ISSN 2350-1049

International Journal of Recent Research in Interdisciplinary Sciences (IJRRIS)
Vol. 10, Issue 3, pp: (1-7), Month: July - September 2023, Available at: www.paperpublications.org

STUDIES ON THE USE OF LACTIC ACID BACTERIA FOR THE BIO-CONTROL OF FOOD SPOILAGE MYCOTOXIGENIC FILAMENTOUS FUNGAI

Adeoye, B.A. [1]*, Labbo, Z,[1] Shanu M. A[2],Makanjuola, D.O[5]., Ahmadu J. H.[1]., Egbulefu, C.S.[1], Mbochi C. A[2], Nyam, P. N.[3], Fadesire, A. K[4]., Olukotun, G.B[1]

[1]National Biotechnology Development Agency, Musa Yaradua Express Way, Lugbe, FCT, Abuja, Nigeria

[2]Nutrition and Dietetics dept., School of health, Makarfi, Kaduna State, Nigeria.

[3] Department of Social Sciences, SIIHST Makarfi Campus Kaduna State University

[4]Department of Nutrition and Dietetics, SIIHST Makarfi Campus Kaduna State University

[5]Department of Environmental Science, college of Environmental Studies, Kaduna Polytechnic, Nigeria

DOI: https://doi.org/10.5281/zenodo.8105307

Published Date: 01-July-2023

Abstract: **In recent years, fungal spoilage has been documented as an important issue for the agriculture-based food industries, leading to food sensory defects, food waste, economic losses and public health concern through the production of mycotoxins especially in the developing countries. As such, there has been a corresponding search for safer natural products and the demand for less processed and chemically treated foods. The aim of this study was therefore to evaluate the *in vitro* antifungal effect of some strains of Lactic Acid Bacteria (LAB). Some spontaneously fermented cow milk product was screened for potential LAB using pour plating technique using Man Rogosa Sharpe (MRS) agar incubated at 37°C in anaerobic conditions. Afterwards, the cell-free supernatant (CFS) of the pure, promising LAB were recovered by centrifugation of broth-grown cultures to determine its antifungal activity using halo diffusion agar test. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were determined for each LAB isolate. The CFS of the two pure LAB strains was used respectively as an *in vitro* natural biocontrol agent against *Aspergillus niger* (FS06) and *Fusarium avenaceum* (FS43). *L. casei* LG4 showed the highest antifungal effect against all essayed strains, while *L. plantarum* LG7 showed less effect. The results suggest that the application of the CFS from these strains in food could reduce the postharvest spoilage of foods and it thus offers promising steps for the development of biocontrol of fruits (oranges).**

Keywords: **fungal spoilage, agriculture-based food industries, public health, Lactic Acid Bacteria (LAB).**

1. INTRODUCTION

In Nigeria and Africa as a whole, a lot of food is lost along the food production supply chain emanating from harvesting time, climatic conditions, pre-harvest and post-harvest practices as well as storage conditions. Mycotoxigenic fungi have for long been identified as the main agent in this deterioration and subsequent health hazards associated with the consumption of some fruits and vegetables (Andrea, 2019). In many societies, fruits play a vital role in human nutrition and diets by supplying necessary growth factors such as vitamins and essential minerals which help to live a healthy life by preventing diseases (Joanne and Beate, 2012). Nigeria is blessed with abundant arable land that could be used for the production of various types of fruits, however, major parts become unwholesome and lost in the field, storage, transit,

handling processes of the crop from the grower to the whole sale dealer/ retailer and to consumers. The high proliferation of microorganisms in these food materials due to the nutritional composition are first often seen on the fruit surfaces where they progressively multiply and thereafter penetrated into the sub-surfaces. While, aside causing spoilage, some microorganisms like fungi are toxigenic in nature (Oluwadara *et al*., 2018). Spoilage generally refers to any change in the condition of food in which the food becomes undesirable or unacceptable for human consumption, whereas, food materials become toxigenic when it becomes ladened with microbial toxins that are able to initiate diseases in human and animals.

Generally speaking, fungi and especially the filamentous fungi pose a serious problem in both the food industry and agriculture food chain. They are responsible for causing many contaminations of food, feeds, causing crop diseases and contributing to serious economic loss especially in the humid climatic areas (Garner *et al*., 2016). They are reported to be capable of biosynthesis of toxic secondary metabolites (the mycotoxins) of which several have been found to be carcinogenic. Among the reported instance are fumonisin B1, aflatoxin B1, ochratoxin A, aflatoxins, fumonisins, ochratoxin A, toxin T-2, patulin, aflatoxin B1, ochratoxin A, zearalenone, aflatoxins, patulin etc. (Santini *et al*., 2015). Among the filamentous fungi, *Aspergillus* and *Fusarium* are the major fungal genera associated with food contamination reportedly due to their ability to colonize and grow in a wide range of environmental conditions. Furthermore, these species are also known to produce mycotoxins, the secondary metabolites produced by the mycelial structure of filamentous fungi that have no biochemical significance in fungal growth but have the potential to elicit undesirable effects on human and animal health, following consumption of contaminated food or feedstuffs (Marin *et al.*, 2013). Hence, mycotoxin contaminated fruits result in illness and economic losses once they are ingested through foods such as fruits. Lactic Acid Bacteria (LAB) as a microbial-based control has increased because LAB has some inherent abilities to inhibit spoilage organisms due to their active metabolites which are "Generally regarded as safe" (GRAS). This GRAS status has conferred safety confidence in them making them widely used in many food processing industries (Salam *et al*., 2021). They are able to produce some bio-preservation metabolites as secondary metabolites such as hydrogen peroxide, lactic acid, acetic acid etc. which resultantly play very important roles as antimicrobial agents against pathogenic microorganisms and their toxins (Rahmeh *et al*., 2019). The discovery of this mechanism become very significant because of the possibility of exchanging chemical sources of microbial control with biological origin. Therefore, the aim of this research was to screen some indigenous lactic acid bacteria (LAB) strains from some local, spontaneously fermented dairy products for in vitro test against some food spoilage and toxigenic filamentous fungi.

2. MATERIALS AND METHODS

Materials and Culture Media

The culture media used in this study included; Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), and Man–Rogosa–Sharpe agar (MRS-A) and broth (MRS-B), Deionized distilled water and Sodium Chloride (NaCl) were acquired from suppliers in good and standard conditions.

Sample Sampling Site

The sampling site for processed milk was temporary settlement at the outskirt of Iddo, along Lugbe-Giri road while the fruits (Oranges) were collected at the major fruit market in Lugbe all in the Federal Capital Territory, Abuja, Nigeria. This market is a major fruit market and depot for orange in the area which brought to the market from states such as Enugu, Kogi, Ondo and Edo.

Sample Collection

A total of ten milk samples in batches of four were randomly collected from different locations and twenty-six samples of diseased oranges was collected for two different lots of the oranges brought into the market at intervals of several days. Spoiled or diseased oranges were identified by physical examinations.

Isolation of Microorganisms

Isolation and Maintenance of Lactic Acid Bacteria

In total, 135 Lactic acid bacteria (LAB) strains were isolated from fermented dairy product (Nono) prepared by some household women in the collection areas and analysed in the laboratory using pour-plate method according to the methods of Yang *et al*., 1992. From the thoroughly homogenized collected nono sample, 1 ml aliquot was aseptically added to 9ml pre-sterilized peptone water and serially diluted to 10^{-6}. The 10^{-4} and 10^{-6} dilution were pour-plated in molten MRS/M17

agar respectively. The inoculated plates were incubated at 37^0C for 48 hours. After the incubation, discrete colonies that developed were studied for Lactic acid bacteria morphologies. Gram stain was performed on the pure cultures with LAB characteristics in order to select the Gram-positive bacteria and they were then sub-cultured on sterile media. The obtained pure culture was maintained on media slants at 4^0C until further needed.

Isolation and Maintenance of Filamentous Fungi

The fungi used were *Aspergillus niger* and *Fusarium avenaceum* which were the dominant fungi associated and isolated from the spoilt oranges from the studied area.

Preparation of Samples

The samples with obvious disease conditions were cut from the advancing edges of lesion by using a sterilized knife after sterilizing the surface of the fruit with 85% ethanol and rinsing with sterile distilled water. The cut lesion was then homogenized in sterilized peptone water with stirring using sterile glass rod. Serial dilution up to 10^{-4} was made and aliquot 1ml of 10^{-2} and 10^{-4} were respectively introduced into media plates of already prepared Potato Dextrose Agar (PDA) containing Chloramphenicol (30mg/l) to prevent the growth of bacteria and were incubated at ambient room temperature (25 – 30° C) for 7 days. After 7 days, growth of fungal colonies on the agar were studied (Samuel *et al.*, 2019).

Identification of the Isolated Fungi Isolates

The method of Fawole and Oso (1995) was used for the identification of the fungi. In the procedure, a drop of Lactophenol cotton blue stain was placed on a clean slide and using a mounted needle, a small portion of the mycelium from the overnight-grown fungal cultures was removed and placed in the drop of the stain. The mycelium was spread carefully on the slide with the aid of two mounted needles. A cover slip was gently lowered on it, and excess liquid was wiped by putting the slide between two folds of filter paper with a gentle pressure around the cover slip. The slide was then examined under the microscope at low and high-power objectives (XI0 and X40) of the microscope (Olympus model). The respective morphological characteristics of the fungi such as type of hyphae (whether septate or non-septate), asexual reproductive structure (whether borne sporangia or conidia, in chain or single) were observed and recorded accordingly.

Pathogenicity Assay

Five healthy oranges were surface-disinfected and three holes of about 2mm were made with a flame-sterilized and cooled wire loop. A portion of the laboratory-grown young fungi isolate was inoculated into the holes in triplicate respectively. Uninoculated samples was set aside to serve as control. Then both inoculated and uninoculated orange samples were separately placed in sterilized *Bama* bottles with the opening loosely covered with cotton wool to allow oxygen access to the experimented fungi. The set-up was kept for 7-14days at ambient temperature (25-30°C). After the Seven days (7-14days), both the inoculated and uninoculated oranges were observed for growth of fungal colonies and were correspondently matched with the original colonies used as source of inoculum (Darvas, 1987).

Preservation and Maintenance of Fungal Isolates

The isolated and screened moulds were grown for five days on PDA slant and sterilized 25% glycerol was aseptically added to cover the slants and were maintained at 40C until further needed.

Screening of Lactic Acid Bacteria for Bio-active Compound Production

All the lactic acid bacteria were screened for their antifungal ability by growing them in MRS broth until the late exponential and stationary phase at incubation temperature of 37°C for 48 hours to obtain cell-free supernatants. The CFS were then obtained by centrifugation (8000 x g x 5 min) and filtration using 0.45 µm-pore size filter. The radial growth inhibition method was used for the test and in the procedure, the modified PDA plates (supplemented with 0.5% Chloramphenicol to suppress the bacterial growth) were prepared and 10µL of a freshly-prepared solution containing about 1x 10^6 spores/mL of test organisms (*Aspergillus sp*. and *Fusarium sp*.) were spread on the plate, a hole with a diameter of 6 mm was made aseptically with a sterile cork borer tip at the centre of the plate, and an aliquote 20µL of the CFS solution was introduced into the holes respectively. Control plates were prepared by adding the same concentration of sterile MRS broth (Azizi *et al*., 2017). The set-up was incubated for 72 hours after which the respective inhibition percentage was determined by measuring the radial growth of the hyphae. All the assays were performed in triplicate.

ISSN 2350-1049

International Journal of Recent Research in Interdisciplinary Sciences (IJRRIS)
Vol. 10, Issue 3, pp: (1-7), Month: July - September 2023, Available at: www.paperpublications.org

3. RESULTS AND DISCUSSION

Table 1 shows the number of fermented milk samples collected from Twelve (12) sampling areas (A-L).

Table 1: Sample Collection for Lactic Acid Bacteria Isolates

Sample Point	Total Samples	Total LAB Obtained
A	5	05
B	5	11
C	5	06
D	5	13
E	5	09
F	5	21
G	5	15
H	5	02
I	5	17
J	5	04
K	5	12
L	5	03

It also shows the total number of Lactic Acid Bacteria. The total lactic acid bacteria obtained ranged from 2 from sample site H to the highest number of 21 from site F. Several lactic acid bacteria have been isolated from fermented milk and other milk products. Those isolated and reported from traditionally fermented vegetables by FAO/WHO. (2002) were shown to have some antagonistic activity against toxigenic and non-toxigenic filamentous fungal strains. In a similar work by Wang *et al*., 2021, showed that some lactic acid bacteria strains used as starter have antimicrobial activity which were statistically significant. Lactic acid has been found to occur in fermenting products where their growth and fermentation process cause a range of metabolites production with antimicrobial effects which are attributed to the destabilization of the membrane, inhibition of the synthesis of cell wall enzymes, interference of proton gradients and the induction of the formation of reactive oxygen (Pradhan and Kadyan, 2020).

Table 2: Occurrence and Plate Counts of Fungi Obtained at the Sample Sites

Sample Point	Total Plate Count	Dominant Fungi Obtained
A	$1.1x10^3$	*Aspergillus niger*
		Alternaria sp.
		Penicillium sp.
B	$1.5x10^2$	*Mucor sp.*
		Fusarium avenaceum
		Rhizopus sp.
C	$2.0x10^2$	*Aspergillus niger*
		Mucor sp.
		Fusarium avenaceum
D	$1.9X10^2$	*Aspergillus niger*
		Penicillium sp.
E	$1.4x10^2$	*Aspergillus niger*
		Fusarium avenaceum

Table 2 presents the total plate count from five diseased oranges sampled and the dominant fungi isolated thereof. The result revealed that sampling points C and E of the samples infected had Aspergillus sp. and Fusarium sp. respectively, while A, B, F and G had either of the two fungi and the fungi counts in all the samples ranged from $1.4x10^2$ in sample E to 1.1x10 in sample A.

The observations in this result agrees with the fact that LAB occur in fermented foods where they are regarded as safe and have been reported to be widely distributed as well as being used in the food industries due to their essential bio-preservative roles they play (Agriopoulou *et al*., 2020).

Several works have shown that LAB are able to colonize and out-compete other microorganisms in their environment owing to the production of some metabolites including hydrogen peroxide, lactic acid, acetic acid and low molecular weight substances (diacetyl, fatty acids, reuterin, reutericyclin), antifungal compounds (phenyl lactate, propionate, hydroxyphenyl lactate) and bacteriocins with antagonistic properties (Castellano *et al*., 2020).

Table 3: The Pathogenicity Test Results

Number of Sample Infected	Fungi/Control	No. Produced Disease
8	*Aspergillus niger*	7
8	*Fusarium avenaceum*	6
8	*Control*	0

Table 3 shows the pathogenicity test results. The results showed that seven out of the eight orange sample infected with the laboratory-grown *Aspergillus niger* produced disease after seven days of incubation which is 87.5%. In a similar manner, six of the eight orange samples infected with *Fusarium avenaceum* produce disease, representing 75%. The table showed that all the fungal isolates were able to infect the healthy oranges while the control did not produce any disease. The occurrence of these species is to be expected because they have severally been reported to be found widely distributed in soil, subterranean and aerial plant parts, plant debris, and other organic matter as well as in water from where they could colonize and cause diseases (Nelson *et al*., 1994).

Table 4: The Antifungal Activity of the Cell-free Supernatants of LAB strains.

Fungal Strain	LAB Cell-free Supernatant	
	L. casei	*L. plantarum*
Aspergillus niger (FS06)	++	+++
Fusarium avenaceum (FS43)	+	++

Table 4 shows the antifungal activity of the cell-free supernatants obtained from the LAB strains against the toxigenic filamentous fungi of Aspergillus and Fusarium genera. The antifungal activity was express as; + = means of 8mm of inhibition zone between the well and the fungal growth, ++ = means of 8-10mm, and +++ = means above 10mm inhibition zone between the well and the fungal growth. From the result, *L. plantarum* had the highest zone of inhibition against *Aspergillus niger* while *L. casei* has the lowest against *Fusarium sp*. Generally, the quantitative results indicate that the Asperillus strain isolated and used in this study was more sensitive to the CFS than the Fusarium strain. Several authors have proved the fact that the growth of some filamentous fungi found in fermented foods are being inhibited by the activity of lactic acid bacteria (Sezer *et al.*, 2013). The inhibitory mechanisms have been found to be mainly due to their ability to produce some metabolic products such as acetic acid, Hydrogen peroxide, lactic acid, bacteriocins e.t.c that destroys the cell membrane and amino acid absorption ability of the fungi (Emmanuel *et al.*, 2021).

4. CONCLUSION

The presence and frequency of occurrence of these molds on food is of great health concern due to their inherent ability to produce toxins. Therefore, this research was carried out to evaluate the antifungal property of lactic acid bacteria against the fungi with the aim of controlling them using non-chemical approach. The research will therefore contribute to the knowledge that lactic acid bacteria could be isolated from the wild and used for a systematic control of toxigenic molds.

5. RECOMMENDATION

The research field of antifungal LAB is yet to reach any advance scale. It is the inhibitory effects of lactic acid bacteria cell-free supernatants that has often been demonstrated at laboratory scale. Therefore, there is need for more research in the areas of identification of the supernatant composition and fractions that are effective as the antimicrobial agent.

Also, since fungal colonization of foods has now been rated as a world-wide challenge from both the economic and health points of view, there is the need to urgently develop further the optimization and upscaling of the lactic acid bacteria extracts that proved to be effective at controlling the fungal food pathogens since they are both safe and eco-friendly.

REFERENCES

[1] Andrea Patriarca (2019): Fungi and mycotoxin problems in the apple industry; Current Opinion in Food Science; Volume 29, Pages 42-47.

[2] Joanne, L. S. and Beate, L. (2012): Health Benefits of Fruits and Vegetables; Adv Nutr. 2012 Jul; 3(4): 506–516.

[3] Oluwadara Oluwaseun Alegbeleye, Ian Singleton, and Anderson S. Sant'Ana (2018): Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: A review; Food Microbiol.; 73: 177–208.

[4] Garner D., Kathariou S. (2016): Fresh produce–associated Listeriosis outbreaks, sources of concern, teachable moments, and insights. *J. Food Protect.*;79(2):337–344.

[5] Santini, A., Raiola, A., Meca, G. and Ritieni, A. (2015): Aflatoxins, Ochratoxins, Trichotecenes, Patulin, Fumonisins and Beauvericin in Finished Products for Human Consumption: Review Article - Journal of Clinical Toxicology; Volume 5, Issue 4.

[6] Marin, S., Ramos, A.J., Cano-Sancho, G., Sanchis, V. (2013): Mycotoxins: occurrence, toxicology, and exposure assessment. Chem Toxicol 60: 218-237.

[7] Salam A. Ibrahim, Raphael D. Ayivi, Tahl Zimmerman,[1] Shahida Anusha Siddiqui, Ammar B. Altemimi, Hafize Fidan, Tuba Esatbeyoglu, and Reza Vaseghi Bakhshayesh (2021): Lactic Acid Bacteria as Antimicrobial Agents: Food Safety and Microbial Food Spoilage Prevention; Foods.; 10(12): 3131.

[8] Rahmeh, R., Akbar, A., Kishk, M., Al-Onaizi, T., Al-Azmi, A., Al-Shatti, A., Shajan, A., Al-Mutairi, S., Akbar, B. (2019): Distribution and antimicrobial activity of lactic acid bacteria from raw camel milk. *New Microbes New Infect.*; 30:100560.

[9] Yang, R., Johnson, M.C., Ray, B. (1992): Novel method to extract large amounts of bacteriocins from lactic acid bacteria. *Appl Environ Microbiol.*;58:3355–3359.

[10] Samuel Mailafia, God'spower Richard Okoh, Hamza Olatunde K. Olabode, and Ramatu Osanupin (2017): Isolation and identification of fungi associated with spoilt fruits vended in Gwagwalada market, Abuja, Nigeria; Vet World. 2017 Apr; 10(4): 393–397.

[11] Fawole, M.O. and Oso, B.A. (1995): Laboratory manual of microbiology. Spectrum Books Limited Ibadan, Owerri, 71-81.

[12] Darvas, J. M. (1987): "Fungi associated with pre-and postharvest diseases of avocado fruit at Westfalia Estate, South Africa," *Phytophylactica*, vol. 19, no. 1, pp. 83–86.

[13] Azizi, F., Najafi, M.B.H., Dovom, M.R.E. (2017): The biodiversity of Lactobacillus spp. from Iranian raw milk Motal cheese and antibacterial evaluation based on bacteriocin-encoding genes. *AMB Express* 7(1):1–10.

[14] FAO/WHO. *Guidelines for the Evaluation of probiotics in food.* Vol. 30. Ontario, Canada: WHO; 2002. WHO working group report on drafting guidelines for the evaluation of probiotics in food.

[15] Wang, Q., Yang, L., Feng, K., Li, H., Deng, Z., Liu, J. (2021): Promote lactic acid production from food waste fermentation using biogas slurry recirculation. *Bioresour. Technol.* 2021, *337*, 125393.

[16] Pradhan, D. and Kadyan, S. (2020): Antifungal Lactic Acid Bacteria (LAB): Potential Use in Food Systems. In *Novel Strategies to Improve Shelf-Life and Quality of Foods*, 1st ed.; Apple Academic Press: Palm Bay, FL, USA, 2020; Chapter 4; pp. 73–94.

[17] Agriopoulou, S., Stamatelopoulou, E., Sachadyn-Król, M., Varzakas, T. (2020): Lactic Acid Bacteria as Antibacterial Agents to Extend the Shelf Life of Fresh and Minimally Processed Fruits and Vegetables: Quality and Safety Aspects. *Microorganisms* 2020, *8*, 952.

[18] Castellano, P., Ibarreche, M.P., Massani, M.B., Fontana, C., Vignolo, G.M. (2017): Strategies for Pathogen Biocontrol Using Lactic Acid Bacteria and Their Metabolites: A Focus on Meat Ecosystems and Industrial Environments. *Microorganisms* 2017, *5*, 38.

[19] Nelson, P.E., Dignani, M.C., Anaissie, E.J. (1994): Taxonomy, biology, and clinical aspects of Fusarium species; Clin Microbiol Rev. 1994;7(4):479.

[20] Sezer, C., Guven, A., Bilge Oral, N., Vatansever, L. (2013): Detoxification of aflatoxin B1 by bacteriocins and bacteriocinogenic lactic acid bacteria. *Turk. J. Vet. Anim. Sci.* 2013, *37*, 594–601.

[21] Emmanuel Edoghogho Imade, Solomon Esharegoma Omonigho, Olubukola Oluranti Babalola and Ben Jesuorsemwen Enagbonma (2021): Lactic acid bacterial bacteriocins and their bioactive properties against food-associated antibiotic-resistant bacteria; *Annals of Microbiology* volume 71, Article number: 44.