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Control of *Brochothrix thermosphacta* in Pork Meat Using *Lactococcus lactis* subsp. *lactis* I23 Isolated from Beef

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Abstract

This study evaluated the antimicrobial activities of two lactic acid bacteria, *Lactococcus lactis* subsp. *lactis* I23 and *Lactococcus lactis* subsp. *lactis* E91, against *Brochothrix thermosphacta* in pork meat during storage at ambient temperature (30°C) for 168 h. The LAB strains and the spoilage organism were inoculated on fresh pork samples at 1×10^6 $CFUg^{-1}$. Results showed about 3 log reduction in the spoilage organism in LAB inoculated samples after 48 h of storage. The spoilage organism showed susceptibility to antimicrobial action of *Lactococcus lactis* subsp. *lactis* I23. There was reduction in the count of Enterobacteriaceae, and no detection of Staphylococcus in the samples inoculated with *Lactococcus lactis* subsp. *lactis* I23 strain. Count of Staphylococcus was between 2.04 and 3.11 log in the untreated samples, and detection was not observed until 72 h of storage. Conclusively, growth of *Brochothrix thermosphacta* was effectively controlled by nisin producing *Lactococcus lactis* subsp. lactis I23 in fresh pork meat and this could enhance the shelf life of the product.

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1. Introduction

Pork is one of the most widely consumed meat product in Nigeria. Effort to improve its safety is of immense importance as a result of poor handling of fresh pork meat by sellers, especially when sales of the product are not exhausted on the day of slaughter; this may increase the risk of spoilage by opportunistic organisms such as *Brochothrix thermosphacta*, thereby affecting the quality of the product adversely. *B. thermosphacta* has been recognised as contributing a significant proportion of the spoilage microbiota of meat, including pork [1-3]. Hence, effort to curtail its growth in pork meat is required to promote safety. The use of bio-preservative agents such as lactic acid bacteria (LAB) to control the spoilage organism *B. Thermo-sphacta* in pork meat may constitute an economically viable approach towards reducing spoilage, and thereby help to avoid associated wastage.

Spoilage of raw meat accounts for major annual losses to processors and retailers [4]. One approach that could be adopted in extending the storage and shelf life of fresh meat is to introduce antimicrobials, preferably the naturally occurring antimicrobials from LAB to the surface of the meat.

The use of LAB commonly associated with meats as protective cultures may demonstrate antagonism towards pathogenic and spoilage organisms in meat preservation [5]. The ability of LAB to inhibit the growth of undesirable bacteria may be due to the production of organic acids, hydrogen peroxide, carbon dioxide, acetaldehyde, diacetyl or bacteriocins [6]. Thus using LABs or their metabolic products in the preservation of food is often referred to as "*biopreservation*" [6].

Olaoye and Dodd [5] reported the use of bacteriocinogenic *Pediococcus acidilactici* in the biopreservation of *tsire*, a Nigerian stick meat, against the spoilage and pathogenic organisms, *Listeria monocytogenes* and *Salmonella typhimurium*. Olaoye and Onilude [7] also investigated the use of *P. pentosaceus* LIV 01 and *P. acidilactici* FLE 01 in the preservation of fresh beef in Nigeria. Olaoye *et al.* [8] used two strains of LAB *Pediococcus pentosaceus* GOAT 01 and *Lactobacillus plantarum* GOAT 012 in the biopreservation of fresh goat meat, and concluded that the use of LAB resulted in the extension of shelf life of the meat product by some days when compared to uninoculated control samples.

B. thermosphacta is aerobic or facultative aerobic, which forms part of the spoilage organisms commonly associated with meat and may result in off-flavours, slimy, pack swelling and/or greening [9,10]. While the control of this spoilage organism in beef products has been variously reported [4,11-14], there is limited information on its control in pork meat. Research findings are thus required on its control in pork as a result of the high consumption of the product in Nigeria. The present study, therefore, aimed at evaluating the inhibition of *B. thermosphacta* by cultures of LAB including *Lactococcus lactis* subsp. *lactis* I23 and *L. lactis* subsp. *hordinae* E91 that have been isolated from a previous study [15].

2. Materials and Methods

2.1. Source of pork meat

The pork meat used in the present report was obtained from a retail market in Ibadan, Oyo State, Nigeria. It was taken to the laboratory on ice crystals for immediate use.

2.2. Lactic acid bacteria and spoilage organism

The cultures of LAB used in this study included *L. lactis* subsp. *lactis* I23 and *L. lactis* subsp. *hordinae* E91 that were isolated in a previous study [15]. The authors reported that the former produced bacteriocin nisin (approximately 610 bp) while the latter produced considerable quantity of lactic acid and diacetyl in an *in vitro* assay. The spoilage organism *B. thermosphacta* NCIMB 10018 used was obtained from the laboratory culture collection unit of Microbiology Department, University of Ibadan, Nigeria; it was maintained on Brain Heart Infusion (BHI, UK) agar at 30°C. The *Lactococcus* strains were maintained on M17 agar (Oxoid, UK) at the same temperature. Frozen cultures were maintained in broth media containing 20% glycerol at -70°C.

2.3. Determination of antimicrobial agents

Determination of antimicrobial agents (lactic and acetic acids) produced by the Lactococcus strains, was carried out using high performance liquid chromatography (HPLC) based method [16-18]. Growth supernatants of M17 broth of the Lactococcus strains were screened for antimicrobial agents in HPLC, using the standards of lactic and acetic acids for monitoring of retention time [18]. Quantification of the antimicrobial agents was done by extrapolation from the standard graphs obtained using different standard concentrations of the agents. The colony forming units per millilitre ($CFUml^{-1}$) of the Lactococcus strains were obtained by serial dilution techniques in the growth media. Concentrations of the antimicrobial agents were initially estimated as grams per millilitre (gml^{-1}) but, finally, expressed as grams per 10^7 colony forming units ($g\ 10^7\ CFU^{-1}$).

2.4. Testing of inhibition by *Lactococcus strains* against *Brochothrix thermosphata*

Pork meat was sliced into thin pieces (5.9×4.2×0.5 cm), weighing 12.4±1.1 g each; they were soaked in filter sterilised glucose solution (10% wv^{-1}) for about 10 min to stimulate the growth of Lactococcus [19]. Each piece was then inoculated with 6 log $CFUg^{-1}$ of each of *Brochothrix thermosphacta* NCIMB 10018 and of Lactococcus using the following treatments: Llac01, inoculated with *L. Lactis* subsp. *lactis* I23; Llac02, inoculated with *L. lactis* subsp. *hordinae* E91; Llac03, inoculated with the mixed cultures of *L. lactis* subsp. *lactis* I23 and *L. lactis* subsp. *hordinae* E91; and U-SAM, uninoculated control sample. The samples were stored at 30°C for 168 h. Sterilised meat samples served as control to set baseline for the microbial count of the spoilage organism. All samples were prepared in three replicates.

2.5. Microbiological counts of pork the during storage

Pork samples were taken during the storage period for microbial analysis. Ten grams (10 g) of samples were homogenized in standard stomacher bags (BA 6141, Seward, West Sussex, UK) containing 90 ml maximum recovery diluent (MRD; Basingstoke, UK) for 3 min at 230 rpm, using a Seward Stomacher (Model 400 circulator, P/4/518, Leighton Buzzard, UK) to give an initial 1:10 dilution. One tenth millilitre (0.1 ml) aliquot of appropriate dilutions was spread plated in duplicates for respective

microbial counts of total viable bacteria, TVC (PCA, Oxoid, UK) incubated at 30°C for 24 h; *Staphylococcus* (MSPRA, Oxoid) 24 h at 37°C; yeasts and moulds (Rose Bengal Chloramphenicol agar, RBCA, Oxoid, UK) 72 h at 25°C; and LAB (MRS agar, Oxoid, UK) 48 h at 30°C. Also 1 ml aliquot of appropriate dilutions of the samples were pour plated for viable counts of Enterobacteriaceae (violet red bile glucose agar, VRBGA) 48 h at 30°C [20].

Characteristic colonies emerging from respective media were counted, and results were expressed in logarithmic of colony forming units per gram of sample.

2.6. Confirmation of colonies

Colonies that emerged from respective growth media were confirmed phenotypically by biochemical tests and the analytical profile index (API) kits, API STAPH (Staphylococcus), API 20E (Enterobacteriaceae) and API 50CHL (lactic acid bacteria). Usage of the kits was according to manufacturer's instructtions. Data obtained from the respective API kits were used in the online software at Biomerieux website (www.apiweb.biomerieux.com) to confirm identities of colonies.

2.7. pH determination

Ten grams of the meat samples were homogenized in standard stomacher bags (BA 6141, Seward, UK) containing 100 ml sterile deionised water (pH 6.8±0.12), using a Seward Stomacher (Model 400 circulator, P/4/518, Leighton Buzzard, UK). pH was recorded using a pH meter (pH 212 Microprocessor, Hanna Instruments, USA).

2.8. Statistical analysis

The data obtained, which depended on the different treatments with Lactococcus strains, were analysed using the means of three replicates of each sample. They were subjected to analysis of variance, and differences between the means were evaluated by Duncan's multiple range test using the SPSS software (ver. 10.01). Significant differences were expressed at $p<0.05$.

3. Results and Discussion

The LAB strains used in this study included *L. lactis* subsp. *lactis* I23 and *L. lactis* subsp. *hordinae* E91, which were isolated from beef in a previous study [15]. The authors showed by PCR that the former encodes gene for production of bacteriocin nisin of about 610 bp. They further demonstrated that the two organisms produced lactic acid and diacetyl above 18 g 10^7 CFU^{-1} and 30 µg 10^7 CFU^{-1}, respectively, after incubation at 18 h in growth media. These properties may thus position the organisms as good candidates of starter cultures for biopreservation of food products, especially in meat processing. Hence, the biopreservative abilities of the two LAB strains (*L. lactis* subsp. *lactis* I23 and *L. lactis* subsp. *hordinae* E91) were evaluated against a spoilage organism, *B. thermosphata*in fresh pork. Additionally, counts of Entero-bacteriaceae and *Staphylococcus* were monitored in the LAB inoculated pork samples during storage in the present study.

Counts of LAB in the pork samples during the storage period are shown in Table 1. LAB counts increased with storage period and those inoculated with the LAB cultures had higher counts than uninoculated samples (U-SAM); counts in the U-SAM samples were observed to differ significantly from others ($p<0.05$). Increase of above 3 logs was noted in the LAB inoculated samples over the initial count of 6 logs. Similar reports were made by Djenane et al. [12] and Castellano and Vignolo [9] in separate studies after inoculation of meat samples with *Lactobacillus sakei* and *L. Curvatus*, respectively. Fall et al. [21] also reported increase in LAB growth during storage after inoculation of shrimps with culture of *Lactococcus piscium*.

pH values of the pork samples generally decreased with storage period, and value 4.7 or lower was recorded in the LAB inoculated pork samples when stored up to 48 h and longer (Table 2).

The decrease in pH may obviously be attributed to production of organic acids by the Lactococcus cultures, and this could be very useful in the control of spoilage and other unwanted organisms in meat.

Table 1. Counts (log CFU) of lactic acid bacteria in pork samples during the storage period

	Pork samples			
SP(h) †	Llac01	Llac02	Llac03	U-SAM
0	6.73±0.16^{a}	6.11±1.02^{a}	6.12±0.21^{a}	3.17±1.20^{b}
24	8.91±0.10^{a}	6.98±0.82^{ab}	7.81±0.86^{a}	4.90±0.28^{b}
48	10.02±0.17^{a}	9.32±1.29^{ab}	9.13±0.77^{ab}	5.42±0.27^{b}
72	10.23±0.58^{a}	9.76±1.01^{a}	9.32^{a}±0.05	7.81±1.28^{a}
96	10.16±0.21^{a}	9.50±0.13^{a}	9.88^{a}±0.12	7.99±1.23^{a}
120	10.31±1.02^{a}	10.12±0.82^{a}	9.10^{ab}±0.13	8.78±0.14^{b}
144	9.72±0.12^{a}	9.18±1.72^{a}	10.12±0.23^{a}	7.82±0.24^{b}
168	9.42±0.28^{a}	9.25±0.51^{a}	9.99±0.25^{a}	7.58±1.28^{b}

†storage period (SP)

Llac01, sample inoculated with *L. lactis* subsp. *lactis* I23; Llac02, sample inoculated with *L. lactis* subsp. *hordinae* E91; Llac03, sample inoculated with mixed cultures of *L. lactis* subsp. *lactis* I23 and *L. lactis* subsp. *hordinae* E91; U-SAM, uninoculated sample.

Values are means of three replicates. Values across rows with different superscripts letters are significantly different ($p<0.05$).

Table 2. Changes in pH of pork meat samples during the storage period

	Pork samples			
SP(h)†	Llac01	Llac02	Llac03	U-SAM
0	5.69±0.27^{a}	5.76±0.02^{a}	5.72±0.93^{a}	5.78±0.86^{b}
24	5.23±0.22^{b}	5.57±0.42^{a}	5.10±0.88^{b}	6.10±0.68^{a}
48	4.90±0.17^{c}	4.98±0.55^{b}	5.12±0.73^{b}	5.94±0.06^{b}
72	4.65±0.19^{c}	4.67±0.59^{c}	4.70±0.48^{c}	5.89±0.27^{b}
96	4.70±0.31^{c}	4.69±0.32^{c}	4.76±0.64^{c}	5.45±0.09^{c}
120	4.66±0.83^{c}	4.71±0.35^{c}	4.81±0.79^{c}	5.54±0.24^{c}
144	4.69±0.88^{c}	4.63±0.38^{c}	4.64±0.95^{c}	5.57±0.75^{c}
168	4.72±0.47^{c}	4.75±0.50^{c}	4.70±0.02^{c}	5.43±0.07^{c}

†SP, Storage Period; Llac01, sample inoculated with *L. lactis* subsp. *lactis* I23; Llac02, sample inoculated with *L. lactis* subsp. *hordinae* E91; Llac03, sample inoculated with mixed cultures of *L. lactis* subsp. *lactis* I23 and *L. lactis* subsp. *hordinae* E91; U-SAM, uninoculated sample.
Values are means of three replicates. Values across rows with different superscripts letters are significantly different ($p<0.05$).

Spoilage of raw meat has been reported to account for major annual losses to processors and retailers [4], and hence, application of biopreservative agents such as Lactococcus may help in the prevention of such losses.

Result of evaluation of the biopreservative effect of Pediodoccus strains against *B. Thermosphacta* in pork indicated that there was decrease in the count of the spoilage organism during storage, especially in the samples inoculated with *L. lactis* subsp. *lactis* I23 alone or co-treated with *L. lactis* subsp. *hordinae* E91 (Table 3).

Table 3. Counts of *Brochothrix thermosphacta*in pork samples during the storage period

	Pork samples			
SP(h)	Llac01	Llac02	Llac03	U-SAM
0	6.15±0.17^{a}	6.42±0.73^{a}	6.30±0.27^{a}	6.21±0.65^{a}
24	2.65±0.85^{a}	7.86±0.23^{b}	2.92±0.62^{a}	7.76±0.08^{c}
48	ND	6.34±1.02^{a}	ND	8.23±0.82^{b}
72	ND	6.21±0.27^{a}	ND	8.94±0.17^{b}
96	ND	5.86±0.87^{a}	ND	10.07±0.32^{b}
120	ND	5.61±0.05^{a}	ND	9.18±0.38^{b}
144	ND	6.02±0.45^{a}	ND	9.32±0.13^{b}
168	ND	6.48±0.73^{a}	ND	9.61±0.04^{b}

Values are means of three replicates. Values across rows with different superscripts letters are significantly different ($p<0.05$).
SP, Storage Period; Llac01, sample inoculated with *L. lactis* subsp. *lactis* I23; Llac02, sample inoculated with *L. lactis* subsp. *hordinae* E91; Llac03, sample inoculated with mixed cultures of *L. lactis* subsp. *lactis* I23 and *L. lactis* subsp. *hordinae* E91; U-SAM, uninoculated sample; ND, non-detectable.

Count of the spoilage organism was below the detectable limit (<2 log $CFUg^{-1}$) from 48 h of storage in samples inoculated with *L. lactis* subsp. *lactis* I23.

However, inoculation of pork samples with *L. lactis* subsp. *hordinae* E91 alone did not produce any significant reduction in the count of the spoilage organism. This indicates that the spoilage organism's susceptibility to the antimicrobial effect of *L. lactis* subsp. *lactis* I23 may not be due to production other antimicrobial agents beside bacteriocin nisin. This was confirmed in an *in vitro* assay after neutralisation of broth supernatants of Lactococcus strains to eliminate the effect of antimicrobial agents that may be present except bacteriocin (Figure 1). Hence, the spoilage organism was shown to be susceptible to the bacteriocin nisin produced by *L. lactis* subsp. *lactis* I23 [18]. The growth and survival of *B. Thermosphacta* at low pH has been attributed to the spoilage organism ability to produce organic acids [22,23]. This could therefore be responsible for the non-antagonism of *L. lactic* subsp. *hordinae* E91 alone against the spoilage organism. Similar results were reported by Maragkoudakis *et al.* [24] where the growth of *B. Thermosphacta* was not affected by production of antimicrobial agents such as organic acids and diacetyl by LAB, but rather bacteriociocins nisin and enterocin produced by the species of *Lactococcus* and *Enterococcus* respectively. Ercolini *et al.* [25] further demonstrated effective antimicrobial activity of purified nisin against *B. thermosphacta* in meat during storage. Hence, the protective activity against this spoilage organism, as observed in the present study, could be very significant towards curtailing spoilage and extending shelf life in fresh pork meat especially in Nigeria. However, other spoilage organisms that may be associated with pork meat should be taken into serious consideration in the eventual proposition of LAB starter cultures for biopreservation of meat products.

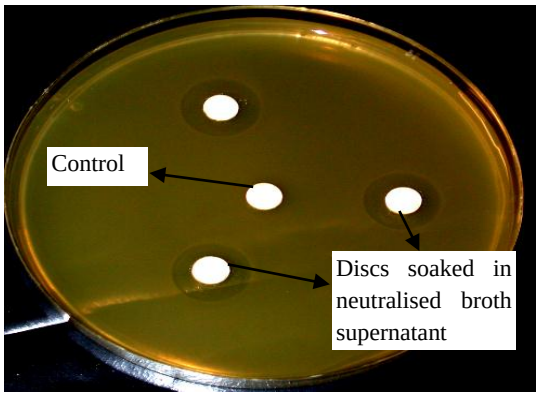

Figure 1. Antagonistic activity of the neutralised broth supernatant of nisin producing *L. lactis* subsp. *lactis* I23 against *B. thermosphacta* NCIMB 10018.

Table 4. Counts of Enterobacteriaceae and *Staphylococcus* in pork samples during the storage period

	Enterobacteriaceae in pork samples				Staphylococcus in pork samples			
SP(h)†	Llac01	Llac02	Llac03	U-SAM	Llac01	Llac02	Llac03	U-SAM
0	3.93±0.55^{a}	3.87±0.89^{a}	3.45±0.73^{a}	3.87±0.72^{a}	ND	ND	ND	ND
24	3.76±0.31^{a}	3.48±013^{a}	3.28±0.72^{a}	4.21±0.62^{a}	ND	ND	ND	ND
48	3.35±0.16^{a}	3.19±0.71^{a}	2.47±0.26^{a}	5.01±1.02^{b}	ND	ND	ND	ND
72	3.24±1.12^{a}	2.92±0.14^{a}	2.31±0.49^{a}	6.16±0.12^{b}	ND	ND	ND	2.04
96	3.733±0.18^{a}	3.99±0.32^{a}	2.05±0.22^{a}	6.48±0.38^{b}	ND	ND	ND	2.36
120	4.53±0.81^{a}	3.83±0.25^{a}	2.79±0.65^{a}	6.77±0.74^{b}	ND	ND	ND	2.79
144	4.12±0.14^{a}	4.23±0.81^{a}	3.62±0.42^{a}	7.16±0.62^{b}	ND	ND	ND	2.94
168	5.87±0.43^{a}	4.54±0.65^{a}	4.74±0.31^{a}	7.57±0.27^{b}	ND	ND	ND	3.11

Values are means of three replicates. Values across rows with different superscripts letters are significantly different ($p<0.05$).
†SP, Storage Period; Llac01, sample inoculated with *L. lactis* subsp. *lactis* I23; Llac02, sample inoculated with *L. lactis* subsp. *hordinae* E91; Llac03, sample inoculated with mixed cultures of *L. lactis* subsp. *lactis* I23 and *L. lactis* subsp. *hordinae* E91; U-SAM, uninoculated sample; ND, non-detectable.

The results of Enterobacteriaceae and Staphylococcus counts in the pork meat samples are represented in Table 4. Counts of Enterobacteriaceae decreased in the pork samples inoculated with *Lactococcus* cultures compared to their U-SAM counterparts, and the decrease was sustained up to 96 h of storage after which increase started setting in. Lower counts were recorded in the samples co-inoculated with *L. lactis* subsp. *lactis* I23 and *L. lactis* subsp. *hordinae* E91 compared to the others, but counts were generally higher in the U-SAM samples. *Staphylococcus* was not detected in the pork samples inoculated with *L. Lactis* strains, unlike the uninoculated counterparts where count of the organism was between 2.04 and 3.11 log during storage; there was no detection in the uninoculated control samples until 72 h (Table 4). Hence, inoculation of fresh pork samples with the mixed cultures of *L. lactis* subsp. *lactis* I23 and *L. lactis* subsp. *hordinae* E91 proved more effective against the spoilage organism than when used individually. More than 1 log decrease occurred in the LAB inoculated samples while increase of up to 4 logs was noticed in the U-SAM samples. *Staphylococcus* counts were below 2 logs in pork samples inoculated with Lactococcus cultures throughout the storage period while counts increased to about 3 logs in the U-SAM samples.

Inhibition recorded by the Lactococcus strains against Enterobacteriaceae and Staphylococcusin the pork samples could be attributed to production of antimicrobial agents [25,26]. Similar findings were reported by other researchers [7,27].

Counts of total viable bacteria (TVC) were lower in the pork samples inoculated with Lactococcus cultures compared to the U-SAM samples during storage (Table 5) counts reduced from 24 h to 96 h of storage in the inoculated samples while increase was recorded in the U-SAM counterparts. This therefore confirmed that the Lactococcus cultures were effective in the reduction of TVC in fresh pork meat yeast and moulds were also lower in samples inoculated with Lactococcus cultures compared to U-SAM, indicating that the LAB cultures had antimicrobial effect on the yeast and moulds.

Table 5. Counts of total viable bacteria and yeasts and molds in pork samples the during storage period

	Total Viable Bacteria				Yeasts and Moulds			
	Pork samples				Pork samples			
SP(h)	Llac01	Llac02	Llac03	U-SAM	Llac01	Llac02	Llac03	U-SAM
0	5.79±0.03^{a}	5.69±0.62^{a}	6.06±0.75^{a}	5.47±0.21^{a}	3.83±0.14^{a}	3.73±0.86^{a}	3.65±0.83^{a}	3.76±0.26^{a}
24	5.42±0.21^{a}	5.37±0.32^{a}	5.06±0.63^{a}	6.54±0.87^{c}	4.14±0.23^{a}	4.85±0.27^{a}	4.24±0.13^{a}	5.32±0.81^{a}
48	4.85±0.12^{a}	5.02±0.91^{b}	4.32±0.07^{a}	7.33±0.42^{c}	4.45±0.43^{a}	4.23±1.02^{a}	4.22±0.27^{a}	5.22±0.16^{a}
72	4.48±1.09^{a}	4.73±0.72^{b}	4.18±0.25^{a}	7.97±0.92^{c}	5.83±0.72^{a}	3.99±1.31^{b}	4.42±0.38^{b}	6.12±0.13^{c}
96	3.97±0.91^{a}	4.01±0.53^{b}	4.35±0.76^{b}	8.24±0.84^{c}	5.49±0.43^{a}	4.21±0.76^{b}	4.65±0.72^{b}	6.43±0.21^{c}
120	4.43±0.65^{a}	4.53±0.21^{b}	5.14±0.24^{b}	9.64±0.57^{c}	4.99±0.62^{a}	4.44±0.33^{a}	4.44±0.29^{a}	6.83±1.02^{b}
144	5.52±0.92^{a}	5.49±0.28^{b}	5.54±0.45^{b}	9.52±0.35^{c}	5.07±0.21^{a}	4.87±0.45^{a}	4.60±0.21^{b}	6.64±1.72^{c}
168	6.24±1.20^{a}	6.17±0.92^{a}	6.23±0.72^{a}	10.25±0.23^{c}	5.84±0.32^{a}	5.26±0.86^{a}	4.75±0.82^{b}	7.73±0.38^{c}

Values are means of three replicates. Values across rows with different superscripts letters are significantly different ($p<0.05$).
SP, Storage Period; Llac01, sample inoculated with L. *lactis* subsp. *lactis* I23; Llac02, sample inoculated with *L. lactis* subsp. *hordinae* E91; Llac03, sample inoculated with mixed cultures of *L. lactis* subsp. *lactis* I23 and *L. lactis* subsp. *hordinae* E91; U-SAM, uninoculated sample.

Similar observations were reported by Casaburi*et al.* [28] where growth of yeast and moulds was reduced by the action of LAB starter cultures in sausages the during storage. Also Erkmen [29] reported reduction in yeast and moulds in a Turkish sausage after inoculation with LAB strains as protective cultures. Furthermore, Olaoye *et al.* [8] noted reduction in the counts of Y & M in fresh goat meat samples that were inoculated with LAB cultures during storage.

The results of this study showed that nisinproducing *L. lactis* subsp. *lactis* I23 exhibited antimicrobial activity against the spoilage organism, *B. Thermosphacta*, in the fresh pork samples during the storage period. Production of antimicrobial agents, especially organic acids, produced no significant antagonism against the spoilage organism. The use of LAB cultures of *L. lactis* subsp. *lactis* I23 and *L. lactis* subsp. *hordinae* E91 also led to decrease in the growth of Enterobacteriacea and Staphylococcus and TVB, especially when combination of the two LAB cultures was used.

4. Conclusion

From the microbiological quality observed during storage, it was concluded that shelf life of fresh pork meat could be extended for up to three days with the use of *L. lactis* subsp. *lactis* I23 culture alone or combination with *L. lactis* subsp. *hordinae* E91 as biopreservative agents.

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6. Conflict of interest

Authors declare that there is no conflict of interest

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