The Biobrio ISSN : 2393-9508 e-ISSN: 2582-4902
An International Quarterly Journal of Life Sciences
Website: www.thebiobrio.in SJIF 2023:8.16
10 (3&4): 1109-1113, 2023

Screening of Lipolytic activity containing isolates of *Lactobacillus* sps. isolated from cow milk

Swikriti Kumari & Arun Kumar
University Department of Zoology, B.N.Mandal University, Madhepura, Bihar, India

Received : 26th August, 2023 ; Accepted : 27th September, 2023

ABSTRACT

The present study was conducted to determine lipolytic activity of isolates of *Lactobacilli* isolated from cow milk. Altogether, seven isolates were isolated belonging to *Lactobacillus brevis* and *Lactobacillus fermentum*. Isolate no. Lb1, Lb2, Lb3 and Lb4 belong to *Lactobacillus brevis* and isolate no. Lf1, Lf2 and Lf3 belongs to *Lactobacillus fermentum*. All isolates were gram positive, catalase, oxidase and indole negative, Glucose lactose and mannitol positive, sucrose fructose and raffinose negative. All isolates showed positive KIA test. Lipase production was maximum at a time interval of 15 minutes. Among *L. brevis,* isolate no. Lb2 showed maximum lipase production (115 unit) and minimum lipase production was observed in isolate no. Lb4 (103 unit). Among isolates of *L. fermentum,* maximum lipase production was observed in isolate no. Lf3 (130 unit) and minimum lipase production was observed in isolate no. Lf2 (120 unit).

Key Words :- Lipase activity, Isolates, *Lactobaciili*, Food and Pharmaceutical industries.

***Corresponding author :** prf.arunkumar@gmail.com

INTRODUCTION

Some species of *Lactobacilli* are able to produce the enzyme proteases which hydrolyse protein. These enzyme belongs to class Serine and catalyse the hydrolysis of triglycerides to glycerol and free fatty acids over the oil-water surface (Liu *et al.* 2006). Lipases are also produced by several plants and animals but lipase from microbes are most preferred in industrial applications. These enzymes are involved in several biological processes that are associated with metabolism of dietary triglycerides to inflammation. Lipases from *Lactobacilli* are most important biocatalyst of industrial significance, therapeutic application and as flavouring agents in food industries.

Lactobacilli are dominant microflora of milk which are gram positive, catalase negative, oxidase negative, indole negative and rod shaped bacteria. These bacteria produces a variety of compounds such as lactic acid, diacetyl, vitamins, bacteriocin, β-galactosidase and lipase. *Lactobacilli* improves immune system, protect from pathogens and are anticancerous. Due to these features, *Lactobacilli* are being used as probiotics. Probiotics drinks such as Lassi, Shrikhand, Yakult, etc. contains different species of *Lactobacilli*. Probiotics drinks in India is prepared by several industries such as Nestle, Amul, Sudha, Mother dairy, Yakult Donane, etc. Amul is considered as largest food brand in India. Dahi and Lassi are the common probiotics drinks produced by Amul. In February 2007, Amul developed probiotic ice-cream which was accepted in probiotic category in Feb. 2011. Mother dairy has also launched some probiotic products by the trade name b-active probiotic dahi, b-active probiotic lassi and nutrifit. Yakult Donane India Pvt. Ltd. established in 2007 with the collaboration of Yakult Japan and

Donane France. It is producing a probiotic drink with the brand name Yakult. This drink is fermented milk which consist of *Lactobacillus acidophilus* and *Bifidobacterium*. 65ml Yakult contain 6.5 billion bacteria.

MATERIAL & METHODS

Sample Collection:

Cow milk was collected from different dairy firms and villages of Madhepura district in plastic bottles and brought to the laboratory.

Culture of Bacteria:

Milk samples were diluted up to 10^{-5} dilution in distilled water. Diluted samples were inoculated in MRS medium and incubated at 37^0C for 24 hours.

Subculture:

Subculture of single colony from culture plate was picked up and inoculated in fresh MRS medium to get pure culture.

Gram Staining: Gram staining technique was developed by Hans Christian Gram and on the basis of this staining technique, Bacteria are divided into two categories: Gram positive and Gram Negative. Two different stains used in this technique are Gentian Violet and Safranin.

Gram staining technique is as follows:

- **a.** Smear is prepared on clean slide and is fixed by warming on spirit lamp.
- **b.** Gentian violet is flooded over the slide and kept for 1 minute.
- **c.** Iodine is flooded over slide.
- **d.** Slide is now washed with Ethyl alcohol or acetone.
- **e.** Safranin solution is flooded over slide.
- **f.** Now, slide is washed with running water.
- **g.** Slide is air-dried and viewed under microscope.

Bio-Chemical Test:

Biochemical tests were performed for catalase, oxidase, indole and Kligers Iron agar test.

1. **Catalase test:** For Catalase test, one loopful culture was taken on a sterile plain slide and one drop of 3% H_2O_2 was added on it. It was mixed thoroughly. Appearance of bubble or froth indicate positive result. If not any bubble or froth appears, the result is negative.
2. **Oxidase test:** Two drops of reagent are dropped in agar plate. The appearance of blue color shows positive result. The reagent for oxidase test is prepared as follow: 1%N, N, N', N'-tetramethyl-p-phenylenediamine dihydrochloride is dissolved in distilled water.
3. **Indole test:** Some bacteria have Enzyme Tryptophanase which break down Tryptophan to produce Indole. Production of Indole is tested using Kovac's reagent.

   Bacterial inoculum was taken from culture and inoculated in Peptone water in a test tube. Culture was incubated at 37^0C for 48 hours. 0.5ml Kovac's reagent was added and shaken gently. A red color ring shows positive result.
4. **Kliger's Iron Agar test:** For this test, Kliger Iron Agar medium was prepared.

   Medium and 15 test tubes were sterilized in autoclave at 15lb/inch^2 pressure for 15 minutes. Slant were prepared in each test tube. Each culture was inoculated in slant by stabbing the butt and streaking the slant. Slant and butt were observed for alkalinity or acidity.

Sugar fermentation test: Sugar fermentation test was performed for the fermentation of glucose, fructose, sucrose, lactose, mannitol, raffinose and arabinose. For sugar fermentation test, 1% sugar was supplemented in peptone broth and the phenol red was added as indicator. All isolates were separately inoculated in test tube containing medium and incubated for 3-4 days. Colour change was observed. If the colour changes from pink to yellow, the result was considered as positive otherwise negative.

Detection of Lipase activity:

For the detection of Lipase activity substrate was prepared from Olive oil and polyvinyl alcohol. 50ml

of Olive oil and 150ml of polyvinyl alcohol were mixed together and vortexed for 3 minutes. 5ml substrate and 5ml phosphate buffer were added in a conical flask. 1ml pure culture from MRS medium was transferred in a conical flask. The conical flash was then kept at 30^0C for 10 minutes. After 10 minutes 15ml ethanol (95%) was added. Liberated fatty acid was titrated against 0.1M NaOH, phenolphthalein was used as indicator. 1µ mol. Fatty acid is equivalent to 1 unit of enzyme Lipase.

RESULT

Altogether, 7 isolates belonging to 2 species of *Lactobacilli* were isolated from milk samples. Isolate code and species are listed in Table 1.

Table 1- List of species and its isolate code

Species	Isolate Code
L. brevis	Lb1, Lb2, Lb3
L. fermentum	Lf1, Lf2, Lf3

All isolates were Gram positive, Catalase, Oxidase and Indole negative. KIA test reveals that all isolates showed yellow acidic in initial test and red alkaline in final test. The result of biochemical test is mentioned in Table 2, result of Sugar fermentation test is mentioned in Table 3 and the result of KIA test is mentioned in Table 4.

Table 2- Gram staining and Biochemical test of all isolates

Species	Isolates	Gram Staining	Catalase	Oxidase	Indole
L. brevis	Lb1	+ve	-ve	-ve	-ve
	Lb2	+ve	-ve	-ve	-ve
	Lb3	+ve	-ve	-ve	-ve
	Lb4	+ve	-ve	-ve	-ve
L. fermentum	Lf1	+ve	-ve	-ve	-ve
	Lf2	+ve	-ve	-ve	-ve
	Lf3	+ve	-ve	-ve	-ve

Table 3- Sugar fermentation test of all isolates

Species	Isolates	Glucose	Lactose	Sucrose	Fructose	Mannitol	Raffinose
L. brevis	Lb1	+ve	+ve	-ve	-ve	+ve	-ve
	Lb2	+ve	+ve	-ve	-ve	+ve	-ve
	Lb3	+ve	+ve	-ve	-ve	+ve	-ve
	Lb4	+ve	+ve	-ve	-ve	+ve	-ve
L. fermentum	Lf1	+ve	+ve	-ve	-ve	+ve	-ve
	Lf2	+ve	+ve	-ve	-ve	+ve	-ve
	Lf3	+ve	+ve	-ve	-ve	+ve	-ve

Table 4- KIA test of all isolates

Species	Isolates	KIA Test	
		Initial	Final
L. brevis	Lb1	Yellow/Acidic	Red/Alkaline
	Lb2	Yellow/Acidic	Red/Alkaline
	Lb3	Yellow/Acidic	Red/Alkaline
	Lb4	Yellow/Acidic	Red/Alkaline
L. fermentum	Lf1	Yellow/Acidic	Red/Alkaline
	Lf2	Yellow/Acidic	Red/Alkaline
	Lf3	Yellow/Acidic	Red/Alkaline

Lipase Activity: Lipase activity was maximum at a time interval of 15 minutes and it decreased at higher and lower time interval. Isolates no. Lf3 of *Lactobacillus fermentum* showed maximum lipase production (130 unit) at a time interval of 15 minutes while maximum production of Lipase (115 unit) was observed by isolate no. Lb2 of *Lactobacillus brevis*. Result is shown in fig. 01.

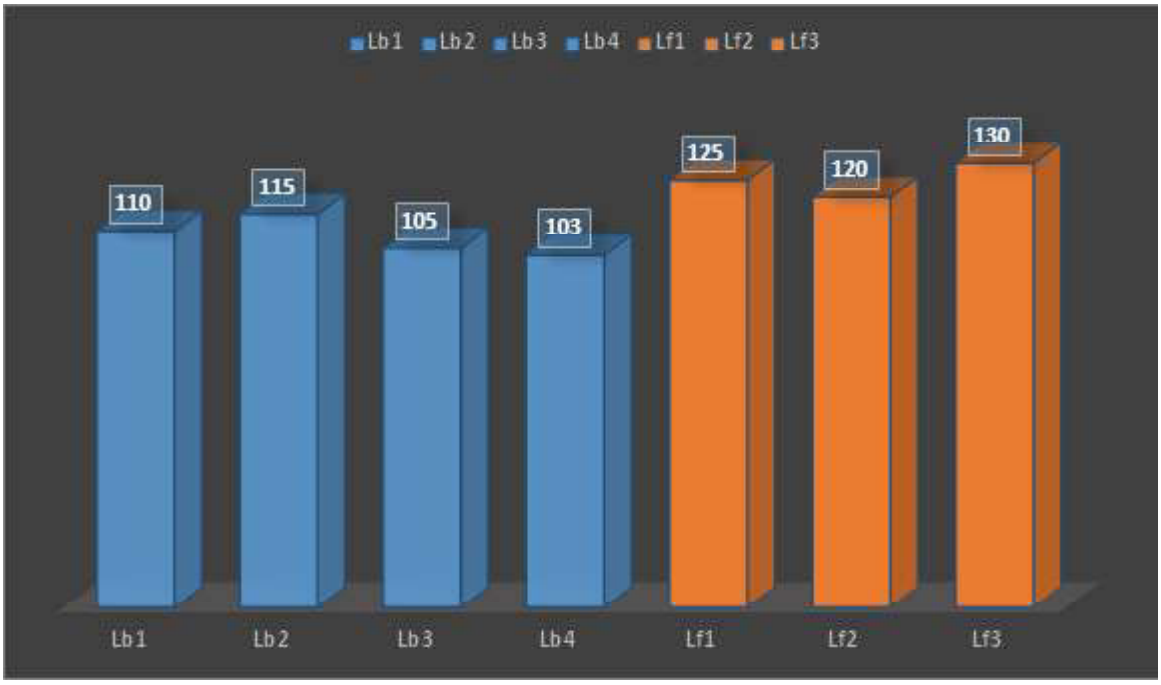

Fig. 1 Lipase activity of all isolates

DISCUSSION & CONCLUSION

Study of lipolytic activity in several microorganisms including Bacteria and Fungi was reported by several authors like Aravindan *et al.* (2007), Gupta *et al.* (2004), Liu *et al.* (2006). In the present study, lipolytic activity of *Lactobacillus* sps. Was examined. Isolates of two species of *Lactobacillus* i.e. *Lactobacillus brevis* and *Lactobacillus fermentum* were identified for lipolytic activity. Isolate of *Lactobacillus fermentum* produced 130 unit at a time interval of 15 minutes while *Lactobacillus brevis* produced 115 unit at the same time interval. Microorganisms such as *Bacillus* sp., *Pseudomonas* sp. and *Saccharomyces* sp. could degrade fat, oil and greases are aerobic (Ruiz *et al.* 2005, Hachemi *et al.* 2017) but in present study the bacteria used were anaerobic. Markossian *et al.* (2000) reported that *B. thermoleovorans* can utilize 93% olive oil during 7 hour fermentation. Lipolytic activity of reported bacteria is very high in comparison to present study.

In the present study, lipase activity of *Lactobacillus* isolates isolated from cow milk was examined. Altogether, 7 isolates belonging to two species of *Lactobacilli* were examined. Isolate no. Lb1, Lb2, Lb3 and Lb4 identified as *Lactobacillus brevis* and isolate no. Lf1, Lf2 and Lf3 were identified as *Lactobacillus fermentum*. All isolates were gram positive, catalase oxidase and indole negative, Glucose lactose and mannitol positive, sucrose fructose and raffinose negative. All isolates showed positive KIA test.

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