

## BIOCHEMICAL CHARACTERIZATION OF LACTIC ACID BACTERIA ISOLATED FROM FISH AND PRAWN

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### Summary

*Lactic acid bacteria (LAB) were isolated from various samples of fresh and frozen fish and prawn. Thirteen species of Lactobacillus were identified among the 64% isolates. Among them, L. plantarum was the dominant species. The remaining 36% isolates of Lactobacillus could not be assigned to any species with the available taxonomic schemes.*

### Introduction

Lactic acid bacteria (LAB) are characterized as Gram - positive, usually non-motile, non - sporulating bacteria that produce lactic acid as a major or sole product of fermentative metabolism. Kandler and Weiss have classified *Lactobacillus* isolates from temperate regions according to their morphology, physiology and molecular characters [7]. Schleifer classified LAB based on the molecular characteristics [16]. LAB from food

and their current taxonomical status have been described by many [6, 13, 14, 19]. Ringoe and Gatesoupe have prepared a review of the LAB present in fish intestine [13]. Taxonomic studies on LAB from poikilothermic animals are rare [6, 13].

The aim of the study was the isolation of LAB from fresh and frozen fish and prawn and their classification based on the morphological and biochemical characteristics.

### Materials and Methods

**Isolation of LAB.** Fresh fish (22 Spp), frozen fish (15 Spp), fresh and frozen prawn (5 Spp) were procured from retail markets and cold storage in Kochi, packed in iceboxes and transferred to the laboratory within 2 h. MRS agar and broth were used for enumeration and culture of LAB [3]. The samples of fish (skin with muscle) and prawns (peeled) were homogenized in a

stomacher blender using saline, serially diluted and pour plated on MRS plates. The MRS plates overlaid with MRS agar and incubated in 5 % CO<sub>2</sub> at 37 °C for 48–72 h. Well - isolated colonies with typical characteristics namely pure white, small (2-3 mm diameter) with entire margins were picked from each plate and transferred to MRS broth.

### Identification of the bacterial strains.

The cultures were identified according to their morphological, cultural, physiological and biochemical characteristics [7, 18]. The used tests were: Gram reaction; production of catalase, cytochrome oxidase and hydrogen peroxide; growth at 15 °C and 45 °C in 1 week; acid production from carbohydrates (1 % w/v) - L-arabinose, cellobiose, D-fructose, D-galactose, esculin, lactose, maltose, melezitose, melebiose, mannitol, D-mannose,

raffinose, rhamnose, D-ribose, salicin, sorbitol, sucrose, trehalose and D-xylose in MRS broth devoid of glucose and beef extract with chlorophenol red as indicator; production of acid and gas from 1 % glucose (MRS broth without beef extract); methyl red and Voges-Proskauer test in MRVP medium; H&L test in O/F medium; production of ammonia from arginine; nitrate reduction in nitrate broth; indole production in tryptone broth and growth on acetate agar.

## Results and Discussion

The LAB isolates were classified into the genera *Streptococcus*, *Leuconostoc*, *Pediococcus* and *Lactobacillus* based on their morphology and biochemical characters [18]. Table 1 shows the distribution of different genera of LAB in fresh and frozen fish and prawn. Of the cultures, 60 % in fresh fish, 65 % in fresh prawn and 80 % each in frozen fish and prawn belonged to the genus *Lactobacillus*. The predominant *Lactobacillus sp.* was further classified to the species level [7]. The differentiating characteristics of *Lactobacillus* species are given in Table 2. Each strain showed variation in their sugar fermentation

pattern. The species identified showed above 80 % or more similarity to the ATCC type cultures. Only tests that gave reproducible results were included in the classification scheme. The species identified were *L. plantarum* (138 isolates), *L. brevis* (66), *L. divergens* (28), *L. gasseri* (24), *L. rhamnosus* (21), *L. fermentum* (20), *L. viridescens* (10), *L. farciminis* (7), *L. buchneri* (7), *L. acidophilus* (5), *L. alimentarius* (4), *L. animalis* (4) and *L. reuteri* (3). A significant fact is that 217 cultures (36.2 %) were found to belong to the genus *Lactobacillus* but could not be assigned to any particular species by these characters.

Table 1. The percentage distribution of different genus of LAB in fresh and frozen fish/prawn samples.

Sample	Lactic acid bacteria (%)			
	<i>Streptococcus</i>	<i>Leuconostoc</i>	<i>Pediococcus</i>	<i>Lactobacillus</i>
Fresh fish	20	10	10	60
Fresh prawn	20	10	5	65
Frozen fish	5	5	10	80
Frozen prawn	10	5	5	80

It is interesting to note that majority of the *Lactobacillus sp.* that have been isolated from fresh and frozen fish/prawns were those

species which were commonly found on meat, animals and human [7]. There were a few reports of isolation of LAB from fresh and

Table 2. Differentiating characteristics of *Lactobacillus* species.

No	<i>Lactobacillus</i> spp	Morphology	Growth at			Acid and gas from glucose	NH <sub>3</sub> from arginine	Sugar fermentation													
			15 °C only	45 °C only	15 and 45 °C			Arabinose	Cellobiose	Mannitol	Mannose	Melebiose	Raffinose	Ribose	Salicin	Lactose	Melezitose	Rhamnose	Sorbitol	Xylose	Trehalose
1.	<i>L. plantarum</i>	SR	+	-	+	-	-		+	+	+	+	+	+	+						
2.	<i>L. brevis</i>	SR	+	-	+	+	+	+						+		+	-	-	-	+	
3.	<i>L. divergens</i>	SR	+	-		+	+		+		+	-	-		+	-				+	+
4.	<i>L. gasseri</i>	SR	-	+		-	-	-	+		+			-	+			-	+		+
5.	<i>L. rhamnosus</i>	SR	-	-	+	-	-		+	+	+	-			+	+	+		+	+	+
6.	<i>L. fermentum</i>	SR			+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+
7.	<i>L. viridescens</i>	SR			+			-	+	+	+	-	-	-	-	+	+	-	-	-	+
8.	<i>L. farciminis</i>	SR			+		+	-	+	+	+	+	-	+	+	-	+	-	+	+	+
9.	<i>L. buchneri</i>	SR	+			+	+	-	+	-	-	+	-	+	-	+	-	-	-	+	-
10.	<i>L. acidophilus</i>	SR	-	-				+	+	+	+	-	-	-	+	+	+	-	-	+	+
11.	<i>L. alimentarius</i>	C	+					+	+	+	+	+	-	+	+	-	+	-	+	+	+
12.	<i>L. animalis</i>	C	-	-				+	+	+	+	+	+	+	-	+	+	-	+	+	-
13.	<i>L. reuteri</i>	R			+	+	+	+	-	-	-	+	-	+	-	+	-	-	-	+	-

Legend: 80 % or more of strains are positive (+); 80 % or more of strains are negative (-).

seawater fish [2, 12]. *L. plantarum* have been isolated from herring, Arctic krill and chilled channel catfish fillets [4, 17]. The *L. divergens* is now classified as *Carnobacterium divergens* [1]. According to them, the *Carnobacterium* cultures would not grow on acetate agar. However, the *L. divergens* cultures we have isolated grew well on acetate agar and so did not conform to the classification adopted by Collins et al for *C. divergens* [1].

Finding that *Lactobacillus* formed the major flora in fish substantiated the observations of several other workers [1, 4, 5, 8-12, 15, 20, 21].

However, Maugin and Novel found that *Lactococcus* was the major flora isolated from fish [9].

The occurrence of "typical" lactobacilli as described by Kandler and Weiss [7] were rare in fish and prawn. This signified the need for a proper classification scheme for LAB from tropical fish and prawn. In our studies we attempted to classify LAB on the basis of the available classification schemes. However further studies are needed in order to include other atypical *Lactobacillus* cultures in the classification scheme.

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## **БИОХИМИЧНА ХАРАКТЕРИСТИКА НА МЛЕЧНО–КИСЕЛИ БАКТЕРИИ, ИЗОЛИРАНИ ОТ РИБА И СКАРИДИ**

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### **Резюме**

*Млечно-кисели бактерии са изолирани от редица проби свежа и замразена риба, и скариди. Тринадесет вида *Lactobacillus* са определени, представляващи 64 % от общото количество. Сред тях преобладаващият вид е *L. plantarum*. Останалите 38 % от изолатите *Lactobacillus* не могат да бъдат отнесени към определен вид посредством използваните таксономични методи.*