



# **Antimicrobial Effects of Lactic Acid Bacteria on Food-Borne Pathogens Isolated from Some Fruits Sold in Ibadan, Nigeria**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. Author JOA conceptualized methodology and prepare the original draft. Author JPA did the data curation and investigation. Author STO did the supervision and validation. Author STO did the reviewing and editing work. All authors read and approved the final manuscript.*

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## **ABSTRACT**

This study was conducted to determine the antimicrobial effect of Lactic acid bacteria on food-borne pathogens isolated from some fruits sold in Ibadan, Nigeria. *Lactobacillus* species isolated from healthy fruits include *L. casei*, *L. brevis*, *L. desidosus*, *L. jenseni*, *Lactiplantibacillus plantarum* and *Fructilactobacillus* spp. while *Aeromonas hydrophylia*, *Enterobacter aerogene*, *Escherichia coli*, *Salmonella typhii*, *Shigella dysenteriae*, *Pseudomonas fluorescens*, *Bacillus megaterium*, *Candida valida*, *Saccharomyces cerevisiae*, *Rhizopus stolonifer* were isolated from spoilt fruits using pour plate technique and biochemical test. Gram-negative isolates were 100% resistant to Cefuroxime, Amoxicillin/Clauvulanate, and Ampicillin while 93.75% of the isolates were highly sensitive to Ofloxacin. Gram-positive isolates were 100% resistant to Cloxicillin and highly sensitive to Ofloxacin and Gentamycin using an antibiotic disc. *Lactiplantibacillus plantarum* had the highest amount of

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Lactic acid (5.6 g/l) while *L. casei* had the lowest yield (3.6 g/l) at 48 hours. *L. casei* had the highest amount of hydrogen peroxide (0.00036 g/l) while *Lactiplantibacillus plantarum* and *Fructilactobacillus* spp. had the lowest yield (0.00021 g/l) at 48 hours. The highest amount of diacetyl (3.01 g/l) was produced by *L. jenseni* while the lowest amount was observed in *L. brevis* (0.43 g/l) at 48 hours. The maximum inhibitory activity was observed in *L. brevis* against *B. megaterum* with a diameter of 18mm zone of inhibition while the minimum activity by *L. desidosus*, *L. jenseni*, *Fructilactobacillus* spp. was observed against *B. subtilis* and *Lactiplantibacillus plantarum* against *K. pneumoniae* with diameter 8 mm zone of inhibition. The antimicrobial compounds produced by the Lactic Acid Bacteria had antimicrobial effects on food-borne pathogens.

**Keywords:** Antimicrobial effect; food-borne pathogens; lactic acid bacteria; fruits.

## 1. INTRODUCTION

“Lactic acid bacteria (LAB) are gram-positive organisms, anaerobic cocci or rods in shape; do not form spores, which produce lactic acid during fermentation [1].

“Fresh fruits and vegetables contain essential vitamins that human body needs for proper growth and development. They have low energy content and high nutritional and therapeutic properties” [2]. Reduction in degenerative diseases such as diabetes, colon cancer, obesity and heart infections to the barest minimum could be achieved by taking fruits and vegetables.

“Animals, insects, soil, water, dirty equipment, human handling, etc. could be sources of contamination by pathogenic organisms. Fruits are characterized by high nutritional composition and moisture content but have low pH which predisposes them to attack by disease causing fungi, hence, rots are inevitable, making them unsafe for consumption due to the presence of mycotoxins” [3,4].

There are various antimicrobial compounds produced by Lactic acid bacteria; these include organic acids (lactic acid), diacetyl, hydrogen peroxide, bacteriocins, etc. which have propensity to inhibit growth and toxin production of several microorganisms” [5, 6]. “Competition of lactic acid bacteria with pathogenic microorganisms for available nutrients could be adduced as a reason for their antimicrobial effect [7].

There is a high prevalence of food-borne pathogens on fruits due to contamination coupled with high antibiotic resistance of pathogens leading to food-borne illness which is hazardous to human health. Lactic acid bacteria are well known to produce antimicrobial compounds

which inhibit microorganisms and are also useful in preventing diseases. In this study, exploring the antimicrobial potentials of LAB from sources such as apples, pineapple, and soursop is necessary. Therefore, this study was conducted to determine the antimicrobial effects of Lactic Acid Bacteria on food-borne pathogens isolated from some fruits sold in Ibadan, Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

In this study, a total of 300 fruits were purchased and selected randomly, fifty (50) apples, pineapple, and soursop from each market (Bodija and Oje) in Ibadan, Oyo State, Nigeria. The healthy fruit samples were collected into labeled sterile containers without washing and transported to the laboratory for microbial analysis. The fruits were stored for two weeks at ambient conditions; the unhealthy (spoil) fruits were obtained from the healthy fruits when spoilage occurs.

### 2.2 Sample Preparation

Samples were prepared according to the method of Akoachere [8-10] with slight modifications. Twenty-five grams of each sample was weighed and homogenized by blending in 225 mL of sterile distilled water. One millilitre of the homogenate was introduced into 9 mL of the distilled water in a test tube, labeled 1:10 ( $10^{-1}$ ) dilution and serially diluted to five other test tubes labeled  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$ ; the procedure was repeated for each sample and the blender was carefully cleaned and disinfected in between samples to prevent any cross-contamination. The isolation and characterization of the probable lactic acid bacteria and food-borne pathogens as presented in Alimi et al. (Submitted for publication) [11].

## 2.3 Antibiotic Susceptibility Test

“This is a test carried out to determine whether or not an infectious agent is sufficiently sensitive to particular antibiotics to permit it for treatment.

Antibiotics disc was used for the study. Mueller Hinton agar plates were swabbed with test organisms with density  $10^8$  cfu/ml (equivalent to 0.5 McFarland standards) and then loaded with antibiotics disc using sterile forceps. Loaded plates were left for 30 minutes at room temperature for compound diffusion and then incubated for 24 hours at  $37^\circ\text{C}$ . The diameter (in mm) of zone of inhibition were measured and interpreted in accordance with Clinical and Laboratory Standards Institute Standard guidelines” [12].

## 2.4 Quantification of Antimicrobial Compounds Produced by Lactic Acid Bacteria

The test organism was grown in MRS broth for 48 hours and centrifuged at  $4000 \times g$  for 15 minutes.

### 2.4.1 Lactic acid

The quantification of lactic acid bacteria was done following AOAC [13]. “This was done by measuring 25 ml of cell free supernatant broth was measured into a beaker while three drops of phenolphthalein were added. To the burette, 0.1 M NaOH was added slowly to the broth until there is a color change to pink.

### 2.4.2 Hydrogen peroxide

“The amount of hydrogen peroxide was determined according to AOAC [13]. To 25 ml of the supernatant broth 20 ml of diluted Sulphuric acid was added. Potassium permanganate (0.1M) was used for the titration until decoloration was observed (endpoint)”.

0.1M Potassium permanganate = 1.070 mg of  $\text{H}_2\text{O}_2$

### 2.4.3 Diacetyl

“Diacetyl was quantified following standard analytical procedure of AOAC [13].

To a conical flask containing 25 ml of the supernatant broth, 7.5 ml of hydroxylamine

solution was introduced for the residual titration was done with 0.1M HCl to a greenish-yellow endpoint”

## 2.5 Screening for Antagonistic Activity of Cell-Free Supernatant

Lactic acid bacteria were inoculated into de Man Rogosa Sharpe (MRS) broth and incubated at  $30^\circ\text{C}$  for 48 hours in a candle jar. After 48 hours of incubation, cell-free supernatants were obtained by centrifuging at 4000 rpm for 15 minutes at  $4^\circ\text{C}$  the antagonistic effect of the cell-free supernatant on bacteria isolated from spoiled fruits was determined using the agar-well diffusion assay (AWDA). Mueller Hinton agar plates and Potato Dextrose Agar plates were inoculated with bacterial isolates (*B. coagulans*, *B. pulmilus*, *B. megaterium*, *Bacillus subtilis*, *B. licheniformis*, *P. cepacia*, *P. putida*, *P. fluorescens*, *A. hydrophylia*, *E. aerogenes*, *E. coli*, *K. pneumoniae*, *S. dysenteriae*, *S. typhi*), and fungal isolates (*C. valida*, *S. cerevisiae*, *R. stolonifer*) respectively using sterile swab well of 7mm were cut into the agar plate and 100 $\mu\text{l}$  of cell-free supernatant was dispensed into each well and incubated aerobically for 24 hours and 3 days for Bacterial and fungal isolates respectively at  $30^\circ\text{C}$ . The inhibitory effects were determined by measuring the diameters of zone of inhibition around the wells [14].

## 3. RESULTS AND DISCUSSION

This study was conducted to determine the antimicrobial effect of Lactic Acid Bacteria on food-borne pathogens isolated from some fruits sold in Ibadan, Nigeria. The antibiotic resistance patterns of Gram negative bacteria isolated from spoiled fruits are shown in Table 1. All the isolates were 100% resistant to Cefuroxime (CRX), Amoxicillin/Clavulanate (AUG) and Ampicillin (AMP) while majority of the isolates were highly sensitive to Ofloxacin (OFL) followed by Gentamycin (GEN) and Ciprofloxacin (CPR). All the isolates are resistant to three to four antibiotics as *Pseudomonas putida* isolated from apple was sensitive to Nitofurantoin (NIT) with 33 mm zone of inhibition. While the antibiotics resistance pattern of Gram positive bacteria isolated from spoiled fruits are shown in Table 2. All the isolates were 100% resistant to Cloxacillin (CXC) and highly sensitive to Ofloxacin (OFL) and gentamycin. The isolates are resistant to at least one antibiotic. *Bacillus coagulans* had the highest sensitive of 27 mm to Ceftriaxone (CTR).

The pathogens isolated from spoilt fruits in this study varied in their sensitivity to different antibiotics. The reason for antibiotic resistance could be due to chromosomal or plasmid resistance of the microorganism cell constituents [15]. The high resistance to ampicillin (100%) recorded in this study is similar to that obtained by Issa [16] and Yakubu [17] where 100% resistance to ampicillin were obtained from isolates in milk and processed meat respectively and Endang [18] who recorded a similar high resistance (87.0%) in isolates obtained from salted fish. The low resistance of isolates to Ofloxacin, Ciprofloxacin and Gentamicin in this

study is in agreement with the study reported by Yakubu [19] and Rahimi [20] where over 80.0% of isolates from various sources were found to be susceptible to each of these antimicrobial agents. Recently, antibiotic-resistant microbial species increased including moulds and yeasts, they are becoming resistant also to preservatives such as sorbate and benzoate as well as chemical detergents [21].

The antimicrobial compounds produced by Lactic Acid Bacteria were quantified using standard methods. *Lactiplantibacillus plantarum* produced the highest amount of Lactic acid (5.6 g/l) at

**Table 1. Antibiotic susceptibility test of gram-negative bacteria isolated from spoilt fruits**

Isolate (Location)	CAZ	CRX	GEN	CPR	OFL	AUG	NIT	AMP
Antibiotic/Zone of inhibition (MM)								
<b>OJE Market</b>								
<i>Klebsiella pneumoniae</i> (P)	21 (S)	0 (R)	21 (S)	26 (S)	23 (S)	0 (R)	0 (R)	0 (R)
<i>Aeromonas hydrophylia</i> (P)	0 (R)	0 (R)	0 (R)	19 (I)	18 (S)	0 (R)	12 (R)	0 (R)
<i>Enterobacter aerogenes</i> (S)	18 (I)	0 (R)	0 (R)	19 (I)	23 (S)	0 (R)	24 (S)	0 (R)
<i>Aeromonas hydrophylia</i> (A)	21 (S)	0 (R)	0 (R)	27 (S)	27 (S)	0 (R)	0 (R)	0 (R)
<i>Pseudomonas putida</i> (A)	29 (S)	0 (R)	21 (S)	26 (S)	29 (S)	0 (R)	33 (S)	0 (R)
<i>Eschericia coli</i> (A)	30 (S)	0 (R)	20 (S)	24 (S)	27 (S)	0 (R)	0 (R)	0 (R)
<i>Salmonella typhi</i> (A)	27 (S)	0 (R)	23 (S)	24 (S)	24 (S)	0 (R)	0 (R)	0 (R)
<i>Salmonella typhi</i> (P)	0 (R)	0 (R)	15 (S)	18 (I)	20 (S)	0 (R)	0 (R)	0 (R)
<i>Shigella dysenteriae</i> (S)	23 (S)	11 (R)	19 (S)	13 (R)	20(S)	0(R)	0(R)	0(R)
<i>Shigella dysenteriae</i> (P)	22 (S)	11 (R)	18 (S)	20 (I)	20(S)	11(R)	0(R)	0(R)
<b>BODIJA Market</b>								
<i>Enterobacter aerogenes</i> (S)	0 (R)	0 (R)	0 (R)	25 (S)	14 (I)	0 (R)	0 (R)	0 (R)
<i>Pseudomonas cepacia</i> (S)	28 (S)	0 (R)	21 (S)	28 (S)	28 (S)	0 (R)	20 (S)	0 (R)
<i>Enterobacter aerogenes</i> (P)	0 (R)	0 (R)	20 (S)	19 (I)	20 (S)	0 (R)	0 (R)	0 (R)
<i>Pseudomonas fluorescens</i> (S)	0 (R)	0 (R)	25 (S)	19 (I)	25 (S)	0 (R)	15 (I)	0 (R)
<i>Aeromonas hydrophylia</i> (S)	0 (R)	0 (R)	21 (S)	14 (R)	18 (S)	0 (R)	0 (R)	0 (R)
<i>Pseudomonas cepacia</i> (A)	24 (S)	0 (R)	21 (S)	23 (S)	23 (S)	0 (R)	0 (R)	0 (R)

CAZ: Cefazidime; CRX: Cefuroxime; GEN: Gentamicin; CPR: Ciprofloxacin; OFL: Ofloxacin; AUG: Amoxycillin/Clavulanate; NIT: Nitrofurantoin; AMP: Ampicillin; P: Pineapple; A: Apple; S: Soursop; R: Resistance; S: Sensitive; I: Intermediate

**Table 2. Antibiotic susceptibility test of gram-positive bacteria isolated from unhealthy fruits**

Isolate (Location)	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG
Antibiotic/Zone of inhibition (MM)								
<b>OJE Market</b>								
<i>Bacillus coagulans</i> (S)	23 (S)	11 (R)	21 (S)	27 (S)	0 (R)	0 (R)	20 (S)	15 (I)
<i>Bacillus pulmilus</i> (A)	18 (I)	0 (R)	17 (S)	13 (I)	0 (R)	0 (R)	26 (S)	0 (R)
<i>Bacillus polymyxa</i> (P)	19 (I)	17 (I)	17 (S)	22 (I)	0 (R)	0 (R)	23 (S)	16 (I)
<b>BODIJA Market</b>								
<i>Bacillus licheniformis</i> (S)	21 (S)	0 (R)	17 (S)	24 (S)	0 (R)	0 (R)	21 (S)	0 (R)
<i>Bacillus megaterum</i> (P)	11 (R)	9 (R)	13 (I)	17 (R)	17 (I)	0 (R)	12 (I)	15 (I)
<i>Bacillus subtilis</i> (A)	20 (I)	16 (I)	17 (S)	13 (R)	15 (I)	0 (R)	21 (S)	19 (S)

CAZ: Cefazidime; CRX: Cefuroxime; GEN: Gentamicin; CTR: Ceftriaxone; ERY: Erythromycin; CXC: Cloxicillin; OFL: Ofloxacin; AUG: Amoxycillin/Clavulanate P: Pineapple; A: Apple; S: Soursop; R: Resistance; S: Sensitive; I: Intermediate

48 hours while *Lacticaseibacillus casei* had the lowest yield (3.6 g/l) at 48 hours; *Lacticaseibacillus casei* produced the highest amount of hydrogen peroxide (0.00036 g/l) while *Lactiplantibacillus plantarum* and *Fructilactobacillus spp.* produced the lowest yield (0.00021 g/l) at 48 hours. The highest amount of diacetyl (3.01 g/l) was produced by *Lactobacillus jensenii* while the lowest amount was observed in *Levilactobacillus brevis* (0.43 g/l) at 48 hours as shown in Table 3.

The antagonistic activity of antimicrobial compounds produced by Lactic acid bacteria against pathogenic organisms isolated from spoiled fruits is shown in Table 4. Among the pathogenic organisms examined, the maximum inhibitory activity was observed in

*Levilactobacillus brevis* against *B. megaterium* with 18 mm zone of inhibition while the minimum inhibitory activity by *Lactobacillus desidosus*, *Lactobacillus jensenii*, *Fructilactobacillus spp.* was observed against *B. subtilis*, and *Lactiplantibacillus plantarum* against *K. pneumoniae* with 8mm zone of inhibition.

Most of the antimicrobial compounds produced by Lactic acid bacteria isolated from healthy fruits shows antagonistic activity against Gram-positive, Gram-negative bacteria and fungi with *L. brevis* showing the highest zone of inhibition of (18mm) against *B. megaterium* while the lowest zone of inhibition (8mm) was observed in *L. desidosus*, *L. jensenii*, *Fructilactobacillus spp.* against *B. subtilis*, and *Lactiplantibacillus spp.* against *K. pneumoniae*. The Gram-negative

**Table 3. Quantity of antimicrobial compounds produced by Lactic Acid Bacteria**

Isolates	Antimicrobial compounds (g/l)		
	Lactic acid	Hydrogen peroxide	Diacetyl
<i>Lacticaseibacillus casei</i>	3.6	0.00036	1.72
<i>Levilactobacillus brevis</i>	5.4	0.00029	0.43
<i>Lactobacillus jensenii</i>	5.3	0.00032	3.01
<i>Lactobacillus desidosus</i>	5.3	0.00021	2.88
<i>Lactiplantibacillus plantarum</i>	5.6	0.00021	0.77
<i>Fructilactobacillus spp.</i>	4.6	0.00032	0.86

**Table 4. Antagonistic activity of antimicrobial compounds produced by Lactic acid bacteria against pathogenic organisms isolated from unhealthy fruits**

Organisms	<i>L. brevis</i>	<i>L. casei</i>	<i>L. desidosus</i>	<i>L. jensenii</i>	<i>Lactiplantibacillus plantarum</i>	<i>Fructilactobacillus spp.</i>
<b>Gram -positive bacteria (Zone of inhibition in mm)</b>						
<i>B. coagulans</i>	-	13	9	-	-	-
<i>B. pulmilus</i>	-	-	-	-	-	-
<i>B. megaterium</i>	18	-	11	14	10	13
<i>Bacillus subtilis</i>	-	-	8	8	12	8
<i>B. licheniformis</i>	15	-	12	14	14	9
<b>Gram- negative bacteria</b>						
<i>P. cepacia</i>	-	-	-	12	-	-
<i>P. putida</i>	-	-	-	-	13	9
<i>P. fluorescens</i>	-	-	-	-	-	10
<i>A. hydrophylia</i>	-	-	-	-	-	-
<i>E. aerogenes</i>	-	10	-	9	-	-
<i>E. coli</i>	-	-	-	-	-	-
<i>K. pneumoniae</i>	-	-	13	-	8	-
<i>S. dysenteriae</i>	-	-	13	14	10	13
<i>S. typhi</i>	10	-	-	-	12	-
<b>Fungi (Yeast and moulds)</b>						
<i>C. valida</i>	10	14	11	11	12	10
<i>S. cerevisiae</i>	-	12	10	11	10	13
<i>R. stolonifer</i>	11	10	-	-	14	-

organisms namely *Pseudomonas cepacia*, *Pseudomonas fluorescens*, *Aeromonas hydrophilia*, *Eschericia coli* showed slight or no inhibition compared to Gram-positive organisms. This is in accordance with earlier reports which revealed that different strains of LAB possess more active inhibition against Gram-positive organisms compared with Gram-negative organisms [22]. This may be due to the presence of an outer protective membrane in Gram-negative organisms, which covers the cytoplasmic membrane and peptidoglycan layer of the cells. It is responsible for preventing molecules such as antibiotics [23].

Lactic acid bacteria are widely known to have antimicrobial compounds which are active against closely related bacteria and other pathogens [24]. It was reported by Ogunbanwo [25] that selected *Lactobacillus* strains (*L. plantarum* F1 and *L. brevis* OG1) produced bacteriocin, which showed inhibitory activity against Gram-positive and Gram-negative strains (*E. coli*, *E. faecalis*, *B. cereus*, *S. aureus*, *Sh. dysentery*, *Sh. flexneri* and *Listeria monocytogenes*). A similar trend of the result was reported by Navarro [26] reported similar results of antimicrobial activity of *L. plantarum* against both Gram-negative and Gram-positive bacteria with an inhibition zone between 9-22mm. It was also reported that there was a poor antimicrobial activity of the cell-free supernatant of *L. brevis* against Gram-positive and Gram-negative strains with a diameter of inhibition zone between 7-15mm [27].

#### 4. CONCLUSION

The antimicrobial compounds produced by the Lactic Acid Bacterial had antimicrobial effects on food-borne pathogens.

#### SUPPLEMENTARY MATERIALS

Supplementary material is available in the following link:

<https://journalsajrm.com/index.php/SAJRM/libraryFiles/downloadPublic/5>.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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