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SHORT COMMUNICATION

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Production of esterase by *Fusarium culmorum* grown in the presence of different concentrations of di(2-ethylhexyl) phthalate and calcium ion in submerged fermentation

Producción de esterasa por *Fusarium culmorum* crecido en presencia de diferentes concentraciones di(2-etilhexil) ftalato y de ion calcio en fermentación sumergida

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ABSTRACT

Phthalates are plastic additives used as plasticizers in the manufacture of flexible plastic materials. These compounds, such as di(2-ethylhexyl) phthalate (DEHP) are toxic substances to the environment and human health, and have been detected as environmental pollutants. In this study, the activity of esterase produced by *Fusarium culmorum* grown in media supplemented with different concentrations of DEHP (1.5, 3 and 4 g/L) and calcium ion (0.1 and 0.5 g/L) in submerged fermentation was characterized by biochemical tests and polyacrylamide gel electrophoresis. The greatest esterase activity was observed in cultures grown in media added with 3 g of DEHP/L and 0.5 g of calcium ion/L, followed by those cultures grown in media containing 1.5 g of DEHP/L. Whereas cultures grown on 4 g of DEHP/L had the lowest esterase activity. In general, cultures added with 0.5 g of calcium ion/L had the maximum enzymatic activity and the highest enzymatic

productivity in the media tested. High concentration of DEHP were used as the sole source of carbon and energy by *F. culmorum*. It is suggested that the calcium ion favored the esterase production during the DEHP biodegradation. However, further studies are needed to understand the relationship between calcium ion, fungal growth, and enzyme production to provide the appropriate concentration of this ion in the culture medium.

Keywords: Calcium ion, di(2-ethylhexyl) phthalate, esterase, *Fusarium culmorum*, submerged fermentation.

RESUMEN

Los ftalatos son aditivos plásticos utilizados como plastificantes en la fabricación de materiales plásticos flexibles. Estos compuestos, dentro de los cuales se encuentra el di(2-etilhexil) ftalato (DEHF), son sustancias tóxicas para el medio ambiente y para la salud humana, y han sido detectados como contaminantes ambientales. En este estudio, se caracterizó la actividad de esterasa producida por *Fusarium culmorum* crecido en medios suplementados con diferentes concentraciones de DEHF (1.5, 3 y 4 g/L) y de ion calcio (0.1 y 0.5 g/L) en fermentación sumergida, empleando análisis bioquímicos y electroforesis en gel de poliacrilamida. La mayor actividad de esterasa se observó en los cultivos crecidos en medios adicionados con 3 g de DEHF/L y 0.5 g de ion calcio/L, seguidos de aquellos cultivos crecidos en medios que contenían 1.5 g de DEHF/L. Mientras que cultivos crecidos en 4 g de DEHF/L tuvieron la más baja actividad de esterasa. En general, se demostró que los cultivos adicionados con 0.5 g de ion calcio/L tuvieron la máxima actividad enzimática y la mayor productividad enzimática en los medios estudiados. Esta investigación muestra que *F. culmorum* empleó altas concentraciones de DEHF como única fuente de carbono y energía. Se sugiere que el ion calcio favoreció la producción de esterasa durante la biodegradación de DEHF. Sin embargo, es necesario realizar más estudios para comprender la relación entre el ion calcio, el crecimiento de hongos y la producción de enzimas para adicionar una adecuada concentración de este ion en el medio de cultivo.

Palabras clave: Di(2-etilhexil) ftalato, esterasa, fermentación sumergida, *Fusarium culmorum*, ion calcio.

1. INTRODUCTION

Phthalic acid esters (phthalates) are used as plastic additives (plasticizers), which are incorporated during plastic manufacture to give flexibility to plastic products. These substances are present as pollutants in the environment because they are released from plastics materials, and have been reported to be toxic to mammals (Wang and Qian, 2021). Due to this toxicity, it is crucial to develop effective, ecological, and economical techniques for environmental remediation. In this context, microorganisms are a promising alternative for efficient and effective plastic additive removal. Di(2-ethylhexyl) phthalate (DEHP) is a high molecular weight phthalate, which is widely used as a plasticizer in the manufacture of PVC products (e.g., shoes, raincoats, and medical devices) (Sánchez, 2021). DEHP is in the list of priority pollutants of the US Environmental Protection Agency (Yang *et al*., 2018). Microorganisms have been reported to be able to degrade DEHP at different concentrations

(ranging from 1 to 3 g/L). Fungi genera such as *Fusarium*, *Aspergillus*, and *Penicillium* have been reported with potential to degrade DEHP, due to their ability to produce esterase (Pérez-Andrés *et al*., 2017; Xu *et al*., 2020; Hernández-Sánchez *et al*., 2019; Sánchez *et al*., 2020; Sánchez, 2021). In general, microbial degradation of DEHP seems to proceed via hydrolysis of ester bonds by an esterase to their corresponding monoesters, which breakdown to simultaneously form phthalic acid and alcohols to ultimately generate short chain acids, which would eventually enter the Krebs cycle (González-Márquez, *et al*., 2019; Loftus *et al*., 2020; Sánchez, 2021; Hernández-Sánchez et al., 2024). Therefore, fungi use DEHP as a source of carbon and electrons, providing them with cellular materials and an energy source, respectively. It has been reported that *F. culmorum* is a highly effective fungus for biodegradation of high concentrations of DEHP (Ahuactzin-Pérez *et al*., 2016; Ferrer-Parra *et al*., 2018; González-Márquez *et al*., 2019; Hernández-Sánchez *et al*., 2024). González-Márquez *et al*. (2020) reported that DEHP degradation by *F. culmorum* was enhanced by the addition of calcium nitrate as nitrogen source, concluding that calcium ion increased the esterase production. It has been reported that calcium is crucial in fungal cell signaling, and is involved in the regulation of a wide range of biological activities (e.g. sporulation, branching, tip growth, hyphal reorientation, sexual reproduction, etc.) (Gadd, 1994; Hyde and Heath, 1995; Jackson and Hardham, 1996; Grinberg and Heath, 1997; Torralba and Heath, 2001). Fungal growth originates from hypha cell physical state. Studies on fungal growth have showed that hypha grows at its tip, with turgor being one of the main forces responsible of growth at the hyphal tip. In addition, the actin cytoskeleton and calcium gradients regulate initiation and maintenance of tip growth. The process of apical growth of hyphae involves the migration of wall vesicles transporting raw materials, which are eventually exocytosed at the hyphal tip (Torralba and Heath, 2001; Lew, 2011), leading ultimately to the mycelial colonization of the substrate.
In this study, the activity of esterase produced by *F. culmorum* grown in media supplemented with different concentrations of DEHP (1.5, 3 and 4 g/L) and calcium ion (0.1 and 0.5 g/L) in submerged fermentation was characterized by biochemical tests and polyacrylamide gel electrophoresis.

2. MATERIALS AND METHODS

2.1. Organism and inoculum preparation

F. culmorum from the culture collection of the Research Centre for Biological Sciences at Universidad Autónoma de Tlaxcala (CICB, Tlaxcala, Mexico) was used. This fungus was isolated from an area polluted by plasticizers (Aguilar-Alvarado *et al*. 2015). Cultures of *F. culmorum* grown on Petri dishes containing malt extract agar (Merck, Mexico) and incubated for 7 d at 25 °C were used as a source of primary inoculum. Three fragments of mycelium (10 mm in diameter) were taken from the colony periphery, which were used to inoculate Erlenmeyer flasks (125 mL) containing 40 mL of V8 juice-$CaCO_3$ agar medium (González-Márquez *et al*., 2019). Flasks were incubated at 25 °C for 5 d to promote spore production, which were collected using a solution of Tween 80 (0.01%, v/v), and then reinoculated into fresh V8 juice-$CaCO_3$ agar medium and incubated at 25 °C for 5 d for spore production, which were used as inoculum in all the experiments.

2.2. Culture media and culture conditions

Six culture media were prepared containing DEHP as carbon source and $Ca(H_2PO_4)_2*H_2O$ as calcium ion source as follows (in g/L): 1) 1.5 of DEHP + 0.1 of Ca^{2+} (0.63 of $Ca(H_2PO_4)_2*H_2O$), 2) 1.5 of DEHP + 0.5 of Ca^{2+} (3.15 of $Ca(H_2PO_4)_2*H_2O$), 3) 3 of DEHP + 0.1 of Ca^{2+}, (0.63 of $Ca(H_2PO_4)_2*H_2O$), 4) 3 of DEHP + 0.5 of Ca^{2+} (3.15 of $Ca(H_2PO_4)_2*H_2O$), 5) 4 of DEHP + 0.1 of Ca^{2+} (0.63 of $Ca(H_2PO_4)_2*H_2O$), and 6) 4 of DEHP + 0.5 of Ca^{2+} (3.15 of $Ca(H_2PO_4)_2*H_2O$). A medium containing mineral salts (in g/L); $Mg(NO_3)_2$, 0.18; K_2HPO_4, 0.19; $FeSO_4*H_2O$, 0.006; KCl, 0.07; and $MgSO_4*7H_2O$, 0.07 was added for 1 g of DEHP/L to all culture media.
Culture media containing 1.5, 3 and 4 g of DEHP/L were supplemented with 150, 300 and 400 µL of tween 80, respectively, and were also added with the corresponding concentration of salts (as described above). The pH of the medium was adjusted to 6.5 using 1 M NaOH/HCl after autoclaving at 121 °C.
Experimental assays were performed in Erlenmeyer flasks (125 mL) containing 50 mL of sterile culture medium, which were inoculated with a suspension of *F. culmorum* spores to a final concentration of 1×10^7 spores/mL. Cultures were incubated at 25 °C in an orbital shaker set to120 rpm. Samples were taken at 12-h intervals for 3 d.

2.3. Esterase assay and determination of enzymatic parameters

Esterase analysis was assessed in the cell-free culture supernatant obtained by vacuum filtration of the samples. Esterase activity (expressed in U/L) was determined using ρ-nitrophenyl butyrate (ρNPB) as substrate according to Ferrer-Parra *et al*. (2018). Briefly, the assay mixture contained 100 µL of supernatant and 900 µL of the substrate. Change in absorbance of samples were measured at 405 nm using a UNICO spectrophotometer (S-2150, Dayton, NJ., USA). One unit enzyme activity (U) was defined as the amount of activity required to release 1 µmol of *ρ*-nitrophenol per min from ρNPB under the assay conditions. The enzymatic parameters, namely, esterase productivity (P), and maximum enzymatic activity (E_{max}), were determined as reported previously (Canavati-Alatorre *et al*., 2016; Ahuactzin-Pérez *et al*., 2016).

2.4. Esterase analysis by zymography

The polypeptide profiles with esterase activity were determined using polyacrylamide gels (SDS-PAGE) (Leammli, 1970). Samples were loaded on 14% separating and 4% stacking PAGE gels and electrophoresed under non-reducing conditions. 1 mL of supernatant was centrifuged, and the resulting pellet was diluted in 15 µL of distilled water and 10 µL of loading buffer. Then, 20 µL of this mixture was loaded into the well. Precision Plus Protein™ Dual Xtra (Bio-Rad) was used as molecular marker. Electrophoresis was carried out in a Mini-Protean Tetra Cell chamber (Bio-Rad) at 110 volts for 3 h. After electrophoresis, the gels were washed and then incubated at 25 °C in a substrate buffer, until the esterase bands become visible. Buffered substrate contained 3 mM α-naphthylacetate, 1 mM Fast Red TR (Sigma) and 100 mM phosphate buffer at pH 7.5 (Medina-Flores *et al*., 2020; Ferrer-Parra *et al*., 2018). Images of the gels were digitized using a Gel Doc EZ Imager (Bio-Rad), and

bands in the lanes were detected and quantified by density using Image Lab Version 6.0.0 (Bio-Rad).

2.5. Statistical analysis

All assays were performed in triplicate. It was verified that the data met the normality assumptions (through the Shapiro-Wilk and Kolmogorov-Smirnov tests) and homoscedasticity (through the Levene and Bartlett tests). Data were analyzed using a factorial analysis of variance (ANOVA). The statistical analysis system (SAS free software trials) program was used with a significance level of $p < 0.05$.

3. RESULTS

Esterase activity of *F. culmorum* produced in media containing different concentrations of DEHP (1.5, 3 and 4 g/L) and calcium ion (0.1 and 0.5 g/L) was evaluated. Table 1 shows that the highest enzymatic activity was observed in culture medium supplemented with 3 and 0.5 g/L of DEHP and calcium ion, respectively. In general, the greatest esterase activity was observed in cultures grown in media added with 3 g of DEHP/L, followed by those cultures grown in media containing 1.5 g of DEHP/L. Cultures grown on 4 g of DEHP/L had the lowest esterase activity. Low concentration of calcium ion (0.1 g/L) favored the esterase activity in cultures supplemented with 1.5 g of DEHP/L after 60 h of growth, however, there was no difference between those concentrations of calcium ion (0.1 and 0.5 g/L) tested in media containing 4 g of DEHP/L. Whereas the enzymatic activity was increased when 0.5 g of calcium ion/L were added to the medium supplemented with 3 g of DEHP/L.

Table 1. Esterase activity (U/L) of *F. culmorum* produced in culture media supplemented with different concentrations of DEHP and Ca^{2+} during 72 h of incubation.

	Culture media (g/L)					
Time	**DEHP, 1.5 Ca^{2+}, 0.1**	**DEHP, 1.5 Ca^{2+}, 0.5**	**DEHP, 3 Ca^{2+}, 0.1**	**DEHP, 3 Ca^{2+}, 0.5**	**DEHP, 4 Ca^{2+}, 0.1**	**DEHP, 4 Ca^{2+}, 0.5**
12	27.39^{b}	22.14^{b}	14.98^{b}	49.53^{a}	7.12^{c}	14.92^{b}
24	32.14^{b}	40.51^{b}	31.29^{b}	99.18^{a}	22.72^{b}	21.16^{b}
36	89.87^{c}	91.36^{bc}	126.82^{b}	191.95^{a}	71.66^{c}	89.11^{c}
48	108.88^{bc}	103.14^{bc}	134.91^{b}	207.12^{a}	78.33^{c}	139.04^{b}
60	125.12^{ab}	106.47^{bc}	127.89^{ab}	148.11^{a}	87.41^{c}	54.21^{c}
72	125.19^{ab}	72.71^{c}	111.08^{b}	142.87^{a}	74.21^{c}	53.36^{c}

Means in a row without a common superscript letter differ ($P < 0.05$) as analyzed by one-way ANOVA and the Tukey's test.

Table 2 shows that *F. culmorum* produced the maximum enzyme activity (207.1 U/L) in medium supplemented with 3 g of DEHP/L and 0.5 g of calcium ion/L, and the lowest E_{max} was obtained in medium supplemented with 4 g of DEHP/L and 0.1 g of calcium ion/L (87.4 U/L). The greatest enzyme productivity (P) (4.31 U/L/h) was also shown in medium

containing 3 g of DEHP/L and 0.5 g of calcium ion/L, followed by that P observed in medium added with 3 g of DEHP/L and 0.1 g of calcium ion/L (2.82 U/L/h), and that P obtained in medium supplemented with 4 g of DEHP/L and 0.5 g of calcium ion/L (2.9 U/L/h). The lowest P was shown in media added with 1.5 g of DEHP/L for both concentrations of the calcium ion tested (1.74 U/L/h and 1.77 U/L/h for 0.1 g and 0.5 g of calcium ion/L, respectively), and in medium containing 4 g of DEHP/L and 0.1 g of calcium ion/L (1.46 U/L/h).

Table 2. Maximum enzymatic activity and enzymatic productivity of *F. culmorum* in culture media supplemented with different concentrations of DEHP and Ca^{2+} during 72 h of incubation.

	Culture media composition (g/L)					
Parameters	**DEHP, 1.5 Ca^{2+}, 0.1**	**DEHP, 1.5 Ca^{2+}, 0.5**	**DEHP, 3 Ca^{2+}, 0.1**	**DEHP, 3 Ca^{2+}, 0.5**	**DEHP, 4 Ca^{2+}, 0.1**	**DEHP, 4 Ca^{2+}, 0.5**
E_{max} (U/L)	125.19^{bc}	106.47^{b}	134.91^{b}	207.1^{a}	87.4^{c}	139.04^{b}
P (U/L/h)	1.74^{c}	1.77^{c}	2.81^{b}	4.31^{a}	1.46^{c}	2.9^{b}

Means in a row without a common superscript letter differ ($P < 0.05$) as analyzed by one-way ANOVA and the Tukey's test. E_{max}, maximum enzyme activity; P, enzyme productivity.

Fig. 1. shows esterase zymogram of *F. culmorum* grown in medium containing 1.5 g of DEHP/L and 0.1 g of calcium ion (a), and 0.5 g of calcium ion (b). Eight bands (25.4, 29.9, 32.4, 38.1, 41.1, 64.5, 79.2 and 99.5 kDa, approximately), and seven bands (25.4, 29.9, 32.4, 41.1, 64.5, 79.2 and 99.5 kDa, approximately) with esterase activity were observed in the gels for cultures grown on 1.5 g of DEHP/L and 0.1 (Fig. 1a), and 0.5 (Fig. 1b) g of calcium ion/L. Fig. 1a shows a band with molecular weight of approximately 99.5 during all the fermentation. Two bands (25.4 and 32.4 kDa approximately) were observed from 24 h and until the end of the fermentation. Two bands with molecular weights of 38.1 and 64.5 kDa approximately were shown at 36 h of incubation. The bands with molecular weights of approximately 41.1 and 79.2 kDa were slightly stained at 60 and 72 h of growth. An additional band of 29.9 kDa approximately was observed at the end of the fermentation. Fig. 1b shows a band with a molecular weight of approximately 99.5 kDa during all the fermentation. A band with molecular weight of approximately 29.9. kDa was slightly stained at 36 h of incubation. Two additional bands of approximately 41.1 and 79.2 kDa were observed from 48 h and until the end of the fermentation. The bands with molecular weights of approximately 32.4 and 64.5 kDa were detected at 60 and 72 h of fermentation. A band of 25.4 kDa approximately was detected at 72 h of growth. It is shown that esterase zymograms of *F. culmorum* grown in both concentration of the calcium ion had similar bands of esterase activity, except that band of 38.1 kDa approximately was only observed in the gels for cultures grown in 0.1 g of calcium ion/L (Fig. 1).

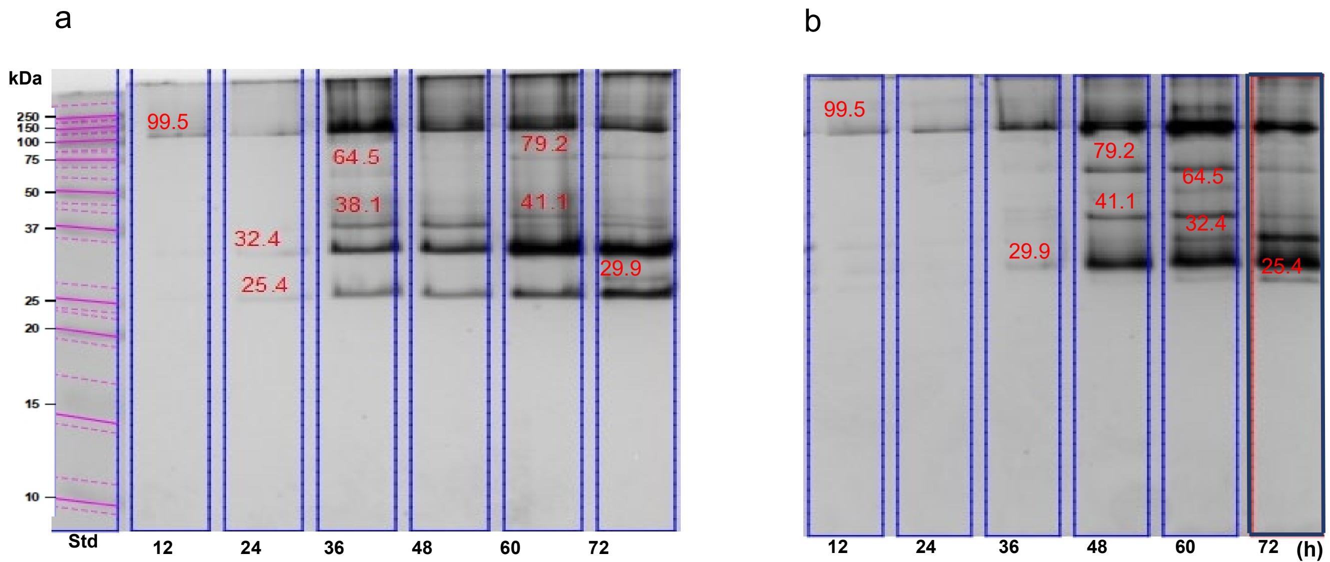

Fig. 1. Esterase zymogram of *F. culmorum* grown in medium containing 1.5 g of DEHP/L and 0.1 g of Ca^{2+}/L (a), and 1.5 g of DEHP/L and 0.5 g of Ca^{2+}/L (b), showing bands with esterase activity. The migration of the molecular mass standards (kDa) is indicated in the Std lane.

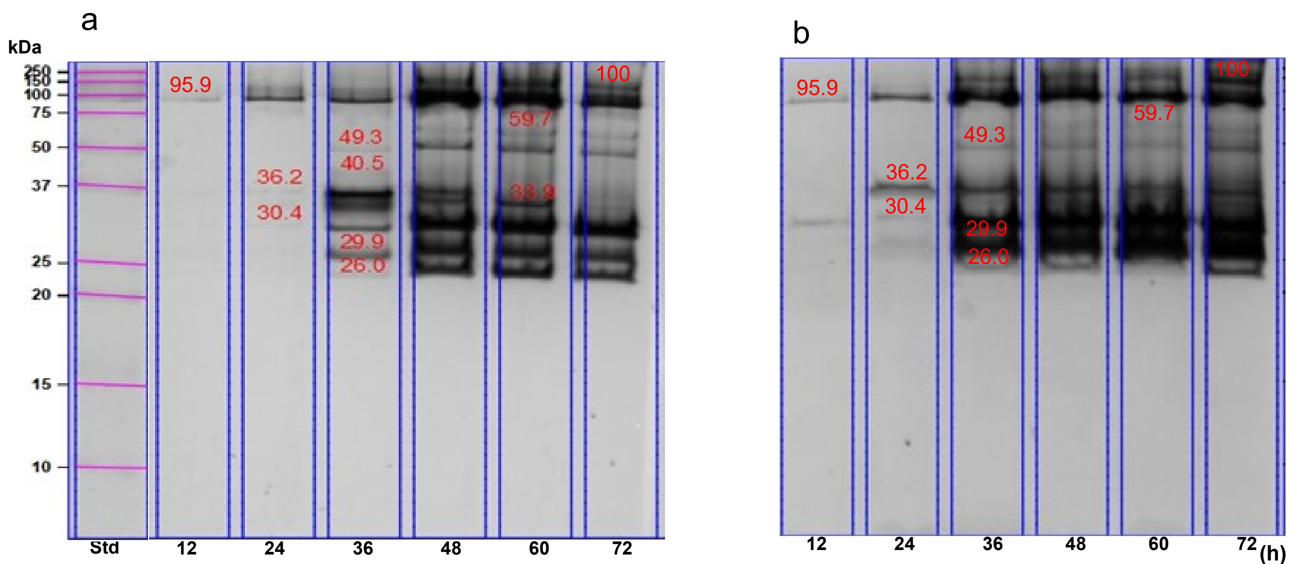

Fig. 2. Esterase zymogram of *F. culmorum* grown in medium containing 3 g of DEHP/L and 0.1 g of Ca^{2+}/L (a), and 3 g of DEHP/L and 0.5 g of Ca^{2+}/L (b), showing bands with esterase activity. The migration of the molecular mass standards (in kDa) is indicated in the Std lane.

Fig. 2. shows the gels for esterase activity of *F. culmorum* grown in medium containing 3 g of DEHP/L and 0.1 g of calcium ion (2a), and 3 g of DEHP/L and 0.5 g of calcium ion (2b). Ten bands (26, 29.9, 30.4, 33.9, 36.2, 40.5, 49.3, 59.7, 95.9, and 100 kDa approximately), and eight bands (26, 29.9, 30.4, 36.2, 49.3, 59.7, 95.9, and 100 kDa approximately) with esterase activity were observed for cultures grown in 0.1 (Fig. 2a), and 0.5 (Fig. 2b) g of calcium ion/L, respectively.

Fig. 2a shows a band with molecular weight of approximately 95.9 kDa during all the fermentation. Two bands with molecular weights of 30.4, and 36.2 kDa approximately were observed from 24 h and until the end of the fermentation. A slightly stained band of 40.5 kDa approximately was detected at 36 h, whereas a band of 49.3 kDa approximately was revealed between 36 and 72 h. Additionally, two bands with molecular weights of 26 and 29.9 kDa approximately were detected from 36 h and until the end of the incubation period. A band of 33.9 kDa approximately was observed at 60 h, whereas a band of 59.7 kDa approximately was slightly stained at 60 and 72 h of fermentation. A band with a molecular weight of 100 kDa approximately was observed from 48 h until the end of the fermentation. Fig. 2b shows two bands of 30.4 and 95.9 kDa approximately from 12 h and until the end of the fermentation. Two bands with molecular weights of 30.4 and 36.2 kDa approximately were detected from 24 h and until the end of the incubation period. Four bands (26, 29.9, 49.3 and 100 kDa approximately) were observed from 36 h and until the end of the fermentation. A slightly stained band with a molecular weight of 59.7 kDa approximately was detected between 48 and 72 h. Similar bands of esterase activity were observed in both concentrations of the calcium ion tested, however; bands with molecular weights of 33.9 and 40.5 kDa approximately were no detected in gels for 0.5 g of calcium ion/L.

Fig. 3. shows esterase zymogram of *F. culmorum* grown in medium containing 4 g of DEHP/L and different concentrations of calcium ion. Five bands with esterase activity were observed in gels for cultures grown in 0.1 g of calcium ion/L (25.4, 36.5, 39.9, 64.4, and 100 kDa approximately) (Fig. 3a), and for cultures grown in 0.5 g of calcium ion/L (31.1, 36.5, 39.9, 64.4, and 100 kDa approximately) (Fig. 3b).

Fig. 3a shows two bands with molecular weights of approximately 39.9 and 100 kDa from 24 h until the end of the fermentation. An additional band of 36.5 kDa approximately was observed from 36 h and until the end of the incubation period. Two slightly stained bands (25.4 and 64.4 kDa approximately) were revealed between 48 and 72 h. Fig. 3b showed two bands in the gel (39.9 and 100 kDa approximately) from 24 h and until the end of the fermentation. Additionally, two bands (31.1 and 36.5 kDa approximately) were observed from 36 h and until the end of the incubation period. A slightly stained band (64.4 kDa approximately) was revealed at 60 and 72 h.

Bands of similar molecular weights were observed in both concentrations of the calcium ion studied. No band was revealed at 12 h at any of the calcium ion concentrations analyzed.

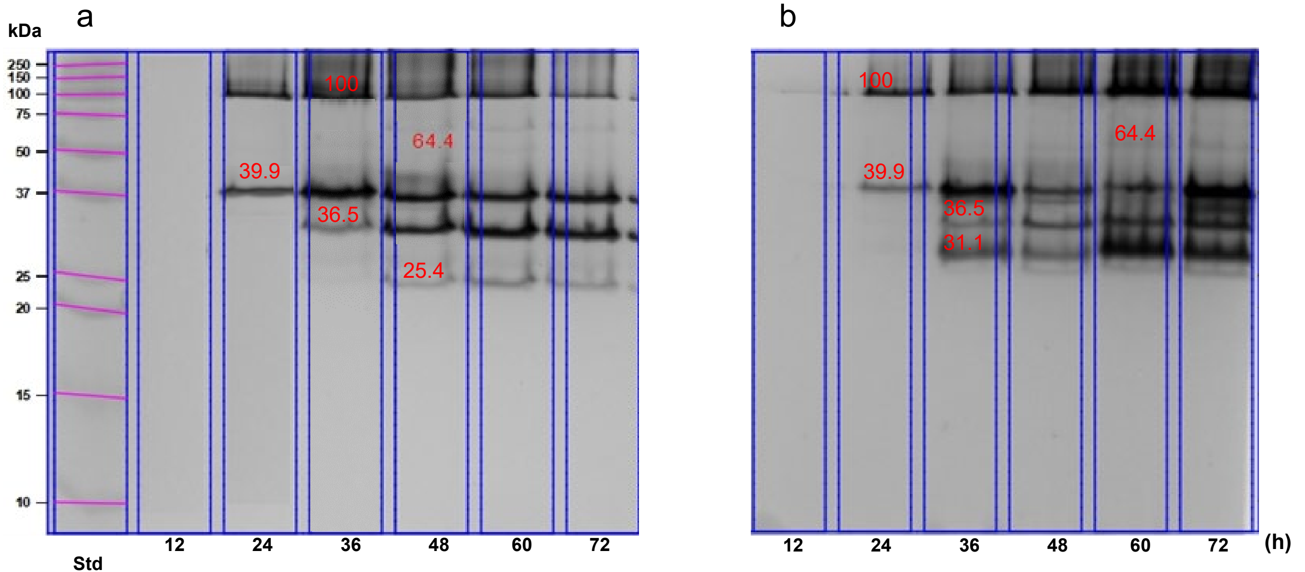

Fig. 3. Esterase zymogram of *F. culmorum* grown in medium containing 4 g of DEHP/L and 0.1 g of Ca^{2+}/L (a), and 4 g of DEHP/L and 0.5 g of Ca^{2+}/L (b), showing bands with esterase activity. The migration of the molecular mass standards (in kDa) is indicated in the Std lane.

4. DISCUSSION

In the present research, *F. culmorum* was grown in media supplemented with high concentrations of DEHP (1.5, 3 and 4 g/L) and different concentrations of calcium ion (0.1 and 0.5 g/L). Our results showed that *F. culmorum* was able to growth and produce esterase in all the media tested. In general, the greatest esterase activity was observed in cultures grown in media added with 3 g of DEHP/L, followed by those cultures grown in media containing 1.5 g of DEHP/L, however, cultures grown in 4 g of DEHP/L had the lowest esterase acyivity. In general, it was shown that cultures added with 0.5 g of calcium ion/L had the maximum enzymatic activity and the highest enzymatic productivity in the media tested. In previous studies, *F. culmorum* was grown in medium supplemented with DEHP (1.5 g/L) under submerged fermentation conditions, and it was found that esterase activity was enhanced in cultures added with $Ca(NO_3)_2$ as nitrogen source in comparison to those cultures in which $NaNO_3$ was used (González-Márquez *et al*., 2019; 2020). In the present study, higher concentrations of DEHP were used to growth *F. culmorum* than those concentrations of DEHP previously used (Ahuactzin-Pérez *et al*., 2016; 2018; Ferrer-Parra *et al*., 2018; González-Márquez *et al*., 2019: González-Márquez *et al*., 2020), however; it was also found that calcium favored esterase activity. Other studies have reported that amylase activity of *Bacillus* sp. was enhanced by metal ions such as calcium (Saxena and Singh, 2011). Several studies have shown that calcium ion is crucial in several biological activities of fungal cell, such as tip growth, hyphal branching, hyphal reorientation, among others (Gadd, 1994; Hyde and Heath, 1995; Jackson and Hardham, 1996; Grinberg and Heath, 1997; Torralba and Heath, 2001). In particular, the actin cytoskeleton and calcium gradients regulate initiation and maintenance of tip growth (Torralba and Heath, 2001; Lew,

2011; 2019). It has been reported that metal ions such as Ca^{2+}, Mn^{2+}, Fe^{2+}, Mg^{2+}, Cu^{2+}, among others can either activate or inhibit the enzymatic activity by interacting with amine or carboxylic acid group of the amino acids components of enzyme (Riordan, 1977; Ishida *et al*., 1980; de Cassia-Pereira *et al*., 2017). In this context, the enhancement of esterase activity in cultures added with calcium ion could be related to the importance of calcium ion for fungal growth, and/or could be due to the interaction between the functional groups and the amino acid constituents of esterase. In the present study, in general; the molecular weights of bands with esterase activity were similar for cultures added with either 0.1 or 0.5 g of calcium ion/L for each concentration of DEHP studied. It was observed that cultures grown in medium supplemented with 1.5 and 3 g of DEHP/L had a higher number of bands with esterase activity in medium containing 0.1 g of calcium ion/L than in those cultures grown in medium adding with 0.5 g of calcium ion/L. It could be because a high concentration of calcium could favor the formation of polymeric enzymes, making it difficult to pass through the pores of the gel. It has been reported that esterases involved in phthalate degradation have a molecular weight of around 15 and 58 kDa and that some of them can have a polymeric structure (Sánchez, 2021). In the present research, the bands with activity of esterase with high molecular weights (e.g. 100, 99.5 and 84.4 and 79.2 kDa) might have a polymeric structure. In particular, Huang *et al*. (2019) studied the DEHP degradation by *Gordonia sp*. 5F and detected an esterase of 58 kDa, which had a hexameric structure in solution with a molecular weight of 370 kDa approximately. In the present research, eight bands with esterase activity (25.4, 29.9, 32.4, 38.1, 41.1, 64.5, 79.2 and 99.5 kDa, approximately) were detected in media added with 1.5 g of DEHP/L and 0.1 g of calcium ion/L. Whereas, González-Márquez *et al.* (2020) reported that five esterase isoforms (26.4, 31.7, 43, 73.6 and 125 kDa) were detected when *F. culmorum* degraded DEHP (1.5 g/L) using calcium nitrate as nitrogen source, in which study no additional concentration of calcium ion was used.

5. CONCLUSION

F. culmorum was able to use high concentration of DEHP as sole source of carbon and energy. It is suggested that the calcium ion favored the esterase production during the DEHP degradation. However, further studies are needed to understand the relationship between the calcium ion, fungal growth, and enzyme production to provide the appropriate concentration of this ion in the culture medium. The use of efficient DEHP-degraders is important for the DEHP bioremediation process. Furthermore, the development of new strategies to increase the production of microbial enzymes is crucial to establish efficient methods for biodegradation of DEHP. Therefore, studies on the effect of calcium ion on enzymatic activity to enhance DEHP biodegradation has much to offer to mitigate the effects of DEHP pollution and the risk of health impacts.

AUTHOR CONTRIBUTION

Alejandro Chamorro-Mejía performed the experimental work and analyzed data. Lidia Patricia Jaramillo-Quintero supervised the research. Carmen Sánchez planned the experiments, conceived the initial idea, supervised the research, analyzed data, and wrote the manuscript. All authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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