ABSTRACTS (PH D THESIS)

Termite ectoparasitic fungi in Japan: distribution, prevalence, and molecular detection

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Termites are widely spread eusocial insects with 3,106 known species, of which 183 are considered pests and 83 cause significant damage to wooden structures. In the natural environment, subterranean termites live in soil with relatively high humidity. These conditions can expose termites to parasites such as fungi. The relationships between termites and fungi are generally divided into two categories: symbiotic mutualism and pathogenic relationships. Termite-fungus interaction is a topic that has attracted researchers for more than fifty years and extensive research has been carried out to reveal the power of entomopathogenic fungi against termite attacks, and 20 species have been tested for their pathogenicity, but none of the researches has focused on ectoparasitic fungi. Ectoparasitic fungi are fungi that attach to and live on the body surface of their hosts. There are 22 species of ectoparasitic fungi obligate parasite to termite, of which *Laboulbeniopsis termitarius* Thaxt and *Antennopsis gallica* Buchli & Heim are the most commonly found on termite cuticle. Ectoparasitic fungi have been reported to have the ability to reduce a termite's lifespan. However, the effects of ectoparasitic fungi on termite activity remain unclear due to their inability to grow in laboratory conditions. On the other hand, ectoparasitic fungi as a whole can be found in a wide distribution region, from tropical to temperate areas, but none of the reports has mentioned Japan, while the detection of ectoparasitic fungi are mainly based on manual observation.

Therefore, the present study aims to find *L. termitarius* and *A. gallica* species associated with *Reticulitermes speratus* (Kolbe), which is a termite widely distributed in Japan. Furthermore, the PCR-based assay to detect termite-associated ectoparasitic fungi was created for effective detection of the ectoparasitic fungi together with trials for cultivation of the fungi in the laboratory. Furthermore, as termite colonies are often infected by multiple ectoparasitic fungi, including the two tested species, a multiplex PCR assay was further designed to allow simultaneous detection of *L. termitarius* and *A. gallica*.

Laboulbeniopsis termitarius Thaxt is the most commonly found ectoparasitic fungi on termite body with varieties of termite species as its hosts. It is reported to be found in wide areas, but none of the report has mentioned about Japan. *Laboulbeniopsis termitarius* morphologically can be characterized by the unique shape and size. They have small body size, barely longer than termite setae, and attach to body surface of termites. *Laboulbeniopsis termitarius* can be identified from three main body structures namely: foot cell, stalk, and sporogonium. The other ectoparasitic fungi is *Antennopsis* sp. In 1952 Buchli and Heim described *Antennopsis gallica* on *Reticulitermes lucifungus* Rossi in southern France, proposed the genus *Antennopsis,* and placed it in a new order, Gloeohaustoriales, of the class Hyphomycetes. Presently the genus *Antennopsis* contains three species: *Antennopsis gallica* Heim and Buchli, *A. grassei* Buchli and *A. gayi* Buchli. Each species of *Antennopsis* is specific to a specific termite species.

The ectoparasitic fungi *L. termitarius* and *A. gallica* were collected from the body surface of *Reticulitermes speratus* (Kolbe) (Isoptera: Rhinotermitidae), collected from Uji, Kyoto Prefecture, Japan. These are the first record of these fungi from Japan. Moreover, the distribution of *L. termitarius* and *A. gallica* in *Reticulitermes* spp. colonies in Japan was observed. Meanwhile, the infection rate and strength of *L. termitarius* and *A. gallica* were discussed with references to effects of environmental factors at the collection sites. In total of 63 colonies of *Reticulitermes* spp. were collected from seventeen locations (from Hokkaido Prefecture to Okinawa Prefecture) in Japan. Five hundred workers and twenty soldiers from each colony were examined individually to see the infection of *L. termitarius* and *A. gallica*. The survey showed that *L. termitarius* distributed in whole Japan and *A. gallica* had a little bit restricted distribution. The infection rate of workers of *Reticulitermes* spp. varied among all locations: 0.10 - 16.10% for *L. termitarius* and 0 - 66.40% for *A. gallica*. No infected soldiers was observed. The negative relationship between temperature and infection rate was speculated in both the fungi. Rearing the colonies in the laboratory might result in the spreading of the fungi in the colonies. The both fungi grew on any body parts of the termites. The trials for isolation and cultivation of *L. termitarius* and *A. gallica* with eight media did not succeed under the laboratory conditions.

Visual observation under a dissecting microscope is a common method for screening for such fungi, it generally requires a large number of termites and is thus very time consuming. Therefore, development of a fast and efficient protocol to detect fungi infection on the termite *R. speratus* was targeted. Species-specific primers were designed based on sequence data, and amplified using a number of universal fungus primer pairs that target partial sequences of the 18s rRNA gene of the two fungi. To detect these fungi in a robust yet economic manner, we then developed a multiplex nested PCR assay using species-specific primers. Results suggested that both fungi could be successfully detected, even in cases where *L. termitarius* was at low titer (e.g., a single thallus per termite). The new method described here is recommended for future surveys of these two fungi, as it is more sensitive, species-specific, and faster than visual observation, and is likely to facilitate a better understanding of these fungi and their dynamics in host populations.

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