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XYLAN AND CARBOXYMETHYLCELLULOSE DIGESTIVE ENZYMES ISOLATED FROM THE ABDOMEN OF THE SMALL SOLDIER OF THE TERMITE MACROTERMES SUBHYALINUS.

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ABSTRACT

By low pressure Chromatographic techniques two isoform enzymes (Ab-CX_1 and Ab-CX_2) were isolated from the abdomen of the small soldier of the termite *Macrotermes subhyalinus*. These enzymes are bifunctional because they have the ability to hydrolyze both xylan and carboxymethylcellulose substrates. Their detection by electrophoresis under native conditions revealed a protein band for each. the relative molecular weights determined by SDS-PAGE and gel filtration suggest that the enzyme Ab-CX_1 is tetrameric and Ab-CX_2 dimeric. The physicochemical characterization of the enzymes Ab-CX_1 and Ab-CX_2 shows that they are acids with a stability at pH 4.6-5.6. They exhibit maximum activity at a temperature of 50 and 55°C respectively for the enzymes Ab-CX_1 and Ab-CX_2. The Cu^{2+} cation have inhibitory effects while Mn^{2+}, Na^+, K^+. Also, we find that EDTA has no effect on the catalytic activity of these biocatalyses.

KEYWORDS: Carboxymethylcellulose, xylan, *Macrotermes Subhyalinus*, abdomen, Carboxymethylcellulase, xylanase.

INTRODUCTION

Termites are highly developed social insects which comprise the order Isoptera. Within this order, there are six families, of which five are classed as the lower termites (Mastotermitidae, Kalotermitidae, Hodotermitidae, Rhinotermitidae and Serritermitidae). Termites can be classified into morpho-physiologically distinct castes called queens, kings, alate forms (reproductive members), workers, soldiers, and immature forms. Termites of the sub-family Macrotermitinae, also known as fungus-growing termites, cultivate symbiotic fungi of the genus *Termitomyces* on fungal combs, which are synergistically maintained in nests of the termite mounds.[1,2] Termites are soil insects that efficiently decompose microbial symbionts.[3] Termites live in lignocellulosic material with the aid of their associated termite mounds, which they build themselves, and these mounds are very predominant in Africa and Asia due to the warmness nature of these two continental regions.[4] Termites participate in nutrient recycling by grinding, decomposition, humification, and mineralization of cellulosic resources and their variants, because they have appropriate digestive mechanisms capable of metabolizing different biopolymers found in wood, fruits, tubers, crops, and soil components.[5]

The cellulases are usually classified as endoglucanases, exoglucanases and β-glucosidases. Endoglucanases cleave randomly glycoside bonds within the cellulose molecule, hydrolyzing more quickly amorphous regions, where there are fewer and/or weaker hydrogen bonds. The exoglucanases act on the extremities of cellulose chain, not only mainly producing cellobiose but also glucose and cellotriose. The β-glucosidases, also known as cellobiases, catalyze the hydrolysis of cellobiose released by exoglucanases and endoglucanases.[6] The complete hydrolysis of xylan requires the synergistic action of different xylanolytic enzymes (xylanase, b-xylosidase, a-L-arabinofuranosidase, a-D-glucuronidase and acetyl xylan esterase).[7] Xylanases act on hemicelluloses that are the branched heteropolymers consisting of pentose sugars (D-xylose and L-arabinose). Xylans are a diverse and complex group of polysaccharides with the common feature of a backbone of β-(1 → 4)-linked xylose residues, in which sidechains are attached at the C2 and C3 positions of D-xylosyl.

These substituents can normally be acetyl, 4-0-methyl-D-glucuronosyl or L-arabinosyl groups.[8,9]

Knowing that termites mainly feed on plant material, what is the potential of their enzyme to degrade lignocellulotic materials. In this study, we highlight the presence of bifunctional cellulasic and xylanasic enzymes in the abodmen of the small soldier of the termite Macrotermes subhyalinus Macrotermitinae

1-MATERIAL AND METHODS

1-1-Enzyme assay and determination of protein

Under the standard test conditions, xylanase or cellulase activity was assayed spectrophotometrically by measuring the release of reducing sugars from Birchwood xylan or carboxyméthylcellulose (CMC). The reaction mixture (0.38 ml) contained 0.2 ml of 0.5% xylan or CMC (w/v) dissolved in 20 mM acetate buffer (pH 5.0) and 0.1 ml enzyme solution. After 30 min of incubation at 45°C, the reaction was terminated by adding 0.3 ml of dinitrosalicylic acid solution followed by 5 min incubation in a boiling water bath. The product was analysed by measuring the optical density at 540 nm.

One unit (U) of enzyme activity was defined as the amount of enzyme capable of releasing one µmol of reducing sugar per min under the defined reaction conditions. Specific activity was expressed as units per mg of protein (U/mg of protein). Protein concentrations were determined spectrophotometrically at 660 nm by method of Lowry[10] using bovine serum albumin as a standard.

1-2-Purification strategy

All the purification procedure was carried out in the cold room (4°C). The crude extract of the termite salivary glands was loaded onto an anion-exchange chromatography using a DEAE-Sepharose Fast Flow column (2.5 cm x 4.5 cm), equilibrated with 20 mM sodium acetate buffer (pH 5.0). The column was washed at a flow rate of 90 mL/h with two bed volumes of equilibration buffer to remove unbound proteins. Bound proteins were then eluted with a stepwise salt gradient (0.1, 0.2, 0.4 and 2 M) of NaCl in 20 mM sodium acetate buffer (pH 5.0), and fractions of 2 mL were collected. The fractions containing the enzyme were pooled and extensively concentrated by adding ammonium sulfate to 80 % final saturation. After centrifugation at 13.000 g for 30 min, the precipitate was dissolved in 20 mM sodium acetate buffer (pH 5.0) and the resulting solution was passed through a Sephacryl S-200 HR column (1.6 X 65 cm) that had been equilibrated with the same buffer, at a flow rate of 30 ml/h; fractions of 1 ml were collected. Fractions of 1 mL were collected and, to the pooled active fractions, solid sodium thiosulphate was slowly added to give a final concentration of 1.7 M and the resulting enzyme solution was subsequently applied on a Phenyl Sepharose 6 Fast Flow column (1.5 cm x 3.2 cm) previously equilibrated with 20 mM sodium acetate buffer (pH 5.0) containing 1.7 M of sodium thiosulphate salt. The column was washed with a reverse stepwise gradient of sodium thiosulphate concentrations (from 1.7-0 M) dissolved in the same sodium acetate buffer at a flow rate of 78 mL/h and fractions of 1 mL were collected. The pooled active fractions were dialyzed overnight against 20 mM sodium acetate buffer (pH 5.0) and constituted the purified enzyme solution.

1-3-Polyacrylamide gel electrophoresis (PAGE)

Electrophoresis was carried out by using the Laemmli[11] method on 10% (w/v) acrylamide gels under denaturing and non-denaturing conditions. Under denaturing conditions, samples were incubated for 5 min at 100°C with SDS-PAGE sample-buffer containing 2-mercaptoethanol. Under non-denaturing conditions, samples were mixed just before running in sample-buffer without 2-mercaptoethanol and SDS. Silver staining was used to localize protein bands (Blum *et al*., 1987). The standard molecular weights (Bio-Rad) comprising myosin (209 kDa), β- galactosidase (124 kDa), carbonic anhydrase (34.8 kDa), BSA (80 kDa) and inhibiteur trypsique de soja (45.0 kDa) were used.

1-4-Native molecular weight determination

The purified enzyme was applied to gel filtration on a Sephacryl S-200 HR column (0.8 cm x 35 cm) equilibrated with 20 mM sodium acetate buffer (pH 5.0) to estimate the native molecular weight. Elution was done at a flow rate of 0.2 mL/min and fractions of 0.5 mL were collected. Standard molecular weights (SIGMA) used for calibration were beta-amylase (206 kDa), cellulase (26 kDa), bovine serum albumin (66.2 kDa), ovalbumine (45 kDa) and amyloglucosidase (63 kDa).

1-5-Effect of pH

The effect of pH on the activity of the purified enzyme was determined by performing the hydrolysis of xylan or carboxymethylcellulose in a serie of buffers (100 mM) at various pH values (3.6–10.0). Buffers used were sodium acetate (pH 3.6–5.6), phosphate citrate (2.6-7.0), glycin-NaOH (8.0 and 10.0) and sodium phosphate (5.6-8.0). pH values of each buffer were determined at 25°C.

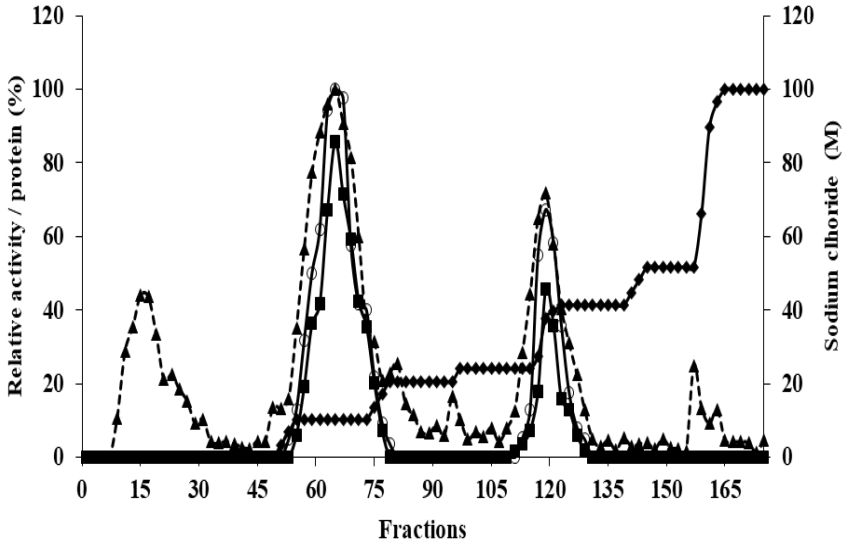
1-6-Effect of pH

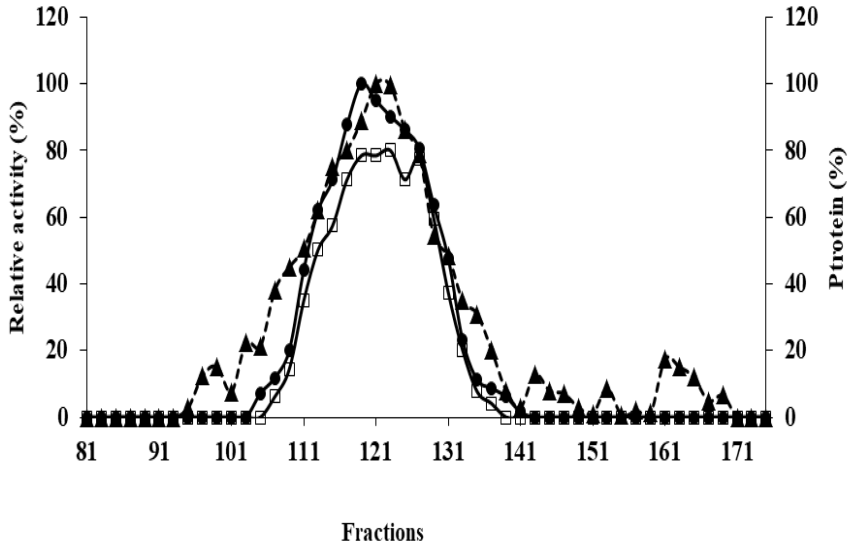
The effect of pH on the activity of the purified enzyme was determined by performing the hydrolysis of xylan or carboxymethylcellulose in a serie of buffers (100 mM) at various pH values (3.6–10.0). Buffers used were sodium acetate (pH 3.6–5.6), phosphate citrate (2.6-7.0), glycin-NaOH (8.0 and 10.0) and sodium phosphate (5.6-8.0). pH values of each buffer were determined at 25°C.

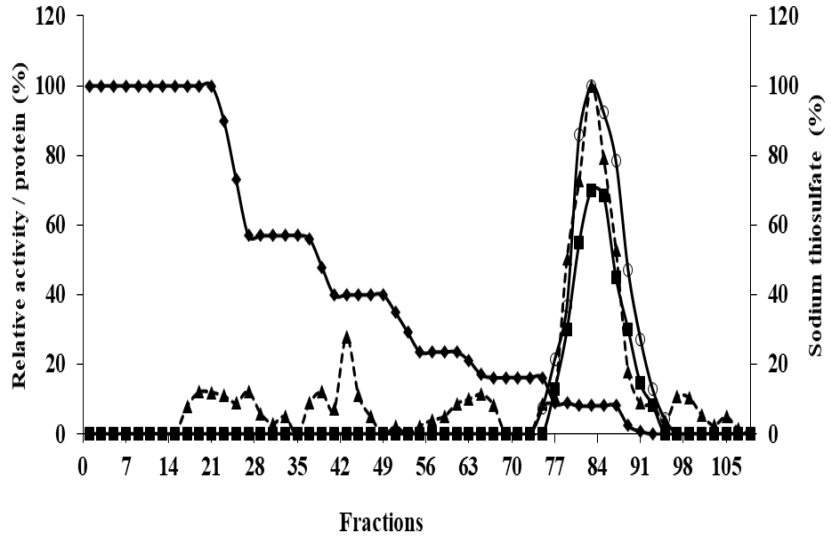
1-7-Effect of temperature

The effect of temperature on purified enzyme activity was performed in 100 mM sodium acetate buffer (pH 5.0) over a temperature range of 30 to 80°C by using xylan or carboxymethylcellulose (0.5%) as substrate under the enzyme assay conditions.

2-RESULT AND DISCUSSION







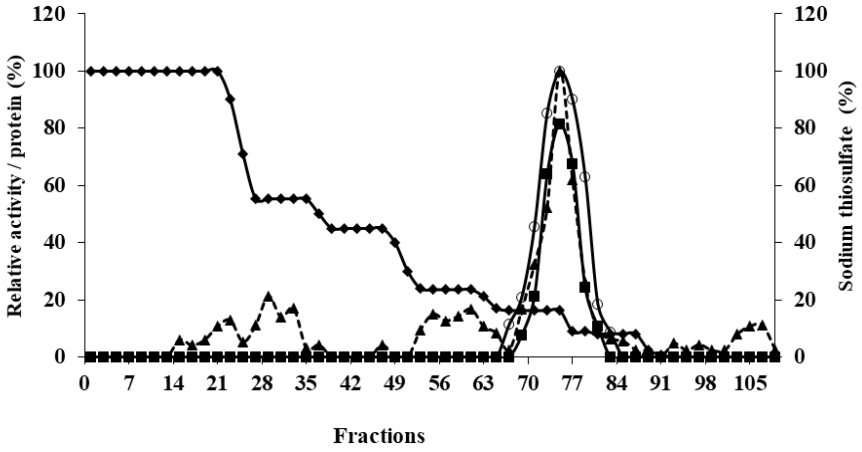


Figure 1: Purification profile of little soldier abdomen of the termite Macrotermes subhyalinus carboxyméthylcellulase and xylanase. (A) Anion exchange chromatography on ANX-Sepharose 4 Fast Flow; (B) Gel molecular exclusion chromatography Sephacryl on S-100 HR; (C) and D Gel hydrophobic chromatography on Phenyl- Sepharose CL-4B. Carboxymethylcellulase activity (■), xylanase activity (○), chloride sodium or sodium thiosulfate (♦), protein (▲).

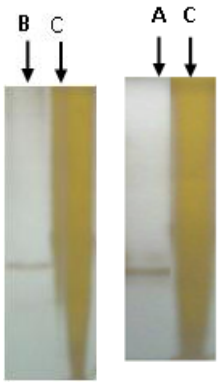


Figure 2: Polyacrylamide gel electrophoresis in native conditions of the carboxyméthylcellulase and xylanase from the little soldier abdomen of the termite *Macrotermes subhyalinus*. (A) Ab-CX$_1$, (B) Ab-CX$_2$ and (C) Crude extract.

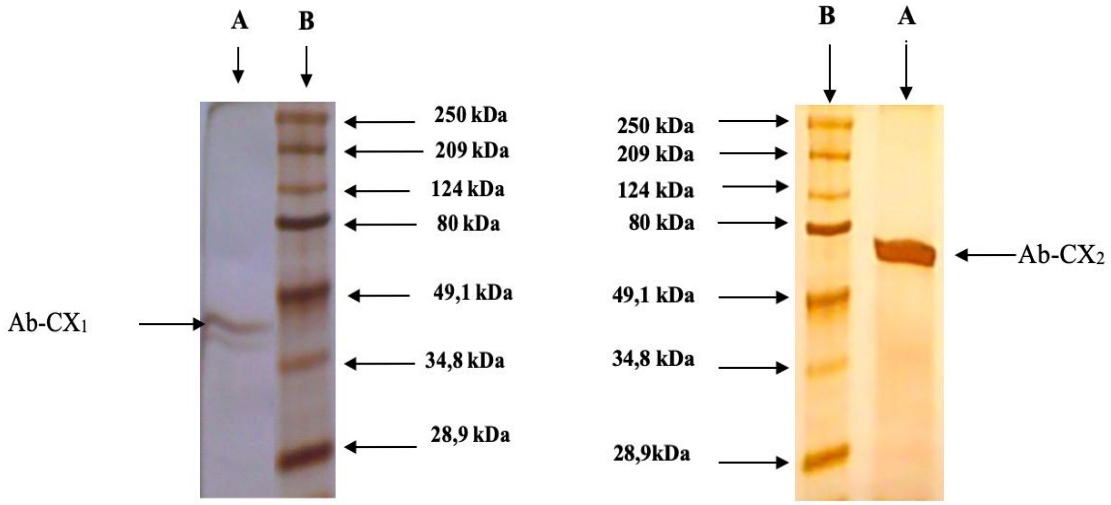


Figure 3: Polyacrylamide gel electrophoresis in native (I, Ab-CX$_1$), (II, Ab-CX$_2$) and denaturing (III, Ab-CX$_1$), (IV, Ab-CX$_2$) conditions of the carboxyméthylcellulase and xylanase from the little soldier abdomens of the termite *Macrotermes subhyalinus*. Lanes 1 crude extract lane 2 and 4 purified enzyme, lane 3 molecular weight markers.

Table 1: Purification procedure of a carboxymethylcellulase and xylanase from little soldier salivary glands of the termite *Macrotermes subhyalinus*.

Purification steps	Total protein (mg)	Total activity (UI)	Specific Activity (UI/mg)	Yield (%)	Purification Fold
Crude extract Abdomen					
Carboxymethylcellulase	202.51±8.62	25.71±9.57	0.13±0.07	100	1
Xylanase	202.51±8.62	30.42±9.32	0.15±0.04	100	1
Anx-Sepharose 4 Fast-flow					
Carboxymethylcellulase Ab-CX_1	52.33±7.28	15.23±3.22	0.29±0.09	59.23±2.35	2.33±0.02
Xylanase Ab-CX_1	52.33±7.28	17.18±2.18	0.32±0.06	56.47±1.87	2.13±0.05
Carboxymethylcellulase Ab-CX_2	26.13±3.62	12.05±1.68	0.46±0.06	46.86±1.09	3.53±0.02
Xylanase Ab-CX_2	26.13±3.62	19.46±1.53	0.46±0.04	63.97±1.24	3.06±0.01
Sephacryl S-100 HR					
Carboxymethylcellulase Ab-CX_1	2.17±1.02	5.29±0.99	2.44±0.2	20.57±2.06	18.57±1.47
Xylanase Ab-CX_1	2.17±1.02	7.01±1.07	3.22±0.2	23.04±2.45	21.53±2.01
Phényl-Sepharose CL-4B					
Carboxymethylcellulase Ab-CX_1	0.51±0.07	2.02±0.41	3.96±0.21	7.85±2.74	30.48±3.75
Xylanase Ab-CX_1	0.51±0.07	2.55±0.18	5±0.69	8.38±1.66	42.79±1.63
Carboxymethylcellulase Ab-CX_2	0.32±0.04	1.78±0.98	5.56±1.46	6.92±0.89	42.79±1.63
Xylanase Ab-CX_2	0.32±0.04	3.04±1.10	9.25±1.51	9.99±0.93	63.33±2.44

Table 2: Some physicochemical characteristics of the carboxymethylcellulase and xylanase from little soldier salivary glands of the termite *Macrotermes subhyalinus*.

	Ab-CX_1		**Ab-CX_2**	
Physicochical properties	**CMCase**	**Xylanase**	**CMCase**	**xylanase**
Optimum pH	5.0	5.6	4.6	5.0
pH stability range	4.6 – 5.6		4.6 – 5.6	
Optimum temperature (°C)	50	55	50	
Activation energy (kJ / mol)	40.93±2.55	73.80±3.67	62.92±4.39	42.56±1.89
Activation Energy	1.66±0.02	1.81±0.05	1.66±0.02	1.95±0.05
Molecular weight (kDa)				
Mobility in SDS-PAGE	41.39±3.68 43.43±2.71		72.18±4.58	
Gel filtration	163.51±6.55		139.42±8.42	

Table 3: Effect of chemical agents on the carboxymethylcellulase and xylanase from little soldier abdomen of the termite *Macrotermes subhyalinus*.

Reagent	**Concentration (mM)**	**Relative Activity (%)**			
		Xylanase Activity		**Carboxymethylcellulase activity**	
		Ab-CX_1	**Ab-CX_2**	**Ab-CX_1**	**Ab-CX_2**
None	0	100	100	100	100
K^+	1	**105±1.25**	**107.30±2.05**	**109±2.13**	101±1.44
	5	**122.18±1.09**	**119.14±1.78**	**118±1.45**	**121±1.11**
Na^+	1	**110±2.12**	**105±2.24**	100.55±2.67	100.78±1.52
	5	**114.46±2.87**	**117.21±1.99**	**121±2.34**	**109±2.03**
Mn^{2+}	1	**145.82±3.09**	129±176	**120.25±1.65**	**118.94±2.32**
	5	**205.15±6.78**	**196.03±4.10**	**181.70±1.69**	**170.71±2.05**
Sr^{2+}	1	100±1.05	**107.09±2.04**	**109.9±2.29**	**105.67±1.79**
	5	99.05±1.38	100.02±1.38	90.05±1.87	97.0±1.24
Fe^{2+}	1	**108.8±2.04**	**106.32±1.66**	101.2±2.08	**116.89±1.48**
	5	**113.44±2.41**	94.40±1.34	**104.5±2,33**	**107.40±2.01**
Ca^{2+}	1	**105±1.82**	100.76±1.57	**106.78±1.09**	**102.17±1.89**
	5	98.54±2.15	96.81±2.48	**115.93±2.11**	**103±2.17**

Ba^{2+}	1	100±1.04	100.60±1.50	100±1.72	98.14±2.23
	5	**106.61±1.25**	96.16±3.17	**112±1.45**	99.07±2.01
Cu^{2+}	1	38,13±2.77	61.49±2.86	49.12±2.09	53.29±3.78
	5	19.20±2.12	28.30±1.09	12.41±2.34	17.16±2.12
Zn^{2+}	1	108.5±2.07	**118.49±2.26**	100.5±2.55	**114.49±1.87**
	5	89.33±1.38	97.48±1.03	90.22±2.67	96.02±1.43
Mg^{2+}	1	102±1.55	101.49±2.76	**109.42±1.88**	**107.31±2.11**
	5	100.10±2.01	100.43±2.25	100.76±2.42	**104.35±2.73**
NH_4^+	1	**111.65±2.26**	102.72±1.04	**112.61±1.12**	**109.13±2.29**
	5	102.54±2.48	**106.33±2.59**	**106.07±1.54**	**102.41±2.24**
Ni^{2+}	1	**109.23±1.72**	98.40±1.04	**103,60±2,02**	96.42±2.76
	5	91.58±2.24	96.27±2.53	92.10±1.69	75.65±1.57
EDTA	1	105±1.03	**107.67±2.05**	101.7±1.37	98.19±2.09
	5	98.87±1.85	100.90±3.21	97.10±1.35	100.12±1.44

DISCUSSION

Two enzymes capable of hydrolyzing both carboxymethylcellulose and xylan were purified to electrophoretic homogeneity from the crude enzyme extract from the abdomen of the small soldier of the termite *Macrotermes subhyalinus*. The first purification step (anion exchange chromatography on the Anx-Sepharose 4 Fast-Flow gel) revealed two enzyme peaks Ab-CX_1 and Ab-CX_2 in the crude enzyme extract from the abdomen (Fig 1). By the same token, the purification of digestive enzyme (xylanase) from *Trichoderma inhamatum* allowed to highlight 2 peaks of enzymatic protein activity thanks to the use of ion exchange chromatography according to the work of Sylva *et al*.[12] After two other separation steps (molecular exclusion chromatography on Sephacryl S-100 HR gel, hydrophobic interaction chromatography on Phenyl-Sepharose CL-4B gel), two protein bands were revealed on polyacrylamide gel in native condition showing their state of purity (Fig 2). The final chromatographic step on hydrophobic interaction gel was decisive. Indeed, it allowed to eliminate by elution, using salt gradients, the proteins of no interest. Native electrophoresis revealed a protein band for each pool of activity. Under denaturing conditions, the relative molecular weights determined in SDS-PAGE and gel filtration suggest that the Ab-CX_1 enzyme is tetrameric and Ab-CX_2 is dimeric. The specific activities of the Ab-CX_1 and Ab-CX_2 enzymes throughout the purification steps show an affinity of the enzymes for the xylane substrate (Table 1). According to Khandeparker and Numan,[13] the degradation of xylan networks located between the microfibrils of cellulose by xylanases could allow cellulases to access and degrade the microfibrils included in the deep structure of cellulose. Thus, degradation of the deep structure of the cellulose microfibrils may help xylanases or cellulases to access and degrade the xylan chains in the deeper structure. These enzymes (Ab-CX_1 and Ab-CX_2) are mesophilic (50°C-55°C) and stable in acidic pH (4.0-5.6) (Table 2). Such xylanases and cellulases were isolated from three isolates of native endophytic fungi according to studies by Yopi and Melliawati.[14] Also, a purified xylanase from the fungus Aspergillus flavus ARC-12 expresses its maximum activity at pH 6.0 and temperature 50 °C.[15]

These two enzymes purified from the abdomen of the small soldier of the termite Macrotermes subhyalinus have the particularity of being bifunctional, unlike the three xylanases and two cellulases isolated from the worker of the same species.[16,17] The feeding behaviour in these termites shows that the workers take nutritional care of the toy soldiers. This bifunctional activity of enzymes in the abdomen of the toy soldier could lead to competition in the digestion of plant material in order to prevent its degradation.

The purified isoforms of the abdomen of the small soldier of the termite Macrotermes subhyalinus can have various origins. Indeed, the work of Tokuda.[18] and Zhang.[19] showed that the enzymes endogenous or secreted by the termite itself are distributed in the salivary glands and in the middle intestine (abdomen). Those that are synthesized by the microbial flora housed in the original rectal rumen are referred to as exogenous enzymes. In this sense, recent biochemical and molecular studies carried out on the enzymes of termites of the Rhinotermitidae family.[20,21,22,23] and also in the Kalotermitidae.[24,25,26] reported that the presence of cellulases in the salivary glands implies that they are synthesized by the termite itself. Also, these enzymes can be found in the abdomen and are responsible for certain digestive hydrolyses. From the above, it is thought that the little soldier of the termite *Macrotermes subhyalinus* synthesizes its own cellulases and xylanases in addition to those it could acquire from symbionts. The study of effectors on xylanase and carboxymethylcellulase activities indicates the activating effect of zinc, manganese, sodium and potassium ions at different concentrations of 1 and 5 mM (Table 3). This activating effect of manganese is similar to that observed by.[27] for *Bacillus amyloliquefaciens* xynalases. EDTA has no significant effect on xylanasic and carboxymethylcellulase activities. This result suggests that these enzymatic activities do not require the presence of ions for the catalytic act as EDTA is an ion chelator.

CONCLUSION

In the abdomen of the small soldier of the termite *Macrotermes subhyalinus* two isoforms Ab-CX_1 and Ab-CX_2 by low pressure chromatographic techniques. These enzymes are acidic, mesophilic and have the ability to hydrolyze both xylan and carboxymethylcellulose, making them bifunctional enzymes. Based on data from the literature, the enzymes Ab-CX_1 and Ab-CX_2 could be of bacterial origin in favor of symbiosis or secreted by the termite itself. This assertion shows the established digestive symbiosis between termite castes and symbiotic microorganisms.

REFERENCE BIBLIOGRAPHIQUE

1. Hyodo F, Tayasu I, Inoue T, Azuma IJ, Kudo T. Differencial role symbiotic fungi in lignin degradation and food provision for fungus-growing termites (*Macrotermitinae: Isoptera*). *Functional Ecology*, 2003; 17: 186-193.
2. Taprab Y, Ohkuma M, Johjima T, Maeda Y, Moriya S, Inoue T, Suwanarit P, Noparatnaraporn N. Kudo T. Molecular phylogeny of symbiotic basidiomycetes of fungus-growing termites in Thailand and their relationship with the host. Biosci. Biotechnol. Biochem, 2002; 66: 1159-1163
3. Ohkuma M. Termite symbiotic systems: Efficient bio-recycling of lignocellulose. Applied Microbiology and Biotechnology, 2003; 61(1): 1-9.
4. Makonde HM, Mwirichia R, Osiemo Z, Boga HI, Klenk HP. Pyrosequencing-based assessment of bacterial diversity and community structure in termite guts, mounds and surrounding soils. Springer Plus, 2015; 4(1): 471.
5. Hartke TR, Baer B,. The mating biology of termites: a comparative review. Animal. Behaviour, 2011; 82: 927–936.
6. Fischer R, Ostafe R, Twyman RM. Cellulases from insects. In: Vilcinskas, A. (Ed.), Yellow Biotechnology II: Insect Biote.chnology in Plant Protection and Industry. Advances in Biochemical Engineering/Biotechnology, vol. 136. Springer, Berlin Hei-delberg, Berlin, 2013; 51–64.
7. Beg QK, Kapoor M, Mahajan L, Hoondal GS. Microbial xylanases and their industrial applications: a review. Appl Microbiol Biotechnol, 2001; 56: 326–338
8. Wakiyama M, Tanaka H, Yoshihara K, Hayashi S, Ohta K Purification and properties of family-10 endo-1,4-beta-xylanase from Penicillium citrinum and structural organization of encod- ing gene. J Biosci Bioeng, 2008; 105(4): 367–374.
9. Dodd D, Cann IKO. Enzymatic deconstruction of xylan for biofuel production. Glob Chang Biol Bioenergy, 2009; 1: 2-17.
10. Lowry OH, Rosebrough NF, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem, 1951; 193: 265-75.
11. Laemmli UK. (1970). Cleavage of structural proteins during the assembly of head of bacteriophage T-4. Nature (London), 1970; 227: 680-685.
12. Silva LAO, Terrasan CRF, Carmona EC. Purification and characterization of xylanases from *Trichoderma inhamatum*. Electronic Journal of Biotechnology, 2015; (18): 307–313.
13. Khandeparker R, Numan MT. Bifunctional xylanases and their potential use in biotechnology. Journal Ind. Microbiology Biotechnology, 2008; 35: 635–644.
14. Yopi, W Tasia, and R Melliawati. Cellulase and Xylanase Production from Three Isolates of Indigenous Endophytic Fungi. Earth and Environmental Science, 2017; 101: 012-035.
15. Archana Gautam, Kumar Amit, and Dutt Dharm Production and Characterization of Cellulase-free Xylanase by *Aspergillus flavus* ARC-12 and its Application in Pre-bleaching of Ethanol-Soda Pulp of *Eulaliopsis binate*. Res. J. Biotech, 2017; 12(8): 63-71.
16. Faulet MB. Purification, caractérisation physico-chimique et moléculaire des xylanases de l'ouvrier du termite *Macrotermes subhyalinus* et de son champignon symbiotique *Termitomyces sp*. Thèse de l'Université d'Abobo-Adjamé, 2006; 180.
17. Séa TB, Saki SJ, Coulibaly AF, Yeboua AF, Diopoh KJ. Extraction, purification et caractérisation de deux cellulases du termite *Macrotermes subhyalinus (Termitidae).* Agronomie Africaine, 2006; 10: 57-65.
18. Tokuda G, Saito H, Watanabe H. A digestive β-glucosidase from the salivary glands of the termite, *Neotermes koshuensis* (Shiraki): Distribution, characterization and isolation of its precursor cDNA by 5_- and 3_-RACE amplifications with degenerate primers. Insect Biochemistry Molecule Biology, 2002; 32: 1681–1689.
19. Zhang S, Mo J, Teng L, Cheng M, Cheng J. Inter-colonial variation in the compositions of the frontal gland secretion of *Coptotrmes formosanus* (Isoptera: Rhinotermitidae). Sociobiology, 2004; 47(2): 553–561.
20. Watanabe H, Nakamura M, Tokuda G, Yamaoka I, Scrivener AM, Noda H. Site of secretion and properties of endogenous endo- β -1,4-glucanase components from *Reticulitermes speratus* (Kolbe), a Japanese subterranean termite. Insect Biochemistry and Molecular Biology, 1997; 27: 305–313.
21. Watanabe H, Noda H, Tokuda G. Lo N. 1998. A cellulase gene of termite origin. Nature, 1998; 394: 330-331.
22. Tokuda G, Lo N, Watanabe H, Slaytor M, Matsumoto T, Noda H. Metazoan cellulase genes from termites: intron/exon structures and sites of expression. Biochim. Biophys. Acta, 1999; 1447: 146–159.
23. Watanabe H, Tokuda G. Animal cellulases. Cell. Mol. Life Sciences, 2001; 58: 1167–1178.
24. Lo N, Tokuda G, Watanabe H, Rose H, Slaytor M, Maekawa K, Bandi C, Noda H. Evidence from

multiple gene sequences indicates that termites evolved from woodfeeding cockroaches. Curr Biol, 2000; 10: 801-804.
25. Thompson GJ, Kitade O, Lo N, Crozier RH. Phylogenetic evidence for a single, ancestral origin of a 'true' worker caste in termites. Journal of Evolutionary Biology, 2000; 13: 869–881.
26. Nakashima K, Watanabe H, Saitoh H, Tokuda G, Azuma JI. Dual cellulose-digesting system of the wood-feeding termite, *Coptotermes formosanus* Shiraki. Insect Biochemestry Molecular Biologie. Jul, 2002; 32(7): 777-84.
27. Kumar S, Haq I, Prakash J, Singh SK, Mishra S, Raj A. Purification, characterization and thermostability improvement of xylanase from *Bacillus amyloliquefaciens* and its application in pre-bleaching of kraft pulp. Biotech, 2017; 3(7): 20.