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Insect Control Using Chitinolytic Soil Actinomycetes as Bio-control Agents

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ABSTRACT

Several actinomycetes species were isolated from United Arab Emirates soil using conventional microbiological techniques. Out of thirty-eight isolates, only three were selected according to their high production of the chitinase enzyme. The three isolates were identified to the species level using cultural, physiological, biochemical and chemotaxonomical characteristic. Isolates 1, 7 and 40 were identified as *Actinoplanes philippinensis*, *Actinoplanes missouriensis* and *Streptomyces clavuligerus*, respectively. Chitinolytic activities of the three isolates were tested against *Drosophila melanogaster* development as percentage of successful pupal formation. The three actinomycetes were individually applied against *D. melanogaster*, and then in combinations of two and all three actinomycetes isolates were used to study synergistic and antagonistic effects between them. The application of either *A. philippinensis* or *A. missouriensis* gave a good effect, shown as lowest pupal formation percentages, being 39.43 and 31.75%, respectively. At the same time, *S. clavuligerus* gave the least effect, being 55.71% compared to control treatment. In the combination of two isolates experiment, *S. clavuligerus*, which gave the least activity when applied individually, was synergized by the presence of *A. philippinensis* (27.35%) and *A. missouriensis* (33.24%). Meanwhile, the combination of *A. philippinensis* and *A. missouriensis*, gave an indication of antagonism (51.06%) shown as high percentage of successful pupal formation. The combination of all of three isolates experiment showed no antagonism (37.47 ± 2.48%).

Key Words: Biological control; Actinomycetes; Insects; *Drosophila;* Chitinase; Synergism

INTRODUCTION

As the environmental contamination by toxic chemicals increases, alternative approaches for controlling pest populations have become research priorities. These have included biological or ecological control methods for limiting the destructive impacts of pest populations, especially in agriculture (Nakas & Hagedorn, 1990; Canaday, 1995; Hokkanen & Lynch, 1995).

Several varieties of microorganisms including fungi, bacteria, nematodes and viruses that are antagonistic to insects have been reported as strategies to biologically control them. Actinomycetes play an important role in the biological control of insects through the production of insecticidally active compounds against the house fly *Musca domestica* (Hussain *et al.,* 2002). The mortality of larval and pupal stages, were very high reaching up to 90% after actinomycetes treatments (Hussain *et al.,* 2002). Actinomycetes were effectively used against *Culex quinquefasciatus* (Sundarapandian *et al.,* 2002).

Actinomycetes are an important group of microorganisms, not only as degraders of organic matter in the natural environment, but also as producers of antibiotics and other useful compounds of commercial interest (Saugar *et al.,* 2002; Bentley *et al.,* 2002; Basilio *et al.,* 2003). In addition, actinomycetes are important for the production of enzymes, such as chitinase (eg. *Streptomyces viridificans*), cellulases (eg. *Thermonospora* spp.), peptidases, proteases (*Nocardia* spp.), Xylanases *(Microbispora* spp.), ligninases (*Nocardia autotrophica*), amylases (*Thermomonospora curvata*), sugar isomerases (*Actinoplanes missouriensis*), pectinase, hemicellulase and keratinase (Solans & Vobis, 2003).

To select non-streptomycete actinomycetes by reducing the numbers of streptomycete actinomycetes on isolation plates, *Streptomyces* phages was applied (Kurtböke *et al.,* 1992; Long & Amphlett, 1996). The isolation of *Streptomyces* phages are of practical importance for a variety of reasons such as the problems they cause to fermentation industries (Chater, 1986), their value for typing streptomycetes in taxonomic studies (Korn-Wendish & Schneider, 1992), their use for the detection and understanding of host controlled restriction-modification systems (Diaz *et al.,* 1989), their utilization as tools for genetic exchange and analysis in *Streptomyces* spp. (Herron & Wellington, 1990), the study of their general and molecular biology (Lomovskaya *et al.,* 1980) and ecology (Williams *et al.,* 1987).

Chitinase is originally an enzyme used by insects to degrade the structural polysaccharide "chitin" during the molting process (Zhang *et al.,* 2002). The largest chitinase activity among bacteria has been observed in species of

Streptomyces, Serratia, Vibrio and *Bacillus* (Reguera & Leschine, 2001). Chitinase enzyme is very important in the biological control of insects (Reguera & Leschine, 2001) and plant pathogenic fungi (El-Tarabily *et al.,* 2000; El-Tarabily, 2003).

Species of *Streptomyces* showed high multiplicity of chitinase genes (Williamson *et al.,* 2000; Saito *et al.,* 2003), as in the case of *Streptomyces coelicolor* and *Streptomyces griseus* (Itoh *et al.,* 2003). However, screens for antagonism have focused primarily on bacteria, fungi, viruses and nematodes (Collier *et al.,* 2001). There is a lack of published information with regard to the use of actinomycetes particularly, rare non-streptomycete actinomycetes, as biocontrol agents of insect pests.

Accordingly, the major objectives of the present study were to: (i) isolate streptomycete actinomycetes using the normal soil dilution plate method; (ii) isolate non-streptomycete actinomycetes using the *Streptomyces* phage method; (iii) screen the isolates for their ability to produce chitinase and ß-1,3 glucanase; (iv) apply the most promising chitinase producing actinomycetes as bio-control agents on *D. melanogaster* under laboratory conditions; and (vi) identify the most promising chitinase producing actinomycetes using conventional identification methodologies.

MATERIALS AND METHODS

Isolation of streptomycete and non-streptomycete actinomycetes from tomato rhizosphere soil. Rhizosphere soil samples were collected from tomato fields at ten random sites from Al-Ain city, 140 Kilometers east of Abu Dhabi, United Arab Emirates and were placed into plastic bags. The soil collected was a light brownish yellow sandy soil. In the laboratory, soil samples were mixed to ensure uniformity, passed through a 3-mm sieve to remove debris and stored in sealable plastic bag at 15°C. Soil pH was found to be 7.5 (0.01 *M* $CaCl_2$).

For the isolation of streptomycete actinomycetes, three 10 g replicates of each rhizosphere soil sample were dispensed into 100 mL of sterile 1 g L^{-1} agar solution in deionized water containing 20 g glass beads (3 mm in diameter). The soil suspension was placed in an ultra-sonicator (Virsonic 60, the Virtis Company, Inc., Gardiner, NY, USA) at a frequency of 55,000 cycles sec^{-1} for 20 sec, and then shaken on a gyratory shaker (Model D-30938, Gesellschaft Für Labortechnik mbH, Burgwedel, Germany) at 250 rpm for 30 min at 28°C. Ten-fold serial dilutions (10^{-2} – 10^{-5}) were made in sterile deionized water and 0.2 mL aliquots were spread with a sterile glass rod over the surface of inorganic salt starch agar (SNA) in sterile plastic, 9 cm diameter Petri-plates. Cooled (45°C) agar was amended with cycloheximide (50 mg L^{-1}) and nystatin (Sigma Chemical Co., St. Louis, USA) (50 mg L^{-1}) immediately prior to pouring plates. Ten plates were used per dilution and dried in a laminar flow-cabinet for 20 min before incubation at 30°C in the dark for 10 days.

In order to facilitate the recovery of non-streptomycete actinomycetes, three polyvalent *Streptomyces* phages (El-Tarabily *et al.,* 1996) were used to reduce the numbers of *Streptomyces* species in the dilution tubes and in turn on isolation plates (Kurtböke *et al.,* 1992). The phage suspension was prepared by combining high-titre phage suspension (X 10^{12} pfu mL^{-1}) of each polyvalent *Streptomyces* phages. A second series of five plates was inoculated with 0.2 mL aliquots of the phage-treated soil suspension, dried in a laminar flow cabinet and incubated for 3 weeks in the dark at 30°C. Plates without phages were used as control treatments.

All isolates were transferred onto oatmeal agar plates supplemented with 0.1% yeast extract (OMYEA) (Williams & Wellington, 1982) and stored in 20% (v/v) glycerol at -20°C (Wellington & Williams, 1978).

Qualitative determination of chitinase production by actinomycetes. The aim of this experiment was to screen the streptomycete and non-streptomycete actinomycete isolates for their capacity to produce chitinase enzyme on colloidal chitin agar (CCA) (Gupta *et al.,* 1995). Five-day-old isolates grown on (SNA) were inoculated onto CCA and incubated for 5 days until zones of chitin clearing were seen around and beneath the colonies. Clear zone diameters were measured (mm) and used as an indicator of chitinase activity. Large diameters represented high activity and small diameters represented low activity. According to the results obtained from the qualitative test, three highly active chitinase-producing isolates (HC) were selected.

Quantitative determination of chitinase and ß-1,3-glucanase. Individual 250-mL Erlenmeyer flasks containing 50 mL of minimal synthetic medium (MSM) (Tweddell *et al.,* 1994) amended with 2 mg mL^{-1} of colloidal chitin were prepared. Flasks containing colloidal chitin were inoculated with 2 mL of a 20% glycerol suspension of each actinomycete isolate and incubated on a rotary shaker at 250 rpm for 7 days. After incubation, the suspensions from each flask were centrifuged for 30 min at 12000 *g*. The supernatant was filtered through sterile Millipore membranes (pore size 0.22 µm, Millipore Corporation, MA, USA), collected in sterile tubes and was used as a source of the crude enzymes.

Chitinase specific activity was determined according to the method described by Tweddell *et al.* (1994) and modified by Singh *et al.* (1999). Chitinase activity was calculated by measuring the release of N-acetyl-D-glucosamine (NAGA). The reaction mixture contained 1 mL of culture supernatant from a colloidal chitin amended MSM and 1 mL of colloidal chitin (10 mg mL^{-1}) in 50 m*M* sodium acetate buffer at pH 6.8 and was incubated in a water bath for 1 h at 50°C. After boiling for 15 min the mixture was centrifuged at 2500 *g* for 20 min. The concentration of NAGA in the supernatant was determined by the procedure of Reissig *et al.* (1955). Specific activity (U = 1 unit of chitinase) was defined as the amount of the

enzyme that released 1 µmol of NAGA mg^{-1} protein h^{-1}.

The specific activity of ß-1,3-glucanase was determined by measuring the amount of reducing sugars liberated using dinitrosalicylic acid solution (DNS) (Miller, 1959). The reaction mixture contained 1 mL of laminarin solution (Sigma) (10 mg mL^{-1}) in 0.2 *M* acetate buffer (pH 5.4). The mixture was incubated in a water bath at 40°C for 1 h and the reaction terminated by adding 3 mL of DNS solution. Boiling for 10 min developed the color of the end product. Reducing sugar concentration was determined by optical density at 530 nm using a scanning spectrophotometer (UV-2101/3101 PC; Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan). Glucose was used as the calibration standard. Specific activity (U=1 unit of ß-1,3-glucanase) was defined as the amount of the enzyme that released 1 µmol of glucose mg^{-1} protein h^{-1}. The protein content of the enzyme solution was determined with the Folin phenol reagent (Lowry *et al.,* 1951).

Identification of the chitinase-producing actinomycete genera to species level. Identification of the three actinomycetes to species level was based on morphological, cultural, physiological and chemotaxonomical characteristics as presented in Bergy's Manual of Systematic Bacteriology (Williams *et al.,* 1989).

Collection and rearing of *D. melanogaster*. Wild type *Drosophila melanogaster* was collected using fermented fruit traps**.** All *Drosophila* stages were kept under 25 ± 2 ºC (Strickberger, 1962).

Collection and counting of *Drosophila* eggs. *Drosophila* eggs were collected by covering solidified media with muslin fabric. Adult male and female *Drosophila* were allowed into the bottle for oviposition on the muslin fabric. Insects were left for 24 h and then transferred to a fresh culture bottle. The fabrics with the medium were carefully removed and the muslin with the eggs was lifted. A female *Drosophila* may lay about 50-75 eggs/day (Strickberger, 1962). Eggs were counted using binocular microscope and were gently removed with a spatula.

Application of actinomycetes on insects. The three most promising actinomycete isolates (coded: 1, 7, & 40), which produced the highest level of chitinase activity, were tested to evaluate their potential and ability to inhibit the growth and development of *Drosophila* under controlled laboratory conditions. The three actinomycete isolates were included singly or in various combinations with each other to determine whether biological control could be enhanced by combining these actinomycete isolates.

In total, there were fifteen treatment combinations, as follows:

(1) Water control (insect only),
(2) *Drosophila* + autoclaved actinomycete number 1,
(3) *Drosophila* + actinomycete number 1,
(4) *Drosophila* + autoclaved actinomycete number 40,
(5) *Drosophila* + actinomycete number 40,
(6) *Drosophila* + autoclaved actinomycete number 7,
(7) *Drosophila* + actinomycete number 7,
(8) *Drosophila* + autoclaved actinomycete 7 + autoclaved actinomycete 40,
(9) *Drosophila* + actinomycete 7 + actinomycete 40,
(10) *Drosophila* + autoclaved actinomycete 1 + autoclaved actinomycete 7,
(11) *Drosophila* + actinomycete 1 + actinomycete 7,
(12) *Drosophila* + autoclaved actinomycete 1 + autoclaved actinomycete 40,
(13) *Drosophila* + actinomycete 1 + actinomycete 40,
(14) *Drosophila* + autoclaved actinomycete number 1 + autoclaved actinomycete number 40 + autoclaved actinomycete number 7,
(15) *Drosophila* + actinomycete number 1 + actinomycete number 40 + actinomycete number 7.

For application, actinomycetes were cultured on OMYEA and incubated at 30ºC until sporulation. Spores were then suspended in 10 mL of sterilized distilled water by scraping each plate separately. Controls for each actinomycete isolate were prepared by taking the same amount of autoclaved spore suspension.

After preparing the spore suspensions, seven replicates (each contain 7 mL) for each active and heat-killed (autoclaved) actinomycetes were added to 1.5 g of *Drosophila* media and mixed thoroughly. *Drosophila* eggs were counted as described above and placed gently on the prepared spores + media. Experiments were kept at 25ºC for approximately 7 days, until pupation. Successful pupations were counted at the end of the experiment.

Statistical analysis. All treatments were arranged in a randomized complete block design for all experiments. Data were subjected to analysis of variance (ANOVA) and significant differences between means were determined using Fisher's Protected LSD Test at $P = 0.05$. Superanova® (Abacus Concepts, Inc., Berkeley, California, USA) was used for all analyses.

RESULTS

Isolation and enumeration of streptomycete and non-streptomycete actinomycetes from tomato rhizosphere soil. Twenty-three *Streptomyces* isolates were isolated from SNA plates without the application of *Streptomyces* phages, whilst fifteen non-streptomycete actinomycetes were isolated from SNA plates with the application of *Streptomyces* phages. The use of *Streptomyces* phages with high polyvalency significantly ($P<0.05$) reduced the numbers of streptomycetes on the isolation plates with a concurrent increase in the numbers of non-streptomycete actinomycetes isolated (Table I).

Qualitative determination of chitinase production by actinomycetes. Of the thirty-eight isolates, only three isolates (one streptomycete and two non-streptomycete actinomycetes) were ranked as HC isolates (Fig. 1) and chosen for further study. These isolates produced large clear zones on CCA (Figs. 2, 3, 4). These three isolates (Isolates

Fig. 1. Plates of colloidal chitin agar (CCA) inoculated with actinomycete isolates with large clear zones of chitinase enzyme production

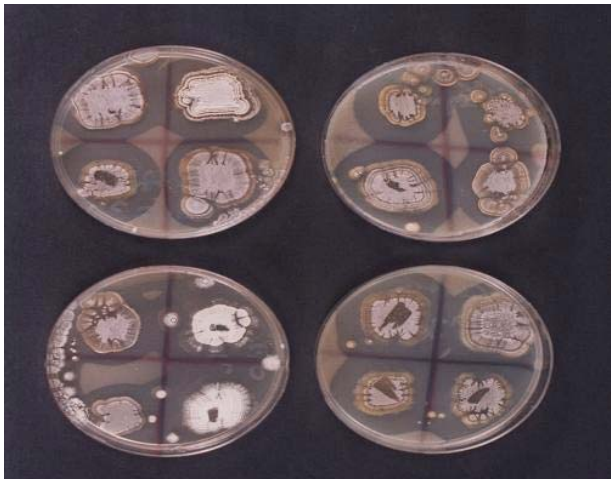


Fig. 2. A plate of colloidal chitin agar (CCA) inoculated with isolate # 1 with clear zone of chitinase enzyme production

1, 7 and 40) were chosen for further studies.

Quantitative determination of chitinase and ß-1,3-glucanase. The three most promising isolates, coded 1, 7 and 40 grew well on the MSM containing colloidal chitin, and laminarin. Their chitinase specific activities were 5.23U, 4.77U and 4.65U, whilst the ß- 1-3 glucanase specific activities were 0.52U, 0.44U and 0.39U, respectively.

Identification of isolates 1, 7 and 40. Identification of the three actinomycetes to species level was based on morphological, cultural, physiological and chemotaxonomical characteristics. Isolate 1 was identified as *Actinoplanes philippinensis* Couch, while isolate 7 was identified as *Actinoplanes missouriensis* Couch. In the mean time, isolate 40 was identified as *Streptomyces clavuligerus* Higgens and Kastner.

Fig. 3. A plate of colloidal chitin agar (CCA) inoculated with isolate # 7 with clear zone of chitinase enzyme production

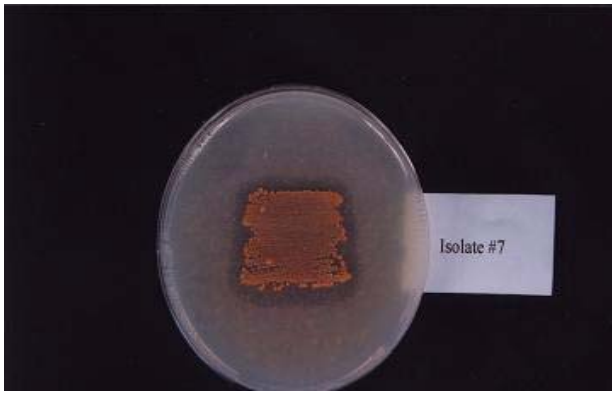
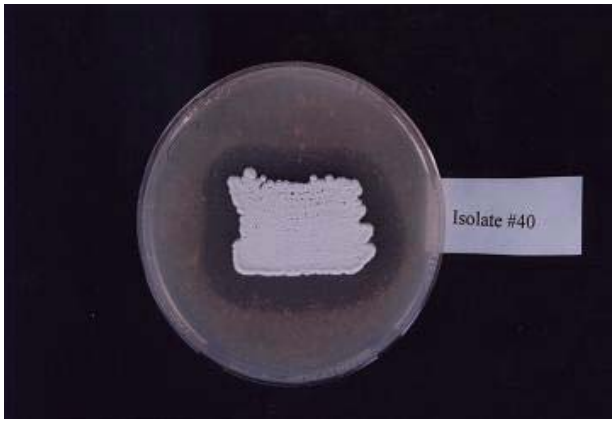


Fig. 4. A plate of colloidal chitin agar (CCA) inoculated with isolate # 40 with clear zone of chitinase enzyme production



Application of the most promising chitinase producing streptomycete and non-streptomycete actinomycetes as insect biological control agents. The most effective biocontrol treatment of *D. melanogaster* was the synergistic interaction of *A. philippinensis* and *S. clavuligerus* (isolates 1 and 40), which reduced pupal formation by 27.35 ± 3.95%. When used individually, *A. philippinensis*, *A. missouriensis* and *S. clavuligerus* reduced pupal formation by 39.43 ± 2.06%, 31.75 ± 3.79% and 55.71 ± 5.56%, respectively. When *A. philippinensis* and *A. missouriensis* (isolates 1 and 7) were combined, an antagonistic interaction was observed with 51.06 ± 5.15% of pupal formation compared to when *A. philippinensis*, alone (39.43 ± 2.06%) and *A. missouriensis* alone (31.75 ± 3.79%) (Table II) were used. When all three isolates were used in combination, the pupal formation was 37.47 ± 2.48% (Table II).

There were no significant differences between pupal formation in the water control (91.13 ± 3.15%), the autoclaved spores of *S. clavuligerus* (83.09 ± 5.61%) and *A. missouriensis* (89.57 ± 2.68%; Table II). However, the

Table I. Comparison of colony forming units (cfu) of streptomycete and non- streptomycete actinomycetes from starch nitrate agar plates with and without the treatment with polyvalent *Streptomyces* phages.

Number of colony forming units of streptomycetes			
Without phage		With phage	
cfu / plate	cfu / g. dry weight soil	cfu / plate	cfu / g. dry weight soil
42.35 ± 2.25*	$10.52 \times 10^3 \pm 1.22$	9.31 ± 1.1	$1.44 \times 10^3 \pm 0.35$
Number of colony forming units of non- streptomycete actinomycetes			
5.41 ± 0.81	$1.03 \times 10^3 \pm 0.13$	13.4 ± 1.25	$3.21 \times 10^3 \pm 0.45$

(*) Values are means ± the standard error. The reduction of streptomycetes and the increase of non-streptomycete actinomycetes in soil after treatment with the polyvalent *Streptomyces* phages were significant (P<0.05) using Fisher's protected PLSD Test.

Table II. A comparison between the applications of actinomycetes, *Actinoplanes philippinensis* (isolates 1), *Actinoplanes missouriensis* (isolate 7) and *Streptomyces clavuligerus* (isolate 40), individually, in combinations of two and all three together on the fruit fly *Drosophila melanogaster*. Controls were autoclaved spores suspended in the same amounts of distilled water. A water control was included for proper comparison. Statistical analysis shows Means ± Standard Errors. All treatments were significantly ($P < 0.05$) different from their controls

Treatments	Pupal Formation (%)
Water control (insect only)	91.13 ± 3.15 [f]
Drosophila + autoclaved actinomycete 1	73.81 ± 2.72 [de]
Drosophila + actinomycete 1	39.43 ± 2.06 [b]
Drosophila + autoclaved actinomycete 40	83.09 ± 5.61 [ef]
Drosophila + actinomycete 40	55.71 ± 5.56 [c]
Drosophila + autoclaved actinomycete 7	89.57 ± 2.68 [f]
Drosophila + actinomycete 7	31.75 ± 3.79 [ab]
Drosophila + autoclaved actinomycetes 7 + 40	81.78 ± 5.75 [ef]
Drosophila + actinomycetes 7 + 40	33.24 ± 2.97 [ab]
Drosophila + autoclaved actinomycetes 1 + 7	87.29 ± 2.62 [f]
Drosophila + actinomycetes 1 + 7	51.06 ± 5.15 [c]
Drosophila + autoclaved actinomycetes 1 + 40	69.04 ± 5.01 [d]
Drosophila + actinomycete 1 + 40	27.35 ± 3.95 [a]
Drosophila + autoclaved actinomycetes 1 + 40 + 7	85.93 ± 5.09 [f]
Drosophila + actinomycetes 1 + 40 + 7	37.47 ± 2.48 [ab]

Values with the same letter are not significantly (P> 0.05) different according to Fishers protected LSD test.

autoclaved spores of *A. philippinensis*, did reduce the amount of pupa formed (73.81 ± 2.72%) compared to the water control (91.13 ± 3.15%).

The application of individual isolates is presented in Table II. The water control gave 91.13 ± 3.15%. Autoclaved spores controls data are shown as the percentage of successful pupal formation being 73.81 ± 2.72% for *A. philippinensis*, 83.09 ± 5.61% for *S. clavuligerus* and 89.57 ± 2.68 for *A. missouriensis*. The active spores of *A. philippinensis* and *A. missouriensis* gave the lowest pupal formation percentages, 39.43 ± 2.06% and 31.75 ± 3.79%, respectively. *S. clavuligerus* was the least effective of the three giving 55.71 ± 5.56% reduction in pupal formation compared to control treatments.

To study the synergistic or antagonistic effects of the three actinomycetes, isolates were introduced to the insect medium in a combination of treatments of two and all three together. The data of this experiment are shown in Table II. *S. clavuligerus* which gave the least activity when applied individually was synergized by the presence of *A. philippinensis* (27.35 ± 3.95) and *A. missouriensis* (33.24 ± 2.97).

Meanwhile, the combination of *A. philippinensis* and *A. missouriensis*, gave an indication of antagonism (51.06 ± 5.15) shown as high percentage of successful pupal formation. Whilst, the application of all isolates together showed no antagonism (37.47 ± 2.48) compared to control treatments.

A highly significant (at $P < 0.05$) difference between the combination treatment of isolates *A. philippinensis* + *S. clavuligerus* (27.35 ± 3.95) and all the other treatments was observed. This makes this combination, the best treatment of all.

DISCUSSION

The present study appears to be a first record of an insect being controlled by a rare slow-growing chitinolytic non-streptomycete actinomycete belonging to genus *Actinoplanes*. The two isolates of *A. philippinensis* and *A. missouriensis* used in the present study produced high levels of chitinase and were capable of reducing the insect population under laboratory conditions. Meanwhile, the inability of the autoclaved preparation of each chitinase-producing isolate to kill the insect indicates that the reduction in the insect population by the chitinase-producing isolates may be associated with their chitinase production.

The process of cuticular chitin deposition is coordinated with the ecdysteroid regulated molting (ecdysis) during insect metamorphosis. Major protein subunits of the chitin-synthase were proven to be integral membrane proteins on the epidermal cell layer underlying the procuticle region of the integument in insects (Tellam *et al.*, 2000).

Chitin synthase genes in an insect like *Drosophila* were elucidated by Gagou *et al.* (2002) where they identified them (DmeChSA and DmeChSB) to be on the other side of centromere of the third chromosome. Chitin formation inhibition is usually achieved either by affecting the catalytic site of the synthase or by interfering at the sulfhydril-sensitive sites of the synthase during polymerization of the ß-1,4-*N*-acetyl-D-glucosamine residues. Whilst, chitin formation can be inhibited by diverse groups of compounds (cyromazine) and can generally be degraded rapidly by chitinase group of enzymes (Zhang *et al.*, 2002; Tripathi *et al.*, 2002).

In the present study, the actinomycetes *A. philippinensis* (isolate 1), *A. missouriensis* (isolate 7) and *S. clavuligerus* (isolate 40) were effective producers of chitinase and caused extensive reduction in *D. melanogaster*

population under controlled laboratory conditions. In addition, all three isolates were effective against the insect when applied individually or in combinations, and significantly reduced the percentage of pupal formation under the same conditions.

Since the cuticle of insect species consists largely of chitin, it was postulated that chitinase produced by these isolates could be involved in insect control. Therefore, the production of chitinases was used as the criteria for the selection of potential biocontrol agents of insects. Microbial chitinolytic enzymes have been considered important in the biological control of many insects because of their ability to interfere with chitin deposition (Tripathi *et al.,* 2002).

The application of chitinase producing actinomycetes to the rearing medium of the fruit fly, *D. melanogaster*, had a significant effect on their mortality. The actinomycete isolates were all considerably effective compared to their controls. Both *A. phlippinensis* and *A. missouriensis* have significantly reduced insect pupal formation when applied to the medium individually. Similar data were generated by Regev *et al.* (1996), Bream *et al* (2001) and Sampson and Gooday (1998) on their work on the effect of the endogenous chitinase activity of Bt against caterpillars of *Spodoptera litoralis* and the larval midges of *Culicoides nubeculosus*. In the present study, synergistic or antagonistic effects of the three actinomycetes isolates alone or in combinations showed that *S. clavuligerus*, which gave the least individual activity was synergized in the presence of *A. philippinensis* or *A. missouriensis*. In contrast, the combination of *A. philippinensis* and *A. missouriensis* was antagonistic as shown by the high percentage of successful pupal formation. There was no antagonism observed when the three actinomycetes were applied together.

Most studies that investigated the activities of soil actinomycetes, have mainly examined the role of streptomycete actinomycetes. In contrast, our study highlights the importance of using selective isolation techniques, such as the use of polyvalent *Streptomyces* phages, for the selective isolation of non-streptomycete actinomycetes and to investigate their effect on insect development. Apart from actinomycetes obtained by the application of the conventional soil dilution method, other rare slow-growing non-streptomycete actinomycetes were easily obtained using the phages technique. It is important to note that the two techniques mentioned above yielded different genera of actinomycetes. The use of the phages techniques in the present study, clearly indicate that the application of phage increased the recovery of *Actinoplanes*, *Micromonospora*, *Rhodococcus*, and *Nocardia* species, whilst the majority of the colonies obtained by the soil dilution method were found to belong to the genus *Streptomyces*.

Although this study has concentrated on actinomycetes at the expense of other groups of potential microbial biocontrol agents such as bacteria, fungi and viruses, it has yielded some interesting information on the taxonomy and biological activity of streptomycete and non-streptomycete actinomycetes. This study highlights the potential of using chitinase-producing actinomycetes for the biological control of insects having chitin as a major component of their cuticle in the Arabian Gulf area or elsewhere.

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