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In vitro Inhibition of *Fusarium* by Lactic Acid Bacteria (LAB): Implication of Yam Disease Control for Economic Growth in Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author RMO designed the study, carried out the analysis, performed the statistical analysis and wrote the first draft. Author PCO designed the study, supervised the analysis, read the first draft and edited it. Author RA provided the materials for the work, financed the work and edited the final write up. All authors read and approved the final manuscript.

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ABSTRACT

Yam is an important crop in Nigeria, where it is produced both as food and cash crop. *Fusarium* rots of yam are among the most important postharvest pathogens of yam worldwide, causing a lot of postharvest losses in stored yam tubers. Lactic acid bacteria (LAB) lower the pH and create an environment that is unfavorable to pathogens and spoilage organisms. *In vitro* inhibition of *fusarium* spp by LAB was investigated; mono-culture and multi-culture were used. The inhibition tests were carried out with pure cultures of LAB and *fusarium* spp. The pure culture of actively growing *Fusarium* was used to inoculate Potato Dextrose Agar medium aseptically and then incubated at room temperature for 72h. The diameter of the growing *Fusarium* was measured, after which less than a loop full of actively growing (18-24 h) LAB isolates were used to inoculate

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the medium containing the growing *Fusarium* at a known distance in the same plate. The whole set up was incubated at 30^0C and inhibition zones on *Fusarium* by the LAB were observed 24 hourly for 96h. The tests were carried out for mono-culture and multi-culture in triplicate. The inhibition zone ranged from 43 to 100% in mono-culture plate and multi-culture plate ranged from 40 to 113%. The slightly larger inhibition in the multi-culture plate may be due to much pressure on the *Fusarium*. Hence LAB may be used to control rot caused by *Fusarium*in in stored yam, which can improve yam tuber storage for better economic growth.

Keywords: Yam; lactic acid bacteria; Fusarium; inhibition; postharvest.

1. INTRODUCTION

Yams (*Dioscorea* spp.) are an important food, source of income generation and basis of culture in West Africa [1,2] About 5 million hectares are used for the cultivation of yams in about 47 countries in tropical and sub-tropical regions of the world. Nigeria uses about 3 million hectares of land for yam production and hence the highest (68 percent) yam producer in the world. Unfortunately, it is also the highest yam loser in the world. Annually 30 per cent of its production is lost [2].These losses occur mostly during storage. Microorganisms especially the fungi are important agents of deterioration and spoilage of this crop during storage. *Fusarium* rots of yam are among the most important postharvest problems worldwide causing a lot of postharvest losses in stored yam tuber [3].

Lactic acid bacteria produce various compounds such as organic acids, diacetyl, hydrogen peroxides and bacteriocins or bactericidal proteins during lactic fermentations [4,5]. Lactic acid bacteria lower the pH and create an environment that is unfavorable to pathogens and spoilage organisms [6]. There are various traditional methods for storage of crops such as curing, chemical control and sprout control in storage. Traditional and modern yam barns however, have been shown to lead to storage losses both quantitatively and qualitatively. Thiabendazole (TBZ) has been effectively used to control yam rot and has been registered as the only effective chemical against dry rot [7]. The resistance of *Fusarium* spp such as *Fusarium moniliforme* and *Fusarium solani* to TBZ as reported by Longerfield [8] has called for alternative means of handling this disease. In addition TBZ is highly toxic and environmentally hazardous hence the need for biological control of yam rot as this is non-toxic and environmentally friendly or safe.

This research aims at investigating the use of Lactic acid bacteria (LAB) for the control of postharvest rot of yams caused by *Fusarium* spp.

2. MATERIALS AND METHODS

2.1 Sources of Materials

2.1.1 Source of *Fusarium* spp

A plug of infected portion of yam was grown on Potato Dextrose Agar (PDA). Sub-culturing was carried out several times to obtain a pure culture which was identified using fungi atlas and other morphological characterization. Further culturing on Saboraut Dextrose Agar (SDA) and on Complete Medium (CM).

2.1.2 Source of Lactic Acid Bacteria (LAB)

The ogi-used in this work was produced using the method of Ngoddy and Ihekeronye [9] Maize grains (*Zea mays*) were cleaned and steeped in clean water for two days in a pot. The water was decanted and the grains wet-milled and sieved with muslin cloth. The pomace was discarded and the starch suspension was allowed to sediment during which natural fermentation was allowed to take place for 2-3 days. The fermented water was inoculated on acidified nutrient agar (NA) and subsequently on de Man Rogosa Sharpe (MRS) aseptically and incubated in an incubator at 25℃ for 2 days (48 hours). Each isolated colonies were further subculture until pure culture was obtained, Gram staining and biochemical characteristics were done for proper identification [10].

2.1.3 Inhibition of *Fusarium* spp with LAB

A loop full of LAB (S=LAB with small colony, M=LAB with medium colony LAB with small colony and L=LAB with large colony) each was used to inoculate a pure culture of well-established *Fusarium* spp lawn at equidistant. The reaction was noted 24 hourly for 96 hours. Small whitish colony (LABS) was further sub-cultured and used for further inoculation and reactions recorded.

2.1.4 Inhibition test with pure cultures

Inhibition test were carried out with *Fusarium* spp pure cultures established on Potato Dextrose Agar (PDA). The diameter of the growing *Fusarium* spp was measured, after which less than a loop full of 18-24 h experimental LAB isolates were used to inoculate the medium containing the growing *Fusarium* spp at a known distance in the same plate. The whole set up was incubated at 30℃ and changes in the growth of both the *Fusarium* spp and the LAB were observed 24 hourly for 96h. The tests were carried out for monoculture and multi culture in triplicate.

2.1.5 *In vitro* Inhibition tests by different LAB dilutions

A concentrate of the LAB was made using Normal saline (0.9% NaCl). Double dilutions (of 1/10, 1/20, 1/40, 1/80, 1/160 and 1/320) were then made from the concentrate as described by Okigbo and Omodamiro [11]. These dilutions were used to test for the inhibitory effect of LAB on *Fusarium* spp in-vitro. The dilution that gave the highest inhibition was taken as the working dilution.

2.2 Statistical Analysis

The collected data in this research work were evaluated for significant differences (5% probability level) in their means with Analysis of Variance (ANOVA). Fisher's Least Significant Differences (LSD) was used for means separation to determine significant differences using Statistical Analysis System (SAS) 2002-2008.

3. RESULTS AND DISCUSION

There were three distinct whitish colonies tagged S=small whitish colony; M=medium whitish colony and L=large whitish colony, on the NA as well as on the de Man Rogosa Sharpe (MRS) media as shown in Plate 1. Table 1 shows the results of the biochemical analysis for the characterization and identification of the isolated LAB. The probable identities of the Lactic acid Bacteria were *Lactobacillus acidophilus*, *Lactobacillus plantarum* and *Lactobacillus casei.* The Photomicrographs of these organisms are shown in plates 2, 3 and 4 for *Lactobacillus acidophilus* (S) *Lactobacillus plantarum* (M) and *Lactobacillus casei* (L) respectively.

Plates 5, 6, 7 and 8 shows pure cultures of *Fusarium* spp on different media *Fusarium* pure culture SDA, Matured growing *Fusarium* spp on CM, Young growing *Fusarium* spp on PDA and Matured growing *Fusarium* spp on PDA respectively for further identification. Further culturing on Saboraut Dextrose Agar (SDA) indicated white mycelia that had pink pigmentation at maturity. On Complete Medium (CM) the white mycelium developed purple pigmentations at maturity confirming the identity of the fungus as *Fusarium nygamai*. The growth was very rapid growth (which is part of characteristics feature of *Fusarium* spp*).*

The probable *Fusarium* spp was identified as *Fusarium nygamai*. Plates 9 and 10 shows inhibition of *Fusarium nygamai* by LAB. Table 2 shows the diameter of *F. nygamai* after inoculation, there was decrease in the diameter from 48hrs. Table 3 shows the results of the inhibitory effect of each of the three colonies on the *Fusarium*. Fig. 1 shows the Percentage inhibition of *F. nygamai* by LAB, *L. plantarum* accounted for 47-97% inhibition, *L. casei* induced 41-77% inhibition and *Lactobacillus acidophilus* caused 39-76% inhibition within the same period) all the colonies shows some levels of inhibition but the small colony gave the highest inhibition (88.06%), hence it was used for further inhibitory studies. All these goes to show that LAB has an inhibitory effect on the growth of *F. nygamai.*

Table 4 contains the results of the inhibition of *Fusarium nygamai* by *L. plantarum, L. acidophilus* and *L. casei* in mono-culture and multi-culture. The inhibition zone ranged from 43-100% in mono-culture while the mixed culture plate it had a range from 40-113%. The slightly larger inhibition in the mixed culture plate may be as a result of much pressure on the *Fusarium nygamai*, induces by the growth of other microorganisms.

L. plantarum showed the highest inhibition of 100%, in mono culture plates and 113%, in multi culture plate against *Fusarium nygamai* after 96 h incubation while *L. acidophilus* and *L. casei* inhibited with lower inhibition zones (60% & 63%) for both organisms in mono-culture and multi-culture respectively. However after 72 h, the inhibition zone for *L. acidophilus* was significantly higher ($p<0.05$) than that of *L. casei* while at 96 h the inhibition zones for both organisms had the same values.

The effect of dilution on the inhibition of *Fusarium nygamai* by *L. plantarum* is shown in Table 5 After 24h of inoculation; dilution 1:160 had the highest percentage inhibition (33.3%) ($p<0.05$). Dilution 1:40 induced an inhibition zone of 30.0% which was significantly higher than other dilutions ($p<0.05$) except for 1:160. Observed inhibition zone was lowest for 1:20 and 1:80 (20% inhibition in both cases). However at 48 h, the 1:40 dilution showed the highest inhibition of 66.7% ($p<0.05$) followed by dilution 1:10 which gave 60% inhibition. The other dilutions were observed to induce longer inhibition zones. The same pattern was observed for incubation periods of 72 h and 96 h. The 1:40 dilution gave the highest inhibition zones, while the inhibitory effects of 1:160 dilution was the lowest.

Klein [12] and Stiles [13] all reported inhibitory effects of *Lactobacillus plantarum* against both Bacteria and Fungi which are pathogenic microorganisms of food spoilage. Oyetayo et al. [5] had reported the inhibitory effect of *Lactobacillus acidophilus* on pathogen in acetic acid rich food. Oyetayo et al. [5] also reported inhibitory effect of *Lactobacillus casei* in their work on safety and protective effect of *Lactobacillus casei* and *Lactobacillus acidophilus* on food.

Table 1. Biochemical analysis of small, medium and large whitish colony of lactic acid bacteria (LAB)

Biochemical reactions	**Small whitish colony***		**Medium whitish colony****		**Large whitish colony*****	
Gram RXN	Gram +ve rod		Gram +ve rod		Gram+ve rod	
Asculin hydrolysis	+		+		+	
Nitrare reduction	-		-		-	
Arginine hydrolysis	-		-		-	
Catalase	-		-		-	
Sporulation	Non-spring		Non-sporing		Non-sporing	
Motility	Non-motile		Non-motile		Non-motile	
Gas & Acid production	**Gas**	**acid**	**Gas**	**acid**	**Gas**	**acid**
Glucose	-	+	-	+	-	+
Arabinose	$	-	$	-	$	-
Galactose	$	+	$	+	$	+
Lactose	+	+	+	+	$	+
Manitol	-	-	-	+	$	+
Maltose	$	+	$	+	$	+
Rafinose	$	-	$	+	$	+
Sarbitol	$	-	$	+	$	+

** Lactobacillus acidophilus** Lactobacillus Plantarium***Lactobacillus casei*
$ = not determined

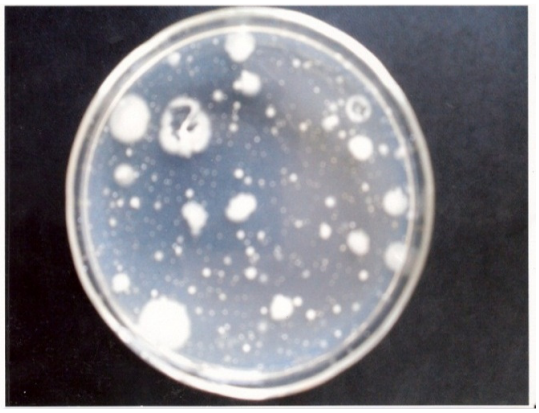


Plate 1. Small; Medium and Large whitish colonies

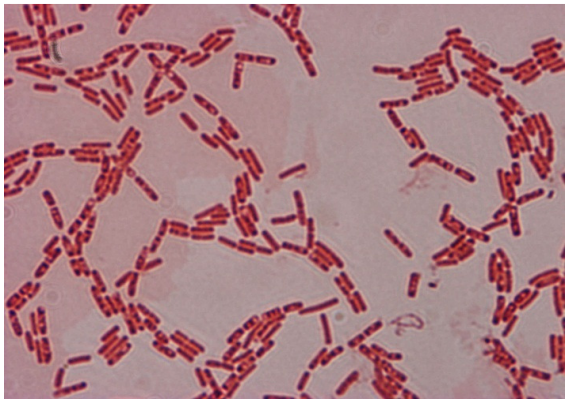


Plate 2. *Lactobacillus acidophilus* ×100

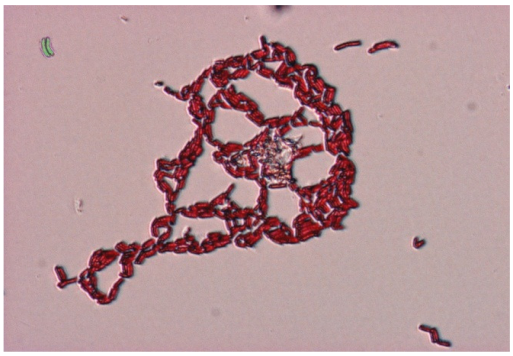


Plate 3. *Lactobacillus plantarum*×100

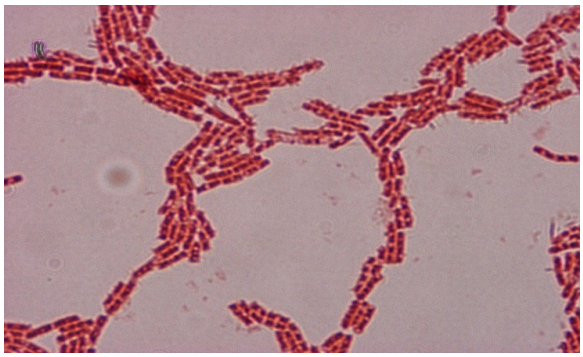


Plate 4. Lactobacillus casei ×100

Table 2. Diameter of *Fusarium nygamai* after inoculation with LAB

Duration (Hour)	Fusarium nygamai Diameter(mm)		
	Small colony*	Medium colony**	Large colony***
0hr	30.0	30.0	30.0
24hrs	30.2	30.2	30.2
48hrs	26.0	29.0	28.0
72hrs	22.0	26.0	25.0
96hrs	16.0	24.0	23.0

Lactobacillus acidophilus **Lactobacillus Plantarium*Lactobacillus casei*

Table 3. Zone of inhibition of the *Fusarium nygamai* by LAB

Duration (Hour)	Zone of inhibition (mm)		
	Small colony (mm)	Medium colony (mm)	Large colony (mm).
0 hr	0.0	0.0	0.0
24hrs	0.2	0.2	0.2
48hrs	4.2	1.2	2.2
72hrs	8.2	4.2	5.2
96hrs	14.22	6.2	7.2

Lactobacillus acidophilus **Lactobacillus Plantarium*Lactobacillus casei*

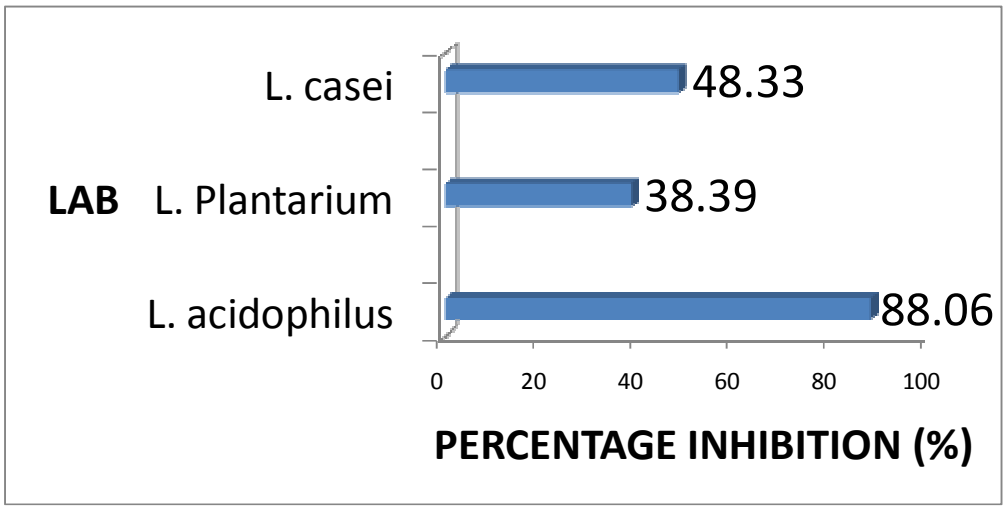


Fig. 1. Percentage inhibition of *Fusarium nygamai* by LAB

The work of Yang and Clausen [14] shows that *L. casei* and *L. acidophilus* supernatant retained antifungal activity against *T. viride* (80%) at pH 6.4. In the work of Vina and Carol [15], LAB was evaluated to determine if the same antimicrobial properties can be used to inhibit mould fungi that typically colonized wood. Based on biomass measurement cell-free supernatants from *Lactobacillus acidophilus* grown in MRS broth inhibited 95-100% growth of three mould fungi and one strain fungus associated with wood–based building material.

Plate 5. *Fusarium* spp pure culture SDA

Plate 6. Matured growing *Fusarium* spp on CM

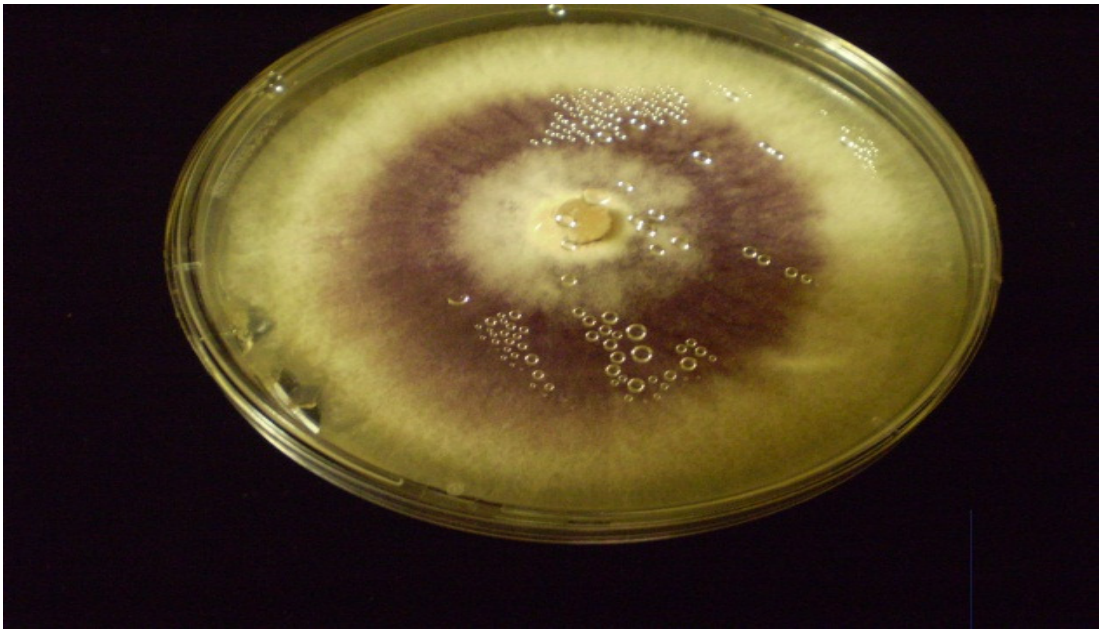


Plate 7. Young growing *Fusarium* spp on PDA

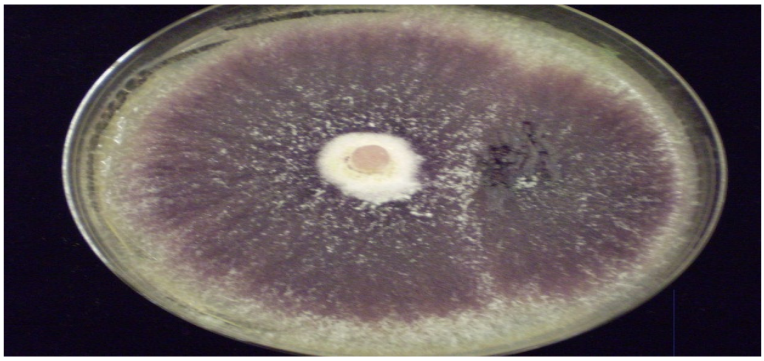


Plate 8. Matured growing *Fusarium* spp on PDA

Table 4. Zone of inhibition of Fusarium *nygamai* by LAB in Mono-culutre and Multi-culture plates

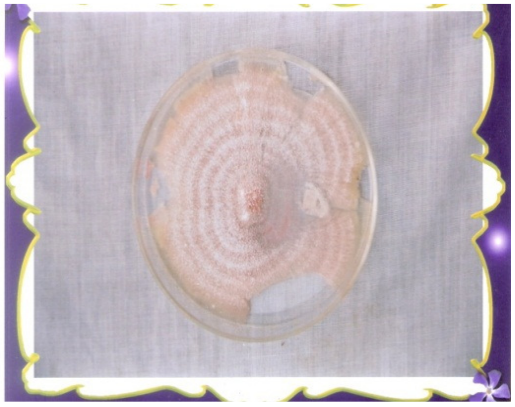
Duration (Hours)	Diameter of inhibition (%)							
	Mono-	Culture			Multi-	culture		
	LAB S	LAB M	LAB L	$LSD_{(0.05)}$	LAB S	LAB M	LAB L	$LSD_{(0.05)}$
24	Nsd	nsd	Nsd	nsd	Nsd	nsd	nsd	nsd
48	60^{a}	46^{b}	43^{b}	2.446	67^{a}	43^{b}	40^{b}	0.04
72	93^{a}	50^{c}	53^{b}	0.020	103^{a}	53^{c}	60^{b}	1.312
96	100^{a}	60^{b}	60^{b}	2.579	113^{a}	63^{b}	63^{b}	0.035
Total	163	156	156	-	283	159	163	-

nsd = no significant difference (all at 0% inhibition)
Means with the same superscript in each column are not significantly different (p>0.05) from one another

Table 5. Effect of Dilution on the Inhibition of *Fusarium nygamai* by Lactic Acid Bacteria

Dilution	Percentage (%)			
	24h	48h	72h	96h
1:10	20.00^{ed}	60.00^{b}	70.00^{b}	76.66^{b}
1:20	23.33^{c}	46.66^{c}	53.33^{c}	70.00^{c}
1:40	30.00^{b}	66.66^{a}	80.00^{a}	86.66^{a}
1:80	20.00^{cd}	33.33^{d}	36.66^{d}	36.66^{d}
1:160	33.33a	26.66^{e}	33.33^{d}	33.33^{e}
$LSD_{0.05}$	4.738	0.8569	4.381	0.028

Means with the same superscript in each column are not significantly different (p>0.05) from one another



Plates 9. Inhibition of *Fusarium* by LAB *(Front view)*

Plates 10. Inhibition of *Fusarium* by LAB *(Back view)*

4. CONCLUSION

The study shows that LAB could be used for the control of postharvest rot of yams caused by *Fusarium* spp *(Fusarium nygamai*) hence the reduction in the yam losses due to *Fusarium nygamai* rots. Further work to be done in the mode of application of this findings and chemical evaluation of the yam.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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