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Full Length Research Paper

Efficacy of lactic acid bacteria in the reduction of trimethylamine-nitrogen and related spoilage derivatives of fresh Indian mackerel fish chunks

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Different strains of lactic acid bacteria (LAB) such as *Pediococcus acidilactici*, *Pediococcus pentosaceous*, *Streptococcus thermophilus, Lactococcus lactis, Lactobacillus plantarum, Lactobacillus acidophilus* and *Lactobacillus helveticus* were procured from the NCL (National Chemical Laboratory), Pune, India. These LAB cells were individually coated on the dressed fresh mackerel fish chunks and incubated at 37°C for two days. Different quality indices such as trimethylamine nitrogen (TMA-N), total volatile basic nitrogen (TVB-N), peroxide value (PV) and free fatty acids (FFA) were estimated for 2 days period. *P. acidilactici* cell coat reduced 40 mg% of TMA-N, 47.66 mg% of FFA, 97 mg% of PV and 97 mg% of PV when compared with control. The TMA-N content was reduced to 20 mg% by *S. thermophilus* cell coat on the treated fish when compared with control, while no reduction was observed on TVB-N. Out of the seven LAB tested for TMA-N reduction, *Lb. helveticus* and *Lc. lactis* reduced 150 mg% identically. All the LAB grew in increasing and decreasing trends using available carbohydrate present in the fish. For, FFA reduction, *Lb. plantarum* showed the highest (188%) as compared to *P. pentosaceous* and *P. acidilactici* followed by *S. thermophilus*. *P. pentasaceous* and *P. acidilactici* showed the highest PV reduction as 98 and 83 milli equivalent fat/kg of fats, when compared with *Lb. plantarum* in the control. Out of seven LAB tested for quality indices reduction, *Lb. helveticus*, *Lc. lactis* and *P. acidilactici* were the best LAB to control TMA-N and TVB-N, respectively. *Lb. acidophilus*, showed the best in the reduction of FFA. Therefore, this study confirms that LAB can be used to preserve freshfish through controlling spoilage bacteria and amines for a short period of time. Inplace of chemical preservatives, LAB may be use to extend the shelf life of fish.

Key words: Lactic acid bacteria, reduction of spoilage indices, trimethylamine nitrogen (TMA-N), total volatile basic nitrogen (TVB-N), peroxide value (PV), free fatty acids (FFA), mackerel chunks.

INTRODUCTION

Fish meat is highly perishable because it contains more protein, polyunsaturated fatty acids, natural enzymes and low stroma protein with higher digestibility (Goodrick, 1987). Natural enzymes present in the fish generate autolytic changes in it that produce amines, aminoacids and glucose for bacterial growth. Bacteria convert the nitrogenous compounds such as ammonia, aldehyde, hydrogen sulphide and indole into various other derivatives under putrefaction (Barrett et al., 1985). Most of the marine fishes contain a non-protein nitrogenous substance called trimethylamine oxide (TMAO). Certain bacteria that occur naturally on the skin, gut of fish and in sea water can break down TMAO to trimethylamine (Brettar et al., 2002). The

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amount of TMA produced is a measure of the activity of spoilage bacteria in the flesh and so is an indicator of spoilage (Dalgaard et al., 2006). TMA is responsible for the fishy odor in fish and its reduction by spoilage bacteria further leads to ammonical odour (Ghaly et al., 2010). The total amounts of ammonia, dimethylamine and trimethylamine are called the total volatile base (TVB) nitrogen, commonly used to estimate fish spoilage. The biogenic amines index (BAI) is one of the tools for seafood freshness. BAI is the amount of the biogenic amines such as histamine, putrescine and cadaverine that increases steadily after the death of the fish due to bacterial action on amino acids (Dalgaard et al., 2006). There is a greater consumer resistance against using chemicals as fish preservative agent due to the formation of undesirable compounds. This may lower the shelf life of fish that leads to unacceptability to the contemporary consumers. Under bio-preservation, combined coating of lactic acid bacteria (LAB) in Tilapia (*Oreochromis niloticus*) fillets had decreased both total volatile basic nitrogen (TVB-N), trimethylamine nitrogen (TMA-N) and thiobarbituric acid (TBA) values, etc (Ibrahim and Salha, 2009). Hu Yongjin et al. (2007) used mixed LAB cultures in the sausage prepared from silver carp to inhibit the growth of spoilage microbes and the accumulation of histamine, cadaverine, putrescine, tryptamine and tyramine. LAB such as *Pediococcus pentasaceous* and *Pediococcus acidilactici* were used to prevent the growth of fish-borne bacteria in mackerel fish chunks (Kannappan and Manja, 2004). LAB coating also deplete salts and nitrites present in the fish (Chang et al., 2004). Therefore, in this study, an attempt has been made to ascertain the reduction of major quality indices such as trimethylamine oxide (TMA-O), TVB-N, peroxide value (PV), and free fatty acids (FFA) by coating various LAB cells in fresh Indian mackerel fish chunks.

MATERIALS AND METHODS

Regeneration of lactic cells

Different strains of LAB such as *P. acidilactici* NCIM 2292 (National Collection of Industrial Microorganism) *P. pentosaceous* NCIM 2296, *Streptococcus thermophilus* NCIM 2412, *Lactococcus lactis* NCIM 2114, *Lactobacillus plantarum* NCIM 2085, *Lactobacillus acidophilus* NCIM 2287 and *Lactobacillus helveticus* NCIM 2126 were procured from the National Chemical Laboratory (NCL), Pune, India. One milliliter of viable LAB cultures was inoculated separately into 25 ml of MRS broth (Deman et al., 1960), shaker incubated at 37°C for two days at 250 rpm. The cells were harvested by Super speed RC-5B Refrigerated centrifuge at 10,000 rpm for 15 min (47378 g force) and washed thrice in sterile saline.

Indian mackerel

Rastrelliger kanagurta (Indian mackerel-cuvier) belonging to the family Scombridae has been employed for investigation. It is a pelagic, fatty fish which was chosen for the study on a very fresh condition, approximately 17.0 to 19.0 cm in length and 90 to 125 g in size.

Preparation of mackerel fish chunks for coating LAB

The fishes were dressed into chunks of 3.0 cm thickness and washed in 5.0 ppm chlorinated water and again washed with sterile distilled water. The scores were made on the fish chunks by a sterile blade to 2.0 mm depth. Fourty grams chunks were transferred to the sterile petri plate asceptically and then 5.0 ml of various LAB cells of 10^6cfu/ ml were coated (12 ml/100 g) individually by pipette on the fish chunks (40 g). The petri plates were closed and sealed by parafilm and stored at 37°C (Kannappan and Manja, 2004). The changes of various spoilage indices were observed for the period of two days excluding the treatment day (the day 0). Lactic acid bacteria were estimated by using de Man, Rogosa and Sharpe (MRS) agar (Hi-media, Mumbai) medium (Deman et al., 1960). A control study was conducted without LAB treatment on the chunks for two days.

Estimation of quality indices

Changes of trimethylamine-nitrogen (TMA-N) and total volatile base Nitrogen (TVB-N) were carried out with Conway Micro diffusion Method (Beatty and Gibbons, 1937). The changes of free fatty acids and peroxide value were carried out using AOAC (1980).

pH measurement

After ascertaining different LAB and the various native spoilage bacteria, the pH was estimated thrice using a combination electrode attached to a pH meter.

Analysis of data

The significance of differences among treatments for each day of storage was determined by analysis of co-variance (Edwin, 1986) using the statistical Package for social sciences (SPSS) program for Windows, version 6.1.2.

RESULTS AND DISCUSSION

Table 1 show the growth values of seven LAB on the variation of trimethylamine and other spoilage indices on fish at 37°C. *S. thermophilus* grew to 7.87 log with pH 7.81 on the second day of storage. The TMA-N was reduced to 20 mg% by *S. thermophilus* cells coat on the treated fish (Figure 1) as compared to control, while TVB-N showed 120 mg% on the second day of storage with no difference in the control (Table 1). FFA was reduced to 174 mg% as compared to control. PV was 19.23 mg% in the treated fish with 79 mg% difference. *P. acidilactici* cell coat reduced to 40 mg% of TMA-N in the fish on the second day of storage as compared with control, and TVB-N was reduced to 20 mg% on the second day of storage. FFA reduced to 47.66 mg% by *P. acidilactici* in the treated fish with 100 mg% difference in the control. *P. acidilactici* cell coat reduced 97 mg% of PV in the treated fish as compared with control.

P. pentosaceous growth was observed in fluctuating trend with the change in pH. The TMA-N was reduced to 40 mg% in the fish during the second day of storage as

Table 1. Effect of coating various lactic acid bacterial cells in the variations of spoilage indices of fresh mackerel fish at 37°C.

Storage period (days)	LAB	LAB growth (log_{10} cfu/ g)	TMA-N (mg %)	TVB-N (mg %)	FFA (% of oleic acid)	PV (milli equivalent fat/kg of fat)	pH
1	*S. thermophilus* (St)	8.04	140 ± 1.0	280 ± 1.5	20 ± 1.0	19.23 ± 0.1	6.95
2		7.87	100 ± 0.8	120 ± 1.5	1.0 ± 1.6	2.72 ± 0.2	7.81
1	*Pediococcus acidilactici* (Pa)	8.07	60 ± 1.5	120 ± 2.0	50.76 ± 1.0	15.5 ± 1.0	6.68
2		7.27	60 ± 1.8	100 ± 2.0	47.66 ± 1.2	1.20 ± 1.0	7.40
1	*Pediococcus pentosaceous* (Pp)	8.20	120 ± 1.3	110 ± 2.0	46.66 ± 1.5	3.15 ± 1.0	7.23
2		8.00	140 ± 1.0	110 ± 1.9	10.38 ± 0.2	3.03 ± 1.0	7.52
1	*Lactobacillus acidophilus* (Lb a)	8.20	120 ± 1.0	140 ± 1.7	27 ± 0.1	1.30 ± 1.1	7.01
2		7.84	90 ± 1.6	100 ± 1.7	10 ± 0.1	2.89 ± 1.2	7.26
1	*Lactobacillus helveticus* (Lb. h)	7.65	20 ± 1.5	120 ± 1.3	48.37 ± 0.1	20 ± 1.3	6.99
2		7.77	10 ± 1.3	60 ± 2.0	56.40 ± 0.1	11.1 ± 1.0	7.75
1	*Lactobacillus plantarum* (Lb.p)	8.20	80 ± 1,5	220 ± 2.0	9.4 ± 0.1	40 ± 1.0	7.00
2		8.09	60 ± 1.6	160 ± 1.6	6.4 ± 0.1	6.25 ± 1.0	7.20
1	*Lactococcus lactis* (Lc l)	8.25	20 ± 1.2	60 ± 1.7	42.3 ± 0.1	13.33 ± 1.1	7.50
2		8.00	10 ± 1.9	40 ± 1.5	28.2 ± 0.1	1.90 ± 1.0	7.8
Variation of spoilage indices of mackerel chunks at 37ºC as control							
1	NE	NE	160 ± 1.0	120 ± 1.0	197.4 ± 1.1	98 ± 1.5	6.70
2	NE	NE	160 ± 1.1	20 ± 1.2	197.4 ± 1.1	99 ± 1.0	7.80

Values are mean ± SD of 3 replications; NE: not exercised.

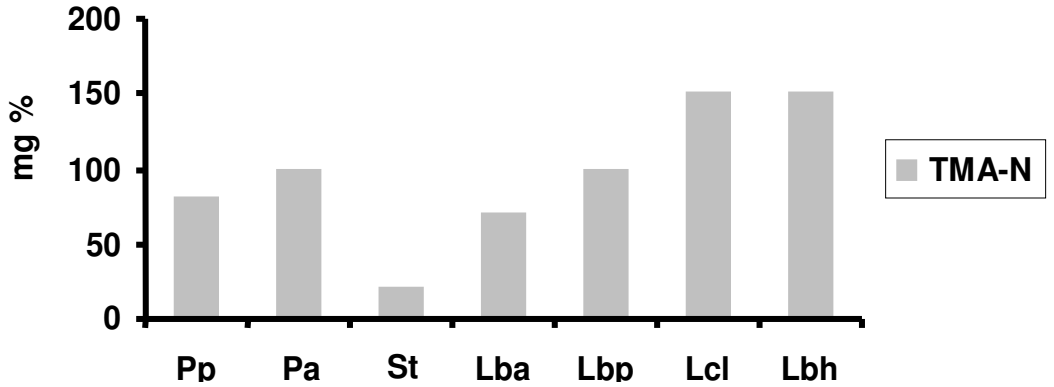

Figure 1. Variation of TMA-N in fresh mackerel chunks by coating with various LAB for 2 days at 37°C.

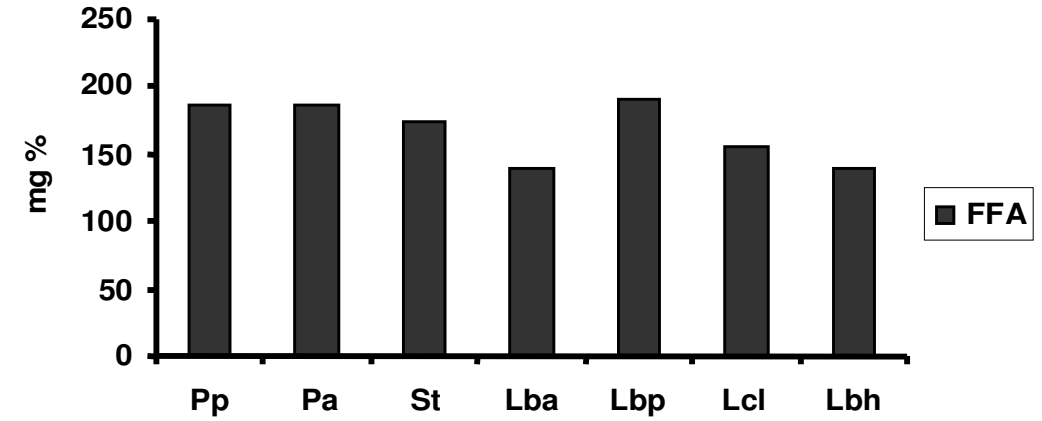

Figure 2. Variation of TVB-N in fresh mackerel chunks by coating with various LAB for 2 days at 37°C.

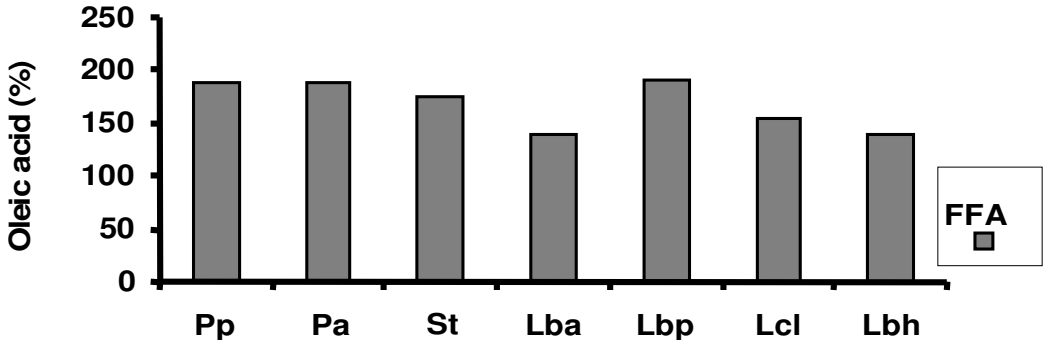

Figure 3. Variation of FFA in fresh mackerel chunks by coating with various LAB for 2 days at 37°C.

compared to control, while TVB-N was reduced to 10 mg% (Figure 2). FFA was reduced to 187 mg% on the treated fish by *P. pentasaceous* cells coat as compared to control, while the value of PV reduced to 96 mg%. TMA-N was reduced by *Lb. acidophilus* cell coat (about 70 mg %) as compared with control, while TVB-N was 20 mg% on the second day of storage. On the second day of storage, *Lb. helveticus* cell coat reduced 10 mg% of TMA-N on fish with the difference of 150 mg% as compared with control. At the same time, TVB-N was reduced to 60 mg% with 60 mg% difference. FFA was determined as 141.0 mg% on the fish with a difference of 56.4 mg% by *Lb. helveticus*, while PV was 88 mg% (Figure 3). *Lb. plantarum* cell coat reduced TMA-N 100 mg% on the second day of storage with the difference of 60 mg% of TMA-N in the control, while TVB-N reduced to 160 mg% on the second day of storage. On the second day of storage, FFA was reduced to 191 mg% by *Lb. plantarum* coat as compare with control, while PV showed 92 mg% reduction. *Lc. lactis* cell coat reduced the TMA-N content to 140 mg% in fish on the second day of storage. But TVB-N was reduced to 80 mg% on the second day of storage. FFA showed 42.30 mg% in the *Lc. lactis* treated fish with 155.0 mg% difference when compared with the control, while PV showed 13.3 mg% in the treated fish on the third day with 85 mg% difference (Figure 4). The pH values of fish did not change to alkaline condition during the test. Based on the analysis of co-variance, it has been found that the treatments of the entire seven LAB are not significantly different (F_{cal}: 0.516, F_{tabled}: 2, 17 at 5% level were equal to 2.70, H_o was rejected). However, the spoilage indices such as TMA-O, TVB-N, FFA and PV are significantly different (F_{cal}: 13.40, F_{tabled}: 3, 17 degrees of freedom was 3.20).

In this study, all LAB reduced the fish spoilage indices. Since LAB has been used to inhibit spoilage bacteria in meat (Ammor et al., 2006), the reduction of spoilage indices may be due to inhibition of fish spoilage bacteria such as species of *Shewanella* and *Pseudomonas,* which are responsible for the conversion of spoilage indices. Trimethylamine may also be produced by *Pseudomonas putrefaciens, Photobacterium* spp. and some *Moraxella* like bacteria present in the fish (Kritty et al., 1977). LAB also produces many extracellular compounds like lactic acid, hydrogen peroxide and diacetyl compounds which facilitate for the improved biochemical quality of fish, reduced microbiota and safety of frozen fish fillets (Ibrahim and Salha, 2009).

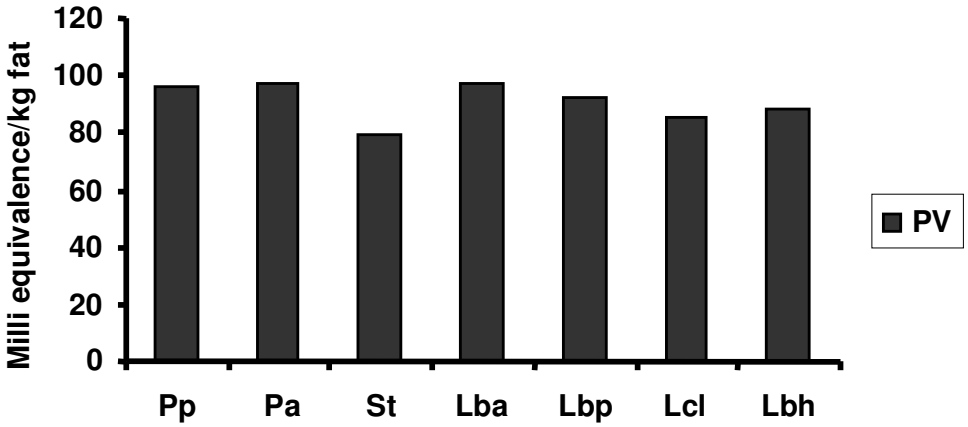

Figure 4. Variation of peroxide value in fresh mackerel chunks by coating with various LAB for 2 days at 37°C.

Lb. helveticus show maximum (3 times) reduction in TMA-N, followed by *Lc. lactis* and *Lb. plantarum* as compared to control. But *Pediococcus* species did not show reduction in TMA-N. The general limit of TMA-N in fish (10 to 15 mg%) which was usually regarded a limit (Saito et al., 1959) and 50 to 70 mg of TVB-N/100 g in fish can be considered inedible. The PV value should be much below 10 for fresh fish. Except for very fatty fish, for a good quality fish, TBA value of < 2 has been recommended (Burt, 1976). In Germany, maximum permissible limits of biogenic amines for fish and fish products is limited to 200 mg/kg, whereas it is only 100 mg/Kg in Canada, Finland and Switzerland (Lange and Wittmann, 2002). But coating LAB cultures like *Lactobacillus* and *Lactococcus* species had reduced maximum TMA-N formation in mackerel fish chunks. *S. thermophilus* showed the effective and highest reduction in TVB-N as compared to other LAB. Reduction of total volatile bases in fish may be due to the combined reduction of total ammonia, dimethylamine and trimethylamine. Reduction in both TMA-N and TVB-N in tilapia fish fillet was observed by treating with combined LAB cultures at 2% level by Ibrahim and Salha (2009). In this study, the control fish was recorded a high value of TVB-N (120 mg% sample); while its value was reduced to 160 mg% by treating with *S. thermophilus.* Besides, TVB-N content sharply decreased in all other LAB treatments, except by *P. acidilactici.* Uniform reduction of FFA was observed by *P. acidilactici*, *Lb. plantarum* and *Lc. lactis* as the case may be in other LAB treatment. It has been proved that mixed LAB cultures decreased the growth of spoilage bacteria in fish and suppressed the accumulation of biogenic amines such as histamine, cadaverine, putrescine, tryptamine and tyramine (Hu Yongjin et al., 2007).

Reduction of TMA-N by coating LAB is certainly equal to inhibition of spoilage bacteria. Reduction of PV in fish indicates less oxidation of fish fat in the meat. *P. acidilactici* and *S. thermophilus* showed max reduction in the PV values. LAB also produced hydrogen peroxide as extracellular product which usually inhibits many food borne bacteria such as *Enterococcus faecalis*, *Enterococcus faecium, Listeria ivanovii*, *Staphylococcus aureus*, *Yersinia enterocolitica* and *Aeromonas hydrophila* (Ayano et al., 2003). In the reduction of TVB-N, *Lc. lactis* was shown to be most effective (80 mg) followed by identical effect by *Lb. plantarum* and *Lb. helveticus* (60 mg), and the least effect was shown by *P. pentosaceous.*

Out of the seven LAB tested for FFA reduction, *Lb. plantarum* showed the highest. Debevere and Boskou (1996) observed inhibition of TMA-N and TVB-N in cod fish fillets kept in modified atmospheric package. Out of seven LAB tested for TMA-N reduction, on the first day, *Lb. helveticus* and *Lc. lactis* showed the highest reduction followed by *Lb. plantarum*. For TVB-N reduction on the first day, *P. acidilactici* showed the highest reduction as compared to *Lb. plantarum,* followed by *Lc. lactis.* For FFA reduction, on the first day, *P. acidilactici* showed the highest reduction followed by *Lb. acidophilus* and *Lc. lactis*. Out of the seven LAB tested for PV reduction, on the first day, *Lb. acidophilus* showed the highest reduction followed by *P. acidilactici* and *Lb. helveticus*. Therefore, it was concluded that, out of seven LAB tested for reduction of quality indices, *Lb. helveticus* and *Lc. lactis* were the best to control TMA-N, while *P. acidilactici* was the best to control TVB-N and the same was also the best LAB in controlling FFA. *Lb. acidophilus* was the best to control PV in fresh mackerel chunks. Thus, the present study has provided evidences that coating lactic acid bacteria could help in improving fish meat quality by inhibiting fish-borne spoilage bacteria and producing a wide range of inhibitory compounds (organic acids, hydrogen peroxide, diacetyl and bacteriocins). In addition, they often have the generally recognized as safe (GRAS) status granted by the USFDA. This study further could be extended to elasmobranches and shell fishes, provided LAB do not change the organoleptic and

nutritional qualities of fish.

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