

Full Length Research Paper

Identification of Lactobacilli Isolated from Mangrove Biotopes of East Coast of India.

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The *Lactobacilli* is gaining more and more important nowadays due to its antimicrobial, antioxidant, anticancer activity. Moreover the lactic acid produced by the *Lactobacilli* is used in many industries such as food, pharmaceutical, textile, environment and agriculture. By understanding the importance of Lactobacilli a new attempt was made to isolate lactic acid bacteria from the mangroves biotopes of east coast of India. In this study six predominant species of lactobacilli were isolated. Morphological characters, biochemical characters, motile nature, carbohydrate fermentation test revealed the presence of the species such as *L.delbreukii*, *L.lactis*, *L.casei*, *L.xylosus*, *L.plantarum*, and *L.curvatus*. Hence this research identified the predominant species of lactobacillus from the mangrove biotopes and the potentiality of such species will be studied in future.

Keyword: Marine lactobacilli, Mangrove, *Lactobacillus*, Microbial diversity, Genus diagnosis.

INTRODUCTION

Only a few studies are available on isolation and taxonomy of lactic acid bacteria from marine environments and those studies are mostly confined to cultured fish (Ringo and Gatesoupe, 1998; Gatesoupe, 1999). A novel marine lactic acid bacterium was isolated from living and decomposed marine organisms of the Japan seas and identified as *Marinilactibacillus psychrotolerans* (Morio et al., 2003). Franzmann et al., (1991) isolated two species of lactic acid bacteria (*Carnobacterium* sp., and *C. alterfunditum*) of possible seawater origin from the waters of Ace Lake in Antarctica (Masuda et al., 1988). To the best of our knowledge, there were no lactobacilli from mangrove environment have been so far isolated and identified. Hence this study was undertaken in order to isolate and identify the lactobacilli from different mangrove biotopes in east coast of India.

MATERIALS AND METHODS

Chemicals

All analytical reagents and media components were purchased from Hi-media (Mumbai, India).

Microbial cultures

The dominant colonies of marine lactobacilli isolated from mangrove sediments were sub-cultured on MRS (deMan- Rogosa- Sharpe) medium, prepared with 50% seawater and stored at 4°C for further studies.

Identification

Identification was done with dominant lactobacilli strains following the keys given in Bergy's manual (1974). The cultures were identified according to their morphological, cultural, physiological and biochemical characteristics.

Morphological characteristics

Studies on nutrient agar

Morphological and cultural characteristics such as growth, pigmentation, optical characteristics, Shape, size, margin, and elevation of colonies were studied on nutrient agar plates. Distribution and growth were determined using nutrient broth medium.

Table 1. Biochemical reactions of the species of genus *Lactobacillus*

Biochemical Tests	Strain					
	<i>L. delbrueckii</i>	<i>L. lactis</i>	<i>L. casei</i>	<i>L. xylosum</i>	<i>L. plantarum</i>	<i>L. curvatus</i>
Fructose	+	+	+	+	+	+
Galactose	-	+	+	+	+	+
Glucose (acid)	+	+	+	+	+	+
Glucose (gas)	-	-	-	-	-	-
Gluconate	-	-	+	+	+	+
Lactose	-	+	+	d	+	+
Maltose	d	-	d	+	+	+
Mannitol	-	-	+	+	+	-
Melizitose	-	-	+	-	d	-
Melibiose	-	-	-	-	+	d
Raffinose	-	-	-	-	+	D
Ribose	-	-	+	+	+	+
Xylose	-	-	-	+	d	-
Esculin	-	-	+	-	+	+
Oxidase	-	-	-	-	-	-
Catalase	-	-	-	-	-	-
Gelatin liquified	+	+	+	+	+	+
Nitrate reduction	-	-	-	-	-	-
Casein hydrolysis	-	-	-	-	-	-
Indole	-	-	-	-	-	-
H ₂ S	-	-	-	-	-	-

+ positive, - negative, d- variable

Motility and Staining characteristics of bacteria

Motility agar medium (or) soft agar medium was used to study motility of bacteria. The Gram's staining kits were used to gram's staining.

Biochemical characteristics

Starch hydrolysis, Lipid hydrolysis, Gelatin hydrolysis, Casein hydrolysis, Esculin hydrolysis, Carbohydrate fermentation (Acid and gas production), Hydrogen sulfide production, Nitrate reduction, Catalase reaction, Oxidase test, Indole production

RESULTS AND DISCUSSION

The genus *Lactobacillus* is the largest one that includes many species of lactic acid bacteria. The species are heterogeneous with substantial differences in their phenotypic, biochemical, physiological and genotypic characteristics. *Lactobacilli* were classically identified based on morphological and biochemical criteria, referring to Bergey's manual. However, these often rely

on the expression of variable phenotypes, which may not always be expressed under test conditions (Nour, 1998). At present, the genus *Lactobacillus* consists of more than 60 described species with three physiological groups namely obligately homofermentative, facultatively heterofermentative and obligately heterofermentative (Lortal *et al*, 1997). They are genetically quite diverse and their DNA guanine plus cytosine (G+C) content ranges from 32 to 54% (Kandler and Weiss, 1986). This is about twice as large as that normally accepted for a well-defined genus (Nour, 1998).

The biochemical characteristics used for identification of *Lactobacilli* may suggest some ideas in relation to the occurrence of the strains in nature. About 80% of *Lactobacilli* examined in this study had the capacity to ferment lactose and galactose (Table 1). Generally, most *Lactobacilli* are able to ferment lactose, by uptake of this disaccharide by a specific permease and splitting it by β -galactosidase for further phosphorylation of galactose and glucose (Kandler, 1983). Because, lactose is present only in milk and milk derivatives, it is possible that these strains have evolved from environments related with mammals, as was suggested for other lactose positive *Lactobacilli* (Garvie, 1984). Lactose may be present in the environment as a waste; resulting from

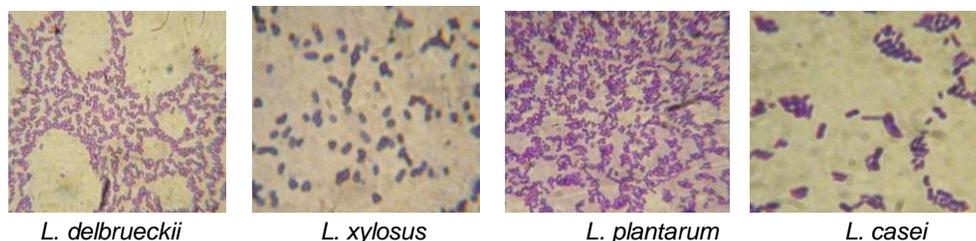


Figure. 1. Dominant lactobacilli species, (magnification 100X)

livestock production, and disposed effluents from dairy factories.

Genus diagnosis

Lactobacilli are generally characterised as gram-positive, non-spore forming, non-motile rods or cocci (Hammes and Vogel, 1995). They are aerotolerant or anaerobic and strictly fermentative. Glucose was fermented predominantly to lactic acid (homofermentatively) or equimolar amounts of lactic acid, CO₂, and ethanol and acetic acid (heterofermentatively). The predominant *Lactobacillus* sp. was further classified to the species level. The differentiating characteristics of *Lactobacillus* species are given in Table 1. Each strain showed variation in their sugar fermentation pattern. Only tests that gave reproducible results were included in the classification scheme. The species identified were *Lactobacillus delbrueckii*, *L. lactis*, *L. casei*, *L. xylosus*, *L. plantarum*, and *L. curvatus* (Figure.1).

Species diagnosis

Lactobacillus delbrueckii

Rod shaped, 0.5- 0.8 µm by about 2-9µm, with rounded ends occurring singly and in short chains. Internal granulations are revealed by methylene blue stain. Non motile. Colony normally rough becoming smooth and compact in the presence of Tween-80 (Rogosa and Mitchell, 1950) and non-pigmented. Lactic acid is the major product without gas from glucose and other carbohydrates (generally 85% or more). Lactic acid produced homofermentatively. Ammonia generally produced from arginine. Calcium pantothenate, niacin and riboflavin are required; Thiamine, pyridoxal, folic acid or vitamin B₁₂ are not required. No growth was observed at 15° C, but at 45° C and frequently at 50-52° C with optimum at 40-44° C.

Lactobacillus lactis

Rods, less than 2 µm wide, are often appearing as long

forms with a tendency to grow in threads, often strongly curling, occurring singly or in pairs in young, vigorous cultures. Generally, granules are demonstrable with methylene blue stain. They are non- motile. Colony is normally rough, 1-3 mm in diameter and non-pigmented being white to light gray. Lactic acid is produced by homofermentation, but negative, weak or variable reactions with esculin hydrolysis. Lactic acid is the major product without gas from glucose and other carbohydrates (generally 85% or more). Ammonia not produced from arginine. They require calcium pantothenate, niacin, riboflavin, vitamin B₁₂; but thiamine, pyridoxal or pyridoxamine, folic acid and thymidine are not required. There is no growth at 15° C; at 45° C or even 50-52° C; optimum growth at 40-43° C.

Lactobacillus xylosus

Lactobacillus xylosus was homofermentative organism, producing L (+) - lactic acid. It ferments xylose but not arabinose and is thus differentiated from *L. arabinosus*. There is no gas production from glucose, but from gluconate. Ribose, when fermented, yields lactic and acetic acids without gas. Thiamine is not required. Variable growth at 45° C. Lactic acid is the major product without gas from glucose and other carbohydrates (generally 85% or more).

Lactobacillus plantarum

Rods with rounded ends straight, generally 0.9–1.2 µm long, are occurring singly, in pairs or in short chains. Motility and flagellation ordinarily absent but motile. Surface colonies are about 3 mm wide, raised, round, smooth, compact, white and occasionally light or dark yellow. Growth in broth results in an even heavy turbidity. Some strains ferment arabinose and some ferment both arabinose and xylose. DL- Lactic acid is produced. Growth occurs on gluconate with CO₂ production. Ribose is fermented to lactic and acetic acids, where other pentoses are fermented, the products are the same as for ribose. Nitrates are reduced to nitrate at the pH of 6.0 or higher. Ammonia is not produced from arginine. Growth occurs in media with 4%

sodium taurocholate. Growth takes place at 15° C, generally not at 45° C, optimal usually 30° C - 35° C. Calcium pantothenate and niacin are required; but thiamine, pyridoxal or pyridoxamine, folic acid, vitamin B₁₂, thymidine or deoxyribosides, riboflavin are not required.

Lactobacillus casei

Short and long rods, generally less than 1.5 µm wide, often with square ends and tending to form chains. Flagella are absent and non-motile. Pour plate and deep colonies are smooth, lens or diamond-shaped, white to light yellow. Growth occurs in broth with heavy turbidity. Sorbitol and sorbose usually fermented. Maltose and sucrose often slowly fermented and negative variants may sometime be selected from a positive population. Glycogen and starch are not attacked. Ribose is fermented to lactic and acetic acids without CO₂ production; and inducible growth with 4% gluconate is rapid and abundant with CO₂ production. Ammonia is not produced from arginine. Riboflavin, folic acid, calcium pantothenate and niacin are required, and pyridoxal or pyridoxamine, are stimulatory. Thiamine, vitamin B₁₂ and thymidine are not required.

Lactobacillus curvatus

Curved, bean-shaped rods, rounded ends, 0.7 -0.9 µm by 1.0 – 1.2 µm, occurring in short chains or closed rings of generally four cells or horseshoe shaped forms and non-sporulating. Some strains are at first motile; but lost on subculture. Colonies are generally somewhat smaller than those of *Lactobacillus plantarum* but usually of the same appearance. It is homofermentative: acid and no gas from glucose; DL-lactic acid is the chief product. Esculin is hydrolysed (98 %). There is no growth in 4 % taurocholate. Growth occurs at 15° C, no growth at 45° C, optimal range 30 - 37° C.

SUMMARY AND CONCLUSION

The lactic acid bacteria are predominant normal micro flora playing significant physical and ecological role. They are not a single taxonomic entity, but a group of bacteria with similar properties. They are highly suitable for probiotic therapeutics of pharmaceutical interest.

Identification of lactobacilli isolated from mangrove biotopes of east coast of India to date are few. Hence, dominant lactobacilli strains were isolated from mangrove habitat and identified following the keys Bergy's manual. The strains were identified based on their morphological, cultural, physiological and biochemical characteristics. 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; HandL 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. The species were identified by using above mentioned tests and they were *Lactobacillus delbrueckii*, *L. lactis*, *L. casei*, *L. xylosus*, *L. plantarum*, and *L. curvatus*.

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