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Production, purification and characterization of Lipase isolated from salt water *Halobacterium sp*

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ABSTRACT: *Halobacterium* are salt loving microorganism, which thrives in the salt lake, and seawater where there is an elevated salt concentration. They have the capacity to balance the osmotic effect of the outside environment and resist against the dehydrating effects of the salts. Their novel characteristics and capacity for large scale culturing make *Halobacterium* potentially valuable for biotechnology. In the present study the *Halobacterium* sp. was isolated from seawater sample and from paddy field nearer to seashore they are characterized by morphological and biochemical characteristics. The *Halobacterium* sp. was subjected to screening for enzyme production like amylase, cellulose, lipase and protease. Lipase enzyme was produced in the laboratory scale it was purified by dialysis method and using ion exchange chromatography. The enzyme activity and concentration were determined using the standard laboratory protocols. The enzyme kinetics studies were also carried out along with the SDS-PAGE using BSA as a standard marker and it was found out that the obtained lipase enzyme was 66 KD in its molecular weight.

Keywords: Seawater - Halobacterium, - lipase enzyme Production and Characterization – determination of molecular weight.

I. INTRODUCTION

Halo bacterium is salt – loving microorganisms that inhabit hypersaline environments [1]. These bacteria has a unique feature of possessing the purple membrane, specialized regions of the cell membrane that contain a two dimensional crystalline lattice of a chromoprotein, bacteriorhodopsin They have the capacity to balance the osmotic pressure of the environment and resist the denaturing effects of the salts [2]. They are able to live in salty conditions by preventing dehydration of their cytoplasm. They do this by either producing large amounts of an internal organic solute or by concentrating a solute from their environment [3]. *Halobacterium* is motile spore producing aerobic microorganism found distributed all over the world in hypersaline environments, many in natural hypersaline brines in arid, coastal, even deep sea locations, as well as in artificial salterns used to mine salts from the sea. Their novel characteristics and capacity for large scale culturing make *Halobacterium* potentially valuable for biotechnology [4]. The genus *Halobacterium* consists of several species of archaea with an obligate aerobic metabolism which require an environment with a high concentration of salt [5] .

A lipase is a water-soluble enzyme that catalyzes the hydrolysis of ester bonds in water–insoluble, lipid substrates. Lipases are ubiquitous throughout living organisms, They have considerable physiological significance and industrial potential, and are being employed for the synthesis of drug intermediates and pharmaceutically important molecules. The lipases used for various purpose are usually fungal or bacterial origin [6]. They are produced as an extra-cellular product by the bacterial species and attract industrial attention. The activity of the enzymes in general appears to be stabilised by immobilisation in suitable matrices. The lipase may be immobilised in hydrogels (alginate, carrageenan, agarose etc) or in the solgels (silicate, celite etc). Many researcher have studied on various species of Halobacterium from salt lake [7] sea water and salt mine. Apart from these, many biomolecules synthesized by the halobacterium have been reported by many authors [8,9,10,11&12] Likewise, many workers have studied the proteome of *Halobacterium salinarum* using proteomics technique and also detected 14 protein molecules. Meanwhile, a very few literature available on the lipase production by halobacterium isolated from sea water. Hence, the present works deals with the isolation and charaterisation of *Halobacterium* from the sea water. These isolated species was then subjected to the enzyme screening for the enzymes like amylase, cellulase, protease and lipase. Lipases have a large variety of applications mainly in the detergent, cosmetic, drug, leather, paper, and food industry and in several bioremediation processes. So owing to their vast and varied applications newer microbes are to be screened for production of lipases of desirable properties.

The enzyme kinetics studies like effect of temperature, pH, activator, inhibitor, and substrate concentration was also studied along with the determination of molecular weight of the lipase enzyme using SDS-PAGE.

II. MATERIAL AND METHODS:

2.1. Collection of water samples

Surface seawater (W1) samples were collected in a sterilized container from the sea at a depth of 1m and 6 km away from seashore at Tiruchendur in Southern Tamilnadu, India. Similarly, water sample (W2) was also collected from paddy field nearer to the seashore area approximately about 4 km away from the sea.

2.2. Estimation of total salt concentration and pH of the water samples

About 10 ml of each samples (W1 and W2) were taken in a two separate clean and dry test tube whose weight is determined previously and they were allowed to evaporate till there is no trace of water droplets inside the test tubes. The weight of the test tubes with the salts was measured and the difference between the weight of the test tube with salt and the empty test tube gives the weight of the salts present in the water samples. The pH of the water samples (W1 and W2) was determined using pH meter and vlues were recorded.

2.3. Isolation of *Halobacterium* from the samples W1 and W2

The samples W1 and W2 were serially diluted in the range of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} in a series of test tube. The *Halobacterium* was cultured in the nutrient medium with increased concentration of Na Cl. The pH of the medium was adjusted to 7 – 7.4, and the composition of the nutrient medium with essential ingredients were given in the Table-1. The samples were inoculated under aseptic condition in the petriplates containing nutrient agar medium using spread plate technique and are incubated at 37°C for 48 hours [13].

Table – 1. Composition of nutrient medium for bacterial growth

Sl.No.	Essential Ingredients	Concentration (g/100ml)
1.	Peptone	0.5
2.	Yeast extract	0.3
3.	NaCl	2.0
4.	Agar	2.0

2.4. Preparation of pure culture of *Halobaterium sp.*

The nutrient agar slant was prepared in test tubes and the isolated colony obtained in the petriplates were taken carefully without contamination (leaving out mixed culture colony) and streaked in the nutrient agar slant in the in a zigzag manner using the inoculation loop. Then test tubes were plugged with cotton plugs and incubated at 37°C for 48 hours.

2.5. Morphological studies of *Halobaterium* using Gram staining

The morphological studies like colony appearance, cell shape, and size on the obtained pure cultures were carried out using Gram staining method. The stained slides were observed for the morphological characteristics of the culture under the compound microscope.

2.6. Determination of respiration type of *Halobaterium*

The nutrient agar deep tube was prepared and the pure culture was inoculated deeply into the solidified medium using inoculation needle under sterile condition. After inoculation, the upper surface of the medium was covered with the molten wax with 0.5cm thickness and incubated for 48 hours at 37°C.

2.7. Biochemical characterization of *Halobaterium*

Different biochemical tests were performed to characterize and to confirm the isolated species as *Halobacterium*, such as, catalase test, indole production test, methyl red and vogas proskauer (MR – VP) test, citrate utilization test and acid production test.

2.8. Screening for enzyme production by *Halobacterium*

The pure culture of *Halobacterium* species was subjected to screening for enzyme production like, amylase, protease, cellulase and lipase. The essential ingredient for the enzyme-screening medium is given in the Table - 2.

Table – 2. Composition of enzyme screening medium

Sl.No	Essential ingredients	Concentration (g/1000ml)
1	**Amylase screening medium**	
	Beef Extract	3.0
	Peptone	5.0
	Na Cl	7.0
	Starch	10.0
	Agar	20.0
2	**Lipase screening medium**	
	Peptone	2.5
	Casein	2.5
	Yeast Extract	3.0
	Tributyrin	10.0
	Na Cl	7.0
	Agar	20.0
3	**Protease screening medium**	
	Skim Milk powder	10.0
	Na Cl	7.0
	Agar	20.0
4.	**Cellulase screening medium**	
	Beef Extract	3.0
	Peptone	5.0
	Na Cl	7.0
	Cellulose (CMC)	10.0
	Agar	20.0

The different types of medium used for the screening of enzyme production were sterilized using autoclave. The medium was distributed equally in the Petriplates and allowed it to get solidified, after few minutes of solidification, the pure culture was inoculated in a zigzag streak using inoculation loop and incubated at 37°C for 24 – 48 hours.

2.9. Assay of Lipase enzyme production

The lipase enzyme was produced using the H*alobacterium* isolated from the sea water in the sterilized lipase production medium. The composition of lipase production medium is indicated in the Table – 3.

Table – 3. Composition of lipase productionmedium

Sl.No.	Essential ingredients	Concentration (g/1000ml)
1.	Peptone	2.5
2.	Casein	2.5
3.	Yeast Extract	3.0
4.	Tri butyrin	10ml
5.	Na Cl	7.0

The pH of the sterilized medium was adjusted to 7.4 and isolated strains of *Halobacterium* were inoculated under aseptic condition using inoculation loop in the medium and incubated at 37°C for 48 hours in a mechanical shaker.

2.9.1. Extraction of lipase enzyme

After considerable growth of bacteria in the lipase production medium, the lipase enzyme produced as an extracellular product by the bacteria was extracted from the medium. The lipase production broth was centrifuged at 6000 rpm for 10 minutes to remove the cell debris and the supernatant was collected in the separate test tubes after centrifugation and filtered using Watt Mann Filter Paper.

The obtained filtrate was subjected for 70% saturation (for 100ml of sample about 44.2g of ammonium sulphate salt gives 70% saturation) with ammonium sulphate by adding the salt slowly in the conical flask containing the filtrate kept in the magnetic stirrer. The content was left undisturbed for one hour, and then it was centrifuged and the pellets were collected and dissolved in the 20 mM Tris buffer. About 1.5 ml of crude enzyme extract was taken in the vials and stored for the future use at 4°C.

2.9.2. Purification of lipase enzyme

The obtained crude enzyme was then purified by Dialysis method and Ion exchange chromatographic process.

2.9.3. Dialysis of crude extract of Enzyme

The obtained crude extract of enzyme was first purified by dialysis process, which is based on the principle of osmosis. About 2 litres of 50 mM Tris hydrochloride is required for the process. The cellulose acetate membrane, which can retain the molecules, whose molecular weight is greater than 10 to 12 KD is used as a dialysis membrane. The pretreatment of dialysis membrane is required to perform the dialysis process.

2.9.4. Pretreatment of Dialysis membrane

About 2 to 2.5 inches of dialysis membrane was taken and it was soaked for 10 minutes in a boiling water bath at 100°C. After 10 minutes of boiling, 2gm of sodium carbonate was added to the boiling water containing the dialysis membrane and allowed to boil for few minutes. Then the dialysis membrane was transferred to the fresh boiling water bath at 100°C for 10 minutes.

2.9.5. Dialysis of enzyme

The pretreated dialysis membrane was taken and made into a pouch like structure with the help of thread tied at the one end of the membranes. To the pouch the crude extract of enzyme was added and it was sealed at both the ends using threads. The sealed membrane with the enzyme was then dipped into the 50 mM Tris HCl and allowed to stand for overnight undisturbed at 4°C. After incubation, the entire content was brought to room temperature and the dialysis process was repeated for 2 times with fresh Tris HCl buffer. To enhance the dialysis process the entire dialysis set up was kept in the magnetic stirrer. About 1.5 ml of dialyzed sample was taken in vials and stored at 4°C for future use.

2.9.6. Purification of lipase using Ion exchange chromatography

The dialyzed sample was further purified using ion exchange chromatography with 25 mM Tris HCl and 1 M NaCl. These ion exchange solvents with different molarities were prepared and distributed in to six different test tubes as shown in the Table – 4.

Table – 4. Ion exchange solvents with different Molarities

Test Tube No.	Tris HCl (ml)	NaCl (ml)	Distilled water (ml)
1.	0.25	0.25	9.50
2.	0.25	0.50	9.25
3.	0.25	0.75	9.00
4.	0.25	1.00	8.75
5.	0.25	1.25	8.50
6.	0.25	1.50	8.25

The enzyme, which is retained in the resin bed, was eluted by the solutions of different molarities, which are added one by one, and the filtrate was collected in the sterile test tubes.

2.9.7. Determination of activity of lipase enzyme using titrimetric method

The activity of the lipase enzyme obtained (Crude enzyme sample, Dialyzed enzyme sample, and the enzyme purified further by ion exchange chromatography sample) was determined by titrimetric method.

2.9.8.Determination of enzyme concentration by Lowry's method

The concentration of the extracted enzyme was determined by the Lowry's method.

2.10. Enzyme Kinetic studies of lipase enzyme using titrimetric method

The kinetics of enzymes like effect of temperature, pH, substrate concentration, inhibitor and activator were studied using the standard laboratory protocol.

2.10.1. Effect of temperature

A series of test tubes labeled with different incubation temperature (4°C, room temperature, 37°C, 55°C, and 100°C) including Blank. About 2.5ml of distilled water was added to each test tube along with the addition of 1.0ml of Tris HCl buffer and 0.3ml of Tributyrin (substrate). The test tubes were incubated at room temperature for 10 minutes and then about 0.1ml of enzyme was added to each test tubes except blank and incubated at 37°C for 30 minutes. About 3 ml of ethanol was added after the incubation period to the test tubes and the enzyme activity was determined.

2.10.2. Effect of pH

A series of test tubes labeled with different pH values such as 3, 5, 7, 9, and 11 along with blank. About 2.5ml of distilled water, 1ml of buffer with different pH values and 0.3 ml of Tributyrin (substrate) were added to the test tubes and incubated at room temperature for 10 minutes. Then 0.1ml of enzyme was added to all the test tubes except blank and incubated at 37°C for 30 minutes. 3.0ml of ethanol was added to the test tubes after the completion of incubation period and the enzyme activity was determined.

2.10.3. Effect of substrate concentration

A series of test tubes labeled with different substrate concentrations like 50µl, 100µl, 150µl, 200µl, 250µl, 300µl, 350µl, 400µl, 450µl, and 500µl were taken. To each test tubes about 2.5ml of distilled water, 1ml of Tris HCl buffer, and Tributyrin (substrate- with different concentration) were added. The test tubes were incubated at room temperature for 10 minutes and about 0.1ml of enzyme was added to all the test tubes except blank. After the addition of enzymes the test tubes were incubated at 37°C for 30 minutes. About 3.0ml of ethanol was added after the incubation period to the test tubes and the enzyme activity was determined.

2.10.4.Effect of activator

A series of test tubes labeled with different concentration of activator ($ZnCl_2$ 1mg/ml) like 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1.0ml were taken and 2.5ml of distilled water, 1 ml of Tris HCl buffer and 0.3ml of Tributyrin (substrate) to each test tubes and they were incubated at room temperature for 10 minutes. After that about 0.1ml of enzyme was added to all the test tubes except blank and the activator of different concentration (Table -) again incubated at 37°C for 30 minutes. About 3.0ml of ethanol was added after the incubation to each test tubes and the enzyme activity was determined.

2.10.5. Effect of inhibitor

A series of test tubes labeled with different inhibitor (Stock solution:EDTA 1mg/ml) concentration like 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1.0 ml were taken, and 2.5ml of distilled water, 1ml of Tris HCl buffer and 0.3ml of Tributyrin (substrate) were added to each test tubes.

All the experimental samples were incubated at room temperature for 10 minutes. After incubation of the content about 0.1ml of enzyme (except blank) and inhibitor of different concentration were added to each test tubes and incubated at 37°C for 30 minutes. About 3.0ml of ethanol was added after the incubation and the enzyme activity was determined.

2.11. Determination of molecular weight of lipase enzyme using SDS-PAGE

The molecular weight of the lipase enzyme obtained from *Halobacterium* species was determined by SDS-PAGE, where BSA serves as a control and the reagents used for SDS-PAGE were indicated in Table – 5.

Table – 5. Reagents used for SDS-PAGE

SI.No	Essential ingredients	Concentration
1	**Stock acrylamide solution**	
	Acrylamide 30%	3g
	Bis acrylamide 0.8%	0.08g
	Water	10ml
2	**Separating gel buffer pH 8.8**	
	1.875M Tris HCl	2.27g
	Water	10ml
3	**Stacking gel buffer pH 6.8**	
	0.6m Tris HCl	0.726g
	Water	10ml
4	**Electrolyte buffer pH 8.2 – 8.4**	
	0.05M Tris	1.2g
	0.192M Glycine	2.88g
	0.1% SDS	0.2g
	Water	200ml
5	**Ammonium persulphate solution**	
	Ammonium per sulphate	0.6g
	Water	10ml

About 2ml of separating gel buffer was mixed with 3.5 ml of stock acrylamide solution and to which 4.5ml of water was added. The separating gel mixture was then degassed and about 20µl of freshly prepared ammonium persulphate (APS) solution was added for the polymerization reaction to form gel. After the addition of APS the separating gel mixture was immediately poured into the tightly clamped SDS plates. The surface of the separating gel was washed with water in order to form a smooth even surface and after polymerization of separating gel, the water was removed and ensures that there is no trace of water in between the glass plates. Then the stacking gel mixture of water 3.5ml, stacking gel buffer 0.5ml and stock acrylamide solution of 1ml was vortexed and degassed, to that about 7µl of APS solution was added and poured above the separating gel

within the glass plates and the comb was inserted immediately to form the wells for loading the samples in the gel and it was left undisturbed to get solidified by the polymerization reaction. After solidification of gel the comb was removed carefully and the wells was washed with electrolyte buffer and the electrophoresis tank was filled with the electrophoresis buffer. The samples were then loaded in the wells and it was electrophoresed at 50 to 100 volts where standard BSA serves as a marker. After electrophoresis the gel was stained with staining buffer and kept for overnight. It was then destained and the presence of enzyme bands was observed.

III. RESULTS AND DISCUSSION IV.

3.1. Estimation of total salt concentration and pH of the water samples

The sea water sample (W1) contains 4g of salts/ 100 ml of water and the water sample (W2) collected from the paddy field nearby sea shore area was found to contain 3.1g of salts / 100ml of water. The pH of the water sample W1 and W2 were recorded as 8.65 and 10.19 respectively.

3.2. Isolation of *Halobacterium*

Numerous colonies like yellow pigmented, red pigmented, white spongy, white radial, dirty white colonies were observed from the water samples. The isolated *Halobacterium sp.* from the seawater samples was found as radial white spongy gram negative bacillus colonies. Ninety five extremely halophilic species have been isolated from distinct saline regions of Turkey. Likewise, a novel species of salt water bacterial namely *Halobacterium jilantaiense* was isolated [7]

3.3. Morphological characteristics of *Halobacterium*

The isolated *Halobacterium* strains were appeared as rod like bacillus, gram-negative organism. They found as radial white spongy colonies. The results of the morphological study of the obtained colonies were indicated in the Table – 6.

All the colonies isolated both from the seawater and paddy field water was gram negative bacilli. They are motile and aerobic in nature, requires oxygen for their metabolism [14, 15, & 16].

Table – 6. Morphological characteristics of *Halobacterium*

Sl.No	Sample	Colony Appearance	Cell shape	Gram staining
1	W1	White colony	Bacilli	Gram Negative
2	W1	Dirty white colony	Bacilli	Gram Negative
3	W2	Spongy white colony	Bacilli	Gram Negative
4	W2	Milky white colony	Bacilli	Gram Negative
5	W2	Yellow pigmented colony	Bacilli posses terminal spores	Gram Negative
6	W2	White colony	Bacilli	Gram Negative

3.4. Determination of type of respiration in *Halobaterium sp.*

It was observed that the colonial growth was absent in the test tubes sealed with the waxes due to the aerobic nature of the colony which requires oxygen for its respiration [17 & 18]. Both the aerobic and anaerobic colonies were isolated from water samples W1 and W2. The results were indicated in Table – 7.

Table – 7. Determination of type of respiration in *Halobacterium sp.*

Sl.No	Sample	Colony Appearance	Respiration type
1	W1	White colony	Aerobes
2	W1	Dirty white colony	Aerobes
3	W2	Spongy white colony	Aerobes
4	W2	Milky white colony	Facultative anaerobes
5	W2	Yellow pigmented colony	Aerobes
6	W2	White colony	Facultative anaerobes

3.5. Biochemical characteristics of Halobacterium sp.

The isolated pure cultures were subjected to various biochemical characterization [19 & 20] and the results were indicated in Table –8. The bacterial colonies isolated from the water samples W1 & W2 were showed positive results almost for all the biochemical tests except a few carried out in the present study. The results of the morphological and biochemical characteristic studies have confirmed the *Halobacterium sp.*

Table 8. Biochemical characteristics of *Halobacterium sp.*

Name of Biochem-ical test	Eperimental Samples					
	W1 (1)	**W1 (2)**	**W2 (3)**	**W2 (4)**	**W2 (5)**	**W2 (6)**
Catalase test	+	+	+	+	+	+
Indole productio n test	+	+	+	+	+	+
Methyl Red test	+	+	+	-	+	+
Vogas proskauer test	-	-	-	+	-	-
Citrate utilization test	+	+	+	+	+	+
Acid Productio n test	+	+	+	+	+	+

3.6. Screening for enzyme production by *Halobaterium sp.*

The inoculated petri dishes were observed after 24 – 48 hours of incubation for the presence of clear zone along the colony growth and the results of the enzyme like amylase, cellulose, lipase and protease productions by the organism were tabulated in Table –9.

The inoculated colonies produce the clear zone only in the lipase and protease-screening medium and not in the amylase and cellulose production medium. This indicated that the isolated *Halobacterium* strains produce both the protease and lipase enzymes and a well distinct zone was observed in the lipase production medium than in the protease production medium indicated that the organisms have produced more amount of lipase. The lipase has been isolated from Fusarium solani and Streptococcus sp. [21, 22, 23, & 24]

Table – 9 screening of various types of enzymes produced by *Halobacterium sp.*

Sample No.	Colony morphology	Cellulase	Amylase	Protease	Lipase
1 W1	White colonies	-	-	+	+
5 W2	Yellow pigmented colonies	-	-	+	+

3.7. Purification of lipase enzyme

The crude enzyme obtained was purified by dialysis with cellulose acetate membrane which retains the enzyme particles with size ranged from 10 to 12 KD, and the enzyme was further purified by ion exchange chromatography and stored.

3.8. Determination of activity of lipase using titrimetric method

The results of the activity of the crude extract of the enzyme and dialyzed sample were indicated in Table – 10 and the sample purified by ion exchange chromatography were determined by titrimetric method and the results were indicated in Tables – 11. There was no difference in the activity of crude extract and dialyzed enzyme. Whereas, in case of enzyme purified by Ion Exchange Chromatography, the enzyme activity was gradually increased with increase in the concentration of enzyme (100000 to 300000 units /ml enzyme) and after saturation level there was no increase in the activity, instead it declined [25, 26 &27].

Table – 10. Determination of activity of lipase present in the crude extract and dialyzed sample using titrimetric method

S.No	Enzyme activity (units/ml enzyme)	
	Crude Extract	**Dialyzed Enzyme**
B	0	0
1W1	30000	30000
5W2	20000	20000

Table – 11. Determination of activity of lipase enzyme purified by ion exchange chromatography by titrimetric method

Sl. No	Solvent	Tris HCl (ml)	NaCl (ml)	Dis. water (ml)	Enzyme activity (units/ml)
1	1	0.25	0.25	9.50	100000
2	2	0.25	0.50	9.25	100000
3	3	0.25	0.75	9.00	200000
4	4	0.25	1.00	8.75	300000
5	5	0.25	1.25	8.50	200000
6	6	0.25	1.50	8.25	100000

3.9. Determination of concentration of lipase enzyme using Lowry's method

The concentration of the lipase in crude extract, sample after dialysis and purified using ion exchange chromatography was determined by Lowry's method [28] and results were indicated in Table – 12. The concentrations of enzyme in the crude extract, dialyzed enzyme and purified by ion exchange chromatography were determined spectrophotometrically and recorded as 0.12µg/ml, 0.18µg/ml and 0.10µg/ml respectively.

Table – 12 Determination of concentration of lipase enzyme present in crude extract, dialyzed sample and purified sample by ion exchange chromatography using Lowry's method

Effect of temperature on lipase activity		Effect of pH on lipase activity	
Temp range °C	Enzyme activity (units/ml enzyme)	pH	Enzyme activity (units/ml enzyme)
B	0	B	0
4	200000	3	200000
RT	300000	5	300000
37	300000	7	800000
55	400000	9	600000
100	200000	11	100000
Effect of substrate concentration on lipase activity			
Substrate concentration (µl)		Enzyme activity (units/ml enzyme)	
B		0	
50		200000	
100		300000	
150		500000	
200		500000	
250		900000	
300		1100000	
350		1200000	
400		1200000	
450		1200000	
500		1200000	
Effect of activator on lipase activity		Effect of inhibitor on lipase activity	
Sl.No	Enzyme activity (units/ml enzyme)	SI.No	Enzyme activity (units/ml enzyme)
B	0	B	0
1	700000	1	900000
2	1500000	2	800000
3	1600000	3	700000
4	1800000	4	700000
5	2200000	5	400000

3.9. Enzyme kinetics of lipase enzyme obtained from *Halobacterium*

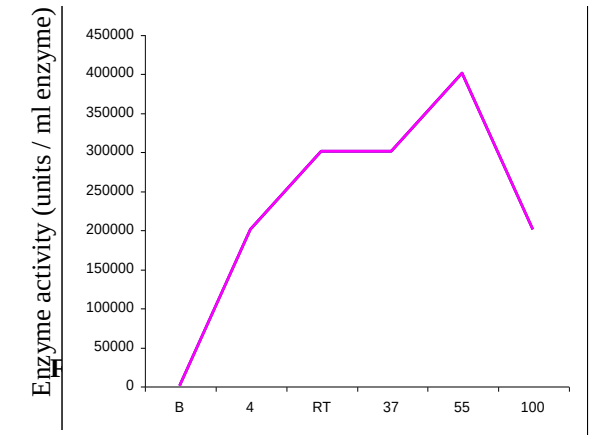
The enzyme kinetic studies were carried out to determine maximum activity of enzyme at optimum conditions. Especially the effect of temperature (Fig.1), pH (Fig.2), substrate concentration (Fig.3), inhibitor (Fig.4), and activator (Fig.5) on lipase activity have been indicated in Table - 13.

Table – 13. Effect of Temperature, pH, substrate concentration, activator and inhibitor on lipase enzyme obtained from *Halobacterium*

SI.No	Vol. of std BSA (ml)	OD Values at 600 nm		
		Crude Extract	Dialyzed Sample	Purified sample by IEC
B	0	0	0	0
1	0.2	0.108	0.012	0.012
2	0.4	0.148	0.020	0.020
3	0.6	0.258	0.050	0.050
4	0.8	0.324	0.056	0.056
5	1.0	0.368	0.086	0.086
T	0.1	0.172	0.084	0.044

The optimum temperature and optimum pH at which the maximum activity of the enzyme was predicted for the lipase enzyme as 55°C, and 7 respectively. Whereas, in case of substrate concentration, the enzyme activity increases steadily with increase in the concentration of substrate up to 300µl and the activity of enzyme remains constant further increase in the substrate concentration.

Fig .1 Effect of temperature on lipase isolated from *Halobacterium sp.*



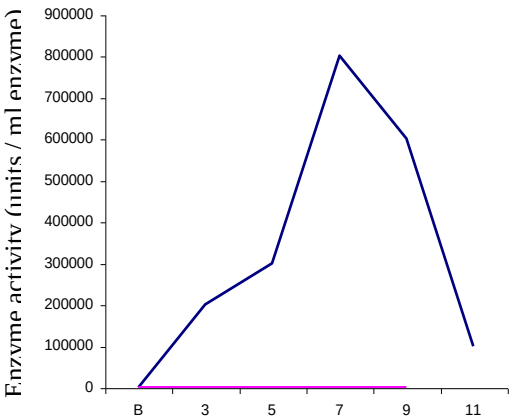


Fig. 3. Effect of substrate concentration on lipase isolated from *Halobacterium*

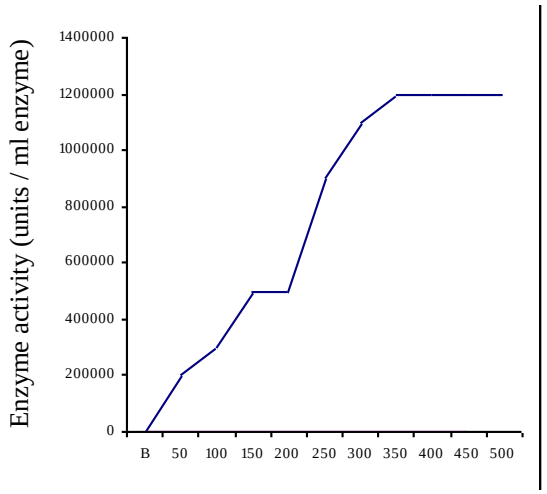


Fig.4. Effect of activator on lipase isolated from *Halobacterium* sp.

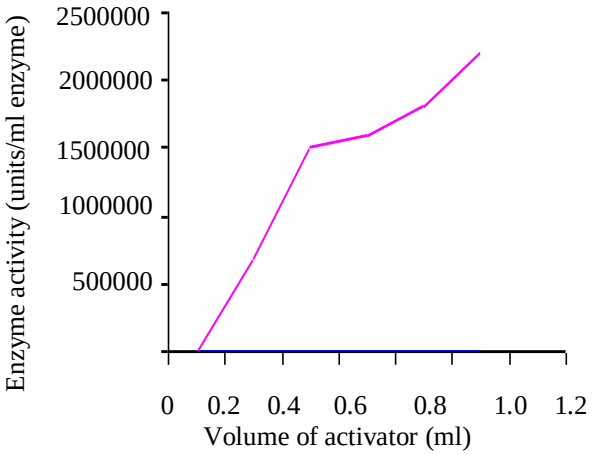
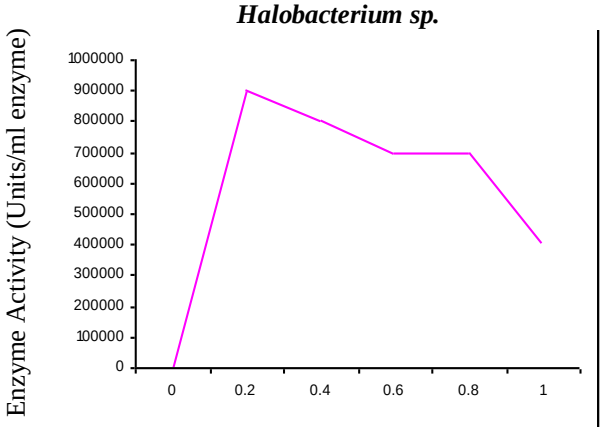


Fig .5. Effect of inhibitor on lipase isolated from *Halobacterium sp.*



The inhibitor (EDTA) inhibits the activity of the enzyme whereby the enzyme activity gradually decreases with increase in the concentration of inhibitor. Similarly, a gradual and steady enhancement in the enzyme activity was observed with increase in the concentration of the activator ($ZnCl_2$) in the present investigation. Similar studies have been carried out in Streptococcus sp, and it was reported that, the optimum temperature and range of pH value for maximum activity of lipase as 37 ^{0}C and 8 – 8.4 respectively.

3.10. Determination of molecular weight of lipase using SDS-PAGE

The molecular weight of the lipase isolated form Halobacterium sp. was found to be 66 KD, which was determined using standard BSA as a marker in the SDS polyacrylamide gel electrophoresis. Similarly, the molecular weight of lipase isolated from Streptococcus sp. was reported as 44.7 KD [6].

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