Antigenic Relationship between Two Bio-Agents and Pathogenic *Fusarium semitectum* Using Two Serological Techniques

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**ABSTRACT**

Wheat (*Triticum aestivum* L.) is an important crop cultivated successfully worldwide and in Egypt, which could be infected by *Fusarium* sp. Fungicides are generally used to control this pathogen. Nowadays, *Trichoderma* spp. and *Pseudomonas fluorescens* are used as biocontrol agents. In this study, a serological technique was carried out to explain the interaction between *Trichoderma viride*, *P. fluorescens* and the pathogenic fungus *Fusarium semitectum* using crossed-immunoelectrophoresis (CIE) and double diffusion reaction (DDR). In homologous reactions, the antigenic structures of *F. semitectum*, *T. viride*, and *P. fluorescens* were 7, 5 and 4 precipitin bands in CIE, while 2, 2, and 3 precipitin bands were detected in DDR, respectively. In heterologous reactions, antigens of *T. viride* electrophoresed against antibodies of *F. semitectum* gave two common bands between them in either of the used methods, while *P. fluorescens* antigens gave only one common precipitin antigen when electrophoresed against antibodies of the pathogen using both methods. This clearly indicated that *T. viride* was able to recognize to the pathogenic fungus *F. semitectum*, which showed more common antigens.

Keywords: biocontrol, *Fusarium*, *Pseudomonas*, serological techniques, *Trichoderma*

**INTRODUCTION**

Wheat (*Triticum aestivum* L.) is one of the most important crops cultivated successfully in Egypt and worldwide. The importance of this crop is as a basic staple food for humans, in addition to its straw, which serves as important fodder for animals. This crop is subjected to infection by several diseases such as rusts, loose smut, powdery mildew, root rot, and wilt. These diseases cause tremendous loss by *Fusarium* sp. in different areas of the world (El-Nashar et al. 2000; Andres-Ares et al. 2004; Strausbaugh et al. 2004; El-Shamy 2006; Muthomi et al. 2008).

*Fusarium semitectum* is a widespread species often isolated from plants with complex disease and also known to be toxigenic (Zaccardelli et al. 2006; Pratt and Tewold 2009). The control of this pathogen depends mainly on chemical fungicides that pollute the environment, disturb the ecological balances for all living microorganisms, and cause harmful effect for beneficial microorganisms (Hooda and Grover 1983). Biological control is increasingly becoming an important component of plant disease management and offers solutions to many of the persistent problems in agriculture (Cook and Baker 1983). The biological control of soil-borne plant pathogens by antagonists i.e. *Trichoderma* spp., *Gliocladium* spp., *Bacillus subtilis* and *P. fluorescens* is likely to be the best alternative to conventional chemical control methods.

Immunological techniques for the detection and identification of particular microorganisms are of great value because of the specificity of the reaction between the antigens of the organisms and the corresponding antibodies (Abs), which are produced in the serum of the animals inoculated with the organism (Ouchterlony and Nilsson 1978). Serological differences were frequently found between *formae* and races of fungi (Iannelli et al. 1982; Ala El-Dein and El-Kady 1985). The common features of these attempts were a few common antigens for comparison either between microorganisms or between pathogens and plants (El-Kazza et al. 1994, 1997; Meyer et al. 2000; Eibel et al. 2005; El-Shamy 2006).

The main objective of this study is to evaluate the potential use of two serological techniques, namely crossed-immunoelectrophoresis (CIE) and double diffusion reaction (DDR) to illustrate the relationship between two biocontrol agents i.e. *T. viride*, *P. fluorescens* and the pathogenic fungus, *F. semitectum*, causing rot and wilt diseases.

**MATERIALS AND METHODS**

**Experimental design**

This experiment consisted of three treatments where the antigen obtained from *F. semitectum*, *T. viride*, or *P. fluorescens* were injected in male Boscat rabbits (3 kg each; Faculty of Agriculture, Benha University, Benha, Egypt) and their antisera were obtained. The antigen-antiserum interaction was examined by CIE and DDR.

**Crossed-immunoelectrophoresis**

1. **Preparation of antigens**

The fresh biomass of either the pathogenic fungus or the biocontrol agents used were ground in a mortar with 20 g glass beads under N2 gas and diluted with HCl-Tris buffer (0.05 M) at pH 7.2 and kept overnight at 5°C in a refrigerator. The extractions were centrifuged at 10,000 rpm for 20 min. The supernatant of each was collected and protein content was adjusted to 20 mg/ml before injection into male Boscat rabbits according to Lowry et al. (1951).

2. **Immunization and production of antisera**

Antigens of either the bio-control agents or the pathogenic fungus were mixed with incomplete Freund adjuvant (1:1 ratio). The mixed antigens-incomplete adjuvant was administrated intramuscularly and subcutaneously at male Boscat rabbits. Each rabbit received a course of 10 injections (two per week); the volume of the...
antigens of gous reactions, two common bands were detected when antigens of their corresponding Abs, tectum precipitin bands were detected when antigens of reactions using CIE. In homologous reactions, 7, 5 and 4 Table 1 Number of precipitin bands detected in homologous and heterologous reactions of F. semitectum T. viride and P. fluorescens using double diffusion reaction.

<table>
<thead>
<tr>
<th>No. of precipitation peaks detected among the isolate</th>
<th>F. semitectum</th>
<th>T. viride</th>
<th>P. fluorescens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusarium semitectum</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Trichoderma viride</td>
<td>2</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>1</td>
<td>-</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2 Number of precipitin bands detected in homologous and heterologous reactions of F. semitectum T. viride and P. fluorescens using crossed-immuno-electrophoresis technique.

<table>
<thead>
<tr>
<th>No. of precipitation peaks detected among the isolate</th>
<th>F. semitectum</th>
<th>T. viride</th>
<th>P. fluorescens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusarium semitectum</td>
<td>7</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Trichoderma viride</td>
<td>2</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 3 Comparison between Double diffusin (DDR) technique and Crossed immuno-electrophoresis (CIE) technique.

<table>
<thead>
<tr>
<th>Homologous reactions</th>
<th>CIE technique</th>
<th>DDR technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusarium semitectum</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Trichoderma viride</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Table 1 and Fig. 1 reveal homologous and heterologous reactions using the DDR test. In homologous reactions, antigens of F. semitectum, T. viride and P. fluorescens showed 2, 2 and 3 bands when electrophorized against their corresponding Abs. In heterologous reactions, antigens of T. viride gave two common bands when electrophorized against F. semitectum Abs, while one common band was detected when antigens of P. fluorescens electrophorized against F. semitectum Abs. Homologous reactions were carried out for each of F. semitectum, T. viride, and P. fluorescens to detect their antigenic structure, while heterologous reactions were performed to detect the common antigens between them.

Table 2 and Fig. 2 reveal homologous and heterologous reactions using CIE. In homologous reactions, 7, 5 and 4 precipitin bands were detected when antigens of F. semitectum, T. viride and P. fluorescens were electrophoresed against their corresponding Abs, respectively. In heterologous reactions, two common bands were detected when antigens of T. viride were electrophoresed against F. semitectum Abs, while one common band was detected between antigens of P. fluorescens and F. semitectum Abs.

In several instances, it has been found that the relationship between organisms controlled by antigenic substances is termed a common antigen. The greater antigen simila-
Antigenic relationship between two bio-agents and pathogenic Fusarium semitectum. Emara et al.

References


Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. Biological Chemistry 193, 265-275


Fig. 1 Precipitation bands showing the antiserium reaction with its homologous and heterologous antigen using Ouchterlony DDR test. F. semitectum (F), P. fluorescens (P), T. viride (T), FP, and, FT, respectively.

Fig. 2 Precipitation bands showing the antiserium reaction with its homologous and heterologous antigen using the CIE technique. F. semitectum (F), P. fluorescens (P), T. viride (T), FP, and, FT, respectively.