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Diversity of endophytic actinobacteria isolated from medicinal plants and their potency as pancreatic lipase inhibitor

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Abstract. *Fitri L, Meryandini A, Iswantini D, Lestari Y. 2017. Diversity of endophytic actinobacteria isolated from medicinal plants and their potency as pancreatic lipase inhibitor. Biodiversitas 18: 857-863.* Endophytic actinobacteria from medicinal plants have high diversity and potency to produce secondary metabolites with various biological functions, including the activity of lipase inhibitor. As it has been widely known that inhibiting fat absorption through the activity of pancreatic lipase inhibitor is one of the most common treatments for weight loss. This study aimed to assess diversity and capability of endophytic actinobacteria in producing pancreatic lipase inhibitor from medicinal plants that were traditionally used as antiobesity. Medicinal plants used in this research were rhizome of *Alpinia galanga*, *Kaempferiae galanga*, *K. rotunda, Zingiber cassumunar*, and leaves of *Murraya paniculata*. The endophytic actinobacteria were isolated, purified, and assayed for lipase inhibitory activity. The selected isolates were molecularly identified based on 16S rRNA gene and compared for their close relationship with reference strains available in the GenBank. A total of 35 endophytic actinobacteria has been isolated from the five medicinal plants examined. The isolates showed to have various morphological diversity based on their colony and microscopic observations, and also to have various lipase inhibitory activity. The inhibition activity ranged from 6.1 to 96.5%. There were five selected endophytic actinobacteria which have lipase inhibitory activity higher than 90%. At the same concentration of sample (1300 ppm), the crude extract of AEBg12 showed slightly higher activity (95.3%) compared with orlistat (93.6%), used as a positive control. The partial sequences of 16S rRNA analyses showed that AEBg4 (1421 bp) had the highest similarity with *Streptomyces* sp. S170 (93%), followed by its close relationship with *S. lannensis* strain SR3-58 (93%), and *S. lannensis* JCM 16578^T (92%). Both AEBg10 (1051 bp) and AEBg12 (1010 bp) had the highest similarity with *Streptomyces* sp. S170 by 99% and 98%, respectively. The AEKp9 (1367 bp) had a close relationship with *Streptomyces* sp. DLDG2 (93%) and *S. bellus* NBRC 12844^T (93%). While for AELk3 (1033 bp), the similarity with *Streptomyces* sp. NRLL B-24869 was 98%. These research data can be considered as new information, regarding the capability of endophytic actinobacteria from medicinal plants, which have potency as antiobesity, through their pancreatic lipase inhibitory activity.

Keywords: Endophytic actinobacteria, pancreatic lipase inhibitor, medicinal plant, *Streptomyces*

INTRODUCTION

Obesity is an excess of abnormal weight gain that occurs due to excessive of fat accumulation. In that case, there is an energy balance disorder, as a number of calories entering the body are much more than the required ones (Muwakhidah and Tri 2008). In 2014, more than 14 million children and 1.9 billion adults were reported overweight, and more than 600 million from those were obese (WHO 2016). Obesity is closely associated with other diseases, such as hyperlipidemia, a disease that occurs due to excessive fat deposition. Several diseases such as coronary heart, diabetes mellitus type II and hypertension can be related to obesity (Ranti et al. 2013). Obesity can be prevented by consuming the antiobesity drug, such as orlistat, which acts as pancreatic lipase inhibitory activity (Sukhdev and Singh 2013). Several plants, e.g. *Kaempferiae rotunda*, *Alpinia galanga*, *Kaempferiae galanga*, *Zingiber cassumunar*, and *Murraya paniculata*, had been reported to produce pancreatic lipase inhibitor activity 65.1%, 56.2%, 37.6%, 29.17%, and 25.66%, respectively (Iswantini et al. 2010, 2011; Pradono et al. 2011).

Actinobacteria is known to produce bioactive compounds which have various biological functions including enzyme inhibitor. Endophytic microbes including actinobacteria have the capability to produce similar bioactive compounds with the host plant (Pujiyanto and Ferniah 2010). *Streptomyces* spp. was isolated from *Lolium erenne*, and able to produce diketopiperazine which functions as an antibiotic (Strobel et al. 2004). *Streptomyces aureofaciens*, an endophytic actinobacterium from ginger plant, was reported to produce arycoumarine which had antitumor activity (Taechowisan et al. 2007). Moreover, endophytic actinobacteria from *Alpinia galanga*

was reported to have antimicrobial activity (Taechowisan and Lumyong 2003). *Streptomyces* spp., endophytic actinobacteria isolated from *Tinospora crispa*, able to produce α-glucosidase inhibitory compound (Pujiyanto et al. 2012). While, *Streptomyces toxytricini*, a nonendophytic actinobacterium, could produce pancreatic lipase inhibitory compound, commercially known as orlistat, a derivative of lipstatin (Ballinger and Peikin 2002). Other *Streptomyces* which are able to produce pancreatic lipase inhibitors is *S. variabilis* strain PO-178, *Streptomyces* sp. MTCC 5219 and *Streptomyces* sp. NR 0619 (Kekuda et al. 2011; Tokdar et al. 2011; Mutoh et al. 1994). Actinobacteria as producer of various bioactive compounds have great potential for industrial application (Golinska et al. 2015). However, the potency of endophytic actinobacteria from medicinal plants as antiobesity has never been explored.

This study aimed to assess diversity and potency of endophytic actinobacteria from medicinal plants which traditionally used as antiobesity, in producing pancreatic lipase inhibitor.

MATERIALS AND METHODS

Isolation of endophytic actinobacteria

Medicinal plants used in this research were rhizome of *A. galanga*, *K. galanga*, *Z. cassumunar*, *K. rotunda* and leaves of *M. paniculata*. These plants were chosen based on the study reported by Iswantini et al. (2010; 2011), and Pradono et al. (2011). The plant samples were obtained from Medicinal Plant Collection Garden, Tropical Biopharmaca Research Center, Institut Pertanian Bogor (Institut Pertanian Bogor), West Java, Indonesia. The samples were selected from healthy plants, 12 months old plant for rhizome, and young leaves for the leave samples. The samples were washed with sterile distilled water then surface-sterilized by soaking in 70% alcohol for 1 min, followed by 1% hypochlorite solution for 5 min, and 70% alcohol for another 1 min, and finally rinsed with sterile distilled water. The surface-sterilized samples were crushed, then a total of 1 mL of sample suspensions were inoculated on Humic acid Vitamin B (HV) agar medium, incubated at room temperature for 14-30 days. Actinobacterial colonies were purified using International *Streptomyces* Project (ISP) 2 media, which in 1 L consisted of 4 g yeast extract, 10 g malt extract, 4 g glucose, and 18 g agar), and kept for the screening of pancreatic lipase inhibitory activity.

Activity test of pancreatic lipase inhibitor

Actinobacteria were inoculated into a 200 mL flask containing 50 mL ISP 2 medium, and incubated in an incubator shaker at 150 rpm for 10 days, at a temperature of 30°C. Cell biomass were separated by centrifugation at 6000 rpm for 30 minutes. The supernatant was used for testing the activity of pancreatic lipase inhibitor. The activity test was carried out by using the method of Etoundi et al (2010), with some modifications. The substrate was prepared by adding 1% (v/v) triolein, and 1% (v/v) tween 80 into 0.1 M phosphate buffer (pH 8). A total of 800 µl triolein mixture was added into a test tube containing 200µL swine lipase (L3126 Sigma) (0.5 g enzyme in 15 mL of 0.1 M phosphate buffer pH 8) and 200µL supernatant. The solution was mixed and its absorbance was measured using UV-Vis spectrophotometer at 450 nm wavelength. The solution was incubated for 30 min at 37°C, and the absorbance was measured as above with three replications. The percentage of pancreatic lipase inhibitory activity was calculated by using following formula:

Inhibition of pancreatic lipase = A - B / A x 100

A = pancreatic lipase activity, B = pancreatic lipase activity after incubation

Morphological characterization of endophytic actinobacteria

Morphological characterization of 5 actinobacterial isolates with high inhibition activity was observed on ISP 2, ISP 3 without the addition of trace salts solution, and ISP 4 media. Microscopic observation was conducted using light microscope at a magnification of 100 and 400 x.

Identification of endophytic actinobacteria based on 16 rRNA gene

Identification of selected isolates was conducted based on 16S rRNA gene sequences. DNA extraction was carried out using Genomic DNA Mini Kit (Geneaid) followed by amplification of the 16S rRNA gene using primer 20F (5'GATTTTGATCCTGGCTCAG3') and 1500R (5'GTTACCTTGTTAC-GACTT3') for AEBg4 and AEKp9 isolates, primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 16Sact1114R (5'-GAGTTGACCCCGGCRGT-3') for AEBg10, AEBg12, and AELk3 (Martina et al. 2008). The amplification reaction was performed by using Thermal cycler (model 480 Perkin-Elmer, USA). The amplified product was visualized on 1% agarose gel at 80 volts for 45 min. The PCR product then stained using ethidium bromide for 15 min and visualized using UV light transilluminator. DNA bands that appeared were documented using Gel Doc. PCR product sequences were performed by DNA Sequencing Service Company. All of the 16S rRNA gene sequences then compared to the GenBank database, NCBI BLAST (http: //www.ncbi.nlm.nih.gov/). Phylogenetic analysis was performed using MEGA 6 (Molecular Evolutionary Genetics Analysis, Version 6). The phylogenetic tree was constructed using the neighbor-joining method and MEGA 6.0 software (Tamura et al. 2013). The topology of phylogenetic tree construction was evaluated using bootstrap analysis with 1000 replications.

RESULTS AND DISCUSSION

Endophytic actinobacteria diversity

A total of 35 endophytic Actinobacteria were isolated from 5 different plants, that was rhizome of *A. galanga*, *K. galanga*, *Z. cassumunar*, *K. rotunda*, and leaves of *M. paniculata* with 6, 5, 9, 12, and three isolates, respectively.

The number of endophytic actinobacteria was dominant in the rhizome of *K. rotunda* (12 isolates), while from leaves of *M. paniculata* was only three isolates. Endophytic actinobacteria obtained from this work showed to have a various morphological colony, e.g. color of aerial and substrate mycelia, pigmentation, elevation of the colony, as described in Table 1.

The number of actinobacteria from the rhizome of medicinal plants tested was higher than that from the leaves (*M. paniculata*). This study was in line with Passari et al. (2015), who were able to obtain 42 endophytic actinobacteria from 7 medicinal plants and different organs (roots, flowers, leaves and petiola). The highest number of endophytic actinobacteria was found in the roots (52.3%) with *Streptomyces* as the dominant genus. Similar phenomena were also reported by Taechowisan et al. (2003), where the actinobacteria were dominant in rhizome (212 isolates), followed by leaves (97 isolates), and stem (21 isolates). Pujiyanto et al. (2012) found 65 endophytic actinobacteria from medicinal plants. A total of 45 isolates were found from the roots, 14 isolates from rhizomes, then followed by the stem and leaves with 3 isolates respectively.Actinobacteria are often found lived and colonized in soil. The possible explanation of this phenomenon is that the existence of endophytic actinobacteria in plants were probably originated from soil, followed by root colonization, then the actinobacteria may migrate to other plant parts.

Table 1 Morphological characteristic of endophytic actinobacteria isolates in ISP-2 medium and pancreatic lipase inhibition activity

Medicinal plants	Isolates code	Color of aerial mycelium	Color of substrate mycelium	Soluble pigment	Elevation	Inhibition activity (%)
A. galanga	AELk1	White	Dark brown	-	Flat	51.2
	AELk2	Grey	Light yellow	-	Flat	61.2
	AELk3	Bluish green	Dark green	-	Convex	93.8
	AELk4	White	Cream	-	Flat	48.6
	AELk5	White	Cream	-	Convex	57.2
	AELk6	White	Peach	-	Convex	60.5
Total of isolates		6				
K. galanga	AEKc1	White	Cream	-	Convex	57.9
	AEKc3	White	Cream	Cream	Flat	63.7
	AEKc4	Greenish grey	Light green	-	Convex	83.1
	AEKc8	White	Cream	Cream	Flat	77.1
	AEKc9	White	Cream	-	Flat	6.1
Total of isolates		5				
Z. cassumunar	AEBg1	Grey	Cream	-	Convex	67.3
	AEBg2	Greenish grey	Dark brown	-	Convex	54.6
	AEBg4	Greenish grey	Brown	-	Convex	96.5
	AEBg5	Grey	Brown	-	Convex	70.8
	AEBg8	White	Fawn	-		71.4
	AEBg9	Brownish grey	Cream	-	Convex	35.4
	AEBg10	Grey	Brown	-	Convex	95.3
	AEBg11	White	Brownish cream	-	Convex	78.2
	AEBg12	Grey	Brown	-	Convex	95.6
Total of isolates		9				
K. rotunda	AEKp2	Grey	Cream	-	Convex	43.6
	AEKp3	White	Orange	Yellow	Convex	15.1
	AEKp4	Grey	Yellow	Yellow	Convex	46.1
	AEKp6	White	Milky white	-	Convex	54.2
	AEKp7	White	Yellow	Cream	Convex	77.9
	AEKp8	Brownish grey	Cream	-	Convex	82.0
	AEKp9	Greenish white	Cream	-	Convex	92.4
	AEKp10	Greenish grey	Greenish grey	-	Convex	58.1
	AEKp11	White	Cream	-	Flat	79.6
	AEKp14	Greyish white	Brown	-	Convex	53.2
	AEKp15	Greenish brown	Brown	-	Convex	71.5
	AEKp16	Brown	Cream	-	Convex	79.3
Total of isolates		12				
M. paniculata	AEKm1	White	Cream	-	Flat	15.4
	AEKm2	Grey	Brown	-	Convex	31.5
	AEKm3	Grey	Dark brown	-	Flat	76.7
Total of isolates		3				
	Total of isolates				35	

Note: AELk = *A. galanga* endophytic actinobacteria, AEKc = *K. galanga* endophytic actinobacteria, AEBg = *Z. cassumunar* endophytic actinobacteria, AEKp = *K. rotunda* endophytic actinobacteria, AEKm = *M. paniculata* endophytic actinobacteria

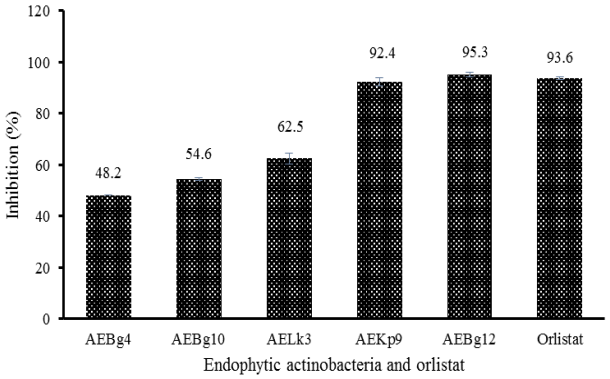

Figure 1. The avarage value of pancreatic lipase inhibitor activity from a crude extract of endophytic actinobacteria. Note: Isolate codes refer to Table 1.

Based on morphological colony observation, most isolates of endophytic actinobacteria indicated a close relationship with *Streptomyces* group, which can be commonly found in roots, stems, and leaves. *Streptomyces* spp. are filamentous bacteria, belonging to Actinomycetales, that is widely distributed in the various ecological environment. Discovery of the diversity of endophytic actinobacteria from *K. galanga*, *Z. cassumunar*, *K. rotunda* and *M. paniculata* have never been reported before. Moreover, there is no available data regarding their potency as producer of pancreatic lipase inhibitor as described in this paper. Therefore, the data described here can be considered as new information.

Pancreatic lipase inhibitory activity

Pancreatic lipase inhibitory activity test was carried out for 35 endophytic actinobacteria isolates. The result showed that all isolates had various lipase inhibitory activity, the lowest inhibition value was 6.1% and the highest value was 96.5%. Table 1 shows that endophytic actinobacteria had various pancreatic lipase activity. The activity was obtained from isolates (9 mm in colony diameter) which grown in ISP2 media for 10 days. Various lipase inhibitory activity showed by the tested isolates might be related with the different in their growth rate. Five isolates that have high activity value were AEBg4, AEBg10, AEBg12 obtained from *Z. cassumunar*, AELk3) from *A. galanga*, and AEKp9 from *K. rotunda*, with an inhibition value 96.4%, 95.6%, 95.3%, 93.8% and 92.4%, respectively.

The crude extract (1300 ppm) from 5 selected isolates gave various lipase inhibitory activity. AEBg12 showed higher pancreatic lipase inhibitory activity (95.3%) than orlistat (93.6%), as the positive control. Meanwhile, 4 other isolates, e.g. AEBg4, AEBg10, AELk3 and AEKp9 showed lower activity i.e. 48.2%, 54.4%, 62.5% and 92.4%, respectively (Figure 1). The data confirm that endophytic actinobacteria from the medicinal plants tested were able to produce pancreatic lipase inhibitor, as it is also produced by their host plants. It has been reported that *K. rotunda*, *A. galanga* and *Z. cassumunar*, showed pancreatic lipase inhibitory activity, i.e. 65.1% 56.2% and 29.17%, respectively (Iswantini et al. 2010; 2011; Pradono et al. 2011). Here, the discovery of endophytic actinobacteria from those plants is considered as the first reported data.

One of the mechanisms to prevent obesity is to inhibit the absorption of fatty acid by inhibiting the activity of pancreatic lipase on digestive organs. This is caused by the increase of pancreatic lipase activity which can increase the number of monoglycerides and fatty acids that are absorbed by the body, and it can lead to obesity (Iswantini et al. 2010). Orlistat, a derivative of lipstatin, is produced by *S. toxytricini* (Weibel et al. 1997), a non-endophytic actinobacterium. At the recommended therapeutic dose of 120 mg three times a day, orlistat inhibits dietary fat absorption around 30%. Although the use of orlistat is clinically approved for the treatment of obesity, orlistat may have some side effects, such as liquid stool, abdominal bloat and abdominal cramp (Ballinger and Peikin, 2002). At the same concentration (1300 ppm), our study showed that crude extract of selected endophytic actinobacteria (AEBg12) from *Z. cassumunar* had a higher percentage in inhibiting pancreatic lipase activity than orlistat. However, the characteristics of the active compound with pancreatic inhibitory activity from AEBg12 has not yet been elucidated.

Streptomyces sp. NR 0619 that was found in the soil in Yamaga-machi, Oita prefecture Japan, could also produce Panclicins A, B, C, D and E. Panclicins knew to inhibit swine pancreatic lipase, with IC50 values 2.9, 2.6, 0.62, 0.66, and 0.89 μM (Mutoh et al. 1994). *Streptomyces* sp. MTCC 5219 from the soil in India was reported to produce (E)-4-Aminostyryl, which is another type of pancreatic lipase inhibitor substance (Tokdar et al. 2011). The study of Kekuda et al. (2011) showed that *Streptomyces* isolated from soil in Karnataka region, India has the ability as an antiobesity with the highest inhibition value 61.67%. The information coming out from this work, regarding the pancreatic lipase inhibitory activity from endophytic actinobacteria isolated from medicinal plants which traditionally known for treating obesity is regarded as newly reported data.

Morphological characteristics of selected endophytic actinobacteria isolates

The five selected endophytic actinobacteria, i.e. AEBg4, AEBg10, AEBg12, AELk3 and AEKp9 showed variations on colony morphology (Table 2), when grown in ISP 2 for 14 days. The aerial mycelium of isolates had a wide range of colors, e.g. greenish gray, gray, bluish green, white, turqouise. Actinobacteria could be distinguished from other bacteria by looking at the shape of the colony in a solid medium. The five selected endophytic actinobacteria did not produce soluble pigment when grown in ISP 2, ISP 3 and ISP 4 media.

Actinobacteria colonies looked stiff (Figure 2), in contrast with bacterial colonies which often looked soft and some of them are slimy in agar. The five tested isolates have various spore chain characteristics. Two isolates have a spiral-shaped spore chain morphology, whereas the other three isolates do not have spiral-shaped spore. Our work also examined that the five isolates of endophytic

actinobacteria were Gram-positive, produced an abundance of aerial mycelia. Morphological observation of colonies on several media showed that the isolates grew well in ISP2, modified ISP 3 without the addition of trace salts component, and ISP 4 media. Aerial and substrate mycelium of AEBg4, AEBg10 and AEBg12 showed the same color in those three different media, whereas AELk3 and AEKp9 showed differences in color. Based on the above morphological colony and microscopic observation, the tested isolates showed to have the characteristics of *Streptomyces* genera, as described by Shirling and Gottlieb (1966). The observed *Streptomyces* morphological characters of the tested isolates are also strengthened by their molecular data, based on 16S rRNA gene sequence analysis, as described below.

Identification of endophytic Actinobacteria isolates based on 16S rRNA gene

The partial sequences of 16S rRNA analyses showed that AEBg4 (1421 bp) had the highest similarity with *Streptomyces* sp. S170 (93%), followed by a close relationship with *S. lannensis* strain SR3-58 (93%), and *S. lannensis* JCM 16578^T (92%). Both AEBg10 (1051 bp) and AEBg12 (1010 bp) had the highest similarity with *Streptomyces* sp. S170 by 99% and 98%, respectively. While AEKp9 (1367 bp) had the highest similarity with *Streptomyces* sp. DLDG2 (93%), and AELk3 (1033 bp) had 98% similarity with *Streptomyces* sp. NRLL B-24869 (Table 3).

Streptomyces lannensis was obtained from stingless bees (*Tetragonilla collina*) in Thailand (Promnuan et al. 2013). *Streptomyces bellus* was reported as an antibiotic producer (Margalith and Beretta 1960). Until now, the study of pancreatic lipase inhibitor produced by *S. lannensis* has never been reported.

Percentage of 16S rRNA gene sequence homology less than 97.5% could be a different species or new species (Stackebrandt and Goebel 1994), but further research is needed to confirm for the species novelty by using polyphasic taxonomy. These methods include genotyping analysis, chemotaxonomic and phenotyping data. Genotype information is based on the percentage of mol G+C, restriction patterns, genome size, the size of DNA, and DNA-DNA hybridization; whereas chemotaxonomic information can be found on the cell wall components such as peptidoglycan and teicoat acid. Phenotype analysis including, physiology, biochemistry, and morphological characteristic of an organism (Prakash et al. 2007).

The phylogenetic tree showed that AEBg4, AEBg10 and AEBg12 isolates belonged to one group. The AEBg4 was closely related with *Streptomyces* sp. S170, while both AEBg10 and AEBg12 were closely related to *Streptomyces lannensis* strain TW1K20. Moreover, AEKp9 and AELk3 had a close relationship with *Streptomyces* sp. DLDG2 and *Streptomyces* sp. CU16-I, respectively. As the outer group, *Bacillus subtilis* represented Gram-positive non-actinobacteria (Figure 3). Based on the phylogenetic tree, it showed that AEBg4, AEBg10, AEBg12, AELk3 and AEKp9 belong to the same genus, i.e. *Streptomyces*. On the phylogenetic tree, *S. toxytricini*, the known producer of pancreatic lipase inhibitor was also showed.

Table 2. Morphological characteristics of endophytic actinobacteria isolates that produce pancreatic lipase

Isolate	Medium	Growth characterization		
		Aerial mycelium	Substrate mycelium	Soluble pigment
AEBg4	ISP 2	Greenish grey	Brown	-
	ISP 3	Greenish grey	Brown	-
	ISP 4	Greenish grey	Brown	-
AEBg10	ISP 2	Grey	Brown	-
	ISP 3	Grey	Brown	-
	ISP 4	Grey	Brown	-
AEBg12	ISP 2	Grey	Brown	-
	ISP 3	Grey	Brown	-
	ISP 4	Grey	Brown	-
AELK3	ISP2	Bluish green	Dark green	-
	ISP 3	White	Cream	-
	ISP 4	Grey	Cream	-
AEKP9	ISP2	Greenish white	Cream	-
	ISP 3	Greyish blue	Cream	-
	ISP 4	turquoise	Cream	-

Note: Note: Isolate codes refer to Table 1.

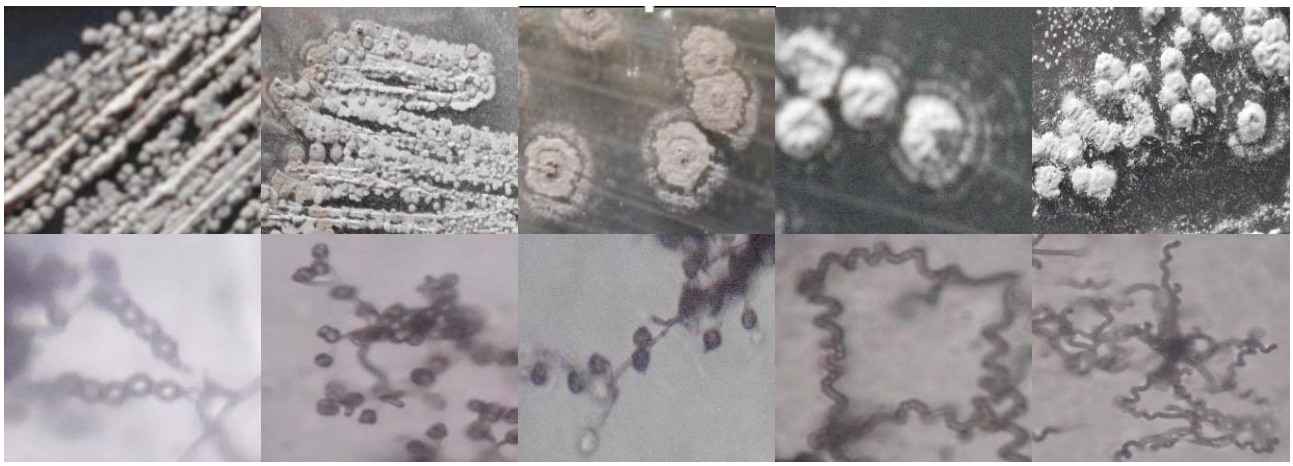

Figure 2. Various morphological characteristics of endophytic actinobacteria grown in ISP2 medium for 14 days (Magnification 400 x). Note: Note: Isolate codes refer to Table 1.

Table 3. BLAST.N results of 16S rRNA gene of endophytic actinobacteria on medicinal plants

Isolates code	Closest species	Identities (Isolates/GenBank)	Similarity	Accession number
AEBg4	*Streptomyces* sp. S170	1329/1423	93%	KF_772663.1
	S. lannensis strain SR3-58	1328/1423	93%	LC_128345.1
	S. lannensis JCM 16578^T	1328/1423	92%	NR_113181.1
AEBg10	*Streptomyces* sp. S170	1038/1051	99%	KF_772663.1
	S. lannensis strain SR3-58	1037/1051	99%	LC_128345.1
	S. lannensis strain SR3-109	1037/1051	99%	LC_126314.1
AEBg12	*Streptomyces* sp. S170	994/1011	98%	KF_772663.1
	Streptomyces sp. strain 10116b	993/1011	98%	KX_573753.1
	S. lannensis strain TW1K20	993/1011	98%	KT_820003.1
AEKp9	*Streptomyces* sp. DLDG2	1283/1373	93%	GQ_357959.1
	S. bellus NBRC 12844^T	1255/1334	93%	NR_041222.1
	Streptomyces sp. 21214	1282/1373	93%	KF_956675.1
AELk3	*Streptomyces* sp. NRLL B-24869	1017/1034	98%	JQ_247519.1
	Streptomyces sp. CU16-1	1017/1034	98%	AB_444070.1
	S. bellus strain HBUM175137	1017/1034	98%	FJ_532419.1

Note: Isolate codes refer to Table 1.

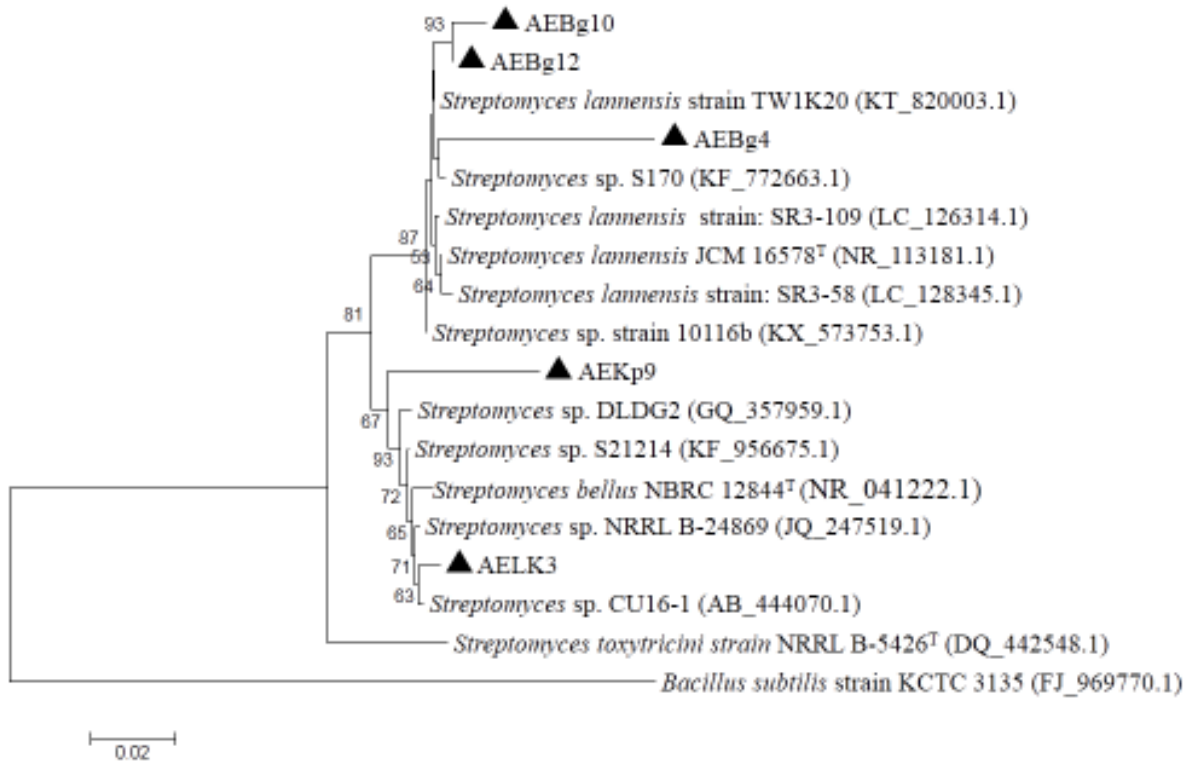

Figure 3. Phylogenetic tree of 16S rRNA gene of endophytic actinobacteria on medicinal plants as a potential pancreatic lipase inhibitor with software MEGA 6 (bootstrap analysis with 1000 replication). Note: Isolate codes refer to Table 1.

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