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Araştırma Makalesi / Research Paper

Aspergillus sojae Tarafından Üretilen Poligalakturonazın Kısmi Saflaştırılması için Kromatografik Bir Yaklaşım

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ÖZ

Bu çalışmanın amacı, *A. sojae* mutantından poligalakturonaz üretilmesi ve ham ekstraktın kromatografik yöntemlerle kısmi saflaştırılmasıdır. Peptitlerin konfirmasyonu için ilk basamak olarak, jel içinde sindirilmiş sodyum-dodesil-sülfat-poliakrilamid-jel-elektroforezi (SDS-PAGE) jellerinde matriks-yardımlı lazer desorpsiyon/iyonlaştırmalı-uçuş zamanlı-kütle spektrometresi (Maldi-TOF MS) analizi yapılmıştır. Poligalakturonaz üretimi için, katı-faz ve derin fermentasyonlarda üç farklı karbon kaynağı kullanılmıştır. Ham ekstrakt ilk olarak iyon değişim kromatografisi (IEXC) ile saflaştırılmıştır ve ardından bunu boyut eleme kromatografisi izlemiştir. Derin [acı portakal kabuğu, şeker pancarı melası ve $(NH_4)_2SO_4$] ve katı-faz (buğday kepeği, şeker pancarı ve HCl) fermentasyonlarından elde edilen ham ekstraktlar yüksek seviyede poligalakturonaz enzim aktivitesi (sırasıyla 95.22 and 50.27 U/mL) göstermiştir. IEXC toplanmış fraksiyonunun (180, 200 ve 220 mM tuz fraksiyonları) boyut elemesi, en yüksek verimi (%36) ve saflaştırma katını (2.00) göstermiştir. SDS-PAGE'den elde edilen olası poligalakturonaz bantları jel içinde sindirilmiş ve peptit konfirmasyonu için Maldi-TOF-MS ile analiz edilmiştir.

Anahtar Kelimeler: *Aspergillus sojae*, Poligalakturonaz, Maldi-TOF MS, Boyut eleme kromatografisi, İyon değişim kromatografisi

A Chromatographic Approach for Partial Purification of Polygalacturonase Produced by *Aspergillus sojae*

ABSTRACT

The aim of this study was to produce polygalacturonase from *A. sojae* mutant and partially purify the crude extract by chromatographic methods. As a preliminary step for the confirmation of its peptides, matrix-assisted laser-desorption-ionization-time-of-flight mass spectrometry (Maldi-TOF MS) analysis was performed on in-gel digested sodium-dodecyl-sulphate-polyacrylamide-gel-electrophoresis (SDS-PAGE) gels. Three different carbon sources were employed in submerged and solid-state fermentations for the production of polygalacturonase. Crude extract was first purified by ion-exchange chromatography (IEXC) and followed further by size exclusion chromatography. Crude extracts obtained from sub-merged [of bitter orange peel, sugar beet molasses and $(NH_4)_2SO_4$] and solid-state [of wheat bran, sugar beet and HCl] fermentation exhibited high levels of polygalacturonase enzyme activity (95.22 and 50.27 U/mL, respectively). Size exclusion of IEXC pooled fraction (180, 200 and 220 mM salt fractions) revealed the highest yield (36%) and purification fold (2.00). The likely polygalacturonase bands from SDS-PAGE were in-gel digested and analyzed by Maldi-TOF MS in route for peptides confirmation.

Keywords: *Aspergillus sojae*, Polygalacturonase, Maldi-TOF MS, Size exclusion chromatography, Ion exchange chromatography

INTRODUCTION

Pectin and other pectic substances are complex polysaccharides that are responsible for the firmness of plant tissues [1]. Pectinases are a complex and diverse group of enzymes that degrade pectic substances. They have wide application area in food industry such as clarification and reduction of bitterness of fruit juices, clarification of wine, coffee and tea fermentations, extraction of vegetable oils, curing of coffee and cocoa, refinement of vegetable fibers and manufacture of pectin-free starch [2, 3].

The two groups of pectinases include de-esterification and depolymerizing enzymes depending on the mode of action. Depolymerizing pectinases include hydrolases and lyases which cleave the chain either randomly (endo-) or act on the terminal end (exo-). Among the hydrolases, polygalacturonase (EC 3.2.1.15), also known as pectin depolymerase, (PG) hydrolyses pectic acid into oligo- and monogalacturonates using random (endo-PG) and terminal (exo-PG) modes of action, respectively [3]. The various biological source of commercial PG can be listed as: *Aspergillus*, *Penicillium*, *Erwinia*, *Bacillus*, *Saccharomyces*, *Kluyveromyces*, and *Fusarium*. Among them, *Aspergillus niger* is the main producer due to its GRAS (generally regarded as safe) status [4].

The production of PG have been studied before from various agricultural residues such as wheat and soy bran [5, 6], lemon peel [7], sugar beet [8], corn [9], orange peel and pulps [6, 10]. The two common fermentation techniques to produce commercial pectinase preparations are solid-state fermentation (SSF) and submerged fermentation (SmF) [11]. Production of PG from *A. sojae* using different fermentation media and types has been recently studied elsewhere [12-15]. On the other hand, purification of PG is also important in terms of understanding its properties, structure and functional mechanism as well as eliminating interfering compounds such as melanin like color compounds from the crude extract prior to commercial application. The cost effective purification process requires selection of minimum number of separation steps with high specific activity and purity [16, 17]. The combination of different column chromatographic techniques such as gel filtration, ion exchange or affinity chromatography have been reported for the purification of PG from various fungal and bacterial sources [18-22].

It is also necessary for the purified PG to be confirmed for its purity and identified for its protein and peptide sequences. Gel electrophoresis is a common approach to separate the protein mixtures and estimate the molecular masses of proteins. The coupling of either one or two dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) with mass spectrometry becomes a powerful method for the identification of peptides using database search algorithms [23]. Matrix-assisted laser-desorption-ionization-time-of-flight mass spectrometry (Maldi-TOF MS) has been a rapid, sensitive, accurate and widely used instrument for mass fingerprinting of peptides derived from in-gel digested SDS-PAGE gels [24, 25].

To the best of our knowledge, purification of PG produced from *A. sojae* mutant and identification of its peptides using Maldi-TOF MS have not been reported in the literature. Therefore, the aim of this study was to produce PG from *A. sojae* mutant using different culture media and fermentation techniques and partially purify the crude extract by a combination of ion exchange (IEXC) and size exclusion chromatographic (SEC) methods followed by the Maldi-TOF MS analysis for the in-gel digested SDS-PAGE gels as a preliminary step for confirmation of its peptides. This information in fact can be used as a source for future enzyme engineering. Besides, the purification methods used in this study can be considered as a mean of downstream processing of this particular enzyme.

MATERIALS and METHODS

Reagents and Materials

Bitter orange peel and wheat bran were purchased from a local market in Monterrey, Mexico. Sugar beet molasses was purchased from a local market in Bremen, Germany (Goldsaft, Grafschafter Krautfabrik, Mackenheim, Germany). Polygalacturonic acid (Mr = 25000-50000, No. 81325), D-(+)-galacturonic acid monohydrate (No. 48280-F), were purchased from Sigma-Aldrich GmbH (Schnelldorf, Germany). Endo-polygalacturonidase (E-PGALUSP) enzyme was purchased from Megazyme International Inc. (Wicklow, Ireland). All other chemicals of analytical grade [phosphate buffered saline, sodium acetate, $(NH_4)_2SO_4$, HCl, sodium phosphate, ethanol, acetone, glucose, glycerol, Tween 80, $MnSO_4.H_2O$, $CuSO_4.5H_2O$, peptone, NaCl, KCl, $MgSO_4$, $FeSO_47H_2OKH_2PO_4$, agar powder and malt extract] were from Sigma-Aldrich GmbH (Schnelldorf, Germany), Fluka (Steinheim, Germany) and Applichem (Darmstadt, Germany). Trypsin (T6567, proteomics grade) from porcine pancreas and alpha cyano-4-hydroxycinnamic acid were purchased from Sigma (Schnelldorf, Germany). Iodoacetamide and dithiothreitol (DTT) were purchased from Acros organics (Geel, Belgium) and Applichem (Darmstadt, Germany), respectively.

Instrumentation

A microplate spectrophotometer (Epoch, BioTek Instruments, Inc., USA) was employed in the protein and enzyme activity analyses. On the other hand, for size exclusion chromatography analyses Äkta Explorer 100 (GE Healthcare, United Kingdom) equipment with Superos 10/300 GL chromatographic column was employed. The buffers were PBS and sodium phosphate (10 mM, pH 7.2) with 150 mM of KCl at a flow rate of 0.5 mL/min. Mass spectrometry experiments were performed with an Autoflex III Smartbeam MALDI TOF/TOF (Bruker Daltonics, Bremen, Germany) which used internal MASCOT software (Matrix Science, London, UK). The instrument was operated in positive ion reflectron mode, where the ions were generated

using 337-nm nitrogen laser. For each spectrum, ~3000 laser shots were averaged for best representation of data at a laser frequency of 100 Hz. Spectra acquisition was performed using Flexcontrol 3.0 and FlexAnalysis 3.0 software. A mixture of α-cyano-4-hydroxycinnamic acid (CHCA) in ethanol-acetone (20:80, v/v) was used as the matrix at a concentration of 5 mg/mL. The peptide and fragment ion mass tolerances, which indicate the fit of theoretical mass with the experimental measurement were 200 mg/kg and 0.6 Da, respectively. The charge state was 1+ and mass range of the analytes was set to 700-3500 Da. Uniprot and Swissprot protein sequence databases were employed in the peptide search under all taxonomy. Other database search parameter was carbamidomethylation (C) as fixed global modification.

Propagation and Fermentation

A. sojae ATCC 20235 mutant M3 strain was used in this study [26]. This strain was propagated according to the procedure defined by Mata-Gomez et al. [14]. The spore suspension was employed as inoculum in fermentation processes using three carbon sources: (I) Submerged fermentation using bitter orange peel (10 g/L), sugar beet molasses (60 g/L), and $(NH_4)_2SO_4$ (8 g/L) [27], (II) solid-state fermentation using bitter orange peel (2%), wheat bran (8%) and 7 mL of 50 mM HCl [12], (III) solid-state fermentation using wheat bran (7 g), sugar beet (3 g) and 16 mL of 0.2 N HCl [14]. The fermentation media were inoculated with spore suspensions of 4×10^5 spore/mL, 10^4 spore/mL and 2×10^7 spore/mL and enzymes were recovered following incubation at 30°C/5 days, 22°C/4 days and 30°C/7 days, respectively. All crude extracts were dialysed and concentrated using amicon centrifugal filter tubes with a 3 kDa cut-off size membrane (Merck Millipore Ltd., Ireland) using sodium acetate buffer (pH 5.5). Protein contents and enzyme activities were determined according to the micro-scale protocols of Bradford [28] and Panda et al. [29], respectively. Here the PG activity was based on the enzyme reaction with the polygalacturonic acid as substrate and galacturonic acid as standard. According to this procedure, 243 µL aliquots (200 µL substrate plus 43 µL enzyme sample) of the reaction mixture were incubated for 10 minutes at 40°C and treated afterwards with copper reagent (250 µL) and arsenomolybdate reagent (500 µL), transferred into a microplate, and absorbances were measured at 620 nm. One unit of enzyme activity was defined as the amount of enzyme that catalyzes the release of 1 µmol of galacturonic acid (product) per mL of sample (enzyme) per minute under the assayed conditions. All dilutions were performed with sodium acetate buffer (pH 5.5).

Purification of Polygalacturonase and SDS-PAGE Electrophoresis

The first step of purification included ion exchange chromatography (IEXC) where desalted protein was loaded on a weak basic anion exchanger column (Vivapure diethylamine-D, Sartorius GmBH, Goettingen, Germany) equilibrated with sodium acetate buffer (100 mM, pH 5.5). The elution was performed with 5 mL of NaCl solution in sodium acetate buffer (100 mM, pH 5.5) with a stepwise gradient from 25 to 220 mM (25-50-75-100-120-140-160-180-200-220 mM). Each fraction was analyzed for its protein content and enzyme activity. The purification steps were confirmed using SDS-PAGE electrophoresis procedure developed by Laemmli [30]. Depending on the SDS-PAGE results, the PG active IEXC fractions that exhibit similar protein bands were pooled (as mentioned in Results & Discussion section) and 100 µL of each sample was injected into size exclusion chromatography equipment. Similar to IEXC, each SEC fraction was analyzed for its protein content, enzyme activity and confirmed using SDS-PAGE. All the analysis including fermentation and partial purification of the enzyme were performed at the Centro de Biotecnología-FEMSA, Departamento de Biotecnología e Ingeniería de Alimentos, Tecnológico de Monterrey, México.

In-gel Digestion and Maldi-TOF/TOF MS Analysis

In-gel digestion of SDS-PAGE bands, which were cut and divided into smaller pieces of approximately 1 mm, were performed according to the procedure of KinterSherman [31]. Mass spectrometry experiments were performed at Izmir Institute of Technology at the Center for Mass Spectrometry and Proteomics Studies, Izmir, Turkey.

RESULTS and DISCUSSION

The protein contents and enzyme activities of fermentations with different carbon sources are presented in Table 1. According to the results, crude extract from third procedure (SSF with wheat bran and sugar beet as carbon source) produced highest protein amount (0.50 mg/mL) with PG activity of 50.27 U/mL. This was followed by SmF producing 0.25 mg/mL protein and 95.22 U/mL PG activity. It has been reported that SSF systems supported with different carbon sources such as glucose, sucrose or galacturonic acid produce comparatively higher PG than SmF systems [32]. Since the crude extract from SmF was difficult to handle for further processing due to mainly viscous nature of the broth it was not considered for further study.

A recent research by Demir and Tari [33] has characterized the stability of this enzyme (produced by SSF) under different conditions. The study revealed that PG from a mutant *A. sojae* ATCC 20235 strain produced by SSF was an acidic pectinase displaying its optimum activity on polygalacturonic acid in the pH range of 4.0-5.0 and at a temperature of 40°C. Moreover, it showed stability in the pH range of 3.0-7.0 by retaining at least 65% of its activity and thermostability was found to be between 40-50°C. These results were compatible with the studies of PG from *A. niger* produced by SSF [34-36]. The study concluded that further purification studies of PG from a mutant *A. sojae* ATCC 20235 strain produced by SSF were important as its biochemical properties were found to be as satisfactory as the commercial pectinases. The enzyme has a high

potential to be considered in the clarification of fruit juices and wine.

Table 1. Protein content and enzyme activity results of fermentations with different media

	Protein Content (mg/mL)	Enzyme Activity (U/mL)
Procedure 1: Bitter orange peel (10 g/L), sugar beet molasses (60 g/L) and $(NH_4)_2SO_4$ (8 g/L)	0.25	95.22
Procedure 2: Bitter orange peel (2%) and wheat bran (8%) and 7 mL 50 mM HCl	0.12	7.16
Procedure 3: Wheat bran (7 g) and sugar beet (3 g) and 16 mL 0.2 N HCl	0.50	50.27

In this study, polygalacturonase produced in solid-state fermentation (3rd procedure) was purified by combination of two different separation techniques such as ion exchange and size exclusion chromatographic methods. Studies on the combination of these chromatographic techniques for PG purification have been reported elsewhere [20, 37]. To be easily adsorbed by the Vivapure diethylamine-D column, the dialyzed and previously concentrated crude extract was diluted 3-fold in sodium acetate buffer (pH 5.5) to 0.85 mg/mL protein content and 130.02 U/mL PG activity.

The protein contents, PG activities and specific activities of ten different salt concentrations that were employed in the IEXC purification are shown in Figure 1 and SDS-PAGE results are presented in Figure 2.

The IEXC step purified the crude extract to a yield (recovery) of 59% which was the ratio of total enzyme amount of all IEXC salt fractions to that of crude extract (Table 2).

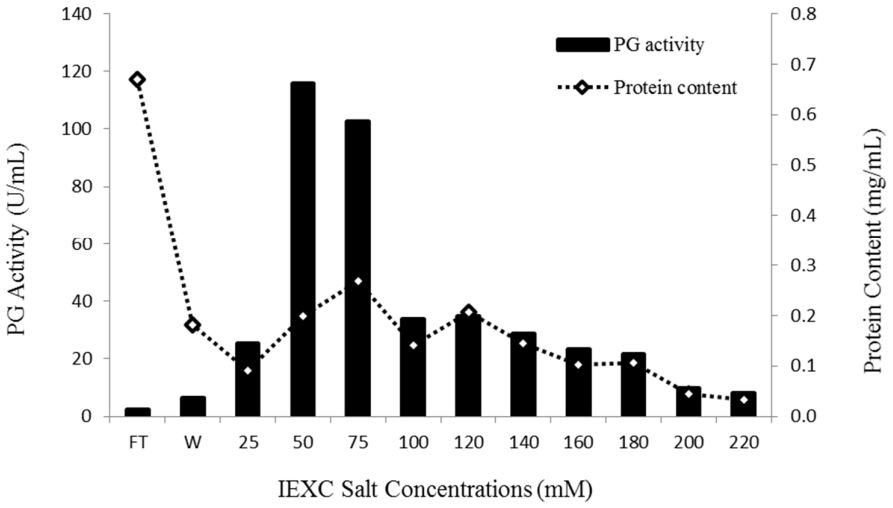

Figure 1. Protein contents, PG activities and specific activities of ten different salt concentrations employed in the IEXC purification

The purification fold and specific activity were found as 2.0 and 300 U/mg, respectively. However according to the fractional results, 50 mM IEXC fraction revealed the highest specific activity (579 U/mg) and purification fold (3.8) among all other fractions. The SDS-PAGE results confirmed PG bands (encircled in Figure 2 for 120 mM IEXC fraction) for IEXC fractions between 120-220 mM at a molecular weight of 37 kDa. Molecular weight of different polygalacturonases were reported to vary from 35 to 79 kDa [3]. According to the SDS-PAGE results, 50-75-100 mM, 120-140-160 mM and 180-200-220 mM fractions were pooled and concentrated using Amicon ultra centrifugal tubes and further purified with SEC. The SEC chromatogram revealed four peaks coded as “a”, “b”, “c” and “d” (Figure 3).

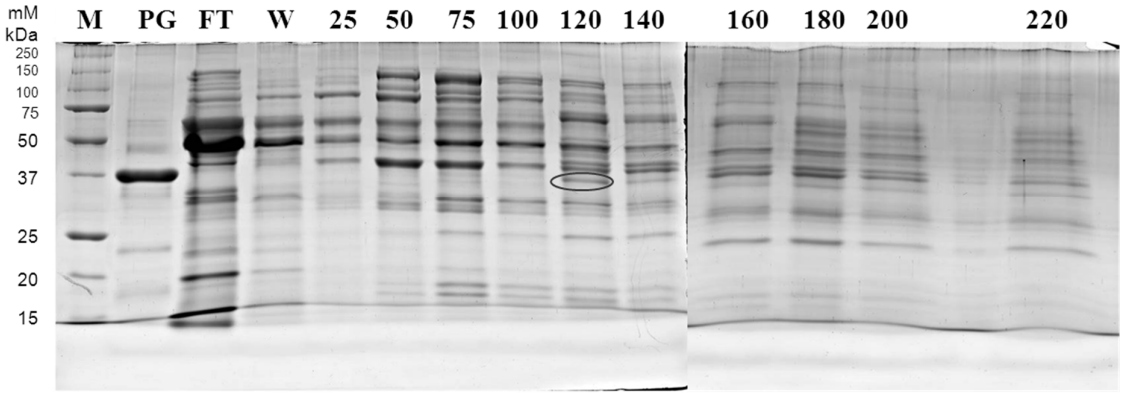

Figure 2. SDS-PAGE results of IEXC fractions: Lane 1: (M) molecular weight marker, Lane 2: (PG) commercial polygalacturonase marker, Lane 3: (FT) flow through, Lane 4: (W) wash, Lane 5: 25 mM IEXC fraction, Lane 6: 50 mM IEXC fraction, Lane 7: 75 mM IEXC fraction, Lane 8: 100 mM IEXC fraction, Lane 9: 120 mM IEXC fraction, Lane 10: 140 mM IEXC fraction, Lane 11: 160 mM IEXC fraction, Lane 12: 180 mM IEXC fraction, Lane 13: 200 mM IEXC fraction, Lane 14: 220 mM IEXC fraction. (PG band was encircled for 120 mM IEXC fraction)

Table 2. Results of IEXC and SEC Procedures

Step	Total Protein (mg)	Total Enzyme (U)	Specific Activity (U/mg)	Yield (%)	Purification Fold
Crude Extract	8.47	1300.22	154		
IEXC	2.52	756.14	300	59	2.00
SEC Fractions (mM)					
50-75-100	0.22	40.18	184	9	1.20
120-140-160	0.43	28.22	65	17	0.43
180-200-220	0.11	33.03	307	36	2.00

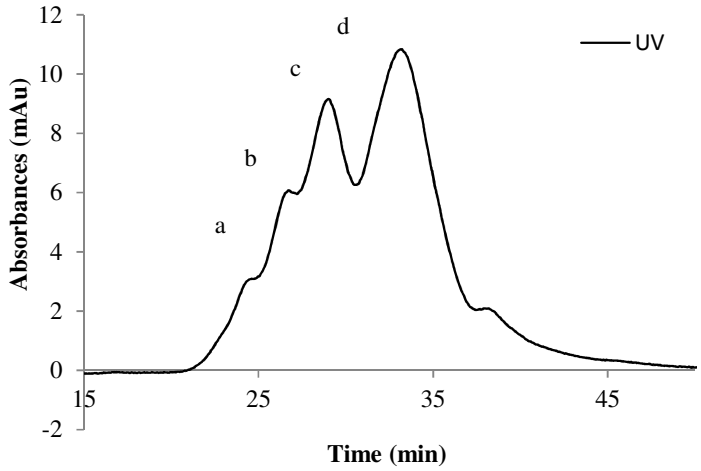

Figure 3. SEC chromatogram of IEXC pooled fractions (mAu: milli Absorbance unit)

Among these four peaks, peak coded as “c” revealed PG activity for all IEXC pooled fractions according to the PG activity analyses. SDS-PAGE results confirmed PG band only for the 2nd (120-140-160 mM) and 3rd (180-200-220 mM) IEXC pooled fractions (Figure 4).

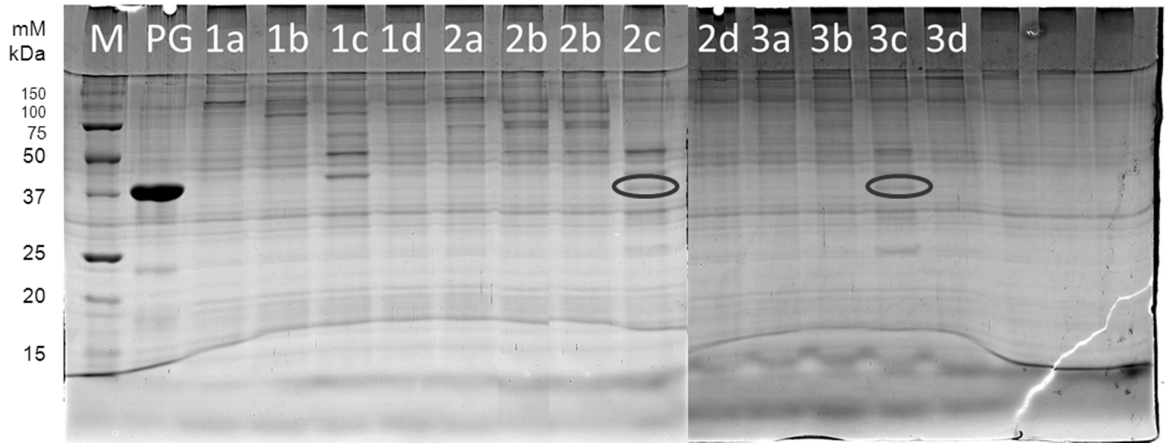

Figure 4. SDS-PAGE results of SEC fractions: Lane 1: (M) molecular weight marker, Lane 2: (PG) commercial polygalacturonase marker, Lane 3: (1a) SEC fraction "a" of 50-75-100 mM IEXC pooled fraction, Lane 4: (1b) SEC fraction "b" of 50-75-100 mM IEXC pooled fraction, Lane 5: (1c) SEC fraction "c" of 50-75-100 mM IEXC pooled fraction, Lane 6: (1d) SEC fraction "d" of 50-75-100 mM IEXC pooled fraction, Lane 7: (2a) SEC fraction "a" of 120-140-160 mM IEXC pooled fraction, Lane 8: (2b) SEC fraction "b" of 120-140-160 mM IEXC pooled fraction, Lane 10: (2c) SEC fraction "c" of 120-140-160 mM IEXC pooled fraction, Lane 11: (2d) SEC fraction "d" of 120-140-160 mM IEXC pooled fraction, Lane 12: (3a) SEC fraction "a" of 180-200-220 mM IEXC pooled fraction, Lane 13: (3b) SEC fraction "b" of 180-200-220 mM IEXC pooled fraction, Lane 14: (3c) SEC fraction "c" of 180-200-220 mM IEXC pooled fraction, Lane 15: (3d) SEC fraction "d" of 180-200-220 mM IEXC pooled fraction. (PG bands were encircled for 2c and 3c SEC fractions with PG activity).

The specific activities of each IEXC pooled fraction after SEC purification were calculated as 184 U/mg, 65 U/mg and 307 U/mg for the 1st (50-75-100 mM), 2nd (120-140-160 mM) and 3rd (180-200-220 mM) fraction, respectively (Table 2). In addition to its high specific activity, SEC purification of 3rd IEXC pooled fraction revealed the highest partial yield (36%) and purification fold (2.00). According to a study by Dogan and Tari [38], PG from *A. sojae* ATCC 20235 strain was purified with a recovery of 25.5% and 6.7 fold purification by three-phase partitioning in which the enzyme solution was mixed with ammonium sulphate and tert-butanol. In another study by Nagai et al. [37] the two step purification including cation exchange and size exclusion chromatography for *Aspergillus awamori* resulted in only 3.04% recovery with a higher purification fold (345) and specific activity (487 U/mg) than the findings in this study. On the other hand, using the same purification methods, Jacob et al. [21] have reported better purification parameters (57.1% recovery, 54.9 fold purification and 504.8 U/mg specific activity) for *Streptomyces lydicus*. The two step gel filtration purification produced 27.06% yield with 12.34 fold purification and 61.35 U/mg specific activity for *Rhizomucor pusillus* [39]. Ethanol precipitation combined with gel filtration produced 5.01% yield with 6.52 fold purification and 54.3 U/mg specific activity for *Aspergillus niger* [40]. In comparison to the multi-step purification of Contreras EsquivelVoget [18] (vacuum concentration-acetone precipitation-Sepharose Q column-Sephacryl S-100 column), PG from *Aspergillus kawachii* was purified with better yield (50%) and purification fold (470) than the results in this study. Purification by treating the crude extract with activated charcoal powder resulted in better yield (69.8%) and purification fold (34.8) but lower specific activity (128 U/mg) for *Aspergillus awamori* [16]. There is a variation in the purification folds, yields and specific activities in literature depending on the type of strain and purification methods used.

Due to its high specific activity, yield and purification fold, SDS-PAGE band corresponding to the SEC peak "c" of the 3rd IEXC pooled fraction (180-200-220 mM) (marked in circle in Figure 4) was cut and in-gel digested together with a commercial PG marker band. The Maldi-TOF MS data were evaluated using Mascot software (Matrix Science, London, UK). The commercial PG band spectra shown in Figure 5A revealed dominant peaks such as 1162.463 m/z, 1985.867 m/z and 2137.058 m/z. These signals were statistically matched to endo-PG from *Aspergillus aculeatus* with a score of 131 in the SwissProt database of Mascot search and the calculated mass was 38.961 Da. The matched peptides in the aminoacid sequence given in Table 3 are shown in bold. On the other hand, the spectra of the sample indicated no sequence homology to endopolygalacturonase (Figure 5B).

This misdetection of peptides might be due to the insufficient concentration of proteins in the sample. Moreover, combination of Maldi-TOF MS technique with the high resolving power of 2D-electrophoresis that allows the separation of proteins according to both molecular weight and isoelectric point could provide more powerful means of identifying and separating complex protein mixtures [41]. Although this was the first attempt to identify PG peptide sequence from *A. sojae* using Maldi-TOF MS, in literature, several researchers were able to identify the masses or peptide sequences of their extracts from different bacterial and fungal sources. For instance, Yuan et al. [42] have reported the

verification of gel filtration purified recombinant endo-PG I from *Pichia pastoris* through the trypsin digestion of SDS-PAGE bands and using liquid chromatography-electrospray ionization-tandem mass spectrometer (LC-ESI-MS), were able to find four peptides corresponding to the aminoacid sequence of endo-PG I. Similarly, an extracellular PG from *Rhizopus oryzae* was purified and its molecular mass and peptide sequence was identified with the aid of ESI-QTOF MS (Electrospray Ionization Quadrupole Time-of-flight Mass Spectrometry) [43]. Moreover, Rodrigo et al. [44] have determined the masses of peptides in purified PG fractions extracted from different tomato varieties using Maldi-TOF MS.

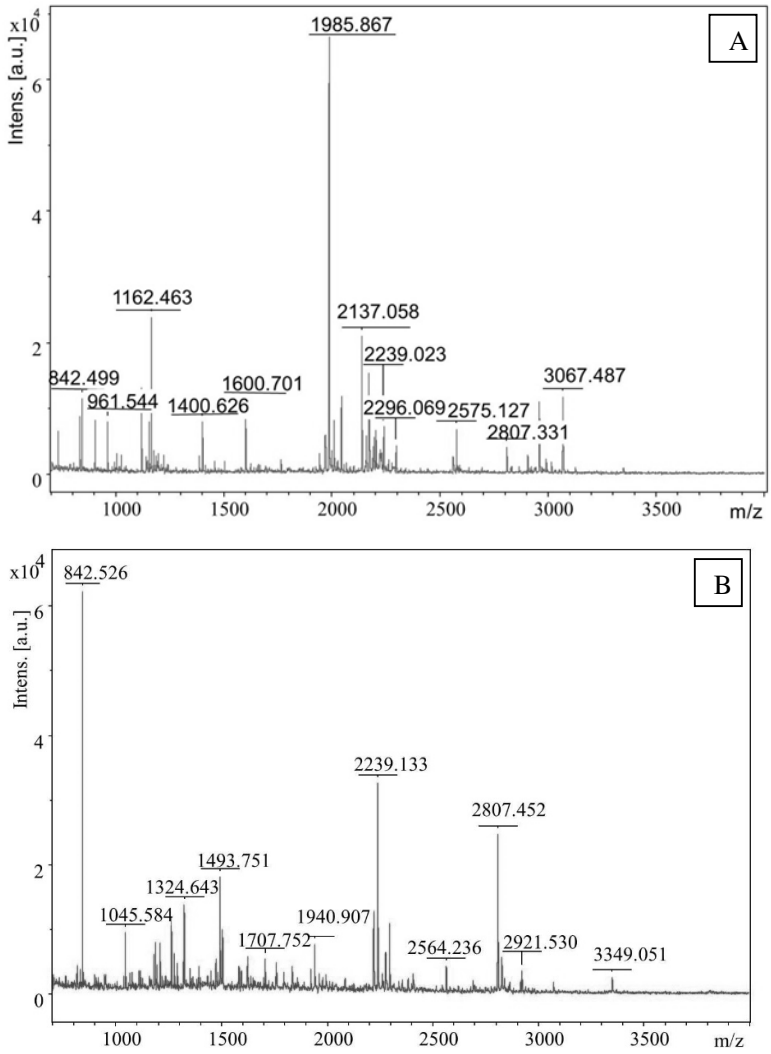

Figure 5. MALDI Mass spectra of (A) SDS-PAGE band of commercial polygalacturonase and (B) SDS-PAGE band of SEC fraction “c” of 160-180-200 mM IEXC pooled fraction (3c)

Table 3. Amino acid sequence of Endopolygalacturonase from *Aspergillus aculeatus*.

1	MHLNTTLLVS	LALGAASVLA	SPAPPAITAP	PTAEEIAKRA	TTCTFSGSNG
51	ASSASKSKTS	CSTIVLSNVA	VPSGTTLDLT	KLNDGTHVIF	SGETTFGYKE
101	WSGPLISVSG	SDLTITGASG	HSINGDGSRW	WDGEGGNGGK	TKPKFFAAHS
151	LTNSVISGLK	IVNSPVQVFS	VAGSDYLTLK	DITIDNSDGD	DNGGHNTDAF
201	DIGTSTYVTI	SGATVYNQDD	CVAVNSGENI	YFSGGYCSGG	HGLSIGSVGG
251	RSDNTVKNVT	FVDSTIINSD	NGVRIKTNID	TTGSVSDVTY	KDITLTSIAK
301	YGIVVQQNYG	DTSSTPTTGV	PITDFVLDNV	HGSVVSSGTN	ILISCGSGSC
351	SDWTWTDVSV	SGGKTSSKCT	NVPSGASC		

CONCLUSION

It can be concluded that polygalacturonase from *A. sojae* mutant can be produced by both solid-state and sub-merged fermentations using different fermentation media. The particular crude extract from solid-state fermentation was also partially purified by two-step chromatography approach using ion exchange (IEXC) and size exclusion chromatographic (SEC) methods. An attempt to peptides confirmation was performed using SDS-PAGE electrophoresis and Maldi-TOF MS. The findings reported here can be used to establish the initial trials on the identification of the purified *A. sojae* polygalacturonase. To the best of our knowledge, purification of PG produced from *A. sojae* mutant and identification of its peptides using Maldi-TOF MS have not been reported in the literature.

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CONFLICT OF INTEREST

There are no conflicts of interest among the authors.

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