

# Effect of Hydroquinone and Naphtoquinone on the Purified Bifunctional Endoxylanase from the Salivary Glands of the Major Soldier of Termite *Macrotermes subhyalinus* (Insecta: Isoptera)

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**Abstract:** The GX endoxylanase, which are responsible for the degradation of xylan and carboxymethylcellulose, isolated from the salivary gland of the major soldier of termite *M. subhyalinus*, has been characterized with a view to highlighting the effect of defensive substances present in salivary secretions in the major soldier on the performance of digestive enzymes. The carboxymethylcellulase (Cel) and xylanase (xyl) activities of the enzyme GX are inhibited by hydroquinone and naphthoquinone at different concentrations. cel and xyl are inhibited by concentrations of hydroquinone at 2 and 3 mM respectively. however, these activities were inhibited by naphthoquinone at a concentration of 3 mM at levels ranging from 20 to 60 % (w/v). This shows that the presence of these substances could be at the origin of the diet by stomodeal trophallaxy of the major soldier by the worker of the same termite in *M. subhyalinus*.

**Keywords:** *Macrotermes subhyalinus*, xylanase, carboxymethylcellulase, hydroquinone, naphthoquinone

## 1. Introduction

Termites are insects known for the damage they cause in plantations [1]. However, because of their numbers and diet, these insects contribute to the management of ecosystems by promoting the recycling of organic matter and the formation of humus, the physicochemical properties and the functions of the soil. This could be an indicator of soil restoration by incorporating nutrients into the soil through the decomposition of plant residues, and a tool of choice to address the problems of soils damaged by bush fires in many tropical countries in general and in particular Côte d'Ivoire [2], [3]. In addition, termites are characterized by a good structured social organization consisting of breeding castes consisting of the king and queen and neutral castes represented by soldiers and workers. Their diet consists mainly of wood and the components they digest into simpler molecules that can be assimilated by the body, thanks to the enzymes they possess in particular the cellulolytic and xylanolytic enzymes [4], [5], [6]. These enzymes are synthesized either by the termite (salivary glands and midgut) [7], [8], [9], or by the symbiotic microorganisms present in its digestive tract [10], [11], most of the work on extracellular enzymes produced by Fungi have in fact mainly affected basidiomycetes.

Despite this, the major soldiers are unable to feed themselves. They feed on stomodeal trophallaxy thanks to the workers. And yet, the major ones come from the

transformation of the worker [12], [13]. Regarding their enzymatic equipment, very little work has been done because of its low glycosidic activity compared to that of the worker [14], [6], [15]. Olagbemiro et al. (1988), reported the presence of defense substances of the family of quinones in the salivary glands of the major soldier. So would not these substances be the cause of soldier's inability to self-feed. What is the interest of trophallactic in the soldier? Or in other words, what is the impact of defences on the ability of soldier's hydrolases?

The objective is to characterize the xylanolytic and cellulolytic enzymes of the major soldier in order to highlight the effect of the defensive substances present in the salivary secretions of the major soldier on the performance of the digestive enzymes. This was done in order to determine the origin of the stomodeal trophallactic diet of this insect by the worker.

## 2. Materials and methods

### 2.1. Chemical Product

Substrates such as carboxymethylcellulose and birch wood xylan are chemicals from Sigma Chemical Company (St. Louis, MO, USA). The other products are of analytical grade.

## Reagents preparation

Reagents used by the method of Lowry et al. (1951) consist of solution A: Folin-Ciocalteus reagent diluted by half in 0.1 N sodium hydroxide; of solution B: sodium carbonate (2%, w / v) prepared in 0.1 N sodium hydroxide; solution C1: copper sulfate (0.5%, w / v) prepared in distilled water; of solution C2: sodium tartrate of sodium and potassium (1%, w / v) prepared in distilled water and solution D: prepared extemporaneously from solutions C1, C2 and B in proportions of 0.1; 0.1; 10 (v / v / v). Chromogenic reagents used by the Bernfeld method (1955) were prepared. Two (2) g of Di Nitro Salicylic acid (DNS) and 3.2 g of sodium hydroxide were dissolved in 70 ml of distilled water. Then, 60 g of potassium sodium double tartrate was gradually added to this mixture under heating and stirring. The final volume was made up to 200 ml with distilled water.

## 2.2. Biological Materials

Purified enzyme GX from the major soldier salivary glands of the *M. subhyalinus* were preserved at a temperature of - 20 °C [9].

## 2.3. Measurement of glycosidic activities

In order to measure, under standard conditions, the carboxymethylcellulase and xylanase activities present in the enzymatic purified extract of the salivary glands of the major soldier of the termite *M. subhyalinus*, the quantity of reducing sugars released was determined according to the method of Bernfeld (1955) using the 3,5- dinitrosalicylic acid (DNS). The reaction medium consisting of enzymatic solution (100 µl) and carboxymethylcellulose (CMC) or xylan substrates (0.5%, w / v, 200 µl) was incubated in a water bath for 30 min at 45.degree. Then, 300 µl of a DNS solution was added to stop the enzyme reaction. The new reaction medium was then homogenized and heated on a steam bath for 5 minutes and then cooled for 10 minutes at room temperature (25 °C). Absorbance was measured at 540 nm on a spectrophotometer against a control (containing all the products except for the enzyme solution) after adding 2 ml of distilled water. This absorbance was then converted to micromoles of reducing sugars by means of a calibration line obtained using a glucose solution (2 mg / ml). The enzyme unit (EU) or International Unit (IU) has been defined as the amount of enzyme that catalysis the hydrolysis of one µmole of substrate per min under the conditions described above. Specific activity was expressed in µmole of reducing sugars released per min and per mg protein (IU / mg protein) [18], [9].

## 2.4. Protein assay

Concerning the evaluation of the specific activities of the enzymes and the elution profiles of the proteins after chromatography, the protein concentrations were determined according to the method of Lowry et al. (1951) using Folin- Ciocalteus reagent. Each protein aliquot (20 to 100 µl) was diluted in 2 ml of solution D. The mixture was stirred and incubated for 10 min at room temperature. Then, 200 µl of solution A were added thereto. The reaction medium was stirred and allowed to stand for 30 minutes to

allow the development of the coloring. One (1) ml of distilled water was added thereto and then the absorbance of the test was measured at 660 nm on the UV-visible spectrophotometer (Spectronic Genesys 5) against a control made under the same conditions but not containing protein extract. Absorbance was converted to mg of protein using a calibration line obtained with a stock solution of bovine serum albumin (BSA) (0.2 mg / ml).

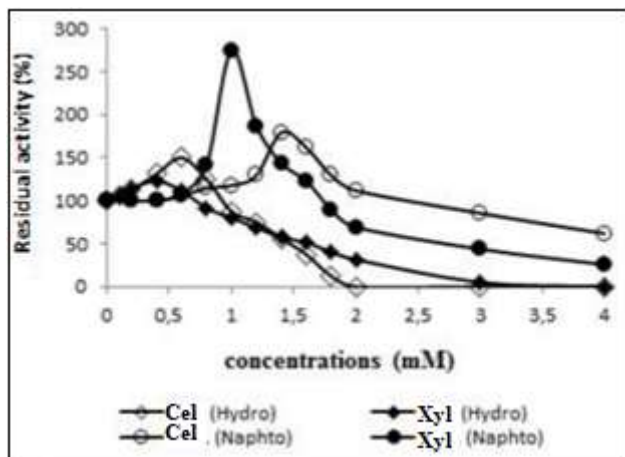
## 2.5. Effect of quinones on the activity of purified enzymatic extracts

Free radicals that can cause the destruction of DNA, proteins and amino acids [20]. These observations would suggest that the stomodeal trophallactic phenomenon would eventually allow the major soldier to receive pre-degraded food by the worker's enzymes to complete the enzymatic activities of the digestive tract.

The effect of the chemical agents on the purified enzyme was determined by incubating the GX enzyme for 2 h at room temperature (25 °C) in the presence of hydroquinone or naphthoquinone at different concentrations (0.1 to 4 mM). After incubation, the residual activity was determined by the standard enzyme assay using xylan from Birchwood or CMC as substrate. The activity of enzyme assayed in the absence of the chemical agents was taken as 100 %.

## 3. Results and discussion

Cel and Xyl respectively represent the carboxymethylcellulase and xylanase activities of the GX enzyme. Cel and xyl are activated by hydroquinone, respectively at concentrations of between 0 and 0.6; 0 and 0.4 mM. Beyond these concentrations, the two enzymatic activities are inhibited. Cel and Xyl are inhibited by concentrations of hydroquinone at 2 and 3 mM respectively (Figure 1). When the naphthoquinone is used at the concentration of 3 mM in the reaction medium, the activities Cel and Xyl, respectively of the enzyme GX are inhibited at levels ranging from 20 to 60 % (w/v) (Figure 1). This result shows that these substances can be effectors of biocatalysts. It is therefore at the origin of the weak hydrolytic activity of the enzymes of the soldiers characterized by the above - mentioned authors. Ollinger & Brunmark (1991) revealed that quinones are mutagenic and toxic substances. The inhibitory action of these substances is thought to be due to the formation



**Figure 1:** Effect of hydroquinone and naphthoquinone on the carboxymethylcellulase and xylanase activities of the GX enzyme of the total grindings of the major soldier of *M. subhyalinus*

#### 4. Conclusion

Hydroquinone and naphthoquinone were able to inhibit the xyl and cel activities of the endoxylanase from the salivary glands of major soldier of *M. subhyalinus*. The presence of these substances in the salivary secretions would be one of the causes of the weak activity of the enzymes of the soldier. This, in relation to those of the worker and could be one of the negative factors at the origin of the stomodaeum trophallaxy diet of the great soldier by the termite worker.

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