THE SCREENING OF BACTERIAL INSECTICIDES AGAINST *Musca domestica* L. (DIPTERA: MUSCIDAE)

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ABSTRACT

In this study, a total of 396 bacterial isolates were obtained from soil samples from Eskişehir, Turkey and these were screened for their antimicrobial and insecticidal activity. Only the isolates displaying weak or no activity, a total of 322, were selected for insecticidal activity studies. Two of these (39.02 and 42.04) showed insecticidal activity on *Musca domestica* larvae and on adult flies with topical application. The insecticidal activity of the isolates was compared to that of standard insecticidal compounds produced by actinomycetes (antimycin A, cycloheximide and nikkomycin Z) and insecticidal compounds producing collection strains (*Streptomyces avermitilis* NRRL 8165 and *S. pactum* NRRL 5230). In the primary screening program, the mortality rates of isolates 39.02 and 42.04 on *M. domestica* larvae were 95% and 75%, respectively. The insecticidal compounds produced by the isolates 39.02 and 42.04 were more effective by topical application than ingestion on adult flies. However, they did not show any insecticidal activity on the pupae and all pupae hatched into adult flies. The genotoxic potentials of the crude extracts from the active isolates were studied with the Umu test using *Salmonella typhimurium* NM2009 and NM3009 strains. The results show that all of the compounds did not have mutagenic activity. An 16S rRNA analysis of the isolates 39.02 and 42.04 shows that they belong to genera *Achromobacter* and *Streptomyces*, respectively.

KEYWORDS: *Achromobacter*, Insecticidal activity, *Musca domestica*, *Streptomyces* sp., Umu Test System

INTRODUCTION

Insect pests cause considerable damage on planted crops in agriculture. Because of their high efficiency, synthetic compounds are primarily used for the control of these pests. However, intense utilization of the synthetic chemicals has caused serious problems, such as food contamination, environmental pollution, an accumulation of insecticide residues, induction of pesticide resistance, and toxicity. To prevent these problems during control of the pests, natural products and their semi-synthetic derivatives are considered as an ideal remedy. Several natural products, which could be used in an environmental friendly and safe way have been developed as insecticidal agents. Although there are some insecticidal products isolated from higher plants [1,2] and mushrooms [3-5], studies in this area have focused on the insecticidal metabolites of different microbial groups [6-16].

Although there have been some strains obtained from marine samples in the literature [14, 17], most of the insecticidal microorganisms have been isolated from terrestrial environments or insects [15, 16, 18-21]. To date, the most successful microbial biocontrol agent has been the insecticidal bacterium *Bacillus thuringiensis*, which accounts for more than 90% of the world biopesticide market [22]. Most of the insecticidal compounds are known primarily for their antimicrobial activity [23-25]. They include respiration inhibitors (antimycin A, patulin, and piericidins), protein synthesis inhibitors (cycloheximide and tenuazonic acid) and membrane active agents (dextruxin, beauvericin and some polyene macrolide antibiotics) [26]. The aim of the present study was to screen insecticidal compounds which do not have antimicrobial activity by bacterial isolates from the soil samples from Eskişehir, Turkey.

MATERIALS AND METHODS

Microorganisms and Chemicals

All of the 131 soil samples used in the experiments were collected from Eskişehir, Turkey (Fig 1). In the soil sampling, after cleaning the surface of the soil, samples were taken from 10 cm depths using alcohol disinfected spatula. The soil samples were subsequently stored in plastic bags at 4 °C until they reached the laboratory which was no more than 16 h later. All the microorganisms were isolated by dilution plate techniques on starch-casein agar,

yeast extract malt extract agar and tripton yeast extract agar. The test microorganisms for antimicrobial activity (*Staphylococcus aureus* B-767, *Staphylococcus epidermidis* B-4377, *Bacillus subtilis* B-558, *Escherichia coli* B-3008, *Enterobacter aerogenes* B-3567, *Salmonella typhimurium* B-4440, *Zygosacharomyces rouxii* Y-229, *Candida albicans* Y-12983) and the microorganisms which were used as the positive control (*Streptomyces avermitilis* NRRL 8165, *Streptomyces pactum* NRRL 2939, *Streptomyces pactum* NRRL 5230) were obtained from the United States Department of Agriculture, Agricultural Research Service, Peoria, Illinois–USA. Antimycin A was obtained from Calbiochem (San Diego-USA). Cycloheximide, 4-nitroquinoline 1-oxide (4NQO), O-Nitrophenyl-ß-D-galactopyranoside (ONPG), 5-bromo-4chloro-3-indol-ß-D-galactosidase (Xgal), and sodium dodecyl sulfate (SDS) were purchased from Sigma (Germany). Nikkomycin Z was obtained from Dr. Hans Peter Fiedler (Tübingen University, Germany). All the media used in the experiments were sterilized at 121°C for 15 min.

Determination of Antimicrobial Activity

The antimicrobial activity of bacterial isolates was screened using the agar piece method [27]. Briefly, cylindrical agar plugs from bacterial isolates propagated on a yeast extract malt extract medium were cut out with a cork borer (6 mm). The agar plugs were placed on the surfaces of Petri dishes seeded with test organisms. After an incubation period at 27 ºC, clear inhibition zones around the agar plugs were recorded for confirmation of antibiotic activities. Six bacteria and two yeasts were used as test microorganisms. Nutrient agar and malt extract agar were used as growth media for the bacteria and yeasts, respectively. Bacterial isolates which do not have antimicrobial activity were selected for further insecticidal activity studies.

Determination of Insecticidal Activity

Screening studies were carried out on the larva, pupae and adult flies of *Musca domestica* L. (Diptera: Muscidae) by the method of Fabre et al. [28]. A screening study was performed at two stages.

At the primary screening stage, bacterial isolates were screened against to third larval instar of *M. domestica* for insecticidal activities. Bacterial isolates were grown on GAPY medium (20 g starch, 10 g glucose, 5 g neopeptone, 5 g biosoyase, 1 g $CaCO_3$, 15 g agar, 1000 ml distilled water; 15 ml per 55 mm diameter Petri dish) at 27 ºC for 7 days [28]. At the end of the incubation period, half of the media on which the bacteria had developed (7.5 ml) was given as food to 10 third larval instar of *M. domestica*. The larvae fed on the media which included bacterial metabolites. Uninoculated GAPY medium kept at 27 ºC for 7 days was used as a negative control. In the negative controls, larvae consumed the majority of the medium by the 3rd day and all of larvae were transformed into pupae by the 4th day. On the 8th and 9th days the pupae hatched into flies. We could expect about half of the adult flies to die by day 12 and almost all by day 14. The criteria used to show the insecticidal activity of the bacterial isolates was death of at least 6 of the 10 larvae after 1 week, at least 6 of the 10 pupae not hatching, the early death of the adult flies, or a combination of these parameters [28]. As positive controls, not only Antimycin A, Nikkomycin Z and Cycloheximide were included in the GAPY medium at

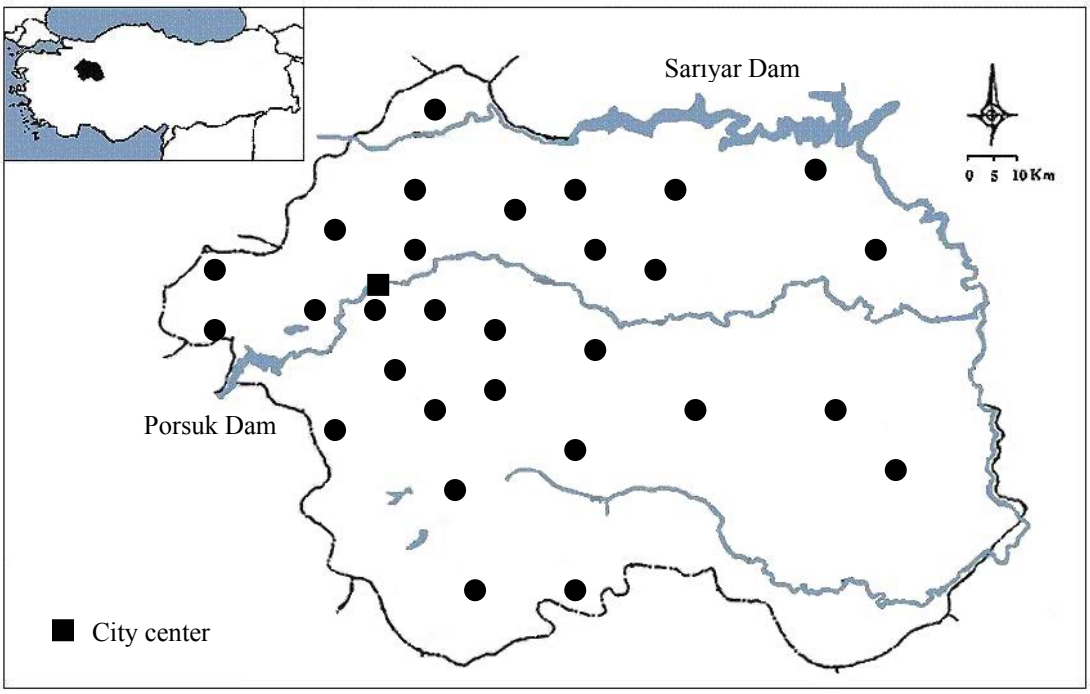

FIGURE 1 - The sampling areas in Eskisehir, Turkey.

different concentrations, but also insecticidal compounds producing standard strains such as *Streptomyces avermitilis* NRRL 8165 and *S. pactum* NRRL 5230 propagated on the GAPY medium were used.

At the secondary screening stage, to determine the type of activity, crude extracts of active isolates were tested on larvae and adult *M. domestica* by way of topical application and drinking.

Preparation of Crude Extracts

Whole agar medium which had developed bacteria isolate was extracted twice with 30 ml of methanol. Methanolic extract was evaporated to a dark red residue *in vacuo*. The residue was dissolved in 3 ml of distilled water and this solution was then extracted with 1:1 (v/v) chloroform. The aqueous and organic phases were separated and the chloroform phase was dried *in vacuo*. Chloroform residues were dissolved to a final concentration of 40 mg/ml in acetone [28].

Topical Application

1 µl of aqueous extracts and 5 µl of acetone preparations ½ diluted with acetone were used for topical application for larvae and adults. The topical activity was measured to apply the solution of the samples on the thorax of 10 adult flies previously anaesthetized and on the cuticle of 10 larvae. The mortal activity on larvae was determined on the 3rd day or by the pupae not developing into flies after treatment. As for the adult flies, the criterion used for describing activity was mortality by the third day [28]. Acetone and commercial insecticides, such as Antimycin A and Cycloheximide, were also used as negative and positive controls, respectively.

Ingestion Tests

500 µl of aqueous solution and an acetone preparation were diluted in 1/20 distilled water. The aqueous extracts were used directly. A total of 10 ml solution was given to 10 adult flies to drink *ad libitum*. After 72 hours, the dead flies were counted [28].

Phylogenetic analysis of isolates

The spore surface and the spore chain morphology of the active isolates were examined using a light microscope (CH40, Olympus) and a scanning electron microscope (Jeol JXA-840). A diaminopimelic acid isomer analysis of whole-cell hydrolysates was performed by the Lechevalier and Lechevalier method [29].

DNA extraction

1 ml of culture was centrifuged and resuspended in 1 ml of TE (Tris-HCL, 50 mM; EDTA, 20 mM; pH 8.0). A lysis solution, 0.38 ml, was added, followed by 0.40 ml of sodium perchlorate solution. Phenol-chloroform was added into the 2 ml centrifuge tube, and the culture was extracted. The aqueous upper phase was transferred into another tube and extracted with chloroform-isoamyl alcohol. After this, 2 ml of 95% ethanol was added to the aqueous phase, and the DNA was spooled out, washed in 80% ethanol, and air dried. The DNA was resuspended in 0.1x SSC (15 mM sodium chloride, 15 mM sodium citrate; pH 7.0). RNase was added to a final concentration of 1 mg/ml. The mixture was extracted once again with chloroform-isoamyl alcohol and centrifuged; the aqueous phase was transferred to another tube, and SSC was added (1x, final concentration). The DNA was then dissolved in 500 µl of TE. [30].

16S ribosomal RNA (rRNA) sequencing

Oligonucleotide primers with specificity for eubacterial 16S rRNA genes (forward primer 27: 5'-AGAGTTTGATC MTGGCTCAG3'and reverse primer 5'-GGTTACCTTGTT ACGACTT-3') were used to amplify 16S rDNA [31]. PCR fragments were purified using a Qiaquick Gel Extraction Kit (Qiagen, Valencia, CA) and an ABI 373A sequencer (MACROGENE Inc., Korea). Sequencing data was analyzed by comparison to 16S rRNA genes in the Ribosomal Database Project and EMBL-GeneBank databases, and aligned manually with the MEGA 4.1 Beta software.

PCR amplification

The PCR amplification was performed on a Gene Amp PCR System 9700 thermal cycler. The PCR amplification reaction mixtures consisted of template DNA (100 ng), 50 mM KCl, 10 mM Tris-HCl (pH9.0), 0.1 % (wt/vol), 1.5 mM $MgCl_2$ 100 nM primer, and 1.5 U of Taq polymerase (Promega). Amplification was performed with an initial denaturation step of 4 min at 94ºC and then 30 cycles of 1 min denaturation at 94ºC, 30 s at 57ºC for primer annealing, and 1.5 min at 70ºC for primer extension. A 7 min extension and cooling to 4ºC completed the reaction sequence.

Genotoxicity Test

To determine the genotoxic potential of the crude extracts of the active strains, the Umu test system was performed as recommended by Oda *et al.* [32-34]. This test system is a colorimetric method using *Salmonella typhimurium* NM2009 and NM3009 strains. At least a 2-fold or greater increase in ß-galactosidase activity above the control level was accepted as positive results for the extracts. The extracts were used in 10-5000 µg/ml concentrations. The results were compared with enzyme activity values provided by 4NQO substance used as positive mutagen (Fig 2).

All three experiments were performed simultaneously and the results are given as an average of the experiments.

RESULTS AND DISCUSSION

Determination of Antimicrobial and Insecticidal Activity

The ability of the actinomycetes to produce secondary metabolites that have biological activity such as antibiotic, antitumor, antiparasitic, herbicidal, insecticidal, and so forth is well documented [23]. It is known that some antimicro-

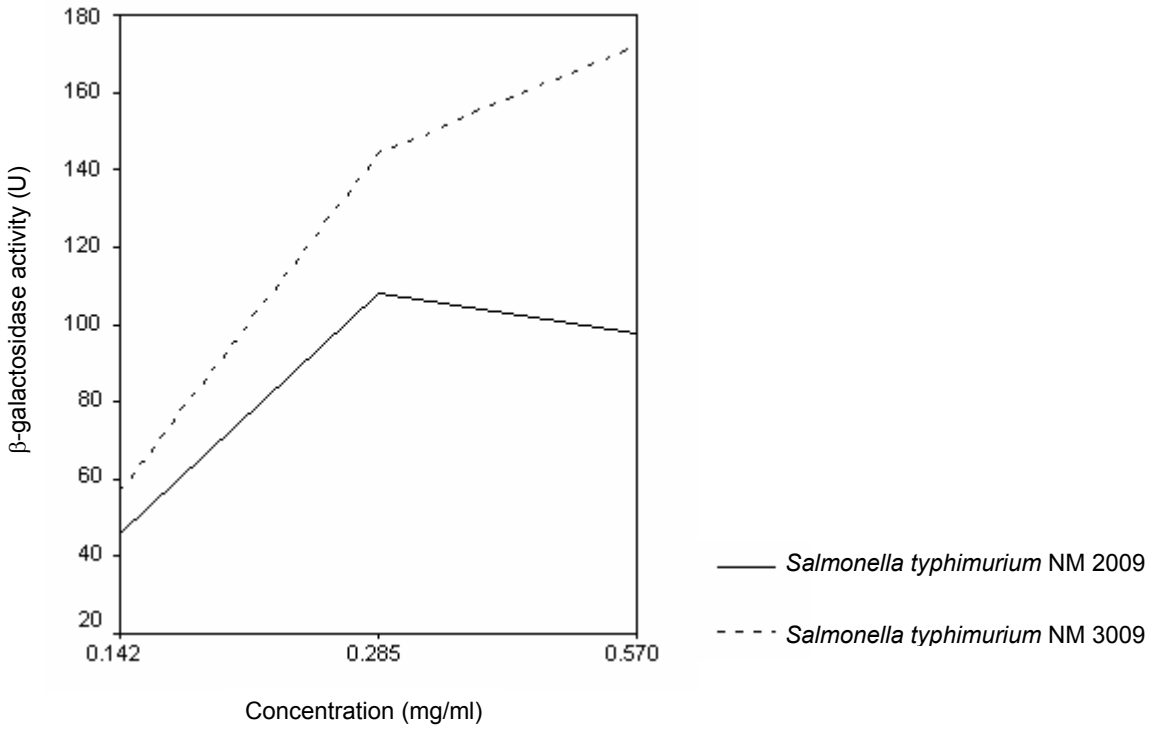

FIGURE 2 - β-galactosidase activity of positive mutagen (4NQO) in different concentrations

bial compounds also possess insecticidal activity like antimycin A, cycloheximide and sreptothricin [28]. As an alternative approach in this study, only bacterial isolates which were devoid of any antimicrobial activity were selected for the screening of the insecticidal activity to enhance the probability of discovering new insecticides. A total of 396 bacteria isolates were screened for their antimicrobial activity against test microorganisms. The isolates displaying weak or no activity, a total of 322, were selected for the insecticidal activity studies.

For control of the sensitivity and reliability of the used screening method, three insecticidal compounds and three standard strains producing insecticidal compounds were also included in the study. The results of the positive controls, known insecticidal drugs included in GAPY media and standard strains, are shown in Table 1 and 2, respectively. Cycloheximide and nikkomycin Z did not show any activity against adult flies at 100 µg / ml concentrations. Similarly, larvae of the *M. domestica* were tolerant to Antimycin A at the same concentration (Table 1). This data corroborates previous findings by Fabre et al. [28]. The standard control strains all showed insecticidal activity except *S. pactum* NRRL 2939 (Table 2).

TABLE 1 - Insecticidal activity of certain chemicals on *Musca domestica*

Chemicals	Activity on larvae (µg / ml)	Activity on adults (µg / ml)
Antimycin A	> 100[a]	20
Cycloheximide	30	>100
Nikkomycin Z	100	>100

[a] Minimal concentration to kill 60% of the larvae or shorten the lives of adult flies

TABLE 2 - Activity of standard strains against *Musca domestica*

Collection Strains	Products of strain	Activity on larvae	Activity on adults
Streptomyces pactum NRRL 5230	Piericidins	+	+
Streptomyces avermitilis NRRL 8165	Avermectins	+	+

+ : killing 60% of the larvae or shorten the lives of adult flies

TABLE 3 - Lethal effects of active isolates and positive controls on larvae and adult flies in the secondary screening

Experimental Groups	Mortality on larvae[a] (%)	Mortality on adult (%)	
	Topical application	Ingestion	Topical application
Isolate 39.02	100	60	100
Isolate 42.04	80	60	80
Cycloheximide	80	80	80
Antimycin A	100	80	90

[a] **All values were obtained from organic phases.**

Insecticidal activity is not a common property among bacterial isolates. In other screening studies, isolates with a proportion of insecticidal activity have been reported as 0.75% [28],) 1.39% [31], 2.3% [17], and 12.08% [14]. In the present study, only 2 of 322 isolates (39.02 and 42.04) were determined as active for insecticidal activity (0.62%) in the primary screening program. Both of the isolates have insecticidal activity, especially on *M. domestica* larvae. The mortality of isolates 39.02 and 42.04 was determined as 95% and 75% on *M. domestica* larvae, respectively. Both of the active isolates had a higher activity than those of the standard control strains. *S. avermitilis* NRRL 8165 and *S. pactum* NRRL 5230 showed only 62.5% and 2.5% activity respectively in the screening studies. The highest mortality percentages for insect larvae by actinomycetes isolates in other studies were reported as 80% [35], 30-60% [36], 30-100% [31] and 100% [12, 37]. Therefore, the mortality of our isolates 39.02 and 42.04 on *M. domestica* larvae can be accepted as satisfactory. On the other hand, isolates 39.02 and 42.04 did not cause the death of the pupae in the primary screening studies. All of the pupae were hatched into adult flies using these two isolates. They have not shown any activity on the hatching of the pupae.

Some abnormalities, such as very soft-bodied larvae, sclerotized and compressed larvae and perished larvae and the prolongations in larval duration were reported in a previous screening study [35]. In the current study, such abnormalities or prolongation were not observed in the larval duration. The insecticidal activity type for both of the active isolates was lethal.

Information about the type of activity and the solubility of the active compounds was obtained in the secondary phase screening studies. At this stage, topical application was performed on the larvae and adults and an ingesting test was performed on the adults only. Organic phases were determined as active on larvae and adult flies in all experiments. Water phases were not effective on larvae or adult flies in both topical applications and ingestion tests. Results obtained in the secondary phase experiments can be seen in Table 3. The insecticidal activity level of the active isolates were comparable to those of the control chemicals, cycloheximide and antimycin A. Isolate 39.02 yielded higher activity when compared to that of isolate 42.04 in the topical application. The lethal activity of isolate 39.02 was very impressive. All of the larvae and adult flies died by the topical application of the acetone solution of crude extract (40 mg/ml) of this isolate. In general, topical application was more effective than ingestion on adult flies. However, the flies were also in contact with the active compounds while drinking water. Therefore, the death of the flies may have occurred because of contact with active material during ingestion. We conclude that active components from these isolates might be developed as efficient and naturally-derived insecticides against dipteran pests in agricultural systems.

Phylogenetic analysis of isolates

The metabolites of microorganisms may be a viable alternative to synthetic insecticides as a part of integrated pest management endeavors. Bacteria have an important place among known secondary metabolites producers. There have been many reports concerning their insecticidal activity and different isolated compounds. Therefore, screening studies focusing on bacteria have priority for the isolation of unknown substances. The assignment of the isolates 42.04 and 39.02 to the genus *Streptomyces* and *Achromobacter* was supported by 16S rRNA. A comparison of the almost complete 16S rRNA gene sequence of the tested strains with corresponding streptomycete sequences from the GenBank database show that both isolates lay in the evolutionary clade of *Streptomyces* allied taxa (Fig. 3). A high similarity value (higher than 99%) has been observed in *Streptomyces* sp.42.04 (accession number GQ475300) in 16S rRNA gene sequences with *S. bikiniensis* NBRC 14598, *S. violaceorectus* NBRC 13102, and *S. cinereoruber* subsp. *cinereoruber* NBRC 12756 as well as *S. argenteolus* NBRC 12841.

The strain *Achromobacter* sp. 39.02 (accession number GQ475298) has been observed to have a high similarity value (higher than 98%) with *A. marplatensis* strain B2, 97% with *A. insolitus* LMG 6003 and *A piechaudii* EY3860, and 96% identity with *Bordetella avium* (AF177666).

The results indicate that there was no close relationship between the isolate strains and it is necessary to conduct further studies on sub species. Therefore, our work constitutes a contribution towards increasing the number of reports for *Streptomyces* and *Achromobacter* sequences in the database.

In the last decades, many insecticidal compounds from actinomycete strains have been isolated and characterized to control pests and parasites. Valinomycin [24], Respirantin [10], Indanomycin [12], Avidin and Streptavidin [38], Tartrolone C [39] and Meilingmycin [40] are just some of the samples for the insecticidal compounds isolated from actinomycetes. Avermectins and milbemycins are the most

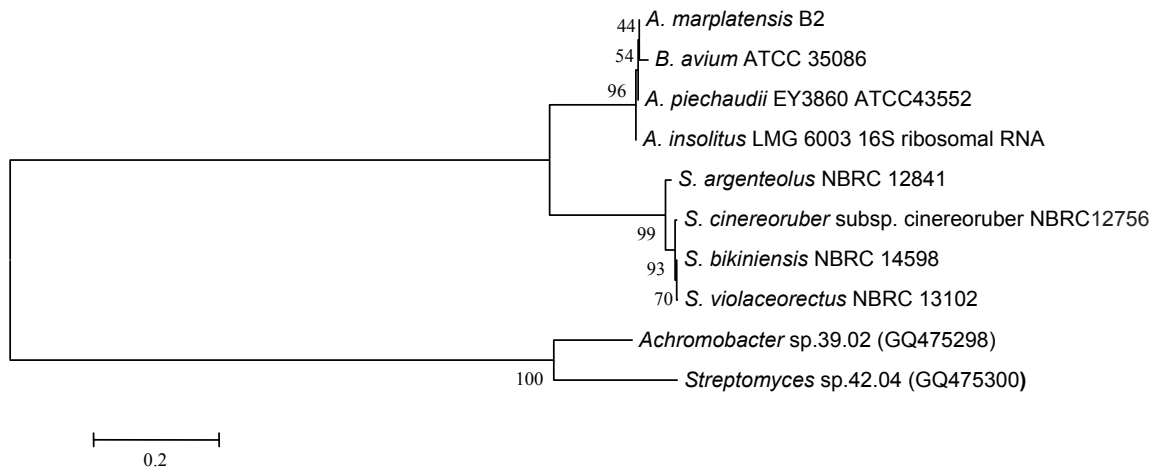

FIGURE 3 - Phylogenetic tree for 16S rRNA gene sequences of isolates 39.02 and 42.04 and representatives of the Streptomyces, Achromobacter and Bordetella available in the database

popular and commercially available bioinsecticides from actinomycetes.

The insect-pathogenic bacterium *Achromobacter nematophilus* is known as symbionts of insect-pathogenic nematodes of the *Steinernema* and *Heterorhabditis* families [41, 42]. It has been observed that after infecting an insect the nematode burrows through the intestinal wall of the insect and releases the bacteria into the hemolymph. The bacteria avoid the immune response of the insect, multiply rapidly and kill the insect host within 24-48 h, providing suitable conditions for nematode reproduction. Axenic nematodes can still infect an insect but are much less virulent than the *Xenorhabdus* sp.-carrying nematodes [41, 43]. Different toxins were purified from a culture broth of this insect-pathogenic bacterium [43, 44]. In addition, the larvicidal nature of a structural subunit of *X. nematophila* pilin was also reported [45].

Biological control studies are mostly focused on *Bacillus thuringiensis* isolates. Therefore, the biocontrol potential of our *Streptomyces* and *Achromobacter* isolates can be accepted as a first and important step in developing biological preparations against agricultural pests.

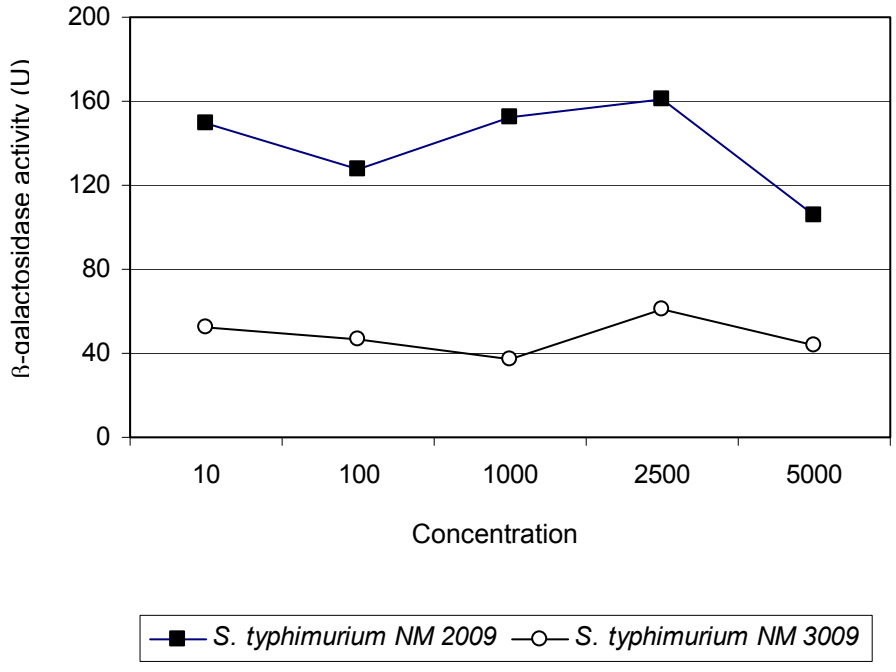

FIGURE 4 - β-galactosidase activity of isolate 39.02 crude extract in different concentrations

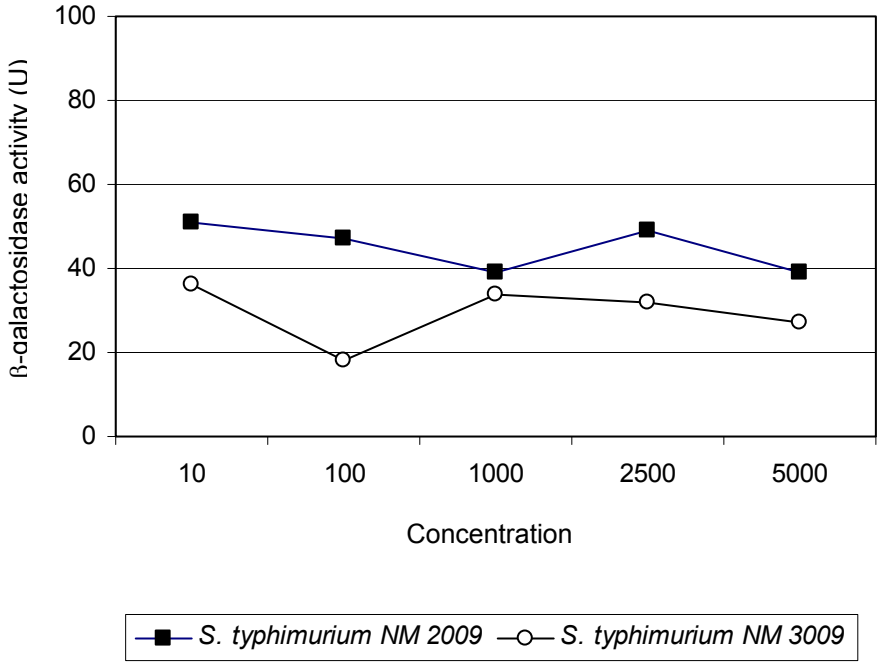

FIGURE 5 - β-galactosidase activity of isolate 42.04 crude extract in different concentrations.

Genotoxicity Test

We are in contact with agrochemicals directly or indirectly. Finding safe to use compounds to non-target organisms, low toxicity and short duration in the environment are the main goals of the new screening studies [35]. As a result, we have to know the toxicity level of the newly-isolated bioactive compounds. The method we used for the genotoxic investigation of the crude extracts of the active isolates is one of the short-time tests [32]. The values obtained as a result of the Umu test system are shown in Figures 4 and 5. As shown in these Figures, the fallen β-galactosidase activity was exhibited by both crude extracts. Therefore, both tested extracts cannot be defined as positive mutagenic agents due to the extracts showing β-galactosidase activity.

The HPLC profile of the crude extracts of the active isolates was very complex. However, we determined that the crude extracts of the isolates do not include the most popular insecticidal compounds produced by actinomycetes, avermectin, piericidin and valinomycin (unpublished data). Thus, in order to determine the produced bioactive metabolites, whether novel or otherwise, purification, characterization and identification of the compounds are needed. Further testing for purification and chemical characterization of insecticidal metabolites, optimization of the production conditions, and identification of the active isolates species level would be the focus of future studies.

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REFERENCES

[1] Elliott, M., Farnham, A.W., James, N.F., Needham, P.H., Pulman, D.A. and Stevenson, J.H. (1973) A photostable pyrethroid. *Nature*. 246, 169-170.

[2] Gebre Amlak, A. and Azerefegne, F. (1999) Insecticidal activity of chinaberry, endod and pepper tree against the maize stalk borer (Lepidoptera: Noctuidae) in southern Ethiopia. *Int. J. Pest. Manag*. 45, 9-13.

[3] Mier, N., Canete, S., Klaebe, A., Chavant, L. and Fournier, D. (1996) Insecticidal properties of mushroom and toadstool carpofores. *Phytochem*. 41(5), 1293-1299.

[4] Wang, M., Triguéros, V., Paquereau, L., Chavant, L. and Fournier, D. (2002) Proteins as active compounds involved in insecticidal activity of mushroom fruitbodies. *J. Economic Entomol*. 95(3), 603-607.

[5] Triguerosa, V., Lougarrea, A., Ali-Ahmeda, D., Rahbéb, Y., Guillot, J., Chavanta, L., Fourniera, D., and Paquereaua, L. (2003) *Xerocomus chrysenteron* lectin: identification of a new pesticidal protein. *Biochim Biophys Acta* 1621, 292– 298.

[6] Bucher, G.E. (1981) Identification of bacteria found in insects, In: Burges, H.D. (ed.). *Microbial control of pests and plant diseases 1970-1980*, Academic Press, London, pp. 7-33.

[7] Ohana, B., Margalit, J. and Barak, Z. (1985) A method for identifying colonies of bacteria possessing mosquito larvacidal effects. *Appl. Microbiol. Biotechnol.* 21, 250-251.

[8] Klein, M.G. (1988) Pest management of soil-inhabiting insects with microorganisms. *Agric. Ecosys. Environ.* 24, 337-349.

[9] Hanula, J.L. and Andreadis, T.G. (1988) Parasitic microorganisms of Japanese beetle (*Coleoptera*: *Scarabaeidae*) and associated scarab larvae in Connecticut soils. *Environ. Entomol.* 17, 709-714.

[10] Urushibata, I., Isogai, A., Matsumoto S. and Suzuki, A. (1993) Respirantin, A novel insecticidal cyclodepsipeptide from *Streptomyces. J. Antibiotics*. 46, 701-703.

[11] Sathiyamoorthy, P. and Shanmugasundaram, S. (1996) Preparation of cyanobacterial peptide toxin as a biopesticide against cotton pests. *Appl. Microbiol. Biotechnol*. 46, 511-513.

[12] Zhang, D., Nair, M.G., Murry, M. and Zhang, Z. (1997) Insecticidal activity of indanomycin. *J. Antibiotics.* 50, 617-620.

[13] Forsyth, G., and Logan, N.A. (2000) Isolation of *Bacillus thuringiensis* from Northern Victoria Land, Antarctica. *Lett. Appl. Microbiol.* 30, 263-266.

[14] Xiong, L., Li, J. and Kong, F. (2004) *Streptomyces* sp. 173, an insecticidal microorganisms from marine. *Lett.Appl. Microbiol.* 38, 32-37.

[15] Aslan, I., Çoruh, S., Özbek, H., Yaman, M. and Şahin, F. (2005) *Brevibacillus agri*, a pathogenic bacterium of *Malacosoma neustria* (Lepidoptera: Lasiocampidae). *Fresen. Environ. Bull*. 14 (2), 98-100.

[16] Katı, H., Sezen, K. and Demirbağ, Z. (2007) Characterization of a highly pathogenic *Bacillus thuringiensis* strain isolated from common cockchafer, *Melolontha melolontha. Folia Microbiol.* 52, 146-152.

[17] Hu, Z.Y., Liou, S.Z., Huang, Y.J., and Su, W.J. (2000) A rapid screening assay for insecticidal antibiotic produced by marine Actinomycetes. *Marine Sci. Bull.* 19, 36–41.

[18] Zhang, D.F. (1996) Recent developments in research and utilization of microorganisms. *J Anhui Agric. Sci.* 24 (Suppl.1), 44-46.

[19] Fuxa, J.R. and Kunimi, Y. (1997) Microorganisms interacting with insects. In: Hurst, C.J. (ed.) *Manual of Environmental Microbiology*, ASM Press, Washington DC, pp. 509–519.

[20] Leonard, G.C. and Julius, J.M. (2000) Review biopesticides: a review of their action, applications and efficacy. *Pest Manag. Sci.* 56, 651–676.

[21] Ertürk, Ö. and Demirbağ, Z. (2005) The first study on bacterial flora and biological control agents of *Yponomeuta malinellus* Zell. (*Lepidoptera*: *Yponomeutidae*) *Advances in Food Sciences*. 27 (2), 72-76.

[22] Liu, X. and Li, S. (2005) Fungal secondary metabolites in biological control of crop pests, In: An, Z. (ed.) *Handbook of Industrial Mycology*, Marcell Dekker, New York, p. 723.

[23] Desphande, B.S., Ambedkar, S.S. and Shewale, J.G. (1988) Biologically active secondary metabolites from *Streptomyces*. *Enzyme Microb. Technol.* 10, 455-473.

[24] Heisey, R.M., Huang, J., Mishra, S.K., Keller, J.E., Miller, J.R., Putnam, A.R. and D'Silva, T.D.J. (1988) Production of valinomycin, an insecticidal antibiotic, by *Streptomyces griseus* var. *flexipertum* var. nov. *J. Agric. Food Chem.* 36, 1283-1286.

[25] Shopotova, L.P. and Shenin, Y.D. (1993) New natural insecticide from actinomycetaceae *Streptomyces lipmani*. Isolation, physicochemical and biological properties and identification. *Russian J.Appl.Chem.* 66, 1074-1076.

[26] Demain, A. L. (1983) New application of microbial products. *Science*. 219, 709-714.

[27] Ishikawa, T., Date, M., Ishikura, T. and Ozaki, A. (1971) Improvement of kasugamycin-producing strain by the agar piece method and the prototroph method, *Folia Microbiol.* 16, 218-224.

[28] Fabre, B., Armau, E., Etienne, G., Legendre, F. and Tiraby G. (1988) A simple screening method for insecticidal substances from Actinomycetes. *J. Antibiotics*. 41, 211-219.

[29] Lechevalier, M.P. and Lechevalier, H.A. (1970) Chemical composition as a criterion in the classification of aerobic Actinomycetes. *Int. J. Sys. Bacteriol.* 20, 435-443.

[30] Kieser, T., Bibb, M.J., Buttner, M.J., Charter, K.F. and Hopwood, D.A. (2000). Practical Streptomyces genetics. The John Innes Foundation, Norwich, United Kingdom, pp. 170-171.

[31] Sujitwanit, A., Chunthong, P., Piluk, J., Tantithanagorngul, W., Tolieng, W.W., Palaga, P., Sangvanich, P., Petsom, A. and Pinphanichakarn, P. (2007) Screening for insecticide-producing *Streptomyces* isolates from Thai soil. *The 19th Annual Meeting of the Thai Society for Biotechnology "TSB2007: Biotechnology for Gross National Happiness"* pp. 245-250.

[32] Oda, Y., Yamazaki, H., Watanabe, M., Nohmi, T. and Shimada, T. (1993) Highly sensitive Umu test system for the detection of mutagenic nitroarenes in *Salmonella typhimurium* NM 3009 having high o-acetyltransferase and nitroreductase activites, *Environ. Mol. Mutagen.* 21, 357-364.

[33] Oda, Y., Yamazaki, H., Watanabe, M., Nohmi, T. and Shimada, T. (1995) Development of high sensitive Umu test system. *Mutation Res.* 334, 145-156.

[34] Oda, Y., Yamazaki, H., Shimada, T. (1999) Role of human N-acetyltransferases, NAT1 or NAT2, in genotoxicity of nitroarenes and aromatic amines in *Salmonella typhimurium* NM 6001 and NM 6002. *Carcinogenesis*. 20 (6), 1079-1083.

[35] Ghazal, S.A., Bream, A.S., Abd El-Aziz, Z.K. and Ibrahim, S.Y. (2001) Preliminary studies on insecticidal activity of Actinomycete strains propagated on solid and broth media using *Musca domestica* (Diptera: Muscidae). *Med. Fac. Landbouww. Univ. Gent*. 66/2b, 559-570.

[36] Bream, A.S, Ghazal, S.A., Abd El-Aziz Z.K. and Ibrahim S.Y. (2001) Insecticidal activity of selected Actinomycete strains against the Egyptian cotton leaf worm *Spodoptera littoralis* (Lepidoptera: noctuidae). *Med. Fac. Landbouww. Univ. Gent.* 66/2a, 503-512.

[37] Reddy, K., Ewett, G., Fatig, III R., Brockman, M., Hatton, C., Savickas, P. and Hashaá, H. (1991) New insecticidal metabolites from soil isolate W719. *J. Antibiotics*. 44 (9), 962-968.

[38] Markwick, N.P., Christeller, J.T., Dochterty L.C. and Lilley, C. M. (2001) Insecticidal activity of avidin and streptavidin against four species of pest Lepidoptera. *Entomologia Experimentalis et Applicata*. 98, 59-66.

[39] Lewer, P., Chapin, E.L., Graupner, P.R., Gilbert, J.R. and Peacock, C. (2003) Tartrolone C: A novel insecticidal macrodşolide produced by *Streptomyces* sp. CP1130. *J. Nat. Prod.* 66, 143-145.

[40] Zhuang, Y.P., Chen, B., Chu, J. and Zhang, S. (2006) Medium optimization for meilingmycin production by *Streptomyces nanchangensis* using Response Surface Methodology. *Process Biochem*. 41, 405-409.

[41] Poinar, G. O. and G. M. Thomas (1966) Significance of *Achromobacter nematophilus* in the development of the nematode, DD136 (*Neoaplectana* sp. *Steinernematidae*). *Parasitology*. 56, 385-390.

[42] Thomas, G. M. and G. O. Poinar (1979) *Xenorhabdus* gen. nov., a genus of entomopathogenic nematophilic bacteria of the family *Entomobacteriaceae*. *Int. J. Syst. Bacteriol.* 29, 352-360.

[43] Ryu, K.G., Bae, J.S., Yu, Y.S. and Park, S.H. (2000) Insecticidal Toxin from *Xenorhabdus nematophilus*, symbiotic bacterium associated with entomopathogenic nematode *Steinernema glaseri* . *Biotechnol. Bioprocess Eng*. 5, 141-145.

[44] Brown, S.E., Cao, A.T., Hines, E.R., Akhurst, R.J. and East, P.D. (2004) A novel secreted protein toxin from the insect pathogenic bacterium *Xenorhabdus nematophila*. *J Biol Chem.* 279, 14595–14601.

[45] Khandelwal, P., Choudhury, D., Birah, A., Reddy. M.K., Gupta, G.P. and Banerjee, N. (2004). Insecticidal pilin subunit from the insect pathogen *Xenorhabdus nematophila*. *J. Bacteriol*. 186 (19), 6465–6476.

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