Preliminary

Effects of Physiological Stresses on Respiration Rates and Methane Emission Rates by the Termite, *Coptotermes formosanus* Shiraki

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Introduction

Methane is present at concentrations of 1.6–1.7 ppm in the atmosphere, and is comprised in the important trace gases accounting for the "greenhouse" effect. Methane is emitted into the atmosphere primarily from the fermentative degradation of organic materials.

It has been estimated that termites are responsible for 2–30% of the global methane emission[1-5], which various uncertainties would not allow accurate estimation. The varied methane emission rates obtained in the laboratory studies may result in the unreliable data on the responsibility of termites. However, effects of laboratory artificial conditions on the physiological activities of termites such as methane emission rates and nitrogen fixation rates have been reported by only a few researchers[6,7]. To assess the effects of artificial stresses on the physiological activities of termites in more detail must contribute to the re-estimation of the role of termites in geo-environment.

In this paper, we describe the effects of physiological stresses, i.e. food differences and fungi infestation, on respiration rates and methane emission rates by the termite, *Coptotermes formosanus* Shiraki, one of the major pest species in Japan.

Materials and Methods

Matured workers of *Coptotermes formosanus* Siraki taken from a laboratory colony maintained in the dark at 28± 2°C and >85% RH were used in the present investigation. As food materials sapwood meals of akamatsu (*Pinus densiflora* Sieb. et Zucc.) (WM), cellulose powder (Fibrous cellulose powder: Whatman BioSystems Co. Ltd.) (CP) and cellulose powder with 0.5% uric acid (Nakarai Tesque Co. Ltd.) (CP-U) were served. One minute's contact of workers of *C. formosanus* with a commercial sheet formulation of a entomopathogenic fungus, *Beauveria brongniartii* (Saccardo) Petch (Bio-Lisa: Nitto Denko Co. Ltd.), was condected for the fungus infestation. The sheet has been reported for its strong pathogenic activity against *C. formosanus*[8].

Fifty sound workers of *C. formosanus* were put in an acrylic cylindrical container (55 mm in diameter and 60 mm in height) with a plaster bottom on which a plastic cup (18 mm in diameter and 10 mm in height) containing WM or CP or CP-U was set. Starvation control (without any food) was also employed. Fifty fungus-infested workers were fed on WM. The assembled containers were kept in the dark at 28±2°C and >85% RH. Survival rates and weight changes of workers were observed at 1 day, 3 days, 5 days, 14 days, 21 days, 28 days, 35 days and 42 days after setting-up.

Respirations rates and methane emission rates were measured as follows: at the above mentioned periods randomly collected 30 workers were transferred into an clean glass vial (50 ml) with a silicon rubber rid, which has been already determined for its exact volume, and the vial was kept in the dark at 28±2°C and >85% RH for one hour. Then the vial was hand-shaken for 30 sec to get an even distribution of CO_2 and methane. The 0.10 ml head-space gases were obtained through the rid, and analyzed by GC (Shimadzu GC-14B) equipped with methanizer (Shimadzu MTN-1) and FID-detector. Analyzing conditions; column: Porapak Q 80/100 (Shinwa MFG: P/N ZP-6), column temp.: 32°C, injection temp.: 50°C, detector temp.: 32°C. Respiration rates and methane emission rates per hour and per gram of termite workers were calculated from the obtained concentrations of CO_2 and methane in the samples and the air.

Ten workers were also randomly collected from the test containers at the same time intervals, and the existences of three protozoan species, *Pseudotrichonympha grassii* Koidzumi, *Holomastigotoides hartmanni* Koidzumi and *Spirotrichonympha leidyi* Koidzumi, in the hindguts were qualitatively observed by an objective microscope.

Results and Discussion

Workers fed on WM, CP and CP-U showed the similar survival curves until 28 days, and the rates were finally decreased to 45–65% at the end of the test (42 days). On the other hand, almost all starved workers were died after 42 days. Few survivors were observed in workers infested with *B. brongniartii* after 7 days.

Average masses of a single worker fed on WM, CP and CP-U were drastically decreased within 7 days from 4.5–5.0 mg to 3.8–4.2 mg (approximately 20% loss). After that the masses were gradually decreased to reach 3.4–3.6 mg at 42 days. Starved workers also showed the similar curve until 35 days, but the mass was drastically increased between 35 days and 42 days, might be related to the cannibalism in termite societies.

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Respiration rates, expressed as CO_2 emission rates, were gradually decreased all through the test period from 15–18 μmol/h/g termite to 12 μmol/h/g termite, and did not show any food-depending characteristic. Toyoshima *et al.* reported that workers of *C. formosanus* respired 65 μmol/h/g termite immediately after the collection[9]. Although experimental conditions and analyzing methods are completely different between two investigations, it might be possible that the respiration activity drastically drops down in the fairly short period when workers are transferred from the nest to the artificial conditions in termites.

Methane emission rates also showed the drastic changes in the very early stage of the test. In cellulose-based foods, CP and CP-U, the rates remarkably rose up once from 180 nmol/h/g termite to 320 nmol/h/g termite and 120 nmol/h/g termite to 200 nmol/h/g termite, respectively, within 7 days, and drastically dropped down to 20 nmol/h/g termite and 50 nmol/h/g termite, respectively during next 7 days. After that the rates were relatively stable. Workers fed on WM showed relatively stable changes all through the test period (40–190 nmol/h/g termite). Taking account of the facts that methanogenic bacteria only inhabit in the bodies of the smallest protozoa, *Spirotrichonympha leidyi* Koidzumi, in *C. formosanus*[7] and that the protozoa is seemed to rely on the other protozoan species nutritionally[10], the drastic changes of the rates in workers fed on cellulose-based foods are likely to associate with the qualitative and quantitative changes of the protozoan fauna. However, no qualitative difference was observed among WM, CP and CP-U until 14 day.

From the result, it is assumed that the most important factor affecting physiological activities of workers of *C. formosanus* is the transfer from the nest itself. This is well supported by a phenomenon called "social facilitation", meaning that social interactions may enhance termite activity to overcome physiological stresses. As shown in our study, the effects occur immediately after the transfer, probably within 24 hours or less.

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