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Secondary metabolites of the culturable endophytic fungus *Penicillium chrysogenum* and their plant growth regulator activity

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Short communication

Secondary metabolites of the culturable endophytic fungus *Penicillium chrysogenum* and their plant growth regulator activity

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Running title: SECONDARY METABOLITES OF *PENICILLIUM CHRYSOGENUM*

Abstract - This study reports the isolation of two natural compounds, zearalenone and hyrtiosulawesine, from the endophytic fungus *Penicillium chrysogenum* isolate SR192 which is associated with the invasive plant species *Solanum rostratum*. In general, both isolated compounds exhibited a dose-dependent stimulatory effect on the growth of four weed species when concentrations ranging from 5 to 100 $\mu\text{g mL}^{-1}$ were tested. However, suppressive effects were observed when the concentration was increased to 500 $\mu\text{g mL}^{-1}$, suggesting a biphasic response. Our findings indicate that these compounds have the potential to be further investigated as environment-friendly plant growth regulatory agents.

Keywords: Hyrtiosulawesine, *Penicillium chrysogenum*, *Solanum rostratum*, zearalenone

Introduction

Fungal endophytes are microbes that symbiotically inhabit the inner tissues of their hosts for part or all of their life cycles without causing visible pathogenic symptoms and potentially modulating plant reproductive capacity, growth dynamics, and resilience to abiotic and biotic stressors (Rho et al. 2018, Ripa et al. 2019). They have been identified as important sources of novel biologically active compounds like steroids, alkaloids, terpenoids, phenols, quinones, isocoumarins, lignans, and lactones (Dwibedi and Saxena 2019, Kuchkarova et al. 2020). These

compounds have significant potential for further research in medical applications or as eco-friendly agrochemicals, owing to their diverse range of biological properties, including antiviral, anticancer, immunosuppressive, antidiabetic, antioxidant, antimicrobial, phytotoxic, and plant growth regulatory activities (Koul et al. 2016, Kuchkarova et al. 2024a).

The increasing global population and land scarcity underscore the importance of plant growth regulators (PGRs) in sustainable agriculture. Despite significant advancements in the PGR industry between 2003 and 2022, existing research overlooks industry trends, challenges, and innovation opportunities. PGRs demonstrate considerable market potential, bolstering global crop production and contributing to sustainable development (Rademacher, 2015).

Numerous endophytic fungal species can be found in *Solanum rostratum*, and these species produce bioactive secondary metabolites (Kuchkarova et al. 2024b). An endophytic fungus called *Penicillium chrysogenum* SR192 isolated from the invasive plant *S. rostratum* produces secondary metabolites with significant plant growth regulatory activity (Kuchkarova et al. 2020). The study aimed to isolate and identify biologically active compounds with potential plant growth regulatory effects. Further, this study provides a theoretical basis for the responsible use of secondary metabolites from *S. rostratum* endophytic fungi.

Material and methods

The endophytic fungal isolate SR192 was obtained from the leaf of the invasive *Solanum rostratum* growing in suburban Urumqi, Xinjiang Province, China. The fungal isolate was identified as *P. chrysogenum* (Accession No. OM698376), based on sequence analysis of the rDNA ITS. The sequence fragment was identified using the basic local alignment search tool BLAST (<http://www.ncbi.nlm.nih.gov/>) of the NCBI and Mycobank (<https://www.mycobank.org>) databases. The isolate SR192 is preserved at the State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi, China (Kuchkarova et al. 2024a).

The endophytic fungal isolate SR192 was cultivated for 21 days at 28 °C and 120 r/min on a rotary shaker in a 14.4-liter potato dextrose broth (PDB) medium. Then, the broth was fully extracted with ethyl acetate four times, and the ethyl acetate extracts were evaporated by using a rotary evaporator (IKA RV8, Germany) under reduced pressure at 40 °C to harvest a brown oily gum (2.5 g). For fractionation, the dried extract was processed by silica gel (200-300 mesh, Qingdao Haiyang Chemical Factory, Qingdao, China) column chromatography (CC) with a n-hexane/ethyl acetate gradient (100:0, 90:1, 70:1, 50:1, 25:1, 12:1, 6:1, 4:1, 2:1 and 1:1 v/v) to afford ten fractions (F.1-10). All the fractions underwent preliminary screening for plant growth regulatory activity on the test plant *Amaranthus retroflexus* for promotive activity at 20 µg mL⁻¹ and inhibitory activity at 500 µg mL⁻¹. Fractions F.8 and F.9 with significant growth regulatory activity were combined (0.5 g, brown gum) and subjected to column chromatography on silica gel (300-400 mesh) using a gradient elution with ethyl acetate followed by ethyl acetate/methanol mixture (9:1, 6:1, 4:1, 2:1, 1:1, and 0:1 v/v), resulting in separation into 12 subfractions (F.9.1-F.9.12). Subfraction F.9.10 was further purified by column chromatography on Sephadex LH-20 (H&E Co. Ltd., Beijing, China) using chloroform/methanol (1:1) as an elution solvent, yielding 12 subfractions. Then, following the same method, subfraction F.9.10.5 was purified again by using CC on Sephadex LH-20 to obtain compound 1 (4.7 mg) and compound 2 (3.9 mg). Thin-layer chromatography (TLC) was applied to determine the purity of natural compounds (Toshmatov et al. 2024). Sulfuric acid/ethanol (5:95 v/v) mixture was sprayed onto fraction spots on the TLC plates, and then heated at 105 °C for visualization. ¹³C NMR, ¹H NMR, and 2D NMR

spectra were recorded on Varian MR-400 and VNMRS-600 NMR spectrometers with tetramethylsilane (TMS) as an internal standard (Harris et al. 2001).

The plant growth regulatory activity of isolated compounds was screened using two monocot plants, *Lolium perenne*, and *Setaria viridis*, and two dicot plants, *A. retroflexus* and *Medicago sativa*. Before use, the seeds of the test plants were surface-sterilized with 75% ethanol. The two compounds were diluted with methanol to obtain 5, 20, 100, and 500 $\mu\text{g mL}^{-1}$ concentrations. Subsequently, Petri dishes containing a layer of filter paper were filled with 0.5 mL of the corresponding methanol solution. The test seeds (10 seeds/dish) and 0.5 mL of distilled water were added to the corresponding Petri dishes once the methanol had evaporated entirely. Afterwards, Petri dishes were kept at 25 °C in the dark and sealed with parafilm. After seven days of seedling growth, the lengths of the roots and shoots were measured. Each bioassay was done in three replicates. Ultimately, thirty plant seedlings per concentration were selected for measurement (N =30). Data were analyzed using Fisher's LSD test at a significance level of $P < 0.05$ to compare differences among treatments, and results were expressed as mean \pm standard error (SE) (Kuchkarova et al. 2024b).

Results and discussion

Penicillium chrysogenum is a valuable source of new and known natural products, and numerous studies have investigated its biological activities (Chen et al. 2022, Lu et al. 2024). Furthermore, Anh et al. (2024) isolated and identified one new and nine known compounds from the methanol extract of the marine fungus *P. chrysogenum* VH17, and they were discovered to possess antimicrobial and cytotoxic activities. In this study, two compounds were isolated from the subfraction (F9.10) of the SR192 fungal extract. The spectral data of the isolated compounds (PMR, ^{13}C NMR, HSQC, and HMBC) were compared with published literature (Yang et al. 2008, Sauleau et al. 2006), leading to their identification as zearalenone and hyrtiosulawesine.

Zearalenone (1), light-orange powder (MeOH), $\text{C}_{18}\text{H}_{22}\text{O}_5$. ^1H NMR spectrum (600 MHz, CD_3OD , δ , ppm, J/Hz): 1.37 (3H, d, $J = 6.2$, H-11'), 1.56 (2H, m, H-4', 8'), 1.63 (1H, m, H-5'), 1.77 (2H, m, H-4', 5'), 2.06 (1H, m, H-8'), 2.11 (1H, m, H-3'), 2.16 (1H, m, H-9'), 2.29 (2H, m, H-7', 9'), 2.65 (1H, m, H-3'), 5.71 (1H, dd, $J = 15.0, 10.3, 3.9$, H-2'), 6.22 (1H, d, $J = 2.3$, H-3), 6.38 (1H, d, $J = 2.3$, H-3), 6.98 (1H, dd, $J = 15.7, 1.8$, H-1'). ^{13}C NMR spectrum (150 MHz, CD_3OD , δ , ppm): 21.0 (C-11'), 22.0 (C-8'), 23.3 (C-4'), 32.1 (C-9'), 35.9 (C-5'), 37.5 (C-7'), 43.8 (C-3'), 74.4 (C-10'), 102.9 (C-3), 104.0 (C-1), 109.5 (C-5), 133.0 (C-2'), 134.5 (C-1'), 144.8 (C-6), 164.0 (C-4), 166.3 (C-2), 172.7 (C-12'), 213.8 (C-6').

Hyrtiosulawesine (2), a yellow powder (MeOH), $\text{C}_{20}\text{H}_{13}\text{N}_3\text{O}_3$. ^1H NMR (600 MHz, CD_3OD , δ , ppm): 6.82 (1H, d, $J = 8.8$ Hz, H-6'), 7.14 (1H, dd, $J = 8.8, 1.5$ Hz, H-7), 7.32 (1H, d, $J = 8.3$ Hz, H-7'), 7.55 (1H, d, $J = 8.8$ Hz, H-8), 7.57 (1H, s, H-5), 8.02 (1H, s, H-4'), 8.16 (1H, d, $J = 4.9$ Hz, H-4), 8.43 (1H, d, $J = 4.9$ Hz, H-3), 8.89 (1H, s, H-2'); ^{13}C NMR (150 MHz, CD_3OD , δ , ppm): 106.76 (C-5), 108.01 (C-4'), 113.27 (C-7'), 113.83 (C-6'), 113.90 (C-8), 116.05 (C-3'), 118.47 (C-4), 119.82 (C-7), 122.61 (C-5a), 129.87 (C-3'a), 132.35 (C-7'a), 132.48 (C-4a), 137.31 (C-8a), 137.31 (C-9a), 137.39 (C-3), 139.09 (C-2'), 140.36 (C-1), 152.65 (C-6), 154.45 (C-5'), 189.86 (C-8'), (Fig. 1).

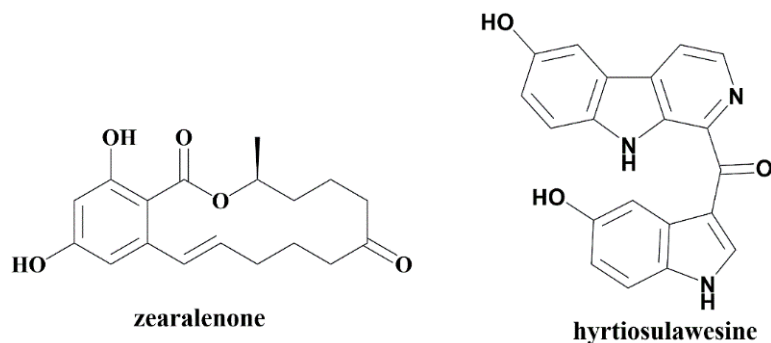


Fig. 1. Structures of compounds isolated from *Penicillium chrysogenum* extract.

Compound 1, zearalenone, is a mycotoxin primarily synthesized by the fungal genera *Aspergillus* and *Fusarium*. It has also been isolated from a marine strain of *Penicillium* sp., obtained from the surface of drifting cotton clothing collected on Namhae Island, Gyeongnam, Korea (Yang et al. 2008). Compound 2, hyrtiosulawesine, was originally isolated from the *Hyrtios erectus* Red Sea marine sponge and studied for its antiphospholipase A₂ activity (Sauleau et al., 2006). More recently, Toshmatov et al. (2024) reported its isolation from the seeds of the invasive plant *S. rostratum* and evaluated its phytotoxic activity on *A. retroflexus* and *L. perenne* seedlings. Our current research expands the scope by evaluating the biological activity of hyrtiosulawesine on four weed species. Two dicot plants, *A. retroflexus*, and *M. sativa*, as well as two monocot plants, *L. perenne*, and *S. viridis*, were used to screen the plant growth regulatory effects of the two compounds at concentrations of 5, 20, 100, and 500 $\mu\text{g mL}^{-1}$ (Figs. 2 and 3).

The growth of the seedlings generally exhibited a dose-dependent pattern as the concentration of the compound increased. Compounds 1 and 2 significantly accelerated the root growth of *A. retroflexus* by 51.53% and 40.49%, and shoot growth by 29.54% and 27.27%, respectively, at a concentration of 5 $\mu\text{g mL}^{-1}$. At 20 $\mu\text{g mL}^{-1}$ and higher concentrations of both compounds, the promotion of growth was reduced, and even suppressed by compound 2 at 500 $\mu\text{g mL}^{-1}$. Both compounds observably promoted root elongation of *M. sativa* by 87.38% and 92.23%, respectively, at 5 $\mu\text{g mL}^{-1}$. At 20 $\mu\text{g mL}^{-1}$, the promotion was reduced to 45.63% and 77.67%, respectively. In contrast, the opposite trend was found in the case of shoot lengths. These results showed that compound 1 inhibited the shoot lengths of *M. sativa* by 0.77%, 3.85%, 14.62%, and 15.00% at 5 - 500 $\mu\text{g mL}^{-1}$ concentrations, respectively. Moreover, the plant growth regulatory effect of compound 2 showed different results at all concentrations. For instance, at 5 and 20 $\mu\text{g mL}^{-1}$, shoot lengths of *M. sativa* slightly increased by 10.77% and 2.69%, respectively, while they were suppressed by 15.77% and 28.46% at higher concentrations of 100 and 500 $\mu\text{g mL}^{-1}$, respectively.

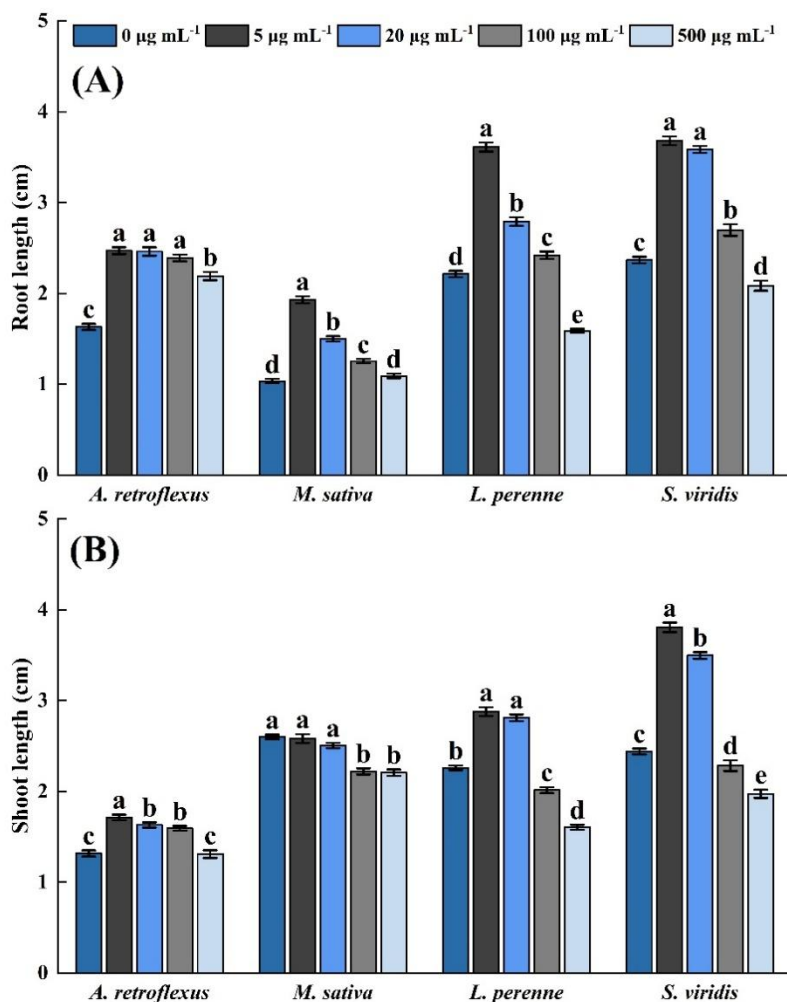


Fig. 2. Plant growth regulatory effects of zearalenone (compound 1) isolated from *Penicillium chrysogenum* extract on the root length (A) and shoot length (B) of *Amaranthus retroflexus*, *Medicago sativa*, *Lolium perenne*, and *Setaria viridis* plant seedlings. Each value is the mean of three replicates \pm standard error. Different letters indicate significant differences among different concentrations at $P < 0.05$ level according to Fisher's LSD test.

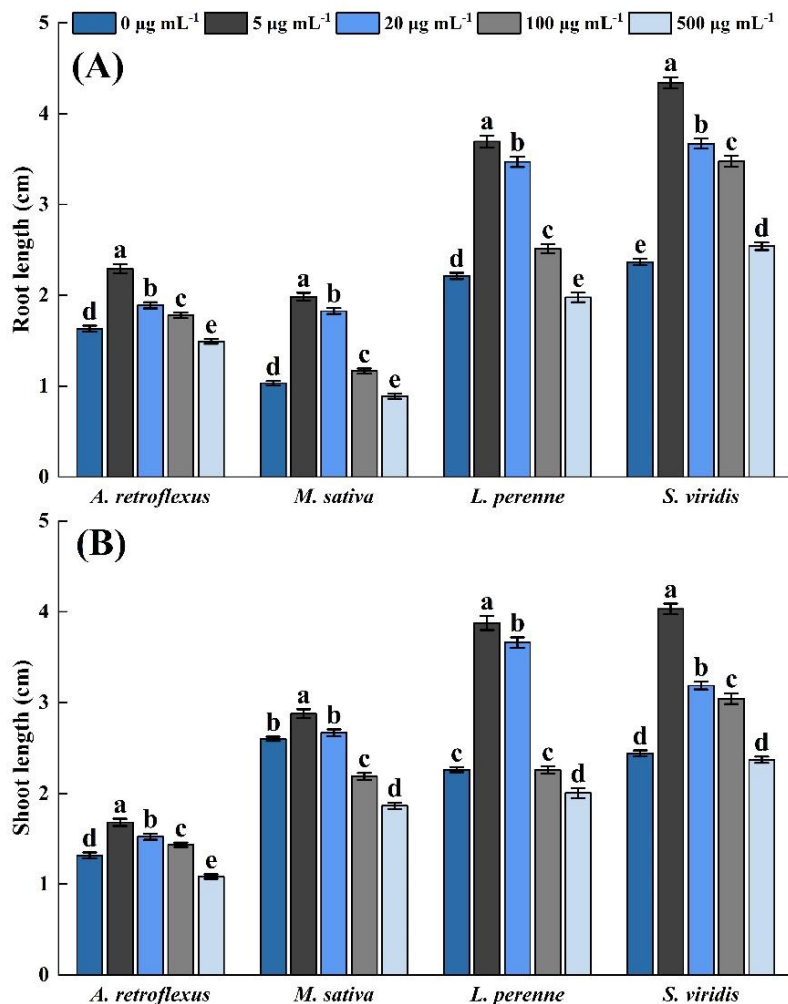


Fig. 3. Plant growth regulatory effects of hyrtiosulawesine (compound 2) isolated from *Penicillium chrysogenum* extract on the root length (A) and shoot length (B) of *Amaranthus retroflexus*, *Medicago sativa*, *Lolium perenne*, and *Setaria viridis* plant seedlings. Each value is the mean of three replicates \pm standard error. Different letters indicate significant differences among different concentrations at $P < 0.05$ level according to Fisher's LSD test.

At 5 $\mu\text{g mL}^{-1}$, compounds 1 and 2 significantly increased *L. perenne* and *S. viridis* root growth by 63.35%, 66.97%, 55.27%, and 83.12% respectively, demonstrating significant stimulant effects. Compound 2 greatly promoted *L. perenne* and *S. viridis* shoot growth at 5 $\mu\text{g mL}^{-1}$ by 71.68% and 65.16%, and at 20 $\mu\text{g mL}^{-1}$ by 61.94% and 30.74%, respectively. However, as the compound concentrations increased, the promoting effect gradually decreased, and the inhibitory effect gradually increased (Figs. 2 and 3).

Compound 2, hyrtiosulawesine, exhibited stronger stimulatory effects on root and shoot growth of *M. sativa* and *S. viridis* at 5 and 20 $\mu\text{g mL}^{-1}$ than zearalenone, suggesting its potential as an environmentally friendly plant growth regulator. The compounds isolated from *S. rostratum*, which included hyrtiosulawesine, showed a moderate phytotoxic effect on the dicot *A. retroflexus*, but exhibited a strong promotive effect on the monocot *L. perenne* (Toshmatov et al. 2024).

In conclusion, this study demonstrates that the endophytic fungus *Penicillium chrysogenum* SR192, isolated from the invasive plant *Solanum rostratum*, produces two bioactive secondary metabolites, zearalenone and hyrtiosulawesine, with significant plant growth regulatory activity. Both compounds exhibited dose-dependent effects, stimulating root and shoot growth at lower concentrations and inhibiting growth at higher concentrations. This is the first report of the isolation and characterization of these compounds from *P. chrysogenum* SR192, underscoring the potential of endophytic fungi from invasive plants as valuable sources of novel plant growth regulators. Further research on the mechanisms and field applications of these compounds could contribute to sustainable agricultural practices.

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Author contribution statement

Nigora Kuchkarova – fungus and compounds isolation, writing original draft, methodology, investigation; Caixia Han - visualization, data curation, formal analysis; Zokir Toshmatov - methodology, validation, software, formal analysis; Sandra Ozuzu - visualization, resources, data curation; Asma Shah- supervision, visualization; Chi Zhang - supervision, project administration, funding acquisition. Hua Shao – writing, reviewing, editing, supervision, project administration, funding acquisition.

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