

BACTERICIDAL POTENTIAL OF CRUDE BACTERIOCINS FROM LACTIC ACID BACTERIA ISOLATED FROM MOUSE GUT AGAINST SOME FOOD SPOILAGE BACTERIA

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ABSTRACT

The cells and secondary metabolites from Lactic Acid Bacteria (LAB) provide a biological food preservative suitable as an alternative to the use of chemical in extending food shelf life. The present research was aimed at examining the potential of crude bacteriocin from LAB isolated from the gut of mouse as a bio-control measure against the commonly encountered food spoilage bacteria. Compared to lactic acid bacteria (LAB) from other sources, including fermented foods, LAB from the mouse gut show better persistence and gut microbiota regulation. Their special adaptations to the gastrointestinal environment of mice increase their efficacy as probiotics by strengthening the intestinal barrier and boosting immune responses. Using standard microbiological methods, LABs were isolated from the gut of a mouse on De Mann Rogosa Agar (MRSA), characterized and identified. Crude bacteriocin was obtained from broth culture of the isolates and tested for antibacterial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus megaterium*, *Bacillus cereus* and *Staphylococcus aureus* using agar well diffusion method. Four species of LAB were obtained and identified, three of which were *Lactobacillus* species 1, 3, and 4 while one was a *Lactococcus* species. Crude bacteriocin from the *Lactococcus* species was the most effective against all the tested bacteria except *B. megaterium*, crude bacteriocin from *Lactobacillus* species 1 and 3 were not effective against *E. coli* and *B. megaterium* while the crude bacteriocin from *Lactobacillus* species 4 was active against only *S. aureus*. *Staphylococcus aureus* was the most sensitive to the crude bacteriocins with an average zone of growth inhibition of 16.50 mm, followed by *B. cereus* (14.00 mm) and *P. aeruginosa* (13.50 mm), the least inhibition zone was in *E. coli* while *B. megaterium* was resistant (0.00 mm). This study shows that bacteriocin derived from Lactic Acid Bacteria (LAB) isolated from mouse gut demonstrates potential as a natural food preservative by effectively inhibiting various food spoilage bacteria.

Keywords: Lactic Acid Bacteria, Biopreservative, Bacteriocins, *Lactobacillus*, *Lactococcus*, Food Spoilage Bacteria

INTRODUCTION

Lactic acid bacteria (LAB) are versatile microbes from the firmicutes group, including genera like *Streptococcus*, *Bifidobacterium*, *Lactococcus*, and *Lactobacillus*, known for their benefits in food processing (Makela *et al.*, 2021). Their ability to produce growth-inhibitory substances, such as organic acids and bacteriocins, contributes to improved gut health, enhanced immune function, and disease prevention (Tannock, 2021 and Bacigalupo *et al.*, 2020). LAB maintain gut microbiota balance, inhibit pathogens, and enhance essential vitamins (Davis *et al.*, 2022). Bacteriocins produced by LAB modulate gut health and regulate microbial populations (Lozupone *et al.*, 2021). Dysbiosis can disrupt gut health, but LAB can restore balance by competing with harmful bacteria (Ley *et al.*, 2020; Kleerebezem and Vaughan, 2020). LAB from mouse gut can be isolated for use as probiotics, promoting health and preventing pathogen growth (Huang *et al.*, 2020). Mouse gut LAB (lactic acid bacteria) is unique due to their distinct microbiota composition, influenced by adaptation and environmental factors (Huang *et al.*, 2020). These LAB differ from those found in fermented foods in their specific strains and functional properties (Kleerebezem and Vaughan, 2020). While LAB in food mostly improve flavor and preservation, LAB in the mouse gut is essential for immune system and gut health modulation, which reflects their adaptation to the host's particular environment. (Davis *et al.*, 2022). Compared to lactic acid bacteria (LAB) from other sources, including fermented foods, LAB from the mouse gut show better persistence and gut microbiota regulation. Their special

adaptations to the gastrointestinal environment of mice increase their efficacy as probiotics by strengthening the intestinal barrier and boosting immune responses. (Gänzle, 2022; O'Sullivan *et al.*, 2023 and Gänzle, 2023).

Previously used LAB species with antibacterial potential are the species of *Lactococcus*, *Pediococcus*, *Streptococcus*, *Bifidobacterium* and *Lactobacillus*. These bacteria have ability to produce bacteriocins with potential to inhibit the growth of food spoilage and foodborne pathogens (Pérez-Burillo *et al.*, 2021; Reid *et al.*, 2020 and Gänzle, 2021). The functioning of LAB however depends on their adherence to the gut which promotes the synthesis of metabolites that support gut health (Morrison and Preston, 2021). The alarming inherent menace associated with food wastage due to spoilage bacteria, production of toxins in food during spoilage, toxicity of chemical used as food preservatives, and the health implication of consumption of foodborne pathogens have led to an insatiable demand for natural metabolites as food preservatives. This has necessitated the call for a search for novel bacteriocin producing bacteria that are indigenous and effective against commonly encounter food spoilage bacteria. The availability of these indigenous strains of biological preservative producing LAB isolates will tremendously act as measures that enhance local food processing and preservation, eliminate or reduce the use of toxic chemical as preservatives and alleviate the occurrence of foodborne diseases. This research is therefore aimed at evaluating the bacteriocinogenic property of LAB isolated from the gut of mouse against some food spoilage bacteria

MATERIALS AND METHODS

Collection and Preparation of Mouse and Test Bacteria

A mouse was obtained from the Biochemistry Department in Kwara State University, Malete and transported to the laboratory, the mouse was killed by euthanasia using chloroform, a method that ensures rapid and humane paralysis (Henderson *et al.*, 2013). Following euthanasia, the gut was aseptically removed and processed to isolate LAB.

The food spoilage organisms were isolated through sub-culturing from existing culture from the Department of Microbiology, University of Ilorin. This involves selecting colonies of interest from previous culture obtained from spoiled food samples. The culture was maintained on agar slant for further research. The test bacteria obtained were *Pseudomonas aeruginosa* and *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, and *Bacillus megaterium*

Microbial Analysis

Isolation of Lactic Acid Bacteria from the Mouse Gut

The isolation of bacteria was accomplished using the streak plate method (El Kahlout *et al.*, 2018). The aseptically removed gut was dissected and a portion of the ileum was cut and lifted with a sterile forcep into a test tube where it was washed using sterile peptone water. The aliquot obtained was serially diluted in ten folds to the tenth power. One milliliter of the final dilution was plated on MRS (Man, Rogosa, and Sharpe) agar plates. The plates were incubated for 72 hours at 37°C in an anaerobic jar. Lactic Acid bacterial counts were determined using colony-counter in colony forming unit (CFU) on MRS agar. Due to mixed growth colonies on the plates, a close observation of morphological characterization of distinct pure colonies was made. Distinct colonies were sub cultured onto freshly prepared plates. Slants were used to maintain pure colonies for further study. (Eni, 2010 and Makinde *et al.*, 2020).

Identification of the Isolated Lactic Acid Bacteria

Morphological, colonial and biochemical were used to characterize and identify the isolates. Gram staining and biochemical tests for catalase and oxidase production, and sugar fermentation were carried out (Mannan *et al.*, 2017; El Kahlout *et al.*, 2018).

Crude Bacteriocins Extraction

In a 250 ml Erlenmeyer flask with 100 ml of MRS broth, fresh cultures of each isolate were inoculated separately. The inoculation was autoclaved for 72 hours at 37°C. To acquire the cell-free culture supernatant (CFS), each sample was centrifuged at 6,000 rpm for 15 minutes at 4°C after incubation. The cell-free supernatant obtained was mixed with ammonium sulfate to neutralize the organic acid (70 %

saturation), as it optimizes the precipitation of bacteriocins, enhancing yield and activity. This concentration has been shown to effectively separate active components while minimizing the loss of bioactivity (Gänzle, 2022). The mixture was placed in a cold incubator with rotary shaker for two hours at 4°C at 250 rpm. A rotary evaporator operating at 40°C was used to concentrate the crude bacteriocin obtained under vacuum (Islam *et al.*, 2020). The crude bacteriocin extracts obtained from LAB isolates were tested against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, and *Bacillus megaterium*. Positive controls, such as known bacteriocins like nisin, and negative controls, such as MRS broth alone, were included in the agar well diffusion assay to assess the antimicrobial activity accurately. (Gänzle, 2021; Kaur & Kaur, 2021 and Kaur & Kaur, 2022).

Determination of Antibacterial Activity of Bacteriocin

Antibacterial activity of the bacterial isolates against all the food spoilage bacteria tested was done using the agar well diffusion method (Islam *et al.*, 2020). Precisely one of each test bacterium (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, and *Bacillus megaterium*) was grown overnight (18 hours) in a peptone broth to obtain actively growing cells, the broth culture was diluted favorably before 1 ml of the appropriate dilution was seeded onto Muller Hilton agar plates. Thereafter, 1ml of the cell-free crude bacteriocin was added to wells bored onto the surface of the agar plates with the aid of a sterile 6 mm cork borer. The plates were allowed to rest for penetration and even distribution before they were incubated for 24 hours at 37°C. The diameter of the inhibition zone surrounding the wells measure in mm was used to determine the antibacterial activity.

Statistical analysis was performed using one-way ANOVA to assess differences among treatment means, followed by Duncan's Multiple Range Test (DMRT) for post-hoc multiple comparisons at a significance level of $p < 0.05$. Means were first ranked, and pairwise differences were evaluated against studentized range-based critical values (q) multiplied by the standard error of the mean ($SE = \sqrt{(MSE/n)}$), where MSE was the ANOVA error term and n the number of replicates.

RESULTS AND DISCUSSION

The morphological and biochemical characteristics with sugar fermentation ability of the isolates are presented in Table 1. The isolates were Gram positive rods and coccus, negative for catalase, indole, oxidase activity, and citrate utilization. All the isolates were good fermenters of more than three out of all the sugar tested.

Table 1: Morphological and Biochemical Characteristics of the Lactic Acid Bacteria Isolates

Isolates	Colony Color	Gram Reaction	Morphology	Nitrate Reduction	Catalase	Indole	Oxidase	Citrate Utilization	Glucose	Lactose	Sucrose	Maltose	Mannitol	Fructose	Raffinose	Arabinose	Xylose
LAB 1	White	Gram-positive	Rod-shaped, non-motile, in chains	-	-	-	-	-	+	+	+	+	-	+	-	+	-
LAB 2	White	Gram-positive	Short rods	-	-	-	-	-	+	-	-	+	+	+	-	-	-
LAB 3	White	Gram-positive	Cocci shaped, appear in pairs	-	-	-	-	-	+	+	+	+	+	+	+	+	+
LAB 4	White	Gram-positive	Short cocci-shaped, occurs in pairs	+	+	-	-	+	+	+	+	+	+	+	-	+	+

Antibacterial Activity of the Crude Bacteriocin against the Test Bacteria

The crude bacteriocin extracts obtained from the LAB isolates were active against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus* but not active on *Bacillus megaterium*. The highest zone of inhibition was recorded on *P. aeruginosa* (13.33 ± 0.58) followed by *S. aureus* (14.33 ± 1.53) and *B. cereus* (14.00 ± 0.00) by *Lactobacillus* species 1. The crude bacteriocin from the *Lactococcus* species (LAB 2) produced inhibitory effect on *E. coli* (13.67 ± 0.58) and *B. cereus*

(14.67 ± 0.58). Crude bacteriocin from *Lactobacillus* species 3 (LAB 3) inhibited *S. aureus* (17.33 ± 0.58), *P. aeruginosa* (12.67 ± 0.58) and *B. cereus* (11.67 ± 0.58). The crude bacteriocin from *Lactobacillus* species 4 (LAB 4) was only able to inhibit the growth of *S. aureus* (13.33 ± 0.58). None of the crude bacteriocins from the extracts was active against *B. megaterium* growth. The antibacterial activity of crude bacteriocins from the LAB2 and 3 were more potent and the activities were significantly different. Statistical analysis through Duncan tests confirmed the significance of the obtained results between treatments.

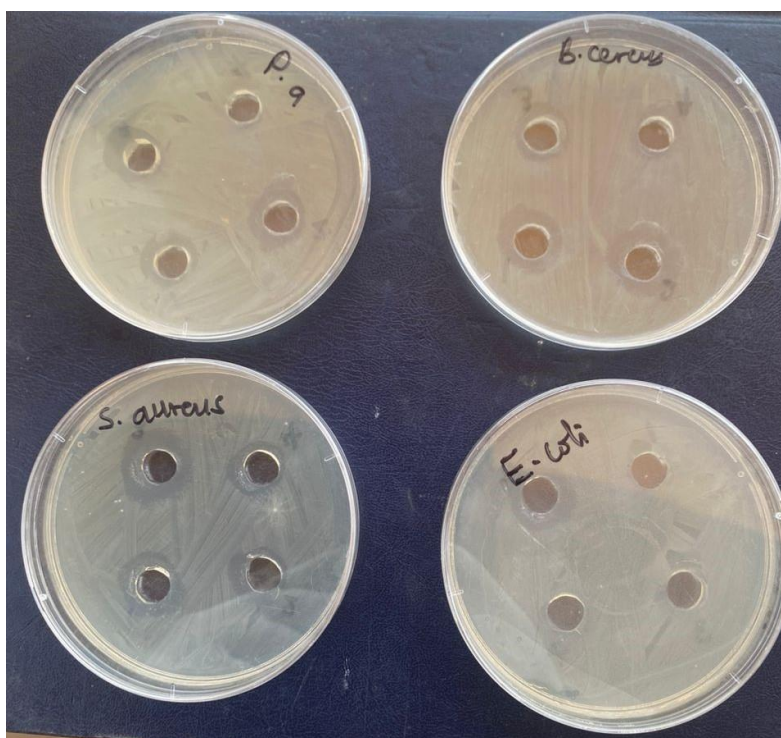


Plate 1: Pictorial representation of the antibacterial activity of the LAB isolates against *Pseudomonas aeruginosa*, *B. cereus*, *S. aureus*, and *E. coli*

Table 2: Antibacterial Activity Crude Bacteriocins from LABs Diameter of inhibition zone (mm)

LAB Isolates	Organisms				
	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>B. cereus</i>	<i>B. megaterium</i>
<i>Lactobacillus casei</i>	13.33 ± 0.58^c	14.33 ± 1.53^a	0.00 ± 0.00^a	14.00 ± 0.00^c	0.00 ± 0.00
<i>Lactobacillus fermentum</i>	12.00 ± 0.00^b	12.50 ± 0.71^a	13.67 ± 0.58^b	14.67 ± 0.58^c	0.00 ± 0.00
<i>Lactococcus lactics</i>	12.67 ± 0.58^{bc}	17.33 ± 0.58^b	0.00 ± 0.00^a	11.67 ± 0.58^b	0.00 ± 0.00
<i>Pediococcus pentosaceus</i>	0.00 ± 0.00^a	13.33 ± 0.58^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00

Mean values are presented as mean \pm SEM (standard error of the mean), with n = 3 independent biological replicates.

Superscript letters within each column indicate statistically significant differences among means ($p < 0.05$), determined

by one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) using studentized range (q) values for $n = 3$

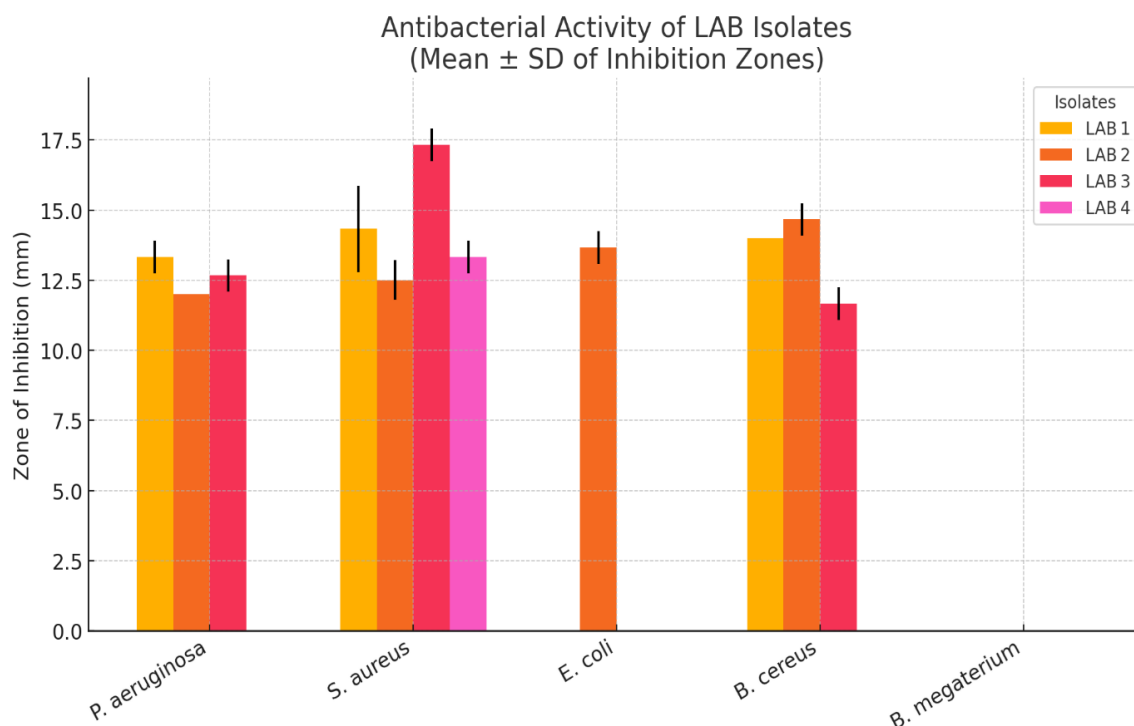


Figure 1: Antibacterial Activity of LAB Isolate

Discussion

The current study focused on the isolation of Lactic Acid Bacteria with potential probiotic functions explored from Mouse gut. In the present investigation, four potential probiotic isolates were identified as three *Lactobacillus* species (LAB 1, LAB 3 and LAB 4) and a *Lactococcus* species (LAB 2). The isolation of these bacteria from the mouse gut supported previous report on the presence of and the dominant population of this group of bacteria in the gut of monogastric animals including mouse. The LAB are free living dwellers of the gut (Duar *et al.*, 2017), previous reports have also isolated *Lactobacillus* species (*Lactobacillus acidophilus* and *Lactobacillus fermentum*) from human, pigs, hamsters, and horses' gut (Duar *et al.*, 2017).

Their association with the gut could suggest their potential for use as probiotic organisms to promote gut health (Sorescu *et al.*, 2020), the isolated LAB species could also serve in particular in developing probiotic bacteria for the mouse species because probiotic bacteria sometimes could be host-adapted and have a high ecological fitness in their host or related species. Moreover, this higher fitness is relevant in the process of outcompeting the pathogens.

The ability of the LAB species to perform conversion of several types of simple and disaccharide sugar into acid suggest a wide fermentative potential that could be of advantage in the dairy and other industries for food processing. The organic acid formed as a result of the conversion of sugar into lactic acids and other acids could be used as food preservative to extend shelf life. The acids produced often lowers or reduce pH and thus, could inhibit the growth of many bacteria spoilage bacteria which are normally neutrophiles. Morphological and biochemical characteristics of this LAB isolates were in tandem with previous report from similar work (Sorescu *et al.*, 2019 and Sorescu *et al.*, 2020).

In conformity to the observation of Sorescu *et al.* (2020), the isolated from the mouse gut, can be important for developing probiotic compounds for the same mouse species because they are host-adapted and have a high ecological fitness. Moreover, this higher fitness is relevant in the process of outcompeting the pathogens.

Differentiation of *Lactobacillus* species was performed as described before by Sorescu *et al.* (2019) and Sorescu *et al.* (2020) mainly on the basis of some morphological characters, some cultural characters and especially, biochemical characters.

Bacteriocins which are ribosomal synthesized proteins have similar actions to antibiotics and could serve same purpose in food. The crude bacteriocins from the LAB isolated in this study were very active to inhibit the growth of all the tested food spoilage bacteria except *Bacillus megaterium*. Previous authors have reported the antibacterial activity of bacteriocin against commonly encountered foodborne bacteria (Adedayo and Abdulkareem, 2024; Mohammaddoos *et al.*, 2015). The ability of the crude bacteriocins to inhibit the growth of *Pseudomonas aeruginosa* represents a significant threat to both public health and food safety, primarily due to its intrinsic resistance to a broad range of antibiotics. This resistance is largely attributed to the production of hydrolytic enzymes, such as β -lactamases, and the presence of multiple virulence-associated genes (Breidenstein *et al.*, 2011). *Pseudomonas aeruginosa* shows natural resistance to bacteriocins like nisin due to its outer membrane barrier and efflux mechanisms, highlighting the need for more effective antimicrobial agents (Gharsallaoui *et al.*, 2016). *Pseudomonas aeruginosa* is now emerging as opportunistic pathogen with ability to form biofilm in food pipes during processing. Hence, having a bacteriocin that could limit its growth incorporated into foods during production and

distribution lines will enhance shelf life and product safety (Adedayo and Abdulkareem, 2024).

Escherichia coli, *Staphylococcus aureus* and *Bacillus cereus* were also very sensitive to the crude bacteriocins. *Bacillus megaterium* may resist LAB due to its thick cell wall and potential bacteriocin-neutralizing mechanisms, while *Lactococcus spp.* likely demonstrated higher levels of activity by producing a number of bacteriocins that targeted several locations. (Cotter et al., 2005). These bacteria were mostly foodborne pathogens and are often occurring in spoiled food, and especially in ready to eat food. Toxin producing species of these bacterial release poisonous metabolites into foods during spoilage in store or prior to consumption after processing. The heterotrophic nature of these bacteria makes them ready tools for food spoilage during which they release certain metabolites into food rendering it unfit for consumption. They are also reported to have genes for antibiotic resistance and virulence factors that enhance pathogenicity. The presence of methicillin resistance *Staphylococcus aureus* was reported in open displayed ready to eat fish and fruit during distribution line (Adedayo and Yusuf, 2024; Adedayo et al., 2024). The close association of the test organisms to human enhances their contamination of food and invariably they get consumed. *Bacillus cereus* though a soil dweller, has been isolated from food as spoilage and pathogenic bacteria (Ayilara et al., 2023).

The bacteriocin genic property of the crude extracts from the isolated *Lactobacillus* and *Lactococcus* species against the *Pseudomonas aeruginosa*, *B. cereus*, *S. aureus*, and *E. coli* suggests that the isolates or their metabolites if incorporated into food during processing could prevent the growth of the bacteria. However, the crude bacteriocins were not able to inhibit the growth of *B. megaterium* though it inhibited the growth of *B. cereus*, which is a closely related organism. The probable reason for this might be a subject for future research. The activity of the crude bacteriocin was both on the closely related Gram-positive *S. aureus* and *B. cereus* as well as the Gram-negative *P. aeruginosa* and *E. coli* indicating a wide spectrum of activity. Bacteriocins are usually more effective on closely related strains than more distant strains but the observations in this study deviates from the former reports by been potent on many species of food spoilage bacteria. The bacteriocins in pure form could be engaged in the food processing and distribution lines to replace the use of common antibiotics in food. Because they can inhibit pathogens and prolong shelf life, crude bacteriocins from lactic acid bacteria are used as natural food preservatives. They also remain stable in real-world settings like heat, pH changes, and cold storage, and encapsulation techniques further increase their efficacy and controlled release in food systems (Fotso Techeu et al., 2022; Mohammed et al., and 2024; Perez et al., 2023). The ripple effect of which will include safer food, extended shelf life and reduction in the development and spread of community acquired antibiotic resistance through consuming food.

CONCLUSION

The study isolated *Lactobacillus* and *Lactococcus* species in this study were bacteriocin producers and the crude bacteriocins from the LABs were variously active against the tested food spoilage bacteria except *Bacillus megaterium* which showed resistant to all the crude bacteriocins from the isolated LAB. In this study, the probiotics produced by the lactic acid bacteria isolated from mouse gut are considered to be effective for antibacterial agents and safe to the host animal. The availability of these indigenous strains of biological preservative producing LAB isolates will

tremendously act as measures that enhance local food processing and preservation, eliminate or reduce the use of toxic chemical as preservatives and alleviate the occurrence of foodborne diseases

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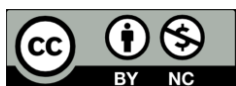
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