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# Inhibitors of Cellulase Activities According to the Trophic Group of Termites (Insecta: Isoptera) from Daloa (Côte d'Ivoire)

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

The presence of termites in the cocoa plantations and quarries of Côte d'Ivoire poses a threat to the producers of this sector. Producer yields are insufficient to cover the strong market demand. This situation leads to food insecurity for the population. Knowledge of the specific inhibitory molecules of digestive enzymes of termites is necessary to enhance the effectiveness of insecticides to optimize crop production. The present study was aimed to characterize termite cellulases according to the trophic group. Specifically, the influence of chemical agents on the

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cellulase activities of four humivorous (*Cubitermes fungifaber*) and xylophagous termites (*Nasutitermes latifrons*, *Microcerotermes fuscotibialis* and *Amitermes guineensis*) collected in Daloa during the October period was investigated. Thus, the cellulase activities were measured by the spectrophotometric method in the absence and in the presence of the concentrations of 1 and 5 mM of various chemical agents. The chemical agents used behaved differently on cellulase activities. Thus, $Cu^{2+}$, $Pb^{2+}$ and EDTA inhibited the cellulase activity of *M. fuscotibialis* more than 90% at concentrations of 1 and 5 mM, respectively, indicating the presence of a metalloprotein. On the other hand, that of the other two xylophagous species was slightly inhibited. In addition, the cellulase activity of C. Fungifaber was inhibited at the two respective concentrations by $Cu^{2+}$ at about 70%. In conclusion, $Cu^{2+}$, $pb^{2+}$ and EDTA can be used in the formulation of some specific insecticides against humivorous and xylophagous termites.

*Keywords: Chemical agents; cellulases; termites; cultures; Côte d'Ivoire.*

## 1. INTRODUCTION

In terrestrial ecosystems, termites have important functions. Thus, numerous works carried out in Ivory Coast showed the damage caused by these insects on the oil palm [1], the rubber tree [2], in the mango orchards [3] and the cacao tree [4].

The strong expansion of termites as pests of cultivated plants is due to their great ability to degrade the constituents of wood (polysaccharides, lignin, tannins, etc.) thanks to the digestive enzymes they possess, notably cellulases, which are responsible for the degradation cellulose [5]. Studies conducted by Blei et al. On the determination of the physicochemical properties of cellulases of soldiers of *Macrotermes subyalinus* [6,7,8] and termite workers according to their trophic group (publication in progress) have shown that Behave differently Faced with this situation, strengthening the efficacy of insecticides is therefore necessary to guarantee crop production.

The present study was aim**ed** to characterize termite cellulases according to the trophic group. More specifically, it will be necessary to determine the chemical agents capable of inhibiting the cellulase activities of four species of humivorous (*Cubitermes fungifaber*) and xylophagous termites (*Nasutitermes latifrons*, *Microcerotermes fuscotibialis* and *Amitermes guineensis*) collected at Daloa in order to know the inhibitors.

## 2. MATERIALS AND METHODS

### 2.1 Biological Material

The biological material consists of the species of Humivorous termites (*Cubitermes fungifaber*) and xylophagous (*Amitermes guineensis*, *Nasutitermes latifrons, Microceroterms fuscotibialis*) collected in plantations of cacao, coffee and teak of Daloa (Côte d'Ivoire*)*.

### 2.2 Methods

#### 2.2.1 Sampling technique

Termites were first harvested from dead woods and soil with equipment (such as daba, machete) and kept in perforated boxes to let air through to keep them alive. Then, some termites of each species were kept in labeled eppendoffs containing 70% alcohol to identify them. The identification of different species of termites collected, was carried out using a binocular loupe. Several manuals have been used to identify them [9]. And other termite samples were brought to the laboratory to be stored at -20ºC in a freezer for analysis of their enzyme equipment.

#### 2.2.2 Technique for obtaining enzymatic crude extracts

Five hundred and fifty (550) workers of various termite species were washed with distilled water and dewatered on whatmann paper No.1. These samples were ground in a porcelain mortar containing 30 ml of NaCl (0.9%, w / v). The ground material obtained was centrifuged at 13,750 rpm for 30 minutes at a temperature of 4ºC in a 5427R centrifuge. The supernatant obtained constituted the enzymatic crude extract of the workers (*A. guineensis*, *C. fungifaber*, *N. latifrons*, *M. fuscotibialis*).

#### 2.2.3 Measurement of cellulase activity

For the measurement of cellulase activity, the dosage of reducing sugars was carried out by the Bernfeld method [9] using 3,5-dinitrosalicylic acid (DNS). The reaction medium consisting of 80 µl of 20 mM acetate buffer pH 5.0, 100 µl of enzymatic solution and 200 µl of substrate (Carboxymethylcellulose, 0.5%, w / v) was used. This reaction medium was incubated in a water bath at 37ºC. for 30 minutes. Then, 300 µl of a DNS solution was added to stop the enzymatic reaction. It was then homogenized and heated on a steam bath for 5 minutes and then cooled for 10 minutes at room temperature (25ºC). Absorbance was measured at 540 nm spectrophotometer (Gilson) against a control (containing all products except the enzyme solution) after adding 2 ml of distilled water. This absorbance was then converted into micromoles of reducing sugars by means of a calibration line obtained using a glucose solution (2 mg/ml).

#### 2.2.4 Influence of chemical agents on enzymatic activities

The effect of chemical agents on enzyme activity was studied by pre-incubating the enzymatic crude extract of each termite species for 2 hours at room temperature (25ºC) in the presence of different chemical agents such as salts of potassium chloride (KCl), sodium chloride (NaCl), barium chloride ($BaCl_2$), copper sulphate ($CuSO_4$), potassium iodide (KI), lead acetate (pb( $C_2H_3O_2$) $_2$), ethylene diamine tetra acetate (EDTA) and hydroymethylamino methane (tris), at concentrations of 1mM and 5mM, respectively. Cellulase related activities were measured under standard conditions.

## 3. RESULTS AND DISCUSSION

### 3.1 Results

The results of Figs. 1, 2, 3 and 4 show the sensitivity of the enzymatic activity in the presence of some metal ions ($Na^+$, $K^+$, $Ba^{2+}$, $Cu^{2+}$, $Pb^{2+}$, $I^-$), EDTA and Tris. For the 1 mM and 5 mM concentrations, the $Na^+$, $K^+$, $Ba^{2+}$ and Tris agents have virtually no effect on the cellulase activity of *C. fungifaber*, *A. guineensis* and *N. latifrons* (Figs. 1, 3 and 4). However, at the concentration of 5 mM, Tris and $Ba^{2+}$ ion activate the cellulase activities of termites *M. fuscotibialis* and *N. latifrons* respectively at 31 and 11% (Figs. 2 and 4). In addition, $Cu^{2+}$ and EDTA are present as inhibitors (Figs. 1, 2, 3 and 4) at concentrations of 1 and 5 mM, respectively. However, the cuprous ion ($Cu^{2+}$) inhibits the cellulase activity of *M. fuscotibialis* by more than 95% compared to the metal ions used (Fig. 2). In addition to the $Cu^{2+}$ ion and EDTA, the iodide I- ion inhibited the cellulase activities of *C. fungifaber*, *M. fuscotibialis* and *N. latifrons* (Figs. 1, 2 and 4) while it has no effect on that of *A. guineensis* (Fig. 3). In addition, the Pb2 + ion inhibited respectively the cellulase activities of *C. fungifaber*, *M. fuscotibialis and N. latifrons* at 42,70 and 36% at the concentration of 5 mM (Fig. 4). On the other hand, it activate**d** by 8% the cellulase activity of *A. guineensis* at concentrations of 1 and 5 mM, respectively (Fig. 3).

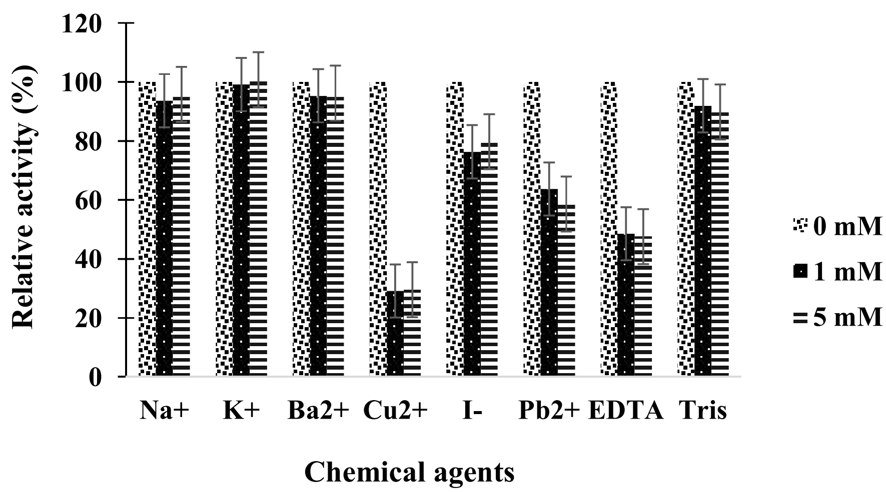


**Fig. 1. Cellulase relative activity of termite *C. fungifaber* as a function of the concentration of chemical agents**

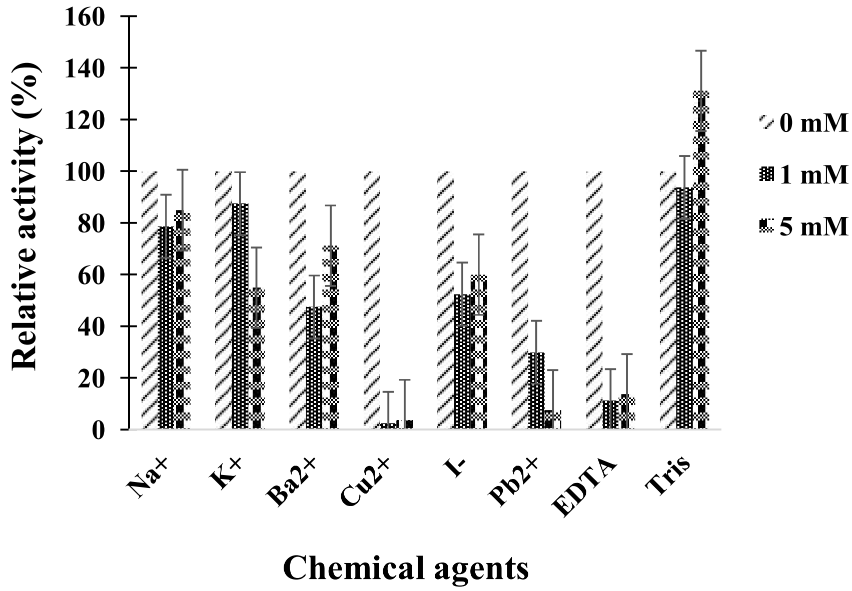


**Fig. 2. Relative cellulase activity of termite *M. fuscotibialis* as a function of the concentration of the chemical agents**

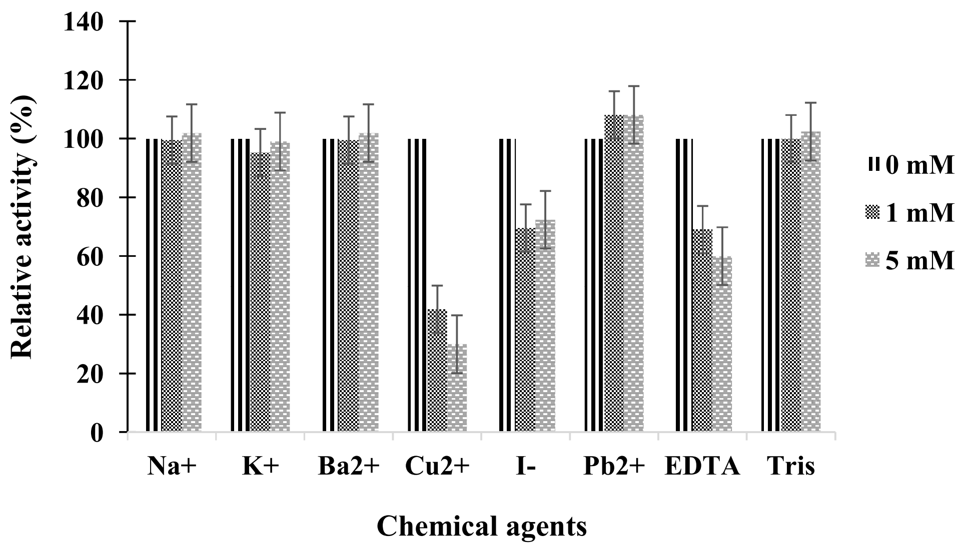


**Fig. 3. Cellulase relative activity of termite *A. guineensis* as a function of the concentration of chemical agents**

## 3.2 Discussion

Sodium and potassium chloride salts influence the change in enzymatic activity, certainly through interaction with regulatory sites [10]. According to Roy et al. [11]. Na $^+$ and K + ions have an effect on enzymatic activity due to changes in electrostatic binding that would affect the tertiary structure of the enzyme. Comparison of enzymatic activity with other studies has shown that the endoglucanic activity of *Aspergillus flavus* increases with increasing concentration of $Na^+$ and $K^+$ cations [12]. However, this activity was strongly influenced by

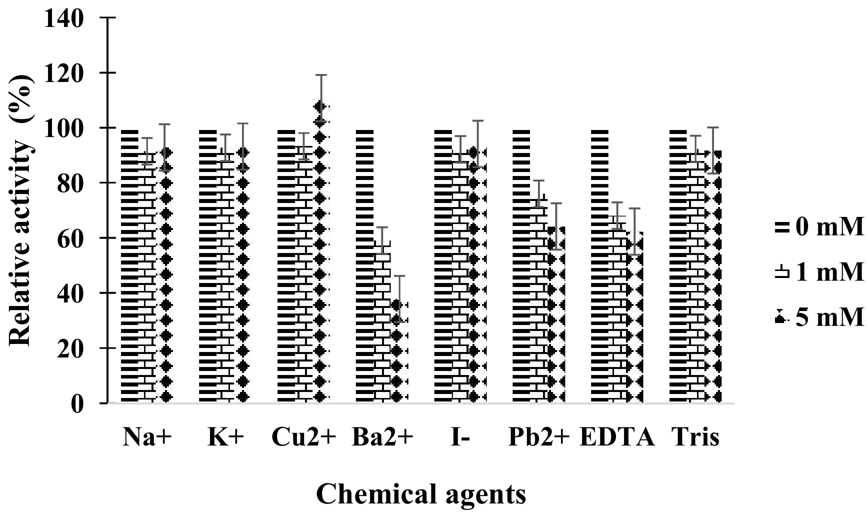


**Fig. 4. Cellulase relative activity of *N. latifrons* termite as a function of the concentration of chemical agents**

the presence of the $K^+$ ion with a concentration of 1 mM (85% of the activity). In the case of this study, the $K^+$ and $Na^+$ ions have practically no effect on the activity. A study of bacterial cellobiohydrolases reported a slight improvement in activity with $Ba^{2+}$ [13,14], and a fungal cellulase was inhibited by $Ba^{2+}$. For their part, Zhu et al. [15] showed that the $Ba^{2+}$ ion could lead to a moderate increase in the activity of a Geobacillus esterase and deep with a concentration of 10 mM. All these results are in the same direction as those reported in this study with the different species studied because these same effects of barium were found. This behavior of the $Ba^{2+}$ ion on the cellulase activity could be due to the composition of the amino acids or to the presence of certain ions in the catalytic site of the enzyme. Inhibition of cellulase activity by $Cu^{2+}$ ion are consistent with those obtained by several authors. Thus, Roy et al. [16] have shown in previous studies that $Cu^{2+}$ has significant inhibition on endoglucanases in *Myceliophthora thermophila* D-14 (ATCC48104A). Similarly, Deb et al. [17] show inhibition of enzymatic activities in *Bacillus amyloliquefaciens* P-001 by a number of metal ions, including $Cu^{2+}$ copper ion. According to these authors, this divalent ion behaves as a non-competitive inhibitor of enzymatic activity. Copper does not attach to the active site as competitive inhibitors. It is rather related to a side group of the enzyme thus modifying the structure of the enzyme. Also, the way it folds changes the active site [18]. In addition, the indirect reduction of enzymatic activity following the interaction of the toxic part of copper with the microorganisms affects the enzymatic production [19]. Thus, the metals can bind to the substrate or react with the substrate enzyme complex [20]. Inhibition of lead is thought to be due to the presence and fusion between lead and thiol groups of the enzyme [21, 22], since lead increases the activity of other enzymes. Thus, lead varies the characteristics of these enzymes or stop the activity of their inhibitors [21]. This is the case of the species *A. guineensis* whose activity increases in the presence of lead. This is in agreement with the studies of Seregin & Ivanov [21]. Lead inhibits enzymatic activities as a whole and achieves an inactivation constant of between 10-5 and 2 x 10-4 M, which means that 50% of enzyme activities are inhibited in this concentration range [21]. $Pb^{2+}$ is therefore a potent inhibitor [10]. These results corroborate with the results obtained for *C. fungifaber*, *N. latifons* and *M. fucotibialis* species. The inhibition of enzymatic activity by EDTA in the four species studied is explained by the complexation of certain metal ions necessary for the activation and stabilization of the enzyme [23]. These enzymes are metalloproteinic in nature. The low activity suggests that the metal ion has a very high affinity for the enzyme or that the ion is difficult to access in the assay because of steric constraints or amino acid residues because the inhibitory effect of this compound depends on the relative stability of the EDTA-ion complex compared to that of the ion-enzyme complex. Previous studies on endoglucanases, Lee et al. [24] on *Bacillus amyloliquefaciens* DL-3 showed activity inhibition with the presence of EDTA. These results are identical to this study. On the other hand, other studies have shown that at concentrations of 1 and 5 mM, EDTA had no effect on the amylase activity of Archaea. Therefore, this result deduces that the enzyme is not a metalloprotein [25]. Which is not consistent with that of this study.

## 4. CONCLUSION

The divalent ion $Cu^{2+}$ and the EDTA ion chelator are presented as inhibitors of the cellulase activities of the 4 species studied. Moreover, the $Pb^{2+}$ ion inhibits the cellulase activity of *Microceroterms fuscotibialis*. The $Cu^{2+}$ and $Pb^{2+}$ ions as well as the EDTA ion chelator can be used in the formulation of certain specific insecticides for strengthening the fight against humivorous and xylophagous termites.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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