Original

Studies on Digestive System of Termites*

I. Digestion of Carbohydrates by Termite *Coptotermes formosanus* SHIRAKI

Kazumitsu KANAI**, Jun-ichi AZUMA**
and Koichi NISHIMOTO***

Abstract——To clarify the role of protozoa in termites, various carbohydrates were given to the termite *Coptotermes formosanus* SHIRAKI. Protozoa were normally maintained during 8 weeks with cellulose and Avicel. However, no protozoa were observed with amylose, cellobiose, sucrose, maltose, glucose and fructose. Termites devoid of protozoa survived as well as with cellulose. This seems to indicate that protozoa take part in digestion of native cellulose and termites themselves are able to utilize these disaccharides and monosaccharides without aid of protozoa. Almost all termites died within 8 weeks with xylan, glucomannan and arabinogalactan. This seems to indicate that termites cannot utilize these hemicelluloses efficiently.

Introduction

The symbiotic relationship between protozoa and lower termites was first proposed by Cleveland[1]. He indicated termites can not live on wood after they were artificially defaunated by incubation at high temperature (35°C), exposure to pure oxygen, or starvation. Recently, the postulation that termites completely rely on protozoa to digest wood was commented on by several investigators. Bready and Friedman[2], and Yokoe[3] reexamined Cleveland's experiment and concluded that his experiment was not enough to prove the symbiotic relationship, because damage to the termites should occur under the condition of his experiment. Mauldin *et al.*[4] showed that *Coptotermes formosanus* decomposed ^{14}C-labelled cellulose and the label was incorporated into fatty acids even after the protozoa were removed from termites by treatment with oxygen, and suggested that termites have their own cellulolytic activity. Yamaoka and Nagatani[5] also reported that *Reticulitermes speratus* have strong carboxymethylcellulase activity in their salivary glands, and proposed a hypothesis that decomposition of cellulose was achieved initially by protozoa followed by enzymes of termites.

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** Research Facility for Wood Protection.
*** Research Section of Wood Chemistry.

Apart from the symbiotic relationship between protozoa and termites, two theories have been proposed about the digestive process of cellulose in termites. Trager[6] found that the extract from the gut of *Zootermopsis angusticollis* yielded glucose from cellulose, and proposed " the glucose theory ". Hungate[7], however, proposed " the acetic acid fermentation theory ", from the results that protozoa in *Zootermopsis angusticollis* could not only decompose cellulose to glucose but also ferment it to acetic acid, hydrogen and carbon dioxide. Yamin[8] supported the acetic acid fermentation theory of Hungate, from the result that ^{14}C-labelled acetic acid was produced by protozoa when ^{14}C-labelled cellulose was added to pure cultures of *Trichomitopsis termopsidis*. Mauldin *et al.*[9] also supported "the acetic acid fermentation theory" from the result that ^{14}C was incorporated into amino acid when ^{14}C-labelled acetate was added to the diet of termites.

Thus, work so far on the digestive system of termites has given ambiguous results and it seems necessary to investigate the role of protozoa in termites. The purpose of this study was to clarify the role of protozoa in the gut of termites, *Coptotermes formosanus*, using various carbohydrates and acetic acid as diet. The changes in protozoan fauna and activities of glycosidases including cellulase were also investigated.

Experimental

Materials

Sodium salt of carboxymethylcellulose (CMCNa, substitution degree 25–30%), amylose (molecular weight of approx. 16,000), and *p*-nitrophenyl α- and β-D-glycosides except *p*-nitrophenyl α-L-arabinofuranoside were obtained from Nakarai Chemicals Co., Ltd. *p*-Nitrophenyl α-L-arabinofuranoside was from Sigma Chemical Co., Ltd. Arabinan from sugar beet was from Koch-Light Laboratories, Ltd. Avicel SF was from Funakoshi Chemical Industries Co., Ltd. Carboxymethylcellulose (CMC, Capacity: 0.68 meq/gm) was from Wako Pure Chemical Industries Co., Ltd. Acetyl-galactoglucomannan was prepared as described previously[10]. All the other reagents used were analytical reagent grade.

Preparation of crude enzyme solution

Two hundred termites in 2 ml of 0.05 M sodium acetate buffer, pH 4.6, were sonicated at 0°C for 4×30 sec. The homogenate was centrifuged at $15000 \times g$ for 20 min at 5°C to remove solid materials. The supernatant was carefully taken and used as a crude enzyme solution.

Assay of enzymes

(a) Aryl α- and β-glycosidases: The nine enzyme activities such as α-glucopyranosidase, β-glucopyranosidase, α-galactopyranosidase, β-galactopyranosidase,

α-mannopyranosidase, β-mannopyranosidase, α-xylopyranosidase, β-xylopyranosidase and α-arabinofuranosidase were assayed by utilizing appropriate *p*-nitrophenyl D-glycopyranosides and L-glycofuranoside. The reaction mixture contained 0.1 ml enzyme solution and 0.9 ml of 0.65 mM substrate solubilized in 0.05 M sodium acetate buffer, pH 4.6. The mixture was incubated on a Monod-shaker at 37°C for 10 min before the addition of 5.0 ml 0.6 M sodium carbonate. The liberated *p*-nitrophenol was determined spectrophotometrically at 410 nm. One unit of the enzyme was defined as the amount which liberated 1 μmole of *p*-nitrophenol per min. The activity of the enzyme was expressed as the number of units per 1000 termites.

(b) Cellulases: Cellulases were assayed by utilizing Avicel, CMC and CMCNa as substrate. The reaction mixture contained 0.1 ml enzyme solution, 0.25 ml 1% substrate in distilled water, 0.1 ml 0.5 M sodium acetate buffer, pH 4.6, and 0.55 ml distilled water. The mixture was incubated on a Monod-shaker at 37°C for 24 hr. Reducing sugar formed after incubation was determined as glucose by the Somogyi-Nelson method after boling the solution in water bath for 10 min. One unit of the enzyme was defined as the amount which liberated 1 μmole of glucose from the substrate under the conditions described above. The enzyme unit was expressed as units per 1000 termites.

Effect of diet on termite and protozoa

Colonies of *Coptotermes formosanus* were collected in Wakayama city and kept for about ten years with *Pinus densiflora* at 26°C. Three kinds of representative protozoa, *Pseudotrichonympha grassii, Holomastigotoides hartmanni* and *Spirotrichonympha leidyi* were found to be present in the hindgut of the workers kept in our laboratory. The protozoan fauna was essentially similar to that observed in termites collected in the field. An acrylic cup (6 cm in inner diameter) with a hole (1 cm in diameter) at the bottom

Table 1. Compounds used as diets.

	Compound
Polysaccharides	cellulose[a], Avicel, CMC, CMCNa, xylan[b], glucomannan[c], arabinogalactan[d], amylose, amylopectin, arabinan[e], mannan[f], pectin[g], pullulan, inulin[h], dextran[i], laminarin
Disaccharides	cellobiose, sucrose, maltose, trehalose[j]
Monosaccharides	glucose[k], fructose[l], xylose[m], mannose[n], arabinose[o], galactose[p]
Other	acetic acid 10%, 1%

a: Whatman CF11 cellulose powder. b: 4-*O*-Methylglucuronoxylan from beech. c: Acetylgalactoglucomannan from pine[10]. d: Arabinogalactan from larch. e: Arabinan from sugar beet. f: Mannan from yeast. g: Pectin from lemon peel. h: Inulin from Dahlia tubers. i: Dextran T-2000. j: α,α-Trehalose. k: D-Glucose. l: D-Fructose. m: D-Xylose. n: D-Mannose. o: L-Arabinose. p: D-Galactose.

was used as a rearing vessel. The hole was sealed with hard gypsum. This rearing vessel was put on a defatted cotton seat which was moistened with water, so as to supply a proper amount of water to termites. Fifty termite workers were fed in each rearing vessel with diet listed in Table 1, for 8 weeks in the chamber thermostated at 26°C, and the following four items were recorded; (1) number of termites, (2) weight of termites, (3) number of new soldiers transformed from workers through cast differentiation, (4) change of protozoan fauna. Changes in number and weight of termites were respectively expressed as percentage of the number and weight before the experiment. Cast differentiation was judged by the transformation of the mandible. Twenty termites were arbitrally picked up and protozoan fauna was determined. For comparison the same experiment was performed without diet and with wood meal of *Pinus densiflora*. Water-insoluble diets were directly given on the rearing vessels, while water-soluble diets were given 10% aqueous solution absorbed in asbestos.

Results and Discussion

At first, wood meal of *Pinus densiflora* was selected as a diet, because timbers of this wood have been used to keep termites in our laboratory and therefore this wood was thought to be the most desirable diet for termites. The results are shown in Fig. 1. Seventy-five % of termites survived after rearing for 8 weeks. Initially the weight of termites decreased but later became almost constant (about 80% of

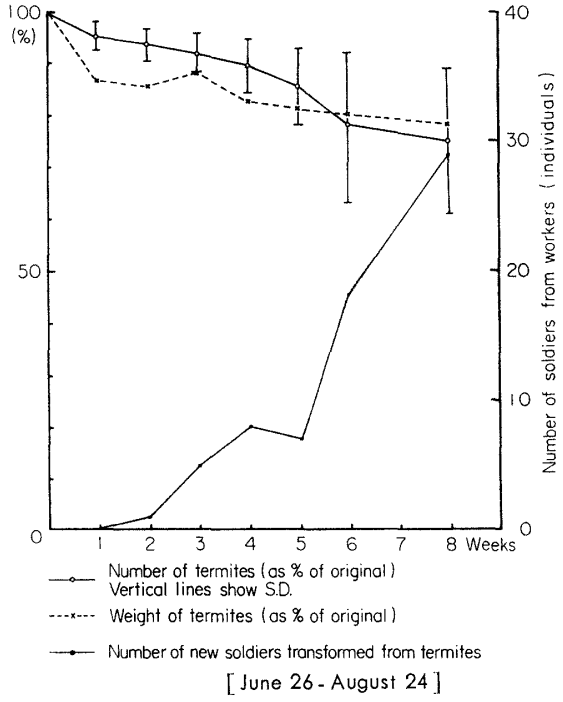

Fig. 1. Effect of wood meal on termites

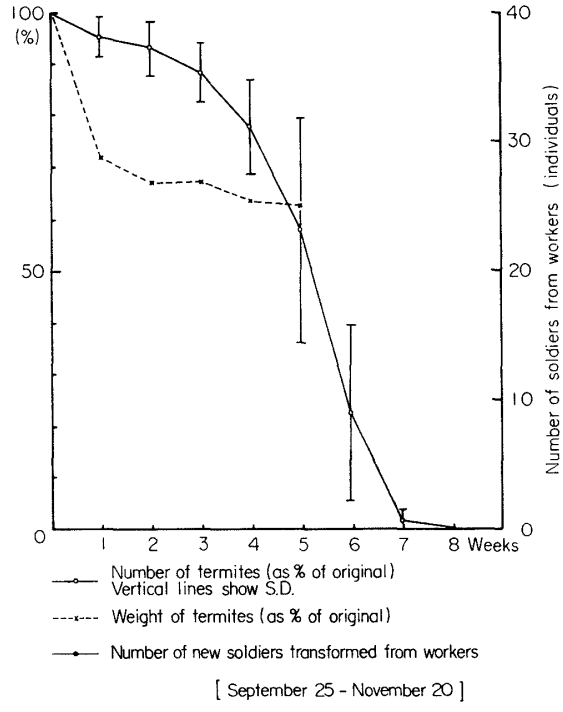

Fig. 2. Effect of starvation on termites.

original weight). Cast differentiation was first observed after rearing for 2 weeks, and 29 new soldiers appeared at the end of experiment (8 weeks).

Next the starvation experiment was performed. Termites were reared without any diet other than water. The results are shown in Fig. 2. In contrast to the results described above, the number of surviving termites decreased rapidly within 5 weeks, and no termites survived after rearing for 8 weeks. Initially the weight of termites decreased rapidly, but it became almost constant after 4 weeks. No cast differentiation was observed. From these results it may be expected that significant changes in the number and weight of termites should occur depending upon the diet. This was verified by using 27 compounds as diet including 16 polysaccharides, 4 disaccharides, 6 monosaccharides and acetic acid.

Fig. 3 shows the results obtained by acetyl-galactoglucomannan. The number and weight loss of termites were almost the same as with starvation. Cast differentiation was observed only once. Quite similar profiles of decrease in weight and number of termites were observed in 4-*O*-methylglucuronoxylan, arabinogalactan, amylopectin, mannan, pectin, pullulan, inulin, dextran, laminarin, trehalose, xylose, mannose, arabinose, galactose and acetic acid. These results may indicate that termites cannot use these compounds. It is interesting to note that so-called hemicelluloses consisting of 23 to 38% of wood cannot be used by termites when they are the sole diet.

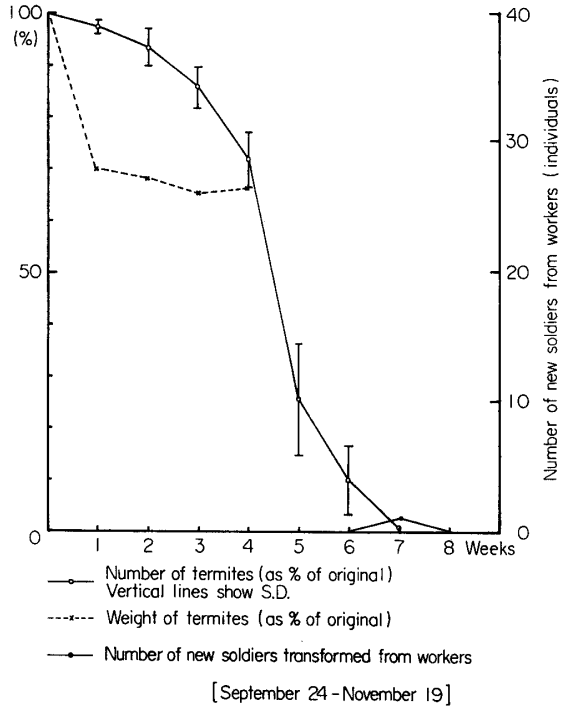

Fig. 3. Effect of glucomannan on termites.

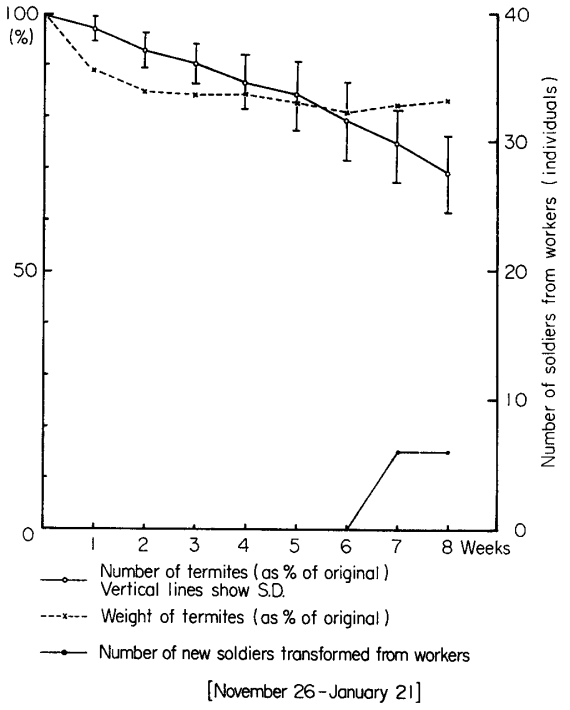

Fig. 4. Effect of cellulose on termites.

These results prompted us to clarify the possibility that termites could utilize cellulose, the most abundant carbohydrate in wood as a sole diet. In this line of consideration, we used two celluloses and two cellulose derivatives, Avicel, cellulose, CMC and CMCNa as diet and number and weight of termites which survived were determined. Typical results obtained by cellulose are shown in Fig. 4. Sixty seven % of termites survived after rearing for 8 weeks. Cast differentiation was initially observed after rearing for 7 weeks, and 6 soldiers were observed at the end of the experiment. In the case of CMC, 65% of termites survived after rearing for 8 weeks, and 10 soldiers were observed at the end of the experiment; cast differentiation being initially observed after rearing for 4 weeks. In the case of CMCNa, however, no termite could survive longer than 5 weeks, and no cast differentiation was observed. These results suggest that CMCNa cannot be utilized by termites. Yaga[11] reported that some inorganic salts including sodium salts were toxic to termites. Therefore sodium in CMCNa may be toxic to termites.

Although starch content in wood is extremely low[12], its importance in the rearing of *Lyctus* sp. is stressed by Parkin[13]. Since the effect of starch on termite is so far unknown, we intended to clarify the role of starch in the digestion process of termites. Fig. 5 shows the results obtained with amylose. Seventy two % termites survived at the end of the experiment, corresponding to the results obtained with wood meal. The weight of termites decreased gradually to 85% of the original weight during

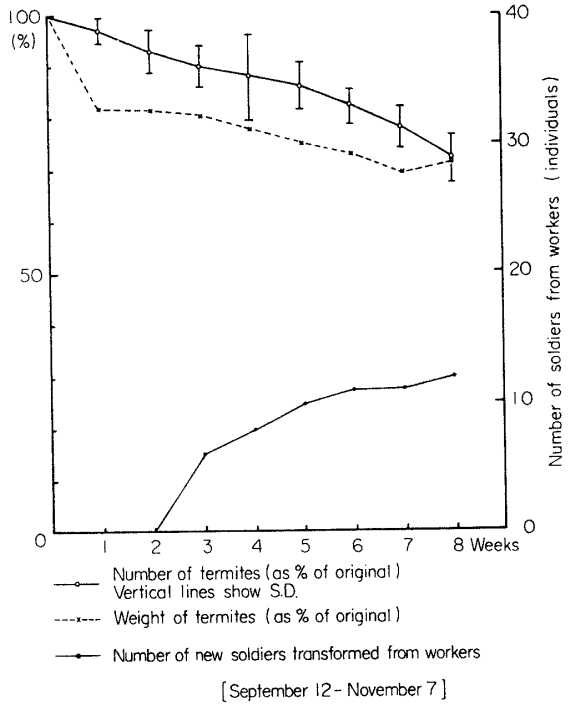

Fig. 5. Effect of amylose on termites.

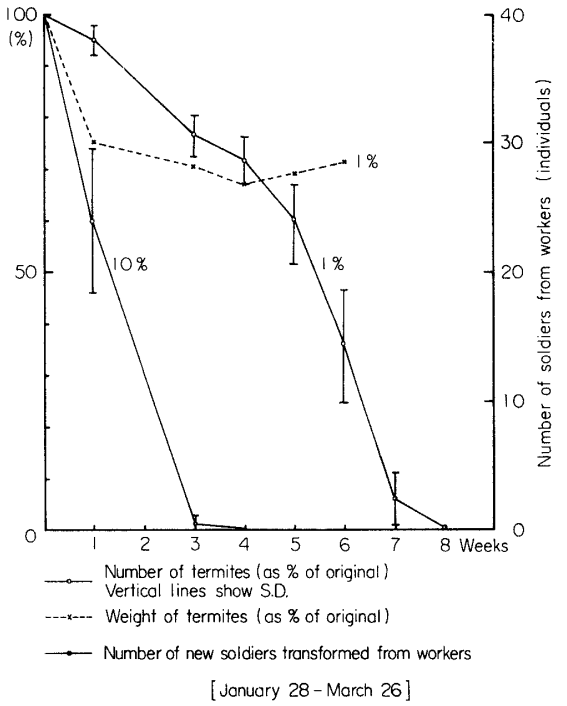

Fig. 6. Effect of acetic acid on termites.

8 weeks. Cast differentiation was observed after rearing for 3 weeks, and 12 workers had been transformed into soliders by the end of the experiment. Essentially the same results were obtained with sucrose, glucose and fructose. These results strongly suggest that termites can utilize these compounds. It should be emphasized that termites could not utilize amylopectin, dextran and pullulan. These polysaccharides differ from amylose in that they contain $\alpha(1\rightarrow6)$-linked glucose residues. This may indicate the lack of the enzyme which can split $\alpha(1\rightarrow6)$-glucoside linkage in termites.

Lastly, the possibility of utilization of aqueous acetic acid by termites was checked, since " the acetic acid fermentation theory " proposed by Hungate was recently resurrected by Yamin[8]. The results are shown in Fig. 6. When 10% acetic acid was used as a sole diet, all termites died within 3 weeks. In the case of 1% acetic acid, the results were almost the same as those presented in Fig. 2. These results clearly indicate that acetic acid is not utilized by termites when given *per os.*

The changes in weight of termites cannot be used as an index to show whether or not the compound is utilized by termites because even in the case of a usable compound, such as amylose, the changes in weight showed a pattern similar to starvation.

Effect of diet on the protozoan fauna

The change of protozoan fauna during the experiment is shown in Table 2. Termites reared with wood meal, cellulose, CMC, and Avicel still retained protozoa through 8 weeks. However, termites reared with amylose, cellobiose, sucrose, maltose, glucose and fructose had lost all of the three protozoa species after 8 weeks. Although termites lost *Pseudotrichonympha grassii* within a week, the other two species of protozoa were sometimes partially retained for more than 4 weeks. Other compounds in Table 2 caused changes in the protozoan fauna similar to those caused by starvation, although most of termites died within 8 weeks.

From the results presented above, the compounds in Table 1 can be divided into three groups (Table 3). The first group was designated as Type 1. In the presence of the compounds in Type 1, survival of termites was achieved even after rearing for 8 weeks, with maintenance of protozoa. These compounds are considered to be utilized by termites with aid of protozoa. The second group was designated as Type 2. In the presence of the compounds of this type, survival of termites was achieved without protozoa. These compounds are considered to be utilized by termites without the aid of any protozoa. The third group was designated as Type 3. Under the presence of the compounds of this type, most termites died within 8 weeks as was observed in the absence of any diet. These compounds are not considered to be used by termites.

CMC is the only exception in Type 1, which lost only *Pseudotrichonympha grassii* among the three protozoa species after rearing for 8 weeks.

Table 2. The change of protozoan fauna with diets.

Diet	1 week			4 weeks			8 weeks		
	P	H	S	P	H	S	P	H	S
Polysaccharides									
Cellulose	++	++	++	++	++	++	++	++	++
Avicel	++	++	++	++	++	++	++	++	++
CMC	−	++	++	−	++	++	−	+	+
CMCNa	−	++	++	/	/	/	/	/	/
Xylan	−	++	++	−	++	++	−	−	−
Glucomannan	−	+	++	−	+	++	/	/	/
Arabinogalactan	−	++	++	−	+	+	/	/	/
Amylose	−	++	++	−	++	++	−	−	−
Amylopectin	−	++	++	−	+	+	/	/	/
Arabinan	−	+	+	−	−	−	/	/	/
Mannan	−	++	++	−	−	−	/	/	/
Pectin	−	++	++	−	+	+	/	/	/
Pullulan	−	++	++	−	±	±	−	−	−
Inulin	−	++	++	−	−	−	/	/	/
Dextran	−	++	++	−	−	−	/	/	/
Laminarin	−	++	++	−	−	−	/	/	/
Disaccharides									
Cellobiose	−	++	++	−	++	++	−	−	−
Sucrose	−	++	++	−	+	+	−	−	−
Maltose	−	++	++	−	+	+	−	−	−
Trehalose	−	++	++	−	−	−	−	−	−
Monosaccharides									
Glucose	−	++	++	−	±	−	−	−	−
Fructose	−	++	++	−	+	+	−	−	−
Xylose	−	+	+	−	±	−	−	−	−
Mannose	−	++	++	−	−	−	−	−	−
Arabinose	±	++	++	−	−	−	−	−	−
Galactose	−	+	+	−	−	−	−	−	−
Others									
Acetic acid	−	++	++	−	−	−	/	/	/
Wood meal	++	++	++	++	++	++	++	++	++
Starvation	−	+	+	−	−	−	−	−	−

P: *Pseudotrichonympha grassii*, H: *Holomastigotoides hartmanni*, S: *Spirotrichonympha leidyi*, ++: more than 10 termites out of 20 had the protozoa, +: 9–5 termites had the protozoa, ±: 4–1 termites had the protozoa, −: no termite had the protozoa, /: no termite survived.

Above results seem to indicate that the termite *Coptotermes formosanus* depends almost exclusively on cellulose among the carbohydrates in wood, while hemicellulose can scarcely be used by termites. Even if hemicelluloses are utilized by termites, they are considered to be of only minor importance. Three kinds of protozoa, es-

Table 3. Three types of diets.

Type	Diets
Type 1	wood meal, cellulose, Avicel, CMC
Type 2	amylose, cellobiose, sucrose, maltose, glucose, fructose
Type 3	glucomannan, arabinogalactan, xylan, amylopectin, arabinan, mannan, pectin, pullulan, inulin, dextran, laminarin, trehalose, xylose, mannose, arabinose, galactose, CMCNa, acetic acid

pecially *Pseudotrichonympha grassii*, are considered to participate in the digestion process of cellulose in the gut of termites. *Holomastigotoides hartmanni* and *Spirotrichonympha leidyi* were always detected, whenever *Pseudotrichonympha grassii* was present. CMC is considered to be utilized with the aid of *Holomastigotoides hartmanni* and *Spirotrichonympha leidyi*. These two results seem to indicate that cellulose may be at first partially decomposed by *Pseudotrichonympha grassii*.

Effect of diet on glycosidases and cellulases are summarized in Table 4. Activities of β-glucosidase, β-mannosidase and β-xylosidase in the case of Type 2, tended to be lower than those in the case of Type 1. This seem to indicate that these three β-glucosidases have some relationship with protozoa. Other α- and β-glycosidases and cellulases did not change clearly, so a clear relationship between these enzymes and protozoa does not seem to exist. The possibility that these enzymes are secreted from termites themselves cannot be ruled out. When termites were reared with cellulose, the activities of α-glucosidase, β-glucosidase, α-mannosidase and β-mannosidase were different from the values reared with wood meal. This seems to indicate

Table 4. Effect of diet on enzyme activity.

Diet / Enzyme	Type 1				Type 2					
	Wood meal	Avicel	Cellulose	CMC	Fructose	Sucrose	Maltose	Glucose	Cellobiose	Amylose
α-glucosidase	391.6	141.5	603.8	51.8	257.6	388.7	141.5	304.8	500.3	150.0
β-glucosidase	527.3	354.2	353.1	33.9	238.6	245.0	354.2	215.1	282.9	150.8
α-galactosidase	157.0	35.1	50.6	17.3	116.0	19.0	21.9	24.7	32.8	24.6
β-galactosidase	119.6	102.4	111.0	40.3	149.5	63.3	72.5	59.2	77.1	111.9
α-mannosidase	258.7	506.6	100.8	34.5	119.0	208.7	361.1	335.3	367.5	100.8
β-mannosidase	369.8	278.9	192.8	85.1	154.7	109.3	166.8	174.2	258.2	192.8
α-xylosidase	0.0	9.2	15.0	11.5	9.2	12.8	0.0	13.2	20.1	11.1
β-xylosidase	32.8	27.0	34.5	6.9	17.3	16.7	0.0	20.7	20.1	17.5
α-arabinosidase	22.4	7.5	15.5	0.6	5.8	4.0	32.0	4.6	17.3	2.0
Avicelase	217.2	200.7	225.4	165.6	198.5	234.2	132.9	176.7	229.2	230.0
CMCase I*	187.3	203.0	231.2	222.8	204.4	249.3	140.2	187.8	257.1	222.6
CMCase II**	338.0	298.7	309.1	264.1	250.2	284.1	307.5	224.1	273.9	294.1

(units/1000 individuals), * substrate used was CMC, ** substrate used was CMCNa.

that the condition of termites reared with cellulose became somewhat unnatural even though they retained all species of protozoa. Thus other polysaccharides such as hemicelluloses may be necessary for termites to maintain their normal condition.

Cast differentiation from workers to soldiers

The phenomenon of cast differentiation from workers to soldiers was observed more frequently with compounds utilized by termites than with unusable compounds. However, even with the usable compounds there were some cases where no cast differentiation was observed within 8 weeks. Repeated experiments indicated that even with the same compounds, more and faster cast differentiation occurred from May to September, but little cast differentiation occurred from January to March (unpublished results). The termites were reared in a chamber thermostated at 26°C, so they were not influenced by the temperature outside. The results above may seem to indicate termites noticed the change of season through factors other than temperature, and cast differentiation became easier in summer than in winter. This result is very interesting, because termites became active and more soldiers are necessary in summer in natural conditions. The results presented above seem to indicate the possibility that some of the soldiers are supplied from workers.

Conclusion

Amylose, cellobiose, maltose, glucose and fructose are considered to be enough to maintain life of termites without protozoa. Protozoa especially *Pseudotrichonympha grassii,* participate in the decomposition of native cellulose in wood. The hypothesis that protozoa yield a single compound from cellulose has to be taken into consideration, because termites themselves are able to utilize the above carbohydrates as diet. The digestion products of these carbohydrates may be absorbed by termites directry or after they are further decomposed by the enzymes secreted by termites themselves. Further studies on the digestion products of these carbohydrates by protozoa are indispensable in order to clarify the relationship between protozoa and termites.

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