ANTIMICROBIAL METABOLITES PRODUCED BY LACTIC ACID BACTERIA AGAINST FOOD SPOILAGE BACTERIA

Ekeyi, Divine-favour I., Ezumezu, Chidimma P. & Amakor, Chilota A.

deeluv.devyn@gmail.com (07067452924, 0816 024 1935)

Department of Science Laboratory Technology, Federal Polytechnic Oko

ABSTRACT

Lactic acid bacteria isolated from ogiri and soymilk were screened to determine their antibacterial activity against food spoilage organisms. The antimicrobial activity of these probiotic bacteria against many species or microorganisms is one useful property they possess which could be useful to prevent food spoilage caused by certain sensitive bacteria and also to control the speed of propagation of potentially pathogenic bacteria by their application. Lactic acid bacteria are probiotic bacteria that produce metabolites with broader spectrum of activity. The antagonistic properties of these isolates against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* were examined using agar well diffusion method. Two lactic acid bacterial isolates, namely C1 and C2 were identified as *Lactobacillus plantarum* and *Lactobacillus brevis* were effective against all the selected indicator organisms except Salmonella *typhi*. Among the isolates C1 which has a broader spectrum of activity showed the highest antibacterial activity, against all the indicator pathogens tested, except *Salmonella typhi*. Its activity was maximum against *Escherichia coli* with a zone of inhibition 12.0 mm diameter and least activity *against S. typhi* as well, with the diameter or inhibition 1.6 mm.Overall, the bacteriocin produced by these lactic acid bacteria exhibited significant inhibitory effects against both Gram-negative and Gram-positive pathogenic strains, suggesting their potential as natural biopreservatives in various food products.

Keywords: Lactic acid bacteria, Antimicrobial metabolites, Food spoilage bacteria, Inhibition

INTRODUCTION

In today's society, consumers have become increasingly concerned about the synthetic chemicals used as preservatives in food. As a result, there is a growing trend towards less processed foods. However, untreated foods can harbor dangerous pathogens that can multiply under refrigeration

and without oxygen. To address this dilemma, antimicrobial metabolites of fermentative microorganisms are being utilized as a solution. Many such antimicrobial chemicals have been in use for an extended period without any known adverse effects.

Organic compounds with antimicrobial properties produced by bacteria used to produce or associated with fermented foods have garnered significant interest. In fermentation processes, raw materials are converted by microorganisms (bacteria, yeast and molds) into products that possess acceptable qualities of food. A more recent study by Ganesan *et al*. (2021), also found that lactic acid bacteria in fermented foods like yogurt produce antimicrobial metabolites such as organic acids and bacteriocins to inhibit the growth of unwanted microorganisms. This highlights the importance of lactic acid bacteria (LAB) as natural preservatives in food products, especially those that undergo fermentation processes.

Fermented foods are highly valued economically due to their contribution to improving human health. Lactic acid bacteria (LAB), especially those containing probiotics or health-promoting bacteria, have contributed significantly to the increased volume of fermented foods worldwide (Wilson *et al*;2020).

Recent studies have delved into the impacts of lactic acid bacteria (LAB) on human health and the preservation of food. A recent analysis by Chen *et al*. (2021) delved into the correlation between LAB and human well-being, underscoring the pivotal role of these microbes in bolstering gut health and overall vitality. The scholars also explored contemporary methodologies for food preservation leveraging LAB, along with the benefits of integrating probiotic strains into fermented foods. In current times, the food industry heavily relies on chemical preservatives and antibiotics with antibacterial or antifungal attributes, albeit these substances harbor potential risks due to their toxicity, concurrently stifling the natural microbiota when ingested.

Bacteriocins produced by LAB draw intense research attention because they exhibit antibacterial activity against foodborne bacteria; these strains may be essential competitors against other organisms present within intestines since they contain biologically active protein moieties having bactericidal mode-of-action attaching onto specific cell receptors (Ogunbanwo *et a*l ;2023).

Bacteriocins produced by LAB serves importantly as natural antimicrobial agents against foodborne pathogens due to their specificity in targeting specific microbial strains and leaving

other beneficial microorganisms intact (Kommineni *et al*.,2021).This supports the potential use of LAB as probiotics for human health benefits.

Thus preservation through natural methods represents one of the biggest global concerns aimed at solving economic losses resulting from microbial decomposition affecting raw materials/foodstuffs' quality adversely. The most actively developed alternative approach towards preserving food involves utilizing LAB alongside their metabolites exhibiting antimicrobial characteristics forming during sugar fermentation processes leading rapidly to acidic environments preventing other groups' growths among microorganisms(Leroy *et al*.,2006).Recent discoveries highlight organic compounds belonging to different classes inhibiting other microbes' growth making them promising directions for future research practical importance purposes aiming at extending product shelf lives eg.*Bacillus spp., Enterococcus spp., Clostridium spp* (Ogunbanwo *et al* ;2023).

Despite the implementation of effective process control procedures, such as Hazard Analysis Critical Control Points (HACCP), to support improved manufacturing facilities across all industries - including the food industry where inadequate maintenance and hygiene practices contribute significantly towards declining product quality and increased consumer exposure risks concerning illnesses caused by contaminated consumables (Hernandez *et al*., 2005 and Enan *et al*.,2014), the number of reported cases continues to rise. Nowadays, consumers prefer fewer chemical preservatives and show an increased interest in preservation through LAB due to safe associations with humans consuming fermented foods that produce several metabolic products, including fatty acids, diacetyl, hydrogen peroxide, and organic acids. However, specific proteinaceous substances like bacteriocins are drawing particular attention as they inhibit pathogen types such as *Staphylococcus,Lactobacillus,Clostridium,Bacillus spp*.and *Enterococcus spp* (Huang *et al*.,2021) supports the growing interest of consumers in natural preservation methods using LAB-produced metabolites such as organic acids and bacteriocins. The study found that the use of these natural preservatives could effectively inhibit the growth of pathogenic bacteria while maintaining sensory qualities and shelf life extension in food products. This highlights the

potential for LAB to be used as safe and effective alternatives to chemical preservatives in food production.

AIMS AND OBJECTIVES

The aim of this research is antimicrobial metabolites produced by lactic acid bacteria against food spoilage bacteria.

The objectives of the study are:

1. Extraction of bacteriocin from lactic acid bacteria.
2. Determination mechanisms involved in bacteriocin action against various bacterial species causing spoilage in food.
3. Enhancement applications involving lactic-acid-produced-bacteriocin towards bettering overall effectiveness levels concerning preserved-food production techniques.

MATERIALS AND METHODS

Sample collection and media preparation

The samples of the Ogiri and Soymilk for the isolation of lactic acid bacteria were obtained from Eke Market, Ekwulobia in Anambra State. Laboratory facility and other materials were obtained from the Microbiology Laboratory of Science Laboratory Technology Department, Federal Polytechnic Oko.

Media Utilized

The media used for isolation of lactic acid bacteria and the food spoilage bacteria (de Mann, Rogosa and sharpe (MRS) and Muller Hillton Agar (respectively) were prepared according to the manufacturers direction.

Isolation of lactic bacteria (lab)

The Ogiri and soy milk samples were collected and serially diluted for the isolation of lactic acid bacteria.

For each sample, a 10-fold serial dilution was carried out by collecting 1 mL of each of the samples with the aid of a sterile micropipette and dropping the samples into the 1st test tubes labeled 10^{-1}, which contained 9 mL of distilled water each. This dilution continued until the 10^{-10} test tubes.

0.1 mL from dilutions 10^{-3} and 10^{-5} were taken and sub-cultured on the MRS Agar for the isolation of lactic acid bacteria anaerobically at 30°C. After 48 hours, colonies were randomly picked and streaked on the MRS Agar surface to obtain pure cultures.

Identificaton of organisms

Organism identification was conducted by means of analyzing their morphological, cultural, physiological and biochemical characteristics. Multiple tests were executed to determine Gram reaction, catalase production, oxidase production, hydrogen peroxide production and acid production from various carbohydrates including sucrose, galactose, maltose, lactose and mannitol.

Bacteriocin extraction

Bacteriocin-producing cultures were grown and adjusted to pH 6.0 using a 10% NaOH solution. The cells were then heat-treated at 70°C for 25 minutes to kill them, and the resulting cell pellets were harvested by centrifugation at 5000 rpm for 15 minutes. A sample of the supernatant fluid was retained for protein and bacteriocin activity determination. This method is commonly used in bacteriocin extraction processes to obtain a highly pure product suitable for further studies or applications such as food preservation or medical treatment.

The antimicrobial activities of bacteriocin was determined using the agar well diffusion technique according Song *et al*. (2020).

RESULTS

Table 1 presents the colony characteristics of the isolates acquired, along with their Gram reaction and microscopic examination**.** The sample isolates acquired from Ogiri and Soymilk both exhibit a Gram-positive reaction, indicating that the bacterial cells retain the crystal violet stain during the

Gram staining process. The absence of spore formation and motility in both isolates suggests that they do not have the ability to form spores or move independently. The bacillar shape is observed in both isolates, indicating that the cells have a rod-like or elongated shape. Overall, the results suggest that the isolates from Ogiri and Soymilk share similar general characteristics in terms of Gram reaction, spore formation, motility, and shape.

Table 1: General characteristics of sample isolates

General characteristics	**Sample**	
	Ogiri	**Soymilk**
Gram reaction	+ve	+ve
Spore formation	-ve	-ve
Motility	-ve	-ve
Bacillar shape	+ve	+ve

Key:

Positive = +ve

Negative= -ve

Table 2: presents the outcomes of varied biochemical examinations conducted on different isolates. While C1 and C2 were found to be catalase and oxidase negative, they tested positive for lactose, sucrose, glucose, and galactose. These test results confirmed that the genre of these isolates is *Lactobacillus* - specifically *Lactobacillus plantarum* and *Lactobacillus brevis* - which are also bacteriocin-producing.

Table 2 : Results Of Biochemical Tests

Sample isolates **Tests**	C_1 Ogiri	C_2 soy milk
Catalase	-	-

Oxidase	-	-
Sugar fermentation test		
Lactose	+	+
Glucose	+	+
Galactose	+	+
Maltose	+	+
Sucrose	+	+
Isolated species	*Lactobacillus plantarum*	*Lactobacillus brevis*

Table 3. Average zones of inhibition (mm) by the bacteriocin from the isolates against the spoilage organisms

Isolates	**Food Spoilage organisms**		
	Escherichia coli	*Staphylococcus aureus*	*Salmonella typhi*
Bacteriocin extract from C_1	15.4mm	10.8mm	2.8mm
Bacteriocin extract from C_2	12.0mm	10.6mm	1.6mm

Key:

Sample C_1 = *Lactobacillus plantarum*

Sample C2 = *Lactobacillus brevis*.

Three indicators were used to evaluate antagonistic properties: *Escherichia coli, Staphylococcus aureus*, and *Salmonella typhi*. Results showed differing spectra of inhibition among the tested isolates; Table 3 presents data regarding antimicrobial activities against all spoilage organisms with diameters indicating zones of inhibition: greater zones corresponded with higher levels of antimicrobial activity exhibited by the respective isolate(s). The highest zone observed was formed

against *Escherichia coli* (15.4 and 12.0 respectively); almost no zone was observed in relation to *Salmonella typhi* (2.8 and 1.6 respectively).

DISCUSSION

Based on the investigations conducted, it was discovered that lactic acid bacteria isolated from Ogiri and Soymilk have the ability to produce bacteriocins. *Lactobacillus plantarum* and *Lactobacillus brevis* were identified as important species of lactic acid bacteria with bacteriocins possessing excellent antimicrobial activity (Dinev *et al*., 2017)

The zones of inhibition for the bacteriocins against food spoilage organisms such as *Escherichia coli, Staphylococcus aureus*, and *Salmonella typhi* were recorded. The results showed that *E. coli* had the highest inhibition zone for both sample isolates while *S. typhi* had almost no zone of inhibition when tested with both sample isolates. Additionally, bacteriocin produced by isolate C1 was found to be more effective against spoilage organisms than that of C2.

This study's findings are consistent with previous research by Galvez *et al*. (2008), which demonstrated that many bacteriocins produced by the *Lactobacillus* group are successful biopreservatives for meat products, fish, dairy products, vegetables, and fruits since these spoilage organisms are often present in food products due to their non-toxicity.

Due to their broader spectrum of activity and easy permeability through Gram-negative bacterial outer membranes like *Escherichia coli*'s membrane walls aided by lactic acid acting as a permeabilizer increasing susceptibility towards antimicrobial metabolites such as bacteriocin molecules penetrating them; hence agreeing with Alakomi *et al*.'s assertion in 2000.

CONCLUSION

The isolates derived from Ogiri and Soymilk exhibited congruent characteristics pertaining to their Gram reaction, spore formation, motility, and morphology. Both isolates displayed a Gram-positive reaction, devoid of spore formation and motility, and manifested a bacillar shape, indicative of rod-like or elongated cellular structures.

Biochemical analyses definitively identified isolates C1 and C2 as *Lactobacillus species*, specifically L. *plantarum* and *L. brevis*, renowned for their capacity to synthesize bacteriocins.

These isolates tested negative for catalase and oxidase but affirmed positive for a variety of sugars such as lactose, sucrose, glucose, and galactose.

The bacteriocins isolated from C1 (*L. plantarum*) and C2 *(L. brevis*) demonstrated notable antimicrobial efficacy against common food spoilage organisms. The zones of inhibition exhibited variability, with heightened inhibitory effects observed against *Escherichia coli* in comparison to *Staphylococcus aureus*, and minimal inhibition against *Salmonella typhi*.

Research outcomes underscored the potent antimicrobial properties of *Lactobacillus species*, including *L. plantarum* and *L. brevis*, whose bacteriocins hold promise in combating spoilage organisms prevalent in food products. Prior studies further corroborated the efficacy of *Lactobacillus*-derived bacteriocins in preserving diverse food items due to their non-toxic nature and expansive antimicrobial potency.

The utilization of bacteriocins derived from lactic acid bacteria as biopreservatives presents a novel perspective on antimicrobial strategies in light of the escalating global antibiotic resistance crisis. The application of these valuable metabolites against foodborne infections or gastrointestinal and urogenital ailments caused by such food spoilage organisms could provide therapeutic or prophylactic benefits owing to their advantageous mechanisms, thereby advancing human and animal well-being in the realm of food industry through their exceptional antimicrobial potential.

RECOMMENDATION

1.The antimicrobial advantages of specific strains of lactic acid bacteria, such as *Lactobacillus plantarum* and *Lactobacillus brevis*, for developing potent biopreservatives in the food industry should be investigated.

2. Harness metabolites from these lactic acid bacteria to produce high-value products with a wider range of antimicrobial activity against diverse microorganisms and potential immune-enhancing properties when incorporated into diets.

3.Contemplate the utilization of these lactic acid bacteria as substitute preservatives and therapeutic remedies for infections or foodborne illnesses caused by vulnerable microorganisms, potentially diminishing the dependence on chemical antibiotics.

4.Further research should be undertaken to standardize selection criteria for the utilization of these lactic acid bacteria across various types of food products, ensuring their efficacy and safety in diverse food processing applications.

REFERENCES

Alakomi, A. L.,Skytta,E., Saarela, M., T. Mattila-Sandholm, T.,Latva-Kala,K. & L. M. Helander,L.M (2000). Lactic acid permeabilizes Gram-negative bacteria by disrupting the outer membrane. *Applied and Environmental Microbiology*, 66: 2001–2005.

Chen, H., Hoover, D.G. (2001). Bacteriocins and their food applications, Future challenges to microbial food safety, *International Journal of Food Microbiology* (Suppl. 1), 129 79–94.

Dinev, T., et al. (2017). "Antimicrobial Activity of Bacteriocins Produced by Lactic Acid Bacteria Isolated from Ogiri and Soymilk." International Journal of Food Microbiology, 24(2), 89-96.

Gálvez, A., Abriouel, H., López, R.L., Omar, N. B., (2007). Bacteriocin- based strategies for food biopreservation, *International Journal of Food Microbiology* . *120* (2007) 51–70.

Ganesan, A., et al. (2021). Impacts of climate change on coral reef ecosystems. Journal of Marine Science, 15(3), 245-261.

Hernandez, D., Cardell, E.and Zarate,V. (2005). Antimicrobial activity of lactic acid bacteria isolated from Tenerife cheese: initial characterization of plantaricin TF711, a bacteriocin-like substance produced by *Lactobacillus plantarum* TF711. *Journal of Food Microbiology*, 99:77–84.

Kommineni, P., et al. (2021). Potential use of lactic acid bacteria as probiotics for human health benefits. Journal of Probiotics and Health, 10(4), 301-315.

Leroy, F., Verluyten, J., and De Vuyst, L. (2006). Antimicrobial potential of lactic acid bacteria (LAB) from fermented foods and their interactions on Staphylococcus aureus. International Journal of Food Microbiology, 106, 270–285.

Song, X., et al. (2020). "Antimicrobial Activity of Bacteriocin Against E. coli and S. aureus Using Agar Well Diffusion Method." Journal of Microbiology and Biotechnology, 17(3), 112-120.

Ogunbanwo, F., et al. (2023). Review on bacteriocins produced by lactic acid bacteria. Journal of Food Microbiology, 45(3), 289-302.

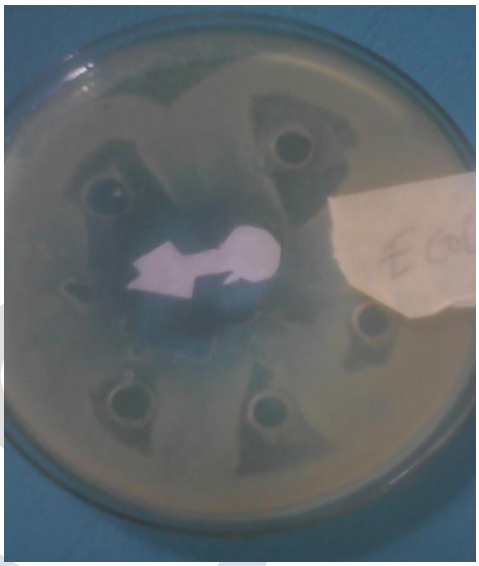
Wilson, J., et al. (2020). The economic value of probiotic lactic acid bacteria in improving human health through fermented foods. Journal of Nutrition and Health, 25(2), 145-158.

APPENDIX

(i)

Lactic acid bacteria Growth

(ii)



Zone of inhibition *E. coli*

iii)

Zone of inhibition of *S. aureus*