



## Growth and cutinase activity of *Fusarium culmorum* grown in solid-state fermentation

### Crecimiento y actividad enzimática de cutinasa de *Fusarium culmorum* en fermentación sólida

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#### ABSTRACT

Cutinase is a very versatile enzyme due to the wide range of substrates that it can use. It has application in several biotechnological areas, acting as biocatalysts in the food industry, in detergents as well as in biodegradation of polymers and other toxic substances. In this research, glucose consumption, protein content, biomass and cutinase production by *Fusarium culmorum* were evaluated. Cutinolytic activity was observed using gel zymography. This fungus was grown on culture media containing glucose and different concentrations of apple cutin (0, 1, 3 and 10 g/L) in solid-state fermentation. *F. culmorum* showed the highest production of biomass, protein and cutinase in medium supplemented with 10 g of cutin/L. Glucose uptake was inversely proportional to the cutin concentration. Medium lacking cutin showed the fastest glucose consumption. Heavily stained bands were observed in the gel with increasing concentrations of cutin after 72 h, showing a molecular weight of 50 kDa. These results shown that apple cutin induced cutinase production and was also used as carbon source. It was also observed that glucose did not act as a repressor of cutinase production.

Keywords: cutinase, *Fusarium culmorum*, solid-state fermentation.

## RESUMEN

La versatilidad de la cutinasa es debida a los diversos sustratos que puede emplear. Estas enzimas tienen aplicación en distintas áreas biotecnológicas, lo que las convierte en atractivos biocatalizadores para la industria alimentaria, de detergentes, así como en la degradación de polímeros y sustancias tóxicas. En este trabajo se evaluó el consumo de glucosa, contenido de proteína, la producción de biomasa y cutinasas por el hongo *Fusarium culmorum* crecido en medios conteniendo glucosa y diferentes concentraciones de cutina de manzana (0, 1, 3 y 10 g/L) en fermentación sólida. La actividad cutinolítica fue observada empleando zimografía. *F. culmorum* mostró la mayor producción de biomasa, proteína y cutinasas en el medio adicionado con 10 g de cutina/L. El consumo de glucosa fue inversamente proporcional a la concentración de cutina. La glucosa se consumió más rápido en el medio sin adición de cutina. El aumento en la concentración de cutina de manzana incremento la intensidad en la banda mostrada en el zimograma a partir de las 72 h, cuyo peso molecular fue de 50 kDa. Estos resultados muestran que la cutina de manzana indujo la producción de cutinasa y que fue usada como fuente de carbono. La glucosa no actúa como un represor de la producción de cutinasas.

Palabras clave: cutinasa, fermentación sólida, *Fusarium culmorum*.

## 1. INTRODUCTION

Cutinases (E.C 3.1.1.73) are extracellular and inducible enzymes which break down cutin, the major component of vegetal cuticle in higher plants, which is composed of esterified fatty acids (C<sub>16</sub> or C<sub>18</sub>) (Castro-Ochoa *et al.*, 2012). Cutinases are predominantly produced by phytopathogenic fungi with the ability to grow on cutin as sole carbon source. These enzymes can be induced by cutin (Purdy & Kolattukudy, 1973; Pio & Macedo 2007; Degani, 2015). Cutinases are also able to catalyze the synthesis and hydrolysis of short chain synthetic esters, triglycerides and polyesters (Pio & Macedo 2005; Degani, 2015). Cutinase production is regulated by several conditions, especially the carbon sources and the fermentation system (Fraga *et al.*, 2012). Solid-state fermentation (SSF) shows a great potential in fungal enzymes production over commonly used submerged fermentation processes. SSF offers some advantages for organisms to growth, such as similarity with natural habitat, higher productivities, lower cost, lower effect of carbon repression and it has potential application in biotechnological areas or industrial processes in which low water activity is required (Viniestra-Gonzalez *et al.*, 2003; Macedo & Fraga 2007). On the other hand, cutinases have been widely used in different industries, like food, detergents, agrochemicals, biofuel and in plastic degradation (Macedo & Pio 2005; Speranza *et al.*, 2011; Fraga *et al.*, 2012). Cutinases are enzymes that belong to the group of esterases and are important in bioremediation processes. Filamentous fungi of the genus *Fusarium* have been widely studied for producing cutinases that have the ability to degrade environmental pollutants such as phthalates (Kim *et al.*, 2005). Particularly, it has been reported that *F. culmorum* produced esterase and degraded high concentrations (1000 mg/L) of di (2-ethyl hexyl) phthalate, a plasticizer widely used in the manufacture of plastics (Ahuactzin-Pérez

*et al.*, 2016). Chen *et al.* (2013) reported that the traditional method to measure cutinase activity is to use cutin as substrate and detect the cutin fatty acid monomers released by hydrolysis. In the present research, *Fusarium culmorum* was evaluated for glucose consumption, protein content, biomass and cutinases production. The fungus was grown on culture media containing glucose and different concentrations of apple cutin (0, 1, 3 and 10 g/L) in solid-state fermentation. Cutinolytic activity was observed using gel zymography.

## **2. MATERIALS AND METHODS**

### **2.1. Microorganism**

A strain of *F. culmorum* from the culture collection from the Research Centre for Biological Sciences (CICB) at Universidad Autónoma de Tlaxcala, Mexico, was used. This strain was isolated in a recycled paper industry (Aguilar-Alvarado *et al.*, 2015). The strain was grown on malt extract agar (Difco) at 20 °C and stored at 4 °C for its conservation.

### **2.2. Cutin extraction**

Cutin was extracted from the cuticle of apples (Golden Delicious) using the method described by Macedo & Pio (2005). Apples were peeled and peels were placed in a beaker, and then a buffer solution of sodium oxalate at pH of 3.5 was added. Peels were boiled for 30 min in order to separate the pulp remaining from the peels. Peels were filtered and freeze-dried at -40 °C (for 12 h) and then lyophilized. A solution of methanol and dichloromethane (1:1 v/v) was added to the lyophilized material and then left overnight. Cutin was obtained in the supernatant after filtering the solution and then cutin powder was obtained by rotary evaporation at 45 °C.

### **2.3. Culture media preparation**

Four culture media were prepared: 1) glucose yeast extract (GYE), 2) GYE + 1 g of cutin/L, 3) GYE + 3 g of cutin/L, and 4) GYE + 10 g of cutin/L. GYE medium contained (in g/L) 10 glucose, 5 yeast extract, 0.6 KH<sub>2</sub>PO<sub>4</sub>, 0.5 MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.4 K<sub>2</sub>HPO<sub>4</sub>, 0.25 CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.05 FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.05 MnSO<sub>4</sub>, and 0.001 ZnSO<sub>4</sub>·7H<sub>2</sub>O. The pH of the medium was adjusted to 8.5. Flasks of 125 mL containing 0.5 g of polyurethane foam (PUF) (cubes of 0.5 x 0.5 x 0.5 cm<sup>3</sup>) were added with 15 mL of culture medium.

### **2.4. Biomass production**

Flasks containing culture medium and PUF cubes were autoclaved at 120 °C for 15 min, cooled to room temperature and then inoculated with three mycelial plugs (of 10 mm diameter) taken from the periphery of 7-d-old colonies of *F. culmorum* grown on malt extract agar. Cultures were incubated at 22 °C for 5 days, samples were taken at 16-h intervals in triplicate. Biomass was separated from the supernatant by filtration, kept in the oven for 24 hours to dry and weighted (Ahuactzin-Pérez *et al.*, 2016).

## 2.6. Glucose consumption analysis

Glucose assay was carried out using 3,5-dinitrosalicylic acid (DNS) and detected by spectrophotometry at 575 nm. Reaction mixture contained 60  $\mu$ L of the supernatant, 2 mL  $\mu$ L of DNS and 940  $\mu$ L distilled. The samples were incubated in a water bath for 5 minutes and immediately transferred to cold water for another 5 minutes to stop the reaction. The spectrophotometer was calibrated using 2 mL DNS and 1 mL of distilled water (Miller, 1959).

## 2.6. Protein production

Supernatants were used to determine the protein production using Bradford (1976) protein assay. 960 mL of Bradford reagent (Bio-Rad) added to a test tube with 40 mL of each supernatant. Protein production was evaluated by using a Jenway spectrophotometer at 595 nm.

## 2.7. Cutinase activity and zymography

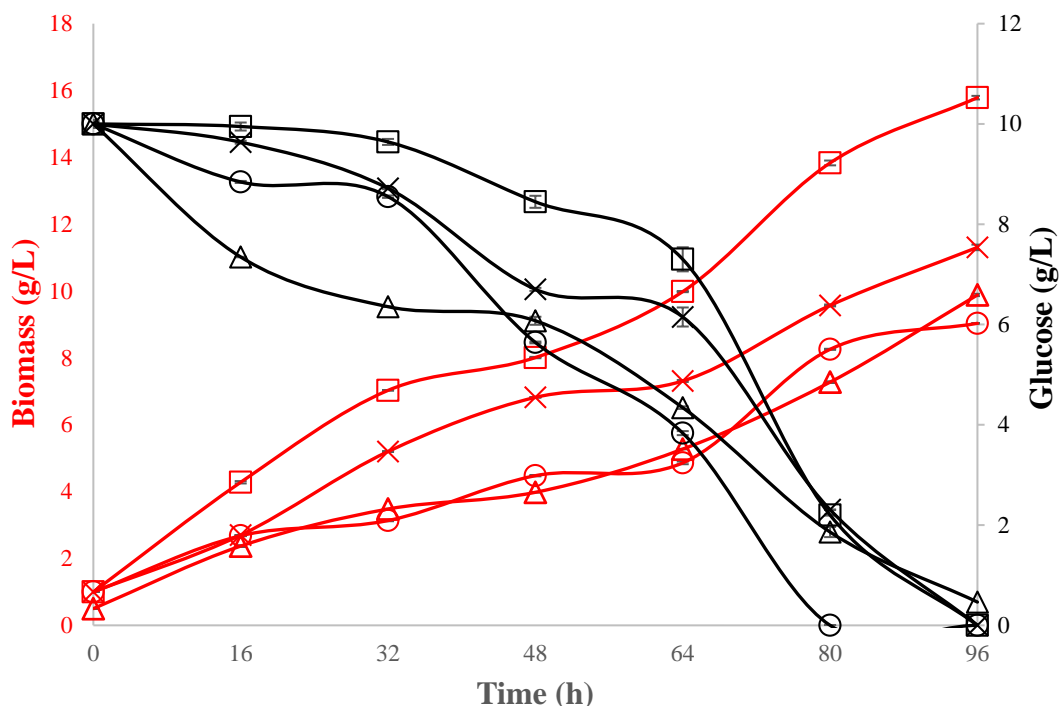
Cutinase activity was evaluated by spectrophotometry at 405 nm by adding 900  $\mu$ L of substrate and 100  $\mu$ L of supernatant sample. The reaction mixture contained 19.6  $\mu$ L of p-nitrophenyl butyrate (pNPB), 1.108 mL acetonitrile, 11.108 mL distilled water, 4.6  $\mu$ L Triton X-100 and was diluted with phosphate buffer (pH 7.5) in order to obtain 100 mL of substrate (Córdoba-Sosa *et al.*, 2014). Cutinase activity was measured in the supernatant and detected using zymography. The polypeptide profiles of the samples with cutinase activity were analyzed by electrophoresis in 0.1% polyacrylamide gels with sodium dodecyl sulfate (SDS-PAGE) (Laemmli, 1970). 12% and 4% acrylamide gels were used as separation and packaging gels, respectively. Precision Plus Protein™ Dual Xtra Standards (Bio-Rad) was used as molecular weight marker. Samples were tested on gels of 0.75 mm in a Mini Protean electrophoresis system Tetra Cell (Bio-Rad) at 100 volts for 1.30 h. Gels were incubated with 20 mM Tris-HCl buffer (pH 6.5) and Triton X100 (2%) for 10 min at 4 °C. After incubation solution was poured and the top of the gels were rinsed with deionized water. Subsequently, gels were incubated with agitation for 3 hours at room temperature in a solution containing 3 mM of  $\alpha$ -naphthyl acetate, 1 mM Fast Red TR (Sigma) and 100 mM phosphate buffer (pH 7.5) (Karpushova *et al.*, 2005). Finally, cutinase activity was detected by the appearance of red-colored bands in the gels.

## 3. RESULTS

### 3.1. Biomass production and glucose consumption

The results for biomass production and glucose concentration are shown in Figure 1. The highest biomass production was obtained in medium containing 10 g of cutin/L and the lowest was obtained in medium lacking cutin.

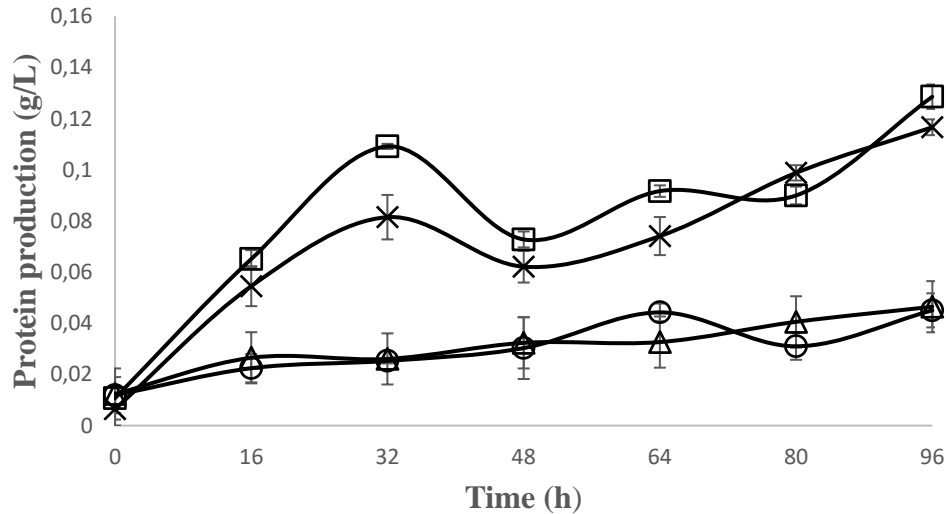
The fastest glucose consumption was observed in the medium supplemented with 1 g of cutin/L and the slowest was showed in medium containing 10 g of cutin/L.



**Fig. 1.** Biomass production (red lines) and glucose consumption (black lines) by *F. culmorum* grown on media supplemented with 0 ( $\Delta$ ), 1 ( $\circ$ ), 3 ( $\times$ ) and 10 ( $\square$ ) g of cutin/L.

### 3.2. Protein production

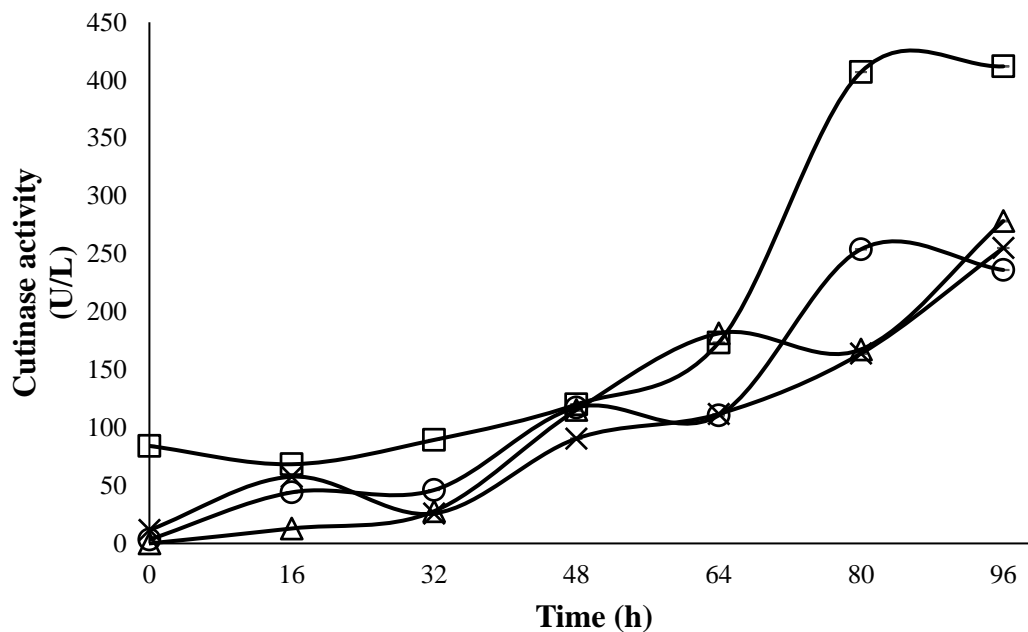
The results for protein production (Fig. 2.) showed that *F. culmorum* produced the maximum protein in the culture medium containing 10 g of cutin/L, having similar results in medium supplemented with 3 g of cutin/L. Both culture media, added with 0 and 1 g of cutin/L showed the lowest protein production with a maximum production of approximately 0.04 g/L.



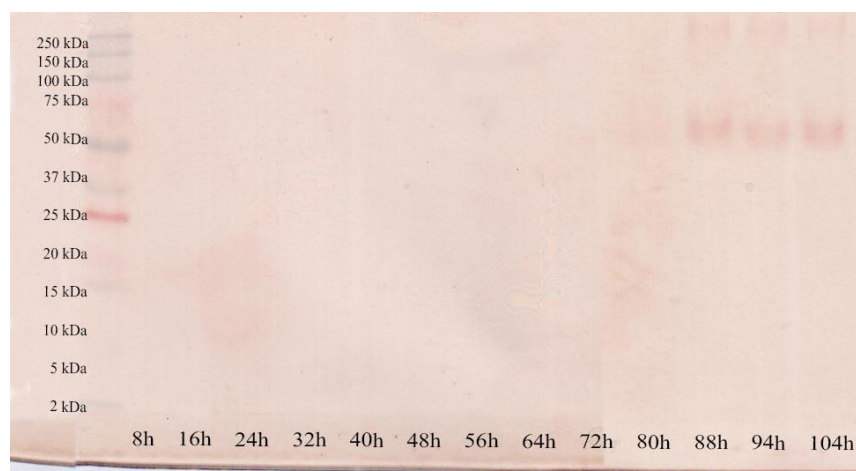
**Fig. 2.** Protein production of *F. culmorum* grown on media supplemented with 0 ( $\Delta$ ), 1 ( $\circ$ ), 3 ( $\times$ ) and 10 ( $\square$ ) g of cutin/L in SSF

### 3.3. Cutinase activity and zymograms

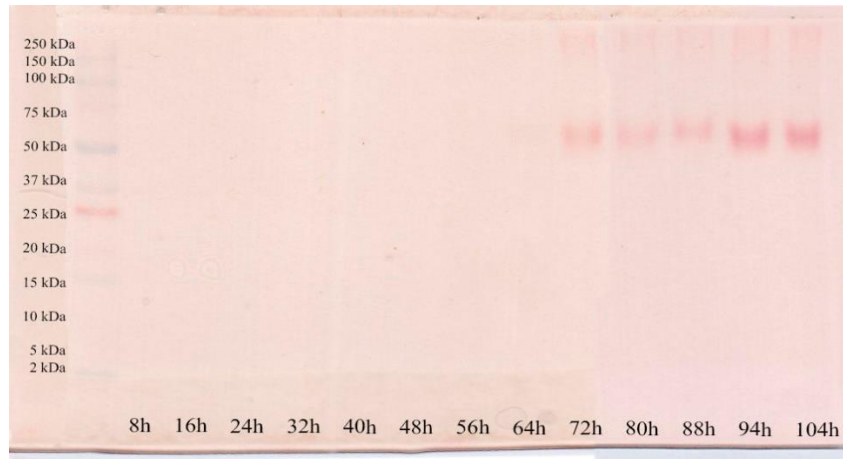
The cutinase activity increased according to the amount of cutin added to the culture media. The concentration of 10 g of cutin/L showed the highest enzymatic activity in comparison with the other concentrations (Fig. 3.). This was confirmed with the zymograms. The zymogram for 0 g/L (Fig. 4.) showed four bands slightly stained, they represent the fermentation samples taken from day 4 and 5. Since there was no cutin added to this culture media, *F. culmorum* did not show an increase in its cutinase production. Figs 5 and 6 showed the zymograms of *F. culmorum* grown on media supplemented with 1 and 3 g of cutin/L, respectively. The color intensity in the bands of the zymogram for 10 g/L (Fig. 7.) was higher than the rest of the zymograms (Figs. 5, 6). In general, bands appeared from hour 64 to 104, which represent the fermentation samples taken from day 3 to 5.



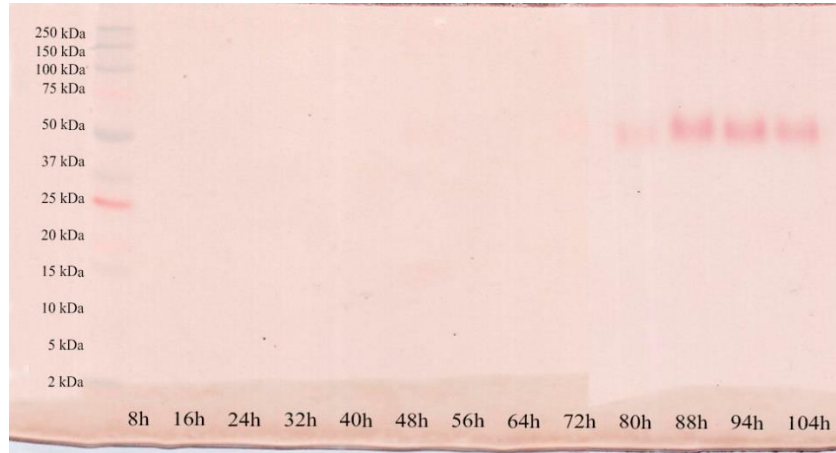
**Fig. 3.** Cutinase activity of *F. culmorum* grown on media supplemented with 0 (Δ), 1 (○), 3 (×) and 10 (□) g of cutin/L in SSF.



**Fig. 4.** Zymogram of *F. culmorum* grown on media supplemented with 0 g of cutin/L. Lightly stained bands can be observed from hour 80 to 104.

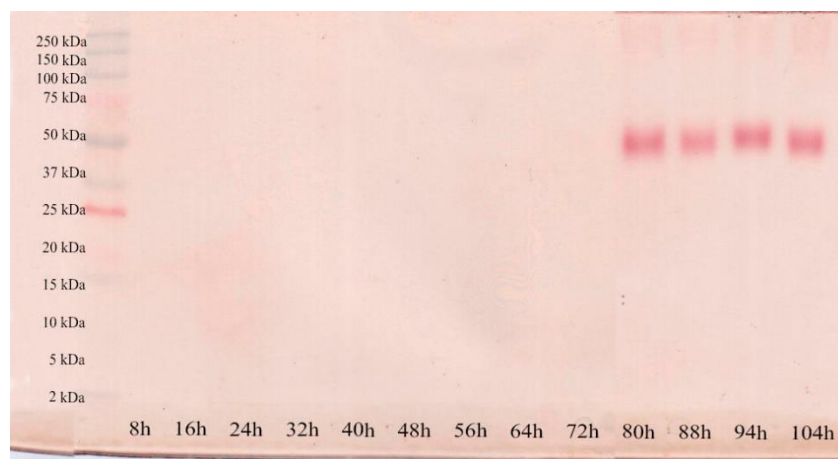


**Fig. 5.** Zymogram of *F. culmorum* grown on media supplemented with 1 g of cutin/L. Bands become more visible and can be observed from hour 64 to 104.



**Fig. 6.** Zymogram of *F. culmorum* grown on media supplemented with 3 g of cutin/L. Bands are completely visible and can be observed from hour 72 to 104.





**Fig.7.** Zymogram of *F. culmorum* grown on media supplemented with 10 g of cutin/L. Bands are intensively stained and can be observed from hour 80 to 104.

#### 4. DISCUSSION

Cutinases hydrolyze various substrates and catalyze several reactions, thus natural cutin has been used as a growth substrate to demonstrate cutinases identity (Chen *et al.*, 2013). Evaluation of cutinase activity in *Fusarium solani f. sp. pisi* has confirmed that these enzymes are produced by filamentous fungi when grown in media containing cutin or its hydrolysate (Purdy & Kolattukudy, 1973; Lin & Kolattukudy 1978; Degani, 2015). Similarly, our results confirmed that *F. culmorum* was able to grow in media supplemented with natural cutin and use it as a carbon source, since it showed higher biomass production as cutin concentration increased in the growth medium. On the other hand, the increase in protein content was directly related to the increase in biomass production and enzymatic activity detected in supernatant samples. This suggests that the fungus produced soluble proteins to metabolize the simple carbon source (glucose) and once it was fully consumed, *F. culmorum* produced cutinolytic enzymes, allowing the degradation of cutin. Aguilar-Alvarado *et al.* (2015) studied *F. culmorum* grown on media containing di (2-ethyl hexyl) phthalate (DEHP) and demonstrated the ability of this organism to degrade esterified compounds, resulting in an increase in biomass and esterases production in the media containing the highest concentration of DEHP (1500 mg/L). Ahuactzin-Pérez *et al.* (2016) demonstrated that *F. culmorum* used DEHP as a carbon and energy source even when the media contains high concentration of glucose (10 mg/L) and DEHP (1000 mg/L). Furthermore, due to the difficulty of assessing the enzymatic activity of cutinase using radiolabeled cutin as substrate (Purdy & Kolattukudy, 1973; Davies *et al.*, 2000). It was decided to detect cutinase activity by simpler methods using native cutins as substrate (Castro-Ochoa *et al.*, 2012), and 4-nitrophenyl esters as commercial substrates (Degani *et al.*, 2006). Degani (2015) reported that cutinase activity increases according to the amount of cutin added to the culture media. Hawthorne *et al.* (2001) demonstrated that the enzymatic activity is regulated by the source and concentration of cutin, which is in accord with the results shown here, since *F. culmorum* cutinolytic activity raised by increasing cutin concentration. Macedo & Pio (2005) reported that *F. oxysporum* showed an increase

in the cutinolytic activity determined by spectrophotometry using *p*NPB when a concentration of 4 g of cutin/L was added to the media. Several techniques have been developed in order to identify cutinases in SDS-PAGE gels (Castro-Ochoa *et al.*, 2012; Karpushova *et al.*, 2005; Yang *et al.*, 2013). The use of these and other techniques have revealed fungal cutinases of about 22 kDa (Castro-Ochoa *et al.*, 2010; Degani, 2015). Soliday & Kolattukudy (1976) reported that *Fusarium roseum culmorum* produced cutinases and non-specific esterases that hydrolyzed cutin and *p*-NPB in a similar way to those cutinases from *F. oxysporum*, which molecular weight was 24.3 kDa. In this paper, we were able to observe by zymography, esterase activity bands between 72 and 104 in the four different media, with a molecular weight of approximately 50 kDa.. Thus, this could be a new isoform produced by *F. culmorum*. The zymograms that correspond to the media supplemented with cutin showed bands with a higher color intensity when compared with the control. These results show that apple cutin induced the production of cutinases by *F. culmorum*, and that apple cutin was used as carbon source. It was also observed that glucose did not act as a repressor of cutinase production.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## REFERENCES

Aguilar-Alvarado Y., Báez S.R., Martínez C.C., Ahuactzin P.M., Cuamatzi M.M. & Sánchez C. 2015. Mycelial growth and enzymatic activities of fungi isolated from recycled paper wastes grown on Di(2-ethylhexyl) phthalate. Polish Journal of Environmental Studies. 24(5): 1897-1902.

Ahuactzin-Pérez M., Tlecuitl-Beristain S., García-Dávila J., González-Pérez M., Gutiérrez-Ruiz MC. & Sánchez C. 2016. Degradation of di (2-ethyl hexyl) phthalate by *Fusarium culmorum*: Kinetics, enzymatic activities and biodegradation pathway based on quantum chemical modeling. Science of the Total Environment. DOI: 10.1016/j.scitotenv.2016.05.169.

Bradford M. M. 1976. A Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*. 72: 248–254.

Castro-Ochoa D., Peña-Montes C. & Farrés A. 2010. Producción y características de cutinasas: alternativa interesante para la biocatálisis a nivel industrial. *Revista Especializada en Ciencias Químico-Biológicas*. 13(1): 16-25.

Castro-Ochoa D., Peña-Montes C. & Farrés A. 2012. ANCUT2, an extracellular cutinase from *Aspergillus nidulans* induced by olive oil. *Revista Especializada en Ciencias Químico-Biológicas*. 13(1): 16-25.

Chen S., Chen J. & Wu J. 2013. Cutinase: Characteristics, preparation and application. *Biotechnology Advances*. DOI:10.1016/j.biotechadv.2013.09.005.

Córdoba-Sosa G., Torres J.L., Ahuactzin-Pérez M., Díaz-Godínez G., Díaz R. & Sánchez, C. 2014. Growth of *Pleurotus ostreatus* ATCC 3526 in different concentrations of di (2-ethylhexyl) phthalate in submerged fermentation. *Journal of Chemical, Biological and Physical Sciences*. 4(5): 96-103.

Davies K. A., De Lorono I., Foster S.J., Li D., Jhonstone K. & Ashby A.M. 2000. Evidence for a role of cutinase in pathogenicity of *Pyrenopeziza brassicae* on brassicas. *Physiological and Molecular Plant Pathology*. 57: 63-75.

Degani O. 2015. Production and purification of cutinase from *Fusarium oxysporum* using modified growth media and a specific cutinase substrate. *Advances in Bioscience and Biotechnology*, 6: 245-258.

Degani O., Salman H., Geptein S. & Dosoretz C. 2006. Synthesis and characterization of a novel cutinase substrate, 4-nitrophenyl (16-methyl sulfone ester) hexadecanoate. *Journal of Biotechnology*. 121: 346-350.

Fraga P., Carvalho P. & Macedo A.G. 2012. Production of cutinase by *Fusarium oxysporum* on Brazilian agricultural by-products and its enantioselective properties. *Food and Bioprocess Technology*. 5: 138-146.

Hawthorne B.T., Rees-George J. & Crowhurst R.N. 2001. Induction of cutinolytic esterase activity during saprophytic growth of cucurbit pathogens, *Fusarium solani* f. sp. *cucurbitae* races one and two (*Nectria haematococca* MPI and MPV, respectively). *Federation of European Microbiological Societies Microbiology Letters*. 194: 135-141.

Karpushova A., Brummer F., Barth S., Lange S. & Schmid R.D. 2005. Cloning, recombinant expression and biochemical characterisation of novel esterases from *Bacillus* sp. associated with the marine sponge *Aplysina aerophoba*. *Applied Microbiology and Biotechnology*. 67: 59-69.

- Kim Y.H., Ahn J.Y., Moon S.H. & Lee J. 2005. Biodegradation and detoxification of organophosphate insecticide, Malathion by *Fusarium oxysporum* f. sp. *pisi* cutinase. *Chemosphere*. 60: 1349-1355.
- Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. 15,227(5259): 680-685.
- Lin T. & Kolattukudy P. 1978. Induction of a biopolyester hydrolase (Cutinase) by low levels of cutin monomers in *Fusarium solani* f. sp. *Pisi*. *Journal of Bacteriology*. 133: 942-951.
- Macedo G. & Fraga L. 2007. Production of cutinase by *Fusarium oxysporum* in solid-state fermentation using agro-industrial residues. *Journal of Biotechnology*. 131(2): S211-S241.
- Macedo G.A. & Pio T.F. 2005. A rapid screening method for cutinase producing microorganisms. *Brazilian Journal of Microbiology*. 36(4): 388-394.
- Miller G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*. 31(3): 426-428. DOI: 10.1021/ac60147a030.
- Pio T.F. & Macedo G. A. 2007. Optimizing the production of cutinase by *Fusarium oxysporum* using response surface methodology. *Enzyme Microbial Technology*. 41: 613-619.
- Purdy R.E. & Kolattukudy P.E. 1973. Depolymerization of hydroxy fatty acid biopolymer, cutin, by an extracellular enzyme from *Fusarium solani* f. *pisi* isolation and some properties of the enzyme. *Archives of Biochemistry and Biophysics*. 159: 61-69.
- Soliday C.L. & Kolattukudy P.E. 1976. Isolation and characterization of a cutinase from *Fusarium roseum culmorum* and its immunological comparison with cutinases from *F. solani pisi*. *Archives of Biochemistry and Biophysics*. 176: 334-343.
- Speranza P., Carvalho P. & Macedo A.G. 2011. Effects of the different solid state fermentation substrate on biochemical properties of cutinase from *Fusarium* sp. *Journal of Molecular Catalysis B: Enzymatic*. 72:181-186.
- Viniegra-González G., Favela-Torres E., Aguilar C.N., Romero-Gómez S.J., Díaz-Godínez G. & Augur C. 2003. Advantages of fungal enzyme production in solid state over liquid fermentation systems. *Biochemical Engineering Journal*. 13:157-167.
- Yang S., Xu H., Yan Q., Liu Y., Zhou P. & Jiang Z. 2013. A low molecular mass cutinase of *Thielavia terrestris* efficiently hydrolyze poly(esters). *Journal of Industrial Microbiology and Biotechnology*. 40: 217-226.