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Biopreservation of Horse Mackerel Fillet using *Lactobacillus plantarum* (ATCC 8014) and *Lactobacillus sakei* (ATCC 15521)

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Abstract Antagonistic properties of LAB allied to their safe history of use in traditional food fermented products meke them very attractive to be used as biopreservatives. *L. plantarum* (ATCC 8014) and *L. sakei* (ATCC 15521) exhibited strong inhibition against *S. aureus* (ATCC 25923), *E. faecalis* (MTCC 2729) and *P. aeruginasa* (MTCC 3163). The varying growth of *S. aureus* encountered in all samples suggests bacteriostatic rather than bacteriocidal impact of the hurdles. T_3 resulted in the lowest Staphylococcus count (7.58 log cfu/g) and FFA values which may be due to the combined effect of several hurdles like acidifications by LAB, low temperature and vacuum. The lowest value of TVB-N (24.21mg%) was encounted in T_4 suggesting a significant ($p< 0.05$) combination effect in reducing TVB-N Content. Combination of LAB and vacuum seems to have positive effect ($p <0.05$) in reducing the PV in comparison to the control samples. So the product inoculated with LAB acceptable till 12 days.

Keywords Lactic acid bacteria, Biopreservation, Inhibition of *Staphylococcus aureus.*

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Introduction

One of the concerns in food industry is the contamination by pathogens, such as *Salmonella, Campylobacter jejuni Escherichia coli* 0157 : H7, *Listeria monocytogenes,Staphylococcus aureus* and *Clostridium botulinum,* which are frequent cause of food borne diseaseas [1]. Among these food borne pathogens. *Staphylococcus aureus* is reported to be the causative agent of a wide panel of infections ranging from superficial lesions to life -threatening septicaemia [2]. The organism has subsequently been incriminated in incidents involving a wide range of food vehicles including seafood, meat, dairy, cream-filled bakery, poultry and egg products as well as salads and canned mushrooms and is commonly associated with food borne diseases.

Inhibition of growth of food pathogens is therefore necessary and presently consumers prefer natural but effective preservation of food. An attractive and alternative approach to chemical preservatives [3] is biopreservation. It involves the use of non-pathogenic microorganisms and/or their metabolites to improve microbiological safety and extend the shelf life of foods [4]. Lactic acid bacteria (LAB) and their antagonistic substances, called bacteriocins, known to be inhibitory towards various pathogenic bacteria and spoilage microorganisms during growth and refrigerated storage in food products, are generally regarded as safe (GRAS) [5]. Therefore, the objective of the study is to evaluate the inhibitory effect of two lactic cultures viz. *Lactobacillus plantarum,* ATCC 8014 and *Lactobacillus sakei*, ATCC 15521)on *Staphylococcus* aureus (ATCC 25923) inoculated on horse mackerel fillets.

Materials and Methods

Bacterial strains and culture media

Lactobacillus plantarum (ATCC 8014) and *Lactobacillus sakei* (ATCC 15521) (Hi-media, India) were used in the present study. *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (MTCC 1563), *Enterococcus faecalis* (MTCC 2729), *Pseudomonas aeruginosa* (MTCC 3163) and *Salmonella enterica* (MTCC 3224) were used as pathogenic organisms (indicator) to screen the inhibitory effect of the lactic cultures.

Screening

Screening of the two lactic acid bacteria (LAB) cultures for bacteriocin production was done through well diffusion assay (WDA), as described by Nath et al. [6] against indicator organisms by preparing neutralized cell-free supernatant (NCFS) as described by Nath et al. [6].

Inhibition of *Staphylococcus aureus* (ATCC 25923) on horse mackerel fillets by Lactic cultures

Fresh Horse Mackeral *(Megalopsis cordella)* with an average weight of 500 g was purchased from the fish market in South Kolkata and were transferred to the laboratory in iced condition, decapitated and filleted by hand. Cubes were cut from the fillets such that the final weight of each piece was approximately 10 g for microbiological analysis. The study was conducted as a completely randomized design with four treatments, two types of packaging (normal and vacuum packaging) and stored at 6 ± 1^0C temperature. There were five sampling intervals. The fillets were subjected in triplicate for microbial and sensory analyses at 3 days interval starting from day 0 for *S. aureus* (ATCC 25923) count by spread plating with appropriate dilutions on Baird Parker Agar Plate [7] and results expressed as log cfu/g. The treatments were prepared aseptically maintaining good hygienic practices and stored at 6 ± 1^0C a follows :

Only *S. aureus,* under aerobic packaging condition (C_1), only *S. aureus,* under vacuum packaging condition (C_2), *S. aureus + L. plantarum,* under aerobic packkaging condition (T_1), *S. aureus + L. sakei,* under aerobic packaging condition (T_2), *S. aureus + L.plantarum,* under vacuum packaging condition (T_3), *S. aureus + L. sakei*, under vacuum packaging condition (T_4)..

An overnight culture of *S. aureus* (ATCC 25923) was diluted in sterile peptone buffer to obtain a viable cell population of approximately 10^6 CFU/ml as determined by using Mcfarland Standard. For the cubes of treatment T_1,T_2, T_3 and T_4, 1ml of the fresh concentrated culture of *L. plantarum* (ATCC 8014) (T_1 and T_3) and *L. sakei* (ATCC 15521) (T_2 and T_4) containing a population of $I \times 10^7$ cfu/ml was added and distributed with a dropper onto the surface of the fillets, as described by Chowdhury et al. [8] and stored at 6 ± 1^0C. All the treatments were surface inoculated by *S. aureus* (ATCC 25923) [8]. The fillets of treatment C_2, T_3 and T_4 of each set were vacuum packed by using INDVAC packaging machine as described by Chowdhury et al. [8].

Total volatile base nitrogen (TVB-N), Peroxide value (PV) and Free fatty acid (FFA) of the lipid and pH of the sample was determined by the method as described by Nath et al. [9]. All of the data were checked for normal distributions with normality plots prior to two-way analysis of variance (ANOVA), to determine significant differences among means at $\alpha = 0.05$ level, using statistical tools of R software.

Results and Discussion

Both the test strains *L. plantarum* (ATCC 8014) and *L. sakei* (ATCC 15521) exhibited zone of inhibition against *S. aureus* (ATCC 25923), *E. faecalis* (MTCC 2729) and *P. aeruginosa* (MTCC 3163) during agar overlaying technique suggesting a spectrum of inhibition of both Gram positive and Gram negative bacteria. However, the NCFS of both the LAB strains did not show any antimicrobial activity against the indicator strain by WDA.

To study the inhibitory effect of the lactic cultures on the growth of pathogens, *S. aureus* was chosen as an indicator strain because. *S. aureus* (ATCC 25923) demonstrated the highest inhibition rate by both the LAB strains as was also reported by Diop et al. [10]. Although the inhibitory effect of the LAB

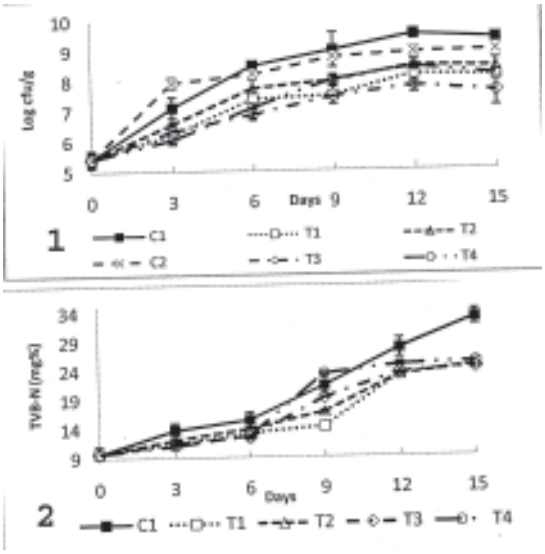

Fig. 1. Inhibition of *Staphylococcus aureus* (ATCC 25923) at 6 ± 1^{0}C by lactic cultures. **Fig. 2.** Changes in TVB-N of Horse Mackerel fillet treated with lactic cultures at 6 ± 1^{0}C.

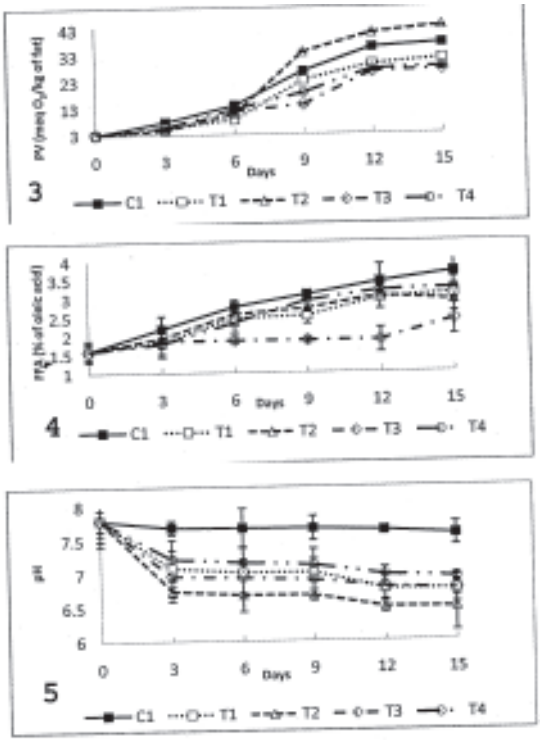

Fig. 3. Changes in PV of Horse Mackerel fillet treated with lactic cultures at 6 ± 1^{0}C. **Fig. 4.** Changes in FFA value of Horse Mackerel fillet treated with lactic cultures at 6 ±1^{0}C. **Fig. 5.** Changes in pH value of Horse Mackerel fillet treated with lactic cultures at 6 ± 1^{0}C.

strain may be due to several reasons including competition for nutrients and oxygen, competition for attachment or adhesion sight and production of a wide range of inhibitory substances such as organic acids, acetoin, diacetyl, hydrogen peroxide, reuterin and bacteriocins indicated by Olaoye [11], the antimicrobial activity of the presently selected strains may not be due to the production of bacteriocin, as no inhibitory effect was detected during WDA of the NCFS. The choice of the temperature 6 ± 1^{0}C for storage is significant considering the fact that 20% of commercial and residential refrigerators maintain temperature >10^{0}C [12]. The horse mackerel fillet was selected as a ready to cook product, which posses chances of post-processing contamination and being subjected to abused refrigeration temperature of 6 ± 1^{0}C.

It is evident that application of LAB and packaging of Horse Mackerel fillet in vacuum resulted in reduction in the final count of *S. aureus* (ATCC 15923), although in varying rates after 15 days at 6 ± 1^{0}C suggesting a bacteriostatic rather than bacteriocidal impact of the hurdles. In C_2, the final count of *S. aureus* was 8.95 (log cfu/g), 0.4 log cycle less than C_1 (9.35 log cfu/g) after 15 days of storage. This result is similar to the findings of Plaatjies et al. [13] who reported approximately 60% lower *Staphylococcus* growth at 5^{0}C under vacuum. In T_1,there was almost 1.2 log cycle reduction in final count of *S. aureus* which may be because of the production of the lactic acid by the *L. plantarum* as Charlier et al. [14] reported. He further reported that the optimal pH for growth of *S. aureus* is close to neutrality and medium acidification resulting from lactic fermentation by LAB seems to be one of the main factors involved in the inhibition of *S. aureus* growth. Application of vacuum (T_3) resulted in the lowest *Staphylococcus* count (7.58 log cfu/g) among all the treatments which may be due to the combined effect of several hurdles like acidification by LAb, low temperature and vacuum, as suggested by Ananou et al. [15]. Addition of *L. sakei* ATCC 15521 resulted in reduction of *S. aureus* count

nearly 1.0 and 1.2 log cycles in T_2 and T_4 samples respectively.

The changes in TVB-N values of T_3 and T_4 samples were significantly lower ($p < 0.05$) compared to control samples. The sharp increase in TVB-N value was observed in both T_1 and T_3 samples on 12th day of storage which may be because of extended lag phase of the spoilage micro-organisms [8] as result for competitive inhibition by LAB as well as acidification. Although T_2 sample exhibited significantly lower ($p < 0.05$) TVBN value (24.65 mg%) compared to control (32.9 mg%), the lowest value of TVB-N (24.21 mg%) was encountered in T_4 suggesting a significant ($p<0.05$) combination effect of *L. sakei* ATCC 15521 and vacuum. Sudalayandi and Manja [16] reported that, out of seven LAB tested for quality indices reduction, *Lb. helveticus, Lc. Lactis* and *Pediococcus acidilactici* successfully controlled TVB-N.

Although T_1, T_2, T_3 and T_4 all exhibited a significant resuction in PV ($p<0.05$), T_1 and T_2 crossed the limit of acceptability (20 meq O_2/kg of fat) on 9th day of storage (23.45 meq O_2/kg and 34.14 meq O_2/kg respectively) and to that of T_3 and T_4 was on 12th day and finally, highest reduction in PV was observed T_3 reaching a final value of 27.27 meq O_2/kg of fat as compared to control (37.38 meq O_2/kg) which may be due to combination of *L. plantarum* and vacuum have positive effect ($p>0.05$) in reducing the PV. As T_2 exhibited maximum rise in PV (43.91 meq O_2/kg), even higher than the control (37.38 meq O_2/kg) at the end of day 15 of storage study, it can be inferred that in spite of *L. sakei* being an important starter culture for the production of fermented meat products; it may dominate the spoilage in association of vacuum packaged processed meat products as reported by Schilinger and Holzapfel [17].

FFA contributes to off flavor and causes textural alteration by complexion with muscle proteins. The final FFA value for aerobic treatments viz. C_1, T_1 and T_2 samples were 3.68% of oleic Acid. 3.11% of oleic Acid and 2.93% of oleic Acid respectively, with no significant variation ($p>0.05$) among the samples. Whereas, T_3 and T_4 the FFA values reached 2.41% of oleic acid and 3.25% of oleic acid respectively with T_3 yielded the best result which may be supported by the findings of Sudalayandi and Manja [16] leading to conclude that vacuum has a significant effect in reducing the FFA value only in presence of *L. plantarum.* They reported that, *L. plantarum* reduced FFA from 9.4 to 6.4 % of oleic acid in Indian Mackerel chunks during 2 days storage at 37^{0}C.

Lowering trend of pH observed in all treatments with significantly ($p< 0.05$) low values of pH observed in T_2 samples (6.49), although vacuum has no significant effect in lowering pH ($p<0.05$). Such medium acidification may have contributed to the inhibition of *S. aureus.*

From the microbial and biochemical analyses, It may be concluded that the test strains behaved as spoilers both in aerobic and anaerobic condition after 12th day of storage at 6 ± 1^{0}C. Before that, both *L. plantarum* (ATCC 8014) and *L. sakei* (ATCC 15521) may be used for inhibition of pathogenic organisms like *S. aureus,* although complete elimination may not be possible. The nature of inhibition was bacteriostatic rather than bacteriocidal. The best preservation was observed by the combination of *L. plantarum* (ATCC 8014) and vacuum (T_3) stored under 6 ± 1^{0}C till 12th days of storage. Therefore, the lactic cultures may be effective as biopreservative of horse mackerel fillet only for a limited period of maximum 15 days beyond which the changes in biochemical characteristics of the fillet is expected to occur.

References

1. Nath S, Chowdhury S, Sarkar S, Dora KC (2016) *Bacillus*- a potential biopreservative for seafood safety. Accepted for publication in proceedings : Nat Sem on Nutri and Wellness. 7-8 Nov 2016. The Physical Education Unit, Palli Siksha Bhavana (Inst Agric), Vishwa-Bharati, India.
2. Kluytmans JAJW, Wertheim HFL (2005) Nasal carriage of Staphylococcus aureus and prevention of nosocomial infections. Infection 33 : 3—8.
3. Sharma N, Kapoor R, Gautam N, Kumari R (2011) Purification and characterization of bacteriocin produced by *Bacillus subtilis* R 75 isolated from fermented chunks of mung bean *(Phaseolus radiatus).* Food Technol Biotechnol 49 : 169—176.
4. Nath S, Chowdhury S, Dora KC, Sarkar S (2014) Role of biopreservation in improving food safety and storage. Int J Engg Res Appl 4 (Version 3) : 26—32.
5. Parada JL, Caron CR, Medeiros ABP, Soccol CR (2007) Bacteriocins from lactic acid bacteria : purification, properties and use as biopreservatives. Braz Arch Biol Technol

50 : 512—542.
6. Nath S, Dora KC, Sarkar S, Chowdhury S (2015) Detection and characterization of antimicrobial peptide produced by *Bacillus subtilis* EPTB23 isolated from curd. TAJAS 10 : 115—123.
7. Hisar SA, Kaban G, Hisar O, Yanik T, Kaya M (2005) Effect of *Lactobacillus sakei* Lb706 on behavior of *Listeria monocytogenes* in vacuum-Packed Rainbow Trout Fillets. Turk J Vet Anim Sci 29 : 1039.
8. Chowdhury S, Raychaudhuri U, Nath S, Dora KC (2016) Shelf life of lactic acid bacteria inoculated vacuum packed *Tenualosa ilisha* (Hamilton, 1822) at low temperature. Ind J Fish 63 : 156—161.
9. Nath S, Chowdhury S, Dora KC (2014) Effect of lactic acid bacteria application on shelf life and safety of fish fillet at 6 ± 1^{0}C. IJAR 2 : 201—207.
10. Diop MB, Dauphin RD, Tine E, Ngom A, Destain J, Thonart P (2007) Bacteriocin producers from traditional food products. Biotechnol Agron Soc Environ 11 : 275.
11. Olaoye OA, Ntuen IG (2011) Spoilage and preservation of meat : A general appraisal and potential of lactic acid bacteria as biological preservatives. IRJOB 2 : 33—46.
12. Chowdhury S, Raychaudhuri U, Dora KC (2012) Competitive inhibition of *Staphylococcus aureus* in vacuum packed fish fillet by lactic acid bacteria. TAJAS 7 : 108—113.
13. Plaatjies Z, Lues JFR, Buys EM, Venter P (2004) Staphylococcal growth in fresh vacuum-packed red meat at various storage conditions. Proc: 8th world Cong on Environm Hlth. 22-27 Feb 2004. Document Transformation Technologies, Durban, South Africa.
14. Charlier C, Cretenet M, Even S, Loir TL (2009) Interactions between *Staphylococcus aureus* and lactic acid bacteria : An old story with new perspectives. Int J Food Microbiol 131 : 30.
15. Ananou S, Maqueda M, Martinez-Bueno M, Valdivia E (2007) Biopreservation, an ecological approach to the safety and shelf-life of foods. In : Commun Curr Res and Educational Topics and Trends in Appl. Microbiol (A. Mendez-Vilas Eds, Formatex, Spain), pp 475.
16. Sudalayandi K, Manja (2011) Efficacy of lactic acid bacteria in the reduction of trimethylamine-nitrogen and related spoilage derivatives of fresh Indian mackerel fish chunks. Afr J Biotechnol 10 : 42.
17. Schilinger U, Holzapfel WH (2003) Culture Media for Lactic Acid Bacteria. In : Culture Media for Food Micro-biology, Corry JEL, Curtis GDW and Baird RMAmsterdam, Elsevier, pp 127—140.