

Isolation, Characterization, and Herbicidal Activity of *Streptomyces* spp. from Diseased Potato Scab Tubers

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Abstract

Background

A highly effective and environmentally friendly method of controlling weeds is biological herbicides, which typically constitute of naturally secondary metabolites, such as bioherbicidal metabolites produced by *Streptomyces* sp. However, the isolation of phytotoxic compounds from pathogenic *Streptomyces* has not been fully studied.

Results

Here, a total of 50 strains of *Streptomyces* genera were isolated from the potato tubers with typical common scab (CS) symptoms using the culture-dependent method. The radish seedling test indicated that 32 fermentation broths of potato common scab-associated *Streptomyces* could produce phytotoxic metabolites that affect the normal growth of radish seedlings' radicles. Of note, two potential new pathogens (NKY-4 and L7-2) of potato scab were discovered by combining the methods of radish seedlings, potato tuber slices, and PCR detection of pathogenic genes *txtAB*. Moreover, the phytotoxic test demonstrated that the fermentation broths of 31 strains exhibited phytotoxic activities against at least one of the tested weeds (*Echinochloa crusgalli*, *Digitaria sanguinalis*, and *Abutilon theophrastis*). Furthermore, one novel metabolite and three known compounds, including new N-(2,5-dihydroxyphenyl)-3-acetamide-4-hydroxybenzamide (**1**), thaxtomin A (**2**), nicotinic acid (**3**) and pyrrole-2-carboxylic acid (**4**) were isolated from *S. bottropensis* (AMCC400023). Among them, compound **2** exhibited strong phytotoxic activity against *E. crusgalli*, *D. sanguinalis*, and *A. theophrast* with IC₅₀ values of 0.99, 0.78, and 1.95 µg/mL, respectively, which was comparable to those of the positive control 2,4-D.

Conclusions

Based on the results of these findings, phytotoxic metabolites from the potato scab pathogen may be one of the effective ways to develop new biological herbicides.

Background

Weeds are one of the most universal and challenging problems faced by agricultural production worldwide, causing detrimental effects on both crop yields and production costs. Generally, the control of weeds mainly includes physical control and the use of chemical or biological herbicides [1]. Physical weeding is mainly completed by manual and mechanical means, which is labor- and time-intensive and with adverse effects on the soil [2]. At present, chemical herbicides have high efficiency in controlling weeds, but alter the balance of environmental ecosystems. Problems specifically manifest as herbicide resistance in agrestal weeds, accumulation of toxic substances changes the soil and water environment,

and dangerous to human health [3, 4]. Therefore, the search for environmental-friendly biological herbicides has drawn much more attention [5].

Biological herbicides are secondary metabolites or microbial agents that are produced by microorganisms such as fungi, bacteria, and actinomycetes [6]. The rationale for biological herbicides is to cause plant phytotoxic activities that affect the normal growth of plants through the phytotoxins produced by microorganisms [7]. *Streptomyces* is rich in natural products with structurally diverse and phytotoxic activities, which might be used to create as a good candidate for biological weed control [8]. For instance, the compound rubiginone D2 with strong herbicidal activity was purified and identified from *Streptomyces* sp. KRA18-249, which was isolated from a natural recreational forest [6], and herbicidins A and B produced by *S. saganonensis* also had potent herbicidal activity [9].

Up to now, more than 900 *Streptomyces* species have been described, and relatively few species have the capacity to cause plant diseases, such as elicit scab symptoms on potato, radish, beet, and other tap root crops [10, 11]. Among them, *S. scabies* was the first species to be identified as the cause of common scabs on potatoes, which adversely affects its quality [12]. Thaxtomin A (TA), the main phytotoxin produced by the pathogen *S. scabies*, not only induced scab symptoms on the surface of healthy potato stems, but also exhibited potent herbicidal activity against weeds [13, 14]. In addition, the severity of potato scab outbreaks is rising, causing new pathogens *Streptomyces* spp. are constantly being discovered, which are due to continuous potato cropping and the increasing resistance of pathogens against bactericides [15]. Therefore, identifying undiscovered new pathogens from potato scabs and isolating phytotoxins has become a fundamental and urgent issue.

In this study, we isolate and purify cultivable actinobacteria from potato tubers with lesions by using culture-dependent, assessed the pathogenicity and herbicidal activity of culturable actinobacteria. Additionally, we described the isolation, and structural characterization of secondary metabolites produced by one pathogenic for pathogen (*S. bottropensis* AMCC400023) with herbicidal activity.

Materials and methods

Sample collection

Potato samples with typical CS symptoms were collected from Anhui Academy of Agricultural Sciences, Hefei, China (GPS: 31°53′ N, 117°20′ E) and Wuwei, China (GPS: 37°94′ N, 102°61′ E), respectively, in December 2020 and September 2021.

Isolation of actinobacteria

The potato tubers with typical CS symptoms were selected for isolation of actinobacteria. The samples were washed to discard soil residues, then placed on sterile filter paper and air dry naturally in dark conditions. For each isolation, small pieces (1 cm² × 0.5 cm) were cut from typical potato scab lesions with a sterile scalpel. Then, the cut lesion tissue was separately placed in 10 mL 75% ethanol for 20-30s

[16], followed by soaking them in sterile water for three times and fully homogenized separately in 10 mL of sterile water. Lastly, an aliquot of 100 µL serial dilution (10^{-1} , 10^{-2} , 10^{-3}) of homogenates was spread to twelve different actinobacteria-selective media types [17] (Table S1), including actinobacteria isolation agar (AIA), cellulose-casamino acid (CCM), B4 medium, Gause's No. 1 (G1), glucose-yeast-malt (GYM), luria bertani (LB), ISP medium No.2 (ISP2), ISP medium No.4 (ISP4), oatmeal agar (OMA), Reasoner's 2A agar (R2A), starch casein agar (SCA), minimal medium (MM). All isolation media were supplemented with a final concentration of 75 mg/L nystatin, 25 mg/L nalidixic acid, 100 mg/L cycloheximide, and 50 mg/L potassium dichromate to suppress Gram-negative bacterial and fungal growth [18]. Incubation proceeded at 28°C for 1–4 weeks until colonies appeared. Typical *Streptomyces* colonies were purified by single spore methodology and transferred onto Gause's No.1 agar. All isolates were preserved on slants at 4 °C or stored in 10% glycerol (-80 °C).

Identification and phylogenetic analysis of isolates

Isolates were cultivated on G1 medium at 28 °C for one week, then initially identified by their phenotypic characteristics including the characteristics of colonies on plates and production of diffusible pigment. All isolates were further identified based on 16S rRNA gene sequencing [19]. The 16S rRNA gene of isolates was amplified using 2 × Taq Plus Master Max $\times$ (Day Plus) and the specific primer pair 27F (5'-TCCTCCGCTTATTGATATGC-3') / 1492R (5'-GGTTACCTTGTTACG ACTT-3'), with the following PCR conditions: 95°C for 3 min, and 33 cycles of (95°C for 20 s, 56°C for 20 s, 72°C for 90 s), followed by 72°C for 70 min. In addition, the *trpB* and *rpoB* gene of four strains (NKY-15, NKY-17, L7-2 and NKY-4) was amplified using the specific primer pair *trpB*-F (5'-GCGCGAGGACCTGAACCACACCGGCTCAC-3') / *trpB*-R (5'-TCGATGGCCGGGATGATGCCCTCGG-3') and *rpoB*-F (5'-GAGCGCATGACCACCCAGGACGTCGAGGC-3') / *rpoB*-R (5'-CCTCGTAGTTGTGACCCTCCACGGCATGA-3') [20]. The PCR products were sent to Tsingke Biotechnology Co., Ltd. (Nanjing, China) for sequencing, then the sequencing results were submitted to EzTaxone server [21] (<https://www.ezbiocloud.net/>) for comparison with type material sequences. The 16S rRNA gene sequences obtained in this study were deposited in the GenBank database (accession numbers OR186221-OR186270). The *rpoB* gene and *trpB* gene of four strains (NKY-15, NKY-17, L7-2 and NKY-4) sequences obtained in this study were deposited in the GenBank database (accession numbers PP212887- PP212890 and PP212891- PP212894).

Pathogenicity tests

Virulence assays of isolated strains on radish seedlings were performed according to the reported method [16]. The radish seeds were washed with sterile water and placed in culture dish moistened with sterile filter paper to induce germination for 24 h. 30 seeds were allowed to grow for 4 days (at 24 °C) in Petri dishes (9 cm diameter) containing 5 ml of Actinomyces fermentation broth. Then root length of each seedling was recorded. The negative control was sterile water and the positive control was *S. bottropensis* AMCC400023.

The maceration ability of the isolated strains was evaluated by performing the potato tuber slice assay as previously described [22]. Firstly, the fresh potato tubers (no lesion on the surface) were washed with

water, then soaked and disinfected in 75% anhydrous ethanol for 5 minutes, washed in sterilized tap water. The 2 cm thick potato slices with a diameter of 15 mm were placed on moistened filter paper in 90 mm Petri dishes. Five strains (AMCC400023, L7-2, L7-7, NKY-4, and G1-67) with potent inhibitory activity on radish seedlings were selected to grow on G1 medium for one week at 28°C. Following incubation, agar plugs (5 mm diameter) containing sporulating colonies were sampled and placed at the centre of each tuber slice. Potatoes were incubated at 28°C for 7–10 days and observed for severity of browning and necrosis of potato tuber slice. The G1 solid culture medium and AMCC400023 were used as the negative and positive controls, respectively. All experiments were repeated in triplicate.

The *txtAB* gene was involved in the biosynthesis of the main toxin (thaxtomins) of potato scab pathogens [23]. Some genes including *nec1* and *tomA* were also somehow required for the virulence. But the prevalence of CS pathogens without *txtAB* genes was either very rare [24]. We used the most common pathogenic genes *txtAB* to perform pathogenicity validation on the isolated strains. Polymerase chain reaction was used to amplify *txtAB*, and the specific primer pair were txtAB-F (5'-CCACCAGGACCTGCTCTTC-3') / txtAB-R (5'-TCGAGTGGACCTCA CAGATG-3') [23]. Finally, the reactions were analyzed by 1.2% agarose gel electrophoresis.

Screening for phytotoxic activities

32 strains with inhibitory activity to radish seedlings were selected for small-scale fermentation to screen for further test of herbicide activity. The strains were grown in 150 mL G1 liquid medium in 250 mL Erlenmeyer flask at 28 °C, 180 rpm for 7 days. The fermentation broth of cultures was obtained by filtering through cotton gauze, and screened for potential herbicidal activity against two monocotyledonous weeds, *E. crusgalli* and *D. sanguinalis*, and a dicotyledonous weed, *A. theophrasti* by the method described in the previous literature [25]. 30 pregerminated seeds with consistent status were selected to place in Petri dishes (9 cm diameter) with filter paper, and filled with 5 ml of Actinomyces fermentation broth. Distilled water and 2,4-dichlorophenoxyacetic acid (2,4-D, Shanghai Chuangsai Technology Co., Ltd.) were used as negative and positive controls, respectively. After 2–3 days, the length of the radicle of each weed seed was measured.

Isolation and characterization of secondary metabolite

Based on bioassay-oriented, the strain *S. bottropensis* AMCC400023 with significant herbicidal activity was selected for identification of compounds in this study. The strain AMCC400023 was inoculated into a 250 mL Erlenmeyer flask containing 150 mL of SCM3 liquid medium and incubated at 28 °C (180 rpm) for 3 days. The seed inoculates were then transferred to 400 mL of the same medium in 1000 mL Erlenmeyer flasks, and cultured at 28 °C (180 rpm) for 7 days. The fermentation broth (16 L) was filtered through four layers of gauze, and the supernatant extracted three times with EtOAc. The ethyl acetate fractions were concentrated in vacuo with the aid of a rotary evaporator to obtain crude extract (6.0 g).

The crude extract was fractionated into seven fractions (100:0, 100:1, 100:2, 100:4, 100:8, 100:16, and 100:32, v/v) by silica gel column (200–300 mesh) using a dichloromethane (CH₂Cl₂) and methanol (MeOH) gradient. Fraction 5 was further purified on a silica gel column, and followed by fractionation on

a Sephadex LH-20 column (MeOH) to yield compound **1** (120 mg) and subfraction (R1). The subfraction R1 was recrystallized from methanol, yielding an orange-yellow crystals **2** (148 mg). The fraction (Fr 4) was repeatedly purified on silica gel using CH₂Cl₂/MeOH (100:1, 100:2, 100:4) to give compound **3** (10 mg). Fr 2 was fractionated by silica gel column chromatography (CH₂Cl₂/MeOH) and recrystallized from methanol to give compound **4** (30 mg).

Structural identifications of the compounds were determined via spectroscopic analysis. NMR spectra were measured with Agilent II DD2 spectrometers (Agilent, USA) at 600 MHz for ¹H and 150 MHz for ¹³C, and the 2D spectra (COSY, HMQC, HMBC and DETP) were obtained by using standard Agilent software.

Phytotoxic assay of compounds

Compounds **1–4** were determined for phytotoxic activity against *E. crusgalli*, *D. sanguinalis* and *A. theophrasti* by using Petri dish bioassay [26]. All test compounds were dissolved in acetone and diluted to 100 µg/mL with distilled water. The 2,4-D was used as the positive control.

Statistical analysis

Statistical analysis was performed using origin 2021 software, considering a significance level of 95%. For the bio-herbicidal assay on tested seeds, each treatment was composed of 30 seeds with three replicates of 10 seeds.

Results

Isolation of actinomycetes strains from the potato tubers with typical common scab

Here, a total of 50 isolates were isolated and purified from the scab lesions on potatoes using 12 different isolation media. As shown in Figure S1, the number of actinomycetes isolated from different media exhibited appreciable differences. Among them, G1 were the most successful media for isolating actinobacterial strains with the number of 24 strains (about 48%), followed by SCA with 10 strains (20%). The number of strains obtained from the following six culture media were relatively small, including B4 (4 strains), AIA (4 strains), GYM (3 strains), CCM (2 strains), R2A (2 strains) and MM (1 strain). However, no strains were isolated from LB, ISP2, ISP4 and OMA media.

Identification and phylogenetic analysis

PCR amplification and sequencing analysis of the 16S rRNA gene was performed on 50 isolated actinomycetes, then the results of sequences were analyzed using BLAST and Ezbiocloud database. All isolates were identified as the genus *Streptomyces* belonging to the family Streptomycetaceae, which belongs to 12 species (Table S2). Among them, *S. pratensis* (32% of strains) and *S. violascens* (24%) were the dominant species. Notably, the 16S rRNA gene sequences were analyzed by EzTaxon indicated that some isolates showed relatively low similarities to the type strains of the corresponding genera.

Using 16s rRNA, *trpB* and *rpoB* to analyse *Streptomyces* spp. with low similarity (NKY-15 and NKY-17), the results indicate that the strains NKY-15 and NKY-17 were in separate branches (Figure S2, S3), which indicated potential new species.

Pathogenicity assay of the *Streptomyces* isolates

The pathogenicity of selected *Streptomyces* spp. isolates was determined via the following three assays, including radish seed assay, potato tuber slice assay and detection of *txtAB* gene. A total of 63% (32 strains) of the selected *Streptomyces* spp. were positive in the radish seed assay (Fig. 1). Among them, 5 strains exhibited outstanding inhibitory activity against radish seedlings with an inhibition rate of 60%-70%, which were comparable to that of the positive AMCC400023 with an inhibition rate of 70%. A moderate inhibitory activity was found for 5 strains with an inhibition rate of 40%-60%. In addition, 21 strains showed relatively weak phytotoxic activity against radish seed with the inhibition rate of less than 40%. Furthermore, thaxtomin A (TA) is considered the primary phytotoxin produced by the pathogen *Streptomyces scabies*, which is the most important pathogenicity factor of potato common scab. Results of PCR analysis with the primer pair txtAB-F/txtAB-R showed that three strains (L7-2, L7-7 and NKY-4) were positive (Fig. 2A). In the potato tuber slice assay, two isolates (L7-2 and NKY-4) caused CS lesions on potato tubers (Fig. 2B). Similar to the positive AMCC400023, the strains L7-2 and NKY-4 could successfully colonize on the surface of potato tuber slice with different degree. Specially, the obvious black brown necrotic lesions were observed on potato slice. Therefore, the strains L7-2 and NKY-4 were positive in all three pathogenicity assays, which indicated that they were potential species causing potato scab disease (Figure S2, S3).

Phytotoxic assay of fermentation broth of actinomycetes

32 bioactive actinomycetes in the radish seed assay were evaluated for their herbicide activity against the seedlings of *E. crusgalli*, *D. sanguinalis* and *A. theophrasti* using Petri dish bioassays. As shown in Fig. 1, 31 of the 32 isolates (97%) exhibited herbicidal activities against at least one of the growth of weeds roots. Among them, three strains (NKY-12, SCA2, and SCA10) showed strong phytotoxic activity against *E. crusgalli* with the inhibition rate of 100%. Ten strains (L7-12, NKY-N, NKY-12, GS-49, GS-67, SCA-2, SCA4, SCA7, SCA10, and AMCC400023) also had strong inhibitory effects on the growth of *Digitaria sanguinalis* root, with the inhibition rate of over 60%. Besides, 14 strains presented moderate phytotoxic activity against *A. theophrasti* with the inhibition rate of over 50%. Especially, 18 isolates exhibited inhibitory effects on the growth of both monocotyledonous weeds (*E. crusgalli* and *D. sanguinalis*) and a dicotyledonous weed (*A. theophrasti*).

Structural characterization of secondary metabolites from AMCC400023

Four pure compounds (Fig. 3) were purified from SCM3 liquid medium fermentation product of *Streptomyces* sp. AMCC400023, and their structures were analyzed by NMR and EI-MS techniques,

including one new compound N-(2,5-dihydroxyphenyl)-3-acetamide-4-hydroxybenzamide (**1**) and three known compounds thaxtomin A (**2**) [27], nicotinic acid (**3**) [28] and pyrrole-2-carboxylic acid (**4**) [29].

N-(2,5-dihydroxyphenyl)-3-acetamide-4-hydroxybenzamide (**1**): brown powder; HR-ESI-MS: m/z : 301.0824 $[M-H]^-$, calculated for $C_{15}H_{14}N_2O_5$ 302.0903, which was consistent with ^1H and ^{13}C NMR data (Table 1 and Figures S4-S10). The ^1H NMR spectrum of **1** exhibited three phenolic hydroxyl group signals δ_H [8.82 (brs, 1H), 9.04 (brs, 1H) and 10.58 (brs, 1H)], two amide group signals δ_H [9.93 (s, 1H) and 9.14 (s, 1H)], and a methyl group signal δ_H 2.11 (s, 1H). ^{13}C NMR and DEPT135 spectrum showed two amide carbon signals (δ_C 164.5, and 169.1), twelve olefinic carbons signals (δ_C 109.1, 111.1, 115.3, 116.3, 121.9, 124.1, 124.9, 126.3, 126.8, 140.3, 149.9 and 151.2), and one methyl carbon signal (δ_C 23.6). The HMBC spectrum of **1** exhibited correlations (Fig. 3) from H-2 (δ_H 8.36) to C-6 (δ_C 124.1), and C-4 (δ_C 151.2), from H-3 (δ_H 9.39) to C-2 (δ_C 121.9), C-4, and C-7 (δ_C 169.1), from H-4 (δ_H 10.58) to C-3 (δ_C 126.3) and C-4, from H-5 (δ_H 6.95) to C-1 (δ_C 124.9), C-3, from H-6 (δ_H 7.57) to C-2 and C-4, and from H-8 (δ_H 2.11) to C-7, together with ^1H - ^1H -correlation spectroscopy (COSY) correlations (Fig. 3) observed between H-5 and H-6 indicated that compound **1** possessed trisubstituted benzene and acetamide moieties. Further analysis of HMBC data, the correlations from H-1' (δ_H 9.14) to C-6' (δ_C 109.1), C-2' (δ_C 140.3), and C-7' (δ_C 164.5), from H-3' (δ_H 6.69) to C-1' (δ_C 126.8) and C-5' (δ_C 149.9), from H-4' (δ_H 6.39) to C-2' and C-6, from H-5' (δ_H 8.82) to C-4' (δ_C 111.1), C-5', and C-6', and from H-6' (δ_H 7.33) to C-2', C-4', together with ^1H - ^1H -correlation spectroscopy (COSY) correlations (Fig. 3) observed between H-3' and H-4' revealed the presence of trisubstituted benzene and phenolic hydroxyl groups. Accordingly, the structure of **1** was established as a new compound, and N-(2,5-dihydroxyphenyl)-3-acetamide-4-hydroxybenzamide was proposed as its trivial name.

Table 1
 ^1H and ^{13}C NMR data for compounds **1** in
DMSO- d_6 .

Position	δ_{C}	δ_{H} , mult, J in Hz
1	124.9, C	
2	121.9, CH	8.36, s
3	126.3, C	
4	151.2, C	
5	115.3, CH	6.95, d (8.4)
6	124.1, CH	7.57, d (8.0)
7	169.1, C	
8	23.6, CH ₃	2.11, s
1'	126.8, C	
2'	140.3, C	
3'	116.3, CH	6.69, d (8.6)
4'	111.1, CH	6.39, dd (2.8, 5.4)
5'	149.9, C	
6'	109.1, CH	7.33, d (2.3)
7'	164.5, C	
4-OH		10.58, br s
2'-OH		9.04, br s
5'-OH		8.82, br s
3-NH		9.93, s
1'-NH		9.14, s

Thaxtomin A (**2**): orange-yellow crystals; HR-ESI-MS: m/z : 461.1401 $[\text{M} + \text{Na}]^+$, calculated for $\text{C}_{22}\text{H}_{22}\text{N}_4\text{O}_6$ 438.1539; ^1H NMR (600 MHz, CD_3OD) δ : 1.64 (1H, dd, J = 14.1, 8.8 Hz, H-10_b), 2.61 (1H, dd, J = 14.2, 6.3 Hz, H-10_a), 2.81 (3H, s, N-CH₃), 3.03 (3H, s, N-CH₃), 3.11 (2H, d, J = 13.6 Hz, H-17), 3.87 (1H, dd, J = 8.4, 6.6 Hz, H-11), 6.71 (3H, m, H-19, H-21, H-23), 6.95 (1H, s, H-2), 7.18 (1H, t, J = 7.9 Hz, H-6), 7.23 (1H, t, J = 8.1 Hz, H-22), 7.69 (1H, d, J = 8.0 Hz, H-7), 7.83 (1H, d, J = 7.8 Hz, H-5). ^{13}C NMR (150 MHz, CD_3OD) δ : 168.5 (C, C-16), 167.0 (C, C-13), 159.2 (C, C-20), 143.9 (C, C-4), 141.3 (C, C-9), 137.5 (C, C-18), 132.6 (CH,

C-2), 131.3 (CH, C-22), 122.9 (CH, C-23), 121.1 (CH, C-6), 120.0 (C, C-8), 119.3 (CH, C-5), 118.8 (CH, C-7), 118.5 (CH, C-19), 116.0 (CH, C-21), 110.7 (C, C-3), 88.2 (C, C-14), 64.8 (C, C-11), 43.8 (CH₂, C-17), 34.3 (CH₃, C-15), 33.7 (CH₂, C-10), 28.6 (CH₃, C-12).

Nicotinic acid (**3**): white crystals; HR-ESI-MS: *m/z*: 124.0391 [M + H]⁺, calculated for C₆H₅NO₂ 123.0320; ¹H NMR (600 MHz, CD₃OD) δ: 7.55 (1H, dd, *J* = 5.0, 7.7 Hz, H-5), 8.40 (1H, d, *J* = 7.9 Hz, H-4), 8.72 (1H, d, *J* = 3.7 Hz, H-6), 9.12 (1H, s, H-2). ¹³C NMR (150 MHz, CD₃OD) δ: 125.3 (CH, C-5), 129.1 (C, C-3), 139.3 (CH, C-4), 151.5 (CH, C-6), 153.7 (CH, C-2), 168.2 (CH, C-7).

Pyrrole-2-carboxylic acid (**4**): brownish crystals; HR-ESI-MS: *m/z*: 110.0247 [M-H]⁻, calculated for C₅H₅NO₂ 111.0320; ¹H NMR (600 MHz, CD₃OD) δ: 6.16 (1H, s, H-4), 6.83 (1H, s, H-3), 6.92 (1H, s, H-5). ¹³C NMR (150 MHz, CD₃OD) δ: 124.8 (CH, C-5), 124.2 (C, C-2), 116.4 (CH, C-3), 110.6 (CH, C-4).

Phytotoxic activities of compounds

The phytotoxic activities of the test compounds (**1–4**) against radicle growth of *E. crusgalli*, *D. sanguinalis* and *A. theophrasti* were shown in Table 2. The results showed that compound **2** exhibited strong phytotoxic activity against *E. crusgalli*, *D. sanguinalis* and *A. theophrasti* with the inhibition rate of 100%, which was same as that of the positive 2,4-D at a concentration of 100 µg/mL. However, the new compound **1** had weak inhibitory activity against above three weeds with the inhibition rate of less than 30%. Similarly, the compound **4** exhibited weak phytotoxic activity against two monocotyledonous weeds (*E. crusgalli*, *D. sanguinalis*) with the inhibition rate of less than 40%. Further testing was conducted on the herbicidal activity of compound **2** with different concentrations. The results also showed that the metabolite **2** presented strong phytotoxic activity with the IC₅₀ values of 0.99, 0.78, and 1.95 µg/mL, respectively, which were almost comparable with those of the positive 2,4-D with IC₅₀ values of 0.88, < 0.1, and < 0.1 µg/mL (Fig. 4).

Table 2
Inhibitory effects of compounds on the growth of weeds roots (%).

Compounds	<i>E. crusgalli</i>	<i>D. sanguinalis</i>	<i>A. theophrasti</i>
1	9.6 ± 6.8	19.3 ± 8.1	20.8 ± 7.9
2	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
3	NI	22.3 ± 8.4	NI
4	20.0 ± 7.2	31.3 ± 6.5	NI
2,4-D	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Results are presented as the mean ± standard; “NI” means not inhibited; the concentration for the test is 100 µg/mL;			
2,4-D was the positive control.			

Discussion

Potato common scab (CS) scab was a soil-borne disease caused by the typical phytopathogenic *Streptomyces* sp. and was one of the most devastating diseases in potatoes, reducing both crop growth and quality [30]. In this study, 50 potato-associated *Streptomyces*, including two potentially new species, were isolated from the potato tubers with typical common scab and identified by morphological and molecular biological methods. As reported previously, a new species was defined by a 16S rRNA gene sequence homology below 98.7% [31, 32]. Specifically, based on 16s rRNA and the high-resolution *Streptomyces* sp. housekeeping genes: *trpB* (tryptophan synthase subunit beta) and *rpoB* (RNA polymerase subunit beta) [33] the phylogenetic trees indicated that *Streptomyces* spp. with low similarity (NKY-15 and NKY-17) were in separate branches and might potential new species.

Here, twelve selective isolation media were employed to isolate as many actinobacteria as feasible. Among them, the G1 and SCA media were the most effective as regards the number of obtained isolates. The G1 and SCA media, which were used to find saccharolytic bacteria, including actinobacteria [18, 34, 35], were mostly made of soluble starch which facilitated the mycelial extension and cell growth of actinomycetes as a sole carbon source [19].

Till now, approximately 30 species of *Streptomyces* causing CS have been identified worldwide [36], of which *S. scabies*, *S. acidiscabies*, and *S. turgidiscabies* were the most characterized [36, 37]. Noticeably, the strains NKY-4 and L7-7 were positive in pathogenicity assays, which have never been reported to cause potato scab, indicating that might be newly discovered pathogen of potato scab disease. However, 38% of all the isolates were negative in all pathogenicity assays, indicating that nonpathogenic *Streptomyces* were also colonizing potato tubers, which was consistent with the previous reports [16, 38]. Our results show that about 63% (32 strains) of the tested *Streptomyces* spp. were positive in the radish seed assay, but most of which were negative in other two pathogenicity assays. This indicates that although thaxtomin family, which is encoded by *txtAB* gene, is considered as a major player toward plant pathogenicity, other phytotoxic may exist in different species of *Streptomyces* and can affect the normal growth of radish seedlings [39]. Other *Streptomyces* phytotoxic specialized metabolites have been reported, such as two phytotoxic nigericin and geldanamycin were isolated from plant pathogen *Streptomyces* sp. 11-1-2 [40], desmethylmensacarcin is a novel phytotoxicity was isolated from pathogen *S. niveiscabiei* [41]. The herbicidal activity of 32 strains of *Streptomyces* spp. exhibiting inhibitory activity on the root length of radish seedlings was further tested against three weeds. The results revealed that 31 *Streptomyces* strains showed inhibitory activity against at least one of the weeds' roots. This provides new ideas for the development of novel herbicides of microbial-pathogen origin.

Glufosinate was the first microbial-origin herbicide developed and synthesized as a lead compound by the secondary metabolite of bilanafos from *Streptomyces* [42]. We investigated the secondary metabolites from the pathogen *S. bottropensis* AMCC400023, which resulted in the isolation of compounds **1–4**. Among them, compound **2** displayed strong inhibitory activity against *E. crusgalli*, *D.*

sanguinalis, and *A. theophrasti*, with IC₅₀ values of 0.99, 0.78, and 1.95 µg/mL, respectively. Indeed, compound 2 (TA) was the main phytotoxin synthesized by the potato common scab-causing pathogen *Streptomyces* spp., which has been reported to be phytotoxic to both broadleaf and the dicotyledonous weed, *A. theophrasti* [43]. However, this study found for the first time that compound 2 (TA) showed potential herbicidal activity against two monocotyledonous weeds, *E. crusgalli* and *D. sanguinalis*. Moreover, it was common to find that the mechanisms of action of phytotoxin was an impact on plant chlorophyll content, lipid peroxidation, and electrolytic leakage [6]. Therefore, *Streptomyces* was one of the important sources of lead compounds in natural herbicides.

Conclusion

In summary, 50 actinomycetes were isolated and identified from potato samples using culture-dependent methods. Sequences analysis demonstrated that all actinomycetes were attached to the *Streptomyces* genera. 32 strains exhibited a certain degree of virulence as determined by a pathogenicity assay on radish plants. Furthermore, potential new pathogens NKY-4 and L7-2 were positive in three pathogenicity assays. The results of phytotoxic tests showed that 31 extracts (97%) exhibited phytotoxic activities against at least one of the tested weeds. In addition, one novel metabolite and three known compounds were purified from pathogen *S. bottropensis* AMCC400023. Compound 2 displayed outstanding phytotoxic activity against *E. crusgalli*, *D. sanguinalis*, and *A. theophrasti* with IC₅₀ values of 0.99, 0.78, and 1.95 µg/mL, respectively. Therefore, our results suggest that metabolites produced by *S. bottropensis* AMCC400023 with herbicidal activity may be a new bioherbicide candidate or leads molecule for a more efficient herbicide.

Abbreviations

CS	Common scab
PCR	Polymerase chain reaction
2,4-D	2,4-dichlorophenoxyacetic acid
TA	Thaxtomin A
AIA	Actinobacteria isolation agar
CCM	Cellulose-casamino acid
G1	Gause’s No. 1
GYM	Glucose-yeast-malt
LB	Luria bertani

ISP2	ISP medium No.2
ISP4	ISP medium No.4
OMA	Oatmeal agar
R2A	Reasoner's 2A agar
SCA	Starch casein agar
MM	Minimal medium
CH ₂ Cl ₂	Dichloromethane
MeOH	Methanol
COSY	¹ H- ¹ H-correlation spectroscopy correlation
HMBC	¹ H detected heteronuclear multiple bond correlation
DEPT	Distortionless enhancement by polarization transfer

Declarations

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Author contribution

Ying-lao ZHANG and Shu-xiang ZHANG conceived and designed research. Shu-ping SHI and Yi ZHANG conducted experiments. Zhong-di HUANG and Cai-ping YIN analyzed the data. Zhong-di HUANG wrote the manuscript. All authors read and approved the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this manuscript, its supplementary information files and in GenBank database. The 16S rRNA gene sequences obtained in this study were deposited in the GenBank database (accession numbers OR186221-OR186270). The *rpoB* gene and *trpB*

gene of four strains (NKY-15, NKY-17, L7-2 and NKY-4) sequences obtained in this study were deposited in the GenBank database (accession numbers PP212887- PP212890 and PP212891- PP212894).

Ethics approval and consent to participate

The research conducted in this study did not involve any animal subjects, therefore obtaining consent to participate was not applicable.

Consent for publication

Not applicable.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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References

1. Kim H. J., Bo A. B., Kim J. D., Kim Y. S., Khaitov B., et al. 2020. Herbicidal Characteristics and Structural Identification of the Potential Active Compounds from *Streptomyces* sp. KRA17-580. *J. Agric. Food Chem.* **68**, 15373–15380.
2. Bruciene I., Buragiene S., Sarauskis E. 2022. Weeding Effectiveness and Changes in Soil Physical Properties Using Inter-Row Hoeing and a Robot. *AGRONOMY-BASEL*, **12**, 1514.
3. Dai P., Yan Z., Ma S., Yang Y., Wang Q., et al. 2018. The herbicide glyphosate negatively affects midgut bacterial communities and survival of honey bee during larvae reared in vitro. *J. Agric. Food Chem.* **66**, 7786–7793.
4. Cho K. M., Shin S. C., Bo A. B., Umurzokov M., Jia W., et al. 2022. Isolation, Screening, and Molecular Identification of *Streptomyces* sp. W-200 and Its Bioherbicidal Activity in Weed Control. *Weed & Turfgrass Science*, **11**, 277–287.
5. Westwood J. H., Charudattan R., Duke S. O., Fennimore S. A., Marrone P., et al. 2018. Weed management in 2050: perspectives on the future of weed science. *Weed Sci.* **66**, 275–285.
6. Umurzokov M., Lee Y. M., Kim H. J., Cho K. M., Kim Y. S., et al. 2022. Herbicidal characteristics and structural identification of a potential active compound produced by *Streptomyces* sp. KRA18–249. *Pestic. Biochem. Phys.* **187**, 105213.
7. Mirjalol U., Choi J. S., Farrukh R., Kim Y. S., Cho K. M., et al. 2021. Herbicidal activity of KRA16-334 broth filtrate on *Sicyos angulatus*. *Weed & Turfgrass Science*, **10**, 437–443.

8. Shi L. Q., Wu Z. Y., Zhang Y. N., Zhang Z. G., Fang W., et al. 2020. Herbicidal secondary metabolites from actinomycetes: structure diversity, modes of action, and their roles in the development of herbicides. *J. Agric. Food Chem.* **68**, 17–32.
9. Arai M., Haneishi T., Kitahara N., Enokita R., Kawakubo K. 1976. Herbicidins A and B, two new antibiotics with herbicidal activity. I. Producing organism and biological activities. *J. Antibiot (Tokyo)*. 1976, **29**, 863-869.
10. Wang L. Q., Wang M. Y., Fu Y. D., Huang P. J., Kong D. K., et al. 2020. Engineered biosynthesis of thaxtomin phytotoxins, *Crit. Rev. Biotechnol.* **40**, 1163-1171.
11. Loria R., Kers J., Joshi M. 2006. Evolution of plant pathogenicity in *Streptomyces*. *Annu. Rev. Phytopathol.* **44**, 469-87.
12. Lambert D. H., Loria R. 1989. *Streptomyces scabies* sp. nov., nom. rev. *Int. J. Syst. Bacteriol.* **39**, 387–392.
13. Wolfe J. C., Neal J. C., Harlow C. D. 2016. Selective Broadleaf Weed Control in Turfgrass with the Bioherbicides Phoma macrostoma and Thaxtomin A. *Weed Technology.* **30**, 688–700.
14. Sarwar A., Latif Z., Zhang S., Hao J., Bechthold A. 2019. A potential biocontrol agent *Streptomyces violaceusniger* AC12AB for managing potato common scab. *Front. Microbiol.* **10**, 202.
15. Cui L. X., Yang C. D., Jin M. J., Wei L. J., Yang L. P. 2021. Identification and biological characterization of a new pathogen that causes potato scab in Gansu Province, China. *Microb. Pathogenesis.* **161**, 105276.
16. Chalupowicz L., Tsrer (Lahkim) L., Hazanovsky M., Erlich O., Reuven M., et al. 2022. Isolation and characterization of *Streptomyces* spp. from potato and peanut in Israel. *Plant. Pathol.* **71**, 1870–1879.
17. Li S., Dong L., Lian, W. H., Lu C. Y., Xu L., et al. 2021. Exploring untapped potential of *Streptomyces* spp. in Gurbantunggut Desert by use of highly selective culture strategy. *Sci. Total. Environ.* **790**, 148235.
18. Cui P., Wu H., Jiang T., Tao J., Zhu Z., et al. 2022. Diversity and antibacterial potential of the Actinobacteria associated with *Apis mellifera ligustica*. *Front. Microbiol.* **13**, 1056176.
19. Long Y., Zhang Y., Huang F., Liu S., Gao T., et al. 2022. Diversity and antimicrobial activities of culturable actinomycetes from *Odontotermes formosanus* (Blattaria: Termitidae). *BMC Microbiol.* **22**, 80.
20. Fyans, J.K., Bown, L., Bignell, D. R. D. 2016. Isolation and Characterization of Plant-Pathogenic Streptomyces Species Associated with Common Scab-Infected Potato Tubers in Newfoundland. *Phytopathology.* **106**, pp.123-131.
21. Kim O. S., Cho Y. J., Lee K., Yoon S. H., Kim M., et al. 2012. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol.* **62**, 716-721.
22. Zoledowska S., Motyka A., Zukowska D., Sledz W., Lojkowska E., et al. 2017. Population structure and biodiversity of *Pectobacterium parmentieri* isolated from potato fields in temperate climate.

Plant Dis. **102**, 154-164.

23. Dees M. W., Somervuo P., Lysøe E., Aittamaa M., Valkonen J. P. 2012. Species' identification and microarray-based comparative genome analysis of *Streptomyces* species isolated from potato scab lesions in Norway. *Mol. Plant. Pathol.* **13**, 174-186.
24. Park, D. H., Yu, Y. M., Kim, J. S., Cho, J. M., et al. 2003. Characterization of streptomycetes causing potato common scab in Korea. *Plant Dis.* **87**, 1290–1296.
25. Wu Z., Liu F., Ke S., Zhang Z., Hu H., et al. 2023. Secondary metabolites from *Aspergillus sparsus* NBERC_28952 and their herbicidal activities. *Plants*.**12**, 203.
26. Cai Z. F., Zhang W. L., Cao Y. Y., Du X. H., Heterocycl J. 2022. Synthesis and herbicidal activities of 2-phenylpyridine compounds containing alkenyl moieties. *J Heterocyclic Chem.* **59**, 1247–1252.
27. King R. R., Lawrence C. H., Clark M. C., et al. 1989. Isolation and characterization of phytotoxins associated with *Streptomyces scabie*. *J. Agr. Food Chem.* **13**, 849-850.
28. Cheng M. J., Tseng M., Chen I. S., et al. 2009. Secondary metabolites from the culture broth of actinomycete *Acrocarpospora* sp. firdi 001 and their antimicrobial activity. *J. Chil. Chem. Soc.* **54**, 198-200.
29. Lewis E. A., Adamek T. L., Vining L. C., et al. 2003. Metabolites of a blocked chloramphenicol produce. *J. Nat. Prod.* **66**, 62-66.
30. Hao J. J., Ashley K. 2021. Irreplaceable role of amendment-based strategies to enhance soil health and disease suppression in potato production. *Microorganisms.* **9**, 1660.
31. Rossi-Tamisier M., Benamar S., Raoult D., Fournier P. E. 2015. Cautionary tale of using 16S rRNA gene sequence similarity values in identification of human-associated bacterial species. *Int. J. Syst. Evol. Microbiol.* **65**, 1929-1934.
32. Molina-Menor E., Gimeno-Valero H., Pascual J., Peretó J., Porcar M. 2021. High culturable bacterial diversity from a European desert: the tabernas desert. *Front. Microbiol.* **11**, 583120.
33. Yang, Z., Qiao, Y., Konakalla, N.C. et al. 2023. *Streptomyces* alleviate abiotic stress in plant by producing pteridic acids. *Nat Commun* **14**, 7398.
34. Weeraphan T., Somphong A., Poengsungnoen V. 2023. Bacterial microbiome in tropical lichens and the effect of the isolation method on culturable lichen-derived actinobacteria. *Sci Rep.* **13**, 5483.
35. Sapkota A., Thapa A., Budhathoki A., Sainju M., Shrestha P., et al. 2020. Isolation, characterization, and screening of antimicrobial-producing actinomycetes from soil samples. *Int. J. Food Microbiol.* **2020**, 2716584.
36. Wei Q., Li J., Yang S., Wang W. Z., Min F. X., et al. 2022. *Streptomyces rhizophilus* causes potato common scab disease. *Plant Dis.* **106**, 266-274.
37. Biessy A., Filion M. 2022. Biological control of potato common scab by plant-beneficial bacteria. *Biol. Control.* **165**, 104808.
38. Jordaan E., Van der Waals J. E. 2016. *Streptomyces* species associated with common scab lesions of potatoes in South Africa. *Eur. J. Plant. Pathol.* **144**, 631-643.

39. Li Y., Liu J., Díaz-Cruz G., Cheng Z., Bignell D. R. 2019. Virulence mechanisms of plant-pathogenic *Streptomyces* species: an updated review. *Microbiology*. **165**, 1025–1040.
40. Díaz-Cruz, G.A., Liu J. Y., Tahlan K., Bignell, DRD. 2022. Nigericin and geldanamycin are phytotoxic specialized metabolites produced by the plant pathogen *Streptomyces* sp. 11-1-2. *Microbiol. Spectr.* **10**, e02314-21.
41. Lapaz M. I., López A., Huguet-Tapia J. C., Pérez-Baldassari F. M., Iglesias C., et al. 2018. Isolation and structural characterization of a non-diketopiperazine phytotoxin from a potato pathogenic *Streptomyces* strain. *Nat. Prod. Res.* **33**, 2951-2957.
42. Hoerlein G. 1994. Glufosinate (phosphinothricin), a natural amino acid with unexpected herbicidal properties. *Rev. Environ. Contam. T.* **138**, 73-145.
43. Wolfe J. C., Neal J. C., Harlow C. D., Gannon T. 2016. Efficacy of the bioherbicide Thaxtomin A on smooth crabgrass and annual bluegrass and safety in cool-season turfgrasses. *Weed Technol.* **30**, 733-742.

Figures

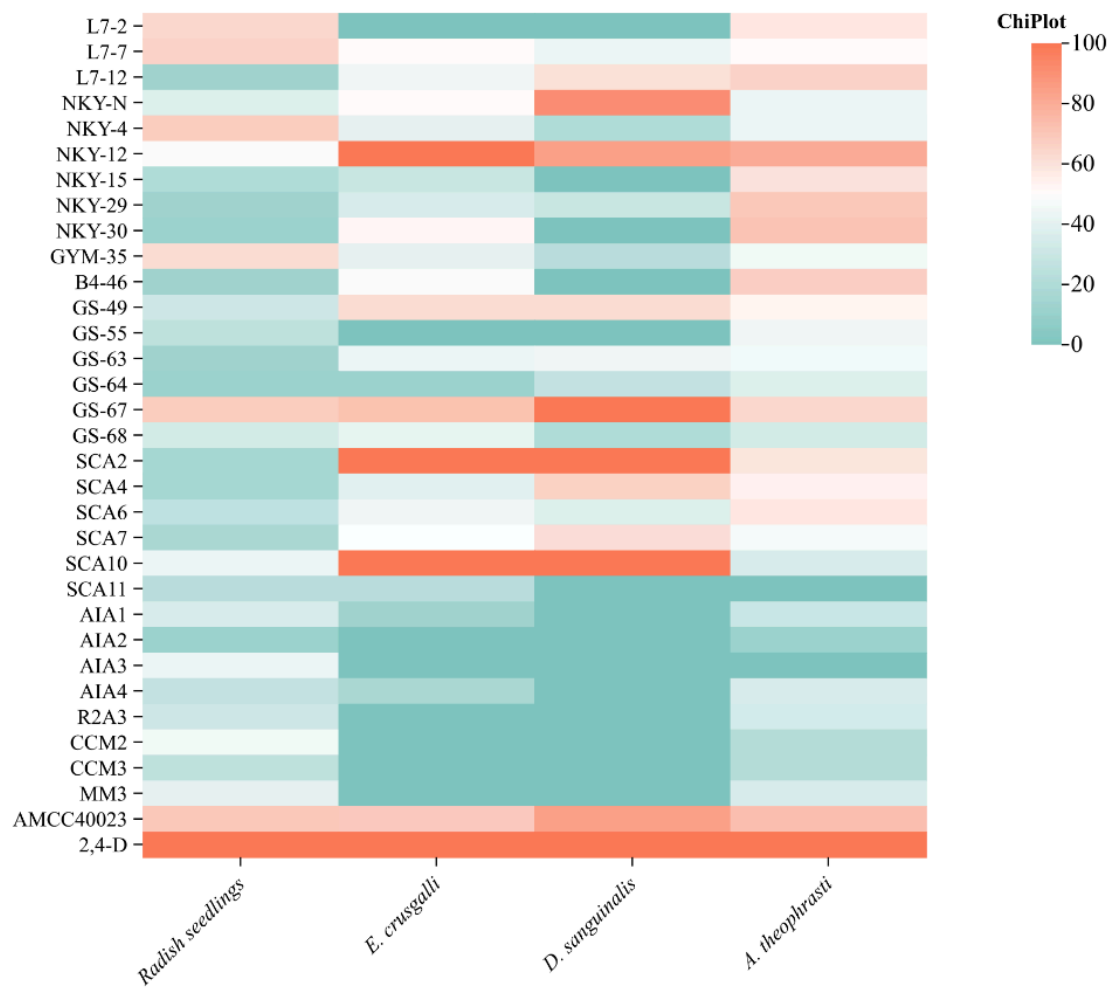


Figure 1

The heatmap depicts inhibitory effects of the fermentation broth on the growth of radish seedlings and weeds roots (%).

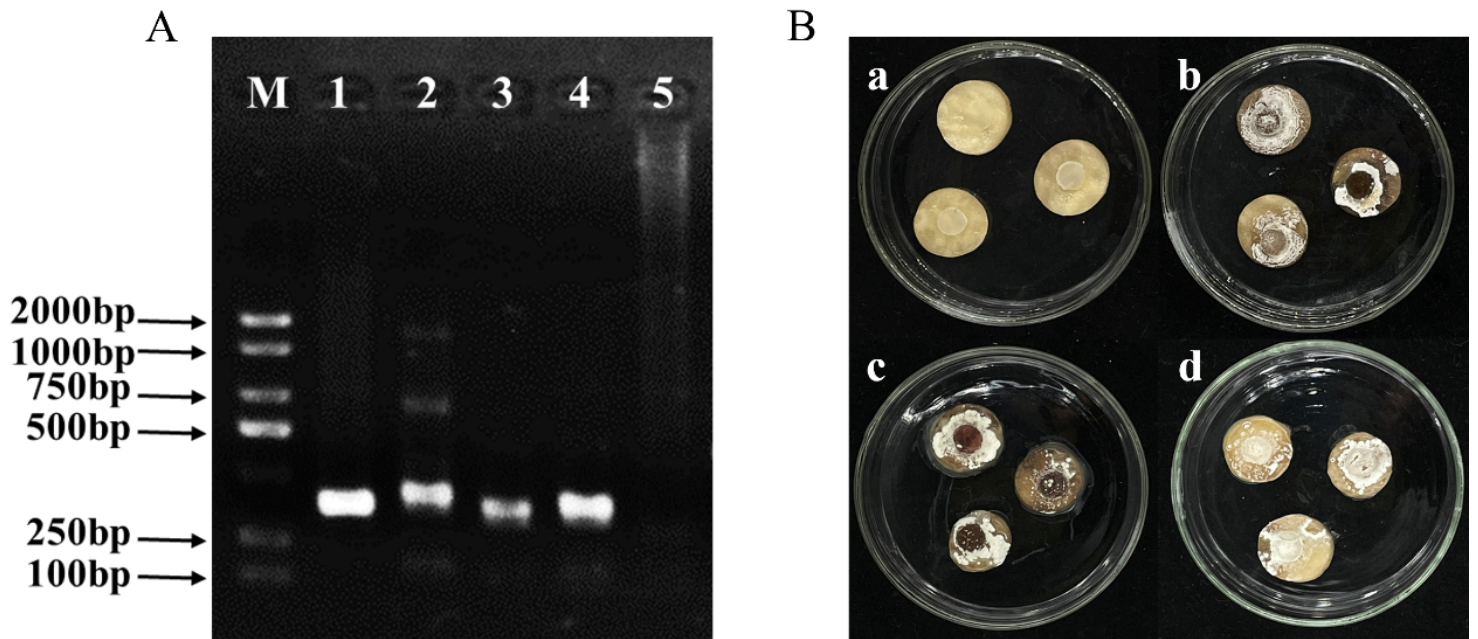


Figure 2

Pathogenicity test of isolate. (A) PCR detection electrophoresis results of pathogenic genes *TxtAB*. (B) Pathogenicity verification results of small potato chip. (A) DL2000 Marker (M); Strain AMCC400023 (1); Strain L7-2 (2) Strain L7-7 (3); Strain NKY-4 (4); Strain GS-67 (5). (B) GS media (a); Strain AMCC400023 (b); Strain NKY-4 (c); Strain L7-2 (d).

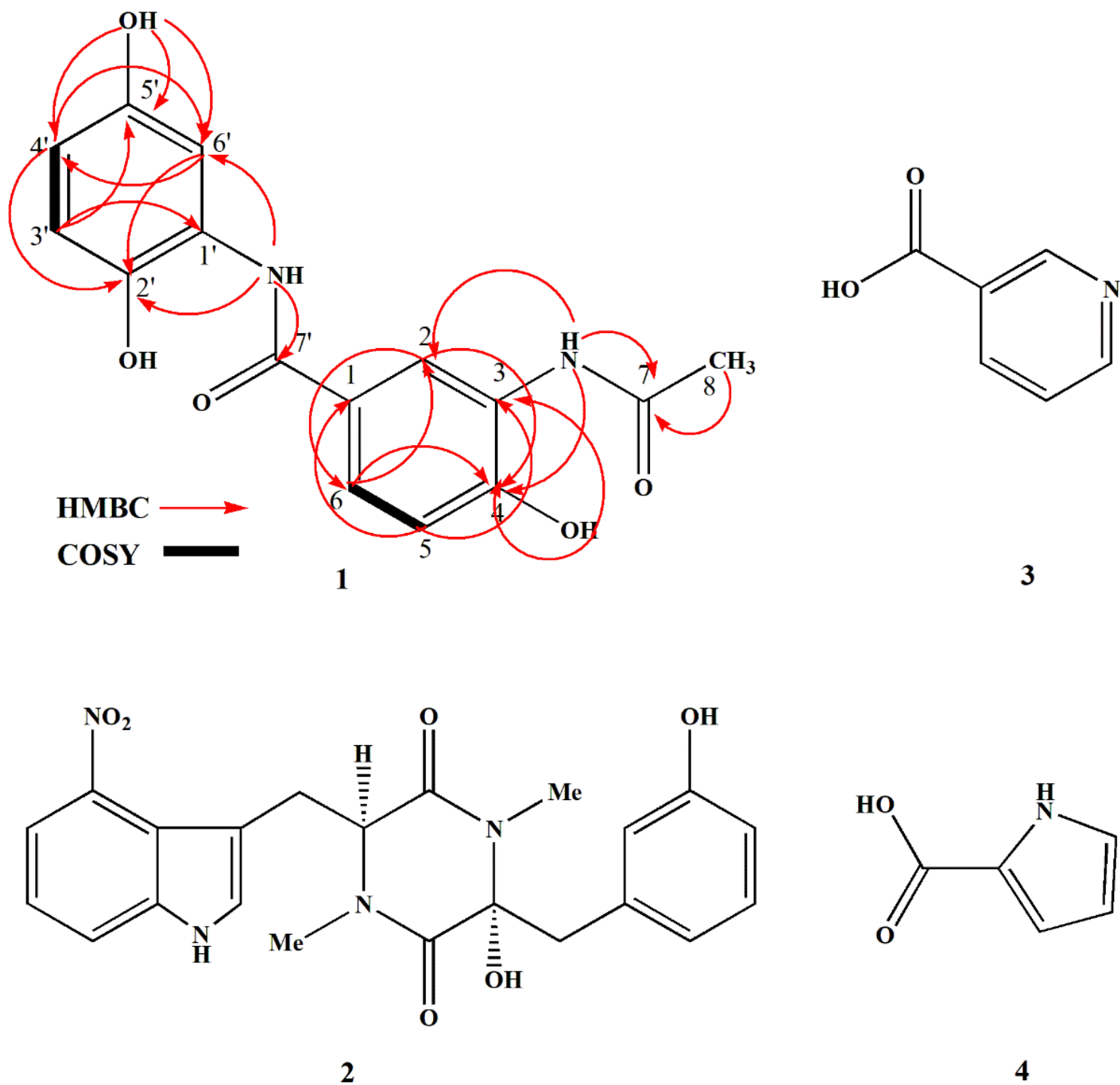


Figure 3

The secondary metabolites of strain *S. bottropensis* AMCC400023.

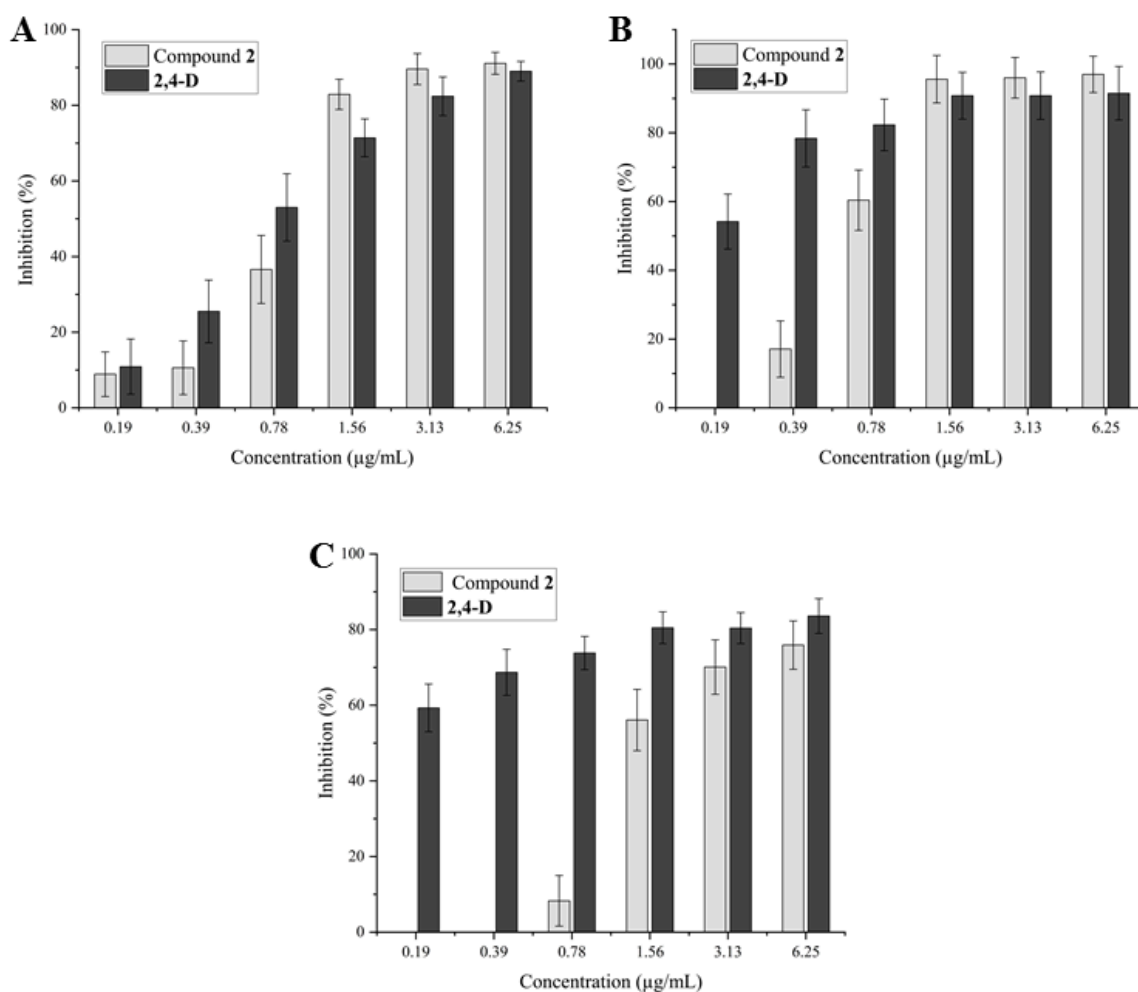


Figure 4

IC₅₀ values of compound **2** in inhibiting the growth of weeds roots.

The 2,4-D as positive control. (A) *E. crusgalli*; (B) *D. sanguinalis*; (C) *A. theophrasti*.

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