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Pentaene macrolides AB023a and takanawaene C produced by *Streptomyces xanthocidicus* strain S3 for controlling pepper anthracnose

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Abstract

A natural product library consisting of the culture extracts of 814 actinomycete strains was screened for antifungal compounds that disrupt the cell integrity of plant pathogenic fungi using an adenylate kinase (AK) assay system. The culture extract of *Streptomyces xanthocidicus* strain S3 exhibited high AK activity against various plant pathogens. The active ingredients, AT-1 and AT-2, were isolated from the culture extract using a series of chromatographic procedures. Based on MS, UV, and NMR spectrometric analyses, the structures of AT-1 and AT-2 were determined as the pentaene macrolides, AB023a and takanawaene C. AB023a and takanawaene C displayed broad-spectrum antifungal activity against *Aspergillus oryzae*, *Botrytis cinerea*, *Colletotrichum coccodes*, *C. gloeosporioides*, *C. orbiculare*, *Cylindrocarpon destructans*, and *Fusarium oxysporum* f. sp. *lycopersici*, showing minimum inhibitory concentrations of 1–32 µg/mL. Treatment of AB023a and takanawaene C successfully inhibited anthracnose development on pepper plants in a concentration-dependent manner without phytotoxicity. The disease control efficacy of both compounds was comparable to that of the commercial fungicide chlorothalonil. Collectively, these results suggest that the polyene macrolides produced by *S. xanthocidicus* strain S3 can be used as natural fungicides for plant disease control.

Keywords *Streptomyces xanthocidicus*, *Colletotrichum coccodes*, Pentaene macrolide, Pepper anthracnose, Plant disease control agent

Introduction

Colletotrichum species, causal pathogens of anthracnose, have a broad host range and are known to be widely distributed in temperate regions [1]. Generally, *Colletotrichum* spp. can attack leaves, stems, and both pre- and post-harvest fruits to cause dark, sunken, circular spots, leaf blight, and defoliation, leading to direct marketable yield and economic losses [2, 3]. Synthetic fungicides have been intensively used to reduce the loss incurred from *Colletotrichum* spp. However, the increased environmental risks associated with their use and the emergence of fungicide-resistant strains have raised public concerns, prompting the development of new control measures. One such measure involves the utilization of natural

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product-based fungicides with a novel mode of action and enhanced efficacy.

Natural products are a rich source of biologically active compounds that have the potential to be developed into new medicinal and agricultural drugs [4–6]. As a source of fungicidal agents, they offer several merits in terms of biocompatibility, degradability, and structural diversity in comparison to synthetic compounds [7]. The discovery of streptomycin from *Streptomyces griseus* and penicillin from *Penicillium notatum* has opened new avenues in which natural products are perceived as sources of drugs [8]. Early research has led to the discovery of numerous novel antibiotics and the development of commercial antifungal and antibacterial agents. By the end of 2013, the FDA had approved 547 natural products, including unmodified natural products, semisynthetic derivatives, and synthetic structures with natural product-derived pharmacophores [9]. In addition, antifungal agents originating from natural products have been used to protect crops in agricultural fields. Kasugamycin is used per se as an active ingredient in commercial fungicides, and strobilurin has become a lead compound in numerous QoI fungicides, one of the two most popular fungicide classes used in agricultural fields [10]. Blasticidin S, kasugamycin, validamycin, streptomycin, polyoxin, midiomycin, and oxytetracycline have been marketed as fungicides for plant disease control [11].

Targeting the integrity of the fungal cell envelope, which consists of cell walls and membranes, has emerged as a crucial strategy for the discovery of potent antifungal agents owing to its lethal effect against fungal pathogens [12, 13]. As part of our efforts to find natural antifungal agents to control plant diseases, we adopted an adenylate kinase (AK) assay to screen a natural product library for disruptors of fungal cell envelopes. An AK-based cell lysis assay enables the screening of antifungal compounds targeting cell envelopes via detection of ATP leakage from cells. Therefore, high AK activity indicates substantial damage to the cell envelope, leading to increased ATP release from the cell. In the screening process, the culture extract of *S. xanthocidicus* strain S3 showed potent AK activity compared with that of caspofungin, which inhibits fungal 1,3- β -glucan synthase, an essential component of fungal cell wall biosynthesis [14]. In this study, we identified active ingredients of the culture extracts of *S. xanthocidicus* strain S3 via chromatographic and spectroscopic analyses and examined their antifungal activity against plant pathogenic fungi and disease control efficacy on anthracnose in pepper plants.

Materials and methods

Construction of a microbial culture extract library

Microorganisms were isolated from soil samples collected in Jeju island. Soil samples (1 g) collected from 5 to 20 cm depth of the soil surface were mixed with 10 mL of sterilized water in 15 mL falcon tube. The tubes containing mixtures were then shaken at 28 °C at 200 rpm for 1 h. The soil suspensions were filtered using Whatman no. 1 filter paper. The filtered soil suspensions were serially diluted using tenfold up to 10^{-5} . Soil samples containing 100 μ L of tenfold serial dilutions were plated onto humic acid vitamin agar medium (1.0 g humic acid, 0.5 g Na_2HPO_4 , 1.71 g KCl, 0.05 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02 g CaCO_3 , 0.5 mg thiamine-HCl, 0.5 mg riboflavin, 0.5 mg niacin, 0.5 mg pyridoxine-HCl, 0.5 mg inositol, 0.5 mg Capantothenate, 0.5 mg *p*-aminobenzoic acid, 0.25 mg biotin, 50 mg cycloheximide and 18 g agar in 1 L water, adjusted to pH 7.2). The plates were incubated at 28 °C for 4–14 days. Microbial colonies with different morphological type which appeared on the plates were selected and transferred into trypticase soy agar (TSA). The isolates were stored in 20% glycerol suspension at -80 °C. To construct of a microbial culture extract library, selected microorganisms were cross-hatch streaked three TSA plate (20 mL of medium in a 9 cm diameter). After incubation for 3–5 days at 28 °C, the cultures were thinly sliced and extracted with 50 mL of methanol in a sonication bath for 90 min. The culture extracts were centrifuged at $10,000 \times g$ for 30 min, and evaporated under reduced pressure. The concentrated culture extracts were dissolved in 1 mL dimethyl sulfoxide (DMSO).

Adenylate kinase assay

The AK assay was performed as described previously [13, 15]. Adenylate kinase assay was evaluated using conidial suspension of *Fusarium oxysporum* f. sp. *lycopersici* (4×10^6 conidial/mL). 25 μ L of the conidial suspension was added into each well of a 96-well plate containing 25 μ L of quadruple-strength potato dextrose, 48 μ L of sterile water, and 2 μ L of each microbial extract. Caspofungin (40 μ g/mL) and DMSO (2%) were used as positive and negative controls, respectively. The 96-well plate was incubated at 28 °C for 4 h and then putted a plate at room temperature for 1 h. The mixture (50 μ L) from each well of 96-well plate was transferred to a white-walled 96-well plate and treated with AK detection reagent (50 μ L) (Toxilight kit, Lonza, Basel, Switzerland). After incubation for 15 min, relative luminescence units (RLU) were measured using a multi-label plate reader Victor 3 (Perkin Elmer Inc., Waltham, MA, USA).

16S rRNA gene sequencing and phylogenetic analysis of the S3 strain

The actinomycete, strain S3, was grown on TSA at 28 °C for five days. The boiling method described by Metsä-Ketelä et al. [16] was used for the extraction of genomic DNA from strain S3. The colonies were transferred to a microtube and suspended in 100 µL sterilized water. The cell suspension was boiled in a 100 °C water bath for 15 min and centrifuged at 12,225×g for 10 min. The supernatant was transferred to a microtube and used as a template for PCR. Primers used for amplification of the 16S rRNA gene of strain S3 were as follows: forward primer fD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer rP2 (5'-ACGGCTACCTTGTTACGACTT-3'). PCR amplification was performed using 2×PreMIX-HF (Macrogen Co., Seoul, Korea). PCR reaction mixtures consisted of 100 ng template DNA, primers fD1 and rP2 (each at a concentration of 10 pmol), 0.2 U Taq-HF DNA polymerase, Taq-HF buffer, 4 mM MgCl₂, and a mixture of 2×deoxynucleoside triphosphates (0.4 mM each). The final volume of the mixture was adjusted to 20 µL by adding triple distilled water. The PCR mixture was subjected to an initial denaturing step at 94 °C for 5 min and thermocycling conditions of 25 cycles consisting of 1 min at 94 °C, 1 min annealing at 56 °C, and 1 min and 30 s extension at 72 °C. Finally, the reaction sequence was terminated after a 3 min extension at 72 °C. The amplified product was examined via electrophoresis on a 1% agarose gel and visualized using UV fluorescence after staining with ethidium bromide. Purification of the amplified product was performed using the LaboPass PCR product purification kit (CosmoGen, Korea) according to the manufacturer's instructions.

The 16S rRNA gene sequence of strain S3 was analyzed using an ABI Prism 3700 automatic DNA sequencer (Applied Biosystems, CA, USA) and aligned using the DNASTAR software program (DNASTAR Inc., WI, USA). The aligned sequences were compared with *Streptomyces* species submitted to the GenBank database using BLAST network services at the National Center for Biotechnology Information (NCBI). Phylogenetic analysis of the 16S rRNA gene sequences of strain S3 was carried out using MEGA software and the neighbor-joining method [17]. Bootstrap values are expressed as percentages of 1,000 replicates.

Production and purification of active compounds from the strain S3

The S3 strain was cultured on TSA for five days at 28 °C. Single colonies on plates were selected and pre-cultured in 1-L Erlenmeyer flasks containing 100 mL trypticase soy broth at 28 °C for three days on a rotary shaker at

200 rpm. The culture was used as an inoculum (1% of culture volume) for a 2.5 L culture using 25 flasks that each contained 100 mL production medium (30 g glucose, 30 g soybean meal, 5 g cottonseed flour, 1 g yeast extract, 3 g CaCO₃, and 1 L distilled water, pH 8.0). After incubation at 28 °C for five days in a rotary shaker (200 rpm), the culture broth (2.5 L) was centrifuged at 10,000×g for 30 min and extracted with an equal volume of *n*-butanol. The *n*-butanol extracts were concentrated under reduced pressure and the remaining residue dissolved in 1 L of 2% aqueous methanol.

The crude *n*-butanol extract was purified using flash column chromatography with a 500 mm×50 mm i.d. glass column packed with Diaion HP-20 resin (Mitsubishi Chemical, Tokyo, Japan) and a 200 mm×30 mm i.d. glass column packed with 20–63 µm Lichropep RP-18 resin (Merck, Darmstadt, Germany). The crude extract was poured and absorbed onto Diaion HP-20 resin and then eluted with a stepwise gradient of 0, 20, 40, 60, 80, and 100% methanol in water, then washed by acetone. The eluted fractions were evaporated *in vacuo* and their antifungal activities against *F. oxysporum* f. sp. *lycopersici* evaluated using the disk diffusion method [18]. The active 100% methanol fraction was dried and redissolved in 500 mL of 2% aqueous methanol, and then subjected to C18 flash column chromatography. The column was eluted with a stepwise gradient of 0, 20, 40, 60, 80, and 100% methanol in water. The active 80% methanol fraction was concentrated *in vacuo*, dissolved in a small amount of methanol, and further purified using a high performance liquid chromatography (HPLC) system (Varian, Palo Alto, CA, USA) equipped with J'sphere ODS-H80 column (250×10 mm, 4 µm; YMC Co., Ltd., Kyoto, Japan). The HPLC analysis was conducted at a flow rate of 2 mL/min using a linear gradient elution of 20–95% methanol in water over 10 min, followed by isocratic elution with 95% methanol in water for 20 min. Each peak was monitored at 254 nm and collected individually. Finally, two antifungal peaks AT-1 (2.9 mg) and AT-2 (2.1 mg) were obtained at respective retention time of 17.7 min and 19.1 min, respectively.

Spectroscopic analyses of purified antifungal compounds

The ultra-violet (UV) absorption spectra of the antifungal compounds were measured in an Optizen POP UV-Vis spectrophotometer (Mecasys Co., Ltd., Daejeon, Korea). The high resolution mass spectrometry (HR-MS) data of the antifungal compounds was obtained using a Q-ToF mass spectrometer (Waters, Milford, MA, USA) with a negative electrospray ionization (ESI) mode. For analysis of nuclear magnetic resonance (NMR), the active compounds were dissolved in dimethyl sulfoxide-*d*₆ (99.9% D; Cambridge Isotope

Laboratories, Andover, MA, USA). The ^1H and ^{13}C NMR spectra were obtained using a Varian 500 MHz NMR spectrometer.

Broth microdilution assay of AB023a and takanawaene C on various plant pathogenic fungi

The minimum inhibitory concentration (MIC) values of AT-1 and AT-2 were evaluated for various fungal and oomycete plant pathogens using the CLSI M38-A protocol in a 96-well plate [19]. Spore suspensions (1×10^5 spores/mL) of *Alternaria brassicicola*, *Aspergillus oryzae*, *Botrytis cinerea*, *Cladosporium cucumerinum*, *Colletotrichum coccodes*, *C. gloeosporioides*, *C. orbiculare*, *Cylindrocarpon destructans*, *Fusarium oxysporum* f. sp. *cucumerinum*, *F. oxysporum* f. sp. *lycopersici*, *Rhizopus stolonifer* var. *stolonifer*, and *Phytophthora capsici* were added to potato dextrose broth in a 96-well plate. Compounds were serially diluted two-fold to yield different concentrations of 1–128 $\mu\text{g/mL}$. The MIC value of each compound was determined by visual observation of microbial growth at 1–3 days after treatment.

Pepper anthracnose control efficacy test of AB023a and takanawaene C

The disease control efficacy of AT-1 and AT-2 was evaluated against pepper anthracnose caused by *C. coccodes*. Pepper seeds (*Capsicum annuum* L. cv. Nockwang) were sown in 72-hole plastic tray (hole size: W58 \times L58 \times H63 mm) containing the sterilized commercial soil (Baroker, Seoul Bio Co., Eumsung, Korea) and grown in a greenhouse at 25 ± 3 °C until the four-leaf stage of pepper seedlings (about 4 weeks). Test compounds were dissolved in methanol (25 mg/mL) and diluted to concentrations of 1, 10, 50, 100, and 500 $\mu\text{g/mL}$ in 0.05% Tween 20 solution. Each solution was sprayed onto the leaf surface of pepper plants. The commercial fungicide chlorothalonil and 2% methanol were used as positive and negative controls, respectively. After 24 h after treatment, the spore suspension (4×10^6 conidia/mL) of *C. coccodes* was sprayed on the leaf surface of pepper plants. Next, the inoculated plants were placed in a dew chamber at 28 °C for 24 h and thereafter transferred to greenhouse. At seven days after inoculation, disease severity (diseased leaf area / total leaf area $\times 100\%$) was estimated using an image analysis program Matrox Inspector 2.2 [20]. Data ($n = 12$) were analyzed by one-way analysis of variance (ANOVA) and the significance of mean differences between treatments was determined by Duncan's least significant range test ($P < 0.05$) using R software (version 4.2.3; available online: <https://www.r-project.org/>) [21].

Results

Adenylate kinase activity of *Streptomyces xanthocidicus* strain S3

Among 814 actinomycete culture extracts, the S3 culture extract was one of the most effective in inducing AK release from the mycelia of *F. oxysporum* f. sp. *lycopersici*. The S3 extract showed potent AK-releasing activity that was 86.6% similar to that of caspofungin (positive control) (Additional file 1: Fig. S1). S3 extract, like caspofungin, could disrupt the integrity of the fungal cell wall. Therefore, strain S3 was selected for further studies to identify its active ingredients and antifungal efficacy against plant pathogenic fungi.

Identification of *Streptomyces xanthocidicus* strain S3.

The partial 16S rRNA region (1335 nucleotides) of the S3 strain was analyzed by comparison with sequences in GenBank through nBLAST (<http://www.ncbi.nlm.nih.gov/> BLAST). In the phylogenetic analysis of 16S rRNA gene sequences (Additional file 1: Fig. S2), the S3 strain was most closely related to *Streptomyces xanthocidicus* IFO 13469 (99.48% sequence identity). Thus, the S3 stain was identified as *S. xanthocidicus* and deposited in GenBank under the accession number of KF041002.

Structure elucidation of AB023a and takanawaene C

Through a series of chromatographic steps, the antifungal compounds AT-1 (2.9 mg) and AT-2 (2.1 mg) produced by the S3 strain were obtained as yellow powders. The UV spectra of AT-1 and AT-2 in methanol showed the characteristic strong UV absorption maxima of 317, 331, and 349 nm indicating the presence of conjugated pentaene moiety (Fig. 1). The HR-ESI-MS of AT-1 and AT-2 showed intense $[\text{M}-\text{H}]^-$ molecular ions at m/z 549.3434 (calculated m/z 549.3427) and m/z 577.3766 (calculated m/z 577.3740) corresponding to molecular formula of $\text{C}_{31}\text{H}_{50}\text{O}_8$ and $\text{C}_{33}\text{H}_{54}\text{O}_8$, respectively (Fig. 1). The ^1H and ^{13}C NMR data of AT-1 and AT-2 are summarized in Table 1. The UV, MS, and NMR analytic data for AT-1 and AT-2 were identical to those of the previously reported pentaene macrolides AB023a and takanawaene C, respectively (Fig. 2) [22, 23].

In vitro and in vivo antifungal activities of AB023a and takanawaene C

The MICs of AB023a and takanawaene C against fungal and oomycete plant pathogens are summarized in Table 2. Both AB023a (MICs of 2–16 $\mu\text{g/mL}$) and takanawaene C (MICs of 1–2 $\mu\text{g/mL}$) showed broad-spectrum antifungal activity against all tested fungi except *R. stolonifer* var. *stolonifer*. Among tested fungi, three *Colletotrichum* species and *B. cinerea* were most sensitive fungi against AB023a. The MIC values of takanawaene C were 1–2 $\mu\text{g/}$

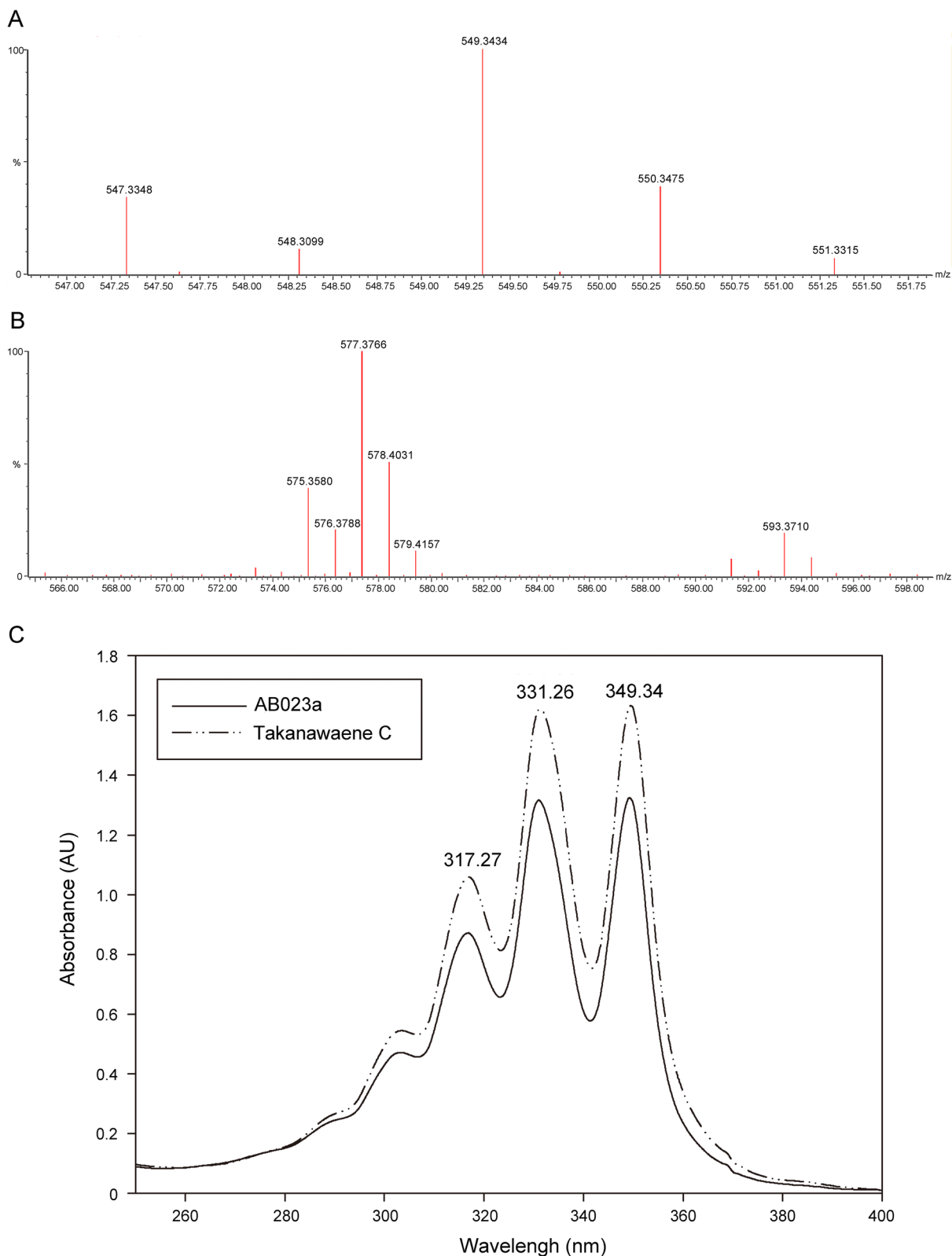


Fig. 1 Mass spectra and UV spectroscopic data of AB023a and takanawaene C. Mass spectra of **A** AB023a and **B** takanawaene C. **C** UV spectra of AB023a and takanawaene C

Table 1 ^1H and ^{13}C NMR spectral data for AB023a and takanawaene C (δ in ppm and J in Hz) in $\text{DMSO-}d_6$

Carbon no	AB023a		Takanawaene C	
	δ_{C}	δ_{H}	δ_{C}	δ_{H}
1	173.7		174.2	
2	46.1	2.26 (1H, m)	45.9	2.30 (1H, m)
3	72.0	3.71 (1H, m)	71.8	3.74 (1H, m)
4	41.5	1.32 (2H, m)	41.7	1.34 (2H, m)
5	71.4	3.45 (1H, m)	71.4	3.44 (1H, m)
6	38.8	1.23 (2H, m)	38.8	1.25 (2H, m)
7	22.4	0.83 (1H, m), 1.71 (1H, m)	22.5	0.83 (1H, m), 1.72 (1H, m)
8	40.4	1.23 (2H, m)	40.1	1.25 (2H, m)
9	71.2	3.51 (1H, m)	71.4	3.52 (1H, m)
10	43.4	1.27 (2H, m)	43.6	1.29 (2H, m)
11	69.6	3.80 (1H, m)	69.5	3.80 (1H, m)
12	38.2	1.23 (2H, m)	38.3	1.25 (2H, m)
13	67.7	3.45 (1H, m)	67.7	3.44 (1H, m)
14	45.4	1.63 (1H, m)	45.4	1.62 (1H, m)
15	73.5	3.63 (1H, m)	74.5	3.60 (1H, m)
16	136.5	5.59 (1H, dd, 15.35, 8.52)	136.7	5.57 (1H, dd, 15.22, 8.66)
17	130.3	6.11 (1H, m)	130.4	6.08 (1H, m)
18	132.9–131.2	6.25 (1H, m)	132.9–131.1	6.22 (1H, m)
19	132.9–131.2	6.25 (1H, m)	132.9–131.1	6.22 (1H, m)
20	132.9–131.2	6.25 (1H, m)	132.9–131.1	6.22 (1H, m)
21	132.9–131.2	6.25 (1H, m)	132.9–131.1	6.22 (1H, m)
22	132.9–131.2	6.25 (1H, m)	132.9–131.1	6.22 (1H, m)
23	132.9–131.2	6.25 (1H, m)	132.9–131.1	6.22 (1H, m)
24	129.5	6.11 (1H, m)	129.7	6.08 (1H, m)
25	136.2	5.87 (1H, dd, 15.31, 6.65)	136.1	5.82 (1H, dd, 15.22, 6.87)
26	38.2	2.34 (1H, m)	36.0	2.45 (1H, m) ^a
27	74.4	4.65 (1H, m)	80.1	4.59 (1H, dd, 8.60, 3.45)
28	13.2	1.06 (3H, d, 7.01)	13.8	1.12 (3H, d, 6.90)
29	10.4	0.88 (3H, d, 6.69)	10.5	0.88 (3H, d, 6.79)
30	15.9	1.01 (3H, d, 6.81)	15.6	1.00 (3H, d, 6.65)
31	18.7	1.18 (3H, d, 5.97)	28.7	1.95 (1H, m)
32			19.7	0.83 (3H, d, 6.96)
33			15.4	0.85 (3H, d, 7.04)

^a Signals are partially obscured by solvent

mL indicating that takanawaene C is a more potent anti-fungal compound than AB023a. On the other hand, both compounds showed no significant activity against the oomycete *P. capsici* (MIC > 128 $\mu\text{g/mL}$) (Table 2).

The efficacy of AB023a and takanawaene C in controlling pepper anthracnose was evaluated. Both compounds were effective to protect pepper plants from an attack of *C. coccodes* (Fig. 3). Treatment of AB023a and takanawaene C significantly inhibited anthracnose development on pepper plants in a concentration-dependent manner. Disease severity was 2.36% in untreated pepper plants, whereas significant reduction in lesion area on pepper leaves started to

appear in plants treated with 50 $\mu\text{g/mL}$ of each compound (Fig. 3). At the highest concentration of 500 $\mu\text{g/mL}$, AB023a and takanawaene C completely controlled the development of pepper anthracnose without phytotoxic effect (Fig. 3). The disease control efficacy of both compounds was comparable to that of the commercial fungicide chlorothalonil (Fig. 3).

Discussion

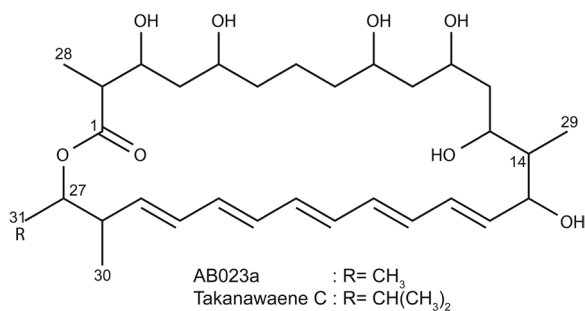
As part of our efforts to find natural antimicrobial agents to control plant diseases, we screened a natural product library consisting of microbial culture extracts for fungal

Table 2 Minimum inhibitory concentrations (MICs) of AB023a and takanawaene C against various plant pathogenic fungi and oomycetes

Plant pathogenic fungi and oomycetes	MIC ^a (μg/mL)	
	AB023a	Takanawaene C
<i>Alternaria brassicicola</i>	16	2
<i>Aspergillus oryzae</i>	8	1
<i>Botrytis cinerea</i>	2	1
<i>Cladosporium cucumerinum</i>	8	1
<i>Colletotrichum coccodes</i>	2	1
<i>Colletotrichum gloeosporioides</i>	2	1
<i>Colletotrichum orbiculare</i>	2	1
<i>Cylindrocarpon destructans</i>	8	1
<i>Fusarium oxysporum</i> f. sp. <i>cucumerinum</i>	8	2
<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	8	2
<i>Phytophthora capsici</i>	> 128 ^b	> 128
<i>Rhizopus stolonifer</i> var. <i>stolonifer</i>	> 128	> 128

^a After incubation for 1–3 days, the lowest concentration that completely inhibited growth of the plant pathogenic fungi and oomycetes was determined.

^b The > 128 value indicates that growth of the test fungi was not inhibited at concentrations above 128 μg/mL

**Fig. 2** Structures of AB023a and takanawaene C produced by *Streptomyces xanthocidicus* strain S3

cell envelope disruptors using an AK-based cell lysis assay. In this study, the culture extract of *S. xanthocidicus* strain S3 showed the AK-releasing activity similar to that of caspofungin (a fungal 1,3-β-glucan inhibitor) (Additional file 1: Fig. S1). Compounds with AK releasing activity disrupt cell membrane permeability and integrity, and cause leakage of intracellular contents, and leading to cell death (strong antifungal activity). Therefore, the polyene macrolides AB023a and takanawaene C could be the active

ingredients for the promising AK-releasing activity of the S3 culture extract.

AB023a and takanawaene C are 28-membered pentaene macrolides. Various 28-membered pentaene macrolides have been reported so far. Filipin [24], fungichromin [25], chainin [26] and elizabethin [27] are methylpentaenes having a methyl group substituted at C-16. Strevertene [28] with a carboxyl group substituted at C-14 has also been reported. Bortolo et al. [23] first isolated and characterized AB023a and AB023b from the broth of *Streptomyces* sp. SD581. AB023a and AB023b are 28-membered pentaene macrolides with no substituents on the conjugated double-bond chain, and the two compounds differ only by a substitution at C-27. Later, takanawaenes A, B, and C were reported to have a proton or methyl group at C-14, with differences in methyl, ethyl, and isobutyl groups at C-27 [22]. These macrolides were reported to disrupt the fungal plasma membrane; they bind ergosterol, a major fungal sterol in the plasma membrane, and cause cell death via membrane pore formation [29].

In previous reports [30, 31], the polyene macrolides AB023a and takanawaene C have been described as broad-spectrum antifungal agents. Similarly, in our study, both compounds showed inhibitory activity against a wide range of plant pathogenic fungi (Table 2). Notably, the fungal growth of *Colletotrichum* spp. was completely inhibited at very low concentrations of 1–2 μg/mL (Table 2). Furthermore, takanawaene C showed two- to eight-fold lower MIC values than AB023a (Table 2), suggesting that the alkyl substituents at C-27 could play an important role in determining antifungal property of 28-membered pentaene macrolides. Kim et al. [31] reported the antifungal activity of AB023a and takanawaenes A/B/C against *Aspergillus niger*, *Mucor racemosus*, *Candida albicans*, and *S. cerevisiae* using a paper disk-agar diffusion assay. Although these polyene macrolides differed in methyl, ethyl, and isobutyl groups at C-27 position, there was no substantial difference in antifungal activity [31]. Therefore, further studies are required to determine structure–activity relationship of AB023a and takanawaenes.

Despite the long-standing use of various polyene macrolides in the clinical and food industries, their recognition as fungicides for plant disease control remains limited. These compounds are susceptible to inactivation or degradation under harsh conditions commonly encountered during crop cultivation, including high temperatures,

(See figure on next page.)

Fig. 3 In vivo disease control efficacies of AB023a, takanawaene C, and chlorothalonil against anthracnose caused by *Colletotrichum coccodes* in pepper plants. **A** anthracnose development on pepper leaves treated with different concentrations of the antifungal compounds. Disease severity (diseased leaf area/total leaf area × 100%) of leaves treated with **B** AB023a, **C** takanawaene C, **D** chlorothalonil at the indicated concentrations. Disease severity was rated on 7 days after inoculation. Different letters on the graph indicate significant differences between treatments (n = 12 replicates; P < 0.05)

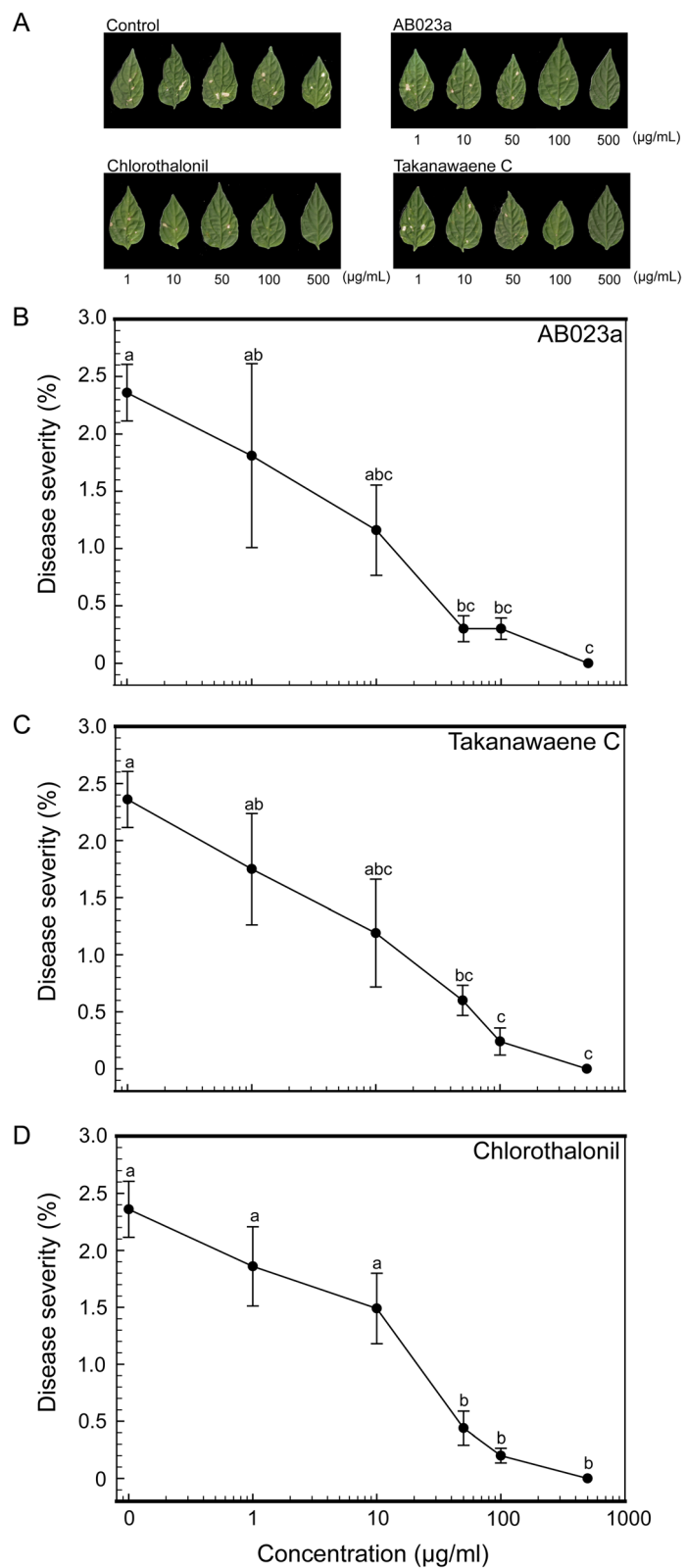


Fig. 3 (See legend on previous page.)

dry soil, exposure to heavy metals, and ultraviolet radiation. This vulnerability arises from their inherent structural characteristics [32]. Phytotoxicity of polyenes can be another limiting factor to their use as control agents of plant disease [33]. Nevertheless, recent reports have shown promising results for several pentaene macrolides as control agents for plant diseases. Strevertene A, strevertene B [34] and fillipin III [24] were reported to strongly inhibit tomato Fusarium wilt caused by *F. oxysporum* f. sp. *lycopersici* at concentrations of 1 and 10 µg/mL, respectively. More recently, fungichromin isolated from the culture filtrate of *S. padanus* strain PMS-702 was reported to be effective against downy mildew on cucumber leaves caused by *Pseudoperonospora cubensis* [35]. These results demonstrate the potential of pentaene macrolides as agricultural fungicides for plant disease control. In this study, treatment with 100 µg/mL AB023a and takanawaene C showed a control efficacy of 87.3% and 89.8% against anthracnose on pepper plants, respectively. Furthermore, even seven days after treatment, both compounds effectively controlled disease development. In addition to this disease control effect, they did not show any phytotoxicity on pepper plants, even at a dose of 500 µg/mL, the highest concentration tested. These results demonstrate that AB023a and takanawaene C have a certain level of structural stability and selectivity and could be used as plant disease control agents.

To our knowledge, this is the first study to evaluate the potential of AB023a and takanawaene C as disease control agents *in planta*, although further studies on their formulation and application methods are required for their development as commercial fungicides.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13765-023-00813-2>.

Additional file 1: Figure S1. Adenylate kinase (AK) assay of the culture extract of strain S3 against *Fusarium oxysporum* f. sp. *lycopersici*. Error bar represent standard deviation. **Figure S2.** Neighbor-joining phylogenetic tree based on 16S rRNA gene sequence showing the phylogenetic relationships between *Streptomyces xanthocidicus* strain S3 and related *Streptomyces* species. Bootstrap values of 1000 replications are shown at the branch points. Scale bar, 0.01 substitutions per nucleotide position.

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Author contributions

JB: formal analysis, investigation, methodology, conceptualization, writing—original draft, writing—review, and editing. JEK: formal analysis, investigation, conceptualization. JJK: funding acquisition, writing—original draft, writing—review, and editing. BSK: funding acquisition, conceptualization, supervision, validation, writing—original draft, writing—review, and editing. All authors contributed to the article and approved the submitted version.

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Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Competing interests

The authors declare no competing interests.

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