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ORIGINAL RESEARCH

Effect of surfactant Tween 80 on growth and esterase production of *Fusarium culmorum* in liquid fermentation

Efecto del surfactante Tween 80 en el crecimiento y la producción de esterasa de *Fusarium culmorum* en fermentación líquida

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ABSTRACT

Tween 80 is a widely used non-ionic surfactant that is added to culture media to make hydrophobic substrate available to microorganisms. Because of this surfactant widespread use, it is important to understand how it affects microbial growth and enzyme production. In this work, the effect of different concentrations (100, 400 and 600 µl/L; v/v) of Tween 80 (as the sole carbon source) on the biomass production, esterase activities (assessed through biochemical tests and zymographic assays) and protein content of *Fusarium culmorum* grown in liquid fermentation was determined. The specific growth rate (µ), biomass yield ($Y_{X/S}$), esterase yield ($Y_{E/X}$), esterase productivity (P), maximal enzymatic activity (E_{max}), and specific rate of enzyme production (q_p) were also estimated. A control medium added with glucose was used. The highest µ was showed in the medium added with 100 µl of Tween 80/L. However, the greatest esterase activity was observed in those media containing the highest concentrations of Tween 80 (400 µl/L and 600 µl/L; v/v). These results show that Tween 80 was used as the sole carbon and

energy source and it also induced the esterase production by *F. culmorum*. Tween 80 concentration is positively correlated with the number of esterase isoforms produced by this fungus. The higher the Tween 80 concentration (400 µl/L and 600 µl/L, v/v), the more number of esterase isoforms will be induced. However, lower concentration (100 µl/L) of Tween 80/L did not show a significant effect on the induction of the esterase activity.

Keywords: Esterase, fungal growth, *Fusarium culmorum*, liquid fermentation, Tween 80.

RESUMEN

Tween 80 es un tensoactivo no iónico ampliamente utilizado que se añade a los medios de cultivo para hacer que el sustrato hidrófobo esté disponible para los microorganismos. Debido al uso generalizado de este surfactante, es importante conocer su efecto sobre el crecimiento microbiano y la producción de enzimas. En este trabajo, se determinó el efecto de diferentes concentraciones (100, 400 y 600 µl/L; v/v) de Tween 80 (como única fuente de carbono) sobre la producción de biomasa, la actividad de esterasa (evaluada mediante pruebas bioquímicas y ensayos zimográficos) y el contenido de proteína de *Fusarium culmorum* crecido en fermentación líquida. También se estimaron la tasa de crecimiento específico (µ), rendimiento de biomasa ($Y_{X/S}$), rendimiento de esterasa ($Y_{E/X}$), productividad de esterasa (*P*), actividad enzimática máxima (E_{max}) y la tasa específica de producción de esterasa (q_p). Un medio adicionado con glucosa fue usado como testigo. La mayor µ se mostró en el medio con 100 µl de Tween 80/L. Sin embargo, la mayor actividad de esterasa se observó en aquellos medios que contenían las concentraciones más altas de Tween 80 (400 µl/L y 600 µl/L; v/v). Estos resultados muestran que *F. culmorum* utilizó Tween 80 como única fuente de carbono y energía, y que indujo la producción de esterasa. La concentración de Tween 80 se correlaciona positivamente con el número de isoformas de esterasa producidas por este hongo. Una mayor concentración de Tween 80 (400 µl/L y 600 µl/L, v/v), indujo un mayor número de isoformas de esterasa. Sin embargo, una concentración más baja (100 µl/L) de Tween 80/L no mostró un efecto significativo sobre la inducción de la actividad de esterasa.

Palabras clave: Crecimiento de hongos, esterasa, fermentación líquida, *Fusarium culmorum*, Tween 80.

1. INTRODUCTION

Surfactants are amphiphilic compounds constituted by a hydrophobic and a hydrophilic moiety, which have the characteristics of solubility and emulsification as well as the abilities to decrease surface and interfacial tensions. The addition of

surfactants in the medium is a common practice since it makes hydrophobic substrates available to the organism. In fact, the rate of hydrolysis of organopollutants improves by using surfactant (Muff *et al*., 2020). In general, surfactants are classified into cationic, anionic and nonionic by the chemical nature of the hydrophilic part. Particularly, Tween 80 (polysorbate 80, polyoxyethylene sorbitan monooleate, $C_{64}H_{124}O_{26}$) is a nonionic surfactant widely used in pollutant biodegradation studies (Bustamante *et al*., 2012; Cheng *et al*., 2017). This surfactant is added to the culture medium to make hydrophobic substrate available to microorganisms (Ahuactzin-Pérez *et al*., 2016; Ahuactzin-Pérez *et al*., 2018a; 2018b; 2018c; González-Márquez *et al*., 2019a). Due to the widespread use of Tween 80, it is important to understand how this surfactant affects microbial growth and enzyme production. Some studies have reported that microorganisms are capable of utilizing Tween 80 as a sole source of carbon (Minami, 1958; Chakrabarty *et al*., 1970; Nielsen *et al*., 2016; Nguyen *et al*., 2018).

Fungi are of great importance for bioremediation process due to the highly efficient intracellular and extracellular enzymatic system (Sánchez, 2020). For example, *Monascus* sp. strains produced protease, esterase and lipase during polyurethane biodegradation (El-Morsy *et al*., 2017). Furthermore, *Pestalotiopsis microspore* showed an increase of the serine hydrolase activity when it was grown on polyester polyurethane as its sole carbon source (Russell *et al*. (2011). In addition, it was found that the ascomycetes *Gloeophyllum trabeum* and *Zalerion maritimum* were capable to degrade microplastics (Krueger *et al*., 2015; Paço *et al*., 2017). Ameen *et al*. (2015) demonstrated that a consortium of ascomycete strains were capable to degrade low density polyethylene, exhibiting lignin peroxidase, laccase and manganese peroxidase activities. It has been reported that *Penicillium citrinum* produced polyesterase and was able to hydrolyze PET pellets (Liebminger *et al*. 2007). Furthermore, Ronkvist *et al*. (2009) reported that *Thermomyces* (formerly *Humicola*) *insolens* had catalytic activities of cutinase when grown on (7%) PET films as substrate.

In particular, some ascomycete fungi from the genus *Fusarium* have been reported to be highly efficient organopollutant degrading organisms due to their secretion of enzymes such as esterase (Chhaya and Guspte, 2013; Aguilar-Alvarado *et al*., 2015; Bouchiat *et al*., 2015, Ahuactzin-Pérez *et al*., 2016, 2018a; 2018b; 2018c; Gonzalez-Marquez *et al*., 2019). *Fusarium culmorum* has been reported as an organism able to degrade phthalates (Ahuactzin-Pérez *et al*., 2016; Ahuactzin-Pérez *et al*., 2018a; 2018b; 2018c; González-Márquez *et al*., 2019a) and apple cutin (González-Márquez *et al*., 2019b).

In this work, the effect of different concentrations (100, 400 and 600 μl/L; v/v) of Tween 80 on the biomass production, esterase activities and protein content of *F. culmorum* grown in liquid fermentation were determined. Esterase activity was assessed by biochemical test, and in SDS/PAGE gels, it was detected by zymography.

2. MATERIALS AND METHODS

2. 1. Organism

F. culmorum Fc1-CICBUAT from the microbial collection from the Research Centre for Biological Sciences (CICB) at the Autonomous University of Tlaxcala (UAT) (Tlaxcala, Mexico) was used in this study. This strain is deposited at the Collection of the Mexico's National Center for Genetic Resources (CNRG-INIFAP) (Jalisco, Mexico).

2.2. Culture media and culture conditions

Four liquid culture media were prepared containing the following components (in L^{-1}): 1) Mineral medium (MM) + 10 g glucose, 2) MM + 100 µl of Tween 80, 3) MM + 400 µl of Tween 80, 4) MM + 600 µl of Tween 80. Mineral medium had the following components (in L^{-1}): 3.0 g $Na(NO_3)_2$; 1.0 g K_2HPO_4, 0.5 g $MgSO_4.7H_2O$, 0.5 g KCl, and 0.01 g $FeSO_4.7H_2O$. The pH was adjusted to 6.5 using either 0.1 M HCl or 0.1 M NaOH. Flasks of 125 mL containing 50 mL of culture medium were autoclaved at 121 ºC for 15 min, cooled to room temperature and then inoculated with a suspension of *F. culmorum* spores to a final concentration of 10^7 spores/mL as reported by González-Márquez *et al*. (2019a). Cultures were incubated with shaking at 120 rpm on an orbital shaker at 25 °C for 5 days. Samples were collected at 12-h intervals.

2.3. Specific growth rate calculation and protein content evaluation

The mycelial biomass in the liquid cultures was collected by vacuum filtration and the specific growth rate (µ) was determined by fitting the biomass data across time using the logistic equation. The biomass yield ($Y_{X/S}$) was estimated as the coefficient of the linear regression of biomass concentration *versus* substrate concentration in grams of biomass/g of substrate consumed (Ahuactzin-Pérez *et al*. 2016). The protein content of samples was quantified using a Bradford assay (Bradford, 1976), where 200 µl of Bradford reagent (BIORAD) was added to 100 µl of supernatant and 700 µl of sterile distilled water, mixed, and incubated at room temperature for 10 min. The absorbance of the solutions was measured at 595 using a spectrophotometer UNICO (S-2150 series Dayton, NJ, USA).

2.4. Esterase assay and pH measurements

Esterase activity was quantified in the supernatant using *p*-nitrophenyl butyrate (*p*NPB) as substrate according to Ferrer-Parra *et al*. (2018). The esterase yield per unit of biomass ($Y_{E/X}$), esterase productivity (P), maximal enzymatic activity (E_{max}), and specific rate of enzyme production (q_p) were estimated as previously described (Ahuactzin-Pérez *et al*. 2016). One unit enzyme activity (U) was defined as the amount of activity required to release 1 µmol of *p*NPB per min from *p*NPB under the assay conditions. Volumetric and esterase specific activities were expressed in U/L and U/mg of protein, respectively. The polypeptide profiles with esterase

activity were determined using polyacrylamide gels (PAGE) (Leammli 1970). Esterase activity was assayed by zymography according to Ferrer-Parra *et al*. (2018). Briefly, samples were loaded on 20% separating and 4% stacking PAGE gels and electrophoresed under non-reducing conditions. After electrophoresis, the gels were washed and then incubated overnight at 25 °C in a substrate buffer. ProteinTM Dual Precision Xtra Plus Standards (BIORAD) was used as molecular markers. The gels were imaged using a Gel Doc EZ Imager (BIORAD), and bands in the lanes were assessed for their densities using Image Lab Version 6.0.0 (BIORAD). The pH was measured every 12 h in the supernatant of the cultures using a digital potentiometer (Conductronic, Mexico).

2.5. Statistical analysis

All assays were performed in triplicate. Statistical analyses were conducted using one-way ANOVA followed by Tukey's *post hoc* test using SigmaPlot version 12.0 (Systat Software Inc, San Jose, CA, USA).

3. RESULTS

3.1. Biomass production and pH of the cultures

Figure 1 shows biomass production by *F. culmorum* in media containing different concentrations of Tween 80 (100, 400 and 600 µl/L; v/v) and glucose (control medium) as carbon sources. The highest X_{max} and µ were showed in the medium added with glucose, followed by the media added with Tween 80 (Fig. 1, Table 1). In those media added with Tween 80, in general, the greatest X_{max} and µ were observed in the medium added with 100 µl of Tween 80/L, followed by the media added with 400 µl of Tween 80/L and 600 µl of Tween 80/L (Fig 1., Table 1). The highest $Y_{x/s}$ was showed in the medium containing 100 µl of Tween 80/L followed by the media containing 400 µl of Tween 80/L and 600 µl of Tween 80/L. The lowest $Y_{x/s}$ was observed in the medium containing glucose as carbon source (Table 1).

On the other hand, the pH showed a slight increase from the starting 6.5 to 6.55 after 120 h of fermentation in those media containing glucose and 100 µl of Tween 80/L. However, the pH of the cultures added with 400 µl of Tween 80/L and 600 µl of Tween 80/L increased from 6.5 to 7.5 after 120 h of growth (Fig. 2).

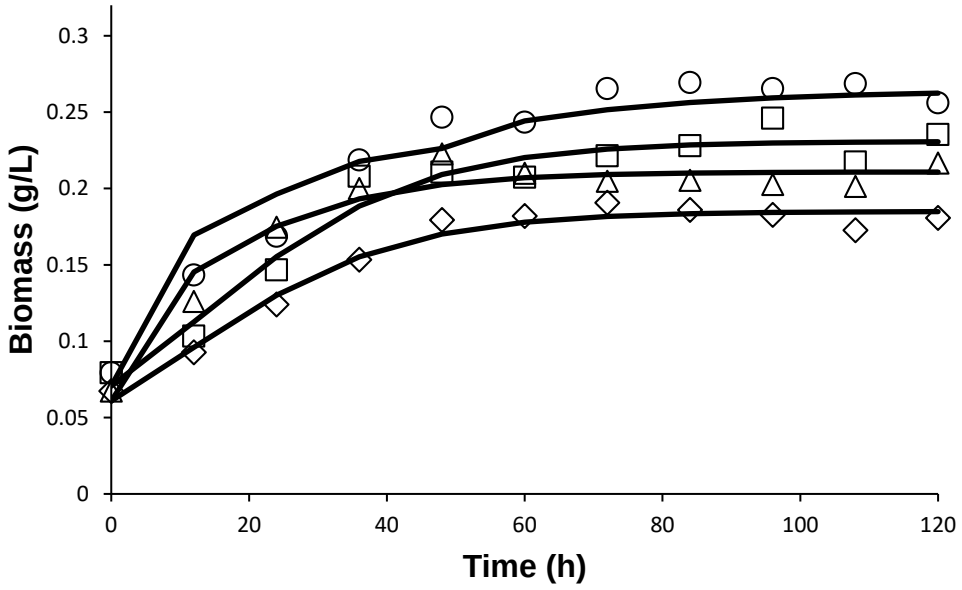

Fig. 1. Biomass production by *F. culmorum* grown in glucose-supplemented medium (10 g/L) (○) and in Tween 80-supplemented media in liquid fermentation. 100 μl/L (□), 400 μl /L (△) and 600 μl/L (◇).

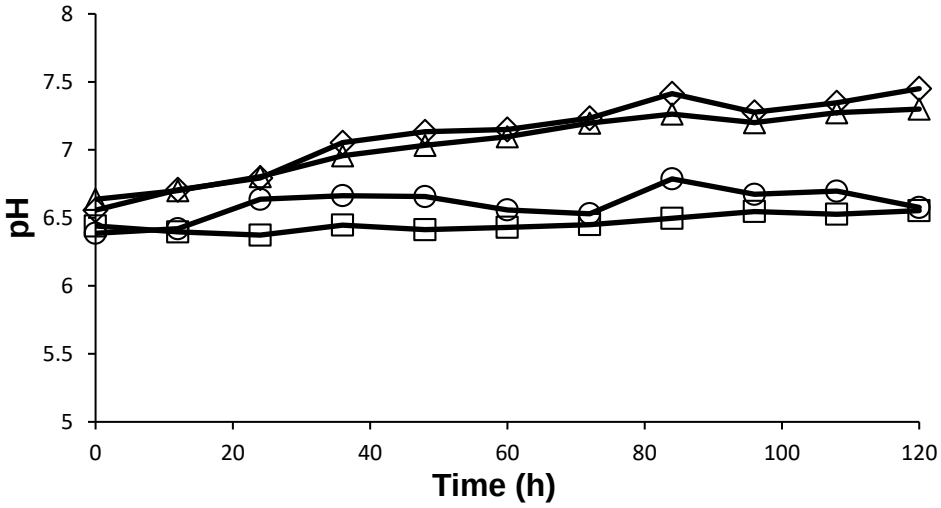

Fig. 2. pH of *F. culmorum* grown in glucose-supplemented medium (10 g/L) (○) and in Tween 80-supplemented media in liquid fermentation. 100 μl/L (□), 400 μl /L (△) and 600 μl/L (◇).

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Table 1. Growth parameters of *F. culmorum* grown in glucose-supplemented medium and in Tween 80-supplemented media in liquid fermentation.

Growth parameter	Glucose medium	Tween 80 (100 μl/L)	Tween 80 (400 μl/L)	Tween 80 (600 μl/L)
X_{max} (g/L)	0.26^{a} ±0.04	0.23^{b} ±0.03	0.21^{b} ±0.04	0.19^{c} ±0.04
μ (h^{-1})	0.0014^{a} ±0.001	0.0012^{b} ±0.001	0.009^{c} ±0.001	0.009^{c} ±0.001
$Y_{X/S}$ (g*X*/g*S*)	0.02^{c} ±0.001	2.3^{a} ±0.001	0.52^{b} ±0.001	0.32^{b} ±0.001

Values are expressed as mean±SEM (n=3); means within the same column not sharing common superscript letters (a, b and c) differ significantly at 5% level. Kinetic parameters (X_{max} and μ) of the logistic equation were evaluated using a non-linear least squares fitting program. The biomass yield ($Y_{X/S}$) was estimated as the coefficient of the linear regression of biomass concentration *versus* substrate concentration.

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

Figure 3 illustrates the protein content of the cultures during the fermentation. It is observed that those media added with 400 μl of Tween 80/L and 600 μl of Tween 80/L had higher protein content than the medium containing glucose. The lowest protein content was observed in the medium supplemented with 100 μl of Tween 80/L.

Figure 4 shows that the highest esterase activity was showed in those media containing 400 μl of Tween 80/L and 600 μl of Tween 80/L. In general, esterase specific activity increased after 48 and 72 h in those media supplemented with 400 μl of Tween 80/L and 600 μl of Tween 80/L, respectively, whereas the media added with glucose and 100 μl of Tween 80/L had the lowest esterase specific activity. Esterase specific activity increased at the end of the fermentation process in all the media tested (Fig. 4). The highest esterase volumetric activity was also observed in those media added with the highest concentrations of Tween 80 (400 and 600 μl/L), followed by the medium added with glucose (Table 2). The lowest esterase volumetric activity was showed in the medium added with 100 μl of Tween 80/L (Table 2). The greatest enzymatic yield parameters ($Y_{E/X}$, P and q_p) were observed in those media supplemented with 400 and 600 μl of Tween 80/L followed by the media containing glucose and 100 μl of Tween 80/L (Table 2).

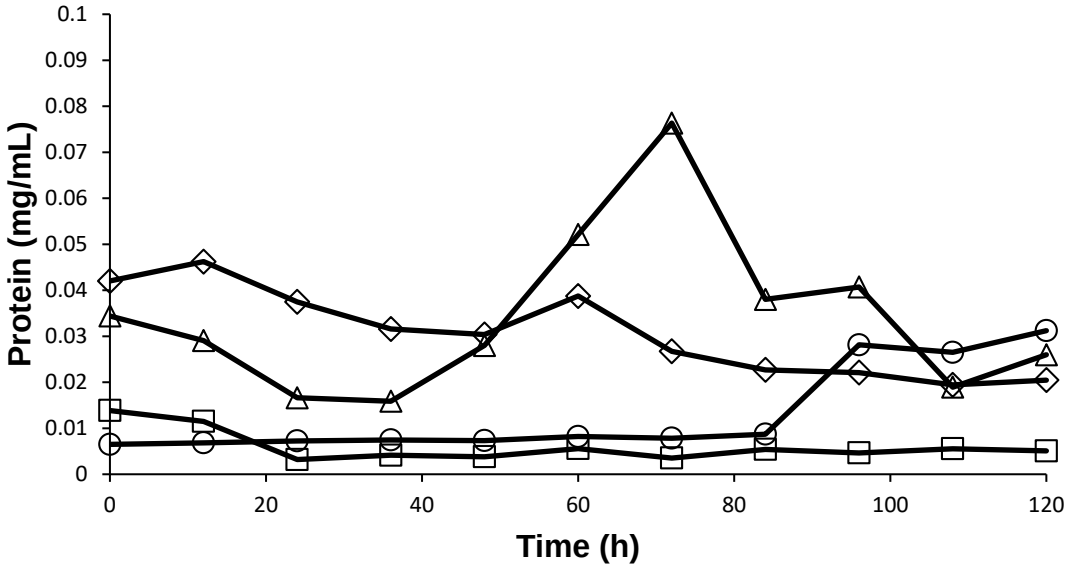

Fig. 3. Water-soluble protein content of *F. culmorum* grown in glucose-supplemented medium (10 g/L) (o) and in Tween 80-supplemented media in liquid fermentation. 100 µl/L (□), 400 µl /L (△) and 600 µl/L (◇).

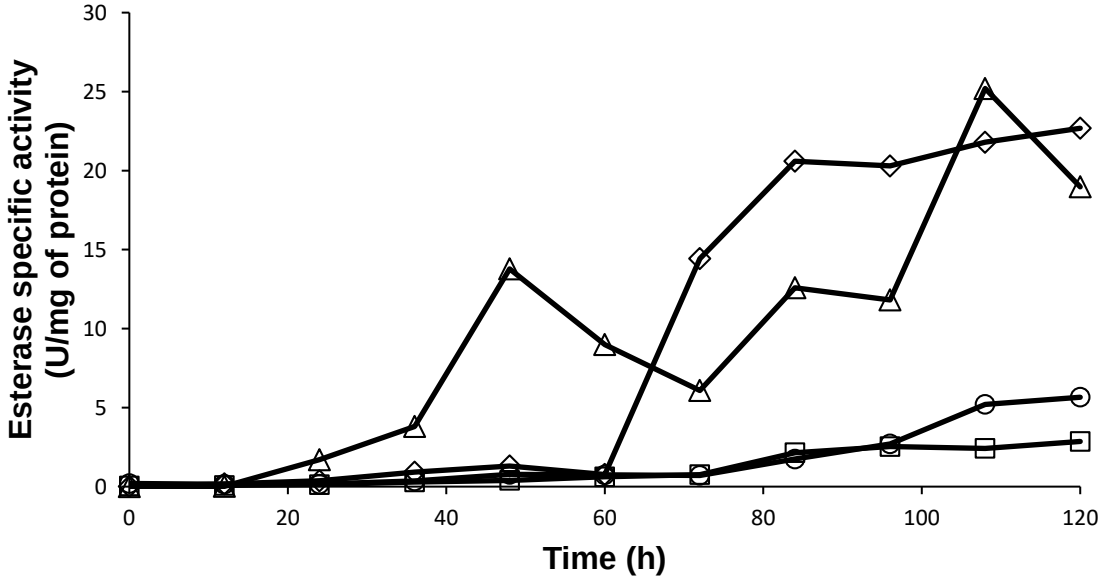

Fig. 4. Esterase specific activity of *F. culmorum* grown in glucose-supplemented medium (10 g/L) (o) and in Tween 80-supplemented media in liquid fermentation. 100 µl/L (□), 400 µl /L (△) and 600 µl/L (◇).

Table 2. Enzymatic yield parameters of esterase of *F. culmorum* grown in glucose-supplemented medium and in Tween 80-supplemented media in liquid fermentation.

Enzymatic yield parameters	Glucose medium	Tween 80 (100 μl/L)	Tween 80 (400 μl/L)	Tween 80 (600 μl/L)
E_{max} (U/L)	176.6^{b} ±0.3	14.5^{c} ±0.4	492.8^{a} ±0.4	464.3^{a} ±0.4
$Y_{E/X}$ (U/g*X*)	679.2^{b} ±0.8	63.0^{c} ±2.1	2346.6^{a} ±2.1	2443.6^{a} ±2.1
P (U/L*h)	1.47^{b} ±0.02	0.12^{c} ±0.01	4.10^{a} ±0.02	5.52^{a} ±0.04
q_p (U/g*X*h)	0.95^{b} ±0.001	0.07^{c} ±0.001	21.1^{a} ±0.002	22.0^{a} ±0.002

Values are expressed as mean ± SD (n=3); means within the same column not sharing common superscript letters (a, b and c) differ significantly at 5% level.

3.3. Detection of esterase by zymography

Figure 5 shows the esterase zymogram of *F. culmorum* grown in glucose-containing medium. One band with esterase activity was detected after 48 and 84 h with a molecular weight of 31 kDa approximately. Two esterase isoforms (33 and 63.8 kDa) were observed after 96 h and until the end of the fermentation period (120 h). The esterase zymogram of *F. culmorum* grown in medium supplemented with 100 µl of Tween 80/L is shown in Fig. 6. Two bands (31.3 and 61.9 kDa) with esterase activity were observed during the first 12 h of the fungal growth. One of them (31.3 kDa) was observed throughout the fermentation period. It is likely that those esterase isoforms shown in the medium added with glucose (Fig. 5) are the same as those observed in the medium added with 100 µl of Tween 80/L (Fig. 6).
The addition of 400 µl of Tween 80/L induced the production of five esterase isoforms (26, 31.6, 44.5, 64.6 and 222.6 kDa), which were observed after 48 h and until at the end of the fermentation period (120 h). An esterase isoform having a molecular weight of 31.6, and two esterase isoforms (26 and 64.6 kDa) were observed after 12 h and 36 h, respectively and throughout the fermentation period (Fig. 7). Figure 8 illustrates the esterase zymogram of *F. culmorum* grown in medium containing 600 µl of Tween 80/L. Nine bands (27.3, 33.4, 41, 48, 56.1, 70.6, 146.4, 150.4, 236.7 kDa) with esterase activity were observed after 84 and 120 h of fermentation. Isoforms with a molecular weight of 146.4 and 33.4 were observed after 36 h and 72 h of growth, respectively, which were present until the end of the fermentation period.

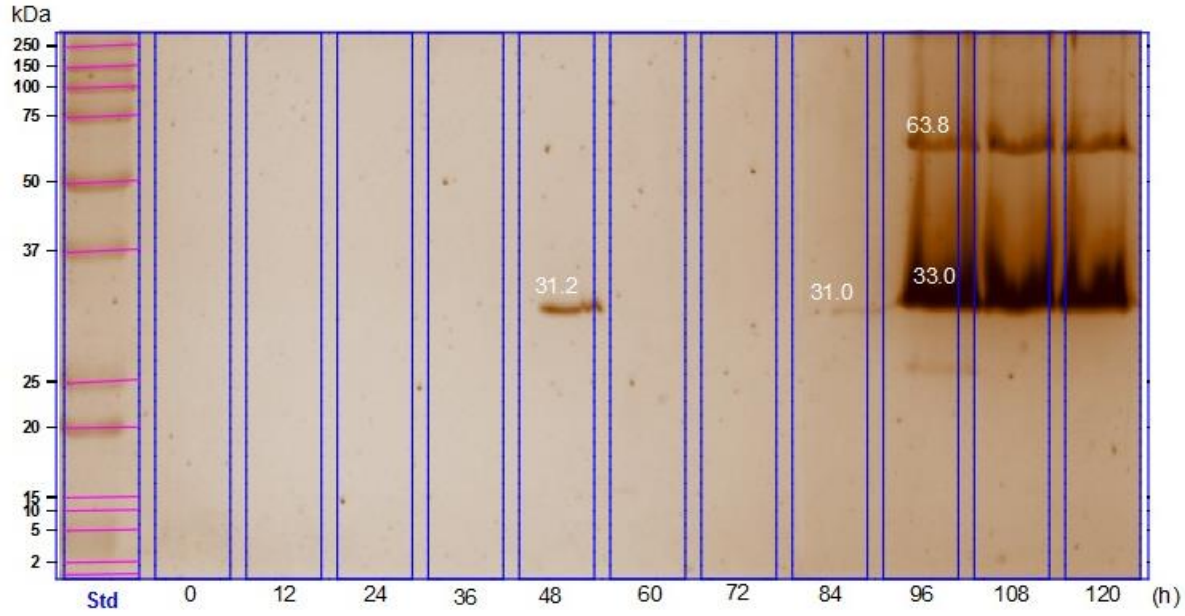

Fig. 5. Esterase zymogram of *F. culmorum* grown in glucose-supplemented (10 g/L) medium in liquid fermentation.

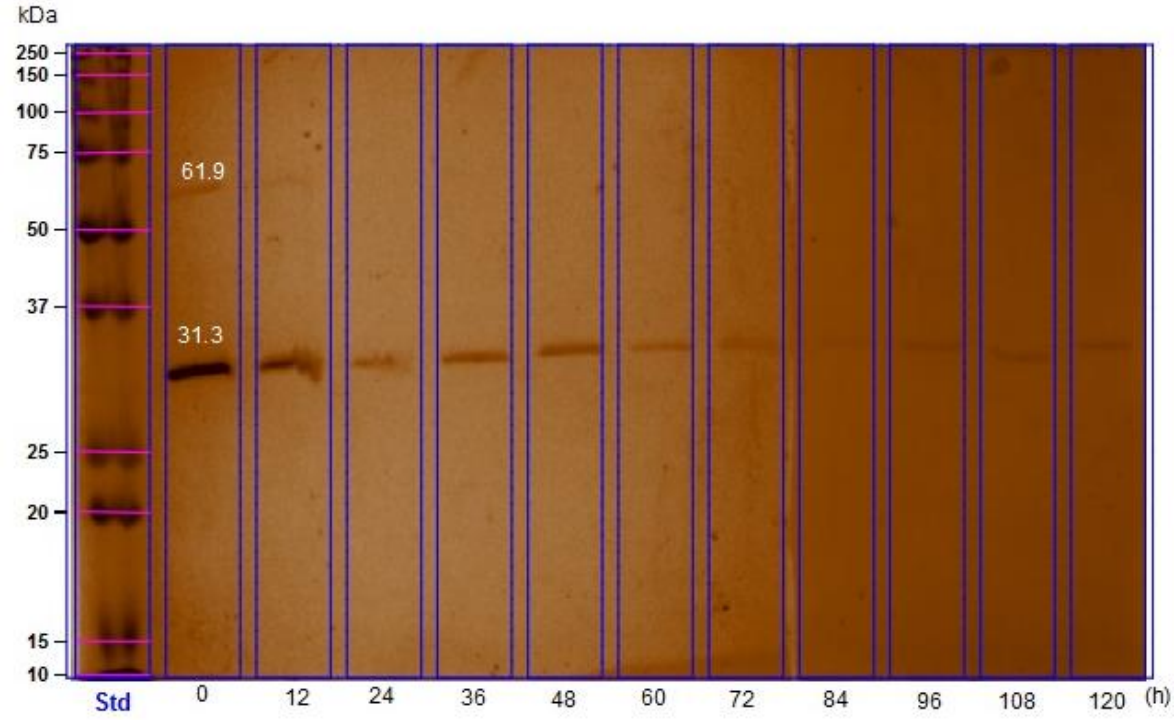

Fig. 6. Esterase zymogram of *F. culmorum* grown in Tween 80-supplemented (100 μl/L) medium in liquid fermentation.

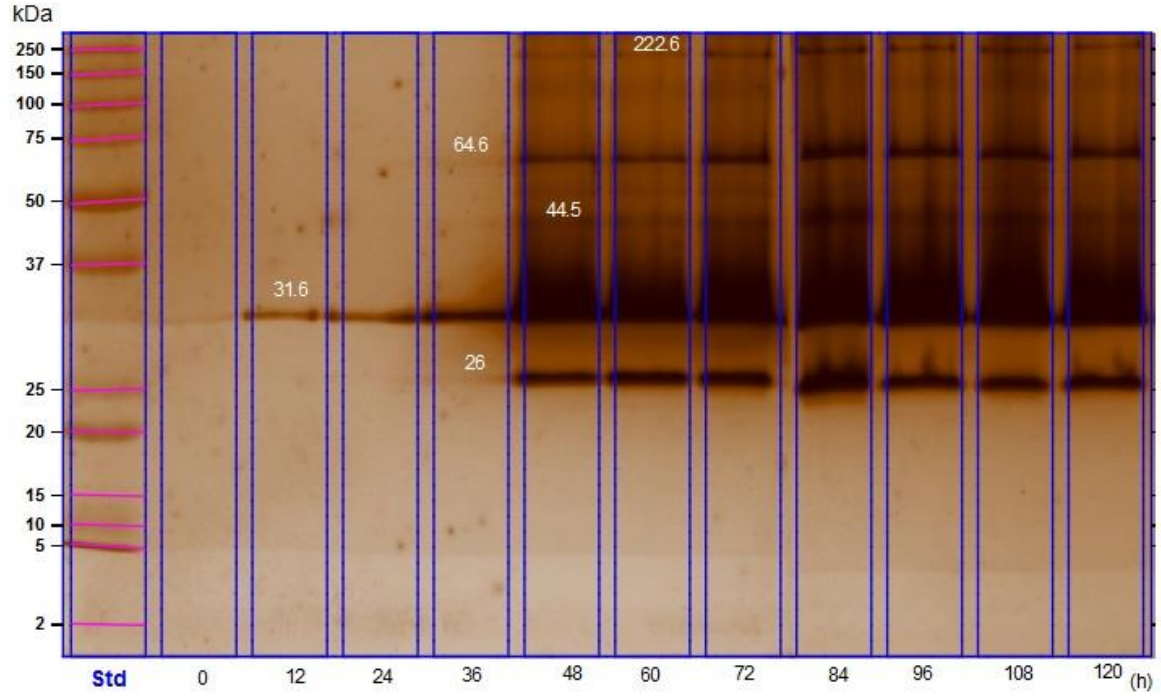

Fig. 7. Esterase zymogram of *F. culmorum* grown in Tween 80-supplemented (400 μl/L) medium in liquid fermentation.

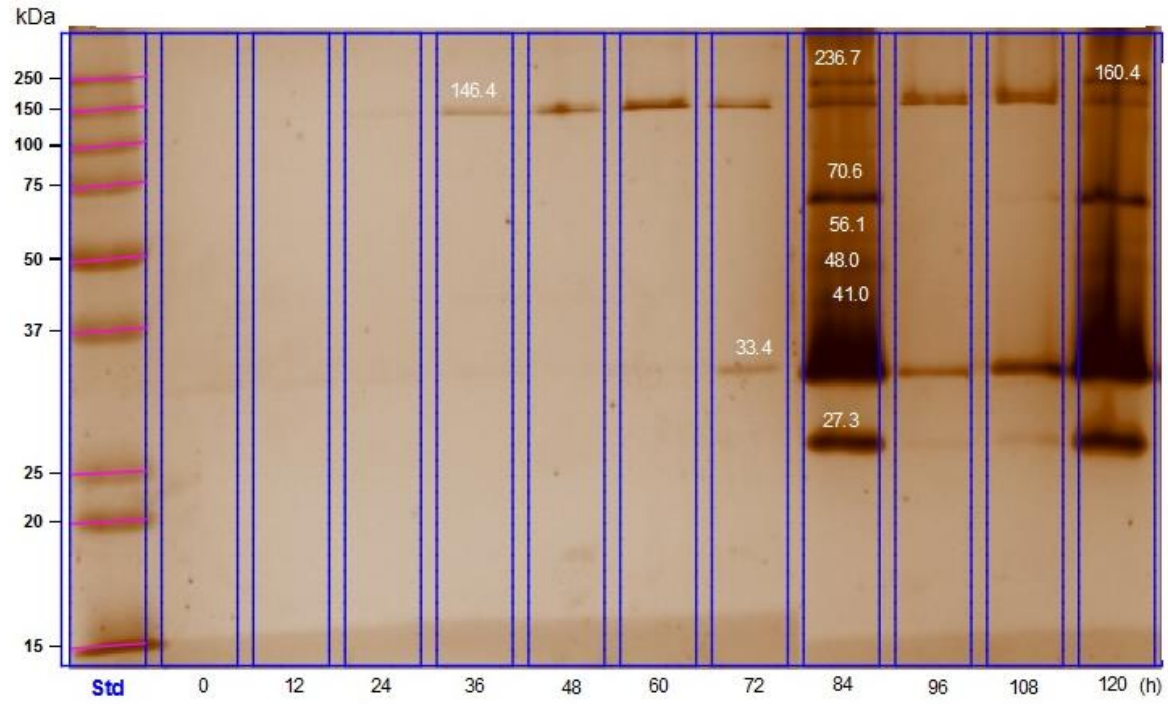

Fig. 8. Esterase zymogram of *F. culmorum* grown in Tween 80-supplemented (600 μl/L) medium in liquid fermentation.

4. DISCUSSION

Several studies have reported that Tween 80 affects microbial growth and enzyme production. Akpinar & Ozturk (2017) showed that Tween 80 induced laccase production in *Pleurotus* eryngii grown under solid state conditions. It has been suggested that Tween 80 increases permeability of the cell membrane, facilitating enzyme secretion and that it could also protect the enzyme structure and its activity (Akpinar & Ozturk 2017). However, in the present research, it has been demonstrated that Tween 80 can be used as sole source of carbon and energy by *F. culmorum*. Nguyen *et al*. (2018) reported that *Acinetobacter baumannii* was capable of utilizing Tween 80 as its sole carbon and energy source. It was suggested that the mechanism for polysorbate utilization is via β-oxidation, which would involve cleavage of oleate ester linkage by an esterase. The cleavage of the ether linkages would form primary alcohol. Alcohols would be subsequently oxidized to aldehydes and then to carboxylic acids which would enter the Krebs cycle to be mineralized to CO_2 and H_2O (Nguyen *et al*., 2018).

Li *et al*. (2018) reported that Tween 80 increased the biomass and the intracellular polysaccharide content in *Lentinula edodes* grown under liquid fermentation conditions. Furthermore, it has been reported that *Mycobacterium smegmatis* was capable to produce tween hydrolyzing esterase (Tomioka, 1983). Elsayed *et al*. (2012) evaluated the effect of different concentration of Tween 80 (0.1-0.75%; v/v) on laccase activity, and it was found that laccase showed its maximum value at a concentration of 0.1% (Tween 80) and increased by about 44% than control (without Tween 80). Cao *et al*. (2020) reported that Tween 80 enhanced the degradation of benzo[a]pyrene as well as the enzymes production (lignin peroxidase and laccase) by the fungus *Lasiodiplodia theobromae*. In addition, it was also found that Tween 80 was degraded by *L. theobromae* (Cao *et al*., 2020). Teodoro *et al*. (2018) reported that the addition of Tween 80 to the culture medium enhanced laccase activity during the degradation of bisphenol A by *Pleurotus sajor-caju*. Nielsen *et al*. (2016) found that the addition of Tween 80 (0.1%) to growth media increased the growth rate of *Staphylococcus aureus* batch cultures, and it also increased the total biomass when *S. aureus* was grown as biofilms. In contrast, Tween 80 had no effect on batch cultures of *Listeria monocytogenes*, it slowed the growth rate of *Pseudomonas fluorescens*, and it led to formation of less biofilm by both *L. monocytogenes* and *P. fluorescens* (Nielsen *et al*. 2016). Okagbue *et al*. (2001) studied the production of extracellular α-glucosidase and β-glucosidase by the yeast *Aureobasidium pullulans* in liquid medium and found that the addition of Tween 80 to a 24 h old culture led to a three-fold increase in yield of extracellular α-glucosidase. Liu *et al*. (2000) found that Tween 80 enhanced the lipase activity during the degradation of ketoprofen ester by *Candida rugose*.

The present research shows that Tween 80 was used as the sole carbon and energy source by *F. culmorum*, inducing the esterase production. It was demonstrated that Tween 80 concentration is positively correlated with the number of esterase isoforms produced by this fungus. The higher the Tween 80 concentration (400 µl/L and 600 µl/L, v/v), the more number of esterase isoforms will be induced. However, lower concentration (100 µl/L) of Tween 80/L did not show a significant effect on the induction of the enzyme activity.

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REFERENCES

Aguilar-Alvarado Y., Báez-Sánchez M. R., Martínez-Carrera D., Ahuactzin-Pérez M., Cuamatzi-Muñoz 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. 15 (24): 1897-1902. https://doi.org/10.15244/pjoes/58808.

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. Science of the Total Environment. 566–567: 1186-1193. https://doi.org/10.1016/j.scitotenv.2016.05.169.

Ahuactzin-Pérez M., Tlecuitl-Beristain S., García-Dávila J., González-Pérez M., Gutiérrez-Ruíz M. C. & Sánchez C. 2018b. A novel biodegradation pathway of the endocrine-disruptor di(2-ethyl hexyl) phthalate by *Pleurotus ostreatus* based on quantum chemical investigation. Ecotoxicology and Environmental Safety. 147: 494-499. https://doi.org/10.1016/j.ecoenv.2017.09.004.

Ahuactzin-Pérez M., Tlecuitl-Beristain S., García-Dávila J., Santacruz-Juárez E., González-Pérez M., Gutiérrez-Ruíz M. C. & Sánchez C. 2018a. Mineralization of high concentrations of the endocrine disruptor dibutyl phthalate by *Fusarium culmorum*. 3Biotech. 8: 42–51. https://doi.org/10.1007/s13205-017-1065-2.

Ahuactzin-Pérez M., Tlecuitl-Beristain S., García-Dávila J., Santacruz-Juárez E., González-Pérez M., Gutiérrez-Ruíz M. C. & Sánchez, C. 2018c. Kinetics and pathway of biodegradation of dibutyl phthalate by *Pleurotus ostreatus*. Fungal Biology. 122(10): 991-997. https://doi.org/10.1016/j.funbio.2018.07.001.

Akpinar M., & Ozturk U. R. 2017. Induction of fungal laccase production under solid state bioprocessing of new agroindustrial waste and its application on dye decolorization. 3 Biotech, 7(2): 98. https://doi.org/10.1007/s13205-017-0742-5.

Ameen F., Moslem M., Hadi S., & Al-Sabri, A. E. 2015. Biodegradation of low density polyethylene (ldpe) by mangrove fungi from the Red Sea Coast. Progress in Rubber Plastics and Recycling Technology, 31(2): 125-143. https://doi.org/10.1177/147776061503100204.

Bouchiat R., Veignie E., Grizard D., Soebert C., Vigier M. & Rafin C. 2015. Ability of filamentous fungi to degrade four emergent water priority pollutants. Desalination and Water Treatment. 57(15): 6740-6746. https://doi.org/10.1080/19443994.2015.1013508.

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. https://doi.org/10.1016/0003-2697(76)90527-3.

Bustamante M., Durán N. & Diez M. C. 2012. Biosurfactants are useful tools for the bioremediation of contaminated soil: a review. Journal of Soil Science and Plant Nutrition. 12(4): 667-687. https://doi.org/10.4067/s0718-95162012005000024.

Cao H., Wang C., Liu H., Jia W. & Sun H. 2020. Enzyme activities during Benzo[a]pyrene degradation by the fungus *Lasiodiplodia theobromae* isolated from a polluted soil. Scientific Reports, 10(1). 865. https://doi.org/10.1038/s41598-020-57692-6.

Chakrabarty A. N., Adhya S. & Pramanik M. K. 1970. The hydrolysis of Tween 80 by Vibrios and Aeromonads. Journal of Applied Bacteriology. 33: 397-402. https://doi.org/10.1111/j.1365-2672.1970.tb02212.x.

Cheng M., Zeng G., Huang D., Yang C., Lai C., Zhang C., & Liu Y. 2017. Advantages and challenges of Tween 80 surfactant-enhanced technologies for the remediation of soils contaminated with hydrophobic organic compounds. Chemical Engineering Journal. 314: 98-113. https://doi.org/10.1016/j.cej.2016.12.135.

Chhaya U. & Gupte A. 2013. Possible role of laccase from *Fusarium incarnatum* UC-14 in bioremediation of Bisphenol A using reverse micelles system. Journal of Hazardous Materials. 254-255: 149-156. https://doi.org/10.1016/j.jhazmat.2013.03.054.

El-Morsy E. M., Hassan H. M. & Ahmed E. 2017. Biodegradative activities of fungal isolates from plastic contaminated soils. Mycosphere 8: 1071-1087. https://doi.org/10.5943/mycosphere/8/8/13.

Elsayed M. A., Hassan M. M., Elshafei A. M., Haroun B. M. & Othman A. M. 2012. Optimization of cultural and nutritional parameters for the production of laccase by *Pleurotus ostreatus* ARC280. British Biotechnology Journal. 2(3): 115-132. https://doi.org/10.9734/BBJ/2012/1305.

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 submerged fermentation. Mexican Journal of Biotechnology. 3(1): 82–94. https://doi.org/10.29267/mxjb.2018.3.1.82.

González-Márquez A., Loera-Corral O., Santacruz-Juarez E., García-Dávila J., Tlecuitl-Beristain S., Viniegra-González G. & Sánchez C. 2019a. Biodegradation patterns of the endocrine disrupting polluntant di (2-ethyl hexyl) phthalate by *Fusarium culmorum*. Ecotoxicology and Environmental Safety. 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. Mexican Journal of Biotechnology. 4(4): 50-64. https://doi.org/10.29267/mxjb.2019.4.4.50.

Krueger M. C., Hofmann U., Moeder M. & Schlosser D. 2015. Potential of wood-rotting fungi to attack polystyrene sulfonate and its depolymerisation by *Gloeophyllum trabeum* via hydroquinone-driven Fenton chemistry. PLoS One. 10: e0131773. https://doi.org/10.1371/journal.pone.0131773.

Leammli U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227(5259): 680-685. https://doi.org/10.1038/227680a0.

Li Q., Lei Y., Hu G., Lei Y. & Dan D. 2018. Effects of Tween 80 on the liquid fermentation of *Lentinus edodes*. Food Science and Biotechnology. 27(4): 1103-1109. https://doi.org/10.1007/s10068-018-0339-8.

Liebminger S., Eberl A., Sousa F., Heumann S., Fischer-Colbrie G., Cavaco-Paulo A. & Guebitz G. M., 2007. Hydrolysis of PET and bis-(benzoyloxyethyl) terephthalate with a new polyesterase from *Penicillium citrinum*. Biocatalysis and Biotransformation 25, 171–177. https://doi.org/10.1080/10242420701379734.

Liu Y. Y., Xu J. H. & Hu Y. 2000. Enhancing effect of Tween 80 on lipase performance in enantioselective hydrolysis of ketoprofen ester. Journal of Molecular Catalysis B: Enzymatic, 10(5): 523-529. https://doi.org/10.1016/s1381-1177(00)00093-x.

Minami K. 1958. Isolation of a Soil Coccus capable of utilizing ‘Tween 80’ as a sole source of carbon. Nature 181: 430-431. https://doi.org/10.1038/181430b0.

Muff J., MacKinnon L., Durant N. D., Bennedsen L. F., Rügge K., Bondgaard M. & Pennell K. D. 2020. Solubility and reactivity of surfactant-enhanced alkaline hydrolysis of organophosphorus pesticide DNAPL. Environmental Science and Pollution Research. 7(3): 3428-3439. https://doi.org/10.1007/s11356-019-07152-0.

Nguyen N. T., Tran T. N. & Ha T. B. N. 2018. Characterization of *Acinetobacter baumannii* strain PS3 in degradation of food emulsifiers. In: Proceedings of the 2nd

International Conference on Applied Sciences (ICAS-2) AIP Conf. Proc. 1954: 030013-1–030013-4. https://doi.org/10.1063/1.5033393.

Nielsen C. K., Kjems J., Mygind T., Snabe T. & Meyer R. L. 2016. Effects of Tween 80 on growth and biofilm formation in laboratory media. Frontiers in Microbiology. 7: 1878. https://doi.org/10.3389/fmicb.2016.01878.

Okagbue R. N., Mwenje E., Kudanga T., Siwela M. & Sibanda T. 2001. Isolation of *Aureobasidium pullulans* from Zimbabwean sources and glucosidase activities of selected isolates. South African Journal of Botany, 67(2): 157-160. https://doi.org/10.1016/s0254-6299(15)31114-5.

Paço A., Duarte K., da Costa J. P., Santos P. S. M., Pereira R., Pereira M. E., Freitas A. C., Duarte A. C. & Rocha-Santos T. A. P. 2017. Biodegradation of polyethylene microplastics by the marine fungus *Zalerion maritimum*. Science of the Total Environment. 586: 10-15. https://doi.org/10.1016/j.scitotenv.2017.02.017.

Ronkvist A. S. M., Xie W., Lu W. & Gross R. A. 2009. Cutinase-catalyzed hydrolysis of poly(ethylene terephthalate). Macromolecules. 42: 5128-5138. https://doi.org/10.1021/ma9005318.

Russell J. R., Huang J., Anand P., Kucera K., Sandoval A. G., Dantzler K. W., Hickman D., Jee J., Kimovec F. M., Koppstein D., Marks D. H., Mittermiller P. A., Núñez S. J., Santiago M., Townes M. A., Vishnevetsky M., Williams N. E., Vargas M. P., Boulanger L. A., Bascom-Slack C. & Strobel S. A. 2011. Biodegradation of polyester polyurethane by endophytic fungi. Applied and Environmental Microbiology. 77, 6076-6084. https://doi.org/10.1128/aem.00521-11.

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

Teodoro T. L., Oliveira F., Poffo C., Braga L. P., Arbigaus A., Rampinelli J. R., Wisbeck E., Bonatti-Chaves M. & Furlan S. A. 2018.The influence of Tween 80 on laccase production by *Pleurotus sajor-caju* and the efficiency of crude enzyme broth in the removal of bisphenol-A. Arquivos do Instituto Biológico. 85: 1-10, e1022017. https://doi.org/10.1590/1808-1657001022017.

Tomioka H. 1983. Purification and characterization of the tween-hydrolyzing esterase of *Mycobacterium smegmatis*. Journal of Bacteriology. 155(3): 1249-1259. https://doi.org/10.1128/JB.155.3.1249-1259.1983.