

Occurrence and Distribution of Lactic Acid Bacteria in Fish and Fishery Environments

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Occurrence and distribution of Lactic acid bacteria (LAB) in fresh and frozen fishes of internal trade, fresh marine fishes as well as brackish and aquaculture farm fishes were studied vis-a-vis total bacterial counts and total coliforms. Selected LAB strains were studied for assessing biochemical and growth characteristics. It was found that the LAB counts were always 1-2 log cycles less than the total plate counts in the case of fishes from internal trade. While the LAB were present in fresh and brackish water fishes, these were not detected in Ocean fresh fish. About 90% of the LAB strains isolated were characterised as *Lactobacillus* of which 41% were streptobacteria and 14% thermobacteria. Nearly 80% of the LAB cultures tested for antibacterial properties against *E. coli*, *Staphylococcus aureus* and *Bacillus cereus*, none could inhibit the growth of *E. coli* and *S. aureus*. But 13 out of 15 cultures inhibited the growth of *Bacillus cereus*.

Key words: Lactic acid bacteria, Antibacterial property, Fresh and Frozen fish, TPC, coliforms

Lactic Acid Bacteria (LAB) are widely distributed in nature. These play a very important role in fermentations of vegetable, fish, meat and dairy products. Production of organic acids by these bacteria contributes to the desired taste and flavour of fermented products and also makes the substrate unfavourable for the proliferation of spoilage and other undesirable microorganisms. The LAB have been isolated from fresh, smoked and marinated fish as well as the intestinal contents of fish (Blood, 1975; Molin *et al.* 1983; Pilet *et al.* 1995). Lalitha *et al.* (1994) reported on the use of LAB for fish sauce fermentation. Abraham *et al.* (1994) studied the influence of certain parameters like salt and pH on lactic fermentation of under utilized fish.

As part of the CIFT, FAO network project study on the improved utilization

of low value fish detailed investigations on the occurrence and distribution of LAB in fish, shellfish and fishery environments were undertaken. Some of the salient findings are presented in this paper.

Materials and Methods

Fish, prawn and frozen fish from local markets of Cochin and Ocean fresh/farm fresh fish and prawn from fishing vessels operating off Cochin/aquaculture farms at Chellanam and Thrissur were the material used in the study. The fishes from local markets included 6 samples of *Nemipterus* sp., 4 samples of Tuna (*Thunnus obesus*), 8 samples of *Otolithus* spp., 6 samples of Indian mackerel (*Rastrelliger kanagurta*), 3 samples of Baracuda (*Sphyrna jello*) and 8 samples of pomfret (*Parastromateus*

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niger). Ocean fresh Indian mackerel and oil sardine (*Sardinella longiceps*) were obtained from country crafts operating off Cochin. From aquaculture farms at Chellanam, pearlspot (*Etroplus suratensis*) and tiger prawn (*Penaeus monodon*) were collected. Rohu (*Labeo rohita*) and catla (*Catla catla*) were obtained from fresh water aquaculture farms at Thrissur. Both seawater and freshwater from the farm were sampled for the LAB.

Frozen fish samples tested included 1 sample each of oil sardine, spotted flat fish *Otolithus* spp., tilapia and baracuda.

Total bacterial count (TPC), total coliforms and *Escherichia coli* were determined by standard methods (USFDA 1978). De Man Rogosa and Sharpe (MRS) agar (De Man *et al.*, 1960) was used for enumeration and isolation of LAB. TPC was determined using Tryptone Glucose Agar (TGA). The plates were incubated at room temperature ($28 \pm 2^\circ\text{C}$) for 48 h and counts taken. Total coliforms and *E. coli* were determined by standard 3 tube, most probable number (MPN) method. In the case of LAB the samples were pour plated with MRS agar. An overlay with the same media was made once the plates were set. The plates were incubated at 37°C in a CO_2 incubator set at 5% CO_2 for 3 days (NAPO automatic CO_2 incubator, precision scientific, USA). Pure white and small (2-3 mm dia) colonies were counted as LAB.

Results and Discussion

Fig. 1 shows the distribution of LAB vis-a-vis total plate count, and coliforms in different fishes from the local markets in Cochin. LAB was present in all the samples of fish tested, so also coliforms.

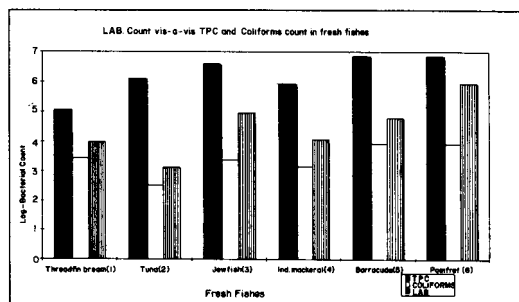


Fig. 1. LAB count vis-a-vis TPC and Coliforms count in fresh fishes.

- (1) *Nemipterus* spp. (2) *Thunnus obseus*
- (3) *Otolithus* spp. (4) *Rastrelliger kanagurta*
- (5) *Sphyraena jello* (6) *Parastromateus niger*

It was noted that the LAB counts always were about 1-2 log cycles less than the TPC. At the same time the LAB counts were always higher by 0.5-1.5 log cycles with respect to total coliforms. These results indicated that the LAB formed a significant part of the total bacterial population of the fishes available in local markets.

Table 1 gives the details of the LAB counts and total plate count in 5 samples of frozen fish from local retail outlets of Cochin. The total LAB counts were

Table 1. Total plate counts and LAB counts of frozen fishes from local trade at Cochin

Sl. No.	Fish	TPC/g	LAB/g
1	Oil sardine (<i>Sardinella longiceps</i>)	2.70×10^3	1.30×10^4
2	Nachaka (<i>Scatophagus argus</i>)	1.59×10^5	1.39×10^5
3	Pallikora (<i>Otolithus</i> spp.)	1.75×10^7	1.60×10^5
4	Tilapia (<i>Oreochromis mossambicus</i>)	6.40×10^5	2.50×10^5
5	Baracuda (<i>Sphyraena jello</i>)	4.70×10^5	2.10×10^4

Table 2. TPC and LAB in Ocean fresh fishes cultured fishes and prawn

Sl. No.	Fish	TPC/g	LAB/g
Marine fishes			
1	Indian mackerel (<i>Restrelliger kanagurta</i>)	2.50x10 ³	Nil
2	Oil sardine (<i>Sardinella longiceps</i>)	4.63x10 ⁴	Nil
Cultured fishes			
1	Rohu (<i>Labeo rohita</i>)	2.08x10 ⁴	1.32x10 ²
2	Catla (<i>Catla catla</i>)	7.24x10 ³	2.71x10 ²
3	Pear spot (<i>Etroplus suratensis</i>)	4.30x10 ⁴	6.63x10 ³
4	Tiger prawn (<i>Penaeus monodon</i>)	1.37x10 ⁵	4.14x10 ³

almost in the same range as the total aerobic plate counts except for frozen *Otolithus* spp., where the LAB counts were comparatively very less and for sardines it was one log cycle higher than TPC. This is in contrast to the observations made in the case of fresh fish from local markets where a greater population of total aerobic bacteria was found than the LAB counts. But generally the bacteria found in association with fish except Gram positives were susceptible to freezing (Thampuran & Gopakumar, 1993). The LAB being Gram positive more or less survived freezing. That may be the reason for higher population of LAB in frozen fish compared with fresh fish. The TPC and LAB counts in Ocean fresh fish, cultured fish and prawn are presented in Table 2. While the LAB were totally absent in Ocean fresh fishes, they were present in appreciable numbers in cultured fishes and prawn indicating that the LAB are seldom present in marine ecosystem. A total of 234 pure LAB cultures were isolated and studied. Based on their

fermentative properties they were classified into hetero-fermentative and homofermentative. Heterofermentative cultures were always less compared with homofermenters. Out of the 234 samples only 62 were heterofermentative and the rest were homofermentative.

Nearly 90% of the LAB cultures isolated were characterised as *Lactobacillus* spp. which included both homofermentative and heterofermentative rods. Others were mostly heterofermentative cocci, identified as *Leuconostoc* spp. Rarely some homofermentative cocci were also isolated which were identified as streptococcus.

The homofermentative group among *Lactobacillus* cultures constituted 55% of all the *Lactobacillus* isolated. Based on the growth temperature requirements, the homofermentative *Lactobacillus* were grouped into (1) thermobacteria (growing at 45°C and not at 15°C) and (2) strepto bacteria (growing both at 45°C and 15°C) Forty one percent of the *Lactobacillus* isolated were strepto-bacteria and 14% thermobacteria.

Salt tolerance of LAB strains were studied over a range of 0-10% of NaCl in MRS broth at room temperature (28±2°C). Of the 30 cultures tested, all could grow in presence of 1-4% NaCl, about 80% of the cultures grew in presence of 5-7% of NaCl but only very few cultures could tolerate NaCl conc. higher than 7%. None of the cultures could grow in the presence of 10% salt (NaCl).

Table 3 shows the list of sugars tested for studying the fermentation potential of the LAB cultures isolated.

Table 3. Fermentation of sugars

Number of cultures tested = 25

Sl. No.	Sugars	No. of cultures capable of fermentation	% of cultures capable of fermentation %
1	Glucose	25	100
2	Lactose	20	80
3	Sucrose	20	80
4	Maltose	20	80
5	Sorbitol	20	80
6	Starch	20	80
7	Xylose	10	40
8	Fructose	10	40
9	Ribose	10	40
10	Salicin	10	40
11	Melesitose	8	32
12	Cellobiose	8	32
13	Galactose	7	28
14	Mannitol	5	20
15	Arabinose	5	20
16	Raffinose	4	16
17	Melibiose	7	28
18	Inulin	8	32

Twenty five LAB cultures randomly chosen from the total 234 cultures were used for the fermentation of sugars. All the cultures were capable of fermentation of glucose. Nearly 80% of the cultures could ferment lactose, sucrose, maltose, sorbitol and starch. Xylose, fructose, ribose and salicin were fermented by about 40% of the cultures. Only a smaller percentage of the cultures tested could ferment the rest of the sugars listed.

Table 4 gives the data on production of titrable acidity by chosen number of LAB cultures. Of the 19 cultures tested only 7 cultures could produce a titrable acidity equivalent to 7 ml of 0.01 N within an incubation period of 8 days.

Table 4. Production of titrable acidity in MRS broth and glucose

Total No. of LAB cultures tested = 19

Number of cultures producing titrable acidity (ml of 0.01 N NaOH)/ml of culture

No. of days of incubation	Above 10 ml	Between 5 ml and 10 ml	Less than 5 ml
2	4	3	12
5	6	5	11
8	7	4	8
10	7	4	8

Antibacterial properties of 15 randomly selected LAB cultures belonging to *Lactobacillus brevis*, *L. buchneri*, *L. viridescens*, *L. casei* and *L. plantarum* were tested against three species of pathogenic/indicator bacteria by agar well diffusion method (Kavanagh, 1963) (Table 5). None of the cultures of *E. coli* and staphylococcus tested could be inhibited by the LAB cultures. But except *L. viridescens*, all the LAB cultures tested could exhibit antibacterial property against *Bacillus cereus* to varying extent.

The antibiotic property of the LAB could be due to lowering of pH (acidity), production of H_2O_2 or production of bacteriocin like substances. In this study none of the cultures tested produced H_2O_2 as evidenced by test, using catalase. The effect of low pH was tested by studying the antibiotic property of the supernatant before and after neutralization with alkali and it was found that the antibiotic property against bacillus strains was not due to the low pH. Hence it was concluded that the antibiotic property exhibited by the LAB cultures tested was due to production of bacteriocin. This was further elucidated by testing the antibiotic property, after

Table 5. Antibacterial property of LAB cultures against selected pathogenic indicator bacteria

Test strain	<i>Escherichia coli</i>			<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	
	EC 101	EC 102	EC 103	EC 104	SA3B	BC 3/3
<i>Lact. brevis</i>	-	-	-	-	-	++
Tu-6, Tu-9	-	-	-	-	-	++
<i>Lact. buchneri</i>						
C3	-	-	-	-	-	+
C2	-	-	-	-	-	++
<i>Lact. viridescens</i>						
C1	-	-	-	-	-	-
C7	-	-	-	-	-	-
<i>Lact. casei</i>						
K2	-	-	-	-	-	+
K6	-	-	-	-	-	+
K5	-	-	-	-	-	+
K8	-	-	-	-	-	+
AC5	-	-	-	-	-	+
<i>Lact. plantarum</i>						
B1	-	-	-	-	-	+
O1	-	-	-	-	-	+
H2	-	-	-	-	-	+

Note: ++ Inhibitory zone above 10 mm dia, + Inhibitory zone present, - No inhibitory zone present.

treating the neutralised culture broth by (1) heating in boiling water bath for 10 min and (2) by treating the broth with protease enzyme for 30 min. It was found that the antibiotic action of the culture broth disappeared after the above two treatments, thereby confirming that the antibiotic property was due to the action of bacteriocins.

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