ПРИКЛАДНАЯ БИОХИМИЯ И МИКРОБИОЛОГИЯ, 2013, том 49, № 2, с. 181–189

UDC 577.154.3

INHIBITORY EFFECT OF COMPONENTS FROM *Streptomyces* SPECIES ON α-GLUCOSIDASE and α-AMILASE OF DIFFERENT ORIGIN

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Received April 26, 2012

The search for the effective and safe α-glucosidase and α-amylase inhibitors from Actinomycetaceae being antidiabetic agents is actual problem. Twenty one *Streptomyces* spp. of soil samples collected from different places of China were screened for the ability to produce this kind of inhibitory activities. Fermentation broth of isolated strains had absorbance between 350–190 nm. The *Streptomyces* strains PW003, ZG636, and ZG731 were characterized by special absorption at 280, 275, and 400 nm, respectively. Ten of the collected actinomycete strains had the ability to inhibit α-glucosidase or/and α-amylase and the fermentation broth of the same strain had inhibitory activity varied greatly depending on the enzyme source. In the process to screen the leading compounds used as antidiabetic agents, human α-glucosidase and α-amylase were revealed as the best used in trail compared with the same enzymes from other sources. Active α-glucosidase inhibitor was isolated from *Streptomyces* strain PW638 fermentation broth and identified as acarviostatin I03 by MS and NMR spectrometry. Its IC_{50} value was 1.25 and 12.23 μg/mL against human intestinal *N*-terminal maltase-glucoamylase and human pancreatic α-amylase, respectively.

DOI: 10.7868/S0555109913020104

α-Glucosidase inhibitors were used to treat type II diabetes and obesity by suppressing the absorption of glucose and reducing postprandial hyperglycemia [1]. Current interest in these compounds has been extended to a diverse range of diseases including lysosomal storage disorders, cancer and AIDS [2]. It has been well acknowledged that microbial extracts and phytochemicals are potential alternatives to synthetic inhibitors against α-glucosidase. The use of kinetic data combined with variations in potential inhibitor structural information can elucidate the enzyme activity [3]. Such knowledge is fundamental to the discovery of lead compounds, because of their promising therapeutic potential.

The genus *Streptomyces* was described by Waksman and Henrici [4] for aerobic and spore forming Actinomycetes and emended by Kim et al. [5]. These microorganisms remain an important source of drug research for the future. *Streptomyces* were able to degrade relatively complex and recalcitrant plant and animal residues. Currently, about 10,000 antibiotics had been discovered from microorganisms. It had been estimated that approximately two thirds of these naturally occurring antibiotics were isolated from Actinomycetes [6]. All *Streptomycetes* strains were acid fast negative and Gram stain positive. They differed greatly in their morphology, physiology, and biochemical activities, producing the majority of known antibiotics.

The main digestible carbohydrates in the human diet are starch and sucrose [7]. Human intestinal *N*-terminal maltase–glucoamylase (MGAMnt, EC 3.2.1.20) is a membrane anchored glycoprotein of the intestinal epithelial cells and can be solubilized by papain digestion [8]. The enzyme is more heat-stable than the other disaccharidases [9] and is the key enzyme which catalyses the final step in the digestive process of carbohydrates. Hence, α-glucosidase inhibitors can reduce postprandial plasma glucose levels and suppress postprandial hyperglycaemia, because of retarding the liberation of *D*-glucose of oligosaccharides and disaccharides from dietary complex carbohydrates and delay glucose absorption [10]. Glucosidase inhibitors delay the breakdown of carbohydrates in the small intestine and thus diminish the postprandial increase of blood glucose in diabetic subjects [11, 12].

In the course of screening for new bioactive compounds, studies are currently oriented towards the isolation of new *Streptomyces* species from uncommon habitats. It has been reported that *Streptomyces* species such as *S. hygroscopicus* [13], *S. griseus* [14], *S. fradiae* [15], and *S. lavendulae* [16] produce many bioactive compounds, while there are also many *Streptomyces* species that have not yet been shown to produce them. To select new *Streptomyces* species, several methods have been developed to identify this genus of bacteria. These include selective plating technique [17], construction of genetic marker systems [18], a combination of chemical markers, the presence of L,L-diami-

nopimelic acid and the absence of characteristic sugars in the cell wall [19]. In addition, 16S rRNA sequence data have proved invaluable in *Streptomycetes* systematic, in which they have been used to identify several newly isolated *Streptomyces* species [20, 21].

In order to develop physiological functional compounds for use as antidiabetic agents, much effort has been expended in the search for effective α-glucosidase inhibitors from natural materials. In a series of our studies on extracting inhibitor from *Streptomyces* species, we previously reported that acarviostatins from *Streptomyces coelicoflavus* ZG0656 were a new class of α-glucosidase inhibitors [22].

The aim of the study was to isolate of 21 *Streptomyces* spp. of soil samples collected from different places of China and to test them for ability to inhibit the activity of MGAMnt and human pancreatic α-amylase (HPA, EC 3.2.1.1). Active α-glucosidase inhibitor, acarviostatin I03, was prepared from the *Streptomyces* strain PW638 broth. In the process to screen the lead compounds for use as antidiabetic agents, mammalian α-glucosidase and α-amylase were revealed as the best to be used in trail.

MATERIALS AND METHODS

Biological material. *Streptomycetes* were collected from the following places of China: (i) 3 strains were isolated from a wet black mud sample collected at Anshan, Liaoning province; (ii) 7 strains were collected directly from soil samples at campus of the University of Nankai (CN); (iii) 3 strains were collected in the cave of Tai mountain; (iv) 4 strains were isolated from a paddy field, Gutian, Fujian province; (v) 4 strains were isolated from a soil sample, Shenzhou, Fujian province.

Sample collection, isolation and storage of *Streptomyces* spp. For each collected sample, 3.0 g of soil were suspended in 100 mL of 0.85% NaCl and allowed to stand for 15 min. Three different dilutions (1 : 10, 1 : 100 and 1 : 1000) were prepared using sterile saline solutions in a total volume of 10 mL. An aliquot of 0.1 mL of each dilution was plated on Gause's No. 1 synthetic medium [23]. Plates were incubated at 28°C, and monitored after 48, 72, and 96 h. Representative colonies were selected and streaked on new plates of Gause's No. 1 synthetic medium. The isolated *Streptomyces* species were preserved on Gause's No. 1 synthetic medium plates at 4°C until use. This procedure led to pure colonies of *Streptomyces*. The isolated *Streptomyces* strains were maintained as suspensions of spores and mycelial fragments in 10% glycerol (v/v) at 4°C in the Nankai University Collection of Pharmaceutical Sciences (China).

Genus identification and morphological characteristics. Visual observation of both morphological and microscopic characteristics using light microscopy and Gram staining were performed. All morphological properties were observed on Gause's No. 1 synthetic medium and used for classification and differentiation.

Preparation of the fermentation complex. The culture of *Streptomyces* strain was filtered by hollow cellulose membrane (MOTIMO, China) with 100000 MWCO (Molecular Weight Cutoff) and the mycelium was discarded. The impurities were removed by ultrafiltration using hollow cellulose membrane with 360 and 10000 MWCO (MOTIMO, China). The effluent liquid passed through a D301R macroporous resin column (300 × 40 mm) (The Chemical Plant of Nankai University, China) to partly remove pigments, followed by a column of 001*7 cation-exchange resin (200 × 20 mm), washed with water, eluted with 0.1 M ammonia (The Chemical Plant of Nankai University, China). Then about a ninefold volume of EtOH was added to the concentrated eluate, and the supernatant was discarded after centrifugation at 3000 g, 10 min. The pellet was lyophilized to give the fermentation complex.

Purication and structure analysis of the *Streptomyces* strain PW638 fermentation complex. The *Streptomyces* strain PW638 fermentation complex was dissolved in water and filtered through a 0.45 μm membrane (Sangon Biotech, China), then separated by semi-preparative reversed phase HPLC using a stainless steel column filled with Kromasil C_{18} (250 × 10 mm, i.d., 10 μm) at 25°C. The mobile phase was MeCN: water (10 : 90) at a fow rate of 5 mL/min with UV detection at 205 nm. The active fraction containing inhibitors was collected at 10.1 min. This fraction was further puried on the Waters (USA) Spherisorb S5 SCX semi-preparative column at 25°C with water: ammonia: acetic acid (1000 : 8 : 8) as the mobile phase. One active fraction containing inhibitors was collected with peak at 9.7 min.

Mass spectrometric detection was performed on a ThermoFinnigan LCQ Advantage mass spectrometer (USA) equipped with an ESI source and a mass range up to m/z 2000. Positive ion mode was employed, and the spray voltage was set at 4.5 kV. The capillary voltage was fixed at 5.0 V, and its temperature was maintained at 220°C. The solvent was nebulized using N_2 as both the sheath gas and the auxiliary gas at a flow rate of 0.8 and 0.08 L/min, respectively. NMR spectrometry was also used in the identification. The MS and NMR data then compared with the known α-glucosidase inhibitors.

The α-glucosidase inhibitory activity assay. Fifty μL of dissolved fermentation complex and 50 μL of 0.1 M phosphate buffer, pH 6.9, containing α-glucosidase solution (1.0 U/mL) were incubated in 96 well plates at 37°C for 10 min. After pre-incubation, 100 μL of 5.0 mM maltose in 0.1 M phosphate buffer (pH 6.9) was added to each well at 5 s interval. The reaction mixtures were incubated at 37°C for 15 min. 50 μL aliquots were taken and added to 100 μL of glu-

Table 1. Sources of *Streptomyces* strains investigated in the current research

Strain	Color of fermentation	pH	The fermentation product dry weight, mg/mL	Mycelial dry weight, mg/mL	Location	Gram-stain properties
PW409	Grey	9.3	6.16	2.40	Tai moutain, Shandong	+
ZG434	Light yellow	5.9	7.74	2.56	Shenzhou, Fujian	+
ZG728	Yellow	9.2	5.64	2.30	Shenzhou, Fujian	+
PW638	Light yellow	5.7	4.56	1.96	Nankai university, Tianjin	+
ZG243	Yellow	5.7	6.94	2.57	Shenzhou, Fujian	+
ZG031	Light yellow	8.1	5.44	2.34	Shenzhou, Fujian	+
PW609	Light pink	9.3	5.02	2.19	Nankai university, Tianjin	+
ZG624	Grey	9.0	3.92	1.60	Tai moutain, Shandong	+
ZG727	Light yellow	7.6	5.08	2.11	Tai moutain, Shandong	+
PZB126	Yellow	4.6	5.72	2.39	Nanjing, Jiangsu	+
PW852	Black	8.7	6.32	2.53	Nanjing, Jiangsu	+
PW698	Yellow	5.4	5.38	2.27	Nankai university, Tianjin	+
ZG731	Dark yellow	8.6	5.50	2.35	Anshan, Liaoning	+
ZG642	Yellow	7.9	5.94	2.43	Anshan, Liaoning	+
ZG084	Light yellow	7.1	4.20	1.72	Anshan, Liaoning	+
ZG159	Yellow	8.5	5.44	2.29	Gutian, Fujian	+
ZG737	Light yellow	8.9	4.74	2.07	Gutian, Fujian	+
ZG574	Dark green	9.0	6.10	2.38	Gutian, Fujian	+
ZG419	Yellow	6.9	5.04	2.18	Gutian, Fujian	+
ZG636	Light yellow	6.1	6.40	2.63	Nanjing, Jiangsu	+
PW003	Yellow	6.5	8.10	3.06	Nankai university, Tianjin	+

cose oxidase assay reagent (Sigma, USA) in a 96 well plate. Reactions were developed for 20 min and OD_{490} was measured by microarray reader and compared to the control which had 50 μL of buffer solution instead of the dissolved fermentation complex. The α-glucosidase inhibitory activity was expressed as inhibition (%) and calculated as $(OD_{control} - OD_{test})/OD_{control} \times \times 100\%$. Each test was performed 3 times and the mean value was used to indicate the inhibitory activity of the fermentation broth.

The α-amylase inhibitory activity assay. 500 μL of dissolved fermentation complex and 500 μL of 0.02 M sodium phosphate buffer, pH 6.9, with 0.006 M NaCl containing α-amylase (0.5 U/mL) were incubated for 10 min at 37°C. After pre-incubation, 500 μL of 1% starch solution in the same buffer was added to each tube at 5 s interval. The reaction mixtures were incubated at 37°C for 10 min. The reaction was stopped in a boiling water bath for 5 min and probes were cooled to room temperature. 50 μL aliquots were taken and added to 100 μL 4% iodine solution in a 96 well plate. Reaction was developed for 20 min and OD_{540} was measured by microarray reader and compared to the control which had 50 μL of buffer solution instead of the dissolved fermentation complex. The α-amylase inhibitory activity was expressed as inhibition (%) and calculated as $(OD_{control} - OD_{test})/OD_{control} \times 100\%$. Each test was performed 3 times and the mean value was used to indicate the inhibitory activity of the fermentation broth.

RESULTS

Taxonomy of the isolated *Steptomyces* strains. Samples collected from different regions of China, were spread on Gause'No.1 synthetic medium. All isolated strains were Gram positive, non acid fast, non-motile, aerobic actinomytes (Table 1). The aerial mycelium was absent and well-branched yellowish or brown substrate mycelium grew on medium, showing the expected straight or flexuous forms, hooks, open loops and coils, which were used, among other features, to establish differences between them. The mature single spores on the tips of substrate mycelium were observed (0.6–0.9 μm) (Fig. 1a). It was revealed that the morphology of the spore chains varied depending on the species. The diameter of the substrate mycelium was between 0.4 and 0.7 μm (Fig. 1b). It was also observed that some strains produced diffusible pigments in the surround in medium and some of them produced melanoid pigment.

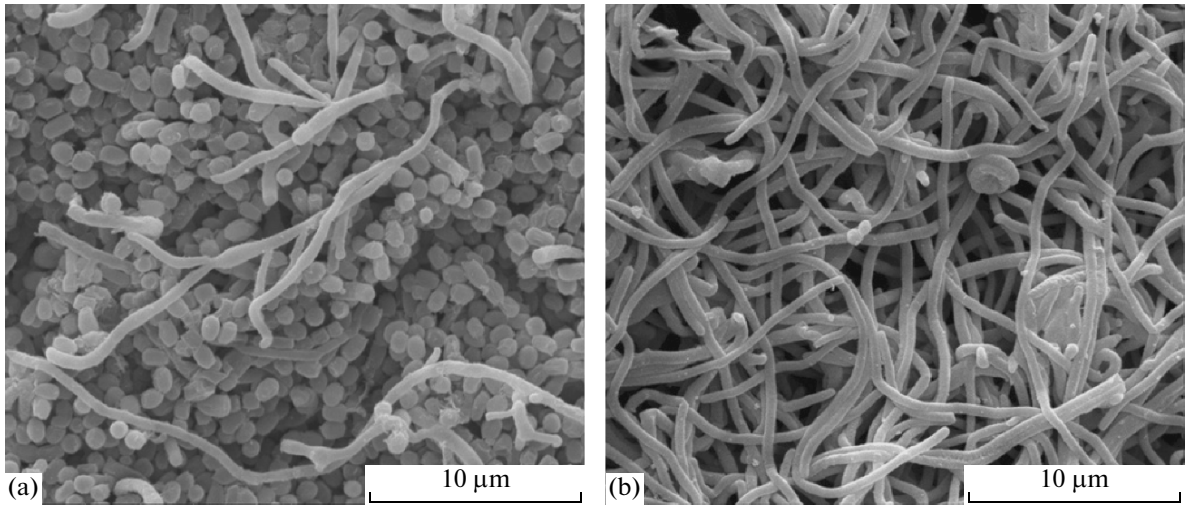

Fig. 1. Scanning electron micrograph of the *Streptomyces* strain PW609 spores (a) and the strain PW409 mycelia (b).

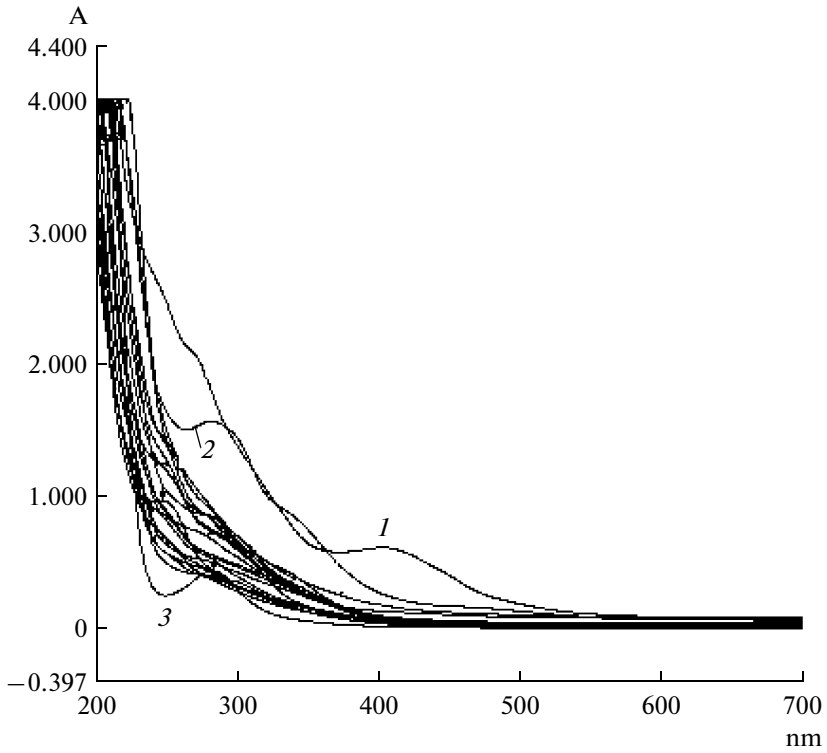

Fig. 2. Wavelength scan of the fermentation complex (1 mg/mL) of different *Streptomyces* strains between 190–700 nm. *1* – ZG731 strain; *2* – PW003 strain; *3* – ZG636 strain.

Fermentation of the *Steptomyces* strains. After incubation at 28°C for 7 days by growing in shake flasks of Gause'No.1 synthetic medium broth, cultural characteristics of the *Steptomyces* strains were determined and the most color of fermentation broth was yellow, three of them gave black, pink and green pigments. The solid contents of fermentation products from all the kinds of strains were around 5.0 mg/mL (Table 1). By observing the changes of the mycelial dry weight, it was around 2.0 mg/mL. It was found that the fermentation product dry weight had positive relative with mycelial dry weight. The pH of culture influences the fermentation process. During the course of fermentation, for the of most of isolated strains the fermentation broth pH decreased to 4.9, maybe due to the production of organic acids.

Wavelength scan of the *Steptomyces* fermentation complex. After concentration, fermentation broth

Table 2. Inhibitory activities of the fermentation complex from different *Streptomyces* strains on HPA and rat intestinal α-amylase

Strain	Inhibitory activity (HPA)			Inhibitory activity (rat intestinal α-amylase)		
	10 mg/mL	1.0 mg/mL	0.1 mg/mL	10 mg/mL	1.0 mg/mL	0.1 mg/mL
PW409	2.7	2.2	2.4	7.3	4.6	4.2
ZG434	89.1	74.4	30.5	60.9	41.0	26.7
ZG728	2.3	2.3	2.1	6.5	4.2	4.1
PW638	1.7	1.6	1.8	1.7	1.6	1.8
ZG243	82.9	6.0	2.2	44.4	8.7	4.2
ZG031	65.5	24.0	3.0	64.2	23.8	6.7
PW609	96.3	94.0	79.5	75.0	63.6	46.4
ZG624	2.4	2.2	2.1	7.2	4.9	4.4
ZG727	2.3	2.1	2.0	5.0	4.6	4.5
PZB126	75.1	73.9	46.3	53.5	47.8	31.1
PW852	2.9	2.2	2.0	6.8	6.1	4.6
PW698	2.6	2.3	2.2	6.0	5.4	4.7
ZG731	2.9	2.2	2.0	8.1	4.6	4.5
ZG642	5.5	2.3	2.2	11.5	5.5	5.1
ZG084	44.3	2.3	2.2	17.4	5.4	4.7
ZG159	4.5	2.3	2.2	7.5	5.6	5.2
ZG737	3.0	2.2	1.9	5.5	4.9	4.4
ZG574	1.7	1.6	1.6	1.8	1.7	1.6
ZG419	7.8	2.2	2.0	13.3	4.9	4.6
ZG636	3.3	2.3	2.2	6.8	4.8	4.1
PW003	2.3	2.2	2.0	4.7	4.5	4.4

passed through ultrafilters and the macroporous resin to obtain coarse powder after precipitation of eluate by ethanol. Double distilled water was used to dissolve the powder. The UV-Vis spectra of the fermentation complex from different *Steptomyces* strains (1 mg/mL) were recorded in the wavelength range of 190 to 700 nm. Acquired absorption spectrum data are showed in Fig. 2, all the samples had absorbance between 190–350 nm. The *Steptomyces* strains PW003, ZG636, and ZG731 had peaks around 280, 275 and 400 nm, respectively.

The α-amylase inhibitory activity assay. The isolated *Steptomyces* strains were tested for their ability to inhibit α-amylase, HPA and rat intestinal α-amylase (EC 3.2.1.1). The fermentation complex was used for that. As can be observed in Table 2, 6 *Steptomyces* strains (ZG434, ZG243, ZG031, PW636, PZB126, ZG084) showed the inhibitory effect on α-amylase: at 10 mg/mL it was 89.1, 82.9, 65.5, 96.3, 75.1, 44.3% on HPA, respectively, and 60.9, 44.4, 64.2, 75.0, 53.5 and 17.4% on rat intestinal α-amylase, respectively. The inhibitory action on the HPA and rat intestinal α-amylases did not exhibited significant differences but the inhibitory activity of 6 *Steptomyces* strains on rat intestinal α-amylase was relatively weaker than on HPA at the same concentration. Increasing the concentrations of the fermentation complex resulted corresponding increase in α-amylase inhibitory activity. The inhibitory activities of the *Steptomyces* strain ZG434 on rat intestinal α-amylase were found to be 60.89% (10 mg/mL), 40.99% (1 mg/mL) and 26.73% (0.1 mg/mL), respectively (Table 2).

The α-glucosidase inhibitory activity assay. MGAMnt and rat intestinal α-glucosidase (EC 3.2.1.20) were used to investigate the inhibitory activity of the *Steptomyces* fermentation complex. We found that the α-glucosidase inhibitory activities varied widely among the tested strains (Table 3). Six *Steptomyces* strains (PW409, PW609, PW638, PZB126, PW852, PW698) had inhibitory effect on the α-glucosidase: at 10 mg/mL it was 34.0, 63.2, 64.5, 62.6, 17.4 and 49.1% on MGAMnt, respectively, and 82.0, 60.5, 72.0, 56.3, 81.3 and 31.2% on rat intestinal α-glucosidase, respectively. The inhibitory effects of the *Steptomyces* strains PW409 and PW852 was similar for both enzymes. An increased concentration of the strain fermentation complex resulted to higher α-glucosidase inhibitory activity. The inhibitory activity of the *Steptomyces* strain PW638 fermentation complex on rat intestinal α-glucosidase was found to be 77.2%

Table 3. Inhibitory activities of the fermentation complex from different *Streptomyces* strains on MGAMnt and rat intestinal α-glucosidase

Strain	Inhibitory activity (MGAMnt)			Inhibitory activity (rat intestinal α-glucosidase)		
	10 mg/mL	1.0 mg/mL	0.1 mg/mL	10 mg/mL	1.0 mg/mL	0.1 mg/mL
PW409	34.6	21.4	10.1	82.0	79.1	35.9
ZG434	14.6	6.6	9.7	2.3	2.6	4.5
ZG728	11.1	4.8	4.4	18.2	15.8	4.1
PW638	64.5	59.5	51.4	77.2	60.5	26.8
ZG243	32.0	25.0	6.3	6.8	11.2	6.5
ZG031	37.6	19.4	11.6	6.3	2.6	0.9
PW609	63.2	59.2	53.3	60.5	44.4	11.9
ZG624	11.1	10.0	7.7	15.3	11.6	6.5
ZG727	11.1	8.9	6.4	18.5	11.6	5.4
PZB126	62.6	57.1	44.3	56.3	32.6	5.2
PW852	17.4	13.0	12.2	81.3	67.8	9.8
PW698	49.1	35.5	30.2	31.1	9.6	3.4
ZG731	18.6	18.2	13.2	14.5	9.8	6.4
ZG642	3.6	0	0	5.5	3.9	1.4
ZG084	7.2	6.3	4.6	12.4	10.1	5.3
ZG159	10.2	6.5	6.2	13.5	8.8	10.0
ZG737	2.4	1.6	6.3	11.6	5.9	5.5
ZG574	6.6	6.8	4.0	8.3	4.6	3.4
ZG419	4.7	16.8	6.4	6.1	7.2	6.8
ZG636	6.7	4.0	8.3	9.2	15.3	14.7
PW003	1.7	16.3	17.4	13.1	8.7	11.1

(10 mg/mL), 41.0% (1 mg/mL) and 26.7% (0.1 mg/mL), respectively (Table 3).

Purication and structure analysis of fermentation complex from *Steptomyces* strain PW638. The *Steptomyces* strain PW638 fermentation complex (0.5 g) was dissolved in water, separated by semi-preparative Kromasil C_{18}, the active fractions were collected at 10.1 min (Fig. 3a). The fractions were further purified on the Spherisorb S5 SCX semi-preparative column, one active fraction (9 mg) was collected with peak at 9.7 min (Fig. 3b). It had inhibitory activity against the α-glucosidase. The purity of this compound was determined by HPLC analysis.

The pale white powder was treated by completely acidic hydrolysis followed by monosaccharide analysis with the 1-phenyl-3-methyl-5-pyrazolone precolumn derivatization HPLC method [25], the result proved that it contained glucose unit. The molecular formula was deduced as $C_{37}H_{64}NO_{28}$ by combined high resolution positive ESI-MS (Anal. Found $[M + H]^+$ 969.3537, Calcd 970.3537). The molecular mass of the compound was determined by a ThermoFinnigan LCQ Advantage mass spectrometer. The mass spectrum showed one major peak at m/z 969.4 (Fig. 4). Its sodium adducts were revealed at m/z 991.4. The signals at m/z 807.3, 645.3 and 483.4 were due to the loss of one to three glucose units from the molecule 829.3, 667.2 and 505.4 (M + Na), respectively. The precursor ion at m/z 969.4 fragmented to generate the abundant peak at m/z 304.3 corresponding to cleavage of the glycosidic bonds, suggesting that no glucose was linked to the non-reducing end. In the further analyses, NMR was performed to identify the structure of the active compound (data not shown). Compared with the MS, 1H NMR and 13C NMR spectrum of the known α-glucosidase inhibitors, the data showed that the purified α-glucosidase inhibitor from the *Steptomyces* strain PW638 fermentation broth was acarviostatin I03 speculatively (Fig. 4). The IC_{50} value of acarviostatin I03 was 1.25 μg/mL and 12.23 μg/mL against MGAMnt and HPA, respectively. Acarbose and acarviostatin I03 showed similar inhibitory activity against α-glucosidases and α-amylase. They may have the same molecular mechanism to inhibit the two enzymes.

DISCUSSION

Streptomycetes are known to be a rich source of bioactive compounds, notably antibiotics and enzymes.

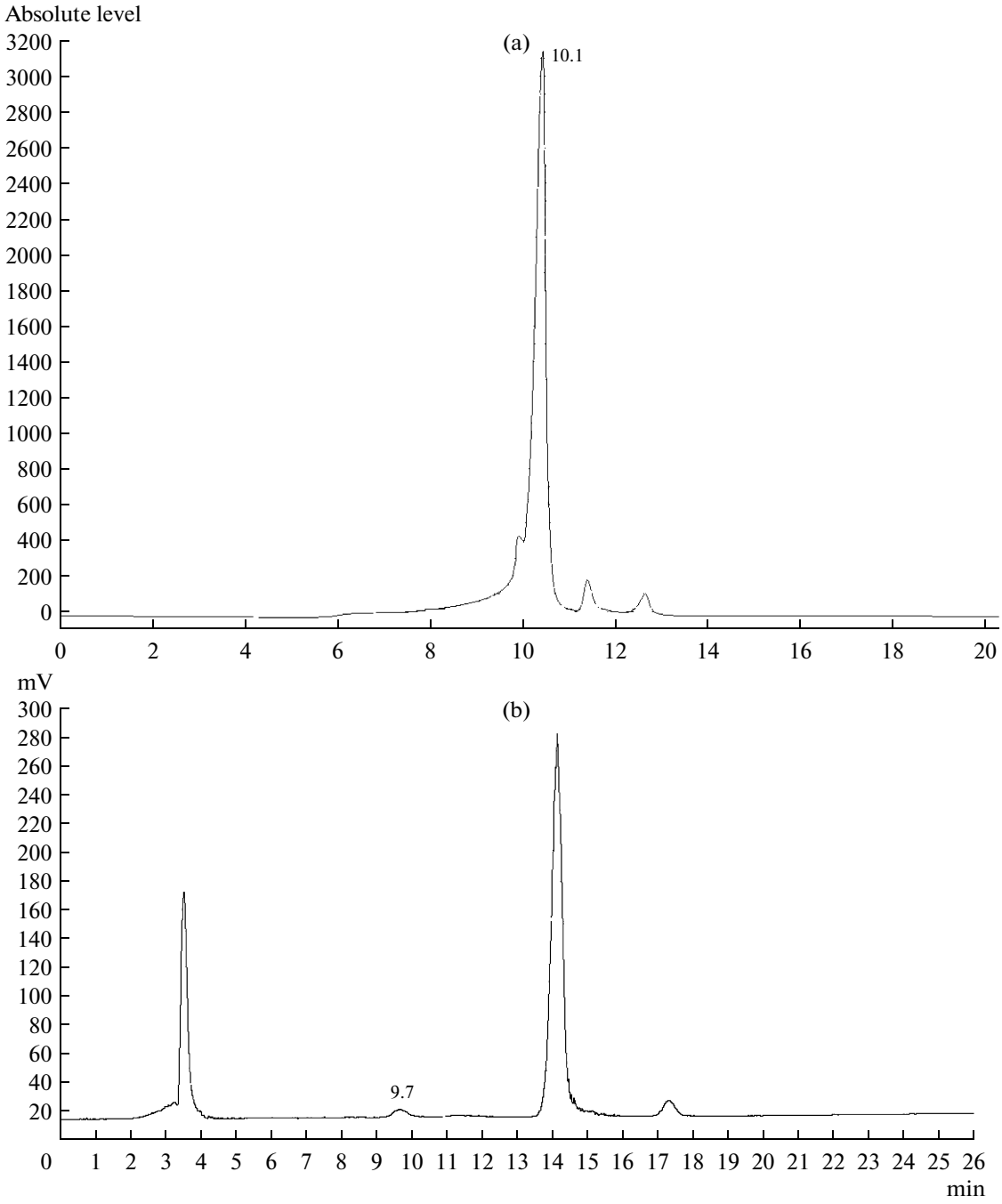

Fig. 3. HPLC of the *Streptomyces* PW638 fermentation complex on semi-preparative Kromasil column (a) and HPLC of active fraction obtained from (a) on Spherisorb S5 SCX (b).

They differ greatly in their morphology, physiology, and biochemical activities, producing the majority of known antibiotics. The phenotypic and phylogenetic data presented in this study provide clear evidence that 21 isolated microorganisms belong to different *Streptomyces* strains. Polyhydroxy glucosidase inhibitors are a widely diverse class of compounds frequently isolated from *Streptomyces*, they have significant therapeutic use or potential to manage diabetes [24]. Inhibitors of mammalian intestinal α-glucosidase have become exciting candidates to slow down the digestion of carbohydrates and in turn mitigate postprandial hyperglycemia. Therefore, investigation of such agents from *Streptomyces* species has become more important and researchers are competing to find new, effective and safe therapeutic agents for the treatment of diabetes. Dibutyl phthalate, an α-glucosidase inhibitor from *S. melanosporofaciens* [26] and 4 acarviosin containing

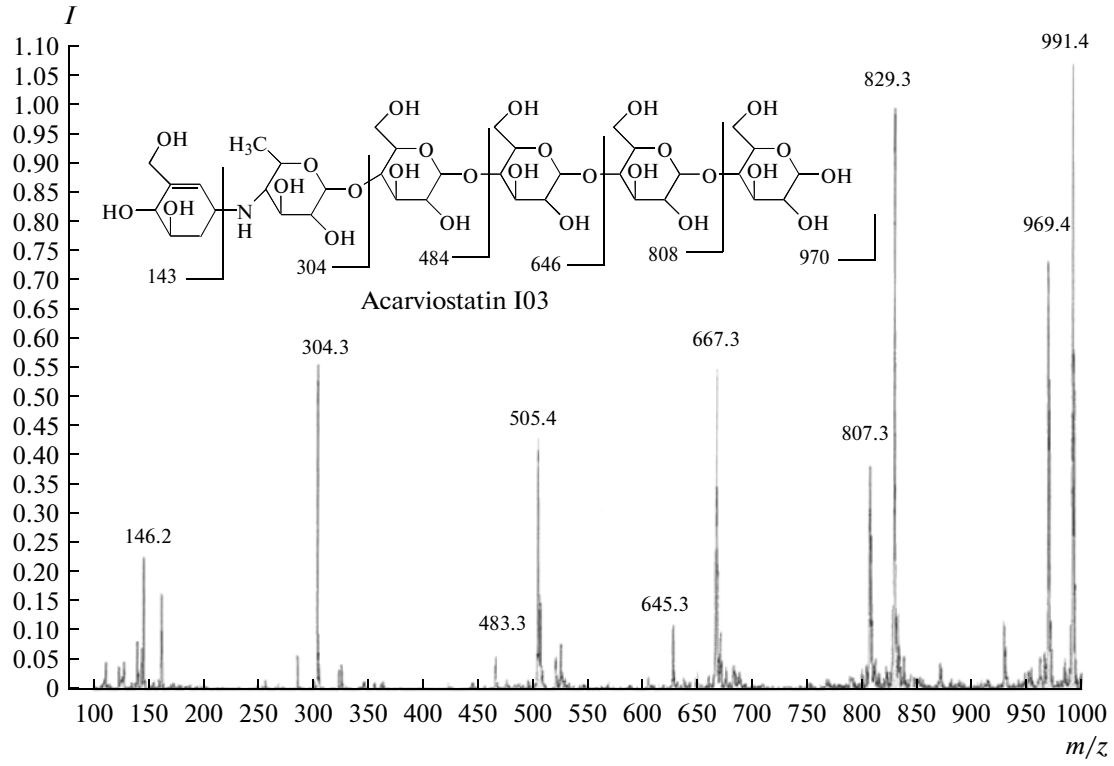

Fig. 4. Positive ESI-MS spectrum profile and chemistry structure of acarviostatin I03 obtained from HPLC active fraction (Fig. 3b).

oligosaccharides identified from *S. coelicoflavus* ZG0656 are potent inhibitors of α-amylase [27]. Among the valuable members of antidiabetic agents, most of them belong to C_7N-aminocyclitol family. Aminocyclitols are a group of microbially derived bioactive natural products with important clinical and agricultural applications [28, 29].

α-Glucosidase is widely distributed in microorganisms, plants, and animal tissues, and the substrate specificity of α-glucosidases is known to differ greatly depending on their source. The bacterial, yeast and insect enzyme, named α-glucosidase I, shows higher activity toward such heterogeneous substrates as sucrose and *p*-nitrophenyl-α-glucoside, and none or less toward such homogeneous substrates as maltooligosaccharides, implying that α-glucosidase I recognizes the glucosyl structure in the substrate. It has been reported that compounds with α-glucosidase inhibitory activity were preliminarily screened on yeast α-glucosidase (Sigma G7256) [30] and (Sigma G6136) [31]. The mold, plant, and mammalian enzyme, named α-glucosidase II, hydrolyzes the homogeneous substrates more rapidly than the heterogeneous ones, indicating that this class of α-glucosidases recognizes the maltose structure. The inhibitory activities of 2, 4, 6-tribromophenol and 2, 4-dibromophenol purified from red alga *Grateloupia elliptica* against rat intestinal sucrase and maltase were reported [32, 33]. Some α-glucosidase II enzymes also attack such α-glucans as soluble starch and glycogen.

α-Glucosidases I and II have greatly different substrates. The inhibitors which have effect on α-glucosidase I maybe fail to inhibit α-glucosidase II. Inhibitors of mammalian intestinal α-glucosidase are exciting candidates to slow down the digestion of carbohydrates and play the role in the human body. An increased concentration of the *Streptomyces* strain fermentation complex resulted in an increase in α-glucosidase inhibitory activity, and we suggest that there is a conformational change derived from binding of inhibitor to the enzyme. Among fermentation complex of the analyzed *Streptomyces* strains, a favorable correlation between MGAMnt and rat intestinal α-glucosidase inhibitory effects was not found. This was especially true for the *Streptomyces* strains PW409 and PW852, which had high enzyme inhibitory activity on rat intestinal α-glucosidase, but showed almost no effect on MGAMnt. The fermentation complex of the *Streptomyces* strains PW609 and PZB126 inhibited α-glucosidase and α-amylase at the same time. This may indicate that their fermentation complex contains the same component inhibiting both enzymes or it has 2 components, one inhibiting α-glucosidase and the other inhibiting α-amylase. The *Streptomyces* strains ZG434, ZG243, and ZG031 were able to inhibit only α-amylase, while strains PW698, PW409, PW638, and PW609 inhibited only α-glucosidase. Thus, in the process to screen out a physiological functional food or lead compounds for use as antidia-

betic agents, human α-glucosidase and α-amylase are the best to be used.

The present study introduces novel sources for the prevention of diabetes diseases. We found that 10 of the isolated *Streptomyces* strains had the ability to inhibit α-glucosidase or/and α-amylase and purified α-glucosidase inhibitor, acarviostatin I03, from the *Streptomycetes* strain PW638.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (21002052), the Tianjin Municipal Science and Technology Commission, China (10JCYBJC14300) and the Fundamental Research Funds for the Central Universities of China (65011161).

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