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STUDIES ON POTENTIAL APPLICATION OF REPRESENTATIVE PROMISING ISOLATES OF *LACTOBACILLUS* FOR PREPARATION OF SOFT DRINK LIKE LASSIE

R.D.Kamble, G.R. Pathade[1]
Department of Biotechnology Engineering,
Tatyasaheb Kore Institute of Engineering and Technology,Warananagar.
[1] Department of Microbiology, Ferguson College, Pune **Email:rdkbt@yahoo.com**

Abstract

About 25 different isolates of *Lactobacillus* spp. were obtained from surfaces of different plant parts like fruits, leaves, flowers and buds. Collected surface washings of plant parts, prepared in sterile saline were subjected for microscopic examination, for presence of Lactobacilli. They were enriched and isolated by using selective solid media. Isolated cultures were maintained in laboratory after characterization and identification. Homofermentative and heterofermentative patterns of prominent isolates were studied. Some were found to be low acidity producing isolates. Such isolates of *Lactobacillus* used for preparation of soft drink like Lassie.

Key words :- *Lactobacillus,* Homofermentative, Heterofermentative, Lassie.

INTRODUCTION

Lactic acid bacteria are a group of bacteria which are heterogeneous possesses diverse type of properties. They are Gram positive, non-sporulating cocci or rods, Catalase negative, usually non-motile, obligate fermenters, producing mainly lactic acid and sometimes also volatile acids and CO_2.Lactobacillus is one of the genus of Lactic acid bacteria group. Lactobacilli are Gram positive occurring in chains fermenting milk to produce lactic acid, mostly non-motile, non-sporulating. Lactobacilli are saccharoclastic, usually lactate is not fermented. Lactobacilli are micro-aerophilic. Some are strictly anaerobic organisms, growth enhanced by anaero-biosis or educed oxygen pressure. Nitrate reduction highly unusal, Gelatin not liquefied, casein not digested, Indole and Hydrogen sulphide not produced. Catalase and cytochrome negative, few strains decompose peroxide by a pseudocatalase, Benzene reaction negative. Complex nutritional requirements for amino acids, peptides, nucleic acid derivatives, vitamins, salts, fatty acids or fatty acid esters and fermentable carbohydrates. Growth temperature range 2-53^{0}C, optimum generally 30-40^{O}C.Lactobacilli are found in dairy products, grain products, meat and fish products, water, fruits and fruit juices, pickled vegetables, sour dough. Lactobacilli have adapted to specific ecological conditions and are generally not found outside their specialized habitats. In nature, all plant surfaces contain Lactobacilli in low numbers [1] and also grow luxuriantly with other lactic acid bacteria in many decaying plant material, especially decayed fruits. Hence, Lactobacilli are also important for the production and spoilage of fermented vegetable feed and

food (e.g. silage, mixed pickles) and beverages (e.g. Beer, Wine, Fruit Juices). Prominently isolated species from different plant sources are *Lactobacillus plantarum,Lactobacillusbrevis,Lactobacillus coryniformis, Lactobacillus casei,Lactobacilluscurvatus,Lactobacillus sake, Lactobacillus fermentum [2, 3], Lactobacillus delbrueckii subsp.Delbueckii* is the characteristic thermophilic organism found in potato and grain mashes fermented at 40-55^{0}C. It is also used in fermentation of millet mash to produce Bantu beer and and in industrial production of lacticacidfrom molasses.

MATERIALS AND METHODS

Different plant materials like fruits, leaves, flowers and buds were collected from Karad region.(Table 1)All the plant materials were collected in sterile beaker. Then immediately suspended in sterile saline. Shake thoroughly and kept for 1 hr, with the help of pipette 1 to 2ml of surface washings was directly collected. Gram staining of surface washings was performed so as to observe rods like Lactobacillus. Those washings showed typical Gram positive rods like morphology (characteristic of Lactobacilli) were subjected for enrichment using following selective media like MRS (deMan, Rogosa and Sharpe),APT (Atypical Peptone Tryptone) and LBS (Lactobacillus selective).1 to 2 ml of surface washings were inoculated in 10ml of enrichment media and incubated under microaerophilic conditions in Shaker incubator at 30^{0}C for 48hrs.After enrichment enriched material was observed microscopically for enrichment of Gram positive rods. The enriched material was suitably diluted into sterile saline and were streaked on solid media. So as to get isolation under microaerophilic conditions at 30^{0}C for 48 hrs to get practicable colonies. The typical and well isolated colonies were subjected to the studies like colony characteristics, microscopic examination (Gram nature and morphology), Catalase and Benzidine tests and presence of metachromatic granules. The isolate showing properties like Gram positive rods, Catalase negative or pseudocatalase positive and show presence of metachromatic granules were tentatively taken as Lactobacillus isolates and preserved at 4^{0}C on MRS agar stabs in triplicates after sealing with paraffin wax and regular passages were given monthly as and when required. Isolates were inoculated into glucose broth containing chloramphenicol red incubated at 30^{0}C for 48 hrs. The production of only acid in the medium was taken as indication of Homofermentative pattern while both gas and acid is taken as Heterofermentative pattern. Esculin agar with MRS (deMan, Rogosa and Sharpe) base and caseinate agar with each isolate of Lactobacillus. The extent of acidity produced in milk with respect to time was measured by Milkactivity test. In the milk activity test after every 6hrs pH of the medium and titrable amount of acid produced as lactic acid per ml of milk was calculated.As per following relationship,

1 ml 0.1N NaOH $\equiv$ 7 mg of Lactic acid.

The lactobacilli isolates were then classified as the moderate and high

acidity producing isolates on the basis of extent of lactic acid produced per ml of milk. One of each of low moderate and high acid forming representative isolates were selected and milk activity test was repeated using 200 ml skimmed milk and 10 ml precultured milk as a starter and after optimum time of maximum acidity production. The contents were homogenized, sweetened (with sufficient amount of sterile sugar solution) and flavorings were added as preservative and kept at room temperature to test the keeping quality by smell, odor and organoleptic testing for taste and on the basis of keeping quality the suitability of cultures for such product formation was assessed.

RESULTS AND DISCUSSION

Screening of different plant materials for isolation of Lactobacilli. Table 1 indicates that material from plants was used for isolation of Lactobacilli viz. Rose, Jaswand, Guava, Spinach, Mango. From these plants flowers, leaves, fruits and buds were used. Sterile saline water washings of different plant material were inoculated in the enrichment media i.e. MRS, APT, LBS. One loopful from the enrichment media was streak inoculate on respective solid media i.e. MRS, APT and LBS agar, the colonies developed after incubation were studied for their characteristics. Gram nature and morphology of cells from colonies indicated that they were gram positive in nature and morphology ranged from coccobacillary rods, short rods to long rods and all showed presence of metachromatic granules. Each isolate was characterized and identified. It is evident that in total twenty five Lactobacillus isolates were obtained from eight different plant material surfaces and they were identified as *Lactobacillus marinus, lactobacillus bavaricus, Lactobacillus casei Rhamnosus, Lactobacillus acidophilus, Lactobacillus fructosus, Lactobacillus fermentum, Lactobacillus reuteri, Lactobacillus crispatus, lactobacillus delbruecki, Lactobacillus alimenarius* i.e.11 different species.

It is further evident that from Table 2 that out of twenty five Lactobacillus isoloates three isolates viz. isolate number 14, 15 & 16 were heterofermentative and produced acid & gas from glucose while remaining twenty two isolates were found to be homofermentative and produced only acid from glucose fermentation.

Table 1:- Types of Lactobacilli found in different plant material used

Sr. no.	Plant name	Plant part used	No.of types of Lactobacillus	Identification
1	*Rosa indica*	Flowers, leaves	2	*L.marinus, L.sharpee*
2	*Hibiscus rosasinesis*	Flowers, leaves	2	*L. casei, L. ruminis*
3	*Psidium guyava*	Fruits, leaves	3	*L. acidophilus, L. fructosus L. ruminis*

4	*Spinacea oleracea*	Leaves	6	*L. reuteri, L. crispatus, L. delbruekii, L. alimentarius L. ruminis, L. plantarum*
5	*Magnifera indica*	Buds, leaves	1	*L. plantaum*

Table 2:- Physiological characteristics of isolates

Isolate no.	Glu	Suc	Lact	Xyl	Gala	Mal	Cellob	Treh	Melli	Arab
1	A	A	A	---	---	---	---	A	---	A
2	A	A	A	---	A	---	A	---	---	A
3	A	A	A	---	A	---	---	---	A	A
4	A	A	A	---	---	---	---	---	---	A
5	A	A	A	---	---	A	A	A	---	A
6	A	A	A	---	A	---	---	A	---	A
7	A	A	A	---	---	---	---	---	---	A
8	A	A	A	---	A	A	---	A	---	---
9	A	A	A	---	A	A+G	---	A	---	---
10	A	A	A	---	---	A	---	A	---	---
11	A	A	A	---	---	---	A	A	---	A
12	A	A	A	A	---	A	A	A	A	---
13	A	A	A	---	---	---	---	A	A	A
14	A+G	A	A	A	---	A	---	---	A	---
15	A+G	A	A	A	---	---	---	---	A	---
16	A+G	A	A	A	---	A	---	---	---	---
17	A	A	A	A	---	A	---	A	---	---
18	A	A	A	A	---	A	A	A	A	---
19	A	A	A	A	---	A	---	---	---	A
20	A	A	A	A	---	A	A	---	A	---
21	A	A	A	A	---	A	---	---	A	---
22	A	A	A	A	---	A	---	A	A	---
23	A	A	A	A	---	A	---	A	---	A
24	A	A	A	A	---	A	---	A	---	---
25	+	A	A	A	---	A	A	A	---	---

Key:-Glu- Glucose; Suc- Sucrose; Lact- Lactose; Xyl- Xylose; Gala- Galactose;Mal- Maltose;Cellob- Cellobiose; Treh- Trehalose; Melli- Mellibiose;Arab- Arabinose;A:- Acid productionA+G:-Acid and gas production---:- No acid and gas production

As Lactobacillus members have great significance as starters in the fermented foods especially milk products, the milk activity test of Lactobacillus members possesses paramount significance.It is evident from Table 3 that in milk activity test all the Lactobacillus isolates were inoculated in the milk at pH 7.0 and Lactobacillus members showed variety to pH drop in the milk as function of acidity production due to fermentation of milk sugar.The pH drop ranges from 6.0 to 4.0 pHs. pH drop to 6.0 was shown by isolates 10, 12, & 20.While pH dropto pH to 5.0 was shown by isolates 1, 5, 8, 11, 21, while pH drop to 4.0 was shown by isolates 2, 3, 4, 6, 7, 13, 14, 15, 16, 17, 18, 19, 22, 23, 24, & 25 which showed that most of the isolates produced high acidity, some produced moderate acidity & few produced low or mild acidity in the milk test. The incubation periods for above said acidity ranged from 22 to 44 hrs. Maximum acidity (pH 6.0) in case of isolate no. 10 was found after 22 hrs of incubation while in case of isolate no. 20 it was after 28 hrs. Remaining isolates showed production of maximum acidity after 44 hrs of incubation.

Table 3 : Milk activity test

Isolate no.	InitialpH (immediate after inoculation in milk)	pH at which maximum activity produced	Incubation in hrs.	Maximum titration reading (ml of 0.1N NaOH req.)	Amount of lactic acid produced per ml of milk)
1	7.0	5.0	44	5.0	3.5
2	7.0	4.0	44	5.8	4.06
3	7.0	4.0	44	4.0	2.8
4	7.0	4.0	44	8.0	4.6
5	7.0	5.0	44	6.0	4.2
6	7.0	4.0	44	6.6	4.62
7	7.0	4.0	44	5.1	3.56
8	7.0	5.0	44	2.7	1.89
9	7.0	4.0	44	2.5	1.75
10	7.0	6.0	44	2.7	1.89
11	7.0	5.0	44	5.5	3.85
12	7.0	6.0	44	1.1	0.77
13	7.0	4.0	44	1.5	5.24
14	7.0	4.0	44	3.4	3.38
15	7.0	4.0	44	8.0	5.60
16	7.0	4.0	44	9.1	6.37
17	7.0	4.0	44	6.5	4.55
18	7.0	4.0	44	5.5	3.85
19	7.0	4.0	44	4.8	3.36
20	7.0	6.0	44	2.8	0.96
21	7.0	5.0	44	2.3	1.65
22	7.0	4.0	44	4.3	3.09
23	7.0	4.0	44	3.6	2.52
24	7.0	4.0	44	6.0	4.2

25	7.0	4.0	44	4.0	2.8

It was evident that amount of acidity in terms of lactic acid produced by Lactobacillus isolates ranged from minimum of 0.77 mgs/ ml in case of isolate -12 to maximum for isolate-16 viz.6.37 mgs/ ml. Further studies (Table 4) on milk activity test & it's extension for testing feasibility of some representative isolates viz. Low acid producing (Isolate no. 12) , moderate acid producing(Isolate no.5) ,and high acidity producing (Isolate no. 16) isolates indicated that, isolate no. 5 produced a soft milk drink(Lassie) Of desired quality i.e. it produced milk acidity and this acidity is retained further for about 24 hrs at 30^0C without any increase or decrease on development of any undesirable aroma and flavor. While in case of isolate no. 13 the produced formed was of food aroma and flavor and in case of isolate no. 16 product formed was sourer. These results indicated that culture like isolate no. 5 could be a promising candidate for production of soft milk drinks.

Table 4: Milk activity testing for selected isolates

Isolate no.	**Extent of acidity**	**Type of aroma and flavor in the product form**	**Keeping quality at 30^0C in hrs**
5	Moderate	Rich & desired	Moderate acidity formation in 24 hrs
12	Low	Flat	Remained
16	High	Sour	More acidity and sourness produced

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