



PRODUCTION AND CHARACTERIZATION OF LIPASE ENZYME FROM *LACTOBACILLUS*

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ABSTRACT

Lactobacilli are considered to be one of the most successes in production of lipase enzyme. Lipase is an enzyme the body uses to break down fats in food so that they can be absorbed in the intestine. It also allows cell nutrient and cell waste to move through the walls of the cells in our body. For the isolation and production of lactobacillus a curd sample is taken and isolation carried out by serial dilution plate agar method. *Lactobacillus* are found to be prominent on curd as a enriched media hence the sample was taken for the better result. The isolation of the bacteria *Lactobacillus* was carried on two media i.e. Nutrient Agar Media (NAM) and Lactobacillus Selective Base Agar (LBS), six strains were isolated i.e. LC1, LC2, LC3*, LC4, LC5, LC6. LC3* was taken as the main strain for the production of lipase enzyme from *Lactobacillus*. The isolated strains LC3 were identified by morphological and biochemical characterization to confirm as *Lactobacillus*. The *Lactobacillus* strain (LC3*) was selected for lipase production on LBS agar media. *Lactobacillus* strain LC3 gave maximum production of lipase i.e. 176IU/ml in selective media at 37°C of 48 hrs incubation and minimum in Nutrient broth i.e. 38IU/ml.

KEY WORDS: Lipase, *Lactobacillus*, Morphology.

INTRODUCTION

Enzymes are proteins that have catalytic functions indispensable to maintenance and activity of life. All chemical reactions occurring in a living organism are dependent on the catalytic actions of enzymes, and this is why enzymes are called Biotransformation. At present, there are about 4,000 kinds of enzymes whose actions are well known. Enzymes function as highly selective catalysis in such a way that they selectively catalyze specific reactions (reaction specificity) and specific materials (substrate specificity). (Garlapati et al., 2010).

A lipase is an enzyme that catalyzes the hydrolysis of fats (lipids). Lipase is produced by the pancreas, liver, intestine, tongue, stomach, and many other cells. In particular, *Candida albicans* has a large number of different lipases, possibly reflecting broad lipolytic activity, which may contribute to the persistence and virulence of *C. albicans* in human tissue. (Tan et al., 2003).

Lipase can hydrolyze ester bond of long chain fatty acid which is mainly component of oil. The sources of lipase enzyme are generally found in nature such as plants, animals, yeast, fungi and bacteria, for example, *Candida rugose*, *Fusarium oxysporum* f. sp. *Lin*, *Candida Antarctica*, *Rhizopus oryzae*, *Lactobacillus spp.* *Bacillus stearothermophilus* *Burkholderia* sp. Bacterial lipases are important enzymes applications in various industries,

because of friendly for environment, non-toxic and no harmful residues. For instant, there are widely uses in dairy industry and pharmaceutical industry, detergent and surfactant, taste or flavor industry, agricultural industry, chemical, cosmetic and perfume. (Loo et al., 2006).

Lactobacillus, area group of rod-shaped, Gram-positive, non-spore-forming bacteria of the family Lactobacillaceae, *Lactobacillus* is a type of bacteria. These are "friendly" bacteria that normally live in our digestive, urinary, and genital systems without causing disease. *Lactobacillus* is also in some fermented foods like yogurt and in dietary supplements. s. Various species of *Lactobacillus* are used commercially during the production of sour milks, cheeses, and yogurt, and they have an important role in the manufacture of fermented vegetables (pickles and sauerkraut), beverages (wine and juices), sourdough breads, and some sausages. (Boekema et al 2007).

Lactobacillus is used for treating and preventing diarrhea, including infectious types such as rotavirus diarrhea in children and traveler's diarrhea. It is also used to prevent and treat diarrhea associated with using antibiotics. (Azim et al., 2001).

In the last decades, the interest in microbial lipase production has increased, because of its large potential in

industrial applications as additives for foods (flavour modification), fine chemicals (synthesis of esters), waste water treatment (decomposition and removal of oil substances), cosmetics (removal of lipids), pharmaceuticals (digestion of oil and fats in foods), leather (removal of lipids from animal skins) and medicine. (Ramani *et al.*, 2010).

Lipases are employed *in situ*, and sometimes together with other enzymes, during the elaboration of bread, cheese, and other foods to improve their shelf-life and their rheological properties, or to produce aromas. Moreover, they are used *ex situ* to produce flavors, and to modify the structure or composition of acylglycerols by inter- or trans esterification, in order to obtain acylglycerols with an increased nutritional value, or suitable for parenteral feeding (Nadia *et al.*, 2010).

Lipase are used in pharmaceutical and agrochemical industries for the modification or synthesis of antibiotics, anti-inflammatory compounds, pesticides, etc., and for the production of enantiopure compounds or the resolution of racemic mixtures (Hasan *et al.*, 2006).

Technology for utilizing enzymes for the improvement of our life is a key feature of biotechnology. Enzymes are produced by microorganisms. These microorganisms can be modified to produce enzymes with much better yield properties and purity. Such GMMs (genetically modified micro-organisms) are however not part of the final enzyme product. After fermentation, the micro-organisms are inactivated and separated from the fermentation broth (containing the enzyme) by a series of separation / filtration steps. The enzyme itself is not - and cannot be - a GMM because enzymes are substances and not organisms. Enzymes can be produced from any living organism, either by extracting them from their cells or by recovering them from cell exudates. (Sebdani *et al.*, 2011).

MATERIALS AND METHODS

Collection of Raw Material

The raw material used for the production of lipase was curd sample, collected from local area of Dehradun (U.K.). The sample was collected in a sterile stainless steel container, brought to the laboratory and carried the isolation of *Lactobacillus*.

Isolation of *Lactobacillus* from curd sample

The *Lactobacillus* was isolated by serial dilution plate agar method from curd sample. Weigh 1g of the curd sample and inoculate in 9ml sterile distilled water (10^{-1}) and make the dilution up to 10^{-5} . Mix the sample in vortex shaker and transfer 1ml to second dilution and 1ml to sterile petri plates. Pour the sterile nutrient agar media, swirl in clockwise and anti-clockwise direction. Solidify the plates and incubated at 37°C for 24-48 hrs. After incubation observe for the growth of small, circular, creamish colonies of *Lactobacillus*.

Screening of *Lactobacillus*

The isolated strains were screened for the production of lipase enzyme. Prepare 250 ml of *Lactobacillus* selective agar base media in a conical flask. Sterilize at 15 p.s.i for 15 minutes. Cool the media and pour into sterilized petri plates. Keep the plates for solidification and then streak the isolated strains. Incubate the plates at 37°C for 24 hours. After incubation observe the plates for growth of large and whitish colonies of *Lactobacillus*.

Maintenance of culture

The isolated bacterial culture of *Lactobacillus* was maintained on nutrient agar media (NAM) slants and stored at 4°C in refrigerator.

Development of the inoculum and production of lipase from *Lactobacillus* in selective media (*Lactobacillus* selective broth)

For the development of inoculum 1 ml culture of *Lactobacillus* was transferred from stock to 100 ml sterile nutrient broth and *Lactobacillus* selective broth. For the production of lipase take 100ml of nutrient broth and *Lactobacillus* selective broth in a 250 ml conical flask. Sterilize the media at 15 p.s.i for 15 minutes, cooled and added 1% inoculum ($A_{410} = 0.5$) of *Lactobacillus*. Incubated the flask at 37°C at 150rpm for 72hrs and the lipase production was checked for every 24 hours.

Lipase enzyme assay

Lipase activity was assayed in the LBS broth by using *p*-nitro phenol as a standard curve. The LBS broth was centrifuged at 10,000rpm for 10 min and collect the supernatant. To the supernatant, lipase activity was carried out by using 0.05 M Tris-HCl buffer, pH 8.5. To 2.9 ml of Tris-HCl buffer (0.05 M, pH 8.5), added 60 µl of the substrate (*p*-NPP, 9 mM). Incubated the reaction mixture at 55°C in a water bath for 10 min in order to remove the turbidity and 40 µl of enzyme was added thereafter. The reaction mixture was again incubated at 55°C in water bath for 10 min. The reaction was stopped by chilling at -40°C. A standard curve of *p*-Nitrophenol was plotted at the selected concentrations (100-1000 µg/mL) of Absorbance 410nm of test sample.

One unit (U) of lipase activity was defined as amount of enzyme required to release one micromole of *p*-NPP from the substrate (*p*-NPP) per minute by one mL of the enzyme preparation.

RESULTS AND DISCUSSION

Isolation of *Lactobacillus* from curd sample

In the present study the isolation of *Lactobacillus* was successfully done by serial dilution plate method from the curd sample. 6 bacterial strains named as LC1, LC2, LC3, LC4, LC5 and LC6 were isolated on Nutrient agar media. The isolated strains were identified by morphological and biochemical characterization to confirm as *Lactobacillus*. The isolated strain LC3* was

confirmed *Lactobacillus* on the basis of biochemical test (Table,1&2).

Screening of *Lactobacillus*

The isolated strains LC3 were screened for lipase production on *Lactobacillus* selective base agar media. The strains gave large cremish colonies at 37°C of 48hrs incubation.

Media optimization of Lipase from *Lactobacillus* strains LC3

The *Lactobacillus* strains LC3 were further optimized for production of lipase enzyme on two media i.e. Nutrient agar and *Lactobacillus* selective base agar media. The *Lactobacillus* strains LC3 gave maximum production on *Lactobacillus* selective base broth i.e. 176 IU/ml at 37°C of 48hrs incubation in aerobic conditions and Nutrient broth gave 38 IU/ml at 37°C of 48hrs. The lipase activity was checked every 24 hrs (Table,4 & 5).

Table 1: Isolation of *Lactobacillus* from curd sample.

Strain No.	Morphological characterization of colonies			Gram Staining
	Color	Shape	Size	
LC1	Cream	Circular	Small	Gram +ve Cocci
LC2	Cream	Circular	Large	Gram +ve Cocci
LC3*	white	Circular	Small	Gram +cocco bacilli
LC4	Yellow	Irregular	Small	Gram +ve Cocci
LC5	Pale yellow	Round	Small	Gram -ve Rod
LC6	orange	Glistening	Large	Gram -ve Rod



Fig 1: Isolation of *Lactobacillus* from curd sample.



Fig 2: Screening of selective isolated strain LC3* from Curd sample for Lipase production on *Lactobacillus* selective base agar media.

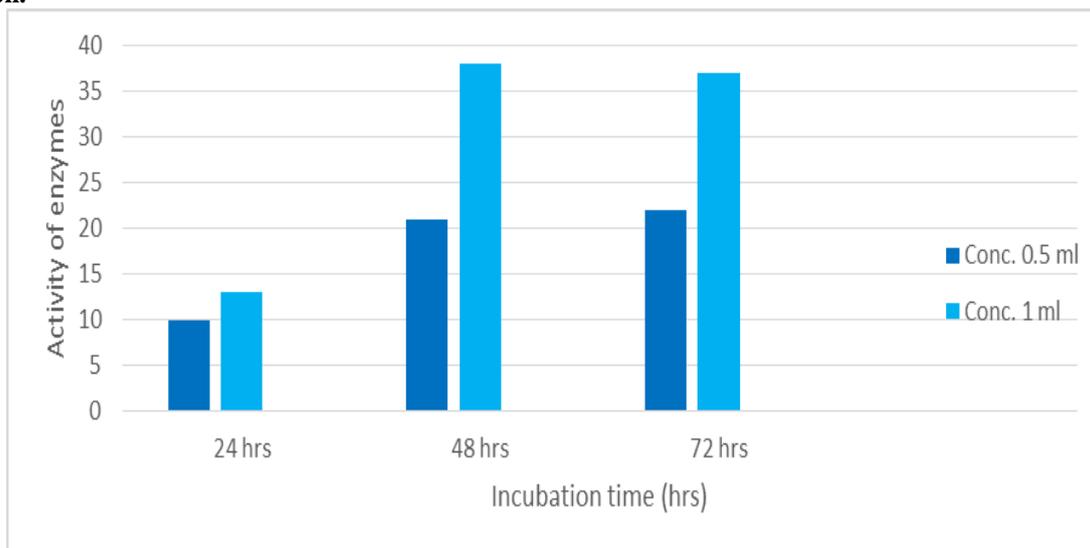
Table 2: Screening of selective isolated strain LC3* from Curd sample for Lipase production on *Lactobacillus* selective base agar media.

STRAIN NO.	COLOUR	LIPASE PRODUCTION
LC3	Whitish colonies	Presence of <i>Lactobacillus</i>
LC2	Cremish colonies	Absence of <i>Lactobacillus</i>

Table 3: Biochemical Characterization of isolated strains LC3 *Lactobacillus*.

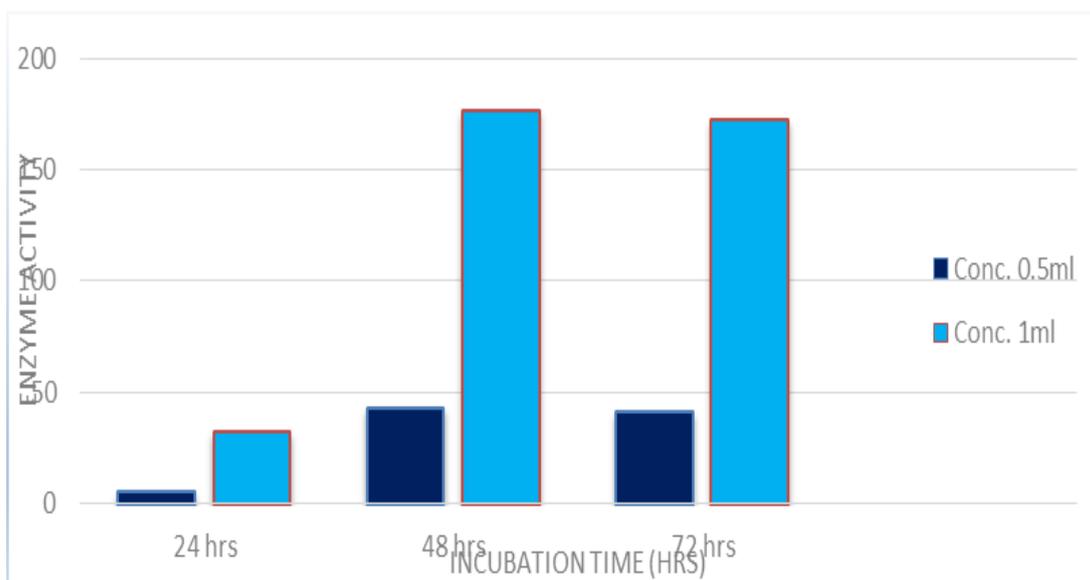
Strain No	Biochemical test				
	H ₂ S Production	Oxidase test	MR Test	VP Test	Indole Test
LC3	-ve	+ve	-ve	-ve	-ve
	Starch hydrolysis	Fermentation Lact., Dext., Sucr.	Citrate Test	Urease Test	Catalase Test
	-ve	+ve	-ve	-ve	-ve

Table 4: Production of Lipase enzyme from *Lactobacillus* strain(LC3*) cultivated in Nutrient Broth in aerobic condition.



Graph 1: Production of Lipase enzyme from *Lactobacillus* strain (LC3*) cultivated in Nutrient Broth in aerobic condition.

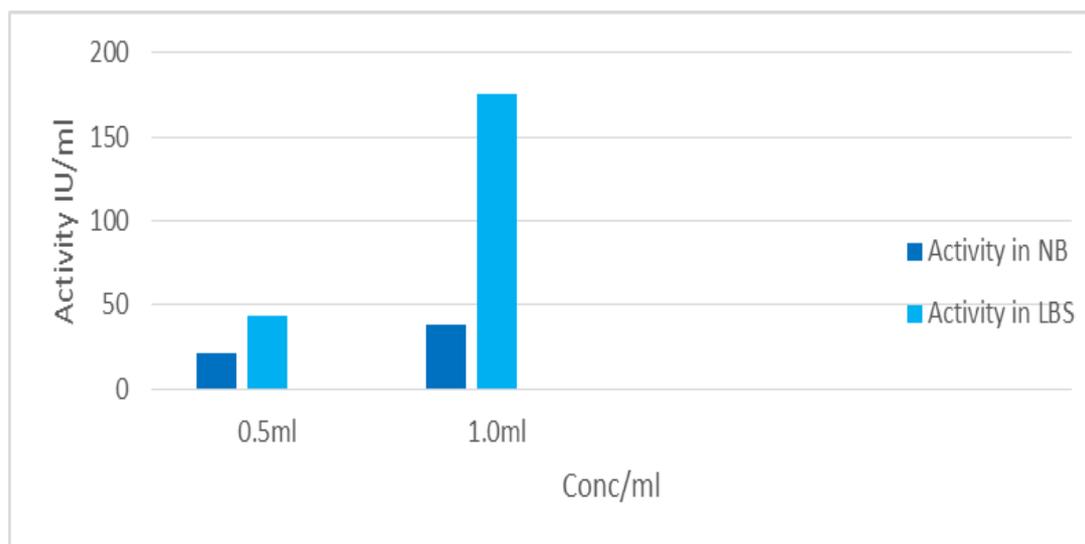
Table 5: Production of Lipase enzyme from *Lactobacillus* strain (LC3*) cultivated in Lactobacillus selective base Broth in aerobic condition.



Graph 2: Production of Lipase enzyme from *Lactobacillus* strain (LC3*) cultivated in Lactobacillus selective base Broth in aerobic condition

Table 6: Comparative study of Lipase enzyme activity from *Lactobacillus* strain (LC3*) cultivated in Lactobacillus selective base Broth and Nutrient Broth in aerobic condition.

The comparative study of production of lipase enzyme was carried out from *Lactobacillus* LC3 strain in aerobic condition of Lactobacillus selective base Broth and Nutrient broth at 37°C for 48hrs incubation time. The optimum density of lipase was measured at 410nm.



Graph: 3 Comparative study of Lipase enzyme activity from *Lactobacillus* strain (LC3*) cultivated in Lactobacillus selective base Broth and Nutrient Broth in aerobic condition.

CONCLUSION

In the present study main focus of the research was to evaluate maximum dairy residue for the production of enzyme because dairy residues are easily available and low in cost. The *Lactobacillus* strains LC3 was isolated from curd, screened and biochemically identified. The production of lipase enzyme was best on selective media in aerobic conditions. So the results from current study provided a basis for production enzyme and antimicrobial compounds by *Lactobacillus*. However there is a need of such more studies on the enzyme production by using new recombinant DNA technology for commercial scale enzyme production.

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