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Antidiabetic components from a mangrove actinobacterial culture in Vietnam

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ABSTRACT

In our quest for mangrove microbial candidates with antidiabetic potential in Vietnam, actinobacterial strains were isolated and screened for inhibitory activity against carbohydrates digesting enzymes α-amylase and α-glucosidase. As a result, *Streptomyces* sp. strain S-X10.2 emerged as a microbe of interest since its ethyl acetate extract exhibited inhibition rates of 62.66 ± 2.50 and 58.95 ± 1.27% against α-glucosidase and α-amylase, respectively, at test concentration of 500 µg/mL. Using column chromatography integrated with bioassays, **XA1** was separated from the ethyl acetate extract and appeared as the most abundant as well as the most bioactive among purified products of the chemical isolation. **XA1** showed α-amylase and α-glucosidase inhibitory activities with IC_{50} values of 294.62 and 335.22 µg/mL, respectively. Further GC/MS identification revealed the presence of saturated and unsaturated fatty acids in **XA1**, with oleic acid (27.70%), palmitic acid (23.70%), and linoleic acid (21.80%) as the most major compounds. These results revealed evidence of bioactive fatty acids in ethyl acetate extract of an actinobacterial strain's fermented culture. Furthermore, they contributed to drawing a new aspect in exploiting natural products from mangrove microorganism.

Keywords: *Streptomyces*, actinobacteria, antidiabetic, fatty acids.

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INTRODUCTION

Mangroves are complex ecosystems that occur in tropical and subtropical intertidal estuarine zones. They are home to a rich biodiversity due to their high salinity and organic matter content. Mangroves are a rich source of bioactive microorganisms, which can be used in medicine, agriculture, and other fields [1]. Mangrove-derived microorganisms include actinomycetes, bacteria, cyanobacteria, algae, and protozoa. These can produce various medicinal compounds, including enzymes, antitumor agents, insecticides, vitamins, and immunosuppressants [2].

A diverse group of actinomycetes was recorded as mangrove inhabitants, which have been known as producers of secondary metabolites. These compounds may exhibit a variety of biological activities, including antibacterial, antifungal, antiviral, and anticancer, as well as agents for treating neurodegenerative diseases and diabetes [3]. Several reports from different geographical locations worldwide have described the occurrences of actinomycetes in diverse mangrove habitats [4]. This relatively large distribution of actinomycetes species throughout the world's mangrove ecosystems appears to reason of mining them efficiently. The bacterial group is considered to be the dominant force behind the discovery of new and exciting chemistry. Up to 2021, 519 natural products from mangrove-derived microorganisms were isolated, among them, exceeding one third are *Streptomyces* derived [4].

Alpha-glucosidase and α-amylase are digestive enzymes involving in the breaking down of carbohydrates into simpler sugars for nutritional absorption in the body [5, 6]. The inhibition against these enzyme targets can significantly reduce the post-prandial increase of blood glucose and, therefore, can be an important strategy for managing blood glucose levels in type 2 diabetic and borderline patients [7]. Currently, there is renewed interest in microorganisms-derived medicines and functional foods modulating physiological effects in preventing and curing diabetes [8, 9]. Accordingly, α-glucosidase and α-amylase inhibitors from these natural sources may offer an attractive strategy for the control of hyperglycemia. Our present study aimed to screen and isolate natural compounds with *in vitro* antidiabetic potential from a mangrove actinobacterial strain in Vietnam.

MATERIALS AND METHODS

General chemicals

Chemicals were purchased from Sigma-Aldrich (USA), and solvents for column chromatography were in analytical grade (Merck, Germany). Ethyl acetate for extraction was purchased from Xilong Scientific Co., Ltd (China). Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F_{254} plates (Merck, Germany), and spots were detected under UV illumination 254 nm and sprayed with H_2SO_4 10% reagents followed by heating. Column chromatography was performed using silica gel 60 (0.04–0.063 mm; 0.063–0.2 mm) purchased from Merck (Germany).

Actinobacterial strain

Actinobacterial strain S-X10.2 was isolated from mangrove sediment collected in Northern Vietnam (Sampling site: N 20°12.226', E 106°33.009'). The strain was cultured on inorganic salts starch agar (ISP4) composed of (g/L): soluble starch - 10.0, K_2HPO_4 - 1.0, $MgSO_4{\cdot}7H_2O$ - 1.0, NaCl - 1.0, $(NH_4)_2SO_4$ - 2.0, $CaCO_3$ -2.0, $FeSO_4{\cdot}7H_2O$ - 0.001, $MnCl_2{\cdot}7H_2O$ - 0.001, $ZnSO_4{\cdot}7H_2O$ - 1.0 and agar - 15.0 (pH 7.2–7.4) and is currently preserved at Laboratory of Experimental Biology - Institute of Chemistry.

The strain shows 98.5% similarity to *Streptomyces* sp. MA66 [accession: MH699171.1] and *Streptomyces* sp. STR22 [accession: KF803368.1] based on 16S rDNA sequence analysis. Morphologically, strain S-X10.2 appeared in ISP4 agar medium with grayish colonies, 1.5–2.2 mm in diameter, after 5 days of incubation (37°C). Physiologically, it was found to produce melanine pigment, utilizing glucose, fructose and lactose as carbon sources. The optimal temperatures and pHs for

the growth of S-X10.2 ranged from 30 to 37°C and from 6.5 to 7.5, respectively. Besides, the strain was found to be able to grow at a salinity of 5%. As a result of these identified properties, the actinobacterial strain was determined as a *Streptomyces* sp.

Preparation of microbial crude extract

Streptomyces sp. S-X10.2 was primarily inoculated in Erlenmeyer flasks containing ISP4 broth (30°C, 110 rpm, 7 days) to prepare bacterial cell suspension. The cell suspension was added to rice pots containing sterile brown rice as the growth substrate with a ratio of 1 mL:50 g (v/w) for actinomycete inoculum production. The inoculum was sealed and incubated (30°C, 12 days), and extracted with ethyl acetate (Xilong Scientific, China). After rotary evaporation at reduced pressure (45°C at 40 rpm) (Eyela, Japan), the ethyl acetate crude extract of S-X10.2 was collected.

Isolation of microbial metabolites

The ethyl acetate crude extract of strain S-X10.2 (80 g) was chromatographed on a silica gel column (0.063–0.2 mm, Φ = 10 cm) eluting with a gradient of n-hexane/ethyl acetate in different ratios 20:1, 10:1, 5:1, 1:1 (v/v), which resulted in four fractions of organic phase symbolizing as A1-A4, respectively. By thin-layer chromatography, fractions A2 and A3 appeared similar and were combined as fraction A23. The fraction A23 was further chromatographed on a silica gel column (0.04–0.063 mm, Φ = 3 cm) eluting with n-hexane/ethyl acetate (20:1, v/v), resulting in 15 sub-fractions symbolizing as A23/1–A23/15. Among these, subfraction A23/1 was purified on a silica gel column (0.04–0.063 mm, Φ = 1.5 cm) eluting with a gradient of n-hexane/ethyl acetate (100:1, v/v) to obtain compound **XA1**.

Structural characterization

To determine the structures of **XA1**, mass and NMR spectra data were analyzed. ^{1}H- and ^{13}C- nuclear magnetic resonance (NMR) spectra were measured on a Bruker Avance III D (Bruker, USA) spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C using tetramethylsilane as an internal standard. Spectra of heteronuclear multiple quantum coherence (HSQC) and heteronuclear multiple bond correlation (HMBC) were further recorded (Bruker, USA) to support data for structure elucidation, particularly the carbon multiplicity and correlated carbon positions.

Gas chromatography

The classical method for preparing fatty acid methyl esters from glycerolipids, including saponification and methylation, was applied [10]. For analyzing fatty acid components of **XA1**, gas chromatography/mass spectroscopy (GC/MS) was carried out with an Agilent 7890 A (USA) gas chromatograph fitted with an HP-5 MS fused silica capillary column (60 m, 0.25 mm, i.d., 0.25 µm film thickness) and a flame ionization detector. Helium was used as the carrier gas and set at 1 $mL.min^{-1}$. Each component's relative percentage was estimated based on the GC peak area (FID response).

Enzyme inhibitory assays

The α-amylase inhibitory activity was assayed as previously reported by Hansawasdi et al., (2000) [11]. Briefly, test samples were pre-incubated with porcine pancreatic α-amylase solution (Sigma-Aldrich, USA) (37°C, 5 min), and then incubated with substrate starch azure solution (10 mg/mL) for reaction (37°C, 10 min). Acetic acid solution was added to stop the reaction. The absorbance was measured at 595 nm (Tecan, Switzerland).

The inhibitory activity against *α*-glucosidase was measured according to Tadera et al., (2006) [12] with minor modifications. A mixture of α-glucosidase (Sigma-Aldrich, USA) and a test sample in phosphate buffer was preincubated at 37°C for 15 min. Then, substrate *p*-NPG (1 mM) was added to the mixture and further incubated (37°C, 20 min). The reaction was stopped by adding 50 µL of Na_2CO_3 (0.1 M). The absorbance was recorded at 405 nm (Tecan, Switzerland).

Blanks were prepared without the test sample and enzyme and replaced by equal

amounts of buffer (0.05 M Tris-HCl buffer, pH 6.9 at 20°C).

Acarbose (Sigma-Aldrich, USA), a well-known inhibitor of α-amylase and α-glucosidase, was used as positive control in both assays.

RESULTS AND DISCUSSION

Structure elucidation of compound XA1

Compound **XA1** was obtained as a colorless viscous liquid. The ^{1}H- and ^{13}C-NMR DEPT data suggested that **XA1** is a glycerolipid. In detail, the ^{1}H-NMR spectrum of **XA1** revealed a multiplet-signal of one proton of oximethine group at δ_H 5.11 (1H, m, H-2), two other multiplet-signals of oximethylene 1-CH_2 and 2-CH_2 groups at δ_H 4.17 and 4.02 ppm, respectively. Besides, the ^{1}H-NMR spectrum of **XA1** showed a characterized signal at δ_H 2.20 (6H, m), indicating the existence of three methylene groups: 2'-CH_2, 2''-CH_2, and 2'''-CH_2. A *cis* double bond in the side chain was indicated by two signals at δ_H 5.23 (4H, s) and δ_H 5.33 (s) ppm. The ^{13}C-NMR spectrum of **XA1** showed signals of 3 carbons of carbonyl group at δ_C 173.25, 173.22 and 173.18 ppm, 6 carbons of 3 double bonds at δ_C 130.18, 129.98, 129.97, 129.94, 129.68 and 128.07 ppm, one carbon of oximethine group at δ_C 68.91 and 3 carbons of methyl groups at δ_C 14.03, 14.07 and 22.63 ppm.

Further HMBC spectral data of **XA1** showed the correlations from protons of oximethylene at δ_H 4.17 and 4.02 ppm, and a proton of oximethine at δ_H 5.11 to carbons of carbonyl groups at δ_C 14.03, 14.07, and 22.63 ppm. Additionally, correlations of proton of methylene at δ_H 2.20 and carbons at δ_C 14.03, 14.07 and 22.63 ppm were observed. However, the HMBC correlations from protons of double bonds to carbons of carbonyl groups and from protons of methyl groups to carbons of double bonds were not observed, indicating the positions of double bonds at the middle of side chains.

Based on the above-analyzed spectral data, the structure of **XA1** was determined as a glycerolipid compound. Additional GC-MS analysis was applied to determine its individual fatty acid compositions. The results were summarized in Table 1.

Table 1. Chemical composition of **XA1** from *Streptomyces* sp. S-X10.2

Peak	Rt.	Constituents		Percentage (%)
		Characteristics	Name	
1	9.72	12:0	Lauric acid	2.96
2	10.768	14:1n-9	cis-9-tetradecenoic acid	0.99
3	12.659	14:0	Myristic acid	2.10
4	12.942	15:1n-7	trans-palmitoleic acid	4.07
5	13.9	15:0	Pentadecanoic acid	1.27
6	16.094	16:1n-9	cis-7-hexadecenoic acid	7.55
7	16.738	16:1n-7	Palmitoleic acid	0.75
8	17.557	16:0	Palmitic acid	23.70
9	20.151	17:0	Heptadecanoic acid	1.59
10	24.163	18:2n-9	Linolenic acid	21.80
11	24.408	18:1n-9	Oleic acid	27.27
12	25.281	18:3n-3	α-Linolenic	1.65
13	32.257	18:0	Stearic acid	0.35
14	41.048	20:0	Arachidic acid	0.21
15	48.406	24:0	Lignoceric acid	0.35
			Others	3.39

As shown in Table 1, the GC/MS analysis revealed a mixture of saturated and unsaturated fatty acids, with unsaturated compounds (64.08%) about two times higher than saturated constituents (32.53%). Among these, palmitic acid, linolenic acid, and oleic acid were identified as major components with 23.70, 21.80, and 27.27%, respectively. These results generally indicate the possible existence of diverse individual fatty acids varying from C_{12} to C_{24} either in the side chains of glycerolipids or as free fatty acids in XA1. On the other hand, previous studies highlighted palmitic acid, linoleic acid, and oleic acid as predominant fatty acid components of filamentous fungal species [13, 14]; however, to the best of our knowledge, this is the first time similar fatty acid composition of a *Streptomyces* was mentioned.

Evaluation of *in vitro* antidiabetic activities

In order to assay *in vitro* antidiabetic activities, α-amylase and α-glucosidase inhibitory properties of the actinobacterial extract and its fractions and isolated compound were investigated. Tables 2 shows the inhibitory percentage and the IC_{50} values of these assessments. Both actinobacterial crude extract and XA1 showed inhibition potential against the tested enzyme. While the ethyl acetate extract (at a test concentration of 500 µg/mL) exhibited inhibition rates of 62.66 ± 2.50 and 58.95 ± 1.27% against α-glucosidase and α-amylase, respectively, XA1 displayed inhibitory activity against these enzyme with with IC_{50} values of 294.62 and 335.22 µg/mL, respectively. Previously, certain fatty acids were reported with ability to inhibit α-glucosidase and α-amylase, including oleic acid and linoleic acid, with oleic acid being the most effective [15]. Our present result contributed to claim the role of fatty acids in carbohydrate digestion and their possible potential in regulating postprandial hyperglycemia in diabetes mellitus.

Table 2. Inhibitory activities against α-amylase and α-glucosidase of components from *Streptomyces* sp. S-X10.2

Test samples	Conc. (µg/mL)	Inhibition (%)		IC_{50}, µg/mL	
		α-amylase inhibitory activity	α-glucosidase inhibitory activity	α-amylase inhibitory activity	α-glucosidase inhibitory activity
DMSO	-	0	0	-	-
Positive control	500	89.22 ± 1.42	60.35 ± 2.10	23.27	196.45
S-X10.2's EtOAc extract	500	62.66±2.50	58.95 ± 1.27	372.54	442.91
Fraction A23	500	41.39 ± 1.81	37.24 ± 3.15	> 500	> 500
Subfraction A23/1	500	50.93 ± 2.74	43.53 ± 1.85	498.40	> 500
XA1	500	67.05 ± 1.68	64.15 ± 2.93	294.62	335.22

CONCLUSION

In conclusion, our study reported an *in vitro* activity-guided isolation of possible antidiabetic glycerolipid and fatty acid component from mangrove actinomycete *Streptomyces* sp. strain S-X10.2. GC-MS analysis revealed the presence of multiple saturated and unsaturated fatty acids in the EtOAc extract of the strain. In particular, palmitic acid, linolenic acid, and oleic acid were identified as major components, each with approximately 20%. These results revealed evidence of bioactive fatty acids in ethyl acetate extract of a mangrove actinobacterium's fermented culture. They suggested further considerations of these microbial components in antidiabetic treatment.

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