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Research Article

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Perspective of biological fight against fungal germs contaminant corn (*Zea mays*): Case of the use of lactic bacteria

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ABSTRACT
Moulds are contaminants of foodstuffs, particularly cereals. They produce toxins with harmful effects on consumer health. The objective of this work was to select isolates of lactic acid bacteria (LAB) from maize (*Zea mays*), with antifungal activity, for a probable biological control of fungal germs contaminating maize. Thus, the microbiological analysis of the fermented maize paste allowed the isolation of forty (40) isolates of lactic bacteria, including 3 bacilli (7.5%) heterofermentative, 10 bacilli (25%) homofermentative and 27 cocci (67.5%) homofermentative. The comparison test between mycotoxin-producing moulds of the genus Aspergillus (flavus and niger), Fusarium sp and Penicilium sp and each isolate of lactic acid bacteria, carried out on MRS agar (CondiLab, Spain), revealed their inhibitory effect on the growth of these moulds from maize. Of the 40 LAB isolates obtained during the 3 days of fermentation, 34 (85%) isolates showed antagonistic activity on the 4 fungal germs tested. The isolates LABT0.2, LABT0.7, LABT1.6, LABT1.7, LABT2.10, LABT3.2, LABT3.4, LABT3.6, LABT3.9 and LABT3.10 were the ones with very good antifungal activity, with inhibition diameters ranging from 5.0 ± 0.0 to 7.1 ±0.14 cm. In sum, this study revealed the existence of lactic acid bacteria with antifungal activity, which can be exploited for biological control of cereal contaminants in general, and those of maize in particular, with a view to guaranteeing the safety of food for human consumption.
Keywords: Mycotoxins, lactic acid bacteria with antifungal activity, biological control, food safety, maize (*zea mays*).

INTRODUCTION

Maize (*zea mays*) is a tropical plant of the Gramineae family, widely used in human and animal food (poultry, pigs, cattle) (Boukar, 2017). In Côte d'Ivoire, maize is grown in several agro-ecological regions. Constituting the staple food of many Ivorian populations, maize is also used as a raw material in certain industries such as breweries, soap factories and oil mills. After harvesting, maize is generally preserved in small-scale storage facilities for varying lengths of time, depending on human and animal consumption needs. However, during storage, maize grains are contaminated by fungal pathogens. These fungi, which cause considerable crop losses and deterioration of grain quality, are linked to diseases such as fusariosis and aspergillosis, involving moulds of the genera Fusarium and Aspergillus (Munkacsi et al., 2007). Indeed, these strains produce mycotoxins, substances that are pathogenic for humans and animals (Dupuy, 1994). These substances lead to losses of cereals and their derivatives estimated at 5 to 10% worldwide (Pfohl-Leszkowicz et al., 1999; De Muynck et al. 2004). The fight against the contamination of food products by mycotoxins constitutes a major stake for all the operators of the food industry. The use of chemical products is currently the most widely used technique to control these harmful moulds. However, the intensive and indiscriminate use of these products has led to contamination of the biosphere and the food chain (FAO/WHO, 2015). Also, decontamination by physical treatments such as irradiation-ionisation, thermal irradiation, adsorption aiming to reduce the number of mycotoxins in food, impact on the nutrients and vitamins of the food by releasing free radicals that cause cancer and cellular ageing (Assaoui, 2018). This suggests the search for means of biological decontamination of food products in general, and those made from maize in particular, contaminated by toxigenic moulds. Biological decontamination against maize mycotoxins, through the use of numerous micro-organisms including yeasts, fungi and bacteria, inducing transformation and/or enzymatic degradation of toxins into less toxic products, are increasingly used (Bhatnagar, 1998; Delos et al. 2013; Assaoui, 2018). This study focused on a biological control trial using antifungal lactic acid bacteria (LAB)

from maize, which are the best candidates to meet these requirements (Gerez et al. 2009), and can be an alternative to the use of chemicals and physical treatments, simply to ensure the safety of food for human consumption.

MATERIALS AND METHODS

Collection of maize samples

Five (5) kg of maize (*zea mays*), at a rate of one kilogram of maize grains per vendor, were purchased in June 2020, at the large market of Adjamé in the District of Abidjan (Côte d'Ivoire), from 5 different vendors chosen at random and far from each other. The maize grains, once collected, were put in hermetically sealed sterile plastic bags and transported to the laboratory for analysis.

Processing of maize grains into flour and fermentation of maize paste

Two hundred and fifty (250) grams of maize kernels, weighed with a METTLER electronic balance with a precision of 0.001, are sorted, cleaned, dehulled and washed with distilled water. These grains are then soaked in distilled water (1/2 : m/v) for 48 hours in a jar. The moistened grains are drained at room temperature and then ground using a mechanical mill. The flour obtained is defibrated by sieving with water through a muslin cloth. The starch milk collected is subjected to decantation for 4 hours (Louembé et al 2003). The maize paste obtained after decantation is mixed with distilled water (m/v) and fermented spontaneously in a hermetically sealed jar for 72 hours at room temperature in the dark (Nche et al 1994).

Search for lactic acid bacteria isolates in fermented maize dough

The isolation of lactic acid bacteria during the fermentation of fermented maize dough was performed according to the method described by Vermeiren et al. For this purpose, 10 g of fermented flour were taken at 24-hour intervals for 72 hours and homogenised in 90 mL of buffered peptone water (BioRad, France), which constitutes the enrichment broth. A decimal dilution ranges from 10-2 to 10-8 was performed with a triptone salt solution (BioRad, France). MRS agar (Condilab, Spain) supplemented with 0.1% nystatin was streaked with 100 µL of each dilution. The inoculated Petri dishes were incubated at 30°C for 48 hours in an oven (MEMMERT, Germany). The catalase test described by Prescott et al. (2003), the detection of cytochrome oxidase according to the method of Kovacs et al. (1995), the determination of the fermentative type of the presumptive colonies described by Badis et al. (2005) and the Gram staining test were used to investigate the biochemical characteristics of the LAB isolates.

Screening of LAB antagonistic to moulds from maize grains

The antagonistic activity of LAB isolates was carried out on moulds of the genus *Aspergillus* (*flavus* and *niger*), *Fusarium* sp and *Penicilium* sp, previously isolated from maize seeds (Yobouet, 2020). The screening of lactic acid bacteria isolates with antifungal activity was carried out by the double layer or overlay method as described by Magnusson et al. (2003) with some modifications. The LAB isolates were first streaked (two) 2 cm on MRS agar (Condilab, Spain), previously poured into Petri dishes, and then the whole set was incubated anaerobically at 30 °C for 48 hours. The colonies obtained were then covered with 10 mL of Sabouraud medium (BioRad, France), previously inoculated with the spores of the different moulds and maintained in supercooling. After 72 h of incubation at 30 °C, the diameters of the inhibition zones around each streak of lactic acid bacteria isolates were measured.

Statistical analysis of the data obtained

Analysis of variance (ANOVA) followed by Tukey's test with R software version 3.1 was used to compare the means of the diameters of the inhibition zones. Differences are considered significant for values of $P < 0.05$.

RESULTS AND DISCUSSION

Isolation and determination of biochemical characteristics of lactic acid bacteria isolates from fermented maize pulp

The microbiological analysis of the fermented maize pasta, the macroscopic and microscopic tests (Gram staining, colony appearance), as well as the biochemical tests (catalase, oxidase and the search for the fermentative type) carried out on presumptive lactic bacteria colonies revealed the presence of 40 lactic bacteria isolates. The presumptive colonies of lactic acid bacteria, during the fermentation time, are mostly in the form of cocci (Figure 1).

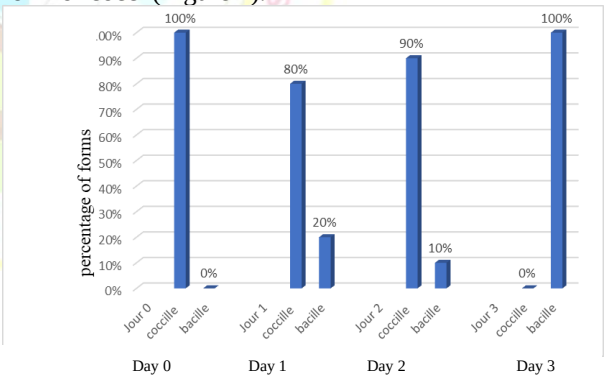

Figure 1. Distribution of LAB forms during the fermentation of maize dough

Of these isolates, 3 bacilli (7.5%) are heterofermentative, 10 bacilli (25%) are homofermentative and 27 cocci (67.5%) are homofermentative (Figure 2).

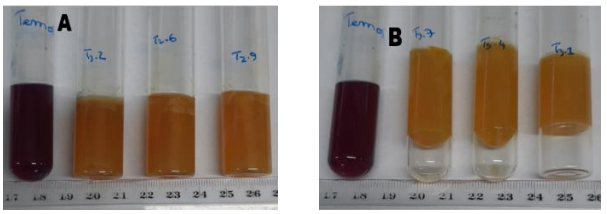

Figure 2. Photographs of agar tubes showing the fermentative type of lactic acid bacteria isolates from fermented maize paste

A : homofermentative type ; B : heterofermentative type ; T : negative control

Antifungal activities of lactic acid bacteria isolates from fermented maize dough

In this study, we hypothesized that LAB could inhibit the development of fungi isolated from the same substrates. The antifungal activity of the lactic acid bacteria by overlay was carried out for all 40 isolates of lactic acid bacteria from fermented maize dough, against the four mould strains previously isolated from maize grains (*Aspergillus niger*, *Aspergillus flavus*, *Fusarium* sp and *Penicillium* sp). Screening of the antifungal activity of the LAB isolates against these spoilage fungi showed that out of 40 LAB isolates obtained during the 3 days of fermentation, only 34 (85%) inhibited the growth of the indicator fungal strain. The diameter of the clear zone around the two lines of each LAB isolate varied from 0.3 ± 0^{d} cm to 9.0 ± 0.0^{a} cm (Figure 3).

At day D0, the lactic acid bacteria isolate LAB T0.2 and LAB T0.7, obtained before the fermentation of the maize dough, were the ones that showed a very good antagonistic activity on the tested fungal germs, with diameters of the inhibition zones varying from 3. 4 ± 0.14^{b} cm to 6.0 ± 0.0^{a} cm for isolate LABT0.2 ; and from 3.30 ± 0.28^{b} cm to 5.50 ± 0.07^{a} cm for isolate LAB T0.7. However, isolate LABT0.2 was more active on *Apergillus niger* (5.2 ± 0.28^{a} cm), *Aspergillus flavus* (5.5 ± 0.28^{a} cm) and *Fusarium* sp (6.0 ± 0.0^{a} cm) (Table 1).

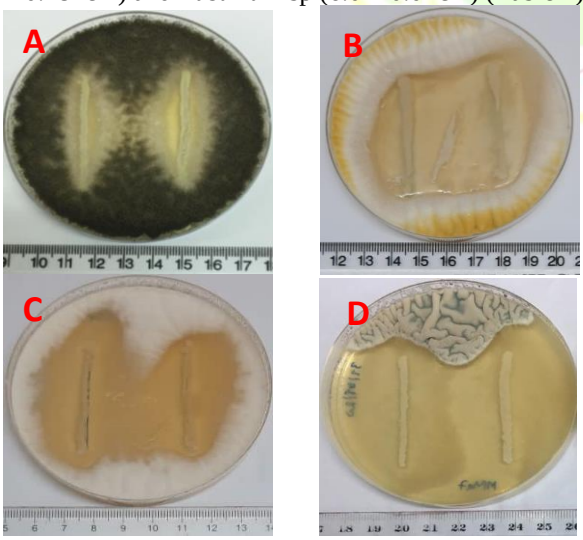

Figure 3. Antifungal activity of LAB isolated from fermented maize by the overlay test

A: Overlay of LAB T1.9 by *Aspergillus niger*; B: Overlay of LAB T0.2 by *Aspergillus flavus*; C: Overlay of LAB T1.6 by *Fusarium* sp; D: Overlay of LAB T2.6 by *Penicillium* sp

At the end of fermentation (D3), all LAB isolates showed very good activity on *Fusarium* sp. However, isolates LABT3.2, LABT3.4, LABT3.6, LABT3.9 and LABT3.10 showed very good activity on the 4 germs tested, with inhibition diameters ranging from 5.4± 0.14^{c} to 6.7±0.14^{a} cm for LABT3. 2, from 4.5 ± 0.28^{c} cm to 7.0 ± 0^{a} cm for LABT3.4, from 5.1 ± 0.14^{c} cm to 7.1 ±0.14^{a} cm for LABT3.6, from 4.6 ± 0.14^{b} cm to 7.1 ±0.14^{a} cm for LABT3.9 and from 5.4 ±0.28^{b} cm to 7.1 ±0^{a} cm for LABT3.10 (Table 4).

The isolates LABT1.6 and LABT1.7, obtained after 24 h of fermentation (D1), also show a very good antagonistic activity on the 4 fungal germs tested with inhibition diameters varying from 3.50 ± 0.14^{c} cm to 7.10 ± 0.14^{a} cm for isolate LABT1. 6 and from 4.5 ± 0.0^{b} cm to 6.9 ± 0.14^{a} cm for isolate LABT1.7. However, both isolates were more active on Fusarium sp and Penicillium sp (Table 2).

Table 1. Table of diameters of the inhibition zones of LAB isolates at day 0 on the fungal germs tested

N° isolates	Shapes / Type fermentation	*Aspergillus niger*	*Aspergillus flavus*	*Fusarium sp*	*Penicillium sp*
LABT 0.1	Coccus/ Homo	4.90 ± 0.14^{b}	4.80 ± 0.14^{b}	5.65 ± 0.21^{a}	2.90 ± 0.14^{c}
LABT 0.2	Coccus/ Homo	5.2 ± 0.28^{a}	5.5 ± 0.28^{a}	6.0 ± 0.0^{a}	3.4 ± 0.14^{b}
LABT 0.3	Coccus/ Homo	4.2 ± 0.0^{b}	3.9 ± 0.14^{b}	6.1 ± 0.14^{a}	3.1 ± 0.14^{c}
LABT 0.4	Coccus/ Homo	6.50 ± 0.28^{a}	3.95 ± 0.07^{c}	4.90± 0.14^{b}	2.90 ± 0.14^{d}
LABT 0.5	Coccus/ Homo	2.7 ± 0.28^{c}	4.4 ± 0.28^{b}	5.8 ± 0.14^{a}	6.1 ± 0.14^{a}
LABT 0.6	Coccus/ Homo	5.4 ± 0.28^{a}	3.0 ± 0.0^{c}	4.0 ± 0.0^{b}	5.0 ± 0.0^{a}
LABT 0.7	Coccus/ Homo	5.50 ± 0.28^{a}	3.30 ± 0.28^{b}	5.15 ± 0.07^{a}	3.50 ± 0.0^{b}
LABT 0.8	Coccus/ Homo	5.00 ± 0.0^{b}	2.85 ± 0.21^{c}	6.20 ± 0.14^{a}	3.30 ± 0.14^{c}
LABT 0.9	Coccus/ Homo	1.7 ± 0.14^{d}	9.0 ± 0.0^{a}	5.5 ± 0.0^{b}	2.6 ± 0.14^{c}
LABT 0.10	Coccus/ Homo	3.9 ± 0.14^{b}	3.2 ± 0.14^{c}	5.6 ± 0.14^{a}	0± 0.0^{d}

Also, of the 10 lactic acid bacteria isolates obtained on the second day of fermentation, only isolate LABT2.10 was very active on both Aspergillus flavus (6.9 ± 0.14^{a} cm) and Fusarium sp (7.1 ± 0.14^{a} cm) (Table 3).

In sum, of these 34 lactic acid bacteria isolates with antagonistic activity on the fungal germs tested, the isolates LABT0.2, LABT0.7, LABT1.6, LABT1.7, LABT2.10, LABT3.2, LABT3.4, LABT3.6, LABT3.9 and LABT3.10 are those with very good antifungal activity. From this point of view they could be used for a probable biological control of cereal contaminants in general and those of maize in particular.

Table 2. Table of diameters of the inhibition zones of LAB isolates at day 1 on the fungal germs tested

N° Isolates	Shapes / Type fermentation	*Aspergillus niger*	*Aspergillus flavus*	*Fusarium sp*	*Penicillium sp*
LAB T1.1	Coccus/ Homo	3.9 ± 0.14^{b}	3.2 ± 0.14^{c}	5.6 ± 0.14^{a}	0 ± 0^{d}
LAB T1.2	Coccus/ Homo	3.5 ± 0.28^{b}	4.2 ± 0.14^{b}	5.9 ± 0.14^{a}	4.1 ± 0.14^{b}
LAB T1.3	Coccus/ Homo	2.7 ± 0.14^{c}	5.1 ± 0.14^{b}	6.4 ± 0.14^{a}	4.6 ± 0.14^{b}
LAB T1.4	Coccus/ Homo	4.0 ± 0.14^{b}	5.6 ± 0.14^{a}	0.0 ± 0^{c}	6.0 ± 0.0^{a}
LAB T1.5	Coccus/ Homo	2.3 ± 0.14^{d}	3.6 ± 0.14^{c}	7.0 ± 0^{a}	4.2 ± 0.14^{b}
LAB T1.6	Bacill / Homo	4.55 ± 0.07^{b}	3.50 ± 0.14^{c}	7.10± 0.14^{a}	6.70 ± 0.14^{a}
LAB T1.7	Coccus/ Homo	4.5 ± 0^{b}	4.9 ± 0.14^{b}	6.9 ± 0.14^{a}	6.8 ± 0.14^{a}
LAB T1.8	Bacill / Homo	2.2 ± 0.14^{d}	4.5 ± 0^{c}	7.3 ± 0.14^{a}	6.0 ± 0^{b}
LAB T1.9	Coccus/ Homo	2.0 ± 0.0^{d}	4.4 ± 0.14^{b}	5.2 ± 0.14^{a}	3.8 ± 0.14^{c}
LAB T1.10	Coccus/ Homo	9.0 ± 0^{a}	4.0 ± 0^{d}	6.5 ± 0^{b}	5.1 ± 0.14^{c}

Table 3. Table of diameters of the inhibition zones of LAB isolates at day 2 on the fungal germs tested

N° isolates	Shapes / Type fermentation	*Aspergillus niger*	*Aspergillus flavus*	*Fusarium sp*	*Penicillium sp*
LABT 2.1	Coccus/ Homo	2.1 ± 0.14^{c}	5.5 ± 0.28^{b}	6.3 ± 0.14^{a}	6.1 ± 0.14^{ab}
LABT 2.2	Coccus/ Homo	3.9 ± 0.14^{c}	4.9 ± 0.14^{b}	0 ± 0^{d}	5.8 ± 0.14^{a}
LABT 2.3	Coccus/ Homo	3.7 ± 0.14^{c}	6.0 ± 0.0^{a}	6.0 ± 0.0^{a}	5.0 ± 0.0^{b}
LABT 2.4	Bacill / Homo	4.9 ± 0.14^{b}	6.4 ± 0.14^{a}	0.0 ± 0.0^{c}	6.0 ± 0.0^{a}
LABT 2.5	Coccus/ Homo	2.30 ± 0.14^{c}	5.35 ± 0.21^{b}	6.50 ± 0.0^{a}	2.20 ± 0.14^{c}
LABT 2.6	Coccus/ Homo	4.65 ± 0.07^{c}	6.90 ± 0.14^{a}	5.70± 0.14^{b}	3.50 ± 0.28^{d}
LABT 2.7	Coccus/ Homo	3.6 ± 0.14^{b}	4.5 ± 0.0^{a}	0 ± 0^{c}	0 ± 0^{c}
LABT 2.8	Coccus/ Homo	2.4 ± 0.0^{c}	4.4 ± 0.28^{b}	6.9 ± 0.14^{a}	1.3 ± 0.0^{d}
LABT 2.9	Coccus/ Homo	4.1 ± 0.14^{c}	5.1 ± 0.14^{b}	7.2 ± 0.14^{a}	3.2 ± 0.14^{d}
LABT 2.10	Coccus/ Homo	5.0 ± 0^{b}	6.9 ± 0.14^{a}	7.1 ± 0.14^{a}	5.3 ± 0.14^{b}

Table 4. Table of diameters of the inhibition zones of LAB isolates at day 3 on the fungal germs tested

N° isolates	Shapes/ Type fermentation	*Aspergillus niger*	*Aspergillus flavus*	*Fusarium sp*	*Penicillium sp*
LAB T3.1	Bacill/ Hetro	5.3 ± 0.14^{b}	5.1 ± 0.14^{b}	6.9 ± 0.14^{a}	0 ± 0^{c}
LAB T3.2	Coccus/ Homo	6 ± 0.14^{b}	5.4 ± 0.14^{c}	6.5 ± 0^{a}	6.7 ± 0.14^{a}
LAB T3.3	Coccus/ Homo	6.1 ± 0.14^{b}	0.3 ± 0^{c}	6.5 ± 0^{ab}	6.9 ± 0.14^{a}
LAB T3.4	Bacill/ Hetero	5.4 ± 0.14^{b}	4.5 ± 0.28^{c}	7.0 ± 0^{a}	6.4 ± 0.14^{a}
LAB T3.5	Bacill / Homo	5.0 ± 0^{c}	5.8 ± 0.14^{b}	6.4 ± 0.14^{a}	5.5 ± 0^{b}
LAB T3.6	Bacill / Homo	5.1 ± 0.14^{c}	5.6 ± 0^{b}	7.1 ± 0.14^{a}	7.0 ± 0^{a}
LAB T3.7	Bacill/ Hetero	6.0 ± 0.14^{b}	5.5 ± 0^{c}	6.9 ± 0.14^{a}	0 ± 0^{d}
LAB T3.8	Coccus/ Homo	5.6 ± 0.14^{c}	5.4 ± 0^{c}	7.2 ± 0.14^{a}	6.4 ± 0.14^{b}
LAB T3.9	Bacill / Homo	6.5 ± 0.28^{a}	4.6 ± 0.14^{b}	7.1 ± 0.14^{a}	6.8 ± 0.14^{a}
LAB T3.10	Coccus/ Homo	5.4 ± 0.28^{b}	5.9 ± 0.14^{b}	6.8 ± 0.14^{a}	7.1 ± 0^{a}

Homo: Homofermentative; Bacill: Bacillus; Hetero: Heterofermentative

Cereals are foodstuffs that are frequently contaminated by moulds. Contamination can occur before harvest, in the field, during drying, or during grain storage. Fungal contamination is one of the main causes of cereal grain deterioration, impacting on variations in grain technological parameters and leading to considerable losses (Atalla et al., 2003; Molinie et al., 2005). The different genera of moulds contaminating foodstuffs are *Fusarium* sp and especially *Aspergillus* sp and *Penicillium* sp (Doguiet, 2017; Abdellah, 2004), which are known to produce mycotoxins such as ochratoxin A (OTA), citrinin (CIT) and aflatoxin B (AFB) during grain storage by moulds of the genera *Aspergillus* and *Penicillium* (Pitt, 2000). Cases of food poisoning related to maize flour contaminated with mycotoxins have been reported by Fofana-Diomande et al. (2019). Also, the biological effect of these toxins causes damage to human organs such as the liver, kidneys and brain. Found in finished by-products, these toxins can be mutagenic, teratogenic, carcinogenic and immunotoxic (Abdalah, 2004). The present work, devoted to the selection of antifungal lactic acid bacteria, reveals their capacity to release probable antifungal substance (Doguiet, 2017). Indeed, recovery tests have shown that the growth of moulds is effectively inhibited by the presence of lactic acid bacteria isolates. This antifungal activity of lactic acid bacteria isolates would explain the decrease in mould growth. In a previous study, Muhialdin et al (2018) evaluated the antifungal activity of 870 LAB

isolates from Malaysian fermented foods against bakery spoilage fungi, namely Aspergillus niger, Aspergillus flavus MD3, Penicillium roqueforti MD4; and the LAB isolates showed activity against other selected fungi. This would imply that the fermentative process of certain foods, such as fermenting corn dough, would favour its decontamination in mycotoxins, under the action of isolates of lactic acid bacteria holding antifungal compounds involved in this antifungal activity. The use of chemical preservatives in food products would justify the implementation of this study on the possible use of these LAB isolates as biopreservatives against food pathogens. Foods that can serve as a source of beneficial and varied LABs for consumers should be increasingly popularised. From this point of view, bacterial isolates inhibiting all targeted fungal strains appear to be particularly interesting strains to be used as biopreservatives in the preservation of cereals in general and maize in particular.

CONCLUSION

The objective of the present study was to select isolates of lactic acid bacteria with antagonistic effects on the growth of toxigenic moulds, for probable biological control of maize moulds. The results show that fermented maize dough contains antifungal LAB isolates that can be used as food additives. These LAB isolates are reported to contain components that inhibit the development of foodborne pathogens, suggesting that they can replace chemical additives and provide attractive and diversified food products. Also, the proven inhibitory effect of this bacterial flora shows a real possibility of biotechnological application, for the biopreservation of food in general and maize in particular. This would contribute to ensuring food safety by producing food suitable for human consumption.

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