

## Original Research Article

Comment [u1]:

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

Comment [u2]: The author can adopt this topic for detailed information: **Antimicrobial activity of lactic acid bacteria against food pathogen isolated from selected fruits sold in Major Markets in Ibadan, Oyo State, 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 *Aeromonashydrophylia*, *Enterobacteraerogene*, *Eschericia coli*, *Salmonella typhii*, *Shigelladysentriae*, *Pseudomonas fluorescens*, *Bacillismegaterium*, *Candida valida*, *Saccharomyces cerevisiae*, *Rhizopusstolonifer* 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. megaterium* 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.

Comment [u3]: Italics. State briefly the method of isolation, identification of the food borne pathogen and the LAB isolates use before result. What method did you use for the antibiotic susceptibility?

**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 rodshaped 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<sub>2</sub>, 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°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 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

**Comment [u4]:** The author need to be more detailed with the method of isolation. Method of identification of the LAB should be detailed too. The different biochemical test such as catalase, indole, gram staining etc need to be stated. This will lead to knowing the probable microorganisms identified in this study. How did the author get the food borne pathogen? Was it freshly isolated or obtained from collection centre?  
This section should be subtitled and be arranged orderly such as:  
Sample collection  
Sample preparation  
Isolation of LAB from healthy fruits  
Isolation of LAB from unhealthy fruits  
Biochemical identification of the Isolates food borne pathogens and the LAB isolates.  
Determination of Lactic Acid, Hydrogen peroxide and diacetyl of the LAB isolates  
Antibiotic Susceptibility of the Food borne pathogens  
Screening for antagonistic activity of the LAB Isolates.

**Comment [u5]:** This should be well spelt out.

**Comment [u6]:** This method is not detailed enough. Compare this with the screening of antagonistic activity of cell-free supernatant. Is the author saying the same thing?

**Comment [u7]:** Determination of Lactic acid, Hydrogen peroxide and diacetyl produced by the LAB

**Comment [u8]:** Write explicitly on each method use for each metabolites

**Comment [u9]:** Was this test carried out against resistance food borne pathogen or on all the food borne pathogen isolated and identified?

**Comment [u10]:** Agar Well Diffusion Assay (AWDA)

**Comment [u11]:** Write out the different species of bacteria and fungi use for this study

for Bacterial and fungal isolates respectively at 30°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 spoiled fruits are shown in Table I. All the isolates were 100% resistant to Cefuroxime (CRX), Amoxycillin/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 Nitofurantoine(NIT) with 33mm zone of inhibition. While the antibiotics resistance pattern of Gram positive bacteria isolated from spoiled 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 spoiled 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 *Lactocaseibacillus casei* had the lowest yield (3.6 g/l) at 48 hours; *Lactocaseibacillus 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 spoiled fruits are shown in Table 4. Among the pathogenic organisms examined, the maximum inhibitory activity was observed in *Levilactobacillus brevis* against *B. megaterium* 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 spoiled 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 hydrophilia</i>       | 0 (R)  | 0 (R) | 0 (R)  | 19 (I) | 18 (S) | 0 (R) | 12 (R) | 0 (R) |

**Comment [u12]:** The author need to be more organized with the report. The author need to first give details on the colony count of isolated obtained from the samples. The author should report on the result obtained from identification of isolates. The biochemical table needs to be presented. The author should report the result according to the pattern stated in materials and methods.

**Comment [u13]:** The author need to state the following: give any information on the number of samples obtained from each market. give the colony count of organisms isolated the percentage of occurrence of isolates obtained from healthy and unhealthy fruits.

|                                    |        |        |        |        |        |       |        |       |
|------------------------------------|--------|--------|--------|--------|--------|-------|--------|-------|
| <b>(P)</b>                         |        |        |        |        |        |       |        |       |
| <b>Enterobacteraerogenes (S)</b>   | 18 (I) | 0 (R)  | 0 (R)  | 19 (I) | 23 (S) | 0 (R) | 24 (S) | 0 (R) |
| <b>Aeromonashydrophylia (A)</b>    | 21 (S) | 0 (R)  | 0 (R)  | 27 (S) | 27 (S) | 0 (R) | 0 (R)  | 0 (R) |
| <b>Pseudomonas putida (A)</b>      | 29 (S) | 0 (R)  | 21 (S) | 26 (S) | 29 (S) | 0 (R) | 33 (S) | 0 (R) |
| <b>Eschericia coli (A)</b>         | 30 (S) | 0 (R)  | 20 (S) | 24 (S) | 27 (S) | 0 (R) | 0 (R)  | 0 (R) |
| <b>Salmonella typhi (A)</b>        | 27 (S) | 0 (R)  | 23 (S) | 24 (S) | 24 (S) | 0 (R) | 0 (R)  | 0 (R) |
| <b>Salmonella typhi (P)</b>        | 0 (R)  | 0 (R)  | 15 (S) | 18 (I) | 20 (S) | 0 (R) | 0 (R)  | 0 (R) |
| <b>Shigelladysentriae (S)</b>      | 23 (S) | 11 (R) | 19 (S) | 13 (R) | 20(S)  | 0(R)  | 0(R)   | 0(R)  |
| <b>Shigelladysentriae (P)</b>      | 22 (S) | 11 (R) | 18 (S) | 20 (I) | 20(S)  | 11(R) | 0(R)   | 0(R)  |
| <b>BODIJA MARKET</b>               |        |        |        |        |        |       |        |       |
| <b>Enterobacteraerogenes (S)</b>   | 0 (R)  | 0 (R)  | 0 (R)  | 25 (S) | 14 (I) | 0 (R) | 0 (R)  | 0 (R) |
| <b>Pseudomonas cepacia (S)</b>     | 28 (S) | 0 (R)  | 21 (S) | 28 (S) | 28 (S) | 0 (R) | 20 (S) | 0 (R) |
| <b>Enterobacteraerogenes (P)</b>   | 0 (R)  | 0 (R)  | 20 (S) | 19 (I) | 20 (S) | 0 (R) | 0 (R)  | 0 (R) |
| <b>Pseudomonas fluorescens (S)</b> | 0 (R)  | 0 (R)  | 25 (S) | 19 (I) | 25 (S) | 0 (R) | 15 (I) | 0 (R) |
| <b>Aeromonashydrophylia (S)</b>    | 0 (R)  | 0 (R)  | 21 (S) | 14 (R) | 18 (S) | 0 (R) | 0 (R)  | 0 (R) |
| <b>Pseudomonas cepacia (A)</b>     | 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*, *Aeromonashydrophylia*, *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    |
|---|--------|--------|--------|--------|-------|-------|--------|--------|
| <b>ANTIBIOTIC/ZONE OF INHIBITION (MM)</b> |        |        |        |        |       |       |        |        |
| <b>OJE MARKET</b>                         |        |        |        |        |       |       |        |        |
| <b>Bacillus coagulans (S)</b>             | 23 (S) | 11 (R) | 21 (S) | 27 (S) | 0 (R) | 0 (R) | 20 (S) | 15 (I) |
| <b>Bacillus pulmilus (A)</b>              | 18 (I) | 0 (R)  | 17 (S) | 13 (I) | 0 (R) | 0 (R) | 26 (S) | 0 (R)  |
| <b>Bacillus polymyxa (P)</b>              | 19 (I) | 17 (I) | 17 (S) | 22 (I) | 0 (R) | 0 (R) | 23 (S) | 16 (I) |
| <b>BODIJA MARKET</b>                      |        |        |        |        |       |       |        |        |
| <b>Bacillus licheniformis</b>             | 21 (S) | 0 (R)  | 17 (S) | 24 (S) | 0 (R) | 0 (R) | 21 (S) | 0 (R)  |

|                              |        |        |        |        |        |       |        |        |
|------------------------------|--------|--------|--------|--------|--------|-------|--------|--------|
| <b>(S)</b>                   |        |        |        |        |        |       |        |        |
| <b>Bacillus megaterum</b>    | 11 (R) | 9 (R)  | 13 (I) | 17 (R) | 17 (I) | 0 (R) | 12 (I) | 15 (I) |
| <b>(P)</b>                   |        |        |        |        |        |       |        |        |
| <b>Bacillus subtilis (A)</b> | 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:** Amoxicillin/ClavulanateP : 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].Galalet al., 2012 also

Comment [u14]:

Comment [u15]: Incomplete statement

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

#### 4. Conclusion

The use of antimicrobial compounds produced by Lactic acid bacteria could help to curtail multiple drug 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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