Single method of purification for endoglucanase from *Aspergillus niger* by polyelectrolyte precipitation

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Abbreviations: PVS, poly vinyl sulfonate; CHS, Chitosan; CE, commercial endoglucanase; PF, purification factor; %R, percentage of recovery

Abstract

The precipitation of endoglucanase from *Aspergillus niger* with synthetic and natural electrically charged polymers -poly vinyl sulfonate (PVS) and chitosan (CHS)- was characterized and applied to a simple method of purification of an enzymatic extract obtained from fungal culture under solid-state fermentation (SSF).

The kinetics of complex formation was determined. The results of the kinetic profile obtained for CHS and PVS indicated an exothermic mechanism for the formation of the non-soluble complex. CHS exhibited a marked stabilizing effect on endoglucanase.

The enzyme precipitated successfully with both polymers. The precipitation method applied to commercial endoglucanase and the fungal extract showed similar patterns with high purification factors. The recovery of the activity in the re-dissolved precipitate from the fungal extract was close to 40% at pH 5.3 using PVS (1% w/w) as precipitating agent and the purification factor was near 9. The purification factor of endoglucanase in the precipitate of the enzymatic extract from SSF with CHS (0.05% w/v) was around 7. These parameters make this precipitation method appropriate to be included in the last stages of a downstream process, with advantages such as simplicity, scalability and ability to concentrate and stabilize the enzyme.

Keywords: endoglucanase, anionic polyelectrolyte, cationic polyelectrolyte, precipitation, bioseparation

1. Introduction

Cellulases are hydrolases which comprise a complex of endoglucanase (3.2.1.4), exoglucanase and β -glucosidases (Sohail et al., 2009). Cellulases have a wide range of applications in various industries such as textile, laundry, pulp and paper, food and feed products, as well as in bioethanol production (Bhat, 2000; Dave et al., 2013; Silva et al., 2013). Cellulases have great potential in the saccharification of lignocellulosics into fermentable sugars which can be used for the production of bioethanol (Boggione et al., 2016; Maki et al., 2009; Pandey et al., 2016). It is critical to consider the conditions that contribute to enzyme stability for the design and development of a biotechnological process where the function of the enzyme is relevant. Highly polluting chemical processes can be replaced by environmentally-friendly biotechnological methodologies using cellulases.

Endoglucanase is one of the main components of the enzyme complex (Sohail et al., 2009). Traditional methods for endoglucanase purification are the precipitation with ammonium sulphate, ion-exchange chromatography, affinity chromatography and gel filtration (Bakare et al., 2005; Bischoff et al., 2006; Calza et al., 1985; Gupta et al., 2012; Kaur et al., 2007; Murashima et al., 2002; Saha, 2004; Yin et al., 2010). The latter is costly, time-consuming and ammonium sulphate cannot be disposed into the environment. Besides, chromatography is not a suitable technique to be used in a downstream process from the economic point of view. Therefore, a new protocol which considers the costs and reduces the environmental impact is needed.

In the downstream processing of enzymes, the choice of the purification technique should ensure the preservation of the enzyme structure associated with its biological function. One technique for large-scale protein separation involves the addition of polyelectrolytes to achieve selective protein separations.

Proteins interact strongly with both synthetic and natural polyelectrolytes (Romanini et al., 2007). These interactions are modulated by a broad array of variables such as pH and ionic strength, and may result in soluble complexes (Morawetz and Hughes Jr, 1952; Xia and Dubin, 1994), complex coacervation (Ahmed et al., 1994), precipitation (Sternberg and Hershberger, 1974), or gelation (Petit et al., 1995).

Several interactions such as electrostatic, hydrophobic, hydrogen bonds may occur between proteins and polymers, resulting in protein folding and complexation (Radeva, 2001). Polyelectrolyte-protein complex formation includes recovery and protein purification (Roy et al., 2005), stabilization and immobilization of enzymes (Manrich et al., 2008), modification of protein–substrate affinity (Mattiasson et al., 1998).

The phase boundaries of different proteins can be used in systems containing several proteins as a means

of selecting the optimal pH and ionic strength for maximum yield and purity. The light scattered by the samples, detected through turbidity measurements, appears to be the simplest and most sensitive technique to follow the progress of any kind of aggregation (Radeva, 2001).

Precipitation by the formation of polymer-protein complex is a novel alternative potentially applicable in order to recover endoglucanase from *Aspergillus niger*. This method has different advantages over traditional ones, such as simplicity, low cost and speed.

The first aim of this work was to investigate the formation of the polyelectrolyte-endoglucanase complex. Two polymers were selected to evaluate complex formation: anionic poly vinyl sulfonate (PVS) and cationic chitosan (CHS). The mechanism of polymer-endoglucanase interaction and the conditions for precipitation were determined in order to obtain the optimum conditions for precipitation.

The second goal of this work was to apply the above mentioned information to design a simple downstream process that requires a low amount of chemicals and is able to recover and concentrate endoglucanase from a solid culture medium.

2. Materials and methods

2.1 Chemicals and Materials

Commercial endoglucanase (CE) from *Aspergillus niger* was purchased from Sigma-Aldrich (USA).

Solutions of CE were prepared in 50 mM citrate buffer (pH 5.3). PVS of 170000 Da molecular mass, 1.267 g/cm³ density was purchased from Sigma-Aldrich (USA) and was used in solutions of 1% w/w in 50 mM citrate buffer (pH 2.7).

CHS was purchased from Sigma-Aldrich (USA) with a minimum deacetylation degree of approximately 75% , and used without further purification. Solutions of CHS (0.05% w/v) in 50 mM phosphate buffer (pH 5.1) were prepared. All the other chemicals were of analytical grade. Carboxymethylcellulose (CMC) was acquired from Sigma-Aldrich (USA).

Soy hull pellets, a by-product of soybean processing, were used in this work as support for SSF.

2.2 Determination of endoglucanase activity and total proteins

Endoglucanase activity was determined by the enzymatic capacity to hydrolyze CMC (Miller, 1959) , which releases reducing sugars. Under certain conditions, 3,5-dinitrosalicylic acid (DNS) is reduced by the generated sugars and changes its color from orange to red. The reduced DNS can be detected by

measuring absorbance at 560 nm. The reaction was carried out by mixing 900 μ L of 1% w/v CMC solution with an adequate aliquot of enzymatic sample at 50°C for 10 min. Then, 1 mL of 1% w/v DNS was added and maintained at 100°C for 10 min and the absorbance was measured at 560 nm. Blanks without enzyme and without CMC were assayed as controls. A calibration curve of glucose was carried out under the same experimental conditions as the samples. One unit of endoglucanase activity is the amount of enzyme that liberates 1 μ mol of glucose/min under the above mentioned conditions (Bansal et al., 2012; Gouka et al., 1997). Total protein concentration was estimated by the Warburg and Christian method (Warburg and Christian, 1941).

2.3 Endoglucanase turbidimetric titration curves with polymers at different pH and ionic strengths

The non-soluble polymer-endoglucanase complex was monitored by turbidimetric titration (Cooper et al., 2005; Kokufuta et al., 1981). CE solutions with a fixed protein concentration were titrated at 20°C in a glass cell with the polymer solution in the absence and presence of NaCl. Both protein and polyelectrolyte solutions were adjusted to the same pH value in order to avoid pH changes in the course of the titration. Complex formation was monitored by measurement of the absorbance at 420 nm vs. the added amount of polymer. Stoichiometric polymer/protein ratio was defined as the minimum ratio needed to achieve a full protein-precipitation by forming a non-soluble complex. Stoichiometric polymer/protein ratio was obtained from the turbidimetric curves as the minimal polymer concentration required to achieve a plateau. Data have been expressed as mg of polyelectrolyte per mg of CE.

The time required to form the complex was determined by measuring the time demanded in order to obtain the maximum absorbance (Cooper et al., 2005).

Mixtures of different polymer-protein mass ratios were titrated by adding NaOH or HCl. The absorbance determined at 420 nm was plotted against pH (Nath et al., 1995).

2.4 Endoglucanase precipitation with PVS and CHS from commercial enzyme

A solution of PVS and CE at suitable ratio of mg of polymer per mg of CE (0.0108 mg PVS/mg CE) in 50 mM citrate buffer (pH 2.7) was prepared. The resulting precipitate was incubated at 15°C for different time periods, centrifuged at 815 g for 10 min and re-dissolved in 50 mM citrate buffer (pH 5.3). Endoglucanase activity was determined in the re-dissolved precipitate.

A mixture of 0.05% w/v CHS and 30 mg/mL of CE in 50 mM phosphate buffer (pH 5.1) was prepared at a final ratio of 0.0013 mg CHS/mg CE. The formed precipitate was incubated at 15°C for different time periods and centrifuged at 1700 g for 10 min. The supernatant was removed and the pellet was re-dissolved in 50 mM phosphate buffer (pH 5.1) by adding 1 M NaCl.

2.5 Endoglucanase production by *Aspergillus niger* under SSF

Strains of *A. niger* NRRL3 were provided by the culture collection of Agricultural Research Service, USDA. The production of endoglucanase was performed under SSF according to Jecu (2000) with some modifications. The microorganism was propagated on a potato-dextrose agar medium (PDA) at 30°C for five days. The spores were dislodged from the PDA under gentle agitation with sterile distilled water. Spore numbers were estimated by microscopic counting using a Thoma chamber. The culture medium was constituted by: 1.4 g/L (NH₄)₂SO₄, 2.0 g/L KH₂PO₄, 0.50 g/L MgSO₄ . 7 H₂O, 0.40 g/L CaCl₂, 0.0060 g/L FeSO₄ . 7H₂O, 0.0020 g/L MnSO₄ .H₂O, 0.0014 g/L ZnSO₄, 0.020 g/L CoCl₂ .6 H₂O, 0.30 g/L urea, 0.25 g/L yeast extract. This medium was autoclaved at 121°C for 15 min and was inoculated with 1 × 10⁶ spores/mL after it reached room temperature. SSF was carried out in 250 mL Erlenmeyer flasks with 20 g of soy hull pellets used as growth support and carbon source, which were sterilized and 50 mL of culture medium were added. The medium was homogenized and incubated for three days at 30°C. The enzymatic extract was obtained by the addition of 30 mL of 50 mM phosphate buffer (pH 5.1) to each reactor, compressed with a syringe, filtered and centrifuged at 3000 g for 10 min (Latifian et al., 2007).

2.6 Analysis of purification process

The purification factor and recovery of endoglucanase were determined.

The purification factor (PF) was defined as the number of times that the specific activity increases in each fraction. The PF was calculated by comparing the endoglucanase specific activity in the re-dissolved precipitate with the endoglucanase specific activity in the control as follows:

$$PF = \frac{SAp}{SAc}$$

where *SAp* is the endoglucanase specific activity in the re-dissolved precipitate and *SAc* is the endoglucanase specific activity in the control. Endoglucanase specific activity was calculated as the endoglucanase activity divided by total protein concentration.

Percentage of recovery (%R) represents the percentage of retained activity with respect to the initial sample and it was calculated as the endoglucanase activity in the re-dissolved precipitate divided by the endoglucanase activity in the control as follows:

$$\%R = \frac{Ap}{Ac}$$

where Ap is the endoglucanase activity in the re-dissolved precipitate and Ac is the endoglucanase activity in the control.

2.7 SDS-PAGE and zymogram analysis

SDS-PAGE was performed in a 15% (w/v) polyacrylamide gel in accordance with the method described by Laemmli (1970). Coomassie Blue R-250 staining was performed for visualization of proteins on the polyacrylamide gel. For visualization of endoglucanase activity bands on the gel, the method of Medve et al. (1998) was employed with some modifications. The zymogram analysis of endoglucanase was performed by adding 0.15% (w/v) CMC into the polyacrylamide gel. Following SDS-PAGE, the gel was washed twice at room temperature with a washing solution which contained 50 mM acetate buffer and 25% isopropanol (pH 4.8) to remove the sodium dodecyl sulfate. The enzyme was renatured in a 50 mM acetate buffer (pH 4.8) containing 5 mM β -mercaptoethanol by stirring the gel overnight at 4°C. Then, the gel was transferred to a 50 mM acetate buffer (pH 4.8) for 2 h and incubated at 50°C for another 2 h. The gel was stained in 0.1% (w/v) Congo Red for 30 min and destained with 1 M NaCl. Endoglucanase activity was visible as clear bands against a red background.

3. Results and discussion

3.1 Titration of Endoglucanase with Polymers

The isoelectric point of endoglucanase is close to 4.5; therefore, the charge of the enzyme will be positive when the medium pH is lower than 4.5. Figure 1A exhibits the variations of absorbance at 420 nm when CE at different concentrations is titrated with 1% w/w PVS (pH 2.7). Turbidity increased after adding small volumes of polymer. A decrease in absorbance is observed when the polymer concentration is increased using a CE concentration of 45 mg/mL. The plateau was achieved at 0.0042 mg PVS/mg CE when CE concentration was 30 mg/mL. This value corresponds to the stoichiometric polymer/protein ratio and is equivalent to 0.0894 mg PVS/ mg total protein. The hyperbolic pattern suggests that the complex formation involves a saturation process (Fig. 1 A).

A pH above the isoelectric point of endoglucanase (4.5), where the protein is negatively charged, was selected in order to precipitate endoglucanase with CHS. Figure 1B exhibits the titration curves of CE with 0.05% w/v CHS (pH 5.1). The curves presented a sigmoid function. Precipitation increased with an increase in the total polymer concentration and the plateau was reached at 0.0010 mg CHS /mg CE when CE concentration was 30 mg/mL. This value corresponds to the stoichiometric polymer/protein ratio and is equivalent to 0.0220 mg CHS/ mg total protein.

Considering the results obtained, it can be seen that absorbance values at 420 nm are higher when CHS was used as titrant. Therefore, precipitation is more meaningful when CHS is employed and, besides, it occurs at a lower mass ratio of polymer/CE.

The shapes of the titration curves are related to the mechanism of precipitation. The hyperbolic relationship, observed for PVS/CE, suggested a saturation process while the sigmoidal behavior, obtained for CHS/CE, is compatible with a cooperative process.

3.2 Solubility diagrams of endoglucanase with PVS and CHS

Solubility diagrams were carried out at three different mass ratios and for both the commercial enzyme and the polymers. Figures 2 A and B display a solubility diagram of CE with PVS and CHS, respectively, and they also show the pH variation effect on the non-soluble complex formation obtained for a given PVS/CE and CHS/CE fixed ratio.

The different mass ratios showed similar behavior for the PVS/CE complex. Therefore, the insolubility range was not altered by the assayed mass ratios. A substantial increase of absorbance is observed when the pH drops below 3 (Fig. 2 A). This event is consistent with the formation of the PVS/CE non-soluble complex.

For the CHS/CE complex, absorbance values were higher when concentration of CHS was increased.

However, the pH range of precipitation remained constant for the three tested mass ratios.

According to Figure 2 B, the formation of the CHS/CE non-soluble complex is maximal in the pH region from 5 to 7.

3.3 Ionic strength effect on the formation of non-soluble complex

Figures 3A and 3B show the effect of NaCl concentration on the solubility of the non-soluble complex. A decrease of turbidity was observed when NaCl concentration was increased. The turbidity decreased by

more than 50% for a solution of 0.25 M NaCl and it reached values close to zero when a solution of 1M NaCl was used. This result evidences that the precipitate could be re-dissolved at high ionic strength and it confirms the electrostatic nature of the interaction between endoglucanase and both polymers. A similar finding has been reported for the interaction of lipase with polyethyleneimine and chitosan (Bassani et al., 2011).

3.4 Kinetics of non-soluble complex formation

The required time to form the non-soluble complex was assayed at different temperatures. The PVS-CE complex achieved the maximum turbidity in 10 min at 15, 20 and 25°C (Figure 4 A). As shown in figure 4 A, a lower amount of complex is produced when temperature is increased, which indicates that complex formation is exothermic. The kinetic profile is different at 30°C, a drastic increase in turbidity is observed at shorter times when compared with the other temperatures, and then a decrease in turbidity is observed, which is consistent with the dissociation of the complex. The kinetic profile obtained for CHS was similar to that obtained for PVS, thus suggesting an exothermic mechanism for the formation of these non-soluble complex.

3.5 Polymer and salt effects on endoglucanase stability

The variations of enzyme activity in the presence and absence of polymer enable the estimation of their effect on enzyme stability. Certain polymers increase enzymatic activity while others decrease it (Geng et al., 2012; Porfiri et al., 2012). In order to establish whether polymer concentration or salt concentration affect enzymatic stability, endoglucanase activity of a sample without polymer was compared to samples in the presence of PVS and CHS at different concentrations and in the presence and absence of 1 M NaCl. Figures 5 A and 5 B show that enzyme activity was not affected when polymer concentrations were increased. In the presence of salt, enzyme activity increased slightly in most cases.

Figure 6 shows endoglucanase activity as a function of time for the control, the precipitate and the supernatant. The control was prepared from CE in the same conditions as the CE solution used to precipitate. According to Figure 6 A, PVS did not alter endoglucanase activity. The sum of activity in the precipitate and in the supernatant was 6 to 20 % higher than in the control at all the times assayed. The stabilizing effect of CHS was greater than that of PVS since the sum of the precipitate and the supernatant was 26 to 50 % higher than in the control (Figure 6 B).

Therefore, PVS and CHS increase enzymatic activity and they may be considered enzymatic stabilizers.

3.6 Endoglucanase precipitation from commercial enzyme

Table 1 exhibits the purification factors and the percentage of recovery of endoglucanase after precipitation from the commercial enzyme with PVS and CHS. A control from the initial solution of the commercial enzyme was used in order to calculate the purification parameters.

The PFs obtained using PVS as titrant were suitable for all the incubation times tested; they reached a maximum value of 6.3. PF was not significantly modified after 20 min. These results are consistent with our kinetic studies (section 3.4) that showed that PVS-CE complex was fully formed after 10 min. On the other hand, the percentage of recovery (%R) reached a maximum of 37% in the re-dissolved precipitate. The opposite behavior was observed in the precipitation with CHS. PFs were lower in the re-dissolved precipitate and the recovery was over 60%.

3.7 Endoglucanase precipitation from fungal extract

An excellent growth of *A. niger* NRRL3 was achieved on soy hull pellets under SSF and an appropriate endoglucanase activity was obtained. Besides, SSF has the advantage of using agro-industrial waste for the production of enzymes at low cost, leading to high yields (Boggione et al., 2016; Gupta et al., 2015). Precipitation with both polymers from the fungal extract was carried out under the most favourable conditions established previously in this work. Polymer/protein ratios near the stoichiometric polymer/protein ratio obtained from studies with commercial enzyme were selected for the precipitation of endoglucanase from the fungal extract.

For endoglucanase precipitation with PVS, a volume of fungal extract was adjusted to the same pH value of the polymer solution and then 1% w/w PVS in 50 mM citrate buffer (pH 2.7) was added until the selected mass ratio was reached. The mixture was incubated at 15°C for 20 min, centrifuged at 815 g for 10 min and the precipitate was separated from the supernatant. The precipitate was dissolved by the addition of 50 mM citrate buffer (pH 5.3).

For endoglucanase precipitation with CHS, a volume of fungal extract was taken and 0.05% w/v CHS solution was added until the selected mass ratio was reached. The mixture was incubated at 15°C for 50 min, centrifuged at 815 g for 10 min and the precipitate was separated from the supernatant. The precipitate was re-dissolved in 50 mM phosphate buffer (pH 5.1) with 0.50 M NaCl.

Table 2 shows the purification parameters of endoglucanase from fungal extract at different polymer/protein ratios for both polymers. A sample of fungal extract was considered as control. Endoglucanase precipitation from enzymatic extract obtained from fungal culture under SSF showed a similar behavior to that obtained with the commercial endoglucanase for both polymers with outstanding purification values.

When CHS was used as titrant, the PFs were higher in the endoglucanase precipitation from fungal extract than those obtained with the commercial enzyme. However, the percentages of recovery were lower.

In the case of PVS, the same trend was observed but the increase was significant. PFs close to 9 were reached.

3.8 SDS-PAGE and zymogram analysis of the fungal extract and the re-dissolved precipitate

Purification of endoglucanase from fungal extract after precipitation with both polyelectrolytes was analyzed by SDS-PAGE. Figures 7 A and 8 A show the protein pattern of the fungal extract and re-dissolved precipitate using PVS and CHS as titrants, respectively. It is to be noted that the amount of protein in the re-dissolved precipitate decreased with respect to the fungal extract.

Figures 7 B and 8 B show the same samples after staining with Congo Red for visualization of endoglucanase activity. Zymogram analysis displayed at least five bands in the fungal extract. Two major bands with molecular weights of 61 and 47 KDa and minor bands in a molecular-weight range from 20 to 30 KDa were detected in the re-dissolved precipitate using PVS and CHS as titrants. These results are in agreement with previous works which have reported the production of endoglucanases of different molecular weights by other microorganisms (Liu et al., 2011; Sun et al., 2008; Zhou et al., 2008).

4. Conclusions

In this work, the conditions of non-soluble complex formation between commercial endoglucanase and PVS and CHS were determined. Solubility diagrams for both complexes were slightly dependent on polymer concentration. The increase in enzymatic activity observed after precipitation with PVS and CHS allowed us to conclude about the stabilizing effect of both polymers; however, CHS presented a more marked effect. Precipitation curves made at different ionic strengths demonstrated that the interactions between the enzyme and the two polymers are predominantly electrostatic. The kinetics of the precipitate

formation showed that both complexes (PVS-enzyme and CHS-enzyme) are favored at temperatures below 20 °C, the formation of the PVS-enzyme complex being faster (10 min) than that of CHS-enzyme (40 min).

High values of purification factors were obtained when applying the methodology on a commercial endoglucanase. The same trend was observed when the precipitation was carried out on a fungal extract. However, the fungal extract presented higher purification factors (close to 9 fold). These performance parameters make this precipitation method appropriate to be included in the last stages of a downstream process. It is a simple, economical and scalable methodology that uses low quantities of non-toxic and biodegradable polymers such as CHS. Additionally, a concentration and stabilization of the enzyme can be achieved, which represents an advantage over chromatographic methods. If the inclusion of this method in earlier stages were desirable, the recoveries could be enhanced by the re-precipitation of the enzyme by an extra addition of polymer to the supernatant.

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Figure Captions

Fig. 1A: Titration curves for three CE concentrations with 1% w/w PVS. Medium: 50 mM citrate buffer (pH 2.7). Temperature 15 °C.

Fig. 1B: Titration curves for three CE concentrations with 0.05% w/w CHS. Medium: 50 mM phosphate buffer (pH 5.1). Temperature 15 °C.

Fig. 2A: Solubility diagram for the PVS-CE complex. Medium: 50 mM citrate buffer (pH 2.7). Temperature 15 °C.

Fig. 2B: Solubility diagram for the CHS-CE complex. Medium: 50 mM phosphate buffer (pH 5.1). Temperature 15 °C.

Fig. 3A: Ionic strength effect on the formation of CHS-CE complex. [CE] = 30 mg/mL, [CHS] = 0.05 % w/v. Medium: 50 mM phosphate buffer (pH 5.1). Temperature 15°C.

Fig. 3B: Ionic strength effect on the formation of PVS-CE complex. [CE] = 30 mg/mL, [PVS] = 1% w/w. Medium: 50 mM citrate buffer (pH 2.7). Temperature 15°C.

Fig. 4A: Kinetics of PVS-CE complex formation at different temperatures. Medium: 50 mM citrate buffer (pH 2.7). Polymer/protein ratio = 0.0051 mg PVS/mg CE.

Fig. 4B: Kinetics of CHS-CE complex formation at different temperatures. Medium: 50 mM phosphate buffer (pH 5.1). Polymer/protein ratio = 0.0013 mg CHS/mg CE.

Fig. 5A: Enzymatic activity at different concentrations of PVS and in the presence and absence of 1 M NaCl. Medium: 50 mM citrate buffer (pH 2.7).

Fig. 5B: Enzymatic activity at different concentrations of CHS and in the presence and absence of 1 M NaCl. Medium: 50 mM phosphate buffer (pH 5.1).

Fig. 6A: Enzymatic activity as a function of time in the supernatant and re-dissolved precipitate using PVS as titrant. Medium: 50 mM citrate buffer (pH 2.7). Temperature 15°C.

Fig. 6B: Enzymatic activity as a function of time in the supernatant and re-dissolved precipitate using CHS as titrant. Medium: 50 mM phosphate buffer (pH 5.1). Temperature 15°C.

Fig. 7: SDS-polyacrylamide gels followed by staining for visualization of protein (A) and endoglucanase activity (B) in the fungal extract and in the re-dissolved precipitate using PVS as titrant.

(A) SDS–polyacrylamide (15%) gel electrophoresis (Coomassie Blue R-250 staining) of the proteins present in the fungal extract and in the re-dissolved precipitate using PVS as titrant.

A-M, protein molecular weight markers. The molecular weight marker consist of: Phophorylase b (97 KDa); Albumin (66 KDa); Ovoalbumin (45 KDa); Carbonic anhydrase (30 KDa); Trypsin inhibitor (20.1 KDa) , A-lane 1, fungal extract, A-lane 2, re-dissolved precipitate using PVS as titrant.

(B) Zymogram analysis of endoglucanase. B -lane 1, fungal extract, B-lane 2, re-dissolved precipitate using PVS as titrant.

Fig. 8: SDS-polyacrylamide gels followed by staining for visualization of protein (A) and endoglucanase activity (B) in the fungal extract and in the re-dissolved precipitate using CHS as titrant.

(A) SDS–polyacrylamide (15%) gel electrophoresis (Coomassie Blue R-250 staining) of the proteins present in the fungal extract and in the re-dissolved precipitate using CHS as titrant.

A-M, protein molecular weight markers. The molecular weight markers consist of: Phophorylase b (97 KDa); Albumin (66 KDa); Ovoalbumin (45 KDa); Carbonic anhydrase (30 KDa); Trypsin inhibitor (20.1 KDa) , A-lane 1, fungal extract, A-lane 2, re-dissolved precipitate using CHS as titrant.

(B) Zymogram analysis of endoglucanase. B -lane 1, fungal extract, B-lane 2, re-dissolved precipitate using CHS as titrant.

Table 1. Precipitation of endoglucanase . Purification factor (PF) and percentage of recovery (%R) in the re-dissolved precipitate and supernatant at different times. Mass ratios: 0.0108 mg PVS/mg CE; 0.0013 mg CHS/mg CE. T = 15°C.

Table 2. Purification parameters of endoglucanase precipitation from fungal extract at different polymer/total protein (TP) ratios. Percentage of recovery (%R) and purification factor (PF) in the re-dissolved precipitate and supernatant.

Figure 1

A

B

Figure 2

A

B

Figure 3
A

B

Figure 4

A

B

A Figure 5

B

A Figure 6

B

Figure 7

Figure 8

Table 1. Precipitation of endoglucanase. Purification factor (PF) and percentage of recovery (%R) in the re-dissolved precipitate and supernatant at different times. Mass ratios: 0.0108 mg PVS/mg CE; 0.0013 mg CHS/mg CE. T = 15°C.

	t (min)	PF		%R	
		re-dissolved precipitate	supernatant	re-dissolved precipitate	supernatant
PVS	0	4.0 ± 0.6	1.1 ± 0.1	23 ± 3	89 ± 6
	20	6.30 ± 0.08	1.19 ± 0.03	27 ± 2	86 ± 4
	90	6 ± 1	0.84 ± 0.08	30 ± 1	75 ± 5
	240	6 ± 1	0.96 ± 0.07	37 ± 4	81 ± 3
	1440	6 ± 1	1.17 ± 0.09	26 ± 2	95 ± 1
CHS	0	2.7 ± 0.1	1.2 ± 0.1	46.5 ± 0.2	97 ± 4
	45	2.3 ± 0.3	0.85 ± 0.02	61 ± 1	65 ± 5
	90	2.4 ± 0.2	0.99 ± 0.04	57 ± 4	71 ± 1
	240	2.50 ± 0.08	0.97 ± 0.06	52.2 ± 0.2	75 ± 2
	1440	2.03 ± 0.09	1.30 ± 0.03	47 ± 3	98 ± 6

Data are shown as the mean ± standard deviation (SD)

Table 2. Purification parameters of endoglucanase precipitation from fungal extract at different polymer/total protein (TP) ratios. Percentage of recovery (%R) and purification factor (PF) in the re-dissolved precipitate and supernatant.

Polymer	Polymer/TP ratio (mg/mg)	Fraction	Total Protein (mg/mL)	Activity (U/mL)	PF	%R
PVS	0.0845	Control	13.7 ± 0.4	338 ± 6	1	100
		Precipitate	0.51 ± 0.01	109 ± 1	8.7 ± 0.2	32.2 ± 0.6
		Supernatant	12.3 ± 0.3	302 ± 3	0.99 ± 0.02	89 ± 2
	0.1126	Precipitate	0.65 ± 0.01	120 ± 2	7.5 ± 0.2	36.0 ± 0.6
		Supernatant	12.9 ± 0.4	307 ± 2	0.96 ± 0.03	91 ± 2
		Control	13.5 ± 0.4	334 ± 5	1	100
CHS	0.0185	Precipitate	0.58 ± 0.01	60.6 ± 0.8	4.2 ± 0.1	18.2 ± 0.3
		Supernatant	13.2 ± 0.4	309 ± 3	0.94 ± 0.03	92 ± 1
	0.0296	Precipitate	0.55 ± 0.01	94.2 ± 0.8	6.9 ± 0.2	28.2 ± 0.4
		Supernatant	12.9 ± 0.5	299 ± 5	0.94 ± 0.04	89 ± 1

Data are shown as the mean ± standard deviation (SD)