International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 5, No. 2, p. 40-45, 2014

RESEARCH PAPER OPEN ACCESS

Study of pectinolytic activity of *Fusarium oxysporum f.sp albedinis* agent responsible for bayoud in Algeria

Karkachi Noureddine[1*], Gharbi, Samia[2], Kihal Mebrouk[1], Henni Jamal Eddine[1]

[1]*Department of Biology , University of Oran, Algeria*

[2]*Department of Biotechnology University of Sciences Technologies Oran (USTO), Algeria*

Article published on August 05, 2014

Key words: *Fusarium oxysporum* f.sp *albedinis, pathogenicity, pectinmethylesterase (PME), polygalacturonase (PG), citrus pectin.*

Abstract

The present study is devoted to the morphological characterization, pathogenicity of *Fusarium oxysporum* f.sp *albedinis,* which showed morphological variability of isolates. The study of pectin methyl esterase and polygalacturonase activities by reducing sugars dosages of 10 isolates, showed that the PME and PG activities are present in the culture filtrates of isolates and the estimated amount is much higher in the presence of citrus pectin and glucose. We noted that this activity is variable among isolates, high activity is recorded (1.59 10^3 U/mole) in presence of glucose but it was greater than 4 times in the presence of citrus pectin, The PG activity is recorded (1.39 10^3 and 0.44 10^3µegr) in the presence of 1% citrus pectin and glucose respectively.

* **Corresponding Author:** Karkachi Noureddine ✉ noureddinekarkachi@hotmail.com

Introduction

Bayoud is the acclamation the most dangerous disease of date palm (*Phoenix dactylifera L.*); this is a real problem in areas of growing date palms in North Africa. It represents a threat to the free areas. The phytopathogenic fungus, it performs at least a partial degradation of materials, which constitute the cell walls of plants (Reignault *et. al.*2008). *Fusarium oxysporum*, like other pathogenic vascular, easily produce an assortment of enzymes. These enzymes degrade most of the cell wall components (cellulose and polymers consisting essentially of acid anhydrogalacturonic). The work undertaken by (El Moudafar and El Boustani, 2000), show that the growth and development of the causative Bayoud, *Fusarium oxysporum* f.sp *albedinis* cultivated in a mineral medium containing the cell walls of the roots of date palm, as the sole carbon source, are related to the production of extracellular enzymes that degrade the cell walls. The plant cell wall consists of cellulose, hemicellulose, lignin and pectin. Pectin is a family of complex polysaccharides found in the middle lamella of primary cell walls of plants. Pectin and complex hemicelluloses are responsible for the integrity and coherence of plant tissues as well as for the texture of vegetables and fruits. Pectins are an extremely complex and structurally diverse group of polymers (Nitinkumar and Bhushan, 2010); (Al-Najada, 2014). These enzymes are essential for fungal pathogens that do not have specialized penetration structures as well as for necrotrophic pathogens during the late stages of the invasion process (Al-Najada *et. al.*2012; Al-Najada, 2014). The virulence of pathogen strains has been related to total PG activity or specific PG isozymes. The production of PG isozymes by fungi is influenced by many factors such as in vitro and in vivo conditions, nutrients, and fungal strains (Zhang *et. al.*1997). Several studies, concerning the correlation between the *in vitro* enzymatic activities and the pathogenic power, showed that most aggressive isolates are the ones which produce most hydrolytics enzymes. The pathogen is frequently seed-borne, surviving for extended periods internally or externally on chickpea seed. This indicates that fungal metabolism becomes oriented towards synthesis and secretion of a whole arsenal of enzymes that are able to digest almost the complete plant cell wall (Gharbi *et. al.* 2013). The objective of this Article, is studied the morphological variability of isolates, their pathogenicity and highlight the pectinmethylesterase and polygalacturonase activities by colorimetric assay.

Materials and methods

Fungal isolates and growth conditions

Strains of *Fusarium oxysporum* f.sp *albedinis* are isolated from date palm rachis with symptomatic Bayoud. The rachis are cut into small pieces approximately 3 cm, disinfected with 2% sodium hypochlorite for 3 minutes, and followed by several rinses with sterile distilled water. Three fragments placed in Petri dishes containing PDA medium (Potato Dextrose Agar).

Pathogenicity test

After two months old, 10 ml spore suspension (10^6 spores /ml) was injected where young roots are grouped, 50 seedlings are used. The control seedling were treated with 10 ml of sterile distilled water, 10 ml of a suspension of 10^6 spores / ml of *F. oxysporum* f.sp *ciceri* and 10 ml of a suspension of 10^6 spores/ml of *F. oxysporum*. The start of the scoring began as soon as the first symptoms were visible; mortality is recorded at regular intervals for 2 months after the beginning of the first sign of disease, and the scoring is stopped when an isolate reaches 100% mortality. The test will be validated when the mortality exceeds 20%, and control plants do not present signs of disease.

Effect of different carbon sources on the mycelia growth

The isolates of *Foa* were cultivated in Czapek medium ($NaNO_3$ 2g, K_2HPO_4 1g, KCl 0.5g, $MgSO_4$ 0.5g, $FeSO_4$ 0.01g) containing 1% of different carbon sources as glucose, apple pectin and polygalacturonic acid. After 10 days of incubation at 22°C, the growth measurement of isolates was evaluated by determining of mycelium dry weight.

Assay PME activity

The assay method of the activity of this enzyme was based on the increase of the activity due to demethylation of pectin substrate. It is carried out as follows, 1 ml of culture filtrate is added to 5 ml of citrus pectin solution 0.5%, The reaction medium is adjusted to pH 7 with NaOH 0.1 N. The control test, the enzyme preparation is raised to 100 ° C in a water bath for 15 min after it was adjusted to pH 7. The culture filtrates tests and controls were incubated at 30 ° C stirring for 3 hours, then titrated with 0.05 N NaOH. The activity is expressed in U/mole, where one unit corresponds to the volume of filtrate required to obtain the release of a micro-equivalent of H^+/minute, it is then related to the volume of culture filtrate (Hamdy, 2005).

Assay of polygalacturonase activity

The polygalacturonase activity was measured by titration of reducing groups with the iodine-thiosulphate method. The reaction mixture 15 ml contained 12 ml of 0.5% polygalacturonic acid in 0.025 M citrate-phosphate buffer pH 4.0 with 0.02% sodium azide and 3 ml of the culture filtrate. After 2 hours at 30°C, 5 ml of reaction mixture was withdrawn, allowed to react with 5 ml of O.1N iodine solution and the residual iodine titrated with 0.05 N $Na_2S_2O_3$ solutions. The difference in titer values between the control and the treated samples was expressed as enzyme activity. The solution was acidified with 10 ml of N HCI, and the remaining iodine was titrated with 0,1N sodium thiosulphate solution. A blank titration without pectin was carried out at the same time, and the difference in titer gave the amount of 0.1 N Iodine solution reduced by 10 ml of pectin solution(Gharbi *et. al.*2013).

Statistical analyze

Multiple comparisons of means were performed by the Keuls-Newman test at a significance level of *P*= 0.05 using *XlStat* to differentiate the enzyme levels during the time course experiment. Correlation between data for in vitro enzyme production parameters and for pathogenicity parameters was calculated.

Results

The study of the macroscopic and microscopic appearance of isolates of *Fusarium oxysporum* f.sp *albedinis,* it was based on the appearance and color of the mycelium, characterization revealed macroscopic morphological variability within the collection. Different morphological aspects of our isolates were observed. This type is ras majority, this 50%, the downy type that has 16% other isolates have a compact and cottony mycelium having 33% of the collection.

Table 1. Mortality rates by weeks and isolate.

	Percentage mortality per week				
Isolates	2nd	3rd	4th	5th	Total%
KN1	8,0	32,0	51,0	8,2	99,2
KN2	4,0	20,0	40,8	8,2	73,0
KN3	10,0	28,0	26,5	8,2	72,7
KN4	12,0	10,0	10,2	8,2	40,4
KN5	8,0	14,0	10,2	8,2	40,4
KN6	10,0	10,0	12,2	8,2	40,4
KN7	8,0	16,0	18,4	22,4	64,8
KN8	14,0	28,0	12,2	10,2	64,4
KN9	14,0	10,0	12,2	4,2	40,4
KN10	10,0	22,0	22,4	10,2	64,6

We did not observe any sign of wilting or yellowing that characterizes this fusariose in controls, except for a few seedlings are dead. The withering of some control seedlings is not due to the action of *Fusarium*, perhaps, it is related to other factors. The number of seedlings withers varies according to isolates, isolate KN1 has destroyed 49 seedlings or a mortality rate of 99.2% and isolate KN4 caused a mortality of 20 seedlings a rate equal to 40% mortality. The collected

data expressed as percentage of mortality and isolate per week are reported in Table 01.

The mortality rate is varied as a function of time ($p < 0,007$) was significantly at the 5th and 2nd week and significantly higher in the 4th and 3rd week. Mortality did not differ significantly for lot factor ($p = 0,08$). However, the average mortality (22.07 and 17.607) is higher for the 4th and 3rd week (9.793 and 9.583) for the 5th and 2nd week. Test and Newman Keuls showed a distribution of three groups according to significant homogeneity, Group A that includes mortality 4th and 3rd week and Group B is composed of the 5th and 2nd week.

Table 2. Percentage growth of isolates *Fusarium oxysporum*.

Isolate	*citrus pectin*	*polygalacturonic acid*
Foc	96,2	93,9
Fo	99,1	99,8
KN1	92,0	90,9
KN2	88,3	87,7
KN3	99,3	89,6
KN4	68,4	94,2
KN5	99,2	92,9
KN6	50,5	50,4
KN7	99,0	99,0
KN8	96,7	93,7

The ANOVA shows that the mortality rate of 10 isolates inoculated seedlings of date palm to two-leaf stage, are significantly different at the 5% level. The mortality rate is highly significant, the Newman-Keuls test showed that the isolate KN1 has a high mortality rate, by against isolates KN8, KN7 and KN10 have an average mortality rate, whereas isolates KN9, KN5, KN6 KN4 and have a low mortality rate. This statistical test, it appears that isolates fall into four distinct groups. Group 1 contains only isolate KN1, the Group 2 consists of isolates KN2 and KN3, Group 3 is composed of isolates KN8, KN7 and KN8, and finally, the last group contains isolates KN9, KN5, KN6 and KN4.

Influence of substrates on mycelia growth

The average radial growth of isolates was transformed into percentage rate; results are expressed as percentage growth in table 02. The high percentage of growth is seen in *Foc, which reached 99.1% and 93.9% in the presence of citrus pectin and polygalacturonic acid* and there is a slight difference of the isolates growing on different substrates, and ANOVA revealed no significant difference (*p=0,96*).

Assay of PME activity

The incubation in buffered medium supplemented citrus pectin and polygalacturonic acid in the presence of culture filtrates causes acidification of the medium, this activity is measured quantitatively using the conventional technique of reducing power. The PME activity is present in the culture filtrates of isolates and the estimated amount is much higher in the presence of citrus pectin and glucose. We noted that this activity is variable among isolates, high activity is recorded (1.59, 1.36 and 1.1 10^3 U/mole) in KN1, Foc and Fo respectively. In the presence of citrus pectin, and this activity is greater than 4 times detect glucose, low PME activity whatever the substrate (glucose or citrus pectin) is observed in the isolate KN7 (0,01 et 0,02 10^3 U/mole) respectively. We noticed that this activity is present in all isolates of *Fusarium oxysporum* f.sp *albedinis*, *Fusarium oxysporum* and *Fusarium oxysporum f.sp ciceri* (fig 1).

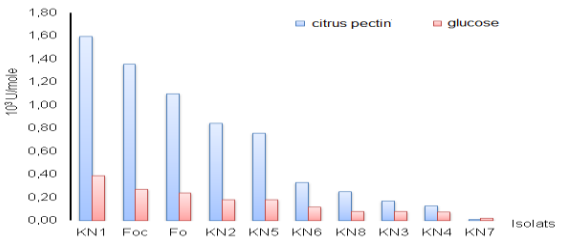

Fig. 1. PME activity in culture filtrates amended 1% glucose and citrus pectin in *Fusarium oxysporum*.

Assay of PG activity.

The activity measurement of the PG of the ten isolates was searched in culture filtrates with glucose and citrus pectin. Indeed, the activity of the PG was estimated after significant degradation of the citrus pectin.

The PG activity is recorded in KN1, *Foc* and *Fo*; equal (1.39 10^3, 0.07 10^3 and 0.02 10^3 µegr) respectively in the presence of 1% citrus pectin. In the presence of 1% glucose, this activity equal (0.44 10^3µeg) in isolate KN1 and (0.02 10^3 µeg) for *Foc* and *Fo* (fig 2).

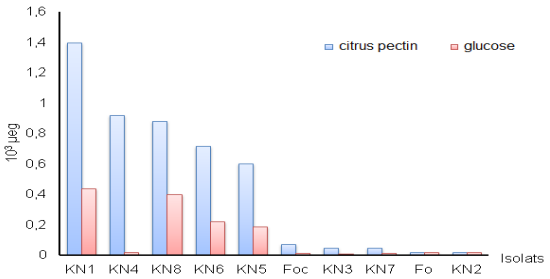

Fig. 2. Polygalacturonase activity in culture filtrates amended 1% Glucose and citrus pectin in Fusarium oxysporum.

Evaluation of pathogenicity and the ability to produce pectin methyl esterase and polygalacturonase in culture filtrates of ten isolates was analyzed by the Spearman correlation test, which showed a strong correlation between pathogenicity and production polygalacturonase.

Discussion

Morphological study of isolates has shown morphological variability; this study is the repeated practice of observing cultures of *Fusarium*, shows minor differences on the pigmentation or the abundance of aerial mycelium. There are always the same morphological types. According to descriptions on *Fusarium oxysporum,* the morphological variability of two isolates of *Fusarium oxysporum f. sp. lycopersici* was investigated both within inter- and intraclonal populations of conidia from young and old cultures. Data show the occurrence of pronounced interclonal variability in conidia from one of the isolates only, whereas the two isolates displayed intraclonal variability (Henni *et. al.*1994a), these authors showed that the pigment is relatively downy morphotype is always present in the freshly made isolates. Macroscopic and microscopic observations have not allowed differentiating between isolates. The number of seedlings wilt compared to control plants estimated the pathogenicity of 10 isolates;this test leads to the definition of special forms (Armstrong *et. al.*1981) (Henni *et. al.*1994b) Variation in pathogenicity was investigated in inter- and intra-clonal progenies of two strains of *Fusarium oxysporum* f. sp. *lycopersici*. Only quantitative variations in virulence (but no race modification) have been noticed. Thirty-two isolates of *Fusarium oxysporum f. sp. lentis* (Fol) were obtained from wilted lentil plants collected from different lentil growing areas in north-western Algeria. Pathogenicity tests were performed on all isolates (Belabid and Fortas, 2002).

The relationships between in vitro production of cell wall-degrading enzymes and aggressiveness of three *Phaeosphaeria nodorum* isolates were investigated. When grown in liquid medium containing 1% cell wall from wheat leaves as the carbon source, the isolates secreted xylanase, α-arabinosidase, β-xylosidase, polygalacturonase, β-galactosidase, cellulase, β-1,3-glucanase, β-glucosidase, acetyl esterase and butyrate esterase. Time-course experiments showed different levels of enzyme production and different kinetics between isolates. A highly aggressive isolate produced more xylanase, cellulase, polygalacturonase and butyrate esterase than did the two weakly aggressive isolates. Xylanase was the most active polymer-degrading enzyme produced, suggesting a key role during pathogenesis by *P. nodorum* (Lalaouia *et. al.*2000). Production of cell wall degrading enzymes (CWDEs) polygalacturonase (PG), pectate lyase (PL), and xylanase was studied in chickpeas (*Cicer arietinum* L. 'P-2245') inoculated with *Fusarium oxysporum* f. sp. *ciceris* (Padwick) Matuo & K. Sato races 0 (mildly virulent, causing a yellowing syndrome) and 5 (highly virulent, causing a wilting syndrome) by the water-culture method. These CWDEs were similarly produced in both syndromes. PG and PL were the only enzymes occurring in roots and stems and attained the highest specific activity,

this being generally higher for race 5 than for race 0. CWDE activities in roots and stems were positively correlated with development of yellowing and wilting. Exceptions to this were PG in stems, which was negatively correlated with the development of yellowing, and PG in roots, which showed a negative trend with development of either syndrome. The levels of CWDEs that significantly correlated with disease development were adequately described by exponential functions of disease progress. Results have implications for the role played by CWDEs in the early and later stages of pathogenesis in chickpea fusarium wilt (Jorge *et. al.*2006).

Refernces

Al-Najada AR, Al-Hindi RR, Mohamed SA. 2012. Characterization of polygalacturonases from fruit spoilage *Fusarium oxysporum* and *Aspergillus tubingensis*. African Journal of Biotechnology **34,** 8527-8536.

Al-Najada AR. 2014. Partial purification and physicochemical characterization of polygalacturonase from *Aspergillus awamori*. Life Science Journal **5,** 253-259.

Armstrong GM, Armstrong JR. 1981. Forms specials and races of *Fusarium oxysporum* causing wilt diseases. *Fusarium* : diseases, biology and taxonomy. States Univ. Press. Univ Park and London **3,** 391-393.

Belabid L, FORTAS Z. 2002. Virulence and vegetative compatibility of Algerian isolates of *Fusarium oxysporum* f.sp *lentis*. Phytopathol. Mediterr **41,** 179–187.

El Moudafar C, El Boustani E. 2000. Relationship between cell wall susceptebility to cellulases and pectinases of *Fusarium oxysporum* and susceptibility of date palm cultivars. Biologia Plantarium **4,** 571-576.

Gharbi S, Karkachi, N, Kihal M, Henni J. 2013. Carbon sources and pH effect on pectinolytic activity production by *Ascochyta rabiei* isolated from chickpea (*Cicer arietinum L.*) in West Algeria. African Journal of Microbiology Research **7,** 3483-3488.

Hamdy HS. 2005. Purification and characterization of the pectin lyase produced by *Rhizopus oryzae* grown on orange peels. Annals of Microbiology **3,** 205-211.

Henni J, Boisson C, Geiger JP. 1994a. Variabilité de la morphologie chez *Fusarium oxysporum f. sp. lycopersici* . Phytopath. medit **33,** 51-58.

Henni J, Boisson C, Geiger JP. 1994b. Variabilité du pouvoir pathogène chez *Fusarium oxysporum f. sp. lycopersici* . Phytopath. medit **33,** 10-16.

Jorge I, Navas-Cortés JA, Jiménez-Diaz RM, Tena M. 2006. Cell wall degrading enzymes in fusarium wilt of chickpea: correlation between pectinase and xylanase activities and disease development in plants infected with two pathogenic races of *Fusarium oxysporum* f. sp. *ciceris*. Can. J. Bot **84,** 1395–1404.

Lalaoui F, Halama P, Dumortier V, Paul B. 2000. Cell wall-degrading enzymes produced in vitro by isolates of *Phaeosphaeria nodorum* differing in aggressiveness. Plant Pathology **49,** 727-733.

Nitinkumar PP, Bhushan LC. 2010. Production and purification of pectinase by soil isolate *Penicillium sp* and search better agro-residue for its SSF. Recent Research in Science and Technology **7,** 36-42.

Zhang JX, Bruton BD, Biles CL. 2010. Polygalacturonase isozymes produced by *Phomopsis cucurbitae* in relation to postharvest decay of cantaloupe fruit. Phytopathology **87,** 1020-1025

Reignault R, Valette-Collet O, Boccara M. 2008. The importance of fungal pectinolytic enzymes in plant invasion, host adaptability and symptom type. Bulletin OEPP/EPPO **33,** 245–247.