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Vol. 14(20), pp. 1753-1756, 20 May, 2015
DOI: 10.5897/AJB2015.14631
Article Number: D0C25D753004
ISSN 1684-5315
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African Journal of Biotechnology

Short communication

Cellulolytic activity of gut extract of subterranean termite, *Odontotermes obesus* Rambur: A pretreatment tool for conversion of lignocellulosic biomass to fermentable sugar for biorefinery industry

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Received 10 April, 2015; Accepted 15 May, 2015

Lignocellulosic biomass is a chief and cheap raw material for bioethanol production. However, pretreatment is a critical and most expensive step in lignocellulosic biomass to biofuel conversion. Biological pretreatments offer an alternative for lignocellulosic biomass conversion using enzyme hydrolysis. Termites are well known for the ability to digest lignocelluloses, using it as a sole food source. To effectively digest ligonocellulose/wood, termites produce an array of enzymes along with the help of microbial and protist symbionts. Subterranean termite, like *Odontotermes obesus*, devoid of protist symbiont in their hind gut capable of digesting cellulose using endogenous cellulases produced naturally by them. The cellulolytic activity of gut extracts of *O. obesus* was evaluated with commercially available carboxymethylcellulose (CMC) for their efficacy in conversion of cellulose to fermentable sugar. It is found that the gut extracts of *O. besus* potentially convert 49 to 75% of CMC into glucose. Hence, we look for novel hydrolytic enzymes in the gut extracts of *O. obesus* for efficient conversion of lignocellulosic biomass to fermentable sugars.

Key words: Termite gut, *Odontotermes obesus*, enzymes, lignocelluloses, hydrolytic enzymes.

INTRODUCTION

Petroleum products are the main transportation fuel. It has been recognized for some time that current use of fossil fuels will not only deplete the world´s oil reservoir but also have serious impact on the environment, leading to increased health risk and global climate change (Panwar et al*.,* 2010). It has been estimated that fossil fuels will be depleted by the year 2100 which makes the need for alternative fuels solutions significant (Saxena et al*.,* 2007). Global warming can mainly be attributed to an increase in CO_2 emissions which have increased by 30% in the past 200 years. Renewable energy causes little or no pollution (European Renewable Energy Council, 2008). Fuel produced by the activity of microorganisms, such as ethanol, methane and hydrogen, are called

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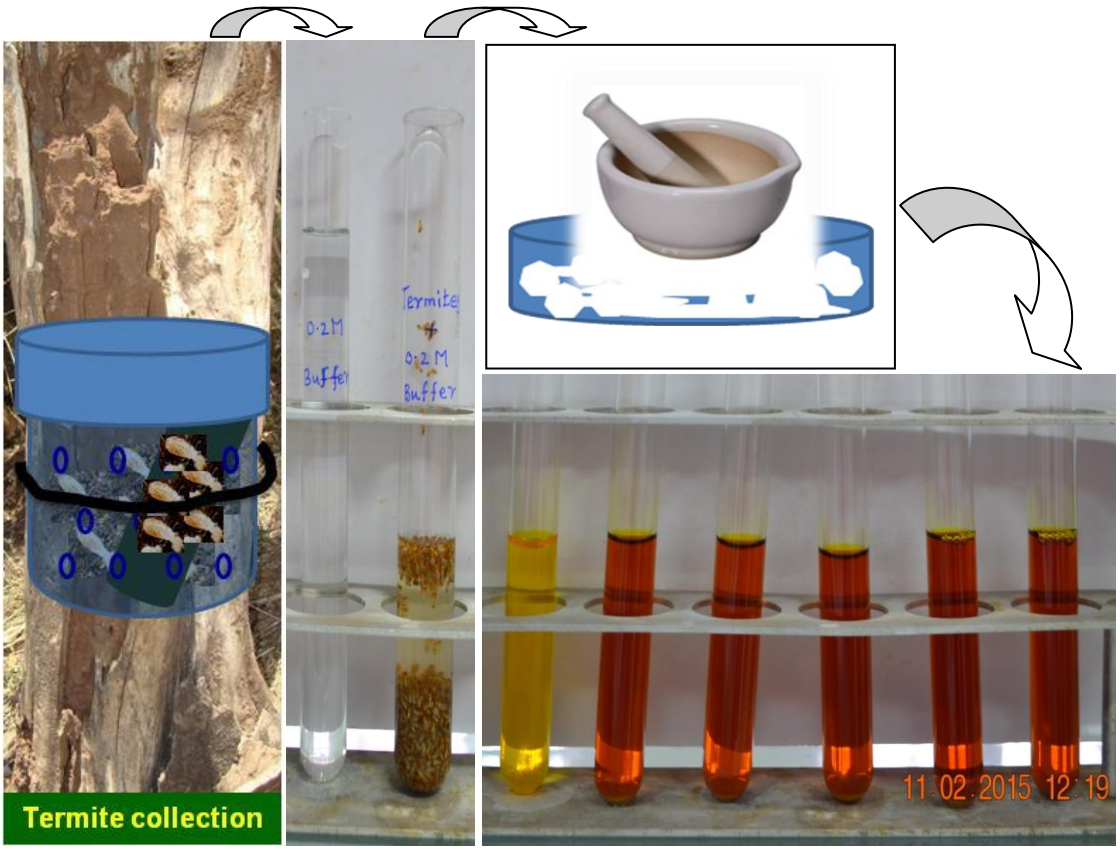

Plate 1. Termite collection and extraction of enzymes and enzyme assay.

biofuels (Drapcho et al., 2008). The use of biomass for biofuel production which can also be used for human consumption (for example, sugar beets, sugar cane and corn) is very controversial and has caused a food-versus-fuel debate worldwide since the amount of such biomass is limited. Therefore, the interest in other types of biomass has emerged in recent years. Lignocellulosic biomass is an example of such biomass. It is available in almost all plants (Hahn-Hägerdal et al., 2007). The production of lignocellulose on earth is about 2 to 5 $\times 10^{12}$ tons every year (Wyman et al., 2005). Pretreatment is a crucial and most expensive step in lignocellulosic biomass to biofuel conversion. Therefore, pretreatment of lignocellulosic biomass by alternative cost effective method is appreciable. Biological conversion of cellulosic biomass to fuels and chemicals offers the high yields to products vital to economic success and the potential for very low costs. Biological pretreatments offer an alternative for lignocellulosic biomass conversion using enzyme hydrolysis (Noah et al*.,* 2013) hence, exploration of such system in nature is needed. Insects include termites have the unique ability to digest lignocellulose with high efficiency, often using it as a sole food source. Termites can digest 74-99% lignocelluloses (Ni and Tokuda, 2013). Earlier studies indicated that novel enzymes with high lignocellulose degradation potential may reside in termite guts (Ni and Tokuda, 2013). A better understanding of lignocellulose digestion by termites may help to overcome challenges in the conversion of lignocellulosic biomass into soluble sugars. Thus, enzyme profiling combined with enzyme activity with reference to cellulose degradation is attempted.

MATERIALS AND METHODS

Termite collection

O. obesus termites were field collected on the *Eucalyptus camadulensis* tree at Forest Campus, Coimbatore, Tamilnadu, India as described in Smith and Koehler (2007) (Plate 1). Its identity was confirmed at National Forestry Insect Collection (NFIC), Forest Research Institute, Dehradun. Briefly, a PVC bucket with holes to allow termite access was tied on the trunk of a tree covered with a PVC lid. A small wood piece of *E. camadulensis* was placed into the bucket as a food source. Termites were migrated into the wood piece placed in the bucket. The bucket containing termite colony was brought into the laboratory. Termites were separated from the wood piece, frozen and kept under refrigeration until dissection. Collections were restricted to a single colony.

Table 1. Cellulase activity of gut extracts of *O. obesus* on CMC.

Parameter	Carboxymethylcellulose (CMC) substrate concentration (mg/ml)				
	1	2	3	4	5
Concentration of crude enzyme (ml)	0.1	0.2	0.3	0.4	0.5
Concentration of product (glucose) mg/ml	0.49 (49%)	1.08 (54%)	1.83 (61%)	2.72 (68%)	3.75 (75%)
Remaining substrate (mg/ml)	0.51	0.46	0.39	0.32	0.25
Specific activity of enzymes (nmol reducing sugar/ termite equivalent/ min)	1.77	2.91	3.06	4.18	5.37

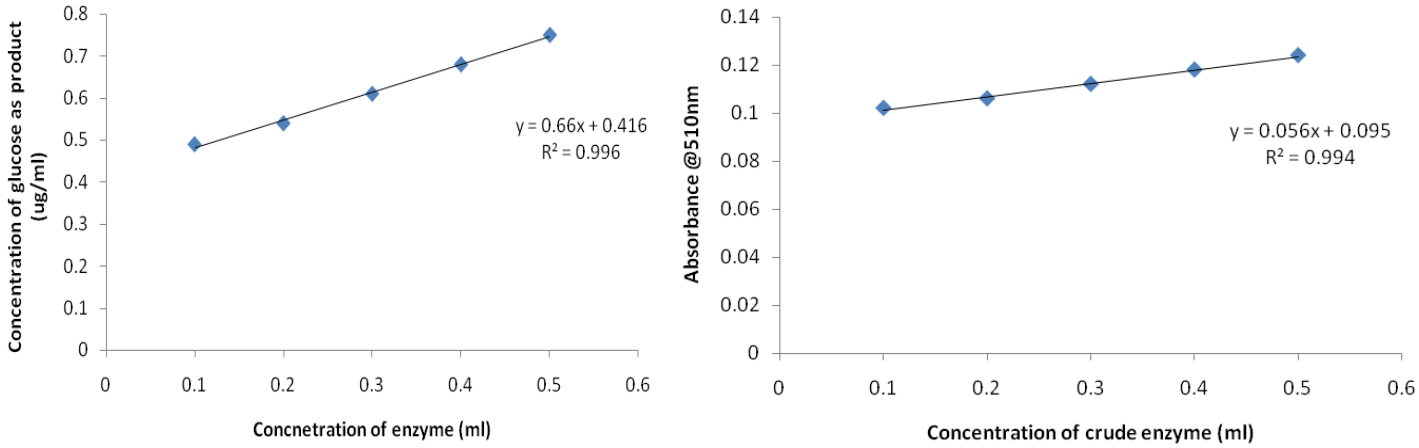

Figure 1. Determination of enzyme activity of gut extracts of *O. obesus*

Termite dissection, enzyme extraction and activities

Enzymes were extracted from collected termites sample as described by Smith et al. (2009). Briefly, each termite's gut was removed in sodium acetate buffer (0.1 M, pH 5.5 for endogluconase assay). An enzyme extract was prepared for whole gut region from 50 termites (100 mg weight) for the endoglucanase assay (Smith et al., 2009). The gut regions were placed into mortar and pestle containing the appropriate buffer after removing the gut contents, and kept on ice. The gut regions were manually homogenized on ice. The homogenates were centrifuged at 20000 *g* at 4°C for 15 min. The supernatants were collected, frozen, and kept at -20°C until enzyme assays. Final concentrations were equivalent to 50 termite gut regions per 10 mL. For the endoglucanase assays, 0.1 to 0.6 ml of tissue extract was combined with 0.9 to 0.4 ml of 2% carboxymethylcellulose solution (CMC, Sigma-Aldrich, in 0.1 M sodium acetate buffer, pH 5.5) in each test tube and allowed to react for 70 min at 23°C. A 1 ml volume of 1% 3,5-dinitrosalicylic acid (DNSA), 0.4M sodium hydroxide and 30% sodium potassium tartrate was added to each tube. The tubes were immediately placed in boiling water for 10 min and then on ice for 15 min. Each cooled test tubes were read at 510 nm using an UV-vis spectrophotometer (VWR Scientific, Hitachi). Control tubes were allowed to react for 10 min to allow for passive mixing of solutions before boiling with DNSA solution (Zhou et al. 2007); standards used were dilutions of glucose. For all replicates, control plates were used to adjust for 510 nm absorbance. The standard graph was prepared using glucose as standard. Based on the standard graph, regression equation was arrived at and concentration of the enzyme was calculated.

Data analysis

Experiments were set up as one-factor designs with a single homogenization for each gut /substrate combination. Thus, the experiment had a single biological replication, a previously accepted method (Nakashima et al., 2002), with technical replicates being used for statistical analysis. The endoglucanase assays had four technical replicates. Enzymatic activities were calculated using the formulae presented in Smith et al. (2009).

RESULTS AND DISCUSSION

Cellulase activity of whole gut extracts of *O. obesus* on commercially available cellulose CMC was evaluated and the results are tabulated in Table 1. The activity of termite gut enzymes in terms of product delivery (glucose) was calculated based on the standard graph made using glucose as standard. The crude enzyme activity was also measured in terms of concentration of glucose as end product of CMC (Figure 1). It was found that the crude enzyme from *O. obesus* digested 49 to 75% of the substrate

within 35 min of reaction time. The activity increases with increase in enzyme concentration. 0.5 ml of crude enzyme digested 3.75 mg/5 mg of CMS within 35 min of reaction period. It was also found that the specific activity of crude enzyme extracted from whole gut of *O. obesus* ranges from 1.77 to 5.37 nmol reducing sugar/ termite equivalent/ min. It was supported with the earlier study made by Smith and Koehler (2007) on the enzyme activity of gut extracts from subterranean termite, *Reticulitermes flavipes* (Kollar) and found specific activity of 2.81 to 5.21 nmol reducing sugar/ termite equivalent/ minute. The activity especially endoglucanase activity was high in fore and hind gut than in the mid gut. The endoglucanase activity started at foregut where amorphous region of cellulose was cleaved followed by crystalline structure at hindgut (Smith and Koehler, 2007). Endoglucanase activities were mainly confined to the hindgut (Smith et al., 2009). The patterns of cellulolytic enzymes in the *O. obesus* appeared to indicate degradation of amorphous and crystalline cellulose along the gut. Endoglucanase activity was observed to be higher in the fore gut than in the mid gut and appeared to increase progressively in the hind gut. Similar endoglucanase patterns have been observed in *Coptotermes lacteus* (Hogan et al., 1988). This indicated that the amorphous and crystalline cellulose were degraded throughout the gut ecosystem of *O. obesus*. Subterranean termite, *O. obesus* is not only more aggressive, but their digestive systems are apparently more capable of digesting wood, particularly crystalline cellulose. This would suggest the possibility of natural cellulose source to wood digestion being available in their gut range. Our findings suggest a processive mechanism of amorphous cellulose degradation started at foregut and digestion of crystalline cellulose at hindgut. This corroborates the need of biotechnological interventions to study in detail about digestion in gut of *O. obesus* for *in vitro* production of endogenous cellulolytic enzymes.

Conclusion

One of the major constraints in bioethanol industry is conversion of lignocellulosic biomass to fermentable sugar, since it is very expensive process. Biological pretreatment methods are cost effective and environment friendly. Hence, use of enzymes from natural resources is warranted. Lower animals especially termites produce array of enzymes to digest lignocelluloses to sugars for their energy needs. We extracted enzymes from gut of subterranean termite, *O. obesus* and evaluated the activity of the extracted enzymes for cellulose degradation. It was found from the study that *O. obesus* can be able to digest 49 to 75% of cellulose into glucose. The specific activity of crude enzyme extracted from whole gut of *O. obesus* ranges from 1.77 to 5.37 nmol reducing sugar/termite equivalent/minute. Therefore, use of cellulolytic enzymes in gut extracts of termite, *O. obesus* in biorefinery industry would be through biotechnological interventions.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

Drapcho CM, Nhuan NP, Walker TH (2008). Biofuels Engineering Process Technology. The McGraw-Hill companies Inc, United States of America.

European Renewable Energy Council. (2008). Bioenergy. Available at: http://www.erec.org/renewable-energy/bioenergy.html

Hahn-Hägerdal B, Galbe M, Gorwa Grauslund MF, Lidén G, Zacchi G (2007). Bio-ethanol–the fuel of tomorrow from the residues of today. Trends in Biotechnology. 24: 12.

Hogan M E, Schulz MW, Slaytor M, Czolu RT, O'Brien RW (1988). Components of termite and protozoal cellulases from the lower termite, *Coptotermes lacteus* Froggatt. Insect Biochem. 18: 45-51.

Jinfeng Ni, Gaku Tokuda. (2013). Lignocellulose-degrading enzymes from termites and their symbiotic microbiota. Biotechnology Advances. 31: 838-850

Nakashima K, Watanabe H, Saitoh H, Tokuda G, Azuma JI (2002). Dual cellulose digesting system of the wood-feeding termite, *Coptotermes formosanus* Shiraki. Insect Biochem. Mol. Biol. 32: 777-784.

Noah Weiss**,** Johan Börjesson**,** Lars Saaby Pedersen**,** Anne S Meyer (2013). Enzymatic lignocellulose hydrolysis: Improved cellulase productivity by insoluble solids recycling. Biotechnology for Biofuels. 6:5 doi:10.1186/1754-6834-6-5

Panwar NL, Kaushik SC, Kothari S (2010). Role of renewable energy sources in environmental protection: A review. Renewable and Sustainable Energy Reviews. 15: 1513-1524.

Saxena RC, Adhikari DK, Goyal HB (2007). Biomass-based energy fuel through biochemical routs: A review. Renewable and Sustainable Energy Reviews. 13: 167-178.

Smith JA, Koehler PG (2007). Changes in *Reticulitermes flavipes* (Isoptera:Rhinotermitidae) gut xylanolytic activities in response to dietary xylan content. Ann. Entomol. Soc. Am. 100: 568-573.

Smith JA, Scharf ME, Pereira RM, Koehler PG (2009). Comparisons of gut carbohydrolase activity patterns in *Reticulitermes flavipes* and *Coptotermes formosanus* (Isoptera: Rhinotermitidae) workers and soldiers. Sociobiology. 53: 113-124.

Wyman CE, Dale BE, Elander RT, Holtzapple M, Ladisch MR, Lee YY (2005). Coordinated development of leading biomass pretreatment technologies. Bioresource Technology. 96, 1959-1966.

Zhou X, Smith JA, Oi FM, Koehler PG, Bennett GW, Scharf ME (2007). Correlation of cellulase gene expression and cellulolytic activity throughout the gut of the termite *Reticulitermes flavipes.* Gene. 395: 29-39