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(RESEARCH ARTICLE)

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Isolation of bacteria producing lipase from copra (*Cocos nucifera* l.) Cake for degradation of vegetable waste oils

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Abstract

Vegetable waste oil consists of long chain triglycerides (LCTs). Waste oil from the kitchen causes clogging of the pipe as they solidify while passing from the kitchen. The waste with oil in the garbage will be drained into the ground and then into ground water which will lead to pollution of the ground water. Lipase is an enzyme used to break down the triglycerides into free fatty acids and glycerol by catalyzing the hydrolysis of the ester bonds in triglycerides. Bacteria Lipase is isolated from copra (*Cocos nucifera L.)* cake. The isolated bacteria were identified by the biochemical characterization and was sequenced by 16sRNA. The lipase production by the bacteria was identified by tween 80. The enzyme activity was identified by titrimetric method. Enzyme was extracted by salting out method and was partially purified by the SDS – PAGE.

Keywords: Lipase; long chain triglycerides; Cocos nucifera; *Staphylococcus epidermidis*

1. Introduction

1.1. Microorganisms

Microorganisms are ubiquitous. They can be found in the deepest ocean strata, in areas with high pollution levels, high air pressure, abnormally high temperatures, and both cold and high temperatures. Microbes are first living organisms and are able to play major role in the breaking down of carbon and energy sources and convert them into amino acids, nucleotides, vitamins, carbohydrates and fatty acids by producing specific enzymes. Microorganisms are employed because they can adapt to a wide range of environmental factors and produce certain enzymes. The food, pharmaceutical, textile, paper, leather, and other industries all use microbial enzymes extensively (Hasan, et al. 2006).

Their uses have been expanding quickly. Hydrolases, which include enzymes with a broad substrate specificity, are the most significant enzymes for industry. Carbohydrates, proteases, pectinases and lipases are classified into hydrolases. They catalyze the hydrolysis of natural organic.

1.2. Lipase

Lipase is an enzyme that breaks do triglycerides into free fatty acids and glycerol by catalyzing the hydrolysis of the ester bonds in triglycerides. Lipases were first discovered in 1856 by Claude Bernard in pancreas. Lipases are being extracted from several species of animals, plants, fungi, yeast and bacteria. Microbial lipases are biotechnologically important enzymes, because of their versatile properties (tolerance to extremes of pH, temperature, metal ions and organic solvents) (KONWAR, 2021).

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Exactly like lipases themselves, lipases have a variety of characteristics. The heat stability of this enzyme is certainly a desired trait. Bacteria are the primary source of thermally resistant lipases, and their structural makeup is related to their thermal toughness. pH and the presence of heavy metals are two factors that affect thermal stability. Every action taken at the level of protein engineering aims to increase the stability of the organism. Immobilizing lipases on substrates and in various types of carriers can significantly increase the resistance of many lipases (Celligoi et al., 2017).

1.2.1. Source of Lipase

Lipases are found in all prokaryotes including bacteria and archaea and eukaryotes including plants, animals and fungi. Microbial lipases are enzymes derived from plants and animals. Bacterial enzymes are more preferred over fungal enzymes because of their higher activities and neutral or alkaline pH optima. Bacterial lipase: A variety of lipases are produced by both Gram-positive. the most important Gram-negative genus is *Pseudomonas*. Gram-positive lipase producers are *Staphylococcus* (especially, S. aureus and S. hyicus), *Streptomyces* and *Bacillus* species. Among Gram-positive bacteria *Bacillus* is most widely used in industries (Jaeger et al., 1994).

1.2.2. Detection of Lipolytic microorganism

The lipase activity was detected by the growth of the microorganisms; production of lipase under suitable growth conditions; lipase activity (Shelley, et al. 1987).

1.2.3. Industrial Application of Lipase

In 2004, the industrial enzyme market was worth roughly $ 2 billion. By 2009, that market is expected to have increased to almost $ 2.4 billion. Due of their potential for biotechnology, lipases make up roughly 4% of this industry, and attention to them has grown (Hasan, et al. 2006).

In comparison to chemical catalysts, lipases as biocatalysts have a number of advantageous characteristics that make them suited for particular applications. Chemical processes are often more non- specific, whereas lipase-catalyzed reactions are highly specific due to their broad substrate specificity and high Regio and/or stereoselective characteristics. The synthesis of undesirable compounds in the waste stream is reduced or stopped than.

2. Materials and methods

2.1. Collection of the sample

The sample copra cake was collected from oil pressing mill gummidipoodi, Chennai.

Isolation and characterization of bacteria producing lipase:

Lipase producing bacteria was isolated by serial dilution of sample on the tributyrin agar. The composition of tributyrin agar (per liter) 5g peptone, 3g yeast extract,

10ml tributyrin and 15g agar. The selected colonies were sub cultured on the nutrient agar. The composition of nutrient agar (per liter) 5g peptone,3g yeast extract, and adjusted to pH 7 with 0.1 NaOH. The isolated bacteria were characterized by gram`s staining and biochemical test such as catalase test and coagulase tests were performed.

2.1.1. Screening of lipase production and confirmation

Tween 80 broth was prepared with the compositions Peptone-10g/L; NaCl

-5g/L; CaCl2·2H2O -0.1g/L; Tween 80 - 10ml/L and the overnight culture was suspended with deionized water and incubated in the broth for 24 hours. The turbid culture broth was centrifuged at 12000 rpm for 15minutes. Supernatant was collected and pellet was discarded. Tween80 agar was prepared and sterilized along with the peri plates in the auto clave. Cut the well with the help of sterile a cork borer and pour the supernatant with the help of pipette. Plates are Incubated at 37°C for 24 hours.

2.2. Assay for Lipase activity

2.2.1. The activity od lipase was estimated using titrimetric method:

10ml of 10% homogenized oil and 10ml of 10% gum acacia were added into conical flask using pipette.2ml of 0.6%cacl2 and 5ml of 0.2 mol/L phosphate buffer (pH-7) were also added. To this 1ml of crude enzyme was added. This Mixture

was incubated on an orbital shaker with 100 rpm for 37℃ for 1 hour. Reaction was stopped by addition of 20ml of acetone: ethanol (1:1) mixture. Each flask containing mixture is titrated against 0.1M NaOH using phenolphthalein as indicator until a pale pink color is appeared and the end point is recorded.

FORMULA

μ mol fatty acid/mL sample = [(mL NaOH for sample—mL NaOH for blank) x N x1000] ÷ volume of reaction mixture (ml)

Optimization of the lipase activity: The activity of lipase is determined at different pH (3,5,7,9,), temperatures (14,25,37,45), different time (24hours,48hours,72 hours and 92 hours) and shaking and non- shaking condition.

Partial Purification of the enzyme Lipase producing media of 20ml of the bacterial strain was taken an was centrifuged at 5000 rpm for 30 minutes. The supernatant was collected and the enzyme was concentrated with ammonium sulphate 0-100%. Fractionated enzyme was then subjected to dialysis process of partial purification with the help of dialysis membrane.

2.3. Molecular determination of lipase by SDS-PAGE

The partially purified lipase by dialysis and ammonium sulphate precipitation molecular weight is determined by SDS-PAGE.

3. Result and discussion

3.1. Gram`s Staining

Bacteria isolated from the copra sample was Gram positive. As the colonies were purple color and cocci shaped when the organism was stained and observed under the microscope (Fig.2)

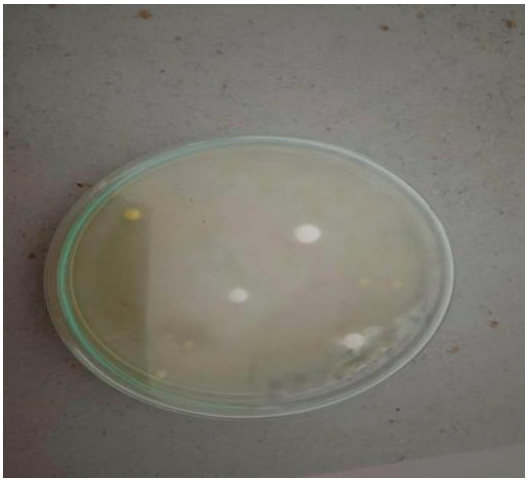

Figure 1 Isolated colonies

3.1.1. Isolation and characterization of bacteria from copra cake

The isolated colonies are obtained by serial dilution of the sample and plating it on the Tributyrin agar plates with 1%palm oil in it. The colonies are small in size, round in shape, pale yellow in color and few colonies were white and small, round shaped. The colony of interest were taken (pale yellow color colonies) were further characterized.

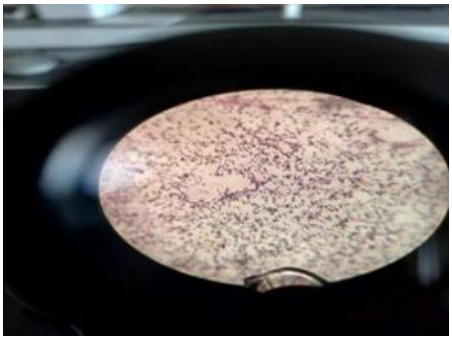

Figure 2 Gram-positive Bacteria

3.2. Catalase Test

This catalase test was positive as there was efflorescence when the organism was introduced into the 3% hydrogen peroxide (Fig:3).

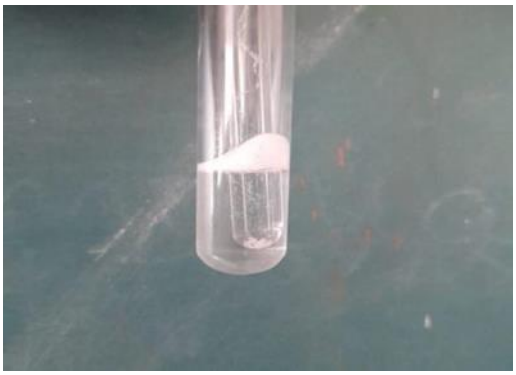

Figure 3 The foam development as a result of Catalase

3.3. Activity

3.3.1. Coagulase Test

This test was positive because when a small number of bacteria is added into the plasma then coagulation was observed.

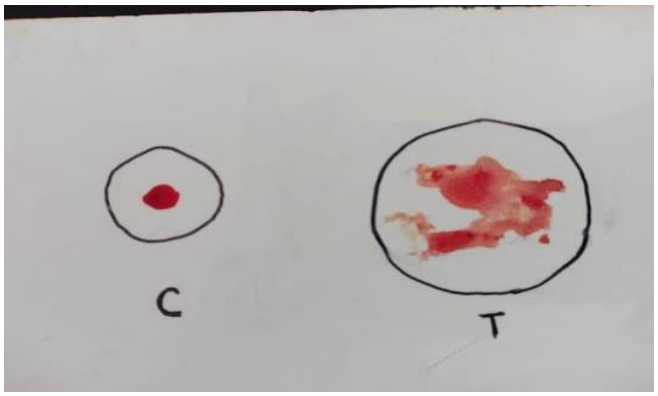

Figure 4 Blood clotting in test (T) as result of Coagulase activity

3.3.2. lipase production and confirmation by microorganism:

After incubating the broth medium of culture in tween 80 broth a white precipitate was formed around the zone confirms that the microorganism isolated is able to produce lipase enzyme.

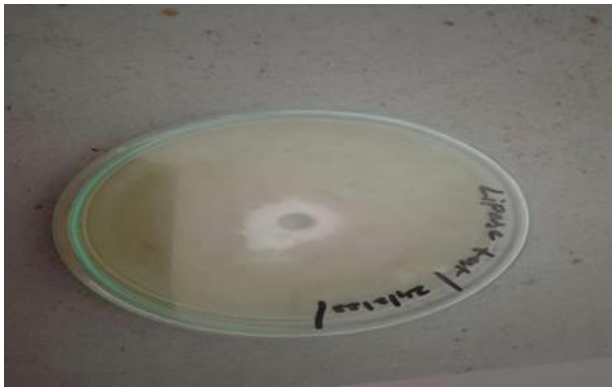

Figure 5 White precipitate around the zone

3.3.3. 16s rRNA sequencing

The bacterial species were confirmed by 16S rRNA sequencing. 16S rRNA was confirmed performing Polymerase chain reactions using primers. The NCBI`s BLAST programs index was used to confirm the sequence was 16S rRNA.

The isolated bacteria were identified as *Staphylococcus epidermidis.*

3.4. Effect of pH in lipase activity

Figure 6 Lipase activity determined by Titrimetric method

Table 1 Effect of pH

	pH 3	**pH 5**	**pH 7**	**pH 9**
DAY 1	37.5	45.7	47.5	45.71
DAY 2	80.5	84.64	88.92	82
DAY 3	51.45	64.37	80	78.33
DAY 4	41.07	52.12	62.08	47

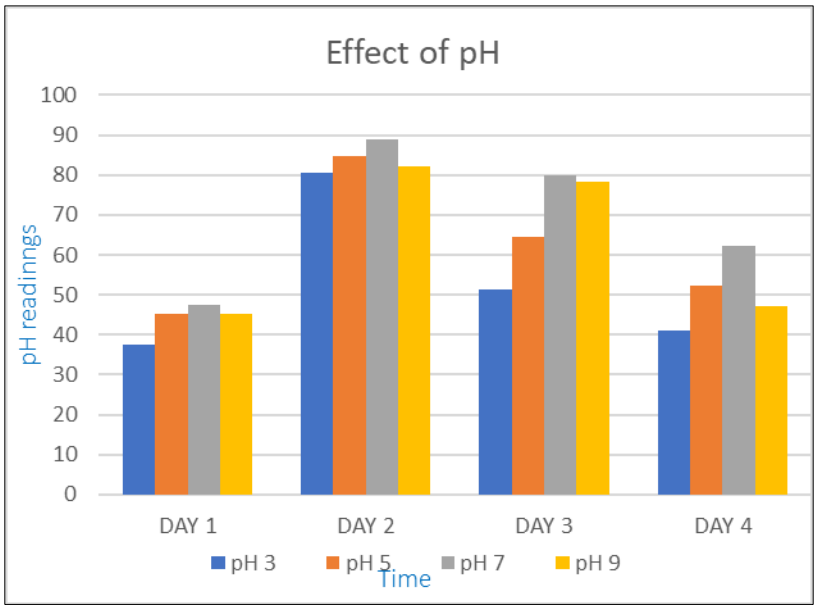

Figure 7 Effect of pH in lipase activity

This result show that the optimum temperature for maximum activity of lipase is at 37oC.

The effect of pH in lipase activity is optimum from the graph is pH 7. There is increase lipase activity seen in day 2.

3.5. Effect of temperature in lipase activity

Table 2 Effect of temperature

	14⁰C	**25⁰C**	**37⁰C**	**45⁰C**
DAY 1	22.34	36.34	53.76	47.74
DAY 2	23.75	73.75	85.04	72.09
DAY 3	36.25	51.08	75.79	62.70
DAY 4	25.8	62.70	73.08	64.98

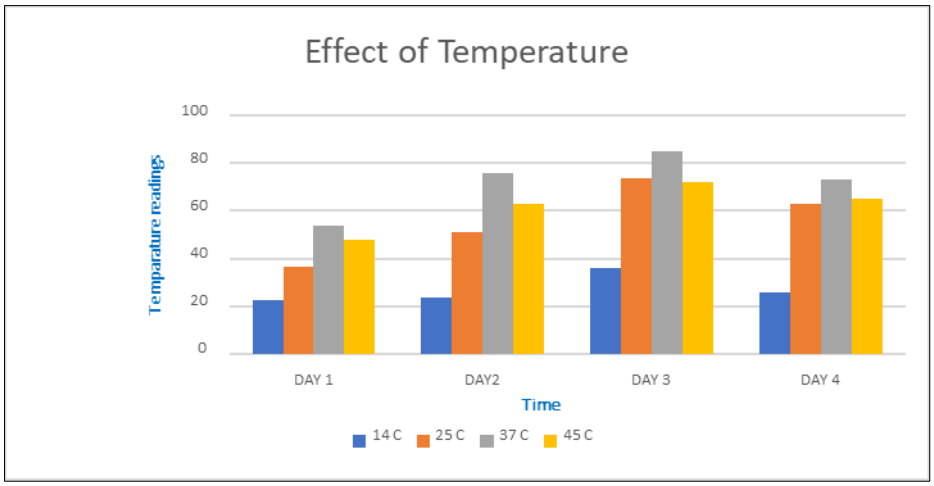

Figure 8 Effect of temperature in lipase activity

3.6. Effect of Shaking and Non- Shaking conditions

Table 3 Effect of shaking and non-shaking

	SHAKING	NON- SHAKING
DAY 1	34.8	18.4
DAY 2	39.79	23.95
DAY 3	43.7	24.37
DAY 4	47.6	25.6

Staphylococcus epidermidis shows optimum growth in shaking condition compared to the non-shaking condition.

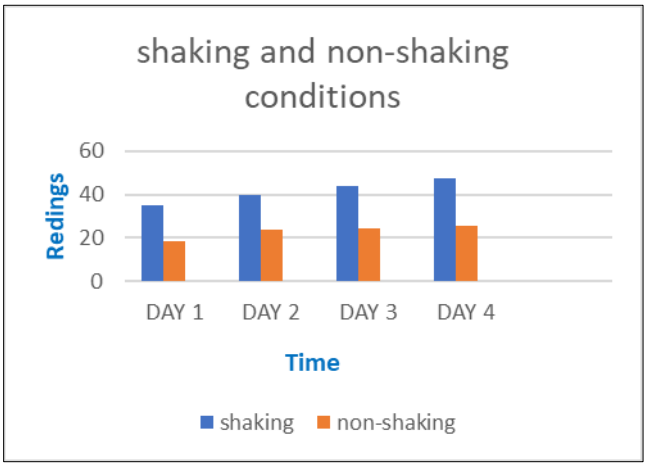

Figure 9 Effect of shaking and non-shaking condition in lipase activity

3.7. Partial Purification in lipase activity

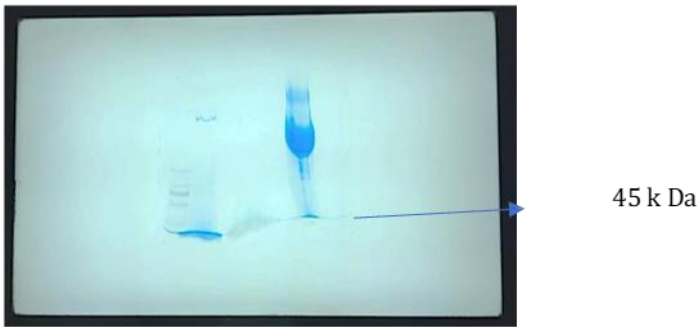

Figure 10 SDS PAGE

The crude enzyme was partially purified by precipitating 40% ammonium sulphate and dialysis process. The molecular weight of the sample was determined by SDS PAGE was 45 k Da.

Application studies: Degradation of vegetable oil. The hair was oiled and a nylon cloth was dipped in enzyme and the hair is wrapped in it. After incubation at 37oC, the degradation of vegetable oil was observed after a couple of days.

Figure 11 Degradation of the vegetable oil in hair

4. Conclusion

Lipase enzyme was isolated from the biological source (copra) for the degradation of the vegetable oil is important in industries and biotechnology. In this study the *Staphylococcus epidermidis* was isolated and the lipase activity was determined by titrimetric method. The lipase activity was checked in different conditions like pH, temperature, shaking and non-shaking conditions and optimal growth parameters were achieved. The enzyme was partially purified by salting out with ammonium sulphate precipitation and by dialysis. The applications were studied by degrading the oil with the partial purified enzyme. Lipase enzyme has been used for the many applications in industries like food, pharma, and detergent industries. Lipase produced from the *Staphylococcus epidermidis* which is copra is also cheap. Lipase has the many applications it has a very great impact in bioplastic production, pharma for drug delivery, in food industry to enhance flavor of food and also in production of biodiesel.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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