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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 *Antrirrhinum 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[4] Cooper *et al.*[5] reported the degradation of cell walls of tomato cultivars by polygalacturonase of *Fusarium oxysporum* f sp *lycoperiscum* and *V. alboatrum*.

Correlations of *in vitro* production of pectic enzymes with pathogenicity have been repeatedly pointed out[6], 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[7] and can be influenced by the composition of the culture medium and the age of the cultures at the time of harvest[8]. With respect of *V. alboatrum*, the situation is further compounded by the fact that the fungus produces both an endo-PG and an exo-PG in culture[3,9] and relative amounts of these two enzymes produced vary with the isolate examined and the culture medium used[8].

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. alboatrum, 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 1. Sources of different fungal isolates.

Species	Locations	Host plants
Fusarium oxysporum	Saudi Arabia	Tomato
Verticillium alboatrum	Netherlands	Potato
Verticillium dahliae	Netherlands	Sunflower
Verticillium tricorpus	Netherlands	Potato
Verticillium nubilum	Netherlands	Potato

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) ethanosulphuric 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[10] was used for (endo-PG) detection. Hexa decyltrimethl bromide was added when a clear zone did not appear for 5-10 minutes. The method

for (exo-PG) activity using the paper chromatography technique was adopted as the procedure devised by Bateman[11].

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[12,7,1,13,9].

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*[14]. 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. tricorpus* 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

Species	Endo - PG	Exo - PG
	Cup plate	Paper chromatography
Fusarium oxysporum	–	–
Verticillium alboatrum	–	–
Verticillium dahliae	–	+
Verticillium tricorpus	–	–
Verticillium nubilum	–	–

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 *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[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 *Antirrhinum majus* could be considered as good indications that those isolates lost their pathogenic activities under long storage conditions.

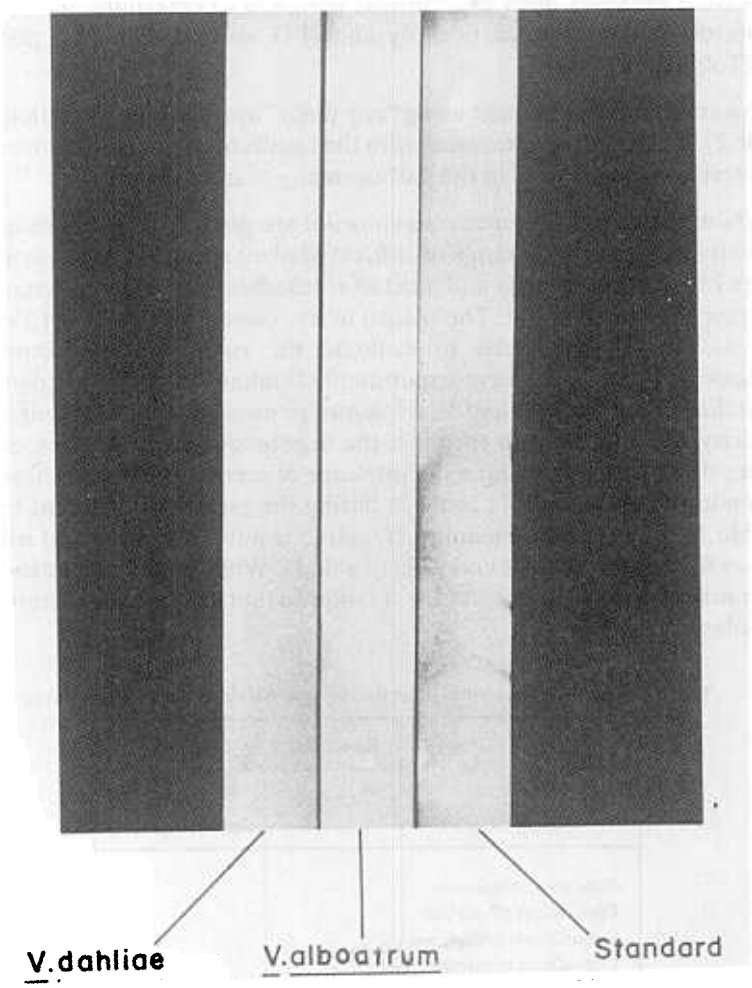

FIG. 1. Paper chromatography test showing exo-PG activity after 8 hours of inoculation.

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علاقة الإفراز الإنزيمي البولي جلاكتيورونيز الخارج خلوي بفقدان الأعراض المرضية في العزلات الفطرية المختلفة

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المستخلص . تمت دراسة مقدرة العزلات الفطرية لفطر فيوزاريم أوكسيسبوريم وأربعة أنواع من جنس فيرتيسيليم هي ألبواتريم ، داهلي ، ترايكوربس و نابيلم في إنتاج إنزيم البولي جلاكتيورونيزالداخلي والخارجي في التجارب المعملية . وكان نشاط البولي جلاكتيورونيز في هذه العزلات باستخدام طريقة "cup plate" سالبًا بيد أن بعض العزلات مثل فيرتيسيليم داهلي و فيرتيسيليم نابيلم أعطت نشاطًا موجبًا لإنزيم البولي جلاكتيورونيز الخارجي وذلك باستخدام طريقة الفصل الكروماتوجرافي .

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