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PURIFICATION OF HALOLIPASE FROM *HALOFERAX LARSENII* (KP636736): AN ARCHAEON ISOLATED FROM GUJARAT, INDIA

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ABSTRACT

Extremely halophilic microbes which inhabit the hypersaline environments such as marine salterns, salted food products, soda lakes, etc. are diverse in morphology, physiology and biotechnological applications. Gujarat has a very long coastline (approximately 1600 km) and there are several locations where the sea water is used for manufacturing marine chemicals. We report here the production and purification of an extracellular lipase from the archaeon *Haloferax laresnii* strain BVM005 (Gen Bank accession no. KP636736) that was isolated from the solar salt works at Okhamadhi, near Dwarka. This isolate had a remarkable characteristic of producing multiple extracellular hydrolyases namely, amylase, caseinase, gelatinase, lipases (hydrolyzed Tween 20, 40, 60 and 80), etc. The *Haloferax larsenii* lipase (HLL) produced in broth culture was precipitated by using isopropyl alcohol and further purified by reverse-phase chromatography (RP-HPLC). Ultra-performance liquid chromatography (UPLC) analysis was used to determine the amino acid profiles. SDS-PAGE and Matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) analyses revealed that HLL was a monomer, with a molecular mass of 31kDa. Compared to Lipolase (Novozymes), HLL displayed an elevated and considerable compatibility with various commercialized laundry detergents, and wash performance analysis revealed that it could remove oil-stains effectively. HLL has a number of attractive properties that make it a potential candidate for the synthesis of non-aqueous peptides and application in detergent industry.

Keywords: *Haloferax larsenii* halolipase (HLL), MALDI-TOF-MS, UPLC, Detergent wash performance, Okhamadhi.

1. INTRODUCTION

Lipases belonging to serine hydrolase family are one of the largest group of enzymes with diverse catalytic functions, classified as triacylglycerol acylhydrolase (EC 3.1.1.3) which catalyzes the hydrolysis of the carboxyl ester bonds in triacylglycerols (TAGs) to produce diacyl-glycerols, monoacylglycerols, fatty acids, and glycerol [1]. In addition, lipases catalyze the hydrolysis, acidolysis, aminolysis, and trans-esterification of other esters as well as the synthesis of esters [2]. They are produced by several microorganisms namely, bacteria, fungi, archaea and eukarya, as well as animals and plants. They have potential applications in bioprocesses due to their activity and stability especially in organic solvents as well as ability to catalyze the synthesis and translocation of ester linkages, mainly under low water content or non-aqueous conditions [3]. The lipases of microbial origin are most interesting, because they are versatile biocatalysts with an application in diverse industries such as meat (egg processing) and fish industry, paper industry (removal of subcutaneous fat), textile industry (processing for the removal of impurities from raw cotton), dairy industry (for the hydrolysis of milk fat and cheese ripening, bakery products, confectionary and cheese flavouring, oil industries, tea processing, wine making, pharmaceuticals (synthesis of chiral drugs and organic synthetic compounds), lipid biotechnology (emulsifiers, cosmetics, flavors, fragrances, detergent industries (removal of fatty food stains and sebum from fabrics), biodiesel production (catalyzes esterification and trans-esterification reaction to produce methyl esters), bioplastics, remediation (removal of lipid rich pollutants) oleochemical industry, waste water treatment, etc. [4-7]. Lipolase (NOVO industrials, Denmark) was the first commercial recombinant lipase industrialized from the fungus *Termomyces lanugiwnosus* and expressed in *Aspergillus oryzae* in 1994 [8].

Halophilic hydrolases such as lipases are reportedly advantageous especially with regard to their stability under extreme conditions of elevated temperature and salinity as well as organic solvent compatibility [9, 10]. Although a large number of halophilic lipases have been

listed on the NCBI's protein database, very few have been purified and characterized in detail, and not much is known about the correlation of their solvent stability with structural features. Structural studies further require purified form of lipase [11]. Halophilic proteins have a predominance of acidic amino acids to maintain their structure in high salinity, making them resistant to aggregation which is a problem with non-halophilic and thermophilic lipases [12]. However, halophilic extremozymes from haloarchaea are scarcely explored, unlike the conventional enzymes from non-halophilic eubacteria [13-17]. Currently, many resources have been investigated worldwide in order to develop biotechnological and industrial applications of extremozymes [18-20]. Halophilic lipases have been reported from *Hfr. mediterranei, Hfr. lucentensis, Haloarcula* sp., *Micrococcus halobitus, Marinobacter litoralis, Natronococcus sp., Halovivax sp.,* etc. [10, 13, 19, 21-23].

The present study is in continuation of our previous work in which six (6) haloarchaeal strains producing extracellular hydrolyases were reported [24]. To the best of authors' knowledge, this work is the first report describing the purification of lipase and biotechnological application of halophilic archaeal strains isolated from solar salterns at Okhamadhi site near Dwarka.

2. MATERIAL AND METHODS

2.1. Substrates, chemicals, reagents

Glyerol tributyrin was procured from sigma Chemical Co, (USA); yeast extract, gum acacia powder, glycine, Tween-80 and all other analytical grade chemicals and reagents were purchased from Hi-Media Laboratories (Mumbai, India) or MERCK Specialties Private Limited (India). Magnesium sulfate and Tris-buffer were purchased from Qualigens Fine Chemicals Limited**.** Olive oil used for lipase production was locally available. Unless otherwise specified, all substrates, chemicals, and reagents were of the analytical grade or highest commercially available purity. The lipase from *Thermomyces lanuginosus* or Lipolase a well-known commercial valuable enzyme was obtained from Novozymes (NOVO Industrials, Denmark) was used as a standard product. It was in liquid form and used after pretreatment with 25 mM potassium phosphate buffer to make half of its initial concentration.

2.2. Screening for lipase producing haloarchaea

The lipase producing haloarchaeal strain was isolated from Okhamadhi salterns (*Hfr. larsenii* BVM005) as described earlier [24] and used for halolipase production. For inoculum preparation, loopful of the stock from slant culture that was grown in the modified Mullakhanbhai and Larsen (M-L) medium [25] (%w/v): 25.0 NaCl; 5.0 $MgCl_2 . 6H_2O$; 0.5 K_2SO_4; 0.2 $CaCl_2$; 0.5 Peptone; 0.5 Yeast Extract; 0.2 Glycine; 2-3.0 agar-agar powder, pH 7.5. The medium for lipase production was the same as the above isolation medium (without agar) that was supplemented with 1.0 % v/v Olive oil and 1 % v/v Tween 80, pH 7.5. The culture was grown at 37°C with shaking at 150 rpm till a constant absorbance of 1.0 was attained at 600 nm to obtain an active growing culture as the inoculum. Then, 50ml of the medium in 250ml Erlenmeyer flask and seeded with 1.0ml of inoculum (2.0 % v/v). The incubation was carried out at 150 rpm (orbital shaker), 37°C for 5 days. Samples were withdrawn at regular time intervals were centrifuged at 8000×g for 15 mins and the enzyme activity was assayed in the cell free supernatant.

2.3. Titrimetric determination of lipase activity using triacylglycerol substrates

Halophilic lipase activity was determined according to the modified method (Potentiometric Titration Method) as described by scientists [26, 27] using Glycerol tributyrin as substrate with few modifications. The substrate emulsions containing 75 mM triacylglycerol and 3% (w/v) gum arabic was dispersed in 2.5 mM Tris-HCl buffer (pH 7.0). The reaction was performed in a glass vessel containing 9.5ml of emulsion and 0.5ml of the enzyme at 37°C. Finally, the lipase activity was measured by the production of free fatty acids and butyric acid was formed as the end product. The alkali consumption was registered as a function of time at pH 7.0 using an automatic Potentiometric Titrator. One unit of enzyme was defined as the amount of enzyme that liberated 1μmol of butyric acid, per minute. All measurements were performed in triplicate. Results are expressed as the average of these triplicate measurements ± the standard error of the mean.

2.4. Protein determination

Protein concentration was measured [28] with bovine serum albumin (BSA) as the standard protein [29]. The absorbance was measured at 595 nm. Protein content (mg/ml) was determined from the sample.

2.5. Specific activity

The specific activity of the purified enzyme after each purification step was determined by number of activity units per milligram of protein.

2.6. Halophilic lipase: purification and characterization

The culture was grown for five days and then centrifugation was carried out at 8000 rpm for 30 minutes to remove the microbial cells. The supernatant containing extracellular lipase was clarified by filtration through a 0.45µm membrane and submitted to the following purification steps. An equal amount of pre-chilled isopropyl alcohol (IPA) was mixed with the chilled filtrate with continuous stirring (2h) and then stored at 4°C in a refrigerator for 2 days. It was then centrifuged at 12,000 rpm for 30 min (4°C) and the pellet obtained was dried completely so as to remove any traces of IPA. The powder was later dissolved in a minimal volume of 20mM phosphate buffer (pH 7.2) and stored at 4°C. The lipase sample was further purified by RP-HPLC.

2.6.1. Analytical RP-HPLC of lipase

The crude lipase was analyzed for homogeneity by RP-HPLC under the following conditions: column: AGILENT (make) reverse phase HPLC (1200 series) with flow rate 1.0ml/min; initial solution A): 0.1% TFA in Milli Q water linear gradient with solution B: 0.09% TFA in (1:9) ACN. 20mg of the haloarchaeal (Lipase) and 1mg of standard lipase were weighed and dissolved in Milli Q water, 500µL of the sample was transferred into HPLC vial and loaded onto the instrument with 100µL injection volume. Protein was detected at 280nm. The lipase activity was determined in the precipitate and supernatant both. The fractions showing the highest lipase activity were pooled and stored at 4°C for SDS-PAGE and further enzyme characterization.

2.6.2. SDS-PAGE for Molecular weight determination and Zymography

For determination of molecular weight, Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on vertical slab gels according to the method [30] using 5% and 12% polyacrylamide for the stacking and resolving gels, respectively. The gel was stained by Silver staining method (Biorad Labs.) and molecular weight (Mr) was estimated by comparison with broad range molecular weight standards (10-250 kDa). Non-denaturing SDS-PAGE was performed using a discontinuous SDS-PAGE gel under non-reducing conditions (for non-denaturing PAGE, 10% separating gel and a 4% stacking gel were used). Electrophoresis was carried out at 110 mV at 4°C. The band corresponding to the lipase was identified by zymography. Zymography was performed by including Tween 80 in the medium and the gel slab was placed on it, after overnight incubation the halo zone was observed after silver staining around the band.

2.6.3. UPLC (Ultra-performance liquid chromatography)

The free amino acids of the lipase from the *Hfr. larsenii* strain was determined by UPLC, using an Acquity® UPLC H-Class System (Waters, Milford, USA) that was equipped with a quaternary solvent manager (QSM), a sample manager with Flow-Through Needle (FTN), a column heater (CH-A) and a photodiode array (PDA) detector. A Waters AccQ·Tag™ (The AccQ•Tag is a derivatizing reagent developed specifically for amino acid analysis) Ultra RP Column, Acquity® UPLC Ethylene-Bridged-Hybrid (BEH) C18 (pore diameter 130 Å, particle size 1.7 µm, inner diameter 2.1 mm, length 100 mm) column was used. 100ul of the sample was taken and transferred into a clean glass vial. To 50ml broth tubes, 2.0ml of 6N HCl was added and the glass vial containing the sample was dipped into the tube. The broth tubes were sealed with parafilm. The tube was placed in a dry bath at 60°C under N_2 gas for 15 minutes, to maintain inertness. Then the temperature was increased to 110°C and incubated for 24 hrs. 200µl of Borate buffer was added to the pellet, vortexed and centrifuged. After centrifugation, 10µl of the supernatant was taken for derivatization. Derivatization was done by taking 10µl of sample, 70µl of Borate buffer, 20µl of Accq Tag ultra-reagent and incubated for 10 mins at 55°C. After incubation 1.0µl was loaded on to the instrument, which was quantified using a Sigma standard. Buffers used were Mobile Phase A: Accq Tag Ultra eluent A1, and Mobile Phase B: Accq Tag Ultra eluent B.

2.6.4. Matrix Assisted Laser Desorption/Ionization-Time of Flight - Mass Spectrometry (MALDI-TOF-MS)

The sample was taken for digestion. The band/spot was cut into small pieces and de-stained using 1:1 ratio of 15mM $K_3[Fe(CN)_6]$ and 50mM Hypo for 15mins, followed by Buffer washes and dehydration using acetonitrile. The sample was then treated with 100mM DTT at 56°C for 1.0 hr followed by 250mM IDA at room temperature in dark for 45min.The sample was then digested with Trypsin and incubated overnight at 37°C. The resulting tryptic peptides were extracted using 0.1% TFA and vacuum dried and dissolved in 5µl of TA buffer. Then 1.5µl of the sample was mixed with

1.5µl of (α-Cyano-4-hydroxycinnamic acid) HCCA matrix and 1.5µl of the mixture was spotted onto the MALDI plate. The peptides obtained were mixed with HCCA matrix (5.0mg/ml α-Cyano- 4-hydroxycinnamic acid in 1:2 ratio of 0.1% TFA and 100% ACN) in 1:1 ratio and the resulting 2.0ul was spotted onto the MALDI plate (MTP 384 ground steel (Bruker Daltonics, Germany). After air drying the sample, it was analyzed on the MALDI TOF/TOF ULTRAFLEX III instrument (Bruker Daltonics, Germany). External calibration was done with standard peptide (PEPMIX Mixture) supplied by Bruker, with masses ranging from 1046 to 3147 Da. Further analysis was done with FLEX ANALYSIS SOFTWARE (Version 3.3) in reflectron ion mode with an average of 500 laser shots at mass detection range between 500 to 5000 m/z. The masses obtained were submitted for Mascot search in "Haloferax" database for identification of the protein. The MALDI data was analyzed using MASCOT software for protein sequencing. The spectrum obtained was analyzed by the online software MASCOT (http://www.matrix-science. com).

2.7. Wash performance analysis of the purified HLL

Wash performance analysis of the purified HLL as a biodetergent additive was evaluated on white cotton cloth pieces which were stained with sauce and fat/greasy butter, and vegetable oil material. The stained cloth pieces were incubated in different wash treatments at 37°C and agitated at 120 rpm for 60 min in 250ml Erlenmeyer flasks containing a total volume of 100 ml of: Tap water, Ariel detergent at final concentration of 7 mg/ml (heat inactivated), and then the detergent was added to each lipase (HLL or Lipolase) solution (at 75 U/mL). After treatment, the cloth pieces were removed, rinsed with distilled water, dried and subjected to visual observation to examine the stain removal effects of the enzymes.

3. RESULTS AND DISCUSSION

3.1. Sampling, isolation, and cultivation of haloarchaea producing lipases

One of the largest integrated marine chemicals complexes in the South East Asia is located at Tata Chemicals Limited (TCL), Mithapur, on the west coast of Gujarat State, India. Water (brine) samples of various densities and colour were collected from Okhamadhi site (owned by TCL) near Dwarka. The haloarchaeal isolates BVM005 hydrolyzed all Tweens (20, 40, 60 and 80). This strain was a moderately halophilic archaeon that required more than 2.5M NaCl for its optimal growth as well as high demand of magnesium (0.02-0.04 M). Optimal growth was observed at 37-40°C and pH 7-8.0. In our previous work we reported six (6) haloarchaeal strains with extracellular hydrolyases [24]. From the two (2) *Haloferax* strains exhibiting lipolytic activity *Hfr. larsenii* BVM005 was selected for lipase production and characterization. A study was carried out on the extracellular lipase (HML) from haloarchaea *Hfr. mediterranei* strain ATS1, isolated from the Sebkha (Medea, Algeria) [19]. Several studies have been carried out in order to isolate new halophilic archaea with lipase activity [9, 31-33]. Ozcan B *et al*. have reported five halophilic archaeal strains that grew in a range from 3.0 to 4.5M NaCl for optimal extracellular esterase activities [33]. The phylogenetic relationship between the halophilic isolate BVM005 with other *Haloferax* strains/ species is shown in (Fig. 1).

3.2. Extracellular lipase production

During the growth of *Hfr. larsenii* BVM005 in submerged fermentation, the extracellular lipase was produced (fig.2) as growth associated metabolite reaching a maximum value of 146.52U/ml (using Tributyrin) at the end of the exponential phase and the early stationary phase. Lipase production using tributyrin as a substrate, and along with other substrates has been reported [34, 36]. Extracellular lipase activity (from *Natronococcus* sp.) increased as the concentration of NaCl increased, reaching a maximum value at 4.0M of NaCl and showing no activity in the absence of salt has been demonstrated by Ozcan B *et al.* [33]. It has been reported previously about similar correlation for growth and enzyme production at the beginning of the stationary phase of growth for strain *Hfr. mediterranei* CNCMM 50101 [19].

3.3. Purification and characterization of *Haloferax larsenii* lipase (HLL)

Purification process of lipase occurs in sequential manner. Lipases have been purified and characterized from diverse microbial and archaeal sources. However, no single technique or generic protocol can be defined as the best. The purification strategy varies from source to source [37]. Lipases have been purified for different purposes ranging from industrial application, medical uses, the 3-D studies. Depending on the purpose for the purification, some factors such as its degree of purity, cost and properties such as temperature and pH stability may be considered. In the present investigation, purification of lipase produced by the halophilic strain,

Hfr. larsenii BVM005 (KP636736) was undertaken with multi-step procedures using isopropyl alcohol precipitation followed by ultra-filtration, Reverse-phase chromatography, Ultra-performance liquid chromatography analysis (for the free amino acid profiles). In the next step by analytical RP-HPLC it got further concentrated and enzyme was concentrated further. The orange pigment produced by the haloarchaea was removed during isopropyl alcohol precipitation. This step removed traces of alcohol as well as the low molecular weight proteins. The protein formed high molecular weight aggregates and hence it was possible to concentrate the 31 kDa protein-aggregates using 100 kDa molecular weight cut off membrane. The purification procedures resulted in a pure protein, giving a single band on a silver-stained SDS-PAGE gel.

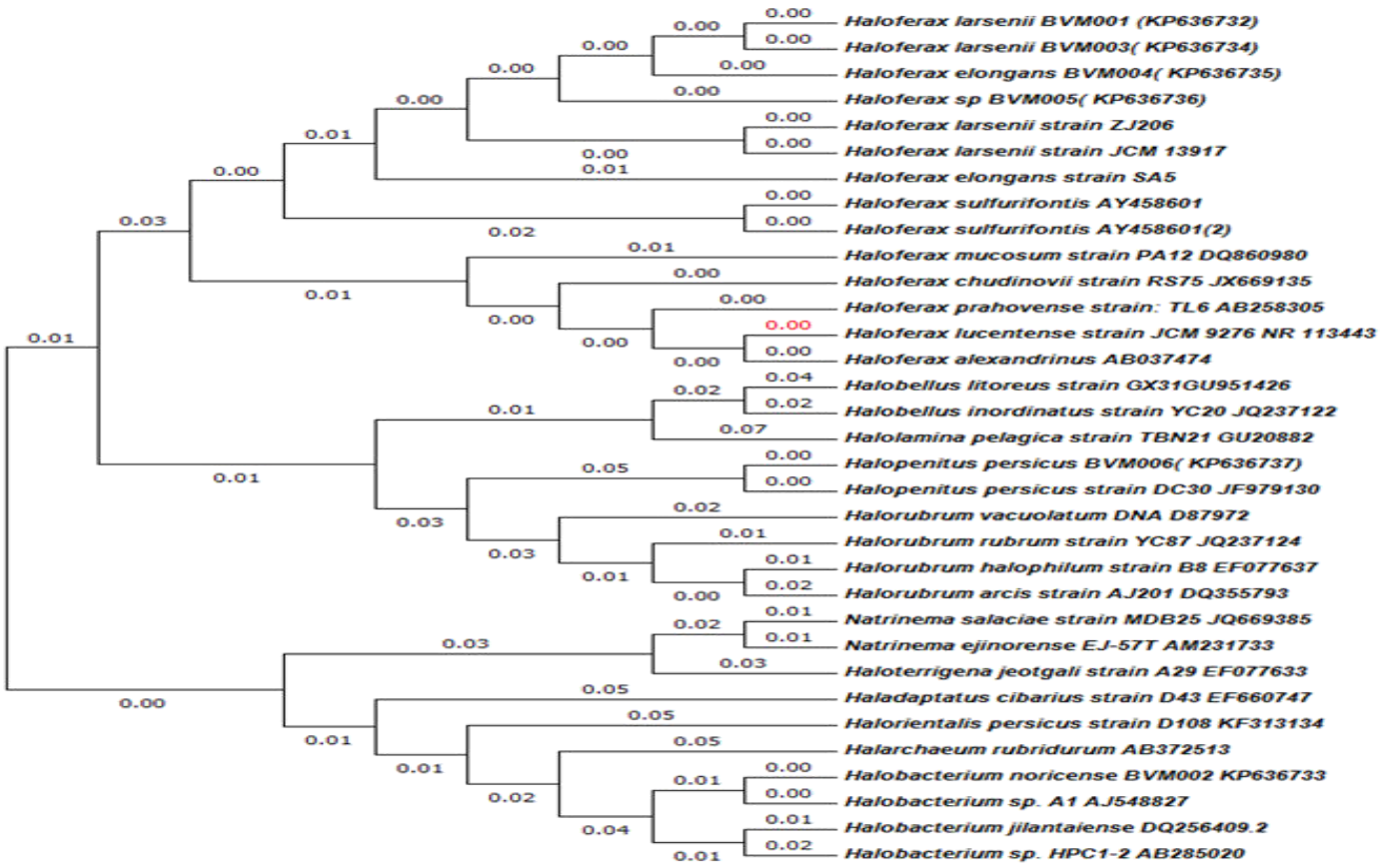


Fig. 1: Phylogenetic tree showing the position of investigated strain *Haloferax larsenii* BVM005

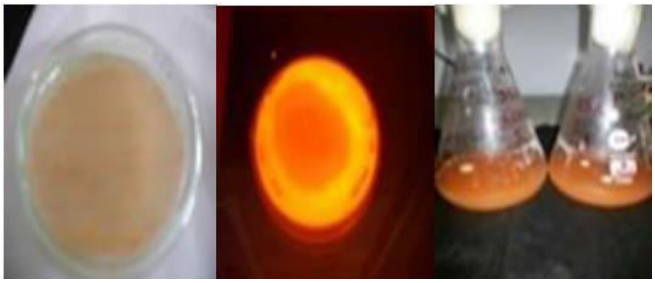


Fig. 2: (a) Isolation of lipase producing haloarchaea; (b) Lipase activity of isolate BVM005 demonstrated by halos (in medium containing Rhodamine B and Tween 80; (c) Lipase production in broth culture.

3.3.1. Isopropyl alcohol precipitation preparation (for crude enzyme powder preparation)

Partial purification of HLL produced by *Hfr. larsenii* BVM005 was carried out by IPA precipitation. Haloarchaeal lipase purified at a ratio 1:3.5 level of IPA gave better halophilic lipase activity. Lipolytic activity with about 2.0-fold increase in corresponding halophilic lipase yield (146.52 U/ml) (fig.3).

3.3.2. Analytical RP–HPLC of purified lipase

Reverse-phase chromatography is known to be an effective method for separating and purifying peptides from protein hydrolysates [38]. The presence of major

protein in the sample was confirmed by analytical reversed-phase HPLC. The little difference between their retention times in standard and sample might indicate a strong similarity of both lipases. The HPLC chromatogram for purified enzyme produced from our isolate indicated the presence of lipase enzyme. The active fractions collected after IPA were pooled and then subjected to HPLC chromatography, the enzyme was eluted as a sharp peak with retention time 7.309 min (Fig.4).

Fig. 3: Partially purified haloarchaeal lipase powder from *Hfr. larsenii* BVM005

3.3.3. SDS-PAGE for Molecular Weight Determination and Zymography

SDS-PAGE analysis showed (Fig. 5) that the purified HLL was a monomeric protein. The size of lipases is diverse including enzymes as small as 19.4 kDa and oligomeric forms of above 300 kDa with subunits around 50 kDa [39]. It is important to remark that when the molecular weight of halophilic proteins is determined by SDS-PAGE technique, it is possible to overestimate their mass because the excess of acidic residues causes an aberrant migration in the gel. The purified enzyme showing lipase activity was observed as a single band corresponding to the molecular weight of approximately 31.0 kDa, which indicated the purified lipase had no other impurity. Taken together, these observations strongly suggested that HLL is a monomeric protein. Comparable with our results, a previous study done with *B. coagulans* BTS-3 have reported lower molecular mass 31.0 kDa lipase [40]. In contrast, purified lipase with molecular weight 45.0 kDa at pH 7.0 and temperature 60°C from *Hfr. mediterranei* CNCMM 5010 has also been reported [19]. A lipase with molecular weight of about 33 kDa by SDS-PAGE at pH 8.0 and temperature 35°C has also been reported [41]. In previous studies, the molecular weight of lipase from halophilic bacteria varied from 23.0 to 62.0 kDa [34]. It was stable at pH 7-7.5. Therefore *Hfr. larsenii* BVM005 haloarchaeal lipase had representative molecular weight of lipases reported from other haloarchaea.

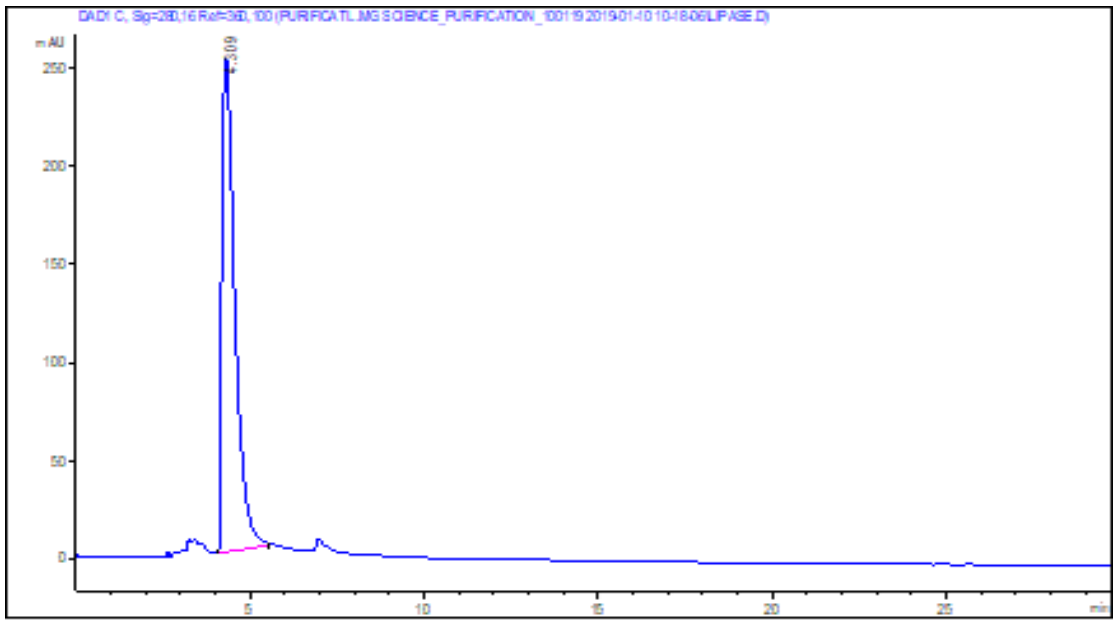
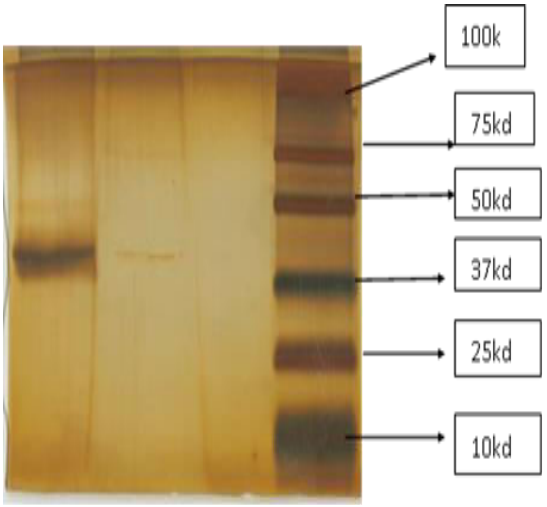


Fig. 4: Chromatogram of the purified HLL produced by *Hfr.larsenii* BVM005

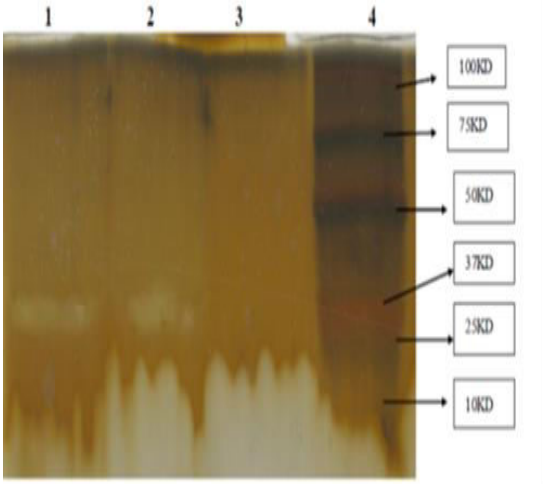


Lane 1. Standard, 2. Sample of halolipase, 3. Negative Control, 4. Molecular weight Marker

Fig. 5: Silver stained SDS-PAGE gel of fractions Std: molecular weight standards10-100 kDa (Bio Rad)

3.3.4. UPLC (Ultra-performance liquid chromatography analysis for the free amino acid profiles of the Halolipase)

Separation and quantification of 23 amino acids in a chromatogram needed more than 35 min using conventional HPLC [42-43]. UPLC not only offers higher peak capacity, greater resolution, good sensitivity and high speed of analysis [44] but also short analysis time. It needs only 8 min for good separation of 26 amino acids, which is only 1/4 of analysis time of HPLC with better resolutions. UPLC analysis method (Fig.7.a) also showed there were many unidentified peaks, namely, there were some amino acids not included in the standard mixture or other compounds that contained an amino group that could react with the AccQTag Ultra reagent. Detailed knowledge regarding the amino acid composition of lipase along with the structures enables new design of enzymes well-suited for future industrial applications.



Lane 1- Standard protein marker, Lane 2- Purified lipase, Lane 3- empty, Lane 4- Marker

Fig. 6: Zymogram analysis of purified lipase from *Hfr. larsenii* BVM005

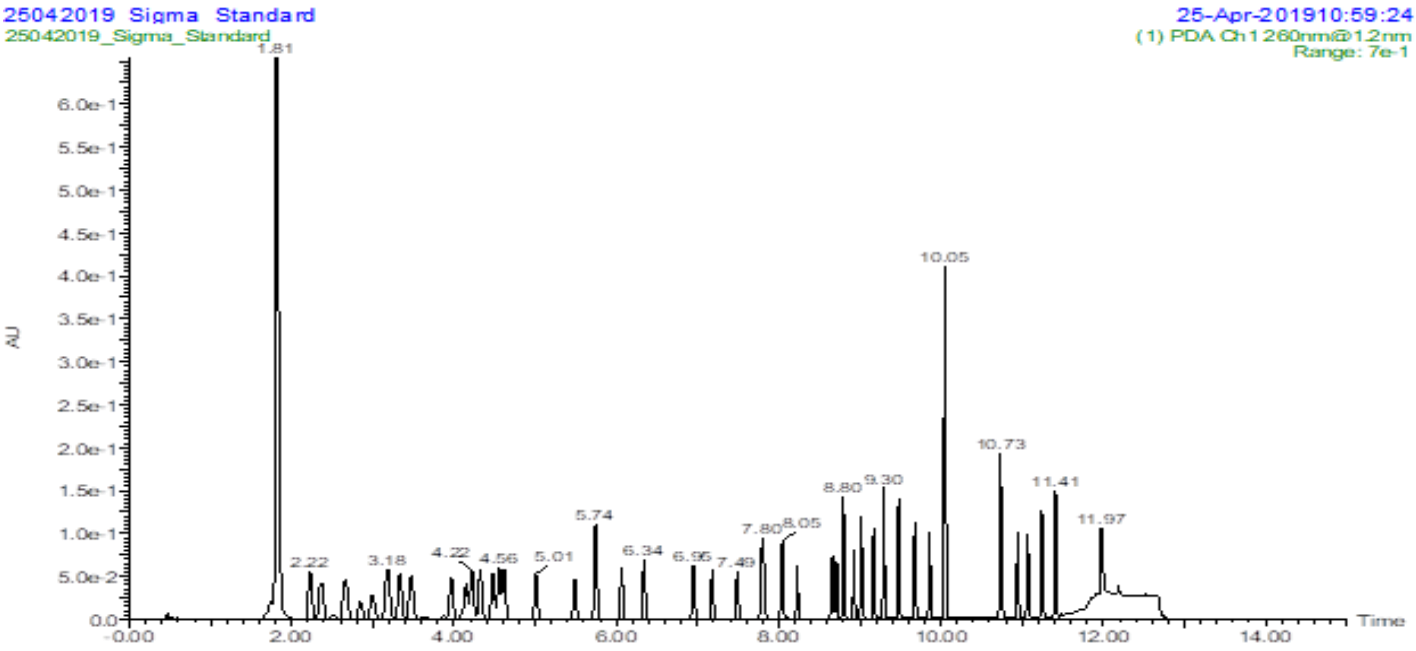


Fig. 7(a): UPLC chromatogram of amino acid of standard Lipolase (Novozyme)

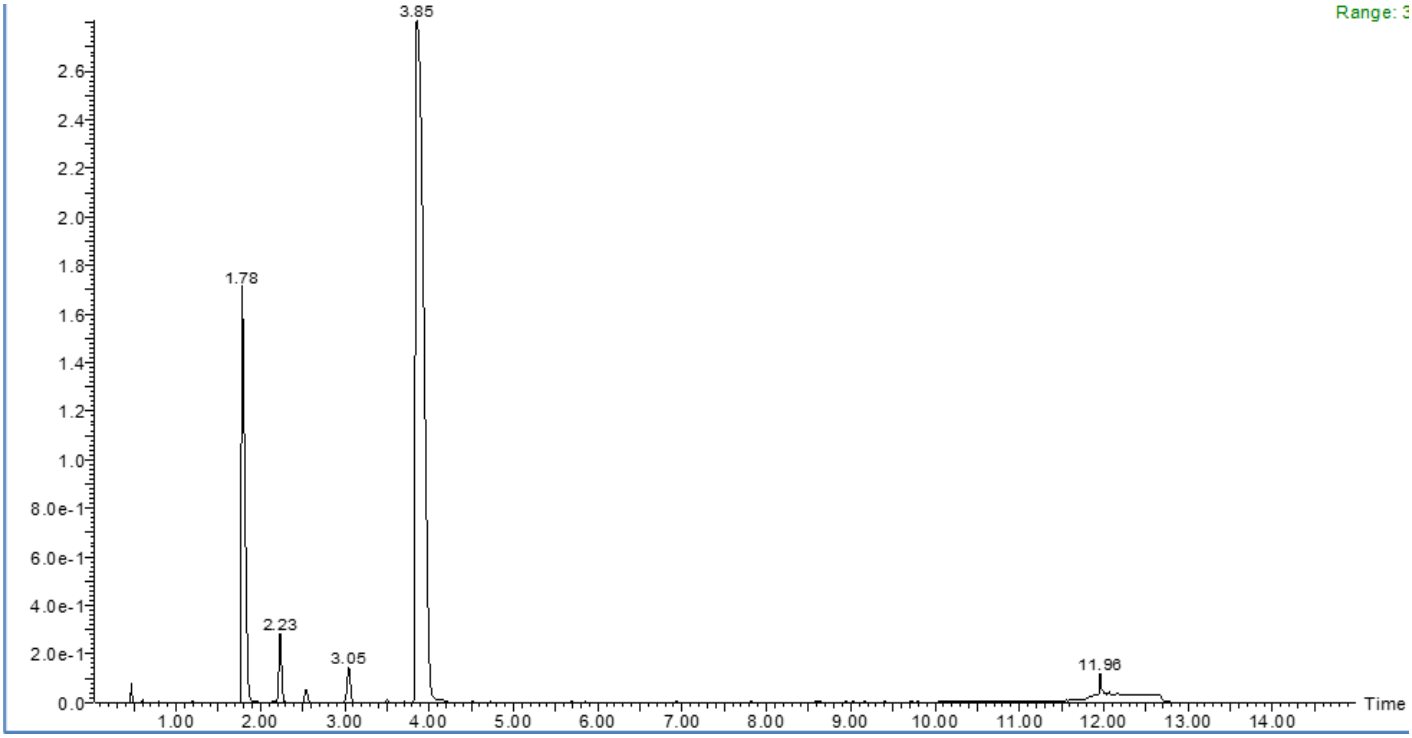


Fig. 7(b): UPLC chromatogram of amino acid of halolipase from *Hfr. larsenii* BVM005

3.4. Matrix Assisted Laser Desorption/Ionization-Time of Flight/Time of Flight Mass Spectrometry (MALDI-TOF-MS)

The purified haloarchaeal lipase was analyzed by MALDI-TOF for sequence identification. MALDI-TOF-MS confirmed that the purified haloarchaeal lipase HLL had 55% of sequence coverage. Together, these results confirm the identity of the purified protein. From the obtained MALDI spectrum (Fig. 8) the peptide masses were interpreted to generate amino acid sequence of lipase purified from *Hfr. larsenii* BVM005 using MASCOT software. The putative amino acid sequence is shown in (Fig.8.b). Together, these results confirm the identity of the purified protein. Similar results [45] has confirmed that the purified lipase from *Yersinia enterocolitica* namely LipC12 had 68.4% of sequence similarity and has 293 amino acids.[46] has pointed out and described method MALDI-TOF-MS, showed an intense peak corresponding to 47.162 kDa from thermostable lipase from *Geobacillus*. 19. Bhattacharya [19] has shown that halophilic lipase HML (*Hfr. mediterranei* strain ATS1) is a monomer with a molecular mass of 45 kDa on analysis using MALDI-TOF-MS.

3.5. Wash performance analysis of purified HLL and Lipolase enzymes

Lipases are used in detergent industries to minimize phosphate-based chemicals in detergent formulations, and reduce or replace synthetic detergents to provide eco-friendly product. In the household laundry it reduces environmental pollution and enhances the ability of detergent to remove tough oil or grease stains. The effect of different commercial detergents on oil removal from the sauce sample stain with greasy material has been shown in (Fig. 9). The supplementation of HLL (*Haloferax laresnii* lipase) in detergent significantly improves the cleansing of the oil/ greasy material and testifies the scope for use of this lipase as biodetergent additive. Recently scientists [19] have also reported that supplementation of HML (*Haloferax mediterranei* lipase) or Lipolase in detergent significantly improves the cleansing of the oil from cotton cloth. In fact, HML facilitated the release of fatty acids in a much easier way than the currently used lipolase (Novoenzyme). Thus, our results are in agreement with the findings mentioned above and that of [47] that reports the use of lipase from *Ochrobactrum intermedium* strain. Scientists [48] have reported the use of *Bacillus methylotrophicus* PS3 strain for lipase production and its application in detergent industry. It has been demonstrated that combination of extracellular lipase from *Haloferax* sp BVM005 with different commonly used detergents could enhance the removal of greasy stains from textile.

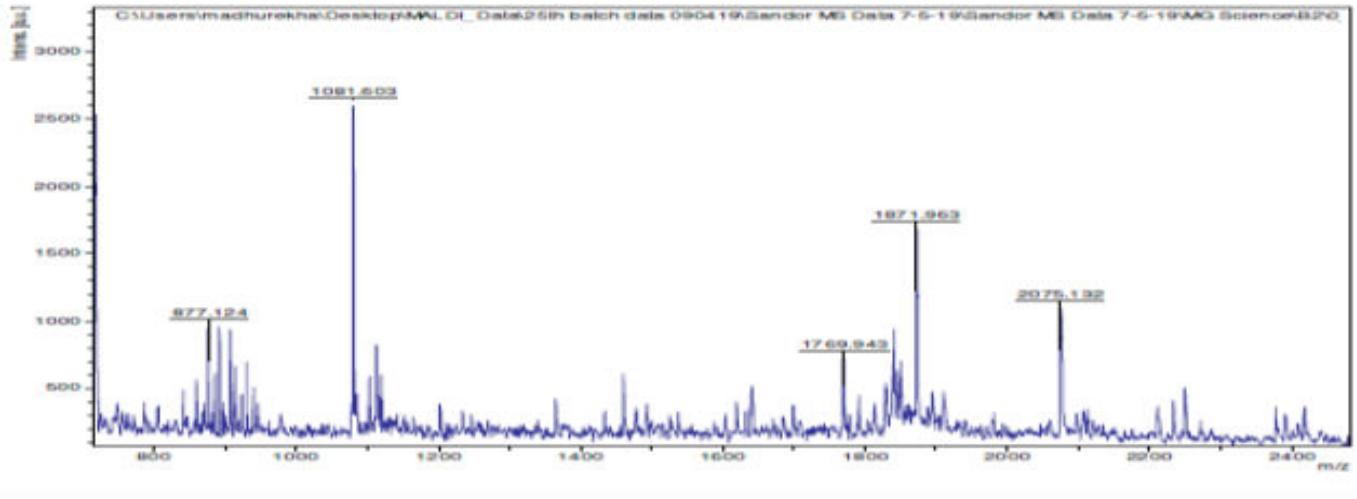
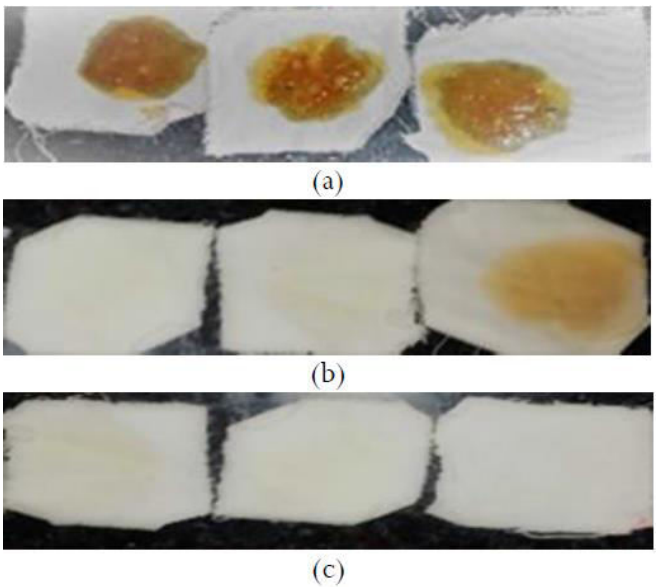


Fig. 8: a) MALDI-TOF-MS spectrum of purified HLL

>Lipase from *Haloferax larsenii* BVM005 (KP636736)
MNDSQGALADREWRLIREDIRSGPMQMALDEVAGETAANGGPRTVRVYSWEPSCLSLGY
GEDPDTVDWEFCEREGIDVTRRPTGGGGIYHDRDGDVAYSIVAPKAELPGDLIDCYHLLCE
PILDAIRSVGIDVDFVDEDVPVIWHPACYLRALHPAHDMVAEGRKIAGNAQYRRRDAVVQ
HGSLTYSVDAETHLGVFDGHDVTPEEFRDRVVGVDELADVSRETFVEAVTDSLADFVDAE
EGSWTDDELDRARTRVEEKYATDEWVRRNPRERQ

Fig. 8: b) Putative amino acid sequence of the purified HLL



(a) Cloth stained with sauce sample with fat/greasy material (b) oil-stained cloth treated with Aerial detergent and washed with distilled water (c) oil-stained cloth treated with Ariel detergent (heat treated) and purified HLL and washed with distilled water.

Fig. 9: Wash performance evaluation of the purified lipase HLL form

4. CONCLUSION

We have isolated and characterized a hydrolytic extremozyme, HLL (*Haloferax larsenii* lipase) from the halophilic archaeon *Hfr. larsenii* BVM005. The purity of the protein was checked by HPLC analysis, which has shown a sharp peak at retention time of 0.739 min. The purified lipase showed a single band when it was subjected to SDS-PAGE, with molecular weight of 31 kDa. HLL was precipitated by using isopropyl alcohol precipitation and subjected to ultrafiltration, Reverse phase chromatography (RP-HPLC), Ultra performance liquid chromatography (UPLC) analysis for the free amino acid profiles. The enzyme was also found to be stable in the presence of various detergents. This HLL (*Haloferax larsenii* lipase) enzyme has the potential for removing oil stains and was compatible with commercial detergents. Furthermore, this lipase has strong potential for commercial exploitation in various biotechnological applications. The purified lipase will be further characterized for structural analysis like amino acid sequencing for homology modeling and prediction of enzyme structure for further uses.

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Conflict of interest

None

6. REFERENCES

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