African Journal of Biotechnology Vol. 12(14), pp. 1657-1664, 3 April, 2013
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB2013.11942
ISSN 1684–5315 ©2013 Academic Journals

Full Length Research Paper

Taxonomy of *Streptomyces* strains isolated from rhizospheres of various plant species grown in Taif region, KSA, having antagonistic activities against some microbial tissue culture contaminants

Mohamed S.H. [1,2], El-Helafiy Seham S.D.[1], Ismail Mona A.[1] and Sadik A.S.[1,3]

[1]Department of Biology, Faculty of Science, Taif University, P.O. Box 888, Taif, Kingdom of Saudi Arabia.
[2]Soil, Water and Environmental Research Institute, Agricultural Research Center, 9 Gamaa St., P.O. Box 12619, Giza, Egypt.
[3]Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, P.O. Box 68 Hadayek Shubra, 11241, Cairo, Egypt.

Accepted 1 March, 2013

This work was designed to identify and taxonomically classify *Streptomyces* strains isolated from the rhizospheres of various plant species; banana, rose, pomegranate and grape plants, having antagonistic activity against some microbial (bacteria and fungi) tissue culture contaminants. *Streptomyces* strains with the most potent antagonistic traits were identified using sequencing of 16S rRNA gene amplicons generated by PCR of DNA and blasting the sequences into GenBank. The *Streptomyces* isolates had antagonism against five identified fungi (*Aspergillus fumigates*, *Aspergillus flavus*, *Aspergillus nidulans* (1), *Aspergillus nidulans* (2), *Cladosporium herbarum*) and against five out of the 11 Gram positive bacilli bacterial contaminants. The three selected *Streptomyces* isolates (14, 15 and 17) were identified as strains of *Streptomyces noboritoensis*, *Streptomyces albolongus* and *Streptomyces griseorubiginosus,* respectively. Isolate 14 for which 1443 nucleotides were obtained was compared with eight universal *Streptomyces* strains and was classified as a new species of gray *Streptomyces*, to be named *Streptomyces noboritoensis* SSMA2-KSA strain. This study recommends conducting further studies on the use of streptomycetes in the biological control in a large scale production.

Key words: Taxonomy, *Streptomyces*, microbial tissue culture contaminants, antagonistic activities, 16S rRNA.

INTRODUCTION

Plant tissue culture technology has allowed for the propagation of large number of plants from small pieces of stock plants in a relatively short period of time (Daniel, 1998). Microbial contamination is a constant problem, which often compromise development of all *in vitro* techniques. About 31 microorganisms from ten different plant cultivars growing in micro-propagation have been isolated, identified and characterized, with yeasts, *Corynebacterium* sp. and *Pseudomonas* sp. being predominant (Leggatt et al., 1994). Odutayo et al. (2004) reported associating the following bacteria *Pseudomonas syringae* pv. *phaseolicoli*, *Bacillus licheniformis*, *Bacillus subtilis*, *Corynebacterium* sp. and *Erwinia* sp. with the contamina-

*Corresponding author. E-mail: dratefsadik@gmail.com. Fax: +966 (02) 724 2296.

tion of *Hibiscus cannabinus* and *Telfaria occidentalis* in Nigeria. 19 microbial contaminants (including 11 bacteria and eight fungi) were found associated with the tissue culture plants and the laboratory environments (Odutayo et al., 2007).

Preventing or avoiding microbial contamination of plant tissue cultures is critical to successful micropropagation. Epiphytic aIKI endophytic organisms can cause severe losses to micropropagated plants at each stage of growth (Cassells, 1991; Leifert et al., 1991). Bacterial contaminants are often difficult to detect because they remain mostly within the plant tissue (Debergh and Vanderschaeghe, 1988; De Fossard and De Fossard, 1988; Viss et al., 1991). Contaminated plants may have no visible symptoms, reduced multiplication and rooting rates or may die (Leifert et al., 1989, 1992). *Streptomyces* sp. is Gram-positive filamentous bacteria that produce and secrete a wide array of biologically active compounds including antibiotics, hydrolytic enzymes and enzyme inhibitors. These characteristics make streptomycetes attractive candidates for biological control agents against soil-borne plant pathogens (Samac and Kinkel, 2001; Hassan et al., 2011). Attempts have been made to develop *Streptomyces* species as fungal root disease control agents, since *Streptomyces* spp. are capable of producing a remarkably wide spectrum of antibiotics as secondary metabolites (Franklin et al., 1989). A rapid method for identifying filamentous soil-actinomycete genera was developed based on 16S rRNA gene (Moran et al., 1995; Cook and Meyers, 2003; Mohamed et al., 2012; Shori et al., 2012). Significantly, the genus *Streptomyces* could be differentiated from all other actinomycete genera.

This study aimed at isolating and identifying some streptomycetes from rhizosphere-soils of some plants used for propagation *via* tissue culture technique and evaluating their filtrates abilities to be used as biopesticides for *in vitro* control of the growth of plant-tissue culture contaminants (bacteria, fungi or yeasts).

MATERIALS AND METHODS

Soil samples collection

Rhizosphere soils of banana, rose, pomegranate and grape plants were collected from different private farms of Taif area. Samples were collected in clean plastic pages. At each rhizosphere, soil samples were randomly collected from five sites around the roots in clean plastic pags and thoroughly mixed together to form one representative sample.

Isolation, purification and maintenance of streptomycetes

Isolation (by plate technique) and purification (by streaking technique) of streptomycetes-like isolates (SLI) was carried out as described by Mohamed (1998) using starch nitrate agar medium (Waksman and Lechevalier, 1961).

Collection of some contaminated-tissue culture plant materials from some tissue culture laboratories

Blank (non-cultivated) as well as contaminated tissue-culture jars containing bacteria-like microorganisms or fungi or both were collected from Tissue Culture Laboratory, Department of Biology, Faculty of Science, Female Branch, Qarwah, Taif University, KSA (Figure 1). The jars were cultivated with different plant materials, that is, banana, rose, pomegranate and grape.

Isolation, purification and identification of the microbial contaminants

In the case of bacteria, isolation, purification and maintenance of bacterial tissue culture contaminants were carried out by plate technique (Mohamed, 1998) using nutrient agar medium (Jacobs and Gerstein, 1960). Inoculated plates were incubated at 37°C for 2 days. Fungal tissue culture-contaminants, were maintained on potato glucose agar medium (Waksman and Lechevalier, 1961), and sent to The Clinical Plant Center, Department of Plant Pathology, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, for identification for genus and/or species.

Antagonistic activities of the purified streptomycetes-like isolates

Antimicrobial activities of purified streptomycetes-like isolates were tested against the 5 identified fungi and the 11 bacterial isolates isolated as tissue culture-contaminants as described by Mohamed et al. (2012). Bacterial strains were cultivated on nutrient agar medium (Jacobs and Gerstein, 1960) and fungal strains on potato agar medium (Waksman and Lechevalir, 1961). Sterile discs of filter-paper Whatman of about 10 mm diameter were made and 3 to 4 discs were saturated with 0.1 ml of a supernatant. Petri-dishes were kept in a refrigerator for one hour to permit diffusion of the supernatant. Inoculated plates were incubated at 30±2°C for 24 to 48 h. The inhibition zones (mm) were determined as described by British Pharmacopoeia (1968). Discs treated with 0.1 ml of un-inoculated starch nitrate broth was used as a control.

Identification of the *Streptomycete* isolates showing high levels of antagonistic activities against tissue culture contaminants

The proposed key of Bergey's Manual of Determinative Bacteriology (1974) as detailed by Mohamed (1998) was used for identification of all purified streptomycetes-like isolates up to genus.

PCR isolation and sequencing of the 16S rRNA gene of a *Streptomyces* isolate

The DNA of the selected *Streptomyces* isolate was extracted as described by El-Domyati and Mohamed (2004) and used as a template for PCR-isolation of 16S rRNA gene using two universal primers (518F: 5' CCA GCA GCC GCG GTA ATA CG3'; 800R: 5' TAC CAG GGT ATC TAA TCC3') (Jacob, 2012). The PCR product of 16S rRNA was firmly packed and was delivered to Lab-Technology® (Macrogen agent in Egypt) on crushed ice. Sequencing was performed using the ABI PRISM BigDyeTM Terminator Cycle Sequencing Kits, ABI PRISM 3730XL Analyzer

Figure 1. Collection of tissue culture contaminants from blank jars (A) and cultivated jars (B). The presence of different fungi approximately covered the medium.

(96 capillary type) sequencer (Applied Biosystems), MJ Research PTC-225 Peltier Thermal Cycler, DNA polymerase (FS enzyme) (Applied Biosystems). Following the protocol provided by the manufacturer, single-pass sequencing was performed on each template using the same primer pairs described in the PCR step. The fluorescent-labeled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples were re-suspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer. The DNA sequences of the PCR product of the 16S rRNA gene of *Streptomyces* sp. (isolate 14) were aligned with the related nucleotide sequences of the universal isolates collected from http://www.ncbi.nlm.nih.gov/, http://www.ncbi.nlm.nih.gov/, using the DNA Star Software Package – Lasergene (Expert Sequence Analysis Software, USA) and MegaAlign program.

RESULTS AND DISCUSSION

Isolation, purification and maintenance of streptomycetes

The actinomycetes especially the strains of *Streptomyces* are the most common antibiotic-producing microorganisms found in soil (Ara et al., 2012). Taddei et al. (2006) reported that the genus *Streptomyces* was represented in nature by the largest number of species and varieties among the family Streptomycetaceae. In this study, 23 SLI were isolated from the rhizosphere-soil samples and 18 SLI were purified. A number of 5, 5, 5, 4 and 4 were derived from banana, rose, pomegranate and grape, respectively. At the level of colour series, isolates 9, 7 and 2 SLI belonged to gray, white and red series. The widespread distribution of actinomycetes in the rhizosphere and non-rhizosphere soils was confirmed *via* several reports of Mohamed (1998), Duangmal et al. (2005), El-Sherbiny (2006), Ismail (2006), Al-Askar et al. (2011), Atta et al. (2011), Mohamed et al. (2012) and Shori et al. (2012).

Isolation, purification and identification of the microbial contaminants from some contaminated-tissue cultural plant materials

George and Sherrington (1984) reported that although aseptic conditions are typically employed for *in vitro* growth “sterile” of plant cells, tissue or organs as they are separated from the mother plant on artificial/synthetic medium, many plant cultures do not stay aseptic *in vitro* as they get contaminated. Contamination with bacteria is generally considered the most serious (Horsch and King, 1983). There are much fewer publications describing yeast and fungal contaminants and their effects on plantlets grown *in vitro* (Enjalric et al., 1988). In this study however, fungal contaminants were the most common on jars to be used for tissue culture. Bacterial contaminants associated with the tissue-cultured plant material were also observed. 11 bacterial contaminants were isolated, purified and identified as Gram positive bacteria. In the case of fungal contaminants, 5 fungal isolates were cultivated on potato dextrose agar (PDA) medium and after appearing, its growth were sent to a specific private laboratory for further purification and identification using specific media. Results show that the fungal isolates were identified as *A. fumigatus*, *A. flavus*, *C. herbarum* and two different strains of *A. nidulans* (1 & 2), (Figures 1, 2 and 3).

Antibiosis activities of the purified streptomycetes-like isolates against the 16 identified bacterial and fungal contaminants

Antibiosis activities of the 18 SLI were determined against the 11 bacterial contaminants. Data show that 2 SLI (10 and 11) were not active against the 11 tested bacterial isolates, as no zone of inhibition were found around the discs saturated with filtrates of these SLI.

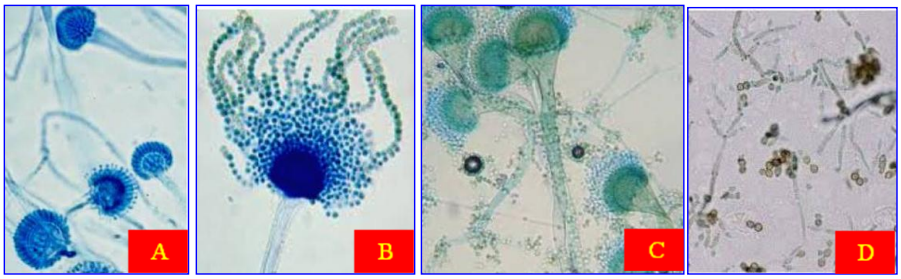

Figure 2. The **f**our identified tissue culture contaminant fungi: *Aspergillus fumigates* (A), *Aspergillus flavus* (B), *Aspergillus nidulans* (1) and *Cladosporium herbarum* (D).

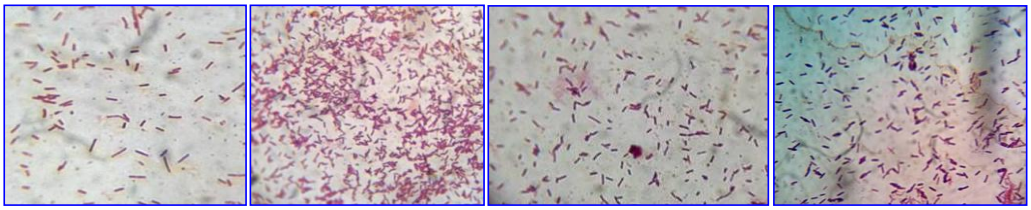

Figure 3. Four out of the eleven Gram positive tissue culture contaminant bacilli.

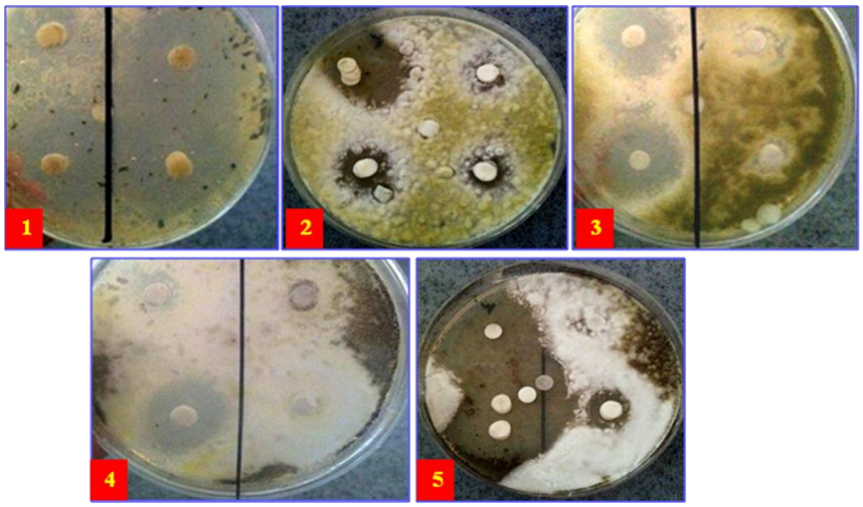

Figure 4. Antibiosis activity of some SLI against the five identified fungal isolates (1 to 5) identified fungal isolates (*A. fumigatus*, *A. flavus*, *A. nidulans* (1), *A. nidulans* (2), *C. herbarum*), 72 h post incubation.

While, 6 SLI were active against one bacterial isolate. A number of 8 SLI (2, 3, 4, 5, 7, 16, 19 and 21) showed antagonistic activities against two bacterial isolates. Two SLI (6 and 18) appeared with activities against 3 bacterial isolates. SLI 14, 15 and 17 were the highest isolates as they were active against 5 out of the bacterial isolates.

Regarding the antibiosis activities of the tested SLI against the five identified fungal isolates (*A. fumigatus*, *A. flavus*, *A. nidulans* (1), *A. nidulans* (2), *Cladosporium herbarum*), results show that thefive fungi were affected by 15, 4, 3, 7 and 10, respectively. Results also confirmed the high activities of the 3 selected SLI (14, 15 and 17), as they were able to inhibit the fungal growth of the 5 tested fungi. These results are in harmony with that of

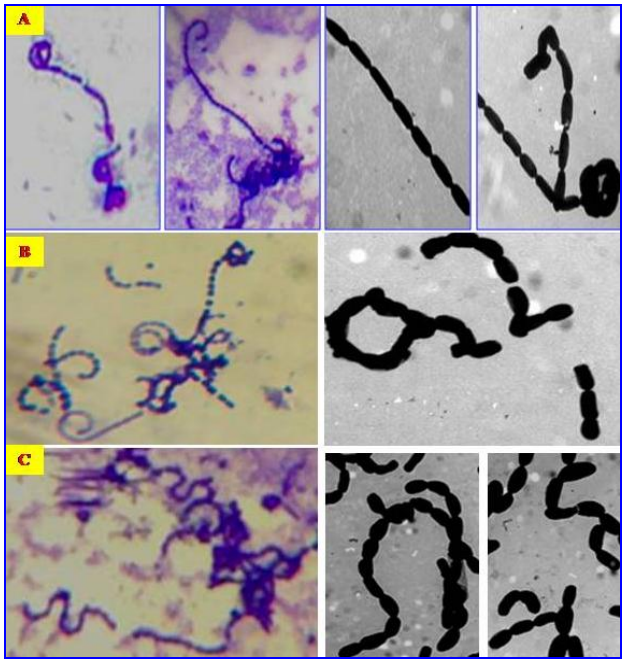

Figure 5. Microphotograph and electron micrograph of SLI No. 14 (A), 15 (B) and 17 (C) showing RF [RF: Rectus-Flexibilis (spores in straight (R) or flexuous (F) chains] and smooth spores surface.

Srividya et al. (2012), who identified a potent actinomycete isolate 9p with broad spectrum antifungal property OCA3; *Collectotrichum gleosporioides* OGC1, against four phytopathogens tested *Alternaria brassiceae Rhizoctonia solani* MTCC 4633 and *Phytophthora capsici* (Figure 4).

Identification of the *Streptomycete* isolates showing highly antagonistic activities

Streptomyces isolate 14

It was found that the *Streptomyces* isolate 14 belonged to the gray colour series, while the vegetative mycelium was pigmented with dark gray. This isolate had RF spore chain with smooth surface. Melanoid pigments were produced on tyrosine agar medium (Shinobu, 1958), peptone - yeast extract iron agar medium (Tresner and Danga, 1958) and tryptone-yeast extract broth medium (Pridham and Gottlieb, 1948). It also had very good growth on Cazpek's agar medium and actively utilized all added sugars as carbon sources for growth. This isolate showed antibacterial and antifungal activities. It was not sensitive to streptomycin antibiotic in the medium. This isolate was tolerant to NaCl up to concentration of 7%. According to the key proposed by Pridham and Tresner (1974), the experimental isolate 14 appeared to be related to *Streptomyces noboritoensis* although there was slight difference in use of L- Rhamnose and sucrose as sole sources of carbon. Therefore, isolate 14 could be considered a as strain of *S. noboritoensis* (Figure 5A).

Streptomyces isolate 15

Results clearly indicate that the *Streptomyces* isolate 15 belonged to the white colour series. Aerial spore chains belonged to section RF; the spores were characterized by smooth surface. Melanoid pigments were detected on the standard media used. This isolate was characterized by excellent growth on Cazpek's agar medium. The physiological characteristics showed that D-glucose, D-xylose, L-arabinose, L-rhamnose, D-mannitol, D-fructose, i-inositol and sucrose were used as carbon sources for growth.

In addition, this isolate showed antimicrobial activities and sensitivity to streptomycin (4 µg ml^{-1}) was observed. However, it was able to grow in the presence of 7% NaCl in the medium. Comparing the cultural, morphological and physiological characteristics of the *Streptomyces* spp. in Pridham and Tresner (1974) study with that of *Streptomyces* isolate 15, this isolate is very likely to be a strain of *Streptomyces albolongus* (Figure 5B).

Streptomyces isolate 17

Results of *Streptomyces* isolate 17 show that, this isolate has gray aerial mycelium (gray colour series), while the vegetative mycelium was pigmented with gray colour. It had straight and long spore chains (section RF) and the spores were characterized by smooth surface without any ornamentation. This isolate was also characterized by excellent growth on Cazpek's agar medium, actively utilized all added sugar, was tolerant to NaCl concentration up to 7%, sensitive to streptomycin (4 µg ml^{-1}) and antagonized some bacterial and fungal used. Considering the description keys proposed by Pridham and Tresner (1974), the tested isolate 17 was closely related *to Streptomyces griseorubiginosus* (Figure 5C).

PCR-isolation and sequencing the 16S rRNA gene of *S. noboritoensis* isolate 14

Identification of actinomycetes using microscopic techniques alone was not enough to ensure certainty. Biochemical methods would be the best method to identify actionmycetes to their species level. But this test consumes a lot of time and chemicals. With the advancement of technology in molecular study, primers had been developed by researchers to target specifically the 16S rRNA sequence of the actinomycetes. Identification of

GGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGTGAAGCCCAGCTTGCTGGGTGGA
TCAGTGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCCCTACTTCGGGATAAGCCTTGGAAAC
GGGGTCTAATACCGGATATGAACGTCTGCCGCATGGTGGGTGTTGGAAAGTTTTTCGGTGGGGGAT
GGGCTCGCGGCCTATCAGCTTGTTGGTGGGGTAATGGCCTACCAAGGCGTCGACGGGTAGCCGGCC
TGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGG
GAATATTGCACAATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTT
GTAAACCTCTTTCAGCAGGGAAGAAGCGTGAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTA
CGTGCCAGCAGCCGCGGTAATACGTAGGGGCCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTA
GGCGGTCTGTCGCGTCTGGTGTGAAATCCCATGGCTCAACTGTGGGCTTGCATCGGGTACGGGCAG
ACTGGAGTGCTGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGA
ACACCGATGGCGAAGGCAGGTCTCTGGGCAGTTACTGACGCTGAGGAGCGAAAAAGAGCGAACAGG
ATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGGCCAGTTCCACTGGTT
CTGTGCCGTAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAG
GAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
ACCAAGGCTTGACATGTACGAGAACGCCCCAGAGATGGGGTTCTCTTTGGACACTCGTACACAGGT
GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCT
TGTCTCATGTTGCCAGCGGGTTATGCCGGGGACTCATGGGAGACTGCCGGGGTCAACTCGGAGGAA
GGTGGGGATGACGTCAAATCATCATGCCCCTTATGTCTTGGGCTTCACGCATGCTACAATGGCCGG
TACAGAGGGCTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATCGGGG
TCTGCAACTCGACCCCGTGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATA
CGTTCCCGGGCCTTGTACACACCGCCCGTCAAGTCACGAAAGTTGGTAACACCCGAAGCTCACGGC
CCAACCCTTTGGGAGGGAGTGGTCGAAGGTGGGACTGGCGATTGGGACTAGTCGAAG

Figure 6. Final nucleotide sequence (1443 nts) of the PCR product of 16S rRNA gene amplified from the DNA of *S. noboritoensis*-isolate 14.

Table 1. Sequences producing significant alignments between *S. noboritoensis*-isolate 14 and the related overseas bacterial strains.

Accession	Description	Query coverage (%)	Max identity (%)
NR_041107.1	*Streptomyces noboritoensis*, strain NBRC 13065	99	89
AY999830.1	*S. noboritoensis*, strain KCTC 9060	97	88
AB184287.1	*S. noboritoensis*, strain NBRC 13065	99	89
NR_041144.1	*S. albolongus*, strain NBRC 13465	99	89
JN609385.1	*S. albolongus*, strain BC-32	97	89
AY999756.1	*S. albolongus*, strain JCM 4716	97	89
AB184425.1	*S. albolongus*, strain NBRC 13465	99	89
NR_042298.1	*S. griseorubiginosus*, strain :LMG 19941	99	88
AB706352.1	*S. griseorubiginosus*	99	89
AB184276.2	*S. griseorubiginosus*, strain: NBRC 13047	99	88

actinomycetes to genus level was made possible in a fast and accurate manner. This had been a great advancement in the area of identification as the ability to obtain the genus of the actinomycetes in just a few hours is now possible. In this study, actinomycetes from the farming soil of Agriculture Research Centre Semongok, Sarawak were isolated. The isolates were later tested for their bioactive compounds and selected isolates were later identified using primers targeting their 16S rRNA sequence (Jeffrey, 2008).

In this study, the nucleotide sequences of 16S rRNA gene were partially determined using the DNA template of the *Streptomyces* sp. (isolate 14). Results show the two sequences of both forward direction matched to each other. l A sequence of about 1443 nucleotides (Figure 6) was obtained and compared with four universal bacterial isolates as mentioned in Table 1 and results appendix (B). These bacteria are *S. noboritoensis*, strain NBRC 13065 (NR_041107.1); *S. noboritoensis*, strain KCTC 9060 (AY999830.1); *S. noboritoensis*, strain NBRC 13065 (AB184287.1); *S. albolongus*, strain NBRC 13465 (NR_041144.1); *S. albolongus*, strain BC-32 (JN609385.1); *S. albolongus*, strain JCM 4716 (AY999756.1); *S. albolongus*, strain NBRC 13465 (AB184425.1); *S. griseorubiginosus*, strain :LMG 19941 (NR_042298.1); *S. griseorubiginosus* (AB706352.1) and *S. griseorubiginosus*, strain: NBRC 13047 (AB184276.2).

Results in Table 1 show that the percent identities between the isolate of this study and that compared with the eight gray *Streptomyces* strains, collected from the GenBank, ranged between 88 and 89%. It is worth to mention that the *Streptomyces* strain of this study covered

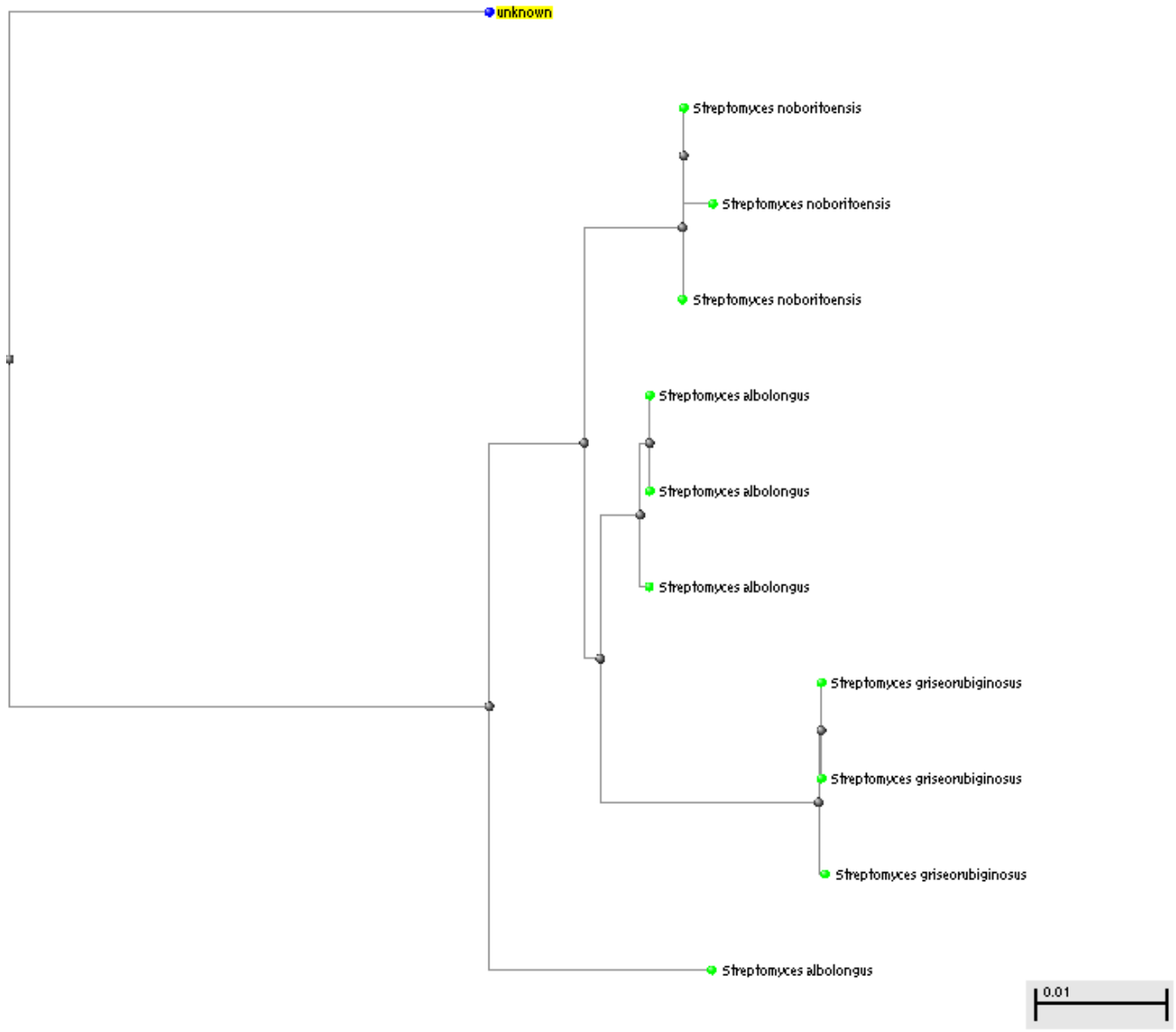

Figure 7. Phylogenetic tree of nucleotide sequence of the PCR product of 16S rRNA gene amplified from the DNA of *S. noboritoensis*-isolate 14 and the related universal bacteria strains.

from 97 to 99% of the sequences of the 8 overseas *Streptomyces* strains.

The phylogenetic tree (Figure 7) showed that the *S. noboritoensis* isolate 14 segregated into a separate cluster. Therefore, it could be classified as a new species of gray *Streptomyces*, and it was suggested to be named *S. noboritoensis* SSMA-KSA strain. These results are in agreement with that of other investigators, using the 16S rRNA gene to characterize Actinomycetes (Moran et al., 1995; Cook and Meyers, 2003; Song et al., 2004; Al-Askar et al., 2011; Jose et al., 2011; Mahasneh et al., 2011; Mohamed et al., 2012; Shori et al., 2012).

REFERENCES

Al-Askar AA, Abdul Khair WM, Rashad YM (2011). *In vitro* antifungal activity of *Streptomyces spororaveus* RDS28 against some phytopathogenic fungi. Afr. J. Agric. Res. 6(12): 2835-2842.

Ara I, Rizwana H, Al-Othman MR, Bakir MA (2012). Studies of actinomycetes for biological control of *Colletotrichum musae* pathogen during post harvest anthracnose of banana. Afr. J. Microbiol. Res. 6(17): 3879-3886.

Atta HM, Bahobail AS, El-Sehrawi MH (2011). Studies on isolation, classification and phylogenetic characterization of antifungal substance produced by *Streptomyces albidoflavus*-143. New York. Sci. J. 4(3):40-53.

Bergey's Manual of Determinative Bacteriology (1974). 8th ed. Buchanan RE. and Gibbons N.E. Williams and Wilkins Company,

Baltimore, USA.758, 764, 768 & 793.
Buchanan R.E. and Gibbons N.E. Williams and Wilkins Company, Baltimore, USA.758, 764, 768 & 793.
British Pharmacopoeia (1968). Biological assay of antibiotics. The Pharmaceutical Press, London, pp.1313-1317.
Cassells AC (1991). Problems in tissue culture: culture contamination. In: Micropropagation: Technology and Application. Kluwer Academic Publications, The Netherlands. Ed. By Deberh, R. Zimmerman. p. 31-44.
Cook AE, Meyers PR (2003). Rapid identification of filamentous actinomycetes to the genus level using genus-specific 16S rRNA gene restriction fragment patterns. International J. Syst. and Evol. Microbiol. 53: 1907-1915.
Daniel RL (1998). The many dimension of plant tissue culture research. Webmaster of Aggie Horticulture Publications, pp. 201-210.
De Fossard RA, De Fossard H (1988). Coping with microbial contaminants and other matters in a small commercial micropropagation laboratory. Acta Hort. 227: 346-351.
Debergh PC, Vanderschaeghe AM (1988). Some symptoms indicating the presence of bacterial contaminants in plant tissue culture. Acta Hort. 255: 77-81.
Duangmal K, Ward AC, Goodfellow M (2005). Selective isolation of members of the *Streptomyces violaceoruber* clade from soil. FEMS Microbiol. Lett. 245(2): 321-327.
El-Domyati FM, Mohamed SH (2004). Molecular genetic characterization of some Streptomyces isolates exhibiting different levels of resistance to the herbicide BASTA. Egypt. J. Gen. and Cytol. 33: 249-286.
El-Sherbiny GME (2006). Isolation, purification and characterization of an actinomycete isolate having the ability to produce an antimicrobial metabolite. Egypt. J. Microbiol. 41(1): 35-47.
Enjalric F, Carron MP, Lardet L (1988). Contamination of primary culture in tropical areas. The case of Hevea brasiensis. Acta Hort. 225: 25-65.
Franklin TJ, Snow GA, Barrett-Bee KJ, Nolan RD (1989). Antifungal, antiprotozoal and antiviral agents, p.137–161. In Biochemistry of rhizosphere with bacteria antimicrobial action, 4th ed. Chapman & Hall Ltd., New York.
George EF, Sherrington PD (1984). Plant Propagation by tissue culture, Eversley, Bassingstoke; Exegetices Ltd. pp. 88-93.
Hassan AA, El-Barawy AM and El Mokhtar NM (2011). Evaluation of biological compounds of *Streptomyces* species for control of some fungal diseases. J. Amer. Sci. 7(4): 752-760.
Horsch RB, King JM (1983). A convert contamination of cultured plant cells, elimination of a *Hyphomicrobium* spp. from cultures of *Datura innoxia* (Mill). Plant cell Tiss. Org. Cult. 2: 21-28.
Ismail A (2006). Numerical assessment of mycelium color in classification of some *Streptomyces* isolates. Int. J. Agric. and Biol. 8(6):872-875.
Jacob HJ (2012). Classification of halophilic heterotrophic bacteria thriving in the Jordanian dead sea littoral zone. J. Biol. Sci. 12: 246-252.
Jacobs MB and Gerstein MJ (1960). Hand book of Microbiology. D. Van Nostrand Co., Inc., New York, pp. 139-207.
Jeffrey LSH (2008). Isolation, characterization and identification of actinomycetes from agriculture soils at Semongok, Sarawak. Afr. J. Biotechnol. 7(20): 3697-3702.
Jose AP, Santhi SV, Jebakumar RD (2011). Phylogenetic-affiliation, antimicrobial potential and PKS gene sequence analysis of moderately halophilic *Streptomyces* sp. inhabiting an Indian saltpan. J. Bas. Microbiol. 51(4): 348-356.
Leggatt IV, Waites WM, Leifert C, Nicholas J (1994). Characterisation of microorganisms isolated from plants during micropropagation. In: Bacterial and Bacteria-like Contaminants of Plant Tissue Cultures. Ishs Acta Horticulturae, 225: *http://www.actahort.org/books/225/index.htm.*
Leifert C, Waites WM, Nicholas JR (1989). Bacterial contaminants of micropropagated plantcultures. J. Appl. Bacteriol. 67: 353 -361.
Leifert C, Ritchie JY, Waites W (1991). Contaminations of plant tissue and cell cultures. World J. Microbiol. Biotechol. 7: 452-469.
Leifert C, Camotta H, Waites WM (1992). Effect of combinations of antibiotics on micropropagated *Clematis, Delphinium, Hosta, Iris* and *Photinia*. Plant Cell Tiss. Org. Cult. 29: 153-160.
Mahasneh IA, Al-Thani RF, Abuannadi MA, Qandil MA (2011). A complete sequence of the 16S rRNA gene of a novel *Streptomyces coelicolor* (AB588124) (QU66c-2002) isolated from the soil of Qatar. Biotechnol. 10: 167-174.
Mohamed SH (1998). Role of actinomycetes in the biodegradation of some pesticides. Ph.D. Thesis, Agric. Microbiol., Dept. Agric. Microbiol., Faculty of Agric., Ain Shams University, Cairo, Egypt 151 pp.
Mohamed SH, Omran WM, Abdel-Salam SM, Al-Shehri AS, Sadik AS (2012). Isolation and identification of some halotolerant actinomycetes having antagonistic activities against some plant pathogens (*i.e*., *Tobacco mosaic virus*, *Aspergillus* sp., *Fusarium* sp.) from soil of Taif Governorate, KSA. Pakist. J . Biotechnol. 9(1): 1-12.
Moran M, Rutherford L. Hodson R (1995). Evidence for indigenous *Streptomyces* populations in a marine environment determined with a 16S rRNA probe. Appl. Environ. Microbiol. 61(10): 3695-3700.
Odutayo OI, Oso RT, Akinyemi BO, Amusa NA (2004). Microbial contaminants of cultured *Hibiscus cannabinus* and *Telfaria occidentalis* tissues. Afr. J. Biotechnol. 3: 301-307.
Odutayo OI, Amusa NA, Okutade OO, Ogunsanwo YR (2007). Determination of the sources of microbial contaminants of cultured plant tissues. Plant Pathol. J. 6: 77-81.
Pridham TG, Gottlieb D (1948). The utilization of carbon compounds by some Actinomycetales as an aid for species determination. J. Bacteriol. 56: 107-114.
Pridham TG, Tresner HD (1974). Family Streptomycetaceae. In; Bergey's Manual of Determinative Bacteriology (1974), 758, 764, 768 & 793, (Buchanan R.E. and N.E. Gibbons, 8th Eds.), Williams and Wilkins Co., Baltmore, USA.
Samac DA, Kinkel LL (2001). Suppression of the root lesion nematodes (*Pratylenchus penetrans*) in alfalfa (*Medicago ativa*) by *Streptomyces* sp. Plant and Soil 235: 35-44.
Shinobu R (1958). Physiological and cultural study for the identification of soil actinomycetes species. Mem. Osaka Univ. B. Nat. Sci. 7: 1-76 (Modified).
Shori GBO, Mohamed SH, Abdel-Salam SM, Sadik AS (2012). Characterization of streptomycetes having antibiosis activities isolated from soil in western region of KSA. Pak. J. Biotechnol. 9(1): 21-38.
Song J, Chan LS, Kang JW, Beak HJ and Suh JW (2004). Phylogenetic analysis of *Streptomyces spp*. isolated from potato scab lesions in Korea on the basis of 16S rRNA gene and 16S-23S rDNA internally transcribed spacer sequence. IJSEM 54(1): 203-209.
Srividya S, Thapa A, Bhat DV, Golmei K and Dey N (2012). *Streptomyces* sp. 9p as effective biocontrol against chilli soilborne fungal phytopathogens. Europ. J. Exper. Biol. 2(1): 163-173.
Taddei A, Rodriguez MJ, Vilchez EM and Castelli C (2006). Isolation and identification of *Streptomyces* spp. from Venezuelan soils: Morphological and biochemical studies. 1. Microbiol. Res. 161(3): 222-231.
Tresner HD and Danga F (1958). Hydrogen sulfide production by *Streptomyces* as a criterion for species differentiation. J. Bacteriol. 76: 239-244.
Viss PR, Brooks EM, Driver JA (1991). A simplified method for the control of bacterial contamination in woody plant tissue culture. *In Vitro* Cell Dev. Biol. 27: 42.
Waksman SA and Lechevalier HA (1961). The actinomycetes Vol. II. Classification, identification and descriptions of genera and species. The Williams and Wilkins, Co. Baltimore, USA.