Preliminary

Lignin Modification by Termite and Its Symbiotic Protozoa*[1]

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Introduction

Wood mainly consists of cellulose, hemicellulose and lignin. Lignin therefore is a member of major biomasses on the earth. So far, it is well established that lignin is degraded by only white rot fungi and some kinds of bacteria. *Coptotermes formosanus* Shiraki is one of the most dangerous pest for wooden constructions in the world, and is considered to degrade cellulose by an association with symbiotic protozoa in the hindgut[1)]. In recent years, lignin degradation by termites has been studied using wood which is containing ^{14}C-labeled lignin by some researchers[2,3)], and the results indicate the minor degradation of lignin. But an individual event in the minor degradation, such as modification of functional group or change of molecular weight, is not detectable in ^{14}C-method. We describe the lignin ingestion and degradation by *C. formosanus* and its symbiotic protozoa by microscopic observations and chemical analyses.

Materials and Methods

Preparation of samples

Milled-wood-lignin (MWL) was used as a test substrate. Wood meal (60 mesh) of akamatsu (*Pinus densiflora* Sieb.et Zucc.) was stepwisely extracted with ethyl alcohol : benzene (1 : 2, v/v) for 48 hr, and 0.25% potassium acetate aq. for 24 hr at 60°C. The extracted wood meal was ultraground with a vibratory ball mill under N_2 atomosphere for 48 hr, and then was extracted with 90% dioxane solution for 48 hr at room temperature. The extract was evaporated to give crude MWL. Holocellulose was prepared by the Wise

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methods[4] from the same wood meal.

Termite fecal lignin (TFL) was obtained by the following methods: About one thousand workers of *C. formosanus* and wood meal of akamatsu (<60 mesh) were put into a container, and the container was kept in the dark at 28°C. The fecal materials were dislodged from the surface of the walls of container every two days. After extraction of termite faeces with diethyl ether (1 hr) and hot water (1 hr at 60°C), the residue was extracted again with 90% dioxane solution for 48 hr at room temperature. The extract was evaporated to give crude TFL.

Effect of lignin on survival of wokers of *C. formosanus*

Two hundreds workers of *C. formosanus* were forced to feed on wood meal of akamatsu, holocellulose and MWL. Surviving individual were counted weekly for 17 weeks.

Microscopic observation of MWL ingested by the protozoa

Morphological observation of MWL ingested by the protozoa in the hindgut of *C. formosanus* was carried out by phase-contrast, epifluorescence and transmission electron microscopes.

Chemical analyses of MWL and TFL

Analyse of MWL and TFL were made by UV and IR spectrometry and GPC (gel permeation chromatography) to discuss the modification of lignin by termite.

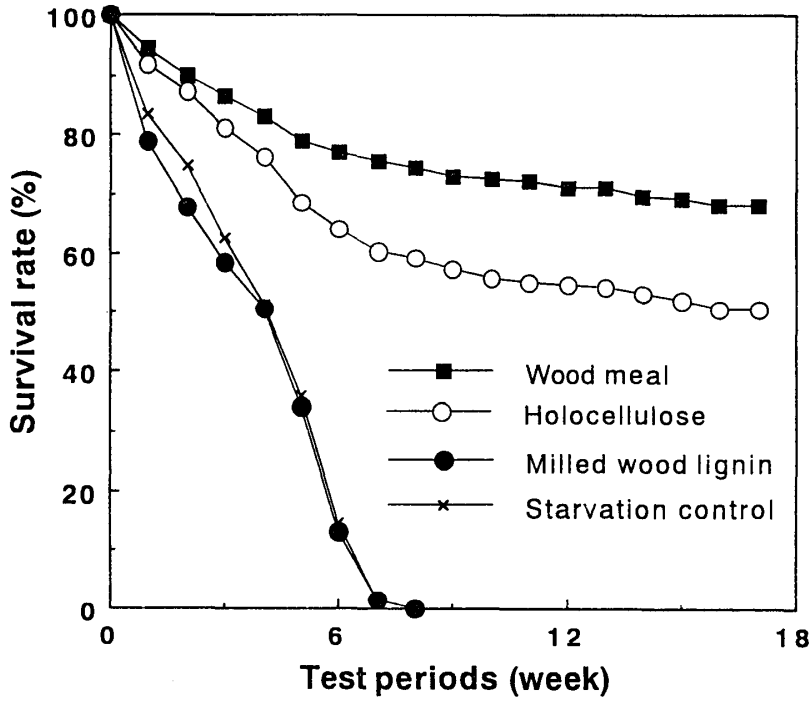

Fig. 1. Time-course of survival rate of *C. formosanus* worker fed on different substrate.

Results and Discussion

Figure 1 shows the survival rate curves of workers on the three kinds of substrates. MWL caused lowest survival rate of workers comparable to that of starvation control. This shows that test insects could not utilize MWL as nutrient. While survival rate of workers fed on wood meal was much higher than that of holocellulose throughout the test period. Therefore, lignin in wood may has a roll to make workers of *C. formosanus* healthy condition, although lignin itself may not be nutritionally contributable. Ingestion of MWL by the protozoa in the hindgut of workers of *C. formosanus* is shown in Fig. 2. As shown in Fig. 2-b,

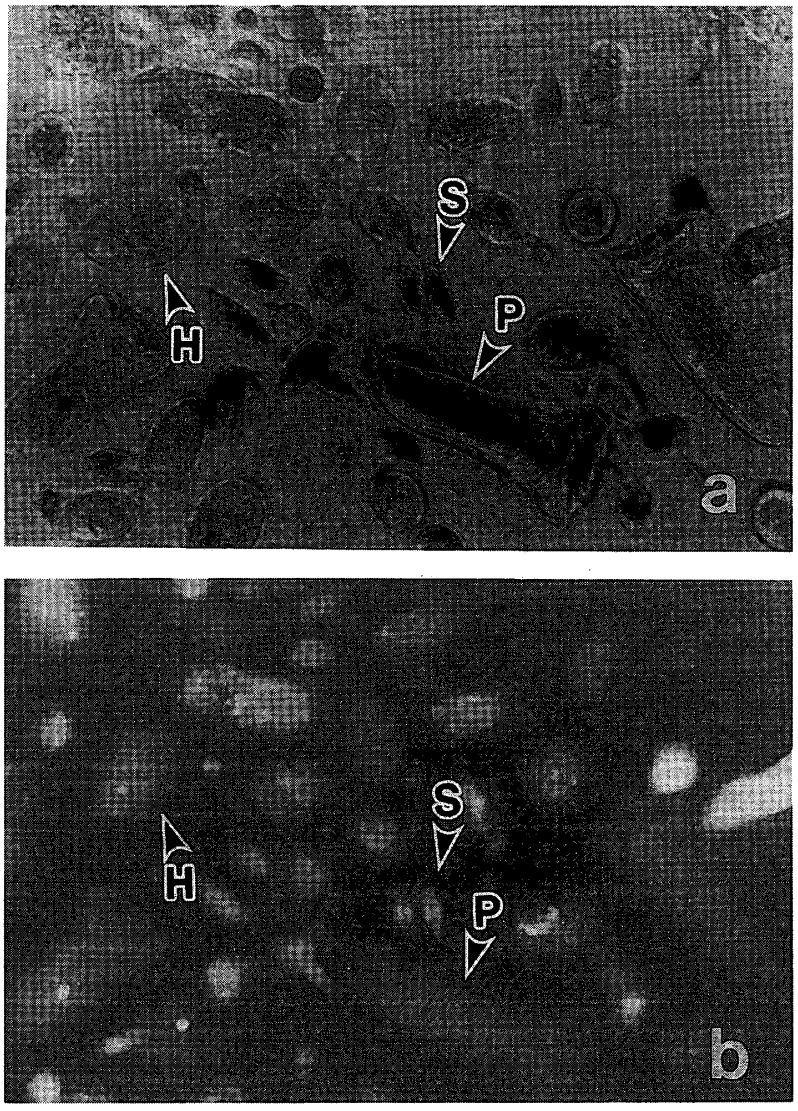

Fig.2. A phase contrast (a) and a epifluorescent (b) micrographs of the protozoa in the hindgut of worker of *C. formosanus* fed on MWL. MWL particles are seen in *H. hartmanni* as brightened substarate in Fig. 2-b.
P: *P. grassii*, H: *H. hartmanni*, S: *S. leidyi*.

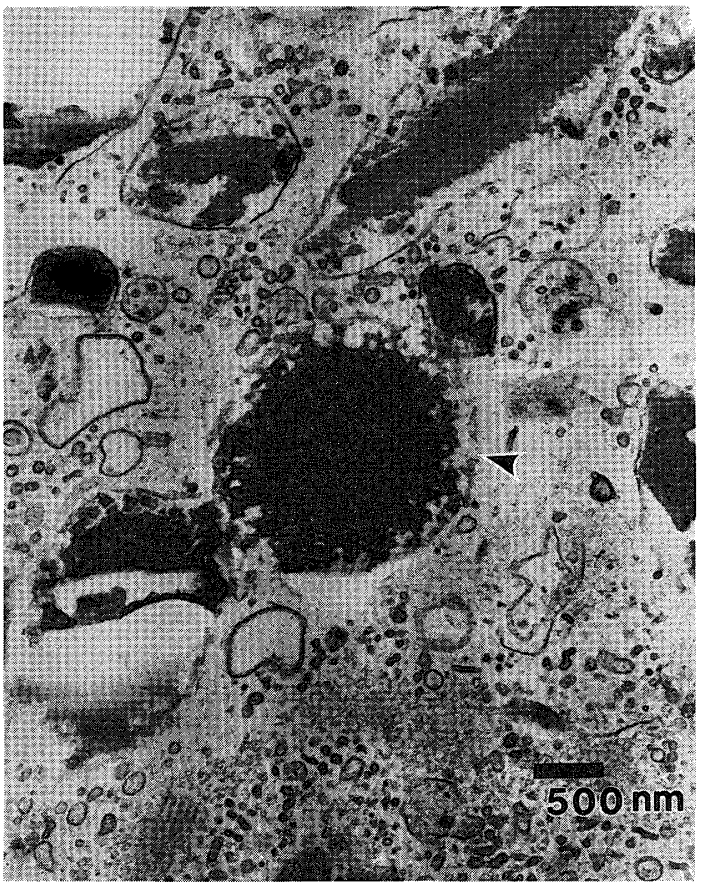

Fig. 3. A transmission electron micrograph of *H. hartmanni* ingesting MWL. The surface of a MWL particle (arrow head) is disintegrated.

middle-sized protozoa, *Holomastigotoides hartmanni* Koidzumi, actively ingested MWL. On the other hand, MWL particle were hardly observed in the bodies of large-sized protozoa, *Pseudotrichonympha grassii* Koidzumi, and small-sized protozoa, *Spirotrichonympha leidyi* Koidzumi. Figure 3 is a transmission electron micrograph of ingested MWL particle into the body of *H. hartmanni*. The surface of the MWL particle might be degraded enzymatically. However more detailed histochemical approach is required to evidence it, since MWL sample used here still contained a small amount of carbohydrate.

No major change of ingested lignin molecular, e.g. side chain or aromatic ring clevages, was observed by IR and UV analyses (Data not shown). However, TFL was found to be composed of lower molecular weight fractions than MWL by GPC analyses for TFL (Data not shown). This suggests an modification of Lignin-Carbohydrate Complex (LCC) structure in the termite gut. Reduction of molecular weight of MWL may be caused by the release of carbohydrate fractions from LCC.

The present results suggest that lignin plays a minor but unknown role in the physiological aspect of the lower termite, *C. formosanus*. However, lignin skelton is not

destroyed but at least some modification may occur in termite body. Middle-sized protozoa, *H. hartmanni* is possibly responsible for this modification.

References

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