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RICH MICROBIAL COMMUNITY ASSOCIATED WITH THE NEST MATERIAL OF *RETICULITERMES FLAVIPES* (ISOPTERA: RHINOTERMITIDAE)

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Subterranean termites live in a soil environment that can contain a rich community of microorganisms and the nest material can be a site of enhanced microbial activity (Holt & Lepage 2000). By using feces and saliva as building materials for nest construction and gallery systems, termites can alter the qualitative and quantitative composition of the resident soil microorganism community from adjacent soils (Jouquet et al. 2005). Keya et al. (1982) suggested that a significant part of the altered microorganism community was mostly composed of cellulose decomposers, but function and origin of the microbial community associated with termite nests remains unclear. In this report we evaluate whether subterranean termites have the ability to import various microorganisms from distant soils or from their own gut microbial community into a microorganism-free soil environment.

In Chouvenc et al. (2008), we investigated the survivorship of groups of 960 *Reticulitermes flavipes* (Kollar) from large two-dimensional arenas filled with sterile sand (washed with 70% ethanol, rinsed with sterile deionized water and oven dried at 60°C for 24 h). At the end of the 90-d experiment, 4 arenas from 2 different termite colonies were dismantled to obtain 2 types of nest material: (1) gallery material including sand mixed with termite fecal material from termites galleries, and (2) non-gallery material including undisturbed sand (not tunneled by the termite) at least 3 cm away from any termite gallery (Fig. 1). A total of 12 soil samples per arena (10 g per sample) including 6 samples for each type of nest material were collected. In order to estimate the microbial communities in the 2 types of nest material, 1 g sub-sample from each original sample was processed following the general procedure described in Elliott & Des Jardin (1998). Serial dilutions of the sub-samples were made (10^{-1} to 10^{-7}) with sterile water and the diluted sub-samples were plated on 5 different selective media to isolate various groups of microbes including (1) overall aerobic bacteria, (2) fluorescent pseudomonads, (3) actinobacteria, (4) overall fungi and, (5) entomopathogenic fungi. All selective media were previously described in Elliott & Des Jardin (1998) except the medium for entomopathogenic fungi (Veen & Ferron 1966). Soil samples collected from a control arena were established with only sand and no termites to confirm the absence of microbes in the sterile sand. Growth of microbes for each dilution and each selective medium was quantified to estimate the overall microbial structure of the 2 nest materials (Table 1). Individual microbe colonies were selected and transferred on 1/5 strength potato dextrose agar. Based on colony morphology of more than 2,000 isolates, we found 432 morphologically distinct isolates including 399 aerobic bacterial isolates, 11 actinobacterial isolates, and 22 fungal isolates. The molecular identification of these isolates and their potential function will be presented elsewhere. Samples from the control arena with no termites showed no microbial growth.

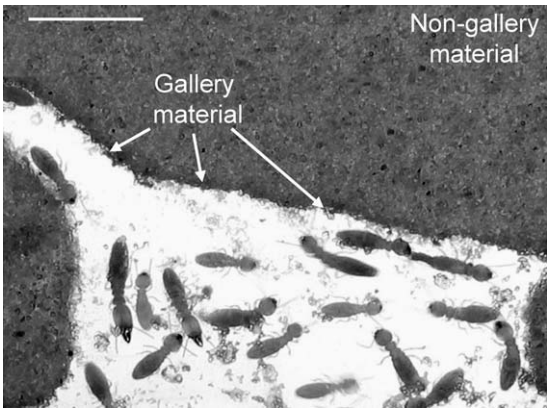

Fig. 1. A group of termites in a two-dimensional arena for 90 d. Arrows show sampling sites for microbioal isolation. Note the dark shade in the gallery wall showing the presence of deposited fecal material. Bar = 1 cm.

Our results showed that when groups of 960 termites are allowed to forage and build a complex gallery system in sterile sand, a large microbial community establishes in the sand wall galleries mixed with fecal material. Because the sampling method cannot take into account the non-culturable microbes, our results suggest that the actual microbial diversity within the termite's gallery system is probably greater than what we could observe. After 90 d in the presence of termites in the galleries, this microbial community moved away from the tunnels into the undis-

TABLE 1. COLONY FORMING UNITS (CFUS) OF VARIOUS MICROBE GROUPS GROWN ON DIFFERENT SELECTIVE MEDIA, PER GRAM OF SAMPLE OF TERMITE-GALLERY MATERIAL AND NON-GALLERY MATERIAL (MEAN ± SE).

Microbe Group	n[a]	Gallery material	Non-gallery material	t-test[b]
Overall aerobic bacteria	24	$3.5 \times 10^7 \pm 7.9 \times 10^6$	$3.9 \times 10^5 \pm 5.2 \times 10^4$	$P < 0.001$
Fluorescent Pseudomonads	24	$7.2 \times 10^2 \pm 3.0 \times 10^2$	8 ± 6	$P < 0.001$
Actinobacteria	24	$1.4 \times 10^5 \pm 2.8 \times 10^4$	$1.1 \times 10^2 \pm 5.2 \times 10^1$	$P < 0.001$
Overall fungi	24	$6.1 \times 10^2 \pm 1.1 \times 10^2$	$4.3 \times 10^1 \pm 1.6 \times 10^1$	$P < 0.001$
Entomopathogenic fungi	12	0 ± 0	0 ± 0	N/A

[a]Total number of samples plated on a given selective media per concentration from serial dilutions.
[b]For a given microbe group based on selective media, comparison of the colony forming units between gallery material and non-gallery material.

turbed sand, although the overall amount of bacteria remains 100-fold less than the fecal-sand mix. Previous studies have shown that actinobacteria can be isolated from the gut of various termites (König et al. 2006). Here we demonstrate that actinobacteria are also part of the microbe community that can colonize the termite nest structure. Fungi represented a very small fraction of the overall microbes enumerated, supporting the observation by Chouvenc et al. (2008, 2009) that termite gut contents, i.e., fecal material, used as building material for the gallery system possess highly fungistatic properties, and do not allow fungi to colonize the termite nest environment. The apparent absence of entomopathogenic fungi support that termites have mechanisms to prevent the spread of harmful fungi within the termite nest (Chouvenc et al. 2010).

Since all the arenas were constructed of sterile sand, it is assumed all the microorganisms originated from the termite guts or from the surface of the cuticle of the termites. This indicates that a complex microbial community is associated with the termites themselves before they were introduced into the arena. We assume that most of the cuticular microbes were acquired from the field nest where they were originally collected, although some could be strict termite-associated microbes and therefore ectosymbionts transmitted vertically. In addition, the gut can be a reservoir that temporarily hosts various microbes, which are later inoculated into various part of the gallery system. Because fungi were sampled in very small quantities, we hypothesize that the termite fecal material within the tunnel wall acts as an inhibitor against specific types of organisms. A broader sampling of termite colonies from diverse origin and molecular identification of the microbes will provide a better understanding of the origin and the function of some of these microbes.

SUMMARY

Field-collected subterranean termites were held in groups of 960 individuals for 90 d in two-dimensional arenas filled with sterile sand. After 90 d, the tunnel walls made of sand mixed with fecal material, and sand from areas not disturbed by termites were compared for the presence of microbes. We show that a rich microbial community associated with the termites can colonize the termite nest environment and is primarily associated with the tunnels.

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