Advances in Biology & BioMedicine

www.advancejournals.org
Open Access Scientific Publisher

Research Article

PURIFICATION AND CHARACTERISATION OF TWO ALPHA-GLUCOSIDASES FROM TERMITE WORKERS MACROTERMES BELLICOSUS (TERMITIDAE: MACROTERMITINAE)

Siaka Binaté[1], Assoi Yapi Désiré Patrice Yapi[1], Gbocho Serge Elvis Ekissi[1], Kouassi Hubert Konan[1] E. J. Parfait Kouadio[1], Lucien Patrice Kouamé[1]
[1]Laboratoire de Biochimie et Technologie des Aliments de l'Unité de Formation et de Recherche des Sciences et Technologies des Aliments de l'Université Nangui Abrogoua, 02 BP 801 Abidjan, Côte d'Ivoire

Correspondence should be addressed to **Kouassi Hubert Konan**

Received October 31, 2015; Accepted January 15, 2016; Published February 15, 2016;

Cite This Article:S., Yapi, A., Ekissi, Konan, K., Binaté, G., Kouadio, E., Kouamé, L.(2016). Purification and characterisation of two alpha-glucosidases from termite workers macrotermes bellicosus (termitidae: macrotermitinae).. Advances in Biology & BioMedicine, 3(1).1-10

ABSTRACT

In present study, purification and characterization of two alpha-glucosidases from termite workers *Macrotermes bellicosus* (Termitidae: Macrotermitinae) were conducted. The purification procedure consisted of anion-exchange, gel filtration, and hydrophobic interaction chromatography. The enzymes designated alpha-glucosidases A1 and A2 had native molecular weights of approximately 191.23± 0.7 and 140.39± 1.2 kDa, respectively, and there functioned as monomeric structures. The two isoforms isolated exhibited maximal alpha-glucosidase activity pH at 5.6. Alpha-glucosidases A1 and A2 hydrolytic activities were maximal at 50 and 45°C, respectively. The purified enzymes pH stabilities were in the range of 5.0-6.0. The enzymes readily hydrolyzed *p*-nitrophenyl-α-D-glucoside, maltose, maltodextrins and required strictly alpha-gluco configuration for activity. They cleaved glucose-glucose alpha-(1–2) linkages better than alpha-(1–4), alpha-(1–1), alpha-(1–3) and β-(1–6) linkages. The catalytic efficiency (V_{max}/ K_M) values for *p*-nitrophenyl-α-D-glucoside, were respectively 277.75±9.2 and 59.51±4, for alpha-glucosidases A1 and A2. Both alpha-glucosidases were inhibited by sulfhydryl-binding reagents. The physiological role of the two alpha-glucosidases in the digestive tract of the termite could be the digestion of di-and oligosaccharides derived from starch. The enzymes could be used as a tool in the structural analysis of D-glucose containing oligosaccharide chains of glycoproteins, glycolipids and starch.

KEY WORDS: Enzyme purification, characterization, alpha-glucosidase, *Macrotermes bellicosus*, termite.

INTRODUCTION

Alpha-glucosidases (alpha-D-glucoside glucohydrolase; EC 3.2.1.20) are a widespread group of enzymes that catalyze the hydrolysis of the alpha-glucosidic bond from the non-reducing end of a chain as well as the alpha -glucosidic bond of free disaccharides [1][2] They also catalyze other aryl- and alkyl- α-glucopyranoside [3]Many known α- glucosidases seem to prefer the alpha-1,4 bonds of maltose or maltooligosaccharides [1]. These enzymes, which are widespread in mammals, plants, and microorganisms, can be classified into three types based on their substrate specificities [4] Type I alpha-glucosidase hydrolyzes heterogeneous substrates (e.g. synthetic alpha-glucosides and sucrose) more rapidly than homogeneous substrates (e.g. maltooligosaccharides), whereas types II and III display higher activity toward homogeneous than toward heterogeneous substrates. Only type III is capable

of hydrolyzing polysaccharide substrates (e.g. soluble starch). Glucosidase enzymes are involved in several biological processes such as the intestinal digestion, the biosynthesis of glycoproteins and the lysosomal catabolism of the glycoconjugates [5]. It has been discovered that many organisms that produce extracellular amylolytic enzymes also produce an intracellular alpha- glucosidase. In this instance, alpha-glucosidase is the final enzyme involved in the metabolism of starch, or perhaps other carbohydrates, to glucose. Intestinal alpha-glucosidases are involved in the final step of the carbohydrate digestion to convert these into monosaccharides which are absorbed from the intestine. Alpha-glucosidase has potential uses in biotechnological processes such as the production of glucose syrup and in brewing industry [3]. In view of the fact that there are several cellulosic wastes that are still remain untapped because of the unavailability of degrading enzymes, this study was therefore carried out with the aim of producing, purifying and characterize alpha-glucosidase enzymes from termite workers *Macrotermes bellicosus* which could ultimately be of industrial importance.

MATERIALS AND METHODS

Chemicals

Substrates: saccharose, maltose, maltotriose, maltotetraose, maltohexaose, maltoheptaose, trehalose, kojibiose, nigerose, isomaltose, cellobiose, Laminaribiose,Arabino-galactane, carboxymethylcellulose, inulin, laminarin, xylan, lichenan, starch, glucose containing, substrates and *p*-nitrophenyl-glycopyranosides were purchased from Sigma Aldrich (St. Louis, MO, USA). DEAE-Sepharose CL-6B, Sephacryl-S200 HR, phenyl Sepharose CL-6B gels were obtained from Pharmacia-LKB Biotech (Uppsala, Sweden). The chemicals used for polyacrylamide gel electrophoresis (PAGE) were from Bio-Rad (Milan, Italy). All other chemicals and reagents were of analytical grade.

Biological material

Workers of the termite *M. bellicosus* originated from the savanna of Lamto (Cфte d'Ivoire). They were collected directly from the nest and then stored frozen at -20°C.

Enzyme samples

The collected termite workers (2 g) were homogenized with 15 ml 0.9% NaCl (w/v) solution in an ultra-turrax and then sonicated as previously described by Rouland *et al.* [6]. The homogenate was centrifuged at 10000 x g for 15 min. The collected supernatant constituted the crude extract. After freezing at −180°C in liquid nitrogen, the crude extract was stored at −20°C.

Enzyme assays

Under the standard test conditions, alpha-glucosidase activity was measured by the release of *p*-nitrophenol from the substrate *p*-nitrophenyl-α-D-glucopyranoside. An assay mixture (275 μl) consisting of a 100 mM acetate buffer (pH 5.6), 1.25 mM*p*-nitrophenyl-α-D-glucopyranoside and enzyme solution, was incubated at 37°C for 10 min. The control contained all reactants except the enzyme. Determination of other *p*-nitrophenylglycosidase activities was carried out under the same experimental conditions. The reaction was stopped by the addition of 1M sodium carbonate (2 ml), and absorbance of the reaction mixture was measured at 410 nm.

Oligo-saccharidase activity was determined by measuring the amount of glucose liberated from oligosaccharide by incubation at 37°C for 10 min in a 100 mM acetate (pH 5.6), containing 10 mM oligosaccharide. The amount of glucose was determined by the glucose oxidase-peroxidase method [7] after heating the reaction mixture at 100°C for 5 min.

Polysaccharidase activity was assayed by the dinitrosalicylicacid procedure [8], using 1% (w/v) polysaccharide (arabino-galactan, carboxymethylcellulose, inulin, lichenan, laminarin, xylan and starch) as substrate. The enzyme (100 μl) was incubated for 30 min at 37°C with 200 μl buffer (100 mM acetate, pH 5.6) and100 μl polysaccharide. The reaction was stopped by addition of 300 μl dinitrosalicylic acid and heating in boiling water for 5 min. The absorbance was read at 540 nm after cooling on ice for 5 min.

One unit 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).

Protein assays

Protein concentrations and elution profiles from chromatographic columns were determined by the Lowry method [9]using bovine serum albumin as a standard.

Purification of enzyme

All the purification procedure was carried out in the cold room. The crude extract of worker *M. bellicosus* was loaded onto an anion-exchange chromatography using a DEAE-Sepharose CL-6B column (2.5 cm x 4.5 cm), equilibrated with 20 mM sodium acetate buffer (pH 5.6). The column was washed at a flow rate of 3 mL/min with two bed volumes of equilibration buffer to remove unbound proteins. Bound proteins were then eluted with a stepwise salt gradient (0.1, 0.3, 0.5, 0.7 and 1 M) of NaCl in 20 mM sodium acetate buffer (pH 5.6), and fractions of 3 mL were collected. Two peak of alpha-glucosidase activity was obtained.

On the one hand, the unbound alpha-glucosidase activity (Peak 1) was submitted to ammonium sulphate precipitation at 80% saturation overnight in a cold room. The mixture was stirred for at least 8 h and centrifuged at 10,000 g for 15 min. The pellet was suspended in 1 mL of 20 mM sodium acetate buffer (pH 5.6) and loaded onto a Sephacryl S-200 HR column (1.6 cm Ч 64 cm), a gel filtration chromatography, equilibrated with the same buffer. Fractions of 1 mL were collected at a flow rate of 0.25 mL/min, and those containing the alpha-glucosidase

activity were pooled. To the pooled active fractions, solid ammonium sulphate was slowly added to give a final concentration of 1.7 M and the resulting enzyme solution was subsequently applied on a Phenyl SepharoseCL-6B column (1.4 cm Ч 5.0 cm) previously equilibrated with 20 mM sodium acetate buffer (pH 5.6) containing 1.7 M of ammonium sulphate salt. The column was washed with a reverse stepwise gradient of ammonium sulphate concentrations (from 0–1.7 M) dissolved in the same sodium acetate buffer at a flow rate of 1 mL/min and fractions of 1 mL were collected. The pooled active fractions were dialyzed overnight against 20 mM sodium acetate buffer (pH 5.6) and constituted the purified enzyme solution.

On the other hand, the bound alpha-glucosidase activity (Peak 2) eluted from DEAE-Sepharose CL-6B at the first step was also subjected to 80% saturation with ammonium sulphate. The precipitate obtained after centrifugation (10,000 g) was dissolved in 1 mL of 20 mM sodium acetate buffer and loaded onto the same Sephacryl S-200 HR column in the same experimental conditions as described above. Alpha Glucosidase activity peak obtained was saturated to a final concentration of 1.7 M ammonium sulphate and loaded onto the Phenyl-Sepharose 6 Fast Flow column in the same procedure as above. Finally, the pooled active fractions were also dialyzed against 20 mM acetate buffer (pH 5.6) and kept refrigerated at 4 °C for assays.

Homogeneity and molecular weight determination

To check purity and determine molecular weight, the purified enzyme was analyzed using SDS-PAGE electrophoresis on a 10% separating gel and a 4% stacking gel (Hoefer mini-gel system; Hoefer Pharmacia Biotech, www.hoeferinc.com), according to the procedure of Laemmli [10] at 10°C and constant current 20 mM. Proteins were stained with silver nitrate according to Blum *et al.* [11]The sample was denatured by a 5 min treatment at 100°C. Electrophoretic buffers contained sodium dodecyl sulfate (SDS) and beta-mercaptoethanol.

The native molecular weight of the enzyme was determined using HPLC gel filtration chromatography. The TSK (Sigma-Adrich) column (2.5 cm Ч 52 cm; QC-PAKGFC 200) was equilibrated with 20 mM acetate buffer(pH 5.6) containing sodium azide 0.5 % (w/v) and calibratedwith beta-amylase (200 kDa), alcohol dehydrogenase(150 kDa), bovine serum albumin (66 kDa), ovalbumin(48.8 kDa) and cytochrome C (12.4 kDa). Fractions of 0.5 ml were collected at a flow rate of 0.5 ml/min.

Temperature and pH optima

The effect of pH on alpha-glucosidase activity was determined by measuring the hydrolysis of p-nitrophenyl-α-D-glucopyranoside in a series of buffers at various pH values ranging from pH 3.6 to 8.0. The buffers used were acetate buffer (100 mM) from pH 3.6 to 5.6 and phosphate buffer (100 mM) from pH 5.6 to 8.0. The pH values of each buffer were determined at 37°C. Alpha-glucosidase activity was measured at 37°C under the standard test conditions. The effect of temperature on alpha-glucosidase activity was followed in 100 mM acetate buffer pH 5.6 over a temperature range of 30 to 80°C using 1.25 mM p-nitrophenyl-α-D-glucopyranoside under the standard test conditions.

pH and temperature stabilities

The stability of alpha-glucosidase was followed over the pH range of 3.6 to 8.0 in 100 mM buffers. The buffers were the same as those used in the study of the pH and temperature optima. After 2 h incubation at 37°C, aliquots were taken and immediately assayed for residual alpha-glucosidase activity. The thermal inactivation was determined at 37 °C and at each enzyme optimum temperature (at pH 5.6). Enzymes in appropriate buffers (pHs) were exposed to each temperature for up to 60 min. The enzyme was incubated in 100 mM acetate buffer pH 5.6. Aliquots were drawn at intervals (10 min) 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.

Determination of kinetic parameters

The kinetic parameters (K_M, V_{max} and V_{max}/ K_M) were determined in 100 mM acetate buffer (pH 5.6) at 37°C. Hydrolysis of *p*-nitrophenyl-α-D-glucopyranoside was quantified on the basis of released *p*-nitrophenol as in the standard enzyme assay. Maltose and Saccharose hydrolysis was quantified by determination of released glucose, determined with oxidase-peroxidase method [7] 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-alpha-D-glucopyranoside(1–10 mM) and oligosacharides (1–20 mM).

Effect of chemical agents

The enzyme was incubated with 1 mM or 1% (w/v) of different chemical agents for 20 min at 37°C (various cations in the form of chlorides). After incubation, the residual activity was determined by the standard enzyme assay using *p*-nitrophenyl-α-D-glucopyranoside as a substrate. The activity of enzyme assayed in the absence of the chemical agents was taken as 100%.

RESULTS AND DISCUSSION

Enzyme purification

The purification procedure of the two alpha-glucosidases purified from termite workers *M. bellicosus* involved three steps including anion-exchange, size exclusion and hydrophobic interaction chromatographies; the results are summarized in Table 1. Two major peaks of alpha-glucosidases activity named A1 and A2 were resolved on DEAE-Sepharose Fast-flow column. Active proteins were eluted respectively at 0.3 M (A1) and 0.5 M (A2) of NaCl (data not shown), indicating the existence of two forms of alpha-glucosidases in termite workers *M. bellicosus* as

reported for multiple forms of alpha-glucosidases from many other sources 99After this step, the two alpha-glucosidases (A1 and A2) were separately loaded onto a gel filtration chromatography using a Sephacryl S-200 HR column. One peak showing an alpha-glucosidase activity was resolved for both of the two activities. The two alpha-glucosidases (A1 and A2) activities were subsequently purified by using an ultimate hydrophobic chromatography on a phenyl-Sepharose 6 Fast Flow column. The active proteins were eluted with 1.0 and 0.5 M of ammonium sulphate, respectively (data not shown). Finally, A1 and A2 were purified with overall yields of 8.55 and 1.31 % and enriched about 50.15 and 30.86 fold, respectively Table 1This low yield could be due to several fractionation steps used. Each isoenzyme showed a single protein band on SDS-PAGE gel electrophoresis staining with silver nitrate (Fig.1). This result confirmed that these enzymes were purified to homogeneity.

Molecular weight estimation

SDS-PAGE profile of purified enzymes is depicted in figure 1. After SDS-PAGE analysis under reducing conditions, each alpha glucosidase from termite workers *M. bellicosus* showed a single protein band. Their relative molecular weights were estimated to be 189.51± 1.2 and 139.4± 0.9 kDa for A1 and A2, respectively (Fig. 1; Table 2). On the other hand, the molecular weights determined by HPLC were 191.23± 0.7 and 140.39± 1.2 kDa for A1 and A2, respectively (Table 2). These results strongly suggest that the purified alpha glucosidases exists as a monomer as describe by Yapi *et al.* [14]. In comparison with other molecular weights of insects purified alpha glucosidases, those from termite workers *M. bellicosus* were higher with regard to alpha glucosidases from *Apis cerana indica* (68 kDa) [15], *Apis mellifera* (98 kDa) [13] and *Diatraca saccharalis* (54 kDa) [16].

Effect of pH and temperature

A1 and A2 hydrolytic activities were maximal at 50 and 45°C, respectively in sodium acetate buffer pH 5.6 Table 2 The pH optimum of 5.6 is in agreement with the general range of most alpha-glucosidases, extracted from insects, exhibits pH optima ranging from 4.5 to 7.0 [17, 18, 19, 20]. At 37 °C, the studied enzymes displayed better stability at pH ranging 5.0-6.0. The pH stability margin is wide, it might be beneficial to synthesis reactions or hydrolysis which uses purified alpha-glucosidase in biotechnology processes.

The thermal inactivation study at pH 5.6 indicated that, alpha-glucosidase A1 remained fully stable for 60 min at 37 °C Figure 1. However, at 50 °C (its optimum temperature) the enzyme was less stable and lost about 50% of its hydrolytic activity after 60 min of pre-incubation. However, alpha glucosidase A2 was less stable at 37 °C and lost about 30% of its hydrolytic activity after 60 min of pre-incubation.

Kinetic parameters values of alpha-glucosidases

Workers *M. bellicosus* alpha-glucosidases did not attack the following *p*nitrophenyl glycosides: beta-glucoside, alpha-and beta-galactoside, alpha-and beta-mannoside, alpha-and beta-xyloside, alpha-and beta-L-arabinoside, alpha-and beta-fucoside Table 3; nor the following oligosaccharide: cellobiose; nor the following polysaccharides: arabinogalactan, carboxymethylcellulose, inulin, lichenan, laminarin, xylan and starch (Table 4). However, they were very active on *p*-nitrophenyl-alpha-D-glucopyranoside, maltose, maltodextrins and saccharose Table 3Table 4. Low activity was observed towards Trehalose, Nigerose, Kojibiose and Isomaltose (Table 4). This result suggests that the two alpha-glucosidases are exo- glycosidases, and have no polysaccharidase activities. Further, these enzymes appear to have a high specificity for the alpha-anomeric configuration of the glucosidic linkage. This pattern seems to reflect the activity of the alpha-glucosidases from *A. mellifera* [13] and from cockroach, Periplaneta Americana [21]

Kinetic parameters values of alpha-glucosidase

The effect of substrate concentration on enzymatic activity was studied with *p*-nitrophenyl-beta-D-glucopyranoside, maltose and saccharose. With the three substrates, alpha glucosidases A1 and A2 obeyed the Michaelis- Menten equation. The K_M, V_{max} and V_{max}/K_M values are shown in table 5. The catalytic efficiency of alpha-glucosidases, given by the V_{max}/K_M ratio was much higher for the *p*-nitrophenyl-alpha-D-glucopyranoside than the maltose and saccharose Table 4.

Effect of chemical agents on enzyme activity

Chemical agents Fe, DTNB, *p*CMB, β-mercaptoethanol and L-cystein showed an inhibitory effect on alpha-glucosidases activities Table 2. These enzymes contain thiol groups in its structure because it is inhibited by agents such as *P*CMB and DTNB. The presence of thiol groups in the essential conformation of the enzyme is shown also by the inhibitory action of the Hg^{2+} ion. Indeed, the reduction of enzyme activity by Hg^{2+} ion indicates that thiol groups are not only located in the active center of the enzyme but these thiol groups participate in the catalytic act [22].

Figure 1: SDS-PAGE analysis of the purified alpha-glucosidases from termite workers Macrotermes bellicosus. The sample was loaded onto a 10 % gel. Lane 1, purified alpha-glucosidase A2, Lane 2, purified alpha-glucosidase A1, Lane 3, numbers on the right indicate

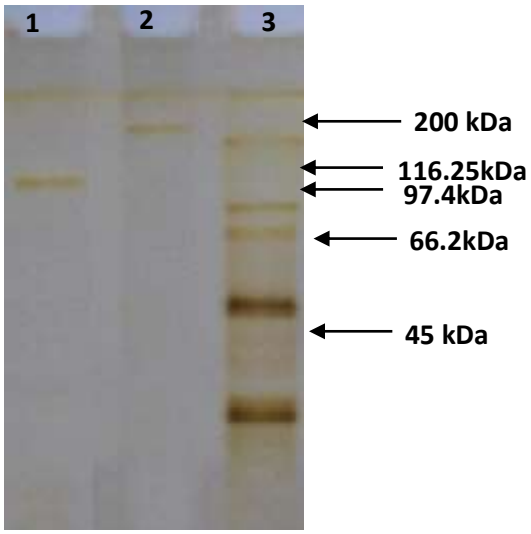

Figure 2: Thermal inactivation of the purified alpha-glucosidases from from termite workers Macrotermes bellicosus.

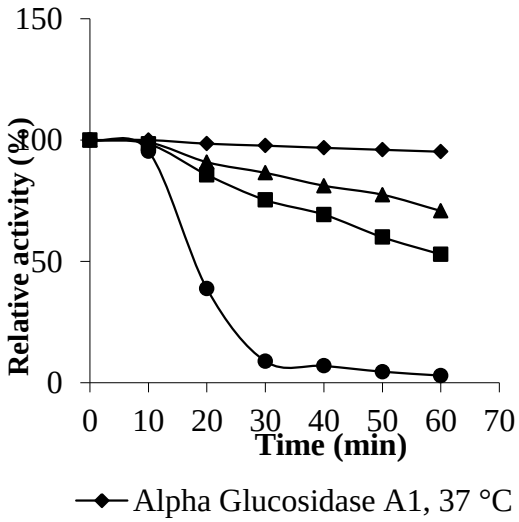

Table 1: Purification procedure of two alpha-glucosidases purified from termite workers Macrotermes bellicosus

Purification steps	Total activity [a](Units)	Total protein (mg)	Specific activity (Units/mg)	Purification fold	Yield (%)
Crude extract	38.02	47.30	0.81	1	**100**
DEAE Sepharose CL-6B					
Alpha glucosidase A1	28.90	3.20	9.03	11.15	**76.01**
Alpha glucosidase A2	5.08	2.76	1.84	2.27	**13.36**
Sephacryl-S200 HR					
Alpha glucosidase A1	8.07	0.22	36.68	45.28	**21.22**

Alpha glucosidase A2	2.95	0.77	3.83	4.73	**7.95**
Phenyl-Sepharose CL-6B					
Alpha glucosidase A1	3.25	0.08	40.62	50.15	**8.55**
Alpha glucosidase A2	**0.50**	**0.02**	**25.00**	**30.86**	**1.31**

Table 2: Some physicochemical characteristics of two alpha-glucosidases purified from termite workers

	Values	
Physicochemical properties	**Alpha Glucosidase A1**	**Alpha Glucosidase A2**
Optimum temperature (°C)	50°C	45°C
Optimum pH	5.6	5.6
pH stability	5.0-6.0	5.0-6.0
Molecular weight (kDa)		
SDS-PAGE	189.51± 1.2	139.4± 0.9
Gel filtration	191.23± 0.7	140.39± 1.2
Activation energy (kJ/mol)	51.88 ± 4.64	47.70 ± 3.09
Q_{10}	1.92 ± 0.15	1.89 ± 0.11
Inhibitor agents	Hg^{2+}, Fe^{2+}, L-cystein DTNB[a], *p*CMB[b], β-mercaptoethanol	Hg^{2+}, $Fe2^{+}$, L-cystein DTNB[a], *p*CMB[b], β-mercaptoethanol

SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.a: 5,5'-dithio-2,2'dinitro-dibenzoïc acid ; b: Sodium *para*-chloromercuribenzoate.

Table 3: Activities of two alpha-glucosidases purified from termite workers Macrotermes bellicosus on synthetic chromogenic substrates

Substrats	Relative activities	
	Alpha Glucosidase A1	Alpha Glucosidase A2
***p*-nitrophenyl-α-D-glucoside**	100	**100**
***p*-nitrophenyl-β-D-lucoside**	0	**0**
***p*-nitrophenyl-β-D-mannoside**	0	**0**
***p*-nitrophenyl-α-D-mannoside**	0	**0**
***p*-nitrophenyl-β-D-galactoside**	0	**0**
***p*-nitrophenyl-α-D-galactoside**	0	**0**
***p*-nitrophenyl-α-D-fucoside**	0	**0**
***p*-nitrophenyl-β-D-fucoside**	0	**0**
***p*-nitrophenyl-α-L-arabinoside**	0	**0**
***p*-nitrophenyl-β-L-arabinoside**	0	**0**
***p*-nitrophenyl-β-D-xyloside**	0	**0**
***p*-nitrophenyl-α-D-xyloside**	**0**	**0**

Glc. Glucose

Table 4: Activities of two alpha-glucosidases purified from termite workers Macrotermes bellicosus on oligosaccharide and polysaccharide substrates

Substrats	Relative activities (%)	
	Alpha Glucosidase A1	**Alpha Glucosidase A2**
Maltose	100	100
Maltotriose	159±4	20±2
Maltotetraose	197±3	12±1
Maltopentaose	80±1	5±1
Maltohexaose	70±2	2±0
Maltoheptaose	48±1	1±0

Trehalose[Glcα(1-1)Glc]	14±1	08±1
Kojibiose[Glcα(1-2)Glc]	29±2	14±1
Nigerose[Glcα(1-3)Glc]	12±1	03±0
Isomaltose[Glcα(1-6)Glc]	09±1	06±1
Saccharose[Glcα(1-2)Fru]	167±3	123±3
CellobioseGlcβ(1-4)Glc	0	0
laminarin	0	0
Arabino-galactane	0	0
Carboxymethylcellulose	0	0
Inuline	0	0
Lichenane	0	0
Xylane	0	0
starch	0	0

Table 5: Kinetic parameters of two alpha-glucosidases purified from termite workers Macrotermes bellicosus towards p-nitrophenyl-α-D-glucopyranoside, Maltose and Saccharose

Substrat	**Alpha Glucosidase A1**			**Alpha Glucosidase A2**		
	K_M (mM)	**V_{max} (U/mg)**	**V_{max}/ K_M (U/mM x mg)**	**K_M (mM)**	**V_{max} (U/mg)**	**V_{max}/ K_M (U/mM x mg)**
p-nitrophenyl-α-D-glucopyranoside	0.20±0.0	55.55±3.7	277.75±9.2	0.35±0.0	20.83±1.9	59.51±4.2
Maltose	1.42±0.2	16.66±1.0	11.73±1.4	0.80±0.2	43.47±2.9	54.33±3.4
Saccharose	0.60±0.1	19.23±1.6	32.05±2.5	1.60±0.2	38.46±2.6	24.03±1.1

CONCLUSION

The two alpha-glucosidases that were purified from workers of the termite *M. bellicosus* (Termitidae, Macrotermitinae) in this study appear to be distinct from other alpha-glucosidases reported so far, in terms of substrate specificity and high affinity towards maltose. Based on our findings, we propose that the physiological role of these alpha-glucosidases in the digestive tract of the termite *M. bellicosus* workers is the digestion of di-and oligosaccharides derived from plant material starch. The enzymes could be used as a tool in the structural analysis of D-glucose containing oligosaccharide chains of glycoproteins, glycolipids and starch.

ACKNOWLEDGMENTS

We are grateful to Professor Mamadou Dagnogo, Director of the Laboratoire de Biologie et de Cytologie, UFR des Sciences de la Nature, for contribution on identification of the specimen (*Rhynchophorus palmarum* larvae) used in the present study.

REFERENCES

[1] Nashiru, O; Koh, S; Lee, Y and Lee D.S: Novel α-glucosidase from extreme thermophile *Thermus caldophilus* GK24. *Journal of Biochemistry and Molecular Biology*, 2001, 34: 347-354.

[2] Noguchi, A; Yano, M; Ohshima, Y; Hemmi, H; Inoharaochai, M; Okada, M; Min, K.S; Nakayama, T and Nichino, T: Deciphering the molecular basis of the broad substrate specificity of alpha- glucosidase from *Bacillus sp. Journal of Biochemistry*, 2003, 134: 543- 550.

[3] Constantino, H.R; Brown, S.H and Kelly, R.M: Purification and characterization of an α-glucosidase from a hyperthermophilic archebacterium, *Pyrococcus furious*, exhibiting a temperature optimum of 105oC and 115oC. *Journal of Bacteriology*, 1990, 172(7): 3654-3660.

[4] Chiba, S: The Handbook of Amylases and Related Enzymes. The Amylase Research Society of Japan, Ed., Pergamon Press, Oxford, 1988, 104-116.

[5] Asano, N; Nishida, M; Kato, A; Kizum, H; Matsu,i K; Shimada, Y; Itoh, T; Baba, M; Azin, M; Moraveg, N and Zareh, D: Self directing optimization of parameters for extracellular chitinase production by *Trichoderma harzianum. Process Biochemistry*, 2007, 34: 563-566.

[6] Rouland, C; Civas, A; Renoux, J and Petek, F: Purification and properties of cellulases from termite *Macrotermes mulleri* (Termitidae, Macrotermitinae) and its symbiotic fungus Termitomyces sp. *Comparative Biochemistry and Physiology*, 1988, 91B: 449-458.

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

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

[9] Lowry, O.H; Rosebrough, N.J; Farr, A.L and Randall, R.J: Protein measurement with the Folin phenol reagent. *Journal of Biology and Chemistry*, 1951, 193: 265-275.

[10] Laemmli U.K: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 1970, 227: 680-685

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

[12] Takewaki, S; Chiba, S; Kimura, A; Matsui, H and Koike, Y: Purification and properties of alpha-glucosidase of honeybee, *Apis mellifera.* Agricultural *and Biological Chemistry*, 1980, 44: 731-740.

[13] Nishimoto, M; Kubota, M; Tsuji, M; Mori, H; Kimura, A; Matsui, H and Chiba, S: Purification and substrate specificity of honeybee, *Apis mellifera*, alpha-glucosidase III. *Bioscience, Biotechnology, and Biochemistry*, 2001, 65: 1640-1616.

[14] Yapi, A.Y.D.P; Konan, K.H; Binatй, S; Kouadio, E.J.P and Kouamй L.P:. Purification and biochemical characterization of a specific alpha-glucosidase from the digestive fluid of larvae of the palm weevil, *Rhynchophorus palmarum* L. *International Journal of Biosciences*, 2015, 7(1): 74-85.

[15] Chanchao, C.H; Pilalam, S and Sangvanich P: Purification and Characterization of alpha-glucosidase in *Apis cerana indica. Insect Science*, 2008, 15: 217-224.

[16] Carneiro, C.N; Isejima, E.M; Samuels, R.I and Silva, C.P: Sucrose hydrolases from the midgut of the sugarcane stalk borer *Diatraca saccharalis. Journal of Insect Physiology*, 2004, 50 (11): 1093-1110.

[17] Frandsen, T.P and Svensson, B: Plant alpha-glucosidases of the Glycoside Hydrolase Family 31 Molecular Properties, Substrate Specificity, Reaction Mechanism and Comparison with Family Members of Different Origin. *Journal of Insect Physiology*, 1998, 37: 1–13.

[18] Ghadamyari, M; Hosseininaveh, V and Sharifi, M: Partial Biochemical Characterization of alpha and beta-glucosidases of Lesser Mulberry Pyralid, *Glyphodes pyloalis* Walker (Lep.: Pyralidae). *Comptes Rendus Biologies*, 2010, 333, 197–204.

[19] Ramzi, S and Hosseininaveh, V: Biochemical Characterization of Digestive alpha-amylase, alpha-glucosidase and beta-glucosidase in Pistachio Green Stink Bug, *Brachynema germari* Kolenati (Hemiptera: Pentatomidae). *Journal of Asia-Pacific Entomology*, 2010, 13: 215-219.

[20] Saberi, R.N; Ghadamyari, M and Motamediniya, B: Biochemical Characterization of alpha- and beta-glucosidases and alpa- and beta-galactosidases from Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier) (Col.: Curculionidae). *Plant Protection Science*, 2012, 48 (2): 85-93.

[21] Kouamй, L.P ; Due, E.A; Niamkй, S.L; Kouamй, F.A and Kamenan, A: Syntheses enzymatiques de neoglucoconjugues catalysees par l'α-glucosidase purifiee de la blatte *Periplaneta americana* (Linnaeus). *Biotechnology, Agronomy, Society and Environment* 2005, 9 (1): 35–*42.*

[22] Yang, C and Liu, W.H: Purification and properties of a maltotriose-producing alpha- amylase from *Thermobifida fusca. Enzyme and Microbial Technology*, 2004, 35: 254-260.