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Carbohydrate Utilization Profiles of *Lactobacillus* Isolates from Marine Waters of Bay of Bengal near Krishnapatnam Port, Nellore District, Andhra Pradesh.

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Short Communication

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ABSTRACT

Ten different Lactobacillus isolates were isolated from marine waters using MRS medium. On MRS broth all the isolates showed uniform growth. All the isolates were Gram +ve, rod shaped bacteria, homofermtative, with creamy smooth round colonies on MRS agar medium. They were Oxidase and Indole negative. Some of the strains are catalase positive. The carbohydrate utilization profile are strain specific. All the isolated strains were unable to utilize Arabinose.

INTRODUCTION

Lactic acid bacteria (LAB) produce lactic acid from fermentable carbohydrates. They are Gram positive, aero-tolerant anaerobes, non-sporulating rods and/ or cocci frequently occurring in chains[1]. They are widespread in nature. The marine strains have much better potential than their terrestrial counterparts because of their unique features. Among lactic acid bacteria lactobacilli have played a crucial role in the production of fermented products for millennia. The genus lactobacillus are beneficial microorganisms of particular interest because of their long history of use[2] . Lactobacilli were among the first organisms used by man for processing food stuffs [3]. Lactic acid bacilli have been basically identified on the basis of cell morphology and analysis of various carbohydrate substrates. Selective culture media and phenotypic tests enable lactobacilli to be differentiated from morphologically similar bacteria[4] . The present study was aimed at isolating and biochemical characterization of lactobacilli from marine waters and studies their carbohydrate utilization profiles.

MATERIALS AND METHODS

Sampling

Sea water samples were collected from bay of Bengal situated near Krishnapatnam port Nellore district. Sea water samples were collected from 10-15 cm depth by using random sampling method. These water samples were collected in sterilized bottles, which were rinsed twice with local sea water before use and immediately kept in freezer box. Samples were analyzed within 24 hrs of acquisition.

Isolation of Lactobacilli from Marine Water

Isolation of Lactobacilli was performed using the methods described by Kodama [6]. Sea water sample were taken directly and used as inoculums. One ml of the inoculums was spread plated on Demann Rogosa Sharpe agar (MRS) medium. The plates were incubated

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at room temperature for 1-2 days. The colonies developed were purified by quadrant streaking and transferred to MRS agar vials overlaid with sterile liquid paraffin.

Microscopic and Biochemical Characteristics of the Isolates

To observe Gram's nature and motility Gram's staining and Hanging drop technique were employed respectively. To confirm the isolates as lactobacilli biochemical tests such as oxidative/fermentative test, catalase test were performed.

Carbohydrate Utilization Profile

The ability of the isolates to utilize 8 different sugars as sole carbon source was examined in carbon utilization medium . Different sugars impregnated in cellulose discs (Hi-media) were added to the liquid medium to the final concentration of 1 %. The test tubes were inoculated with 1 ml of suspension of isolates. The result was recorded as positive when growth was greater than that in the negative control and that equal to or less than that in the negative control as negative.

RESULTS AND DISCUSSION

In the present study lactobacilli species were isolated from marine waters using spread plate method and pour plate method. Spread plate method proved better in isolating lactic acid bacteria. Around 124 number of colonies/ ml were produced on spread plate method and incase of pour plate method 118 colonies / ml were produced. The pure isolates of lactobacilli were subjected to microscopic and biochemical studies.

Based on colony morphology 10 isolates were selected and purified. 10 isolates were subjected to Gram's staining, motility, cell shape, catalase production, oxidative/fermentative tests, indole production, Growth on MRS broth, type of fermentation, H_2S production (Table-1)

Table 1 : General Microscopic and Biochemical characteristics of *Lactobacillus* strains (L1-L10) isolated from marine waters

Isolates	Colony morphology	Result
L1	Gram's staining	Gram +ve
L2	Cell shape	Rod
L3	Motility	-ve
L4	Growth in MRS broth	Uniform turbidity
L5	Type of fermentation	homofermentative
L6	Catalase	+ve
L7	Oxidase	-ve
L8	Indole production	-ve
L9	H_2S production	+ve
L10		

All isolates on MRS medium were creamy to light creamy, smooth round colonies. All the isolates were gram +ve and in rod shape. The isolates showed uniform turbidity in MRS broth. Three isolates were catalase positive and remaining isolates were catalase negative. However all the isolates were homo fermentative, oxidase negative and indole negative. All the isolates produced H_2S.

Assimilation patterns of different carbohydrates were also tested. All isolates showed varying profiles of carbohydrate utilization patterns as shown in Table-2. Glucose was utilized by isolate L-1, L-2, L-4, L-7, L-8, L-9 and L-10. Mannitol was utilized by isolates L-1, L-4, L-8, L-9 and L-10. Fructose was utilized by (L-1, L-4, L-7,3, L-8, L-9 and L-10) Xylose by (L-2, L-3, L-4, L-5, L-6, L-8, L-9 and L-10) and Galactose (L-1, L-4, L-6, L-8,L-9 and L-10). However Sucrose (L-3, L-7 and L-8) and Maltose (L-1,L-4, L-8 and L-9) were utilized by fewer isolates. Whereas none of isolates utilized 'Arabinose' as carbon source (Table-3). This indicates that the utilization of carbon sources is strain specific. Our results corroborated with the studies of Diana-Roxana et al, [7].

The marine environment is rich in nutrient and organic matter therefore we are able to isolate lactobacillus strains easily. In marine environment they are exposed to changes in the solute concentration of their natural habitats [8]. So far only a few strains of lactobacillus strains are known from marine environment [9]. This study may help to understand the use of marine lactobacillus in the manufacture of fermented dairy products.

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Table 2: Assimilation of carbon source by various isolates

S.no.	Name of the isolate	Glucose		Manitol		Sucrose		Fructose		Maltose		Arabinose		Xylose		galactose	
		Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
1	L1	-ve	+ ve	- ve	+ ve	- ve	- ve	- ve	+ve	- ve	+ ve	- ve	- ve	- ve	- ve	- ve	+ ve
2	L2	- ve	-ve	+ ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	+ ve	- ve	- ve
3	L3	- ve	- ve	- ve	- ve	- ve	+ ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	+ ve	- ve	- ve
4	L4	- ve	+ ve	- ve	+ ve	- ve	- ve	- ve	+ve	- ve	+ve	- ve	- ve	- ve	+ ve	- ve	+ve
5	L5	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	+ ve	- ve	- ve
6	L6	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	+ ve	- ve	+ ve
7	L7	- ve	+ ve	- ve	- ve	- ve	+ ve	- ve	+ ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
8	L8	- ve	+ ve	- ve	+ ve	- ve	+ ve	- ve	+ve	- ve	+ ve	- ve	- ve	- ve	+ ve	- ve	- ve
9	L9	- ve	+ ve	- ve	+ ve	- ve	- ve	- ve	+ ve	- ve	+ve	- ve	- ve	- ve	+ ve	- ve	- ve
10	L10	- ve	+ ve	- ve	+ ve	- ve	- ve	- ve	+ ve	- ve	- ve	- ve	- ve	- ve	+ ve	- ve	- ve

+ve =positive, -ve =negative

Table 3: Consolidation of carbon source utilization of isolates

Carbon source	Isolates
Glucose	L-1 , L-3, L-4,L-7,L-8,L-9,L-10
Mannitol	L-1, L-4,L-8, L-9, L-10
Sucrose	L-3, L-7, L-8
Fructose	L-1, L-4, L-7, L-8, L-9, L-10
Maltose	L-1, L-4, L-8, L-9
Arabinose	Nil
Xylose	L-2, L-3, L-4, L-5, L-6, L-8, L-9, L-10
Galactose	L-1 ,L-4 , L-6 ,L-8, L-9, L-10

CONCLUSION

The present study demonstrates the carbohydrate utilization profiles of different marine lactobacillus isolates which were dwindling in natural environmental stress. Our findings suggest that carbohydrate utilization ability was strain specific.

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