BIOLOGICAL CHARACTERIZATION OF SOME MICROORGANISMS OF BIOTECHNOLOGICAL INTEREST

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Abstract

Successful use of biological insecticides depends on their "active substance" quality, which ensures the biological efficacy and performance of bioproducts in field conditions: the active substance have to be virulent and to have ecological competences (epizootiological qualities, multiplication capacity, spreading and persistence in habitats and target pests populations). These parameters are objective criteria for selection of microorganisms that can be successfully included in biological control programs of pests. In this paper are presented results of some laboratory tests aimed to estimate biological parameters specific for entomopathogenic fungi, including the spores germination and spores production on culture media and insects. The biological material used in this study consisted in five Beauveria bassiana entomopathogenic biotypes, isolated from insects belonging to the ord. Coleoptera, Hymenoptera and Diptera in natural epizootic outbreaks, during the years 2008-2010. The results led to the following conclusions: (i) B. bassiana strains having different origins in terms of habitat and host insect exhibit different biological qualities, (ii) the evaluation of test insects mortality induced by B. bassiana doesn’t provide a complete pathological characterization of fungal isolates; to assess the biological control potential of B. bassiana isolates, the virulence have to be correlated with the biological cycle of the fungus and the spores production on the insect cuticle; (iii) the quantification of spore production on insect is also necessary to further evaluate the fungal strains potential to naturally increase of virulence.

Key words: entomopathogenic fungi, biotypes, Beauveria bassiana

INTRODUCTION

Agricultural production is still the main source of food for humankind. Population explosion has determined the increase of agricultural production in response to growing food requirements. The growth in human population around the world affects all people through its impact on the economy and environment. In many cases it was made an irrational exploitation of natural resources leading to ecological imbalance. One of the biggest ecological challenges facing plant protectionists is the development of environmentally friendly alternatives to the extensive use of chemical pesticides for crop diseases and pests control. Improvements in crop management systems can have an important influence on product quality as well.

Uses of synthetic pesticides led to undesirable effects on non-target organisms sharing the ecosystem and decrease the loss of efficacy due to adaptation of pest agents. Moreover, the toxicity of their residues affects the environment and people health too (Keller&Brenner, 2005; NRC, 1996; Robinson et. al, 1980). Research on biological pest control methods can make an important contribution to reducing the use of chemicals for crop protection. (Butt&colab, 2001; Gerhardson, 2002; Lacey&colab., 2001).

Beauveria bassiana (Bals.) Vuill. is an entomopathogenic fungus that grows naturally in soils (Steinhaus, 1956). It is being used as a biological insecticide all over the world in order to control pests such beetles, whiteflies, aphids, mealybugs, grasshoppers etc.

B.bassiana strains vary in their host ranges, having, generally, a wide host range. B.bassiana strains isolated from different hosts inhabiting different geographical regions differ in theirs ecological capacities.
Ecological competences of different strains (epizootiological qualities, multiplication capacity, spreading and persistence in habitats and target pests populations) are objective criteria for selection of those which can be successfully included in biological control programs of pests (Andrei, 1999). Several strains of the entomopathogenic fungi (including 5 strains of B. bassiana presented in this experiment) have been isolated in order to use them for biological insecticides production at the Research-Development Institute for Plant Protection Bucharest. This work aimed at selecting B. bassiana strains favourable growth, sporulation, viability and infectivity responses on different culture media.

**MATERIAL AND METHOD**

Five strains of B. bassiana were used as biological material in this study. All strains were isolated from natural outbreaks, purified and stored on sterilized potato dextrose agar (PDA) slants, at 4°C (Table 1).

<table>
<thead>
<tr>
<th>Fungal strain</th>
<th>Insect</th>
<th>Name and order</th>
<th>Isolated from/at</th>
<th>Natural outbreaks</th>
</tr>
</thead>
<tbody>
<tr>
<td>BbIt</td>
<td></td>
<td>The European spruce bark beetle ( Ips typographus) Coleoptera</td>
<td>Iacobeni / Sept. 2010</td>
<td>Spruce trunk in a coniferous forest</td>
</tr>
<tr>
<td>BbAl</td>
<td></td>
<td>East Asian sawfly ( Aporrectes leucopoda) Hymenoptera</td>
<td>Soldângesti, / June 2010</td>
<td>Elm leaf in a deciduous forest</td>
</tr>
<tr>
<td>BbBrodoc</td>
<td></td>
<td>Leaf beetle (Chroicocephalus sp.) Coleoptera</td>
<td>D.X. Brodoc / July 2011</td>
<td>Cap Kaliakra / Aug. 2009</td>
</tr>
<tr>
<td>BbCr</td>
<td></td>
<td>Leaf beetle (Sciara sp.) Diptera</td>
<td>Topoloveni, Aug. 2008</td>
<td>Pot with wet soil</td>
</tr>
</tbody>
</table>

The strains used in the experiment were grown on plates using three different culture media (Sabouraud, Czapek-Agar and PDA) as monosporal cultures, and incubated at 25°C, under dark conditions. For each strain were prepared separately nine Petri dishes, three plates for each type of culture media (three replications).

The colonies were measured daily during 14 days. Macroscopic observations were made at 10 and 15 days after inoculation and were analyzed the average growth of colonies, their appearance, the sporulation, the emergence of sporulating structures and pigmentation for every strain and for each of the three media.

The experiment aimed also the virulence of these five strains. Insects from order Coleoptera and Lepidoptera (2nd and 3rd larval stage) were selected for this purpose: *Galleria mellonella* L., *Plodia interpunctella* (Hübner) and *Tenebrio molitor* L. The artificial inoculation was made by spraying on insects the *B. bassiana* conidial suspensions. The larval mortality was analyzed after 5 and 10 days. The sporulation rates of each strain on death test insects were analyzed after 30 days using serial dilution method for counting fungal spores.

**RESULTS AND DISCUSSIONS**

The influence of solid media on the development of the B. bassiana strains is presented in Table 2-7. The growth rate was monitored daily by measuring the colony diameter. The highest mycelial growth rate of B. bassiana colonies cultivated on Czapek medium was observed at BbAl strain (Fig. 1). The average fungal colonies diameter was 39.67 mm. The lowest growth rate was recorded for BbIt strain, with an average diameter of 33.33 mm colony growth after 14 days.

![Fig. 1. Growth rate of colonies cultivated on Czapek medium](image)

On Sabouraud medium, the highest mycelial growth rate of colonies was observed at BbCr strain. The average fungal colonies diameter
was 32.23 mm. The lowest growth rate was recorded for BbSc strain, with an average diameter of 19.11 mm colony growth after 14 days (Fig 2).

The highest mycelial growth rate of *B. bassiana* colonies on PDA medium was observed at BbCr strain. The average fungal colonies diameter was 45.50 mm. The lowest growth rate was recorded for BbBrodoc strain, with an average diameter of 29.78 mm colony growth after 14 days (Fig 3).

The highest increase in BbAl mycelial colony diameter was recorded on those cultivated on Czapek medium (39.67 mm), followed by those on PDA (31.39 mm) and Sabouraud (29.33 mm) (Fig 4).

The highest increase in BbLt mycelial colony diameter was recorded on those cultivated on PDA medium (42.33 mm), followed by those on Czapek (30.33 mm) and Sabouraud (26.29 mm) (Fig. 5).
The highest increase in BbCr mycelial colony diameter was recorded on those cultivated on PDA medium (45.50 mm), followed by those on Czapek (35.13 mm) and Sabouraud (32.23 mm) (Fig 6).

The tests were performed on microscope slides containing one of the culture media: agar-water, minimal medium, potato-dextrose-agar, potato-dextrose-1% yeast extract agar, sabouraud-dextrose-yeast extract-agar and complete medium. The culture media influenced the germination of the species studied, verifying within and inter specific variations. B. bassiana development was also tested in different liquid cultures, resulting in different yield of blastospores. In peptone-glucose, the yeald of blastospores was four-fold higher than in glucose-peptone-yeast extract (Bidochna et al., 2004).

Rombach (2006) tested several simple liquid media for B. bassiana submerged conidia. The results proved maximum yields of conidia in medium consisting of sucrose-yeast extract and basal salts and maximum yields of hyphal bodies in a sucrose-yeast-extract medium. More than that, Kmitowa (1979) studied the effect of the quantitative gradient of nitrogenous compounds added to the culture medium has on the growth and pathogenicity of over 30 B. bassiana strains. The author reported that among the B. bassiana strains under study some formed poorly growing and weakly sporulating colonies while others grew rapidly and proliferously forming dense and sporulating colonies. Biomass of some strains exceeded many times that of the others. The pathogenicity of these strains was not correlated with their rates of growth. Colonial morphology of B. bassiana biotypes is presented in Photo 1-8 and Table 2-6.
**BbAl strain growth and sporulation**

![Image](108x397 to 162x451)

Photo 1. Mature colonies of BbAl strain grown on three different culture media: a. PDA; b. Sabouraud; c. Czapek

<table>
<thead>
<tr>
<th>Colony morphology (10 days after inoculation)</th>
<th>BbAl strain on culture media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape</td>
<td>Czapek</td>
</tr>
<tr>
<td>Elevation</td>
<td>flat</td>
</tr>
<tr>
<td>Margin</td>
<td>regular edge</td>
</tr>
<tr>
<td>Opacity</td>
<td>translucent</td>
</tr>
<tr>
<td>Sporulation</td>
<td>unsporulated</td>
</tr>
</tbody>
</table>

**BbIt strain growth and sporulation**

![Image](108x453 to 112x605)

Photo 2. Mature colonies of BbIt strain grown on three different culture media: a. PDA; b. Sabouraud; c. Czapek

<table>
<thead>
<tr>
<th>Colony morphology (10 days after inoculation)</th>
<th>BbIt strain on culture media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape</td>
<td>Czapek</td>
</tr>
<tr>
<td>Elevation</td>
<td>flat</td>
</tr>
<tr>
<td>Margin</td>
<td>filiform</td>
</tr>
<tr>
<td>Opacity</td>
<td>translucent</td>
</tr>
<tr>
<td>Sporulation</td>
<td>sporulated</td>
</tr>
</tbody>
</table>

**BbCr strain growth and sporulation**

![Image](108x625 to 111x877)

Photo 3. Mature colonies of BbCr strain grown on three different culture media: a. PDA; b. Sabouraud; c. Czapek

<table>
<thead>
<tr>
<th>Colony morphology (10 days after inoculation)</th>
<th>BbCr strain on culture media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevation</td>
<td>flat</td>
</tr>
<tr>
<td>Margin</td>
<td>regular curved</td>
</tr>
<tr>
<td>Opacity</td>
<td>translucent</td>
</tr>
<tr>
<td>Sporulation</td>
<td>unsporulated</td>
</tr>
</tbody>
</table>

**Table 2. Colonial morphology of BbAl strain grown on different culture media**

**Table 3. Colonial morphology of BbIt strain grown on different culture media**

**Table 5. Colonial morphology of BbBrodoc strain grown on different culture media**

**Table 4. Colonial morphology of BbCr strain grown on different culture media**

**BbBrodoc strain growth and sporulation**

![Image](108x877 to 111x910)

Photo 4. Mature colonies of BbBrodoc strain grown on three different culture media: a. PDA; b. Sabouraud; c. Czapek

**BbSc strain growth and sporulation**

![Image](108x910 to 111x943)

Photo 5. Mature colonies of BbSc strain grown on three different culture media: a. PDA; b. Sabouraud; c. Czapek

Culture media differentially influenced the growth, colony character and sporulation of B. bassiana tested strains. The results reported by Barnes G.L. (Barnes et al., 1975) proved...
that *B. bassiana* growth and sporulation are influenced by various peptone sources in culture.

Table 6. Colonial morphology of BbSc strain grown on different culture media

<table>
<thead>
<tr>
<th>Colony morphology (10 days after inoculation)</th>
<th>Czapek</th>
<th>Sabouraud</th>
<th>PDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape</td>
<td>circular</td>
<td>circular, gibbous</td>
<td>circular, slightly bulging</td>
</tr>
<tr>
<td>Elevation</td>
<td>plat</td>
<td>hemispheric, fluffy mycelia</td>
<td>flat</td>
</tr>
<tr>
<td>Margin</td>
<td>telliform</td>
<td>entire</td>
<td>regular</td>
</tr>
<tr>
<td>Opacity</td>
<td>translucent</td>
<td>opal</td>
<td>opal</td>
</tr>
<tr>
<td>Sporulation</td>
<td>sporulated</td>
<td>sporulated</td>
<td>sporulated in concentric circles</td>
</tr>
</tbody>
</table>

Pathogenicity tests performed in laboratory conditions and quantified in external sporulation on test-insects (Photo 9) proved that the saprophytic development of *B. bassiana* biotypes vary from one to another strain (Table 7).

From all tested strains, BbSc strain recorded the largest amount of spores, both on *G. mellonella* and *P. interpunctella* larvae.

Table 7. *B. bassiana* strains spore production on test insects

<table>
<thead>
<tr>
<th>B. bassiana strains</th>
<th><em>Galleria mellonella</em></th>
<th><em>Plodia interpunctella</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>spore concentration</td>
<td>ml⁻¹</td>
<td>spore concentration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ml⁻¹</td>
</tr>
<tr>
<td>BbCr</td>
<td>0.525</td>
<td>1.175</td>
</tr>
<tr>
<td>BbSc</td>
<td>0.825</td>
<td>10.9</td>
</tr>
<tr>
<td>BbIt</td>
<td>0.425</td>
<td>0.975</td>
</tr>
<tr>
<td>BbBrodoc</td>
<td>0.675</td>
<td></td>
</tr>
<tr>
<td>BbAl</td>
<td></td>
<td>0.55</td>
</tr>
</tbody>
</table>

CONCLUSIONS

The results of this work show that BbAl and BbCr strains are recommended for further investigation in order to determine the most favourable substrate for mass production of bioformulated products.

Statistically, PDA environment has provided the best carbon source for vegetative development *B. bassiana* strains. *B. bassiana* tested strains, regardless of their origin, has biotechnological potential, related to the ability to degrade various synthetic nutritive substrates and to keep the pathogenicity against insects belonging to different orders.
Spores production tests proved different capacities of B. bassiana biotypes to efficiently exploit the nutritive substratum. B. bassiana strains with different origins in terms of habitat and host insect exhibits different biological qualities. The evaluation of test insects mortality induced by B. bassiana doesn’t provides a complete pathological characterization of fungal isolates; to assess the biological control potential of B. bassiana isolates, the virulence have to be correlated with the biological cycle of the fungus and the spores production on the insect cuticle. The quantification of spore production on insect is necessary to further evaluate the fungal strains potential to naturally increase of virulence.

REFERENCES

[12] Rombach M.C., 1989, Production of Beauveria bassiana (Deuteromycotina Hyphomycetes) sympoduloconidia in submersed culture; Entomophaga 34, pp.45-52.