PATHOGENECITY OF TWO BEAUVERIA BASSIANA INDIGENOUS ISOLATES TOWARDS THE GREATER WAX MOTH GALLERIA MELLONELLA L. LARVAE IN EGYPT

Alia Zayed
Entomology Department, Faculty of Science, Cairo University, Giza, Egypt

ABSTRACT

Bioassays were carried out in the laboratory to determine the virulence of two Beauveria bassiana indigenous isolates on the greater wax moth Galleria mellonella. LC$_{50}$ and LT$_{50}$ values were taken as indices for the efficacy of fungal isolates, designated as Bb1 and Bb2, collected from Ismailia governorate in Egypt and isolated from soil and trap larvae of G. mellonella, respectively. The larvae of G. mellonella were more susceptible to Bb2 than to Bb1 isolate. Four days post-exposure to the fungal treatment, the LC$_{50}$ value of Bb1 was over 4000 times higher than that of Bb2. At a concentration of $2.5 \times 10^6$ spores/ml, the LT$_{50}$ of larvae contaminated with Bb1 was 10 days, whereas for Bb2, significantly lower LT$_{50}$ of 6.4 days (P<0.05) was observed. The variability of the virulence and its relationship to either germination and growth of the fungus or defence mechanism of the insect are discussed in the current work.

Keywords: Beauveria bassiana, isolation, pathogenicity, Galleria mellonella.

INTRODUCTION

The development of insect resistance to chemical insecticides and the concern over the deleterious effects of chemicals on environmental and human safety have provided a strong impulse to the development of microbial control agents for use in integrated control of insect pests. There is an increasing interest in the exploitation of entomopathogenic fungi for the control of insect pests (Inglis et al., 2001). As a part of increasing emphasis on the use of entomopathogenic fungi in biological control programmes, the potential of several species of class Hyphomycetes as microbial insecticides has been reviewed by many authors (Hajek and St. Leger, 1994; Boucias and Pendland, 1998; Butt and Goettel, 2000). Hyphomycetes include the most promising fungal species employed against a variety of different insect pests in different agroecosystems. Several taxa including B. bassiana, Metarhizium anisopliae, and Verticillium lecanii have demonstrated excellent suppression capacity of pest populations.

B. bassiana is the causative agent of the white muscardine disease of many insect species (Tanada and Kaya, 1993), and under certain climatic conditions causes epizootics among field insects and soilborne pests. This fungus represents the first example describing a micro-organism as the agent of a contagious disease. Additionally, it has one of the largest host list among the imperfect fungi. B. bassiana has been isolated from a variety of mites and soil insects, e. g., grubs, lepidopteran pupae, termites, ants, etc. (Keller and Zimmermann, 1989).

The entomopathogenic fungus B. bassiana contains a diverse assemblage of genotypes and probably comprises species complexes. Therefore, it is conceivable to have individual isolates or pathotypes which exhibit a substantially restricted host range (Inglis et al., 2001).

The isolation of indigenous isolates or strains of mycopathogens from different hosts or localities provides control programmes with available and specific tool of controlling certain indigenous pests. In the present work two locally collected isolates of B. bassiana designated as Bb1 and Bb2 were assayed under laboratory conditions against larvae of the model insect G. mellonella L. The pathogenicity of the isolates was measured according to their lethal potential.
MATERIALS AND METHODS

Test Insects

A colony of *G. mellonella* L. was maintained in the laboratory at 23 ± 2 °C. The larvae were reared on sterile bee wax. The bee wax was washed with 0.1% chlorine water, thoroughly rinsed with sterile distilled water, air dried and then kept under – 20°C until used.

Isolation of the pathogens

Trap larvae of *G. mellonella* used as insect baits to capture entomopathogenic fungi from the soil as well as soil samples collected from different localities were used for the isolation. The trap insect technique (Bedding and Akhurst, 1975) was originally used for the isolation of entomopathogenic nematodes from soil. Larvae were first microscopically examined for the presence of any fungal infection.

Whole infected larvae that already showed hyphal growth on their bodies were placed on Sabouraud’s dextrose yeast agar (SDAY) containing 0.01-0.03% chloramphenicol to prevent bacterial contamination conditions. The larvae were incubated at 23±2 °C until adequate growth of fungus is observed, then the fungus was transferred to fresh SDAY medium and incubated for at least 7 days under the same conditions. After sporulation, microscopic examinations of the fungus were accomplished.

Whole living larvae that might be infected with entomopathogenic fungi were surface-sterilised by dipping consequently in 70% ethyl alcohol, 1% sodium hypochlorite, and sterile distilled water; each for 3 minutes. Insects were then cultured under the same conditions as mentioned above.

Soil particles were plated directly onto the surface of the agar medium until fungal hyphae radiate out from the particles. The microscopic investigations were undertaken to identify the grown fungus.

Identification, cultivation and preservation of the fungus

When sporulation took place, the saprophytic contaminants were rejected, and morphological examinations of the desired fungi were carried out. Lacto-phenol blue was used for the microscopic visualisation of the fungus, and glycerol was used as a mounting agent. Mycotaxonomic keys of Samson (1981) and Samson *et.al* (1988) were used for the identification of the fungus.

The isolated *B. bassiana* strains were grown on SDAY for 2 weeks. Fortnightly subculturing of the fungus was followed for fungus maintenance. The selected isolates Bb1 and Bb2 were isolated from soil and trap larvae of *G. mellonella*, respectively, that were originally collected from Ismailia governorate.

Susceptibility of larvae to the fungal isolates

Last instar larvae of healthy *G. mellonella* were used for the bioassays. Fungal suspensions were prepared by flooding the spores from culture plates with sterile distilled water containing 0.05% Tween-80. The clumping of the fungal spores was removed by gently scrubbing the concentrated suspension with a teflon hand homogenizer adapted for 1.5 ml Eppendorf tubes. The spores were counted with the help of an improved Neubauer Haemocytometer. The spore concentrations were then adjusted for 0.156, 0.312, 0.625, 1.25, 2.5, 5, 10, 12.5 and 25 x 10^6 spores / ml in sterile distilled water.

Larvae were exposed to the fungus by the dipping technique. The larvae were placed in a large funnel (125 mm diameter) coated with a folded filter paper, then 30 ml of each fungal suspension were applied. Sterile-distilled water was used instead of fungal suspension for control experiments. After
drying for one minute, larvae were placed individually in 24 multi-well tissue culture plates to avoid cross contamination. Three replicates, each of 24 larvae were used for each concentration and the control experiment. Larvae were offered appropriate and equal pieces of sterile wax as diet. Treated and for control insects were incubated at 25 ± 2 °C.

Larval mortalities were recorded 2, 4, 6 and 8 days post-exposure or up to 100 % mortality. Probit analysis was used to determine the mean lethal concentration (LC50) and time (LT50). The program was modified by Dr. C. Back and kindly provided by Dr. J. Grunewald, University of Tübingen (personal communication).

RESULTS

The results obtained indicate that *G. mellonella* larvae were more susceptible to Bb2 than to Bb1. The LC50 value of Bb1 was over 4000 times higher than that of Bb2 when the mortalities were recorded four days after treatment. This was greatly reduced with the elongation of the exposure period to reach 22 folds after 8 days. As shown in Table 1, the LC50 values of Bb1 are 8.2 x 10^10, 2.3 x 10^8 and 2.2 x 10^7 spores / ml after exposing the larvae for 4, 6 and 8 days to the fungus, respectively. However, the LC50 values of Bb2 are 19 x 10^7, 3.3 x 10^6 and 10^6 spores / ml, at the same exposure periods.

Two concentrations, 2.5 x 10^6 and 5 x 10^6 spores / ml were selected to fit for the comparison between LT50’s of each isolate. At conidial concentration of 2.5 x 10^6, Bb1 isolate induced 50 % larval mortality within a period of 10 days, whereas for Bb2, the LT50 was 6.4 days which was significantly lower than that of Bb1 (P<0.05). At 5x10^6 spores / ml, the LT50 of Bb2, 8.4 days, was significantly lower than that of Bb1, 5.5 days. The LT50 values of Bb1 at 2.5 x 10^6 and 5 x 10^6 spores / ml were not significantly different from each other.

DISCUSSION

The present results show variability in the pathogenicity of both of the tested fungal isolates towards *G. mellonella* larvae. *B. bassiana* isolated from a trap-*G. mellonella* larva was more virulent than that isolated from the soil. Bb2 caused faster mortality rather than Bb1 so that at a concentration of about 2 x 10^7 spores / ml induced 50% larval mortality in about 4 days, whereas Bb1 induced the same effect after 8 days. The passage through the trap host *G. mellonella* might have led to an adaptation of the isolate Bb2 to this host which in turn could increase its virulence towards the insect. The same isolate, Bb2, was previously tested by Darwish and Zayed (2002) against *Musca domestica* larvae, and induced the highest mortality rate among other fungal isolates and species.

The variability in the pathogenicity among strains could be related to the different attachment ways of each isolate spores onto the insect cuticle, mode of germination, or to the suppression of host immune system (Chandler et. al., 1993). The growing manner of the fungal isolates over the surface of the host might also affect their virulence. This was observed by Perkul and Grula (1979) on comparing between the development of pathogenic and low pathogenic mutants of *B. bassiana*, and by Zayed (1998) on characterizing the pathogenicity of different *Verticillium lecanii* isolates.

Bidochka and Kachatourians (1990) studied the correlation of the enzyme production of *B. bassiana* and the associated LT50 to the migratory grasshopper *Melanoplus sanguinipes*. They suggested that differences in the virulence might be related to the production of extracellular proteases. Zayed and Zebitz (1997) assumed that the virulence of an *V. lecanii* isolate might be correlated with its production of the fungal extra-cellular peroxidase.

Therefore, it is imperative to study the way of growth of the selected isolates (i.e. germination, length of germ tube, and penetration) to characterize the virulent isolate. In addition, biochemical
investigations are also required to characterize the cuticle degrading enzymes and their relevance to the variable pathogenicity of the isolates.

Table 1: Virulence of Beauveria bassiana isolates (Bb1 and Bb2) on Galleria mellonella last instar larvae expressed by the mean lethal concentrations (LC\textsubscript{50}) at different exposure periods.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Days after treatment</th>
<th>LC\textsubscript{50} (95% fiducial limits) (spores/ml)</th>
<th>Slope ± SE</th>
<th>( \chi^2 )</th>
<th>degrees of freedom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bb1</td>
<td>4</td>
<td>8.2 x10\textsuperscript{10} (2.1-10) x 10\textsuperscript{10}</td>
<td>0.34 ± 0.1</td>
<td>20*</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2.3 x10\textsuperscript{8} (1.2-4) x 10\textsuperscript{8}</td>
<td>0.33 ± 0.1</td>
<td>39*</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>22 x10\textsuperscript{6} (8-33) x 10\textsuperscript{6}</td>
<td>0.50 ± 0.1</td>
<td>14.7*</td>
<td>7</td>
</tr>
<tr>
<td>Bb2</td>
<td>4</td>
<td>19 x10\textsuperscript{6} (7.2-76) x 10\textsuperscript{6}</td>
<td>0.97 ± 0.1</td>
<td>57.7*</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>3.3 x10\textsuperscript{6} (2.4-4.5) x 10\textsuperscript{6}</td>
<td>1.13 ± 0.2</td>
<td>1.7</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1 x10\textsuperscript{6} (0.67-1.4) x 10\textsuperscript{6}</td>
<td>1.14 ± 0.2</td>
<td>5.2</td>
<td>3</td>
</tr>
</tbody>
</table>

*Significant heterogeneity at P < 0.05

Table 2: Comparison between the mean lethal time (LT\textsubscript{50}) of Beauveria bassiana isolates (Bb1 and Bb2) at two concentrations of spore suspension

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Concentration (spores/ml x10\textsuperscript{6})</th>
<th>LT\textsubscript{50} (95% fiducial limits) (days)</th>
<th>Slope ± SE</th>
<th>( \chi^2 )</th>
<th>degrees of freedom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bb1</td>
<td>2.5</td>
<td>10.5 (8-15)a</td>
<td>3.6 ± 0.7</td>
<td>0.9</td>
<td>2</td>
</tr>
<tr>
<td>Bb2</td>
<td>2.5</td>
<td>6.4 (5.8-7.1)b</td>
<td>4.3 ± 0.5</td>
<td>0.7</td>
<td>2</td>
</tr>
<tr>
<td>Bb1</td>
<td>5</td>
<td>8.4 (8-18)a</td>
<td>4.7 ± 0.8</td>
<td>7.9*</td>
<td>2</td>
</tr>
<tr>
<td>Bb2</td>
<td>5</td>
<td>5.5 (4.5-6)b</td>
<td>3.2 ± 0.4</td>
<td>19*</td>
<td>2</td>
</tr>
</tbody>
</table>

Data in the same column followed by the same letter are not significantly different as indicated by overlapping between the fiducial limits. *Significant heterogeneity at P < 0.05.

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REFERENCES


