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ABUNDANCE OF CULTIVABLE MICROORGANISMS IN VARIOUS SECTIONS OF THE GUT OF WORKER TERMITES *ANACANTHOTERMES AHNGERIANUS*

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ABSTRACT

The abundance and spatial distribution of cultivable physiological groups of microorganisms were investigated in the foregut, midgut, and hindgut of worker termites *Anacanthotermes ahngerianus* collected in the Beruniy District of the Republic of Karakalpakstan, Uzbekistan. The populations of cultivable bacteria, *Clostridium* species, micromycetes, and aerobic and anaerobic cellulose-degrading microorganisms were determined using appropriate culture media. Significant differences in the quantitative distribution of the studied groups were observed across the different gut segments. The abundance of cultivable bacteria was 4.5×10^3 CFU/termite in the foregut, 3.0×10^2 CFU/termite in the midgut, and 6.0×10^3 CFU/termite in the hindgut. *Clostridium* counts reached 1.5×10^2 CFU/termite in the foregut and 6.0×10^2 CFU/termite in the hindgut, while no growth was detected in the midgut. The abundance of micromycetes ranged from 1.5×10^1 to 4.5×10^1 CFU/termite, with the highest value recorded in the midgut. The highest abundance of aerobic cellulose-degrading microorganisms was found in the hindgut (1.1×10^5 CFU/termite), which was approximately 157 times greater than the count in the foregut. In the midgut, anaerobic cellulose-degrading microorganisms predominated, with an abundance of 2.5×10^3 CFU/termite-approximately 1.9 times higher than the number of aerobic microorganisms within this same functional group. The results obtained indicate spatial heterogeneity within the cultured gut microbial community of *A. ahngerianus*, as well as differences in the distribution of its physiological groups.

Keywords: *Anacanthotermes ahngerianus*, Termites, Gut microbiota, Microbial abundance, Cultivable bacteria, Clostridia, Micromycetes, Cellulose-degrading microorganisms, Aerobes, Anaerobes.

1 INTRODUCTION

Termites play a role in the decomposition of plant residues and the transformation of organic matter, and their gut microbiota possesses a diverse metabolic potential related to the utilization of carbohydrates and components of lignocellulosic biomass [1–3]. The termite gut is not a homogeneous environment; individual compartments can differ in terms of substrate availability, oxygen content, pH, and redox conditions. These variations can give rise to specific microbial niches [4–6].

Quantifying cultivable physiological groups makes it possible to determine the distribution of microorganisms capable of growing under specific laboratory conditions and to identify regions of the intestine where certain groups predominate.

Modern research indicates that the termite gut is a spatially organized microbial system, in which distinct compartments differ in their physicochemical conditions, the composition of their microbial communities, and their potential metabolic functions. A comprehensive study of the gut of *Labiotermes labralis* has demonstrated the value of an integrative approach for understanding the processes of polysaccharide conversion within the host–microbiota system [5]. Investigations into the bacterial communities of *Coptotermes formosanus* have likewise revealed differences in the taxonomic composition of microorganisms across the foregut, midgut, and hindgut [7].

Particular attention is focused on cultivable cellulose-degrading bacteria, which are of interest as potential producers of enzymes involved in the hydrolysis of plant polysaccharides. Tinrat et al. [8] isolated bacterial cultures from the termite gut that are capable of breaking down carboxymethyl cellulose, and they characterized the cellulase activity of the most promising strains.

Modern genomic studies are further expanding our understanding of the diversity of bacterial lineages in the termite gut and their potential role in the conversion of lignocellulosic biomass [9-10].

At the same time, metagenomic data on the taxonomic composition and functional potential of the microbiota do not replace the quantitative assessment of viable, cultivable microorganisms.

In this regard, determining the abundance of major physiological groups of microorganisms in different sections of the gut of *Anacanthotermes ahngerianus* is of scientific interest. Comparative culture-based analysis makes it possible to identify the quantitative distribution patterns of microbial communities across various sections of the intestinal tract and to assess their spatial heterogeneity. The data obtained expand our understanding of the structure of the cultivable microbiota in termites and provide a foundation for further investigation into the physiological and enzymatic properties of the isolated cultures.

The objective of this study is to determine the abundance of the primary cultivable groups of microorganisms in the anterior, mid, and posterior sections of the gut of *A. ahngerianus* workers, and to identify the specific features of their spatial distribution.

2 MATERIALS AND METHODS

The subjects of this study were worker individuals of *A. ahngerianus* collected in the Beruniy District of the Republic of Karakalpakstan, Uzbekistan. The gut was divided into the anterior, mid, and posterior sections [8]. The study was conducted in three biological replicates, with 10 individuals selected for each replicate. The corresponding gut sections were dissected aseptically, pooled by section type within each replicate, and homogenized in sterile phosphate-buffered saline. The resulting suspensions were subjected to serial dilution using a sterile 0.9% NaCl solution.

Serial dilutions were plated using the pour-plate method. Meat-peptone agar (MPA) was used to enumerate culturable bacteria; Czapek's medium was used for micromycetes; Hutchinson's medium was used for aerobic cellulose-decomposing microorganisms; and Omeliansky's medium was used for anaerobic cellulose-decomposing microorganisms. Incubation was carried out at 29°C. Colonies were counted on the 3rd day for bacteria, the 7th day for micromycetes, and the 25th day for cellulose-decomposing groups. Results were expressed as CFU per termite and presented as the mean values of three biological replicates.

3 RESULTS

The abundance of culturable microorganisms in *A. ahngerianus* worker termites varied depending on the gut segment. The highest bacterial count was observed in the hindgut, reaching 6×10^3 CFU/termite, while in the foregut, this figure was 4.5×10^3 CFU/termite. In the midgut, the bacterial count was significantly lower - 3×10^2 CFU/termite. *Clostridium* species were detected in the foregut and hindgut at counts of 1.5×10^2 and 6×10^3 CFU/termite, respectively, but were not found in the midgut. The abundance of micromycetes was relatively low: 1.5×10^1 CFU/termite in the foregut, 4.5×10^1 CFU/termite in the midgut, and 3×10^1 CFU/termite in the hindgut. Yeasts were detected only in the hindgut, with a count of 1.5×10^1 CFU/termite.

The distribution of cellulose-degrading microorganisms showed marked differences between the various sections of the gut. The abundance of aerobic cellulose-degrading microorganisms increased from the foregut (7×10^2 CFU/termite) to the midgut (1.3×10^3 CFU/termite) and reached a maximum in the hindgut (5.1×10^5 CFU/termite). Similarly, the maximum abundance of anaerobic cellulose-degrading microorganisms was observed in the midgut, reaching 2.5×10^3 CFU/termite. In the foregut and hindgut, their numbers were significantly lower - 6×10^1 and 1.3×10^2 CFU/termite, respectively (Fig. 1).

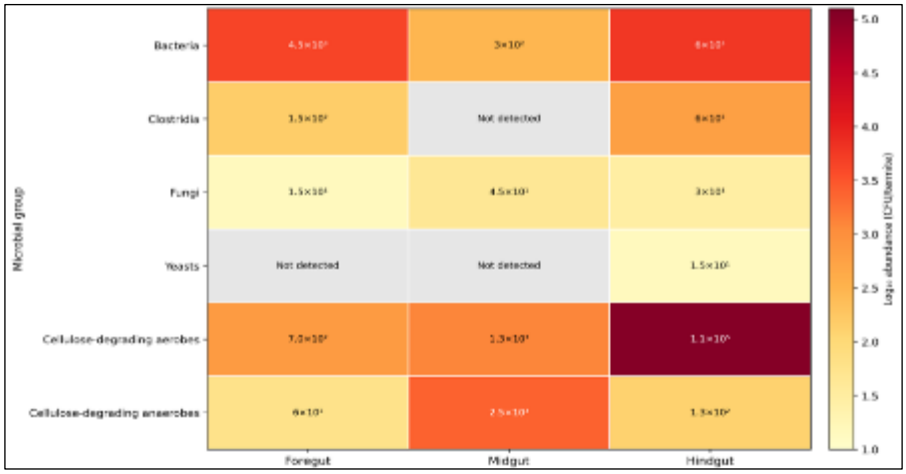


Figure 1: Abundance of cultivable microorganisms in various sections of the gut of worker termites *A. ahngerianus*

Figure 2 shows the growth of microbial colonies isolated from various sections of the gut of *A. ahngerianus* worker termites, cultured on meat-peptone agar (MPA).

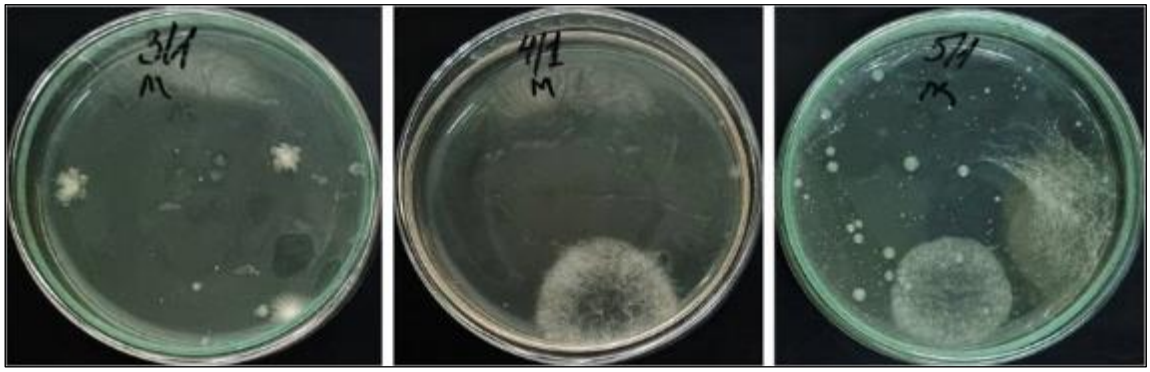


Figure 2: Growth of cultivable microorganisms on meat-peptone agar from various sections of the gut of worker termites *A. ahngerianus*

3/1-foregut; 4/1-midgut; 5/1-hindgut:

Thus, the results of the culture-based analysis indicate a heterogeneous distribution of the studied microbial groups along the intestinal tract of *A. ahngerianus*. The terminal section was characterized by the highest abundance of bacteria and aerobic cellulose-degrading microorganisms, whereas the midsection contained the greatest number of anaerobic cellulose-

degrading microorganisms. These differences suggest a potential spatial differentiation of microbial communities; however, further research into the physicochemical conditions of the intestinal environment is required to determine the underlying causes of these observed patterns.

4 DISCUSSION

The results obtained indicate a pronounced spatial heterogeneity in the cultivable microbial community within the gut of *A. ahngerianus* worker termites.

The abundance of cultivable bacteria was 4.5×10^3 CFU/termite in the foregut, decreased to 3.0×10^2 CFU/termite in the midgut, and reached 6.0×10^3 CFU/termite in the hindgut. Thus, the highest count was recorded in the hindgut, where the bacterial abundance was 20 times greater than in the midgut and approximately 1.3 times greater than in the foregut. The decline in the number of cultivable bacteria in the midgut may be attributed to differences in the availability of nutrient substrates, the physicochemical characteristics of the gut environment, and the ability of specific microorganisms to grow on the culture medium used. However, these factors were not directly measured in the present study and are therefore considered as potential explanations for the observed pattern.

The spatial heterogeneity of bacterial communities in the termite gut is supported by research on other species. Köhler et al. demonstrated that distinct compartments of the *Nasutitermes corniger* gut differ in terms of oxygen concentration, pH, redox potential, and bacterial density. The highest bacterial community density was found in the anaerobic, expanded section of the hindgut [11]. A study on *Labiotermes labralis* also revealed differences in bacterial diversity and the distribution of taxa across various sections of the digestive tract [5].

However, the results of the present study do not entirely align with the patterns described for these species; in *A. ahngerianus*, the lowest number of culturable bacteria was recorded in the midgut. This discrepancy may reflect specific characteristics of the species under study, the composition of the gut contents, and the cultivation conditions employed. Furthermore, the culture-based method accounts only for microorganisms capable of forming colonies on the chosen medium; therefore, the values obtained do not represent the total abundance of the bacterial community.

The number of cultivable Clostridia was 1.5×10^2 CFU/termite in the foregut and 6.0×10^2 CFU/termite in the hindgut. Consequently, the count in the hindgut was four times higher than in the foregut. No growth of organisms from this group was observed in the midgut using the medium employed. Clostridia are of interest as a group of anaerobic bacteria, many of which are capable of participating in the fermentation of organic compounds. In studies of the gut microbiota of termites, members of the class Clostridia have been identified among the primary bacterial groups, with their distribution varying according to the specific gut segment examined. In particular, in the gut of *L. labralis*, Clostridia were found to be abundant in the hindgut segments [5].

In the present study, the increased number of cultured *Clostridium* species in the distal intestinal segment may be attributed to the availability of organic substrates and the specific conditions favoring the growth of anaerobic microorganisms. At the same time, the lack of growth in the midgut should not be interpreted as evidence for the complete absence of *Clostridium* in this part of the intestine. It may instead be due to a low number of viable cells, the specific composition of the culture medium, or the conditions under which the cultures were maintained.

The abundance of culturable micromycetes was relatively low across all examined gut sections. The highest count was observed in the midgut, where it was three times higher than in the foregut and 1.5 times higher than in the hindgut. Unlike the total culturable bacterial population and clostridia, micromycetes did not reach their maximum abundance in the hindgut. This indicates that the distribution of fungal microorganisms does not necessarily coincide with that of bacterial groups. Potential factors underlying these observed differences include the availability of organic compounds derived from feed, as well as the capacity of individual fungal species to remain viable within the intestinal environment.

The most pronounced quantitative differences were observed among cellulose-degrading microorganisms. In the foregut, the count of aerobic microorganisms was 7.0×10^2 CFU/termite, while that of anaerobic microorganisms was 6.0×10^1 CFU/termite; thus, the aerobic group was approximately 11.7 times more abundant than the anaerobic group. In the midgut, the relationship was reversed: the anaerobic group was approximately 1.9 times more abundant than the aerobic group. In the hindgut, the number of aerobic cellulose-degrading microorganisms exceeded the levels found in the anterior and midgut sections by approximately 157-fold and 85-fold, respectively.

5 CONCLUSION

The study reveals that the abundance of cultivable physiological groups of microorganisms in different sections of the gut of *A. ahngerianus* worker termites is characterized by marked spatial heterogeneity.

Thus, the results demonstrate the spatial heterogeneity of the cultivable gut microbiota in *A. ahngerianus*. Differences in the abundance of specific physiological groups of microorganisms - particularly aerobic and anaerobic cellulose-degrading forms - underscore the necessity of studying the microbial communities of different gut sections separately. These findings provide a foundation for further research into the taxonomic composition, physiological properties, and enzymatic activity of the isolated cultures, including their potential role in the decomposition of plant polysaccharides.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflicts of interest regarding this manuscript.

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