African Journal of Biotechnology Vol. 11(8), pp. 2130-2138, 26 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3388
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Antiviral activities of streptomycetes against tobacco mosaic virus (TMV) in *Datura* plant: Evaluation of different organic compounds in their metabolites

Ismet Ara*, Najat A. Bukhari, N. M. Aref, Mahera M. A. Shinwari and M. A. Bakir

Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 22452, Riyadh-11495, Kingdom of Saudi Arabia.

Accepted 19 December, 2011

A total of 20 strains of actinomycetes were isolated from Al-Kharj (K) and Al-Madina (M) areas in Saudi Arabia. Among these strains, six were selected for antiviral activity screening which are K1, K2, K3, M1, M2 and M3. All the selected strains were characterized morphologically to be under the genus *Streptomyces*. Primary and secondary screenings were performed against seven human pathogenic microorganisms such as *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella suis* ATCC 13076, *Shigella sonnei* ATCC 11060 and *Candida albicans* ATCC 1023. In the data, all the obtained six selected strains had shown a positive and very promising result with little variations. The bioactive compounds were extracted from the strains using solvent extraction methods. The tobacco mosaic virus (TMV) was obtained from tomato plants and the extract was prepared using a simple technique of homogenizing in water and filtration. Tobacco mosaic virus and the metabolites of streptomycetes strains were applied to the selected *Datura metel* plant leaves. After incubation for one week, it was found that the viral infection symptoms in the form of local lesions caused by the TMV were notably reduced on the plant leaves in the presence of bioactive metabolites. Further, in this study, gas chromatography-mass spectroscopy (GC-MS) analysis of all the crude metabolites of streptomycetes strains were performed for the determination of different compounds.

Key words: Antiviral activity, tobacco mosaic virus, actinomycetes, *Streptomyces*, *Datura metel*, GC-MS analysis, human pathogenic bacteria.

INTRODUCTION

Actinomycetes population has been identified as one of the major groups of soil population which may vary with the soil type. Soil rich in organic matter is highly suitable for the growth of actinomycetes. Primary and secondary metabolites produced by these organisms are potent, biologically active and remain a powerful source for pharmaceutical discovery of many compounds. Actinomycetes species, especially under the genus *Streptomyces* are producers of approximately two thirds of all bioactive compounds known. Some produce a great variety of compounds which have clinical application on the basis of their activity against different kinds of organisms (Todar, 2009). *Streptomyces* are gram positive (Lechevalier and Lechevaliemr, 1970a,b) free living, saprophytic bacteria which comprise a group of branching unicellular microorganisms. They produce branching mycelium which may be of two kinds (substrate mycelium and aerial mycelium). Among actinomycetes, the *Streptomyces* group is the dominant. The non-streptomycetes are called rare actinomycetes, comprising approximately 100 genera. Members of the actinomycetes, which live in

*Corresponding author. E-mail: ismetara@yahoo.com or iara@ksu.edu.sa. Tel: +966534509242 or +966 1478 9585. Ext. 1639.

Abbreviations: SEM: Scanning Electron Microscope; **TEM**: Transmission Electron Microscope; **TMV**: Tobacco Mosaic Virus.

marine environment, are poorly understood and only few reports are available pertaining to actinomycetes from mangroves (Siva, 2001; Vikineswary et al., 1997; Rathana and Chandrika, 1993; Lakshmanaperumalsamy, 1978).

Streptomyces sp. is capable of producing microbial antibiotics with a wide variety of chemical structures. In particular, approximately 60% of antibiotics developed for agricultural use have been isolated from *Streptomyces* sp. (Tanaka and Ōmura, 1993). These strains continue to provide a wide variety of new antibiotics suggesting that substantial numbers of *Streptomyces* species are strains with novel antibiotic productivity (Okami and Hotta, 1988). In searching for bioactive antibiotics, *Streptomyces* have been isolated from various types of soils (Lee and Hwang, 2002) including rice paddy (Hayakawa et al., 1988), lake mud and water, deciduous forest, tropical forest, wasteland and cave soils (Tanaka and Ōmura, 1993; Jiang and Xu, 1996; Xu et al., 1996; Langó et al*.,* 1999).

Viruses on the other hand are intracellular obligatory parasitic pathogenic agents which infect other living organisms. Human diseases caused by viruses include chickenpox, herpes, influenza, rabies, smallpox and AIDS. Although these are the viruses commonly known, the first virus ever described and from which the term was eventually derived was tobacco mosaic virus (TMV) which was discovered by Martinus W. Beijerinck, a Dutch microbiologist, in 1898.

Most if not all economically important crop plants may become infected with viruses. In most cases, the virus (or viruses) will cause a reduction in yield or quality of the infected crop, but the extent of the economic loss can vary greatly (Walker, 1991).

Tobacco mosaic virus (TMV) is an economically important disease infecting tobacco (*Nicotiana tabacum L*.) and other Solanaceous crops worldwide. TMV infects 199 different species from 30 families of plants; however, Solanaceous crops incur the most dramatic losses from the disease. Tobacco mosaic virus is a very difficult disease to control because it spreads easily. The disease is mechanically transmitted, resulting in quick and effective infection. Once a susceptible plant is infected, symptoms show up in seven to14 days post infection. The first symptom to occur in newly infected plants is vein clearing. A plant exhibiting vein clearing allows the veins in a leaf to be more clearly seen. The vein clearing is seen in the new upper leaves and can be seen more clearly at 40°C than at 25°C (Dawson, 1999). No known mechanisms for vein clearing have been reported. Soon after vein clearing, mosaic symptoms occur in the newer leaves. This mottling consists of irregularly shaped dark green areas of tissue surrounded by light green areas of tissue. The dark green areas are called green islands and contain no virus, while the lighter green areas have virus (Dawson, 1999). The young leaves infected with TMV are often deformed and wrinkled. TMV infection may also cause the production of nonviable seeds.

The idea of using biological control agents for human and animals health protection is one of the most studied areas in the last few years because of the enormous impact on general health maintenance. However, the same cannot be said about plant virus epidemics as they have received less attention than those caused by fungal or bacterial pathogens. The fact that viral diseases are systemic means that the individual diseased plant can be considered as the population unit which simplifies modeling. In this respect, the current study was designed to evaluate the effect and antiviral activity of some actinomycetes biocompound against the TMV that affects many plants causing induced local lesions on leaves. The plant of choice in this study is *Datura metel* which is affected easily by the TMV virus.

MATERIALS AND METHODS

Sample collection

Soil samples were collected in clean sterile plastic bags from two different locations in Saudi Arabia. The first sampling location was the city of Madina (M) which is known for its sandy soil. Collection was made in this city from a residential area. The second location was Al-Kharj (K) which is an agricultural city and thus the soil collected was agricultural soil. At each site, samples were taken from up to 20 cm depth, after removing approximately 3 cm of the soil surface.

Samples preparation and actinomycetes isolation

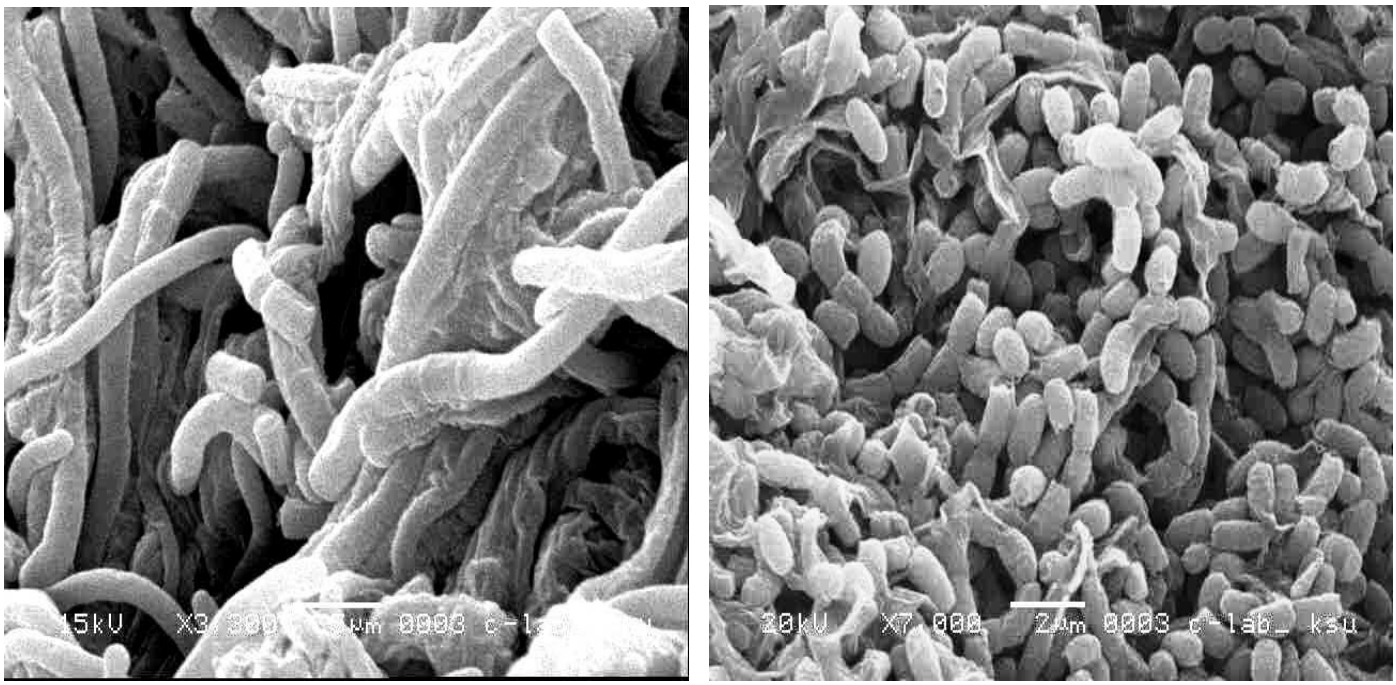
The collected soil samples were sieved and air-dried at room temperature for three days. Actinomycetes were isolated from the air dried soil samples by standard soil dilution plate technique. An 0.5 ml aliquot was taken from each dilution and spread evenly over the surface of the yeast extract-soluble starch agar medium. The inoculated plates were incubated at 30°C for three to four weeks. Repeated streaking method was used to purify the soil actinomycetes colonies on yeast starch (YS) agar medium.

Morphology and biochemical characterization

The strains chosen for this study were gram stained, shape and size were identified under light microscope. The mycelium structure and arrangement of spore on the mycelium were examined under the light and scanning electron microscope (SEM). Various biochemical tests were performed for the characterization of the actinomycetes isolates.

SEM for morphological characterization

Bacterial cells were prepared for SEM (JEOL, JSM, 3060) at King Saud University Central Laboratory, Saudi Arabia according to the initial fixation and dehydration steps previously published (Moore et al., 1992). The cells were fixed at 24°C for 60 min with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) (Sigma-Aldrich Chemie Gmbh, Steinheim, Germany), dehydrated with a serial concentration of ethanol, and then dried on a critical point dryer (HCP-2; Hitachi Co.). The dried cell samples were coated with gold, and examined using a SEM (JEOL, JSM, 3060). (Tamura et al., 1994) (Figure 1).



a b

Figure 1. Electron micrographs (a and b) of selected actinomycetes strains M1 (a) and K1 (b) grown in YS agar. Spore chain morphology showing the strain as *Streptomyces* species. YS, Yeast starch.

Antibacterial compound extraction

The six strains of actinomycetes producing antibacterial compound were each inoculated into flasks containing starch glucose yeast (SGY) broth (10 g starch soluble,10 g glucose,10 g glycerol, 2.5 g corn flower, 5 g peptone, 2 g yeast extract, 3 g $CaCO_3$ and l L distilled water) (Ismet et al., 2002, 2003, 2004). The flasks were incubated at 37°C in a rotary shaker for seven days. The antibacterial compounds were recovered from the broth by solvent extraction method (Westley et al., 1979; Liu et al., 1986). Methanol was added to the flasks containing the mixture of strains and broth in the ratio of 1:1 (v/v). The flasks were then returned to the shaker for 24 h and the contents were filtered to separate the mycelium from the liquid. From the liquid part, methanol was evaporated to dryness in hot air oven and two drops of distilled water was added to the residue obtained. The crude extract obtained was used to determine antimicrobial activity.

Screening of isolates for antagonistic abilities

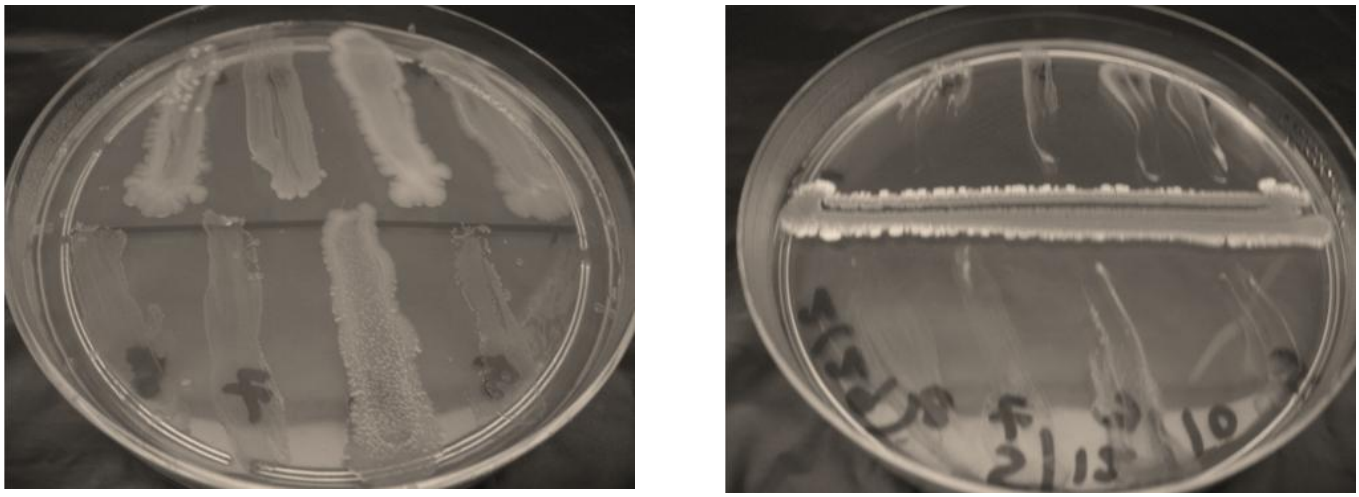
All six selected pure actinomycetes were subjected to primary screening by perpendicular streak method (Egorov, 1985) (Figure 2) against human pathogenic bacteria and yeast. The test bacteria were gram-positive *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 25923 and gram-negative *Streptococcus suis* ATCC 13076, *Pseudomonas aeruginosa* ATCC 27583, *Escherichia coli* ATCC 25922 and *Shigella sonnei* ATCC 11060 (Table 1). The yeast was *Candida albicans* ATCC 10231. Secondary screening for the antimicrobial activity of the crude extract from the actinomycetes strains was performed on the same test pathogens by agar well diffusion method assay. The presence of inhibition zones around the active compound(s) was determined (Figure 3).

Gas chromatography-mass spectroscopy (GC-MS) analysis of crude cell extract

Gas chromatography-mass spectroscopy analysis was performed to identify the active antibacterial, antiviral and antifungal compound in the extract. Identification of several compounds was done by injecting 1 µl of sample into a RT x -5 column (30 × 0.32 nm) of GC-MS model (Perkin Elmer, Clarus 500, USA) and helium (3 ml/min) was used as a carrier gas. The following temperature gradient program was used (75°C for 2 min followed by an increase from 75 to 175°C at a rate of 50°C per min and finally 7 min at 175°C). The m/z peaks representing mass to charge ratio characteristics of the antimicrobial fractions were compared with those in the mass spectrum library of the corresponding organic compounds (Pandey et al., 2010). The chemical components of the extracts were analyzed in the central laboratory of King Saud University, Riyadh, Saudi Arabia. Identification of the chemical constituents of extracts were performed using Perkin Elmer (Clarus 500, USA) gas chromatography coupled with (Clarus 500, USA) mass spectrometer (MS) (Table 2).

Virus isolation and purification

Pure TMV was obtained from Faculty of Agriculture, Ain-Shams University, Cairo, Egypt. It was propagated on tomato plants and purified using chloroform and low speed centrifugation for 20 min at 300 rpm. The filtrate was further centrifuged at high speed for 2 h at 40, 000 rpm (Walker, 1991). The virus preparation was negatively stained by uranyl acetate 2% and examined by transmission electron microscopy (TEM). The size of virus was 35 nm in length and 13 nm in width (Figure 4).



a b

Figure 2. Primary screening by perpendicular streak method. (a) Control plate of seven pathogenic bacteria, (b) Inhibited plate against *Streptomyces* sp. M1.

Table 1. Inhibition zone* showing the antagonistic abilities of the bioproducts derived from the six *Streptomyces* strains chosen for the study.

Isolate code	Pathogenic microorganism						
	Gram positive bacteria		Gram negative bacteria				Yeast
	S. aureus ATCC 13076	*B. subtilis* ATCC 6633	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27583	*S. suis* ATCC 13076	*S. sonnei* ATCC 11060	*C. albicans* ATCC 10231
M1	+++	+++	+	++	+++	++	+++
M2	+++	+++	++	+	++	++	++
M3	++	+++	+++	+	++	++	+++
K1	++	+++	++	+++	++	+	++
K2	++	+++	++	++	++	++	++
K3	++	++	+	++	++	++	++

*Inhibition zone: - No inhibition zone; + (weak), 5 to 10 mm; ++ (moderate), 10 to 15 mm; +++ (strong), 15 to 20 mm.

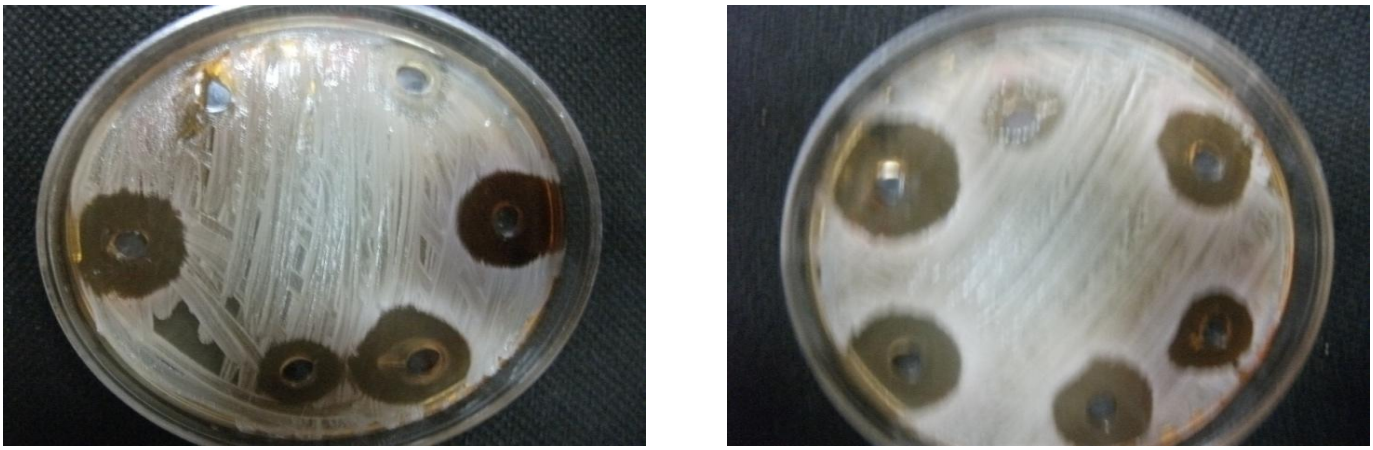


Figure 3. Secondary screening by agar well diffusion method showing the inhibition zone caused by the antagonistic ability of the isolates M1, M3, M3, K1, K2 and K3 bioactive compound against human pathogenic bacteria *B. subtilis*.

Table 2. The GC-MS analysis of the different compounds found in the six *Streptomyces* crude extract with high REV (percentage of compound peak in the whole sample).

S/N	Compound name	M.W.	Formula	REV
Strain K1				
1	2-butanone,3-methoxy-3-methyl	116	$C_6H_{12}O_2$	908
2	Cyclohexanol,4-ethyl-4-methyl-3-(1-methylethyl)-,(1.alpha.,3.alpha.,4.	184	$C_{12}H_{24}O$	901
3	Phytol	296	$C_{20}H_{40}O$	911
4	2-hexanol,3-methyl	116	$C_7H_{16}O$	901
5	2-decenal,(z)	154	$C_{10}H_{18}O$	944
6	Hexadecane,1,1-bis(dodecyloxy)	594	$C_{40}H_{82}O_2$	923
7	12-methyl-e,e-2,13-octadecadien-1-ol	280	$C_{19}H_{36}O$	910
8	9-octadecenal,(z)	266	$C_{18}H_{34}O$	905
Strain K2				
1	2-butanol,3-methyl	88	$C_5H_{12}O$	919
2	Glycerin	92	$C_3H_8O_3$	991
3	4h-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	144	$C_6H_8O_4$	921
4	Triacontane,1,30-dibromo	578	$C_{30}H_{60}Br_2$	923
Strain K3				
1	Glycerin	92	$C_3H_8O_3$	993
2	4h-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl (Bisacrylamide)	144	$C_6H_8O_4$	934
Strain M1				
1	Propanoic acid,2-(aminooxy)	105	$C_3H_7O_3N$	932
2	2-heptanol,(s)	116	$C_7H_{16}O$	926
3	Glycerin	92	$C_3H_8O_3$	989
4	4h-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	144	$C_6H_8O_4$	912
5	1-hexacosene	364	$C_{26}H_{52}$	918
6	1-pentacontanol	718	$C_{50}H_{102}O$	913
7	1-decanol,2-hexyl	242	$C_{16}H_{34}O$	922
8	Dichloroacetic acid,heptadecyl ester	366	$C_{19}H_{36}O_2Cl_2$	918
Strain M2				
1	Glycerin	92	$C_3H_8O_3$	984
2	1,6;3,4-dianhydro-2-deoxy-.beta.-d-lyxo-hexopyranose	128	$C_6H_8O_3$	925
3	2-undecenal	168	$C_{11}H_{20}O$	949
4	2-dodecenal,(e)	182	$C_{12}H_{22}O$	960
Strain M3				
1	4h-pyran-4-one,2,3-dihydro-3.5-dihydroxy-6-methyl	144	$C_6H_8O_4$	942
2	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro	154	$C_7H_{10}O_2N_2$	902

M.W., Molecular weight. GC-MS, gas chromatography-mass spectroscopy.

Virus extract preparation

The extract was prepared by placing 5 g of the dry leaf in a glass jar together with phosphate buffer (pH 7). The mixture was homogenized for several minutes until a fine homogenate was produced (Walker, 1991).

Bioproduct and TMV application on *Datura metel* plant leaves

The whole *D. metel* plant was used for the application and the leaves were divided in to three partitions each containing triplicates. Using a sterile cotton swab, the virus extract was applied alone to the first partition as a positive control. Bioproduct was applied to the

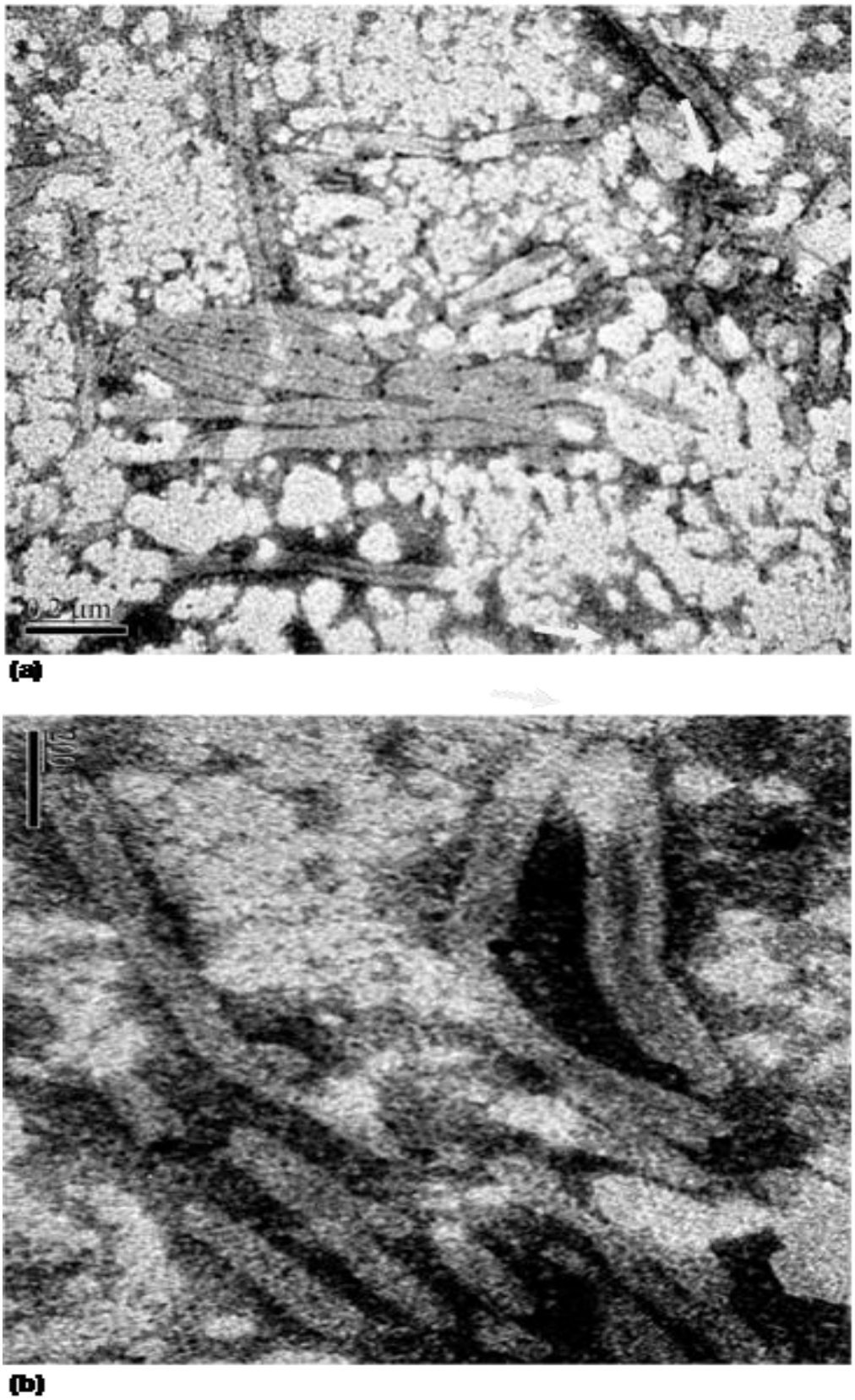


Figure 4. Electron micrograph of particles of TMV virus shown in *D. metel* leaf (a, with white arrow, magnification 60,000x and b, with white arrow, magnification 150,000x) (the arrow indicates the position of the TMV virus). TMV, Tobacco mosaic virus.

second partition as negative control. For the treatment, one volume of crude sap of infected source of virus was mixed with one volume of bioproduct and applied to the third partition of leaves.

Numerical method for identifying the effect of *Streptomyces* bioproduct

The antiviral activities of the isolates against the TMV were determined using the local lesions count method. After applying the bioactive compound to the infected *D. metel* leaves, the number of local lesions caused by the TMV was enumerated in order to deduce the effect of the biocompound extracted from the *Streptomyces* strains chosen for this study.

RESULTS AND DISCUSSION

Description of actinomycetes isolates

All six isolates which exhibited high activity against all the pathogens previously mentioned were selected for further studies. According to Bergey's manual of determinative bacteriology and the laboratory manual for identification of actinomycetes, the isolates were identified as *Streptomyces* sp.

Antagonistic activity of the *Streptomyces* isolates against pathogenic microbes

The *Streptomyces* flora isolated from soil samples collected from two locations in Saudi Arabia, were screened for their potential as a source of antibiotics active against pathogenic microbes. The formation of inhibition zone around the pathogenic strains is indicative of production of secondary metabolites by the *Streptomyces* isolates.

All the isolates were tested for their ability to produce inhibitory substances against seven test microorganisms. The test microorganisms included two gram positive bacteria: *S. aureus* ATCC 25923 and *B. subtilis* ATCC 6633, four gr am negative bacteria: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. suis* ATCC 13076 and *S. sonnei* ATCC 11060 and 1 yeast which was *C. albicans* ATCC 10231. The isolates which exhibited antimicrobial activity against at least two of the test organisms were selected for this study. As shown in Table 1, all six streptomycetes isolates were shown to have a very potent antimicrobial activity against the test organisms. The morphological examination of these isolates, which showed activities against the test organisms indicated that these belong to the *Streptomyces* genus (Waksman, 1961; Shirling and Gottlieb, 1966; Nonomura, 1974; Williams et al., 1983a; Cross, 1989; Goodfellow, 1989; Lechevalier, 1989; Locci, 1989).

All six isolates produced antibacterial substances against both the gram negative and gram positive bacteria in the test. *B. subtilis* ATCC 6633 was inhibited greatly by almost all six isolates. Two of the isolates (M1 and M2) showed strong activity against the tested *S. aureus* ATCC 13076, while the other four strains M3, K1, K2 and K3 appeared to have moderate effect on the same pathogenic bacteria strain. The isolates M1 and K3 were having weak inhibitory effect on *E. coli* ATCC 25922, while M2, K1 and K2 inhibited *E. coli* growth moderately, M3 was the most active against this strain of bacteria. The strains M1, K2 and K3 had moderate effect against *P. aeruginosa* ATCC 27853, unlike the strains M2 and M3 which were very weak in inhibition on the same strain of bacteria, but K1 did affect *P. aeruginosa* ATCC 27853 greatly. The *Streptomyces* strain M2 exhibited moderate activity against *S. suis* ATCC 13076, *S. sonnei* ATCC 11060 and *C. albicans* ATCC 1023. Similarly, M3 was moderate against *S. suis* ATCC 13076 and *S. sonnei* ATCC 11060, but highly active against *C. albicans* ATCC 1023. K2 and K3 inhibits all *S. suis* ATCC 13076 and *S. sonnei* ATCC 11060 and *C. albicans* ATCC 1023 moderately. As for the strain K1, it had a very mild effect on *S. sonnei* ATCC 11060 and moderate effect on both *S. suis* ATCC 13076 and *C. albicans* ATCC 1023. The strain M1 inhibited the growth of tested *S. suis* ATCC 13076 and *C. albicans* ATCC 1023 greatly and was very weak against *S. sonnei* ATCC 11060 (Table 1).

Antiviral activity of the isolates

Six strains of *Streptomyces* isolates (obtained from Madina and Al-Kharj areas of Saudi Arabia) were selected for the control of the test TMV plant virus. The necrotic local lesions on the inoculated leaves were clearly reduced by all six isolates used in the study (Figure 5). Isolates M1 and K3 showed moderate antiviral activities as a few number of the necrotic local lesions did appear during the experiment of the bioproducts. On the other hand, the isolates M3, M2, K1 and K2 showed very promising results in the form of the absence of all the local lesions on the *Datura* plant leaf. In a comparison between the results obtained from this study, the healthy whole plant (Figure 5a), the negative control (Figure 5b) and the positive control (Figure 5c), it is clearly notable that all six isolates which were characterized by good growth on yeast-starch extract medium showed a very promising antiviral activity against TMV (Figure 5d).

The chemical composition of the crude extract

The chemical composition of the crude extracts of all the six streptomycetes strains isolated from Madina and Al-Kharj areas in Saudi Arabia was analyzed using GC-MS. The major components of the extracts are given in Table 2, which demonstrated an interesting concoction of compounds with significant antiviral activity. Similar result has been found (Mahfouz and Mohamed, 2002) for some local *Streptomyces* species isolated from Sinai sandy soil

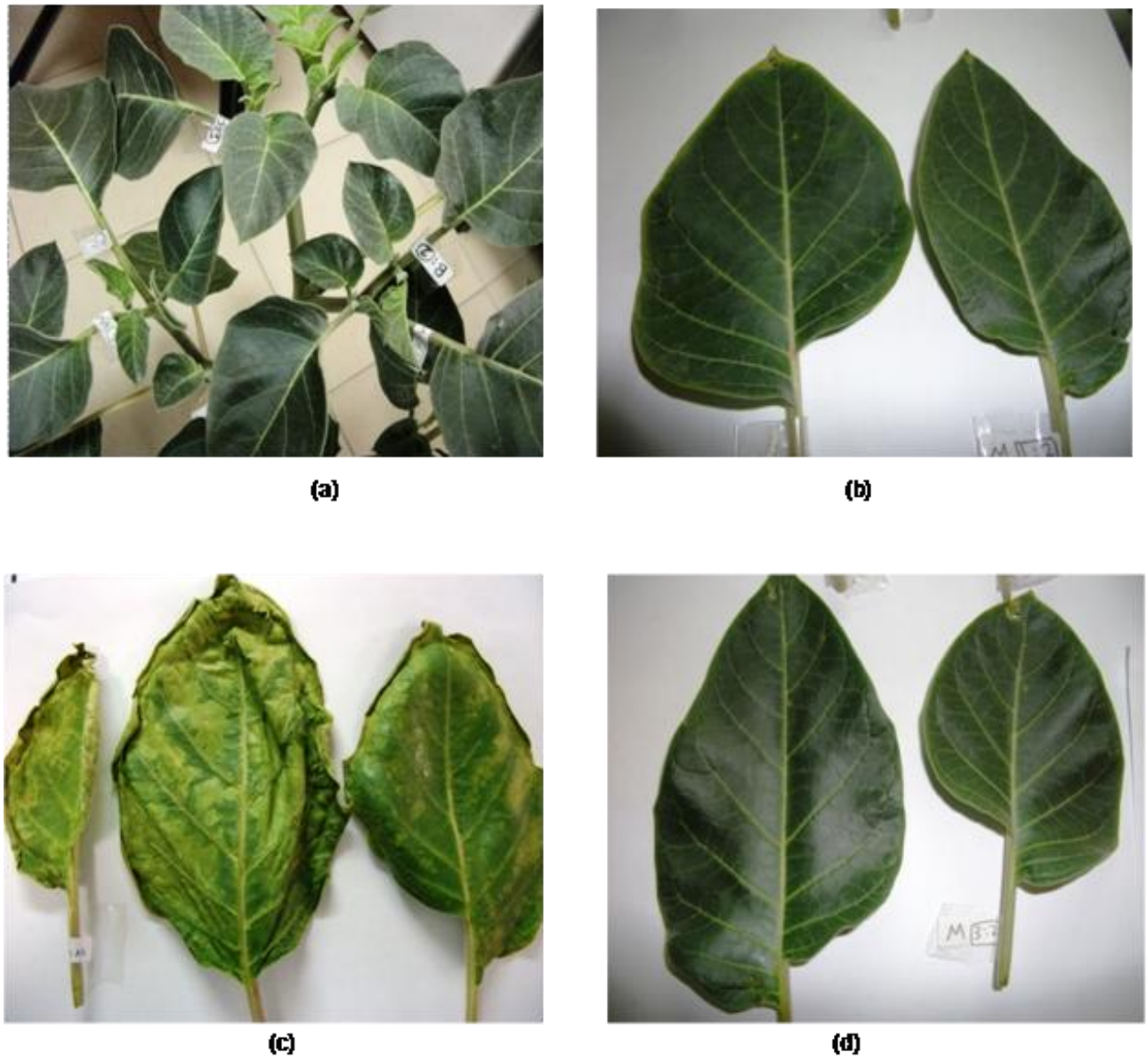


Figure 5. The effect of the bioactive compounds on *D. metel* plant leaves. (a) Whole plant with no treatment; (b) negative control using the bioproduct only; (c) positive control using TMV only as the local lesions appeared clearly on the leaves; (d) result of the TMV and bioproduct mixture treatment as the local lesions are reduced on the leaf. TMV, Tobacco mosaic virus.

against TMV using *D. metel* as a local lesion host which correlates with the present study results.

Conclusion

A further study on these compounds can finalize the relation between these metabolites and the antiviral properties of *Streptomyces* strains. The GC-MS results indicate the possibilities of extraction, purification and identification of such substances as well as support and encourage the idea for using such active streptomycetes as biopesticide for controlling some plant pathogens.

ACKNOWLEDGEMENT

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RGP-VPP-066.

REFERENCES

Cross T (1989). Growth and examination of actinomycetes some guidelines. In: Bergey's Manual of Systematic Bacteriology, Vol. 4, Williams and Wilkins Company, Baltimore, pp. 2340-2343.

Dawson WO (1999). Tobacco mosaic virus virulence and avirulence.

Philos. Trans. R. Soc. London, 354: 645-651.
Egorov NS (1985). Antibiotics, a scientific approach. Mir Publishers, Moscow.
Goodfellow M (1989). Suprageneric classification of Actinomycetes. In: Bergey's Manual of Systematic Bacteriology, Williams and Wilkins Company, Baltimore, 4: 2333-2339.
Hayakawa M, Ishizawa K, Nonomura H (1988). Distribution of rare actinomycetes in Japanese soils. J. Ferment. Technol. 66: 367-373.
Ismet A (2003). Biological, molecular and chemical characterisation of a selected antagonistic *Micromonospora* sp. isolated from mangrove rhizosphere. Ph.D. Thesis. Institute of Biological Sciences, Faculty of Science, University of Malaya, Malaysia.
Ismet A, Paramaswari S, Vikineswary S (2002). Diversity of *Micromonospora* in Malaysian mangrove rhizosphere soil. Malaysian J. Sci. 21: 51-59.
Ismet A, Vikineswary S, Paramaswari S, Wong WH, Ward A, Seki T, Fiedler HP, Goodfellow M (2004). Production and chemical characterization of antifungal metabolites from *Micromonospora* sp. M39 isolated from mangrove rhizosphere soil. World J. Microbiol. Biotechnol. 20: 523-528.
Jiang CL, Xu LH (1996). Diversity of aquatic actinomycetes in lakes of the middle plateau, Yunnan, China. Appl. Environ. Microbiol. 62: 249-253
Lakshmanaperumalsamy P (1978). Studies on actinomycetes with special reference to antagonistic *streptomycetes* from sediments of Porto Novo coastal zone. Ph.D. thesis, Annamalai University, India, p. 192.
Langó ZS, Tóth LG, Kovács G, Cruz–Pizarro L (1999). Identification and biochemical characterization of *Streptomyces galbus* from the sediment of the high–mountain lake La Caldera (Sierra Nevada, Spain). Fol. Microbiol. 44: 651-656
Lechevaliehr A, Lechevaliemr P (1970a). A critical evaluation of the genera of aerobic actinomycetes. In The Actinomycetales. Edited by H. Prauser. Jena: Gustav Fischer Verlag. p. 393405.
Lechevaliemr P, Lechevaliemr A (1970b). Chemical composition as a criterion in the classification of aerobic actinomycetes. Int. J. Syst. Bacteriol. 20: 435-443.
Lechevalier HA (1989). The actinomycetes ill, a practical guide to generic identification of Actinomycetes. Williams and Wilkins Company, Baltimore, Bergey's Manual Syst. Bacteriol. 4: 2344-2347.
Lee JL, Hwang BK (2002). Diversity of antifungal actinomycetes in various vegetative soils of Korea. Can. J. Microbiol. 48: 407-417.
Liu CM, Westley JW, Herman TE, Prasser BLT, Palleroni N, Evans RH, Miller PA (1986). Novel polyether antibiotics X-14873 A, G and H produced by *Streptomyces*. Taxonomy of the producing culture, fermentation, biological and ionophores properties of the antibiotics. J. Antibiot. 39(12): 1712-1718.
Locci R (1989). Streptomyces and related genera. Bergey's Manual of Systematic Bacteriology. Williams and Wilkins Company, Baltimore, 4: 2451-2508.
Mahfouz HT, Mohamed SH (2002). Physiological antagonistic and fingerprinting studies on some halotolerant *Streptomyces* strains. Arab. J. Biotechnol. 5: 103-20.
Moore CW, Del R, Valle J, McKoy A, Pramanik, Gordon RE (1992). Lesions and preferential initial localization of [S-methyl- 3 H] bleomycin A2 on Saccharomyces cerevisiae cell walls and membranes. Antimicrob. Agents Chemother. 36: 2497-2505.
Nonomura H (1974). Key for classification and identification of 458 species of the Streptomyces included in ISP. J. Ferment. Technol. 52: 78-92.
Okami Y, Hotta K (1988). Search and discovery of new antibiotics. In: Goodfellow M, S T Williams and M Mordaski (eds.). Actinomycetes in Biotechnol. Academic Press, London, United Kingdom. pp. 33-67.
Pandey A, Milind M, Naik, Dubey SK (2010). Organic metabolites produced by Vibrio parahaemolyticus strain An3 isolated from Goan mullet inhibit bacterial fish pathogens. Goa, India. AJB, 9(42): 7134-7140
Rathna K, Chandrika V (1993). Effect of different media for isolation, growth and maintenance of actinomycetes from mangrove sediments. Indian J. Mar. Sci. 22: 297-299.
Shirling EB, Gottlieb D (1966). Methods, classification, identification and description of genera and species. The Williams and Wilkins Company, Baltimore, 2: 61-292.
Siva K (2001). Actinomycetes of an Indian Mangrove (Pichavaram) environment: An Inventory. Ph.D. thesis, Annamalai University, India, p. 91.
Tamura T, Nakagaito Y, Nishii T, Hasegawa T, Stackebrandt, E and Yokota A (1994). A new genus of the order Actinomycetales, Couchioplanes gen. nov., with descriptions of Couchioplanes caeruleus (Horan and Brodsky 1986) comb. nov. and Couchioplanes caeruleus subsp. azureus subsp. nov. Int. J. Syst. Bacteriol. 44: 193-203.
Tanaka YT, Ōmura S (1993). Agroactive compounds of microbial origin. Annu. Rev. Microbiol. 47: 57-87.
Todar K (2009). Antimicrobial agents in the treatment of infectious disease.Todars Online Text Book of Bacteriology.
Vikineswary S, Nadaraj P, Wong WH, Balabaskaran S (1997). Actinomycetes from a tropical mangrove ecosystem - Antifungal activity of selected strains. Asia Pacific J. Mol. Biol. Boitech. 5(2): 81-86.
Waksman SA (1961). The Actinomycetes resistant bacteria. J. Arid Environ. 53: 365-371.
Walker DGA (1991). Applied Plant Virology, second edition. Printed in Great Britain by St Edmundsbury press, Bury St Edmunds, Suffolk.
Westley JW, Ecans RH, Sello LH, Troupe N, Liu CM, Blount JF (1979). Isolation and characterization of antibiotic X-1454A, a novel monocarboxylic acid ionophore produced by Streptomyces antibioticus NRRL 8167. J. Antibiot. Tokyo. 32(2): 100-107.
Williams ST, Goodfellow M, Alderson G, Wellington EMH, Sneath PHA, Sackin MJ (1983a). Numerical classification of Streptomyces and related genera. J. Gen. Microbiol. 129: 1743-1813.
Xu LH, Li QR, Jiang CL (1996). Diversity of soil actinomycetes in Yunnan, China. Appl. Environ. Microbiol. 62: 244-248.