**INTRODUCTION**

Cellulose is the most abundant biomass on the earth (Tomme et al., 1995). It has been used as important industrial raw material and renewable energy in human life. Cellulose from higher plants has been exploited for centuries in a wide variety of practical applications. In many industrial applications of cellulose today, enzymatic hydrolysis is becoming increasingly desirable owing to the mild processing conditions, high specificity of the reactions and better control of the processes (Teeri, 1997). However, because most current applications of cellulolytic enzymes are carried out using small amounts of crude enzyme mixtures rather than purified enzymes, the hydrolysis efficiency of cellulose materials is very low and production cost of product is very high. It is thought that one way to resolve this problem is to explore cellulase with high activity from organisms, and then to develop genetic engineering microorganisms (Wang, 1997).

Termites are one group of social insects using cellulose as a food resource, and they play a key role in decomposing dead plant tissue in natural ecosystems. Phylogenetically, termites can be classified into two subgroups: lower termites, which are termites from Mastotermitidae, Hodotermitidae, Kalotermitidae, Rhinotermitidae and Serritermitidae, and higher termites, which are termites from Termitidae (Edwards and Mill, 1986). The lower termites possess symbiotic protozoans in the hindgut on which they depend for digestion of cellulose (Krishna and Weesner, 1969). The higher termites from subfamily Macrotermitinae do not have cellulolytic protozoa in the hindgut and use a synergistic collaborative relationship with the ectosymbiosis fungus *Termitomyces* sp. for hydrolysis of cellulose to glucose (Abo-Khatwa, 1978; Rouland et al., 1988; Hyodo et al., 2003). Extensive hydrolysis of native, crystalline cellulose by fungi usually requires the activity of enzymes of all three functional classes: endoglucanases (endo-1,4-β-glucanases, EC 3.2.1.4); exoglucanases, which include cellobiohydrolase (1,4-
β-β-glucan cellbiohydrolase, EC 3.2.1.91) and exoglucohydrolase (1,4-β-β-glucan glucohydrolase, EC 3.2.1.74); and β-glucosidases (EC 3.2.1.21). Endoglucanases cleave internal glucosidic bonds along the polyglucan chain. Exoglucanases attack the terminus of a polyglucan chain, liberating celllobiose or glucose units from the nonreducing end. β-Glucosidases hydrolyze celllobiose and water soluble cellodextrins to glucose (Breznak and Brune, 1994). However, endogeneous cellulases in termites comprise only multiple endo-1,4-β-glucanase and β-glucosidase components, and there is no evidence that an exo-1,4-β-glucanase is involved in the production of glucose from crystalline cellulose (Slaytor, 1992).

Cellulases from termites are an interesting and important research field. Since Cleveland (1925) proposed that termites could live on a diet of pure cellulose, the distribution and characterization of cellulases derived from the termite and its symbionts have been described extensively in the past decades (Retief and Hewitt, 1973; Potts and Hewitt, 1974; Mcewen et al., 1980; Veivers et al., 1982; Ohkuma, 2001; Watanabe and Tokuda, 2001; Nakashima et al., 2002). However, up to the present few studies have been done on the cellulase activity of termites in China, except one report about the cellulase activity of Reticulitermes flaviceps and Coptotermes formosanus (Zhang et al., 2001). Therefore, in order to find termites with high cellulase activity, the cellulase activity of five common termites in China were compared.

MATERIALS AND METHODS

Chemicals for experiments. Carboxymethyl cellulose (CMC) and salicin were purchased from Shanghai Chemical Reagents Company. All other chemicals in the assay were of the highest purity commercially available.

Termite species. The termites used were Cryptotermes pingyangensis He et Xia (Isoptera: Kalotermitidae), Reticulitermes flaviceps (Oshima) (Isoptera: Rhinotermitidae), R. leptomandibularis Hsia et Fan (Isoptera: Rhinotermitidae), Coptotermes formosanus Shiraki (Isoptera: Rhinotermitidae), and Odontotermes formosanus (Shiraki) (Isoptera: Termitidae). These termites are very important pests in houses and other buildings, dams and dykes, and forests and orchards in China. Cry. pingyangensis was provided by Pingyang Termite Control Station, Pingyang County, Zhejiang province and it is a drywood-inhabiting termite. R. flaviceps was collected from the arboretum of the Huajiachi campus, Zhejiang University. R. leptomandibularis was collected from the Baoshi Mount near West Lake, Hangzhou, and C. formosanus was collected from the village of Laoyancang, Haining City, Zhejiang Province. All of them are termites inhabiting soil or dampwood. O. formosanus was also collected from the Baoshi Mount near West Lake, Hangzhou, and it is a soil-inhabiting and fungus-growing termite. The colonies of termites mentioned above were kept under dark conditions at 25±1°C with 65±5% relative humidity and blocks of Pinus massoniana as their food source.

Preparation of enzyme extracts. The caste of termites used for experiments were healthy adult workers, but pseudoworkers for Cry. pingyangensis. Eighty healthy termites were divided into four groups and starved for 1 day before experiment. After that, they were put into 0.95% physiological saline, thoroughly washed and weighed. One group of termites was then used for an experiment of the whole termite, and the other three groups of termites were divided into the following sections: head, foregut, midgut and hindgut. Finally, the heads, foreguts, midguts, and hindguts of 20 termites as well as 20 whole termites were collected together and homogenized, respectively, in 1.8 ml of 0.1 m sodium acetate buffer (SAB), pH 5.6 in a Bankman homogenizer. The homogenates were centrifuged for 10 min at 15,000×g. Supernatants were collected and kept in refrigerator of −30°C for assay of enzyme activities. Enzyme extracts were stable for at least two weeks at this temperature (Retief and Hewitt, 1973). All operations were carried out at 0–4°C.

Assay of cellulase activities. Two substrates, CMC and salicin, were used for assay of cellulase activity of enzyme extracts. Cellulase activity of enzyme extract against CMC was assayed by incubating 0.5 ml of 1% sodium carboxymethylcellulose in 0.1 m SAB, pH 5.6, with 50 μl enzyme extract for 4 h at 37°C. β-Glucosidase activity of enzyme extracts was assayed by incubating 0.5 ml of 1% salicin in 0.1 m SAB, pH 5.6, with 20 μl enzyme extract for 4 h at 37°C. Glucose produced was estimated using the arsenomolybdate chro-
mogenic reagent of Nelson (1951) according to the method of Somogyi (1937, 1945, 1952). Glucose was used as the standard. The protein content of the extract was determined by the method of Lowry et al. (1951) using bovine serum globulin (Sigma) as the standard. One unit of cellulase activity is defined as the amount of enzyme which produced 1 μmol of reducing sugar (or glucose) per minute.

RESULTS

Activities of cellulase against CMC in five species of termites

Activities of cellulase against CMC in five species of termites are shown in Table 1. As for the whole termite, among the five species of termites tested, R. leptomandibularis and O. formosanus had the largest and the smallest amounts of activity of cellulase against CMC, respectively. There was no significant difference (p<0.05) in the amount of activity of cellulase against CMC between Cry. pingyangensis, R. flaviceps and C. formosanus.

In the head, Cry. pingyangensis had the larger amount of cellulase activity against CMC compared with the other four species of termites. In the foregut, the amounts of cellulase activities against CMC of Cry. pingyangensis, R. flaviceps and R. leptomandibularis were larger than those of C. formosanus and O. formosanus, and the amount of cellulase activities did not show a significant difference (p<0.05) between the former three species of termites or between the latter two species of termites. In the midgut, C. formosanus had the largest amount of cellulase activity against CMC compared with the other four species of termites, in which no significant difference (p<0.05) existed in the amount of cellulase activity. In the hindgut, there was no significant difference (p<0.05) between R. flaviceps and R. leptomandibularis in the amount of cellulase activity against CMC, but the amount of cellulase activity of these two termites was significantly larger than that of the other three termites tested (p<0.05).

As for cellulase activity per gram termite against CMC, the whole worker and foregut and hindgut of R. leptomandibularis showed the strongest cellulose digestibility among the five species of termites tested. However, in the head, the digestibility of R. leptomandibularis cellulase against CMC was similar to that of R. flaviceps, C. formosanus and O. formosanus, and was significantly lower than that of Cry. pingyangensis (p<0.05).

According to the cellulase activity per milligram protein against CMC in the five species of termites, most of the specific activities of cellulase against CMC were found in the foregut and midgut except for R. flaviceps, which had the most specific activi-

### Table 1. Comparison of activities of CMCase in five species of termites in China

<table>
<thead>
<tr>
<th>Enzyme activity type</th>
<th>Termite species</th>
<th>Head</th>
<th>Foregut</th>
<th>Midgut</th>
<th>Hindgut</th>
<th>Whole termite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity per termite</td>
<td>Cry. pingyangensis</td>
<td>288.8 ± 74.1 a*</td>
<td>412.2 ± 95.9 a</td>
<td>73.2 ± 13.0 b</td>
<td>116.3 ± 11.3 b</td>
<td>660.6 ± 4.6 b</td>
</tr>
<tr>
<td>R. flaviceps</td>
<td>127.1 ± 10.0 b</td>
<td>409.3 ± 134.4 a</td>
<td>84.8 ± 34.4 b</td>
<td>280.3 ± 67.2 a</td>
<td>652.5 ± 39.0 b</td>
<td></td>
</tr>
<tr>
<td>R. leptomandibularis</td>
<td>68.8 ± 18.0 b</td>
<td>510.5 ± 126.7 a</td>
<td>80.0 ± 40.6 b</td>
<td>309.6 ± 107.8 a</td>
<td>1,065.7 ± 62.0 a</td>
<td></td>
</tr>
<tr>
<td>C. formosanus</td>
<td>74.4 ± 13.4 b</td>
<td>87.7 ± 18.7 b</td>
<td>152.7 ± 14.0 a</td>
<td>131.9 ± 37.9 b</td>
<td>536.8 ± 221.8 b</td>
<td></td>
</tr>
<tr>
<td>O. formosanus</td>
<td>68.0 ± 11.9 b</td>
<td>143.8 ± 35.6 b</td>
<td>60.5 ± 4.0 b</td>
<td>53.2 ± 4.7 b</td>
<td>301.6 ± 36.3 c</td>
<td></td>
</tr>
</tbody>
</table>

*Means within a column followed by the same letter are not significantly different (p<0.05, ANOVA and multiple t comparison). Each mean±SD is based on 3 replicates.
Activity in the foregut and hindgut.

Activities of β-glucosidase in five species of termites

The data in Table 2 indicate that like the activity of cellulase against CMC, the whole worker of R. leptomandibularis also had the highest amount of activity of β-glucosidase among the tested five species of termites, and was 599.0 U/termite, 1.2–3.5 times higher than that of the other four species of termites. The lowest amount of activity of β-glucosidase was detected in the body of Cry. pingyangensis pseudoorgenerate compared with the other four tested species of termites.

In the head, O. formosanus showed the largest amount of activity of β-glucosidase, and the difference reached a significant level (p<0.05) compared with the other four species of termites tested. However, though there were some differences in the amount of activity of β-glucosidase between Cry. pingyangensis, R. flaviceps, R. leptomandibularis and C. formosanus, the differences were not significant (p<0.05).

In the foregut, the amounts of activity of β-glucosidase in Cry. pingyangensis and C. formosanus were higher than that in R. flaviceps, R. leptomandibularis and O. formosanus. Termites with the highest amount of β-glucosidase activity were C. formosanus in the midgut and R. flaviceps in the hindgut. It was suggested that the amount of activity of β-glucosidase in the different parts of the alimentary canal varied with the species of termites.

As for the β-glucosidase activity per body weight of termite workers, the whole worker of R. leptomandibularis also showed the strongest digestibility of salicin among the five species of termites tested. However, no consistent findings for the different parts of the alimentary tract were found in the digestibility of β-glucosidase. For the head, the termite with the strongest digestibility of β-glucosidase was O. formosanus. Cry. pingyangensis had the strongest digestibility of β-glucosidase in the foregut, and C. formosanus and R. flaviceps had the strongest digestibility of β-glucosidase in the midgut and in the hindgut, respectively.

Data on the β-glucosidase specific activity per milligram protein (Table 2) show that the sites with the highest β-glucosidase specific activity were the foregut and midgut for Cry. pingyangensis and C. formosanus, the head for O. formosanus, the hindgut for R. flaviceps, and the midgut and head for R. leptomandibularis, respectively.

**DISCUSSION**

*Cry. pingyangensis* is a lower dry-wood termite infesting houses and other buildings in southern China. It had the largest amount of activity of cellulase against CMC in the head and activity of β-glucosidase in the foregut compared with the other
four species of termites, but the activity of cellulase against CMC and the β-glucosidase in the head were all lower than that in the foregut. The amounts of activity of cellulase against CMC and the β-glucosidase in the foregut of this termite account for 68.5% and 72% in the whole alimentary canal, respectively. Because Cry. pingyangensis lives only in dry wood with little water, high activities of cellulase in the head and foregut of pseudotermites show this termite has strong digestibility of cellulose. Therefore, if the cellulase of pseudotermites is studied further, we could use its cellulase to degrade wood to glucose or alcohol to gain a new energy resource in the future.

R. flaviceps, R. leptomandibularis and C. formosanus are also lower termites. Although these termites have similar biological habits and inhabit damp-wood or soil, they have different levels of cellulase activity. In the present study, R. leptomandibularis showed the strongest activity of cellulase against CMC in the whole gut, and R. flaviceps showed the strongest activity of β-glucosidase in the hindgut, but in C. formosanus, the highest activity of cellulase against CMC and β-glucosidase appeared in the midgut.

Similar findings were also reported in other species of termites. Inoue et al. (1997) reported that in R. speratus, 77.8% of endo-β-1,4-glucanase activity was found in the salivary glands, and 54.0% of β-glucosidase activity was distributed in the hindgut. In C. lacteus, the cellulase activity was distributed in equal amounts between the foregut and the hindgut (O’Brien et al., 1979). In C. formosanus, β-glucosidase activity was in the salivary glands and the midgut (Itakura et al., 1997), and cellulase activity detected in the salivary glands was 80.8% of the total activity in the digestive system (Nakashima and Azuma, 2000).

O. formosanus is one of the subterranean higher termites that heavily infests severely dams and dykes, and forests and orchards in China. It cultivates a symbiotic fungus garden in the nest that is essential for its survival. The study of Hyodo et al. (2003) showed that O. formosanus depends completely on symbiotic fungi for their carbon source. Compared to the other species of termites tested, it had the largest amount of β-glucosidase activity and the strongest digestibility of salicin in the head. This indicates that the salicin digestion in this species of termite was realized in the head, and the specific activity of β-glucosidase per milligram protein in the head also verified it. As for the activity of cellulase against CMC, the head and every part of the gut all had greater amounts of distribution in this termite, but the cellulase activity decreased gradually from the foregut to the hindgut.

It was suggested that the cellulase against CMC in O. formosanus is from the salivary gland.

Slaytor (1992) suggested that cellulase activity against crystalline cellulose in the termites examined by him was found in the salivary glands and predominantly in the foregut and midgut. Our results from O. formosanus are consistent with the conclusion of Slaytor (1992) and Martin and Martin (1978).

However, the endoglucanase activity in the salivary glands was only 2% in Nasutitermes walkeri (Hogan et al., 1988) and 7–9% in Macrotermes michaelseni (Veivers et al., 1991). In Termes obsesus, the cellulase activity against cellulose suspension, which was prepared from filter paper, even resided completely in the hindgut (Misra and Ranganathan, 1954). Clearly, the distribution of cellulase activity in the alimentary canal had greater differences in different species of higher termites.

Czolij and Slaytor (1988) thought that the salivary glands of termites are devoid of microorganisms or have very low numbers, and enzymes found there must be endogenous. In our study, all five species of termites tested had higher activities of cellulase against CMC and β-glucosidase in the head. It was suggested that the salivary glands of these termites could also secrete endoglucanase and β-glucosidase.

In N. walkeri and the mound-building higher species N. exitiosus, the majority of the activity of cellulase against CMC was located in the epithelia and the luminal contents of the midgut, with lesser amounts in the foregut and mixed-segment and only 8% in the hindgut (O’Brien et al., 1979; Hogan et al., 1988). Kovoor (1970) found that most endo-β-1,4-glucanase activity of Macrocerotermes edentatus workers was in the midgut. In Tri- nervitermes trinervoides, about 70% of the total endo-β-1,4-glucanase was also found in the midgut (Potts and Hewitt, 1973). In M. subhyalinus, the worker midgut contains 99% of all the cellulase activity (endo-β-1,4-glucanase and β-glucosidase) demonstrable in the alimentary canal.
(Veivers et al., 1991). These facts suggested that the midgut is also the secreting site of endogenous cellulase in some species of termites. In *O. formosanus* and *C. formosanus*, the activities of β-glucosidase in the midgut were higher than those in the foregut and hindgut. This indicates that the midgut of these two termites also has the function of cellulase secretion. In fact, the midgut of *C. formosanus* could secrete endogenous cellulase (Nakashima et al., 2002).

At present, contrasting expression sites of cellulase in termites have been clarified. In the higher termite *N. takasagoensis*, expression of the endo-β-1,4-glucanase gene (*NtEG*) was located in midgut columnar cells (Tokuda et al., 1999). In the lower termite *R. speratus*, localization of antigenic endo-β-1,4-glucanase proteins was confirmed in the salivary glands (Watanabe et al., 1997, 1998; Tokuda et al., 1999). Our present study compared the activity of cellulase in the five common species of termites in China. It is necessary to do further study on the expression gene of cellulase in order to utilize the cellulase from these termites to serve humanity in the future.

ACKNOWLEDGEMENTS

We would like to express cordial gratitude to Prof. Tadashi Miyata, Nagoya University, for critical comments and improvement of the earliest version of this manuscript. We also thank Mr. Guoliang Liu, Termite Control Station of Pingyang County, Zhejiang Province, P. R. China, for helping to collect a local population of Cryptotermes pingyangensis. This work was supported by the Scientific Research Foundation for the Returned Overseas Chinese Scholars, Ministry of Education of P. R. China, and the Scientific Research Foundation for the Returned Overseas Chinese Scholars, Zhejiang University.

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Cellulase Activity of Five Termites in China


