NOTES

Association of Actinomycete-Like Bacteria with Soil-Feeding Termites (Termitidae, Termitinae)

DAVID E. BIGHELL, HAKON OSKARSSON, AND JONATHAN M. ANDERSON
Department of Biological Sciences, University of Exeter, Exeter, Devon, United Kingdom EX4 4PS

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Electron microscopy of the hindgut showed that actinomycete-like bacteria were the principal microbial associates of two African species of soil-feeding termites. Elongated cuticular spines provided attachment.

Termites are well known for their associations with symbionts which play a significant role in digestion. The literature has concentrated largely on the primitive families (lower termites) in which the gut harbors cellulose-degrading protozoans, whereas the Termitidae (higher termites, comprising four-fifths of the described species of Isoptera) have been little studied. Within the latter group, the Nasutitermitinae have functionally significant gut bacteria, while the fungus gardens of the Macrotermiteinae are also well-known phenomena (6). The trophic group of soil feeders, however, contains a major proportion of the species in this family. In the humid forests of West Africa and South America, these are among the most abundant insects and evidently play a key role in nutrient cycling, yet virtually nothing is known of their symbiotic associations. We report that actinomycete-like bacteria are prominent among the intestinal organisms of two species of Termitinae. Attachment sites for the filaments are provided by elongated cuticular spines in the posterior hindgut.

Gut microorganisms were examined by scanning electron microscopy and transmission electron microscopy in Procubitermes aburienisis Sjostedt and Cubitermes severus Silvestri (Termitidae, Termitinae). Mounds were flown to the United Kingdom within a few days of collection in an alluvial secondary forest at Rabba, Nigeria, and maintained in sterilized soil or mixtures of sterilized potting compost and millipede frass at 25°C. For scanning electron microscopy gut sections were opened by longitudinal incision and agitated vigorously for 20 s in bacteriological Ringer solution to remove loose mineral debris. Fixation was for 1 h in 2.5% gluteraldehyde without agitation, post-fixed for 1 h in 1% OsO₄, and embedded in Spurr resin.

A previous investigation by light microscopy of gut organisms in C. severus showed that the P3 and P4 regions (Fig. 1) contained large concentrations of nonfilamentous bacteria (2). This was confirmed by our investigation, but scanning electron microscopy showed that fine septate filaments were also abundant in the hindgut posterior to the enteric valve. The filaments formed a dense network extending through the P3 and P4, free of attachment to the gut wall except in the posterior dilation of the colon (designated P4b) where anchorage was provided by about 200 cuticular spines (Fig. 2). The spines were regularly distributed over the surface of the gut wall, about 70 μm apart at the base, and were 180 to 210 μm long and 2 μm in diameter at the base, tapering to the tip. Each spine supported and was almost entirely encased by a complex aggregation of filaments and mineral materials. The filaments, of various diameters

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**FIG. 1. General morphology of the gut. The cecum is vestigial in C. severus. Bar, 1 mm.**
Fig. 2. Scanning electron micrograph of P4b of P. aburiensis opened by longitudinal incision to show cuticular spines (arrowheads) and associated filaments. Bar, 100 μm.
ranging from 0.2 to 2.0 μm, could be seen at higher magnification attached to the surface of the spines (Fig. 3). Sporing structures were apparently absent, but transmission electron microscopy of the microbial complex showed that all cells present were typically procaryotic in ultrastructure, lacking nuclear membranes and mitochondria (Fig. 4). Inoculation of starch-casein or chitin agar plates with small numbers of the cuticular spines showed that a variety of actinomycetes could be readily isolated. Many of the filaments surrounding the spines may therefore be actinomycetes. Interestingly, some of the cells showed a large electron-dense core with a lateral groove (Fig. 4, arrows), which is not characteristic of described actinomycetes (S. T. Williams, personal communication). A number of nonfilamentous bacteria (not shown) and much extracellular secretion were also present.

The length of the spines is unusually great for

![Figure 3](http://aem.asm.org/)

**Fig. 3.** Scanning electron micrograph of part of a single cuticular spine of C. severus, showing the attachment of filaments (arrows). Bar, 2 μm.

![Figure 4](http://aem.asm.org/)

**Fig. 4.** Transmission electron micrograph of microbial complex adjacent to cuticular spine in P. aburiensis. Arrows are explained in the text. Bar, 1 μm.
insect hindguts, both absolutely and also in relation to the gut diameter, which in the P4b does not exceed 400 μm. In situ the spines sloped obliquely in the direction of the rectum (Fig. 1, P5), spines from opposite sides of the gut wall meeting and in some cases interdigiting with one another at the tip. The spines and associated microorganisms therefore formed a matrix across the lumen of the gut through which ingested soil was filtered during peristalsis. The association was observed in both species of termites and did not vary between freshly freighted insects and those maintained for up to 4 months in the laboratory.

Actinomycetes have been isolated occasionally from the guts of wood-feeding termites (3, 8), but no information has been available on their abundance or orientation within the host. The attachment of bacteria to cuticular spines has been reported in the hindgut of the cockroach Blaberus posticus (1), but filamentous forms appeared less numerous than in the present study with termites. The prominence of actinomyete-like bacteria in soil feeders and attachment to retaining structures within the gut suggests a possible role in digestion. Despite assumptions to the contrary (7), we have found no evidence that soil feeders select or degrade the cellular remains of higher plants. Gut content analysis, supported by particle size, organic carbon, and mineral analyses of P. aburienis and C. severus mound material, showed that mineral–humus complexes were the principal organic substances ingested (J. M. Anderson, D. E. Bignell, R. A. Johnson, and T. G. Wood, unpublished data). There is no conclusive evidence that actinomycetes can degrade humus (5), but the ability of many soil-dwelling strains to secrete phenoloxidases has led to the proposition that they assist in humus formation from phenolic complexes derived from plant tissues (4). Thus the presence of actinomycete-like bacteria in the guts of soil-feeding termites raises the possibility that their nourishment includes a significant component from soil organic matter fractions.

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LITERATURE CITED