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Research Article

Research on Extraction and Characterization of Cellulase from Commercial Enzyme Preparation

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Abstract: The extraction of cellulase from commercial enzyme preparation by ammonium sulfate precipitation and its enzymatic characterization were studied. The results were as follows: the conditions of precipitation was 60% saturation of ammonium sulfate, the recovery rate of carboxymethylcellulase (CMCase) and Filter Paper enzyme Activity (FPA) were 73.8 and 71.4%, respectively. The enzyme has optimal temperature of 55°C, optimal pH of 4.8, the ions of Co^{2+}, Mn^{2+} in the buffer lowered the activity of CMCase, but the Cu^{2+} in low concentration activated the CMCase. The ions of Co^{2+}, Mn^{2+} and Al^{3+} in the buffer lowered the activity of FPA, but the $Cu^{2+}$and K^{+} in low concentration activated the FPA; K_m and V_{max} of the enzyme were 2.22×10^{-3}g/mL and 1.11×10^{4} U/h using Lineweaver-Burk graphic method, respectively.

Keywords: Carboxymethylcellulase, cellulase, filter paper activity, enzymatic property, salting out

INTRODUCTION

Cellulose is the most abundant renewable carbon source and can be degraded into glucose by cellulase, by which we may solve the problems of energy, chemicals and food in the world. The cellulase from fungi consist of the following three components: 1, 4-β-glucan glucanohydrolases (endoglucanases, EGs, EC 3.2.1.4), 1, 4-β-D-glucan cellobiohydrolases (exoglucanases, CBHs, EC 3.2.1.91) and β-D-glucoside glucohydrolases (β-glucosidases, EC 3.2.1.21). The whole cellulase activity is often represented by Filter Paper enzyme **(**FPA) including synergistic actions of the three parts. The single endoglucanase activity is often represented by carboxymethyl cellulase (CMCase) activity (Xiaohong *et al*., 2004).

Cellulase production is the most important step in the economical production of ethanol, single cell protein and other chemicals from cellulosic materials (Zhang and Lynd, 2004; Bhat, 2000; Lynd *et al*., 2002; Cherry and Fidantsef, 2003; Angenent *et al*., 2004). These enzyme preparations must work efficiently to convert the dominant polysaccharides to monomers. Currently, high loadings of cellulases are needed to reach 95% conversion of cellulose in pretreated biomass in 3-5 days in a Simultaneous Saccharification and Fermentation (SSF), but the cellulase activity is low and cost is high, especially in crude enzyme preparation for the complexity of its own composition, so the research on extraction and enzyme properties of cellulase is necessary (Chenhe, 2008; Esterbauer *et al*., 1991; Marimuthu *et al*., 2010; Jason *et al*., 2011). In this study, the celluase was extracted from commercial enzyme by salting-out of ammonium sulfate and studied its enzymatic characterization.

MATERIALS AND METHODS

Extraction of cellulase: The commercial crude cellulase powder(Green Biotechnology Co., Ltd. Hangzhou) produced by Trichoderma viride through solid state fermentation were mixed with 10 volumes of water to extract cellulase, stirred slowly at 30°C for 1 h and filtered, then by centrifugation (10,000 g for 15 min). The clarified supernatant was used as the crude enzyme; the crude enzyme was then used for the measurement of cellulase activity.

Enzyme assays: Carboxy Methylcellulase (CMCase) activity was determined using a solution of 2% (w/v) of carboxy methyl cellulose in 50 mM acetate buffer with pH of 4.8, Filter Paper enzyme Activity (FPA) was determined using the method of Ghose, Reducing sugar was determined using 3, 5-dinitrosalicylic acid (DNS) reagent with glucose as a standard (Miller, 1959), respectively. The CMCase and FPA were both expressed as U/g of koji. One Unit (U) of enzyme activity is defined as the amount of enzyme required to liberate 1μmol of product per 30 min.

Salting out of cellulase by ammonium sulfate: The solution of saturated ammonium sulfate was added to

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the crude enzyme and make saturation of ammonium sulfate reach 20, 30, 40, 50, 60, 70 and 80%, respectively. Stand for 12 h at 4°C, then Centrifuged 4000 r/min for 10 min, collected in the supernatant and precipitation of each centrifuge tube and measured CMCase and FPA activity in supernatant, the precipitation was added pH 4.8 of Na2HPO4-citric acid buffer to restore to the original volume to measure the enzyme activity, Recovery of enzyme activity was equal to the total liquid precipitation recovery of enzyme activity divided by the total enzyme activity before Salting out.

RESULTS AND DISCUSSION

Effect of saturation of ammonium sulfate on salting out of CMCase and FPA: After salting out of crude enzyme solution with different concentrations of ammonium sulfate, the supernatant of the FPA of enzyme activity of CMC is shown in Fig. 1.

During 20% to 60% saturation of ammonium sulfate, with the saturation of ammonium sulfate increasing, the CMCase and FPA activity in the supernatant were both decreased. Ammonium sulfate saturation of 60% to 80%, the supernatant had been almost no activity at 60% to 8% saturation of ammonium sulfate. Thus, the optimal saturation of ammonium sulfate of salting out for CMCase and FPA was 60%. The recovery of CMCase and FPA activity were 73.6 and 71.4%, respectively.

Effect of temperature on activity of CMCase and FPA: Using Xinhua No.1 filter paper and CMC-Na as substrate, the CMCase and FPA were measured according to the method mentioned above at 25, 35, 45, 50, 55, 60, 65, 70, 75, 85°C, recpectively and the results shown in Fig. 2.

With the temperature rising, the CMCase and FPA activity increased during 25°C-55°C and reached maximum, the activity of CMCase and FPA was 9377g/U and 266g/U, respectively. Over this temperature, the activity of CMCase and FPA both gradually decreased and was 4177 g/U and 121 g/U at 85°C, respectively. The reason was that the enzyme molecules and substrate increases the frequency of contact with temperature increasing at the low temperature range, speed up the enzymatic reaction. But when the temperature exceeded a certain range, the enzyme protein gradually denaturation and the loss of CMCase and FPA activity for denaturation was faster than the enzymatic effect caused by temperatures. The Optimum temperature (°C) of tea fungal cellulase in crude extract and after precipitation with ethanol was 50°C (Sariri *et al*., 2006), which was lower than that of the cellulase from commercial enzyme preparation by ammonium sulfate precipitation by us, but The optimum temperatures of Bacillus sp. CH43 and HR68 endoglucanase activity were 70°C and 65°C, respectively. (Crispen *et al*., 2000), this showed that the

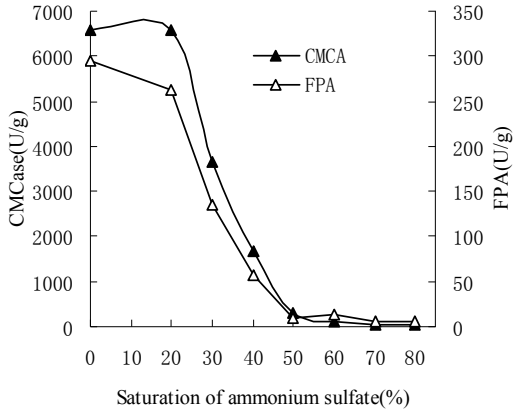

Fig. 1: Effect of saturation of ammonium sulfate on salting out of CMCase and FPA

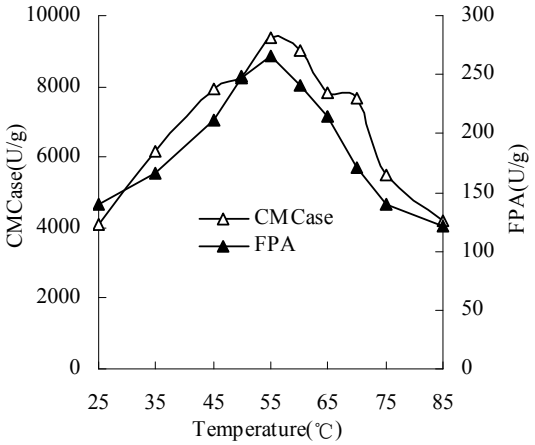

Fig. 2: Effect of temperature on activity of CMCase and FPA

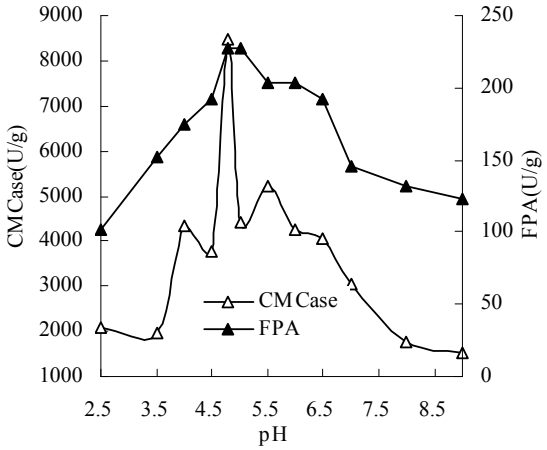

Fig. 3: Effect of pH on activity of CMCase and FPA

cellulase from *Bacillus* sp. were more stable on the heat than that of Trichoderma sp.

Effect of pH on activity of CMCase and FPA: Using Xinhua No.1 filter paper and CMC-Na as substrate, the CMCase and FPA were measured according to the

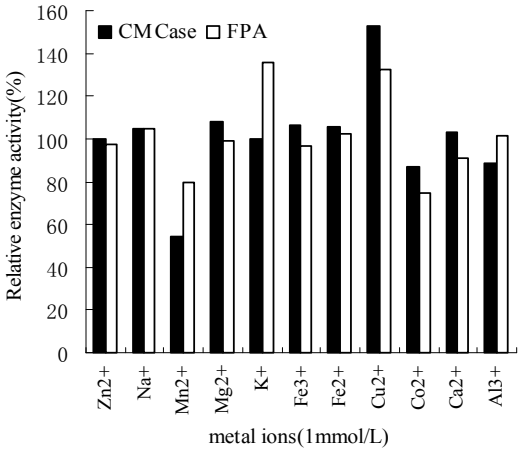

Fig. 4: Effect of metal ions on activity of CMCase and FPA

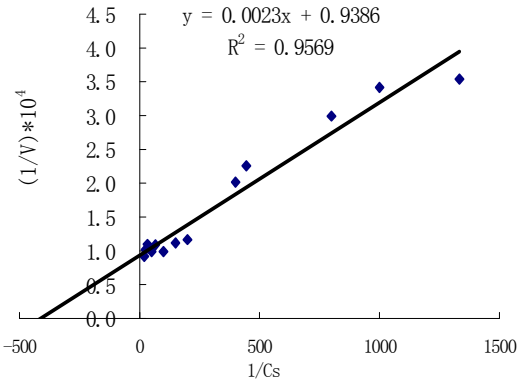

Fig. 5: The Lineweaver-Burk double-reciprocal graph of cellulase

method mentioned above at pH 2.5, 3.5, 4.0, 4.5, 4.8, 5.0, 5.5, 6.0, 6.5, 7.0, 8.0 and 9.0 and the results shown in Fig. 3.

With the pH rising, the CMCase and FPA activity increased during 2.5-4.8 and reached maximum, the activity of CMCase and FPA was 8464 g/U and 228 g/U at pH 4.8, respectively. Over the pH, the activity of CMCase and FPA both gradually decreased and was 1537 and 123 g/U, respectively, because cellulase is protein and have many acidic and alkaline groups such as Amino and carboxyl, which lead to cellulase extremely sensitive to different pH values affecting the protein conformation, enzyme and substrate dissociation state. In addition, pH value will affect the enzyme molecule of some other groups, the dissociation of these groups and the ionization state of enzyme conformation in the active center. The most suitable pH for the enzyme and its substrate varies, but is too acid and too alkaline will cause enzyme denaturation which lead to CMCase and FPA activity reducing. The Optimum pH of tea fungal cellulase in crude extract and after precipitation with ethanol were 7.0 and 7.5, respectively (Sariri *et al*., 2006). CH43 and HR68 cellulase from Bacillus sp. had the highest activity at pH 6.5 for both cellulases in phosphate buffer (Crispen *et al*., 2000). The three cellulase had higher pH than that of the cellulase from commercial enzyme preparation by ammonium sulfate precipitation by us.

Effect of metal ions on activity of CMCase and FPA: 1mmol/L Metal ions including Na^+, K^+, Co^{2+}, Al^{3+}, Zn^{2+}, Mn^{2+}, Ca^{2+}, Mg^{2+}, Fe^{3+}, Cu^{2+}, Fe^{2+} were added in the enzyme-substrate mixture, the results shown in Fig. 4.

The ions of Co^{2+}, Mn^{2+} and Al^{3+}in the buffer lowered the activity of CMCase, but the Cu^{2+} in low concentration activated the CMCase, which showed Co^{2+}, Mn^{2+} and Al^{3+} could inhibited the the activity of CMCase and Cu^{2+} could promote the the activity of CMCase. The ions of Co^{2+}, Mn^{2+} and Al^{3+} in the buffer lowered the activity of FPA, but the $Cu^{2+}$and K^+ in low concentration activated the FPA, The ions of Zn^{2+}, Na^+, Mg^{2+}, Fe^{3+} and Fe^{2+} had no significant influence on CMCase and FPA. Metal ions of Cu^{2+} and Fe^{3+} on cellulose hydrolysis by cellulases from *Penicillium decumbens* had inhibitory effect and showed the inhibition of Fe^{3+} took place on both enzyme and substrate levels (Mingyu *et al*., 2013).

Determination of catalytic kinetic parameters for cellulase: The substrate (CMC-Na) was adjusted to the following final concentration: 0.75, 1.00, 1.25, 1.75, 2.25, 2.5, 6.67, 10, 15, 20, 30 g/L. The initial velocity of enzyme reaction was calculated according to enzyme activity assay method mentioned above. The Lineweaver-Burk double-reciprocal graph was draw by using the reciprocal of substrate concentration (1/S) as the abscissa and the initial velocity of enzyme reaction the reciprocal (1/V) as the vertical axis. The results shown in Fig. 5 (the unit of substrate (CMC-Na) was g/mL and the unit of reaction rate was U/(g.h), The K_m of cellulase was 2.22×10^{-3}g/mL and the maximum reaction rate (V_{max}) was 1.11×10^4U/(g.h) from Fig. 5.

The conditions of salting out for cellulase is 60% saturation of ammonium sulfate, the recovery rate of carboxymethylcellulase (CMCase) and Filter Paper enzyme Activity (FPA) were 73.8 and 71.4%, respectively. The enzyme has optimal temperature of 55°C, optimal pH of 4.8, the ions of Co^{2+}, Mn^{2+} in the buffer lowered the activity of CMCase, but the Cu^{2+} in low concentration activated the CMCase. The ions of Co^{2+}, Mn^{2+} and Al^{3+} in the buffer lowered the activity of FPA, but the $Cu^{2+}$and K^+ in low concentration activated the FPA; K_m and V_{max} of the enzyme were 2.22×10^{-3} g/mL, 1.11×10^4 U/h using Lineweaver-Burk graphic method, respectively.

CONCLUSION

The conditions of salting out for cellulase is 60% saturation of ammonium sulfate, the recovery rate of

carboxymethylcellulase (CMCase) and filter paper enzyme activity (FPA) were 73.8% and 71.4%, respectively. The enzyme has optimal temperature of 55°C, optimal pH of 4.8, the ions of Co^{2+}, Mn^{2+} in the buffer lowered the activity of CMCase, but the Cu^{2+} in low concentration activated the CMCase. The ions of Co^{2+},Mn^{2+} and Al^{3+} in the buffer lowered the activity of FPA, but the $Cu^{2+}$and K^{+} in low concentration activated the FPA; K_m and V_{max} of the enzyme were 2.22×10^{-3}g/mL, 1.11×10^{4} U/h using Lineweaver-Burk graphic method, respectively.

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