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Biopreservation of Tomatoes Using Bacteriocin Produced by *L. Plantarum SJC 103* and *L. Apis Hbam1*

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Abstract
Several species of *Lactobacillus* have been known to produce an antimicrobial peptide known as bacteriocin, which are generally regarded as safe thus making them suitable as food additives and thereby fulfilling consumers demand for chemical free food. The aim of the study was to produce bacteriocin from *Lactobacillus plantarum* SJC103 and *L. apis* HBAM1 and use the bacteriocin produced as a biopresevative of tomatoes. Production of bacteriocin from both Lactobacillus species were performed using broth cultures of the selected isolates, which were centrifuged at 4000 rev/min for 1 hr and then decanted to obtain the supernatant which contained the bacteriocin. The bacteriocin produced was purified using ammonium sulphate precipitation. Microbial analysis was conducted on untreated tomatoes (control), tomatoes treated with bacteriocin, and *Staphylococcus aureus* infected tomatoes treated with bacteriocin and all were stored under the same conditions. The biopreservative potential of bacteriocin from both isolates produced a reduction in colony for the treated tomatoes with 1.10 x 10^7 cfu/g and 2.15 x 10^7 cfu/g for *L. plantarum and L. apis* respectively which were significantly reduced when compared with the *S. aureus* infected tomatoes with 9.25 x 10^7 cfu/g on day 10 of preservation. It was concluded that bacteriocin from Lactobacilli can be harnessed as a natural antibacterial in the preservation of tomatoes.
Keywords: Bacteriocin, biopreservation, tomatoes, partial purification, Lactobacillus

Introduction

Recently, there has been an increase in awareness of consumers on the health risk that can be contracted from the use of chemical preservatives (Silva *et al*., 2018), this has motivated research interest in the use of bacteriocins as an alternative food preservation method in order to meet consumers demand for better quality foods in terms of taste and shelf life. In general, bacteriocins do not alter food quality and can serve as additional technique in food processing, thereby limiting other preservation methods like heat (Barbosa *et al*., 2017). Thus, consumer demand for fruit and food products that maintain their natural aesthetic values are met (Olaimat and Holley, 2012; Elsser-Gravesen, 2014), thereby making bacteriocin a choice option where food preservation is concerned. Furthermore, the addition of bacteriocins to food as a hurdle technology is widespread, but little is known of its application in fruit products (Pei *et al*., 2014). So far, a good number of lactic acid bacteria especially species of *Lactobacillus* and their bacteriocins have been used in the preservation of food and control of pathogens associated with humans (Parada *et al*., 2007). Based on their "food grade quality" and relevance industrially, the bacteriocins produced by lactic acid bacteria have been carefully characterized. Bacteriocins are mostly active against Gram-positive bacteria and can inhibit several spoilage and pathogenic microorganisms of food origin (Barbosa *et al*., 2017).

Tomato is a fruit grown all around the world, it contains high amount of vitamins and minerals and used for cooking and in the production of drinks. It is one of the most important vegetable in most regions of the world and constitutes an important source of food as well as cash in Nigeria (Alao, 2000; Ogbonna *et al*., 2008). Each year, not less than one million hectares has been reported for the cultivation of tomatoes in Nigeria (Etebu *et al*., 2013). However, the aesthetic characteristics of the fruit after harvest is affected by biotic, social and economic factors which reduce its nutritional value and result in about 10-30% of losses (Etebu *et al*., 2013). Tomato contains essential nutrient which makes up a healthy and well balanced diet. The vitamin A content of the yellow tomato variants is higher than those in the red ones, but the latter contain

lycopene, which may have anti-oxidative effect on carcinogenic substances (Seymour *et al*., 2001; Ajayi and Olasehinde, 2009). Generally, the fruit when harvested ripe at room temperature (28 ± 1°C) can store for 5 days, but some tomato fruits referred to as iron tomatoes are so strong that they can store for up to 10 days under favorable conditions (Chuku *et al*., 2008).

Bacteriologically safe fruits and vegetables are essential to maximize the health benefits obtainable from adequate consumption of these produce (Eni *et al*., 2010). Tomato is a highly perishable fruit due to its high water content and as such prone to spoilage by microorganisms. The activities of these microorganisms produce high levels of losses especially after harvesting (Ogundipe *et al*., 2012). The incidence of fruit products contaminated with harmful microorganisms is on the rise, it is therefore vital to study alternative methods to enhance the quality and safety of these products (Barbosa *et al*., 2017) through the use of environmentally friendly techniques that can help curtail spoilage and activities of pathogenic microorganisms in fresh fruits products (Barba *et al*., 2010; Boyacioglu *et al*., 2013). Bacteriocins from lactic acid bacteria could be used as an additional technique in the preservation of fruit products to hinder harmful microorganisms from growing (Barbosa *et al*., 2017).

Materials and Methods

Sample collection

Lactic acid bacteria-*Lactobacillus plantarum SJC103 and L. apis HBAm1* were isolated from *nunu* and *kunu* using DeMann Rogosa Sharpe Agar.

Production of Bacterioicin

The isolates were cultured in MRS broth and incubated at 37°C for 48hrs. After the incubation period, the broth culture of the isolates was centrifuged at 4000rev/min for 1 hour. The cell free supernatant was collected by decantation and neutralized to pH 6 using 1M NaOH which was then used as crude bacteriocin (Ali *et al*., 2016).

Partial Purification of Bacteriocin

The purification process was optimized by precipitating crude bacteriocin-900ml (prepared from 1L MRS broth) with 429.96g of ammonium sulphate up to 70% saturation levels overnight at 4°C (Encor Biotechnology, 2020). The mixture was centrifuged at 4000rev/min for 1 hour and the surface and bottom pellicles were harvested and re-suspended in sodium phosphate buffer (50mM, pH 6.5) (Goyal *et al*., 2018). The solution was filtered using a millipore filter (0.22µm) paper and then air dried in a laminar flow hood.

Bio-preservative Effect of Bacteriocin on Tomato Fruits

Microbial Analysis of Fresh Tomatoes

Fresh tomato fruits were obtained from Malete market, the fruits were immediately transported to the laboratory, after washing with sterile water and 70% ethanol, 25g of tomato was weighed and blended with 225ml of sterile distilled water in a flourish blender, then serially diluted up to 10^{-5}, from the 10^{-4} tube, 0.1ml was spread plated on nutrient agar plates which were then incubated at 35°C for 24 hours to determine the total aerobic bacterial count, the same volume was spread plated on potato dextrose agar plate which was then incubated at 28°C for 48 hours to enumerate the mold and yeast counts, the same procedure was repeated to determine the total number of coliforms present within the fresh fruit using MacConkey agar plates which were then incubated at 35°C for 48 hours. The same analysis was repeated on tomatoes (5 pieces each) stored in two separate sterile plastic containers for 5 and 10 days respectively at room temperature. The experiment was conducted twice and the numbers of colony on each day was recorded.

Effect of Bacteriocin from Selected LAB Isolates on Microflora Present within the Tomato Fruits

Five tomato samples each were packed into three sterile plastic containers and was labelled for day 0, 5 and 10, partially purified bacteriocin (2ml at a final concentration of 10% bacteriocin in sodium phosphate buffer) was sprayed on each tomato sample which were then air dried in a laminar flow hood and stored at room temperature. Twenty five grams from tomato samples labeled for day zero were immediately blended with 225ml of sterile distilled water after the treatment and 1 ml from the tomatoes solution was serially diluted up to 10^{-5}, 0.1ml from the 10^{-4} tube was spread plated on nutrient agar plates to determine the total aerobic bacterial counts, the same volume was spread plated on potato dextrose agar plates to determine the total yeasts and molds and coliform counts was enumerated on MacConkey agar plates and the same analysis was repeated on day 5 and 10 (Ayala-Zaval *et al*., 2008).

Effect of Bacteriocin from Selected LAB Isolates on Tomatoes Inoculated with S. aureus ATCC 25923

The method of Luz *et al.* (2020) was employed with slight modification, the tomato samples were divided into batches, 15 tomatoes were treated with sterile MRS broth (control), with 5 tomatoes in each container labelled for days 0, 5 and 10; another 15 tomatoes were treated with 1 ml of standardized *S. aureus* ATCC 25923 and partially purified bacteriocin with 5 tomatoes in each container labeled for days 0, 5 and 10; while 15 of the fruit sample was only inoculated with *S. aureus* ATCC 25923 with 5 tomatoes in each container labelled for days 0, 5 and 10. Prior to treatments, the tomatoes were sanitized with 70% ethanol and then sterile water. A wound was made on each sample using a sterile pipette tip. One milliliter (1ml) of a standardized solution containing 10^{8}cfu/ml of *S. aureus* ATCC 25923 was sprayed on the tomatoes and dried for 1hr in a laminar flow hood. Finally, each tomato was treated with 2 ml of partially purified bacteriocin at a final concentration of 10% bacteriocin, dried and stored in a sanitized plastic container at room temperature (25°C) and bacterial count (on Mannitol salt agar) was performed as follows: twenty five grams from tomato samples labelled for day zero were immediately blended

with 225ml of sterile distilled water after the treatment and 1 ml from the tomatoes solution was serially diluted up to 10^{-5}, 0.1 ml from the 10^{-4} tube was analyzed for the total *S. aureus* count on mannitol salt agar plates, same analysis was repeated on tomatoes stored for 5 and 10 days respectively. All tests were repeated twice.

Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using IBM SPSS (V21). The data were analyzed using one-way analysis of variance followed by LSD (least significant difference) post-hoc test and independent samples T-test for comparison of means. $P < 0.05$ was considered significant.

Results and Discussion

Results

Microbial Load Present in Fresh Untreated Tomatoes

The microbial load present within the tomato fruits is presented in Fig. 1; the total aerobic bacterial count was 4.3×10^6 cfu/ml and 2.50×10^7cfu/ml on day 0 and 10 respectively, the total yeast and mold counts was zero and 1.01×10^7 cfu/ml on days 0 and 10 respectively, the total number of coliforms was zero and 1.24×10^7 cfu/ml on days 0 and 10 respectively.

Microbial Load Present in Bacteriocin Treated Tomatoes Stored at Ambient Temperature for 10 Days

The effect of bacteriocin on the total aerobic bacteria, coliforms, yeast and molds is presented in Fig. 2; a total of 1.8×10^6 cfu/ml and 5.2×10^6 cfu/ml aerobic bacterial count was recorded on days 0 and 10 respectively for tomatoes treated with bacteriocin from *L. apis* while 2.6×10^6 cfu/ml and 9.2×10^6 cfu/ml aerobic bacterial count was recorded on day 0 and 10 respectively for tomatoes treated with bacteriocin from *L. plantarum*. A total of zero and 3.2×10^6 cfu/ml yeast and mold count was recorded on days 0 and 10 respectively for tomatoes treated with bacteriocin from *L. apis* while zero and 6.8×10^6 cfu/ml yeast and mold count was recorded on day 0 and 10 respectively for tomatoes treated with bacteriocin from *L. plantarum* as presented in Fig. 3 and a total of zero and 2.9×10^6 cfu/ml coliform count was recorded on days 0 and 10 respectively for tomatoes treated with bacteriocin from *L. apis* while zero and 7.2×10^6 cfu/ml coliform count was recorded on day 0 and 10 respectively for tomatoes treated with bacteriocin from *L. plantarum* as shown in Fig. 4.

Biopreservative Effect of Bacteriocin on S. aureus Infected Tomatoes

Table 1 represent the effect of bacteriocin from *Lactobacillus plantarum* SJC103 and *Lactobacillus apis* HBAm1 on *Staphylococcus aureus* infected tomatoes. A total of 1.7×10^7 cfu/g of *S. aureus* was recorded on day 0 on the *S. aureus* infected tomatoes while zero count was recorded on the control and bacteriocin treated tomatoes. On day 5, a total of 4.95×10^7 cfu/g *S. aureus* was recorded on the *S. aureus* infected tomatoes which was significantly higher than the control and bacteriocin treated tomatoes and on day 10, 9.25×10^7 cfu/g of *S. aureus* was recorded on the *S. aureus* infected tomatoes which was significantly higher than the control and bacteriocin treated tomatoes.

Discussion

The bacteriocin from *L. plantarum* SJC103 and *L. apis* HBAm1 were partially purified in order to examine their biopreservative potential on tomatoes. Microbial analysis of tomatoes for the total aerobic, coliform, mold and yeast counts revealed increasing numbers of aerobic bacteria, coliforms, molds and yeasts on days 5 and 10; indicating that microorganisms are the major cause of spoilage of this fruit. This is similar to the work of Obunukwu *et al*. (2018), who reported high incidence of bacteria, fungi and coliform counts in fresh tomatoes stored at ambient temperature for 14 days. Treatment with partially purified bacteriocin from *Lactobacillus plantarum* SJC103 and *Lactobacillus apis* HBAm1 reduced the numbers of aerobic bacteria on days 5 and 10 which was significant when compared with the control. The total coliforms as well as the molds and yeasts were also significantly reduced. This is in agreement with the work of Ayala-Zavala *et al*. (2008) who reported that the treatment of fresh cut tomatoes with natural volatile compounds- 'tea tree oil showed a marked low aerobic plate counts throughout the storage period at 5 °C. Also, Microbial quality expressed as total coliform bacteria was preserved in higher extent on those fruits treated with garlic oil followed by methyl jasmonate-ethanol, tea tree oil, ethanol and methyl jasmonate treatments compared to control fresh-cut tomatoes'.

When tomatoes infected with *S. aureus* ATCC 25923 were treated with bacteriocin from *Lactobacillus plantarum* SJC103 and *Lactobacillus apis* HBAm1, on day 0 the mean bacterial load on the *S. aureus* infected tomatoes were significantly higher compared with the control and treated tomatoes. On day 5, the mean bacterial load on the *S. aureus* infected tomatoes was significantly higher than those of the bacteriocin treated samples, this implies that the treatment was effective on the tomatoes that were treated with the bacteriocin. This is similar to the work of Luz *et al*. (2020) who reported that tomatoes inoculated with *Penicillium expansum* and treated with Cell free supernatant (CFS) showed a visibly improved shelf-life. There was however no significant difference between the bacteriocin treated tomato and the control; since the control were not infected with *S. aureus*, it means the *S. aureus* load on the control were minimal which makes the result obtained when compared with that of the treated tomatoes insignificant. On day 10, the mean bacterial load on the *S. aureus* infected tomatoes was significantly higher when compared to those of the bacteriocin treated tomatoes. This implies that the treatment was still effective on day 10. However, the mean bacterial load on the tomato treated with *L. plantarum* was not significantly different from the control as against that of *L. apis* which produced a mean bacterial load that was significantly different from the control. This is in agreement with the work of Luz *et al*. (2020) who

reported that in 'the control experiment, the percentage of infected tomatoes on day 9 of incubation was 100%, whereas, when CFS fermented by *L. plantarum* TR7 and *L. plantarum* TR71 was used, the values were 29% and 65% respectively'.

Conclusion

From the observations of this study, it can be inferred that locally fermented foods are rich sources of bacteriocinogenic Lactobacilli and the bacteriocin produced by these microorganisms can be used in preserving tomatoes.

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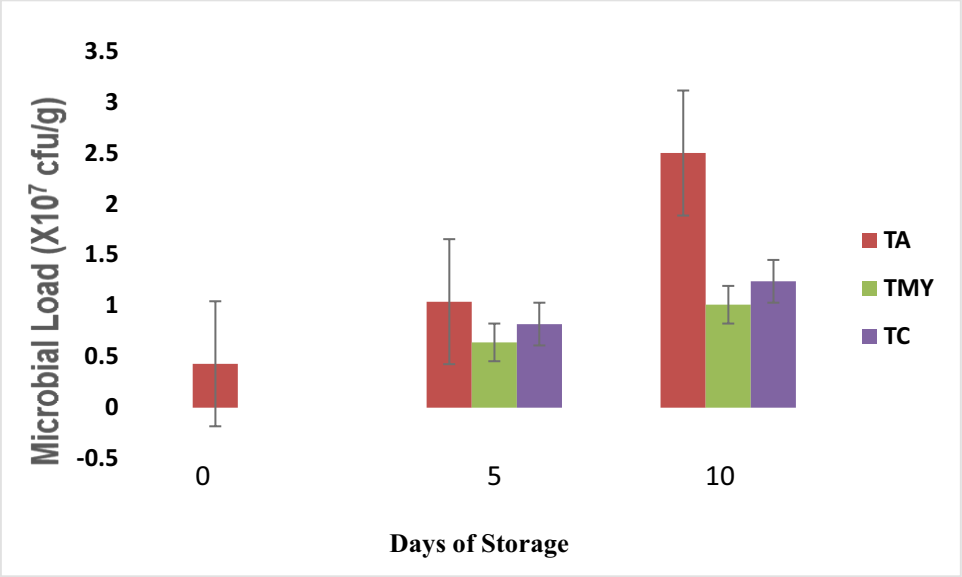

Fig 1: Microbial Load on Fresh Tomatoes

Keys: TA= Total aerobic bacteria, TMY= Total molds and yeasts, TC= Total number of coliforms

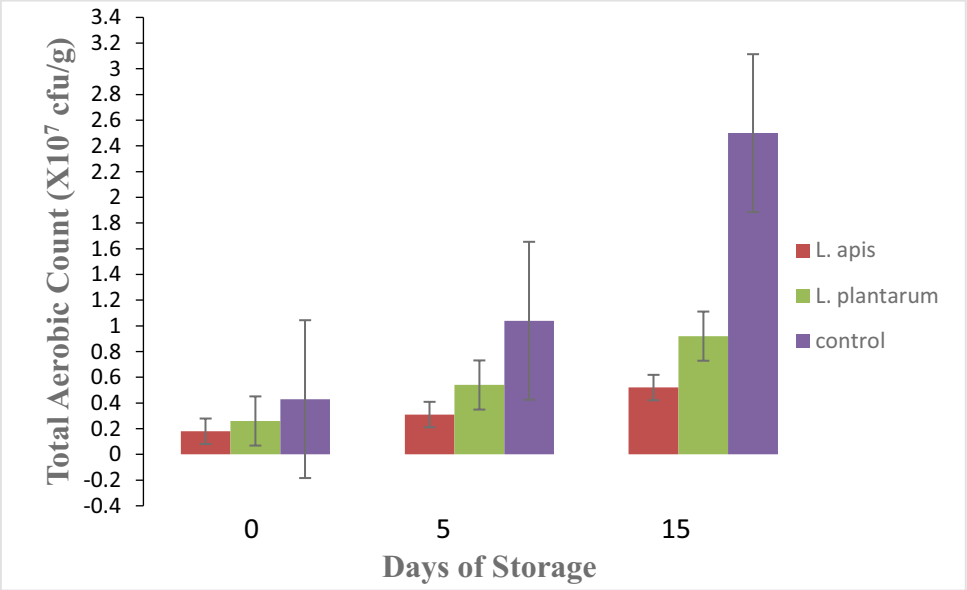

Fig. 2: Total Aerobic Bacteria Count in Bacteriocin Treated Tomatoes

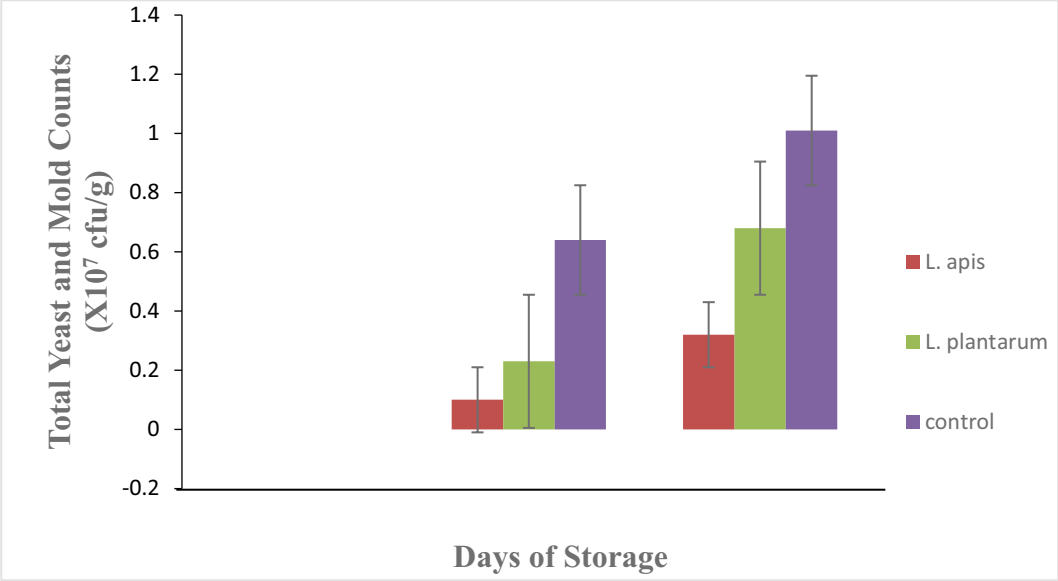

Fig. 3: Total Mold and Yeast Counts in Bacterioicn Treated Tomatoes

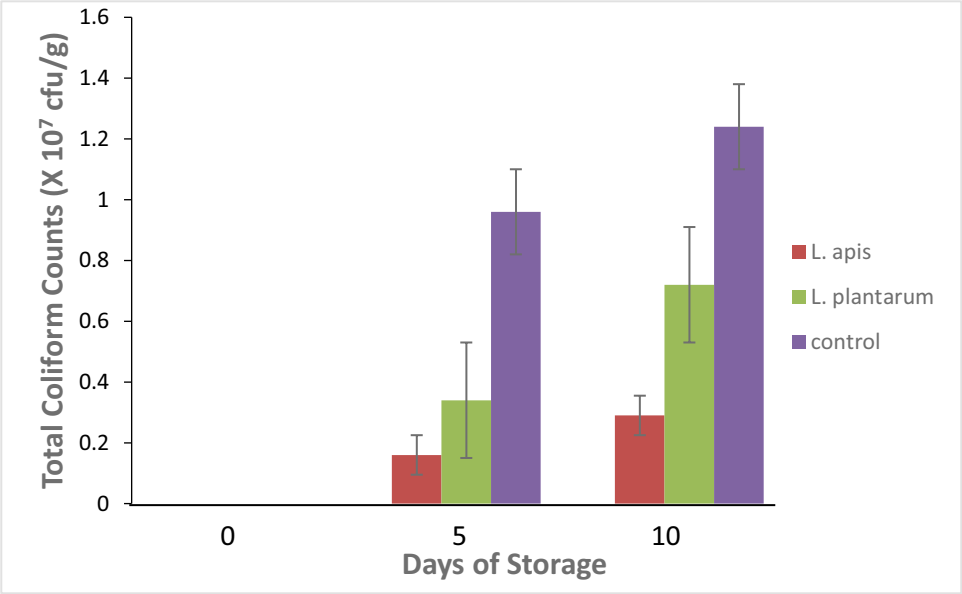

Fig. 4: Total Number of Coliforms Present in Bacteriocin Treated Tomatoes

Table 1: Biopreservative Effect of Bacteriocin from *Lactobacillus apis* HBAm1 and *Lactobacillus plantarum* SJC103 on *S. Aureus* Infected Tomatoes

Days	Control	Staph infected	Treatment with *L. apis*	Treatment with *L. plantarum*
0	0.00 ± 0.00^{a}	1.70 ± 0.30^{a}	0.00 ± 0.00	0.00 ± 0.00
5	1.95 ± 0.25^{a}	4.95 ± 0.35^{abc}	0.25 ± 0.05^{b}	0.80 ± 0.30^{c}
10	4.00 ± 0.30^{ab}	9.25 ± 0.35^{acd}	1.10 ± 0.10^{bc}	2.15 ± 0.35^{d}

Values are expressed as (mean ± SEM) X 10^{7} cfu/g of duplicate reading of the bacterial load on each day. Comparison is made across the row and values with the same superscript in a row are statistically significant at $P < 0.05$.