

Original article

## Assessment of Nutritional Composition and Antifungal Potential of Bacteriocinogenic Lactic Acid Bacteria from “Kati” against Toxigenic *Aspergillus flavus*

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### SUMMARY

In this study, the nutrient contents of “Kati”, a fermented cereal-based food, was revealed and antifungal activity of bacteriocin producing lactic acid bacteria (LAB) from “Kati” was assessed against aflatoxigenic *Aspergillus flavus* (*A. flavus*). The protein content (9.29%) of “Kati” was higher than ( $p < 0.05$ ) wet milled-fermented sorghum (6.17%). During fermentation of milled sorghum to ready-to-eat ‘Kati’, anti-nutrient contents was reduced ( $p < 0.05$ ) from 1.22 to 0.72 mg/100 g, 3.13 to 1.13 mg/100 g and 7.31 to 3.02 mg/100 g for tannin, phenol and phytates, respectively. Molecular technique revealed the identity of isolated LAB as *Lactobacillus pentosus* BS MP-10, *L. paracasei* 4G330, *L. brevis* ABRIINW, *L. casei* KG-5, *L. sakei* strain RFI LAB03, *L. fermentum* JCM 8607, *L. plantarum* KLDS 1.0607, *L. rhamnosus* JCM 8602 and *L. lactis* XLL1734. Among the isolated LAB, *L. plantarum*, *L. lactis* and *L. fermentum* have significant ( $p < 0.05$ ) zones of inhibition of 11.0 mm, 9.1 mm and 7.8 mm, respectively, against aflatoxigenic *A. flavus*. The pronounced antifungal potency of *L. plantarum* cell free supernatant could be attributed to the presence of 3-phenyllactic acid, benzenoacetic acid, plantaricin (bacteriocin) as revealed by gas chromatography/mass Spectrometry (GC-MS). LAB produced metabolites with antifungal property that contributed to shelf life, flavor and nutrient contents of fermented foods.

**Key words:** cereal, proximate, 3-phenyllactic acid, benzenoacetic acid, GC-MS, fermentation

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## INTRODUCTION

“Kati”, which is also called “Egidi” is a customary cereal-based and ready-to-eat food that is indigenous to Akoko town in Ondo State, south-western Nigeria. “Kati” is traditionally prepared from fermented and cooked sorghum or millet slurry, wrapped in *Ficus carica* and *Thaumatococcus daniellii* leaves. It is usually consumed as snacks in hot afternoon by breaking it into cold water (1). A wide range of cereal-based fermented foods and their related products from different countries alleviate food insecurity in different contexts (2): “Dosa” and “Idli” are popular traditional cereal fermented foods in South India and many parts of Sri Lanka (3). “Injera” is an undisputed national food of Ethiopians and it is made from different cereals, including finger millet, sorghum, teff, corn, and barley (3). “Soy sauce” is a blend of soya beans and coarse wheat into fermented dark brown liquid, commonly consumed in Japan and China (4). “Yosa” is an indigenous food consumed in Finland, produces from oat bran pudding that has been cooked in water and fermented with LAB and *Bifidobacteria* sp. (5). “Tarhana” is a Turkish fermented food from wheat, which is rich in protein and vitamins with an acidic and sour taste, having a strong yeasty flavour (5). “Pito” is a cereal-based fermented food prepared from sorghum, millet and maize with varying alcohol content, which is often consumed in Ghana and Southern Nigeria (6). “Ogi”, a popular fermented food from West Africa, is made by lactic acid fermentation of corn, but sometimes, sorghum or millet and soybeans may be added to improve its nutritive value (5). “Masa” is also a Nigeria traditional fermented food product made from maize (3).

Fermentation of cereals into based-foods is a popular custom in Africa that improves the availability of foods (7). With this regard, general awareness of the importance of fermented foods has not only been based on benefits of fermented foods, provision of energy, body maintenance but health benefits of fermenting microorganisms. Some microorganisms involved in fermentation, especially LAB and yeast, are known to produce flavour enhancing compounds, important enzymes, amino acids and antimicrobial substances such as bacteriocin, organic acids, hydrogen peroxide, antifungal peptides and other compounds that contribute to extensive shelf life of food products (8). Fermented food products from cereals are nutritious, with some antifungal

LAB (9). In the findings of Afolabi et al. (10), *Lactobacillus casei*, *Lactobacillus salivarius*, *Lactobacillus jensenii*, *Lactobacillus cellobiosus*, *Lactobacillus plantarum*, *Lactobacillus delbrueckii*, and *Lactobacillus fermentum* were predominantly isolated from traditionally fermented “Kati” and their presence was attributed to organoleptic property of “Kati”. However, there is little or no report on the occurrence of antifungal LAB (bacteriocin producer) from “Kati”. Hence, this research aimed to evaluate the nutrient contents and to assess antifungal activity of bacteriocin producing LAB from “Kati” against aflatoxigenic fungus.

## MATERIALS AND METHODS

### Source of grains

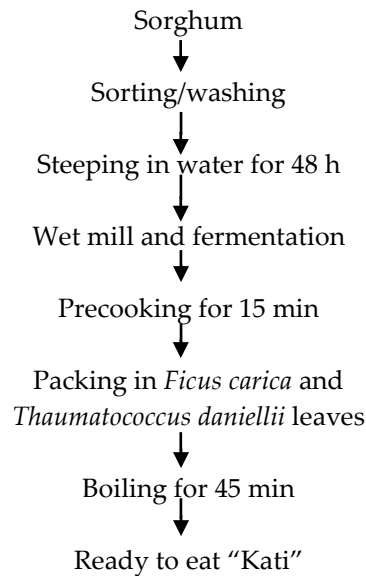
Sorghum (*Sorghum bicolor*) was purchased from a local retail outlet at King’s market, Akure. It was transported in a clean bag to Microbiology laboratory, The Federal University of Technology, Akure (FUTA), Nigeria for further analysis.

### Source of toxigenic *A. flavus*

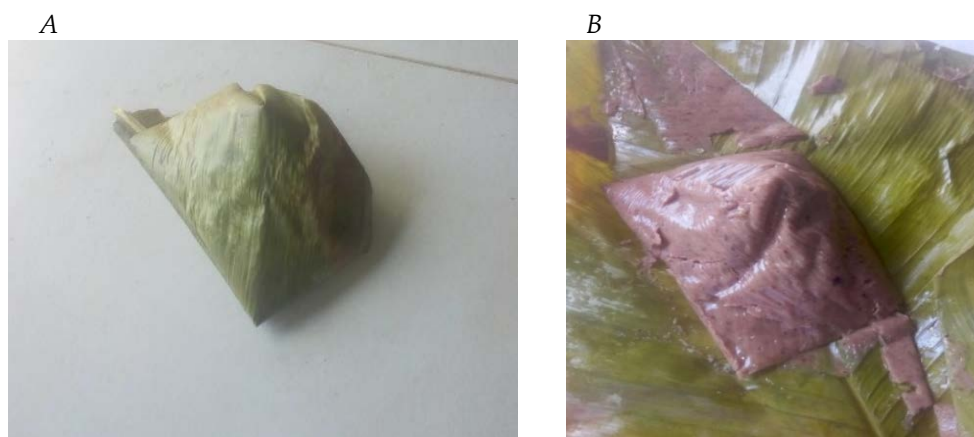
The studied toxigenic *A. flavus* was isolated from damp infested sorghum. Grains were finely grinded with a mill machine (5657 HAAN 1 TYPE ZM1, Retsch GmbH, Haan, Germany). One gram of milled sorghum was dissolved in 9 ml sterile distilled water, serially diluted and aliquots were aseptically dispensed on Potato Dextrose Agar (PDA) plates using spread plate method. PDA plates were incubated for 7 days at  $25 \pm 2^\circ\text{C}$ . Colonies of *A. flavus* were screened and identified based on morphology and mycelia character. The identification of mould was determined by comparing the observed morphological characteristics with those described by Samson et al. (11).

### Preparation of “Kati”

The traditionally fermented gruel was processed according to the method of local producers in Arigidi Akoko with little modification that majorly focused on improving hygiene (Figure 1). Briefly, 500 g of sorghum was sorted and washed with clean water and steeped in 1,000 ml of water at  $28 \pm 2^\circ\text{C}$  for 48 h. Thereafter, water was removed and grains were wet milled. The slurry was allowed to ferment for 24 h, after which it was placed in a vessel, pre-



**Figure 1.** Processes involved during the production of 'Kati'



**Figure 2.** A sample of (a) "Kati" wrapped in *F. carica* and *T. daniellii* leaves (b) unwrapped "Kati"

cooked for 15 min and thoroughly mixed with continuous stirring using a wooden turning stick to obtain a thick paste. The pre-cooked "Kati" was moulded by wrapping in *Ficus carica* and *Thaumatococcus daniellii* leaves (Figure 2). The wrapped "Kati" was cooked in a pot under smoldering fire for 45 min, then offloaded and allowed to cool, after which was ready for consumption. Samples were aseptically taken at each stage of production for further analysis.

#### Isolation of LAB from "Kati"

LAB were isolated from "Kati" by weighing 10 g into 90 ml of bacteriological peptone water (Oxoid,

Basingstoke, UK). After homogenization, a tenfold serial dilution of sample was carried out and 0.1 ml of dilutions (10 - 5) was plated onto de Man, Rogosa and Sharpe (MRS) agar (Oxoid, Basingstoke, UK). The MRS plates were incubated at 37°C under anaerobic condition for 48 - 72 h. Pure colonies were isolated by sub-cultured, stored on MRS slants and maintained at 4°C for further studies. Gram's staining, spore staining, and biochemical tests such as catalase, nitrate reduction, oxidase, urease production, and sugar fermentation were carried out according to the methods described by Cheesbrough (12). Staining reactions and interpretation of biochemical tests were used for the identification of LAB according to Cowan and Steel (13).

### Identification of LAB isolates using 16S rRNA gene amplification

LAB isolates were grown overnight in a liquid MRS broth at 37°C and transferred to Eppendorf tube. The samples were spun at 14,000 rpm for 2 min after which the supernatant was discarded and DNA was extracted using cetyl trimethylammonium bromide (14). Afterwards, DNA was re-suspended in 100 µl of sterile distilled water. DNA concentration of samples was measured on spectrophotometer at 260 nm and the genomic purity was also determined. Agarose gel (1.0%) was used to check DNA and visualized using UV light. The primer used for PCR amplification was 16S universal primer for bacteria; the sequence for the forward primer was 5'AGAGTTTGATCCTGGCTCAG3' and reverse primer was 5'ACGGCTACCTTGTTACGACTT3'. PCR mix was made up of 1µl of 10X buffer, 0.4 µl of 50 mM MgCl<sub>2</sub>, 0.5 µl of 2.5 mM dNTPs, 0.5 µl 5 mM forward primer, 0.5µl of 5 mM reverse primer, 0.05 µl of 5 units/ul Taq with 2 µl of template DNA and 5.05 µl of distilled water. The PCR profile used has an initial denaturation temperature of 94°C for 3 mins, followed by 30 cycles of 94°C for 60 s, 56°C for 60 s, 72°C for 120 s and the final extension temperature of 72°C for 5 min and the 10°C hold for 1 hour. Afterwards, the amplicon was purified before sequencing with 2M sodium acetate (15).

### Determination of nutrient and anti-nutrient contents in "Kati"

The proximate, mineral and anti-nutrient compositions of sorghum, wet milled and fermented sorghum, and the finished "Kati" were determined according to the methods described by Association of Official Analytical Chemists (16). Total carbohydrates content of the samples was calculated by subtracting the percent of moisture, crude protein, crude fibre, crude fat, and ash from 100.

### Screening of *A. flavus* for aflatoxin B1

A test for aflatoxin was carried out according to the method described by Okwu et al. (17). The reverse side of toxigenic *A. flavus* plates were exposed to UV light at 360 nm, and bright greenish-yellow fluorescence indicated the presence of aflatoxin. Aflatoxin analysis was further carried out with thin layer chromatography (TLC) according to Criseo et al. (18) with slight modifications. *A. flavus* in broth (5

ml) was transferred into a 250 ml conical flask; 15 ml of distilled water and 50 ml of chloroform were added and shaken for 1 h. The sample was filtered in a separating funnel, while chloroform layer was allowed to filter through anhydrous sodium-sulphate. The extraction was repeated twice using 50 ml chloroform and the collected filtrate was concentrated. The filtrate of each sample was re-dissolved in 1 ml chloroform and 10 µl of the re-dissolved filtrates were applied on TLC plates. The plates were developed in a solvent system of toluene: ethyl acetate: formic acid (6:3:1 v:v:v) in a chromatographic tank for about 25 min. Pure aflatoxin (0.0025 µg) used as standard was spotted on TLC plates in parallel lane and visualized under the UV light (366 nm).

### Screening of LAB for bacteriocin production

Bacteriocin-producing LAB were screened using the method described by Salasiah et al. (19). Briefly, LAB isolated from "Kati" were grown in MRS broth, incubated at 37°C for 48 h and their cell free supernatant (CFS) was obtained. Thereafter, CFS was centrifuged at 6000 rpm for 10 min and sediment was separated from supernatant by decantation. To rule out the inhibitory effect of other LAB metabolites, apart from bacteriocin, supernatant was filtered (0.22 µm) and 35 µl of CFS aliquot was added to the first well on a prepared solidified MRS agar plate. The remaining CFS was adjusted to pH 6.0 with 1 mol NaOH in order to prevent a likely inhibition due to organic acids. CFS (35 µl) was transferred to the second well. The remaining neutralized CFS was then treated with 1.0 mg/ml of catalase (Sigma, USA) at 25°C for 30 min to exclude the possible inhibitory action of H<sub>2</sub>O<sub>2</sub>. This was filtered and transferred into the third well on the agar plate.

### Gas chromatography/mass spectrometry (GC-MS) analyses of *L. plantarum* CFS

GC-MS analysis of *L. plantarium* CFS as the most effective and bacteriocin producing LAB was qualitatively performed using Varian 4000 GC-MS system (Agilent Technologies, Santa Clara, CA, USA) to show the component of bacteriocin. The ionization voltage was maintained at 70 eV. The injection port temperature was ensured as 250°C and nitrogen flow rate was 1.0 ml/min. The sample (2 µl) was injected in split mode as 10:1 into the GC-MS

equipment. The compounds were separated on a 30 m long capillary column (HP-5MS), which is 0.25 mm in diameter and with 0.25  $\mu\text{m}$  thick stationary phase layer (5% phenyl)-methylpolysiloxane. The instrument was set to an initial temperature of 110°C for 2 min. At the end of this period, the oven temperature was increased to 280°C at an increased rate of 6°C per min and the oven was maintained at this temperature for 9 min. The total analysis time was 55 min and the mass spectral scan was set within 50 - 650 m/z. Identification of chemical structure of secondary metabolites was based upon the interpretation of mass spectra libraries of NIST/EPA/NIH.

#### *In vitro* antifungal activity of LAB against toxigenic *A. flavus*

Antifungal LAB isolated from "Kati" were tested against toxigenic *A. flavus* using agar overlay method described by Lind et al. (20) with slight modifications. Briefly, LAB was cultured in sterile MRS broth and incubated at 35  $\pm$  2°C for 48 h under the anaerobic condition. After incubation, crude culture of each LAB was centrifuged to obtain a CFS. An aliquot of 100  $\mu\text{l}$  from CFS of LAB were aseptically transferred into a separate well of 6.0 mm containing 10 ml of MRS agar. Sterile distilled water (100  $\mu\text{l}$ ) was used as a negative control, while an an-

tifungal drug ketoconazole was used as a positive control. The inoculated plates were incubated at 35 °C and observed for growth for 2 days. After incubation, plates were then overlaid with 10 ml of PDA, inoculated with 100  $\mu\text{l}$  of mould spore (10<sup>6</sup> spores/ml) and incubated at 28  $\pm$  2°C for 72 h. Thereafter, zones of inhibition were recorded in millimeter (mm). The synergistic effect of the best three CFS of LAB was further tested against toxigenic *A. flavus*.

#### Statistical analysis

All experimental studies were performed in replicate (n = 3). Data were subjected to one-way analysis of variance (ANOVA) and values were presented as mean  $\pm$  standard deviation (SD). Tests of significant differences were determined by Duncan's Multiple Range Test at p < 0.05. The statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 23 software undertaken.

## RESULTS

#### Proximate, mineral and anti-nutritive contents of 'Kati'

Table 1 shows nutritive and anti-nutritive composition of "Kati" at the various stages of pro-

**Table 1.** Proximate (%), mineral (mg/100g) and anti-nutrient (mg/100g) contents of fermented sorghum and finished 'Kati'

Test	Sorghum	Wet milled and fermented sorghum	Finished "Kati"
<b>Proximate (%)</b>			
Moisture	44.81 $\pm$ 0.47 <sup>a</sup>	63.70 $\pm$ 0.69 <sup>a</sup>	62.46 $\pm$ 1.44 <sup>a</sup>
Protein	4.47 $\pm$ 0.75 <sup>c</sup>	6.17 $\pm$ 0.49 <sup>b</sup>	9.29 $\pm$ 0.21 <sup>a</sup>
Fat	5.09 $\pm$ 1.11 <sup>a</sup>	3.03 $\pm$ 0.28 <sup>b</sup>	3.04 $\pm$ 0.10 <sup>b</sup>
Fiber	6.55 $\pm$ 0.58 <sup>a</sup>	1.89 $\pm$ 0.24 <sup>b</sup>	2.03 $\pm$ 0.00 <sup>b</sup>
Ash	2.18 $\pm$ 0.69 <sup>a</sup>	2.22 $\pm$ 0.75 <sup>a</sup>	1.19 $\pm$ 0.08 <sup>b</sup>
CHO	36.90 $\pm$ 1.33 <sup>a</sup>	22.99 $\pm$ 0.87 <sup>b</sup>	22.00 $\pm$ 0.98 <sup>b</sup>
<b>Minerals (mg/100 g)</b>			
K	7.63 $\pm$ 0.03 <sup>a</sup>	1.27 $\pm$ 0.03 <sup>b</sup>	1.14 $\pm$ 0.02 <sup>b</sup>
Ca	14.30 $\pm$ 0.03 <sup>a</sup>	5.43 $\pm$ 0.03 <sup>c</sup>	11.30 $\pm$ 0.03 <sup>b</sup>
Na	5.02 $\pm$ 0.03 <sup>a</sup>	4.15 $\pm$ 0.04 <sup>b</sup>	5.43 $\pm$ 0.03 <sup>a</sup>
Mg	4.27 $\pm$ 0.03 <sup>b</sup>	4.40 $\pm$ 0.03 <sup>b</sup>	7.43 $\pm$ 0.03 <sup>a</sup>
<b>Anti-nutrients (mg/100 g)</b>			
Tannin	1.22 $\pm$ 0.03 <sup>a</sup>	0.75 $\pm$ 0.04 <sup>b</sup>	0.72 $\pm$ 0.04 <sup>b</sup>
Phytates	7.31 $\pm$ 0.02 <sup>a</sup>	3.16 $\pm$ 0.04 <sup>b</sup>	3.02 $\pm$ 0.02 <sup>c</sup>
Phenol	3.13 $\pm$ 0.02 <sup>a</sup>	2.23 $\pm$ 0.04 <sup>b</sup>	1.13 $\pm$ 0.02 <sup>c</sup>

Values are mean of replicates (n = 3). Values with different alphabet along the rows are significantly different from each other at  $p < 0.05$

duction. There was an increase in moisture and protein contents of fermented gruel from 44.81 to 62.46% and 4.47 to 9.29%, respectively, when compared to unfermented sorghum. There was a decrease ( $p < 0.05$ ) in ash, crude fibre, fat and carbohydrate contents in sorghum to finished "Kati" from 2.18 to 1.19%, 6.55 to 2.03%, 5.09 to 3.04% and 36.90 to 24.03%, respectively. Potassium, calcium and sodium in unfermented sorghum respectively reduced from 7.63 mg/100 g, 14.3 mg/100 g and 5.02 mg/100 g to 1.27 mg/100 g, 5.43 mg/100 g and 5.51 mg/100 g in wet milled and fermented sorghum. The finished "Kati" had potassium and calcium contents of 1.14 mg/100 g and 11.3 mg/100 g, respectively. An increase ( $p < 0.05$ ) in sodium content (5.43 mg/100 g) was observed, which could be due to deliberate addition of table salt (NaCl) to the finished product. The anti-nutrient contents - tannin, phytate and phenol decreased ( $p < 0.05$ ) from 1.22 mg/100 g, 7.31 mg/100 g and 3.13 mg/100 g in sorghum to 0.75 mg/100 g, 3.16 mg/100 g and 2.23 mg/100 g, respectively after fermentation of wet milled sorghum. The lowest anti-nutritional contents of tannin (0.72 mg/100 g), phytate (3.02 mg/100 g) and phenol (1.13 mg/100 g) were observed in the finished "Kati".

#### Molecular identification of bacterial isolates

Molecular identities of the LAB isolates are

shown in Table 2. The lengths of amplified products were 1676, 1475, 1740, 1508, 1453, 1515, 1471, 1511 and 1449 base pair for *L. pentosus*, *L. paracasei*, *L. brevis*, *L. casei*, *L. sakei*, *L. fermentum*, *L. plantarum*, *L. rhamnosus* and *L. lactis* respectively (Figure 3). Based on the 16SrRNA sequences, *L. pentosus*, *L. plantarum*, *L. brevis*, *L. fermentum*, *L. sakei*, *L. casei*, *L. paracasei*, *L. rhamnosus* and *L. lactis* were confirmed to be *L. pentosus* BS MP-10, *L. paracasei* 4G330, *L. brevis* ABRIINW, *L. casei* KG-5, *L. sakei* RFI LAB03, *L. fermentum* JCM 8607, *L. plantarum* KLDS 1.0607, *L. rhamnosus* JCM 8602 and *L. lactis* XLL1734, respectively.

#### Antifungal activity of bacteriocin producing LAB against aflatoxigenic *A. flavus*

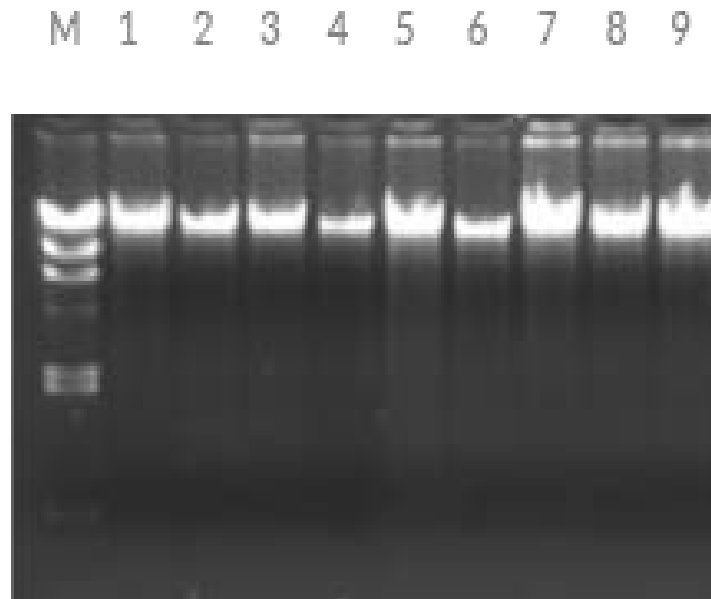
The zones of inhibition displayed by CFS of *L. plantarum*, *L. fermentum*, and *L. lactis* as well as their synergistic combination against toxigenic *A. flavus* were presented in Table 3. *L. plantarum* have the highest zone of inhibition against toxigenic *A. flavus* with 11.0 mm, followed by *L. lactis* and *L. fermentum* with inhibitory zones of 9.1 mm and 7.8 mm, respectively. The major antifungal components detected in the CFS of *L. plantarum* isolated from "Kati" using GCMS include: plantaricin, benzeneacetic acid and 3-phenyllactic acid with their empirical formula and molecular weight (Figure 4).

**Table 2.** Molecular identity of LAB isolated from "Kati"

*Microorganisms	Molecular identity	Genbank accession no.	Similarity (%)
<i>Lactobacillus pentosus</i>	<i>L. pentosus</i> strain BS MP-10	FR871789.1	99
# <i>Lactobacillus plantarum</i>	<i>L. paracasei</i> strain 4G330	MK026811.1	96
<i>Lactobacillus brevis</i>	<i>L. brevis</i> strain ABRIINW	JN368471.1	99
# <i>Lactobacillus fermentum</i>	<i>L. casei</i> strain KG-5	KF263160.1	92
<i>Lactobacillus sakei</i>	<i>L. sakei</i> strain RFI LAB03	EU141957.1	91
# <i>Lactobacillus casei</i>	<i>L. fermentum</i> strain JCM 8607	AB690195.1	96
# <i>Lactobacillus paracasei</i>	<i>L. plantarum</i> strain KLDS 1.0607	EU419597.1	98
<i>Lactobacillus rhamnosus</i>	<i>L. rhamnosus</i> strain JCM 8602	AB690191.1	98
<i>Lactococcus lactis</i>	<i>L. lactis</i> strain XLL1734	MG983982.1	89

\*LAB isolated from "Kati"

#Isolated *Lactobacillus* spp. has more closeness to another LAB strain after using molecular tool



**Figure 3.** Agarose gel electrophoresis plate of LAB isolates

Keys: M = Molecular marker, 1 = *Lactobacillus pentosus* (1676); 2 = *Lactobacillus plantarum* (1471); 3 = *Lactobacillus brevis* (1740); 4 = *Lactobacillus fermentum* (1515); 5 = *Lactobacillus sakei* (1453), 6 = *Lactobacillus casei* (1508), 7 = *Lactobacillus paracasei* (1475), 8 = *Lactobacillus rhamnosus* (1511) and 9 = *Lactococcus lactis* (1449).

**Table 3.** Zones of inhibition (mm) of LAB cell free supernatant and their synergistic effects against toxigenic *A. flavus*

LAB isolates	Zones of inhibition
<i>L. plantarum</i>	11.00 ± 0.43 <sup>b</sup>
<i>L. lactis</i>	9.10 ± 0.16 <sup>c</sup>
<i>L. fermentum</i>	7.80 ± 0.41 <sup>d</sup>
<i>L. rhamnosus</i>	4.00 ± 0.00 <sup>e</sup>
<i>L. paracasei</i>	3.90 ± 0.00 <sup>f</sup>
<i>L. pentosus</i>	3.80 ± 0.01 <sup>f</sup>
<i>L. casei</i>	3.30 ± 0.02 <sup>g</sup>
<i>L. brevis</i>	3.20 ± 0.02 <sup>g</sup>
<i>L. sakei</i>	3.00 ± 0.01 <sup>g</sup>
<b>Synergistic effect</b>	
<i>L. plantarum</i> + <i>L. lactis</i>	6.30 ± 0.04 <sup>d</sup>
<i>L. fermentum</i> + <i>L. lactis</i>	5.10 ± 0.05 <sup>e</sup>
<i>L. plantarum</i> + <i>L. fermentum</i>	4.20 ± 0.10 <sup>e</sup>
<i>L. plantarum</i> + <i>L. fermentum</i> + <i>L. lactis</i>	7.40 ± 0.20 <sup>d</sup>
<b>Ketoconazole</b>	16.10 ± 1.20 <sup>a</sup>

Values are mean of replicates (n = 3). Value with different alphabet along column are significantly different from each other at p < 0.05

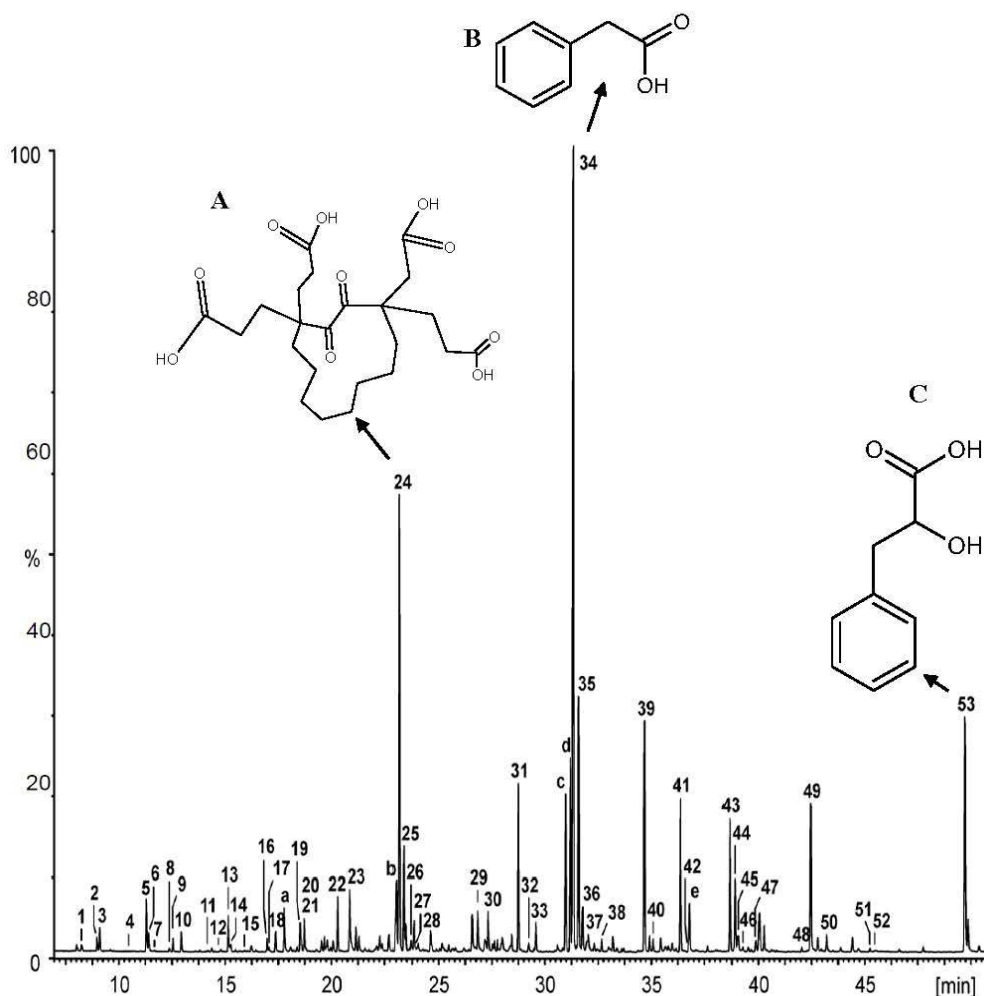


Figure 4: Chromatogram with peak

A: plantaricin C<sub>24</sub>H<sub>36</sub>O<sub>10</sub> (484.542 g/mol), B: benzeneacetic, C<sub>9</sub>H<sub>10</sub>BrNO<sub>2</sub> (244.08 g/mol) and C: 3-phenyllactic C<sub>9</sub>H<sub>10</sub>O<sub>3</sub> (166.17 g/mol) from CFS of *L. plantarum*

## DISCUSSION

The appreciable features of “Kati” could be attributed to its nutritional quality as well as aroma from leaves used for wrapping. Studies have shown that the presence of aromatic (sweet smelling) oil in *T. danielli* leaf enhance flavour in foods (21, 22). The methanol extract of *F. carica* leaf has been reported to possess a strong antibacterial activity against oral bacteria (23). *F. carica* leaf could impact natural antibacterial enhancement to the food. These are medicinal leaves commonly used to treat various illnesses such as gastrointestinal (colic, indigestion, loss of appetite, and diarrhea), respiratory (sore throats, coughs, and bronchial problems), cardiovascular disorders, inflammation and act as antispasmodic remedy (24).

Fermentation reduced the anti-nutritional content of “Kati”. The reduction could be linked to solubility of tannins in water during fermentation (25). Also, some microorganisms produce enzymes that break down anti-nutrient. Phytase produced by *L. brevis* and *Bacillus subtilis* breakdown phytates in fermented foods (26). A similar observation was reported by Esekheigbe and Onimawo (27) when fermentation resulted in significant reduction of anti-nutrients during the fermentation of African walnut. There was an increase in protein content of “Kati”. This could be as a fact that fermentation aids in liberation of nutrients locked (indigestible materials) in plant structures and anabolic synthesis of several complex growth factors by microorganisms (28). However, fermentation reduced fat and carbohydrate contents of “Kati”. This could be a result of



microorganisms utilizing carbohydrate and fat as a source of energy (25). A similar observation was reported by Modupe et al. (29). The researchers reported that microorganisms produce amylase, glucosidase and galactanase to degrade carbohydrates into simple sugars and thus utilize the sugar as energy source, which is also used for other metabolic activities. Fermentation significantly increased all minerals determined in wet milled sorghum except for potassium, which could possibly be due to leaching. A similar observation was reported by Esekheigbe and Onimawo (27). The researchers reported an increase in mineral contents as a result of fermentation and heat treatment on the nutritional composition of African walnut.

The presence of LAB in "Kati", particularly *L. plantarum*, *L. lactis*, *L. casei*, *L. fermentum* and *L. brevis* agrees with findings of Suman et al. (3). Researchers isolated and identified similar bacteria from cereal-based fermented foods/beverages; "Idli", "Dosa", "Injera", "Soy sauce", "Yosa", "Ogi" and "Masa" around the world. Hence, in fermented foods, the presence of LAB and their products improve dietary status and shelf life with various health benefits (2). The importance of LAB has been principally linked to improvement of gastrointestinal health, lactose intolerance, ulcer, cancer and immunosuppression at intestinal and systemic level (30). 16S rRNA gene sequence revealed the identity of nine LAB isolated from "Kati". The genus *Lactobacillus* was predominant, representing 89% of isolates, whereas *Lactococcus lactis* strain XLL1734 represented the remaining 11%. LAB are frequently isolated from locally fermented foods and improve their shelf life due to inhibitory substances. LAB possess a strain-specific spectrum and inhibitory mechanism against different moulds and yeast (31, 32).

The use of CFS from isolated LAB against toxigenic *A. flavus* reflect a remarkable inhibitory potential as an alternative source of antifungal agent. Russo et al. (33) stated that incorporation of CFS into agar plates as antifungal agent was a fast method to establish anti-mycotic potential of LAB. Hence, an *in vitro* study revealed a noteworthy inhibition of toxigenic *A. flavus* by isolated LAB from "Kati". The findings of Ghazvini et al. (34) showed a similar observation as *B. bifidum* and *L. fermentum* significantly reduced mycelia growth of toxigenic *A. parasiticus*. The inhibition of fungal growth by LAB has been attributed to nutritional competition, secondary metabolites, pH or their combinations (35). Of

the various LAB isolated from "Kati", *L. plantarum* KLDS 1.0607 displayed the highest hyphal radial growth inhibition against the toxigenic fungus when compared to *L. fermentum* JCM 8607, *L. lactis* XLL 1734 and their synergistic combination with a close proximity. A similar result was reported by Wang et al. (36) who observed that CFS of *L. plantarum* IMAU10014 was able to completely inhibit the growth of plant pathogen named *Phytophthora drechsleri*. The occurrence and antifungal property of *L. plantarum* has been reported and thus displayed significance in fermentation and bio-preservation (37).

A number of studies have shown that the inhibition of toxigenic fungi by LAB strains involved the reduction of aflatoxins (AFs) through a binding process to the AFs main sites (38). According to Mishra and Das (39), two significant sites with toxic activities of AFs are furofuran and lactone rings. Altering AFs coumarin structure and cleavage of their difuran rings have been reported to detoxify and change the mutagenic properties of AF molecules (40, 41). Hence, antifungal potential of LAB against toxigenic *A. flavus* can be attributed to a wide variety of active antagonistic metabolites like organic acids, carbon dioxide, ethanol, hydrogen peroxide, fatty acids, acetoin, diacetyl, cyclic dipeptides, and bacteriocins (42). The antifungal activity of isolated LAB against toxigenic *A. flavus* could be a result of functional metabolites secreted. Metabolites such as 3-phenyllactic acid, benzenoacetic acid and others produced by *L. plantarum* were revealed by GC-MS. In the studies of Lavermicocca et al. (43), they have linked 3-phenyllactic acid produced by *L. plantarum* to its antifungal activity when tested against species of *Aspergillus*, *Penicillium*, and *Fusarium*. Wang et al. (36) attributed the inhibition of *F. oxysporum*, *P. citrinum* and *P. drechsleri* by *L. plantarum* to antifungal metabolites like 3-phenyllactic acid and benzenoacetic acid, 2propenyl ester produced by the bacteria, which were detected and identified by using HPLC, LCMS, GCMS and NMR.

There was a reduction in antifungal effect by CFS of LAB when they were synergistically combined against toxigenic *A. flavus*. This observation corresponded to the findings of Marcia et al. (44) who recorded no significant antifungal activity when some commercial starters such as *Lactobacillus rhamnosus* CIRM-BIA1759, *Lactobacillus paracasei* CIRM-BIA1761, *Lactobacillus plantarum* CIRM-BIA 1758 were synergistically tested against yeasts and

filamentous moulds and thus suggests a low interaction (metabiosis) between the starter cultures and the antifungal adjunct cultures.

## CONCLUSION

Lactic acid fermentation improved the nutritional quality of “Kati” and reduced their anti-nutritional composition; hence, it can be asserted that “Kati” is an acceptable food with different functionality. LAB isolated from “Kati” inhibited the growth

of toxigenic *A. flavus* through the production of low-molecular-weight antifungal metabolites that created microbial inhibition by competition. The LAB in “Kati” can be used as starter cultures to produce other foods due to their antifungal property, which will improve shelf life of food as well as nutritional values.

## Conflict of interest

None.

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## Procena nutritivnog sastava i antifungalni potencijal bakteriocinogenih bakterija mlečne kiseline iz katija na toksigeni *Aspergillus flavus*

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### SAŽETAK

U ovoj studiji je prikazan nutritivni sastav katija, fermentisane hrane bazirane na žitaricama, kao i antifungalna aktivnost bakteriocina, koji proizvodi bakterije mlečne kiseline, iz katija na aflatoksigeni *Aspergillus flavus* (*A. flavus*). Sadržaj proteina (9,29%) u katiju bio je viši ( $p < 0,05$ ) nego u vlažnom mlevenom fermentisanom sorgumu (6,17%). U toku fermentacije mlevenog sorguma do faze spremnog katija, antinutritivni sadržaj bio je smanjen ( $p < 0,05$ ) od 1,22 na 0,72 mg/100 g, 3,13 na 1,13 mg/100 g i 7,31 na 3,02 mg/100 g za tannin, fenol i fitate, po prikazanom redosledu. Molekularne tehnike pokazale su identitet izolovanih bakterija mlečne kiseline: *Lactobacillus pentosus* BS MP-10, *L. paracasei* 4G330, *L. brevis* ABRIINW, *L. casei* KG-5, *L. sakei* soj RFI LAB03, *L. fermentum* JCM 8607, *L. plantarum* KLDS 1.0607, *L. rhamnosus* JCM 8602 i *L. lactis* XLL1734. Među izolovanim bakterijama mlečne kiseline, *L. plantarum*, *L. lactis* i *L. fermentum* imale su značajne zone inhibicije ( $p < 0,05$ ) od 11,0 mm, 9,1 mm i 7,8 mm, na aflatoksigeni *A. flavus*. Izražen antifungalni potencijal supernatanta *L. plantarum* bez ćelija može se pripisati prisustvu 3-fenilaktične kiseline, fenilsirćetne kiseline, plantaricina (bakteriocina), na šta je ukazala gasna hromatografija – masena spektrometrija (GC-MS). Bakterije mlečne kiseline proizvele su metabolite sa antifungalnim svojstvima, koji su uticali na rok upotrebe, ukus i nutritivni sastav fermentisane hrane.

**Ključne reči:** žitarica, proksimativan, 3-fenilaktična kiselina, fenilsirćetna kiselina, gasna hromatografija – masena spektrometrija, fermentacija