Comparison of Termite Lignocellulases Activity and Enzyme Distribution Patterns across Different Termite Genus

by

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Abstract

In order to guide the further screening for new glycohydrolase and phylogenetic analysis, a comparative study of xylanase and cellulase activity combined gut distribution was performed in diverse termite species. The results of enzyme activity assays had shown that β-glucosidase and xylanase activities for *Macrotermes* sp were significantly higher than other species detected here. *Macrotermes* sp might be a prospecting organism for discovery of novel lignocellulases. Xylanase activity of lower termite *Coptotermes* sp and *Reticulitermes* sp were dominantly contributed by hindgut, otherwise, big proportion of xylanase activity appeared simultaneously in midgut and hindgut of higher termite *Pericapritermes* sp, *Macrotermes* sp and *Odontotermes* sp. In our studies, *Coptotermes* sp was the first time to be found distributing with high β-glucosidase activity in midgut. In addition, the endogenous β-glucosidase mainly soured from salivary/foregut for *Odontotermes* sp, but presented close activity proportions in salivary/foregut and midgut for *Macrotermes* sp, which took us to suppose that cellulase activity and distribution in higher termite are affected by the food available environment.

Key words: enzymatic distribution pattern, xylanase, cellulase, salivary/foregut. midgut, hindgut

Introduction

Termites can digest 74%~99% of the cellulose in the world, are considered to be the most prospecting lignocelluloses decomposing animal (Tayasu *et al.*, 2000;Tokuda *et al.*, 2007;Arakawa *et al.*, 2009).

The main components of termite cellulolytic and hemicellulolytic system are endo-beta-1, 4-glucanase (EC; EG. 3.2.1.4), exo-beta-1, 4-cellobiohydrolases (CBH; EC. 3.2.1.91) and β-glucosidase (EC. 3.2.1.21) for cellulose hydrolyzing, endo-β-D-xylanase or xylanase (EC. 3.2.1.8) for hemicellulose hydrolyzing (Arakawa *et al.*, 2009;Willis *et al.*, 2010). Lower termite glycosyl hydrolases system produce endogenouse cellulases in the salivary glands (Ohkuma, 2008) and endosymbiosis cellulases and hemicellulases sourced from flagellates in the hindgut (Tokuda *et al.*, 1997;Warnecke *et al.*, 2007;Tokuda *et al.*, 2009). On the other hand, higher termites, which do not have symbiotic protozoan in the guts, are considered to secrete endogenouse cellulase in the midgut and bacteria cellulases in the hindgut rather than the salivary galnds, except for those belonging to

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the subfamily Marotermitinae (Arakawa *et al.*, 2009; Matsui *et al.*, 2009).

Until now, the enzyme activity, distribution pattern and expression site of lingocellulases sourced from lower termite already had a lot of researches (Brune and Stingl, 2006;Ohkuma, 2008;Arakawa *et al.*, 2009;Tokuda *et al.*, 2009;Cho *et al.*, 2010;Zhang *et al.*, 2010). Other than lower termite, except few studies focused in the *Nasutitermes* sp, the lignicellulolytic systems for the higher termites were relatively much less researched (Tokuda *et al.*, 2007;Tokuda and Watanabe, 2007;FUJITA *et al.*, 2008;Todaka *et al.*, 2010a). A comparison of cellulolytic and hemicellulolytic system of different termites species is still lacking.

In this paper, we focused on cellulase and xylanase which soucred from five termite genus. This was the first comparative study of hydrolytic activities and their distribution on FPase, xylanase and β-glucosidase in crude extracts of termites above.

Materials and methods

Termites

Colonies of termites were field collected subtropical region of china. Worker-caste termites were utilized throughout the investigation. Classification (family or subfamily) of each termite is indicated in Table 1.

Preparation of crude enzyme

To prepare enzyme extracts, sets of salivary glands and guts including the contents were dissected from termites. One unit (U) of enzyme activity was defined as the amount of enzyme capable of releasing one μmol reducing sugar per min under the defined reaction conditions. Specific activity was express as units per mg of protein (U/ mg protein).

Table 1 Variations in the FPase, xylanase and β-glucosidase activities of five species worker termite collected from different mountains

Species	Collection location	filter paper Activity	Xylanase activity	β-glucosidase activity
***Coptotermes* sp1**[1]	GDLH	0.257±0.011	7.037±0.067	0.416±0.027
***Coptotermes* sp2**[1]	GDMF	0.116±0.002	6.731±0.081	0.303±0.010
***Coptotermes* sp3**[1]	GDLF	0.303±0.055	6.508±0.044	0.538±0.089
***Reticulitermes* sp1**[1]	GDLH	0.124±0.016	10.812±0.070	0.234±0.005
***Reticulitermes* sp2**[1]	GDMF	0.141±0.033	16.126±0.037	0.110±0.000
***Reticulitermes* sp3**[1]	GDLF	0.195±0.009	14.89±0.033	0.124±0.002
Pericapritermes sp1[2]	GDLH	1.945±0.021	0.445±0.036	0.458±0.021
Pericapritermes sp2[2]	GDMF	0.198±0.011	0.303±0.011	0.045±0.005
Pericapritermes sp3[2]	GDLF	0.893±0.005	0.498±0.046	0.176±0.004
Macrotermes sp1[3]	GDLH	0.126±0.015	20.185±0.050	2.644±0.198
Macrotermes sp2[3]	GDMF	0.231±0.018	19.064±0.018	5.111±0.030
Macrotermes sp3[3]	GDLF	0.179±0.109	17.397±0.027	2.937±0.085
Odontotermes sp1[4]	GDLH	0.125±0.009	0.785±0.030	2.103±0.029
Odontotermes sp2[4]	GDMF	0.078±0.007	0.787±0.013	2.026±0.088
Odontotermes sp3[4]	GDLF	0.163±0.009	0.415±0.027	3.099±0.085

Values are means (U/mg protein) of three determinations±SD. The collect location: GDMF, Maofeng Mountain; GDLH, Lianhua Mountain; GDLF, Loufu Mountain. Flagellate-harbouring species are shown in bold; termidis (which lack flagellates) are shown in normal typeface. Number next to species indicates termite families or subfamilies (in the case of termitids), as follows: 1, Rhinotermitidae; 2, Termitinae; 3, Macrotermitinae; 4, Odontotermes

Protein measurement

The protein content of the sample was determined according to the Coomassie Brilliant Blue G-250 method (Lott *et al.*, 1983), using bovine serum as a standard.

Filter paper activity, Endo-β-D-xylanase and β-glucosidase activity

Measurement of filter paper activity (FPA), *endo-β-D-xylanase and β-glucosidase activities* were carried out based on dinitrosalicylic acid (DNS) method, were assayed by measuring the release of deducing sugars from xylan and salicin respectively (Lott *et al.*, 1983;Cai *et al.*, 2008;Eveleigh *et al.*, 2009).

Results and discussion

FPase, endo-β-D-xylanase and β-glucosidase activity

FPase, endo-β-D-xylanase and β-glucosidase activities sourced of whole termite tissue were compared among diverse species by the same methodology (Table 1). FPase activity levels presented closed to each other among two lower termites and two higher termites, except for higher termite *Pericapritermes* sp1 (1.945±0.021U/mg protein) and *Pericapritermes sp3* (0.893±0.005 U/mg protein), had relatively higher FPase activity. There were huge discrepancies in xylanse activity levels among termites examined. The higher termite *Macrotermes* sp showed significant higher xylanse activities (17.000 ~ 20.000 U/mg protein approximated), and the following were the lower termite *Coptotermes* sp (10.000 ~ 6.000 U/mg protein approximated) and *Reticulitermes* sp (6.000 ~ 7.000 U/mg protein approximated). The other two higher termites *Pericapritermes* sp and *Odontotermes* sp presented a tremendous low xylanase activity, which 20 ~ 50 folds smaller than that of *Macrotermes* sp. *Macrotermes* sp and *Odontotermes* sp possessed 5 ~ 10 folds higher β-glucosidase activity than that of the other three termites.

It was found that relatively low levels of cellulase activity were found in higher termite *Nasutitermes takasagonensis* compared to the low termite *Coptptermes formosanus* (Todaka *et al.*, 2010b). In the contrast, our research showed that *Macrotermes* sp possessed higher xylanase and β-glucosidase activity than the other two low termites. Thus, *Macrotermes* sp appeared to be the bioprospecting resources for discovery of novel enzymes. There were significant differences of xylanese and β-glucosidase activity levels presented among three higher termites, while their filter paper digest ability were neck and neck. Both filter paper and crystalline cellulose had been used as preferred cellulase substrates to determine the actual capacity of complete cellulolytic systems (EG, CBH and β-glucosidase) (Tokuda *et al.*, 2005;Willis *et al.*, 2010). Therefore, the study results above might suggest that xylanase activity would not affect the cellulase secretion directly and β-glucosidase is not the key enzymes in the cellulose digest system of termites.

Distribution of FPase, endo-β-D-xylanase and β-glucosidase activities in guts of five termite species

The distribution patterns of the cellulase and xylanase activities throughout the gut and the salivary glands were diverse in five termite species (Fig. 1). For termite *Coptotermes* sp, *Reticulitermes* sp, *Macrotermes* sp and *Odontotermes* sp, the percentage of total FPase activity in three sections almost showed an equal division pattern, 27% in average of salivary/foregut, 35% in average of midgut and 38% in average of hindgut, excepted for the *Coptotermes* sp1 (salivary/ foregut, 19%; foregut, 55%; hindgut, 26%) and *Pericapritermes* sp3 (salivary/foregut, 36%;

foregut, 12%; hindgut, 52%). Our measurement results of FPase activities showed that salivary gland / foregut, midgut and hindgut of diverse termites utilized in this assay could digest nature cellulose independently in a certain extent (Fig. 1-A). For *Coptotermes* sp and *Reticulitermes* sp xylanase activities dominantly soured from hindgut (71% ~ 93%) and were extraordinarily low (2% ~ 9%) in salivary/foregut, excepted for the *Coptotermes* sp2 (salivary/foregut, 13%; midgut, 50%; hindgut, 37%). Whereas, among three higher termites, xylanase showed a slight bigger proportion in midgut (44% in average), secondly was the hindgut (42% in average) (Fig. 1-B). Beta-glucosidase for *Coptotermes* sp and *Odontotermes* sp dominantly secreted from midgut (62% ~81%) and salivary / foregut (89%~90%) respectively, belonged to the endogenous enzymes. In *Macrotermes* sp, besides 34%~41% of β-glucosidase foregut, but in midgut and hindgut also exhibited 36%~45% and 14%~30% of total β-glucosidase activity respectively (Fig. 1-C).

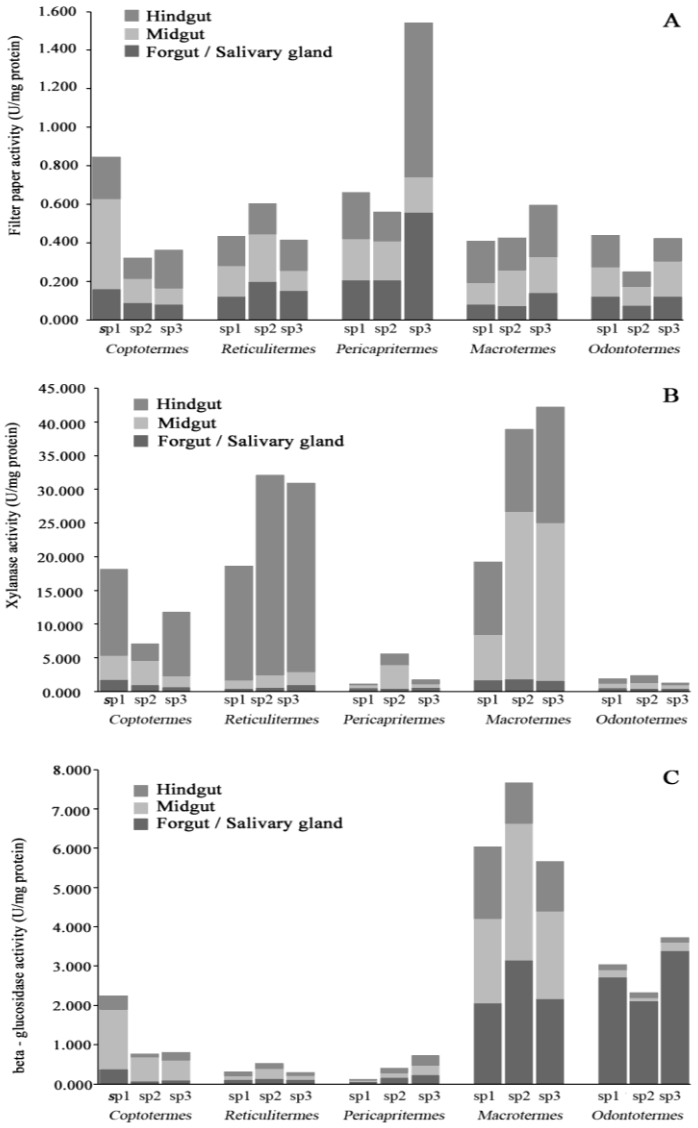

Fig.1 Distribution patterns of PFase, xylanase and β-glucosidase activity of five termite speices.

Three higher termites studied here, high xylanase activities were detected both in midgut and hindgut. In *N. takasagoensis*, β-glucosidase and EG were primary produced in the midgut (Slaytor, 2000;Tokuda *et al.*, 2009). But there still had no researches found higher termite xylanase gene expressed in midgut. All in all, further studied would be necessary to carried out to figure out the origination of the xylanase in midgut of diverse higher termite. A predominant xylanase activity had been found both in hindguts of *Coptotermes* sp and *Reticulitermes* sp (Arakawa *et al.*, 2009). But our research found an interesting result of xylanase activity distribution assay for *Coptotermes* sp: although the total xylanase activity level in *Coptotermes* sp was lower than that in *Reticulitermes* sp, both the percentage of total xylanase activity and activity value in midgut were higher than that of *Reticulitermes* sp. The study results mentioned here made us to suppose that maybe the migut of wood-feeding lower termite in some genus can secrete xylanase when xylanase is shortage in hindgut. The distribution of cellulases activities in the diverse gut regions presented significant

differences among fungus growing higher termites (Tokuda *et al.*, 2005;Tokuda and Watanabe, 2007). Fungus-growing higher termite *Odontotermes formosanus* showed only a trace of crystalline cellulose hydrolysis throughout the gut. In the contrast, the midgut and hindgut of wood-feeding higher *N. takasagonensis* primarily contributed in crystalline cellulose degradation (Todaka *et al.*, 2010a). Additionally, this was the first time to find high proportion of β-glucosidase activities in midgut of *Coptotermes* sp for wood-feeding termite. Thus it is highly likely that the mechanism of cellulose digestion in termites has also altered during evolution (Arakawa *et al.*, 2009) and cellulases component differences were due to the diverse food available from their environments.

Over all, the results here suggested that termite cellulase and xylanase activity and enzyme distribution patterns presented some general characters in a specific genus and some extent variations among different species in one genus. Broader understandings of lignocellulase digestion system in termites and other lignocellulase digested insects would need further researches. We expected that present study can be applied to further investigation of the phylogenesis of termite and discovering a broader range of novel biocatalysts for lignocellulosic biomass conversion.

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