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Comparative Study of Cellulase Activity in *Periplaneta americana*, *Odontotermes obesus* and *Philosamia ricini*

Dip Jyoti HALOI¹, Aparajita BORKOTOKI², Rita MAHANTA³

ABSTRACT [ENGLISH/ANGLAIS]

The common household cockroach (*Periplaneta americana*) and termite (*Odontotermes obesus*) are considered to be a serious pest and eri silkworm (*Philosomia ricini*) is a beneficial insect for human kind, feeding on cellulose rich substances. To have a better insight, how these insects are adapted to feed on cellulose rich diet, an experiment was carried out where cellulase enzyme activity in all the three different insect species was determined both in foregut and midgut homogenates. The cellulase activity was measured by DNSA method using crystalline cellulose substrate. The highest amount of enzyme activity was recorded in both foregut and midgut of cockroach (foregut 18.5 ± 4.29 , midgut 125 ± 15.82) followed by eri silkworm (foregut 0.932 ± 0.035 , midgut 1 ± 0.073) and termite (whole gut 0.519 ± 0.044). The higher cellulolytic activity in the gut of cockroach may be the result of its adaptation to almost all kinds of habitat which might have given them a better survival value than any other insects. Additionally, by enhancing the cellulose assimilation rate in silkworm the quality and quantity of eri silk production can be increased and identification and characterization of crucial insect cellulases may help in the development of insecticidal technologies and lignocellulosic ethanol production which is considered an alternate to fossil fuel in near future.

Keywords: Cellulase, *Philosomia ricini*, *Periplaneta americana*, *Odontotermes obesus*

RÉSUMÉ [FRANÇAIS/FRENCH]

Le ménage commun blatte (*Periplaneta americana*) et les termites (*Odontotermes obesus*) sont considérés comme étant un ravageur important et ERI vers à soie (*Philosomia ricini*) est un insecte bénéfique pour l'espèce humaine, se nourrissant de substances cellulose riches. Pour avoir une meilleure idée, la façon dont ces insectes sont adaptés à l'alimentation sur l'alimentation de cellulose riche, une expérience a été réalisée, où l'activité cellulase enzyme dans les trois espèces d'insectes différentes a été déterminée à la fois dans l'intestin antérieur, l'intestin moyen et de broyats. L'activité cellulase a été mesurée par la méthode utilisant DNSA substrat de cellulose cristalline. Le montant le plus élevé de l'activité enzymatique a été enregistré dans les deux intestin antérieur et l'intestin moyen de la blatte ($18,5 \pm 4,29$ foregut, l'intestin moyen de $125 \pm 15,82$), suivie par ERI vers à soie (intestin antérieur $0,932 \pm 0,035$, $0,073 \pm 1$ intestin) et les termites (intestin toute $0,519 \pm 0,044$). La plus grande activité cellulolytique dans l'intestin de cafard peut être le résultat de son adaptation à presque tous les types d'habitat qui pourrait leur avons donné une valeur meilleure survie que les autres insectes. De plus, en améliorant le taux d'assimilation de cellulose dans des vers à soie de la qualité et la quantité de la production de soie eri peut être augmentée et l'identification et la caractérisation des cellulases insectes cruciales peuvent aider dans le développement de technologies et la production d'insecticides éthanol lignocellulosique qui est considéré comme un carburant de remplacement aux combustibles fossiles dans les dans un avenir proche.

Mots-clés: Cellulase, *Philosomia ricini*, *Periplaneta americana*, *Odontotermes obesus*

Affiliations:

¹ Department of Zoology, Handique Girls' College, Assam, INDIA

² Department of Zoology, Gauhati University, Assam, INDIA

³ Department of Zoology, Cotton College Assam, INDIA

* Email Address for Correspondence/
Adresse de courriel pour la correspondance:
diphaloj@rediffmail.com

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INTRODUCTION

Cellulose, the major constituent of plant cell wall is the most abundant carbohydrate polymer of earth [1]. Cellulose is a fibrous, insoluble, crystalline polysaccharide which is composed of repeated units of D-glucose units [2]. As the human population is increasing the world's energy demand is also increasing. So, there has been an increasing worldwide interest in alternative sources of energy such as agriculture biomass to substitute fossil fuel

based energy resource. [3, 4, 5]. Cellulose degradation requires the synergistic action of three types of glycoside hydrolases (GH): endo- β -1,4-glucanases (EG; EC. 3.2.1.4), exo- β -1, 4-cellobiohydrolases (CBH; EC. 3.2.1.91), and β -glucosidases (EC. 3.2.1.21)[6]. EG enzymes work by random cleavage of β -1,4 glycosidic bonds in the internal portions of cellulose strands to reduce the degree of polymerization of the cellulose chain into smaller subunits. CBH enzymes remove subunits at both reducing

and non-reducing ends of the cellulose chain, releasing either cellobiose or glucose. Due to the inhibition of EG enzymes by accumulation of cellobiose, the presence of β glucosidases to hydrolyze cellobiose to glucose is important for complete degradation of cellulose [7, 8].

Cellulolytic activities were originally thought to be limited to plants, bacteria, and fungi but now a days there is increasing evidence for the existence of animal cellulases, especially in invertebrates [9- 11]. There have been numerous reports on cellulolytic activity in insects [12-15] including identification and cloning of insect cellulases [16-21] Although relevant reviews on cellulolytic activity in insects are available [22,11] broad efforts to quantitatively characterize cellulolytic activity in insects are very limited [23].

Cellulose digestion has been shown in 78 species of insects from 20 families representing eight orders. Taxa in which cellulolytic capacity is common include the thysanuran family Lepismatidae (silverfish & firebrats), which has been elevated to ordinal status and renamed Zygentomabta [24] the isopteran (termites), and the three coleopteran families Anobiidae (furniture beetles & death watch beetles), Buprestidae (metallic wood borers), and Cerambycidae (long horn beetles). Cellulolytic capacity is also common in the orthopteran superfamily Blattoidea (roaches), the coleopteran family Scarabidae (scrab beetle), the dipteran family Tipulidae (crane flies), and the hymenopteran family Siricidae (woodwasp) [25].

The main goal of this study was to compare the cellulolytic activities against crystalline cellulose substrates across a range of insect species belonging to three taxonomic orders which are not given much attention except termite even though they have great potentiality to be used in biofuel ethanol production industry. Fluids from the gut of 3 insect species belonging to the orders Lepidoptera, Isoptera and Dictyoptera, were used to evaluate relative cellulase activities. Our data suggest differences in cellulolytic activity among insect orders, which can be correlated with their feeding strategies and adaptations for their successful survival under evolutionary stress exerted by natural selection process.

MATERIALS AND METHODS

Insect Collection and Dissections

Adults of Cockroach and termite and early 5th instar larva of silkworm were collected from nearby areas. Individuals

were cooled to 4 °C before dissection to slow down the metabolism and to provide easier handling and dissections were performed on ice. In the dissection, gut was collected separately in an attempt to discriminate from the salivary gland. Gut tissues from adult insects were dissected and separated into foregut and midgut, and pooled into separate micro centrifuge tubes with 100 μ l of water.

Estimation of Cellulase Enzyme Activity

Estimation of cellulase enzyme activity is performed by using a modified dinitrosalicylic acid (DNSA) assay [26]. Concentration of the released glucose was measured from a standard glucose curve.

Preparation of Crude Enzyme Sample

Crude enzyme sample was prepared by homogenizing 2.5 g of foregut and midgut insect sample of erisilkworm and cockroach and whole termite in 10 ml 0.1 M Phosphate buffer of pH 7.0. Homogenate was kept overnight in freezer and centrifuged at 10,000 rpm to discard pellet. 5 ml of supernatant was added to 20 ml of ice cold acetone and kept overnight at 4°C to get proteins in precipitate form. The mixture was centrifuged at 10,000 rpm for 15 min. The pellets were air dried and dissolved in 5 ml of 0.1 M phosphate buffer and 5 ml of Tris-HCl buffer of pH 6.0. This crude protein sample was stored at 4°C and used as the enzyme source.

Cellulase Enzyme Assay

Enzyme activity (U/mg) was determined considering one IU equal to 1 μ mol min⁻¹ of glucose formed in the hydrolysis reaction. Reaction mixture was prepared by mixing 100 μ l of the crude enzyme sample with 0.5 ml of crystalline cellulose solution and 0.5 ml 0.1 M sodium acetate buffer (pH 5.0). Then mixture was incubated for 5 h at 50°C C with gentle shaking. After incubation, 2 ml of DNS reagent was added to reaction mixture and incubated in boiling water bath for 15 minutes and then the absorbance was noted at 540 nm.

Statistical Analysis

Statistical analysis of the experimental data was made by analysis of variance (ANOVA).

RESULTS

Cellulolytic Activity in Silkworm Gut

After the estimation of cellulolytic activity in silkworm by DNSA method using crystalline cellulose as substrate the

activity found in foregut was 0.932U/mg of protein and in midgut it was 1 U/Mg of protein.(Table-I and figure-I). Moreover a significant difference was found between the cellulase activity in foregut and midgut which is confirmed by ANOVA test at $p < 0.005$ (Table-I).

Table 1: This table shows cellulase activity in Eri silkworm foregut and midgut

Parameter	Eri Silkworm	
	Foregut	Midgut
MEAN U/Mg of protein	0.932	1
SD	0.16	0.33
SEM±	0.035	0.073
CV(%)	17.16	7.3
	F _c -35.64	
ANOVA	F _{0.05} -4.10(for 1,38 df)	
	<i>Difference in mean is found</i>	

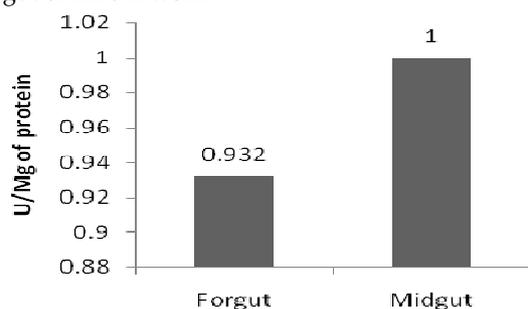
Table 2: This table shows cellulase activity in Termite gut

Parameter	Termite
MEAN U/Mg of protein	0.519
SD±	0.20
SEM±	0.044
CV (%)	38.53

Table 3: This table shows cellulase activity in Cockroach foregut and midgut

Parameter	Cockroach	
	Foregut	Midgut
MEAN U/Mg of protein	18.5	125
SD±	10.47	38.61
SEM±	4.29	15.82
CV(%)	56.59	30.97
	F _c -123.73	
ANOVA	F _{0.05} - 4.96.(for 1,10 df)	
	<i>Significant differences in mean is found</i>	

Figure 1: This table shows cellulase activity in foregut and midgut of Eri Silkworm



Cellulolytic Activity in Termite Gut

Following same procedure the cellulase activity was measured in termite gut. In the termite gut the activity was found to be 0.519U/Mg of protein. (Table-II and Figure-II).

Figure 2: This figure shows cellulase activity in Termite

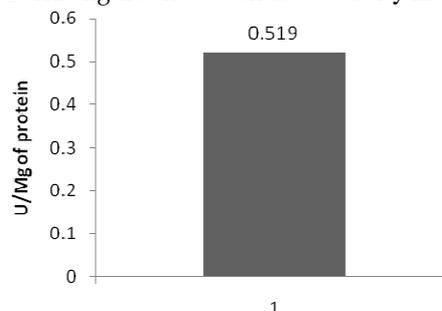


Figure 3: This figure shows cellulase activity in Cockroach

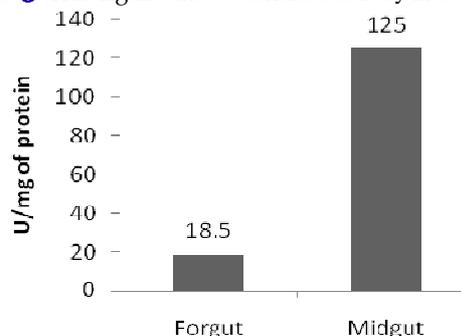


Figure 4: This figure shows cellulase activity in foregut

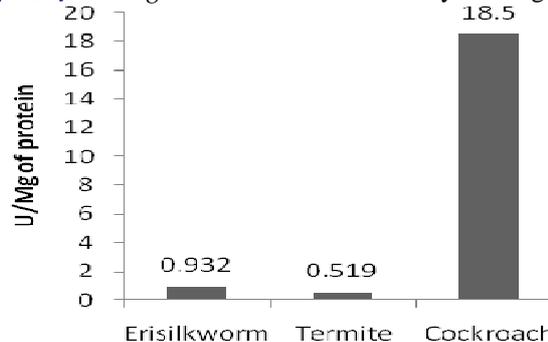
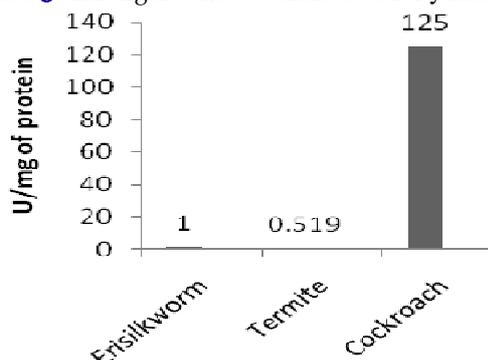


Figure 5: This figure shows cellulase activity in Midgut



Cellulolytic Activity in Cockroach Gut

The cellulolytic activity found in cockroach was 18.5U/mg of protein in foregut and 125 U/mg of protein in midgut respectively (Table-III and Figure-III). Moreover, a significant difference in the level of cellulolytic activity in foregut and midgut was found by ANOVA test at $p < 0.005$ (table-III).

DISCUSSION

Physiological functions of animal cellulases are distinct and depend on their source of secretion. Many insect species have been reported to have their cellulase activity [14]. In a screen for cellulolytic activity in insect gut, we detected high level of activity in both fore and midgut in three insect species belonging to orders dictyoptera, isoptera and lepidoptera. The experiment reports on the detection and preliminary investigation of cellulolytic activity in different parts of the gut from common household cockroach (*Periplaneta americana*), termite (*Odontotermes obesus*) and eri silkworm (*Philosomia ricini*). Except isopteran species no other insect species have traditionally been given much importance for cellulolytic prospecting, probably due to controversial reports of cellulolytic capacity in these insects [27, 28]. The present study in cockroach using homogenates from both foregut (18.5U/mg of protein) and midgut (125 U/mg of protein) revealed a significantly high level of cellulolytic activity than that of termite (0.519 U/Mg of protein) and silkworm (foregut 0.932U/mg of protein ,midgut 1U/mg of protein) gut activity. However, higher cellulase activity in cockroach than that of the eri silkworm and termite species may contribute in the importance of insects other than termite which was mostly discussed as insects to be a good source of cellulase activity. As reported by earlier workers that insects can produce their own cellulase themselves[29,18,30] along with reports on the production from symbiotic organisms harboring in the insect gut and both [31,32], our findings may challenge the traditional view of cellulase activity that cellulose digestion in insects was exclusively mediated by microbial cellulase activity in their hind gut. The reports of the present investigation however, in opposition to previous reports expressed a definite cellulase activity in the foregut and midgut in all the three experimental insect species. Higher cellulolytic activity in gut fluid found against crystalline cellulose suggest the presence of endogenous symbiont-independent cellulase system in all these three categories of insects.

It can be expected that in future detail study of these efficient lignocellulolytic systems will allow identification of novel enzymes possessing features that optimize biotechnological applications for the biofuel industry. Besides, enhancing the cellulose assimilation rate in eri silkworm which is one of the important component of its host plant the quality and quantity of erisilk production can be upgraded. Additionally, identification of crucial insect cellulases may help in the development of insecticidal technologies aimed at inhibiting their vital digestive role [33]. In these regards all the three insect species can be considered as model insects exhibiting effective cellulolytic activity.

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CONFLICT OF INTEREST

No conflict of interests was declared by authors.

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