SCREENING OF SYMBIOTIC *ENTEROBACTERIACEAE* SPECIES FROM THE GUT OF *Macrotermes subhyalinus*AND *Macrotermes bellicosus*TERMITES.

Abstract

Termites play a crucial role in the recycling of organic matter through the activity of symbiotic microorganisms inhabiting their digestive tract. This study focused on the screening of symbiotic Enterobacteriaceae species from the gut of the termites *Macrotermes subhyalinus* and *Macrotermes bellicosus* collected in the shrub savanna of Lamto (Toumodi, Côte d'Ivoire). Different termite castes (workers, minor soldiers, and major soldiers) were subjected to conventional microbiological analyses. Microorganisms were enumerated on selective media, and Enterobacteriaceae isolates were characterized using phenotypic and biochemical methods based on the API 20E identification system. The results showed that aerobic mesophilic bacteria were present in all castes of both termite species, with higher microbial loads observed in workers. Enterobacteriaceae were detected in all castes of *Macrotermes subhyalinus*, whereas they were found only in workers of *Macrotermes bellicosus*. A total of 118 Enterobacteriaceae isolates were obtained, including 97 from *M. subhyalinus* and 21 from *M. bellicosus*. Biochemical characterization led to the identification of seven species belonging to four genera: *Enterobacter*, *Serratia*, *Raoultella*, and *Salmonella*. *Serratia liquefaciens* was the dominant species associated with *M. subhyalinus*. These findings highlight the richness and diversity of Enterobacteriaceae associated with the gut microbiota of the studied termites and emphasize their potential role in plant biomass degradation processes. Furthermore, they provide promising prospects for the biotechnological exploitation of termite-associated symbiotic microorganisms.

Keywords:*Macrotermes subhyalinus*; *Macrotermes bellicosus*; Enterobacteriaceae; gut microbiota; symbiosis; API 20E system; Côte d'Ivoire.

1.Introduction

Termites are eusocial insects that play a fundamental role in the functioning of tropical and subtropical ecosystems through their active involvement in organic matter decomposition and in the biogeochemical cycling of carbon and nitrogen [1 ; 2]. Their remarkable ability to utilize lignocellulosic substrates results from a complex symbiotic association with a dense and diverse microbial community inhabiting their digestive tract [3 ; 2].

The termite gut is considered a natural bioreactor in which symbiotic microorganisms perform the hydrolysis of cellulose and hemicelluloses, the fermentation of released sugars, and nitrogen recycling. These metabolic activities provide the host with a substantial portion of its nutritional requirements and contribute to its adaptation to diets rich in recalcitrant plant materials [2]. This symbiosis represents one of the most remarkable examples of evolutionary cooperation between insects and microorganisms [4].

Higher termites belonging to the family Termitidae, particularly those of the subfamily Macrotermitinae, harbor an essentially prokaryotic gut microbiota, unlike lower termites whose digestive tract also contains flagellated protozoa [5]. In these fungus-growing termites, the tripartite association among the insect host, the symbiotic fungus *Termitomyces*, and gut microorganisms contributes to the exceptional efficiency of plant biomass degradation [6].

The gut microorganisms therefore constitute a metabolically active community whose composition varies according to host species, diet, caste, and environmental conditions [7 ; 8]. Among the various bacterial groups identified within the termite gut microbiota, members of the phylum Proteobacteria, particularly those belonging to the family Enterobacteriaceae, occupy an important position. Several genera, including *Enterobacter*, *Serratia*, *Klebsiella*, *Citrobacter*, and *Raoultella*, have been reported in the digestive tract of various termite species. These bacteria are believed to contribute to the degradation of complex polysaccharides, the fermentation of carbon substrates, and the production of metabolites involved in host nutrition and physiology [8]. Furthermore, some enterobacterial species possess enzymatic activities of biotechnological interest, attracting increasing attention for applications in lignocellulosic biomass valorization, biofuel production, and bioremediation [5].

Despite considerable advances achieved through metagenomic, metatranscriptomic, and phylogenetic approaches, knowledge regarding the diversity of cultivable bacteria associated with African termites remains limited [7 ; 8]. In Côte d'Ivoire, few studies have focused on the characterization of termite-associated symbiotic microorganisms, although *Macrotermes subhyalinus* and *Macrotermes bellicosus* are widespread in savanna ecosystems and play a major ecological role in organic matter recycling [1].

A better understanding of the enterobacterial communities associated with these insects could contribute to a deeper comprehension of microbial symbiosis mechanisms and facilitate the identification of strains with promising biotechnological potential. Therefore, the present study aimed to screen symbiotic Enterobacteriaceae species inhabiting the gut of *Macrotermes subhyalinus* and *Macrotermes bellicosus* collected from the Lamto savanna (Toumodi, Côte d'Ivoire). Specifically, the study sought to evaluate the microbial loads of different termite castes, phenotypically characterize the isolated strains, and identify the Enterobacteriaceae species present in the digestive tract of these two termite species.

2. Materials and Methods

2.1 Biological Material and Study Area

The biological material consisted of three termite castes (workers, major soldiers, and minor soldiers) from two termite species, *Macrotermes subhyalinus* and *Macrotermes bellicosus*, collected from the shrub savanna of the Lamto region (Toumodi, Côte d'Ivoire).

For sample selection, only active termite mounds showing the presence of soldiers and located in areas free from insecticide treatment within a 1-km radius were considered. The termites were transported to the laboratory in sterile containers containing moistened filter paper, maintained at 4°C, and processed within 4 h after collection to minimize post-mortem alterations of the gut microbiota.

2.2 Methods

2.2.1 Dissection and Preparation of the Inoculum

Each sample consisted of ten termites belonging to the same caste. The ten insects were surface-sterilized in 70% ethanol for 5 min and then rinsed three times with sterile distilled water. This external disinfection prevented contamination from cuticular microorganisms.

Dissections were performed aseptically under a sterile stereomicroscope using a sterile stainless-steel blade and forceps inside a vertical laminar-flow cabinet. The entire digestive tract was removed by sectioning between the thorax and abdomen and gently extracting the

gut from the rectum to the crop. After spreading the digestive tract longitudinally on a sterile slide, the gut content was carefully collected. Since the majority of the symbiotic microbiota is located within the gut content, only this material was used for the study. The homogenized gut contents of the ten termites constituted the exudate used for the investigation of symbiotic microorganisms.

2.2.2 Detection and Enumeration of Microorganisms

2.2.2.1 Preparation of the Stock Suspension and Serial Dilutions

To prepare the stock suspension, 1 g of exudate (obtained from the pooled gut contents of ten termites) was aseptically transferred into 9 mL of sterile Buffered Peptone Water (BPW) (AES Laboratoire, Combourg, France). The resulting mixture constituted the stock suspension. Successive decimal dilutions ranging from 10^{-2} to 10^{-11} were prepared from this suspension.

2.2.2.2 Enumeration of Aerobic Mesophilic Bacteria (AMB)

Enumeration of aerobic mesophilic bacteria was carried out according to the AFNOR NF V08-051 standard. Plate Count Agar (PCA) (Oxoid Ltd., England) was used as the culture medium.

One milliliter of each decimal dilution was inoculated into sterile Petri dishes using the pour-plate technique. Approximately 12–15 mL of PCA medium, previously sterilized and maintained at 45°C, was poured into the dishes containing the inoculum. The mixture was homogenized and allowed to solidify at room temperature. After solidification, a second overlay of 5 mL of plain agar was added to prevent the spreading growth of certain bacteria such as *Proteus* spp.

The inoculated plates were incubated at 30°C for 24 h. After incubation, colonies were counted on plates containing between 30 and 300 colonies.

2.2.2.3 Detection and Enumeration of Enterobacteriaceae

Enumeration of Enterobacteriaceae was performed according to AFNOR NF ISO 4832 (July 1991). Eosin Methylene Blue (EMB) agar was used as the selective medium. The presence of inhibitors such as eosin and methylene blue suppresses the growth of Gram-positive bacteria.

One milliliter of the stock suspension and its serial dilutions was inoculated into sterile Petri dishes. Subsequently, 12–15 mL of molten EMB agar maintained at 45°C was poured into each dish and mixed gently. After solidification, a second layer of 4 mL of the same medium was added. Plates were incubated at 30°C for 24 h.

Typical colonies appeared purple or grayish, raised or semi-raised, and measured between 1 and 3 mm in diameter. Colonies were counted on plates containing between 30 and 300 colonies.

2.2.3 Phenotypic Identification of Microorganisms

Following isolation, strains were characterized through the determination of their morphological and biochemical characteristics, allowing comparison with previously identified and well-described microorganisms. Phenotypic identification included the examination of morphological traits, catalase production, oxidase activity, and biochemical characteristics. These rapid analyses enabled classification of isolates at the genus and, in some cases, species level.

2.2.3.1 Morphological Characterization of Isolates

Morphological characteristics of bacterial isolates were determined microscopically using the Gram-staining technique, which provides information on cell morphology, size, and cellular arrangement [9]. Morphological characteristics of yeast isolates were also examined using a light microscope (Nikon Eclipse E800, France).

2.2.3.2 Oxidase Test

For the oxidase test, a colony obtained from a 24-h culture on agar medium was transferred onto an oxidase disc previously placed on a microscope slide. The appearance of a purple coloration indicated the presence of cytochrome oxidase (positive reaction), whereas the absence of color change indicated a negative result.

2.2.3.3 Catalase Test

The catalase test was performed by transferring a colony from an agar medium into hydrogen peroxide using a sterile inoculating loop. The formation of gas bubbles indicated catalase production and a positive reaction, whereas the absence of bubbles indicated a negative result.

2.2.4 Biochemical Identification

Carbon substrate assimilation tests were performed using API 20E identification strips for Enterobacteriaceae. The API system contains culture media designed to detect:

- Enzymatic activities: β-galactosidase (ONPG), lysine decarboxylase (LDC), ornithine decarboxylase (ODC), and urease;
- Fermentative activities using media containing specific carbohydrates and color indicators.

Inoculation and Reading of API Strips

Bacterial cultures grown for 18–24 h on agar media were aseptically collected and transferred into 10 mL of API suspension medium to obtain a turbidity equivalent to McFarland standard 2 (10^8 CFU/mL). Approximately 100 µL of this suspension was transferred into an ampoule of API Medium and homogenized.

The API 20E strips were inoculated by filling the microtubes using a sterile Pasteur pipette and incubated according to the manufacturer's instructions at 30°C. The strips were examined twice daily over a period of 48 h.

The resulting biochemical profiles were interpreted using the APILAB identification software (bioMérieux, France).

3. Results

3.1 Microbial Loads in the Digestive Tract of Termites

Tables 1 and 2 present the microbial loads recorded in the different castes of the two termite species investigated in this study.

Aerobic Mesophilic Bacteria (AMB) were detected in all castes of both termite species. The mean AMB loads in the different castes of *Macrotermes subhyalinus* (workers, minor soldiers, and major soldiers) were $3 \times 10^{10} \pm 3 \times 10^8$ CFU/g, 2.4×10^8 CFU/g, and 6×10^7 CFU/g, respectively. In *Macrotermes bellicosus*, the corresponding loads were 5.1×10^8 CFU/g in workers, 5.5×10^5 CFU/g in minor soldiers, and 2×10^4 CFU/g in major soldiers.

AMB loads were higher in workers of both termite species (3×10^{10} CFU/g) than in soldiers (2.4×10^{8} CFU/g). A significant difference ($P < 0.05$) was observed between AMB and Enterobacteriaceae loads in both workers and soldiers of *Macrotermes subhyalinus*. In contrast, in *Macrotermes bellicosus*, significant differences ($P < 0.05$) were observed among the AMB loads of the different castes. Overall, AMB loads were higher in *Macrotermes subhyalinus* than in *Macrotermes bellicosus*.

Enterobacteriaceae were detected in workers of *Macrotermes bellicosus* at a load of 3×10^{3} CFU/g. However, no Enterobacteriaceae were detected in the minor or major soldiers of this species. In contrast, Enterobacteriaceae were present in all three castes of *Macrotermes subhyalinus* (workers, minor soldiers, and major soldiers), with respective loads of 4.6×10^{4} CFU/g, 2.5×10^{4} CFU/g, and 4.2×10^{3} CFU/g.

No significant difference ($P > 0.05$) was observed between Enterobacteriaceae loads in workers and minor soldiers of *Macrotermes subhyalinus*. However, a significant difference was recorded between the major soldier caste and both the worker and minor soldier castes. A total of 118 Enterobacteriaceae isolates were recovered from the digestive tract of the termite species investigated.

Table 1 : Microbial Loads in the Digestive Tract of the Different Castes of *Macrotermes subhyalinus* (CFU/g)

Microrganisms	**Workers**	**Minor soldiers**	**Mayor soldiers**
AMB	$3.10^{10}\pm3.10^{8}$a	$2{,}4.10^{8}\pm3{,}1.10^{6}$b	$6.10^{7}\pm1{,}2.10^{5}$b
Enterobacteriaceae	$4{,}5.10^{4}\pm3{,}6.10^{3}$a	$2{,}5.10^{4}\pm2{,}7.10^{2}$a	$4{,}2.10^{3}\pm3{,}1.10^{2}$b

Within the same row, values followed by the same letter are not significantly different ($P > 0.05$).

Table 2 : Microbial Loads in the Digestive Tract of the Different Castes of *Macrotermes bellicosus*(CFU/g)

Micoorganisms	**Workers**	**Minor soldiers**	**Mayor soldiers**
AMB	$5{,}1.10^{8}\pm2{,}6.10^{6}$a	$5{,}5.10^{5}\pm2{,}5.10^{4}$b	$2.10^{4}\pm0{,}2.10^{3}$c
Enterobacteriaceae	$3.10^{3}\pm1{,}2.10^{2}$	< 1	< 1

Within the same row, values followed by the same letter are not significantly different (P > 0.05).

3.2 Phenotypic characterization of the different strains isolated from the gut of the two termite species

3.2.1 Distribution of the morphological types of microorganisms

Figures 1 and 2 show the distribution of the different morphological types of microorganisms isolated from the gut of *Macrotermes bellicosus* and *Macrotermes subhyalinus*. The microorganisms were classified into four groups according to their cellular morphology and Gram-staining reaction: Gram-positive rods (GPR), Gram-negative rods (GNR), Gram-positive cocci (GPC), and Gram-negative cocci (GNC).

Overall, Gram-positive rods (GPR) were the predominant morphological group in the gut microbiota of both *Macrotermes bellicosus* and *Macrotermes subhyalinus*, accounting for 70.0% and 51.0% of the isolates, respectively. In both termite species, Gram-negative cocci (GNC) were the least represented group, with proportions of only 0.31% in *M. bellicosus* and 1.12% in *M. subhyalinus*. However, *M. subhyalinus* exhibited higher proportions of Gram-positive cocci (19.2%), Gram-negative rods (15.62%), and Gram-negative coccobacilli (13.03%) than *M. bellicosus*, in which these groups accounted for 9.8%, 9.6%, and 10.27% of the isolates, respectively. These findings indicate that, although the gut microbiota of both termite species is dominated by Gram-positive bacteria, *M. subhyalinus* harbors a more balanced distribution of morphological groups, suggesting greater microbial diversity and potentially higher functional complexity within its gut ecosystem.

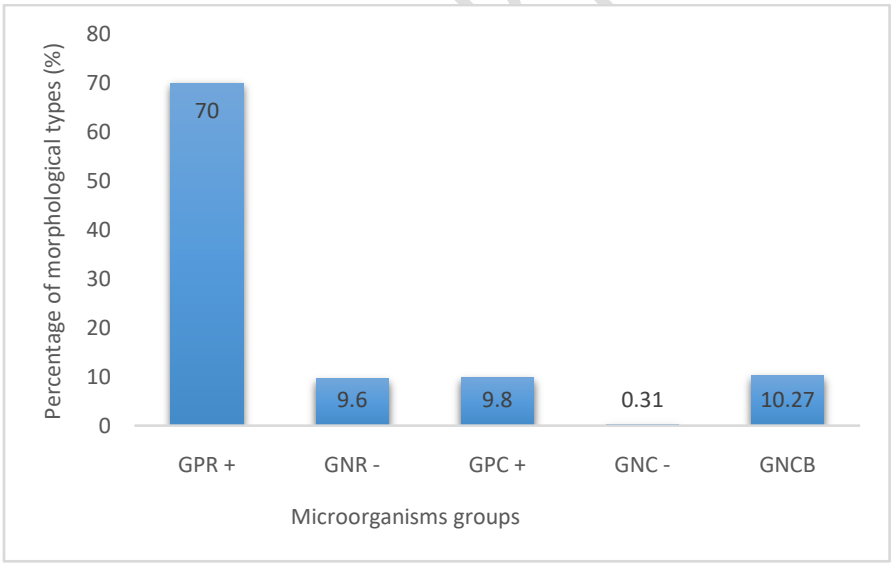

Figure 1 : Distribution of the morphological types of microorganisms in the gut of *Macrotermes bellicosus*

GPR: Gram-positive rods; **GNR:** Gram-negative rods; **GPC:** Gram-positive cocci; **GNC:** Gram-negative cocci ; **GNCB:** Gram-negative coccobacilli.

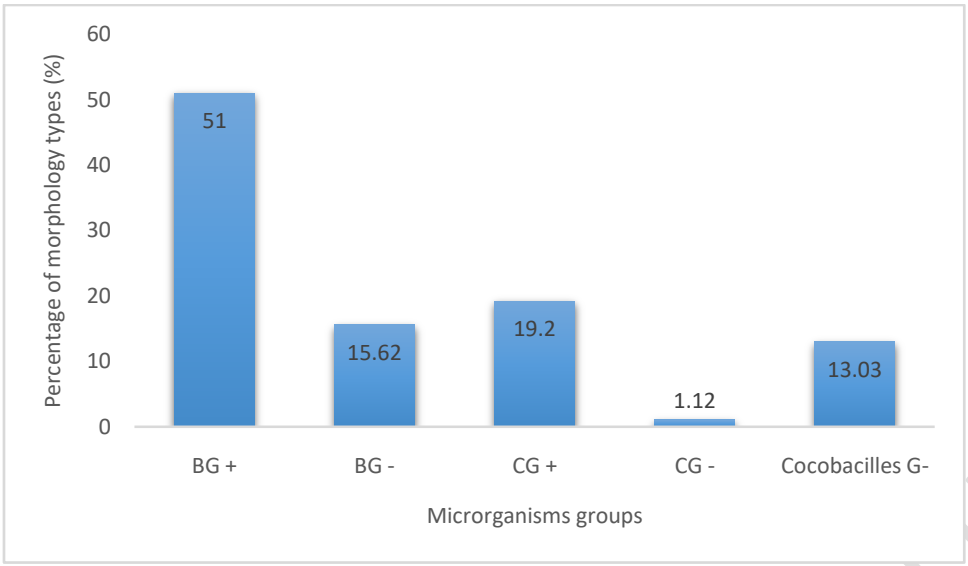

Figure 2 : Distribution of the morphological types of microorganisms in the gut of *Macrotermes subhyalinus*
GPR: Gram-positive rods; **GNR:** Gram-negative rods; **GPC:** Gram-positive cocci; **GNC:** Gram-negative cocci

3.2.2 Diversity of the Identified Enterobacteriaceae

The characterization of the termite gut microbiota led to the isolation of 118 *Enterobacteriaceae* strains, including 97 from *Macrotermes subhyalinus* and 21 from *Macrotermes bellicosus*.

Analysis of carbon substrate assimilation profiles obtained using the API 20E identification system classified the 118 *Enterobacteriaceae* isolates into seven groups (I–VII). The classification was based on the ability of the isolates to utilize specific carbohydrates and produce particular enzymes. Table 3 summarizes the biochemical characteristics of the seven groups of *Enterobacteriaceae* isolated from the gut of *Macrotermes subhyalinus* and the worker caste of *Macrotermes bellicosus*. The identification procedure revealed seven species belonging mainly to the genera *Enterobacter*, *Serratia*, *Salmonella*, and *Raoultella*.

All isolates tested positive for catalase, ONPG, citrate utilization, ornithine decarboxylase (ODC), and the fermentation of glucose, mannitol, sorbitol, sucrose, and amygdalin, whereas all were negative for oxidase and tryptophan deaminase (TDA) activity. These biochemical characteristics confirm their affiliation with the family *Enterobacteriaceae*, which comprises Gram-negative, oxidase-negative bacteria capable of fermenting a wide range of carbohydrates.

Group I, identified as *Raoultella ornithinolytica* (98.25% similarity), was distinguished by its ability to produce indole and urease. Group II, identified as *Salmonella* sp., was characterized by hydrogen sulfide (H_2S) production, a biochemical feature commonly used for the identification of this genus. Groups III and VII corresponded to *Serratia liquefaciens* and *Serratia marcescens*, respectively, two species recognized for their broad metabolic versatility.

Groups IV, V, and VI belonged to the *Enterobacter* complex (*E. cloacae*, *E. asburiae*, and *E. aerogenes*), displaying closely related biochemical profiles but differing in their ability to utilize specific substrates such as inositol, rhamnose, melibiose, and arabinose.

The similarity values, ranging from 97% to 99%, indicate a high level of agreement between the observed biochemical profiles and the identified bacterial species.

Table 3 : Biochemical Characteristics of the Enterobacteriaceae Species Identified in *Macrotermes subhyalinus* and Worker *Macrotermes bellicosus*

Biochemical parameters	**Groups**						
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**
Catalase	+	+	+	+	+	+	+
ONPG	+	+	+	+	+	+	+
ADH	-	+	±	+	+	-	±
LDC	+	+	+	-	-	+	+
ODC	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+
H2S	-	+	-	-	-	-	-
Urée	+	-	-	-	-	-	+

TDA	-	-	-	-	-	-	-
Indole	+	-	-	-	-	-	-
VP	±	-	±	±	±	±	+
Gelatine	-	-	-	±	-	-	-
D-Glucose	+	+	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+	+
Inositol	+	+	±	-	-	±	+
D-Sorbitol	+	+	+	+	+	+	+
Rhamnose	+	+	+	+	-	+	+
Sucrose	+	+	+	+	+	+	+
Melibiose	+	+	+	+	-	+	+
Amygdalin	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	-	+
Oxidase	-	-	-	-	-	-	-
Species identified	*Raoultella ornithinolytica*	*Salmonella* sp.	*Serratia liquefaciens*	*Enterobacter cloacae*	*Enterobacter asburiae*	*Enterobacter aerogenes*	*Serratia marcescens*
% homology	98,25	97	98	99	98	97,64	99

(+) positive ; (-) negative ; (±) response varies depending on the species

ONPG:*o*-Nitrophenyl-β-D-galactopyranoside;**ODC:** L-ornithine decarboxylase; **ADH:** Arginine dihydrolase; **LDC:** Lysine decarboxylase; **H_2S:** Hydrogen sulfide production (from sodium thiosulfate); **TDA:** Tryptophan deaminase; **VP:** Voges–Proskauer test (acetoin production from sodium pyruvate).

3.2.3 Distribution of the Identified Bacterial Species in the Digestive Tract of the Two Termite Species

Table 4 shows the distribution of the *Enterobacteriaceae* species isolated from the intestinal tract of *Macrotermes subhyalinus* and *Macrotermes bellicosus* according to the different termite castes examined. A total of 118 isolates were identified and classified into seven bacterial species. *Serratia liquefaciens* was the most frequently isolated species, with 34 isolates, accounting for 28.81% of the total. It was particularly predominant in the minor soldiers of *M. subhyalinus*, where it represented 48.39% of the isolates, and was also highly abundant in the major soldiers (41.18%).

Enterobacter cloacae (16.10%) and *Enterobacter asburiae* (15.25%) also constituted a substantial proportion of the intestinal microbiota. *Enterobacter cloacae* was mainly isolated from the workers (22.45%) and minor soldiers (25.80%) of *M. subhyalinus*, whereas *Enterobacter asburiae* was detected in all castes except the minor soldiers.

Enterobacter aerogenes accounted for 11.86% of the isolates and was found exclusively in the minor soldiers of *M. subhyalinus* and the workers of *M. bellicosus*. Similarly, *Salmonella* sp. (11.86%) was isolated exclusively from the workers of both termite species. *Raoultella ornithinolytica* represented 11.02% of the isolates and was predominantly recovered from the workers of both species. In contrast, *Serratia marcescens* was the least frequently isolated species (5.08%) and was detected only in the major soldiers of *M. subhyalinus*.

Overall, the worker caste exhibited the highest bacterial species diversity, harboring five different bacterial species in *M. subhyalinus* and four species in *M. bellicosus*. In contrast, the soldier castes displayed a less diverse intestinal microbiota but were characterized by the predominance of specific bacterial species, particularly *Serratia liquefaciens*.

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Table 4 : Percentage Distribution of *Enterobacteriaceae* Species Isolated from the Intestinal Tract of *Macrotermes subhyalinus* and *Macrotermes bellicosus* According to Termite Caste

Species identified	***Macrotermes subhyalinus***			***Macrotermes bellicosus***	**Total**
	Workers	Minor soldiers	Mayor soldiers	Workers	
Enterobacter aerogenes	*0 (0%)	8 (25,8%)	0 (0%)	6 (28,57%)	14 (11,86%)
Enterobacter asburiae	9 (18,36%)	0 (0%)	4 (23,53%)	5 (23,81%)	18 (15,25%)
Enterobacter cloacae	11 (22,45%)	8 (25,8%)	0 (0%)	0 (0%)	19 (16,10%)

Raoultella ornithinolytica	9 (18,36%)	0 (0%)	0 (0%)	4 (19,05%)	13 (11,02%)
***Salmonella* sp.**	8 (16,32%)	0 (0%)	0 (0%)	6 (28,57%)	14 (11,86%)
Serratia liquefaciens	12 (24,49%)	15 (48,39%)	7 (41,18%)	0 (0%)	34 (28,81%)
Serratia marcescens	0 (0%)	0 (0%)	6 (35,29%)	0 (0%)	6 (05,08%)

Number of isolates (%)

4. Discussion

The present study highlights the high microbial density inhabiting the digestive tract of *Macrotermes subhyalinus* and *Macrotermes bellicosus*. Aerobic Mesophilic Bacteria (AMB) were detected in all castes of both termite species, confirming that the termite gut constitutes a particularly favorable ecosystem for the development of an abundant symbiotic microflora. These findings are consistent with those reported by [2] and [5], who described the termite gut as a true biological bioreactor in which microorganisms participate in the degradation of lignocellulosic substrates and nutrient recycling. Similarly, [4] demonstrated that the high bacterial diversity found in termite guts results from a long co-evolutionary relationship between the host and its symbionts.

Microbial loads were higher in workers than in soldiers regardless of the termite species considered. This difference may be related to the functional role of each caste within the colony. Workers are responsible for food collection, fungus comb maintenance, and feeding other colony members, exposing them to a wider diversity of environmental microorganisms. Variations in gut microbiota composition and density among termite castes have also been reported by [10 ; 7 and 11], who demonstrated that dietary and physiological differences among castes strongly influence the structure of intestinal microbial communities. Similar findings were reported by[12], who emphasized the high microbial abundance associated with worker termites.

The higher microbial loads observed in *Macrotermes subhyalinus* compared with *Macrotermes bellicosus* suggest a host-specific influence on microbiota composition as well as ecological and physiological differences between the two termite species. According to [5], the composition of termite gut microbiota is strongly influenced by dietary habits and colony social organization. This observation is also consistent with the findings of [13 ; 8], who identified host phylogeny and feeding habits as major factors shaping microbial communities in higher termites.

Microscopic observations revealed a predominance of Gram-positive bacilli in both termite species. This abundance of rod-shaped bacteria is consistent with observations made in several higher termite species, where Gram-positive bacteria represent an important component of the intestinal microbiota [7 ; 5]. The relatively low proportions of Gram-

negative cocci suggest ecological specialization among the different bacterial groups inhabiting the digestive tract.

The identification of 118 Enterobacteriaceae isolates distributed among four genera (*Enterobacter*, *Serratia*, *Raoultella*, and *Salmonella*) confirms the diversity of Proteobacteria associated with termites. Similar genera have been reported in several termite species and are known to contribute to sugar fermentation, nitrogen metabolism, and lignocellulosic compound degradation [2 ; 8]. The predominance of *Serratia liquefaciens* in *Macrotermes subhyalinus* suggests a particular adaptation of this bacterial species to the physicochemical conditions of the gut environment, as well as its capacity to produce a variety of hydrolytic enzymes involved in the degradation of complex organic compounds [14].

The occurrence of *Enterobacter cloacae, Enterobacter asburiae, Enterobacter aerogenes* (currently reclassified as *Klebsiella aerogenes*), *Raoultella ornithinolytica, Serratia liquefaciens,* and *Serratia marcescens* may be of considerable biotechnological interest. Indeed, several species belonging to these genera possess cellulolytic, xylanolytic, and fermentative activities involved in plant biomass degradation and the production of enzymes with industrial applications [5 ; 8]. Likewise, species of the genus *Enterobacter* are recognized for their contribution to cellulose degradation and nitrogen recycling in several xylophagous insects [15].

The detection of *Salmonella* spp. in the digestive tract of workers from both termite species deserves particular attention. Although some *Salmonella* species are known pathogens, their presence in the termite gut microbiota may also reflect adaptation to this environment or contamination associated with dietary substrates. Additional molecular identification using 16S rRNA gene sequencing or metagenomic approaches would be necessary to clarify their taxonomic status, functional role, ecological significance, and potential biotechnological applications.

Overall, this study demonstrates that the digestive tract of *Macrotermes subhyalinus* and *Macrotermes bellicosus* represents an important reservoir of bacterial diversity. The differences observed among castes and between termite species highlight the influence of host biology on the structuring of symbiotic microbial communities. These findings confirm that the gut of both termite species harbors a diverse community of Enterobacteriaceae that may contribute to lignocellulosic substrate digestion, nutrient recycling, and maintenance of intestinal microbial homeostasis.

Conclusion

The present study revealed the diversity of symbiotic Enterobacteriaceae associated with the digestive tract of *Macrotermes subhyalinus* and *Macrotermes bellicosus* collected from the shrub savanna of Lamto, Côte d'Ivoire. The results showed that aerobic mesophilic bacteria were present in all castes of both termite species, with higher microbial loads observed in workers, reflecting the predominant role of this caste in food acquisition and processing.
Microbiological analyses revealed greater bacterial richness in *Macrotermes subhyalinus* than in *Macrotermes bellicosus*. A total of 118 Enterobacteriaceae isolates were obtained and classified into four genera and seven species:*Raoultella ornithinolytica*, *Salmonella* sp., *Serratia liquefaciens, Serratia marcescens, Enterobacter cloacae, Enterobacter asburiae*, and

Enterobacter aerogenes. Among these, *Serratia liquefaciens* was identified as the dominant species in *Macrotermes subhyalinus*.

The study also highlighted variations in bacterial composition according to termite caste and species, emphasizing the influence of insect physiology and feeding behavior on gut microbiota structure. These findings confirm that the termite digestive tract constitutes a complex microbial ecosystem and a natural reservoir of microorganisms potentially involved in plant biomass degradation.

Beyond their ecological significance, the bacterial species identified may represent a promising source of enzymes and metabolites with biotechnological applications. However, further investigations based on molecular approaches, particularly 16S rRNA gene sequencing and metagenomic analyses, are required to better characterize the actual diversity of microorganisms present, elucidate their metabolic functions, and assess their potential applications in lignocellulosic biomass bioconversion and industrial biotechnology.

Conflict of Interest

The authors declare that they have no competing interests related to the content or the findings reported in this article.

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