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Full Length Research Paper

Repressive efficacy of lactic acid bacteria against the human pathogenic and fish-borne spoilage microbiota of fresh Indian mackerel fish chunks

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Different strains of lactic acid bacteria (LAB) namely *Lactobacillus acidophilus* NCIM 2287, *Lactobacillus plantarum* NCIM 2085, *Lactobacillus helveticus* NCIM 2126 and *Lactococcus lactis* NCIM 2114 were procured from the National Chemical Laboratory (NCL) Pune, India. These LAB cells were individually (10^7 cfu/ml) sprayed using a sterile syringe on the dressed fresh mackerel fish chunks and incubated at 37°C for two days. The growth pattern of each LAB and their antagonism against fish-borne spoilage bacteria namely, specific spoilage bacteria, halophilic bacteria, coliforms, lipolytic, proteolytic bacteria and total plate count were estimated for three days. Pathogenic *Staphylococcus aureus* was inhibited by *Lb. acidophilus* on the second day with 4.30 log difference as compared to control. The growth of specific spoilage bacteria was decreased by *Lb. plantarum* spray on the first day by 1.0 log difference. *Lb. helveticus* inhibited *S. aureus* on the third day by 3.5 log difference. Out of the four LABs tried, *Lb. helveticus* showed the best inhibitory effect against the fish-borne bacteria. All three LABs exhibited inhibition against the fish-borne spoilage bacteria, they may thus be potentially used as bio-preservative bacteria to preserve the whole fish meat or minced meat products etc. for a shorter duration.

Key words: *Lactobacillus helveticus*, *Lactobacillus plantarum*, *Lactococcus lactis, Lactobacillus acidophilus*, antagonism, spoilage bacteria, mackerel fish.

INTRODUCTION

Lactic acid bacteria (LAB) have long been recognized as safe bio-preservative bacteria due to their inhibitory effect on food spoilage bacteria by producing lactic acid (Matamoros et al., 2009) and bacteriocin like inhibitory substances (Zaheer et al., 2010). Bacteriocin from the LAB such as *Enterococcus faecium* showed broad inhibitory effect against a wide range of bacteria including, *Salmonella paratyphoid* (Annamalai et al., 2009). LAB was used in the reduction of trimethylamine-nitrogen and related spoilage derivatives of fresh Indian mackerel fish chunks (Kannappan and Manja, 2011). Supplementation of LAB as probiotic bacteria in broiler improves the quality of meat (Kabir, 2009).

Various LABs have been tested for antagonism against the food-borne pathogens of mackerel fish (Kannappan and Gopikrishna, 2008). LAB, when coated on meat, would increase its shelf life by lowering the pH (James

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and Samuel, 1981). *Lactobacillus* bacteria supplemented milk is used to control the normal intestinal microbiota in individuals suffering from digestive ailments or enteric diseases. Tome et al. (2008) succeeded in controlling growth of pathogenic *Listeria monocytogenes,* using LAB in cold-smoked salmon. Fish spoilage bacteria deteriorate the fish quality and render it inedible (Abbas et al., 2009). The use of beneficial bacteria like LAB for biopreservation therefore is important. Moreover, the consumption of fish treated with chemical preservatives to enhance its keeping quality may have undesirable effects to consumers. Antibacterial activity of the LAB metabolites and LAB in single and combination treatment proved inhibitory against total viable bacterial count and *Staphylococcus aureus, Escherichia coli* and coliform bacteria in frozen Tilapia fish fillets (Ibrahim and Salha, 2009).

Pediococcus pentasaceous and *Pediococcus acidilactici* were used to prevent the growth of fish-borne bacteria on mackerel fish chunks. Various strains of LAB also showed antimicrobial activity against *Enterococcus faecalis, S. aureus* and *E. coli* (Christine et al., 2009). *Lactobacillus rhamnosus GG* and its spent culture inhibited food-borne bacteria such as, *Vibrio parahaemolyticus*, *E. coli, Bacillus cereus* and *S. aureus* isolated from Indian snack foods (Leela et al., 2005). While studies on the application of LAB to control food-borne bacteria are abundantly available in foods such as milk and milk based products, they are scanty in fish and fishery products in India. Therefore, this work was undertaken to study the effect of *Lactobacillus* and *Lactococcus* cultures in reducing or inhibiting the native spoilage bacteria of fresh mackerel fish chunks.

MATERIALS AND METHODS

Mackerel fish and lactic acid bacteria type strains

Rastrelliger kanagurta (Cuvier) of the family Scombridae was used in the study. The fish chosen for the study was very fresh, firm, chilled, and weighed 90 to 125 g. Mackerel fish was dressed and washed in sterile water and later made into chunks. Scores of 0.5 cm depth were made on the chunks using a sterile blade (Kannappan and Manja, 2011). LAB type strains namely *Lactobacillus acidophilus* NCIM 2287 (Lba) *Lactobacillus plantarum* NCIM 2085 (Lbp) *Lactobacillus helveticus* NCIM 2126 (Lbh) and *Lactococcus lactis* NCIM 2114 (Lcl) were procured form National Chemical Laboratory, (NCL-National Collection of Industrial Microorganisms-CSIR) Pune-India.

Estimation of fish-borne spoilage and pathogenic bacteria using specific media

Fish-borne spoilage group bacteria namely proteolytic bacteria (PLB), lipolytic bacteria (LLB), specific fish spoilage bacteria (SSB), halophilic bacteria (HPB), total plate count (TPC), coliforms (Coli), *Staphylococcus aureus* (Staph), mesophilic (MSF) and thermophilic spores (TSF) were estimated. De Man Rogosa and Sharpe agar (MRS) was used for lactic acid bacteria (Deman et al., 1960), milk agar (nutrient agar with 1% milk solids) for proteolytic bacteria, tributyrin agar used for lipolytic bacteria (nutrient agar with 1% tributyrin). Iron agar was used for specific fish spoilage bacteria, halophilic agar for halophilic bacteria, zobel marine agar for total heterotrophic bacteria, violet red bile agar for coliforms, Baird Parker agar for *S. aureus* and dextrose tryptone agar for mesophilic and thermophilic spores were used to determine the total viable bacteria for 3 days. All the media used were obtained from Himedia, Mumbai, India. For thermophilic spores, the plates were incubated at 55°C. All these groups of bacteria were reconfirmed using various bio-chemical tests (Swanson et al., 1992).

Confirmation of coagulase positive *Staphylococcus aureus*

Spread plate method was performed to plate, from pre-determined dilutions, onto Baird parker agar (BPA, Hi- media, Mumbai) supplemented with sterile potassium tellurite egg yolk emulsion. After incubation at 37°C for 48 h, coagulase test was done to typical black grey, bright, smooth colonies with clear zones, and counts were determined accordingly (The oxoid manual, 1998)

Confirmation of coliforms

Total coliforms were determined by the three tube most probable (MPN) method. Lauryl sulphate tryptose broth (Hi-media, India) and brilliant green lactose bile (2%) broth were used for presumptive and confirmation tests for coliforms, respectively. Results were evaluated according to the MPN tables (Harrigan and McCance, 1976).

Preparation and inoculation of LAB cells on mackerel fish

All the LAB strains (1 ml) of 24 h culture were separately inoculated into 25 ml tryptic glucose yeast extract buffer broth (TGE) supplemented with sodium acetate, sodium citrate and potassium hydrogen phosphate, and incubated at 37°C for 24 h. Cells were harvested by centrifugation (10 min with 47378 g) in RC5B super speed refrigerated centrifuge (Du-pont Instruments), and suspended in sterile saline solution (10^7 cfu/ml). Then, 40 g chunks were placed on a Petri plates. Various suspended LABs (5.0 ml each) were sprayed over 40 g mackerel chunks using sterile syringe (Kannappan and Manja, 2011). The LAB inoculums remained as suspension with chunks during the storage. The Petri plates were wrapped with parafilm and kept at 37°C/3 days for incubation (Kannappan et al., 2004)

pH measurement

The pH was measured at different times using pH meter with an electrode (Electronic Corporation of India) in 10 ml aliquots taken from each analysis of various spoilage bacteria and LAB. The pH electrode was calibrated with buffers (Merck) at pH 4.0 and 7.0.

Calculation of percentage inhibition on fish-borne spoilage and pathogenic bacteria

The percentage inhibition was calculated from the values obtained using the following characters. Percentage inhibition = Bacterial

Table 1. Reduction of fish-borne spoilage and pathogenic bacteria in mackerel fish chunks against spraying various lactic acid bacteria.

Period (days)	*Lb. plant*	PLB	LLB	SSB	TPC	HPB	*Coli*	*Stap*	pH	TSF	MSF
Initial	7.39±0.1	5.30±0.2	7.30±1.0	7.3±0.2	5.39± 0.2	6.0±0.2	5.69±1.0	4.84±1.0	5.93±0.1	1.0±0.03	1.0±0.01
I	8.20±0.2	6.0±0.1	6.77±0.2	6.77±0.2	6.35±0.1	7.0±0.2	6.3±0.2	2.0±0.1	7.0±0.2	2.0±0.05	1.0±0.02
II	8.69±0.1	5.0±0.2	7.11±0.1	8.11±0.1	6.0±0.2	5.0±0.2	6.47±0.2	ND	7.2±0.1	ND	ND
III	8.0±0.3	5.0±0.2	6.0±0.2	8.0±0.2	5.0±0.2	4.0±0.2	6.0±0.2	ND	7.5±0.2	ND	ND
Lc. Lactis											
Initial	7.38±0.2	6.0±0.1	5.30±0.2	5.32±0.1	5.69±0.2	6.0±0.1	6.81±0.2	5.3±0.2	6.43±0.1	1.0±0.01	2.0±0.02
I	7.25±0.2	7.0±0.3	5.0±0.1	6.3±0.2	6.0±0.1	7.0±0.1	6.17±0.2	5.77±0.1	7.5±0.2	ND	ND
II	7.07±0.1	6.0±0.2	7.0±0.2	8.04±0.2	5.1±0.2	6.0±0.2	7.0±0.1	2.0±0.1	7.8±0.2	ND	ND
III	7.0±0.2	5.0±0.1	4.0±0.1	7.0±0.1	4.0±0.2	5.0±0.1	6.0±0.2	ND	8.05±0.2	ND	ND
Lb. acidophilus											
Initial	8.3±0.2	6.0±0.1	5.3±0.3	5.2±0.1	5.69±0.1	6.0±0.3	5.0±0.2	5.0±0.2	6.7±0.2	1.0±0.01	1.0±0.01
I	8.2±0.2	6.0±0.2	5.0±0.1	8.38±0.2	6.25±0.3	7.0±0.2	7.0±0.1	6.2±0.2	7.01±0.2	1.0±0.03	1.3±0.02
II	7.84±0.2	6.5±0.2	6.0±0.1	7.69±0.2	6.0±0.3	6.0±0.2	6.69±0.2	3.0±0.1	7.26±0.2	ND	ND
III	7.0±0.2	6.0±0.2	6.0±0.2	7.5±0.2	6.0±0.2	5.0±0.2	6.0±0.2	ND	8.1±0.2	ND	ND
Fish-borne spoilage and pathogenic bacteria in fresh Indian mackerel chunks as control											
Initial		6.0±0.2	7.47±0.3	8.39±0.3	6.6±0.1	7.0±0.2	7.6±0.2	5.6±0.3	6.0±0.2	1.2±0.1	1.3±0.1
I		7.17±0.1	7.25±0.2	8.77±0.3	7.2±0.3	7.89±0.1	7.77±0.3	7.17±0.3	6.71±0.2	1.6±0.2	1.47±0.2
II		7.0±0.2	7.23±0.2	8.17±0.2	7.77±0.2	7.0±0.1	7.39±0.2	6.3±0.2	8.1±0.2	1.0±0.1	1.0±0.1
III		6.0±0.2	7.0±0.2	8.0±0.2	7.0±0.2	6.0±0.2	7.0±0.2	6.0±0.2	8.79±0.2	1.0±0.1	1.0±0.1

Values are $\log_{10}$ cfu/g and Mean ± SD (n = 03), Lbp: *Lb. plantarum*, PLB: proteolytic bacteria, LLB: lipolytic bacteria, SSB: specific spoilage bacteria, HLB: halophilic bacteria, Stap: *S. aureus*, TSF: thermophilic spore formers MSF: mesophilic spore formers. PLB, Proteolytic bacteria, LLB, lipolytic bacteria, HPB, halophilic bacteria, Staph, *Staphylococcus aureus,* TSF, thermophilic spores, MSF, mesophilic, SSB, specific fish spoilage bacteria, TPC, total plate count.

load in the control - Bacterial load in association with LAB divided by bacterial load in the control × 100

Statistical analysis

Student 'T' test was conducted between the pairs of bacteria which were not inhibited by LAB, and the significant growth difference was reported.

RESULTS AND DISCUSSION

Antagonism of *Lb. acidophilus* cells sprayed against fish-borne spoilage and pathogenic bacteria

Pathogenic *S. aureus* was inhibited by *Lb. acidophilus* on the second day by 3.30 log difference as compared to control (Table 1). Immediately after spraying with *Lb. acidophilus*, 17.8% of *S. aureus* load was decreased on the first day and 72.21% on the second day. On the third day, a complete inhibition on *S. aureus* was observed. Mesophilic and thermophilic spore formers were inhibited by *Lb. acidophilus* on the second day of storage by 1.0 log difference as compared to control. Other

Table 2. Inhibitory growth values of various fish-borne spoilage and pathogenic bacteria of fresh mackerel fish chunks (in %) by spraying with different LAB (10^7 cfu/ml).

Storage period in days and LAB	PLB (%)	LLB (%)	HPB (%)	*Coliforms* (%)	*Staph* (%)	TSF (%)	MSF (%)	SSB (%)	TPC (%)
Lc. Lactis									
Initial day	0	29.04	14.28	10.39	4.46	16.66	23.07	36.59	13.78
I	2.37	31.03	11.28	20.59	19.52	100	100	28.16	16.66
II	14.25	3.18	14.28	5.27	68.25	- do -	- do -	1.59	34.36
III	16.66	42.85	16.66	14.28	100	- do -	- do -	12.5	42.85
Lb. plantarum									
Initial day	11.66	2.27	14.28	25.13	13.58	16.66	30.0	12.99	18.33
I	16.31	6.62	11.28	18.91	42.23	25.00	47.0	22.80	11.80
II	28.57	1.65	28.57	12.44	100	100	100	0.73	22.77
III	16.66	14.28	33.34	14.28	100	100	100	0.73	28.57
Lb. helveticus									
Initial day	16.66	33.06	14.28	3.94	7.50	16.66	23.07	33.06	6.51
I	59.00	54.48	11.28	6.04	5.43	37.50	31.98	11.40	2.77
II	100	100	14.28	5.27	20.63	100	100	10.00	6.04
III	100	100	16.66	14.28	100	100	100	11.62	14.28
Lb. acidophilus									
Initial day	3.34	29.00	14.28	34.21	17.80	16.66	23.07	38.02	24.24
I	16.32	31.03	11.28	9.90	72.21	37.50	31.97	4.64	7.08
II	28.57	17.01	14.28	9.47	100	100	100	5.87	22.77
III	16.66	14.28	16.66	6.69	100	-do-	-do-	6.25	28.57

PLB, Proteolytic bacteria, LLB, lipolytic bacteria, HPB, halophilic bacteria, Staph, *Staphylococcus aureus,* TSF, thermophilic spores, MSF, mesophilic, SSB, specific fish spoilage bacteria, TPC, total plate count.

spoilage bacteria were not inhibited by *Lb. acidophilus.* Halophilic bacteria got reduced by 1.0 log from the initial day to third day. *Lb. acidophilus* cells inhibited spore formers on the second day of storage. *Lb. acidophilus* cell count was increased on the first day then decreased on the second and third days.

Gupta et al. (1996) investigated the inhibitory activity of *Lb. acidophilus*-301 against *Salmonella typhi*, *E. coli*, *Proteus vulgaris*, *Yersinia enterocolitica* and *S. aureus* in fermented milk. *Lb. acidophilus* showed wide variations in their activity against these pathogens. Moreover, the metabolites produced by *Lb. acidophilus* were inhibitory to molds (Ibrahim and Salha, 2009). Comparing with the control, on the initial day, reductions of 2.10 log loads among LLB, 3.19 logs among SSB and 2.6 logs among coliforms were achieved by *Lb. acidophilus* cells sprayed but other bacterial reductions were not too high to explain. Specific spoilage bacteria were Gram-negative anaerobes, which are not completely inhibited by *Lb. plantarum*; however, 3.0 log (32.02%) reductions were observed. Nevertheless, there was no significant reductions further (Table 2).

Gilland and Speck (1977) observed 61.20% inhibition on enteropathogenic *E. coli* 022:B4 through co-culturing with *Lb. acidophilus* 4962. The pH of the chunks changed to neutral from alkaline condition. All these reductions among the native spoilage bacteria may be due to LAB cells or their extra cellular bacteriocin like inhibitory substances (BLIS). Table 1 shows the initial values of native spoilage bacteria of fresh mackerel chunks as control. Specific spoilage bacteria were observed to be the highest (8.39 log), followed by coliforms (7.60 log) and lipolytic bacteria (7.47 log). Table 2 shows the growth values of various fish-borne spoilage and pathogenic bacteria of fresh mackerel chunks (in %) against spraying various LABs.

Antagonism of *Lb. plantarum* cells sprayed against the fish-borne spoilage and pathogenic bacteria

Lb. plantarum grew in increasing and decreasing trends, but it did not inhibit PLB and LLB. However, their load got reduced sufficiently on the third day of storage. Growth of specific spoilage bacteria were decreased by *Lb. plantarum* on the first day by 1.0 log difference as compared to

control. As cited earlier, 1.0 log reduction among SSB may assumed to be *Pseudomonas* spp and later *Shewanella* species might have dominated in the fish, due to which the load remained the same. Lipolytic group of bacteria belongs to various species of *Staphylococcus, Clostridia, Flavobacterium*, *Pseudomonas*, *Micrococcus* and *Bacillus*, of which *Staphylococcus*, *Clostridia* and *Micrococcus* were inhibited by *Lb. plantarum*.

Todorov and Dicks (2005) reported about inhibition of Gram-negative bacteria coating with the BLIS produced by *Lb. plantarum*. Here, regarding TPC, 18.33% of reduction was noticed on the initial day, followed by 28.57% on the third day (Table 2). The pH in control samples increased to 7.54 on the third day due to the growth of fish-borne spoilage and pathogenic bacteria. Yusuf and Varadaraj (1999) reported the antibacterial effect of plantaricin produced by *Lb. plantarum* against *B. cereus*, *E. coli* and *S. aureus.* Mami et al. (2008) reported that various LABS including *Lb. plantarum*, inhibited *S. aureus.* In this study, although *Lb. plantarum* reached 8.0 log/g, complete inhibition was not achieved. This might be due to the presence of dominant Gram-negative LLB group of bacteria such as *Flavobacterium* and *Pseudomonas* species. This finding was also evident with the inhibitory potential of *Lactobacillus* and *Streptococcus* cultures against the gram-negative and -positive spoilage microbiota on mutton (Murali et al., 1985). *Lb. plantarum* cells were also inhibited Gram-negative marine *Vibrios* (Chae et al., 2009).

Two and 1.0 logs reduction was observed on TSF and MSF (47.0%) in the initial and on the first day that were completely inhibited thereafter (Tables 1 and 2). Murali et al. (1985) reported complete inhibition of coliforms on mutton sprayed with *Lb. plantarum* alone, as well as in combination with *Streptococcus lactis*. During this study, *S. aureus* was inhibited on the second day with 2.30 log difference from 4.0 logs. Significant growth difference was observed among PLB and LLB (P: 0.0075, T: -3.96), PLB and SPB (P: 0.0013, T: -5.65), PLB and Coli (P: 0.035, T: -2.71), LLB and TPC (P: 0.037, T: 2.66) and SPB and TPC (P: 0.053), SPB and HPB (P: 0.029, T: 2.85) but not among the other pairs.

Antagonism of *Lc. lactis* cells sprayed against fish-borne spoilage and pathogenic bacteria

The growth of *Lc. lactis* was found to show increasing and decreasing trend. Among the spoilage group of bacteria, specific spoilage bacteria were reported (8.17 log) in higher level which included *Shewanella putrefaciens* and *Pseudomonas* group of bacteria. In fresh mackerel, 6.0 log proteolytic bacteria (PLB) and 7.47 log lipolytic bacteria (LLB) were found but spraying with *Lc. lactics* cells, LLB was decreased to 7.0 logs after three days, whereas PLB load remained constant. Total plate count decreased to 1.69 logs from 5.69 log, and coliforms decreased to 1.60 logs on the third day of storage (Table 1) but complete inhibition was not observed.

There was no significant inhibition on the sulphite reducing bacteria since only 1.39 log reductions was observed from their initial 8.39 log. Here, *Lc. lactis* had grown (7.0 log) adequately on the fish chunks utilizing the available carbohydrate. Even then, no further reduction was observed. Halophile count of 7.0 logs was observed on the initial day and this decreased to 6.0 logs on the third day. *S. aureus* was inhibited by *Lc. lactis* on the third day of storage by 4.0 log difference as compared to control but spore formers were inhibited on the first day by 1.4 to 1.6 log difference. Both *Lc. lactis* and *Lb. plantarum* inhibited *S. aureus* and spores in identical fashion (Table 1). *Lc. lactis* did not inhibit coliforms but Murali et al. (1985) observed inhibition of 1.30 log coliforms which was very high as compared to fresh mutton. Coliforms could have been inhibited by spraying with multiple LAB strains in the presence of selected carbohydrates.

Frank and Marth (1977) observed inhibition of *E. coli* by spraying mixed LAB cultures namely, *Streptococcus lactis* and *Streptococcus cremoris*. Balcazar et al. (2009) observed even inhibition on Gram-negative *Aeromonas* by *Lc. lactis.* Similarly, Murali et al. (1985) observed inhibition of 1.0 log load coliforms by spraying with 1:1 ratio of *Lb. plantarum* and *Str. lactis*. It may therefore be possible to inhibit coliforms by multiple strains of LAB. The inhibition of bacteria may be due to LAB or their bacteriocin like substances. Almudena et al. (2004) reported inhibition of *S. aureus* and *Listeria innocua* by the nisin produced by *Lc. lactis* in minced pork meat. Significant growth difference was observed between TPC and *Coli* (P: 0.042, T: -2.58) but not between the other pairs of bacteria.

Antagonism of *Lb. helveticus* cell sprayed against fish-borne spoilage and pathogenic bacteria

Lb. helveticus inhibited mesophilic and thermophlic spore formers on the second day of storage by 1.0 log difference as compared to control (Figure 1). Proteolytic bacteria (Figure 2) and LLB were completely inhibited by *Lb. helveticus* cells on the second day of storage by 1.0 log difference (Figure 3). Of the four LABs tried for inhibition, *Lb. helveticus* was the only bacteria which inhibited PLB and LLB (Table 2). *Lb. helveticus* caused 33.06 and 16.66% inhibition on LLB and PLB, respectively on the first day. On the second day, 54.59% of inhibition of both the groups of bacteria was observed (Table 2). *S. aureus* was inhibited by *Lb. helveticus* on the third day of storage by 3.5 log differences as compared to control (Table 1). Nielsen and Zeuthen (1985) reported inhibition of *S. aureus* by Lactobacillus species. Among sulphite reducing bacteria, 1.30 log reductions

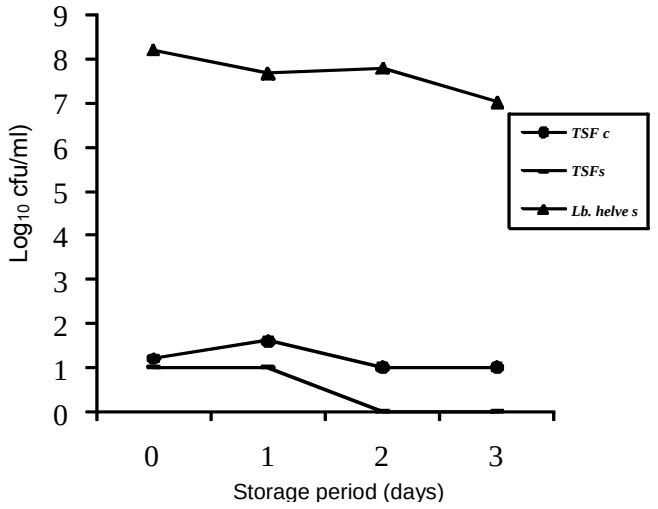

Figure 1. Inhibition of thermophilic spore formers by *Lb. helveticus* in fresh mackerel fish chunks at 37°C (TSFc: thermophilic spore formers control, TSFs: thermophilic spore formers sample, *Lb. helves*: *Lb. helveticus* sample).

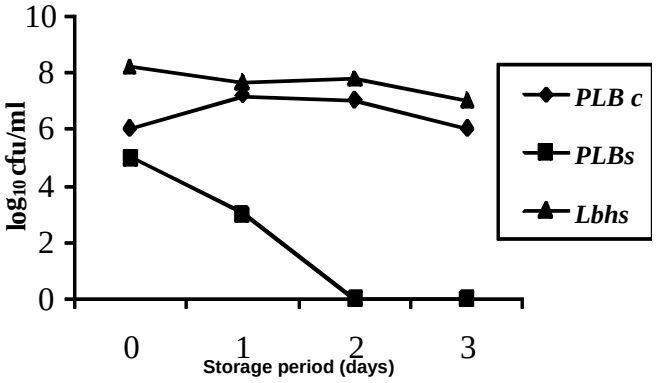

Figure 2. Inhibition of proteolytic bacteria by *Lb. helveticus* in fresh mackerel fish chunks at 37°C. (PLBc: proteolytic bacteria control, PLBs: proteolytic bacteria sample, Lbh s: *Lb. helveticus* sample).

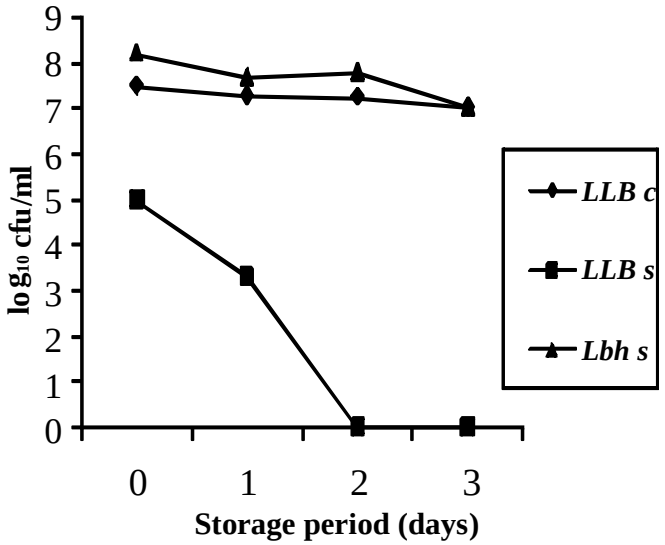

Figure 3. Inhibition of lipolytic bacteria by *Lb. helveticus* in fresh mackerel fish chunks at 37°C. (LLBc: lipolytic bacteria control, LLBs: lipolytic bacteria sample, Lbh s: *Lb. helveticus* sample).

was observed in the *Lb. helveticus* sprayed mackerel as compared to control on the initial day. Among halophilic group, 1.0 log load reduction was observed on the third day by *Lb. helveticus*.

Proteolytic group bacteria consist of various species of Bacillus, Pseudomonas, Staphylococcus and Serratia. Among them, Pseudomonas is a Gram-negative bacteria and a typical fish spoiler which grows in any environment. Pseudomonas might have grown faster than Bacillus, Staphylococcus and Serratia. In this study, *Lb. helveticus* did not out-compete PLB but Jadranka et al. (2009) had reported that feeding *Lb. helveticus* in combination with prebiotics has reduced the enterobacteria and sulphite reducing Clostridia in other animals. This combination also showed enhanced effect in the immune system in other animals. Among halophiles, 1.0 log load growth difference was observed in the chunks as compared to control (Table 1).

Since Halobacterium group bacteria (consisting of Halococcus, Halobacterium and Sarcina) are salt tolerant in nature, normal LAB such as Streptococcus and Pediococcus species might not interact with them but salt tolerant LAB namely Pediococcus halophilus (Tetragenococcus halophilus) would be suitable to out-compete halophiles. Coliforms were also reduced in their load but not inhibited. Significant growth difference was observed between SSB and TPC (P: 0.037, T: 2.67) and SPB and HPB (P: 0.012, T: 3.55) but not among other pairs.

Conclusion

LAB are a very effective biopreservative agent against pathogenic and spoilage bacteria like *S. aureus* and mesophilic spore formers. *Lb. helveticus* was highly effective in controlling mesophilic and thermophilic spore forming bacteria in addition to PLB, LLB and *S. aureus*. After spraying with various LABs on an average, 7.3 to 8.18 log of LAB growth was noticed from the mackerel chunks. This high growth was sufficient enough to out-compete spoilage bacteria. *Lc. lactis* may be the best bacterium to out compete LLB and TPC (42.85%), followed by *Lb. plantarum*. Of all the three LAB bacteria tested against inhibition on spoilage bacteria, only the growth of SSB was reduced by *Lb. plantarum* but the individual group among SSB needs to be probed for LAB antagonism. Since all the three LABs inhibited the fish-

borne spoilage and pathogenic bacteria, these may be effectively used as bio-preservative agent on whole fish, meat and minced meat products, thereby enhancing the safety and quality of fish meat. However, more efforts are required to understand the role of major and minor constituents of fish on post-harvest loses and how it influences the activity of LAB, in order to optimize the shelf-life of fish meat.

Abbreviations

PLB, Proteolytic bacteria; LLB, lipolytic bacteria; SSB, specific fish spoilage bacteria; HPB, halophilic bacteria; TPC, total plate count; coliforms (Coli), Staph, *Staphylococcus aureus*; MSF, mesophilic; TSF, thermophilic spores.

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