Arch Biol Sci. 2018;70(2):349-357 https://doi.org/10.2298/ABS170729052C

Insecticidal activity of endophytic actinomycetes isolated from *Azadirachta indica* against *Myzus persicae*

Yan Chen[1], Jamil Shafi[1,2], Maohai Li[3], Danni Fu[1] and Mingshan Ji[1,*]

[1] *Department of Pesticide Science, Plant Protection College, Shenyang Agricultural University*, 120 Dongling Road, Shenhe District, Shenyang, Liaoning, P.R. China.,110866

[2] *Department of Plant Pathology, University of Agriculture Faisalabad, Sub-Campus Depalpur*, Okara, 6-KM, Lahore Road, G.T. Road, Okara, Punjab, Pakistan, 56300

[3] *Institute of Plant Protection, Jilin Academy of Agricultural Sciences*, No. 303 Kemao West Street, Gongzhuling, Jilin, P.R. China, 136100

***Corresponding author**: jimingshan@163.com

Received: July 29, 2017; **Revised**: December 4, 2017; **Accepted**: December 5, 2017; **Published online**: December 12, 2017

Abstract: In the present study, 85 strains of actinomycetes were isolated from the neem tree (*Azadirachta indica*) and screened for their insecticidal activity against the green peach aphid, *Myzus persicae*. The results showed that crude extracts from 24 strains exerted a contact effect against *M. persicae* with different insecticidal efficacies. Crude extracts from 8 actinomycetes strains exhibited the highest insecticidal activity (above 60%). Out of these 8 strains, 3 isolates that produced the maximum mortalities were screened a second time. The crude extract from strain G30 was the most virulent against the green peach aphid, with LC_{50} and LC_{95} values of 1.680 mg/mL and 4.370 mg/mL, respectively, after 48 h of treatment. The following morphological, culture, physiological and biochemical characteristics of strain G30 were recorded: (i) ovate-orbicular and smooth surface spores with short and curve filaments; (ii) an aerial off-white mycelium with a mustered yellow base; (iii) inability to produce soluble pigments; (iv) the ability to hydrolyze starch but not cellulose; (v) the ability to utilize glycerin and several sugars as a carbon source but not L-rhamnose and sorbitol. Molecular identification of G30 revealed a 99.6% genetic similarity of the 16S rDNA sequence with *Streptomyces albidoflavus*. We conclude that the isolate G30 was *S. albidoflavus* and that the insecticidal activity of its crude extract was sufficiently high to become a candidate for bioinsecticide development.

Key words: *Azadirachta indica*; endophytic actinomycetes; *Myzus persicae*; insecticidal activity; *S. albidoflavus*

INTRODUCTION

Myzus persicae is a cosmopolitan and highly polyphagous phytophagous pest whose host range exceeds 400 plant species, including many economically important agricultural and forest plants. It mainly damages cruciferous, nightshade vegetables and drupe fruit trees [1,2]. *M. persicae* causes damage to its hosts by direct feeding, the production of honeydew and the transmission of plant viruses. It is capable of transmitting over 100 different plant viruses [2]. Chemical pesticides are still the main effective method for controlling green peach aphid. However, the use of chemical pesticides may seriously damage human health and cause environmental pollution. Moreover, due to intensive use of chemical pesticides over many years, widespread and multiple forms of aphid resistance have evolved. *M. persicae* is now reported to be resistant to most classes of insecticides, including organophosphates, carbamates, pyrethroids, cyclodienes and neonicotinoids, making *M. persicae* one of the most resistant species worldwide [3]. According to the WHO, in the next 20 to 30 years chemically based pesticides will lose their efficacy [4,5]. Therefore, scientists are searching for novel strategies and agents for long-term and environmentally safe plant protection [6].

Neem (*Azadirachta indica*) belongs to the Meliaceae family and it originates from India and Burma. It

was hailed as "a tree for solving global problems" by the United States Department of Agriculture [7]. Among the many uses of the neem tree, its insecticidal activity against nearly 400 species of agricultural and forest pests is well-known [8,9]. Many insecticidally active substances have been isolated from seeds, bark and other parts of the neem tree, the most common being azadirachtin, nimbin, nimbidin, nimbidol, nimbinate, quecerétin and salannin [10,11]. Siddiqui et al. [12] reported that all parts of the neem tree are valuable. Because of the antiseptic, bactericidal and insecticidal activity of its leaves, branches, bark, roots and fruits, the neem tree neem is called a "rural pharmacy".

There are over 250 thousand plant species in nature, but only several hundred of them have been studied for their insecticidal activity and the study of their endophytic actinomycetes is more scarce [13]. Various neem formulations have been registered for their efficacy against numerous plant pests [14-16]. Neem extracts are recognized as highly efficient pesticides, but studies on the isolation of endophytic actinomycetes from neem have just started in the past few years and are rarely reported. Plant endophytic actinomycetes have always been an important source of natural active products and thus honored as a "natural treasury" [17]. Therefore, the development of pesticides based on neem endophytic actinomycetes has great potential. Insecticidally active endophytic actinomycetes isolated from neem can overcome the shortcomings of traditional preparation modes (crushing, grinding, and extraction of bioactive compounds from plant organs), and the lack of natural neem. Endophytic actinomycetes are natural internal inhabitants of plants. Although actinomycetes have been known for a long time, their importance only recently became more evident when their role in protecting host plants against insects and diseases was recognized. Actinomycetes have great economic value and widespread practical applications. They contain a wide variety of bioactive metabolites, including antibiotics, enzymes, vitamins, amino acid and steroids [18]. Over 8000 bioactive substances have been found in microbes, and nearly 70% of them are produced by actinomycetes [19].

Thus, screening of insecticidally active substances from the endophytic actinomycetes of neem is of great significance to the development of a novel insecticide. In this study, 85 actinomycete strains isolated from the neem tree were screened for insecticidal activity against green peach aphid. The study was aimed at finding neem endophytic actinomycetes with the highest insecticidal activity and its identification based on morphological, culture, physiological, biochemical characteristics and 16S rDNA sequencing.

MATERIALS AND METHODS

Isolation and purification of endophytic actinomycetes

The leaves and fruits of *A. indica* were collected from Thailand and preserved at 4°C in the Pesticide Science Laboratory of Shenyang Agricultural University. Nuts and leaves of neem were washed in running tap water and then surface-sterilized with 70% ethanol for 1 min, sodium hypochlorite (2.0-2.5% active Cl) for 4 min and 70% ethanol for 30s. The nuts and leaves were then washed three times with sterile distilled water [20]. After washing and sterilizing, neem nuts (4.0 g) and leaves (2.0 g) were ground with a mortar, homogenized in 10 mL deionized water, and 0.1 mL of the homogenate was gradient diluted. Homogenates (100 μL) were painted on Gauserime 1 agar medium plates composed of 20 g of soluble starch, 0.5 g NaCl, 0.001 g $FeSO_4$, 0,05 g $MgSO_4 \cdot 7H_2O$, 1 g KNO_3, 0.5 g K_2HPO_4 and 20 g of agar in 1 L of distilled water, supplemented with 0.005% potassium dichromate to inhibit the growth of fungi and bacteria. The plates were inverted and cultured in constant-temperature incubators at 28°C for 7 days. The strains of different colonial morphology were selected and purified on Gauserime No.1 agar medium [21]. A total of 85 isolates were identified up to genus level by conventional microbiological methods [22]. After purification and preliminary identification, the actinomycetes were numbered and preserved for further use in insecticidal activity screening.

Fermentation and preparation of crude extracts

Fermentation and extraction of crude extracts were performed by the modified method of Zhang et al. [23]. Spore suspensions (10^6~10^7 spores/mL) of different purified strains of actinomycetes were inoculated on Gauserime 1 broth medium and incubated in a

shaking incubator at 180 rpm for 7 days at 28°C. The fermentation liquor was filtered and extracted by an equal volume of ethyl acetate three times. The extracted upper organic phases were mixed and concentrated to dryness by a rotary evaporator (35°C). The crude extracts with active substances were obtained from fermentation liquor.

Primary screening for insecticidal activity

Actinomycete strains were screened according to the method of Luo and Miao [24] with some modifications. Cabbage leaves were cut from plants that contained many aphids. Fifty aphids were kept per leaf by removing extra individuals with a paintbrush. The leaves were placed in Petri dishes (90×14 mm) lined with moisturized filter papers. Different crude extracts from fermentation liquors were dissolved in acetone and diluted with distilled water to obtain quantitative solutions of 20 mg/mL concentration. An aquatic acetone solution (acetone:distilled water=1:10) was used as a control.

The prepared Petri dishes were sprayed with 1 mL of crude extract solutions using a Potter spray tower and settled for 15 s at 24°C. The control was sprayed with 1 mL of aquatic acetone solution. The experiment was performed in three replicates. After spraying, the leaves were air dried for 45 min. The Petri dishes were then covered with sealing film to reduce evaporation of the crude extracts and incubated at 25±1°C. The number of dead aphids was recorded after 24 h and 48 h to calculate the mortalities and corrected mortalities. Aphid death was judged by the immobility of aphids after touching their feet and antenna with a paintbrush.

Secondary screening for insecticidal activity

Strains with corrected mortalities above 70% in the primary screening were further screened by the spray method. The crude extracts from the liquor of these strains were prepared at 5.00 mg/mL, 3.33 mg/mL, 2.50 mg/mL, 2.00 mg/mL and 1.70 mg/mL concentrations. To identify the crude extract with the highest insecticidal activity, insecticidal activities were tested by the same method as described above for primary screening. The experiment was conducted with five replicates and 50 aphids in each replicate. The mortality and corrected mortality were calculated using equations 1 and 2, respectively.

$$\text{Mortality (\%)} = \frac{\text{Number of dead insects}}{\text{Number of insects tested}} \times 100 \quad (1)$$

$$\text{Corrected mortality (\%)} = \frac{\text{Treatment mortality} - \text{Control mortality}}{100 - \text{Control mortality}} \times 100 \quad (2)$$

In addition, the LC_{50} and LC_{95} for these selected strains were also calculated after 48 h of treatment.

Specific identification of the strain with the strongest insecticidal activity

Identification at the genus level of the strain with the highest insecticidal activity was performed by conventional methods on the basis of morphological, physiological and culture characterization [22], while species-level identification was performed by 16S rDNA sequencing. The strain was cultured at 28°C for 7~10 days and the morphological characteristics, including colony morphology, aerial and substrate mycelia, spore morphology and pigmentation were studied. Also, gelatin liquefaction, starch hydrolysis, coagulation of milk and peptone, nitrate reduction, hydrogen sulfide production, cellulose decomposition, nitrogen source utilization and carbon source utilization were evaluated using the methods described by Locci [22].

Molecular identification of the strain with the strongest insecticidal activity was performed on the basis of 16S rDNA gene sequencing analysis. Genomic DNA of the selected strain was extracted following the method described by Kataoka et al. [25] and Mehling et al. [26]. The 16S rDNA gene was amplified by PCR. The PCR system was composed of: 14.8 μL dd H_2O, 2.5 μL 10 buffer, 0.2 μL dNTP, 2 μL Primer A (5'>AGAGTTTGATCCTGGCTCAG<3'), 2 μL Primer B (5'>AAGGAGGTGATCCAGCCGCA <3'), 0.3 μL, 3 U/μl Taq enzyme and 1.0 μL DNA template (Sangon Biotech Co. Ltd, Shanghai, China). The conditions for thermal cycling were as follows: denaturation at 94°C for 3 min, followed by 32 cycles at 94°C for 1 min, annealing at 54°C for 1 min and extension at 72°C for 1 min. At the end of the cycling, the reaction mixture was kept at 72°C for 10 min and then cooled to 4°C

[27]. PCR products (5.0 μL) were separated by 0.8% agarose gel electrophoresis in TBE buffer with DL2000 DNA marker. The electrophoresis was performed for 1 h at 100 V and the results were visualized under UV light after ethidium bromide (EB) staining. PCR amplified products were sequenced by the Shanghai Biochemistry Company using the same primers as above. The 16S rDNA gene sequence was compared for similarity with the reference species of actinomycetes contained in the genomic database using the NCBI-BLAST tool. The neighbor-joining method was employed to construct a phylogenetic tree using MEGA 5.0 software [28].

Statistical analysis

The data were analyzed by one-way ANOVA using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). For specific post hoc comparisons among strains of actinomycetes, Duncan's multiple range tests at a level of significance of $P<0.05$ were carried out. For calculation of LC_{50} and LC_{95}, the mortality data were subjected to probit analysis in SPSS 22.0 software.

RESULTS

Determination of insecticidal activities of crude extracts from actinomycetes

Primary screening

Out of 85 isolates of actinomycetes, the crude extracts from 24 isolates showed contact toxicities to green peach aphids after 24 h. The results given in Table 1 show that the crude extracts from 24 actinomycetes produced certain contact toxicity effects, while 8 of them caused a peach aphid mortality higher than 60%. The insecticidal activities of the crude extracts from G11, G30, G34, and R9 were the highest. After 24 h, corrected mortalities were 89.28%, 78.56%, 92.85% and 71.41%, respectively, while after 48 h the mortalities were 92.75%, 81.88%, 96.38% and 75.36%, respectively. Medium insecticidal activities against green peach aphids were recorded for crude extracts from the G2, G5, G18 and G50 strains. Corrected mortality induced by these extracts ranged between 60.66% and 65.70% after 24 h, and between 62.32% and 68.12% after 48 h. The insecticidal activities of the rest of the 16 strains were below 60%.

Secondary screening

In this investigation, the 3 actinomycetes (G30, G34, and G11) that caused the highest mortality in primary screening were used for the secondary screening. The results presented in Table 2 show that strain G30 was the most effective against *M. persicae* as compared to G34 and G11 ($P<0.05$). Even the corrected mortality at a lower concentration (2.00 mg/mL) caused by G30 was higher than 50%, and the efficacy of its crude extract at a concentration of 5 mg/mL was sufficient to control *M. persicae* (Table 2). The G30 strain showed conspicuous insecticidal activity, with an LC_{50} value of 1.68 mg/mL (95% fiducial limits of

Table 1. Insecticidal activity (means±SE) of crude extracts from selected strains of actinomycetes against *Myzus persicae* after 24- and 48 h-treatments (primary screening).

Strain number	24 h		48 h	
	Mortality (%)	Corrected mortality (%)	Mortality (%)	Corrected mortality (%)
G2	68.00±2.31	65.70±2.47	70.00±1.15	67.39±1.10
G3	53.33±1.76	49.95±1.84	54.67±0.67	50.73±0.96
G5	63.33±1.76	60.66±1.84	65.33±2.91	62.32±3.64
G11	90.00±3.06	89.28±3.23	93.33±2.91	92.75±3.26
G18	66.67±3.53	64.31±3.71	70.67±1.33	68.12±1.82
G20	36.67±1.33	32.12±1.25	40.00±2.31	34.78±3.33
G22	50.00±3.06	46.41±3.54	52.67±1.33	48.55±1.57
G27	46.67±1.33	42.84±1.27	49.33±1.76	44.92±2.61
G30	80.00±2.00	78.56±2.06	83.33±1.76	81.88±2.15
G33	60.00±2.31	57.13±2.22	64.67±1.33	61.60±1.90
G34	93.33±1.76	92.85±1.87	96.67±2.91	96.38±3.20
G41	53.33±1.76	49.98±1.84	58.67±1.33	55.08±1.98
G42	58.67±2.91	55.70±3.38	59.33±1.76	55.79±2.47
G49	23.33±1.76	17.82±1.86	26.67±1.33	20.29±2.39
G50	66.00±2.31	63.56±2.26	68.67±1.33	65.95±1.85
G53	40.00±2.00	35.69±1.94	43.33±1.76	38.40±2.69
G56	56.00±2.31	52.84±2.48	59.33±1.76	55.79±2.47
G59	48.00±2.31	44.27±2.49	52.00±2.31	47.83±3.17
R56	54.67±1.33	51.41±1.28	60.00±2.00	56.52±2.26
R3	62.00±3.06	59.27±3.08	65.33±1.76	62.32±2.39
R9	73.33±2.91	71.41±3.07	77.33±1.76	75.36±2.23
R12	51.33±1.76	47.83±1.84	53.33±1.76	49.27±2.56
R13	61.33±2.91	58.55±3.07	64.00±2.31	60.87±3.00
R6	52.00±2.31	48.55±3.37	54.67±1.33	50.73±2.03
CK	6.67±0.67		8.00±1.15	

CK – 10% aquatic solution of acetone was used as a control treatment.

Table 2. Corrected mortalities (means±SE) of *Myzus persicae* caused by crude extracts from three selected strains (secondary screening).

Strain No.	Concentration (mg/mL)	Corrected mortality (%)	
		24 h	48 h
G30	5.00	79.06±1.43 a	94.61±1.07 a
G34		41.01±3.44 c	35.74±1.44 c
G11		62.03±1.18 b	70.63±1.96 b
G30	3.33	71.31±1.44 a	91.22±1.49 a
G34		33.06±1.33 c	23.68±1.58 c
G11		50.23±1.86 b	54.05±1.71 b
G30	2.50	60.06±0.92 a	78.01±1.35 a
G34		10.39±1.33c	16.72±2.25c
G11		42.89±1.23 b	43.83±1.09b
G30	2.00	50.78±1.24 a	61.97±1.97 a
G34		5.05±0.82 c	12.48±1.04c
G11		26.87±1.32 b	30.63±1.29b
G30	1.70	39.24±1.45 a	46.10±1.74 a
G34		3.38±0.52c	7.93±1.07 c
G11		12.23±1.19 b	18.16±1.22b

Values marked with different letters a, b, c differ significantly among strains within each concentration (Duncan's multiple range test, $P<0.05$).

1.255-1.957 mg/mL), and an LC_{95} value of 4.370 mg/mL (95% fiducial limits of 3.510-7.127 mg/mL) after 48 h of treatment (Table 3).

Morphological and culture characteristics of the G30 strain

Strain G30 was grown on Gauserime 1 medium, potato dextrose medium, Klinefelter medium 1, a medium containing glycerol-asparaginase, glucose-aspartate medium, glucose-yeast extract medium, inorganic salt medium, starch medium, yeast extract, malt extract medium and oatmeal medium. In these media, the mycelia were yellowish-white or dust gray, the base of the mycelia were light yellow, pale mango brown or colorless. The strain grew well on Gauserime 1 medium, Klinefelter 1 medium and inorganic salt starch, whereas moderate growth was observed in the glucose-yeast extract, glycerol-asparaginase and glucose-aspartate media. On the Gauserime 1 medium, strain G30 produced an off-whitish, lush aerial mycelium with a mustered yellow base, but it lacked soluble pigments (Fig. 1A and B). Under the optical microscope, the hyphae were lush green, while the spores were oval shaped with a smooth surface, short and curvy filaments (Fig. 1C and D).

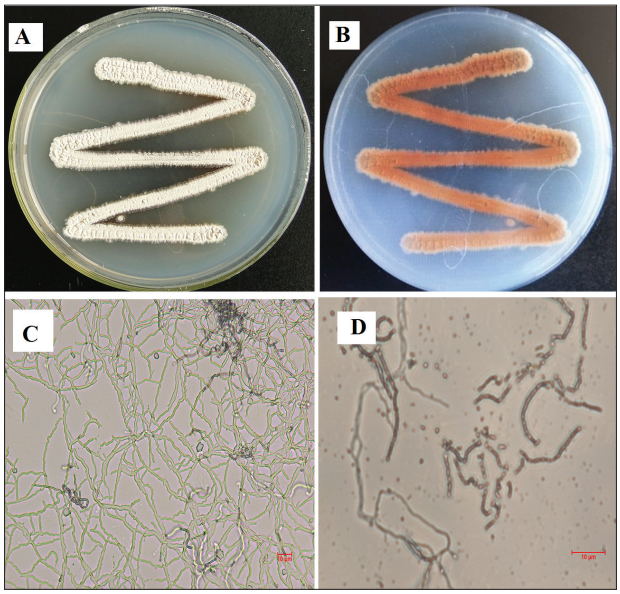

Fig. 1. Morphological features of strain G30. **A** – Morphology of the aerial mycelium cultured on Gauserime 1 medium. **B** – Morphology of the basal mycelium of strain G30 cultured on Gauserime 1 medium. **C** – Hyphal morphology of strain G30 under the optical microscope. **D** – Spore morphology of strain G30 under the optical microscope.

Table 3. Toxicity of various crude extracts against *Myzus persicae* (48 h).

Strain	Regression equation	Concentration (mg/mL)		Chi-square	P value
		**LC_{50}	***LC_{95}		
G30	Y=-0.893+3.961X	1.680 (1.255-1.957)	4.370 (3.510-7.127)	5.523	0.137
G34	Y=-1.816+2.093X	7.379 (5.828-11.191)	45.081 (23.857-145.316)	0.256	0.968
G11	Y=-1.414+2.882X	3.094 (2.868-3.374)	11.516 (8.975-16.643)	2.872	0.412

The numbers in parentheses represent 95% fiducial limits based on a probit analysis.
** LC_{50} is the concentration of crude extract that caused the death of 50% of the population of *Myzus persicae.*
*** LC_{95} is the concentration of crude extract hat caused the death of 95% of the population of *Myzus persicae.*

Physiological and biochemical characterization of the G30 strain

Strain G30 exhibited strong gelatin liquefaction ability, producing milk from peptone instead of solidifying. It showed a strong ability for starch hydrolysis but lacked a hydrogen sulfide production ability. No growth was observed on cellulose. The strain also showed an inability to produce melanin. It exhibited a positive result for nitrate reduction and an efficient use of D-glucose, D-fructose, maltose, lactose, D-mannose and glycerin as the carbon source, while it lacked the ability to utilize sucrose, L-rhamnose and sorbitol as the carbon source (Table 4).

Table 4. Physiological and biochemical characteristics of strain G30.

Characteristics test	Result	Characteristics test	Result
Starch hydrolysis	+	D-glucose	+
Nitrate reduction	+	D-fructose	+
Gelatin liquefaction	+	Maltose	+
Cellulose hydrolysis	-	Lactose	+
Production of hydrogen sulfide	-	D-mannose	+
Milk peptone	+	Glycerin	+
Melanin	-	L-rhamnose	-
Sucrose	-	Sorbitol	-

"+" – positive results; "-" – negative results

Molecular identification of the G30 strain

The PCR product had a clear band of about 1500 bp, showing successful amplification of the 16S rDNA region of strain G30 (Fig. 2). Comparison of the 16S rDNA sequence with the sequences deposited in the NCBI database showed 99% similarity with 11 species of actinomycetes. The phylogenetic tree of strain G30 and related species was constructed using MEGA 6.0, which showed that strain G30 belongs to the genus *Streptomyces* and that it has the highest similarity with *Streptomyces albidoflavus* (Fig. 3).

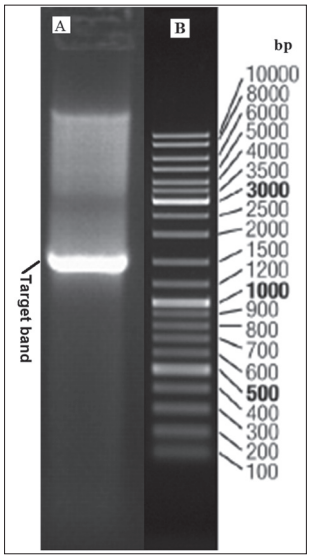

Fig. 2. Agarose gel electrophoresis. **A** – PCR amplified 16S rDNA gene of strain G30. **B** – DNA marker.

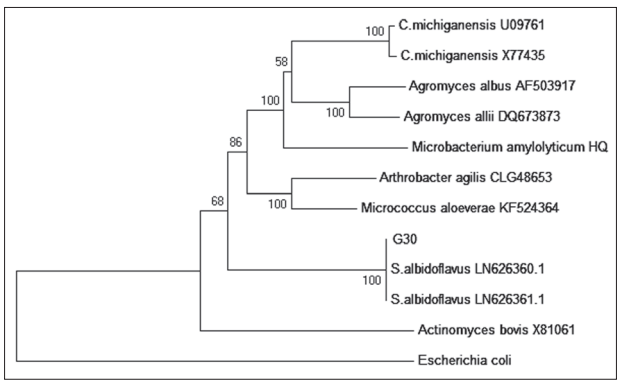

Fig. 3. Phylogenetic tree showing relationships of taxa related to strain G30 built by comparative analysis of the 16S rDNA gene sequences. The tree was built by the neighbor-joining method based on bootstrap analysis of 1000 replicates.

DISCUSSION

Biopesticides produced by microorganisms and used in agriculture have gained significant importance among researchers and the farming community because of their safety as regards non-target organisms and the environment. Moreover, biopesticides have reduced the emergence of natural and cross resistance in plant pests [29]. Actinobacteria, particularly *Streptomyces* spp., have provided a diverse range of secondary metabolites of high commercial significance and continue to be regularly screened for new bioactive compounds [30]. In the present study, a total of 85 actinomycetes were isolated from the neem plant and screened for insecticidal activity against *M. persicae*. All the isolates were identified up to the genus level by a conventional microbiological approach. The morphological, biochemical and physiological characteristics of the selected isolates and comparison with those mentioned in the Manual of Systematic Bacteriology [22] clearly suggest that these isolates belong

to the genus *Streptomyces*. However, the species-level identification of the actinomycetes is not possible by morphological, biochemical and physiological characterization. It requires identification at the genetic level, such as provided by comparison of the 16S rDNA gene sequences with the type strains. Only the G30 strain, which exhibited promising insecticidal activity, was identified to the species level by 16S rDNA gene sequencing.

Of the 85 isolates, the crude extracts from 24 strains showed different contact toxicities against the green peach aphid. In the primary screening, crude extracts from 8 actinomycetes strains showed higher insecticidal activity with corrected mortalities above 60%. Crude extracts from the remaining 16 strains had relatively low insecticidal activities. Similar reports are available for the insecticidal activity of actinomycetes and their products [31-35]. Karthik et al. [36] found that some actinomycete isolates produced strong insecticidal activity. Horikosh et al. [37] screened 300 microbial natural products and found pyripyropene A as a promising insecticidal compound against aphids on cabbage.

During the second screening, the G30 strain showed prominent insecticidal activity with an LC_{50} value of 1.680 mg/mL after 48 h of treatment; however, this LC_{50} value was higher than the previously reported value of Liao et al. [38], who investigated the virulence of three fermentation products of actinomycetes against *M. persicae* and showed that isolate 31-1 was the most effective, having an LC_{50} value of 0.0447 mg/mL and a corrected mortality of 82.9% after 24 h. Similarly, strain G30 displayed a slightly higher LC_{50} value than some of the other natural environment-friendly biopesticides, such as the *Illicium verum* fruit extract (LC_{50}, 0.00014 mg/mL at 72 h) [39] and *Parthenium hysterophorus* (LC_{50}, 1.141 mg/mL at 48 h) [40], but a lower LC_{50} value than the essential oil of *Pogostemon cablin* (LC_{50}, 1.694 mg/mL at 48 h) [41]. The higher LC_{50} value for G30 may be due to impurities present in the crude extract used in this study.

Based on the morphological, biochemical, physiological and molecular characterization, strain G30 belongs to the genus *Streptomyces*. The results of cluster analysis showed the highest similarity between strain G30 and whitish yellow *Streptomyces albidoflavus* that are located on the same branch of the phylogenetic tree. Their homology was high, and clustering analysis had high repeatability. The genus *Streptomyces* is an economically important group of actinomycetes and a key source of copious amounts of biologically active compounds. Several agriculturally important compounds with antibacterial [42], antifungal [31], insecticidal, antiparasitic [43] and antiviral [44] activities have been obtained from *Streptomyces* spp. [45]. Moreover, about 60% of antibiotics and most of the agricultural antibiotics are from the genus *Streptomyces* [46].

The results obtained in the present study on the insecticidal activity of the selected isolates indicate that the crude extract from strain G30 (*S. albidoflavus*) possesses strong insecticidal activity against green peach aphid, and might contain novel insecticidal components. In a recent study [47], a novel polyketide metabolite with insecticidal properties was identified from a marine actinomycete *Streptomyces* sp. AP-123. Similarly, Kaur and Manhas [48] identified *Streptomyces hydrogenans* DH16, which possesses an antifungal, insecticidal and plant growth-promoting potential. Regarding strain G30, further studies on the identification and structure elucidation of the produced active components are required before novel insecticidal compounds are obtained.

Acknowledgments: The financial support by the Ministry of Agriculture of the People's Republic of China to the Department of Pesticide Science, College of Plant Protection, Shenyang Agricultural University as a special fund for agroscientific research in the public interest (2013325) is gratefully acknowledged.

Author contributions: YC and JS were involved in the conception, plan, design and preparation of the manuscript. YC, DF and ML performed the experiments, analyzed the data and interpreted the results. MJ, YC and JS conducted data collection and critical revision of the article. All the authors approved the final version of the article.

Conflict of interest disclosure: The authors declare that there is no conflict of interest.

REFERENCES

1. Bass C, Puinean AM, Zimmer CT, Denholm I, Field LM, Foster SP, Gutbrod O, Nauen R, Slater RWilliamson MS. The evolution of insecticide resistance in the peach potato aphid, *Myzus persicae*. Insect Biochem Mol Biol. 2014;51:41-51.

2. Blackman RL, Eastop VF. Aphids on the world's herbaceous plants and shrubs. Chichester: John Wiley & Sons; 2008. 215 p.
3. Pesticide Environmental Stewardship [Internet]. Washington. Center for Integrated Pest Management. Insecticide Resistance Mechanisms. [cited 2017 Sept 20]; [about 1 screen]. Available from: https://pesticidestewardship.org/resistance/insecticide-resistance/insecticide-resistance-mechanisms/.
4. World Health Organization. Global Plan for insecticide resistance management in malaria vectors. Geneva: WHO; 2012. 44-86 p.
5. Liu N. Insecticide resistance in mosquitoes: impact, mechanisms, and research directions. Annu Rev Entomol. 2015;60:537-59.
6. Gonzalez CA, Reina M, Diaz C, Fraga BSMO. Natural product-based biopesticides for insect control. In: Mander L, Liu HW, editors. Comprehensive natural products. Oxford: Elsevier Science; 2010. p. 237-68.
7. Noel V. Neem: a tree for solving global problems. 1st ed. New York: The Minerva Group, Inc; 2004. 132 p.
8. Li XZS. The toxic effects and mode of azadirachtin on insects. J South China Normal Univ. 1995;17(1):118-22.
9. Jadeja G, Maheshwari RNS. Extraction of natural insecticide azadirachtin from neem (*Azadirachta indica* A. Juss) seed kernels using pressurized hot solvent. J Supercrit Fluids. 2011;56(3):253-8.
10. Xu H. Insecticidal plants and botanical insecticide. Beijing: China Agriculture Press; 2001. 309 p.
11. Xuan TD, Tsuzuki E, Hiroyuki T, Mitsuhiro M, Khanh TDCIM. Evaluation on phytotoxicity of neem (*Azadirachta indica*. A. Juss) to crops and weeds. Crop Protect. 2004;23(4):335-45.
12. Siddiqui BS, Afshan F, Faizi S, Naqvi STR. Two insecticidal tetranortriterpenoids from *Azadirachta indica*. Phytochem. 2000;53(3):371-6.
13. Schulz B, Boyle C, Draeger S, Römmert AK, Krohn K. Endophytic fungi: a source of novel biologically active secondary metabolites. Mycol Res. 2002;106(9):996-1004.
14. Shannag H, Capinera JFN. Efficacy of different neem-based biopesticides against green peach aphid, *Myzus persicae* (Hemiptera: Aphididae). Int J Agr Policy Res. 2014;2(2):61-8.
15. Faraone N, Hillier NKC. Plant essential oils synergize and antagonize toxicity of different conventional insecticides against *Myzus persicae* (Hemiptera: Aphididae). Plos One. 2015;10(5):e0127774.
16. Christopher Cutler G, Ramanaidu K, Astatkie T, Isman MB. Green peach aphid, *Myzus persicae* (Hemiptera: Aphididae), reproduction during exposure to sublethal concentrations of imidacloprid and azadirachtin. Pest Manage Sci. 2009;65(2):205-9.
17. Strobel GDB. Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev. 2003;67(4):491-502.
18. Shi N, Yang RIL. Screening and Primary Study of Actinomycetes with Insecticidal Activity. Chin J Bio Control. 2009;25(1):45-7.
19. Tao S, Zong MS. Ecological distribution of actinomycetes in the southwest part of Shandong province. Shandong Agric Sci. 2006;2:017.
20. Araújo W, Lima AS, Azevedo J, Marcon J, Sobral JLP. Manual: isolamento de microrganismos endofíticos. Piracicaba: Calq; 2002. 86 p.
21. Caruso M, Colombo A, Fedeli L, Pavesi A, Quaroni S, Saracchi MVG. Isolation of endophytic fungi and Actinomycetes taxane producers. Ann Microbiol. 2000;50(1):3-13.
22. Locci R. *Streptomyces* and related genera. In: Williams ST, Sharpe ME, Holt JG, editors. Bergey's manual of systematic bacteriology. Baltimore: Williams & Wilkins; 1989. p. 245-9.
23. Zhang YM, Li HY, Hu C, Sheng HF, Zhang Y, Lin BR, Zhou GX. Ergosterols from the culture broth of marine *Streptomyces anandii* H41-59. Mar Drugs. 2016;14(5):84.
24. Luo L, Miao F. Screening and Identification of two actinomyces strains with insecticidal activity for radish aphid. Agrochem. 2013;2(1):20.
25. Kataoka M, Ueda K, Kudo T, Seki TY. Application of the variable region in 16S rDNA to create an index for rapid species identification in the genus *Streptomyces*. FEMS Microbiol Lett. 1997;151(2):249-55.
26. Mehling A, Wehmeier UFPW. Nucleotide sequences of *streptomycete* 16S ribosomal DNA: towards a specific identification system for *streptomycetes* using PCR. Microbiol. 1995;141(9):2139-47.
27. Lin Y, Du D, Si C, Zhao Q, Li ZP. Potential biocontrol *Bacillus* sp. strains isolated by an improved method from vinegar waste compost exhibit antibiosis against fungal pathogens and promote growth of cucumbers. Biol Control. 2014;71:7-15.
28. Tamura K, Dudley J, Nei MKS. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol. 2007;24(8):1596-9.
29. Vijayabharathi R, Kumari BR, Sathya A, Srinivas V, Abhishek R, Sharma HCS. Biological activity of entomopathogenic actinomycetes against lepidopteran insects (Noctuidae: Lepidoptera). Can J Plant Sci. 2014;94(4):759-69.
30. Nan S, Runlei Ylikun Z. Progress in Actinomycetes with Insecticidal Activities. J Anhui Agric Sci. 2009;11(1):015.
31. Berdy J. Bioactive microbial metabolites. J Antibiot. 2005;58(1):1.
32. Xiong L, Li J, Kong F. *Streptomyces* sp. 173, an insecticidal microorganism from marine. Lett Appl Microbiol. 2004;38(1):32-37.
33. Deng Y, Hu J, Liu L, Wang N, Wang S. Screening for marine actinomycetes producing insecticidal metabolites. J Biotechnol. 2008;136:533.
34. El-khawaga M, Megahed M. Antibacterial and insecticidal activity of actinomycetes isolated from sandy soil of (Cairo-Egypt). Egypt Acad J Biol Sci. 2012;4(1):53-67.
35. Shobha K, Onkarappa R, Goutham S, Raghavendra H. Screening biological activities of a *Streptomyces* species isolated from soil of Agumbe, Karnataka, India. Int J Dug Dev Res. 2012;4(3):104-14.
36. Karthik L, Gaurav K, Rao KB, Rajakumar G, Rahuman AA. Larvicidal, repellent, and ovicidal activity of marine actinobacteria extracts against *Culex tritaeniorhynchus* and *Culex gelidus*. Parasitol Res. 2011;108(6):1447-55.
37. Horikoshi R, Goto K, Mitomi M, Oyama K, Sunazuka T, Omacr S. Identification of pyripyropene A as a promising insecticidal compound in a microbial metabolite screening. J Antibiot. 2017;70(1):272-6

38. Liao W, Ye L, Deng J, Gao JH. Virulence of fermentation products of actinomycetes against *Myzus persicae*. Anhui Agric Sci. 2004;32(6):1191-1202.
39. Zhou BG, Wang S, Dou TT, Liu S, Li MY, Hua RM, Li S, GLin HF. Aphicidal activity of *Illicium verum* fruit extracts and their effects on the Acetylcholinesterase and Glutathione S-transferases activities in *Myzus persicae* (Hemiptera: Aphididae). J Insect Sci. 2016;16(1):11.
40. Reddy SE, Dolma SK, Verma PK, Singh B. Insecticidal activities of *Parthenium hysterophorus* L. extract and parthenin against diamondback moth, *Plutella xylostella* (L.) and aphid, *Aphis craccivora* Koch. Toxin Rev. 2017;https://doi.org/10.1080/15569543.2017.1339281.
41. Chen Y, Li Y, Su Zxian J. Insecticidal and repellent action of pogostone against *Myzus persicae* (Hemiptera: Aphididae). Fla Entomol. 2017;100(2):346-9.
42. Ramesh S Mathivanan N. Screening of marine actinomycetes isolated from the Bay of Bengal, India for antimicrobial activity and industrial enzymes. World J Microbiol Biotechnol. 2009;25(12):2103-11.
43. Pimentel-Elardo SM, Kozytska S, Bugni TS, Ireland CM, Moll H, Hentschel U. Anti-parasitic compounds from *Streptomyces* sp. strains isolated from *Mediterranean sponges*. Mar Drugs. 2010;8(2):373-80.
44. Sacramento DR, Coelho RRR, Wigg MD, Linhares LFTL, Santos MGM, Semêdo LT, Silva AJR. Antimicrobial and antiviral activities of an actinomycete (*Streptomyces* sp.) isolated from a Brazilian tropical forest soil. World J Microbiol Biotechnol. 2004;20(3):225-9.
45. Subramani R, Aalbersberg W. Marine actinomycetes: an ongoing source of novel bioactive metabolites. Microbiol Res. 2012;167(10):571-80.
46. Tanaka Y Omura S. Agroactive compounds of microbial origin. Annu Rev Microbiol.. 1993;47(1):57-87.
47. Arasu MV, Al-Dhabi NA, Saritha V, Duraipandiyan V, Muthukumar CKim SJ. Antifeedant, larvicidal and growth inhibitory bioactivities of novel polyketide metabolite isolated from *Streptomyces* sp. AP-123 against *Helicoverpa armigera* and *Spodoptera litura*. BMC Microbiol. 2013;13(1):105.
48. Kaur T, Manhas RK. Antifungal, insecticidal, and plant growth promoting potential of Streptomyces hydrogenans DH16. J Basic Microbiol. 2014;54(11):1175-85.