

# Purification and characterization of a polygalacturonase from the xylophagous insect *Oncideres albomarginata chamela* (Coleoptera: Cerambycidae)

Alicia Lara-Márquez<sup>1</sup>, Nayeli Soria-Calderón<sup>2</sup>, Maria Guadalupe Villa-Rivera<sup>2</sup>, Everardo López-Romero<sup>1</sup>, Nancy Calderón-Cortés<sup>2\*</sup>

<sup>1</sup>Departamento de Biología, División Ciencias Naturales y Exactas, Campus Guanajuato, Universidad de Guanajuato, Guanajuato, México.

<sup>2</sup>Laboratorio de Ecología Molecular, ENES Unidad Morelia, Universidad Nacional Autónoma de México, Morelia, Mich., México.

## ARTICLE INFO

### Article history:

Received on: October 25, 2021

Accepted on: January 30, 2022

Available online: June 01, 2022

### Key words:

Pectinases,  
Endopolygalacturonase,  
Xylophagous insects,  
Enzyme purification,  
Industrial applications

## ABSTRACT

In this work we purified a polygalacturonase enzyme from the midgut of larvae of the xylophagous insect *Oncideres albomarginata chamela*. The polygalacturonase showed high enzymatic activity (390.7 U/mg) in the crude extract and was purified to apparent homogeneity by means of cation exchange chromatography, hydrophobic interaction chromatography, and gel filtration. The molecular mass of the polygalacturonase was estimated to be 37 kDa by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The enzyme had an optimum pH of 6.0 and an optimum temperature of 50°C. According to the kinetic studies on polygalacturonic acid, the polygalacturonase showed a  $K_m$  of 3.18 mg/ml and  $V_{max}$  of 716.15 U/mg. The enzymatic properties of the purified enzyme correspond to those reported for highly active commercially produced enzymes, highlighting the potential of this insect enzyme to be used in industrial applications.

## 1. INTRODUCTION

Pectin represents an important component of the cell wall of plants, particularly in the middle lamella, it forms a matrix, in which, cellulose microfibrils and hemicellulose are embedded during cell expansion in the early stages of growth [1,2]. Pectin also participates in cell development and structure, morphogenesis, adhesion, porosity, defense, and among others [3,4].

Pectin is a very diverse polysaccharide composed mainly of galacturonic acid residues (GalA) that form different structural domains: homogalacturonan (HG), apioagalacturonan, xylogalacturonan, and rhamnogalacturonan I and II (RGI and RGII, respectively) [2,3]. The HG consists of a backbone of D-GalA residues linked by  $\alpha$ -1,4 glycosidic bonds, some of which can be modified by methylation and/or acetylation, while the other polysaccharides that form pectin are more complex. The RGII is formed by an HG backbone of at least eight 1,4-linked  $\alpha$ -D-GalA residues with multiple branches composed by 12 different types of carbohydrates, making RGII the most complex polysaccharide of pectin, and despite representing a small

part of the pectin (~ 10%), the RGII is important for plant growth and development [3,5]. These polysaccharides are covalently bound and interact with calcium, borate, polyamines, and phenolic compounds and can establish interactions with other cell wall polymers such as hemicellulose, cellulose, and lignin [6].

The enzymes that degrade pectin can be classified into three groups according to their cleavage modes: glycoside hydrolases (GH) (polygalacturonases [PGs], EC 3.2.1.15 and 3.2.1.67), polysaccharide lyases (pectate lyase EC 4.2.2.9, 4.2.2.10 and pectin lyase EC 4.2.2.2), and carbohydrate esterases (CE) (pectin methylesterases, EC 3.1.1.11) [7,8]. In addition to these enzymes that degrade the main structure of pectin, there are other enzymes that participate in the degradation of the structural domains RGI and RGII, and various enzymes considered as accessories that degrade their side chains [7,8].

PGs cleave  $\alpha$ -1,4-D-galacturonic acid linkages by hydrolysis at the HG backbone of pectin. These enzymes are found into GH family 28 (<http://www.cazy.org/>) [9], and they are classified as exo-PGs (EC 3.2.1.67) and endo-PGs (EC 3.2.1.15). The exo-PGs act on the non-reducing end of pectin, while the endo-PGs randomly hydrolyze the inner  $\alpha$ -1,4-D-galacturonic acid linkages [7].

PGs are of great importance in different industrial applications such as wine production, fruit juice clarification, retting and degumming, coffee and tea fermentation, oil extractions, among others [7,10].

\*Corresponding Author:

Nancy Calderón-Cortés, Laboratorio de Ecología Molecular,  
ENES Unidad Morelia, National Autonomous University of Mexico,  
Morelia, Mich., México.  
E-mail: [ncalderon@enesmorelia.unam.mx](mailto:ncalderon@enesmorelia.unam.mx)

Indeed, pectinases comprise 25% of the enzymes that are produced for biotechnological purposes, including food and beverage industries [11]. Hence, there is a growing interest to know the biochemical properties of these enzymes for their use in industrial applications. For example, for PGs, the optimal pH at which the enzymes are active is decisive to define its possible application such as retting of natural fibers and fruit juice clarification. [7].

PGs have been studied extensively in plants, yeasts, fungi, and bacteria [11-14]. However, these enzymes are also present in some insects herbivores [15-18]. Interestingly, PG is one of digestive enzymes with the highest hydrolytic activity in some insect such as herbivore beetles [18-20], which make them key targets for bioprospecting new enzymes with biotechnological potential.

In this study, we purified a polygalacturonase enzyme from the midgut of a xylophagous insect, and characterize some of its enzymatic properties. We used larvae from the borer beetle *Oncideres albomarginata chamela* (Coleoptera: Cerambycidae) given that a previous study indicated that larvae of this species have the ability to digest structural plant cell wall polymers [21]. The studies of the biochemical properties of purified enzymes from insects are needed for prospecting the potential industrial applications.

## 2. MATERIALS AND METHODS

### 2.1. Preparation of Enzymatic Crude Extracts

*O. albomarginata chamela* larvae were collected from branches of the *Spondias purpurea* tree in the Chamela-Cuixmala Biosphere Reserve located in Jalisco (19°30'N and 105°03'W). The larvae were kept at -80°C and thawed on ice to perform the midgut dissections used to prepare the enzyme extracts. The midgut was homogenized with sterile pestle in sodium phosphate buffer (100 mM, pH 6.5) containing complete TM protease inhibitor cocktail (Roche). The homogenate was centrifuged (10,000 × G for 10 min at 4°C), cell debris were discarded, and then the supernatant was filtered in Amicon® Ultra 15 ml centrifugal filter devices with a molecular weight cutoff of 10 kDa (MilliporeSigma) (14,000 × G for 30 min at 4°C) with sodium phosphate buffer (100 mM, pH 6.5). The recovered crude extract was kept at 4°C or stored at -80°C with 10% glycerol.

### 2.2. Measurement of Protein Concentrations

Protein concentrations were determined with Quick Start Bradford Protein Assays (Bio-Rad) by the Bradford method [22]. The standard curve was performed in microassay using the Bovine Serum Albumin Standard Set (Bio-Rad) following the manufacturer's instructions. The absorbance at 595 nm was determined using a Multiskan GO microplate spectrophotometer (Thermo Scientific).

### 2.3. Polygalacturonase Activity Assay

The enzymatic activity was determined by the 3,5-dinitrosalicylic acid (DNS) colorimetric method [23]. The reactions (100 µl of total volume) consisted of 25 µl of sample in the appropriate dilution on a sodium phosphate buffer (100 mM, pH 6) and 75 µl of the substrate (polygalacturonic acid [PGA]) at a concentration of 0.5% (w/v). The reactions were incubated for 20 min at 37°C and stopped by adding 100 µl of DNS followed by an incubation at 90°C for 15 min. Samples were read on a Multiskan GO microplate spectrophotometer (Thermo Scientific) at 540 nm. We used 10 µg of galacturonic acid as a positive control and a sample of crude extract denatured at 100°C during

10 min as a negative control. All reactions were done by triplicate. One unit (U) of pectinase activity was defined as the amount of enzyme producing 1 µmol of reducing sugar per minute; while the specific activity was defined as U/mg protein.

### 2.4. Purification Procedure

For cation exchange chromatography, we used a Unosphere S 1 ml column (Bio-Rad) on the automated NGC quest-plus (Bio-Rad) system. The column was equilibrated with sodium acetate buffer (50 mM, pH 4.5) and the crude extract was applied in the same buffer. Unbound protein was removed with sodium acetate buffer (50 mM, pH 4.5) and the elution of proteins was made by applying a linear gradient of NaCl (0–1 M). In total, 19 ml of crude extract was applied in several rounds of purification on the column. The concentration of proteins in the fractions was measured at 280 nm on the chromatograph, and the activity was determined on microplates using 5 µl of the fraction and 75 µl of 0.5% (w/v) of the substrate PGA in a final volume of 100 µl with sodium phosphate buffer (100 mM, pH 6). The selected fractions were concentrated and changed to the appropriate buffer using Amicon® Ultra 15 ml and 0.5 ml protein filters of 10 kDa molecular weight exclusion (MilliporeSigma), centrifuged at 14,000 × G and 4°C for 30 min.

Hydrophobic-interaction chromatography was carried out using a HiTrap Phenyl FF (high sub, 1 ml) column (GE Healthcare) equilibrated with sodium phosphate buffer (50 mM, pH 6.5) and ammonium sulfate (1 M). Protein elution was performed applying a linear decreasing gradient from 1 to 0 M in sodium phosphate buffer (50 mM, pH 6.5).

Gel filtration chromatography was performed using a HiLoad 16/600 Superdex 75 pg (GE Healthcare) column equilibrated with phosphate buffer (50 mM, pH 6) including NaCl (150 mM) at a flow rate of 1 ml/min. Protein elution was performed with the same buffer and conditions.

### 2.5. Gel Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using mini-protean tris/glycine 4–15% gels (Bio-Rad). Samples were mixed with 2X Laemmli loading buffer (Bio-Rad) and incubated at 100°C during 5 min. Precision Plus Protein Marker (Bio-Rad) and Oriole Fluorescent Gel Stain (Bio-Rad) for protein staining were used; gels were analyzed in ultraviolet light at 254 nm.

For activity analysis by zymogram, samples in duplicate were separated on a 12% SDS-PAGE, staining only one part of the gel with Coomassie Brilliant Blue G-250 (Bio-Rad), leaving the remaining part of the gel unstained to be washed once with 1% Triton X-100 at 4°C for 20 min and twice with 100 mM sodium phosphate buffer (pH 6.5) at 4°C during 30 min each. The gels were covered with a 5% polyacrylamide gel in 100 mM sodium phosphate buffer (pH 6.5) and 1% PGA (w/v), and incubated for 30 min at 37°C. Finally, the gel was stained with ruthenium red 0.05% to reveal the enzymatic activity.

### 2.6. Biochemical Characterization

The optimal temperature and pH as well as the effect of chemicals and ions on enzymatic activity were determined in 100 µl reactions containing 0.5% (w/v) of PGA and sodium acetate buffer (100 mM, pH 6) according to the DNS method as mentioned above. All reactions were prepared by triplicate and incubated at 37°C. The behavior of polygalacturonase during the degradation of PGA as a

substrate in relation to incubation time was analyzed by measuring the enzymatic activity at different times: 0, 5, 10, 15, 20, 25, 30, 60, 80, 100, and 120 min, whilst for the optimal pH tests, the activity of polygalacturonase was analyzed at different pH: pH 4 and 5 (sodium acetate buffer 100 mM), pH 6 and 7 (phosphate buffer 100 mM), and pH 8 and 9 (Tris buffer 100 mM). The reactions were incubated at 37°C for 60 min.

To test the optimal temperature, the polygalacturonase was incubated with 0.5% (w/v) of PGA in phosphate buffer (100 mM, pH 6) at several temperatures (20°C, 30°C, 37°C, 40°C, 50°C, 60°C, and 80°C) for 60 min. The effect of chemicals and ions was determined by analyzing the activity using different concentrations (0, 1, 4, and 10 mM) of  $Mg^{2+}$ ,  $Mn^{2+}$ ,  $Ca^{2+}$ , and EDTA in phosphate buffer (100 mM, pH 6). The metal ions were added as chloride salts. The incubation of reactions was performed for 60 min at 50°C, stopped with DNS and determined as mentioned above.

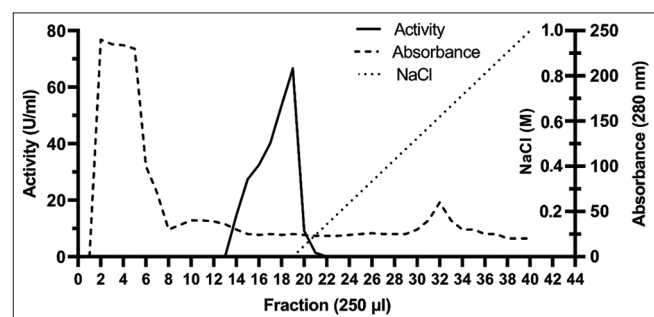
## 2.7. Determination of Kinetic Parameters

PGA at different concentrations (1–6 mg/ml) in 100  $\mu$ l reactions with phosphate buffer (100 mM, pH 6) was used for determining the kinetic parameters of the purified enzyme. Reactions were incubated for 60 min at 50°C, and the specific activity of each concentration was estimated by the DNS method as mentioned above. Data analysis and graphics were performed using the software Hyperbolic Regression Analysis of Enzyme Kinetic Data (Hyper32 v1.0.0.) and GraphPad Prism (GraphPad Software, v.9.2.0 for windows, San Diego, California USA).

## 3. RESULTS

To evaluate the pectinase degradation system of the xylophagous insect *O. albomarginata chamela*, the enzymatic activity was determined in the crude extract of the larvae midgut using PGA as substrate. Pectinase activity showed a specific activity of 390.7 U/mg on PGA.

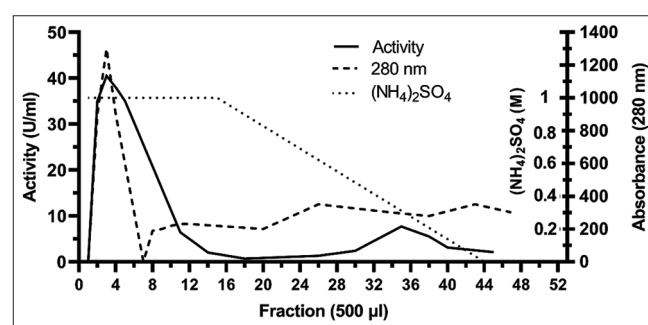
For purification, the crude extract was subjected to cation exchange chromatography [Figure 1], which allowed the removal of contaminants and unwanted proteins from the extract. The most active fractions on PGA (15–19) were subjected to a column of hydrophobic interactions, and in this case, the activity was detected in the fractions (1–12) that did not interact with the matrix [Figure 2]. This purification



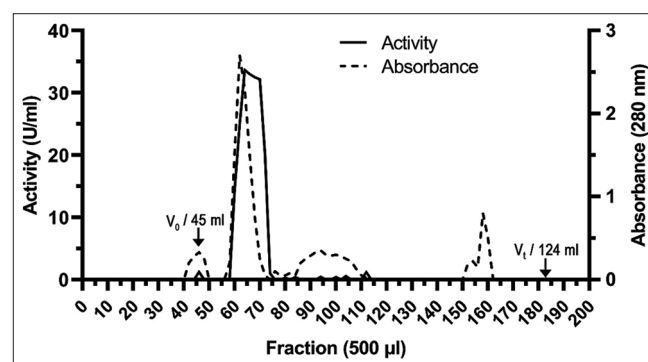
**Figure 1:** Cation exchange chromatography of polygalacturonase from *Oncideres albomarginata chamela*. Crude extract (2ml) from larval midgut was applied to a (8 × 20 mm) Unosphere S column equilibrated with 50 mM sodium acetate buffer (pH 4.5). The elution of the sample was made applying a linear gradient of 0.0–1.0 M NaCl and collecting fractions of 250  $\mu$ l. Most active fractions on PGA (15–19) were pooled for the next step. A total of 19 ml of crude extract were processed in several rounds of purification.

step improved the specific activity by 2.5 fold than that determined in the crude extract [Table 1] and allowed the removal of proteins with cellulase and xylanase activities (data not shown). Finally, the recovered proteins were separated by molecular exclusion on a Superdex 75 pg column. The activity analysis on PGA of the fractions indicates that the active fractions (67–70) correspond to a well-defined protein peak [Figure 3]. The purified polygalacturonase presented a specific activity of 1268 U/mg, which represents 3.2 fold of the specific activity determined in the crude extract and a recovery percentage of 1.2% [Table 1]. According to the analysis by SDS-PAGE, the purified polygalacturonase has a molecular mass of 37 kDa [Figure 4], and as shown in the zymogram, the enzyme is active on PGA.

The activity of the *O. albomarginata chamela* polygalacturonase was detected from 5 min of incubation, and after 60 min, the highest activity was obtained [Figure 5a]. The purified enzyme is active in a range of pH 4.5–6.5, with the greatest activity at pH 6; the enzyme retains approximately 60% of the activity at pH 5, whereas the activity declines completely at pH 7 [Figure 5b]. The polygalacturonase



**Figure 2:** Hydrophobic-interaction chromatography of polygalacturonase from *Oncideres albomarginata chamela*. Active fractions separated by cation exchange chromatography were applied to a column (8 × 20 mm) of HiTrap Phenyl FF equilibrated with sodium phosphate buffer (50 mM, pH 6.5) containing ammonium sulfate (1 M). A linear decreasing gradient of 1.0–0.0 M ammonium sulfate in the same buffer was used for sample elution, collecting 0.5 ml fractions. The fractions with the greatest activity on PGA (1–12) were pooled for the next step.



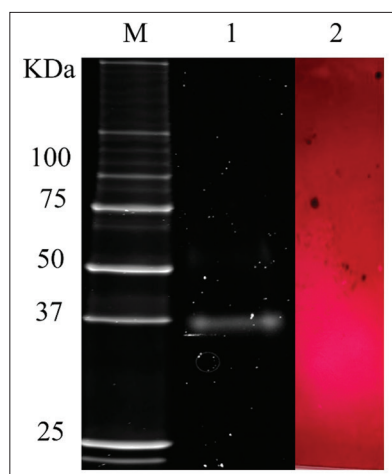
**Figure 3:** Gel filtration chromatography of polygalacturonase from *Oncideres albomarginata chamela*. The enzyme fraction obtained from the hydrophobic-interaction step was applied on top of a column (60 cm × 26 mm) of Superdex 75 pg HiLoad 16/600 equilibrated and eluted with phosphate buffer (50 mM, pH 6) containing NaCl (150 mM). Elution of the sample was made with the same buffer, collecting fractions of 0.5 ml. Fractions 67–70 showing the maximum activity on PGA were pooled and labeled as the purified enzyme.



**Table 1:** Purification of polygalacturonase from the midgut of larvae of *Oncideres albomarginata chamela*

Fraction	Protein (mg)	Total activity (U)	Specific activity (U/mg)	Recovery (%) <sup>a</sup>	Purification (n-fold) <sup>b</sup>
Crude extract	25.8	10021.5	390.7	100	
Cation exchange UNOsphere S	2.1	433.6	207.7	4.3	0.5
Hydrophobic interaction HiTrap Phenyl FF	0.2	202.5	1012.5	2.0	2.6
Gel filtration HiLoad 16/600	0.1	126.8	1268	1.2	3.2

<sup>a</sup>(Total activity stage/total activity first stage)×100. <sup>b</sup>Stage specific activity/First stage specific activity



**Figure 4:** SDS-PAGE of the polygalacturonase purified from the digestive tract (midgut) of the xylophagous beetle *Oncideres albomarginata chamela* stained with Oriole Fluorescent Gel Stain (Bio Rad). M, Precision Plus Protein Unstained Standard (Bio Rad); Lane 1, Purified polygalacturonase; Lane 2, Zymogram on acrylamide gel containing 1% PGA stained with ruthenium red

activity increases from 30°C, with the greatest activity at 50°C, that rapidly decreases at 60°C [Figure 6a].

Regarding metal ions effect on enzymatic activity, the results indicated that  $Mg^{2+}$  decreases the activity of the purified polygalacturonase from 1 mM; the activity slowly decays as its concentration increases. The enzyme was rapidly inhibited by  $Mn^{2+}$  and  $Ca^{2+}$ . EDTA had a negative effect on activity similar to that of  $Mg^{2+}$  [Figure 6b]. According to the Lineweaver-Burk plot, the purified enzyme has  $K_m$  and  $V_{max}$  values of 3.18 mg/ml and 716.15 U/mg, respectively, using PGA as substrate [Figure 7].

#### 4. DISCUSSION

In this work, a high pectinase activity was detected in the midgut extract of *O. albomarginata chamela* larvae (390.7 U/mg). This phenomenon was found previously in the xylophagus insect *Psacothia hilaris*, where the highest activity detected in the larva extract and adult gut corresponds to the pectinolytic activity [24].

The presence of genes encoding PGs of the glycosyl hydrolase family 28 (GHF28), such as endopolygalacturonases has been reported for several herbivorous and xylophagous insects belonging to the orders *Coleoptera*, *Phasmatodea*, and *Hemiptera* [16,17,25]. Among these, the genes encoding GHF28 endopolygalacturonases are highly expressed in the gut tissues of beetles including the species *Anoplophora glabripennis*, *Rhynchophorus ferrugineus*, *Sitophilus oryzae*, *Phaedon cochleariae* [17,26-28], and the

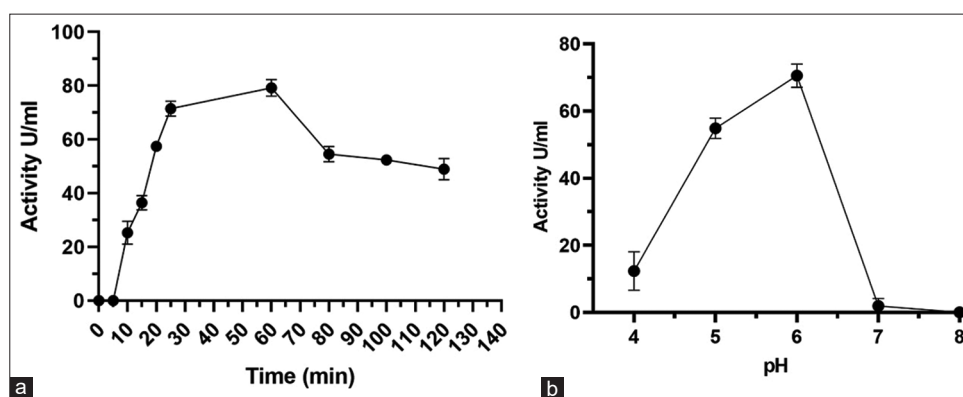
guts of stick and leaf insects *Extatosoma tiaratum*, *Aretaon asperimus*, *Medauroidea extradentata*, *Ramulus artemis*, *Sipylodea sipylus*, and *Peruphasma schultei* [29]; whereas in hemipterans, their salivary glands express GHF28 endopolygalacturonases which are injected during piercing into the plant tissues, probably for softening the plant tissue before oviposition [30,31].

The presence of a diverse family of genes encoding PGs GH28 in chewing beetles and phasmids, their distribution and their phylogenetic analyzes suggest that these insects acquired GH28 PGs by different gene horizontal transfer and genetic duplication events, with subsequent functional diversification that could be important for the evolution of herbivory in these insects [20,26]. For most of beetles, it has been suggested that pectinases are involved in the initial process of degradation of plant cell walls needed for digesting grains and young tissues [20,30,32,33]. In the case of larvae that feed on wood, it is likely that although pectin represents a small portion of the biopolymers of the cell wall, its degradation increases the contact surface by releasing the cells, allowing the access of other digestive enzymes to different cell wall components, as well as to additional nutrients present in the intracellular contents [34,35].

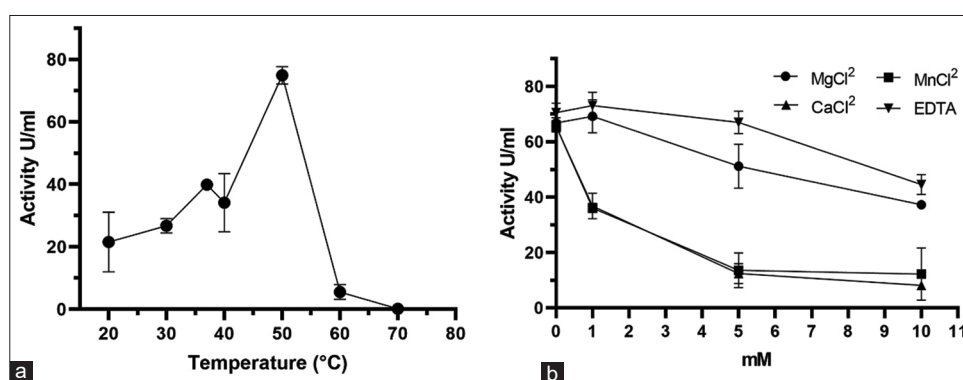
In addition to facilitating the plant cell wall degradation process by other enzymes, the high levels of polygalacturonase expression in insect larvae that feed on plant cell walls suggest that pectin can be used as a source of sugars [17,26], since the treatment of cell walls with endopolygalacturonases purified from other microorganisms in addition to release galacturonic acid (GalA), also released galactose, arabinose, rhamnose, and xylose that form part of pectin [36]. However, there is no evidence about the use of GalA by insects as a source of energy [30].

Due to the great diversity of herbivorous insects, their lifestyles, and the number of genes of plant cell wall degrading enzymes in their genomes, insects have been proposed as valuable sources of enzymes for biotechnological applications [17,37]. In particular, there is a great interest in the discovery of pectinolytic enzymes that can be used in biotechnological applications, since these enzymes are among the most demanded globally, mainly in applications related to food and beverages [11].

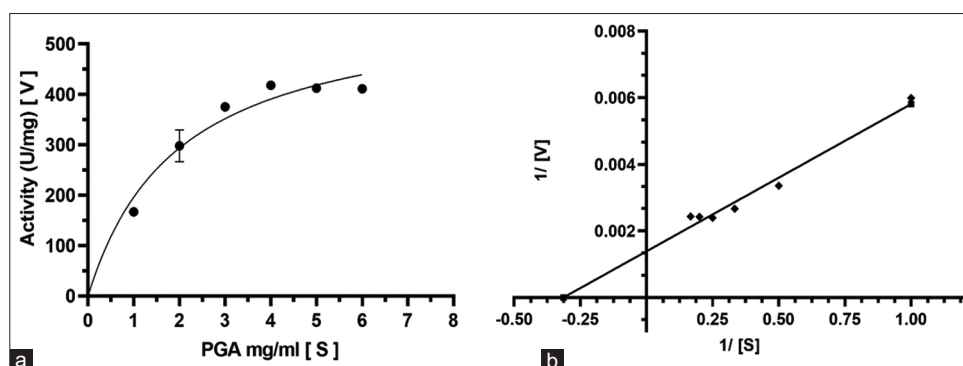
Besides the enzyme production system, other important factors for determining whether an enzyme can be used in biotechnological applications are the enzyme biochemical properties and how they can be adapted to production processes. Previously, it was mentioned that insects are a good source of enzymes for biotechnological purposes; however, there are few reports of purified and/or characterized pectinases. The first purified PGs correspond to those purified from the coleopterans *S. oryzae* and *Diaprepes abbreviatus* [38,39]. In hemipterans, PGs have been partially purified from the salivary glands [30,40], whereas in beetles, two coding sequences for *Apriona japonica* and one for *Sphenophorus levis* have been cloned in



**Figure 5:** Effect of pH and incubation time on PG activity. (a) Standard reactions were incubated for 37°C in 100 mM sodium phosphate buffer at pH 6.5 after the indicated incubation times (0–120 min). (b) Reaction mixtures were incubated at 37°C for 60 min at the indicated pH values with different buffers (sodium acetate for pH 4–5, phosphate for pH 6–7, and Tris for pH 8–9). All other components of the reaction mixture remained unchanged



**Figure 6:** Effect of temperature and divalent cations and EDTA on PG activity. (a) Reaction mixtures were incubated at the indicated temperatures. After 60 min, activity was determined. (b) Enzyme activity was measured for 60 min at 50°C at increasing concentrations of the indicated cations and EDTA



**Figure 7:** Determination of kinetic of PG purified from midgut of *Oncideres albomarginata chamela*. (a) Enzyme activity was measured at 50°C for 60 min as a function of increasing concentrations of the PG as substrate. (b) Lineweaver-Burk plot of data from (a). Results shown are the average from three independent experiments

heterologous expression systems [25,41]. In the latter case, the authors propose that polygalacturonase together with a pectin methylesterase, both produced in *Pichia pastoris* have good potential for industrial applications [41]. According to this, the purification and analysis of some biochemical properties of a polygalacturonase of the xylophagous beetle *O. albomarginata chamela* provide new information useful to identify the best industrial applications for insect enzymes.

Based on the analysis by SDS-PAGE, the polygalacturonase from *O. albomarginata chamela* has a molecular weight (37 kDa), which

corresponds to the molecular weight reported for most microbial PGs (30–80 kDa) [42] and its very similar to the PGs reported for the coleopterans *S. oryzae* and *D. abbreviatus* (38 and 44.5 kDa, respectively) [38,39]. Furthermore, like most PGs, it is a monomeric protein, except for some reports from fungi and plants where they are found as homodimers or heterodimers [43,44].

The purified PG from *O. albomarginata chamela* was inhibited by Ca<sup>2+</sup> and Mn<sup>2+</sup> ions, but it kept more than 50% of activity at high concentrations of Mg<sup>2+</sup> ions and EDTA. The effect of metal ions and

chemicals such as EDTA on purified PGs from bacteria and fungi is highly variable, showing inhibition for some enzymes and no effects in others [45-48]. For coleopterans, the metal ions effect on pectinolytic activity has only been determined for the *R. ferrugineus* crude extract, indicating that  $Mg^{2+}$ ,  $Na^{+}$ , and  $Ca^{2+}$  ions increased pectinolytic activity of this insect species [49]. However, the results of this study are not directly comparable, since no purification of the enzyme was done for *R. ferrugineus*, but they suggest that insect enzymes might exhibit different inhibition properties as reported for microorganisms. Regarding the inhibitory effect of divalent ions such as  $Ca^{2+}$ ,  $Mn^{2+}$ , and  $Mg^{2+}$ , it has been suggested that strong cations can alter the stability of the enzyme [50,51]. An alternative explanation for the inhibition by these ions is the cross-linking of HG chains by  $Ca^{2+}$  and  $Mg^{2+}$  cations, which can limit the amount of substrate available to the enzyme [52].

The optimal pH for the purified PG in this work was 6.0. The previous studies reported that PGs purified from other insects have an optimal pH between pH 4–6 [38,39,41]. This is a more restricted range compared to the range of optimal pH of PGs reported for fungi and bacteria (pH 2–10) (<https://www.brenda-enzymes.org/>), although it is considered that most microbial PGs are active between pH 3.5 and 6.0 [53]. In the case of temperature, the *O. albomarginata chamela* PG is active in a range of 30–50°C, which places it among mesophilic enzymes, like most fungal enzymes [42,54].

Despite the fact that the extremophilic enzymes have attracted much interest to be used in some applications that require stable enzymes at high temperatures [55] or at low temperatures such as wine production [56], most of the pectinases used in biotechnological applications are mesophilic [55]. Among these, acidic enzymes are commonly used for the production of juices, specifically for enzymatic clarification or depectination, which is one of the main applications of pectinolytic enzymes and the largest market for this type of enzyme [57].

Applications such as the production of paper and bioscouring of cotton commonly use alkaline pectinases from fungi or bacteria, according to the chemical processes and conditions needed for these applications [58]. However, in the case of cotton bioscouring, good results have been reported using acidic or neutral pectinases, with additional ecological benefits [59]. In other applications such as fiber processing [60] and biofuels [35], it has been seen that pectinases can significantly participate in the cell wall disassembly, thus finding new sources of highly active enzymes and studying their biochemical characteristics are essential.

The purified polygalacturonase from *O. albomarginata chamela* showed a  $K_m$  value of 3.18 mg/ml. In insects, the  $K_m$  values with PGA as substrate of *Sphenophorus levis* and *D. abbreviatus* are 2.4 and 3.68 mg/ml, respectively [39,41], while the majority of the PGs of microbial origin are found in the range of 0.059–5.0 mg/ml [50,53]. Low  $K_m$  values and high activity are desirable characteristics for use in biotechnological applications [61]. The specific activity of purified PG in this work (1268 U/mg) are higher than that reported for PGs purified from insects [38,39,41] and comparable with the specific activity of PGs of several species of fungi that are commonly used in the development of biotechnological applications [53] (<https://www.brenda-enzymes.org/>). Therefore, according to our results, the polygalacturonase from *O. albomarginata chamela* may be a good candidate for heterologous expression in a friendlier system, allowing the production and purification of the enzyme that can subsequently be tested in important biotechnological applications of the food industry, such as juice production.

## 5. CONCLUSION

Our results demonstrate that the borer beetle *O. albomarginata chamela*, a xylophagous insect whose larvae can digest the polymers of the cell wall of branches of the *S. purpurea* tree, secretes polygalacturonase enzymes that hydrolyze the pectin present in the tissue consumed by the larvae and possibly participate in plant cell wall disassembly, increasing the access to other cell wall and intracellular nutrients. The polygalacturonase purified from the beetle digestive tract is a protein of 37 kDa, with an optimum pH of 6.0 and maximum activity at 50°C, which showed high specific activity (1268 U/mg) and a relatively low  $K_m$  value (3.18 mg/ml). These enzymatic properties suggest that the PG of *O. albomarginata chamela* is a good candidate for its expression in a heterologous expression system to be produced, purified, and tested for different biotechnological applications, particularly those applications at mesophilic conditions.

## 6. ACKNOWLEDGMENTS

This study was supported by CONACyT grant CB-2015-253420 and DGAPA-PAPIIT-UNAM grant IN217420 to NCC. The authors thank to CONACyT for postdoctoral fellowship program 2018 (1) granted to ALM, and to Chamela Biological Station of UNAM for the support to collect biological samples.

## 7. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the present journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

## 8. FUNDING

This study was supported by CONACyT grant CB-2015-253420 and DGAPA-PAPIIT-UNAM grant IN217420 to NCC.

## 9. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

## 10. ETHICAL APPROVALS

Not applicable.

## 11. DATA AVAILABILITY

All data supporting this study are available on request.

## 12. PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

## REFERENCES

1. Cosgrove DJ. Growth of the plant cell wall. *Nat Rev Mol Cell Biol* 2005;6:850-61.
2. Zhang D, Zhang B. Pectin drives cell wall morphogenesis without

- turgor pressure. Trends Plant Sci 2020;25:719-22.
3. Mohnen D. Pectin structure and biosynthesis. Curr Opin Plant Biol 2008;11:266-77.
  4. Voragen AG, Coenen GJ, Verhoef RP, Schols HA. Pectin, a versatile polysaccharide present in plant cell walls. Struct Chem 2009;20:263-75.
  5. Harholt J, Suttangkakul A, Scheller HV. Biosynthesis of pectin. Plant Physiol 2010;153:384-95.
  6. Popper ZA, Fry SC. Xyloglucan-pectin linkages are formed intraprotoplasmically, contribute to wall-assembly, and remain stable in the cell wall. Planta 2008;227:781-94.
  7. Anand G, Yadav S, Gupta R, Yadav D. Pectinases: From microbes to industries. In: Chowdhary P, Raj A, Verma D, Akhter Y, editors. Microorganisms for Sustainable Environment and Healthcare. Amsterdam, Netherlands: Elsevier; 2020. p. 287-313.
  8. de Vries RP, Visser J. Aspergillus enzymes involved in degradation of plant cell wall polysaccharides. Microbiol Mol Biol Rev 2001;65:497-522.
  9. Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B. The carbohydrate-active enzymes database (CAZy) in 2013. Nucl Acids Res 2014;42:D490-5.
  10. Zavala-Páramo MG, Villa-Rivera MG, Lara-Márquez A, López-Romero E, Cano-Camacho H. Applications of fungal pectinases. Encycl Mycol 2021; 2:316-25.
  11. Amin F, Bhatti HN, Bilal M. Recent advances in the production strategies of microbial pectinases-A review. Int J Biol Macromol 2019;122:1017-26.
  12. Niture SK. Comparative biochemical and structural characterizations of fungal polygalacturonases. Biologia 2008;63:1-19.
  13. Chaudhri A, Suneetha V. Microbially derived pectinases: A review. IOSR J Pharm Biol Sci. 2012;2:1-5.
  14. Garg G, Singh A, Kaur A, Singh R, Kaur J, Mahajan R. Microbial pectinases: An ecofriendly tool of nature for industries. 3 Biotech 2016;6:47.
  15. Scully ED, Geib SM, Hoover K, Tien M, Tringe SG, Barry KW, *et al.* Metagenomic profiling reveals lignocellulose degrading system in a microbial community associated with a wood-feeding beetle. PLoS One 2013;8:e73827.
  16. Kirsch R, Kunert G, Vogel H, Pauchet Y. Pectin digestion in herbivorous beetles: Impact of pseudoenzymes exceeds that of their active counterparts. Front Physiol 2019;10:685.
  17. McKenna DD, Scully ED, Pauchet Y, Hoover K, Kirsch R, Geib SM, *et al.* Genome of the Asian longhorned beetle (*Anoplophora glabripennis*), a globally significant invasive species, reveals key functional and evolutionary innovations at the beetle-plant interface. Genome Biol 2016;17:1-18.
  18. Shelomi M, Heckel DG, Pauchet Y. Ancestral gene duplication enabled the evolution of multifunctional cellulases in stick insects (Phasmatodea). Insect Biochem Mol Biol 2016;71:1-11.
  19. Scrivener AM, Watanabe H, Noda H. Properties of digestive carbohydrase activities secreted by two cockroaches, *Panesthia cribrata* and *Periplaneta americana*. Comp Biochem Physiol 1998;119:273-82.
  20. Kirsch R, Gramzow L, Theißen G, Siegfried BD, Ffrench-Constant RH, Heckel DG, *et al.* Horizontal gene transfer and functional diversification of plant cell wall degrading polygalacturonases: Key events in the evolution of herbivory in beetles. Insect Biochem Mol Biol 2014;52:33-50.
  21. Calderón-Cortés N, Watanabe H, Cano-Camacho H, Zavala-Páramo G, Quesada M. cDNA cloning, homology modelling and evolutionary insights into novel endogenous cellulases of the borer beetle *Oncideres albomarginata chamela* (Cerambycidae). Insect Mol Biol 2010;19:323-36.
  22. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-54.
  23. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 1959;31:426-8.
  24. Scrivener AM, Watanabe H, Noda H. Diet and carbohydrate digestion in the yellow-spotted longicorn beetle *Psacothoe hilaris*. J Insect Physiol 1997;43:1039-52.
  25. Pauchet Y, Kirsch R, Giraud S, Vogel H, Heckel DG. Identification and characterization of plant cell wall degrading enzymes from three glycoside hydrolase families in the cerambycid beetle *Apriona japonica*. Insect Biochem Mol Biol 2014;49:1-13.
  26. Antony B, Johny J, Aldosari SA, Abdelazim MM. Identification and expression profiling of novel plant cell wall degrading enzymes from a destructive pest of palm trees, *Rhynchophorus ferrugineus*. Insect Mol Biol 2017;26:469-84.
  27. Kirsch R, Heckel DG, Pauchet Y. How the rice weevil breaks down the pectin network: Enzymatic synergism and sub-functionalization. Insect Biochem Mol Biol 2016;71:72-82.
  28. Kirsch R, Wielsch N, Vogel H, Svatoš A, Heckel DG, Pauchet Y. Combining proteomics and transcriptome sequencing to identify active plant-cell-wall-degrading enzymes in a leaf beetle. BMC Genomics 2012;13:587.
  29. Shelomi M, Danchin EG, Heckel D, Wipfler B, Bradler S, Zhou X, *et al.* Horizontal gene transfer of pectinases from bacteria preceded the diversification of stick and leaf insects. Sci Rep 2016;6:26388.
  30. Shackel KA, Celorio-Mancera MD, Ahmadi H, Greve LC, Teuber LR, Backus EA, *et al.* Micro-injection of lygus salivary gland proteins to simulate feeding damage in alfalfa and cotton flowers. Arch Insect Biochem Physiol 2005;58:69-83.
  31. Celorio-Mancera MP, Allen ML, Powell AL, Ahmadi H, Salemi MR, Phinney BS, *et al.* Polygalacturonase causes lygus-like damage on plants: Cloning and identification of western tarnished plant bug (*Lygus hesperus*) polygalacturonases secreted during feeding. Arthropod Plant Interact 2008;2:215-25.
  32. Cherqui A, Tjallingii WF. Salivary proteins of aphids, a pilot study on identification, separation and immunolocalisation. J Insect Physiol 2000;46:1177-86.
  33. Calderón-Cortés N, Quesada M, Watanabe H, Cano-Camacho H, Oyama K. Endogenous plant cell wall digestion: A key mechanism in insect evolution. Annu Rev Ecol Evol Syst 2012;43:45-71.
  34. Pakarinen A, Zhang J, Brock T, Majjala P, Viikari L. Enzymatic accessibility of fiber hemp is enhanced by enzymatic or chemical removal of pectin. Bioresour Technol 2012;107:275-81.
  35. Latarullo MB, Tavares EQ, Maldonado GP, Leite DC, Buckeridge MS. Pectins, endopolygalacturonases, and bioenergy. Front Plant Sci 2016;7:1401.
  36. Yarullina LG, Akhatova AR, Kasimova RI. Hydrolytic enzymes and their proteinaceous inhibitors in regulation of plant-pathogen interactions. Russ J Plant Physiol 2016;63:193-203.
  37. Pauchet Y, Wilkinson P, Chauhan R, Ffrench-Constant RH. Diversity of beetle genes encoding novel plant cell wall degrading enzymes. PLoS One 2010;5:e15635.
  38. Shen Z, Reese JC, Reeck GR. Purification and characterization of polygalacturonase from the rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae). Insect Biochem Mol Biol 1996;26:427-33.
  39. Doostdar H, McCollum TG, Mayer RT. Purification and characterization of an endo-polygalacturonase from the gut of west indies sugarcane rootstalk borer weevil (*Diaprepes abbreviatus* L.) larvae. Comp Biochem Physiol 1997;118:861-67.
  40. Celorio-Mancera MP, Greve LC, Teuber LR, Labavitch JM. Identification of endo-and exo-polygalacturonase activity in *Lygus hesperus* (knight) salivary glands. Arch Insect Biochem Physiol 2009;70:122-35.
  41. Habrylo O, Evangelista DE, Castilho PV, Pelloux J, Henrique-Silva F.



- The pectinases from *Sphenophorus levis*: Potential for biotechnological applications. *Int J Biol Macromol* 2018;112:499-508.
42. Sharma N, Rathore M, Sharma M. Microbial pectinase: Sources, characterization and applications. *Rev Environ Sci Biotechnol* 2013;12:45-60.
  43. Kant S, Vohra A, Gupta R. Purification and physicochemical properties of polygalacturonase from *Aspergillus niger* MTCC 3323. *Protein Expr Purif* 2013;87:11-6.
  44. Singh P, Dwivedi UN. Purification and characterisation of multiple forms of polygalacturonase from mango (*Mangifera indica* cv. Dashehari) fruit. *Food Chem* 2008;111:345-9.
  45. Khan MM, Choi YS, Kim YK, Yoo JC. Immobilization of an alkaline endopolygalacturonase purified from *Bacillus paralicheniformis* exhibits bioscouring of cotton fabrics. *Bioprocess Biosyst Eng* 2018;41:1425-36.
  46. Yu P, Zhang Y, Gu D. Production optimization of a heat-tolerant alkaline pectinase from *Bacillus subtilis* ZGL14 and its purification and characterization. *Bioengineered* 2017;8:613-23.
  47. Gomes E, Leite RS, da Silva R, Silva D. Purification of an exopolygalacturonase from *Penicillium viridicatum* RFC3 produced in submerged fermentation. *Int J Microbiol* 2009;2009:631942.
  48. Anand G, Yadav S, Yadav D. Purification and characterization of polygalacturonase from *Aspergillus fumigatus* MTCC 2584 and elucidating its application in retting of *Crotalaria juncea* fiber. *3 Biotech* 2016;6:201.
  49. Vatanparast M, Hosseiniaveh V, Ghadamyari M, Sajjadian SM. Plant cell wall degrading enzymes, pectinase and cellulase, in the digestive system of the red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). *Plant Prot Sci* 2014;50:190-8.
  50. Anand G, Yadav S, Yadav D. Production, purification and biochemical characterization of an exo-polygalacturonase from *Aspergillus niger* MTCC 478 suitable for clarification of orange juice. *3 Biotech* 2017;7:122.
  51. Zeng J, Gao X, Dai Z, Tang B, Tang XF. Effects of metal ions on stability and activity of hyperthermophilic pyrolysin and further stabilization of this enzyme by modification of a Ca<sup>2+</sup>-binding site. *Appl Environ Microbiol* 2014;80:2763-72.
  52. Okonji RE, Itakorode BO. Purification and biochemical characterization of pectinase produced by *Aspergillus fumigatus* isolated from soil of decomposing plant materials. *J Appl Biol Biotechnol* 2019;7:1-8.
  53. Jayani RS, Saxena S, Gupta R. Microbial pectinolytic enzymes: A review. *Process Biochem* 2005;40:2931-44.
  54. Hassan B, Ali S. A review on biotechnological impact of pectinases in industries. *J Sci Res Pharm Chem Biol Sci* 2016;1:1-16.
  55. John J, Kaimal KK, Smith ML, Rahman PK, Chellam PV. Advances in upstream and downstream strategies of pectinase bioprocessing: A review. *Int J Biol Macromol* 2020;162:1086-99.
  56. Carrasco M, Rozas JM, Alcaíno J, Cifuentes V, Baeza M. Pectinase secreted by psychrotolerant fungi: Identification, molecular characterization and heterologous expression of a cold-active polygalacturonase from *Tetracladium* sp. *Microb Cell Fact* 2019;18:45.
  57. Li Y, Wang Y, Tu T, Zhang D, Ma R, You S, *et al.* Two acidic, thermophilic GH28 polygalacturonases from *Talaromyces leycettanus* JCM 12802 with application potentials for grape juice clarification. *Food Chem* 2017;237:997-1003.
  58. Rajendran R, Sundaram SK, Radhai R, Rajapriya P. Bioscouring of cotton fabrics using pectinase enzyme its optimization and comparison with conventional scouring process. *Pak J Biol Sci* 2011;14:519-25.
  59. Pušić T, Tarbuk A, Dekanić T. Bio-innovation in cotton fabric scouring-acid and neutral pectinases. *Fibres Text East Eur* 2015;23:98-103.
  60. Nykter M, Kymäläinen HR, Thomsen AB, Lilholt H, Koponen H, Sjöberg AM, *et al.* Effects of thermal and enzymatic treatments and harvesting time on the microbial quality and chemical composition of fibre hemp (*Cannabis sativa* L.). *Biomass Bioenergy* 2008;32:392-9.
  61. Amin F, Mohsin A, Bhatti HN, Bilal M. Production, thermodynamic characterization, and fruit juice quality improvement characteristics of an exo-polygalacturonase from *Penicillium janczewskii*. *Biochim Biophys Acta Proteins Proteom* 2020;1868:140379.

#### How to cite this article:

Lara-Márquez A, Soria-Calderón N, Villa-Rivera MG, López-Romero E, Calderón-Cortés N. Purification and characterization of a polygalacturonase from the xylophagous insect *Oncideres albomarginata chamela* (Cerambycidae: Coleoptera). *J App Biol Biotech.* 2022;10(4):9-16. DOI: 10.7324/JABB.2022.100402