. 2011, Koudela et al. 2023). The sensitivity of prokaryotic and eukaryotic microorganisms to AP is unknown and can be important with respect to the effect of residual AP on soil microbiomes. Residual AP concentrations as high as 0.018 mg/g and 0.120 mg/g at 90 days after application of Milestone herbicide at a rate of 0.123 kg/ha were reported in soils (Tomco et al. 2016). The effect of AP on the growth of soil microorganisms and other microorganisms that come in contact with soil and plant biomass contaminated with AP has not yet been investigated.

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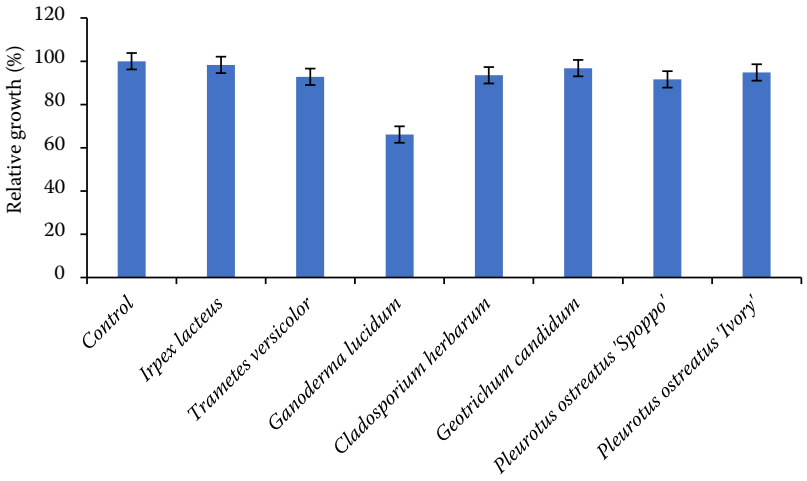

Figure 2. Growth of various microorganisms by 100 ppm aminopyralid (AP) on solid MEA medium. The growth measured was related to a biotic control growing without AP that represented 100% value

We decided to measure the effect of AP on microbial and fungal growth. The effect was measured with fungal organisms, such as the growth of a fungal colony on an MEA medium containing AP in time compared to a control without AP (Figure 2). The experiment included the fungal strains isolated from AP-contaminated soil, *C. herbarum* and *G. candidum*, and a selection of ligninolytic fungi, many of which were regularly detected in soil and can efficiently colonise soil environment, *P. ostreatus*, *I. lacteus*, *T. versicolor*, and *G. lucidum* (Novotný et al. 2001, Borras et al. 2010). Those latter fungi are close to fungal species that decompose plant biomass in soil. Figure 2 shows the relative size of fungal colonies on a solid MEA medium in the presence of 100 ppm AP, related to the growth of each fungus in the absence of AP. A significant growth inhibition was observed only with *G. lucidum*, where the colony size was reduced by 66.1%.

For comparison, the effect of AP at 100 ppm on growth of soil bacterium *B. velezensis* FZB42 and of some fungi was also investigated in a liquid Czapek-Dox medium where it was measured as stationary-phase biomass growth yield against the control without AP (Figure 3). No inhibitory effect of AP on the growth of the microorganisms tested was observed, which confirmed the results obtained on the MEA medium (Figure 2). The biomass growth yield of *G. candidum* in the presence of AP was higher by 27% compared to the growth without AP. The reason for such a behaviour is unknown, but it was also observed with other fungi when different concentrations of AP were tested (Table 1).

The previous experiments showed that fungal and bacterial growth was not very sensitive to 100 ppm AP. We further tested growth inhibition of *C. herbarum*, *G. candidum*, and *P. ostreatus* 'Spoppo' by AP at higher concentrations up to 500 ppm on solid MEA- and in liquid Czapek-Dox media (Table 1). The most sensitive fungus was the strain *P. ostreatus* 'Spoppo', whose growth was reduced by 35.6% at 200 ppm AP. The growth of soil-borne *C. herbarum* and *G can-*

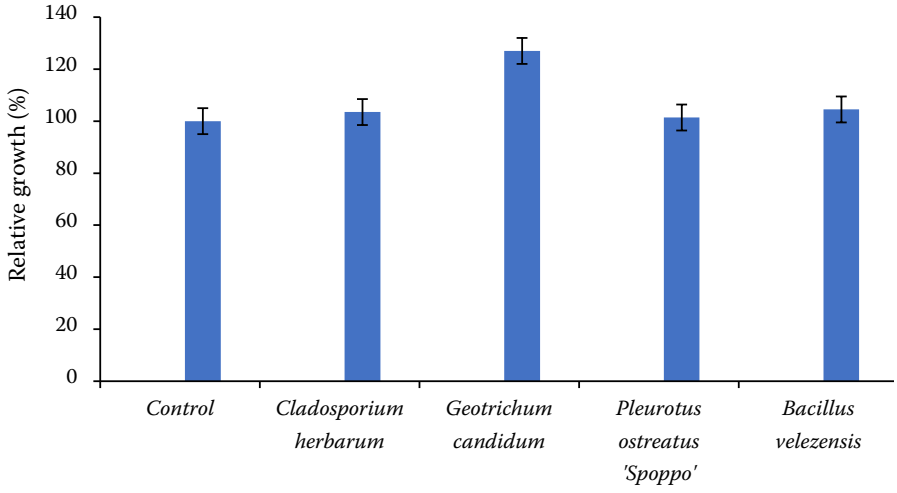

Figure 3. Growth of various microorganisms by 100 ppm aminopyralid (AP) on liquid Czapek-Dox medium. The growth measured was related to a biotic control growing without AP that represented 100% value

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Table 1. Growth of selected fungi at various concentrations of aminopyralid (AP) on solid and liquid media. The growth was related to biotic controls growing without AP that represented 100% value

AP (ppm)	*Cladosporium herbarum*		*Geotrichum candidum*		*Pleurotus ostreatus* ´Spoppo´	
Growth on solid malt extract agar medium						
	colony diameter (5^{th} day) (mm)	relative size of colony (%)	colony diameter (5^{th} day) (mm)	relative size of colony (%)	colony diameter (5^{th} day) (mm)	relative size of colony (%)
0	12.4 ± 0.5	100	31.1 ± 0.5	100	20.2 ± 9.5	100
100	11.6 ± 0.4	93.5	30.1 ± 0.4	96.8	18.5 ± 0.4	91.6
200	11.7 ± 0.4	94.4	30.0 ± 0.4	96.5	13.0 ± 0.9	64.4
300	11.3 ± 0.4	91.1	29.1 ± 0.6	93.6	15.2 ± 0.8	75.2
400	5.9 ± 0.5	47.6	24.0 ±.3	77.2	13.4 ± 0.3	66.3
500	–	–	23.4 ± 0.4	75.2	13.1 ± 0.6	64.9
Growth in liquid Czapek-Dox medium						
	growth yield mg dry biomass per 50 mL of medium	relative value (%)	growth yield mg dry biomass per 50 mL of medium	relative value (%)	growth yield mg dry biomass per 50 mL of medium	relative value (%)
0	613.1 ± 2.3	100.0	236.7 ± 18.6	100.0	410.7 ± 24.9	100.0
100	634.5 ± 13.0	103.5	300.5 ± 31.1	127.0	416.3 ± 5.2	101.4
200	639.3 ± 4.1	104.3	265.7 ± 39.9	112.3	392.0 ± 4.5	95.4
250	–	–	–	–	385.9 ± 9.0	94.0
300	582.1 ± 8.5	94.9	265.7 ± 10.1	112.3	–	–
400	594.6 ± 2.4	97.0	262.3 ± 23.9	110.8	–	–
500	540.5 ± 15.4	88.2	209.8 ± 8.4	88.6	–	–

Colony growth was read on the 5^{th} day after inoculation and is expressed in mm. Dry biomass growth yield was determined two weeks after inoculation. The data of colony diameter and dry weight (DW) growth yield are expressed as average values ± standard deviation

didum was significantly reduced at a concentration of 400 ppm AP by 52.4% and 22.8%, respectively. All three fungi were less sensitive to growth inhibition by AP when growing in a liquid medium compared to the growth in a solid medium (Table 1).

Stimulation of growth in a liquid medium observed in Figure 3 was confirmed for lower AP concentrations in all three fungi (Table 1). Those observations are in accordance with the results obtained with *P. sajor-caju,* where the production of mycelium was increased in the presence of auxins (Mukhopadhyay et al. 2005). This effect's mechanism is unknown but can probably be related to the biological and morphological action of these molecules in fungal organisms (Chanclud and Morel 2016).

Synthetic auxin herbicides are applied in large quantities in today's agrotechnological schemes. The study provides evidence of the poor ability of fungi and bacteria isolated from AP-contaminated soil to degrade AP, which points to the importance of understanding the effects of residual levels of the herbicide persisting long in the environment. Higher concentrations of AP are shown to affect the growth of fungal organisms, which can impact soil microbiomes and their function in nature.

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