Relationship Between Extracellular Polygalacturonase and Pathogenicity Loss in Different Fungal Isolates

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ABSTRACT. The ability of different fungal isolates i.e. Fusarium oxysporum and four species of Verticillium viz V. alboatrum, V. dahliae, V. tricorpus and V. nubilum to produce endo and exopolygalacturonase (PG) in vitro was investigated. The activity of endo-PG by these isolates in pectin salts medium “cup plate” assay, was negative. Active staining of exopolygalacturonase resolved by paper chromatography revealed a degree of variation in enzyme secretion within the isolates, V. dahliae showed a common pattern of exopolygalacturonase enzyme. A pathogenicity test was conducted using Antirrhinum majus seedlings and polygalacturonase secretion is discussed in relation to pathogenicity loss.

Introduction

Most plant pathogens secrete enzymes throughout their existence or upon contact with a substrate. Penetration of pathogens into the cell through the cell wall is brought about by the breakdown of the cell wall by action of these enzymes. The action of pectolytic enzymes has been investigated by several workers\[1,2\], who found that most of the pathological fungal and bacterial manifestations included enzymes involved in the degradation of the pectic constituents of cell walls in plant tissue. In addition, the relationship between pectic enzyme and vascular wilt disease, blockage and middle lamella maceration was reported by Cooper and Wood\[1\]. Mussel and Strouse\[3\] found that the endo-polygalacturonase (endo-PG) is 20 times more efficient than the exo-polygalacturonase (exo-PG) in inducing tomato wilt. V. dahliae Kelb and V. dahliae alboatrum Rembe & Berth cause vascular wilt diseases in a wide
range of wild and cultivated plant species. Cooper et al. reported the degradation of cell walls of tomato cultivars by polygalacturonase of Fusarium oxysporum f sp lycopersicum and V. albostrum.

Correlations of in vitro production of pectic enzymes with pathogenicity have been repeatedly pointed out, many attempts to achieve this correlation have been reported, and the results are as contradictory as would be expected. In vitro production of pectic enzymes by vascular pathogens depends on the isolates used and can be influenced by the composition of the culture medium and the age of the cultures at the time of harvest. With respect of V. albostrum, the situation is further compounded by the fact that the fungus produces both an endo-PG and an exo-PG in culture and relative amounts of these two enzymes produced vary with the isolate examined and the culture medium used.

Four species of Verticillium and F. oxysporum were used in the work described below which deals with their endo and exo-PG production and their role in pathogenesis.

Material and Methods

Five different fungal isolates i.e. Fusarium oxysporum and four species of Verticillium viz V. albostrum, V. dahliae, V. tricorpus and V. nubilum were kept in a refrigerator at 4°C for eight months before used in this study. Their names, locations and sources are given in Table 1. The five isolates were grown on Czapek-Dox agar at 25°C and stocks were maintained at 4°C on corn meal agar slopes.

<table>
<thead>
<tr>
<th>Species</th>
<th>Locations</th>
<th>Host plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusarium oxysporum</td>
<td>Saudi Arabia</td>
<td>Tomato</td>
</tr>
<tr>
<td>Verticillium albostrum</td>
<td>Netherlands</td>
<td>Potato</td>
</tr>
<tr>
<td>Verticillium dahliae</td>
<td>Netherlands</td>
<td>Sunflower</td>
</tr>
<tr>
<td>Verticillium tricorpus</td>
<td>Netherlands</td>
<td>Potato</td>
</tr>
<tr>
<td>Verticillium nubilum</td>
<td>Netherlands</td>
<td>Potato</td>
</tr>
</tbody>
</table>

Polygalacturonase was induced in 250 ml Erlenmeyer flasks containing 100 ml basal salts solution and citrus pectin (0.5% w/v) as sole available carbon source.

To enhance recovery of PG, cultures were grown at pH 5.0 with the non-metabolizable buffer Z-(N-morpholino) ethansulphuric acid (MES) (0.05 m). Flasks were inoculated with two 5 mm agar plugs, removed from 7-14 days Czapek-Doxplates, and incubated on a rotary incubator (150 rev. min – 1, 25°C) for 7 days. Culture filtrates were passed through Whatman No. 1 filter paper and centrifuged (4000g, 10 min) to remove conidia and mycelia.

“Cup plate” assay was used for (endo-PG) detection. Hexa decyltrimethyl bromide was added when a clear zone did not appear for 5-10 minutes. The method
Relationship Between Extracellular Polygalacturonase.

for (exo-PG) activity using the paper chromatography technique was adopted as the procedure devised by Bateman.

To test the pathogenicity of these isolates, seeds of *Antirrhinum majus* (mixed variety) were pregerminated in sterilized sandy soil. After three weeks the identical seedlings were uprooted and suspended in spore suspensions. The spore suspensions were made by submerging ten-day-old petri dishes cultures growing on PDA of the candidate isolates, in sterilized water. For control, seedlings were suspended in distilled and sterilized water.

**Results and Discussion**

Results were obtained from experiments designed to determine the presence of certain pectolytic enzymes, particularly endo-PG and exo-PG on a pectinic salt medium (Table 2).

Negative results were obtained using “cup plate” assay for the detection of endo-PG (Table 2). This is not in agreement with the results of other investigators who implicated secretion of endo-PG in the pathogenicity of many fungi.

*Verticillium* species and *Fusarium oxysporum* are phytopathogenic fungi causing vascular wilt diseases in wide range of cultivated plant species. *V. alboatrum* and *V. dahliae* are of great importance and most of researchers have been concentrated on these species of *Verticillium*. The reason to use these four isolates of *Verticillium* and one isolate *F. oxysporum* to evaluate the role of polygalacturonase in pathogenesis was that in a recent experiment (Bahkali, unpublished data), found that *V. dahliae*, *V. alboatrum* and *V. tricorne* he used for the past studies had lost pathogenicity when he tried to correlate the degree among the species. So experiments were designed to determine the presence of certain pectolytic enzymes, particularly endo-PG and exo-PG (Table 2) during the growth of different fungal isolates (Table 1) on a pectinic medium. Negative results were obtained using “Cup plate” assay for the detection of endo-PG (Table 1). While detection of exo-PG using paper chromatography (Table 2 and Fig. 1) showed that this enzyme secreted only by a single isolate, *V. dahliae*.

**Table 2. Pectolytic enzymes detection in vitro for different fungal isolates**

<table>
<thead>
<tr>
<th>Species</th>
<th>Endo-PG</th>
<th>Exo-PG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cup plate</td>
<td>Paper chromatography</td>
</tr>
<tr>
<td><em>Fusarium oxysporum</em></td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td><em>Verticillium alboatrum</em></td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td><em>Verticillium dahliae</em></td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td><em>Verticillium tricorne</em></td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td><em>Verticillium nubilum</em></td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>
These findings interested us to see if the loss of pathogenicity was resulted from similar alterations in exo-cellular enzymes levels. Enzyme data assay showed that the endo-PG and exo-PG which activities are negative except for exo-PG of \textit{V. dahliae} (Table 2) did not produce any symptoms. This is not in agreement with other findings since the production of endo-PG has been associated with virulence in several plant pathogenic fungi\cite{12,7,15,16,1,13,9}.

The finding that these isolates neither produced endo-PG in axenic culture nor gave rise to symptoms in seedlings of \textit{Antirrhinum majus} could be considered as good indications that those isolates lost their pathogenic activities under long storage conditions.

\textbf{FIG. 1.} Paper chromatography test showing exo-PG activity after 8 hours of inoculation.
References


علاقة الإفراز الإنزيمي البولي جلاكتيرونيز الخارجي خلوي بفقدان الأعراض المرضية في العزلات الفطرية المختلفة

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قسم النبات والأحياء الدقيقة ، كلية العلوم ، جامعة الملك سعود
الرياض ، المملكة العربية السعودية

المستخلص . تمّت دراسة مقدار العزلات الفطرية لنفتر فيوزاريم أوكسيبوريم وأربعة أنواع من جنس فيرسيسيليم هي أكروتريم ، داهلي ، تربكوروس والنايبل في إنتاج إنزيم البولي جلاكتيرونيز الداخلي والخارجي في التجارب العملية . وكان نشاط البولي جلاكتيرونيز في هذه العزلات باستخدام طريقة "cup plate" سالباً بيد أن بعض العزلات مثل فيرسيسيليم داهلي و فيرسيسيليم نايبل أعطت نشاطًا موجباً لإنسام البولي جلاكتيرونيز الخارجي وذلك باستخدام طريقة الفصل الكروماتوجافي .

تم اختبار القدرة المرضية باستخدام بادرات نبات حنك السبع ووجد عدم ظهور أي أعراض مرضية على البادرات . هذا ، وتمت مناقشة عوامل إنزيم البولي جلاكتيرونوز فيما يتعلق بفقدان الأعراض المرضية .