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RESEARCH ARTICLE

Purification and characterization of thermostable cellulase from *Enterobacter cloacae* IP8 isolated from decayed plant leaf litter

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ABSTRACT

Cellulases are important in the hydrolysis of lignocellulosic materials and thereby contribute to biomass conversion into fuels and chemicals. A cellulase-producing bacterium was isolated from decayed plant leaf litter in soil of a botanical garden. Based on morphological, biochemical and 16S rRNA gene sequencing, it was identified as *Enterobacter cloacae* IP8, with gene bank accession number NR118568.1. The bacterial cellulase was purified in a three-step procedure using lyophilization, ion exchange chromatography (QAE Sephadex A-50) and gel filtration (Biogel P-100). Two isoforms of the enzyme were purified 1.21 and 1.23 folds, respectively, with yields of 30 and 29% for isoforms A and B, respectively. Apparent molecular weights of 36.61 ± 1.40 and 14.1 ± 0.10 kDa were obtained for isoforms A and B, respectively, using gel filtration chromatography. Kinetic parameters K_m and V_{max} were 0.13 ± 0.04 mg/ml and 3.84 ± 0.05 U/ml/min, respectively, for isoform A and 0.58 ± 0.06 mg/ml and 13.8 ± 0.10 U/ml/min, respectively, for isoform B. Optimum pH (7.0) and temperature (60 °C) of cellulase activity were determined for both isoforms A and B. Na^+ and Ca^{2+} enhanced the activities of both isoforms. Mg^{2+} inhibited the enzyme activity at concentrations 4–15 mM but, while it stimulated the activity of isoform A at concentrations 15–200 mM, it inhibited that of isoform B at same concentration range. The strong inhibition of the enzyme by ethylenediaminetetraacetic acid (EDTA) confirmed the enzyme as a metalloenzyme. These results reveal the purified cellulase from *E. cloacae* IP8 as a thermostable, acidic to neutral metalloenzyme, suggesting that it has good potential for biotechnological applications.

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Enterobacter cloacae IP8; cellulase; metalloenzyme; thermostable; decayed plant leaf litter; 16S rRNA gene sequencing

Introduction

Cellulose is the most abundant biopolymer in nature constituting major components of plant biomass (Camassola and Dillon 2007). It is a linear polysaccharide polymer of D-glucose units linked by β-1,4-glycosidic bonds (Hikaru et al. 2008). Its crystalline structure is stabilized by intermolecular and intramolecular hydrogen bonds (Hikaru et al. 2008). Cellulose-rich plants biomass exists abundantly as organic wastes in the environment. Cellulases (EC 3.2.1.21) are group of enzymes produced chiefly by fungi, bacteria and protozoans that catalyze the hydrolysis of cellulose to soluble sugars (Bozell and Petersen 2010). The complete hydrolysis of cellulose to glucose is carried out by the synergistic action of three enzyme systems. β-1,4-Endoglucanase (EC 3.2.1.4) randomly attacks the internal glycosidic bonds, resulting in glucan chains of different lengths; the β-1,4-exoglucanase (EC 3.2.1.91) acts on the ends of the cellulose chain and releases β-cellobiose as the end products while the β-D-glucosidase (EC 3.2.1.21) specifically hydrolyses the β-cellobiose giving rise to two glucose molecules (Gautam et al. 2010; Lin et al. 2012). Unlike chemical methods, enzymatic hydrolysis of lignocelluloses offers an attractive method as relatively pure products can be obtained from the hydrolytic process (Dashtban et al. 2009; Maki et al. 2009). Enzymatic conversion of cellulosic biomass is thus a potentially sustainable and efficient approach to develop novel bioprocesses and products. Microbial cellulases are important in this regard and they are investigated for important applications in various bioconversion processes in the biofuel and bioproduct industries. The abundance of cellulosic wastes in the environment has led to search for bacteria with improved cellulolytic activities from their natural environments such as decayed plant leaf litters, which can be used for the processes. Cellulases have widespread applications for the food, brewery, animal feed, textile, detergent, pulp and paper and biofuel industries (Bhat 2000; De Carvalho et al. 2008; Abo-

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State et al. 2010; Ladeira et al. 2015; Onofre et al. 2015). They increase the yield of fruit juices and improve the nutritive quality of bakery products and animal feeds (De Carvalho et al. 2008). They are also used in biopolishing of cotton and other cellulosic fibres in the textile industry (Sukumaran et al. 2005), and help in the conversion of cellulose to glucose and other fermentable sugars which can then be used as substrates for biofuel production (Onofre et al. 2015). Relatively high temperature and pH conditions are often necessary in some industrial processes involving hydrolytic enzymes such as cellulases. It is therefore ideal that cellulases used in industries exhibit high level activities and stabilities over a broad range of temperature and pH and should be active in the presence of organic solvents and various additives (Bhalla et al. 2013), hence the search for novel sources of cellulase with novel properties is important. *Enterobacter cloacae* is the most frequently isolated *Enterobacter* species from man and animals and is commonly found on or in plants, insects and many sources in the environment such as water, sewage and soil. It is a Gram negative, facultatively anaerobic and rod-shaped bacterium (Nishijima et al. 2004). Several bacteria species belonging to the genera *Clostridium*, *Bacillus* and *Pseudomonas* have been implicated in cellulase production (Levoy et al. 1997; Jagtar et al. 2004; Bakare et al. 2005; Lugani et al. 2015). In this study, a strain of *E. cloacae* IP8 isolated from plant leaf litter was identified based on morphological, biochemical and molecular characterization. The bacterium produced cellulase under submerged fermentation conditions which was purified and characterized. This article presents the characteristics of the thermostable cellulase from *E. cloacae* IP8 which demonstrate the viability of the enzyme as good bioresource for industrial and biotechnological applications.

Materials and methods

Isolation and characterization of bacterium

The bacterium used in this study was isolated from decayed plant leaf litters of *Lagerstroemia indica* Linn, in a botanical garden. It was subcultured to obtain pure culture which was maintained at 4 °C in agar slants. The isolate was characterized and tentatively identified using phenotypic methods (morphological, biochemical and physiological characterization (Harrigan and McCance 1976). Molecular characterization and identification of the isolate was then carried out based on the 16S rRNA nucleotide sequencing. The primers used in this study were f25 (5′-AGAGTTTGATCMTGGCTCAG-3′) and r1525 (5′-AAGGAGGTGWTCCARCCGCA-3′). The PCR products were analyzed by agarose gel electrophoresis. ABI 3130 × L Genetic Analyzer (Applied Biosystems, Foster City, CA) was used for the nucleotide sequencing. Sequence similarity search of the Genbank data was done using the National Centre for Biotechnology and Information (NCBI) Basic Alignment Search Tool (BLAST) program.

Submerged fermentation for cellulase production

Submerged fermentation for the production of cellulase from *E. cloacae* IP8 was carried out in a culture medium containing per litre: yeast extract 2 g, KH_2PO_4 1 g, $MgSO_4.7H_2O$ 5 g, NaCl 0.75 g, peptone 20 g and carboxymethyl cellulose (CMC) 2 g. The culture was incubated at 55 °C for 48 h on a rotary shaker at 150 rpm. At the end of incubation, the culture was centrifuged at 6000 g for 30 min. The clear supernatant was collected and used as the crude enzyme extract.

Cellulase assay

Cellulase activity towards CMC was measured by the appearance of reducing ends released by the action of the enzymes on the substrate using modified method of Nelson (1944) and Somogyi (1952). One unit of cellulase activity was expressed as the amount of enzyme that liberated reducing sugar equivalent to 1 μg of glucose per ml/min under the assay condition. The specific activity was expressed as the unit of enzyme activity per mg of protein. Protein concentration of the crude supernatant was determined using Lowry's method (Lowry et al. 1951) with bovine serum albumin (BSA) as standard protein.

Purification of cellulase from E. cloacae IP8

The cell-free supernatant was concentrated by lyophilization and the lyophilized protein was dissolved in 5 ml of 100 mM phosphate buffer pH 7.0. The lyophilizate was applied on QAE Sephadex A-50 (1.0 cm ×10 cm) column (Sigma Aldrich, St. Louis, MO) previously equilibrated with 100 mM phosphate buffer, pH 7.0. Fractions (1.0 ml) were collected at a flow rate of 15 ml/h and bound proteins were eluted with a linear gradient of 0–1.0 M NaCl. Cellulase activity in the fractions and the protein profile were determined. Active fractions were pooled and lyophilized. Size exclusion chromatography was performed on Biogel P-100 (1.0 cm × 48 cm) column. The column which was washed with equilibrating buffer (100 mM phosphate buffer, pH 7.0) was subsequently layered with active fractions from previous study. The elution was carried out using the same buffer at a flow rate of 10 ml/h and 0.5 ml fractions each were collected and assayed for cellulase and protein content. Fractions with cellulase activities were similarly pooled and used for subsequent studies. The concentration of protein during purification was determined by Lowry's method (Lowry et al. 1951) with BSA as standard protein.

Characterization of purified cellulase

Determination of native molecular weight

The apparent molecular weight of the purified cellulase was estimated by gel filtration chromatography on Biogel P-100 (1.0 × 48 cm) column. The column was calibrated previously with lysozyme. The apparent molecular weight of the purified cellulase was estimated by gel filtration on a Biogel P-100 (1.0 × 48 cm) column. The column was calibrated previously with lysozyme (14.5 kDa), α-chymotrypsinogen A (25.6 kDa), ovalbumin (45.0 kDa) and BSA (67.0 kDa). Blue dextran was used to determine the void volume of the column.

Determination of kinetic parameters

The effect of varying concentrations of substrate CMC on the purified cellulase was determined. The apparent kinetic parameters (*V*max and *K*m) were determined for the purified *E. cloacae* IP8 cellulase by incubating an aliquot of the enzyme with varied concentrations of CMC from 0.050 to 0.500 mg/ml in 100 mM of phosphate buffer, pH 7.0. The assays were performed with the enzyme which had been diluted appropriately in 100 mM of phosphate buffer, pH 7.0. The apparent kinetic parameters were estimated from Lineweaver–Burk plots (Lineweaver and Burk 1934). The data obtained were analysed using non-linear regression analysis software GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA).

Effect of pH on cellulase activity

Effect of pH on activity of purified cellulase was determined by assaying for enzyme activity at different pH values ranging from 4.0 to 9.0. The pH was adjusted using 10 mM sodium acetate buffer (pH 4.0–5.5) and 100 mM phosphate buffer (6.0–9.0). The activity of assay was then carried out as previously described.

Effect of temperature on cellulase activity and stability

Optimum temperature of purified cellulase was determined by measuring enzyme activity at various temperatures (35–80 °C). Aliquots (0.05 ml) of the enzyme and substrate were incubated at respective temperature for 20 min before determining cellulase activity according to the standard assay procedure earlier described. Thermal stability of the purified cellulase was examined by incubating the enzyme at different temperatures (50–70 °C). Aliquots were withdrawn at 10 min intervals and the residual activities were measured under standard assay conditions earlier stated. The residual activities were expressed as a percentage of the activities at zero time which was taken to be 100%. The percentage residual activities were plotted against time of incubation.

Effect of cations and ethlenediaminetetraacetic acid *(EDTA)* on cellulase activity

The effect of cations on cellulase activity was determined with K^+, Na^+, Mg^{2+} and Ca^{2+} at different concentrations ranging from 0 to 200 mM. The residual activities were measured in the presence of the salt of each of the ions. For the effect of ethylenediaminetetraacetic acid (EDTA) on the enzyme activity, the concentrations 0, 4, 12, 15, 20, 30, 50, 80, 100 and 120 mM, were used.

Results

Characterization and identification of bacterium

Morphological and biochemical characterization

The bacterium strain was characterized initially based on morphological, physiological and biochemical characteristics (Table 1). The morphological characteristics of the strain were as follows: the cells were Gram negative, rods, non-spore forming and motile. The physiological and biochemical characteristics of the isolated strain IP8 are given in Table 1. The isolate is an aerobic and a catalase-producing strain. It tested positive for gelatin hydrolysis, aesculin hydrolysis and could utilize a wide array of carbohydrates, including maltose, lactose, mannitol, raffinose and arabinose as sole source of carbon. From these various tests, isolate was tentatively identified as *Enterobacter* sp.

Molecular characterization and identification of bacterium

Universal primers were used to successfully amplify 16S rRNA from bacterial isolate IP8, yielding an amplicon of size 1000 bp. The sequence of 16S rRNA from strain IP8 was then analyzed using the BLAST analysis and was found to have 100% homology with several *E. cloacae* reported from different parts of the world in the data bank of the NCBI (Altschul et al. 1990). The 16S rRNA sequence of strain IP8 was also compared with the corresponding sequence of different *E. cloacae* reported from different parts of the world. Sequence analysis revealed that IP8 belongs to *E. cloacae*, as it showed maximum homology (94%) with the complete sequence of *E. cloacae* 16S rRNA (accession no. NR 118568.1) with 43% query cover. Based on above morphological, biochemical and molecular characterizations, the strain IP8 was identified as *E. cloacae*.

Purification of the cellulase from E. cloacae IP8

Preliminary tests conducted to choose the best method for the protein concentration of the cell-free supernatant revealed lyophilization as the best method which gave the highest recovery of 71% with specific activity of 1.40 U/mg protein. This was subsequently adopted for further steps involving concentration. The lyophilizate was applied to the QAE Sephadex A-50 ion exchange chromatography column and the cellulase eluted with linear gradients of 0–1.0 M NaCl. Further purification of the enzyme was carried out using Biogel P-100 gel filtration column revealing two isoforms of the enzyme. The results of the purification procedure are summarized in Table 2. The cellulase was purified 1.21 fold (isoform A) and 1.23 fold (isoform B) with specific activities of 23.6 and 24.6 U/mg, respectively, after Biogel P-100 filtration chromatography. The yield of the enzyme after purification was 30 and 29%, respectively, for isoforms A and B (Table 2).

Table 1. Morphological, biochemical and physiological characteristics of *E. cloacae* IP8.

Characteristics	*Enterobacter cloacae*
Gram reaction	−
Morphology	R
Sporulating	−
Motility	+
Indole utilization	−
Methyl red test	−
Voges Proskauer	+
Citrate utilization	+
H_2S production	−
Glucose	+
Maltose	+
Lactose	+
Mannitol	+
Raffinose	+
Arabinose	+
Sucrose	+
Coagulase test	−
Catalase test	+
Oxidase test	−
ONPG	+
Gelatin hydrolysis	−
Nitrate reduction	+
Esculin hydrolysis	+

Key: +: Positive; −: Negative; R: Rod.

Characterization of purified cellulase

Determination of apparent molecular weight of cellulase

The purified enzyme has a molecular weight of 36.61 ± 1.40 kDa (isoform A) and 14.1 ± 0.10 kDa (isoform B) from estimation by gel filtration on Biogel P-100 column.

Kinetic parameters of purified cellulase

The Michaelis–Menten constants K_m and V_{max} were determined by performing the enzyme assay in the presence of various concentrations of CMC as substrate. The values of K_m and V_{max} of the cellulase were estimated from the Lineweaver–Burk plot to be 0.13 ± 0.04 mg/ml and 3.84 ± 0.05 U/ml for isoform A (Figure 1), and 0.58 ± 0.06 mg/ml and 13.8 ± 0.10 U/ml for isoform B (Figure 2).

Optimum pH of cellulase

The purified cellulase had optimum pH of 7.0 for both isoforms of the enzyme (Figures 3 and 4). It was active over a broad range of pH 5.0–8.0. It retained over

Table 2. Summary of purification protocols of crude cellulase from *Enterobacter cloacae* IP8.

Purification step	Total volume (ml)	Protein concentration (mg/ml)	Enzyme activity (Units/ml)	Total protein (mg)	Total activity (Units)	Specific activity (Units/mg protein)	Yield (%)	Purification fold
Crude enzyme	5	0.68	13.19	3.40	65.95	19.4	100	1.00
Ion exchange on QAE Sephadex A-50								
	8	0.12	6.80	0.96	54.40	56.7	82	2.9
Gel filtration on Biogel P-100								
Peak A	6	0.14	3.30	0.84	19.80	23.6	30	1.21
Peak B	6	0.13	3.20	0.78	19.2	24.6	29	1.23

58.0% of its maximal activity at pH 5.0 for isoform A (Figure 3), and over 61.6% of its maximal activity at pH 5.0 for isoform B (Figure 4).

Optimum temperature and thermal stability of cellulase

The activity of the purified cellulase from *E. cloacae* IP8 was determined at different temperatures ranging from 35 to 80 °C. The optima temperature for the activities of the isoforms of the purified cellulase was 60 °C in both cases (Figures 5 and 6). The isoform A retained 50% of its maximal activity at 70 °C (Figure 5) while isoform B retained 75% of its maximal activity at the same temperature (Figure 6). However, above 70 °C, cellulase activities decreased sharply. The purified cellulase demonstrated thermal stability at a temperature of 60 °C with residual activities of 99 and 70% over a period of 30 and 60 min, respectively, for isoform A (Figure 7). Isoform B exhibited thermal stability at the same temperature with residual activities of 100 and 65% over a period of 30 and 60 min, respectively (Figure 8). At about 60 min exposure to a temperature of 60 °C, less than 36% of residual activity

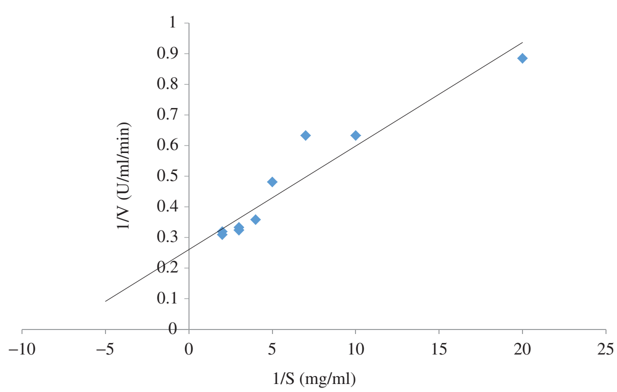

Figure 1. Kinetic parameters (K_m and V_{max}) of the purified cellulase isoform A from *E. cloacae* IP8.

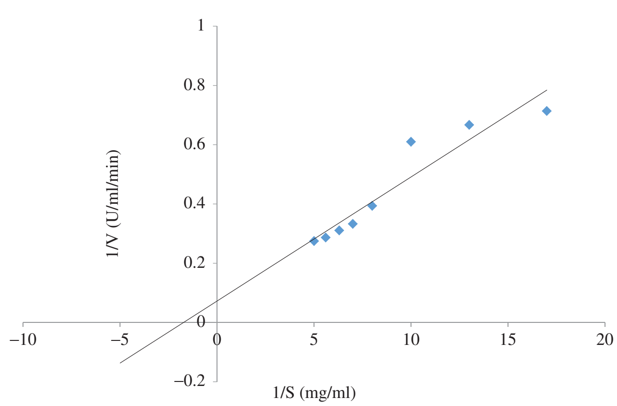

Figure 2. Kinetic parameters (K_m and V_{max}) of the purified cellulase isoform B from *E. cloacae* IP8.

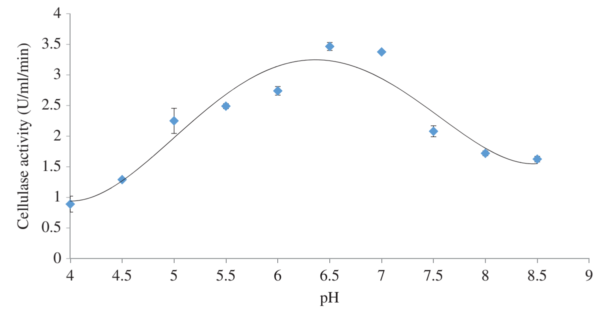

Figure 3. Effect of pH on activity of purified cellulase isoform A from *E. cloacae* IP8.

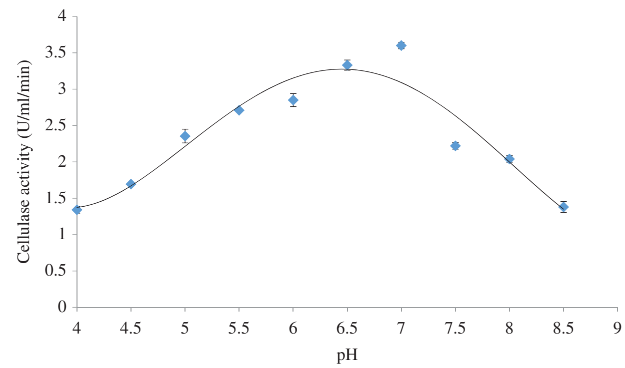

Figure 4. Effect of pH on activity of purified cellulase isoform B from *E. cloacae* IP8.

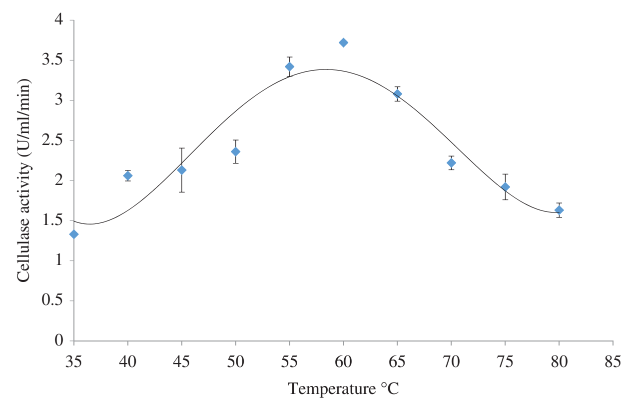

Figure 5. Effect of temperature on activity of purified cellulase isoform A from *E. cloacae* IP8.

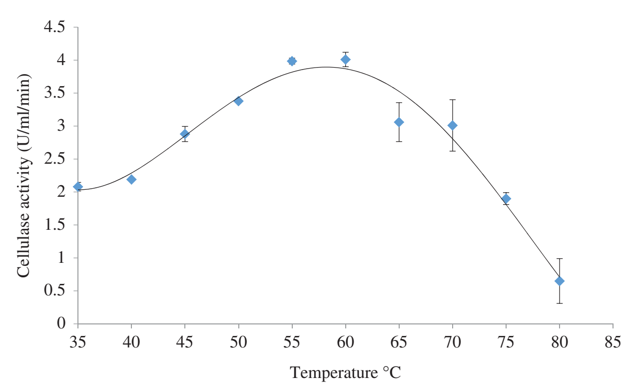

Figure 6. Effect of temperature on activity of purified cellulase isoform B from *E. cloacae* IP8.

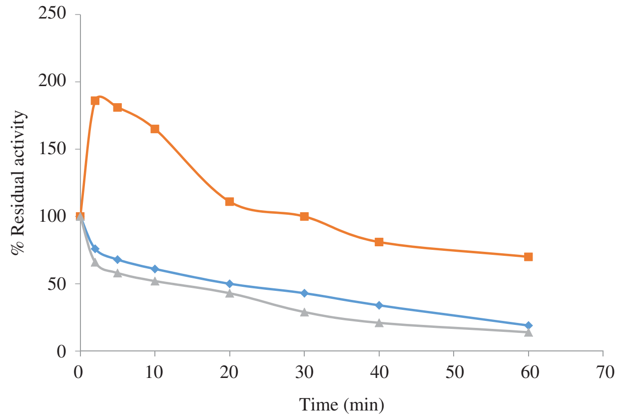

Figure 7. Thermal stability of purified cellulase isoform A from *E. cloacae* IP8. Key: -■- 50 °C; -◆- 60 °C; -▲- 70 °C.

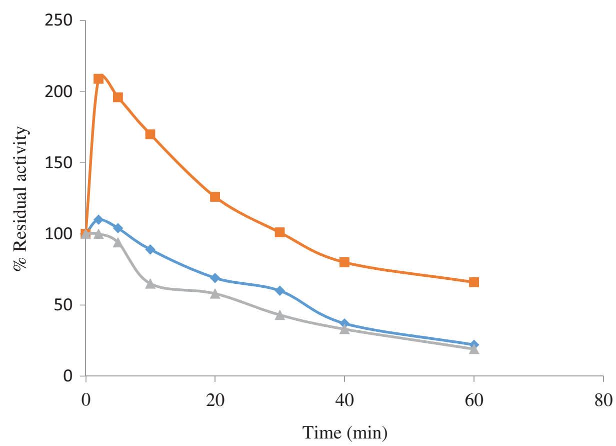

Figure 8. Thermal stability of purified cellulase isoform B from *E. cloacae* IP8. Key: -■- 50 °C; -◆- 60 °C; -▲- 70 °C.

was lost for both isoforms. Furthermore, isoform A had residual activities of 50.2, 99.0 and 31.7% at 50, 60 and 70 °C, respectively after 30 min incubation (Figure 7). Isoform B had residual activities of 66, 100 and 43% at 50, 60 and 70 °C, respectively, after 30 min incubation (Figure 8).

Effect of cations and EDTA on cellulase activity

The effect of various metal ions on the cellulolytic activity of the purified enzyme was tested (Figures 9 and 10). The range of concentrations of cations Ca^{2+} and Na^{+} enhanced the activities of both isoforms of the purified cellulase (Figures 9 and 10). There were significant increases in the residual activities of the purified cellulase up to 200 mM for both isoforms. For Mg^{2+}, concentrations 4–15 mM inhibited the activity of the enzyme, but there were stimulatory effects on the residual activities of the purified cellulase from concentrations 20–200 mM for isoform A (Figure 9).

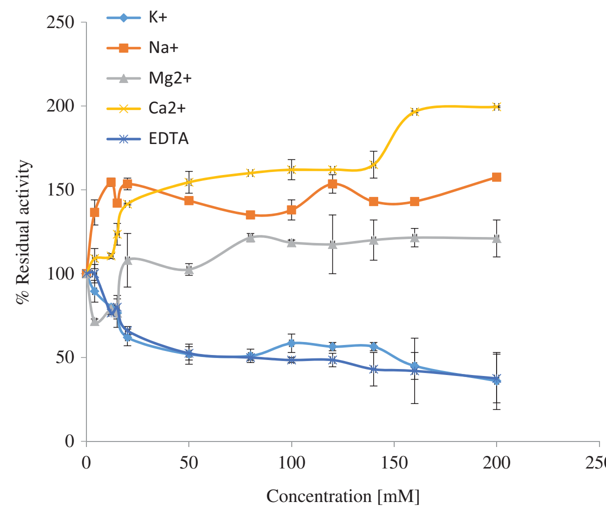

Figure 9. Effect of metal ions and EDTA on activity of purified cellulase isoform A. Key: -◆- K^{+}; -■- Na^{+}; -▲- $Mg2^{+}$; -×- $Ca2^{+}$; -*- EDTA.

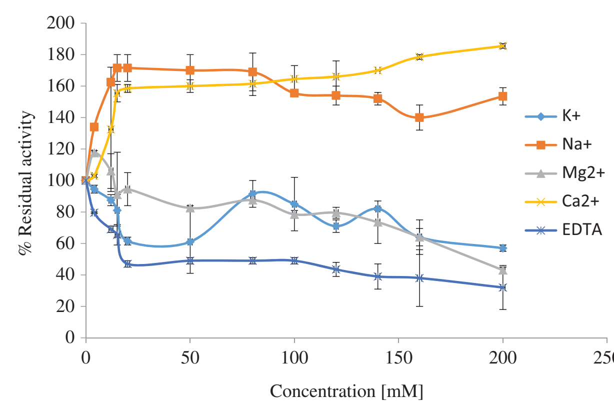

Figure 10. Effect of metal ions and EDTA on activity of purified cellulase isoform B. Key: -◆- K^{+}; -■- Na^{+}; -▲- $Mg2^{+}$; -×- $Ca2^{+}$; -*- EDTA.

However, for isoform B, concentrations 15–200 mM had inhibitory effects on the activity of the purified cellulase (Figure 10). K^{+} inhibited the activities of both isoforms of the purified cellulase across all range of concentrations up to 200 Mm (Figures 9 and 10).

At concentrations above 4 mM, EDTA was inhibitory to the activities of both isoforms of the purified cellulase (Figures 9 and 10).

Discussion

Microorganisms are responsible for the natural recycling of organic carbons such as cellulose and hemicelluloses in the environment (Wang et al. 2008). A bacterium isolated from decayed plant leaf litter of *Lagerstroemia indica* Linn was found to express significant cellulolytic activity under submerged fermentation condition. The bacterium was identified based on morphological and biochemical characterization as *Enterobacter* species. Its identity was confirmed by molecular characterization, based on the 16S rRNA gene sequencing, as a strain of *E. cloacae* with maximum identity of 94% to other *E. cloacae*. Several species of bacteria belonging to genera *Bacillus, Clostridium and Pseudomonas* are reported to exhibit the ability for cellulase production (Levoy et al. 1997; Jagtar et al. 2004; Bakare et al. 2005; Behera et al. 2016). The crude enzyme extract from *E. cloacae* IP8 was concentrated by lyophilization and subsequently purified by ion exchange chromatography on QAE Sephadex A-50 and gel filtration on Biogel P-100, revealing two major peaks and hence two isoforms of the cellulolytic enzyme. The final step of purification yielded 30 and 29%, respectively, of isoform A and B. The molecular weight of the purified cellulase of *E. cloacae* IP8 as determined by from gel filtration column for both isomers were 36.61 ± 1.40 kDa. This result is similar to that of cellulase from a different strain of *E. cloacae* isolated from soil which was found to possess molecular weight of 36 kDa (Muslim and Zaki 2009) and that of *Pseudomonas fluorescence* with molecular weight of 36 kDa (Bakare et al. 2005). Shoseyov and Doi (1990) also reported range of molecular weights of 17–170 kDa for cellulases of different subunits obtained from *Clostridium cellulovorans*. The *K*m values of the purified cellulase were 0.13 and 0.58 mg/ml of CMC, respectively, for isoforms A and B. *K*m value is a measure of the apparent affinity of an enzyme for its substrate. The values of the *V*max observed in this study were 3.84 and 13.8 U/ml/min of CMC for isoforms A and B, respectively. V_{max} is the maximum rate of an enzyme catalyzed reaction. The optimum temperature for the purified cellulase was 60 °C for both isoforms. This result is similar to the findings of Dasilva et al. (1993) on *Bacillus* sp. B.38-2 cellulase and that of Wang et al. (2008) on *Paenibacillus* sp. B39 cellulase, both of which had optimum temperature of 60 °C for activity. However, lower optimum temperature of 35 °C was reported of cellulases from a strain of *E. cloacae* (Muslim and Zaki 2009) and *P. fluorescence* (Bakare et al. 2005). The observed difference in characteristics might be strain-related (Sun et al. 2013). *E. cloacae* IP8 cellulase therefore has potential for industrial applications requiring high temperatures as a result of its relatively high temperature of activity. The two isoforms of the purified cellulase also exhibited appreciable thermal stabilities. The purified cellulase demonstrated thermal stability at a temperature of 60 °C with residual activities of 99 and 70% over a period of 30 and 60 min, respectively, for isoform A and 100 and 65% over a period of 30 and 60 min, respectively for isoform B. Purified cellulase from *E. cloacae* IP8 is thus more stable than that *Bacillus* sp. cellulase which lost 50% of activity when preheated at 50 °C for 10 min (Shikata et al. 1990). Cellulose saccharification processes at elevated temperatures serve to protect both substrates and products of the reaction from microbial contamination and deterioration (Leghlimi et al. 2013). Thermostability is a greatly sought-after characteristic for cellulases in industrial applications such as the bioconversion of lignocellulose (Wang et al. 2008; Li et al. 2008; Liang et al. 2009). Cellulase of *E. cloacae* IP8 may thus be suitable for biomass conversion for biofuel production under harsh conditions. The optimum pH for the purified cellulase in this study was 7.0. A pH value of 7.5 was reported for cellulase of a strain of *E. cloacae* from soil (Muslim and Zaki 2009). The pH value obtained in this study was higher than pH 6.0–6.5 reported for *Bacillus subtilis* YJ1 cellulase (Yin et al. 2010). pH values above and below neutral pH led to decrease in activities of both isoforms of the cellulase from *E. cloacae* IP8. Thus, the purified enzyme can be classified as being active at neutral pH. Purified cellulase activity of *E. cloacae* IP8 was enhanced in the presence of Ca^{2+} and Na^{+} in all range of concentrations, for both isoforms A and B. While Mg^{2+} concentrations 4–15 mM inhibited the activity of the enzyme, it had stimulatory effects on the activities of the purified cellulase from concentrations 20–200 mM for isoform A. However, for isoform B, Mg^{2+} concentrations 15–200 mM had inhibitory effects on the activity of the purified cellulase. However, K^{+} strongly inhibited the activities of both isoforms of the purified cellulase across all range of concentrations up to 200 mM. Structural changes of proteins are known to occur in the presence of metal ions, which inhibit or enhance enzymatic activity by modulating their catalytic sites (Schiffmann et al. 2005). Enhancement of cellulase activity by addition of Ca^{2+} corroborates previously reported enhancement of the substrate binding affinity of cellulase by Ca^{2+} stabilizing the conformation of the catalytic site (Zin et al. 2014). EDTA was found to be inhibitory to the activity of the cellulase used in this study. EDTA as a metal chelating agent probably acts by inactivating the cellulase either by removing metal ions from the enzyme through the formation of coordinating complex or binding inside the enzyme as a ligand, as had been noted by Schmid (1997).

Conclusion

This study has revealed the purified cellulase from *E. cloacae* IP8 as a thermostable, neutral metalloenzyme. The enzyme may be suitable for large scale industrial and biotechnological applications in the bioconversion of agricultural wastes into economically useful products such as fuels and chemicals. Relatively wide pH (5.0–8.0) and temperature (50–70 °C) stability, low *K*m and high *V*max values are indices that may make the enzyme suitable for such applications.

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Disclosure statement

The authors report no declarations of interest.

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