Journal of Animal & Plant Sciences, 2012. Vol. 13, Issue 2: 1747-1757
Publication date: 30/ 3/ 2012, http:/ / www.m.elewa.org/ JAPS; ***ISSN 2071 - 7024***

Purification and biochemical characterization of beta-glucosidase from cockroach, *Periplaneta americana*

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Key words: *Periplaneta Americana*, Purification, Biochemical characterization, Beta-glucosidase

1 SUMMARY

A beta-glucosidase was purified from *Periplaneta americana* by chromatography on ion-exchanges, gel filtration and hydrophobic interaction columns. The preparation was shown to be homogeneous on polyacrylamide gel. The enzyme was purified 9.93-fold to a specific activity of 40.33 (U/ mg of protein) and an overall yield of 1.54 %. SDS-PAGE revealed a single polypeptide of 43.8 kDa, thus indicating apparent homogeneity of the final enzyme preparation. Gel filtration chromatography showed that the enzyme was a 46.5 kDa monomeric protein. The purified enzyme exhibited pH and temperature optima at 3.6 and 55°C, respectively. The beta-glucosidase was stable at 37C and its pH stability was in the range of 3.0-4.0. The enzyme readily hydrolyzed *p*-nitrophenyl-beta-D-glucoside, cellobiose, cellodextrins, *p*-nitrophenyl-N-acetyl-beta-D-glucopyranoside, *p*-nitrophenyl-N-acetyl-beta-D-galactopyranoside and required strictly β-glyco configuration for activity. The specificity constants (V_{max}/ K_M) values for N-acetyl-beta-D-Glucopyranoside, *p*-nitrophenyl-beta-D-glucoside and cellobiose were respectively 31.05 $mM^{-1}s^{-1}$, 12.47 $mM^{-1}s^{-1}$ and 4.59 $mM^{-1}s^{-1}$. It required no metal ion as a co-factor. Zn^{2+}, Ba^{2+}, *p*CMB inhibited the beta-glucosidase activity.

2 INTRODUCTION

Beta-Glucosidase (EC 3.2.1.21; beta-D-glucoside glucohydrolase) hydrolyses terminal, non-reducing 1, 4-linked-beta-glucose residues releasing beta-D-glucose from oligo or polysaccharides (Webb, 1992). Other names for this enzyme are gentiobiase and cellobiase. Beta-Glucosidase has been subdivided into three classes based on substrate specificity. Class 1 includes enzymes with glycosyl beta-glycosidase and aryl beta-glycosidase activity; these enzymes have the ability to hydrolyze cellobiose, lactose, beta-*p*-nitrophenylglucoside, beta-*p*-nitrophenylgalactoside, beta-*p*-nitrophenylfructoside and other similar substrates. Class 2 includes those with only glycosyl beta-glucosidase activity; therefore, they can only hydrolyze substrates such as cellobiose and lactose. Class 3 includes enzymes with only aryl (or alkyl) beta-glucosidase activity; these enzymes would have significant activity towards beta-p-nitrophenylglucoside and similar substrate (Terra and Ferreira, 1994).This enzyme is widely spread in nature, predominantly being produced by microorganisms such as molds, fungi and bacteria (Bayer *et al.* 1998). Beta-glucosidases from fungi and bacteria have been studied extensively (Gueguen *et al*, 2001; Saha *et al*, 2002; Saloheimo *et al*, 2002; Li *et al*, 2002; Wallecha and Mishra 2003). However, little

attention has been paid to beta-glucosidases from insects. However, little attention has been paid to β-glucosidases from insects. The only accounts in the literature refer to enzymes isolated from midgut cells of *Rhynchosciara Americana* (Fungus gnat) larvae (Ferreira and Terra 1983), *Tenebrio molitor* (Mealworm beetle) larvae (Ferreira *et al*, 2001), *Rhynchophorus palmarum* (Palm weevil) larvae (Yapi *et al*, 2008), termites *Macrotermes mulleri* (Rouland *et al*, 1992), *Macrotermes bellicosus* (Matoub, 1993), *Macrotermes subhyalinus* (Kouamé et al. 2005a), *Macrotermes bellicosus* (Binaté *et al*, 2008) and honey bees (*Apis mellifera*) (Pontoh and Low, 2002). Most of these enzymes were shown to have the polyspecificity between gluco-, fructo-, fuco-, galacto- xylo- and arabino-based substrates (Marana *et al*, 2000; Ferreira *et al*, 2001; Li *et al*, 2002; Wallecha and Mishra, 2003; Kouamé *et al*. 2005a). In this study, we attempted to purify and characterize the beta-glucosidase from *Periplaneta americana* (L.) (Insecta: Dictyoptera). In addition, a number of physicochemical and kinetic properties of this enzyme were determined.

3 MATERIALS AND METHODS

3.1 Materials: *Periplaneta americana* were captured from rooms. They were collected directly from the nest and then stored frozen at -20°C. Substrates: Cellobiose, Cellotriose, Cellotetraose, carboxymethylcellulose, inulin, Mannan, *p*-nitrophenyl-glycopyranosides and *p*-nitrophenyl-N-acetyl-glycosides were purchased from Sigma Aldrich. DEAE–Sepharose Fast-flow, Sephacryl S-100 HR, Sephacryl S-200 HR, Phenyl Sepharose 6 Fast-flow gels were obtained from Pharmacia Biotech. Bovine Serum Albumin (BSA) was purchased from Fluka Biochemika. The standard proteins used for molecular weight determination on polyacrylamide gel electrophoresis (PAGE) were provided by Bio-Rad.

3.2 Preparation of crude extract: Weighed *Periplaneta americana* of approximately 30 g were rinsed in cold distilled water and dried with blotting paper. They were ground in a pre-chilled mortar in 60 ml of 20 mM phosphate citrate buffer pH 3.6. The homogenate was subjected to sonication using a TRANSSONIC T_{420} for 10 min and then centrifuged at 6.000 rpm for 30 min. The supernatant filtered through cotton was used as the crude extract.

3.3 Enzyme assays: Under the standard test conditions, beta-glucosidase activity assay was performed at 37 °C for 10 min in 100 mM phosphate citrate buffer pH 3.6 containing 1.5 mM of *p*-nitrophenyl-beta-D-glucopyranoside. After prewarming the mixture (37 °C) for 5 min, the reaction was initiated by adding enzyme solution (50 µl). The final volume was 250 µl. Determination of other *p*-nitrophenylglycosidase activities was carried out under the same experimental conditions. The reactions were stopped by adding 2 ml of sodium carbonate 2 % (w/ v). Enzyme activity was quantified by measuring the released *para*-nitrophenol (*p*NP) at 410 nm using a spectrophotometer GENESIS 5. *p*NP was used as standard. Polysaccharidase activity was assayed by the dinitrosalicylic acid procedure (Bernfeld 1955), using 0.5 % (w/ v) polysaccharide (carboxymethylcellulose, inulin and mannan) as substrate. The enzyme (50 **µ**l) was incubated for 30 min at 37°C with 170 **µ**l buffer (100 mM phosphate citrate, pH 3.6) and 80 **µ**l polysaccharide. The reaction was stopped by addition of 300 **µ**l dinitrosalicylic acid and heating in boiling water for 5 min. The absorbance was read at 540 nm after cooling on ice for 5 min. The oligo-saccharidase activity was determined by measuring the amount of glucose liberated from 10 mM oligosaccharide (cellobiose and cellodextrins) after incubation at 37°C for 10 min in a 20 mM phosphate citrate buffer (pH 3.6). The amount of glucose was determined by the glucose oxidaseperoxidase method (Kunst *et al*., 1984) after heating the reaction mixture at 100°C for 5 min.

One unit (U) of enzyme activity was defined as the amount of enzyme capable of releasing one µmol of *p*-nitrophenol or glucose per min under the defined reaction conditions. Specific activity was expressed as units per mg of protein (U/ mg of protein).

3.4 Protein assays: Protein elution profiles from chromatographic columns and concentrations of purified enzyme were determined by the folin ciocalteus method **(**Lowry *et al*., 1951**)**. Bovine serum albumin was used as the standard protein.

3.5 Purification procedures: All steps in the purification procedure were carried out in the cold room. The crude extract (10 ml) was loaded onto a DEAE-Sepharose Fast-flow (2.3 cm x 8.5 cm) previously equilibrated with 20 mM phosphate citrate buffer pH 3.6. The unbound proteins were removed by washing the gel with two bed volumes of the same buffer pH 3.6. Bound proteins were eluted using a stepwise salt gradient (0, 0.1, 0.5 and 1 M) of NaCl in 20 mM phosphate citrate buffer pH 3.6. Fractions (3 ml each) were collected at a flow rate of 3 ml/ min and assayed for enzyme activity. The active fractions were pooled and submitted to ammonium sulphate precipitation at 80 % saturation overnight in a cold room. The precipitate obtained after centrifugation (6,000 rpm for 30 min) was dissolved in 1 ml of 20 mM phosphate citrate buffer pH 3.6. The resulting enzyme solution was loaded onto Sephacryl S-100 HR column (1.6 cm x 64 cm) equilibrated with the same buffer pH 3.6. Proteins were eluted at a flow rate of 0.25 ml/ min using 20 mM phosphate citrate buffer pH 3.6 and fractions of 1 ml were collected. The pooled fractions from the previous step was saturated to a final concentration of 1.7 M ammonium sulphate and applied on a Phenyl-Sepharose 6 Fast-flow column (1.4 cm x 5.0 cm) previously equilibrated with 20 mM phosphate citrate buffer pH 3.6 containing 1.7 M ammonium sulphate. Proteins were eluted using a reverse stepwise gradient of ammonium sulphate concentration (from 1.7 to 0 M) in the same acetate buffer. Fractions of 1 ml were collected at a flow rate of 0.43 ml/ min and active fractions were pooled. The pooled fractions were dialysed against 20 mM phosphate citrate buffer pH 3.6 overnight in a cold room and constituted the purified enzyme.

3.6 Polyacrylamide gel electrophoresis (PAGE): To check purity and determine molecular weight, the purified enzyme was analyzed using sodium dodecyl sulphate-polyacrylamide gel electrophoresis on a 10 % separating gel and a 4 % stacking gel (Hoefer mini-gel system; Hoefer Pharmacia Biotech, San Francisco, USA), according to the procedure of Laemmli (1970). The samples were denatured by a 5 min treatment at 100°C. Electrophoretic buffers contained sodium dodecyl sulphate and beta-mercaptoethanol. Proteins were stained with silver nitrate according to Blum *et al.* (1987). The standard molecular weight (BIO-RAD) comprising tryptic inhibitor (28.9 kDa), carbonic hydratase (34.8 kDa), ovalbumine (49.1 kDa), bovine serum albumin (80 kDa), beta galactosidase (12.4 kDa), lactate deshydrogenase (140 kDa), Myosine (200 kDa).

3.7 Native molecular weight determination: The molecular weight of the native beta-glucosidase was estimated by gel filtration on a Sephacryl S-200 HR columns (capacity, 0.8 cm x 35 cm; flow rate, 0.2 ml/ min; fraction, 0.5 ml) equilibrated with 20 mM phosphate citrate buffer pH 3.6. The standard proteins (Sigma) used for calibration were beta-amylase from sweet potato (206 kDa), bovine serum albumin (66 kDa), ovalbumin from egg white (45kDa) and cellulase from *Aspergillus niger* (26 kDa). Blue dextran (1000 kDa) and potassium ferricyanide (0.33 kDa) were used to determine the void and total volume, respectively.

3.8 pH and temperature optima: The effect of pH on beta-glucosidase activity was determined by measuring the hydrolyzis of *p*-nitrophenyl-beta-D-glucopyranoside in a series of buffers at various pH values ranging from 2.6 to 8.0. The buffers used were sodium acetate buffer (100 mM) from pH 3.6 to 5.6, phosphate citrate buffer (100 mM) from pH 2.7 to 7.0 and phosphate buffer (100 mM) from pH 5.6 to 8.0. Data from overlapping pH ranges of different buffers were averaged. Beta-glucosidase activity was measured at 37°C under the standard test conditions.

The effect of temperature on beta-glucosidase activity was followed in 100 mM phosphate citrate buffer pH 3.6 over a temperature range of 30 to 80 °C using 5 mM *p*-nitrophenyl-beta-D-glucopyranoside under the standard test conditions.

3.9 pH and temperature stabilities: The stability of the beta-glucosidase was followed over the pH range of 2.6 to 8.0 in 100 mM buffers. The buffers were the same as in the study of the pH and temperature optima (above). After 2 h pre-incubation at 37°C, aliquots were taken and immediately assayed for residual beta-glucosidase activity. The thermal stability of the enzyme was determined at 37 and 55°C after exposure to each temperature for a period from 15 to 210 min. The enzyme was incubated in 100 mM phosphate citrate buffer pH 3.6. Aliquots were withdrawn at intervals and immediately cooled in ice-cold water. Residual activities, determined in both cases at 37°C under the standard test conditions, are expressed as percentage activity of zero-time control of untreated enzyme (Kouamé *et al.*, 2005).

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3.10 Determination of kinetic parameters: The kinetic parameters (K_M, V_{max} and V_{max}/K_M) were determined in 100 mM phosphate citrate buffer pH 3.6 at 37°C. Hydrolysis of *p*-nitrophenyl-β-D-glucopyranoside and *p*-nitrophenyl-N-acetyl-β-D-glucopyranoside were quantified on the basis of released *p*-nitrophenol, similar to the standard enzyme assay. Cellobiose hydrolysis was quantified by determination of released glucose, determined by the oxidase peroxidase method (Kunst et al., 1984) after heating the reaction mixture at 100°C for 5 min. K_M and V_{max} were determined from Lineweaver-Burk plot using different concentrations of *p*-nitrophenyl-β-D-glucopyranoside (0.5-5 mM), *p*-nitrophenyl-N-acetyl-β-D-glucopyranoside (0.5-5 mM) and cellobiose (1-10mM).

3.11 Effect of chemical agents on enzyme activity: The enzyme was incubated with 5 mM or 0.5 % (w/ v) of different chemical agents for 20 min at 37°C. After incubation, the residual activity was determined by the standard enzyme assay using *p*-nitrophenyl-beta-D-glucopyranoside as a substrate. The results were expressed as a percentage of the control without the chemical agents.

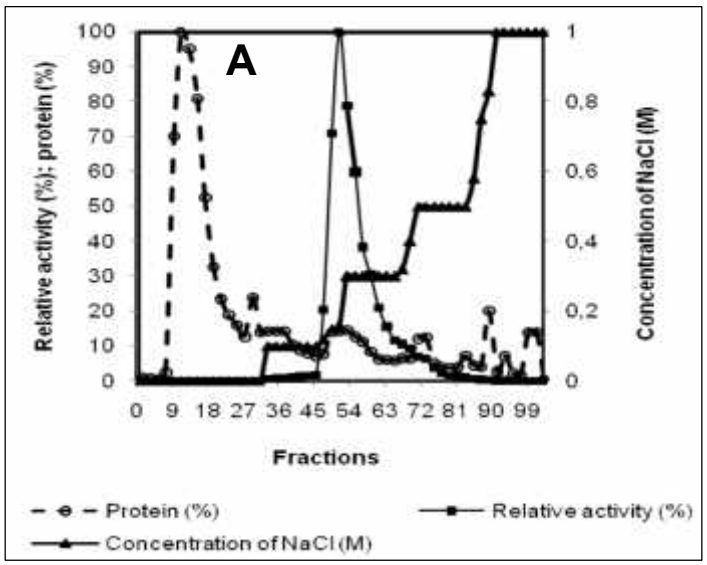
4 RESULTS

4.1 Purification procedures: Purification procedure of beta-glucosidase from *Periplaneta americana* is summarized in table 1. The purification protocol involved three steps of chromatography (Figure 1).

Table 1: Purification procedure of beta-glucosidase from *Periplaneta americana*.

Purification steps	Total protein (mg)	Total activity [a](Units)	Specific activity (Units/ mg)	Yield (%)	Purification factor
Crude extract	102.06	413.92	4.06	100	1
DEAE-Sepharose Fast-flow	19.07	97.26	5.10	69.56	1.26
Ammonium sulphate precipitation (80 %)	1.60	25.71	16.07	25.52	3.96
Sephacryl-S 100 HR	0.65	16.46	25.32	13.40	6.24
Phenyl-Sepharose 6 Fast-flow	0.03	1.21	40.33	1.54	9.93

[a] One unit equals 1 µmol of *p*NP release per min.



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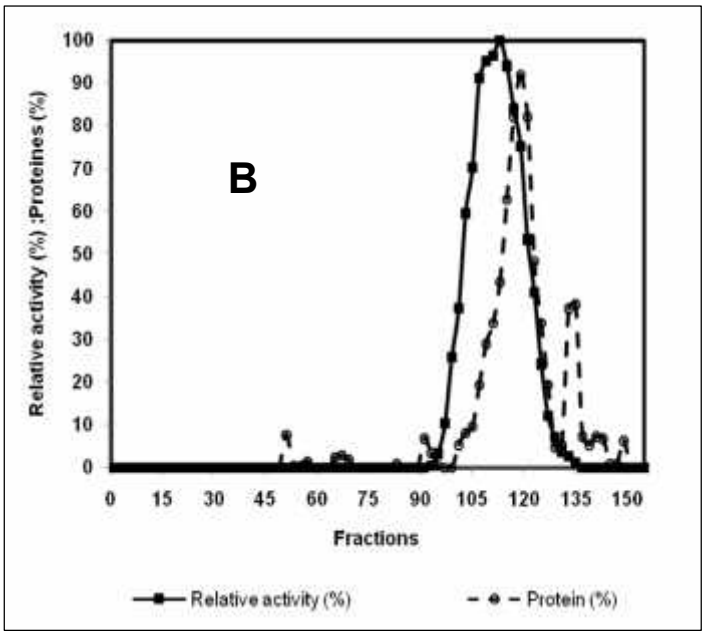

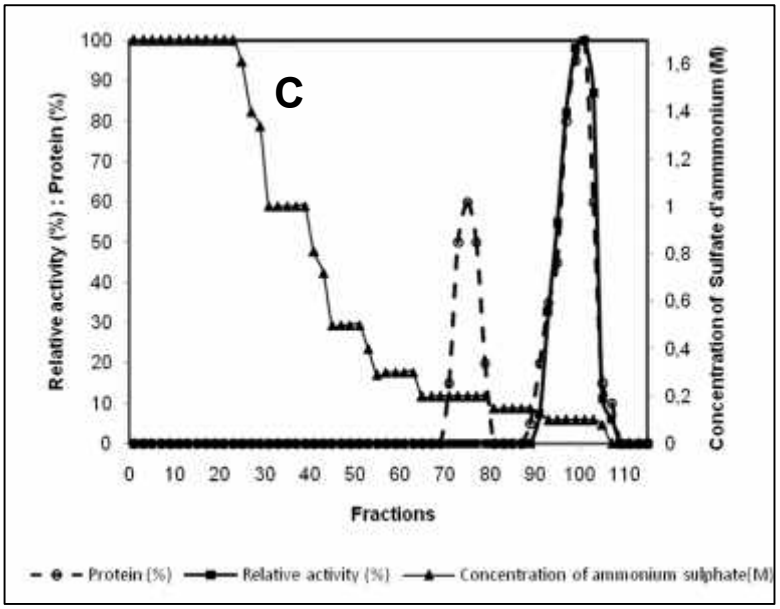

Figure 1: Chromatographic profiles of beta-glucosidase from *Periplaneta americana*. The enzyme activity was measured in 100 mM phosphate citrate buffer pH 3.6 at 37 °C using *p*NP-beta-D-glucopyranoside as a substrate. (A) Anion-exchange chromatography on DEAE-Sepharose Fast-flow. (B) Gel filtration chromatography on Sephacryl S-100 HR. (C) Hydrophobic interaction chromatography on Phenyl-Sepharose 6 Fast-flow.

One major peak of beta-glucosidase activity was resolved on DEAE-Sepharose Fast-flow column, with 0.3 M NaCl concentration in 20 mM phosphate citrate buffer (pH 3.6) (Figure 1A). These fractions were pooled and further separated by Sephacryl S-100 HR. beta-glucosidase activity from *Periplaneta americana* was finally purified by Phenyl-Sepharose 6 Fast-flow hydrophobic interaction chromatography as show in figure 1C. One peak was observed on the chromatogram.

Active fractions were collected as purified beta-glucosidase and used further characterization of the enzyme. The specific activity was 40.33 UI/ mg of protein. The purity of the purified beta-glucosidase increased by 9.93 fold and the yield was 1.54 % (Table 1).Beta-glucosidase from *Periplaneta americana* showed a single protein band on SDS-PAGE gel electrophoresis staining with silver nitrate (Figure 2).

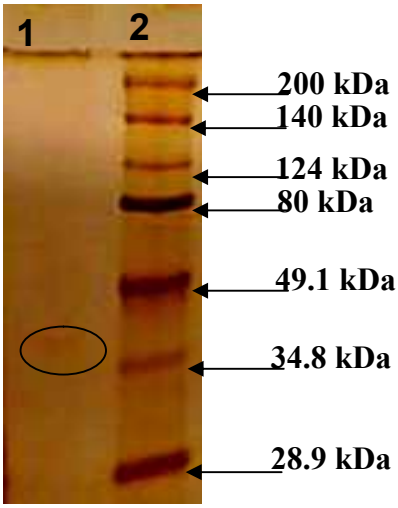

Figure 2: SDS-PAGE analysis of the purified beta-glucosidase from *Periplaneta americana*. The sample was loaded onto a 10 % gel. Lane 1', purified beta-glucosidase, Lane 2', molecular weight markers.

4.2 Molecular weight determination: SDS-PAGE profile of purified enzyme is depicted in figure 2. After SDS-PAGE analysis under reducing conditions, beta-glucosidase activity from *Periplaneta americana* showed a single protein band. Its relative molecular weight was estimated to be 43.8 kDa (Figure 2). Gel filtration chromatography on Sephacryl S-200 HR column showed an approximately molecular weight of 46.5 kDa for native enzyme. These results suggest that the purified enzyme has a monomeric structure.

4.3 pH and temperature optima: The optimum values of pH and temperature for studying beta-glucosidase activity are represented in table 2. The enzyme activity was maximal at 55 °C and at pH 3.6. The best stability was observed in phosphate citrate buffer (data not shown) at a pH range of 3 to 4.

Table 2: Some physicochemical characteristics of beta-glucosidase from *Periplaneta americana*

Physicochemical properties	**Values**
Optimum temperature (°C)	55
Optimum pH	3.6
pH stability	3-4
Molecular weight (kDa)	
SDS-PAGE	43.8
Gel filtration	46.5
Activator agents	Na^{2+},K^{+}, Ca^{2+},EDTA
Inhibitor agents	Zn^{2+}, Ba^{2+}, *p*CMB[a]

a: sodium parachloromercuribenzoate

The thermal denaturation was investigated by incubation of beta-glucosidase at various temperatures for 15 min. The result showed that this enzyme was fairly stable at temperature up to 55 °C. Above this temperature, its activity declined as the temperature increased (Figure 3A).

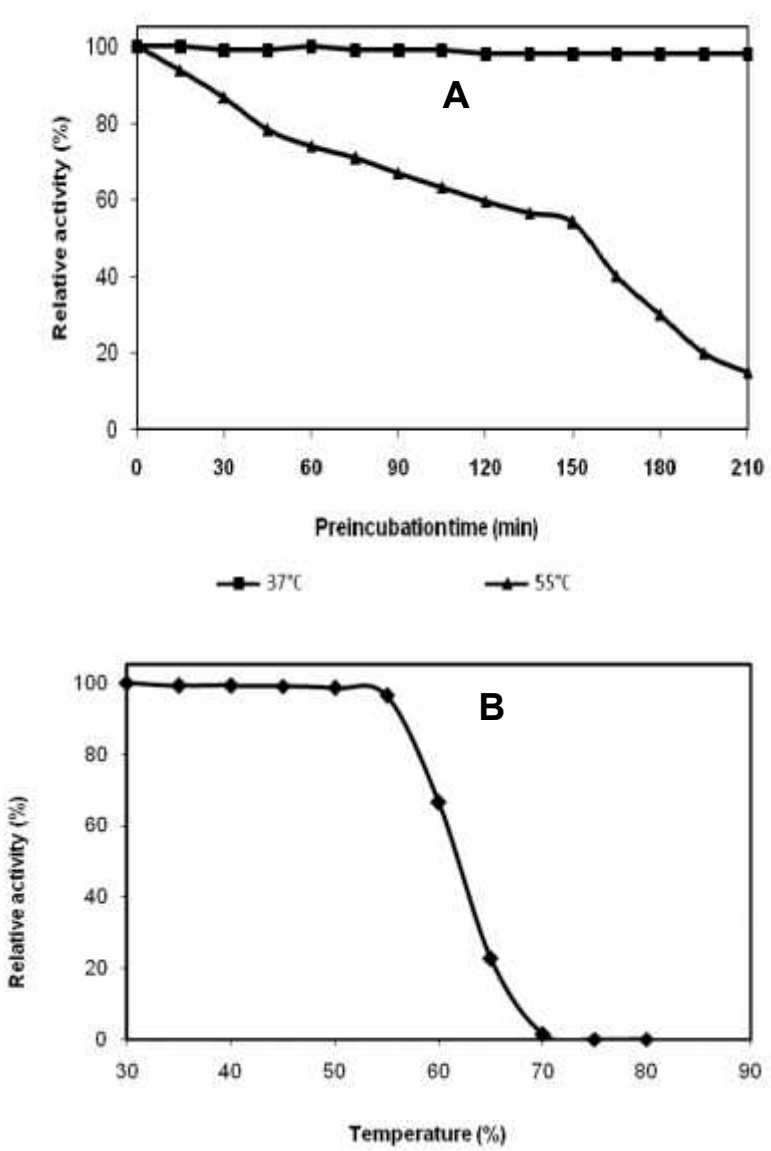

Figure 3: Thermal stability of beta-glucosidase from *Periplaneta americana*. (A) Thermal inactivation, the enzyme was pre-incubated at 37 and 55 °C in 100 mM phosphate citrate buffer (pH 3.6). At the time intervals indicated, aliquots were withdrawn and the residual activity measured at 37 °C under standard assay conditions. (B) Thermal denaturation, the enzyme was pre-incubated at each temperature for 15 min in 100 mM phosphate citrate buffer (pH 3.6). The residual activity is expressed as percentage activity of zero-time control of untreated enzyme.

The thermal inactivation study at pH 3.6 indicated that, beta-glucosidase activity from *Periplaneta americana* remained fully stable for 210 min at 37 °C (Figure 3B). However, at 55 °C (its optimum temperature) the half-life of the enzyme was obtained at 150 min.

4.4 Substrate Specificity: *Periplaneta americana* beta-glucosidase did not attack the following *p*nitrophenyl glycosides: alpha-glucoside, alpha-galactoside, alpha and beta-mannoside, alpha and beta-xyloside, alpha and beta-L-arabinoside, alpha and beta-fucoside; or the polysaccharides carboxymethylcellulose, inulin and (Table 3). However, the enzyme attacked more active on cellobiose, cellodextrins (Table 3), *p*NP-beta-D-glucopyranoside, *p*NP-N-acetyl-beta-D-glucopyranoside and *p*NP-N-acetyl-beta-D-galactopyranoside.

Table 3: Substrate specificity of beta-glucosidase from *Periplaneta americana*. Values given are the averages of at least three experiments

Substrates	Concentration	Relative activity (%)
Oligosaccharide and polysaccharide		
Cellobiose	0.5 % (p/ v)	100
Cellotriose	0.5 % (p/ v)	85.29
Cellotetraose	0.5 % (p/ v)	79.54
carboxymethylcellulose	0.5 % (p/ v)	0
Inulin	0.5 % (p/ v)	0
Mannan	0.5 % (p/ v)	0
Synthetic chromogenic substrates		
*p*NP-beta-D-glucopyranoside	5 mM	100
*p*NP-beta-D-galactopyranoside	5 mM	10.20
*p*NP-N-acetyl-beta-D-glucopyranoside	5 mM	115
*p*NP-N-acetyl-beta-D-galactopyranoside	5 mM	97.34
p-nitrophenyl-beta-D-galactopyranoside	5 mM	0
p-nitrophenyl-alpha-D-galactopyranoside	5 mM	0
p-nitrophenyl-beta-D-mannopyranoside	5 mM	0
p-nitrophenyl-alpha-D-mannopyranoside	5 mM	0
p-nitrophenyl-beta-D-fucopyranoside	5 mM	0
p-nitrophenyl-alpha-D-fucopyranoside	5 mM	0
p-nitrophenyl-beta-D-xylopyranoside	5 mM	0
p-nitrophenyl-alpha-D-xylopyranoside	5 mM	0
p-nitrophenyl-beta-L-arabinofuranoside	5 mM	0
p-nitrophenyl-alpha-L-arabinofuranoside	5 mM	0

The effect of substrate concentration on enzymatic activity was examined using cellobiose, *p*-nitrophenyl-beta-D-glucopyranoside and *p*NP-N-acetyl-beta-D-glucopyranoside. With the three substrates, the enzyme obeyed the Michaelis-Menten equation (Table 4). The K_M, V_{max} and V_{max}/ KM values are reported in Table 4. The catalytic efficiency of beta-glucosidase, given by the V_{max}/ KM ratio is much higher for the *p*NP-N-acetyl-beta-D-glucopyranoside than the cellobiose and *p*NP-beta-D-glucopyranoside.

Table 4: Kinetic parameters of beta-glucosidase from *Periplaneta americana* towards *pNP*-beta-D-Glucopyranoside, N-acetyl-β-D-Glucopyranoside and cellobiose. Values given are the averages of at least three experiments

Substrats	K_M	V_{max}	V_{max} / K_M
*p*NP-beta-D-Glucopyranoside	3.29	41.67	12.47
*p*NP-N-acetyl-beta-D-Glucopyranoside	0.37	11.49	31.05
cellobiose	8.52	39.12	4.59

The Michaelis constants (K_M) and the maximum velocities (V_{max}) are expressed as mM and units/ mg protein, respectively. For cellobiose, the value of V_{max}/ K_M was calculated taking into account that one mol of cellobiose liberates two mol of glucose.

4.5 Effect of metal ions, chelating and reducing agents and detergents: The effect of chemicals on *Periplaneta americana* beta-glucosidase was examined. Most of the chemicals tested did not affect the activity of the enzyme. However, Na^{2+} (Sodium), K^+ (Potassium), Ca^{2+} (Calcium) and EDTA at 5 mM had a slight stimulatory effect. In contrast Zn^{2+} (Zinc), Ba^{2+} (Barium), *p*CMB acted as inhibitors (Table 2).

5 DISCUSSION

A beta-glucosidase was purified to homogeneity from *Periplaneta americana*. The purification protocol involves three steps of column chromatography: anion exchange, gel filtration, and hydrophobic interaction. Although DEAE-Sepharose chromatography led to a low purification factor, this step permitted the elimination of many pigments that are very abundant in the crude extract. The specific activity of the beta-glucosidase is lower than those obtained for the two beta-glycosidases purified previously from *Tenebrio. molitor* larvae midgut (Ferreira *et al*. 2001) and the two beta-glycosidases from the midgut of the sugarcane borer, *Diatraea saccharalis* (Azevedo *et al*., 2003). However, it is higher than those obtained for the beta-glucosidases from *Rhynchophorus palmarum* (YAPI *et al*., 2008) and *T. molitor* larvae lumen (Ferreira *et al*., 2003).

The similarity in the molecular weights determined by denaturing SDS-PAGE and native gel filtration suggest that purified enzyme is likely to be monomeric, as found in beta-glucosidases from *Thermus sp.*IB-21 (Kang *et al*., 2005) and *Rhynchophorus palmarum* (YAPI *et al*., 2008). The enzyme is highly active at pH 3.6, and it is more stable at a pH range of 3.0-4.0. So, the optimum pH is a good compromise between the activity and the stability of the enzyme for hydrolysis of natural substrates and biosynthesis reaction which need to be performed for a long time. This pH is similar to that of beta-glucosidases from *Lactobacillus brevis* (Michlmayr *et al*., 2009). *P. americana* beta-glucosidase was optimally active at 55 °C. This enzyme thermal behaviour is in agreement with insect beta-glucosidase (Kouamé *et al*, 2005; Yapi *et al*., 2008).

A variety of glycosides were tested for their ability to serve as substrates. The *P. americana* beta-glucosidase was inactive on high molecular mass polymer such as carboxymethylcellulose, inulin and mannan. The only substrates that were hydrolyzed by the enzyme were cellobiose, cellodextrins, *p*-nitrophenyl-beta-D-glucopyranoside, *p*NP-beta-D-galactopyranoside, N-acetyl-beta-D-glucopyranoside N-acetyl-beta-D-galactopyranoside. These results suggest that this beta-glucosidase is an exo-glycosidase with a high specificity for the beta anomeric configuration of the glucosidic linkage. This pattern is similar to the activity of the beta-glucosidases from fungi *Sclerotium rolfsii* (Shewale and Sadana, 1981) and *Aspergillus niger* (Watanabe *et al*, 1992). Its inability to cleave alpha-linkages is commonly seen for purified beta-glucosidases. This high substrate specificity suggests that the beta-glucosidase could be used as a tool in the structural analysis of D-glucose containing oligosaccharide chains of glycoproteins, glycolipids, and cellulose.

The results of the kinetic study correlate well with substrate specificity and showed a Lineweaver-Burk plot. Among the substrates used, the purified beta-glucosidase showed the highest catalytic efficiency (V_{max}/ K_M) towards N-acetyl-beta-D-Glucopyranoside. Therefore, this enzyme belongs to the alkyl and/ or aryl-beta-glucoside hydrolysing group (Group 3), according to the classification of Terra and Ferreira (1994). This result indicates that the role of beta-glucosidase in the alimentary canal of *P. americana* is not for the digestion of cellulosic material but is most likely present to hydrolyze glycoside toxins ingested by the cockroach (Terra and Ferreira, 1994).

Various metal cations and potential inhibitors modified the activity of the purified enzyme. The enzyme was indeed greatly inhibited by Zn^{2+}, Ba^{2+} and *p*CMB. Inhibition by *p*CMB, suggests that a sulfhydryl group may be involved in the catalytic center of the enzyme but rather may be essential for maintenance of the three-dimensional structure of the active protein. This result is similar to that for the beta-glucosidases from bacteria *Leuconostoc mesenteroides* (Guegen *et al*. 1997) and fish *Tilapia* intestine (Taniguchi and Takano, 2004) on beta-1, 4 linkage. The chelating agent EDTA did not inhibit *P. Americana* beta glucosidase activity, indicating that divalent cations are not required for enzyme activation. However, Na^{2+}, K^{+}, Ca^{2} did significantly stimulate enzyme activity. Since Na^{2+}, K^{+}, Ca^{2} were not involved in the stability of this enzyme, these specific cations could play a role in the enzyme function (e.g., by modulating its activity according to environmental conditions).

6 REFERENCES

Azevedo TR, Terra WR and Ferreira C: 2003. Purification and characterization of three β-glycosidases from midgut of the sugar cane borer, *Diatrea saccharalis*. *Insect Biochem. Mol. Biol.* 33: 81-92.

Bayer EA, Chanzy H, Lamed R and Shoham Y: 1998. Cellulose, cellulases and cellulosomes. *Curr. Opin. Struct. Biol.* 8: 548-557.

Bernfeld P: 1955. Amylases, α and β. *Methods in Enzymology* 1: 149-158.

Binate S, N'dri D, Toka M. and Kouame LP: 2008. Purification and characterization of two beta-glucosidases from termite workers *Macrotermes bellicosus* (Termitidae, Macrotermitinae). *J. Appl. Biosci.* 10: 461-470.

Blum H, Beier H and Gross B: 1987. Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. *Electrophoresis* 8: 93-99.

Ferreira C and Terra WR: 1983. Physical and kinetic properties of a plasma-membrane-bound β-D-glucosidase (cellobiase) from midgut cells of an insect (*Rhynchosciara americana*) larvae. *Biochem. J.* 213: 43-51.

Ferreira AH, Marana SR, Terra WR and Ferreira C: 2001. Purification, molecular cloning, and properties of a β-glycosidase isolated from midgut lumen of *Tenebrio molitor* (*Coleoptera*) larvae. *Insect Biochem. Mol. Biol.* 31: 1065-1076

Ferreira AH, Terra WR and Ferreira C: 2003. Characterization of a β-glycosidase highly active on disaccharides and of a beta-galactosidase from *Tenebrio molitor* midgut lumen. *Insect Biochem. Mol. Biol.* 33: 253-256.

Gueguen Y, Chemardin P, Labrot P, Arnaud A and Galzy P: 1997. Purification and characterization of an intracellular β-glucosidase from a new strain of *Leuconostoc mesenteroides* isolated from cassava. *J. Microbiol.* 82: 469-476.

Gueguen Y, Chemardin P and Arnaud A: 2001. Purification and characterization of an intracellular beta-glucosidase from a *Candia sake* strain isolated from fruit juices. *Appl. Biochem. Biotechnol.* 95: 151-162.

Kang SR, Cho KK, Ahu JK, Bok JD, Kang SH, Woo JH, Lee HG, You SK and Choi YJ: 2005. Three forms of thermostable lactose-hydrolase from sp. IB-21: cloning expression and enzyme characterization. *Biotechnology*. 116: 337-346.

Kouamé LP, Kouamé AF, Niamke SL, Faulet BM and Kamenan A: 2005a. Biochemical and catalytic properties of two beta-glycosidases purified from workers of the termite *Macrotermes subhyalinus* (*Isoptera: Termitidae*). *Int. J. Trop. Insect Sci.* 25: 103-113.

Kunst A, Draeger B and Ziegenhorn J: 1984. Colorimetric methods with glucose oxydase and peroxydase. In: Bergmeyer HU, editor. *Methods of enzymatic analysis* 6: pp. 178-185. Verlag chemie, weinheim. Verlag chemie, weinheim.

Laemmli UK: 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.

Li YK, Chir J, Tanaka S and Chen FY: 2002. Identification of the general acid/ base catalyst of a family 3 β-glucosidase from *Flavobacterium meningosepticum*. *Biochemistry*. 41: 2751-2759.

Lowry OH, Rosebrough NJ, Farr AL and Randall RJ: 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265-275.

Marana SR, Terra WR and Ferreira C: 2000. Purification and properties of a β-glycosidase purified from midgut cells of *Spodoptera frugiperda* (*Lepidoptera*) larvae. *Insect Biochem. Mol. Biol.* 30: 1139-1146.

Matoub M : 1993. *La symbiose termite-champignon chez Macrotermes bellicosus*. Thèse de doctorat, Université, Paris Val de Marne.

Michlmayr H, Schümann C, Barreira BdSNM, Kulbe KD and Del Hierro AM: 2009. Isolation and basic characterization of a beta-glucosidase from a strain of *Lactobacillus brevis* isolated from a malolactic starter culture. Appl. Microbiol. 6: 12-21.

Pontoh J and Low NH: 2002. Purification and characterization of β-glucosidase from honey bees (*Apis mellifera*). *Insect Biochem. Mol. Biol.* 32: 679-690.

Rouland C, Matoub M, Mora P and Petek F: 1992. Properties of two β-glucosidases purified from the termite *Macrotermes mulleri* and from its symbiotic fungus. *Termitomyces sp. Carbohydr. Res.* 233: 237-245.

Saha R, Roy SB and Sengupta S: 2002. Stabilization and improvement of catalytic activity of a low molar mass cellobiase by cellobiasesucrase aggregation in the culture filtrate of *Termotomyces clypeatus*. *Biotechnol. Progr*: 18: 1240-1248.

Saloheimo M, Kuja-Panula J, Ylosmaki E, Ward M and Penttila M: 2002. Enzymatic properties and intracellular localization of the novel *Trichoderma reesi* beta-glucosidase. *Appl. Environ. Microbiol.* 68: 4546-4553.

Shewale JG and Sadana JC: 1981. Purification, characterization, and properties of β-glucosidase enzymes from *Sclerotium rolfsii*. *Arch. Biochem. Bioph.* 207: 185-196.

Taniguchi YA and Takano K: 2002. Purification and properties of aminopeptidase from *Tilapia* intestine. *Nipp. Suis. Gak*. 68: 382-388.

Terra WR and Ferreira C: 1994. Insect digestive enzymes: properties, compartmentalization and function. *Comp. Biochem. Physiol.* 109: 1-62.

Watanabe T, Sato T, Yoshioka S, Koshuima C and Kuwahara M: 1992. Purification and properties of *Aspergillus niger* β-glucosidase. *Eur. J. Biochem.* 209: 651-659.

Wallecha A and Mishra S: 2003. Purification and characterization of two β-glucosidases from a thermo-tolerant yeast *Pichia etchellsii*. *Biochim. Biophys. Acta* 1649: 74-84.

Webb EC: 1992. Enzyme nomenclature. Recommendation (1992) of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology. Academic Press, San Diego, CA.

Yapi DYA, Gnakri D, Niamke SL and Kouame LP: 2008. Purification and biochemical characterization of a specific β-glucosidase from the digestive fluid of larvae of the palm weevil, *Rhynchophorus palmarum*. *J. Insect Sci.* 8: 65-78.