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Diversification of nitrogen sources to enhance the esterase activity during the degradation of the endocrine disruptor di(2-ethylhexyl) phthalate by *Fusarium culmorum*

Diversificación de las fuentes de nitrógeno para mejorar la actividad de esterasa durante la degradación del disruptor endocrino di(2-etilhexil) ftalato por *Fusarium culmorum*

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ABSTRACT

Di(2-ethylhexyl) phthalate (DEHP) is the most used plasticizer, which is an environmental contaminant and is reported to be an endocrine disruptor in mammals. In this research, the effect of different nitrogen sources (urea, sodium nitrate and magnesium nitrate) on the growth and esterase produced by *Fusarium culmorum* during the degradation of DEHP (1500 mg/L) under submerged fermentation was evaluated. Growth kinetic and enzyme yields parameters were also estimated. These results showed that DEHP induced esterase production by *F. culmorum* and esterase production showed a nitrogen source-dependent

pattern. Number and molecular weight of esterases were similar in the different nitrogen sources analyzed, however, magnesium nitrate was the best nitrogen source for esterase production (21651 U/L), followed by urea (13460 U/L), and sodium nitrate (3762 U/L). Two esterase isoforms appeared during the first 36 h with an average molecular weight of 26 and 32.6 kDa, which might be responsible for the primary DEHP degradation and the rest of the esterases which appeared after 48 h might be involved in the final steps of the biodegradation. Development of new strategies to increase enzyme production is crucial to establish efficient methods for DEHP biodegradation to mitigate the effects of DEHP pollution and the risk of health impacts.

Keywords: DEHP biodegradation, esterase, *Fusarium culmorum*, nitrogen source.

RESUMEN

Di(2-etilhexil) ftalato (DEHF) es el plastificante comúnmente más utilizado, el cual es un contaminante ambiental y se ha reportado como disruptor endocrino en mamíferos. En esta investigación se evaluó el efecto de diferentes fuentes de nitrógeno (urea, nitrato de sodio y nitrato de magnesio) sobre el crecimiento y la esterasa producida por *Fusarium culmorum* durante la degradación de DEHF (1500 mg/L) en fermentación sumergida. La cinética de crecimiento y los parámetros de rendimientos enzimáticos fueron también evaluados. DEHF indujo la producción de esterasa por *F. culmorum*, mostrando un patrón dependiente de la fuente de nitrógeno. El número y el peso molecular de las bandas fueron similares en las diferentes fuentes de nitrógeno, sin embargo, el nitrato de magnesio fue la mejor fuente de nitrógeno para la producción de esterasas (21651 U/L), seguido de la urea (13460 U/L) y del nitrato de sodio (3762 U/L). Durante las primeras 36 h aparecieron isoformas de esterasas con peso moleculares de 26 y 32.6 kDa, que podrían ser responsables de la degradación primaria de DEHF y el resto de las esterasas que aparecieron después de las 48 h podrían estar implicadas en los pasos finales de la biodegradación. El desarrollo de las nuevas estrategias para aumentar la producción de enzimas es crucial para establecer métodos eficientes de la biodegradación del DEHF para mitigar los efectos de la contaminación por DEHF y el riesgo de su impacto en la salud.

Palabras clave: Biodegradación de DEHF, esterasa, *F. culmorum,* fuente de nitrógeno.

1. INTRODUCTION

Phthalate esters are compounds that have alkyl, aryl or di-alkyl esters of 1,2-benzenedicarboxylic acid as molecular structures. These compounds are used worldwide as plastic additives (plasticizers) to provide flexibility to plastic materials at concentrations between 10 and 70% (Sánchez, 2021) and represent approximately 70% of the global plasticizer market (Ren *et al*., 2017). Di(2-ethylhexyl) phthalate (DEHP) is the most used plasticizer in the manufacture of polyvinyl chloride-containing articles, such as medical devices (e.g., infusion tubing, nasogastric tubes, and blood bags), food wrapping, and other commercial and industrial products (Erythropel *et al*., 2014; Pérez-Andrés *et al*., 2017; Sánchez, 2021). DEHP does not adhere strongly to the polymer matrix, as it can be released

into the environment during the manufacturing of plastic products or after their disposal and can be found in soil, groundwater, air and living beings (Lesser *et al*., 2018; Bouattour *et al*., 2020; Shivaraju *et al*., 2020, Sánchez *et al*., 2021; Schaffer *et al*. 2022; Shinohara *et al*. 2024). This substance has been reported as an endocrine disruptor in mammals (Heudorf *et al*., 2007; Ernst *et al*., 2020; Zhu *et al*., 2021), to induce oxidative stress in mouse cells (Tu *et al*., 2020) and may potentially be associated with an increased risk of thyroid cancer (Alsen *et al*., 2021). DEHP has been classified as a priority organic pollutant by regulatory and health protection agencies, including the European Union, the United States Environmental Protection Agency, and the China National Environmental Monitoring Center (Wang *et al*., 2021). It has been reported that microbial degradation is the most effective, low-cost, and eco-friendly method for the remediation of contaminated environments (Fan *et al*., 2018; Bope *et a*l., 2019). The development of promising environmental biotechnologies using microorganisms and their enzymes constitutes a potential resource for pollutant degradation (Olvera-García *et al*., 2017; Sánchez-Sánchez and Sánchez, 2019; Sánchez *et al*., 2020; González-Márquez and Sánchez 2022; González-Márquez and Sánchez 2024; Ocaña-Romo *et al*., 2024). Therefore, identifying efficient DEHP-degrading organisms and understanding the physiological and nutritional requirements for enhanced esterase production are crucial for decontaminating environments polluted with this and other phthalates. Fungal species have much to offer since they possess an exclusive ability to penetrate and colonize hydrophobic substrates and to produce surfactants to increase the bioavailability of the substrate (Sánchez, 2020, Sánchez, 2022). Some fungi, such as *Fusarium* species, have been reported to be highly efficient at degrading di(2-ethyl hexyl) due to their esterase production (Kim *et al*., 2002;2003;2005; Ahuactzin-Pérez *et al*., 2016; Ríos-González *et al*., 2019; González-Márquez *et al*., 2019b; 2020; Loftus et al., 2020; González-Márquez *et al*., 2021; Chamorro-Mejia *et al*., 2024; Sánchez, 2024). Esterases are enzymes that catalyze the cleavage of ester bonds in phthalates to carboxylic acids (Hernández-Sánchez *et al*., 2019; Xu *et al*., 2020). *Fusarium culmorum* was able to efficiently degrade high concentrations of DEHP to CO_2 and H_2O (González-Márquez *et al*., 2019a; Hernández- Sánchez *et al*., 2024). Some studies on the effect of temperature and pH on the microbial degradation of DEHP have been carried out (Li *et al*., 2018; Portillo-Ojeda *et al*., 2020; Lamraoui *et al*., 2020). However, there is no information about the influence of nitrogen sources on the microbial degradation of phthalates. Nitrogen is considered an essential macronutrient for organisms, influencing cellular growth and product formation (Zhang *et al*., 2023). This element is required for the synthesis of nucleic acids, proteins, coenzymes, pigments, etc. (Clarke, 2013). The nitrogen source affects microbial biochemistry and physiology. Therefore, enzyme production requires selecting the suitable nitrogen source for its production. In this study, the effect of urea, magnesium nitrate and sodium nitrate as nitrogen source during DEHP degradation by *F. culmorum* in submerged conditions was studied. Esterase activity was evaluated using zymography analysis and biochemical tests in media supplemented with either DEHP (1500 mg/L) or glucose (as a control). growth kinetic and enzymatic parameters were also calculated for media containing either DEHP or glucose.

2. MATERIALS AND METHODS

2.1. Organism and culture media

Strain of *F. culmorum* from the collection at the Research Centre for Biological Sciences (CICB) at Autonomous University of Tlaxcala (Tlaxcala, Mexico) was employed in this study. Six culture media were prepared containing either DEHP (1.5 g/L) (Sigma; purity grade 99%) or glucose as control medium (10 g/L) (Merck Millipore, Mexico) as carbon sources. Culture media had either $NaNO_3$, $CO(NH_2)_2$ or $Mg(NO_3)_2$ as nitrogen source. The carbon/nitrogen ratio was around 10. Culture medium supplemented with DEHP had the following components (in g/L). K_2HPO_4, 0.32; $FeSO_4.7H_2O$, 0.01; KCl, 0.12; $MgSO_47H_2O$, 0.12, and was also added with 100 µL Tween 80 per litter. Culture medium supplemented with glucose as control medium had the following components (in g/L). K_2HPO_4, 1.2; $FeSO_4.7H_2O$, 0.01; KCl, 0.4 and $MgSO_47H_2O$, 0.46.
DEHP (which boiling point is 385 °C) was incorporated to the culture medium before autoclaving. Media were adjusted to pH of 6.5 after autoclaving using either 0.1 M HCl or 0.1 M NaOH.

2.2. Cultures inoculation and growth conditions

Erlenmeyer flasks of 125 mL containing 50 mL of sterile liquid culture medium were inoculated with 10^7 spores harvested (using 0.01% tween-80 sterile solution) from the surface of a 5-day-old flask culture of *F. culmorum* grown on V8® agar medium (VAM) at 25 °C. VAM contained 226 mL of V8® vegetable juice, 2 g of $CaCO_3$, 15 g of agar and 800 mL of distilled water. Inoculated flasks were incubated for 5 d on a rotary shaker at 120 rpm and 25 °C. Analyses were performed on samples taken at 12-h intervals.

2.3. Growth parameters estimation

Fungal biomass (*X*) was harvested from the flasks by vacuum filtration using filter paper at 20-25 µm of pore-size and then dried at 60 °C. The specific growth rate (µ) was calculated from changes of biomass production in dry weight (g/L) during the fermentation time by using logistic equation.

$$\frac{dX}{dt} = \mu\left[1 - \frac{X}{X_{\max}}\right]X$$

Where µ (h^{-1}) is the maximal specific growth rate, X_{max} is the maximal biomass level reached when $dX/dt = 0$ for $X > 0$.

Solving the differential equation as follows:

$$X = \frac{X_{\max}}{1 + Ce^{-\mu t}}$$

Where, $C = (X_{max} - X_0)/X_0$, and $X = X_0$; the initial biomass value.

Calculation of the kinetic parameters from these equations were carried out using a non-linear least square fitting program (Solver; Excel, Microsoft) as previously reported

(Ahuactzin-Pérez *et al*., 2016). Media added with DEHP showed outliers during the first 12 h due to slow fungal growth at the beginning of the fermentation, which caused over estimation of the biomass weight due to the unused substrate (i.e. mineral salts and DEHP) at the start of the growth. Therefore, a robust nonlinear regression, which is based on the iteratively reweighted least-squares method (Holland and Welsch, 1977; Riazoshams *et al*., 2019) was used for detection and elimination of outliers (Motulsky and Brown, 2006) using the MATLAB® fitting program (R2016 version, The Math-Works, Inc., Natick, MA, USA) (Beck, 2014).

2.4. Analysis of protein content, esterase activity assays, and esterase yield parameters calculation

The concentration of total protein in the cultures was measured by Bradford method (Bradford, 1976). A 100-µL sample of the supernatant was mixed with 700 µL of distilled water and 200 µL of Bradford reagent (BIORAD), mixed well, incubated for 10 min at room temperature and absorbance measurements at 595 nm were undertaken using a UNICO spectrophotometer (S-2150 series DAYTON, NJ, USA). A protein standard curve was performed by measuring the optical density (at 595 nm) of known concentrations of bovine serum albumin (SIGMA) solutions.

Esterases activity was assessed in the supernatant obtained from the filtration of the samples using *p*-nitrophenyl butyrate (*p*NPB) as substrate. Changes in the absorbance were detected at 405 nm using a UNICO spectrophotometer (S-2150 series DAYTON, NJ, USA). The reaction mixture had 100 µL of supernatant and the rest of the components previously reported (Ferrer-Parra *et al*., 2018). In DEHP samples, a small amount of supernatant (i.e. 5, 30 or 50 µL) was used to obtain measurable absorbance values. In glucose samples, 1000 µL of supernatant were used. Glucose samples were centrifuged at 12.000 rpm for 10 min and the supernatant discarded. The filtrate was dissolved in 100 µL of water, which were used in the reaction mixture. The reaction mixture was incubated at 37 ºC for 2.5 and 5 min for media containing DEHP and glucose, respectively, and then the samples were incubated at 4 ºC to stop the reaction. One unit of enzyme activity (U) was defined as the amount of activity required to release 1 micromole of *p*-nitrophenol per minute from *p*NPB at 37 °C and pH 7.5. The esterase specific activities were expressed in U/mg of protein. Maximal enzymatic activity (E_{max}), esterase productivity (P), yield of esterase per unit of biomass produced by the fungus ($Y_{E/X}$), and specific rate of enzyme production (q_p) were evaluated as previously reported (Ahuactzin-Pérez *et al*., 2016).

2.5. Zymographic analysis

Samples were assayed for esterase activity using polyacrylamide gels (PAGE) (Leammli, 1970). The sample was prepared with 90 µL of supernatant and 30 µL of loading buffer for DEHP-supplemented media containing $CO(NH_2)_2$ or $Mg(NO_3)_2$ as nitrogen source, and with 100 µL of supernatant and 34 µL of loading buffer for DEHP-supplemented media containing $NaNO_3$ as nitrogen source. The protein content was very low in the glucose-supplemented media, so concentrated protein samples were used to visualize the bands. In this case, 1 mL of supernatant was centrifuged at 15000 RPM at 2 °C for 5 min, and then the precipitated was dissolved in 15 µL of deionized water and 10 µL of loading buffer. Loading buffer contained 6.25 mL of glycerol, 3.125 mL of 1M Tris buffer (pH 6.8), 0.0025 g of bromophenol

blue dye and 0.5 g of sodium dodecyl sulfate (SDS) (Ferrer-Parra *et al*., 2018). In all the cases, a 20 µl of each sample was loaded in the gel of 1.0 mm, prepared with 14% polyacrylamide and 4% acrylamide as separation and packaging gels, respectively, under non-reducing conditions and run in a Mini Protean electrophoresis system Tetra Cell (BIORAD) at 150 volts for 3 h. After electrophoresis, the gels were washed in buffer 10 mM Tris-HCl pH 3.5 at 7 °C for 10 min and then incubated at 25 °C with shaking overnight in a substrate buffer. The substrate buffer was prepared with 0.056 g of α-naphthyl acetate (SIGMA), 0.044 g Fast Red TR (SIGMA), 2 mL acetone, 2 mL deionized water, which was placed into a 100 mL volumetric flask and make up with phosphate buffer at pH 7.5. The esterase molecular weight was determined using a ProteinTM Dual Precision Xtra Plus Standards (10-250 kD, BIORAD) marker. Esterase activity was revealed by red-colored bands in the gel. The Gel Doc EZ imaging system (BIORAD) was used to digitized gels and bands were identified by their intensity using Image Lab software (Version 6.0.0 BIORAD) (González-Márquez *et al*., 2019a).

2.6. Statistical analysis

All assays were carried out in three independently repeated experiments, and the data are presented as the means ± SEM. Statistical analysis were performed with SAS University edition (SAS Institute, Cary, NC, USA). Analysis of variance was used to test for significant differences between groups followed by Tukey's post hoc test. The significance level was set at $p = 0.05$.

3. RESULTS

3.1. Fungal growth and kinetic growth parameters

Biomass production by *F. culmorum* in medium supplemented with DEHP and in control medium (glucose medium) containing different nitrogen sources is shown in figure 1. In all the nitrogen sources tested, faster growth was observed in the control medium than in the medium containing DEHP. When magnesium nitrate was used as nitrogen source *F. culmorum* entered the stationary phase after 108 and 132 h, in medium containing DEHP and in the control medium, respectively (Fig 1a). *F. culmorum* reached the stationary phase after 120 h and 84 h approximately in DEHP-supplemented medium and in the control, respectively, with urea as nitrogen source (Fig. 1b). *F. culmorum* attained the stationary phase after 108 h approximately in both DEHP-supplemented medium and in the control when sodium nitrate was used as nitrogen source (Fig. 1c). Table 1 shows that the highest µ value was observed in DEHP-supplemented medium containing sodium nitrate, and in the control medium added with urea as nitrogen source. The lowest µ value was showed in DEHP-supplemented medium and control medium containing urea and sodium nitrate as nitrogen sources, respectively. In general, the highest X_{max} was observed in the control media added with the different nitrogen sources, and the lowest X_{max} was observed in DEHP-supplemented media (Table 1).

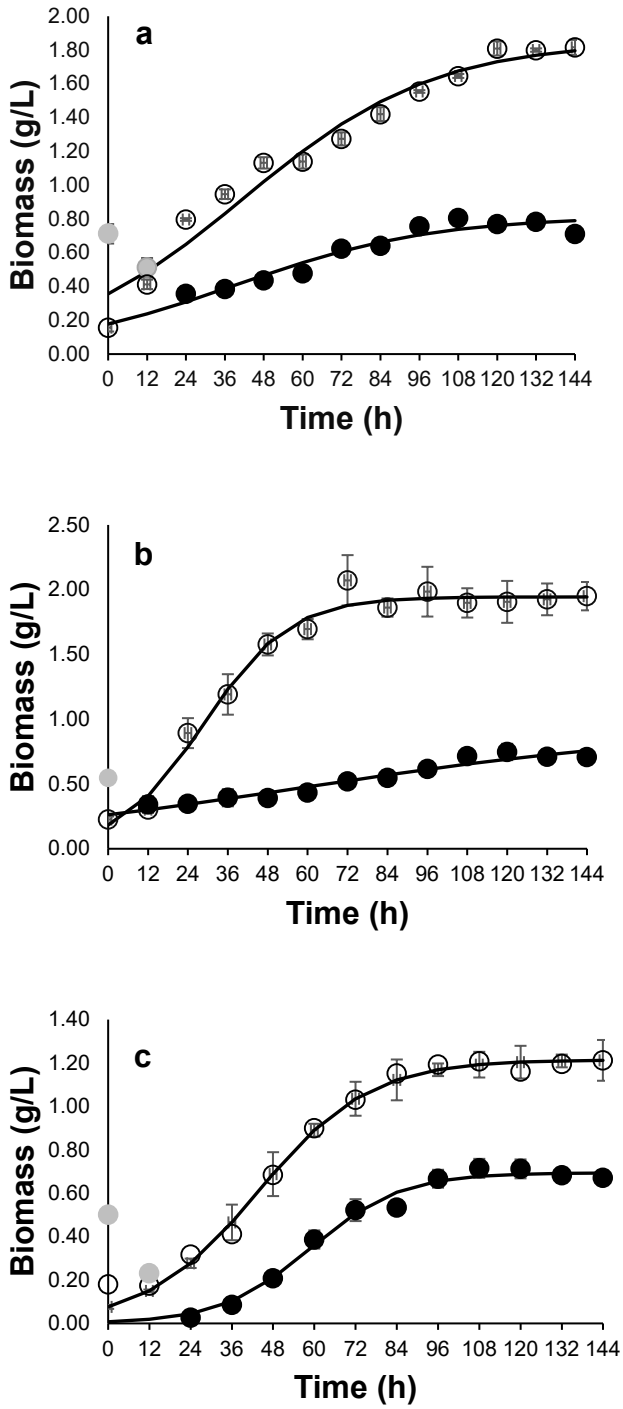

Fig. 1. Biomass production by *F. culmorum* grown in DEHP-supplemented medium (●) and glucose medium (○), containing magnesium nitrate (**a**), urea (**b**), and sodium nitrate (**c**) as nitrogen source in liquid fermentation. Gray data points during the first 12 h are outliers in DEHP-supplemented media.

3.2. Protein content, specific esterase activity and enzymatic production parameters

Protein content and specific esterase activity produced by *F. culmorum* in DEHP-supplemented medium and glucose medium, containing magnesium nitrate as nitrogen source is shown in Fig. 2. *F. culmorum* had higher protein content in the medium containing DEHP as compared to that showed in control medium (Fig. 2a). In general, the protein content was constant during the fermentation in the control medium, however, in the medium added with DEHP, the protein content increased during the first hours of growth reaching the highest value after 48 h and then decreased until the end of the fermentation (Fig. 2a). *F. culmorum* produced the highest specific esterase activity in medium containing DEHP and the lowest was observed in the control medium. In the medium added with DEHP, the specific esterase activity increased during the cultivation reaching the highest value at 108 h (Fig. 2b). Fig. 3 shows the protein content and specific esterase activity produced by this fungus in medium added with DEHP and in control medium, containing urea as nitrogen source. The highest protein content was observed in medium containing DEHP and the lowest in the control medium (Fig. 3a). In medium containing DEHP, the protein content increased during the first 48 h and then decreased gradually until the end of the cultivation (Fig. 3a). Much higher specific esterase activity was observed in the medium added with DEHP in comparison to that observed in the control medium (Fig. 3b). In medium containing DEHP, the specific esterase activity increased after 60 h and reached its highest value after 132 h of cultivation. Fig. 4 shows the protein content and specific esterase activity produced by *F. culmorum* in medium added with DEHP and glucose medium, containing sodium nitrate as nitrogen source. In this case, *F. culmorum* had higher protein content in the control medium than in the medium containing DEHP (Fig. 4a). In the control medium, the protein content increased after 84 h, remaining constant until the end of the fermentation (Fig. 4a). In all nitrogen sources analyzed, a higher specific esterase activity was observed in media containing DEHP than in the respective control medium. In general, of all the media tested, the medium added with DEHP containing magnesium nitrate as nitrogen source, showed the greatest enzymatic production parameters (E_{max}, $Y_{E/X}$, P and q_p), followed by the DEHP-supplemented medium containing urea and the DEHP-supplemented medium containing sodium nitrate. E_{max} value was around 2-fold and 6-fold higher in DEHP-supplemented medium containing magnesium nitrate than those E_{max} values showed in DEHP-supplemented media containing urea and sodium nitrate, respectively. The lowest enzymatic production parameters were observed in those media added with glucose and the different nitrogen sources analyzed (Table 1). These results showed that esterases are induced by growth of *F. culmorum* in DEHP and that magnesium nitrate was the best nitrogen source for esterase production, being sodium nitrate the nitrogen source that less favored the esterase production in media containing DEHP (Table 1).

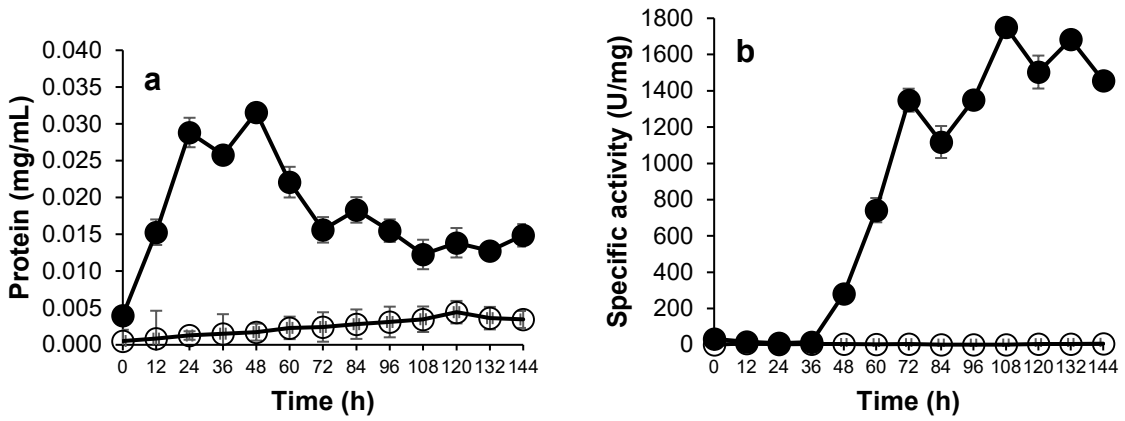

Fig. 2. Protein content (**a**) and specific esterase activity (**b**) produced by *F. culmorum* grown in DEHP-supplemented medium (●) and glucose medium (○), containing magnesium nitrate as nitrogen source in liquid fermentation.

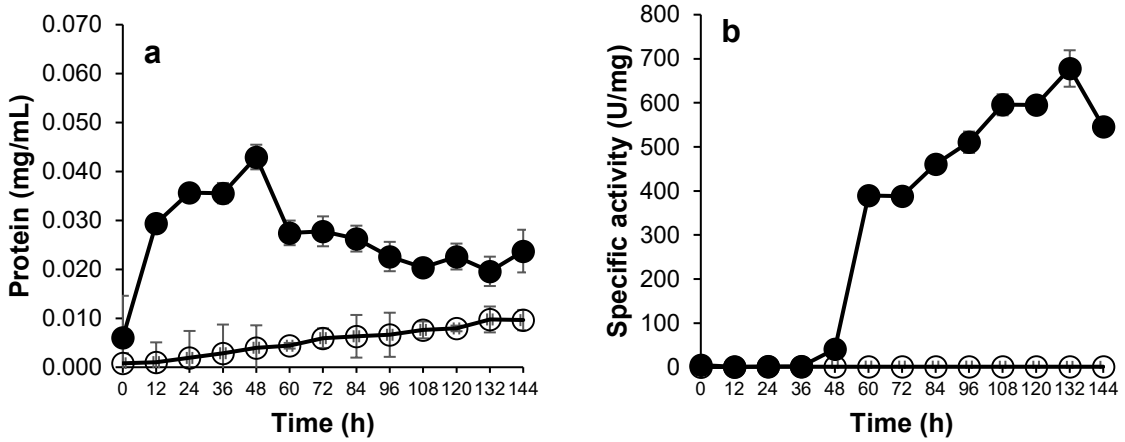

Fig. 3. Protein content (**a**) and specific esterase activity (**b**) produced by *F. culmorum* grown in DEHP-supplemented medium (●) and glucose medium (○), containing urea as nitrogen source in liquid fermentation.

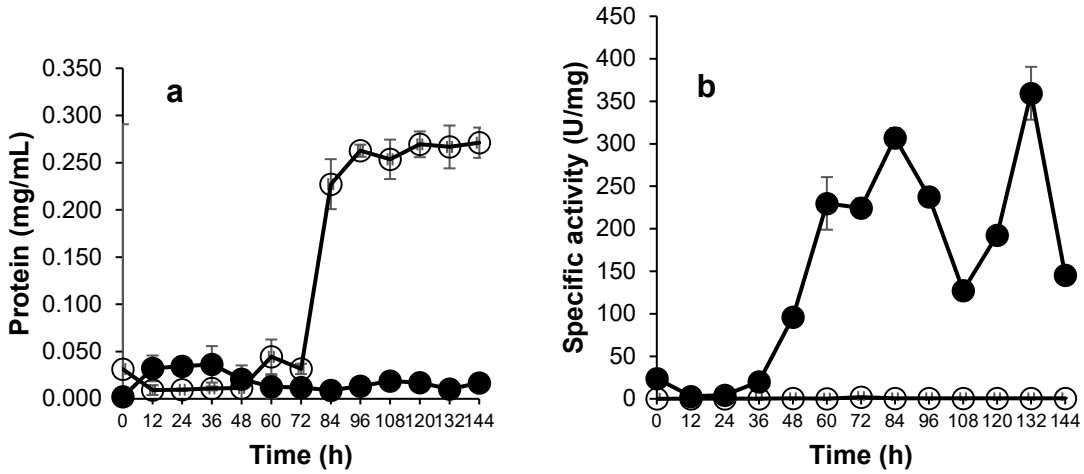

Fig. 4. Protein content (**a**) and specific esterase activity (**b**) produced by *F. culmorum* grown in DEHP-supplemented medium (●) and glucose medium (○), containing sodium nitrate as nitrogen source in liquid fermentation.

3.3. Esterase zymogram patterns

Fig. 5 shows the esterase zymograms of *F. culmorum* grown in DEHP-supplemented medium and glucose medium, containing magnesium nitrate as nitrogen source. Four esterase activity bands were observed in the DEHP-supplemented medium (26.9, 33.5, 45.5. and 75.9 kDa, approx.). Esterase activity bands of molecular weights of 26.9 and 33.5 kDa were observed after 36 h and until the end of the fermentation, while 45.5 and 75.9 kDa lightly stained bands were observed after 48 and 60 h, respectively and until the end of the fermentation (144 h) (Fig. 5a). Three esterase activity lightly stained bands (23, 31 and 110 kDa, approx.) were detected in the control medium. A 110 kDa esterase band was observed during 48 h and 120 h. The 23 and 31 kDa esterase bands appeared after 60 h, a band of 31 kDa was observed after 132 h. The gel patterns in Fig. 6a shows that in DEHP-supplemented medium, containing urea as nitrogen source, 26.3, 32.4 and 44 kDa esterase activity bands were clearly observed after 48 h and until the end of the cultivation. Two esterase bands (26.3 and 32.4 kDa approx.) were also observed after 48 h, while only a faint band (72.2 kDa appox.) was detected during 84 h and 132 h of growth (Fig. 6a). In contrast, five faintly stained bands of esterase activity were observed in glucose medium, three of them (25, 32 and 80 kDa approx.) after 60 h and the 44-kDa and 125-kDa bands were detected after 132 h (Fig. 6b). Esterase zymogram of *F. culmorum* grown on DEHP-supplemented medium and glucose medium, containing sodium nitrate as nitrogen source is shown in Fig. 7. In medium containing DEHP, esterase activity bands of 25 and 32 kDa approx. were observed after 36 h and until 144 h. Two esterase activity faint bands (43 and 66 kDa) were detected after 48 and until 144 h. A faint band of 172 kDa was observed after 60 h and until the end of the fermentation (Fig. 7a). However, three slightly stained esterase activity bands were observed in the control medium (Fig. 7b). In this case, a 24 kDa esterase activity band was observed during the first 36 h, whereas a 30 kDa band was observed through the fermentation and a 90 kDa band was detected after 96 h (Fig.7b).

Table 1. Effect of nitrogen sources on kinetic growth and esterase production parameters of *F. culmorum* grown in glucose medium and DEHP-containing medium in liquid fermentation.

	Nitrogen sources					
	Sodium nitrate		Urea		Magnesium nitrate	
	Glucose	DEHP	Glucose	DEHP	Glucose	DEHP
Growth parameters						
μ (h^{-1})	0.058^{b} ± 0.0005	0.077^{a} ± 0.004	0.079^{a} ± 0.005	0.016^{d} ± 0.002	0.034^{c} ± 0.0008	0.032^{c} ± 0.001
Xmax (g/L)	1.21^{b} ± 0.054	0.71^{c} ± 0.025	2.07^{a} ± 0.11	0.75^{c} ± 0.036	1.82^{a} ± 0.025	0.81^{c} ± 0.015
Esterase parameters						
Emax (U/L)	137.97^{d} ± 0.39	3762^{c} ± 10.49	9.44^{d} ± 0.24	13460.15^{b} ± 211.7	18.83^{d} ± 0.27	21651.41^{a} ± 208.17
YE/X (U/gX)	114.33^{d} ± 4.8	5276.41^{c} ± 176.32	4.58^{d} ± 0.25	18069.91^{b} ± 604.88	10.37^{d} ± 0.088	26867.95^{a} ± 755.8
P (U/L/h)	1.28^{d} ± 0.004	28.5^{c} ± 0.079	0.098^{d} ± 0.002	112.17^{b} ± 1.76	0.13^{d} ± 0.001	150.36^{a} ± 1.44
qp (U/gX/h)	6.61^{d} ± 0.22	410.60^{b} ± 36.58	0.36^{d} ± 0.018	292.42^{c} ± 27.53	0.35^{d} ± 0.009	871.69^{a} ± 12.83

Values are mean ± SEM (n = 3). Means in a row without a common superscript letter differ ($P < 0.05$) as analyzed by one-way ANOVA and the Tukey's test.
μ, specific growth rate; X_{max}, maximum biomass;$Y_{X/S,}$ biomass yield;
E_{max}, maximum enzyme activity; $Y_{E/X}$, enzyme yield; P, enzyme productivity;
q_p, specific rate of enzyme production.

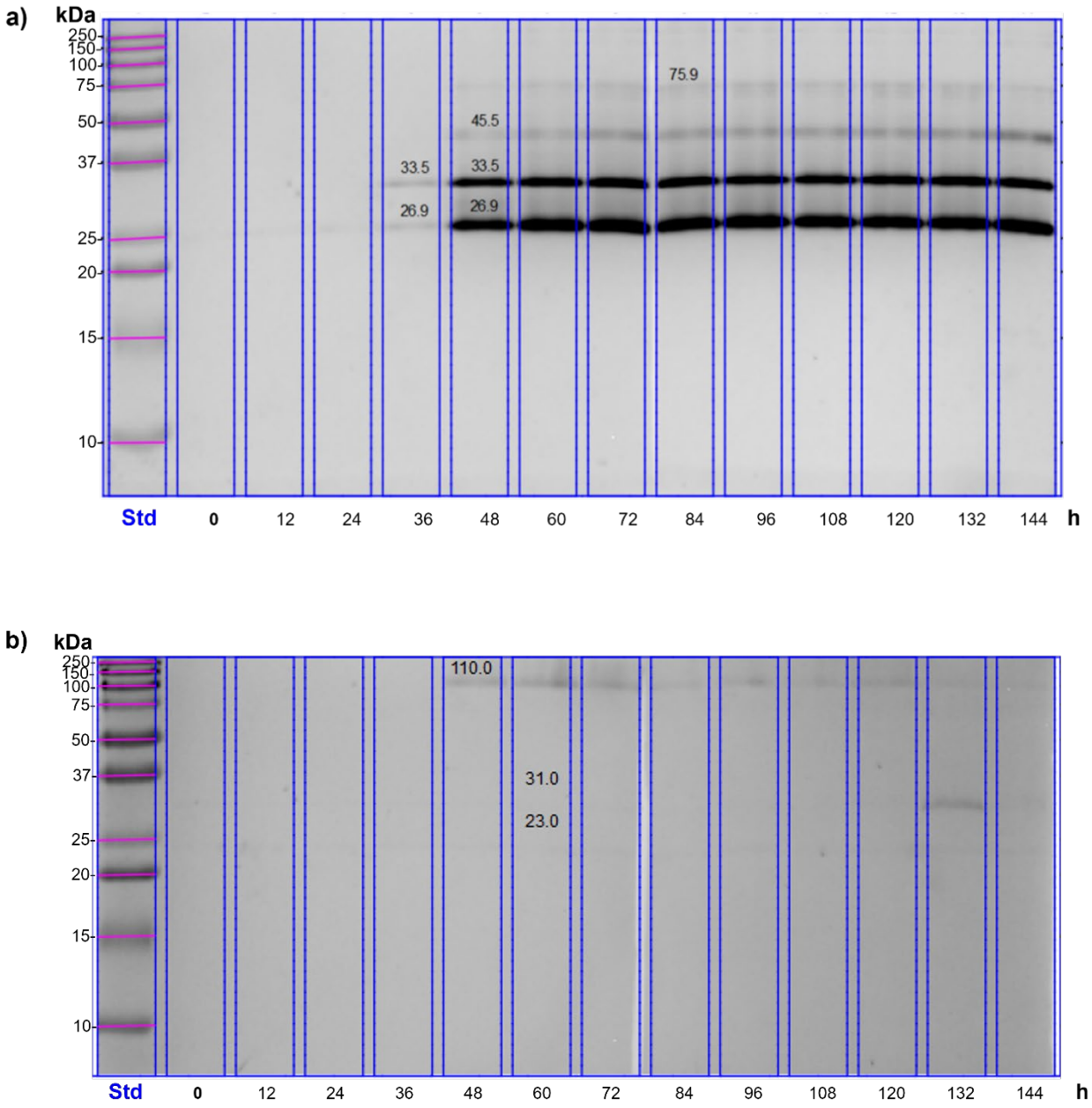

Fig. 5. Esterase zymogram of *F. culmorum* grown on DEHP-supplemented medium (a) and glucose medium (b), containing magnesium nitrate as nitrogen source. Lane 1, protein marker. Lanes 2-14, samples taken from 0-144 h of growth.

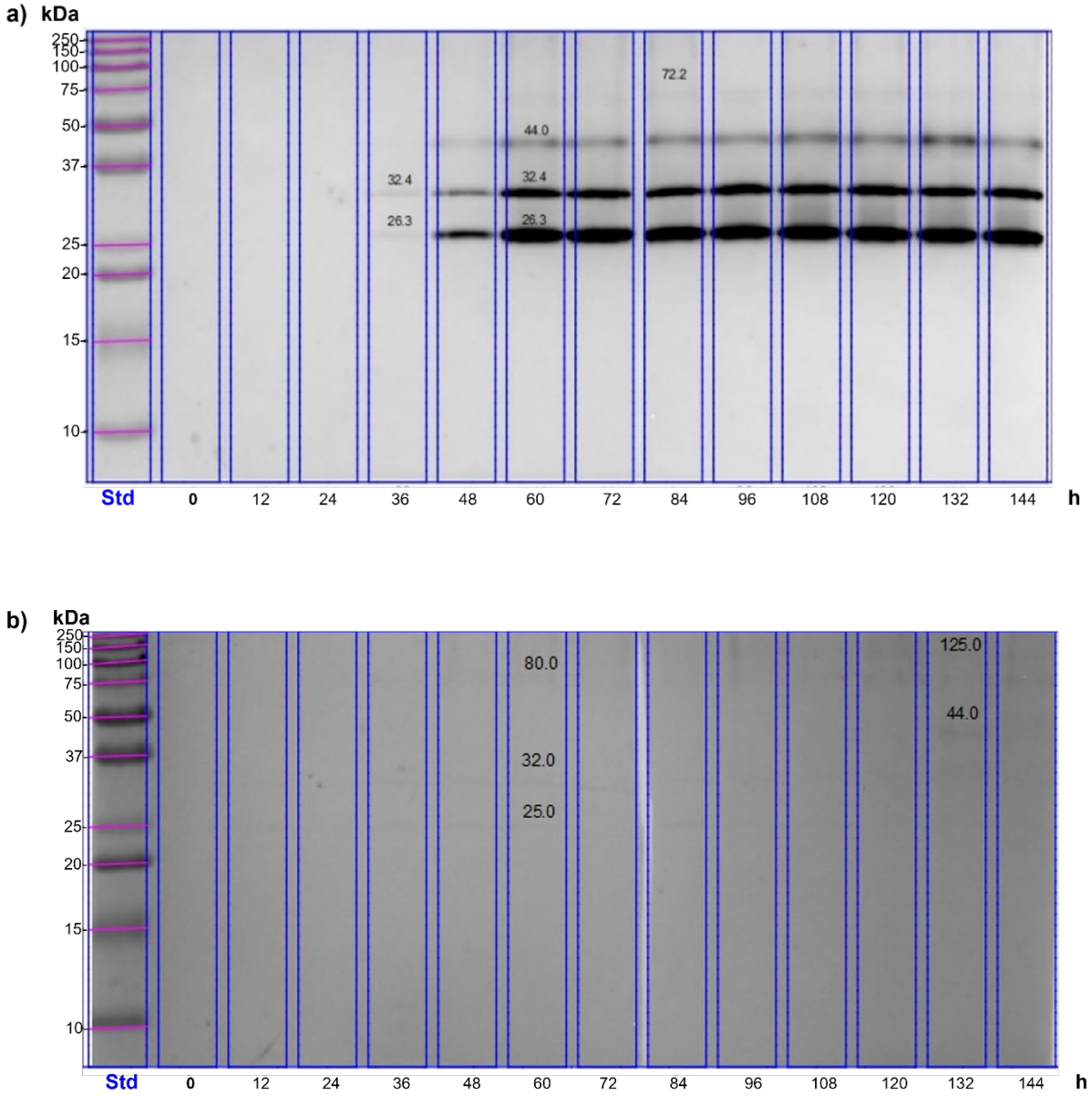

Fig. 6. Esterase zymogram of *F. culmorum* grown on DEHP-supplemented medium (a) and glucose medium (b), containing urea as nitrogen source. Lane 1, protein marker. Lanes 2-14, samples taken from 0-144 h of growth.

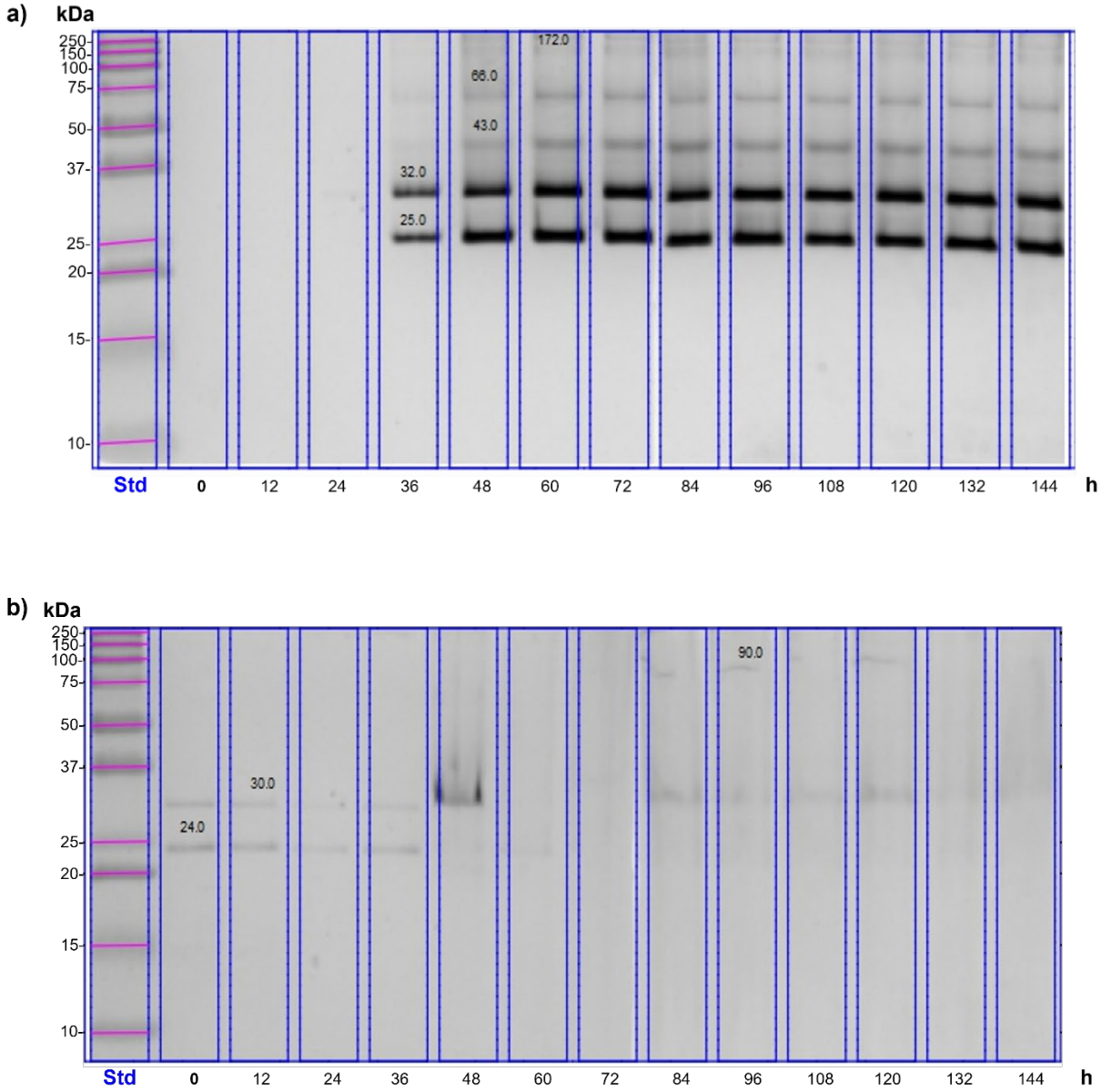

Fig. 7. Esterase zymogram of *F. culmorum* grown on DEHP-supplemented medium (a) and glucose medium (b), containing sodium nitrate as nitrogen source. Lane 1, protein marker. Lanes 2-14, samples taken from 0-144 h of growth.

4. DISCUSSION

In the present study, *F. culmorum* entered the stationary phase after 108 and 132 h, in medium containing DEHP and in the control medium, respectively, when magnesium nitrate was used as nitrogen source. However, this fungus reached the stationary phase after 120 h and 84 h approximately in DEHP-supplemented medium and in the control, respectively, with urea as nitrogen source. Whereas *F. culmorum* attained the stationary phase after 108

h approximately in both DEHP-supplemented medium and in the control when sodium nitrate was used as nitrogen source. Córdoba-Sosa *et al*. (2014) studied the growth of the edible mushroom *P. ostreatus* in a medium containing DEHP (1500 mg/L), glucose as cosubstrate and yeast extract as nitrogen source and found that it entered the stationary phase after 360 h of cultivation. This shows that *F. culmorum* grows faster in DEHP than basidiomycetes such as *P. ostreatus*. Our results are in accord with those obtained previously in which it was observed that *F. culmorum* had higher $Y_{E/X}$, P, and q_p in media containing DEHP than in glucose medium (González-Márquez *et al*., 2020). In the present research, it was observed that esterases are induced by growth of *F. culmorum* in DEHP. Previous studies have also shown that DEHP induced the esterase production in *P. ostreatus* and in *F. culmorum* (Ahuactzin-Pérez *et al*., 2016; González-Márquez *et al*., 2019a; 2020). In the present research, the esterase activity in DEHP-supplemented medium was 4-, 16-, and 25-fold higher in medium added with sodium nitrate, urea and magnesium nitrate, respectively than that obtained previously for *F. culmorum* in DEHP-supplemented medium containing calcium nitrate as nitrogen source (González-Márquez *et al*., 2020). Our studies have shown than the nitrogen source is crucial for esterase production, and for degradation of DEHP by *F. culmorum*. Several studies have also found that nitrogen source influences enzyme production. Lemos *et al*. (2001) reported that nitrogen source affects xylanase production in *Aspergillus awamori*, showing higher endoxylanase and β-xylosidase activities when sodium nitrate was used as the nitrogen source compared to urea, peptone, and ammonium sulfate. Furthermore, Sun *et al*. (2021) reported that protease production was regulated by nitrogen sources, being organic nitrogen sources more beneficial for the production of this enzyme. In addition, Wang *et al*. (2021) evaluated the DEHP degradation (50-300 mg/L) by *Achromobacter* sp., showing that sodium nitrate was the best nitrogen source, followed by ammonium sulfate and sodium nitrite. da Silva *et al*. (2001) studied the biomass production and secretion of hydrolytic enzymes by *Aspergillus nidulans* and *Fusarium oxysporum* and found that metabolism of fungi is regulated by pH and the nitrogen source in correlation to the carbon source.

González-Márquez *et al*. (2019a) studied the DEHP (1000 mg/L) degradation by *F. culmorum* using sodium nitrate as nitrogen source and detected eight esterase (25.7, 29.5, 31.8, 45.9, 66.6, 97.6, 144.5 and 202.9 kDa) activity bands during the degradation process. In addition, González-Márquez (2020) reported that five esterase isoforms (26.4, 31.7, 43, 73.6 and 125 kDa) were detected when *F. culmorum* degraded DEHP (1500 mg/L) using calcium nitrate as nitrogen source. It is observed that the esterase bands of low molecular weight detected previously (González-Márquez *et al*., 2019a; 2020) had similar molecular weight to those found in the present research. The esterase activity bands of high molecular weight (e.g. 96.6, 125, 144.5 and 202.9 kDa) might have a polymeric structure (e.g. threemeric, tetrameric, hexameric structure, etc). It has been reported that esterases involved in phthalate degradation had a molecular weight of around 15 and 58 kDa and that some of them can have a polymeric structure (Sánchez, 2021). 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 approx. In addition, monomeric esterases of 50 and 53 kDa were detected during the DEHP degradation by *Gordonia* sp. YC-JH1 (Fan *et al*., 2018) and *Achromobacter denitrificans* SP1 (Pradeed *et al*., 2015), respectively. Furthermore, Nishioka *et al*. (2006) evaluated the DEHP degradation by *Gordonia* sp P8219 and identified a DEHP mono-2-ethylhexyl phthalate hydrolase, which had a homodimer structure of 32.16 kDa. Previous studies have found that

Fusarium culmorum was able to degrade DEHP to butanediol, CO_2 and H_2O (González-Márquez *et al*. 2019a). Our results showed that in the DEHP-supplemented media containing the different nitrogen sources, two esterase isoforms appeared during the first 36 h with an average molecular weight of 26 and 32.6 kDa, which might be responsible for the first steps in the DEHP degradation, and the rest of the esterases which appeared after 48 h might be involved in the last steps of the DEHP biodegradation.

5. CONCLUSION

These results showed that DEHP induced esterase production by *F. culmorum*, and that esterase production showed a nitrogen source-dependent pattern. The number and molecular weight of esterases were similar in the different nitrogen sources analyzed, however; magnesium nitrate was the best nitrogen source for esterase production, followed by urea, and sodium nitrate. The use of efficient DEHP-degraders is important for the DEHP bioremediation process. However, development of new strategies to increase production of microbial enzymes is crucial to establish efficient methods for DEHP biodegradation to mitigate the effects of DEHP pollution and the risk of health impacts.

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AUTHOR CONTRIBUTION

Brenda Hernández-Sánchez performed the experimental work and analyzed data. Ericka Santacruz-Juárez and Rubén Díaz supervised the research. Jorge Alberto Sánchez-Martínez analyzed data. Carmen Sánchez provided the idea for the project, designed the study, 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.

REFERENCES

Ahuactzin-Pérez, M., Tlecuitl-Beristain, S., García-Dávila, J., González-Pérez, M., Gutiérrez-Ruíz, M.C., Sánchez, C., 2016. Degradation of di (2-ethyl hexyl) phthalate by *Fusarium culmorum*: kinetics, enzymatic activities and biodegradation pathway based on quantum chemical modeling. Sci. Total Environ. 566–567, 1186–1193. http://dx.doi.org/10.1016/j.scitotenv.2016.05.169

Alsen, M., Sinclair, C., Cooke, P., Ziadkhanpour, K., Genden, E., Gerwen, M.V., 2021. Endocrine disrupting chemicals and thyroid cancer: An overview. Toxics. 9 (1), 14. https://doi.org/10.3390/toxics9010014

Beck, A., 2014. Introduction to nonlinear optimization: Theory, algorithms, and applications with MATLAB, MOS-SIAM Ser. Optim. SIAM, Philadelphia, USA.

Bope, A., Haines, S.R., Hegarty, B., Weschler, C.J., Peccia, J., Dannemiller, K.C., 2019. Emerging investigator series: Degradation of phthalate esters in floor dust at elevated relative humidity. Environ. Sci. Proc. Imp. 21 (8), 1268–1279. https://doi.org/10.1039/c9em00050j

Bouattour, Y., Wasiak, M., Bernard, L., Pinguet, J., Richard, D., Le Rouzo-Grèves, M.L., Dhifallah, I., Lambert, C., Pereira, B., Chennell, P., Sautou, V., 2020. Quantification of bis(2-ethylhexyl) phthalate released by medical devices during respiratory assistance and estimation of patient exposure. Chemosphere. 255, 126978. https://doi.org/10.1016/j.chemosphere.2020.126978

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Chamorro-Mejía, A., Jaramillo-Quintero L.P., Sánchez, C., 2024. Production of esterase by *Fusarium culmorum* grown in the presence of different concentrations of di(2-ethylhexyl) phthalate and calcium ion in submerged fermentation. Mex. J. Biotechnol. 9 (4), 1–13.
Clarke, K.G., 2013. Bioprocess engineering: An introductory engineering and life science approach, first ed. Woodhead publishing, United Kingdom.

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. JCBPS 4, 96–103. https://doi/10.5555/20153365486

da Silva, M.C., Bertolini, M.C., Ernandes, J.R., 2001. Biomass production and secretion of hydrolytic enzymes are influenced by the structural complexity of the nitrogen source in *Fusarium oxysporum* and *Aspergillus nidulans*. J. Basic Microbiol. 41 (5), 269–80. https://doi.org/10.1002/1521-4028(200110)41:5%3C269::AID-JOBM269%3E3.0.CO;2-%23

Ernst, J., Grabiec, U., Falk, K., Dehghani, F., Schaedlich, K., 2020. The endocrine disruptor DEHP and the ECS: analysis of a possible crosstalk. Endocr. Connect. 9 (2). 101–110. https://doi.org/10.1530/EC-19-0548

Erythropel, H.C., Maric, M., Nicell, J.A., Leask, R.L., Yargeau, V., 2014. Leaching of the plasticizer di(2-ethylhexyl)phthalate (DEHP) from plastic containers and the question of human exposure. Appl. Microbiol. Biotechnol. 98 (24), 9967–9981. https://doi.org/10.1007/s00253-014-6183-8

Fan, S., Wang, J., Yan, Y., Wang, J., Jia, Y., 2018. Excellent degradation performance of a versatile phthalic acid esters-degrading bacterium and catalytic mechanism of monoalkyl phthalate hydrolase. Int. J. Mol. Sci. 19 (9), 2803. https://doi.org/10.3390/ijms19092803

Ferrer-Parra, L., López-Nicolás, D.I., Martínez-Castillo, R., Montiel-Cina, J.P., Morales-Hernández, A.R., Ocaña-Romo, E., González-Márquez, A., Portillo-Ojeda, M., Sánchez-Sánchez, D.F., Sánchez, C., 2018. Partial characterization of esterases from *Fusarium culmorum* grown in media supplemented with di (2-ethyl hexyl phthalate) in solid-state and liquid fermentation. Mex. J. Biotechnol. 3 (1), 82–94. http://dx.doi.org/10.29267/mxjb.2018.3.1.84

González-Márquez, A., Loera-Corral, O., Santacruz-Juárez, E., Tlécuitl-Beristain, S., García-Dávila, J., Viniegra-González, G., Sánchez, C., 2019a. Biodegradation patterns of the endocrine disrupting polluntant di (2-ethyl hexyl) phthalate by *Fusarium culmorum.* Ecotoxicol. Environ. Saf. 170, 293–299. https://doi.org/10.1016/j.ecoenv.2018.11.140

González-Márquez, A., Loera-Corral, O., Viniegra-González, G., Sánchez, C., 2019b. Production of cutinolytic esterase by *Fusarium culmorum* grown at different apple cutin concentrations in submerged fermentation. Mex. J. Biotechnol. 4 (4), 50–64. https://doi.org/10.29267/mxjb.2019.4.4.50

González-Márquez, A., Loera-Corral, O., Viniegra-González, G., Sánchez, C., 2020. Induction of esterase activity during the degradation of high concentrations of the contaminant di(2-ethylhexyl) phthalate by *Fusarium culmorum* under liquid fermentation conditions. 3 Biotech. 10 (11), 488. https://doi.org/10.1007/s13205-020-02476-y

González-Márquez, A., Sánchez, C., 2022. Tween 80-induced esterase production by *Trichoderma harzianum* in submerged fermentation: An esterase activity assay using α-naphthyl acetate as substrate. Mex. J. Biotechnol: 7 (1), 1–17 https://doi.org/10.29267/mxjb.2024.7.1.1

González-Márquez, A., Sánchez, C., 2024. Mycelial growth and production of laccase and peroxidases by *Pleurotus ostreatus* and *Agrocybe aegerita* in liquid fermentation. Mex. J. Biotechnol: 9(3), 36–49. https://doi.org/10.29267/mxjb.2024.9.3.36

González-Márquez, A., Volke-Sepulveda, T., Díaz, R., Sánchez, C., 2021. Enhanced esterase activity during the degradation of dibutyl phthalate by *Fusarium* species in liquid fermentation. J. Ind. Microbiol. Biotechnol. 48 (9–10), kuab062. https://doi.org/10.1093/jimb/kuab062

Hernández-Sánchez, B., Díaz-Godínez R., Luna-Sánchez S., Sánchez, C., 2019. Esterase production by microorganisms: importance and industrial application. Mex. J. Biotechnol. 4 (1), 25-37. https://doi.org/10.29267/mxjb.2019.4.1.25

Hernández-Sánchez, B., Santacruz-Juárez, E., Figueroa-Martínez, F., Castañeda-Antonio, D., Portillo-Reyes, R., Viniegra-González, G., Sánchez, C., 2024. A novel and efficient strategy for the biodegradation of di(2-ethylhexyl) phthalate by *Fusarium culmorum*. Appl. Microbiol. Biotechnol. 108 (1), 94. https://doi.org/10.1007/s00253-023-12961-y

Heudorf, U., Mersch-Sundermann, V., Angerer, J., 2007. Phthalates: toxicology and exposure. Int. J. Hyg. Environ. Health Res. 210, 623–634. https://doi.org/10.1016/j.ijheh.2007.07.011

Holland, P.W., Welsch, R.E., 1977. Robust regression using iteratively rew eighted least-squares. COMMUN STAT-THEOR M. 6 (9), 813-827. http://dx.doi.org/10.1080/03610927708827533

https://doi.org/10.29267/mxjb.2024.9.4.1
Huang, H., Zhang, X.Y., Chen, T.L., Zhao, Y.L., Xu, D.S., Bai, Y.P., 2019. Biodegradation of structurally diverse phthalate esters by a newly identified esterase with catalytic activity toward di(2-ethylhexyl) phthalate. J. Agric. Food Chem. 67 (31), 8548–8558. https://doi.org/10.1021/acs.jafc.9b02655

Kim, Y.H., Lee, J., Ahn, J.Y., Gu, M.B., Moon, S.H., 2002. Enhanced degradation of an endocrine disrupting chemical, butyl benzyl phthalate, by *Fusarium oxysporum* f. sp. *pisi* cutinase. Appl. Environ. Microbiol. 68 (9), 4684–4688. https://doi.org/10.1128/AEM.68.9.4684-4688.2002

Kim, Y.H., Lee, J., Moon, S.H., 2003. Degradation of an endocrine disrupting chemical, DEHP [di-(2-ethylhexyl)-phthalate], by *Fusarium oxysporum* f. sp. *pisi* cutinase. Appl. Microbiol. Biotechnol. 63, 75–80. https://doi.org/10.1007/s00253-003-1332-5

Kim, Y.H., Min, J., Bae, K.D., Gu, M.B., Lee, J., 2005. Biodegradation of dipropyl phthalate and toxicity of its degradation products: a comparison of *Fusarium oxysporum* f. sp. *pisi* cutinase and Candida cylindracea esterase. Arch. Microbiol. 184 (1), 25–31. https://doi.org/10.1007/s00203-005-0026-z

Lamraoui, I., Eltoukhy, A., Wang, J., Lamraoui, M., Ahmed, A., Jia, Y., Lu, T., Yan, Y., 2020. Biodegradation of di (2-ethylhexyl) phthalate by a novel *Enterobacter* spp. strain YC-IL1 isolated from polluted soil, Mila, Algeria. Int. J. Environ. Res. Public Health, 17 (20), 7501. https://doi.org/10.3390/ijerph17207501

Leammli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227 (1), 680–685. https://doi.org/10.1038/227680a0.

Lemos J.L.S., de A. Fontes M.C., Pereira, N., 2001. Xylanase Production by *Aspergillus awamori* in solid-state fermentation and influence of different nitrogen sources. Appl. Biochem. Biotechnol. 91-93, 681-689. https://doi.org/10.1007/978-1-4612-0217-2_57

Lesser, L. E., Mora, A., Moreau, C., Mahlknecht, J., Hernández-Antonio, A., Ramírez, A. I., Barrios-Piña, H., 2018. Survey of 218 organic contaminants in groundwater derived from the world's largest untreated wastewater irrigation system: Mezquital Valley, Mexico. Chemosphere. 198, 510–521. https://doi.org/10.1016/j.chemosphere.2018.01.154

Li, F., Liu, Y., Wang, D., Zhang, C., Yang, Z., Lu, S., Wang, Y., 2018. Biodegradation of di-(2-ethylhexyl) phthalate by a halotolerant consortium LF. Plos one. 13 (10). https://doi.org/10.1371/journal.pone.0204324

Loftus, M.G., Sánchez C., Moore, D., Robson, G., Trinci, T., 2020. A 21st century miniguide to sporophore morphogenesis and development in Agaricomycetes and their biotechnological potential. Mex. J. Biotechnol. 5(2), 1–50 https://doi.org/10.29267/mxjb.2020.5.2.1.

Motulsky, H.J., Brown, R.E., 2006. Detecting outliers when fitting data with nonlinear regression – a new method based on robust nonlinear regression and the false discovery rate. BMC Bioinformatics. 7, 123 https://doi.org/10.1186/1471-2105-7-123

Nishioka, T., Iwata, M., Imaoka, T., Mutoh, M., Egashira, Y., Nishiyama, T., Shin, T., Fujii, T., 2006. A mono-2-ethylhexyl phthalate hydrolase from a *Gordonia* sp. That is able to dissimilate di-2-ethylhexyl phthalate. Appl. Environ. Microbiol. 72 (4), 2394–2399. https://doi.org/10.1128/AEM.72.4.2394-2399.2006

Ocaña-Romo, E., Rodríguez-Nava, C.O., Sánchez, C., 2024. Oxidases production by *Trametes Versicolor* grown on green waste and on polyurethane foam in solid-state fermentation: a comparative study. Mex. J. Biotechnol. 9 (2), 51–64. https://doi.org/10.29267/mxjb.2024.9.2.51

Olvera-García, C., Díaz-Godínez, G., Sánchez, C., Álvarez-Cervantes, J., Martínez-Carrera, D., Díaz, R., 2017. Laccases from *Pleurotus ostreatus*. Mex. J. Biotechnol. 2 (1), 122–134. https://doi.org/10.29267/mxjb.2017.2.1.112.

Pérez-Andrés, L., Díaz-Godínez, R., Luna-Suárez, S., Sánchez, C., 2017. Characteristics and uses of phthalates. Mex. J. Biotechnol. 2 (1), 145-154. https://doi.org/10.29267/mxjb.2017.2.1.145

Portillo-Ojeda, M., González-Márquez, A., Arteaga-Mejía, M., Sánchez C., 2020. Optimum pH for di(2-ethylhexyl) phthalate degradation by *Fusarium culmorum* in submerged fermentation. Mex. J. Biotechnol. 5 (3), 71-82. https://doi.org/10.29267/mxjb.2020.5.3.71

Pradeep, S., Josh, M.S., Hareesh, E.S., Kumar, S., Benjamin, S., 2015. *Achromobacter denitrificans* strain SP1 produces an intracellular esterase upon utilizing di(2ethylhexyl)phthalate. Int. Biodeterior. Biodegradation. 105, 160–167. https://doi.org/10.1016/j.ibiod.2015.09.007

Ren, L., Lin, Z., Liu, H., Hu, H., 2017. Bacteria-mediated phthalic acid esters degradation and related molecular mechanisms. Appl. Microbiol. Biotechnol. 102 (3), 1085–1096. https://doi.org/10.1007/s00253-017-8687-5

Riazoshams, H., Midi, H., Gebrenegus, G., 2019. Robust Nonlinear Regression with Applications Using R. Wiley, United Kingdom.

Ríos-González, N.S., González-Márquez, A., Sánchez. C., 2019. Growth and esterase activity of *Fusarium culmorum* grown in di(2-ethyl hexyl) phthalate in liquid fermentation. Mex. J. Biotechnol. 4 (1), 51-60. https://doi.org/10.29267/mxjb.2019.4.1.51

Sánchez C., Moore D., Robson, G., Trinci, T., 2020. 21st century miniguide to fungal biotechnology. Mex. J. Biotechnol. 5 (1), 11–42. https://doi.org/10.29267/mxjb.2020.5.1.11.

Sánchez, C., 2020. Fungal potential for the degradation of petroleum-based polymers: An overview of macro- and microplastics biodegradation. Biotechnol. Adv. 40, 107501. https://doi.org/10.1016/j.biotechadv.2019.107501

Sánchez, C., 2021. Microbial capability for the degradation of chemical additives present in petroleum-based plastic products: A review on current status and perspectives. J. of Hazard. Mater. 402, 123534. https://doi.org/10.1016/j.jhazmat.2020.123534

Sánchez, C., 2022. A review of the role of biosurfactants in the biodegradation of hydrophobic organopollutants: production, mode of action, biosynthesis and applications. World J. Microbiol. Biotechnol. 38 (11), 216. https://doi.org/10.1007/s11274-022-03401-6

Sánchez, C., 2024. *Fusarium* as a promising fungal genus with potential application in bioremediation for pollutants mitigation: A review. Biotechnol. Adv. 77, 108476. https://doi.org/10.1016/j.biotechadv.2024.108476

Sánchez-Sánchez, D.F., Sánchez, C., 2019. *Neurospora sitophila* grown in media supplemented with dibutyl phthalate in submerged fermentation: Kinetics of growth and esterase activity. Mex. J. Biotechnol. 4 (1), 61–71. https://doi.org/10.29267/mxjb.2019.4.1.61

Schaffert, A., Karkossa, I., Ueberham, E., Schlichting, R., Walter, K., Arnold, J., Blüher, M., Heiker, J.T., Lehmann, J., Wabitsch, M., Escher, B.I., Bergen. M. V., Schubert, K., 2022. Di-(2-ethylhexyl) phthalate substitutes accelerate human adipogenesis through PPARγ activation and cause oxidative stress and impaired metabolic homeostasis in mature adipocytes. Environ. Int. 164, 107279. https://doi.org/10.1016/j.envint.2022.107279

Shinohara, N., Oguri, T., Takagi, M., Ueyama, J., Isobe, T., 2024. Evaluating the risk of phthalate and non-phthalate plasticizers in dust samples from 100 japanese houses. Environ. Int. 183, 108399. https://doi.org/10.1016/j.envint.2023.108399

Shivaraju, H.P., Yashas, S.R., Harini, R., 2020. Quantification, distribution, effects of di (2-ethylhexyl) phthalate contamination: Risk analysis and mitigation strategies in urban environment. Water Environ. Res. 93 (6), 940-952. https://doi.org/10.1002/wer.1486

Sun, Y., Qian, Y., Zhang, J., Wang, Y., Li, X., Zhang, W., Wang, L., Liu, H., Zhong, Y., 2021. Extracellular protease production regulated by nitrogen and carbon sources in *Trichoderma reesei*. J. Basic Microbiol. 61 (2), 122–132. https://doi.org/10.1002/jobm.202000566

Tu, W., Li, W., Zhu, X., Xu, L., 2020. Di-2-ethylhexyl phthalate (DEHP) induces apoptosis of mouse HT22 hippocampal neuronal cells via oxidative stress. Toxicol. Ind. Health. 36 (11), 844-851. https://doi.org/10.1177/0748233720947205

Wang, P., Gao, J., Zhao, Y., Zhang, M., Zhou, S., 2021. Biodegradability of di-(2-ethylhexyl) phthalate by a newly isolated bacterium *Achromobacter* sp. RX. Sci. Total. Environ. 755, 142476. https://doi.org/10.1016/j.scitotenv.2020.142476

Xu, Y., Minhazul, K. A. H. M., Wang, X., Liu, X., Li, X., Meng, Q., Li, H., Zhang, C., Sun, X., Sun, B., 2020. Biodegradation of phthalate esters by *Paracoccus kondratievae* BJQ0001 isolated from Jiuqu (Baijiu fermentation starter) and identification of the ester bond hydrolysis enzyme. Environ Pollut. 263, 114506. https://doi.org/10.1016/j.envpol.2020.114506

Zhang, X., Hou, X.W., Xu, D., Xue, M., Zhang, J.Y., Wang, J., Yang, Y., Lai, D., Zhou, L., 2023. Effects of carbon, nitrogen, ambient pH and light on mycelial growth, sporulation, sorbicillinoid biosynthesis and related gene expression in *Ustilaginoidea virens*. J. Fungi. 9 (4), 390. https://doi.org/10.3390/jof9040390

Zhu, B.B., Zhang, Z.C., Li, J., Gao, X.X., Chen, Y.H., Wang, H., Gao, L., Xu, D.X., 2021. Di-(2-ethylhexyl) phthalate induces testicular endoplasmic reticulum stress and germ cell apoptosis in adolescent mice. Environ. Sci. Pollut. Res. Int. 28 (17), 21696-21705. https://doi.org/10.1007/s11356-020-12210-z