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Studies on lipase enzyme production by indigenously isolated *Bacillus Cereus (BAIT GCT 127002)* and *Bacillus Cereus (BAIT GCT 127001)*

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ABSTRACT

Microbial lipases holds a prominent place among biocatalysts that act on carboxylic ester bonds. This present study involves about eight strains isolated form slaughter house waste water,Coimbatore producing lipase.Among those strains , two bacterial strains exhibiting high lipase production were identified as *Bacillus Cereus (BAIT GCT 127002)* and *Bacillus Cereus (BAIT GCT 127001)* by both biochemical analysis and 16S rRNA sequencing. Initial studies were done for optimizing lipase production using many components such as time course,Carbon sources ,pH and innoculum volume which revealed maximum lipase acitivity (43.37 U/ml) at 24 hours, using palm oil , at pH 8 and innoculum volume of 1 ml by *Bacillus Cereus (BAIT GCT 127002)*.Further the percentage removal of oil from oil stained fabric was determined using partially purified lipase with or without detergents and the results indicated 20% ,30% removal with use of crude lipase and commercial detergent respectively. Hence, lipase from *Bacillus Cereus(BAIT GCT 127002)* can be regarded as an ideal ingredient that can be used in the laundry detergents.

Keywords

Bacillus Cereus,Microbial lipases,16s rRNA sequencing

Academic Discipline And Sub-Disciplines

Education; Life sciences; Biological Sciences

SUBJECT CLASSIFICATION

Biotechnology

TYPE (METHOD/APPROACH)

METHOD

1.INTRODUCTION

The exploitation of enzymes for plenty of purposes in various industrial fields dates back to the ancient civilization which has then initiated a broad commercial market for the current world. Enzymes are nature's catalysts because of their high specificity and economic advantage without any environmental impacts (Louwrier A. 1998).Their availability is through the fermentation of bio based materials (Godfrey T *et al*, 1996.,Wilke 1999).Microbial enzymes in comparison with the enzymes extracted from plants or animal origin are more in favor for commercial production because of its increased catalytic activity, specificity, thermal and pH stability and ease of genetic manipulation. In addition to all these advantages, Microbial enzyme production doesn't require expensive media for growth of microbes and ensure high yields in short time unlike plants and animals. Microbial enzymes finds wide applications inclusive of all the fields of scientific research, medical diagnostics, cosmetic production, chemical analysis, therapeutic applications and industrial catalysis for special synthesis. (Wiseman A. 1995)

Lipids represent a large part of the Earth's biomass. Lipids are not only the source of energy but also play an important role in structural roles in membranes and signaling activities in all living beings. Lipolytic enzymes are involved in the partitioning and mobilization of lipids within the internal structure of living organisms and also in the transfer of lipids from one organism to another (Beisson *et al* 2000). Lipases are classified as Serine Hydrolases based on their three-dimensional structure. In characteristic with serine proteases, lipases also have a catalytic trial composed of Ser-Asp (Glu)-His on its active site. Lipases are also called triacylglycerol lipases as they catalyze the hydrolysis of ester bonds of triglycerides and release glycerol and free fatty acids. (Winkler FK *et al* 1999, Jaeger *et al* 1993). They constitute a major group of biocatalysts with several unique characteristics such as the ability to display varying degrees of catalytic reactions upon several substrates, the chemo-specific nature which includes high stability towards extreme temperatures, pH, organic solvents and enantioselective and regioselective nature. These unique characteristics have caused its high demand in the commercial market for a wide range of applications and is of intensive interest for scientific research until today.

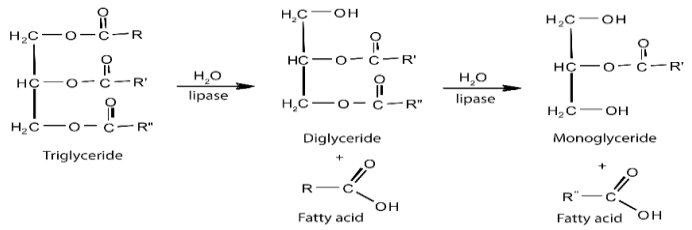


Fig:1-Schematic representation of fatty acid and monoglyceride formation using lipases

Lipases have been isolated and purified from fungal, bacterial, yeast, plant and animal sources. Out of all these, bacterial lipase production is fast, economical and efficient and show ease in mass production. Bacterial Lipases vary in physical, chemical and biological activities with each other. Unfortunately, lipase purification is a complex process and it depends on the structure and origin of lipase. Lipases are employed as additives in Detergent formulations, in cleaning solutions and for other applications in the Textile Industry. Bacterial lipases holds high potential in the textile industry for cloth processing because of its high efficacy and environmental concerns. Phosphate are used in synthetic washing agents which are known to pollute waste waters. Hence, the utility of enzymes such as lipases,esterases and amylases in detergent formulations allows laundering at lower temperatures,and thus reduces energy expenditure. (Davranov K *et al* 1994).

2.MATERIALS AND METHODS:

2.1.Materials:

Nutrient broth, Nutrient Agar, Tributyrin agar, Peptone, Yeast extract were purchased from Hi-media. Oil substrates such as palm oil, olive oil and sunflower oil were obtained from the purchased from the local market.

2.2.Organisms:

Eight bacterial strains were collected from Government College of Technology, Coimbatore, India which were isolated from slaughter house waste water situated in the ukkadam, Coimbatore.The bacterial strains were maintained by periodic sub culturing of samples in Nutrient Broth (NB) media (Hi-Media, Maharashtra, India.).

2.3.Screening of the Isolates for Lipase Activity:

The eight strains were examined for lipase production using Tributyrin agar base containing 3g/l of yeast extract, 5g/l of peptone, and 10ml/l of Tributyrin and 20g/l of agar with pH 7. A loopful of isolate were streaked on the plates,incubated at room temperature and observed for lipolysis after 48 hrs.The amount of lipase production was evaluated by measuring the width of clear zone that is formed due to hydrolysis of Tributyrin.The clear halozone diameters of the colonies were measured in millimeters(Davender Kumar *et al,* 2012).The plates showing maximum halos were considered as positive colonies and were selected for lipase production. Glycerol stocks of the strains were prepared using 50% glycerol and stored at -80^{o}C.

2.4.Culturing and Molecular Anaylsis of Isolates:

The two strains were examined for their morphological characteristics such as gram staining, shape, motility and biochemical analysis as per review of Vashist Hemraj et al, 2013.The results of the genomic analysis (16S rRNA

sequencing) for the bacterial identification using universal primers was obtained from Rajiv Gandhi Centre for Biotechnology.

2.5.Optimization of Fermentation Conditions:

2.5.1.Time period of lipase production:

The study on time course of lipase production was carried out with inoculum of 2% added to 100ml of nutrient broth medium in the 500 ml Erlenmeyer flasks and incubated at 150 rpm on a rotary shaker at 30ºC for 48 hrs.Samples were taken every 12 hrs interval and checked for its lipase activity by titration method. The optimized phase for the highest lipase production was recorded and used for further studies.

2.5.2.Effect of oil as carbon sources:

Different types of oils such as palm oil, sunflower oil, olive oil and Tributyrin were used as substrate along with peptone and yeast extract as nitrogen source .To emulsify the medium, tween 80 was used as surfactant. The estimation of the lipase activity was done on both intracellular and extracellular extract for both the bacterial strains.Sonicate the cell pellet to obtain intracellular extract and centrifuge the innoculted broth to obtain supernatant as extracellular extract. These extracts were used to estimate lipase activity by both titration and pNpp method.

2.5.3. Effect of Inoculum concentration:

Inoculum concentration for lipase production was studied by inoculating culture of different concentration varying from 0.5% to 2% in the optimized medium composition of carbon source. These concentrations were added to the medium containing 100ml of culture and incubated for 24 hours at 150 rpm on a rotary shaker at 30ºC.

2.5.4.Effect of pH on lipase activity:

The optimum pH for enzyme production was identified with different ranging from 7 to 9 without altering other parameters such as inoculum concentration, carbon source and time course. The inoculated broths were kept in a rotary shaker at 30ºC at 150 rpm.

2.6.Enzyme Assay:

Lipase activity was measured by titrimetric method using olive oil as substrate and was emulsified in 100mM potassium phosphate buffer with pH7 .100µl of enzyme was added to emulsion and was incubated for 30 min at 37ºC .The reaction was stopped using acetone: ethanol (1:1).The amount of fatty acids liberated estimated by titrating against 0.05M NaOH using phenolphthalein indicator(E. Sirisha N ,*et al* 2010)

Lipase activity (U/ml) can be calculated from the formula given below.

$$\text{Lipase activity (U/ml)} = \frac{\Delta V \times N}{V(sample)} \times \frac{1000}{30}$$

ΔV= V2 – V1,V1= Volume of NaOH used against control flask, V2= Volume of NaOH used against experimental flask, N= Normality of NaOH,V (Sample) = Volume of enzyme extract.Units of extracellular lipase activity were units per ml (U mL-1) while intracellular Activity was calculated in units per gram (U g-1). One unit of lipase activity was defined as the amount of enzyme that is liberated 1µmol fatty acid min^{-1} at 30ºC and pH under the assay conditions.

2.7.Protein Assay:

The secondary culture was prepared by inoculating with 2% of the overnight primary culture.The culture was centrifuged at 7000 rpm for 15mins. The supernatant discarded and the pellet was stored at -80^{0}C.The pellet was weighed and resuspended in phosphate buffer in the ratio of 5 ml/g of pellet. After resuspension, added lysozyme (100 mg/ml) and kept on ice for 30 mins.The sample was sonicated for 25 mins on ice at 20% amplitude with 9 sec on and off cycles and the sample was centrifuged for 30 mins at 14,000 rpm.Total protein content available in extracellular and intracellular was estimated using Lowry's method. Reagent A:2% Na_2CO_3 in 0.1 N NaOH, Reagent B: 1% sodium potassium Tartrate in H_2O ,Reagent C: 0.5% copper sulfate in H_2O.Both the reagents are mixed by adding 1ml of reagent B,1ml of reagent C, with 48ml of reagent A named as reagent I and reagent II composed of folin-phenol reagent. Add 0.5ml of sample to the 4.5 ml of Reagent I and incubate it for 10 minutes. After incubation,add 0.5 ml of reagent II and incubate for 30 minutes. Measure the absorbance at 660nm by UV-spectrophotometer(Lowry *et al* 1951*)*.

2.8.P-nitrophenol Assay:

The Solution A freshly prepared using 30 mg para-nitrophenyl palmitate in the 10 ml of isopropanol.This solution was emulsified. Solution B consists of 0.1g of Arabic gum,0.4 ml triton-100 emulsified in 1M phosphate buffer at pH 8.Then 2.25 ml of 1:10 dilution of solution A in solution B were pre incubated for 5min.Later 0.25 ml of lipase concentration was added and incubated at 30ºC for 30 min.The reaction was terminated by addition of 0.25 ml of 0.1 M Na_2CO_3 and 2.25 ml of distilled water. Released p-nitrophenol was determined by measuring absorbance at 410nm.One unit of lipase activity defined as the amount of enzyme that caused the release of 1 mole of p-nitrophenol from pNPPin 30 min(Bayoumi,R.A *et al* 2012).

Lipase activity (U/ml) can be calculated from the formula given below

$$\text{Lipase activity (U/ml)} = \frac{\mu molpNPreleased \times Totalvolumeofassay}{Timeofincubation \times Volofenzyme \times Volofsubstrate}$$

One unit of lipase activity was defined as the amount of enzyme that is liberated 1µmol fatty acid min^{-1} at 30ºC and pH under the assay conditions.

2.9.Partial Purification:

Solid Ammonium sulfate was added to 30 % saturation and allowed to stand for 30 min.The precipitate obtained was separated by centrifugation and resulting supernatant was further treated with ammonium sulfate at 50% saturation. Finally it was centrifuged and the precipitate was resuspended in a minimal amount of buffer (50mM Tris HCL at pH 7.0and dialyzed against the same buffer by using successive large volume of buffer. The process was continued till the last trace of ammonium sulphate was removed. The desalted fraction was subjected to protein and enzyme activity assay.

3.APPLICATION OF CRUDE LIPASE IN TEXTILE:

3.1.Pretreatment of Fabric Cloth:

Cotton fabric (5-10 cm) was stained by spotting with 0.5 ml of olive oil: benzene mixture. The quantity of the olive oil on the soiled fabric was100 mg. (C. Hemachander et al.,2000).

3.2.Preparation of Washing Buffer:

The washing solutions were prepared as shown in Table 2. In the composition of buffer, detergent and lipase, 0.05 M Tris HCL buffer (pH7) and detergent solution (1% concentration) were preincubated at 37°C for 10 min followed by the addition of 3ml of lipase. Buffer–lipase, buffer–detergent and buffer were prepared in the same manner and the volume of the final solution was adjusted to 100 ml by adding distilled water(C. Hemachander et al.,2000).Five pieces of the soiled fabric were put into the conical flask containing the washing solutions. The composition was prepared with 1% concentration of detergent (Wheel) and crude lipase enzyme(Table 2).

3.3.Washing Protocol:

Stained fabric was weighed before treatment and washed at 100 rpm for 30 mins.After 30 min of washing, the fabric was rinsed twice with distilled water. The fabric was then air-dried and weighed to check the removal percentage of olive oil stain using different washing solution(C. Hemachander et al.,2000).

Determination of lipase and detergent activity in removal of strain

$$\% \text{ not removed} = \frac{Wb - wa}{wb}$$

% Removal = 100 - % not removed.

Where

$W_b$is the weight of fabric cloth before washing, and

$W_a$is the weight of fabric cloth after washing.

4.RESULTS AND DISCUSSION:

4.1.Screening of Lipolytic Bacteria:

Among the eight strains,two strains showed maximum zone of clearance and glycerol stock was prepared to preserve the strains. It was inferred that maximum the lipase production maximum the zone of clearance due the hydrolysis of Tributyrin oil into glycerol and fatty acids.The zone of clearance was increased from 0.5 cm to 1 cm as incubation time increases from 4 th day to 6thday by strain 1 (fig 1) and whereas in figure 2 shows zone of clearance of 0.6 cm by strain 2 at 4 th day with no further increase in the zone.

4.2.Identification of Lipase Positive Strain by Biochemical Analysis and 16S rRNA Sequencing:

Biochemical analysis confirmed that both the selected bacterial strains were *Bacillus sp.*,(Vashist Hemraj et al., 2013).Further analysis was done by sequencing 16S rRNA of both strain 1 and strain 2 which showed 100% similarity to *Bacillus cereus* in the BLAST research .Then the sequence of the candidate bacterial strain Bacillus strain was submitted to National Centre for Biotechnology (NCBI).

4.2.1.Sequence of Strains:

Strain 1(Bacillus Cereus (BAIT GCT 127002) >SR490-1-16S

TTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAAT
ACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCG
CATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACT
GGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGG
AGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATA
AGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGG
CAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTC
AACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAA
TGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCC
CTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGA
CGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATC
CTCTGACAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTG
TCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAA
GGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACAC
ACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTCG
GATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGA

Strain 2(Bacillus Cereus (BAIT GCT 127001) >SR490-2-16S
TTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAAT
ACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCG
CATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACT
GGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGA
GCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAA
GCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGC
AAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAA
CCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATG
CGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGG
GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTT
TAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGG
GGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGA
CAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGA
GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACT
GCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCT
ACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAG
GCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGA.

4.3.Optimization of Fermentation Conditions:

4.3.1.Effect of Time Course:

The enzyme production was governed by the growth rate of culture. In this study the production of lipase starts in log phase and reaches maximum activity at 24thhour. The two strains,strain 1 and starin 2 are *Bacillus Cereus (BAIT GCT 127002)* and *Bacillus Cereus (BAIT GCT 127001)* respectively showed less lipase activity during 12 hours and 48 hours of incubationcompared to that of 24 hours of incubation.When compared to *Bacillus Cereus (BAIT GCT 127001)*,lipase produced by *Bacillus Cereus (BAIT GCT 127002)* resulted in maximum lipase activityof about 183 U/ml (titration method) at 24hours of incubation(graph 1).

4.3.2.Effect of Carbon Source:

Lipase production is dependent by the type and concentration of carbon source and nitrogen source,the culture pH and inoculum concentration. Studies conducted on the effect of oil as carbon sourcefor lipase production .Olive oil, Sunflower oil, palm oil and Tributyrin oil were used along with nitrogen source such as peptone and yeast extract for producing lipase. It was reported that palm oil (140 U/ml by titration method, 43.36 U/ml by pNpp assay) was found to exhibit maximum lipase activity and protein concentration(3.19 mg/ml)(graph 2) by strain 1 -*Bacillus Cereus (BAIT GCT 127002)*. Its activity was checked with pNppassay(graph 3) and titration method by titrating against 0.1 N NaOH(Table 4).
The partially purified enzyme showing maximum activity by titration method (table 4) were selected to determine its extracellular and intracellular lipase activity by pNpp assay . *Bacillus Cereus (BAIT GCT 127002)* showed maximum lipase activity (43.36 U/ml) using palm oil as substrate.

4.3.3.Effect of Innoculum Volume:

For lipase production *Bacillus Cereus (BAIT GCT 127002)* was chosen and checked for its optimum inoculum volume with increased lipase activity. This present study conducted by varying inoculum volume from 0.5 % to 2 % by keeping carbon (palm oil) and nitrogen source as constant.By using 0.5 ml, 1ml and 2 ml of inoculums volume from 100 ml of culture, the concentration of protein was found to be 4.29 mg/ml, 4.72 mg/ml and 1.42 mg/ml (Graph 4) and lipase activity was found to be 16.8 U/ml, 40.5 U/ml and 40.4 U/ml respectively(Graph 5) .

4.3.4.Effect of pH:

pH of the medium plays an important role in production of enzyme. The present study inferred that at pH 8 *Bacillus Cereus (BAIT GCT 127002)* shows higher protein concentration (5.16 mg/ml) compared to pH 7 (3.38mg/ml)and pH 9 (4.58mg/ml).Graph 6 shows that optimum protein concentration was found to occur at pH 8.pNpp assay was conducted with different pH range from 7-9.At pH 7, pH 8and pH 9, Lipase activity was found to be18.5 U/ml, 41.2U/ml and 33.7U/ml respectively. Graph 7 shows the optimum lipase activity at pH 8.With all optimum conditions lipase production resulted in maximum lipase activity of about 43.37 U/ml.

*4.3.5.Effect of Crude Lipase In Laundry***:**

Lipase enzyme considered as an ideal detergent to improve the removal of fatty food stains from fabrics which are difficult to remove under normal washing conditions.This study was done on cotton fabric using crude lipase and detergent (commercial product -wheel)which showed better results with 20%, 30% and 29% removal of oil stain by crude lipase ,detergent and crude lipase+ detergent respectively(Table 5).

TABLES AND FIGURES:

Table 1: Composition of medium used for bacterial growth for effective lipase production.

Types of oil	Medium 1	Medium 2	Medium 3	Medium 4
Olive oil	0.1 %	-	-	-
Sunflower oil	-	0.1%	-	-
Palm oil	-	-	0.1%	-
Tributyrin	-	-	-	0.1%
Peptone	5%	5%	5%	5%
Yeast extract	3%	3%	3%	3%

Table 2: Composition of washing solution.

Washing solution	Volume (ml)			
	Buffer	Lipase	Detergent	Water
Buffer	40	-	-	60
Buffer + crude Lipase	4	3	-	57
Buffer +Detergent	40	-	50	10
Buffer + Detergent + crude Lipase	40	3	50	7

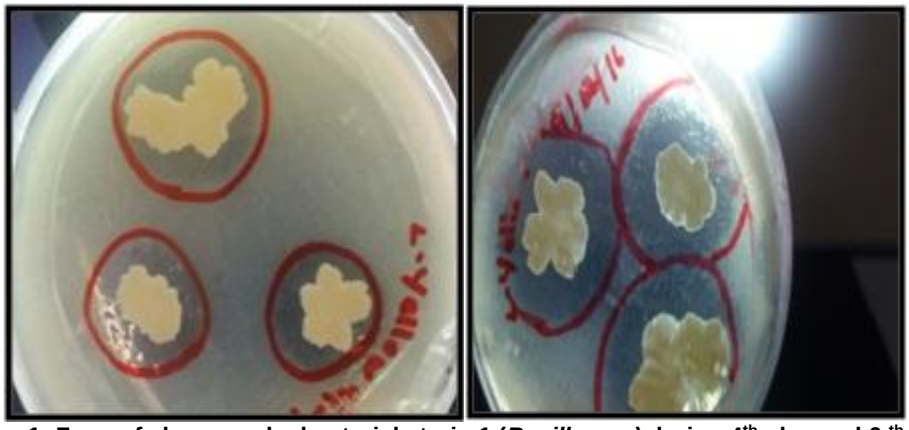


Figure 1: Zone of clearance by bacterial strain 1 (*Bacillus sp)* during 4th day and 6 th day.

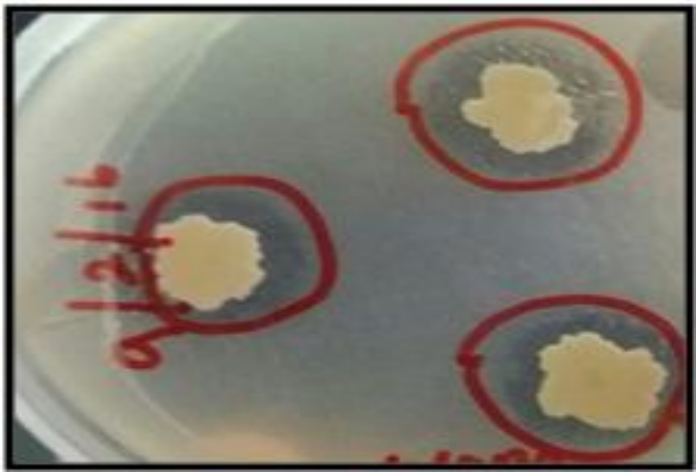


Figure 2: Zone of clearance of bacterial strain 2(*Bacillus sp.,)* during 4th day.

Table 3: Biochemical analysis of the two strains

Tests	Strain 1	Strain 2
Gram's staining	Positive	Positive
Motility	Positive	Positive
Tyrosine	Positive	Positive
Citrateutilization test	Positive	Positive
Urease test	Negative	Negative
VOGES-PROSKAUER	Positive	Positive

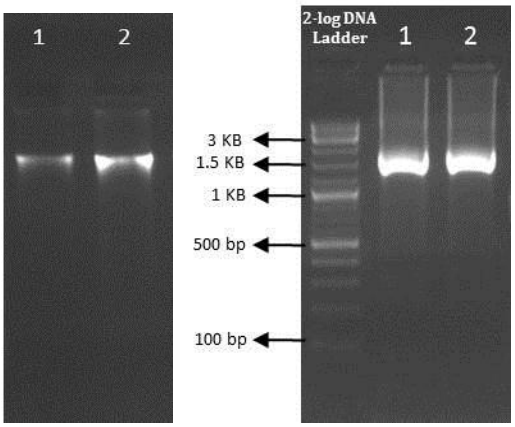
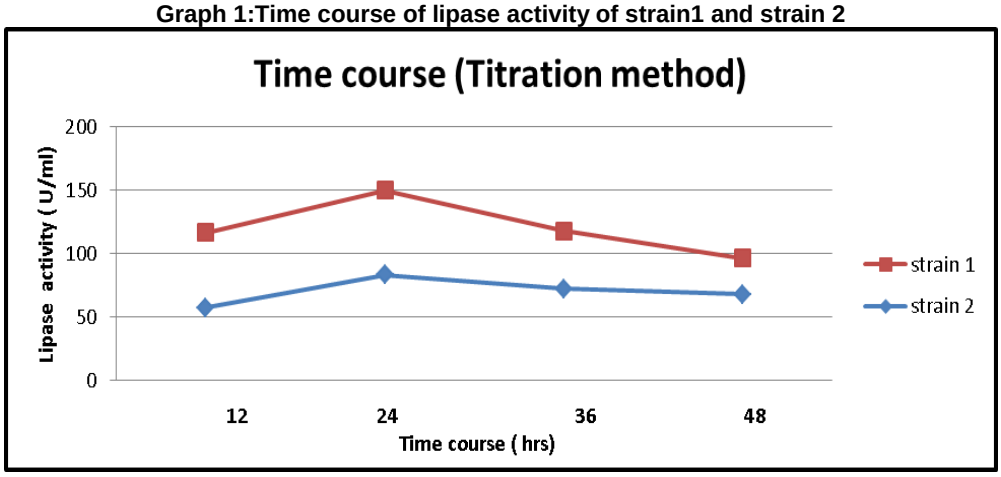


Fig 3:Agarose Gel electrophoresis of PCR products.Lane 1 showing strain 1(*Bacillus Cereus (BAIT GCT 127002)* and Lane 2 *Bacillus Cereus (BAIT GCT 127001).*

Graph 1:Time course of lipase activity of strain1 and strain 2



Graph 1:Protein assay for carbon source for Strain 1 *Bacillus Cereus (BAIT GCT 127002)* and 2 *Bacillus Cereus (BAIT GCT 127001).*

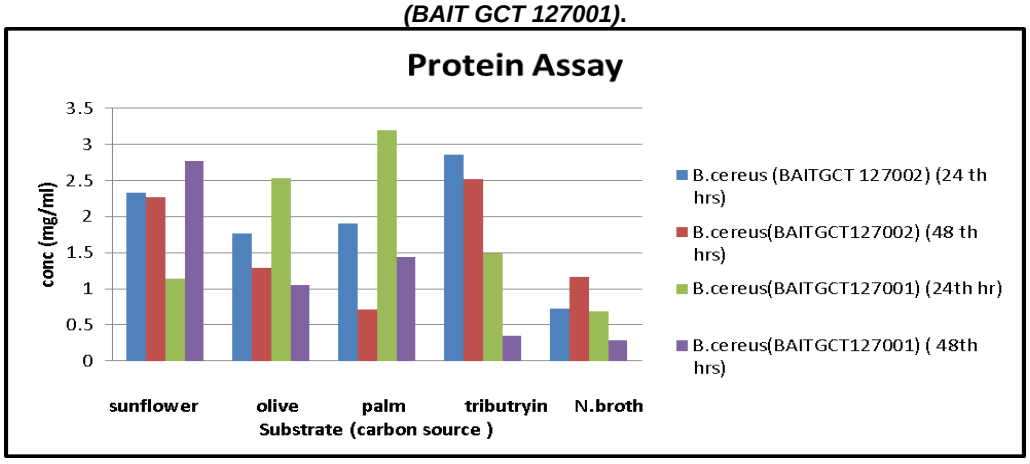
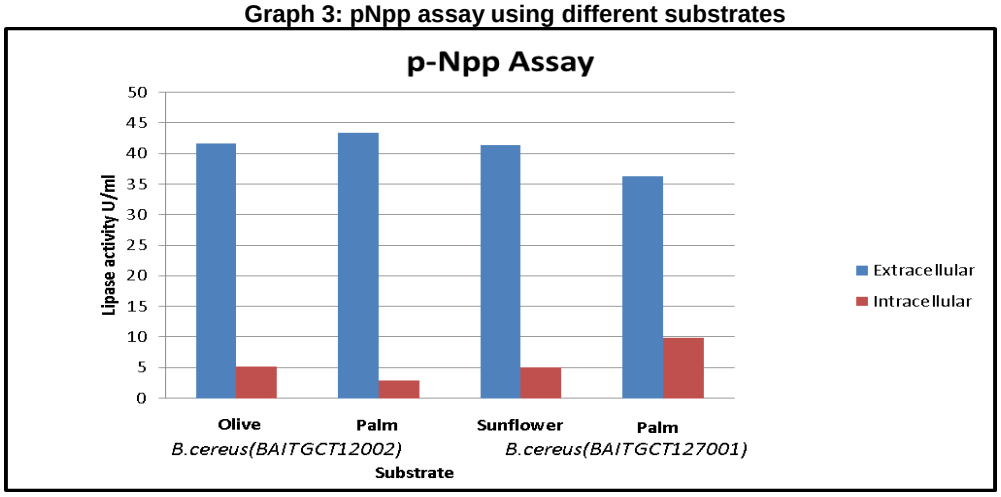


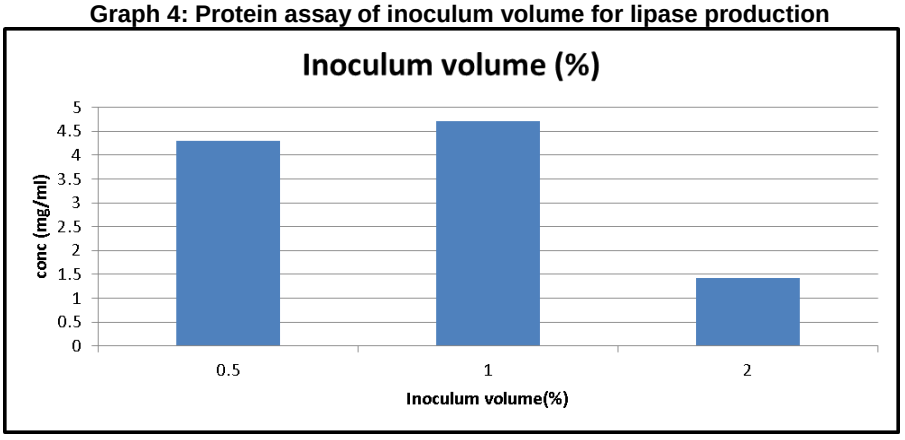
Table 4:Lipase activity of intracellular and extracellular extract by titration method.

TYPES OF OIL	*B. cereus (BAITGCT127002)* (U/ml) 24 th hour	*B. cereus (BAITGCT127001)* (U/ml) 24 th hour	*B.cereus (BAITGCT127002)* (U/ml) 48 th hour	*B. cereus (BAIT GCT127001)* (U/ml) 48 th hour
Nutrient broth	83.32	66.6	68	28.4
Tributryin	50	83.32	44.2	22.6
Olive oil	143.2	66.6	83.32	42.2
Sunflower oil	66.6	100	64.4	50
Palm oil	145	116.6	133.2	83.33

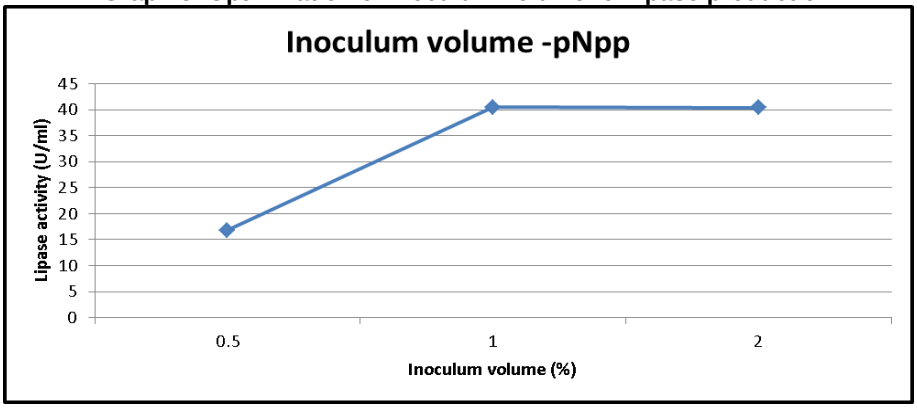
Graph 3: pNpp assay using different substrates



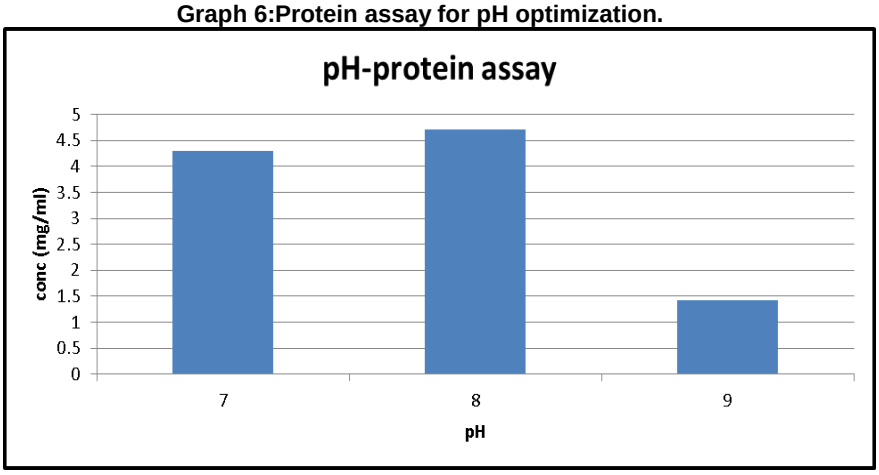
Graph 4: Protein assay of inoculum volume for lipase production



Graph 5: Optimization of inoculum volume for lipase production



Graph 6:Protein assay for pH optimization.



Graph 7: Optimization of pH for lipase production

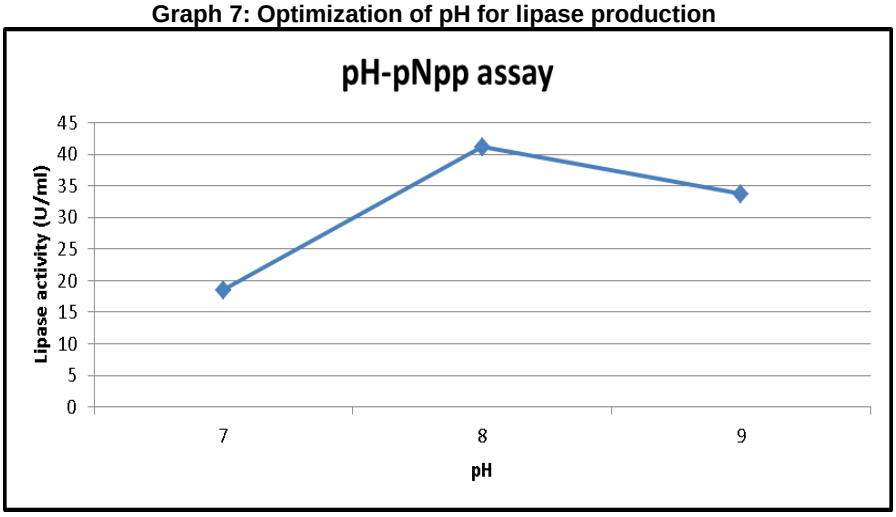


Table5 : Effect of crude lipaseon stained cotton fabric.

Washing solution	% Removal
Buffer	5
Crude Lipase + Buffer	20
Detergent + Buffer	30
Crude Lipase + Detergent +Buffer	29

5.CONCLUSION:

In this study, we observed that the total of 8 strains isolated from slaughter house waste water, produced lipase. Out of the eight strains, *Bacillus cereus (BAITGCT127002)* and *Bacillus cereus (BAITGCT127001)* were found to show large zone of clearance in the tributyrin agar. Analysis of Lipase activity, *Bacillus cereus (BAITGCT127002)* was observed to have maximum lipase activity of about 43.37 U/ml. Therefore this strain was chosen for optimization. The lipase production of *Bacillus cereus (BAITGCT127002)* was optimized with carbon source (palm oil) , pH 8 and inoculum volume 1 ml. Further study was done using crude lipase to check its efficiency to remove oil stain from fabric. This study produced good result with 20% removal of oil stain by crude lipase. .In the future, we will characterize factors of enzyme and application of lipase from *Bacillus cereus (BAITGCT127002)*for laundry and other applications also.

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Author' biography with Photo

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