Original Research Article

Antimicrobial Activity of Lactic Acid Bacteria Against Food-Borne Pathogens from Selected Fruits Sold In Major Markets, Ibadan, Nigeria.

Abstract

This study was conducted to determine the effect of antimicrobial compounds produced by Lactic acid bacteria against food-borne pathogens from selected fruits sold in major markets in Ibadan. The Lactobacillus species isolated from healthy fruits includes *L. casei, L. brevis, L. desidosus, L. jenseni, Lactiplantibacillus spp.* 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. Gram negative isolates were 100% resistant to Cefuroxime, Amoxycillin/Clauvulanate and Ampicillin while majority of the isolates were highly sensitive to Ofloxacin while Gram positive isolates were 100% resistant to Cloxicillin and highly sensitive to Ofloxacin and Gentamycin. *Lactiplantibacillus spp* had highest 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 highest amount of hydrogen peroxide (0.00036 g/l) while *Lactiplantibacillus spp.* and *Fructilactobacillus spp.* had 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 diameter of 18mm zone of inhibition while the minimum activity by *L. desidosus, L. jenseni, Fructilactobacillus spp.* was observed against *B. subtilis and Lactiplantibacillus spp* against *K. pneumoniae* with diameter 8mm zone of inhibition. Antibiotic-resistant pathogenic bacteria and spoilage fungi found in this study could be a major threat to public health.

Keywords: Antibiotic-resistant bacteria, foodborne pathogens, Lactobacillus species

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 fermentation of carbohydrates. They are anaerobic or facultative aerobic *cocci* or rods shaped cells, non-sporulating, and acid-tolerant organisms [1].

Fresh fruits and vegetables are major component of human's 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 of human health significance, and can occur directly or indirectly through animals or insects, soil, water, dirty equipment, and human handling etc. Fruits have low pH, high moisture content and nutrient composition, as a result of these are very susceptible to attack by pathogenic fungi, also causing rots which makes them unfit for consumption by producing mycotoxins [3, 4].

LAB produce an array of antimicrobial compounds, such as organic acids (lactic, citric, acetic, fumaric, and malic acid), hydrogen peroxide, CO , 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 as a result of competition with pathogenic microorganisms for available nutrients [7].

There is high prevalence of food borne pathogens on fruits due to contamination coupled with high antibiotic resistance of pathogens leading to food-borne illness which are hazardous to human health. Lactic acid bacteria is well known to produce antimicrobial compounds which inhibit microorganisms and also useful in preventing diseases. In this study, exploring antimicrobial potentials of LAB from sources such as apple, pineapple and soursop is necessary. Therefore, this study was conducted to determine the antibiotics resistance pattern of pathogens isolated from unhealthy fruits; quantification and antagonistic activity of antimicrobial compounds produced by isolated Lactic acid bacteria against isolated pathogens.

2. Materials and methods

2.1 Sample Collection

The fruits samples such as apple, pineapple and soursop used for this study were randomly purchased from different locations in Bodija and Oje market in Ibadan, Oyo State, Nigeria. The healthy fruits samples were collected into labeled sterile containers without washing and transported to the laboratory for microbial analysis. 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] 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, labelled 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.
Isolation and Characterization of probable lactic acid bacteria and food-borne pathogens were done accordingly [9, 10].

2.3 Antibiotic Susceptibility Test

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 were grown in MRS broth for 48 hours and centrifuged at 4000 x g for 15 minutes.

2.5 Lactic acid, Hydrogen Peroxide and Diacetyl

Lactic acid, Hydrogen Peroxide and Diacetyl of the samples were determined following analytical procedures [12].

2.6 Screening for antagonistic activity of cell-free supernatant

Lactic acid bacteria were inoculated into de Man Rogosa Sharpe (MRS) broth and incubated at 30^{o}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^{o} C, the antagonistic activity of the cell-free supernatant was determined by agar well diffusion assay (AWDA) against foodborne pathogens isolated from spoilt fruits. Mueller Hinton plates and Potato Dextrose Agar plates were inoculated with bacterial and fungal isolates 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 $30^{o}C$. The antimicrobial activity was determined by measuring the diameters of zone of inhibition around the wells [13].

3. Results and discussion

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 increases 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 spp.* 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 spp. and Fructilactobacillus spp.* produced the lowest yield (0.00021 g/l) at 48 hour. 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 are 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 spp* 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 resistance pattern 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	0 (R)	0 (R)	0 (R)	19 (I)	18 (S)	0 (R)	12 (R)	0 (R)

***hydrophylia* (P)**								
***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 resistance pattern 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*). Similar trend of result was reported by Navarro [25] reported similar result of antimicrobial activity of *L.plantarum* against both Gram-negative and Gram-positive bacteria with inhibition zone between 9-22mm. It was also reported that there was poor antimicrobial activity of cell free supernatant of *L. brevis* against Gram positive and Gram negative strains with diameter of inhibition zone between 7-15mm [26]. Galal *et al.*, 2012 also

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 spp.	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 spp.*	*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 use of antimicrobial compounds produced by Lactic acid bacteria could help to curtail multiple drugs resistance. Fruit contamination with antibiotic- resistant bacteria when disposed carelessly into the environment especially water bodies, could lead to water pollution which can be a major threat to public health

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