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Identification and Characterization of Actinomycetes for Biological Control of Bacterial Scab of *Streptomyces scabies* Isolated from Potato

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Abstract
A novel strain of *Streptomyces* in Egypt (known as*Streptomyces scabies*) was isolated from a scabby potato field in different regions of Egypt. The taxonomy of the organism was determined by morphology, biochemistry, and physiological characteristics. Two bacterial strains (actinomycetes) which were isolated from soil sample and showed antagonistic activity towards potato scab were identified using specific polymerase chain reaction (PCR) of 16S rDNA gene. The 16SrDNA sequence analysis showed that the 1st strain belongs to the genus Streptomyces, with closest similarity to *Streptomyces avermitilis* MA-4680 (100% similarity). Sequence similarities between the 1st strain and other Streptomyces species in the same subclade ranged from 98% (with *Streptomyces griseus* NBRC 13350 and *Streptomyces sp.* Wigar10). Keyphenotypic characteristics as well as chemotaxonomic features of the actinomyces were congruent with thedescription of the genus Streptomyces. On the basis of phenotypic and phylogenetic analyses. The 2nd strain identified as *Actinomyces odontolyticus* ATCC 17982(100% similarity). Sequence similarities between the 2nd strain and other Actenomycetes species was 98% (with *Actinomyces odontolyticus*C 505).
The two identified strains showed a high level of antibiosis against pathogenic organism (*Streptomyces scabies*) and achieve a significant control of disease as no sign of disease symptoms are shown on the tested varieties of potato (Cara and Diamond)
Keywords: Actinomycetes, biological control, *Streptomyces scabies.*

1. Introduction

Potato (*Solanum tuberosum* L.) is one of the most important vegetable crops in Egypt, for both local consumption and exportation. It is widely cultivated and could contribute to reducing worldwide food shortages (Han *et al*., 2005). One of the most important potato disease is common scabe caused by *Streptomyces scabies*. It is indigenous in all potato growing areas in the world (Loria *et al*., 2006; Wanner, 2004). Severalspecies of *Streptomyces* can cause PCS but *Streptomyces scabies* is considered to be predominant (Lambert and Loria, 1989). And can infects number of root crops, including radish, parsnip beet and carrot.). The disease has little impact on total potato yield but spoils the appearance, quality, and marketability of the tubers (Johnson and Powelson, 2008). Several methods have been used to control potato scab, including: Planting resistant varieties and agriculture practices such as excess irrigation during tuber formation (Lapwood and Adams , 1975). However all these methods are less effective and durable with seldom preventing disease from occurring but generally reducing its extent of severity 3- Chemical control ,such as (3,5-D,telon) and (polyram and mancozeb).However these chemical increase phytotexicity to freshly cut tuber and decrease in the tuber size and yield. Therefore, we need more research into nonchemical methods of potato production seems to be justified than other. Biological control is suitable alternative of chemical control.
Microorganisms are virtually unlimited sources of novel compounds with many medicinal and agricultural applications. Actinomycetes, among them, hold a prominent position due to their ability to produce numerous different metabolites such as antibiotics, enzymes and inhibitors (Xu *et al*., 2005). Further the discovery of novel antibiotic and nonantibiotic lead compounds through microbial secondary metabolite screening is becoming increasingly important. In recent years, there has been an increasing interest in discovering new agricultural antibiotics for the protection of our living environments. The genus Streptomyces is the largest producer of bioactive compounds (Chun *et al*., 1997; Labeda *et al*., 1997).
Actinomycetes are a gram-positive, aerobic, high GC-content and 0.5–1.0 μm in size. They are filamentous, sporulating colonies and recognized as a transition group between primitive bacteria and fungi (Lo *et al*., 2002). Among the actinomycetes groups, *Streptomyces* are the most popular and found worldwide in soil, and important in soil ecology. They belong to the order Actinomycetales. *Streptomyces* are metabolically diverse and can utilize almost anything as carbon source due to its ability to produce extracellular hydrolytic enzymes, including

sugars, alcohols, amino acids, organic acids, aromatic compounds and other complex substrate such as cellulose, mannan and xylan. They are also well known for their abilities to produce antibiotics and other secondary metabolites (Willey *et al*., 2008). Thus, these microorganisms have been implicated in the antagonism of a wide variety of plant pathogenic bacteria, fungi and nematodes for their potential use as biological disease control agents (Sahilah *et al.,* 2010).

In the context of the information provided above, the objectives of this study have been , to evaluate the potential of these two antibiotic-producing suppressive strains of Actinomycetes to control potato scab disease.

2 . Materials and methods

2.1 Isolation of *Streptomyces scabies*(pathogen)

Samples of soils and tubers of potato cultivars with scab symptoms were collected from fields of different locations of five governorates(Kafr El-Sheikh, El-Gharbiya, El-Minufya, El-Dakahlia and El-Nobaria) in Egypt. Isolation of actinomycetes from the scab lesions was carried out essentially as described byLawrence (1956). Cultural, morphological microscopical and pathological properties were considered to identify the isolated pathogens according to Burgess *et al*. (1994).

2.2 Morphological and physiological characterization *of Streptomyces scabies* (pathogen)

The morphology of the sporophores was examined microscopically, and the color of spores and colonies were observed on oatmeal agar(OMA) after 14 days of incubation at 28°C (Holt, 1994).Production of soluble pigment were observed after 4 days of incubation at 28°C. The ability to utilize the International Streptomyces Project (ISP) sugars was tested on the standard basal medium (Shirling and Gottlieb, 1966).

2.3 Pathogenicity test

Pathogenicity tests were performed on potato cultivars using the methods described by Labruyere (1971).34 Strains were isolated and tested for pathogenicity. Inoculums were prepared by growing test strains for two weeks at 30^{o}C in 50ml tubes containing sterilized veriniculite saturated with a modified Say – solution composed of 20gm of Sucrose, 1.2gmof L-asparagine,0.6gm of K_2HPO_4 and 10 gm of yeast extract per liter of water. The healthy cultivars Cara and Diamond potato were planted in 35cm diameter pots containing sterile clay soil mixed with 20ml of inoculum. Plants were arranged in a growth chamber as a randomized complete block with three replicates. Uninculated controls were included in the tests. Potatoes were harvested after three months and tubers were examined for common scab symptoms.

2.4 Screening and isolation of antagonists

Antagonists were isolated from soil rhizosphere samples of healthy potato plants producing areas at some governorates, Egypt. The used bioagents were isolated on selected media according to the methods recommended by Anonymous (1984), Burgess *et al*. (1994) and Turner *et al*. (1998).

2.5 Biological control of the tested microorganisms on potato scab pathogen

2.5.1 In vitro

The antagonistic activity was estimated by disc diffusion method (Barakate *et al*., 2002). A disc of 5mm in diameter from every microorganisms (from 48 hours old culture) was placed on the surface of OMA plates seeded with potato scab pathogen. The plates were incubated at 28 ºC for 24 h. The inhibition zone around the discs indicated the antagonistic interaction.

2.5.2 In vivo

Pots of 30 cm in diameter were filled with nonsterilized clay-loam soil at rate of 8 Kg per pot. Soil infestation with the pathogen *Streptomyces scabies* (control) and antagonistic microorganisms namely (*Actinomycetes odontolyticus*, *Streptomyces avermitilis*, *Streptomyces griseus*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Trichoderma hamatum* and *Trichoderma koningii*) according to(Michel and Mew,1998).One tuber of either potato cultivars(Cara and Diamond) was planted per pot(a replicate).The same eight treatment were repeated with the other cultivar of potato. Each treatment was represented by six replicates. At harvest
the average weight of tuber and percentage of disease tuber were recorded.

2.6 Statistical analysis.

The obtained data were statistically analysed according to the method of Gomez and Gomez, (1984).

2.7 Molecular characterization of *Streptomyces avermitilis* and *Actinomycetes odontolyticus*

2.7.1 Genomic DNA extraction from actinomycetes and *Streptomyces*. DNA extraction was carried out following the CTAB method according to (Azadeh and Meon, 2009). Ten colonies of bacteria was inoculated into 10 mL nutrient broth and incubated at 28±2°C overnight. One mL of the overnight culture was transferred into a 1.5 mL eppendorf tube and centrifuge for 30 sec at 13,000 rpm The bacteria cells were collected by discarding the supernatant and resuspended in 567 µL TE buffer (10 mM Tris-HCL, pH 7.4, 1 mM EDTA and 1 L distilled water), mixing well by vortexing. Then 30 µL of 10 % SDS, 3 µL of NAOAC (sodium acetate) pH 5.2, 100 µL of 5 M NaCl and 80 µL CTAB-NaCl were added to a total volume of 780 µL and mixed well before incubating for 10 min in water bath at 65°C. An equal volume (780 µL) of chloroform/isoamyl alcohol (24:1) was added to the mixture and centrifuged at 13000 rpm for 5 min to separate the phases. The clear supernatant was transferred into a new eppendorf tube and the aqueous DNA layer was again extracted using phenol/chloroform/isoamyl alcohol (25:24:1). This step was repeated 3 times and the supernatant pooled. The clear supernatant was transferred into new eppendorf tube and 400 µL of isopropanol was added to precipitate the nucleic acid. Finally the DNA was washed with 200 µL of 75% of cooled ethanol and dried at room temperature (28±2°C) before dissolving in 100 µL Sterile Distilled Water and kept at -20°C for further analysis. Electrophoresis was run for identifying the nucleic acids after DNA extraction in 1% of agarose gel and 1% of TBE (Tris base, boric acid, 0.5 M EDTA solution, 1L ddH2O, pH 8.0). The products were mixed with loading dye buffer (MBI Fermentas) in 5:1 ratio and subjected to electrophoresis at 70 volts for 1 h and 45 min. DNA ladder 100 bp (MBI Fermentas) was used as marker. The gel was stained in ethidium bromide solution and the bands visualized and photographed using Sony digital camira.

2.7.2 Primer design and PCR amplification. Oligonucleotide primer for 16S rDNA gene was 16S-1f (5'-GCTAGTTGGTGGGGTAA-3', 17 mer) and 16S-2r (5'- GCCATCTCAGTTCGGATTG-3'; 18 mer) were designed on the basis of the sequence of *E. coli* 16 S gene(corresponding to positions 247 to 263 and 1291 to 1309; *E coli* numbering system) (Wilems and Collins, 1993). Oligonucleotide primer for Glyophosate tolerant gene was AroA f (5'- GCTCTAGAAGTGTTGGAACAATATG-3'; 27 mer) and AroA r (5'-TTACTCGAGTGAGAATTAAATTGATGG-3'; 33 mer) (Sun *et al*., 2005). Amplification reaction for bacteria was performed in 25 µL of total volume containing 2 µL of DNA as a template, 2.5 µL of 10 PCR buffer (Fermentas), 1.5 µL of 25 mM MgCl2 (Fermentas), 0.2 µL of 10 mMdNTPs, 0.1 µL primer oligonucleotides, 0.1 µL Taq polymerase (Fermentas) and 18.5 µL of sterilized distilled water. The amplification was performed in a Thermal Cycler (Biometra®,T3thermocycler) (Syngene, UK) programmed for pre-denaturing of 3 min at 94°C, 30 cycles of 1min at 94°C, 1min at 58°C and 2 min at 72°C. After a final extension of 7 min at 72°C, the samples were cooled to 4°C.

2.7.3 Nucleotide sequence analysis: The PCR products was purified using a commercial kit (QIA Quick PCR purification kit (Qiagen, Valencia, CA), according to manufacturer's instruction. After purification, the PCR products were sent for sequencing services at Sigma Co. Germany. The 16 S gene sequences were aligned using BioEdit software versions 7.0.8 (http://www.mbio.nscu.edu/bioEdit/bioEdit) and searched for sequence similarity to other sequences which are available in the NCBI database at http://www.ncbi.nih.gov using Basic Local Alignment Search Tool (BLAST) algorithm. Multiple sequence alignments were performed on the selected closely related sequence accessions available using bioedit software (http://bioedit.edu/).

2.7.4 Phylogenetic analysis: Phylogenetic analysis was done based on the nucleotides sequences of 16 S gene using draw tree software provided by the Biology Workbench Program (http://workbench.sdsc.edu/).

Number of Data base JYZVMFRR015, K12FNS3P01S.

3. Results and Discussion

3.1 Isolation of *Streptomyces scabies* (pathogen)

Streptomyces scabies Waksman & Henrici occurs worldwide and causes common scab on potato tubers (Hooker, 1981). The type strain of *S. scabies* (ATCC 49173) (Lambert and Loria, 1989) is characterized by gray spores born in spiral chains (sporophores), melanin pigment production on tyrosin-containing medium (peptone iron agar), and utilization of all the diagnostic sugars recommended by the International Streptomyces Project (ISP) (Shirling and Gottlieb, 1966) for identification of *Streptomyces* spp. *S. scabies* does not grow at pH 4.5 (Lambert and Loria, 1989). Subsequently, common scab is less severe in acidic soils and does not usually occur in soils of pH < 5.2. Severity of common scab can also be reduced by maintaining high soil moisture with irrigation and by growing resistant cultivars (Lindholm *et al*., 1997).

3.2 Description of streptomycetes isolated from scabby potato plants. *Streptomyces spp.* were isolated from scabby potato plants collected from fields of different locations from many governorates, Egypt (Table 1). The original scabby tubers showed a range of symptoms from superficial small discrete lesions covering parts of a tuber to large, deep, coalescing raised or pitted lesions covering most or all of a tuber. They also varied in their pathogen city on radish and on potato cultivars.

Table 1: Regions of pathogenic isolates and pathogenicity test.

Region	Isolate source	Scab type	No. of isolates	Pathogenicity	
				Potato tubers	Radish seedling
Kafr El-Sheikh	Tuber	Common	6	+	+
Kafr El-Sheikh	Soil	-	3	+	+
El-Gharbiya	Tuber	Common	8	+	+
El-Gharbiya	Soil	-	4	+	-
El-Minufya	Tuber	Superficial	3	+	-
El-Minufya	Soil	-	1	+	+
El-Dakahlia	Tuber	Common	7	+	+
El-Dakahlia	Soil	-	0	+	+
El-Nobaria	Tuber	Superficial	2	+	+
El-Nobaria	Soil	-	0	+	-

+ Positive reaction
- Negative reaction

3.3 Morphological and physiological characterization *of Streptomyces scabies* (pathogen).
Morphological observation of the 7–15 days old culture of strain isolated from potato scab is revealed that both aerial and vegetative hyphae were abundant, well developed. Long spore chains were developed on the aerial mycelium. The aerial mycelium was observed after the 15th day of incubation in all test media. Cultural characteristics of the strain are shown in Table 2. Aerial mycelium of this strain was abundant, well-developed and varied from brown to white on different tested media. The substrate hyphae varied from pale-yellow to brown or white. Yellow diffusible pigments were produced on Yeast extract–malt extract agar media, and melanin was produced on Peptone- Yeast extract- iron agar.

Table 2: Cultural characteristics of strain *Streptomyces scabies*.

Medium	Color of Aerial mycelium	Color of Substrate mycelium	Production of soluble pigment
Yeast extract–malt extract agar (ISP medium 2)	Brown	Yellow	Yellow
Oatmeal agar (ISP medium 3)	White	White	None
Peptone- Yeast extract- iron agar (ISP medium 6)	Brown	Brown	Melanin
Tyrosine agar (ISP medium 7)	Yellowish White	White	None
Nutrient agar	White	White	None
Czapek's agar	White	White	None

ISP, International Streptomyces Project Shirling and Gottieb (1966).

The physiological and biochemical characteristics of the same strain isolated from potato scab are indicated in Table 3.

Table3: Morphological and Physiological characterization of *Streptomyces scabies*.

Characteristic	Results
Shape of cell	Spiral hyphae
Size of cell	Long
Arial mycelium colour	Grey to brown
Colour of spores	Grey
Gram's staining	+ve
Pigment production agar	brown
Optimum temp.	28-30^{o}
Utilization of sugar:	
Mannitol	++
Fructose	+
Sucrose	++
Arabinose	+
Glucose	++
Fructose	+
Raffinose	+
Rhamnose	+
Starch hydrolysis	-
Gelatin liquefication	+
Indol formation	-
Catalase activity	+
V.P. Test	+
Methyl Red (MR)	-
Production of H_2S	-
Nitrogen reaction	-
Litmus milk	+

+ positive Utilization
+ + strong positive Utilization

These results were agreed with the results shown by (Lindholm *et al*., 1997) which showed that *S. scabie* (type strain ATCC 49173) is characterized by spiral sporophores and gray spores, production of melanin pigment on peptone-iron agar, utilization of all ISP sugars, and pathogenicity on potato. Also color of colonies and production of diffusiblepigments are criteria for species identification.

3.4 Pathogenicity test

Results obtained clear that potato common scabe symptoms varied according to *Streptomyces scabies*.

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3.5 Screening and isolation of antagonists

Preliminary study of 3 *actinomycetes* (*Actinomycetes odontolyticus* , *Streptomyces avermitilis* and *Streptomyces griseus*) , 2 bacteria (*Bacillus subtilis* and *Bacillus thuringiensis*) and 2 fungi (*Trichoderma hamatum* and *Trichoderma koningii*) are isolated.

3.6 Biological control of the tested microorganisms on potato scab pathogen.

3.6.1 In vitro

The isolated organisms 3streptomycesand 2 bacteria and 2 fungi were screened against the plant pathogen *Streptomyces scabies* showed antagonism activity towards it as shown in Table (4).

Table 4: Antagonistic efficiency between isolated microorganisms on potato scab.

Antagonism	Inhibition zone in mm
Actinomycetes odontolyticus	48.9
Streptomyces avermitilis	51.6
Streptomyces griseus	32.0
Bacillus subtilis	24.9
Bacillus thuringiensis	24.3
Trichoderma hamatum	19.6
Trichoderma koningii	22.9

3.6.2 In vivo

At harvest the average weight of tuber and percentage of disease tuber
was determined. The two strains that showed most clearing zone towards *S. scabies* (*Streptomyces avermitilis and Actinomycetes odontolyticus)*were showed a high average weight of tuber and also percentage of disease on tubers (a high biological control of disease) as shown in Table (5).

Table 5: the average weight of tuber and percentage of disease tuber.

Organisms	Average weight of tuber		% of disease tuber	
	Diamond	Cara	Diamond	Cara
Streptomyces scabies	76.67AB	53.57B	96.89A	94.14A
Actinomycetes odontolyticus	79.32 A	58.38 A	0.00 F	0.00 E
Streptomyces avermitilis	78.08 A	53.45 B	0.00 F	0.00 E
Bacillus subtilis	66.79 C	50.35 C	2.147 E	0.00 E
Bacillus thuringiensis	64.28 CD	48.67 D	9.443 D	5.803 D
Streptomyces griseus	74.01 B	53.57 B	31.51 B	22.13 B
Trichoderma koningii	62.04 D	40.15 E	16.27 C	15.16 C
Trichoderma hamatum	70.00 A	50.01 B	50.12 A	23.21 B
L.S.D	3.604 at α 0.05		0.689 at α 0.05	

It is worthy to note that the strains *Streptomyces avermitilis* and *Actinomycetes odontolyticus* showed high protective of antibiosis against
pathogen are subjected to further identification (morphological cultural and biochemical characteristic) (Tables 6 and7).

Table 6: Cultural characteristics of the two antagonistic strains to *Streptomyces scabies*

Medium	Color of Aerial mycelium		Color of Substrate mycelium		Production of soluble pigment	
	Strain 1	Strain 2	Strain 1	Strain 2	Strain 1	Strain 2
Yeast extract–malt extract agar (ISP medium 2)	Brown	Orange-brown	Yellow	Brown	Yellow	Yellow
Oatmeal agar (ISP medium 3)	White to yellow	White to yellow	White	White	None	None
Peptone- Yeast extract- iron agar (ISP medium 6)	Brown	Brown	Brown	Brown	Melanin	Melanin
Tyrosine agar (ISP medium 7)	Yellowish White	Yellow	White	White	None	None
Nutrient agar	White	Brown	White	Yellow	None	None
Czapek's agar	White to yellow	Brown	White	Yellow	None	None

ISP, International Streptomyces Project Shirling and Gottieb (1966).
Strain 1: *Streptomyces avermitilis* strain 2: *Actinomycetesodontolyticus*

Morphological observation of the 7–15 days old culture of strains isolated and showed antagonism against potato scab is revealed that both aerial and vegetative hyphae were well developed. Long spore chains were born on the aerial mycelium. The aerial mycelium was observed after the 15th day of incubation on all test media. Cultural characteristics of the two strains are shown in Table 5. Aerial mycelium of strain1was abundant, well-developed and varied from white to brown on different tested media. The substrate hyphae varied from white to brown. Aerial mycelium of strain 2 was well-developed and varied from white to orange- brown on the tested media. The substrate hyphae varied from white to brown. The diffusible pigment melanin was observed on ISP-6 medium.

Table 7: Morphological and Physiological characterization of the two antagonistic organisms.

Characteristic	Results	
	Strain 1	Strain 2
Shape of cells	Hyphae	Hyphae
Size	Long	Long
Gram's staining	+	+
Sporulation	+	+
Motility	-	-
Growth of KBA Medium	-	-
Optimum temp.	28-30^{o}	30^{o}
Utilization of suger:		
Mannitol	AG	A
Fructose	-	A
Sucrose	AG	AG
Arabinose	-	A
Glucose	A	A
Galactose	A	A
Lactose	-	-
Maltose	A	AG
Dextrose	AG	AG
Glycerol	A	A
Menthol	-	A
Raffinose	-	-
Starch hydrolysis	-	-
Gelatin liquefication	+	+
Indole formation	-	-
Catalase activity	+	+
Lipolytic activity	+	-
V.P. Test	+	+
Methyl Red (MR)	-	-
Production of H_2S	-	-
Nitratereduction	-	+
Milk coagulation	+	+

(+) Positive reaction G : Gas
(-) Negative reaction A : Acid
Strain 1: *Streptomyces avermitilis* strain 2: *Actinomycetes odontolyticus*

These results also were consistent with results obtained for 16SrDNA gene sequences DNA, indicated that one of the two spp. is belongs to actinomycetes and the other is represented as *Streptomyces* sp.

3.7 Identification of the two spp. using 16SrDNA partial sequences DNA.

Two actinomycetes were examined for the specific amplification of 16S rDNA gene sequences (Azadeh and Meon, 2009). As indicated in Table 4, the most active microorganisms against potato scab were strains 1and 2. They were identified as *Streptomyces* species withmore100% sequence similarity. Strain 1and strain 2 was identified up to species level, namely *Streptomyces avermitilis* MA-4680and *Actinomyces odontolyticus* ATCC 17982respectively.

Analysis of the 16SrDNA from Streptomyces species showed that the 1st strain was grouped into a branch with *Streptomyces coelicolor* A3(similarity value of 97%; the close stneighbors. The almost complete 16S rRNA gene sequenceof1st strain was determined in this study and has been deposited in the GenBank datebase. This sequence was subjected to similarity searches against public databases to infer possible phylogenetic relationships of this strain. This analysis revealed that strain was a member of the genus Streptomyces. A neighbor-joining tree (Saitou and Nei, 1987) (Figure 1) based on 16S rDNA gene sequences were constructed to show relationships between the strain and some other related Streptomyces species. Bootstrap analysis was used to evaluate the tree topology of then eighbor-joining data by performing 1000 resembling (Felsenstein, 1985). Thus, based on the results of the above phenotypic and genotypic analyses, strain 1should represent specie of the genus Streptomyces, for which we propose the name *Streptomyces avermitilis* MA-4680.

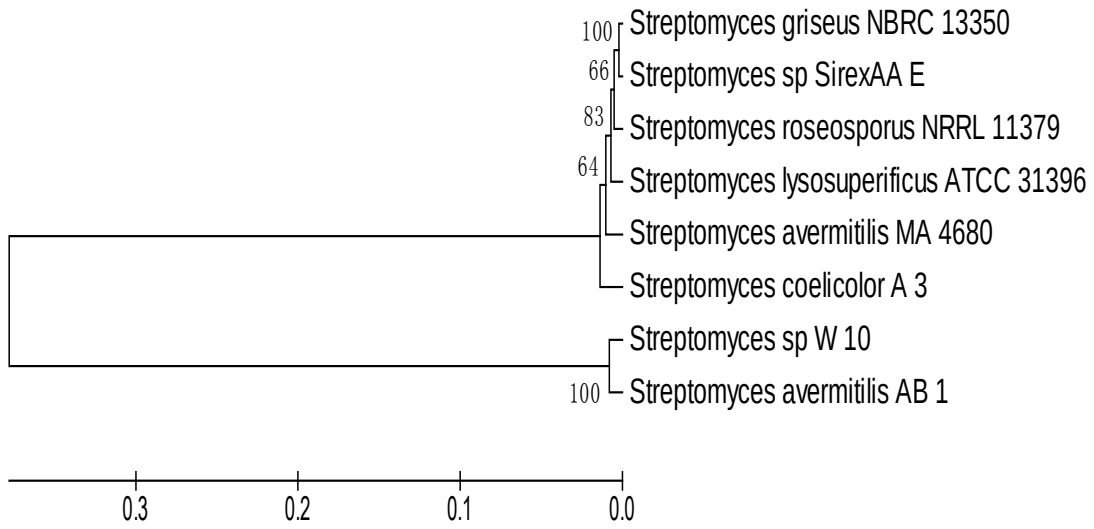

Figure 1. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rDNA sequences, showing the position of strain *Streptomyces avermitilis* MA-4680among phylogenetic neighbors. Numbers on branch nodes are bootstrap values (1000 resamplings).

Description of *Streptomyces avermitilis* MA-4680

Gram positive organism both vegetative and aerial hyphae were abundant and well-developed, spore forming. Aerial mycelium varied from white to brown. The substrate hyphae from white to brown. The diffusible pigment melanin was observed on ISP-6 medium. Gelatin liquefaction, Catalase activity, Lipolytic activity, V.P. Test and Milk coagulation were positive while starch hydrolysis, H_2S and indole production, nitrate reduction were negative. Glucose, mannitol, sucrose, galactose, maltose, dextrose and glycerol are utilized but not fructose, lactose, arabinose, raffinose and mannitol.

The analysis of the 16SrDNA for 2nd strain showed Streptomyces species showed that this strain was grouped into a branch with *Actinomyces odontolyticus* C 505(similarity value of 98%; the closest neighbors.The almost complete 16S rRNA gene sequence of 2nd strain was determined in this study and has been deposited in the GenBank datebase. This sequence was subjected to similarity searches against public databases to infer possible phylogenetic relationships of this strain. This analysis revealed that strain was a member of the genus actinomycetes. A neighbor-joining tree (Saitou and Nei, 1987) as shown in Figure(2) based on 16S rDNA gene

sequences was constructed to show relationships between strain 2 and some other related actinomycetes species. Bootstrap analysis was used to evaluate the tree topology of then eighbor-joining data by performing 1000 resamplings (Felsenstein, 1985). Thus, based on the results of the above phenotypic and genotypic analyses, strain 2 should represent a specie of the genus actinomycetes, for which we propose the name *Actinomyces odontolyticus* ATCC 17982.

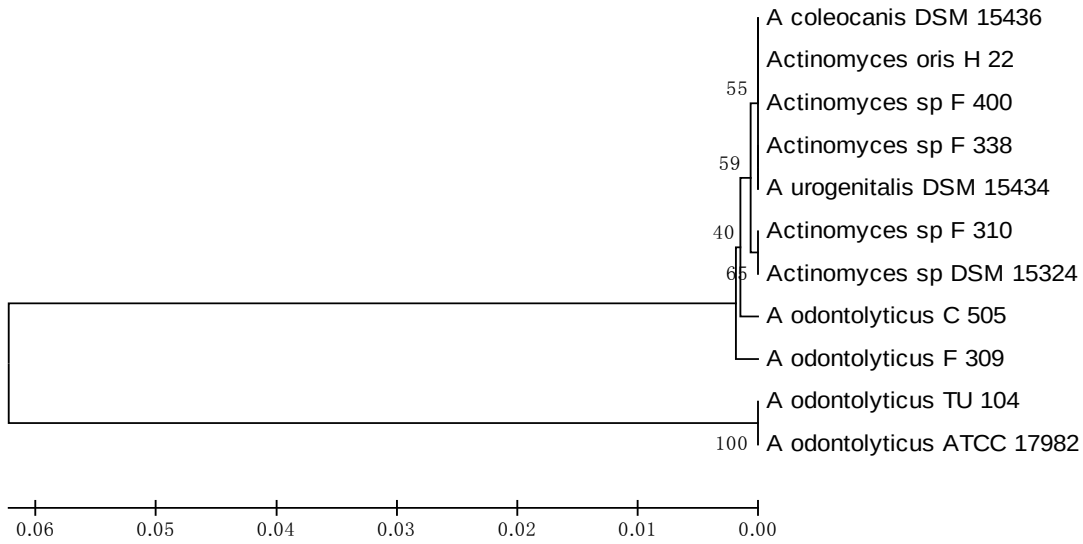

Figure 2. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rDNA sequences, showing the position of strain *Actinomyces odontolyticus* ATCC 17982among phylogenetic neighbors. Numbers on branch nodes are bootstrap values (1000 resamplings).

Description of *Actinomyces odontolyticus* ATCC 17982

Gram positive organism both vegetative and aerial hyphae were abundant and well-developed, spore forming aerial mycelium of strain 2 varied from white to orange- brown on the tested media and the substrate hyphae from white to brown. The diffusible pigment melanin was observed on ISP-6medium.Gelatineliquification, Catalase activity, nitrate reduction, V.P. Test and Milk coagulation were positive while starch hydrolysis, H_2S and indole production were negative. Glucose, mannitol, fructose, sucrose, arabinose, galactose, maltose, dextrose and glycerol are utilizedbut not lactose and raffinose.

Conclusion

The two identified strains *Streptomyces avermitilis* MA-4680 and *Actinomyces odontolyticus* ATCC 17982 are showed high level of antibiosis against pathogenic organism (*Streptomyces scabies*) and achieve a significant control of disease as no sign of disease symptoms are shown on the tested varieties of potato (Cara and Diamond).

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