

Allozyme Patterns of Protozoan Symbionts of *Coptotermes formosanus* (Isoptera: Rhinotermitidae)

by

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ABSTRACT

Polyacrylamide gel electrophoresis with *Coptotermes formosanus* workers revealed five esterase loci. One of these bands (Est-1) was present in guts (alimentary canal) removed from *C. formosanus*, and was not present in the remaining body tissues. To determine the source of this esterase band, termites were selectively defaunated by starvation for one week and six weeks, and by diets of low molecular weight cellulose (LC) with polymerization of 17 or 27. The Est-1 band was not present in starved termites, nor in termites fed LC 17, which has been reported to selectively remove the protozoan *Pseudotriconympha grassii*. Thus, Est-1 appears to be the product of *P. grassii*; while the other two protozoan symbionts *Holomastigotoides hartmanni* and *Spirotrichonympha leidyi* were not found to produce detectable esterase products. From these results, we suggest that whole body samples (with guts) can be used in electrophoretic studies of termite population structure, so long as bands attributable to symbionts are identified.

INTRODUCTION

Coptotermes formosanus Shiraki (Isoptera: Rhinotermitidae) contains three symbiont protozoa in its intestine which aid in cellulose digestion: *Holomastigotoides hartmanni* Kodizumi, *Spirotrichonympha leidyi* Kodizumi, and *Pseudotriconympha grassii* Kodizumi (Kodizumi 1921). Lai (1977) and Yoshimura (1995) described the distribution of these protozoan species in the termite hindgut. Yoshimura (1995) found that *P. grassii* was the most abundant of the three in the anterior part of the *C. formosanus* hindgut, followed by *H. hartmanni* and *S. leidyi* respectively. According to Lai (1977) and Yoshimura (1995), the order of abundance of the protozoan fauna is reversed in the middle and posterior portions of the hindgut, where *S. leidyi* and *P. grassii* are the most and least abundant, respectively. *S. leidyi* is the smallest in size of the three species, while *P. grassii* is the largest.

Gel patterns produced by protein electrophoresis may contain

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products contributed by protozoans if these protozoans are not specifically excluded by removing the alimentary canal. Thus, both Clement (1981) and Wang et al. (1992) recommended excluding the gut and its contents in studies with *Reticulitermes* spp. and *Hodotermopsis* spp., respectively. Broughton and Grace (1994) and Broughton (1995) suggested that mtDNA analysis should be carried out using termite body tissue devoid of the gut to avoid contamination by protozoan fauna. However, the termite gut is sometimes 50% of the body weight (Yoshimura 1995), and the gut itself may produce some specific enzymes. Thus, to completely catalog termite allozyme patterns, it would be best to use samples containing termite guts to maximize information on allozyme patterns, if termite and protozoan patterns can be accurately differentiated.

The objective of this study was to determine allozyme patterns produced by electrophoretic analysis of *C. formosanus* bodies with and without guts, and with their protozoan fauna. Gel patterns contributed by specific protozoan species were identified.

MATERIALS AND METHODS

Production of esterase patterns

The guts were removed from 10 *C. formosanus* workers, and each individual was placed separately into a microtube with 60 μ l of HCL-Tris buffer, and homogenized. Sample homogenates were centrifuged for ten min at 4°C at 10,000 x g. Each gut removed from a termite worker was placed individually into a microtube, and the above procedure was repeated. As a control, 10 termite workers were collected and placed separately in microtubes without dissection and the above procedure was repeated.

Supernatants were loaded into wells of gels made from 7% concentrated polyacrylamide. Gels with samples were run vertically in a chamber of Tris-Glycine buffer at 4°C and 150 constant volts for about two hrs. When the blue front running marker was close to the gel edge, the electrical supply was turned off. Gels were removed from the glass plate and rinsed with distilled water several times. Finally, the gels were incubated in a mixture of alpha and beta naphthyl substrates dissolved in acetone and 0.1M phosphate buffer (pH 6.5) at 30°C for 20-30min. After incubation, the gels were washed with distilled water several times, then stained with Fast Blue BB Salt (Sigma product) for 10 to 20 minutes, until bands were visible.

Identification of *P. grassii* produced bands

To eliminate *P. grassii* from the gut via starvation (Yoshimura 1995), 200 *C. formosanus* workers were placed into a petri dish (Pyrex™ diameter 9cm) without food. Silica-sand (50g) with 10ml (0.2ml H₂O/1g sand) of distilled water were added to keep moisture at a proper level for termites. The workers were held at 28°C for one week. Afterwards, 50 termites were collected and killed by placing them in a refrigerator (-15°C). The remaining individuals were held at 28°C for another five weeks to eliminate the protozoan fauna (Yoshimura 1995). After six weeks, incubation was terminated and termites starved for the two time periods were used for the electrophoretic study. The electrophoresis procedures used in this experiment were the same as those described above.

Confirmation of *P. grassii* produced bands

Selective defaunation of protozoans was used to determine which bands in the gels were produced by specific protozoans. This was accomplished by the methods developed by Yoshimura (1995) and Yoshimura et al. (1992, 1994), using molecular weight cellulose prepared by Dr. J. Azuma, University of Kyoto, Japan. Two types of low molecular weight cellulose (LC) were used to achieve defaunation. The selective defaunation of *P. grassii* can be accomplished by force feeding termites with LC 17 for one week, while *H. hartmanni* and *S. leidy* will remain, without change in their numbers (Yoshimura 1995). LC 17, with an average degree of polymerization of 17, was prepared from Whatman CF-11 cellulose by acetylsis and saponification. LC 27, with an average degree of polymerization of 27, was prepared from Whatman CF-11 cellulose by partial hydrolysis with phosphoric acid.

Six hundred *C. formosanus* workers were collected from wooden traps installed on the University of Hawaii at Manoa campus (Tamashiro et al. 1973) and were randomly divided into four groups of 150 individuals each. The four termite groups were placed into individual petri dishes (Pyrex™ diameter 9cm), each containing 50 grams silica-sand and 10ml distilled water for moisture. One gram of low molecular weight cellulose LC 17 was placed in a container made from a piece of aluminum foil and placed into one petri dish. Likewise, one gram of LC 27 was placed into a second petri dish. As a control, a single Whatman filter paper (diameter 9cm) was placed into the third petri dish as normal food for workers. Nothing was added to the fourth petri dish which served as a control. The petri dishes were placed in a 28°C temperature cabinet. After one week, four workers were removed from

the control treatment, and the guts were dissected from two individuals. Two workers each from the LC 17, LC 27, and starvation treatment were placed into individual centrifuge microtubes, ground with 60 l Tris-HCL buffer (pH 8.0) and centrifuged at 4°C at 10,000 x g for ten min. Gut samples were prepared by placing two removed guts from termites from each treatment into a microtube, and the above described procedures were repeated.

Each running sample (supernates obtained via centrifuging homogenates) was loaded into a well of 7% polyacrylamide gel and electrophoresis was run at 150 volts (DC) and 4°C for two hrs. After electrophoresis, gels were incubated with 0.1M phosphate buffer (pH 6.5) and alpha and beta naphthyl acetate substrates at 30°C for 20-30min. Incubated gels were rinsed with distilled water several times, then stained with Fast Blue BB salt. Banding patterns were recorded from each gel.

RESULTS AND DISCUSSION

Esterase patterns

Esterase gel patterns from whole body termites (control) produced five band zones (Fig. 1). Each band zone was designated as a specific esterase locus from the start of electrophoresis to the end, respectively. Thus, there were five esterase loci present in each control termite sample: Est-1, Est-2, Est-3, Est-4 and Est-5. Comparison of gel

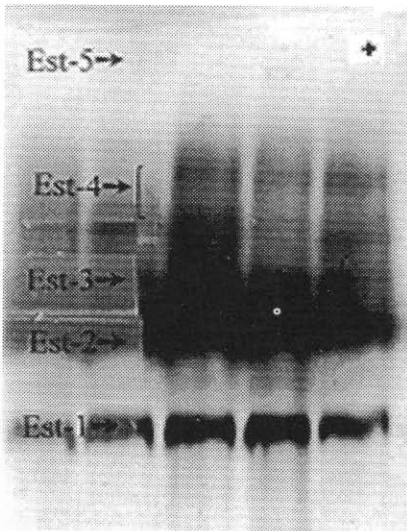


Fig. 1. Esterase gel pattern of intact *C. formosanus* shows five loci.

patterns of termite samples with (control) and without guts as well as samples consisting only of guts (Fig. 2) showed that only the controls and isolated guts have five esterase loci.

Identification of bands produced by *P. grassii*

Samples from the one week and six week starvation treatments failed to produce bands at the Est-1 locus position. There were no distinguishable differences between the banding patterns of these two samples. The gel patterns were the same as those generated by using samples of termites without guts, but differed from those generated using the whole body controls (Fig. 3).

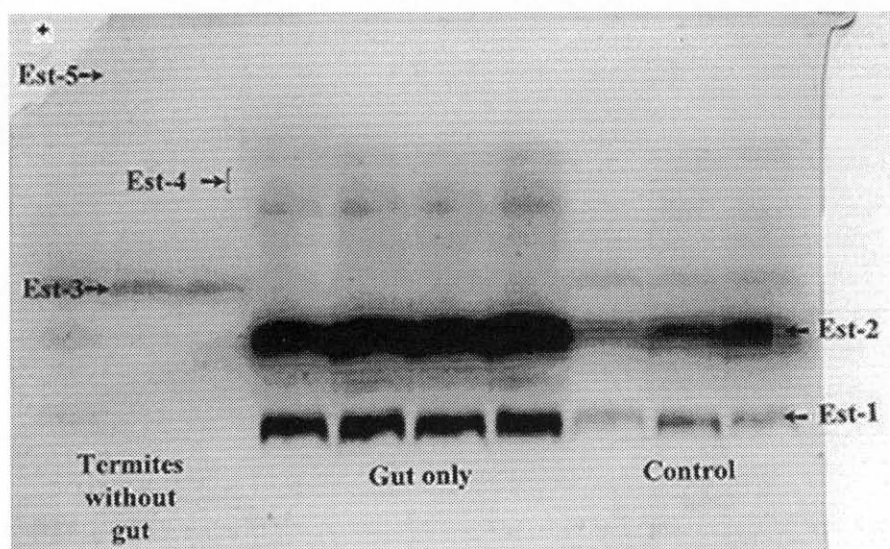


Fig. 2. Comparison of *C. formosanus* with gut removed, termite gut only, and intact termites (control).

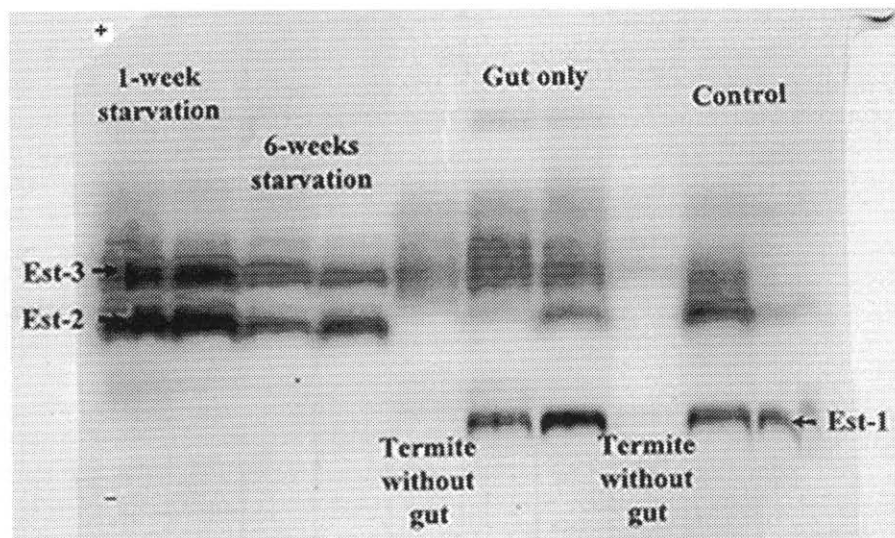


Fig. 3. Comparison of *C. formosanus* esterase gel patterns. Termites starved 1 week or six weeks have the same pattern and are missing Est-1. Termites from which the gut has been removed have the same pattern as starved individuals, except for weak Est-2 activity which suggests that this band may be contributed by gut tissues. The termite gut and intact termites (control) share gel patterns, including Est-1 and Est-2.

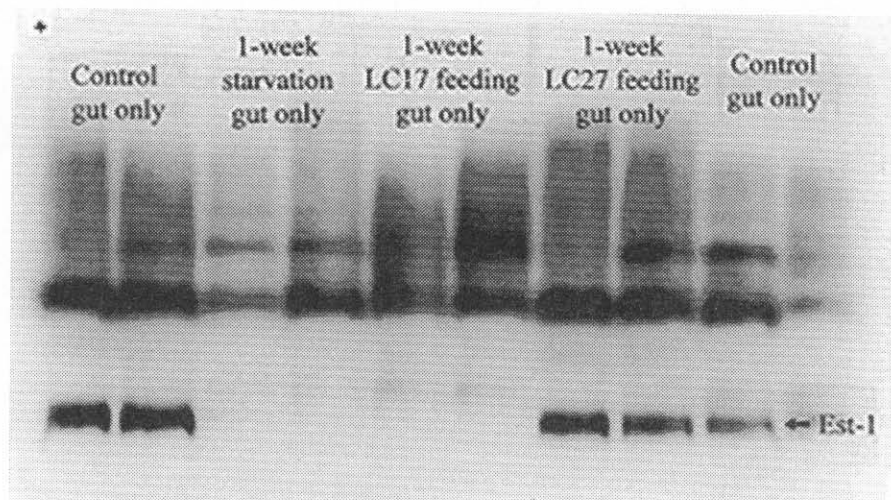


Fig. 4. Esterase gel patterns of *C. formosanus* excised guts removed from termites that received one of four treatments; normal diet (control gut), 1-week starvation, 1-week feeding on low molecular weight cellulose LC 17, 1-week feeding on LC 27. Termites on normal diet and those fed LC 27 for 1 week exhibited bands at the Est-1 locus position, while no Est-1 bands are present in those starved for 1 week or fed LC 17.

Confirmation of bands produced by *P. grassii*

The esterase gel patterns of termites exposed to each cellulose treatment are shown in Fig. 4. Only the termites that received the filter paper and the LC 27 treatment had Est-1 bands. Control termites from which the guts were removed, those that were starved, and those from the LC 17 treatment did not produce Est-1 bands. Esterase gel patterns of the isolated guts from the filter paper fed treatment and the LC27 treatment also had bands present at the Est-1 locus position. However, there were no bands at this position in the gut samples from the starvation and LC 17 treatments. Other than this difference at the Est-1 locus, no other obvious differences were apparent among the treatments.

There was no apparent difference between gel patterns of termites after one week starvation or six weeks starvation. These results strongly suggest that the Est-1 bands are contributed by *P. grassii*, since this protozoan is eliminated by starvation for one week and by a diet of LC 17 cellulose (Yoshimura 1995). *Holomastigotoides hartmanni* and *S. leidyi* are expected to survive both one week of starvation and the LC 17 diet (Yoshimura 1995), and it appears from our gel patterns that these species do not code any detectable esterase products under these conditions of electrophoresis.

These results suggest that it is not necessary to remove the guts of *C. formosanus* to study termite population genetic structure with allozyme electrophoresis. However, bands at the Est-1 position may not represent products coded by termites. The methods described here may also be useful with other enzyme systems to determine which enzymes are coded by other termite species and their intestinal fauna.

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