Original

Studies on Digestive System of Termites III.

Digestibility of Xylan by Termite *Reticulitermes speratus* (Kolbe)

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Abstract——Worker termites of *Reticulitermes speratus* (Kolbe) were fed with various carbohydrates related to wood polysaccharides and 1% acetic acid for 21-days, and changes of survival and weight of termites and protozoan fauna were monitored. The results indicate that the diets can be grouped into four types (Type I to IV) in respect of utilization by protozoa and termite. Type I includes Akamatsu sapwood and cellulose in which large size protozoa, *Trichonympha agilis* and *Teratonympha mirabilis*, take part in digestion. Type II includes CMC and xylan in which smaller size protozoa, *Pyrsonympha* sp. and *Dinenympha* sp., take part in digestion. This was supported by xylanolytic enzyme analysis which indicated that about 70% activity of xylanase still remained in the hindgut of workers after feeding with xylan for 21 days. Type III includes amylose, cellobiose, sucrose, maltose, glucose and fructose which are usable by termites without an aid of the protozoa. Type IV includes the other hemicelluloses and monosaccharides and acetic acid, which are not usable to both termites and protozoa, when used as a sole diet. In conclusion, *R. speratus* is characteristic in digestibility of xylan with involvement of symbiotic xylanolytic protozoa in its hindgut.

Keywords: Termite, digestibility of xylan, *Reticulitermes speratus* (Kolbe), digestive system.

1. Introduction

Wood polysaccharide digestion by termites contributes partly in carbon recycling system on the earth and the symbiosis occurring in lower termites presents an excellent model for characterization of co-operative interactions between different lives. Previous studies demonstrated that wood cellulose was digested by anaerobic flagellate symbionts in the hindgut of lower termites[1–4]. However, Yokoe indicated the endogenous nature of cellulase in *Reticulitermes speratus* (Kolbe)[5], and Yamaoka and Nagatani[6], extending this idea, showed that carboxymethylcellulase (CMCase) was secreted from its salivary gland. They further proposed a hypothesis that cellulose was endocytosed by protozoa with co-operative action of CMCase of termites. However, digestion system of hemicelluloses and

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other carbohydrates in termites is mostly unknown.

Previously, several members of authors shed light on the digestion system of a termite, *Coptotermes formosanus* Shiraki, by feeding the worker termites with various kinds of carbohydrates, and showed that protozoa took part in digestion of native cellulose and termites were able to utilize amylose, cellobiose, sucrose, maltose, glucose and fructose without aid of protozoa, but could not utilize hemicelluloses efficiently[7]. Further, gross characteristics of various carbohydrases of *C. formosanus* were characterized with emphasis of their distribution[8].

In this study, digestion system of the other species of termite, *R. speratus*, was characterized by using various carbohydrates and acetic acid as diet with monitoring survival ratio and weight of the tested worker termites and changes in protozoan fauna. Based on the result, it was concluded that this termite could utilize xylan, and the changes of activities of xylanolytic enzymes were also analyzed to clarify the involvement of protozoa in digestion of xylan.

2. Materials and Methods

2.1 Termites

Worker termites of *R. speratus* grown on Akamatsu woods *(Pinus densiflora* Sieb. et Zucc.) were collected in a field of Uji City and Yoshidayama, Sakyo-ku, Kyoto.

2.2 Analysis of protozoan fauna

By using fine tweezers, the hindgut from one termite was pulled out from the posterior end and its content was forced out in 20 μl of the Trager U solution[9] on a Fuchs-Rosenthal hemocytometer. Then the protozoan fauna was analyzed by optical microscope as described by Yamaoka *et al.*[10]

2.3 Materials used as diets

Six polysaccharides, three disaccharides, six monosaccharides and 1% acetic acid listed in Table 1 were used as diets as described previously[7]. In addition, sapwood meal of Akamatsu wood, which was ground to pass 42 mesh screen, was used as the control diet.

2.4 Feeding experiments

Thirty worker termites were fed with one of diets listed in Table 1 under dark at 26°C for 21 days in an acrylic round-sided cup (i.d., 6 cm) having a hole (o.d., 1.5 cm) at the

Table 1. Compounds used as diets.

	Compounds
Polysaccharides	Cellulose, CMC, Glucomannan, Xylan, Arabinogalactan, Amylose
Disaccharides	Sucrose, Maltose, Cellobiose
Monosaccharides	Glucose, Fructose, Xylose, Mannose, Galactose, Arabinose
Other compound	Acetic acid (1%)

center of the bottom which was sealed with hard medicinal plaster as described previously[7]. Every prearranged days, the following three items were measured; 1) number of living termites, 2) weight of living termites, and 3) protozoan fauna. The number and weight of termites were expressed as percentage of the average values before and after the feeding experiment. Since *R. speratus* contains more than eleven symbiotic protozoa[11] including large amount of small size ones (<50 μm), the counting of the exact number of individual protozoa in many worker termites was not easy in a short time. Therefore, we separated protozoa into five groups, *Trichonympha agilis* (Tr), *Teratonympha mirabilis* (Te), *Pyrsonympha* sp. (P), *Dinenympha* sp. (D) and *Holomastigotes elongatum* (H), and qualitatively checked their existence in the hindgut of worker termites during feeding experiment.

In the separate experiment, workers were fed with xylan and their protozoan fauna and activities of xylanolytic enzyme activities were analyzed quantitatively.

2.5 Crude enzyme preparation

Every week, the hindgut and the residual portion of termite containing salivary gland from ten worker termites were sonicated (20 sec$\times$5) in 1 ml of 50 mM sodium acetate buffer (pH 4.6) kept in an ice-water bath, and the white suspension was centrifuged at 8,000$\times$g for 20 min. The supernatant solution was used as a crude enzyme solution.

2.6 Enzyme assays

Beta-xylosidase activity was assayed by measuring the amount of *p*-nitrophenol liberated after incubation for 4 h at 37°C with 1.625 mM of *p*-nitrophenyl-β-xylopyranoside as described previously[7].

Xylanase activity was assayed by measuring the amount of reducing sugar liberated after incubation for 4 h at 37°C with 1.00% of beech xylan according to the method of Somogyi-Nelson[12] as described previously[7].

One unit of each enzyme activity was defined as the amount of enzyme that liberated 1 μM of *p*-nitrophenol or monomeric sugar as glucose per min. The enzyme activity was expressed as units per one termite.

3. Results and Discussion

3.1 Effects of wood powder and starvation on worker termites

Prior to the diet feeding experiment, it was neccessary to know what happened to worker termites if no diet was fed (starvation experiment) and what was the most desirable diet for worker termites.

First, the starvation experiment was carried out. The result shown in Fig. 1a indicates that the number of survived workers decreased rapidly as incubation progressed and all workers died after incubation for longer than 21 days. The protozoan fauna shown in Table 1 shows that *T. agilis* and *T. mirabilis* were sensitive against starvation completely disappearing during the first week, followed by *H. elongatum* which disappeared after feeding

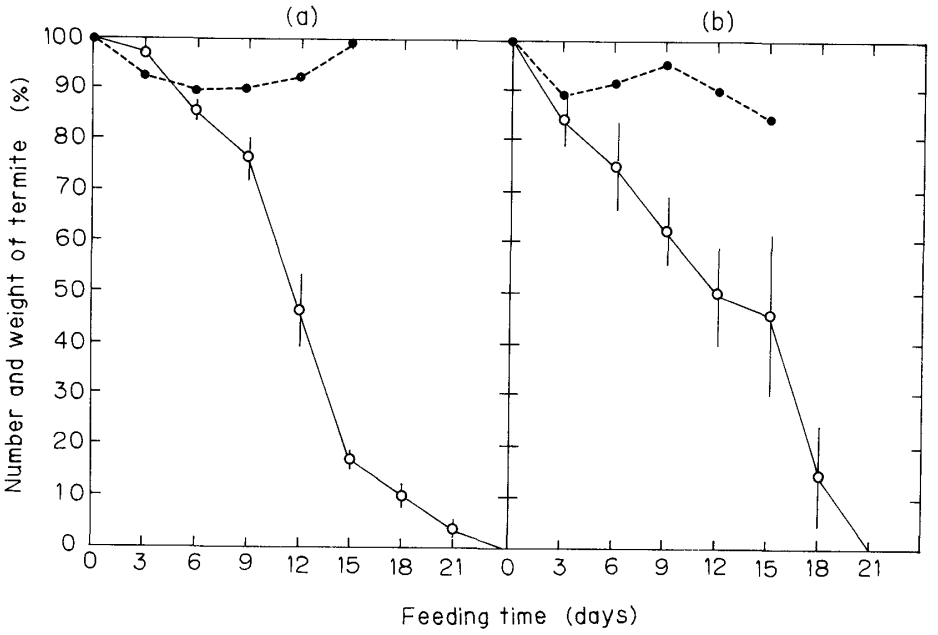

Fig. 1. Effect of starvation (a) and 1% acetic acid (b) on termites. Symbols : —○—, Number of termites (as % of original) ; Vertical lines indicate S.D. ; —●—, Weight of termites (as % of original).

for 2 weeks. Protozoa belonged to *Pyrsonympha* sp. and *Dinenympha* sp. gradually disappeared. *R. speratus* seemes to be more sensitive against dietary condition than *C. formosanus*, because 8 weeks were neccessary for complete death of the latter termite. Based on the starvation experiment, the span of the feeding experiment was decided to 21 days.

Secondly, a sapwood powder of Akamatsu was chosen as a desirable diet close to the natural state because it was noticed that the termites inhabited on a fallen trunk of this species of wood. The result shown in Fig. 2a indicates that 74.0% of the worker termites

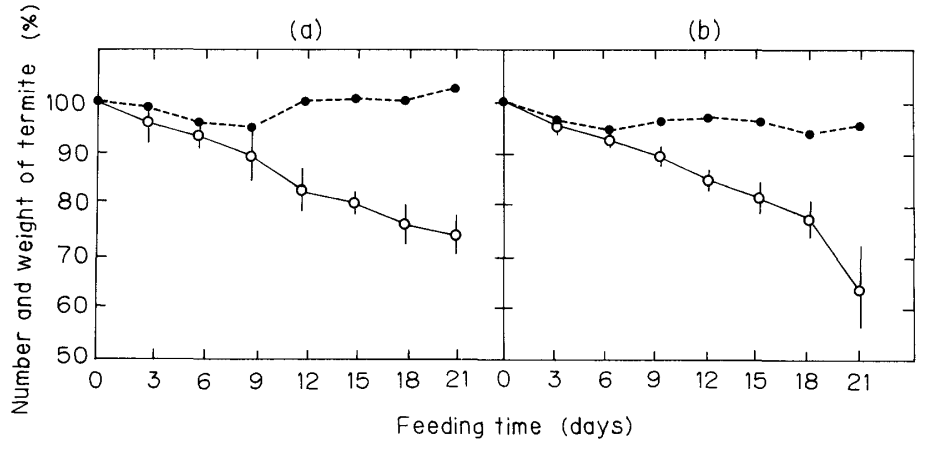

Fig. 2. Effects of Akamatsu wood meal (a) and cellulose (b) on termites. Symbols : —○—, Number of termites (as % of original) ; Vertical lines indicate S.D. ; —●—, Weight of termites (as % of original).

survived after feeding for 3 weeks without loss of their weights. Further, the protozoan fauna shown in Table 2 indicates that all kinds of protozoa were retained throughout the experiment.

These results indicate the validity of the use of Akamatsu wood powder as a control diet. Note that no cast differentiation was observed during the experiment in contrast to the case of *C. formosanus*[7].

3.2 Effects of carbohydrate and acetic acid diets on worker termites

The changes of number and weight of the workers and protozoan fauna during feeding experiment were analyzed by using one of the 16 different diets listed in Table 1. The representative profiles of the number and weight of the workers are shown in Figs. 1–5 and the effects of the diets on the protozoan fauna are summarized in Table 2. Based on the present results, the diets were found to be classified into four types (Type I–IV) in respect of utilization by protozoa and termite as listed in Table 3.

Cellulose powder belongs to the first group (Type I) which showed profiles similar to Akamatsu wood powder as shown in Fig. 2a, b. In this case, 64% of workers survived with retaining all protozoa and 95.7% of their weight after feeding for 21 days. In addition, two large size protozoa (*T. agilis* and *T. mirabilis*) survived throughout the experiment only in the cases of wood powder and cellulose. Previously, Yamaoka reported that *T. agilis* ingested cellulose selectively under culture conditions[13]. Present results indicate that both large size protozoa are involved in the cellulose digestion system in *R. speratus*.

Carboxymethylcellulose (CMC) and xylan belong to the second group (Type II) in which smaller size protozoa, such as *Pyrsonympha* sp. and *Dinenympha* sp., take part in their digestion. In these cases, 65.4% (CMC) and 80.6% (xylan) of workers survived in feeding for 3 weeks with high retaining of their weight, 99.4% (CMC) and 88.8% (xylan) as shown

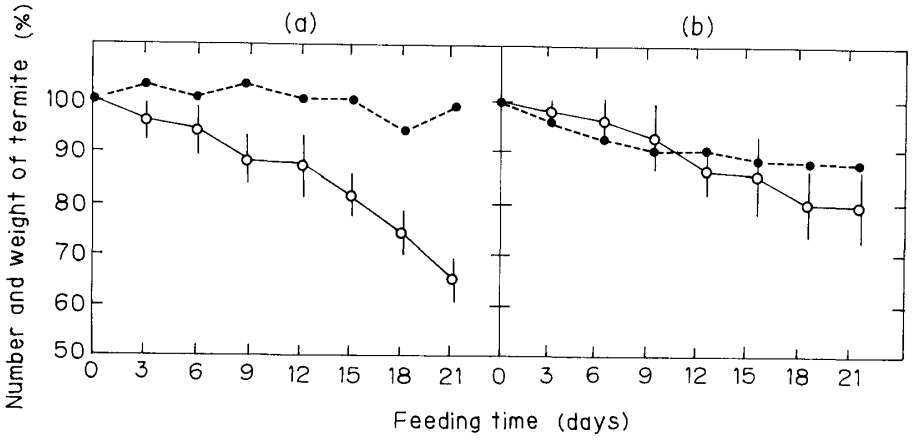

Fig. 3. Effects of CMC (a) and xylan (b) on termites.
Symbols : —○—, Number of termites (as % of original) ; Vertical lines indicate S.D. ; —●—, Weight of termites (as % of original).

in Fig. 3a, b. It must be noted that the survival rate of xylan was 6.6% higher than that of Akamatsu wood powder. However, both CMC and xylan affected the protozoan fauna, and had a characteristic disappearance of large size protozoa within 2 weeks, remarkably

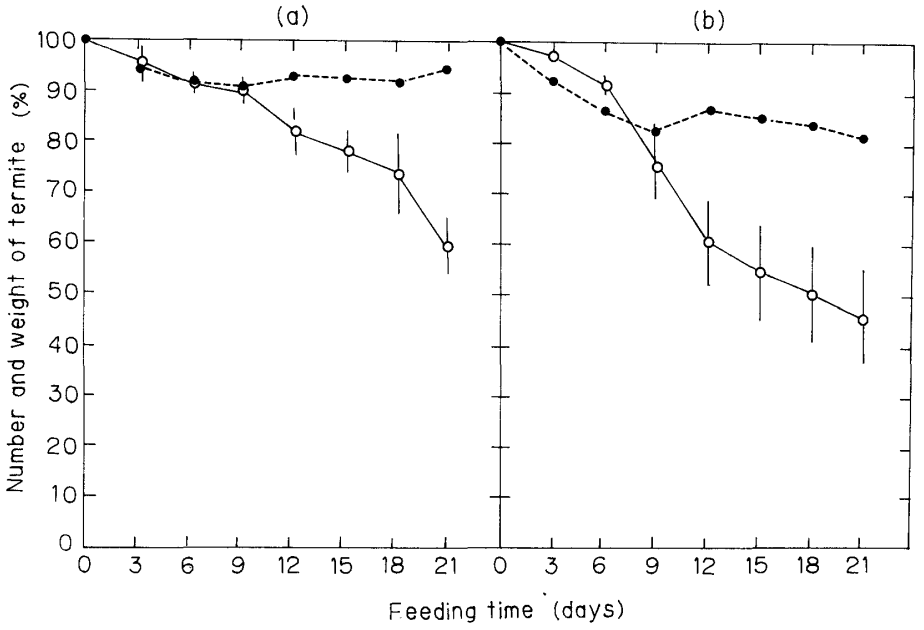

Fig. 4. Effects of amylose (a) and cellobiose (b) on termites.
Symbols: —○—, Number of termites (as % of original); Vertical lines indicate S.D.; —●—, Weight of termites (as % of original).

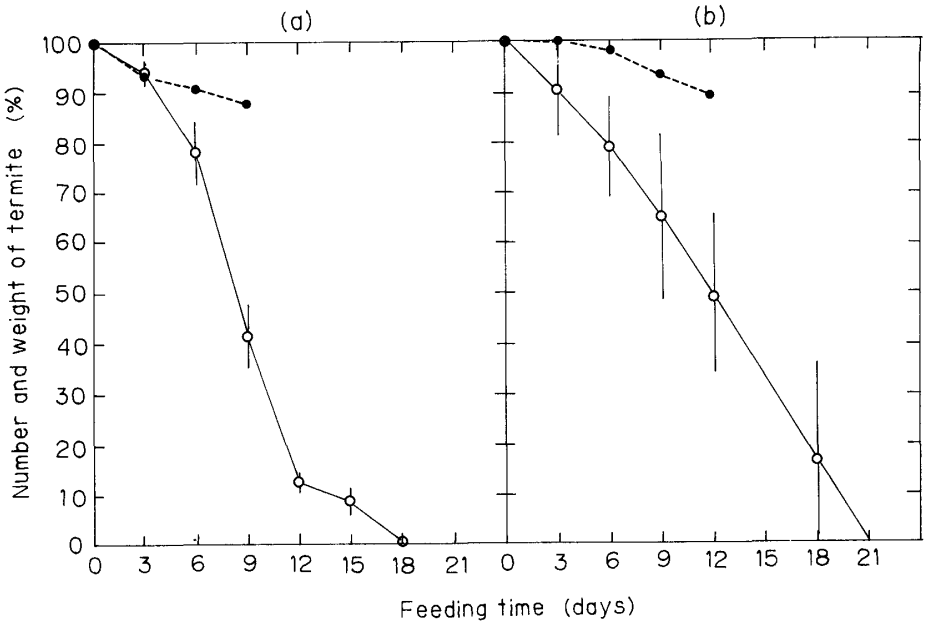

Fig. 5. Effects of glucomannan (a) and xylose (b) on termites.
Symbols: —○—, Number of termites (as % of original); Vertical lines indicate S.D.; —●—, Weight of termites (as % of original).

Table 2. Effects of diet on protozoan fauna.

Diets	6 days					15 days					21 days				
	Tr	Te	H	P	D	Tr	Te	H	P	D	Tr	Te	H	P	D
Wood meal	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##
Cellulose	##	##	##	##	##	##	##	##	##	##	##	##	±	##	##
CMC	##	##	##	##	##	+	+	+	##	##	±	±	±	##	##
Xylan	−	±	##	##	##	−	−	##	##	##	−	−	±	##	##
Amylose	−	−	+	##	##	−	−	±	##	##	−	−	−	±	±
Glucomannan	−	−	−	+	+	−	−	−	−	−	/	/	/	/	/
Arabinogalactan	−	−	−	±	±	−	−	−	−	−	/	/	/	/	/
Sucrose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Maltose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Cellobiose	−	−	+	##	##	−	−	±	##	##	−	−	−	−	−
Glucose	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−
Fructose	−	−	−	±	−	−	−	−	−	−	−	−	−	−	−
Xylose	−	−	−	−	−	−	−	−	−	−	/	/	/	/	/
Mannose	−	−	−	−	−	−	−	−	−	−	/	/	/	/	/
Galactose	−	−	−	+	+	−	−	−	−	−	/	/	/	/	/
Arabinose	−	−	−	−	−	−	−	−	−	−	/	/	/	/	/
Acetic acid (1%)	−	−	−	+	+	−	−	−	±	±	/	/	/	/	/
Starvation	−	−	+	##	##	−	−	−	±	±	/	/	/	/	/

Tr, *Trichonympha agilis*; Te, *Teratonympha mirabilis*; H, *Holomastigotes elongatum*; P, *Pyrsonympha* sp.; D, *Dinenympha* sp.

##, More than five termites out of ten had the protozoa; +, Four to three termites had the protozoa; ±, Two to one termite had the protozoa; −, No termites had the protozoa; /, No termite survived.

Table 3. Classification of diets into four types.

Type	Diete
Type I	Wood meal, Cellulose
Type II	CMC, Xylan
Type III	Amylose, Cellobiose, Sucrose, Maltose, Glucose, Fructose
Type IV	Glucomannan, Arabinogalactan, Xylose, Mannose, Galactose, Arabinose, 1% Acetic acid

different from the case of *C. formosanus*[7]. In the latter case, CMC was effectively used by the workers and retained protozoa throughout 8 weeks-feeding experiment, while xylan could not be used by workers and protozoa. This difference indicates that *R. speratus* has a wider adaptability toward its feed than *C. formosanus*.

Amylose, three disaccharides (cellobiose, sucrose and maltose), and two monosaccharides (glucose and fructose) belong to the third group (Type III) in which the workers survived without help of protozoa. Figure 4a, b shows results of amylose and

cellobiose. When fed for three weeks, 58.7% (amylose) and 46% (cellobiose) of the workers survived with retaining 94.6% (amylose) and 81.4% (cellobiose) of their weights, but complete disappearance of protozoa (Table 2). The other disaccharides and glucose gave similar results ranging 49.4% to 29.4% survival rate and 96.9% to 90.0% weight. Utilization of fructose by termite was not so high as the other members of this type and around 15% of the termites survived after feeding for 3 weeks.

The other carbohydrates shown in Table 1 belong to the fourth group (Type IV) which are considered to be unusable by both termites and protozoa, because all the tested termites died between 2 to 3 weeks with disappearance of protozoa (Table 2). The results obtained with glucomannan and xylose are shown in Fig. 5a, b. These results were in agreement with the previous results obtained on *C. formosanus*[7]. In addition, the present results confirmed the previous result[7] that 1% acetic acid was not utilized by workers when given *per se* as shown in Fig. 1b, although acetate is the major volatile fatty acid in hindgut fluid of lower termites[4]. The present results also indicate that the changes in mean weight of a worker were difficult to interpret, because the weights went down initially but recovered to the initial level after feeding for 15 days. In conclusion, the loss of weight did not have a good correlationship with changes in survival rate and protozoan fauna.

Note that the termite *R. speratus* could not utililize monomeric xylose in spite of its utilization of polymeric xylan. The reason of this observation will be referred later in 3.3.

3.3 Effect of feeding with xylan on protozoa and enzyme activities

As shown in 3.2, the termite *R. speratus* was found to utilize xylan as a diet. This prompted us to further investigate the effects of xylan on protozoa and termites in more detail.

Figure 6 shows the quantitative analysis of the number of protozoa in the hindgut of

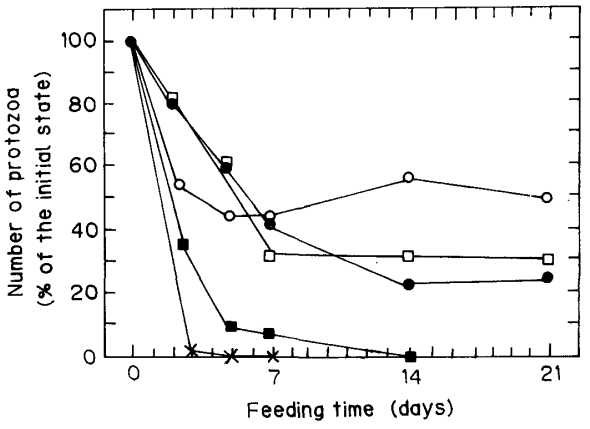

Fig. 6. Effects of xylan on protozoan fauna.
Symbols: —×—, *Trichonympha agilis*; —■—, *Teratonympha mirabilis*; —○—, *Pyrsonympha* sp.; —●—, *Dinenympha* sp.; —□—, *Holomastigotes elongatum*.

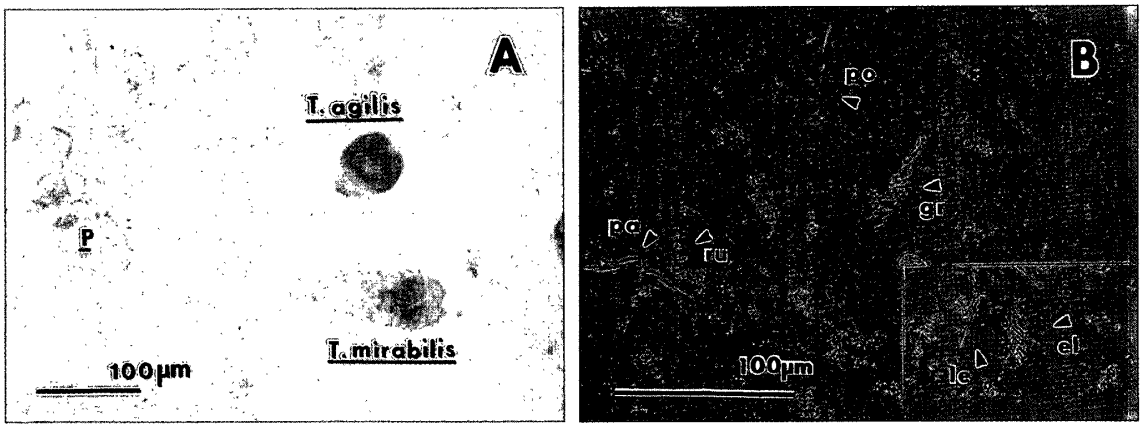

Fig. 7. Photos of protozoa before (A) and after (B) feeding on xylan for 21 days.
Symbols : *T. agilis*, *Trichonympha agilis* ; *T. mirabilis*, *Teratonympha mirabilis* ; P, *Pyrsonympha* sp. ; el, *Holomastigotes elongatum* ; gr, *Pyrsonympha grandis* ; ru, *Dinenympha rugosa* ; le, *Dinenympha leidyi* ; pa, *Dinenympha parva* ; po, *Dinenympha porteri*.

experiment. *T. agilis* was found to disappear within 2 to 3 days feeding. The number of *T. mirabilis* also rapidly decreased during first week and completely disappeared after 2 weeks as already shown in Table 2. There are two *Pyrsonympha* sp., *P. grandis* and *P. modesta*, in the initial state, but the latter species disappeared by feeding with xylan, and about 50% of the former species constantly remained during 7 to 21 days. *H. elongatum* also decreased during the first week, but afterwards about 30% still remained. According to *Dinenympha* sp., there are 6 species in the hindgut of workers, of which 4 species, *D. rugosa*, *D. leidyi*, *D. parva*, and *D. porteri* remained after 21 days as shown in Fig. 7. The total number of these species decreased similar to *H. elongatum* and became constant at about 25% of that of the initial state as shown in Fig. 6. These results strongly indicate that smaller size protozoa take part in degradation of xylan.

Finally, the changes of xylanolytic enzyme activities of the hindgut and the residual portion of workers during 21-days feeding with xylan were stugied and the results are shown in Fig. 8a, b. The distribution and activity of xylanase were found to be markedly different from those of β-xylosidase. At the initial state, 73% of the total activity of xylanase was localized in the hindgut, whereas the residual portion had a similar value of β-xylosidase activity. Further, xylanase activities in the hindgut and the residual portion were about 55 and 8 fold more than β-xylosidase activities, respectively.

As the feeding experiment progressed, the xylanase activity in the hindgut decreased rapidly during the first week and attained rather constant level at about 70% of the initial state after 2 weeks (Fig. 8a) in a good relationship with the change of the other members of the smaller size protozoa (Fig. 6). In the residual portion, however, the xylanase activity decreased to about 20% of the initial state after 2 weeks. These results indicate that degradation of xylan is suggested to be primarily occurred in the hindgut by participation of

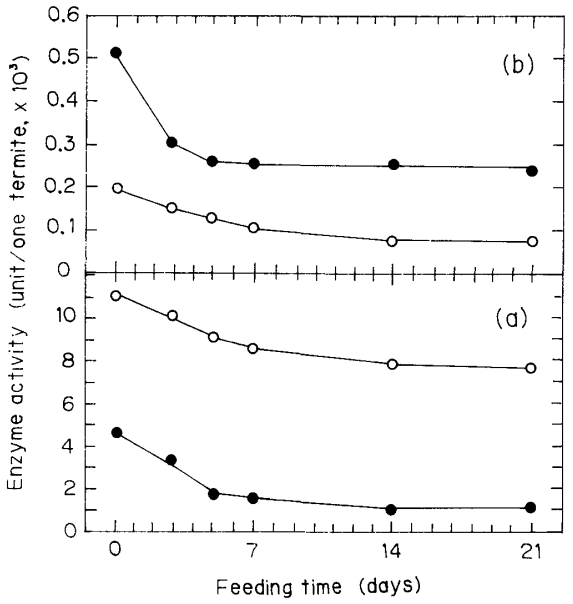

Fig. 8. Effects of feeding with xylan on activities of xylanase (a) and β-xylosidase (b).
Symbols: —○—, hindgut; —●—, residual portion.

symbiotic small size protozoa.

In contrast, the β-xylosidase activity in the hindgut decreased gradually as feeding progressed to attain 35% of the initial state after 3 weeks. Change of β-xylosidase activity in the residual portion was rapid during the first week but about 50% of its activity still remained constant afterwards. These results indicate that β-xylosidase secreted from termite plays a major role in digestive system of *R. speratus*. Its low activity, in comparison with high xylanase activity, may suggest that *R. speratus* utilizes mainly secondary metabolites of xylan which are produced by symbionts and that monomeric xylose produced by β-xylosidase is not effectively utilized by both termite and protozoa resulting in death of termites when they are fed on xylose alone as already shown in 3.2.

Based on the present results, the termite *R. speratus* is concluded to utilize xylan by co-operative action of symbiotic small size protozoa. A further study on purification and property characterization of xylanolytic enzymes of protozoa is now in progress.

Acknowledgements

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