RESEARCH ARTICLE

Antibacterial activity of lactic acid bacteria isolated from Etsako Osuemegbe rice.

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Received: 22 December 2022, Revised: 18 January 2022, Published: 06 February 2023

ABSTRACT

Background: Probiotics have antibacterial effects against pathogenic bacteria in the gut while maintaining the balance of intestinal flora. This study aimed to experiment the antibacterial activity of two Lactobacillus species against pathogenic bacteria. **Methods:** Two different species of *Lactobacillus lactiplantibacillus* plantarum (CIP 103151) and *Limosilactobacillus fermentum* (CIP 102980) that were previously isolated from fermented Etsako Osuemegbe rice were investigated for their inhibitory ability against three (3) organisms (*Escherichia coli, Staphylococcus aureus* and *Salmonella*). Agar overlay method was employed and plates were examined for clear zone of inhibition. **Results:** The *lactiplantibacillus plantarum* inhibited the growth of E. coli, S. aureus, and Salmonella sp with diameter zone of inhibition range between 15.00+0.57 and 18.00+0.77 mm, while *Limosilactobacillus fermentum* inhibited the growth of E. coli, S. aureus, and Salmonella sp with diameter zone of inhibition range between 14.00+1.00 and 18.00+0.50 mm. **Conclusion:** The findings in this study suggest that LAB species can be used as alternate antibiotic if explored due to their ability to inhibit the growth of pathogenic organisms.

Keywords: Lactic Acid Bacteria; Probiotic; Fermentation; Functional food; Antibacterial; Osuemegbe rice

Citation: Musah, S.S., Owuna, J.E., Makut, M.D., Adamu, B.B., Ibrahim G.I, Izebe, K., Kabir, A., Oziegbe, O.S., and Aboh, M.I. (2023).Antibacterial activity of lactic acid bacteria isolated from Etsako Osuemegbe rice. AROC in Food and Nutrition, 2(1), 01-05, https://doi.org/10.53858/arocfn02010105

1.0 Introduction

Nigerians and over half population of people in the world consume rice as staple food [1]. The outer layer of rice, is a by-product of rice milling industry which commonly used as animal feed [2]. Several active compounds including probiotic lactic acid bacteria have been identified in fermented rice which are widely used to enhance the functionalities of some food stuffs and improve their capacity against chronic diseases [3,4].

Fermentation process involved microorganism's metabolic activity as well as increasing the availability of nutrients in raw materials [5]. Biochemical changes associated with microbial metabolism and enzyme actions during fermentation are the main factors affecting the nutritional and production of several active metabolites are produced during fermentation [6]. Probiotic lactic acid bacterial have been widely considered for the treatment and prevention of infectious diseases because of their various mechanisms of action that result in the production of beneficial metabolites with have antagonistic effect against pathogenic bacteria [7]. Lactic acid bacteria produce a variety of antimicrobial organic acids that kill or inhibit growth of pathogenic microorganisms [8]. Homo and hetero-fermentation of glucose yield potent end products such as lactic acid, acetic acid, ethanol and carbon dioxide [9]. Carbon dioxide manifests antimicrobial properties in two ways: firstly, CO2 is itself toxic to some microorganisms, and secondly, creation of an anaerobic atmosphere prevents proliferation of aerobic microorganisms, especially concentrations of 85% nitrogen, 10% hydrogen and 5% carbon dioxide [10].

Furthermore, LAB produces hydrogen peroxide (H2O2) which has a strong oxidizing effect on bacterial cells resulting in destruction or inactivation of pathogenic microorganisms [11]. Lactic acid bacteria (LAB) are group of related bacteria that produce lactic acid as a result of carbohydrate fermentation [12]. They are gram positive, fastidious, acid tolerant, generally non-sporulating, catalase

negative and non-respiring rod or cocci that are associated by their common metabolic and physiological characteristics to produce lactic acid as fermentation metabolites [13,14]. They are nonpathogenic organism reputed as GRAS or Generally Recognized as Safe status. This study aimed to experiment the antibacterial activity of two Lactobacillus species against pathogenic bacteria. Two different species of Lactobacillus *lactiplantibacillus plantarum* (CIP 103151) and *Limosilactobacillus fermentum* (CIP 102980) that were previously isolated from fermented Etsako Osuemegbe rice were investigated for their inhibitory ability against three (3) organisms (*Escherichia coli, Staphylococcus aureus* and *Salmonella*).

2.0 Materials and Methods

2.1 Study Area

This study was carried out in Keffi metropolis of Nasarawa state, Nigeria. Keffi is about 58 km from Abuja, the Federal Capita Territory (FCT), and is about 128 km from Lafia, the capital town of Nasarawa state. Keffi is situated on latitude 8°5'N and longitude 7°50'E, and on the altitude of 850 m above the sea level [15].

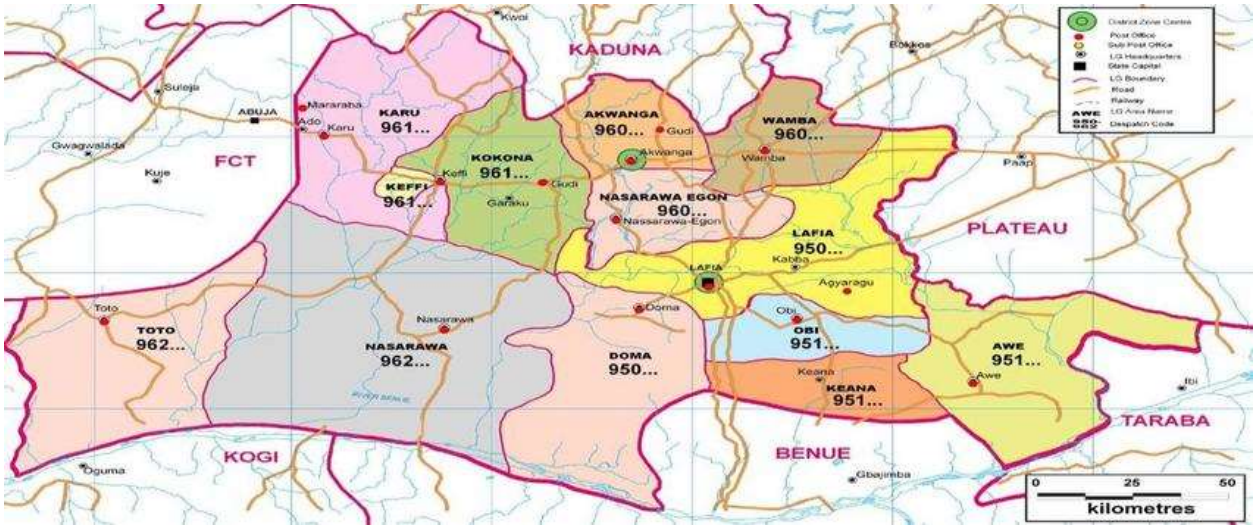

Figure 1: Map of the study area
Source:[16]

2.2 Collection of LABs

In this study, two Lactobacillus strains: *lactiplantibacillus plantarum* CIP 103151 and *Limosilactobacillus fermentum* CIP 102980, that were previously isolated from fermented Osuemegbe rice were obtained from Department of Microbiology and Biotechnology laboratory in National institute for pharmaceutical research development (NIPRD), FCT, Abuja Nigeria.

2.3 Preparation of Lactobacillus strains

Strains of probiotic bacteria were cultivated in MRS medium and three pathogenic bacteria (Staphylococcus aureus, Salmonella, and Escherichia coli) which had been isolated from clinical samples at department of bacteriology laboratory of National institute for pharmaceutical Research and Development (NIPRD) Idu industrial area, karmo, Abuja, where used as test organisms by adopting Agar overlay method for the antibacterial assessment [17]. In this way, the pathogenic samples were subculture into a freshly prepared Muller Hinton broth (oxoid) for 24 hrs at 30°C to under microaerophilic condition to obtain fresh culture, from which 1.5×108 ml of pathogenic bacteria were prepared which was equivalent to 0.5 of broth McFarland in normal saline. Lactobacillus sp. were inoculated in three parts of MRS and incubated at 37°C for 24 hours [18].

Table 1. Bacterial Strains, Media and Culture Condition.

Bacterial Species	Strain	Origin	Media/Atmosphere/Temperature
lactiplantibacillus plantarum	CIP 103151	From fermented rice	MRS/anaerobic/37°C
Limosilactobacillus fermentum	CIP 102980	From fermented rice	MRS/anaerobic/37°C

Source; Makut et al. [19]

 https://doi.org/10.53858/arocfn02010105

2.4 Antibacterial activity Assay of lactic acid bacteria against test organisms

The agar overlay method [17], was employed to determine the ability of the viable lactic acid bacteria strains to inhibit the growth of the indicator pathogens. A loop full of LAB in MRS broth was inoculated on MRS agar plate as a thick line of about 2mm and about 30mm long at a good distance away from the edge of the plates and incubated under microaerophilic condition at 37°C for 24h. After incubation, the MRS agar plates were overlaid with approximately 0.2ml x 104 CFU/ ml of an overnight broth culture of the test pathogens inoculated in 10ml of Mueller Hinton soft agar. The overlay was allowed to set, and incubated at 37°C under aerobic condition. The plates were then examined for clear zone of inhibition around the line of the LAB and the clear zones were measured [20]

2.5 Statistical Analysis

Data generated in this study were analyzed using statistical package for social science (SAS) version 9.4 and presented as means ± standard error of mean. Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were used to determine the significant differences between the rice samples at P= 0.05.

3.0 Results and Discussion

The antibacterial activity of lactic acid bacteria against the test organism is shown in table 2. The *lactiplantibacillus plantarum* inhibited the growth of *E. coli, S. aureus, and Salmonella sp* with diameter zone of inhibition range between 15.00+0.57 and 18.00+0.77 mm, while *Limosilactobacillus fermentum* inhibited the growth of *E. coli, S. aureus, and Salmonella sp* with diameter zone of inhibition range between 14.00±1.00 and 18.00±0.50 mm (Table 2).

Table 2: Antibacterial activity of lactic acid bacteria against the test organism

	Mean diameter in inhibition zone (mm)		
Lactic Acid Bacteria	***E. coli***	***S. aureus***	***Salmonella sp***
SR 5/1	15.00+0.57	19.00+0.77	18.00+0.77
Sd-3/2	14.00±1.00	16.00±0.15	18.00±0.50
Control	3.00±0.00	0.00±0.00	29.00±2.80

SR5/1; *lactiplantibacillus plantarum* CIP 103151, Sd-3/2; *Limosilactobacillus fermentum* CIP 102980, Control: Amoxicillin 500mg

Lactic acid bacteria play a potential alternative to antibiotics in the treatment of inflammatory bowel disease (IBD) [21]. The continuous increase in multiple resistance pathogenic bacteria particularly in the clinical setting has led to the investigation of natural effective alternatives to known antibiotics. Lactic acid bacteria are well known producers of antimicrobial compounds especially bacteriocins which have high antimicrobial activity. As observed in this study, Table 2 showed zones of inhibition and antibacterial activity of lactic acid bacteria and this indicates the efficacy of these *Lactobacillus* species against the test bacteria used.

SR5/1 LABs isolated from the Rice products possess very good antibacterial activity against target bacteria with inhibition zone ranging from 14.00mm to19.00 mm diameter as observed in this study. *Staphylococcus aureus* and *Salmonella specie* shows high sensitive to bacteriocin of *lactiplantibacillus plantarum*. However, *Escherichia coli show a* slight resistant to *lactiplantibacillus plantarum* with an inhibition range of 15.00mm. *Limosilactobacillus fermentum* also exhibit high sensitivity to *Staphylococcus aureus* and *Salmonella specie* while *Escherichia coli also* show a slight resistant to *Limosilactobacillus fermentum*. Amoxicillin that was used as positive control at the concentration of 50µg had antimicrobial activities against all the bacteria used with varied inhibition zones except for *Staphylococcus aureus* that shows complete resistant to the control antibiotic used. It can be concluded that these two Lactobacillus strains had potential antimicrobial compounds against human pathogenic organism and should be further studied for their human health benefits.

Lactic acid bacteria strains are highly recommended to be explored as alternatives to antibiotics since their various mechanisms of growth inhibition against pathogenic bacteria have been extensively documented [22]. Probiotic lactic acid bacteria should be exploited to achieving quality advancement in one health: integrated and unify approach aim at sustainably balance and optimize the health of people, animals and ecosystem [23].

4.0 **Conclusion**

Lactic acid bacteria isolated from fermented Osuemegbe rice have high production of antimicrobial substances as it has shown a

strong inhibition against Gram-negative and Gram-positive bacteria. *Limosilactobacillus fermentum and lactiplantibacillus plantarum* strains produce bacteriocins that inhibit pathogens such as *E. coli, S. aureus* and *Salmonella* specie. The findings in this study suggest that LAB producing bacteriocins can be used as alternate mechanism to inhibit the growth of pathogenic organisms.

Funding: This work received no external funding.

Competing Interests: The authors declared that no conflict of interest exists.

Ethical Approval: Not applicable

Authors contributions: All authors were involved in intellectual content, literature search, manuscript preparation, editing and review. All authors read and approved the final submission of the manuscript.

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