



## RESEARCH PAPER

## OPEN ACCESS

## Screening of fermentative symbiotic microorganisms from digestive tract of *Macrotermes subhyalinus* and *Macrotermes bellicosus*

D.T. Yoro<sup>1</sup>, NK Kouassi<sup>2</sup>, S. Dabonné<sup>3\*</sup>, LP Kouamé<sup>3</sup>, R. Koffi-Nevry<sup>1</sup>

<sup>1</sup>Laboratoire de Biotechnologie et de Microbiologie des Aliments de l'Université d'Abobo-Adjamé (Abidjan, Côte d'Ivoire), 02 BP 801 Abidjan 02, Côte d'Ivoire

<sup>2</sup>Centre National de Recherche Agronomiques KM17 Route de Dabou / 01 BP 1740 Abidjan 01 (Côte d'Ivoire)

<sup>3</sup>Laboratoire de Biocatalyse et des Bioprocédés de l'Université d'Abobo-Adjamé (Abidjan, Côte d'Ivoire), 02 BP 801 Abidjan 02, Côte d'Ivoire

**Key words:** Fermentative microflora, lactic bacteria, *Macrotermes bellicosus*, *Macrotermes subhyalinus*, Symbiotic microorganisms.

doi: <http://dx.doi.org/10.12692/ijb/3.4.27-35>

Article published on April 22, 2013

### Abstract

Diversity of fermentative microorganisms in the digestive tract of *Macrotermes bellicosus* and *Macrotermes subhyalinus*, two species of tropical termites was investigated. Morphological, phenotypic and biochemical parameters were used for this preliminary study. Positive Gram bacteria were revealed on Man Rogosa Sharpe agar MRS and Chalmers agar while sabouraud chloramphenicol agar showed yeasts. Cultural techniques added to the use of API 50 CHL, had allowed the identification of lactic acid bacteria strains of the genera *Lactobacillus*, *Lactococcus* and *Pediococcus*. The API 20 CAUX revealed presence *Candida* and *Cryptococcus* only for *M. bellicosus* worker. These first results highlight the presence of a variety of fermentative microorganisms in the midgut of the two termites.

\*Corresponding Author: S. Dabonné ✉ [sdabonne@yahoo.fr](mailto:sdabonne@yahoo.fr)

## Introduction

With earthworms and ants, termites are one of the most important representatives of the group of organisms which activity significantly modify environment (Lavelle *et al.*, 1997; Lavelle and Spain, 2001). The termite's nests are regarded as the volume of soil and organic resources affected by termites, either directly or through their mandatory or voluntary associations with microorganisms. The flora of the digestive tract, their composition, variability, and their reciprocal interactions with their host, make a digestive system that is still not well known. Termites are social insects that live in an organization structured into families with castes of small soldiers, grand soldiers and workers. They belong to the order of Isoptera. They are arthropods of the insect class known as "white ants" (Klambhampati and Eggleton, 2000). Termites live in nests built in warm regions (Rouland, 1994).

Because of their social life and their distribution over the globe, termites represent the largest biomass of soils in tropical Africa (Woods and Sands, 1978). They are also one of the mayor components of the tropical and subtropical savannas and forests ecosystem from southeastern Asia and Africa. Their diet and their impact on physico-chemical properties of the soil (Woods and Sands, 1978 ; Bignell and Eggleton, 2000 ; Brauman *et al.*, 2000) play a very important role in the recycling of organic matter and formation of humus (Boyer, 1973 ; Bachelier, 1978). So they have an important ecological and economic influence. Previous studies have shown that termites are able to use substrates hemicelluloses without the help of their symbiotic microflora (Rouland and Lenoir-Labe, 1998). Termite's osidases therefore are varied. These enzymes are produced either by the termite itself or by its symbiotic microorganism (Rouland 1986 ; Matoub, 1993 ; Mackenzie *et al.*, 2007).

The enzymatic equipment from several species of African termites with different diets showed that most of these insects are capable of digesting various compounds of plant material. It is due to a symbiotic

relationship between termites and microorganisms involving (protozoa, bacteria, fungi) (Abo-Khatwa, 1978 ; Brauman *et al.*, 2001). Various microorganisms are present in the midgut of all termites (Brauman *et al.*, 2001) with a density of  $10^6$ - $10^7$  cells per  $\mu$ l of intestinal content (Brauman *et al.*, 2001).

We here focused on the microbial diversity of *M. subhyalinus* and *M. bellicosus*. It is now known that they are involved in the complex process of these insects feeding. Due to the diet of termites they could therefore be interesting sources of microorganisms for food process and biotechnological use. This study aims to highlight the diversity of the fermentative microbial population.

## Materials and methods

### Sampling

Termites *M. subhyalinus* and *M. bellicosus* identified by an entomologist were from the savannah of Lamto (Toumodi, Côte d'Ivoire). Twenty (20) insects of each caste (worker, small and grand soldier) were collected without damaging the nests.

### Isolation and enumeration of microorganisms

Twenty (20) insects from each caste (worker, small and big soldier) were separately sterilized in 70% ethanol (w/v) and washed with sterile water. They were then dissected under aseptic conditions using a stainless steel blade and a sterile forceps to extract the contents of the digestive tract. Homogeneous mixing of the contents of the digestive tract of twenty insects was investigated for symbiotic microorganisms. Lactic acid bacteria, yeast and mold were isolated on nutritive and selective broths using classic bacteriology methods (Bahiru *et al.*, 2006). 9 mL of a sterile physiological water solution (AES Laboratory, COMBOURG France) was added to 1 mL of every sample. Serial dilutions ( $10^{-1}$ - $10^{-8}$ ) are performed and 0.1 mL aliquot of the appropriate dilution is directly inoculated in triplicate on the following media.

Man Rogosa Sharpe agar (Laboratory CONDA, Spain) (De Man *et al.*, 1960): (AFNOR, NF ISO 15214), incubated anaerobically at 37°C for 48 h for enumeration of *Lactobacillus* and *Pediococcus*; Bile esculine Azide agar (BEA, ISO 7899/1) incubated at 37°C for 48 h for enumeration of *Enterococcus*; Chalmers agar incubated at 37°C for 48 h enumeration of *Lactococcus*; Sabouraud-Chloramphenicol agar (Laboratory CONDA, Spain) (Norme NF ISO 6611) incubated at 30°C for 2-5 days for enumeration of yeasts; Potato Dextrose Agar (PDA), (Laboratory CONDA, Spain) incubated 25°C at 2-6 days for enumeration of *molds*.

The colonies have been counted on the basis of some morphological and biochemical criteria. Results were expressed by means of  $\pm$  SD. (color of the colonies, Gram coloration, shape, size of the cells, no sporulation, cytochrome oxidase and catalase for the lactic bacteria. Production of catalase and budding for the Yeasts).

#### Phenotypic characterization of lactic acid bacteria

Presumptive lactic acid bacteria strains isolated from different media were assigned to a genus on the basis of key characteristics and tests (Kostinek *et al.*, 2005; Ricciardi *et al.*, 2005 et Bahiru *et al.* 2006). Morphological and arrangement of cells were examined by microscopy. Growth at 10, 45 and 51°C in MRS broth was monitored by spectrophotometer at 600 nm determinate after 1-3 days of incubation. Gas production from glucose was assessed in MRS broth containing Durham tubes. The ability to grow at different pH (3.0, 6.5 and 9.0) was tested. The salt tolerance was evaluated using MRS broth containing

0.5, 5 and 10% (w/v) NaCl. Only the isolates positive Gram, catalase and negative oxydase have been considered as being potential lactic bacteria and have been kept at -80°C in the MRS broth (Laboratory CONDA, Spain) in 20% of glycerol for the ulterior studies.

#### Identification of the *Lactobacillus*

Strains isolated on MRS were identified using the API 50CH galleries and API 50 CHL medium (bioMérieux, l'étoile, France). Tests were performed according to the manufacturer's instructions.

#### Characterization of Yeasts

Yeasts structures were directly observed on agar-agar under the objective (x 40) of the microscope after incubation at 27°C for 24 h to 48 hours. Yeasts selected on Sabouraud-Chloramphenicol were identified through the system API bioMérieux using the galleries API 20 C AUX (bioMérieux, l'étoile, France). Tests were performed according to the manufacturer's instructions.

## Results

#### Enumeration of the fermentative flora

Enumeration of the fermentative flora is resumed in table 1 for *M. Subhyalinus* and Table 2 for *M. Bellicosus*. *Lactobacillus* and *Pediococcus* were the most frequent in both species of termite. *Pediococcus* was lower in little soldier of *M. subhyalinus*. Yeasts were isolated only in the workers of *M. subhyalinus*. No *Enterococcus* or mould was observed.

**Table 1.** Fermentative microflora of the intestinal tract of *M. subhyalinus* (cfu/mL).

Genus	<i>Lactobacillus</i>	<i>Pediococcus</i>	<i>Enterococcus</i>	<i>Lactococcus</i>	Yeasts	Mould
<b>Castes</b>						
worker	2.8 10 <sup>7</sup> ± 0.7 10 <sup>7</sup>	4.2 10 <sup>4</sup> ±1.2 10 <sup>4</sup>	0	3.7 10 <sup>6</sup> ±0.84 10 <sup>6</sup>	2.1 10 <sup>5</sup> ± 0.21 10 <sup>5</sup>	0
Small soldier	3.1 10 <sup>4</sup> ±0.92 10 <sup>4</sup>	1.3 10 <sup>2</sup> ±0.84 10 <sup>2</sup>	0	3.10 <sup>4</sup> ±0.35 10 <sup>4</sup>	0	0
Big Soldier	2.5 10 <sup>4</sup> ±0.77 10 <sup>4</sup>	3.2 10 <sup>3</sup> ±0.49 10 <sup>3</sup>	0	2,8.10 <sup>2</sup> ±0.49 10 <sup>2</sup>	0	0

Each value is an average of three replicates. Values are mean  $\pm$  standard deviation.

### Phenotypic characterization and identification

#### Identification of lactic bacteria

Fermentation and physiological profiles (Table 3) showed that only the presumptive *Lactobacillus* produced gas, so they are heterofermentative. *Lactococcus* and *pediococcus* not producing gas are homofermentative. No *Lactococcus* was observed at 45°C while *Lactobacillus* and *Pediococcus* growth at 10°C. Between pH 3.0 and pH 9.0 *Lactobacillus* was

observed but not *Pediococcus* and *Lactococcus*. At 51°C with 10% NaCl, there was no growth at all. The majority of heterofermentative strains are observed, as *Lactobacilli* that ferment hexoses lactate, acetate (or ethanol) and CO<sub>2</sub>, suggesting that these microorganisms are capable of converting glucose to lactate, acetate or ethanol and CO<sub>2</sub>. Indeed termites can effectively hydrolyze cellulose using their intestinal microflora.

**Table 2.** Fermentative microflora of the intestinal tract of *M. bellicosus* (cfu/mL).

Genres Castes	<i>Lactobacillus</i>	<i>Pediococcus</i>	<i>Enterococcus</i>	<i>Lactococcus</i>	Yeasts	Mould
Worker	3.4 10 <sup>6</sup> ±1.34 10 <sup>6</sup>	1.8 10 <sup>5</sup> ±0.49 10 <sup>5</sup>	0	2.10 <sup>4</sup> ±0 35 10 <sup>4</sup>	0	0
Small soldier	1.8 10 <sup>5</sup> ±0.91 10 <sup>5</sup>	3.10 <sup>3</sup> ±1.06 10 <sup>3</sup>	0	1.7 10 <sup>2</sup> ±0.56 10 <sup>2</sup>	0	0
Big soldier	2.10 <sup>3</sup> ±0.28 10 <sup>3</sup>	2.2 10 <sup>3</sup> ±0.21 10 <sup>3</sup>	0	2.6 10 <sup>2</sup> ±0.07 10 <sup>2</sup>	0	0

Each value is an average e of three replicate, Values are mean ± standard deviation.

Identification of the presumptive bacteria by API 50 CHL led to twenty six strains of lactic acid bacteria. We got on the (twenty six) 26 isolates studied by API 50CHL, (ten) 10 *Lactobacillus*, 6 *Lactococcus*. Seven (7) *Pediococcus* and (three) 3 still unidentified strains.

The tests were performed at 30°C for 24 to 48 hours according to the manufacturer's instructions. The profiles obtained were analyzed in the catalog that contains the API 50CH acidification profiles of several known bacteria. The analysis of these profiles shows that some carbohydrates provide no discrimination within the genus of lactic acid bacteria. They were erythriol, D-arabinose, L-xylose, β-Methyl-xyloside, Dulcitol, inositol, α-Methyl-D-Glucoside, glycogen, xylitol, D-lyxose, D-fucose, L-fucose, L-Arabitol and 2-keto-gluconate that do not ferment and ribose, galactose, D-Glucose, D-Fructose, D-Mannose that all ferment. However, the other sugars distinguish them.

Tables 4 and 5 provide a summary of identifications obtained by the system API 50CHL. According to this system, system, identifications are acceptable when the calculated degrees of homology are greater than 90% (Biomerieux SA, La Balme caves,

Montalieu vercieu, France). Identification of strains from *M. bellicosus* worker and *M. bellicosus* little soldier were conducted with similarities rate equal or less than 90%. They were therefore just suitable for a good identification of the genera *Lactobacillus* and *Pediococcus* but not for the determination of the species. Identification of strains from little soldier, grand soldier, worker of *M. subhyalinus* and strains from grand soldier of *M. bellicosus* are interesting because the degree of homology calculated were greater than 90%. Strains S5 and S13 (grand soldier and worker of *M. subhyalinus*) were determined as being closed to *Pedicoccus pentosaceus*, while strains S10 (*M. subhyalinus* little soldier) and S36 (great soldier *M. bellicosus*) were closed to *Lactobacillus pentosus*. Strains S39 and S41 (*M. subhyalinus* worker) were identified as *Lactobacillus plantarum*. Strain S35 (great soldier *M. bellicosus*) was found to be *Lactococcus raffinolactis*.

#### Identification of yeasts

##### Cultural and morphological characteristics

Cultural characteristics of yeast were observed in liquid medium and on solid medium. The study of cultural characteristics here revealed the

appearance of isolated colonies. It provided evidence that most strains have different cultural characteristics, especially in the genus *Candida* at 80% and the genus *Cryptococcus* at 15.85%. The identification was confirmed by a morphological

study. Morphological characteristics will allow us to describe the shape of the cells and the presence or absence of filaments and their mode of multiplication.

**Table 3.** Characterization of lactic bacteria isolated from intestinal tract of *M. subhyalinus* and *M. bellicosus*.

Characteristics	Lactobacillus	Pediococcus	Lactococcus
Cells shape	Bacillus / coccobacillus	Cocci	Cocci
Grouping mode	Chain, pair, isolated	Pair, tetrad	Isolated, pair, chain
Gram	+	+	+
Catalase	-	-	-
Oxydase	-	-	-
Gas Production	+	-	-
Growth at different Temperatures (T°)			
10	±	-	±
45	±	+	-
51	-	-	-
Growth at different pH			
3	±	-	±
6.5	+	+	+
9	±	±	-
Growth in NaCl (%)			
0.5	+	+	+
5	±	±	±
10	-	-	-

**Table 4.** Identification by API 50CHL of lactic bacteria from *M. subhyalinus*.

castes	Identification API 50CHL	Percentage of identification (%)
Worker	<i>Lactobacillus plantarum</i>	99,9
	<i>Lactobacillus plantarum</i>	94,4
	<i>Lactococcus</i> sp.	89
	<i>Pediococcus pentosaceus</i>	99,9
Small soldier	<i>Pediococcus pentosaceus</i>	99,9
	<i>Lactobacillus pentosus</i>	99,9
	<i>Lactobacillus brevis</i>	92
	<i>Lactococcus raffinolactis</i>	96
Big soldier	<i>Pediococcus pentosaceus</i>	94,4
	<i>Lactobacillus pentosus</i>	96
	<i>Lactobacillus</i> sp.	98

Analysis of morphological characters allows us to classify strains of yeast in a given genre. The strains with some mycelium or pseudomycelium, with blastospore, dividing by budding were closed the genus *Candida* (Figure 1, 2 and 3). The strains L1, L2, L3, L4, L6, L8, L10, L11, L12, and L13 belong to this genus.

Strains L5, L7 and L9 reproducing by fission and do not have mycelium, they can be likened to the genus *Cryptococcus* and *Geotrichum* (Figure 4). However, these results do not allow us to have accurate information on the name of the isolated yeast species.

**Table 5 .** Identification by API 50CHL of lactic bacteria from *M. bellicosus*.

Caste	Identification API 50CHL	Percentage of identification (%)
Worker	Lactobacillus brevis	90
	Lactobacillus acidophilus	80
	Pediococcus spp	99
	Lactococcus sp	96
Small soldier	Lactobacillus curvatus sp curvatus	85,75
	Pediococcus pentosaceus	90
	Lactococcus raffinolactis	99
	Pediococcus spp	80
Grand soldier	Lactobacillus pentosus	95,4
	Lactococcus raffinolactis	92,4
	Pediococcus spp	99

**Table 6.** Taxonomic position of yeasts from *M. subhyalinus* according to the table of API 20 C AUX.

Strains	Taxons	Similarity (%)
L1	Candida Lusitaniae	99,9%
L2	Candida tropicalis	99%
L3	Candida lusitaniae	41,66%
L4	Candida spp	86,12%
L5	Cryptococcus laurentii	89,47%
L6	Candida famata	94,44%
L7	Cryptococcus laurentii	84,21%
L8	Candida norvegensis	99,9%
L9	Cryptococcus spp	80,34%
L10	Candida lusitaniae	75%
L11	Candida Lusitaniae	93,33%
L12	Candida spp	87,4%
L13	Candida tropicalis	92,3%

#### Physiological and biochemical characteristics

The physiological and biochemical characters helped to achieve a complete identification of the yeasts strains using the system of identification of yeasts API 20 C AUX (Biomerieux SA), (Table 6). Strains isolated from the worker of *M. subhyalinus* growth at 37° C. All the strains except strain L9 use glucose.

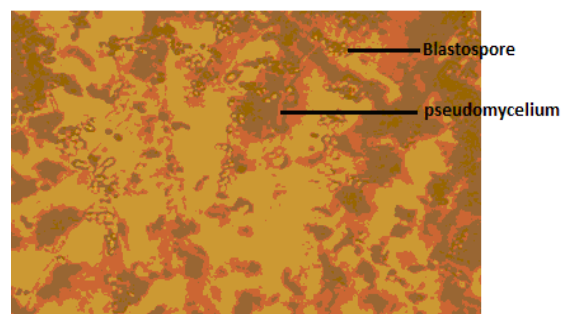
The study tested the assimilation of different substrates has identified ten strains. Four (4) of the belong to *Candida lusitaniae* with similarity percentages ranging from 99.9% to 93.33%, two (2) were found to be *Candida tropicalis* with similarities of 99% to 92.3%, one (1) *Candida famata* with a percentage of 94.4% similarity and one (1) *Candida*

*norvegensis* with a similarity of 99.9%. Seven (7) strains of genera *Candida* and *Cryptococcus* were not enough identified.

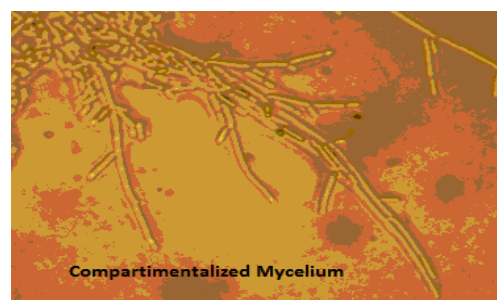
### Discussion

The intestinal microflora of most insects is a complex ecosystem composed of several hundreds of species, subspecies and biotypes bacteria. The density of microorganisms in the intestinal tract was estimated at  $10^6$ - $10^7$  cells/ $\mu$ l. The majority of these bacteria are strictly anaerobic (Brauman *et al.* 2001; Bignell *et al.*, 1980). Using phenotypic and biochemical methods, we have identified some lactic acid bacteria and yeasts in the gut of two species of termites. While galleries identification is not enough discriminatory, their association with phenotypic methods highlighted an effective presence of these microorganisms in the exudates of the intestine of both *M. subhyalinus* and *M. bellicosus*. These analyzes revealed a variety of microorganisms composed mostly of Gram positive as already shown for *Macrotermes michaelsoni* (Mackenzie, *et al.*, 2007). The isolated groups of lactic acid bacteria were recognized as fermentative bacteria. The presence of bacilli and cocci in the intestinal tract of the worker *M. subhyalinus* had been highlighted by Anklin-Mühlemann *et al.*, 1995. Their revealed the high bacterial density ( $10^9$  to  $10^{11}$ ) in the different compartments of the intestinal tract. Anderson (1991) and Bignell (1998) have shown that the diversity of the intestinal microflora is linked to the bacterial specie and the implantation site of the nest. According to some authors the gut of termites must remain completely anaerobic to allow growth of anaerobic bacteria (Graber *et al.*, 2004). It also allows us to explain the strong presence of fermentative bacteria in the intestinal tract of termites studied. However, presence in the intestinal tract of anaerobic strict, anaerobic facultative and aerobic microorganisms had been demonstrated (Kuhnigk *et al.*, 1996). Wenzel *et al.* (2002) also revealed the presence of facultative anaerobic, aerobic and microaerobic bacteria in the gut of the termite *Zootermopsis angusticollis*. They isolated

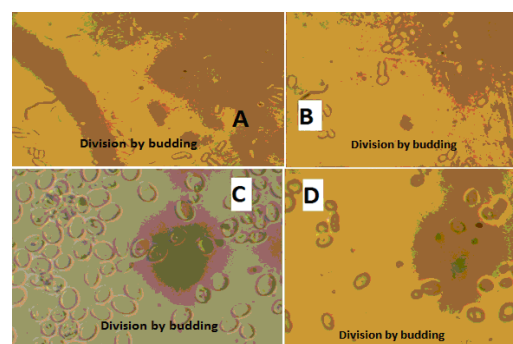
*sp. Microbacterium sp.*, *Kocuria varians*, *Bacillus cereus*, *Bacillus anthracis*, *Brevibacillus brevis* et *Paenibacillus sp.* Higashigushi *et al.* 2006, have also shown the presence of anaerobic, heterofermentative, non-sporulating microorganisms, Gram-positive in the gut of termites *Captotermes formosanus*. They also isolated several kinds of lactic acid bacteria and mainly.



**Fig. 1.** Microscopic observation of blastospore pseudomycelium and the strain of the genus *Candida*.



**Fig. 2.** Compartmentalized Mycelium, strains L8, L10.

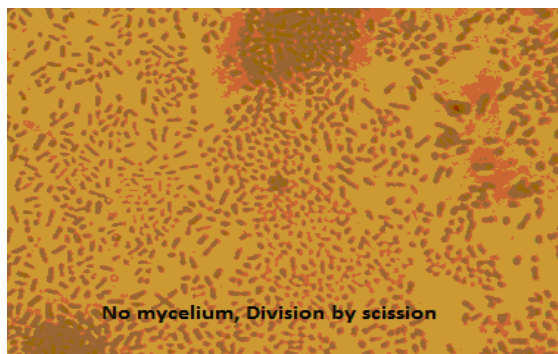


**Fig. 3.** Divisions by Budding ; A : strains L11, L12, L2 ; B : strains L1, L3, L4, L13 ; C : strain L6 ; D : strains L8, L10.

The previous results, reinforces the one we had found and demonstrates that the digestive tract of these termites are very good sources of fermentative



acid bacteria isolated from two species of termites show that the genus *Lactobacillus* is the most predominant in both *M. subhyalinus* and *M. bellicosus*. They constitute 38.46% of the strains identified against 26.92% against 23.07% for *Pediococcus* and *Lactococcus*. Genus *Candida* and genus *Cryptococcus* isolated only in the intestinal tract of *M. subhyalinus* worker call the hypothesis of a linking between the diversity of the symbiotic microorganisms and the caste of the termite.



**Fig. 4.** Strains L5, L7 and L9. Division by scission without mycelium

### Conclusion

This study has revealed that the intestinal tract of *M. subhyalinus* and *M. bellicosus* is an important source of fermentative microorganisms. Some of them still have to be well identified using molecular identification. Their technological properties will be evaluated. They can be used to improve the traditional food ferments performance.

### Acknowledgments

I would like to thank the International Foundation for Science (IFS), which funded this research.

### References

- Abo-Khatwa N.** 1978. Cellulose of fungus growing termites: a new hypothesis on its origin. *Experientia* **34**, 559-560.
- Anderson JM, Knight D, Elliott P.** 1991. Effects of invertebrates on soil properties and processes. In: Veeresh G. K. Rajagopal D, Viraktamath C.A. (Eds.). *Advances in Management and Conservation of soil*. Oxford and IBH Pub. Co. New Delhi, p. 437-484.
- Anklin-Mühlemann R, Bignell DE, Veiver PC, Leuthold RH, Slaytor M.** 1995. Morphological, microbiological and biochemical studies of the gut flora in the fungus-growing termite *Macrotermes subhyalinus*. *Journal of Insect Physiology* **41**, 929-940.
- Bachelier G.** 1978. La faune des sols, son écologie et son action. OSTROM Bondy Initiations, documentations techniques, p. **38**, 391.
- Bahiru B, Mehari T, Ashenafi M.** 2006. Yeast and lactic acid flora of tej, an indigenous Ethiopians honey wine: Variation within and between production units. *Food Microbiology* **23**, 277-282.
- Bignell DE, Eggleton P.** 2000. Termites in ecosystems. In: *Termites: Evolution, sociality, symbioses and ecology*. Abe T, Bignell DE et Higashi M (eds), Kluwer Acad. Publ p. 363-387.
- Bignell DE, Eggleton, P.** 1998. Termites In: Calow, P. (ed) *Encyclopedia of ecology and environmental management*. Blackwell Scientific, Oxford, p. 744-746.
- Bignell DE, Oskarsson H, Anderson JM.** 1980. Specialization of the hindgut wall for the attachment of symbiotic microorganisms, in a termite *Procupitermes aburiensis* (Isoptera: Termitidae. Termitinae). *Zoomorphology* **96**, 103-112.
- Boyer P.** 1973. Action de certains termites constructeurs sur l'évolution des sols tropicaux. *Annal. Sciences. Naturels. Zoo Paris* **15**, 329-498.
- Brauman A, Bignell DE, Tayasu I.** 2000. Soil-feeding termites : biology, microbial associations and digestive mechanisms, p. 233-259.
- Brauman A, Dore J, Eggleton P, Bignell D, Breznak JA, Kane MD.** 2001. Molecular phylogenetic profiling of prokaryotic communities in guts of termites with different feeding habits. *FEMS Microbiology Ecology. Ecol* **35**, 27-36.



- Conway, PL.** 1995. Microbioal ecology of the human large intestine. In Gibson, G.R. 243-265.
- De Man JC, Rogosa M, Sharpe ME.** 1960. Journal of Applied Bacteriology **23**, 130-137.
- Graber JR, Leadbetter JR, Breznak A.** 2004. Description of *Treponema azotonutricium* and *Treponema primitia* sp. nov., the first spirochetes isolated from termite guts. Applied and Environmental Microbiology **70**, 1315-1320.
- Higashiguchi DT, Hussenede C, Grace JK, Berestecky JM.** 2006. *Pilibacter termitis* gen. nov., Sp. nov., a lactic acid bacterium from the hindgut of the Formosan subterranean termite (*Captotermes formosanus*) International Journal of Systematic and Evolutionary Microbiology **56**, 15-20.
- Kambhampati S, Eggleton P.** 2000. Phylogenetics and taxonomy. In: Abe, T., Bignell, D.E. & Higashi, M., (eds.). Termites: Evolution, Sociality, Symbiosis, Ecology. Kluwer Academic Publishers. 1-23.
- Kostinek M, Specht I, Edward VA, Schillinger U, Hertel C, Holzapfel WH, franz CMA.** P. 2005. Diversity and technological proprieties of predominant lactic acid bacteria from fermented cassava used for the preparation of Gari, a traditional African food. Systematic and Applied Microbiology **28**, 927-540.
- Kuhnigk T, Branke J, Krekeler D, Cypionka H, König H.** 1996. A feasible role of sulfate-reducing bacteria in the termite gut. Systematic and Applied Microbiology **19**, 139-149.
- Lavelle P. et Spain A.V.** 2001. Soil ecology. Kluwer Academic Publishers, p. 654 .
- Lavelle P, Bignell DE, Lepage M, Wolters V, Roger P, Ineson P, Heal W, Dillion S.** 1997. Soil function in a changing world: the role of invertebrate ecosystem ingeneers. European Journal of Soil Biology **33**, 159-193.
- Mackenzie LM, Muigai AT, Osir E.O., Lwande W, Keller M, Toledo G, Boga I.H.** 2007. Bacterial diversity in the intestinal tract of the fungus-cultivating termite *Macrotermes michaelseni* (Sjöstedt). African Journal of Biotechnology **6(6)**, 658-667.
- Matoub M,** 1993. La symbiose termite-champignon chez *Macrotermes bellicosus* (Termitidae, Macrotermitinae). Thèse d'Université Paris XII Val de Marne Paris . 199p. pathology. CRC Press, p. 1-24.
- Ricciardi A, Parente E, Piraino, P, Paraggio M, Romano P.** 2005. Phenotypic characterization of lactic acid bacteria from sourdoughs for Altamura bread produced in Apulia (southern Italy). Int. J; microbial **98**, 63-72.
- Rouland C, Lenoir-Labe F.** 1998. Microflore intestinale symbiotique des insectes xylophages: Mythe ou réalité. Cahiers agricultures **7**, 37-47.
- Rouland C.** 1986. Contribution à l'étude des osidases digestives de plusieurs espèces de termites afrcains. Thèse d'Université paris XII Val- de Marne paris, p. 210.
- Rouland C.** 1994. Tropical. Courier de l'environnement, l'INRA n°**23**. 678p.
- Wenzel MI, Schonig M, Berchtold P, Kampfer H, König G.** 2002. Aerobic and facultatively anaerobic cellulolytic bacteria from the gut of the termite *Zootermopsis angusticollis* *Zootermopsis angusticollis*. Journal of Applied Microbiology **92**, 32-40.
- Wood TG, Sands WA.** 1978. The role of termites in ecosystem. In : Brian M. V. (Eds) Production Ecology of ants and termites. Cambridge University Press, Cambridge, p. 245-292.