Original Research Article

Antimicrobial effects of Lactic Acid Bacteria on Food-borne pathogens isolated from some fruits sold in Ibadan, Nigeria.

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, Eschericia coli, Salmonella typhii, Shigella dysentriae, 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, Amoxycillin/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 anantibiotic disc. *Lactiplantibacillus plantarum* had thehighest amount of Lactic acid (5.6 g/l) while *L. casei* had the lowest yield (3.6 g/l) at 48 hours. *L. casei* had thehighest amount of hydrogen peroxide (0.00036 g/l) while *Lactiplantibacillus plantarum.* and *Fructilactobacillus* spp. had thelowest 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 adiameter 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 8mm zone of inhibition.The antimicrobial compounds produced by the Lactic Acid Bacterial had antimicrobial effects on food-borne pathogens.

Keywords: Antimicrobial effect, food-borne pathogens, Lactic Acid Bacteria, Fruits

Introduction

Lactic acid bacteria (LAB) belong to groups of Gram-positive bacteria, with a common characteristic that produces lactic acid as the main product during thefermentation of carbohydrates. They are anaerobic or facultative aerobic coccior rods shaped cells, non-sporulating, and acid-tolerant organisms [1].

Fresh fruits and vegetables are majorcomponents of the human diet because of their nutritional and medicinal properties and low energy content [2]. Several diseases such as Heart disease, colon cancer, obesity, and diabetes etc, can be reduced with a high intake of fruits and vegetables. Raw fruits and vegetables can be contaminated with pathogenic organisms and can occur directly or indirectly through animals or insects, soil, water, dirty equipment, human handling, etc. Fruits have low pH, high moisture content, and nutritional composition and as such susceptible to attack by pathogenic fungi which consequently cause rots and render them unfit for consumption as a result of mycotoxins produced [3, 4].

LAB produces an array of antimicrobial compounds, such as organic acids (lactic, citric, acetic, fumaric, and malic acid), hydrogen peroxide, CO_2, diacetyl, ethanol, reuterin, acetaldehyde, acetoin, ammonia, bacteriocins, bacteriocin-like inhibitory substances (BLIS), and other vital metabolites, which possess strong inhibitory effects on the growth and toxin production of many microorganisms [5, 6]. Furthermore, the antimicrobial effect of lactic acid bacteria is a result of competition with pathogenic microorganisms for available nutrients [7].

There is ahigh 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 arealso 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 (spoilt) 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) are shown in Tables 1-6.

2.3 Antibiotic Susceptibility Test

The antibiotics (µg/disc) used for this study: Ceftazidime (CAZ-30µg), Cefuroxime (CRX-30µg), Gentamicin (GEN-10µg), Ciprofloxacin (CPR-5µg), Cenftriaxone (CTR-30µg), Ofloxacin (OFL-5µg), Erythromycin (ERY-30µg), Cloxicillin (CXC-5µg), Nitrofurantoin (NIT-300µg), Ampicillin (AMP-10µg), Amoxycillin/Clavulinate (AUG-30µg). The antibiotic susceptibility test of the samples was analyzed in accordance with Clinical and Laboratory Standards Institute Standard guidelines [11].

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 x g for 15 minutes.

Lactic acid

The quantity of lactic acid was determined as follows; 25 ml of the supernatant fluid of thetest organism was measured into a beaker, andthree drops of phenolphthalein were added as anindicator. From a burette, 0.1 M NaOH was slowly added to the fluid until a pink colour appeared. Each ml of 0.1M NaOH is equivalent to 90.08 mg of lactic acid [12].

Hydrogen Peroxide

The amount of hydrogen peroxide was determined as follows; 20 ml of diluted Sulphuric acid was added to 25 ml of the supernatant fluid of the test organisms. Titration was carried out with 0.1M Potassium permanganate. Each ml of 0.1M Potassium permanganate is equivalent to 1.070 mg of hydrogen peroxide. The endpoint is reached when there was decolourization [12].

Diacetyl

This was quantified by transferring 25 ml of the supernatant of the test organism into a conical flask and 7.5 ml of hydroxylamine solution was used for the residual titration. Titration was done with 0.1M Hcl to a greenish-yellow endpoint using bromophenol blue as an indicator. The equivalent factor of Hcl to diacetyl is 21.5mg [12].

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°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° C the antagonistic effect of the cell-free supernatant on bacteria isolated from spoilt 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. dysentriae, S. typhi),* and fungal isolates *(C. valida, S. cerevisae, R. stolonifer*) respectively using sterile swab well of 7mm were cut into the agar plate and 100µl 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°C. The inhibitory effects were determined by measuring the diameters of zone of inhibition around the wells [13].

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 spoilt fruits are shown in Table 1. All the isolates were 100% resistant to Cefuroxime (CRX), Amoxycillin/Clauvulanate (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 33mm zone of inhibition. While the antibiotics resistance pattern of Gram positive bacteria isolated from spoilt fruits are shown in Table II. All the isolates were 100% resistant to Cloxicillin (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 27mm 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 [14].The high resistance to ampicillin (100%) recorded in this study is similar to that obtained by Issa [15] and Yakubu [16] where 100% resistance to ampicillin were obtained from isolates in milk and processed meat respectively and Endang [17] 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 [18] and Rahimi [19] 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 [20].

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 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. jenseni* 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 spoilt fruits is shown in Table 4. Among the pathogenic organisms examined, the maximum inhibitory activity was observed in *Levilactobacillus brevis* aga inst *B. megaterum* with 18mm zone of inhibition while the minimum inhibitory activity by *Lactobacillus desidosus, Lactobacillus jenseni, 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. jenseni, Fructilactobacillus* spp. against *B. subtilis,*

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)					
OJE MARKET								
***Klebsiella pneumoniae* (P)**	21 (S)	0 (R)	21 (S)	26 (S)	23 (S)	0 (R)	0 (R)	0 (R)
***Aeromonas hydrophylia* (P)**	0 (R)	0 (R)	0 (R)	19 (I)	18 (S)	0 (R)	12 (R)	0 (R)
***Enterobacter aerogenes* (S)**	18 (I)	0 (R)	0 (R)	19 (I)	23 (S)	0 (R)	24 (S)	0 (R)
***Aeromonas hydrophylia* (A)**	21 (S)	0 (R)	0 (R)	27 (S)	27 (S)	0 (R)	0 (R)	0 (R)
***Pseudomonas putida* (A)**	29 (S)	0 (R)	21 (S)	26 (S)	29 (S)	0 (R)	33 (S)	0 (R)
***Eschericia coli* (A)**	30 (S)	0 (R)	20 (S)	24 (S)	27 (S)	0 (R)	0 (R)	0 (R)
***Salmonella typhi* (A)**	27 (S)	0 (R)	23 (S)	24 (S)	24 (S)	0 (R)	0 (R)	0 (R)
***Salmonella typhi* (P)**	0 (R)	0 (R)	15 (S)	18 (I)	20 (S)	0 (R)	0 (R)	0 (R)
Shigella dysentriae (S)	23 (S)	11 (R)	19 (S)	13 (R)	20(S)	0(R)	0(R)	0(R)
Shigella dysentriae (P)	22 (S)	11 (R)	18 (S)	20 (I)	20(S)	11(R)	0(R)	0(R)
BODIJA MARKET								
Enterobacter aerogenes (S)	0 (R)	0 (R)	0 (R)	25 (S)	14 (I)	0 (R)	0 (R)	0 (R)
***Pseudomonas cepacia* (S)**	28 (S)	0 (R)	21 (S)	28 (S)	28 (S)	0 (R)	20 (S)	0 (R)
***Enterobacter aerogenes* (P)**	0 (R)	0 (R)	20 (S)	19 (I)	20 (S)	0 (R)	0 (R)	0 (R)
***Pseudomonas fluorescens* (S)**	0 (R)	0 (R)	25 (S)	19 (I)	25 (S)	0 (R)	15 (I)	0 (R)
***Aeromonas hydrophylia* (S)**	0 (R)	0 (R)	21 (S)	14 (R)	18 (S)	0 (R)	0 (R)	0 (R)
***Pseudomonas cepacia* (A)**	24 (S)	0 (R)	21 (S)	23 (S)	23 (S)	0 (R)	0 (R)	0 (R)

CAZ: Ceftazidime: **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

and *Lactiplantibacillus spp.* against *K. pneumoniae.*The Gram-negative organisms namely *Pseudomonas cepacia*, *Pseudomonas fluorescens, Aeromonas hydrophylia, 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 [21]. 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 [22].

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)							
OJE MARKET								
Bacillus coagulans (S)	23 (S)	11 (R)	21 (S)	27 (S)	0 (R)	0 (R)	20 (S)	15 (I)
Bacillus pulmilus (A)	18 (I)	0 (R)	17 (S)	13 (I)	0 (R)	0 (R)	26 (S)	0 (R)
Bacillus polymyxa (P)	19 (I)	17 (I)	17 (S)	22 (I)	0 (R)	0 (R)	23 (S)	16 (I)
BODIJA MARKET								
Bacillus licheniformis (S)	21 (S)	0 (R)	17 (S)	24 (S)	0 (R)	0 (R)	21 (S)	0 (R)
Bacillus megaterum (P)	11 (R)	9 (R)	13 (I)	17 (R)	17 (I)	0 (R)	12 (I)	15 (I)
Bacillus subtilis (A)	20 (I)	16 (I)	17 (S)	13 (R)	15 (I)	0 (R)	21 (S)	19 (S)

CAZ: Ceftazidime: **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

Lactic acid bacteria are widely known to have antimicrobial compounds which are active against closely related bacteria and other pathogens [23]. It was reported by Ogunbanwo [24] 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 theresult was reported by Navarro [25] reported similar results of antimicrobial activity of *L.plantarum* against both Gram-negative and Gram-positive bacteria with aninhibition zone between 9-22mm. It was also reported that there was apoor 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 [26].

Table 3: Quantity of antimicrobial compounds produced by Lactic Acid Bacteria

Isolates	**Antimicrobial compounds (g/l)**		
	Lactic acid	**Hydrogen peroxide**	**Diacetyl**
Lacticaseibacillus casei	3.6	0.00036	1.72
Levilactobacillus brevis	5.4	0.00029	0.43
Lactobacillus jenseni	5.3	0.00032	3.01
Lactobacillus desidosus	5.3	0.00021	2.88
Lactiplantibacillus *plantarum*	5.6	0.00021	0.77
Fructilactobacillus spp.	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	*L. brevis*	*L. casei*	*L. desidosus*	*L. jenseni*	*Lactiplantibacillus plantarum*	*Fructilactobacillus spp.*
Gram -positive bacteria(Zone of inhibition in mm)						
B. coagulans	-	13	9	-	-	-
B. pulmilus	-	-	-	-	-	-
B. megaterium	18	-	11	14	10	13
Bacillus subtilis	-	-	8	8	12	8
B. licheniformis	15	-	12	14	14	9
Gram- negative bacteria						
P. cepacia	-	-	-	12	-	-
P. putida	-	-	-	-	13	9
P. fluorescens	-	-	-	-	-	10
A.hydrophylia	-	-	-	-	-	-
E. aerogenes	-	10	-	9	-	-
E. coli	-	-	-	-	-	-
K. pneumoniae	-	-	13	-	8	-
S. dysentriae	-	-	13	14	10	13
S. typhi	10	-	-	-	12	-
Fungi (Yeast and moulds)						
C. valida	10	14	11	11	12	10
S. cerevisae	-	12	10	11	10	13
R. stolonifer	11	10	-	-	14	-

4. Conclusion

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

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