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Antifungal Activity of Lactic Acid Bacteria against Molds Isolated from Corn and Fermented Corn Paste

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Abstract A total of 336 molds were isolated from dried corn, soaked corn and fermented corn paste. The macroscopic and microscopic studies of fungal growth in the following identification media, grouped all the 336 molds into 21 strains. The strains belonged mainly to 4 fungal genera: *Aspergillus, Fusarium, Penicillium* and *Rhizopus.* In addition, the aflatoxinogenic strains were dominant and were mostly isolated from Maroua (63 strains of *Aspergillus flavus*). Moreover, the antifungal activity of 53 Lactic acid bacteria (LAB) isolated from the samples was performed against 21 fungal strains. After a screening test, 06 were selected for their potent antifungal activity and were identified as *Lactobacillus brevis* (2 isolates), *Lactobacillus buchneri* (1 isolate), *Lactobacillus cellobiosus* (1 isolate) and *Lactobacillus fermentum* (2 isolates). During the antifungal tests in solid medium, most of the LAB inhibited the growth of molds but *Lactobacillus brevis* G25 (80 ± 0.5 mm) and *Lactobacillus cellobiosus* (82 ± 0.1 mm) had the greatest antifungal activities after 48 hours against *Aspergillus carbonarius* G23 and *Aspergillus carbonarius* G24. However, the antifungal activity was more efficient in liquid medium and *Lactobacillus brevis* G11 and *Lactobacillus fermentum* N33 totally inhibited the growth of the 21 molds tested in liquid medium. Thus organic acids were identified as substances responsible for the antifungal activity of the LAB. These results show the possibility of exploiting some of these LABs as starters to fight against spoilage molds in fermented corn paste.

Keywords: *antifungal activity, fermented corn paste, lactic acid bacteria, molds*

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1. Introduction

Several molds are known for their ability to grow on a wide range of foods and to alter their nutritional and organoleptic qualities. The most frequent genera implicated in food contamination include: *Aspergillus*, *Penicillium* and *Fusarium* [1]. Their capacity to produce toxic secondary metabolites or mycotoxins is a serious health danger worldwide [2]. In Africa, there is ample evidence of the direct and negative effects of aflatoxins on human health through increased incidence of liver cancer and because of its potential synergistic effect on hepatitis B [3]. Also, studies have pointed out the capacity of some mycotoxins to alter normal immune function when present in food at levels below observable over toxicity [4].

In respect of the dangers which molds and their mycotoxins represent, some food preservation methods, such as addition of benzoic acid and sorbic acid, used as antifungal in food. These acids are usually added in form of water-soluble salts (sodium benzoate and potassium sorbate). However, their consequences such as resistance of the species, the residual level in food, remain the major problems of their use [5]. Moreover, consumers are seeking and demanding for products without chemical preservatives which still maintain good shelf life and safeness. Due to these drawbacks, the concept of biopreservation is gaining popularity. This concept refers to extended shelf life and enhanced safety of foods by growth of the natural or added microflora and their antimicrobial products [6].

Among the microorganisms which can potentially be used as biopreservatives, Lactic Acid Bacteria (LAB) represent a good example and are considered, Generally Recognized As Safe (GRAS) and have traditionally been used in food and animal feed, sauerkraut and silage. A number of studies have focused on inhibition of fungi growth using LAB and their preserving effect relates mainly to the formation of organic acids, hydrogen peroxide, competition for nutrients and production of antimicrobial substances [7].

Although the activity of LAB varies with ecosystems, it would be good that a number of studies be carried worldwide in order to discover new and active LAB.

In the foregoing, the aim of this study was to determine the antifungal activity of LAB strains isolated from corn, fermented paste and to determine the origin of this activity with the hope that they can be applied as biopreservatives.

2. Materials and Methods

2.1. Collection of Samples

The preparation of fermented corn paste in the Sudano-Guinean (Ngaoundere) and Sudano-Sahelian (Maroua and Garoua) zones of Cameroon was done principally by soaking of dried corn, milling soaked corn and fermentation of corn paste for 3 to 5 days (Figure 1). The sampling was performed at different stages of preparation of fermented corn paste. Sampling of dried corn, soaked corn and fermented corn paste were randomly obtained. They were collected in January 2012 during the dry season. For the cities of Maroua and Garoua, 9 samples of dried corn, 9 samples of soaked corn and 9 samples of fermented corn paste were collected. While in town of Ngaoundere, 11 samples of dried corn, 11 samples of soaked corn and 11 samples of fermented corn paste were collected. Each sample weighed 250g with the exception of fermented corn paste which weighed 100g. The soaked corn and fermented corn pastes were introduced in sterile plastic bags and preserved at -18°C prior for analysis. Grains of dried corn were packed in sterile craft papers and preserved in a dry environment.

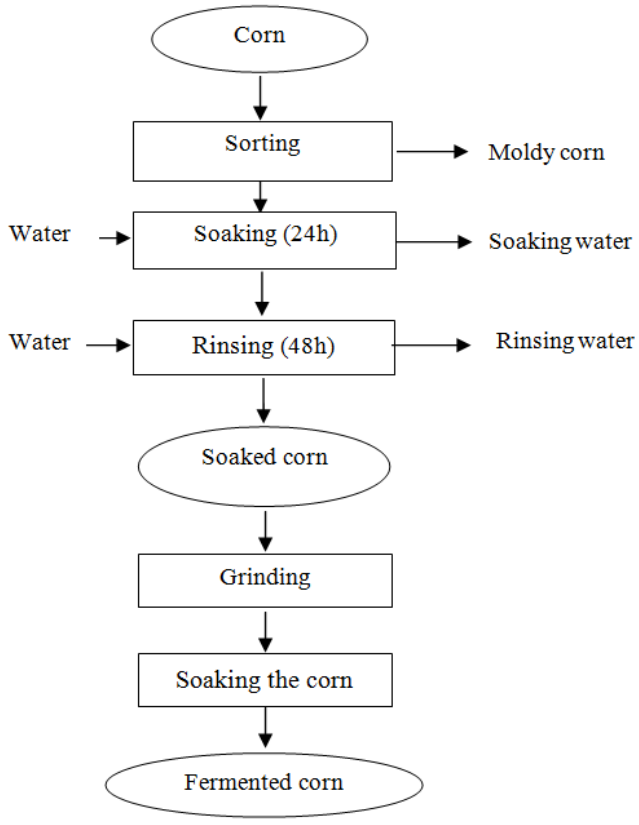

Figure 1. General process used for the preparation of the fermented corn paste

2.2. Evaluation of Contamination of Samples by Molds

To evaluate sample contamination, molds were isolated from dried corn and soaked corn by the direct plating technique as described by Pitt and Hoking [8]. Ten grains of corn per sample were disinfected in aqueous solution of sodium hypochlorite (NaOCl) for one minute at room temperature. Then, they were placed directly on Dichloran 18% Glycerol Agar (DG18). This method permits recovery of the fungi actually growing in the particles. Plates were incubated at 25°C for 5 to 7 days. For the fermented corn paste, the isolation of the molds was carried out according to the method described by Nkwe *et al.* [9]. These strains were kept in distilled water at 4°C prior to further identification.

2.3. Molds Identification

To identify the different molds isolated from dried corn, soaked corn and fermented corn paste samples obtained from the 03 cities under study, the method elaborated by Pitt and Hoking [8] was used. The fungal strains were inoculated onto three media. The three medium were CYA (Czapek Yeast Extract Agar), MEA (Malt Extract Agar) and YES (Yeast Extract Sucrose Agar). After 7 days of incubation at 25°C, the macroscopic and microscopic observations were used to evaluate parameters such as the diameter of the growth, the color of the aerial parts of the mycelium, conidial heads, pigmentation of the base of the mycelium, time of growth and the surface texture of the colonies.

2.4. Determination of the Aflatoxigenic Molds

Isolates of *Aspergillus* and *Penicillium* were cultured on Malt Extract Agar (MEA), Czapek Yeast Extract Agar (CYA), Yeast Extract Sucrose Agar (YES) and Coconut Milk Agar (CMA) at 25°C and 30°C for 7 days and detected by fluorescence of the medium under ultraviolet light [10].

2.5. Isolation of Lactic Acid Bacteria

LABs were isolated in different samples according to the method of Voulgari *et al.* [11]. Briefly, 1 g of the various samples (dried corn, soaked corn and fermented corn paste sample) was homogenized in a stomacher blender for 1 min with NaCl solution (0.85%). The suspension was tenfold serially diluted and appropriate dilutions were plated onto Man Rogosa Sharp (MRS) agar. Plates were incubated at 37°C for 48 hr. After this time, colonies were purified by streaking on the same medium. Only Gram-positive, non-spore forming and catalase-negative bacterial isolates were selected and stored in glycerol (40%) at -20°C for further investigation.

2.6. Biochemical Identification of Selected Lactic Acid Bacteria

The most active isolates were characterized by their carbohydrate fermentation profile using the API 50 CH strips and the API 50 CHL medium according to the manufacturer's instructions (API system, Bio-Merieux, France). Strains were tentatively identified into species level using APIDENT (version 2.0, Bio-Merieux).

2.7. Screening of Lactic Acid Bacteria

This analysis was carried out according to the method of Lind *et al.* [12]. Each isolated LAB strain was tested on mold isolates on MRS medium. To perform this test, the LAB were inoculated by streaking in Petri dishes containing MRS agar. The preparation was incubated for

24 h at 37°C. After this time, the preparation was covered with 5 mL of Potato Dextrose Agar (PDA) medium containing the moulds spores (10^6spores/mL). The whole preparation was incubated for 48 h at 25°C. The presence of a zone of inhibition permits to highlight the active bacteria.

2.7.1. Antifungal Overlay Assay

The LAB isolates obtained were initially screened for their antifungal activity against the mold isolates. The method of Voulgari *et al.* [11] was followed with a minor modification. Reactivated bacterial isolates were grown in MRS broth at 35°C overnight. A total of 20µl of LAB isolate mixed with 30µl of MRS agar was added in a well located at the center of MRS agar. The inoculated plates were incubated for 48 h at 35°C in an inverted position. After incubation, the plates were then overlaid with soft Potato Dextrose Agar containing a final mold spore concentration of 10^6 spores/mL and incubated at room temperature (25°C). The zones of inhibition around the bacterial colonies were recorded after 48 h. The data obtained were used to make the selection of LAB isolates.

2.7.2. Antifungal Activity in Liquid Medium

Only the isolates which showed strong fungal growth inhibition activity in the antifungal overlay assay were used in this test. 45 mL of Lablemco Tryptone broth (LTB) was prepared according to Coallier and Idziak [13] in 100 mL Erlenmeyer flasks and sterilized at 121°C for 15 min. Each flask was inoculated with mold isolates (10^6 spores/mL) and LAB cultures (10^5 CFU/mL) then incubated at 28°C for 8 days. In the same manner, the control flask containing LTB was inoculated with molds (10^6 spores/mL) and incubated in the same conditions as the test. After 8 days of incubation, the percentage of fungal growth was determined by comparing the growth of the control with those treated. The dry weights of molds treated and control molds were measured by infrared (IR) moisture Analyzer (IR 35 Moisture Analyzer). Percentage of mycelium growth inhibition was calculated using the following formula:

$$PI = \frac{Gc - Go}{Gc} x100$$

Where:
PI= Percentage of mycelia growth inhibition; Gc= Mean dry weight for control; Go= Mean dry weight for treated mycelia

2.7.3. Nature of Antifungal POTENTIAL of Lactic Acid Bacteria

This analysis was carried out according to the method of Trials *et al.* [14]. Selected LAB strains were grown in MRS broth for 48 h at 37°C and the cell-free supernatant was obtained by vacuum filtration (Sartorius, 0.45 µm). The supernatant was divided into several fractions (Fraction A, Fraction B, Fraction C, Fraction D and E) and the fractions obtained were subjected to various types of treatment. Fraction A was neutralized with 3 M NaOH to pH 7.0 to eliminate the action of acid [15]. Fraction B was treated with 0.1 mg of catalase/mL at 37°C for 1h in the neutral supernatant (Fraction A) to eliminate the action of hydrogen peroxide (H_2O_2). Fractions C, D and E were treated respectively with proteinase K (20 mg/mL, Eurobio, France), trypsin (40 U/mg, Merck) and α-chymotrypsin (40-60 U/mg) from bovine pancreas. The reaction of catalase and various proteases were stopped by incubating the fractions for 10 min at 65°C in a water bath. Melting Potato Dextrose Agar containing 10^6 conidia/mL was poured in Petri dishes. After solidification, wells were made in PDA and 100 µL of each fraction were placed in the wells to test their inhibition activity against the mycelia growth. The plates were incubated for 30°C for 48h. Inhibition zone formed express a positive activity. The control test was performed with untreated supernatant.

2.8. Statistical Analysis

Means and standard deviations were calculated from 3 independent replicate trials and subjected to analysis of variances using SPSS Statistical package. Differences between means were evaluated using Duncan Multiple Range Test.

3. Results and discussion

3.1. Sample Contamination and Identification of Molds

A total of 336 molds were obtained from dried corn, soaked corn and fermented corn paste. These mold strains were grouped after growth on identification media (CYA, MEA and YES), in 21 molds strains and identified using taxonomic scheme based on morphological characters and microscopic observations. These 21 molds belonged mainly to 4 fungal genera, namely: *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus*. Within the genus *Aspergillus*, toxigenic species such as *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus Niger*, *Aspergillus versicolor* and *Aspergillus carbonarius* were isolated. The presence of toxigenic molds in the samples represents a high carcinogenic risk to the population. Moreover, the presence of both *A. versicolor* and *A. flavus* in some samples is an additional risk of producing some toxins such as AFB_1. Although the Sterigmatocystin produced by *A. versicolor* is 150 times less toxic than AFB_1, it remains a precursor of AFB_1 [8]. The genera of molds isolated in this work are frequently isolated in cereals. In Cameroon, the studies conducted on the contamination of maize by molds also showed the presence of *Aspergillus* and *Penicillium* in corn collected in other regions such as Bamenda, Kumbo, Douala and Yaounde [16].

An evaluation of sample contamination by fungal species (Table 1) showed a high contamination of dried corn and soaked corn. This high contamination of samples by different mold species can be explained by the fact that, cereals, especially maize, are favorite substrates of molds because of their high carbohydrate content recognized as a source of primary energy [17]. Furthermore, the climatic conditions of the study areas (mean temperature at 29°C) related to its geographical location is favorable to their development in the field and during storage of maize. We observed a variation of the fungal diversity when passing from one area to another. Species such as *A. niger*, *A. carbonarius* and *F. solani* were isolated mainly in Garoua while *A. fumigatus* and *Penicillium* sp. were isolated respectively in samples of Maroua and Ngaoundere. This

variation on the contamination could be linked to the difference in temperatures between areas registered during the study period. The average temperatures of 21, 26 and 27°C were recorded respectively in the towns of Ngaoundere, Maroua and Garoua. This explains why the mesophilic strains (25-30°C) were isolated in samples from the cities of Garoua and Maroua while the psychrotrophic molds (0-20°C) as *Penicillium* sp were isolated in the town of Ngaoundere. Furthermore, there is a predominance of toxigenic strains like A*spergillus* genus. A total of 150 *Aspergillus* strains were isolated from the samples collected. In samples of Maroua specifically, there were up to 63 *A. flavus* strains. This high level of contamination of the samples of Maroua by *A. flavus* could be explained by temperature of 26°C, located within the optimal growth temperatures (25-30°C) of *A. flavus*.

Table 1. Frequencies (%) of fungal isolates from dried corn and soaked corn samples from Garoua, Maroua and Ngaoundere

Fungi/City	Number of samples contaminated	
GAROUA	**Dried corn** (n=9)	**Soaked corn** (n=9)
Aspergillus niger	7 (35)	6 (24)
Aspergillus flavus	6 (30)	7 (28)
Aspergillus carbonarius	3 (15)	4 (16)
Fusarium oxysporum	4 (20)	5 (20)
Fusarium solani	0 (0)	3 (12)
MAROUA	**Dried corn** (n=9)	**Soaked corn** (n=9)
Aspergillus flavus	36 (67)	27 (37.5)
Aspergillus fumigatus	0 (0)	18 (25)
Fusarium oxysporum	0 (0)	27 (37.5)
Rhizopus sp.	18 (33)	0 (0)
NGAOUNDERE	**Dried corn** (n=11)	**Soaked corn** (n=11)
Aspergillus versicolor	0 (0)	36 (39)
Penicillium sp	21(29)	0 (0)
Rhizopus sp.	52 (71)	56 (61)

Curiously, no mold was isolated from fermented corn paste. This could be justified by the low pH (pH 3, 2 and 3.6) recorded in fermented corn paste. But the pH alone does not explained this inhibition, because some studies indicate isolation of an acid-tolerant strain of *Penicillium* which could grow actively at pH 1.0 and thrived in pH 0.6 [18]. This makes it possible to envisage the presence of other antifungal compounds such as bacteriocin, hydrogen peroxide, and organic acids such as phenyllactic and hydrophenyllactic acids. Many studies have shown that certain organic acids such as phenyllactic and hydrophenyllactic acids were listed as particularly efficient against fungal growth [19]. However, although molds are absent in paste, it does not exclude the presence of the mycotoxins such as the AFB_1 at the end of spontaneous fermentation. Especially when it is known that the samples were contaminated by the aflatoxigenic molds like *A. flavus* and the production of AFB_1 can be made before the production of the fermented corn pastes, which is in field or during storage [1].

3.2. Aflatoxigenic Molds

Four strains of *Aspergillus* out of the 11 tested, showed aflatoxigenic activity on YES and/or CMA medium (Table 2). The strains of *A. flavus* G14, *A. flavus* G22 and *A. flavus* M15 produced aflatoxins B (Figure 2) on YES and CMA while *A. flavus* G26 produced aflatoxin B only on YES medium. Among the aflatoxin-producing strains, it was found that the city of Garoua recorded 75% of aflatoxin B producing strains, while the city of Maroua recorded 25% of aflatoxin B producing strains. The aflatoxigenic character of some strains of *A. flavus* means that, during the favorable conditions, these molds can produce aflatoxins in dried corn and soaked corn which will then be found in the fermented corn pastes. It should be noted that it is also likely that all strains of *A. flavus* tested are able to produce aflatoxin B. For the production of mycotoxins by the mold can be affected by several factors such as water content, temperature, storage time, the presence of oxygen, carbon dioxide, the substrate composition, the predominance of toxigenic species and microbial interactions [20]. This can represent a difficulty to determine all aflatoxigenic molds in the samples.

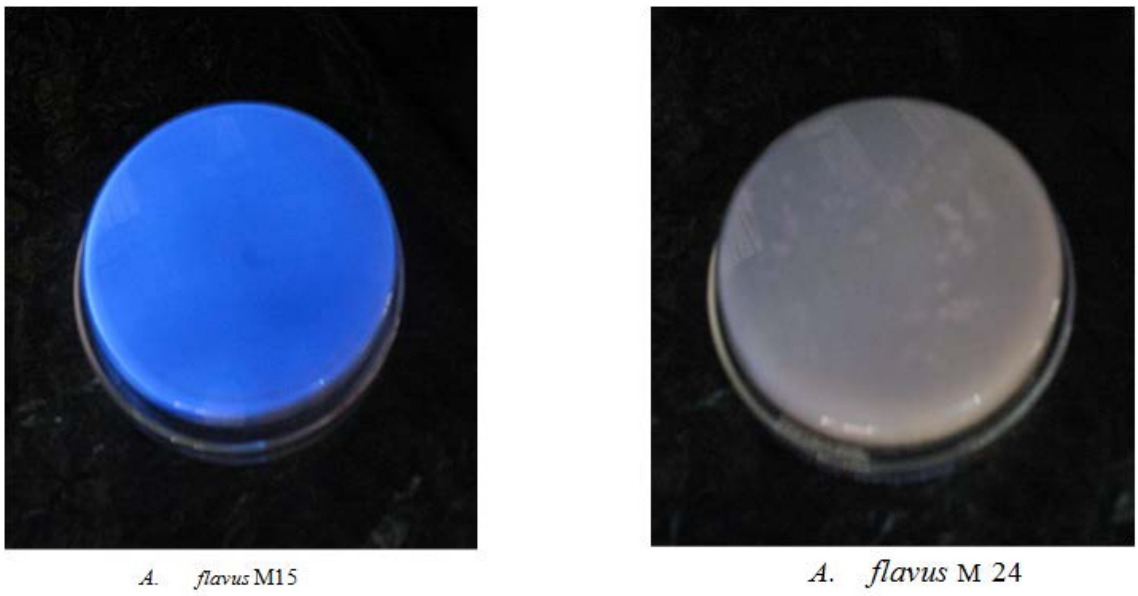

Figure 2. Observation of a blue fluorescence under ultraviolet light underneath the colonies of *A. flavus* M15 and absence of fluorescence underneath the colonies of *A. flavus* M24 on Coconut Milk Agar (CMA) after 7 days at 25°C

Table 2. Aflatoxigenic B potentials of *Aspergillus* and *Penicillium* species isolated from dried corn and soaked corn in Garoua, Maroua and Ngaoundere

	Medium			
Molds	**MEA**	**CYA**	**YES**	**CMA**
Aspergillus niger G12	-	-	-	-
Aspergillus flavus G14	-	-	+	+
Aspergillus niger G21	-	-	-	-
Aspergillus flavus G22	-	-	+	+
Aspergillus carbonarius G23	-	-	-	-
Aspergillus carbonarius G24	-	-	-	-
Aspergillus flavus G26	-	-	+	-
Aspergillus flavus M15	-	-	+	+
Aspergillus flavus M 24	-	-	-	-
Aspergillus fumigatus M 23	-	-	-	-
Penicillium sp N11	-	-	-	-
Aspergillus versicolor N22	-	-	-	-

3.3. Isolation of Lactic Acid Bacteria

A total of fifty-three LAB were isolated from dried corn, soaked corn and fermented corn paste collected in the towns of Garoua, Maroua and Ngaoundere. For samples of the city of Garoua, 33.3% of LAB were isolated from dried corn, 33.3% from soaked corn and 33.3% from fermented corn paste. In Maroua, 33% of LAB were isolated from dried corn, 38.8% from soaked corn and 27.8% from fermented corn mold strain (Table 3, Table 4, Table 5). For the continuation of this work, 06 LAB were selected for their high antifungal activity. In fact, they have induced inhibition zones greater than 8% of the surface of the Petri dish and also because they had an antifungal potential on more than half of the studied molds. Among these molds, 02 were isolated from Garoua (LAB G11 and LAB G25), 02 to Maroua (LAB M11 and LAB M41) and 02 to Ngaoundere (LAB N25 and LAB N33). The strains LAB G11, LAB M11, LAB M41, LAB N25 and LAB N33 had an antifungal potential on 85.7% of molds while LAB G25 had an antifungal potential on 95.2% of molds paste. Finally, in Ngaoundere, 47.0% of LAB were isolated from dried corn, 29.4% from soaked corn and 23.5% from fermented corn paste. Several studies conducted in Cameroon reported the presence of LAB in fermented foods made from corn [21].

Table 3. Antifungal activity of LAB isolated from samples collected in Garoua

	LAB																	
Molds	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
A. carbonarius G 23	+	+	-	-	+	-	-	-	-	-	+	-	-	-	++	+	-	-
A. carbonarius G 24	++	-	-	++	-	-	-	+	-	-	++	-	-	-	+	-	-	-
A. flavus G 14	+	-	-	-	-	-	-	+	-	-	++	-	-	-	++	-	+	-
A. flavus G 22	++	++	-	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-
A. flavus M 15	+	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-
A. flavus M 24	++	++	-	-	+	-	-	-	-	-	++	-	-	-	-	++	+	-
A. fumigatus M 23	++	-	-	++	-	-	-	+	-	-	++	-	-	-	-	-	+	-
A. niger G 12	+	-	-	-	-	-	-	+	-	-	+	-	-	-	+	-	-	-
A. niger G 21	++	-	-	-	-	-	++	-	-	-	+	-	-	-	-	+	-	-
A.versicolor G 26	++	-	-	++	-	-	-	-	-	-	+++	-	-	-	-	+	-	-
A. versicolor N 22	+++	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
F. oxysporum G 12	++	-	-	++	++	-	-	+	-	-	+	-	-	-	-	++	-	-
F. oxysporum G 13	++	-	-	+	-	-	-	-	-	-	+++	-	-	-	-	-	-	-
F. oxysporum G 27	++	+	-	-	+	-	++	+	-	-	+++	-	-	-	-	-	-	-
F. oxysporum G 28	+	-	-	+	-	-	-	-	-	-	+	-	-	-	+	-	-	-
F. oxysporum M 22	+	-	-	+	-	-	++	-	-	-	-	-	-	-	-	-	-	-
F. solani G 25	-	-	-	-	-	-	-	+	-	-	+++	-	-	-	+	-	-	-
Rhizopus sp M 13	-	-	-	-	-	-	++	-	+	-	+	-	+	-	++	-	-	-
Rhizopus sp N 11	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-
Rhizopus sp N 21	++	++	-	-	+	-	-	++	-	-	-	-	-	-	-	+	+	-
Penicillium sp N 11	+	++	-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-

1=LAB G11, 2=LAB G12, 3=LAB G13, 4= LAB G14, 5= LAB G15, 6= LAB G16, 7= LAB G21, 8= LAB G22, 9= LAB G23, 10= LAB 24, 11= LAB 25, 12= LAB G26, 13= LAB G31, 14= LABG32, 15= LAB G33, 16= LAB G34, 17= LAB G35, 18= LAB G36

-: no visible inhibition; **+:** zone of inhibition located between 0.1-3% of the surface of the Petri dish, **++:** zone of inhibition located between 3-8% of the surface of Petri dish and **+++:** zone of inhibition > to 8% of the surface of Petri dish.

Table 4. Antifungal activity of LAB isolated from the samples collected in Maroua

	LAB																	
Molds	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
A. carbonarius G 23	+	-	-	-	++	-	-	-	-	-	+	-	-	+++	+	-	-	-
A. carbonarius G 24	++	-	-	-	++	-	-	-	-	-	++	-	-	++	-	+	-	-
A. flavus G 14	+	-	-	-	-	-	-	-	-	-	++	-	-	+++	-	-	-	-
A. flavus G 22	++	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-
A. flavus M 15	-	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-
A. flavus M 24	+	+	-	-	-	-	+	-	-	+	++	-	-	+++	-	-	-	-
A. fumigatus M 23	-	-	-	-	-	-	+ +	-	-	+	-	-	-	++	+	-	-	-
A. niger G 12	-	-	-	-	++	-	+	-	-	-	-	-	-	-	-	-	-	-
A. niger G 21	++	-	-	-	++	-	-	-	++	-	+	-	-	+	+	-	-	-
A.versicolor G 26	++	-	-	-	-	-	++	-	-	++	-	-	-	++	-	-	-	-
A. versicolor N 22	+++	-	-	-	++	-	+	-	-	-	+	-	-	-	-	-	-	-
F. oxysporum G 12	++	-	-	-	-	-	-	-	++	-	++	-	-	+++	-	-	-	-
F. oxysporum G 13	+	-	-	-	-	-	-	-	+	+	+	-	-	++	+	-	-	-
F. oxysporum G 27	+++	-	-	-	+	-	-	-	-	-	-	-	-	++	-	+	-	-
F. oxysporum G 28	++	-	-	-	++	-	+	-	-	+	-	-	-	+	-	-	-	-
F. oxysporum M 22	++	-	-	-	+	-	-	-	-	-	++	-	-	+	-	-	-	-
F. solani G 25	+	+	-	-	+	-	-	-	+	-	-	-	-	+	-	+	-	-
Rhizopus sp M 13	++	-	-	-	-	-	-	-	++	-	-	-	-	+	-	-	-	-
Rhizopus sp N 11	++	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-
Rhizopus sp N 21	-	-	-	-	-	-	-	-	+	-	+	-	-	++	-	+	-	-
Penicillium sp N 11	+++	-	-	-	++	-	-	-	-	-	++	-	-	+	-	-	-	-

1= LAB M11, 2= LAB M12, 3= LAB M13, 4= LAB M14, 5= LAB M15, 6= LAB M16, 7= LAB M21, 8= LAB M22. 9= LAB M23, 10= LAB M24, 11= LAB M25, 12= LAB M26, 13=LAB 27, 14=LAB M41, 15= LAB M42, 16=LAB M43, 17=LAB M44, 18=LAB M45

-: no visible inhibition; **+:** zone of inhibition located between 0.1-3% of the surface of the Petri dish, **++:** zone of inhibition located between 3-8% of the surface of Petri dish and **+++:** zone of inhibition > to 8% of the surface of Petri dish

Table 5. Antifungal activity of LAB isolated from the samples collected in Ngaoundere

	LAB																
Molds	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
A. carbonarius G 23	-	-	-	-	-	-	-	-	+	++	-	-	+	-	-	+++	-
A. carbonarius G 24	-	-	-	-	-	-	-	-	+	-	++	-	+	-	+	+	-
A. flavus G 14	-	-	-	-	-	-	-	-	+	++	++	-	+++	-	-	+	-
A. flavus G 22	-	-	-	-	-	-	-	-	+	+	-	-	++	-	+	++	-
A. flavus M 15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
A. flavus M 24	-	-	-	-	-	-	-	-	-	+ +	-	-	+	-	+	++	-
A. fumigatus M 23	-	-	-	-	-	-	-	-	-	+	+	-	+++	-	-	+	-
A. niger G 12	-	-	-	-	-	-	-	-	-	++	-	-	-	-	+	-	+
A. niger G 21	-	-	-	-	-	-	-	-	+	+	-	-	++	-	+	+++	-
A.versicolor G 26	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+++	+
A. versicolor N 22	-	-	-	-	-	-	-	-	+	+	-	-	++	-	-	+++	-
F. oxysporum G 12	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
F. oxysporum G 13	-	-	-	-	-	-	-	-	-	-	+	-	+++	-	-	+	++
F. oxysporum G 27	-	-	-	-	-	-	-	-	-	-	+	-	++	-	-	++	+
F. oxysporum G 28	-	-	-	-	-	-	-	-	-	++		-	++	-	-	+++	
F. oxysporum M 22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
F. solani G 25	-	-	-	-	-	-	-	-	-	++	+	-	+	-	-	+	-
Rhizopus sp M 13	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+++	+
Rhizopus sp N 11	-	-	-	-	-	-	-	-	-	-	-	-	++	-	-	+	+
Rhizopus sp N 21	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Penicillium sp N 11	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-

1=LAB N11, 2=LAB N12, 3=LAB N13, 4=LAB N14, 5=LAB N15, 6-=LAB N16, 7=LAB N17, 8=LAB N18, 9=LAB N21, 10=LAB N22, 11=LAB N23, 12=LAB N24, 13= LAB N25, 14= LAB N31, 15= LAB N32, 16= LAB N33, 17= LAB N 34

-: no visible inhibition; **+:** zone of inhibition located between 0.1-3% of the surface of the Petri dish, **++:** zone of inhibition located between 3-8% of the surface of Petri dish and **+++:** zone of inhibition > to 8% of the surface of Petri dish.

Table 6. Biochemical characterization of 6 LAB isolated from the dried corn, soaked corn and fermented corn paste of Garoua, Maroua and Ngaoundere

N°	Sugars	G11	G25	M11	M41	N25	N33
0	0	-	-	-	-	-	-
1	Glycerol	-	-	-	-	-	-
2	Erythritol	-	-	-	-	-	-
3	D-Arabinose	-	-	-	-	-	-
4	L-Arabinose	+	+	+	+	+	+
5	Ribose	+	+	+	+	+	+
6	D-Xylose	+	+	+	+	+	+
7	L-Xylose	-	-	-	-	-	-
8	Adonitol	-	-	-	-	-	-
9	β-méthyl-D-Xyloside	+	+	-	-	-	-
10	Galactose	+	+	+	+	+	+
11	Glucose	+	+	+	+	+	+
12	Fructose	+	+	+	+	+	+
13	Mannose	-	-	-	+	+	+
14	Sorbose	-	-	-	-	-	-
15	Rhamnose	-	-	-	-	-	-
16	Dulcitol	-	-	-	-	-	-
17	Inositol	-	-	-	-	-	-
18	Mannitol	-	-	-	-	-	-
19	Sorbitol	-	-	-	-	-	-
20	α-methyl-D-mannoside	-	-	-	-	-	-
21	α -methyl-D-Glucoside	+	?	+	-	-	-
22	N-acethyl-Glucosamine	+	+	?	?	-	-
23	Amygdalin	-	-	-	-	-	-
24	Arbutin	-	-	-	-	-	-
25	Esculin	-	-	-	+	-	-
26	Salicin	-	-	-	-	-	-
27	Cellobiose	-	-	-	-	-	-
28	Maltose	+	+	+	+	+	+
29	Lactose	-	-	-	-	+	+
30	Melibiose	+	+	+	+	+	+
31	Sucrose	-	-	+	+	+	+
32	Trehalose	-	-	-	+	-	-
33	Inulin	-	-	-	-	-	-
34	Melezitose	-	-	+	-	-	-
35	Raffinose	-	-	+	-	+	+
36	Starch	-	-	-	-	-	-
37	Glycogen	-	-	-	-	-	-
38	Xylitol	-	-	-	-	-	-
39	Gentiobiose	-	-	-	-	-	-
40	D-Turanose	-	-	-	-	-	-
41	D-Lyxose	-	-	-	-	-	-
42	D-Tagatose	-	-	-	-	-	-
43	D-Fucose	-	-	-	-	-	-
44	L-Fucose	-	-	-	-	-	-
45	D-Arabitol	-	-	-	-	-	-
46	L-Arabitol	-	-	-	-	-	-
47	Gluconate	+	+	+	+	+	+
48	2-Keto-Gluconate	-	-	-	-	-	-
49	5-Keto-Gluconate	-	-	+	+	-	-
		L. brevis **(99.9%)**	***L. brevis*** **(99.9%)**	***L. buchneri*** **(98.0%)**	***L. cellobiosus*** **(100 %)**	***L. fermentum*** **(99.1%)**	***L. fermentum*** **(99.1%)**

(+) Positive reaction
(-): Negative reaction
(?): Inconclusive reaction

3.4. Screening of Lactic Acid Bacteria for Antifungal Activity

Among the isolated LAB (53 LAB), 54.71% had antifungal activity on at least one.

3.5. Biochemical Identification of Selected LAB

Identification of the 06 LAB revealed that all belong to the genus *Lactobacillus* (Table 6). These observations are in agreement with those of several authors who have also noticed that the genus *Lactobacillus* is the dominant flora during fermentation of corn [22]. These *Lactobacilli* were identified as *Lactobacillus brevis* G11, *Lactobacillus brevis* G25, *Lactobacillus buchneri* M11, *Lactobacillus cellobiosus* M14, *Lactobacillus fermentum* N25 and *Lactobacillus fermentum* N33. Asmahan and Muna [23] also isolated *L. fermentum* and *L. brevis* during the fermentation of sorghum.

3.6. Antifungal Overlay Assay

Generally, the LAB inhibited the growth of molds on solid medium (Figure 3). However, this antifungal activity varied between the LAB species and the species of molds. It was noted for example that *L. brevis* G11 inhibited 91% of the fungal strains tested then followed by *L. brevis* G25 and *L. cellobiosus* M41 which inhibited 86% of molds tested (Table 7). However, *L. brevis* G25 (82 mm) and *L. cellobiosus* M41 (80 mm) registered the highest diameters of inhibition when tested respectively with *A. carbonarius* G24 and *A. carbonarius* G23. The antifungal activity of these LAB could be related to their ability to produce antimicrobial compounds during fermentation such as organic acids (lactic, acetic, formic, phenyllactic and caproic acids), carbon dioxide, hydrogen peroxide, diacetyl, ethanol and bacteriocins [24]. The amount of these antibacterial substances produced by these lactic acid bacteria may also explain the variation of the diameters of inhibition of fungal growth during fermentation [25]. However, some molds have been resistant to the antifungal activity of one or more LAB namely: *A. Niger* G12, *A. flavus* M15, *A. versicolor* and *Fusarium oxysporum* M22. These molds expressed resistance respectively to 67, 50 and 33% of the LAB tested. Indeed, some microorganisms have the ability to develop a natural resistance or to acquire resistance through mutation or genetic exchange in order to survive and continue their development [26]. The presence of resistant strains to the antifungal activity of LAB is a troublesome result. Furthermore, among the molds identified as being resistant, we noted the presence of AFB_1-producing strains such as *A. flavus* M15 which was resistant to 50% of the bacterial population.

Table 7. Antifungal activity of 06 LAB isolated from samples of dried corn, soaked corn and fermented corn paste of Maroua, Garoua and Ngaoundere

	Diameter of inhibition (mm) ± standard deviation					
Molds	***L. brevis* G11**	***L. brevis* G25**	***L. buchneri* M11**	***L. cellobiosus* M41**	***L. fermentum* N25**	***L. fermentum* N33**
A. niger G12	12±0.1^{a}	24±0.1^{c}	0	0	0	0
F. oxysporum G12	24±1.0^{bc}	32±0.1^{d}	20±0.2^{b}	28±0.1^{d}	26±0.1^{de}	16±0.1^{a}
F. oxysporum G13	44±0.3^{ef}	24±0.1^{c}	46±0.1^{f}	44±0.1f ^{gh}	34±0.1^{fg}	34±0.2^{ef}
A. flavus G14	30±0.1^{cd}	35±0.1^{d}	50±0.1^{g}	50±0.1^{i}	30±0.4^{ef}	40±0.2^{g}
A. niger G21	40±0.1^{e}	38±0.4^{de}	63±0.2^{h}	40±1.0^{fg}	50±1.0^{i}	33±0.3^{def}
A. flavus G22	32±0.1^{d}	42±0.2^{fg}	34±0.2^{d}	22±0.2^{bc}	16±0.1^{b}	35±0.2^{efg}
A. carbonarius G23	44±0.2^{ef}	80±0.5^{J}	72±0.2^{h}	70±0.2^{j}	40±0.1^{gh}	16±0.1^{a}
A. carbonarius G24	27±0.1^{bcd}	40±0.2^{ef}	12±0.1^{a}	82±0.1^{k}	70±0.2^{k}	30±0.2^{cde}
F. solani G25	0	70±0.8^{i}	74±0.2^{h}	25±0.2^{cd}	64±0.2^{jk}	50±0.1^{h}
A. flavus G 26	48±0.4^{f}	36±0.1^{de}	60±0.2^{g}	35±0.1^{e}	45±0.1^{hi}	40±0.1^{g}
F. oxysporum G27	50±0.3^{f}	18±0.1^{b}	25±0.1^{cA}	46±0.2^{hi}	44±0.1^{hi}	28±0.1^{bcd}
F. oxysporum G28	40±1.0^{e}	44±0.2^{fg}	20±0.3^{b}	50±0.2^{i}	40±0.2^{gh}	50±0.6^{h}
Rhizopus sp M13	0	10±0.2^{a}	13±0.1^{a}	20±0.0^{b}	0	14±0.1^{a}
A. flavus M15	12±0.2^{a}	24±0.1^{c}	0	0	0	24±0.1^{b}
F. oxysporum M 22	20±0.1^{b}	0	36±0.2^{de}	10±0.1^{a}	0	30±0.2^{cde}
A. fumigatus M23	50±0.1^{f}	45±0.2^{g}	0	34±0.2^{e}	23±0.1^{cd}	36±0.1^{fg}
A. flavus M24	46±0.2^{ef}	35±0.1^{d}	50±0.5^{f}	36±0.2^{ef}	60±0.2^{j}	25±0.2^{bc}
Rhizopus sp N11	20±0.2^{b}	0	36±0.1^{de}	10±0.1^{a}	0	0
Penicillium sp N 11	40±1.0^{e}	50±0.2^{h}	38±0.1^{e}	40±0.2^{fg}	40±1.0^{gh}	34±1.0^{ef}
Rhizopus sp N21	11±0.1^{a}	0	0	14±0.1^{a}	06±0.1^{a}	0
A. versicolor N22	10±0.1^{a}	20±0.1^{bc}	10±0.2^{a}	0	18±0.1^{bc}	14±0.1^{a}

Values followed by the same letter in the same column are not significantly different (P>0.05)
G: strains from samples of Garoua, M: strains from samples of Maroua, N: strains from samples of Ngaoundere

Figure 3. Typical zone of inhibition produced by LAB against fungi on overlay assay

3.7. Antifungal Activity in Liquid Medium

Unlike studies of Adebayor and Aderiye [27] which showed that, the sensitivity of certain molds (*Penicillium citrinum*) to LAB is similar in liquid medium as in solid medium. The antifungal activity of LAB in liquid medium is higher than in solid medium (Table 8). In a solid medium, *L. buchneri* M11, *L. cellobiosus* M41, *L. fermentum* N25 and *L. fermentum* N33 did not inhibit the growth of *A. niger* G12 after 7 days of incubation whereas, in liquid medium, the LAB inhibited the growth (100%) of *A. niger* G12 after 8 days of incubation. This capacity of the LAB to inhibit fungal growth in liquid medium was also observed by Gerez *et al.* [28]. They showed that, in liquid medium, *L. plantarum* CRL 778, *L. reuteri* CRL 1100, *L. brevis* CRL 772 and *L. brevis* CRL 796 had the capacity to inhibit the growth of certain molds, particularly *A. niger* CH101, *Penicillium* sp C_H 102 and *Fusarium graminearum* C_H 103.

Table 8. Percentage (%) of fungal growth inhibition with LAB

	Percentage of fungal growth inhibition (%)					
Molds	***L. brevis* G11**	***L. brevis* G 25**	***L. buchneri* M11**	***L. cellobiosus* M41**	***L. fermentum* N 25**	**L. *fermentum* N 33**
A. niger G12	100	100	83.89	100	100	100
F. oxysporum G12	100	45.4	100	28.6	98.6	100
F. oxysporum G13	100	88.00	100	100	98.00	100
A. flavus G14	100	100	100	100	0.36	100
A. niger G21	100	74.8	98.7	0	100	100
A. flavus G22	100	55.0	100	0	80.00	100
A. carbonarius G23	100	100	91.9	0	98.8	100
A. carbonarius G24	100	86	100	0	100	0
F. solani G25	100	68.0	100	57.2	99.4	100
A. flavus G26	100	92.9	99.1	100	100	65.9
F. oxysporum G27	100	100	100	0	92.5	100
F. oxysporum G28	100	100	100	100	100	100
Rhizopus sp M13	100	100	100	21.9	100	100
A. flavus M15	100	100	100	0	16.6	100
F. oxysporum M 22	100	100	89.4	63.1	93.4	100
A. fumigatus M23	100	95.2	83.2	0	100	100
A. flavus M24	100	85.7	100	54.2	87.6	100
Rhizopus sp N11	27.2	0	0	100	0	100
Penicillium sp N11	100	100	100	100	100	100
Rhizopus sp N21	100	100	100	82.3	100	100
A. versicolor N22	100	84.2	100	0	100	100

G: strains from samples of Garoua, M: strains from samples of Maroua, N: strains from samples of Ngaoundere.

This high antifungal activity in liquid medium could be explained by the fact that, in liquid medium, the micro-organisms multiply quickly, produce more antifungal compounds which easily diffuse and come into direct contact with the spores of molds [27].

The results obtained on the inhibition of fungal growth in liquid medium are interesting and allow the use of the LAB as starters in corn paste to fight against fungal growth during soaking (fermentation).

3.8. Nature of Antifungal Potential of Lactic Acid Bacteria

Fractions A, B, C and D showed an antifungal potency against the molds (Table 9). This shows that the antifungal activity of LAB is certainly not due to bacteriocins and hydrogen peroxide. However, fraction E that was obtained after treating the supernatant with NaOH showed a total lack of antifungal activity. This lack of activity of the fraction E, allows to conclude that, antifungal activity is due to organic acids produced by the LAB. Several studies have also demonstrated that the antifungal activity of LAB is linked to organic acids [28]. These organic acids (acetic acid, caproic acid, propionic, formic, butyric and n-valeric) produced by the LAB can also act synergistically against the molds. Lavermicocca *et al.* [30] showed that the antifungal activity *of L. plantarum* and *L. sanfrancisco* was respectively linked to 3-phenyllactic acid and caproic acid. The results obtained in this work and those of several studies [29] are contrary to those of Adebayo and Aderiye [27] which showed that bacteriocins produced by LAB isolated in the fermented food products (*Eko, Fufu, Iru*

and *Ogi*) were more responsible for their antifungal potency against *P. citrinum*, *A. flavus* and *A. niger*.

Table 9. Antifungal activity of fractions A, B, C, D and E

	***L. brevis* G11**					***L. Brevis* G25**					***L. buchneri* M11**					***L. cellobiosus* M41**					***L. fermentum* N25**					***L. fermentum* N33**				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
A. niger G12	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F. oxysporum G12	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-
F. oxysporum G13	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-
A. flavus G14	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-
A. niger G21	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-
A. flavus G22	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-
A. carbonarius G23	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-
A. carbonarius G24	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-
F. solani G25	-	-	-	-	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-
A. flavus G26	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-
F. oxysporum G27	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-
F. oxysporum G28	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-
Rhizopus sp M13	-	-	-	-	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	+	+	+	+	-
A. flavus M15	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-
F. oxysporum M 22	+	+	+	+	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	+	+	+	+	-
A. fumigatus M23	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-
A. flavus M24	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-
Rhizopus sp N11	+	+	+	+	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Penicillium sp N11	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-
Rhizopus sp N21	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-
A. versicolor N22	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+	-

+: Visible Inhibition; -: no visible inhibition
Fraction A, B and C: fraction respectively treated with proteinase K, the α-chymotrypsin and trypsin to degrade bacteriocins present in the filtrate;
Fraction D: fraction treated with catalase to eliminate
the hydrogen peroxide present in the filtrate; Fraction E: fraction treated with sodium hydroxide to neutralize the organic acids present in the filtrate.

4. Conclusion

Following this work, 06 LAB reduced the growth of 04 fungal genera (*Penicillium*, *Fusarium*, *Aspergillus* and *Rhizopus*) isolated from corn and fermented corn paste. However, this activity was more efficient in liquid medium than in solid medium. Therefore, it would be interesting to consider the use LAB in fermented corn paste to limit fungal contamination and eliminate toxic substances (AFB_1) during soaking of corn paste in the Sudano-Guinean (Ngaoundere)and Sudano-Sahelian (Maroua and Garoua) zones of Cameroon.

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