ISSN No:-24562165

Extraction and Characterization of Lipase Enzymes from Bacillus Cereus (MS6) and their Medical & Industrial Applications

Mohammed Mohammed Abdu Al-Zazaee*
Mawhob. M. M. Abdu, Doaa Ahmed Mahmoud
Abdul-Aziz Ahmed Mohammed Abdu
Department of Medical Laboratory, Medical Collage, the National University, Taiz, Yemen

Corresponding Author:- Mohammed Mohammed Abdu Al-Zazaee

Abstract:- Lipases are class of enzymes catalyzing aqueous and non-aqueous phases. lipases belong to an important group of biotechnological enzymes, which have got industries dairy, , textile, clinical diagnosis, disease & biomedical importance etc. In this study, we have isolated an extracellular lipase Bacillus cereus MS6 enzymes from the effluent of a local Sewerage Yemen Company of ghee & soap industry (YCGSI) City of Taiz. The lipase production media was optimized of the enzyme and the purification was performed by a series of steps as well as using column Cellulose ion exchange chromatograph, thus SDS- electrophoreses which indicated a single protein band corresponding to a molecular weight of approximately 35 kilo-Dalton. The pH optimum was found to be 9.0 as well temperature at 40 ^{0}C, extracellular lipase that we have isolated and purified in this study has shown thermal and pH stability and activity in comparable with other bacterial extracellular lipases.

Keywords:- *Bacillus Cereus MS6, Lipase Enzymes, Gel Filtration, Characterization, Lyophilized.*

I. INTRODUCTION

Lipases are class of enzymes catalyzing aqueous, non-aqueous phases., microbial cells produce a variety of enzymes, like lipase can hydrolysis of lipids into its component free fatty acids and glycerol [1], which has meet the desires for several industries biodiesel, foods drinks, leather, textile and medicals multipurpose, the microbial origins of lipase enzymes are logically dynamic and proficient also have an extensive range of industrial uses with the manufacturing of altered molecules. the several industrial processes, therefore can attained from several sources, animal, vegetable, and microbiological. [2] These enzymes are active at the interface which distinguishes them from esterase. The extracellular bacterial lipases are of considerable industrial, biomedical application, because of their diverse substrate specificity, stereo-specificity, tolerance against heat and retention of activity in the presence of various organic solvents [2]. Owing to these properties, the bacterial lipases are used in food industry, dairy, detergent, pharmaceutical, textile and chemical trade [3]. organic media containing minute water concentration where most of the lipase reactions shows a highly selectivity, efficiency and they occur under mild conditions. Further, these reactions are also known to occur without added cofactors and with low energy requirements. These properties contribute to reduce industrial conversion cost, and justify the growing interest in lipases [4,5]. The microbial lipases are commercially important because of their unique properties and the ease of bulk extracellular production compared to lipase from other natural sources [6]. Most of the lipase research focuses on the production of extracellular lipase though a wide variety of microorganisms such as fungi, bacteria archaea [7]. Further, among the lipase producing bacteria several species of such as *Bacillus subtitles, bacillus pummels, sphaericus possess* exploited [8,9]. Further Extracted lipase was showed higher thermal stability, higher activity at elevated temperatures as well as more confrontation to chemical denaturation. The need for biotechnological applications seeks lipases that are suitable for use in various extreme conditions as well as ease of production. which look of lipase source [11,12]. In this direction, the production, purification partial lipase Bacillus cereus MS6. We have determined the stability of lipase temperature, optimized conditions with higher stability [10.11]. So, extraction of Bacterial lipase, depends upon a number of factors including carbon and nitrogen sources, pH, temperature, aeration and inoculums size [12]. In this direction, we designate the production, purification and characterization of lipase from *Bacillus cereus* MS6. We have also determined the stability of this lipase temperature, optimized conditions with higher stability. In general Lipase is an enzyme the body uses to break down fats in food so they can be absorbed in the intestines. produced in the pancreas, mouth, and stomach as well as catalyzed growth, blood coagulation, healing, diseases, breathing, digestion, reproduction, and many other biological activities.

ISSN No:-24562165

II. MATERIALS & METHODS

➢ *Sampling & Bacteria Isolation*

Sewage sample used in this study was collected from sewerage Yemen Company of ghee & soap industry (YCGSI) city of Taiz-Yemen. Sewage effluent was collected in 30 ml plastic tubes and refrigerator, the sample was diluted and plated into dishes containing rich media.

➢ *Identification of Bacterial Isolate*

The genomic DNA was isolated as described by with slight modification bacterial primers were used as template for the amplification of 16S rRNA gene using polymerase chain reaction (PCR) [13,14] and partial 16S rRNA gene sequence was quires to the National Center for Biotechnology and the nearest neighbor of the isolate was determined [15,16].

➢ *Optimization Production Parameters*

For the production of the enzyme, olive containing, peptone (0.5, Na2HPO4.7H2O (1.2%), KH2PO4 (0.3%), NH4Cl (0.5%), MgSO4.7H2O (0.2%), NaCl glucose (1%) in 100 ml distilled water. the culture medium was adjusted to 9.0 PH for optimization of production, then inoculated into the conical flask containing 100 ml culture medium and incubated in a rotary shaker at 37^0C ,32 hours, after that the turbidity culture bacterial was centrifuged and supernatant subjected to precipitation and purified by column chromatography.

➢ *Gel Filtration Chromatography*

Crude enzyme after 80% ammonium sulfate precipitation was then subjected to gel filtration chromatography and the column was eluted with the phosphate buffer at a flow rate of 20 ml/hour and elution was experimental by measuring the absorption at 280 nm and the aliquots of fractions were screened for lipase activity and the active fractions containing highest. activity were pooled together, dialyzed and lyophilized. Aliquots of fractions were screened for enzyme activity. The fractions containing highest lipase activity were pooled together, dialyzed and lyophilized.

➢ *Lipase Assay*

Enzyme activity was determined by titrimetric method on the basis of olive oil hydrolysis [17]. One ml sample solution was added to the assay substrate containing 2 ml homogenized, pH 9. The reaction was stopped by adding 4ml of ethanol-acetone mixture (1:.So, liberated free fatty acids were titrated with 0.05 N NaOH until pH 9.0 as indicated by the appearance of light blue color. And activity was determined as a function of reaction incubation time of up to 1h with 5 minutes' time intervals. optimum fermentation growth period was determined by incubating for various time periods of up to 32 hours. pH optimum enzyme was determined at various pH values (6-12).and temperatures (10-100^0C). Similarly, the substrate for activity was determined using different concentrations (0.5 - 4ml). activity lipase was determined calculations using formula [18].

$$\text{Activity} = \frac{(\text{Vol. NaOH}_S - \text{Vol. NaOH}_B) \times N \times 1000}{\text{protein volume} \times \text{Incubation time}}$$

N normality NaOH.

➢ *Protein Assay*

The total amount of protein throughout the experiment was measured according to Lowry's method using Bovine serum albumin (BSA) as standard [19].

➢ *Substrate Concentration on Lipase Activity*

To study the effect of different substrate concentrations on lipase activity, olive oil, coconut oil, sunflower oil & castor oil were used as substrates in the concentration range of 0.5 to 5ml. Further, based on the activity, olive oil was selected as substrate for measuring the lipase activity.

➢ *Effect of Temperature on Lipase Activity*

For determining the effect of temperature on lipase activity, the incubation of the reaction mixture was carried out at different temperatures ranging 10-100 ^{0}C. The sample was incubated for 30 minutes and the aliquots assayed for the enzyme activity.

➢ *Effect of pH on Lipase Activity*

The effect of pH on the activity enzyme was carried out at different pH values in the range 6-12 using appropriate buffers and at a temperature of 40 ^{0}C using olive oil as a substrate.

➢ *Effect of Incubation Period*

The effect of reaction incubation time on the enzymatic activity was carried out by performing the lipase assay at different incubation period in the range 5-60 minutes using olive oil as a substrate.

➢ *Electrophoresis*

The SDS-PAGE was performed using 12% polyacrylamide gels under reducing conditions as described earlier [20]. The protein bands were visualized by staining with Coomassie Brilliant Blue R-250. The molecular weight of the purified enzyme was determined by comparing with standard molecular weight markers in the range 14 to 94 kDa.

III. RESULTS

➢ *Production Lipase Bacillus Cereus MS6*

The optimum production of extracellular lipase from *Bacillus cereus* MS6 was performed using modified broth medium containing 1% olive oil emulsion containing 1% gum Arabic, yeast extract (0.5%), peptone (0.5%), $Na_2HPO_4.7H_2O$ (1.2%), KH_2PO_4 (0.3%), NH_4Cl (0.5%), $MgSO_4.7H_2O$ (0.1%), $MgCl_2$ (0.2%), NaCl (0.5%), glucose (1%) and 100 ml distilled water. The maximum production of lipase was found to be in the olive oil/gum Arabic emulsion after 48 hours of incubation at 37 ^{0}C and at a pH of 9.0. The lipase thus produced by the lipolytic *Bacillus cereus* MS6 was further subjected to purification and

ISSN No:-24562165

characterized for its enzymatic properties such as substrate specificity, pH stability, optimum temperature and substrate concentration as detailed below.

➢ *Purification Technique*

The purification of lipase, which was produced by *Bacillus cereus* MS6 under optimal growth conditions as explained above, was carried out by ammonium sulfate precipitation followed by column chromatography. The culture supernatant (100 ml) was obtained by centrifugation of the culture broth at 9,000 rpm at 4°C for 15 min. Further, the supernatant was subjected to filtration using a Millipore system (membrane pore size of 30µm). The supernatant thus obtained was subjected to ammonium sulfate precipitation. Initially, the supernatant was saturated with 40% ammonium sulfate by slowly adding the salt with stirring and keeping the extract overnight at 4ºC. The precipitate thus obtained was separated by centrifugation at 9,000 rpm for 15 min. The lipase activity was determined both in the precipitate and the supernatant. The lipase activity was found to be in the supernatant fraction and not in the precipitate. To isolate lipase, additional ammonium sulfate was added to the supernatant to bring the saturation level to 80% and kept stirring overnight. The precipitate was separated by centrifugation at 9,000 rpm for 15 min and dissolved in a minimum volume of 0.2 M phosphate buffer and the solution was dialyzed against distilled water for 36 hrs using a bladder membrane. The dialysis obtained from the above step was subjected to gel filtration chromatography using Sephadex G-100 column (2.5 x 50 cm). The column was equilibrated with 0.2 M phosphate buffer, pH 8.0 and eluted with the same buffer at a flow rate of 20 ml/ hour and 2 ml. fractions were collected. The aliquots of fractions were screened for lipase activity and the active fractions were pooled together, dialyzed and lyophilized. The lipase thus purified by Sephadex G-100 chromatography was further subjected to purification by DEAE-Cellulose column chromatography. The lipase thus purified was characterized for various biochemical and enzymatic properties.

➢ *Characterization*

- *Lipase Molecular Weight Determination by SDS-PAGE*

The apparent molecular weight of lipase obtained by *Bacillus cereus* MS6 and purified as above was determined by SDS-polyacrylamide gel electrophoresis using 12% gel and by staining with Coomassie Brilliant Blue [21]. As shown in Fig. 1, the purified lipase showed single band indicating the purity of the isolated enzyme. Based on the comparison of the molecular weight of the standard markers, the apparent molecular weight purified lipase was calculated to be about 35 kDa (Fig 1).

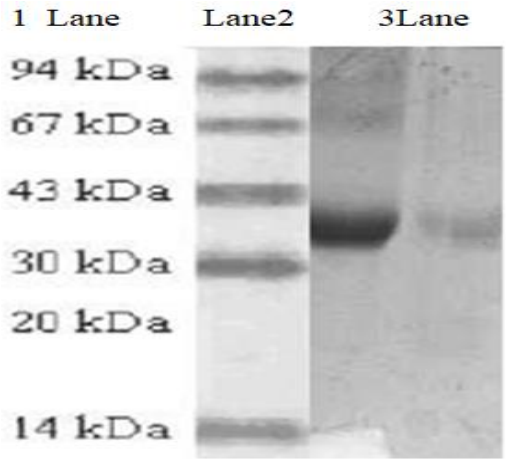

Fig 1 SDS-Electrophoresis was carried out using 12% polyacrylamide gel under reducing conditions at a constant voltage of 80 volts. *Lane 1*, Protein molecular weight standards, *Lane 2*, partially purified Lipase, *Lane 3*, Purified lipase (20 microgram).

➢ *Temperature on Lipase Activity*

The optimum temperature for the isolated *Bacillus cereus* MS6 lipase was determined by measuring the activity in the temperature range of 10 to 100^{0}C (Fig. 2). The activity measurements were carried out using olive oil as a substrate and by incubating for a period of 1 hour. The data shows that the maximum relative activity of the enzyme was found to be about 450 U/ml/min at 40 ^{0}C indicating the temperature optimum for the enzyme. The activity of the enzyme was found to decrease when the temperature was either increased or decreased from this optimum temperature range of 40^{0}C. As shown in the figure, the relative activity value was found to be only about 5 U/ml/min at 80 ^{0}C and which was totally lost at 90 ^{0}C (Fig 2).

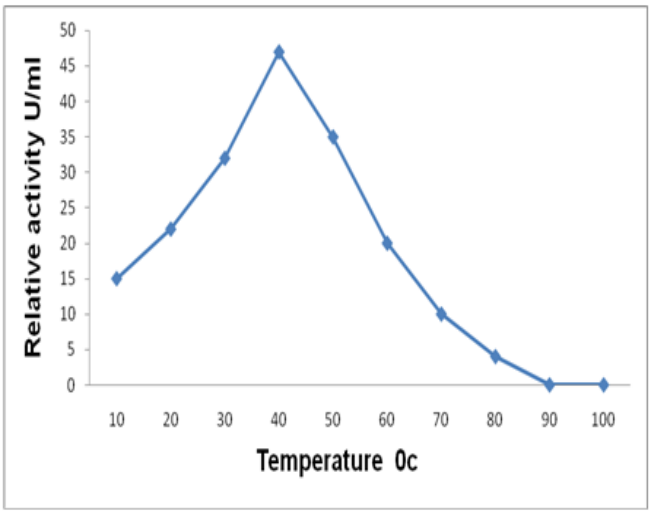

Fig 2 Effect of temperature was determined at various temperatures from 10 to 100 ^{0}C using 2% olive oil as substrate by incubating for a period of 30 minutes at pH 9.0. The relative activity values were plotted versus temperature.

ISSN No:-24562165

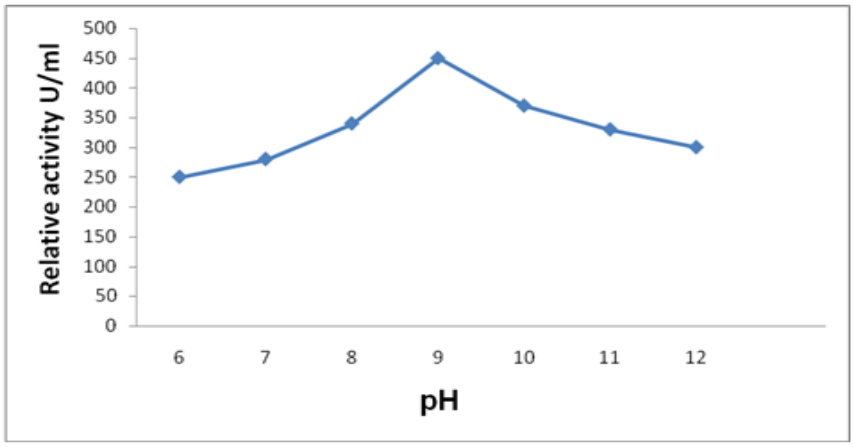

Fig 3 Effect pH on Activity was Determined at Various pH Values (6-12) using 2% olive oil as substrate and by incubating at 40ºC for a period of 30 minutes. The relative activity values were plotted versus pH

Table 1 Purification Steps Lipase Bacillus Cereus MS6 Indicating the Activity, Yield and Fold Purification at Each Step

Purification steps	Volume (ul)	Total Protein(mg)	Enzyme activity U/ml	Total activity (U/ml	Specific activity(U/mg)	Yield %	Fold Purification
Crude enzyme	100	14000	90	9000	0. 64	100	1
Ammonium sulfate saturation 80%	80	8000	187.8	15024	2.4	166.9	3.8
Gel filtration	30	1800	450	13500	15	150	23.4
DEAE-Cellulose	20	400	675	13500	33.8	150	52.8

➢ *Effect of pH on Activity and Stability*

The effect of pH on the enzyme activity was determined by measuring the activity at 40 °C in the pH range of 6–12 using 0.2 M phosphate buffer, tris buffer, and acetate buffer. The reaction mixture included 100 µl of lipase enzyme preparation, one ml of suitable buffer and 1ml of olive oil substrate which was incubated for 1 hour at 40°C. As shown in Fig. 3, the activity of the enzyme varies as a function of pH and which was measured to be about 250 U/ml/min at pH 6.0 and gradually increased and reached a maximum of about 450 U/ml/min at pH 9.0. The activity of the enzyme was found to decrease from pH 9.0 onwards indicating that the pH optimum of the enzyme for olive oil as a substrate was 9.0.

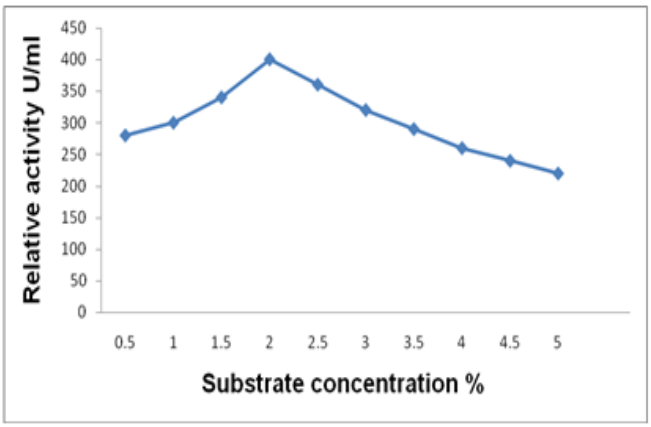

Fig 4 Substrate concentration was determined at a temperature of 40^{0}C and pH 9.0 using various substrate concentrations (0.5 - 5ml) by incubating for 30 minutes. The relative activity values were plotted versus substrate concentration.

➢ *Substrate Concentration Lipase*

The olive oil was found to be the best substrate in comparison with other substrates. The effect of different concentration of olive oil substrate in the range of 0.5 to 6.0 ml was compared with the lipase activity and the data is shown in Fig. 4. As indicated in the figure, the enzyme showed a maximum activity when 2 ml olive oil substrate was used in the incubation mixture. The relative activity of enzyme was found to decrease either with an increase or decrease in the substrate concentration from the optimum value of 2 ml (Fig 4).

➢ *Incubation Time*

The effect of reaction incubation time on lipase activity was determined by incubating the reaction mixture for various time periods of up to one hour using olive oil as substrate and at a temperature of 40 °C (Fig. 5). The relative activity values were measured at five-minutes intervals and the data indicates that the optimum activity was observed at an incubation time of 30 minutes (Fig. 5). Further, as shown in the figure, the relative activity value decreased by about 60% after incubating for one hour. To summarize, in this study, a novel halophilic bacterial strain was isolated from the effluent of Sewerage Yemen Company of ghee & soap industry (YCGSI) city of Taiz. The culture was further screened and enriched by using olive oil containing media plates to obtain the strain with maximum lipolytic activity. The isolated microorganism was characterized and then identified as gram positive, rod-shaped motile organism. The strain was characterized by physiological, chemical and taxonomical approach which revealed that the strain belongs to Bacillus strain. Based on the morphological and biochemical tests, the organism was found to be *Bacillus cereus* MS6 [22]. The production media was inoculated

ISSN No:-24562165

with the isolated bacteria lipolytic and incubated in an orbital shaker at 100 rpm at 37 ^{0}C for 48 hours. The growth characteristics of the strain showed that the maximum production was at a temperature of 37^{0}C although the production could be achieved even up to a temperature to 60^{0}C. The lipase activity was measured using olive oil as a substrate, which was the best among the tested substrates, at an optimum pH of 9.0 and at an optimum temperature of 40 ^{0}C.

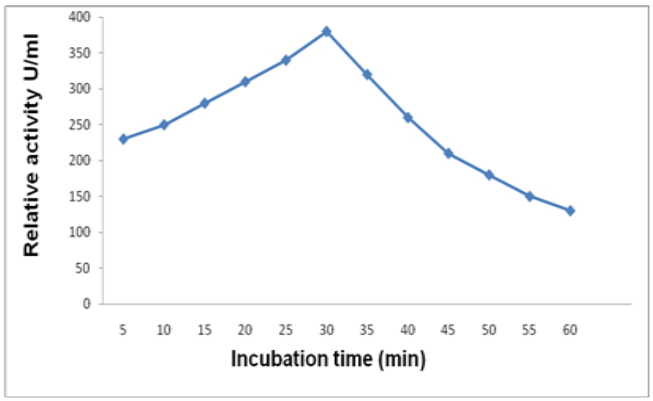

Fig 5 Effect of incubation time. The lipase activity was determined at a temperature of 40^{0}C and pH 9.0 by using various incubation periods of up to one hour using 2% olive oil as substrate. The relative activity values were plotted versus incubation period.

The isolated crude bacterial lipase was subjected to various purification steps and detailed biochemical characterization. During the initial ammonium sulfate precipitation step, most of the activity was found in 80% ammonium sulfate fraction which was further subjected to gel filtration and ion exchange chromatography purification steps. The SDS-PAGE analysis confirmed the purity of the enzyme after purification and the apparent molecular weight of the enzyme was calculated to be 35 kDa. As shown in Table 1, the steps of purification of lipase resulted in the purified enzyme which showed a specific activity of 33.8 units. Overall, the different steps of purification of lipase indicated a 52.8-fold purification. The optimal reaction temperature and pH optimum of lipase studied here was found to be higher or same as that of many bacterial lipases under similar experimental conditions**.** In comparison with other bacterial lipases, the *Arthrobacter Sp* (B G C C #490), lipase from *Bacillus cereus* MS6 showed a value of 40 ^{0}C which was comparable with other bacterial lipases although a higher temperature optimum was reported for lipase of *Pseudomonas fluorescent* and *Bacillus sp* [23]. In that study, a maximum specific activity of 26.6 U/ml/min at pH 9.0 and a temperature optimum of 50 ^{0}C has been reported and the enzyme was found to be stable in the pH range 8.0-10.0 [23], Similarly, a lipase from *Aspergillas Niger* was found to possess an optimum activity in the temperature range of 40-60 ^{0}C and the activity of lipase found to decrease significantly at temperatures above 60 ^{0}C which was lost completely at 90^{0}C. Overall, lipases from *A. niger* strains have been reported to be active between 40 and 55 °C [24,25], while lipase from *Humicola lanuginose* was reported to be active at 45 °C [26]. In another study, lipase from the *Bacillus licheniformis* B-42 and *G.* Stereothermophilus B-78 was reported to possess an optimum temperature of 55^{0}C for lipase production at pH 9.0 [10]. Another study reported that lipase from *Bacillus Pumilus* has a maximum specific activity of 12.8 U/ml at an optimum pH of 8.5 and at a temperature of 55^{0}C [27]. In general, the ability of the *Bacillus cereus* to withstand high salt concentration and varying pH has been established [27].

➢ *Clinical Application of Enzymes*

Gallstones are thought to develop because of an imbalance in the chemical make-up of bile inside the gallbladder. In most cases the levels of cholesterol in bile become too high and the excess cholesterol forms into stones. gallstones are very common further, Lipase's enzymes help a person who has cystic fibrosis, Alzheimer's disease, atherosclerosis then act as a candidate target for cancer prevention and therapy. However, act as diagnostic tool and their presence or increasing levels can indicate certain infection or disease so, lipase enzyme used for that. Many enzymes in the human body have an optimum temperature of approximately 40˚C. anticipate that the lipase enzyme will hydrolyze fats most efficiently at a temperature of about 37 – 40˚**C**, as this is close to our human body temperature (37˚C) and matches the temperature of the digestive organs in which lipase acts, consequently the lipase blood test a type of protein made by pancreas; helps the body digest fats. It's normal to have a small amount of lipase in blood. But, a high level of lipase can mean have pancreatitis, an inflammation of the pancreas, or another type of pancreas disease [28].

➢ *Importance of Lipase Enzymes for Diseases*

Enzymes are the preferred markers in various disease states such as myocardial infarction, jaundice, pancreatitis, cancer, neurodegenerative disorders, etc. They provide insight into the disease process by diagnosis, prognosis and assessment of response therapy and considers it ones an enzyme diagnostic of the branches of enzymology, however it has two main directions use of enzymes as reagents for determination of normal and pathological components in serum, urine, gastric juice and determination of enzyme activity in biological material with diagnostic purpose. Consequently, blood tests to look for elevated levels of pancreatic enzymes, along with white blood cells, kidney function and liver enzymes. Abdominal ultrasound to look for gallstones and pancreas inflammation & Computerized tomography (CT) scan to look for gallstones and assess the extent of pancreas inflammation. So, there many drugs cause high lipase levels such as Narcotics, thiazide diuretics, oral contraceptives, adrenocorticotropic hormone, and cholinergic are commonly associated with hyperlipidemia. However, enzyme may be increased in tumors of the pancreas, or stomach certain stomach conditions. These conditions are usually painful. Gall bladder infection - Inflammation of the gall bladder cholecystitis, may cause increased lipase levels hyperlipidemia, Kidney failure can cause hyperlipidemia. So, there are the different types of lipases an enzyme that helps digest fats. pharyngeal lipase, which is produced in the mouth and is most active in the stomach. hepatic lipase, which is produced by the liver and regulates the level of fats lipids in the blood. On other hand

ISSN No:-24562165

the lipase enzymes used for in industry in baking industry, phospho lipases can be used to substitute or supplement traditional emulsifiers through the degradation of wheat lipids to produce emulsifying lipid situ. Lipase in baking also enhance the flavor of bakery products by liberating short-chain fatty acids through esterification. In addition, lipase used in detergents industries to minimize the use of phosphate-based chemicals in detergent formulations, household laundry reduces environmental pollution and enhances the ability of detergent to remove tough oil or grease stains [28].

IV. DISCUSSION

The extracellular lipase from *Bacillus cereus* MS6 was optimized for the production and purification to the homogeneity. The lipase production and purification was performed by a series of steps including using column DEAE-Cellulose appearance specific activity 33.8 Units with the fold purification achieved was 52.8. purified SDS-electrophoreses which showed band 35 kDa. The pH optimum 9. and 35^0C,. We have also determined the substrate specificity which was found to be 2% olive oil. The extracellular lipase that we have isolated and purified in this study has shown thermal and pH stability and activity in comparable with other bacterial extracellular lipases. Further detailed characterization of the enzyme is needed to determine the usefulness of this lipase for application in food, detergents, chemicals, and pharmaceutical industries so that it could be commercially exploited.[28].

Lipases (triacylglycerol acyl hydrolase, E.C.1.3) hydrolysis; esterification and reversible fats and glycerol [29]. used in low-water for the transformation of commercial esters [30]. the key properties *Bacillus cereus*MS6 lipase to establishing information Since the strains are capable of hydrolyzing of creating comprehension basis for less expensive. Further, a novel haloalkaliphilic *Bacillus cereus* MS6 was enriched media using olive oil as substrate for obtained a maximum production and lipolytic activity under optimum conditions. Therefore, production of lipase from *Bacillus cereus* MS6 was carried out in medium containing 2% olive $NaHPO_4.7H_2O$ 1% NaCl, 1%, 0.2%NH_4Cl and 1% glucose at temperature 37^0C and at pH 9.0. Hence, the rate production until limited period where, reached a maximum production at 48 hours. As well as the inoculums lipase [31].

The production media was inoculated with the isolated bacteria lipolytic and incubated in an orbital shaker at 100 rpm for 32 hours. growth characteristics of the strain showed that the maximum production at temperature of 37^0C although the production could be achieved even up to a temperature of 50^0C. The activity was measured in titrimetric method used 0.05M NaOH which was the best among tested activity enzyme assay under optimum at temperature 40^0C and with stability between 40-60^0C and pH 7.0-9.0 for 2 hours incubation. Isolated crude bacterial lipase was subjected to purification process using the initial ammonium sulfate precipitation, achieved to be 80% ammonium sulfate saturation and fraction precipitation subjected to dialysis with eluted using analysis confirmed the purified lipase where, showed single protein band apparent 35 kDa. Although steps purification of lipase resulted shown activity and specific activity with gel filtration was found to be 400U/ml/minute and 33.8 U/mg respectively with 15=fold whereas, after completed step purification of lipase with DEAE-Cellulose indicated activity and specific activity was found to be 490U/ml/minute and 16.3 with 52.8-fold the data feature shown in table 4. The optimal reaction temperature and pH of lipase studied here was found to be higher or same as that of many bacterial lipases under similar experimental conditions when comparison with other bacterial lipases. Lipase from *Bacillus cereus* MS6 showed a value of 40^0C which was comparable with other bacterial lipases lipase of *fluorescent* and *Bacillus species* [32]. In that study the maximum specific activity was observed to be 26.6 U/ml/minute, pH 9. at 50^0C reported 8.0-10.0 [32]. Similarly, lipase from *Aspergillus Niger* was found to possess an optimum activity 50^0C. So activity found to decrease significantly at temperatures above 60^0C which was lost completely at 90^0C. *Aspergillus* maximum activity 55^0C [33.34] while *lanuginose* maximum activity 45^0C and at pH 9.0. In another study, lipase from the *Bacillus Stereothermophilus* reported to possess an optimum temperature of 55^0C for lipase activity at pH 9.0. Another study reported that lipase from *Bacillus Pumilus* held a maximum specific activity of 12.8 U/ml at an optimum pH of 8. and at temperature of 55^0C [35]. Lipase *Bacillus cereus* MS6 isolated was shown thermal and pH stability and activity in contrast comparable with another bacterial extracellular lipase. However, detailed, characterization of the enzyme was needed to determine the usefulness of this lipase owing to the ever growing need to look for more stable for application in food, detergents, chemicals, and Medicine, pharmaceutical industries as well as the diagnoses of the diseases, so that it could be commercially exploited. In general, the ability of the *lipases Bacillus cereus*MS6 to withstand high salt concentration and varying pH has been established [35].

V. CONCLUSION

The lipase isolated from the effluent of a local Sewerage company of ghee & soap industry has shown thermal and pH stability and activity in comparable with other bacterial extracellular lipases. However, detailed purification and characterization of the enzyme is needed to determine the usefulness of this lipase owing to ever growing need to look for more stable lipases for application in food, detergents, chemicals, clinical disease, diagnosis and pharmaceutical industries so that it can be commercially exploited.

➢ *Acknowledgements*

We extend my thanks to those who financially provided me with the completion this research and thankful to Dr. Ahmed Shmssan Abdullah owner of the National University. Taiz, Yemen, as well as Dr. Mohammed. Ahmed Kahatan & Dr. Mohammed. Abdul-Aziz, rector of the National University.

REFERENCES

[1]. Islam M A, Praveen F, Husain K, Khatun S, Karim M d, Kim G S, Absar N & Haque M d S (2009) Purification and Biochemical Characterization of lipase from the Dorsa part of Cirrhinus Reba Thai Journal of Agricultural Science 42(2), 71-80

[2]. Doo-Sang Park, Hyun-Woo Oh, Sun- Yeon Heo, Won-Jin Jeong, Dong Ha Shin, Kyung Sook Bae & Ho-Yong Park (2007). Characterization of an Extracellular Lipase in Burkholderia sp. HY-10 Isolated from a Longicorn Beetle. Journal of Microbiology 5(45), 409-417.

[3]. Gupta R, Gupta N & Rathi P (2004). Catalytic properties of lipase Extracts from aspergillums' Niger.Apple microbial biotechnology 64, 763-781.

[4]. Lycia M P, Cintia M R, Mario D B & Guillermo R C (2006). Food Technology. Biotechnology 44 (2),247-252.

[5]. Mariana N, Guzman V A, Vargas H, Antezana & Michel S (2008). Lipolytic enzyme production by Halophilic /Halo tolerant Microorganizms isolated from laguna Verde Bolivia.Revista Boliviana De Quimica Mariana 1(25), 14-23.

[6]. Jaeger & Eggert (2002). Lipases for biotechnology. Current Opinion in Biotechnology, Oxford, 13, 390-397.

[7]. Gwen Falony (2006). Production of Extracellure lipase from Aspergillus's Niger by Solid-State fermentation. Food Techno Biotechnology 44 (2), 235-240.

[8]. Roya M S, Mohammad R, Ardakani R G & Ghezelbash Masoud (2011) Phylogenic Characterization of Lipase Producing *Bacillus* Strains Isolated from Persian Gulf Sediments. Australian Journal of Basic and Applied Sciences, 5(4), 121-126.

[9]. Pallovi Pogaku, Suresh A, Srinivas P & Ram Reddy (2010). Optimization of Lipase Production by Staphylococcus SP.Lp12. African Journal of Biotechnology 9(6), 882-886.

[10]. Bayoumi, R. A., El-louboudey, S. S., Sidkey, N. M and Abd-El-Rahman, M. A (2007). Production, Purification and Characterization of Thermo alkalophilic lipase for Application in Bio- detergent industry. Journal of Applied sciences Research, 3 (12) 1752-1765.

[11]. Kambiz M H, Fereshteh E, Baghher Y &FatimahT(2008). Production, Purification and Characterization of Thermoalkalophilic lipase for Application in Bio- detergent industry. Pakistan journal of biological sciences, 11(5), 740-745.

[12]. Nadia N & Nehad Z A (2010). Optimization of lipase synthesis by Mucor racehorses-Production in a triple impeller bioreactor. Malaysian Journal of Microbiology 6(1), 7-15.

[13]. [13] Ausubal F M & Brent R (1992). *Current protocols in Molecular Biology*,New York.

[14]. Mabdhusudan R D & Reddy Y H K (2008). Characterization and identification of Bacillus Cereus GMHS: An efficient picoline degrading bacteria. International Journal of integration Biology, 5 (93) 187-191.

[15]. Al-ZaZaee M M, Neelgund S, Gurumurthy D M & Rajeshwara A N (2011). Identification, Characterization of Novel Halophilic *Bacillus Cereus Ms6: a Source* for Extra Cellular A Amylase. Advances in Environmental Biology 5(5), 992-999.

[16]. Tamura Dudley K J, Nei M & Kumar S (2007). Molecular Evolutionary Genetics Analysis (MEGA4) Software version 4. Molecular Biology and Evolution, 24, 1596-159.

[17]. Selvamohan T, Ajitha R L & Archives P (2010). Isolation and Characterization of lipase-Producing Bacillus strains from oil Mill waste. African Journal of Biotechnology 10 (2), 567-570

[18]. Sharma K & Rathore M (2011). Characterization of physical factors for optimum lipase activity from somebacterial isolates. International Journal Current Trends Sciences &Technology, 1 (2) 51-62.

[19]. Lowry O H, Rosen N J, Rough A L, & Farr Randall R J (1951). Protein measurement with the Fooling phenol reagent. Journal of Biological Chemistry, 1(93), 1951, 265.

[20]. Laemmle (1970). Cleavage of structural proteins during the assembly of head of bacteriophage T4. Nature, 227,680-685.

[21]. Selva MohanT, Palavesam A & Immanvel, G (2008). Isolation and Characterization of lipase-Producing Bacillus strains from oil Mill waste. African Journal of Biotechnology, 7 (15) 2728-2735.

[22]. Pabai F, Kermasha S & Morin (1995). Lipase from Pseudomonas fragi CRDA 323: partial purification, characterization and interesterification of butter fat. Applied microbial biotechnology, 43, 42-51

[23]. Anjana Sharma, Dipa Brdhan & Rahmi Patel (2009).Optimization of physical parameter for lipase production from Arthrobacter SP BGCC490. Indian Journal of Biochemistry and Biophysics, 46, 178-183.

[24]. Kamini N R, Mala J G S & Puvanakrishnan R (1998).Lipase production from *Aspergillus niger* by solid state fermentation using gingelly oil cake. Process Biochemistry,33,505–511.

[25]. Namboodiri V M, Haridasan C& Chattopadhyaya R (2002). Purification and biochemical characterization of a novel thermostable lipase from *Aspergillus niger*. Lipids, 35, 495–502.

[26]. Indu Bhushan., Arvind, Kumar, Yadav and Rajinder, Parshad (2011): Enhancement in th production of lipase from Arthrobacter sp. using fed- batch fermentation strategy. *Asiatic* Journal of Biotechnology Resources, 2 (5) 522-534.

[27]. Rajaram G, Manivasagan P, Thilagavathi B & Saravanakumar A (2011). Purification& Characterization of a Bacitracin Produced by Lactobacillus lactis isolated from Marin Environment. Advance Journal of Food Science and Technology, 2 (2) 138-144.

[28]. Application of enzymatic analysis in medicine 2019-2020, Yildiz technical university department of bioengineering bym 3602 enzyme technology spring
[29]. Meghwanshi, G., Agarwal, L., Dutt, K and Saxena, (2006). Characterization of 1-3 regiospecific lipases from new Pseudomonas and *Bacillus* isolates. Journal of Molecular Catalysis Enzymatic 40, 127-131.
[30]. Treichel, H., Oliveira, D., Mazutti, M., Lucia, M and Oliveira, J. V (2010). A Review of Microbial lipases Production. Food Bioprocess Technology, 3, 182-196.
[31]. Grigiskis, S., Samsonenko, J., Baskys, E. V and Cipinyte, V (2010). Immobilization of lipase from Enterobacter aerogenes E13 on Modified lignins to obtain a novel biocatalyst Chemine Technologija, 1 (54) 47-54.
[32]. Allan, S. Henrik, B. F and Torbenvedel, B (1996). A method of designing α- mutant, with predetermined properties α-amylase variants & detergents containing the variants. Process Biochemistry, 3 (1) 110-210.
[33]. Sharma, K and Rathore, M (2010). Characterization of physical factors for optimum lipase activity from some bacterial isolates**.**International Journal Current Trends Sciences and Technology, 1 (2) 51-62.
[34]. Kamini, N. R., Mala, J. G. S & Puvanakrishnan,R (1998) Process Biochemistry 33,505–511.
[35]. Namboodiri, V. M., Haridasan, C & Chattopadhyaya, R (2002). Purification & biochemical characterization of a novel thermos table lipase from Aspergillus Niger. Lipids, 35, 495–502.