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Research Article

Lipolytic Activity of Lactic Acid Bacteria Isolated from Different Dairy Samples

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ABSTRACT

Lactic acid bacteria isolated from various dairy sampled were screened for lipolytic activity. Among 32 dairy samples, 24 strains of Lactic acid bacteria were obtained on MRS agar, out of them seven strains were screened as lipase producer on tributyrin agar media. These seven isolates were tested morphologically and Biochemically and identified as Lactobacillus sp. To screen best possible bacteria having lipolytic activity, qualitative screening method is performed using disc diffusion method on olive oil agar containing methyl red. Total three strains of Lactobacilli designated as L_{13}, L_{17} and L_{18} were obtained with maximum lipolytic activity. Three strains L_{13}, L_{17} and L_{18} were subjected for lipase production using submerged fermentation process and lipase activity was observed. Strain L_{13} and L_{18} showed maximum lipase activity 5.47 U/ml and 5.19 U/ml, while L_{17} showed minimum lipase activity 3.02 U/ml.

Keywords: *LAB (Lactic acid Bacteria), MRS, Tributyrin, Lipolytic activity etc.*

INTRODUCTION

Lipase is an enzyme that hydrolyze fats and oil. Lipase is produced by pancreas, liver, intestine, tongue, stomach and many other cells in humans. Lipases can hydrolyze ester bond of long chain fatty acid which is main component of oil. The source of lipase enzyme are generally found in nature such as plant, animal, yeast and bacteria (Kumar et al., 2017). Demand for industrial enzyme, particularly of microbial origin is ever increasing in last decade. Lipases, Triacylglycerol hydrolases are important group of biotechnologically relevant enzymes (Padmapriya & Rajeswari, 2011). Fats and oil are important constituents of food. Lipases allow us to modify the properties of lipids by altering the location of fatty acid chain in glycerides and replacing one or more of the fatty acid with new ones. This way a relatively inexpensive and less desirable lipid can be modified to higher value fat (Sharma et al., 2001).

Bacterial lipases are important enzyme having application in various industries such as food, pharmaceuticals, detergent industry because of 'friendly' for environment and nontoxic and no harmful (Sirisha et al., 2010).

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Lactic acid bacteria are friendly bacteria that normally live in our digestive, urinary and genital system without causing disease. LAB are used in dairy industry as well as used for treating and preventing various diseases (Rashmi & Gayathri, 2014).

The beneficial properties of *Lactobacillus* in human including lipolytic activity are strain specific. Hence present work aim to screen for isolation of Lactic acid Bacteria with lipolytic activity from dairy samples collected from traditional milk practioner. Isolates represents the genus *Lactobacillus.*

MATERIALS AND METHODS

Collection of source sample:

For present study, total 32 samples were collected from different area of Nanded district of Maharashtra, India. The dairy samples such as milk and curd were taken in appropriately labelled pre-sterilized glass tubes, brought to laboratory and used for isolation of Lactic acid Bacteria.

Isolation and identification of *Lactobacillus*:

The bacterial strains of Lactic acid Bacteria were isolated by dilution plate agar method using different dairy samples. The source sample was diluted serially 10 fold using 0.85% physiological saline and pour plated on to deMan Rogosa Sharpe MRS agar plate. Plates were incubated at 37^{o}C for 24 to 48 h, anaerobically. After incubation morphologically distinct colony were selected and transferred on to fresh MRS agar medium to obtained pure culture. Then the colonies were observed for their colony morphology and stained using gram staining. Gram positive isolates were tested for motility test, catalase test, indole test, gelatin liquefication test and carbohydrate fermentation test and identified based on Bergay's manual of systematic bacteriology. Further the isolates were maintained on MRS agar slants (Rashmi & Gayathri, 2014).

Primary screening of lipase activity:

All isolated *Lactobacilli* strains were primarily screened using tributyrin agar. All the isolated culture are inoculated into tributyrin agar and kept for incubation at 37^{o}C for 72 h. Lipolytic bacterial colonies showed opaque zone around the colony (potentially positive for lipase activity) were selected for further study (Rashmi & Gayathri, 2014).

Qualitative determination of Lipolytic activity by disc diffusion method:

The isolate capable for lipase production were further screened to isolate the best possible *Lactobacilli* based on agar disc diffusion method. Sterile Methyl red agar plate with olive oil is used for disc diffusion method. Each bacterial isolate were taken one loopful and cultured in to 5 ml liquid medium with composition NaCl 1%; Yeast extract 1%; peptone 2%; Tween-80 1% and sterile olive oil 2%. Paper disc of 5mm was dipped into incubated bacterial cultural for 10-15 min and put into the methyl red agar plate with composition (gm/lit): peptone 10gm; NaCl 5gm; $CaCl_2$ 0.1gm; Agar 20gm; 2.5% tween-80; 5% olive oil and 0.01% methyl red. The culture was incubated for 3 to 5 days. The clear zone was measured in mm and used for further study of enzyme production and activity.

Production of Lipase by fermentation process:

The fermentation was carried out using an production medium composed of peptone (0.5%), Sodium chloride (0.25%), Yeast extract (0.3%), Magnesium sulphate (0.05%) and Olive oil as a substrate with pH 6.0. 2% inoculums of each selected bacterial strain were added to production medium and incubated at 37^{o}C on rotary shaker (120rpm) for 72 hours. After 72 hour of incubation the culture was centrifuged 10,000 rpm for 20 min at 4^{o}C and cell free culture supernatant fluid was used as the source of extracellular enzyme (Patel & Desai, 2018).

Quantitative assay for lipase production and activity:

Lipase activity was measured by volumetric analysis using olive oil as a substrate. 1ml of culture supernatant was added to a reaction mixture containing 2 ml of phosphate buffer with pH 7.0 and 1ml olive oil and incubated at 37^{o}C for 15 min. The reaction was stopped and fatty acid were extracted by addition of 1.0 ml

of acetone: ethanol solution (1:1). Amount of fatty acid liberated was estimated by titration with 0.05N NaOH using phenolphthalein as an indicator (Patel & Desai, 2018).

Lipase activity was calculated as:

Lipase activity (Units /ml)=Volume alkali consumed ×Strength of alkali×1000/ Volume of Sample×time in min.

RESULTS

Isolation and screening of LAB for Lipase activity:

Total 32 different samples of dairy product were considered in this study. Among 32 samples randomly 24 colonies were selected on MRS agar having Gram positive and catalase negative characters. These colonies were plated on tributyrin agar for screening of lipase producer. Among all tested isolate seven isolates showed lipolytic zone on tributyrin agar plates and were designated as L_{13}, L_{14}, L_{15}, L_{16}, L_{17}, L_{18} and L_{19}. Total selected bacterial isolates were further proceeded for morphological and biochemical tests and identified as *Lactobacillus*. Table1 & 2 shows results of various biochemical characteristics.

Qualitative disc diffusion assay:

Lipase producing *Lactobacilli* isolates showed production of clear zone of different diameter on Methyl red olive oil agar plate given in Figure1. Among all isolates strain L_{13}, L_{17} and L_{18} showed maximum zone of clearance as 25mm, 17mm, and 27mm in diameter and L_{14} shows smallest zone of clearance as 9 mm in diameter, while L_{16}, L_{15}, and L_{19} did not show zone of clearance. Strains having zone with highest diameter were further used for production of Lipase.

Production of Lipase:

Thestrains L_{13}, L_{17} and L_{18} of *Lactobacillus* species were selected as higher lipase producer by disc diffusion method and further used for production of enzyme. Lipase production from these isolates showed that the lipase activity increased with time reaching maximum four days and decreased further. Lipase activity was measures by volumetric analysis using olive oil as substrate as in figure 2.

DISCUSSION

In present study, dairy samples were used for isolation of Lipase producing Lactic acid Bacteria from area of Nanded, Maharashtra, India. Lactic Acid Bacteria were isolated using MRS agar and Lipase producing Strains were screened by preliminary screening on Tributyrin agar and lipolytic activity was confirmed by disc diffusion method. Total 24 isolates were obtained on MRS agar as gram positive and catalase negative, among which seven strains were identified as lipase producer using tributyrine agar plate method and designated as strain L_{13}, L_{14}, L_{15}, L_{16}, L_{17}, L_{18} and L_{19} (Rashmi & Gayathri, 2014). All strains were examined for morphological and Biochemical test and identified as *Lactobacillus sp.* as per Bergay's Manual of Systematic Bacteriology. All seven lipase producer LAB isolates were further examined for qualitative lipolytic activity assay using disc diffusion method on olive oil agar containing methyl red. Among Seven lactobacilli strain L_{13}, L_{17} and L_{18} showed maximum lipolytic activity were selected for production of lipase (Patel, & Desai, 2018). The LAB strain L_{18} showed maximum production and lipase activity while strain L_{17} showed less lipase production and activity in the production medium containing olive oil as substrate. Maximum production of enzyme obtained after 96 hour of incubation at 37^{o}C and pH 7.0 on rotary shaker at 120 rpm. Lipase activity was measured by titrimetric method using olive oil as substrate. These results are in accordance with the results of P. Patel B. Desai, (2018). padmpriya et al, (2011).

The enzyme potential for *Lactobacillus sp* is an important factor in food dairy and meat industry. Specific flavor, odor, color and structure of the sausages are due to the characteristics of the raw meat and spices used, natural microflora including lactic acid bacteria (Tanasupawat et al., 2015).

Table1: Morphological and Biochemical characterization of seven LAB isolates which showed lipolytic activity

Sr no	Strain	Gram staining	Motility	Catalase	Gelatin liquification	Indole production	MR test	VP test
1	L_{13}	Positive Rod	Non motile	-	-	-	+	-
2	L_{14}	Positive Rod	Non motile	-	-	-	+	-
3	L_{15}	Positive Rod	Non motile	-	-	-	+	-
4	L_{16}	Positive Rod	Non motile	-	-	-	+	-
5	L_{17}	Positive Rod	Non motile	-	-	-	+	-
6	L_{18}	Positive Rod	Non motile	-	-	-	+	-
7	L_{19}	Positive Rod	Non motile	-	-	-	+	-

-(Negative); + (Positive)

Table 2: Sugar fermentation test

Sr no	Strain	Glucose	Galactose	Lactose	Arabinose	Xylose	Maltose	Sucrose	mannitol
1	L_{13}	A+/G+	A+/G-	A+/G-	A-/G-	A+/G+	A+/G-	A+/G-	A-/G-
2	L_{14}	A+/G+	A+/G-	A+/G-	A+/G-	A+/G+	A+/G+	A+/G-	A-/G-
3	L_{15}	A+/G-	A-/G-	A+/G-	A-/G-	A-/G-	A+/G-	A+G-	A-/G-
4	L_{16}	A+/G+	A-/G-	A-/G-	A-/G-	A-/G-	A+/G+	A+/G-	A-/G-
5	L_{17}	A+/G+	A+/G-	A-/G-	A-/G-	A-/G-	A+/G-	A+/G-	A-/G-
6	L_{18}	A+/G+	A+/G-	A+/G-	A-/G-	A-/G-	A+/G+	A+/G-	A-/G-
7	L_{19}	A+/G+	A-/G-	A+/G-	A+/G-	A+/G-	A+/G+	A+/G-	A-/G-

A+ (Acid positive); A-(Acid Negative); G+(Positive for gas production); G- (negative for gas production

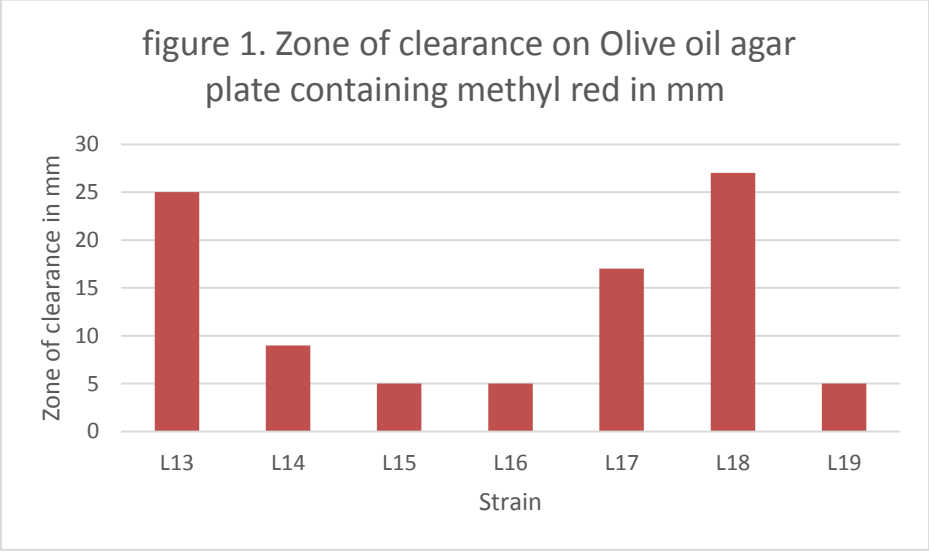

Fig. 1: Qualitative assay by Disc diffusion for lipolytic activity of different LAB isolates: L_{13}, L_{17} and L_{18} showing maximum Zone of clearance

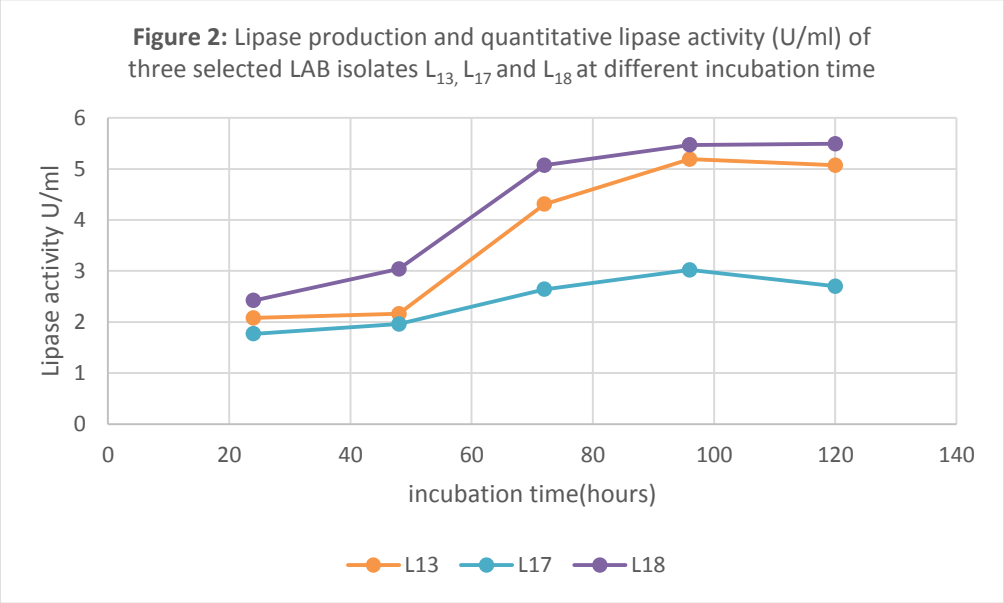

Fig. 2: Lipase production and quantitative lipase activity (U/ml) of three selected LAB isolates L_{13}, L_{17} and L_{18} at different incubation time

CONCLUSION

Result of this study indicate that, some Lactic Acid Bacteria were isolated from different source samples of dairy product collected have significant lipolytic activity. These LAB are identified as *Lactobacilli sp.*. Lipase producing ability is strain specific in regard with LAB. Lipase is one of the important enzyme used in Food industry and medicinal industry. In this context lipase production using microbial source gain much significance, it can be used in diet which confer health benefit.

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