Optimization Of Lipase Enzyme From Novel *Staphylococcus Epidermis* Bacteria From Bakery Waste

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Abstract

Food production is a top priority issue in the world. Bakery products are an item of mass consumption in view of their low price and with rapid growth and changing eating habits of people. The bakery waste has a potential value for producing enzymes. Lipase producers have been isolated mainly from bread waste that contains oils, fats etc. In this paper, *Staphylococcus saprophyticus* bacteria was isolated from bread waste and screened for lipase activity. Parameters like pH, substrate, incubation time, metal ions, carbon source and nitrogen sources, detergents were used for optimization. Among all the parameters the maximum lipase activity of 5.01157U/ml was found in pH8.

Index terms: lipase, *Staphylococcus epidermis,* optimization,

1. Introduction

Food production is a top priority issue, as the lack of food for the continuously growing population is becoming an increasing problem in the world (Zvonko.B.Njezic et al., 2018). On the other hand large amount of food is wasted all around the world every year. So there is an urgent need to reuse them and convert it to useful products. Bakery wastes are a combination of different wastes, and their composition is thus highly variable (McGregor, 2000). Bakery wastes like breads that are removed from the food market become unsellable after but they can consist in any other ingredient like dough, flour , sugar and other edible ingredients. Leftover bread represents an environmental problem, but also a potentially valuable raw material for various purposes. So they can be used for producing enzymes like lipases and is a cheap source. Microbial lipases are enzymes which are obtained from microorganisms which include bacteria, fungi and yeast. They are the preferred potent sources due to several industrial potentials and are used in diary, food, detergent, textile, pharmaceutical, bakery, fat and oil, cosmetic and biodiesel industries (Saxena *et al.,* 1999; Jaeger and Eggert 2002). Microbial enzymes are often more useful than plants or animals enzymes because of the great variety of catalytic activities, their high yieliding and rapid growth of microorganism on inexpensive media. As bread waste is one among the cheapest source for lipase production , the aim of the present study is the production and optimization of lipase producing microorganisms from bread waste.

2. Material and Methods

2.1 COLLECTION OF SAMPLE

Bread waste was collected from a bakery (Kollam) in a sterile plastic bag and brought to the laboratory for further analysis.

2.2 ISOLATION OF LIPASE PRODUCING MICROORGANISM

The serial dilution of thesample bread waste was done in 5 test tubes and plated on tributyrin agar medium by spread plate method and incubated at 37°C for 24 h.

2.3 SCREENING OF STRAINS

The bacterial colonies were then screened by quadrant streaking for obtaining pure culture and the plates were incubated for 24 hours at 37°C .After incubation the colonies were characterized by gram staining and maintained on the nutrient agar slant.

2.4 CHARACTERIZATION OF LIPASE PRODUCING BACTERIAL CULTURE

The characterization of lipase producing bacterial culture was done by biochemical tests. Different Biochemical tests were performed to prove the identity of isolated bacterial culture. Catalase test, glucose fermentation test, mannitol fermentation test and Novobiocin sensitivity test were used for identification.

2.4.1 Catalase test: A loopful of culture was taken in a slide and a small drop of hydrogen peroxide was added to it.

2.4.2 Glucose fermentation test: Medium was prepared using Sugar (0.1g), Yeast extract (0.06g), Magnesium sulphate(0.024g), Dipotassium hydrogen orthophosphate (0.12g), Sodium chloride (0.6g), Ammonium dihydrogen orthophosphate (0.12g) and phenol red (0.01g) in 120ml distilled water. Durham's tube were added to each test tube containing the fermentation media and the culture was inoculated .The test tubes were incubated for 24 hours at 30-40 degree celsius.

2.4.3 Mannitol fermentation test: It was carried out using mannitol fermentation medium which contain Mannitol (0.1g), Beef extract (0.02g), Peptone (0.2g) Sodium chloride (0.1g), Bromocresol purple (0.0003g) in 20ml distilled water with a pH of 6.8.The test tubes containingmannitol fermentation media were inoculated with the culture and incubated for 48 hours.

2.4.4 Novobiocin sensitivity test: Bacterial culture was evenly swabbed on Muller Hinton agar using a sterile swab. One 5µgnovobiocin disc was aseptically placed and pressed firmly on the medium.The agar plates were then incubated for 24 hours at 37degree Celsius.

2.5 OPTIMIZATION

To increase the production and the productivity of the lipase enzyme, different physiological parameters were studied like pH, temperature, incubation time, carbon and organic nitrogen sources. The optimization of lipase production was carried on the distinction of physical and chemical parameters of production medium.

2.5.1 pH: Lipase production was optimized at different pH (5-9).The fermentation media were prepared having different pH such as 5, 6,7,8 and 9 inoculated by the bacterial cultures. The lipase activity was estimated and the optimum pH was selected for maximum lipase enzyme production.

2.5.2 Substrate: Different oils like olive oil, palm oil, coconut oil, sunflower oil and glycerol were used as substrate in the optimization media. The media was incubated at 37°C and the lipase activity was estimated.

2.5.3 Incubation time: Optimization of incubation period was done by the incubation of media for different times. The optimization media were prepared and inoculated with the isolated bacteria in conical flasks. The conical flasks were incubated for different time intervals such as 24, 48, 72 and 96 h.

2.5.4 Carbon source: Optimization media was prepared using Peptone (3g), Ammonium dihydrogen orthophosphate (0.3g), Magnesium sulphate (0.12g), Calcium chloride (0.12g), Sodium chloride (0.75g), palm oil (1ml for each conical flask) in 300ml distilled water.Six conical flasks with 50ml media were taken including control and with different carbon source which include glucose, maltose, mannitol, sucrose and starch.

2.5.6 Organic nitrogen source: Ammonium dihydrogen orthophosphate (0.3g), Sodium chloride (0.75g), Magnesium sulphate (0.12g), Calcium chloride (0.12g) were added to 300ml distilled water which was taken 50ml each in six conical flasks with 1ml palm oil .Each conical flasks had a different organic nitrogen source which include peptone, yeast extract, beef extract, meat extract and malt extract.

2.5.7 Lipase standard:Take 0.2 to 1 ml of the standard dilute to 100 ml with NaOH solution. Read the colour and draw the standard curve

2.6 ASSAY FOR LIPASE ACTIVITY

The incubated cultures were micro-centrifuged at regular intervals. 1ml of the supernatant was taken in test tubes and 500microlitres of triton-x and tris - Hcl were added to it.The tubes were incubated at room temperature for five minutes and pNPP was added.The pNPP solution was prepared by adding solution A (0.001g pNPPin1 ml isopropanol)into solution B(0.01g gum arabic,0.02g sodium deoxycholate). After two minutes of incubation the optical density of the sample is done using spectrophotometry at 400nm.

2.7 LIPASE ACTIVITY ASSAY:-

The crude enzyme was centrifuged and 1ml of the supernatant was taken in a test tube. 1ml of reaction buffer (500µl Triton X and 500µl HCl at pH 9). The mixture was then incubated at 25°C for 5 minutes. After that, 40µl of pNPP was added. The mixture was emulsified for 2 minutes and readings were taken using UV- Vis spectrophotometric analysis.

Lipase Enzyme Activity was found out by the equation:

$$\text{Enzyme Activity} = A \times V/t \times e \times v$$

Where A is the Absorbance; V is the total volume of mixture; t is the incubation time, e is the extinction coefficient and v is the volume of enzyme used.

3 RESULT AND DISCUSSION

Bread waste was collected from a bakery at Kollam in a sterile plastic bag and brought to laboratory for further analysis.

3.1 SERIAL DILUTION

The sample bread waste was serial diluted on five test tubes and spread plated.

3.2 SCREENING

The bacterial colonies from serial dilution were then screened by quadrant streaking and pure cultures were obtained. The lipolytic bacteria was isolated from bakery waste and identified as Staphylococcus epidermis according to Bergey'smanual. The bacterial strain was purified by sub-culturing on agar slant. Characterization of bacterial strain was done by various biochemical tests. The production of lipase activity by staphylococcus sps was screened on tributyrin agar medium which was incubated for 24 hours. *Bacillus* strains were screened for lipolytic activity on agar plates containing tributyrin or Tween 20 or Tween 80 (1%, w/v) and 2% agar-agar (Fakhreddine *et al*., 1998; Zinterhofer *et al*., 1973).

Gram staining was done for identifying the morphology and nature of the bacteria. It was identified as gram positive cocci as it retained the colour of crystal violet.

3.3 BIOCHEMICAL AND MORPHOLOGICAL CHARACTERISTICS

Catalase test, glucose fermentation test, mannitol fermentation test and novobiocin sensitivity test were performed for characterization of the bacterial isolates. The results are listed in the table 4.1.1 below.

3.3.1 Catalase test:

When hydrogen peroxide was added to the culture on slide, bubbles were observed as it positive to catalase test.

3.3.2 Glucose Fermentation Test:

Glucose fermentation test was negative as there was no color change from red to yellow.

3.3.3 Mannitol Fermentation Test:

Mannitol Fermentation Test was negative as there was no color change from violet to yellow.

3.3.4 Novobiocin Sensitivity Test:

Novobiocin Sensitivity Test gave positive result as it showed sensitivity zone around antibiotic disc. In a similar study conducted by Mohammed Badrud Duza and S A Mastan in 2013, lipolytic bacteria were

characterized upto genus level with the help of morphological and different biochemical characteristics. Gram's nature, bacterial colony characters, and motility of the organism were studied for morphological analysis with different biochemical tests.

3.4 OPTIMIZATION

Bacteria was inoculated into the nutrient broth for further characterization. The spectrophotometric reading were done using paranitrophenol standard graph at 400nm (Fig 2.1) and based on that readings were taken. The enzyme was most active between the pH range of 7-9.Maximum lipase activity was 5.011U/ml at pH 8.Constant lipase activity was found at pH9 with concentration 264 (Fig 4.2.2).

3.4.1 Effect of substrate on lipase production

The effect of substrate on lipase production was identified by adding different substrates which include sunflower oil, olive oil, glycerol, palm oil and coconut oil. The enzyme units were calculated and the maximum lipase enzyme activity obtained was 8.59U/ml which was produced when palm oil was used as a substrate on 4th day. Olive oil gave the next highest lipase enzyme activity.Constant lipase activity was a concentration of 184 which was shown by the medium with the substrate sunflower oil (Fig 4.2.3). In a similar study Narasimha *et al*., 2010 observed that olive oil induced the lipase production than the coconut oil.

3.4.2 Effect of incubation on lipase activity

In the present study palm oil induced lipase production than anyother substrates.

With increasing the incubation period lipase activity improved upto 24 hours there onwards declined inthe next 48 hours and showed the maximum lipase activity at 96hrs incubation (Fig 4.2.4).

3.4.3 Effect of carbon source

The effect of carbon source on lipase production was identified by adding glucose, maltose, mannitol, sucrose and starch as carbon sources, palm oil as the substrate, maintained a pH of 8 and was incubated for 96 hours.The optimum enzymeactivity observed was 4.01U/ml formaltose (Fig 4.2.5).

3.4.4 Effect of organic nitrogen source

The effect of organic nitrogen source on production of lipase was identified by adding yeast extract, beef extract, meat extract, malt extract and peptone as organic nitrogen sources,palm oil was the substrate and a pH of 8 was maintained which was incubated for 96 hours.Here, the optimum enzyme activity observed was 3.64U/ml for yeast extract (Fig 4.2.6).

3.5 Lipase assay

In a similar study V.K. Winkler, M. Stuckmann, J. Bacteriol., (1979) concluded that microbial growth was optimized by inoculating bacteria in an autoclaved medium that had pH varying from 5 to 10 by dissolving

components of the minimal medium in the buffer of desired pH. Temperature optimization was carried out by growing bacterial strains at temperature 32–40 °C in a shaking incubator. Effect of media components on lipase activity was measured using photoelectric colorimeter at 610 nm. In the present study the incubated cultures were microcentrifuged at regular intervals. 1ml of the supernatant was taken in test tubes and 500 microlitres of Triton-x and Tris Hcl were added to it. The tubes were incubated at room temperature for five minutes and pNPP was added. The pNPP solution was prepared by adding solution A (0.001g pNPPin 1 ml isopropanol) into solution B (0.01g gum arabic, 0.02g sodium deoxycholate). After two minutes of incubation the optical density of the sample is done using spectrophotometry at 400nm.

4. Legends

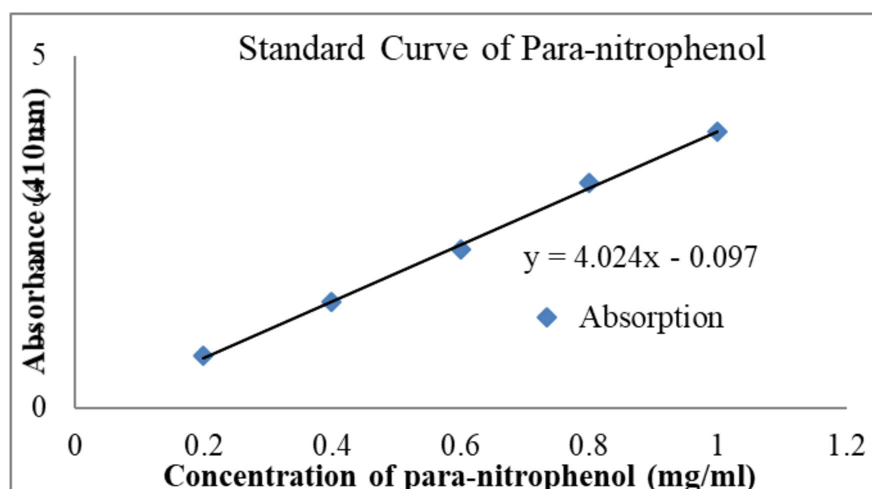
4.1 Tables

4.1.1 Biochemical test

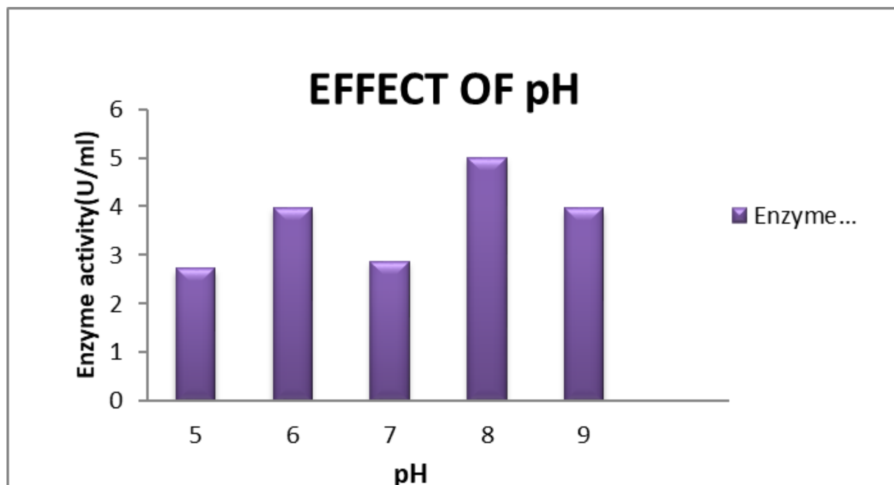
SL.NO.	PARAMETERS	RESULT
1	Gram nature	Positive
2	Morphology	Cocci
3	Motility	Non-motile
4	Catalase test	Positive
5	Glucose fermentation test	Negative
6	Mannitol fermentation test	Negative
7	Novobiocin sensitivity test	Positive

4.2 Figures

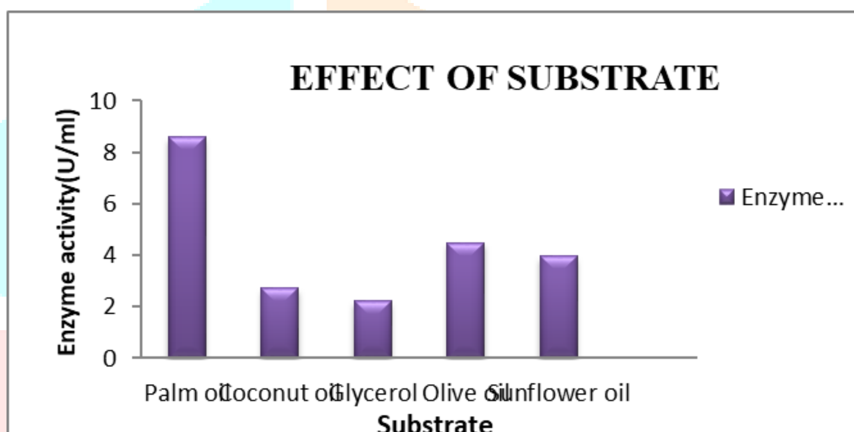
4.2.1 Standard graph of lipase



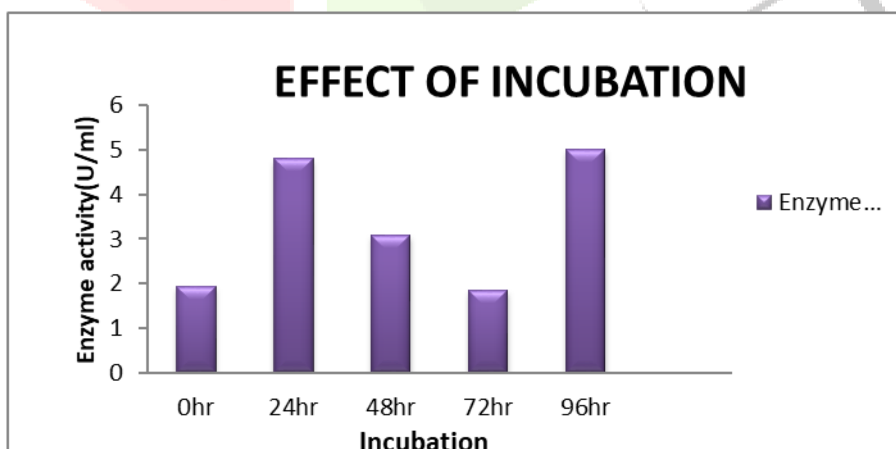
4.2.2 Effect of pH



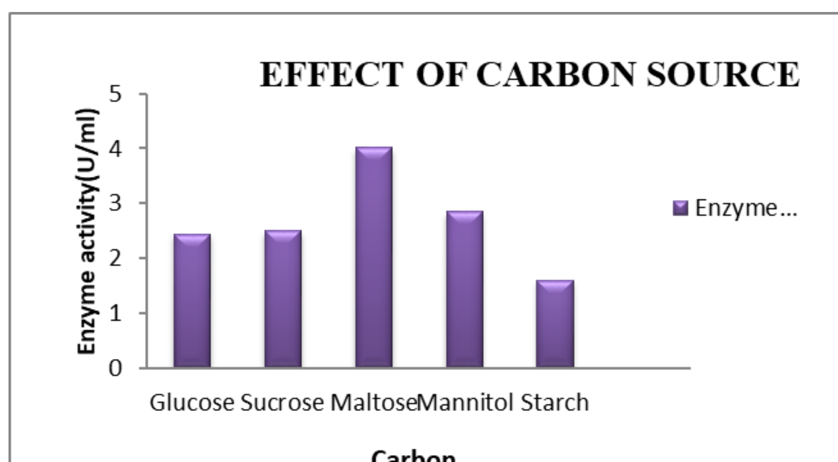
4.2.3 Effect of substrate



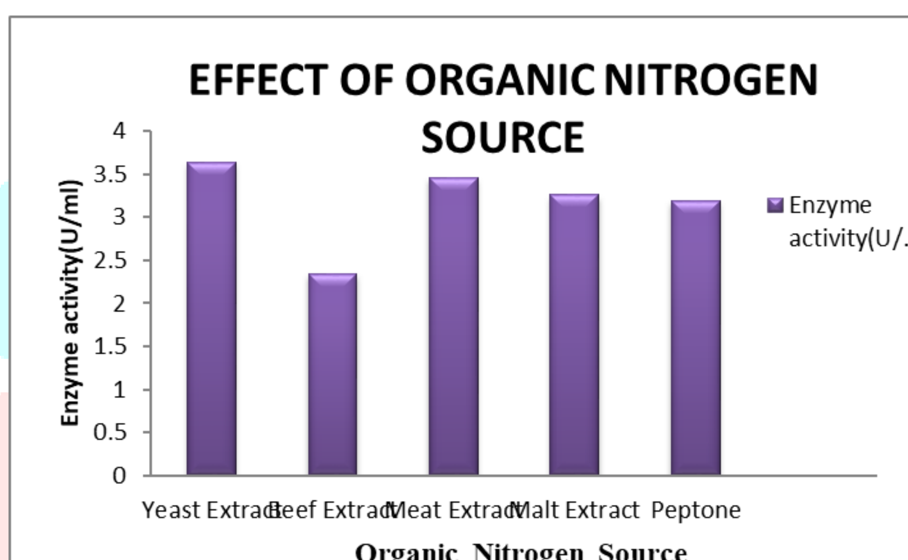
4.2.4 Effect of incubation



4.2.5 Effect of carbon source



4.2.6 Effect of nitrogen source



5. Conclusion

In the present study, optimization of lipase enzyme from novel *staphylococcus epidermis* from bakery waste was done. Bread waste was collected and serially diluted followed by screening and characterization.Characterization was done according to Bergey's manual which included catalase test, glucose fermentation test, mannitol fermentation test and novobiocin sensitivity test. From the above tests the organisms was identified as *Staphylococcus epidermis*. Optimization was carried out under five parameters which are pH, substrate, incubation time, carbon and organic nitrogen sources. High lipase activity was obtained at pH 8 in 96 hours of incubation. Palm oil was the substrate which had maximum lipase activity. The best carbon and organic nitrogen sources were found out to be maltose and yeast extract respectively. Since this study uses bread waste which is a cheap source it opens new horizons of experiments in the extraction of lipase.

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