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Original article (Orijinal araştırma)

Purification and characterization of an esterase from larval *Diplolepis fructuum* (Rübsaamen, 1895) (Hymenoptera: Cynipidae)[1]

Larva dönemindeki *Diplolepis fructuum* (Rübsaamen, 1895) (Hymenoptera: Cynipidae)'dan bir esterazın saflaştırılması ve karakterizasyonu

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Abstract

Diplolepis fructuum (Rübsaamen, 1895) (Hymenoptera: Cynipidae) is one of the important insect species that causes damages on Rosaceae species. With this study commenced in 2018 at the laboratory of Department of Biochemistry, Faculty of Science, Cumhuriyet University to get a biochemical data, an esterase (EC 3.1.1.X) from the larvae of *D. fructuum* was purified using Q Sepharose anion exchange, phenyl Sepharose CL-4B and Sephacryl S100 HR gel filtration chromatography, respectively. The enzyme had 6.94 U/mg protein specific activity, about 29-fold purity, and 8.8% yield. Only one activity band was observed in native-PAGE studies. The molecular weight of the esterase was estimated as 60 kDa using native-PAGE and SDS-PAGE techniques. By the kinetic data, optimum temperature and pH for the enzyme was determined as 40ºC and 9.0, respectively. The enzyme was stable for 4 h at 40ºC and pH 8.0. K_m and V_{max} values were found to be 0.035 mM and 1.41 µmol/mL.min., using 4-nitrophenyl butyrate (p-NPB) as substrate. The enzyme exhibited its highest activities on p-NPB (100%) and 4-nitrophenyl acetate (52%). All of these data indicate that the enzyme might be a typical esterase with different kinetic properties and molecular weight than esterolytic enzymes reported from other insect species.

Keywords: Column chromatography, *Diplolepis fructuum*, esterase, larvae, purification

Öz

Diplolepis fructuum (Rübsaamen, 1895) (Hymenoptera: Cynipidae) Rosaceae türlerinde zararlara yol açan en önemli böcek türlerinden birisidir. Biyokimyasal veri elde etmek için Cumhuriyet Üniversitesi, Fen Fakültesi, Biyokimya Anabilim Dalı laboratuvarında 2018 yılında başlatılan bu çalışma ile *D. fructuum*'un larvasından bir esteraz (EC 3.1.1.X) Q Sefaroz anyon değişim, fenil Sefaroz CL-4B ve Sefakril S 100 HR jel filtrasyon kromatografisini kullanarak saflaştırılmıştır. Enzim 6.94 U/mg protein spesifik aktivite, yaklaşık 29 kat saflık ve %8.80 verime sahipti. Nativ-PAGE çalışmalarında sadece bir aktivite bandı gözlenmiştir. Nativ-PAGE ve SDS-PAGE tekniklerini kullanarak, esterazın molekül kütlesi yaklaşık olarak 60 kDa olarak tahmin edilmiştir. Kinetik datadan, enzimin optimum sıcaklık ve pH'ı sırasıyla 40ºC ve 9.0 olarak belirlenmiştir. Enzim, 40ºC ve pH 8.0'da 4 saat kararlıydı. 4 nitrofenil butirat (p-NPB) substrat olarak kullanılarak, K_m ve V_{max} değerlerinin 0.035 mM and 1.41 µmol/mL.dk olduğu bulunmuştur. Enzim en yüksek aktivitesini p-NPB (%100) ve 4-nitrofenil asetat (%52) üzerinde sergilemiştir. Tüm bu veriler, enzimin diğer böcek türlerinden bildirilen esterolitik enzimlerden farklı kinetik özellik ve molekül kütlesi ile klasik bir esteraz olabileceğini göstermektedir.

Anahtar sözcükler: Kolon kromatografisi, *Diplolepis fructuum*, esteraz, larva, saflaştırma

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Introduction

Arthropods have the highest number of individuals on the earth (Ødegaard, 2000; Canavaso et al., 2001). In addition to this phenomenon, insect-derived diseases are also showing important increases worldwide. For this reason, there are great efforts to control the size of the insect populations showing vector or pest features. Control programs, such as the use of insect growth regulators, depend on the use of the chemical insecticides (Montella et al., 2012). However, repeated applications of the insecticides have led to resistant-insect populations (Shin & Smartt, 2016).

Glutathione-S-transferases, cytochrome P450 monooxygenases, and esterases, especially carboxylesterases, are the important enzymes which have important roles in the metabolic resistance against insecticides (Li et al., 2007). It appears that a common mechanism of the resistance is increased or reduced levels of these enzymes, depending on single or multiple mutations within their genes (Li et al., 2007; Gong et al., 2017).

Carboxylesterases (CarEs) are involved in both in the detoxification processes of the harmful exogenous compounds and in the metabolism of compounds having physiological importance in the metabolism in insects (Ma et al., 2018) and other organisms (Satoh et al., 2002; Satoh, 2005). For this reason, esterases (EC 3.1.1.X) have been given considerable attention (Montella et al., 2012), which was reviewed by Nauen (2007) and Li et al. (2007) due to their roles in insecticide resistance that are develops during pest or vector-control programs. Also, using the inhibition criteria of the insecticide applied is a reliable experimental method to classify esterases. For example, three kinds of inhibitors organophosphates, eserine sulfate and sulfydryl reagent, are used to inhibit carboxylesterase, cholinesterase and arylesterase activities, respectively. The acetylesterases, the fourth class of esterases, are not affected by these chemicals (Dahan-Moss & Koekemoer, 2016). There are available studies on the contribution of esterases from different insect species, such as *Oryzaephilus surinamensis* (Linnaeus, 1758) (Coleoptera: Silvanidae) (Rossiter et al., 2001), *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae) (Yaicharoen et al., 2005), *Aphis gossypii* (Glover, 1877) (Hemiptera: Aphididae) (Tabasian et al., 2010), *Dendrolimus superans* (Buttler, 1877) (Lepidoptera: Lasiocampidae) (Zou et al., 2014), *Anopheles funestus* (Giles, 1900) (Diptera: Culicidae) (Dahan-Moss & Koekemoer, 2016) and *Apis cerana cerana* (Fabricius, 1793) (Hymenoptera: Apidae) (Ma et al., 2018). However, many of these studies have been focused on the esterolytic activity assays without performing an esterase purification study.

Diplolepis fructuum is a member of Cynipidae family containing about 1400 insect species (Ronquist, 1999; Katılmış & Kıyak, 2009). This insect species is capable of making abnormal growths (galls) in the tissues of the plants such as *Rosa canina* (Linnaeus, 1753) from the family Rosaceae, resulting in damage to produce (Lotfalizadeh et al., 2009; Raman, 2011; Akpınar et al., 2017). The level of enzyme expression might be related to the age and life stage of the insects, and this is an important factor to consider during efforts to control harmful insect populations (Dahan-Moss & Koekemoer, 2016). In this context, no studies have been found on the purification of the esterase of *D. fructuum*'s. For this reason, for the first time in this study, an esterase fraction from the larvae of *D. fructuum,* a holometabol insect species, was purified and kinetically characterized to understand its biochemical function.

Materials and Methods

Larvae samples

Diplolepis fructuum (Rübsaamen, 1895) (Hymenoptera: Cynipidae) were collected from the galls on the plant *Rosa canina* L. from different localities in Sivas Province, Turkey between November 2012 and November 2013. Gall samples were also taken to the laboratory and kept in glass bottles. Embryological periods were observed and the larvae samples were obtained by observing the successive embryological periods of the insect. The larvae obtained were preserved at -80ºC until used (Akpınar et al., 2017). From these larvae samples, purification of esterase was attempted using chromatographic techniques in the laboratory as explained below.

Preparation of enzyme extract and purification

The preparation of enzyme extract was performed according to Görgün & Akpınar (2012) with slight modification. The larvae samples (3 g) were homogenized in buffer (buffer A; 50 mM Tris-HCl, pH= 7.4, 1 mM DTT, 1 mM Na4EDTA, 5 mM D-Mannit) with a Wise Tis homogenizer on ice for 5 min at 22,000 rpm. The resulting homogenate was clarified by centrifuging at 10,000 g for 15 min at 4ºC with Sanyo MSE MS 60 ultracentrifuge. The supernatant was obtained and the pellet was re-homogenized in homogenate buffer and then re-centrifuged. The supernatants from both centrifuge steps were combined for purification.

Chromatographic procedures were performed according to Görgün & Akpınar (2012) with modifications. Q Sepharose fast flow column chromatography was the first chromatographic step in the purification studies. The column material was suspended in a column (1 x 20 cm) and ethanol was removed by washing with distilled water. After this procedure, the column was equilibrated with 20 mM Tris-HCl at pH 7.80 (buffer B). The sample was applied into the column and washed with two column volumes of buffer B to elute unbounded fractions. Then, the bound protein fractions were eluted from the column by washing with 0.1, 0.2, 0.4 and 1 M NaCl series of buffer B with a peristaltic pump. The tubes showing esterase activity were combined and concentrated using a Millipore ultra-centrifugal filter unit (MWCO 10 kDa). The concentrated protein fraction was applied into phenyl Sepharose CL-4B hydrophobic interaction column (1 x 20 cm) that was equilibrated with 20 mM Tris-HCl buffer at pH 7.80 containing 0.1 M ammonium sulfate (buffer C). To obtain unbound protein fractions, the column was washed with two column volumes of buffer C then two column volumes of buffer B. The retained proteins in the hydrophobic interaction column were eluted with 40 mL of 10, 20 and 50% isopropanol series in buffer B. The last chromatographic step was Sephacryl S 100 HR gel filtration chromatography (1 x 30 cm) that is equilibrated with buffer B. Activity tubes concentrated from the previous chromatographic step were introduced into the column and elution tubes were collected until protein absorbance reached zero at 280 nm in the spectrophotometer.

Esterase and protein assay

Esterase activity measurements were performed according to Bülow & Mosbach (1987) using 4-nitro phenyl butyrate (p-NPB) as substrate at 405 nm against blank tube in a double beam spectrophotometer. The sample tube consisted of 10 µL of sample, 20 µL of 50 mM p-NPB dissolved in acetonitrile and 970 µL of activity buffer (pH 8.0 Tris-HCl with 4% ethanol). The blank tube contained 980 µL of activity buffer and 20 µL of p-NPB. During chromatographic steps, protein amounts from the elution tubes were recorded at 280 nm absorbance in 1 mL cuvettes. The method of Bollag et al. (1996) was used in the determination of protein amounts of the purification steps, using BSA (bovine serum albumin) as a standard. Every measurement consisted of three repeats.

Kinetic characterization

The method of Görgün & Akpınar (2012) with some modifications was used to obtain the kinetic data. The effects of temperature (between 4 to 60ºC) and pH (from 5.7 to 10) were assessed under standard activity assay conditions by incubating the enzyme solution for 15 min in related parameters. The effect of substrate concentration was evaluated at nine different concentrations between 0.025 and 1.25 mM of p-NPB, using constant amount of enzyme. Substrate chain length were evaluated using 4-nitrophenyl acetate (p-NPA), 4-nitrophenyl butyrate (p-NPB), 4-nitrophenyl dodecanoate (p-NPD) and 4-nitrophenyl palmitate (p-NPP). The stability of the enzyme was also assayed at 40ºC and pH 8.0 between 1 to 4 h. All studies were repeated three times under the standard activity measurements by changing the regarding parameters. All the data obtained were tested statistically using SPSS 11.0 for windows (Görgün & Zengin, 2015). One-way analysis of variance was used to analyze the repeated experiments (mean±SE). Differences between means were evaluated with Tukey's test at 0.05 significance level.

Electrophoretic studies

Native-PAGE studies without using SDS were applied according to Görgün & Zengin (2015). The equal amounts of samples from different purification fractions were loaded onto 10% native gels consisting of only stacking gel. Electrophoresis was performed at a constant 100 mA in Tris-glycine buffer (pH 8.3, 0.025 M Tris and 0.192 M glycine) for 80 min under a cooling system. To detect the esterase bands in the samples, the gels were stained with 1 naphthyl acetate. Later the same gels were stained with Coomassie Brilliant Blue and then silver staining method (Bollag et al., 1996) to follow the progress of different purification stages. Denature SDS-PAGE studies were also conducted on the samples under the conditions mentioned for the native-PAGE studies.

Results and Discussion

The summary of the purification of esterase from the larval stage of *Diplolepis fructuum* can be seen from Table 1. Sequences of Q Sepharose anion exchange, phenyl Sepharose CL-4B and Sephacryl S100 HR gel filtration chromatography were conducted to purify an esterase fraction from the larval stage of *D. fructuum*. These data are presented in Figure 1. A specific activity of 0.246 U/mg protein was found in the homogenate. Using Q Sepharose column, four major protein peaks corresponding to two esterase activity peaks were detected. There was no esterase activity in the unbound protein fraction by the washing with buffer B. Also, elution tubes with 0.1 M NaCl did not show any esterase activities. However, the tubes that have the highest esterase activities were found to be between tubes 22 and 27, obtained by washing with 0.2 M NaCl (in buffer B). The tubes 32 and 33 had minor esterase activities and these fractions have been ignored due to their very little specific activities. This chromatographic step provided about a 6-fold purification and 60% yield with a specific activity of 1.45 U/mg protein. Using DEAE-cellulose anion exchange chromatography, Fahmy et al. (2004) found six esterase forms (from E1 to E6) corresponding to six protein peaks by eluting with sequential NaCl concentrations between 0 and 1 M during the embryogenesis of *Hyalomma dromedarii* (Koch, 1844) (Acari: Ixodidae). Among these bands, E3 that has the highest esterase activity was eluted with 0.2 M NaCl. The chromatographic result of this step was a 5-fold purification with a yield of 5.23%, showing similarities with our results obtained in the ion exchange chromatography step. After this step, they obtained a 19-fold purification parameter of esterase from *H. dromedarii* by gel filtration (Sepharose 6B) chromatography.

Table 1. Purification steps of esterase from the larval period of *Diplolepis fructuum*

Purification step	Volume (mL)	Total protein (mg) (mean±SE)		Total activity (µmol/mL.min)** (mean±SE)		Specific activity (µmol/mL.min./mg) (mean±SE)		Purification factor	Yield (%)
Homogenate	31	166.61±0.28	a*	40.93±1.03	a	0.246±0.06	a	1.00	100.00
Q Sepharose	20	14.74±2.42	b	24.89±0.63	b	1.450±0.04	b	5.90	60.81
Phenyl Sepharose CL-4B	12	3.72±0.75	b	6.31±0.13	c	1.697±0.04	c	6.91	15.42
Sephacryl S100 HR	2.5	0.52±0.03	b	3.60±0.04	c	6.940±0.08	d	28.30	8.80

* Means are for three repeat experiments. Means followed by the same letter are not significantly different at $P \leq 0.05$.

** One unit of esterase activity is defined as the amount of enzyme that catalyze the release of p-nitrophenol (p-NP) per min under assay conditions explained in material and method section.

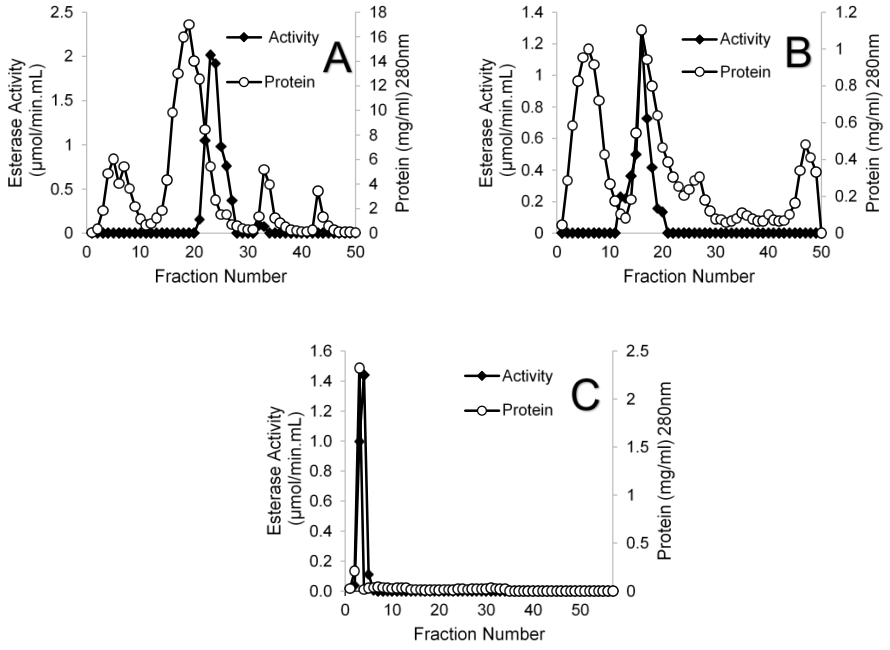

Figure 1. Purification of esterase from *Diplolepis fructuum* larvae by chromatographic series: A) Elution profile of Q-Sepharose of anion exchange chromatography with tubes collected as 4 mL at 3 mL/min flow rate; B) elution profile of phenyl Sepharose CL-4B hydrophobic interaction chromatography with tubes eluted as 4 mL at 2 mL/min flow rate; and C) elution profile of Sephacryl S100-HR gel filtration chromatography with tubes collected as 2.5 mL at 1 mL/min flow rate.

Hydrophobic interaction chromatography has been reported to be useful for the purification of lipolytic enzymes such as lipases and esterases (Bompensieri et al., 1996; Qerioz et al., 2001; Bhardwaj et al., 2001; Görgün & Akpınar, 2012). In the present study, phenyl Sepharose CL-4B column resulted in purification parameters of 1.69 U/mg protein specific activity and about a 7-fold purification of *D. fructuum* larval esterase (Table 1). We obtained three protein peaks corresponding to only one activity peak. These results suggested that this chromatographic step resolved the protein peaks and discharged contaminant proteins, but we had activity losses resulting in a similar specific activity to that of previous chromatographic step (Table 1). In their key study, Arrese & Wells (1994) purified an insect triacylglycerol lipase from the insect fat body of *Manduca sexta* (Linnaeus, 1763) using five different chromatographic steps in which step three was phenyl Sepharose column chromatography, which resulted in a 939-fold purification of the enzyme. Later, the same group reported that this enzyme is an active phospholipase (Arrese et al., 2006).

Sephacryl S100 HR gel filtration chromatography was the last purification step of the present study. Purification parameters of this step were determined as 6.94 U/mg protein specific activity, 8.80% yield, and 28-fold purification (Table 1). In this analysis, the enzyme activity appeared in the first five tubes corresponding to a single protein peak. Despite this finding, electrophoretic data showed that there was a contaminant protein band that lack of esterase activity. The protein content of the purified fraction was 0.52 mg and this contaminant band was also purified with our target enzyme. For this reason, we were not able to get higher purification factor, using Sephacryl S100 chromatography. In the present study, Q Sepharose anion exchange chromatography was able to capture the esterase from the crude homogenate. Hydrophobic interaction chromatography did not show substantial purification factor with the application of the sample obtained from Q Sepharose to phenyl Sepharose CL-4B hydrophobic interaction chromatography. However, this step was important to eliminate contaminant protein bands. The last step, gel filtration chromatography, contained only one protein peak that correspond to one activity peak. However, this protein peak contained two protein bands in denaturing electrophoresis, with one of the protein bands was

contaminant as revealed by native-PAGE studies. There were important statistical differences ($P \leq 0.05$) between the specific activities through the purification process with the chromatographic techniques used in the present study. Purification studies were performed on some insect species, applying the combination of different chromatographic steps, and these studies had both similar and different results to our study. The same sequences of the chromatographic techniques also reported enzyme preparations in different purity. For example, two different researchers dealt with the purification of larval mid gut lipase, using ammonium sulfate precipitation, Sephacryl G-100 gel filtration and DEAE-cellulose anion exchange chromatography. From these studies, a digestive lipase from *Pieris brassicae* (Linnaeus, 1758) (Lepidoptera: Pieridae) larvae was purified with 39.9 U/mg protein specific activity, 18.1% yield and 42-fold purification (Zibaee, 2012), while the second study achieved a 12-fold purification, 8.21% recovery and 5.60 U/mg protein specific activity in *Naranga aenescens* (Moore, 1881) (Lepidoptera: Noctuidae) (Zibaee & Fazeli-Dinan, 2012).

In this study, kinetic data including optimum temperature, optimum pH, K_m and V_{max} values, enzyme stability, effect of the substrate chain length to the enzyme activity were assessed (Figure 2). Optimum temperature and pH were 40ºC and 9.0, respectively. Purified enzyme retained 88% of activity for 4 h at 40ºC and pH 8.0. The relative activities were 100% for p-NPB, 52% for p-PNA, 5% p-NPD, and 1% for p-NPP. This finding suggests that the purified enzyme is more active with short-chain substrates and might be an esterase (Fojan et al., 2000). Using p-NPB as a substrate, K_m and V_{max} values were found to be 0.035 mM and 1.41 µmol/mL.min respectively. The data from the present study has similarities and differences from published reports. The differences that are determined might be a result of the tissue investigated or the purified enzymes are a lipase or esterase. For example, lipolytic enzymes from *Ectomyelois ceratoniae* (Zeller, 1839) (Lepidoptera: Pyralidae) (Ranjbar et al., 2015), *Rhynchophorus palmarum* (Linnaeus, 1758) (Santana et al., 2017) and *Chilo suppressalis* (Walker, 1863) (Lepidoptera, Pyralidae) (Zibaee et al., 2008) exhibited optimum temperatures of 30, 37, and 37-40ºC, and optimum pH of 7, 6.5 and 10, respectively. At the same time, Ranjbar et al. (2015) indicated that the purified enzyme was active for 3 h at 30ºC. Regarding with the substrate specificities, Santana et al. (2017) was assessed the substrates ranging from 10 (p-NPD) to 16 (p-NPP) carbon chain length and they found the highest activity in p-NPP, indicating that the enzyme was a lipase. Esterase (E3) from *H. dromedarii* was showing a great affinity for the short-chain substrate (p-NPA) with a K_m value of 1.43 mM (Fahmy et al., 2004). In *P. brassicae*, V_{max} and K_m values were reported as 30.3 U/mg protein and 2.72 mM p-NPB, respectively (Zibaee, 2012).

In the present study, at the end of the purification experiments, native-PAGE studies were conducted to determine esterase activities both in the homogenate and the purification series, using a native substrate (1-naphthyl acetate). Only one band with esterase activity band and a molecular weight between 60-62 kDa was present in all of the samples (Figure 3A). After defining the location of this band using molecular weight markers, the same gels were stained by silver staining to determine the number of the bands. This showed that purified fraction consisted of only two bands in which one of them was a contaminant protein band with a molecular weight of about 80 kDa that lacked esterase activity (Figure 3B). These experiments were repeated using denaturing SDS-PAGE under the same conditions as used for native-PAGE, and the same findings were obtained (Figure 3C). When compared to the literature, reported molecular weights for the lipolytic enzymes from different purification studies in the insects were 76 kDa in *M. sexta* fat body (Arrese & Wells, 1994; Arrese et al., 2006), 45 kDa in *H. dromedarii* larvae (Fahmy et al., 2004), 72.3 kDa in the midgut of *P. brassicae* (Zibaee, 2012), 84.8 kDa in *D. superans* larvae (Zou et al., 2014), and 25 kDa in the middle gut of *E. ceratoniae* (Ranjbar et al., 2015). Esterases are important enzymes in the living systems to digest both endogenous substrates and exogenous xenobiotics. The present study is the first report on the purification and biochemical characterization of an esterase from *D. fructuum* larvae, which is economically important because it induces galls on *R. canina*. The estimated 60 kDa lipolytic enzyme from *D. fructuum* might be an esterase with slight differences in kinetic properties from lipolytic enzymes reported

for other insect species. The literature data given in above together with our findings suggest that purification parameters obtained might depend on factors such as the insect species under investigation, the tissue of insect, growth stage, expression level of esterases, chromatographic techniques and their application sequences. The kinetic assays that were performed on crude homogenates might show differences with the purified fractions. From the present study, we suggest a method to purify an esterase from *D. fructuum* larvae, a damaging pest of *R. canina*. Likewise, further studies should be undertaken on the purification and characterization of esterases from insect species to inform efforts to manage pest or vectors.

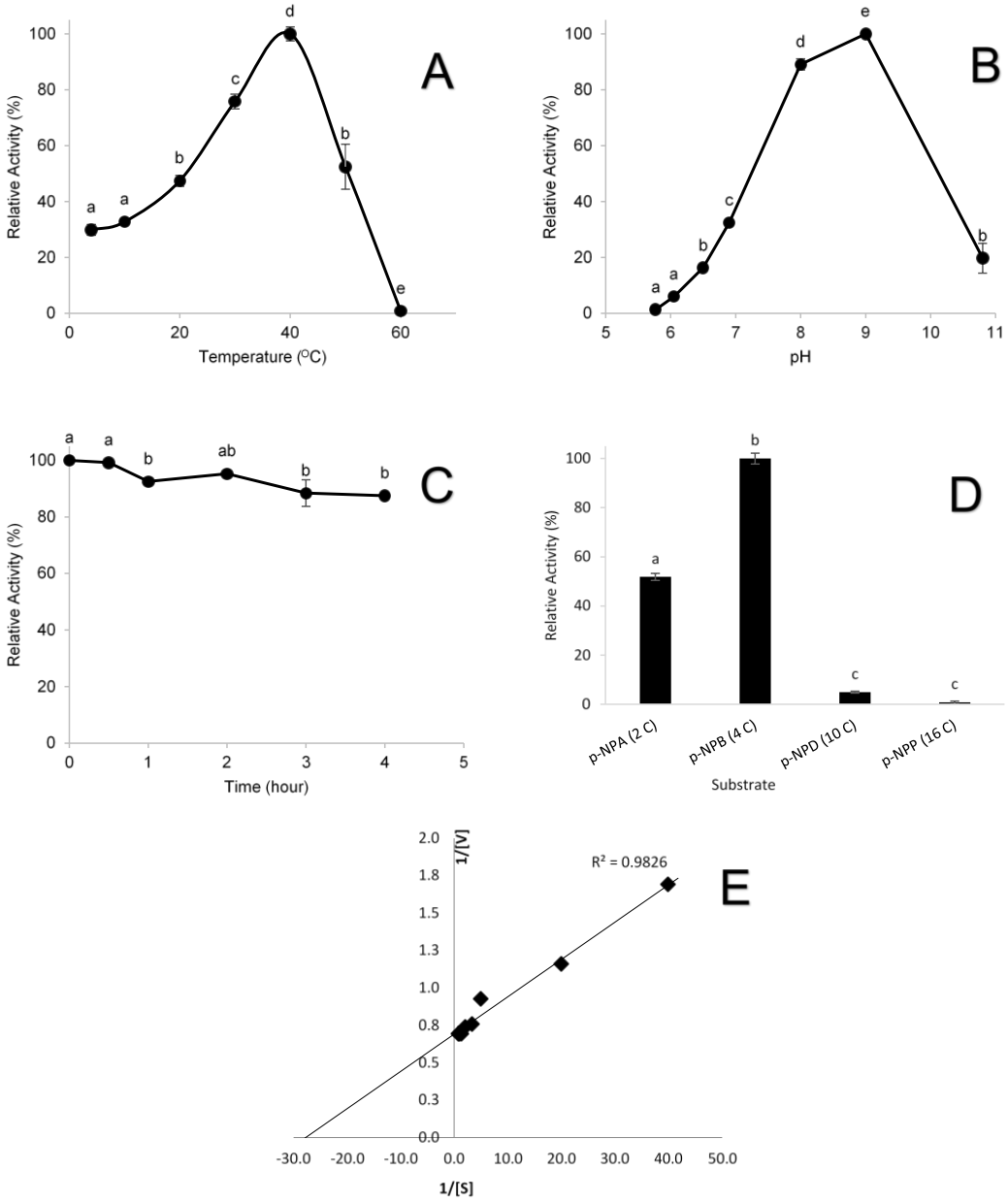

Figure 2. Kinetic characterization of the esterase from *Diplolepis fructuum* larvae: A) Effect of temperature; B) effect of pH; C) stability at 40ºC and pH 8.0; D) effect of substrate chain length; and E) effect of substrate concentration. Values with the same letter are not significantly different at P ≤ 0.05.

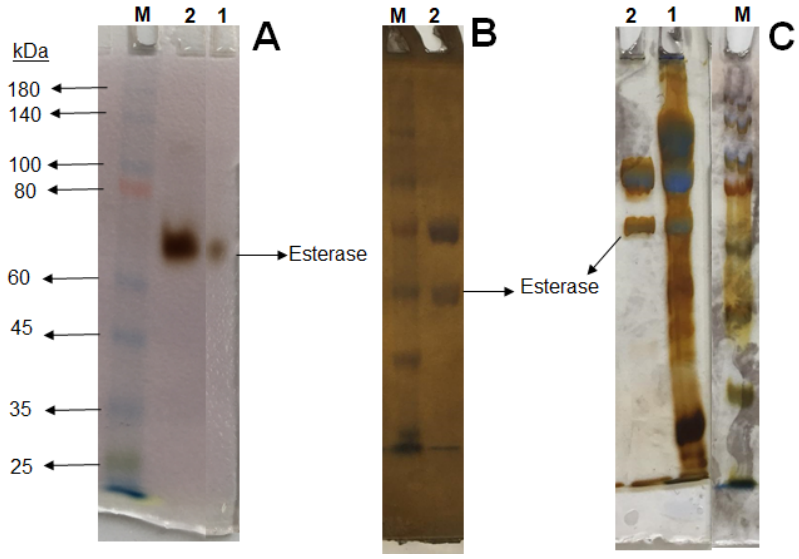

Figure 3. Electrophoretic analyses of the purification steps of esterase from *Diplolepis fructuum* larvae: A) A native-PAGE analysis of homogenate (lane 1) (20 µg protein) and the purified fraction (lane 2) (20 µg protein); B) silver staining of a native-PAGE analysis of the purified fraction (lane 2); and C) SDS-PAGE electrophoretic pattern of homogenate (lane 1) (20 µg protein) and purified fraction (lane 2) (20 µg protein), using coomassie and silver staining. M, molecular weight marker.

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References

Akpınar, M. A., S. Görgün, L. Gençer & A. Aktümsek, 2017. Fatty acid composition of *Diplolepis fructuum* (Rübsaamen, 1895) (Hymenoptera: Cynipidae) during its developmental stages. Journal of the Entomological Research Society, 19 (3): 109-118.

Arrese, E. L. & M. A. Wells, 1994. Purification and properties of a phosphorylatable triacylglycerol lipase from the fat body of an insect, *Manduca sexta*. Journal of Lipid Research, 35: 1652-1660.

Arrese, E. L., R. T. Patel & J. L. Soulages, 2006. The main triglyceride-lipase from the insect fat body is an active phospholipase A1: identification and characterization. Journal of Lipid Research, 47: 2656-2667.

Bhardwaj, K., A. Raju & R. Rajasekharan, 2001. Identification, purification, and characterization of a thermally stable lipase from rice bran. A new member of the (phospho) lipase family. Plant Physiology, 127 (4): 1728-1738.

Bollag, D. M., M. D. Rozycki & S. J. Edelstein, 1996. Protein Methods. A John Wiley and Sons Inc. Publication, 432pp.

Bompensieri, S., R. Gonzalez, R. Kok, M. V. Miranda, I. Nutgeren-Roodzant, K. J. Hellingwerf, O. Cascone & B. C. Nudel, 1996. Purification of lipase from *Acinetobacter calcoaceticus* AAC323-1 by hydrophobic interaction methods. Biotechnology and Applied Biochemistry, 23 (1): 77-81.

Bülow, L. & K. Mosbach, 1987. The expression in *E. coli* of a polymeric gene coding for an esterase mimic catalyzing the hydrolysis of p-nitrophenyl esters. Federation of European Biochemical Societies, 210 (2): 147-152.

Canavaso, L.E., Z. E. Jouni, K. J. Karnas, J. E. Pennington & M. A. Wells, 2001. Fat metabolism in insects. Annual Review of Nutrition, 21: 23-46.

Dahan-Moss, Y. L. & L. L. Koekemoer, 2016. Analysis of esterase enzyme activity in adults of the major malaria vector *Anopheles funestus*. Parasites & Vectors, 9: 110.

Fahmy, A. S., S. S. Abdel-Gany, T. M. Mohamed & S. A. Mohamed, 2004. Esterase and lipase in camel tick *Hyalomma dromedarii* (Acari: Ixodidae) during embryogenesis. Comparative Biochemistry and Physiology Part B, 137: 159-168.

Fojan, P., P. H. Jonson, M. T. N. Petersen & S. B. Petersen, 2000. What distinguishes an esterase from a lipase: A novel structural approach. Biochimie, 82 (11): 1033-1041.

Gong, Y. H., G. M. Ai, M. Li, X. Y. Shi, Q. Y. Diao & X. W. Gao, 2017. Functional characterization of carboxylesterase gene mutations involved in *Aphis gossypii* resistance to organophosphate insecticides. Insect Molecular Biology, 26 (6): 702-714.

Görgün, S. & M. A. Akpınar, 2012. Purification and characterization of lipase from the liver of carp, *Cyprinus carpio* L. (1758), living in Lake Tödürge (Sivas, Türkiye). Turkish Journal of Fisheries and Aquatic Sciences, 12 (2): 207-215.

Görgün, S. & G. Zengin, 2015. Determination of fatty acid profiles and esterase activities in the gills and gonads of *Vimba vimba* (L., 1758). Journal of the American Oil Chemist's Society, 92 (3): 353-360.

Katılmış, Y. & S. Kıyak, 2009. The oak gall wasp *Aphelonyx persica*: a new record from Turkey, with some new host records. Phytoparasitica, 37: 95-97.

Li, X., M. A. Schuler & M. R. Berenbaum, 2007. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annual Review of Entomology, 52: 231-253.

Lotfalizadeh, H., R. Ezzati-Tabrizi & A. Masnadi-Yazdinejad, 2009. *Diplolepis fructuum* (Rübsaamen) (Hym.: Cynipidae) a new host for *Exeristes roborator* (Fabricius) (Hym.: Ichneumonidae) in Iran. Biharean Biologist, 3: 171-173.

Ma, M., H. Jia, X. Cui, N. Zhai, H. Wang & X. Guo, 2018. Isolation of carboxylesterase (esterase FE4) from *Apis cerana cerana* and its role in oxidative resistance during adverse environmental stress. Biochimie, 144: 85-97.

Montella, I. R., R. Schama & D. Valle, 2012. The classification of esterases: an important gene family involved in insecticide resistance-A review. Memorias do Instituto Oswaldo Cruz, Rio *de* Janeiro, 107 (4): 437-449.

Nauen, R., 2007. Insecticide resistance in disease vectors of public health importance. Pest Management Science, 63: 628-633.

Ødegaard, F., 2000. How many species of arthropods? Erwin's estimate revised. Biological Journal of the Linnean Society, 71 (4): 583-597.

Qerioz, J. A., C. T. Tomaz & J. M. S. Cabral, 2001. Hydrophobic interaction chromatography of proteins. Journal of Biotechnology, 87: 143-159.

Raman, A., 2011. Morphogenesis of insect-induced plant galls: facts and questions. Flora, 206: 517-533.

Ranjbar, M., A. Zibaee & J. J. Sendi, 2015. Purification and characterization of a digestive lipase in the midgut of *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae). Frontiers in Life Science, 8 (1): 64-70.

Ronquist, F., 1999. Phylogeny, classification and evolution of the Cynipoidea. Zoologica Scripta, 28: 139-164.

Rossiter, L. C., C. M. Conyers, A. D. MacNicoll & H. A. Rose, 2001. Two qualitatively different B-esterases from two organophosphate-resistant strains of *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) and their roles in fenitrothion and chlorpyrifos-methyl resistance. Pesticide Biochemistry and Physiology, 69: 118-130.

Santana, C. C., L. A. Barbosa, I. D. B. Junior, T. G. do Nascimento, C. B. Dornelas & L. A. M. Grillo, 2017. Lipase activity in the larval midgut of *Rhynchophorus palmarum*: Biochemical characterization and the effects of reducing agents. Insects, 8 (100): 1-7.

Satoh, T., 2005. Toxicological implications of esterases-From molecular structures to functions. Toxicology and Applied Pharmacology, 207: 11-18.

Satoh, T., P. Taylor, W. F. Bosron, S. P. Sanghanı, M. Hosokawa & B. N. La Du, 2002. Current progress on esterases: From molecular structure to function. Drug Metabolism and Disposition, 30 (5): 488-493.

Shin, D. & C. T. Smartt, 2016. Assessment of esterase gene expression as a risk marker for insecticide resistance in Florida *Culex nigripalpus* (Diptera: Culicidae). Journal of Vector Ecology, 41 (1): 63-71.

Tabasian, H., S. Ravan, A. R. Bandani & B. A. Siahsar, 2010. The effect of esterase activity in resistance of *Aphis gossypii* to selective insecticides. Journal of Food, Agriculture & Environment, 8 (3&4): 1108-1112.

Yaicharoen, R., R. Kiatfuengfoo, T. Chareonviriyaphap & P. Rongnoparut, 2005. Characterization of deltamethrin resistance in field populations of *Aedes aegypti* in Thailand. Journal of Vector Ecology, 30 (1): 144-150.

Zibaee, A., 2012. A digestive lipase of *Pieris brassicae* L. (Lepidoptera: Pieridae): purification, characterization, and host plants effects. Archieves of Insect Biochemistry and Physiology, 81 (1): 1-19.

Zibaee, A., A. R. Bandani & S. Ramzi, 2008. Lipase and invertase activities in midgut and salivary glands of *Chilo suppressalis* (Walker) (Lepidoptera, Pyralidae), rice striped stem borer. Invertebrate Survival Journal, 5: 180-189.

Zibaee, A. & M. Fazeli-Dinan, 2012. Purification and characterization of a digestive lipase in rice green semi-looper, *Naranga aenescens* Moore (Lepidoptera: Noctuidae). Signpost Open Access Journal of Entomological Studies, 1: 38-54.

Zou, C.-S., C.-W. Cao, G.-C. Zhang & Z.-Y. Wang, 2014. Purification, characterization, and sensitivity to pesticides of carboxylesterase from *Dendrolimus superans* (Lepidoptera: Lasiocampidae). Journal of Insect Science, 14 (260): 1-6.