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Purification and Characterization of Active Aggregates of an Organic Solvent Tolerant Lipase from *Marinobacter* sp. EMB5

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Abstract

Background: *Marinobacter* is a halophilic bacterial genus and a bio-geochemically important group in sea water, is generally associated with the bioremediation of hydrocarbon-rich effluents released into the salt-rich environment by various anthropogenic activities. It is a well-known lipase producer-the NCBI Protein database shows nearly 520 *Marinobacter* lipases. In this context, its lipase and esterase are considered important. However, their enzymatic properties in purified form have been scantily studied. Since hydrocarbons are acted upon on solvent water interface, the stability and catalytic behavior of *Marinobacter* lipase in non-aqueous media needs to be more specifically investigated.

Aim: *Marinobacter* sp. EMB5 was isolated from Kozhikode, Kerala, India. It was found to be potent lipase producer. The aim of the present work was to obtain a purified preparation and undertake its detailed characterization with respect to above aspects.

Work: The crude *Marinobacter* sp. lipase was purified by Sephacryl S200 gel filtration chromatography. The lipase eluted as a single activity peak in void volume and had a very high molecular mass. Aggregation has not been reported in case of *Marinobacter* lipase, so far, although it is known in other lipases. The aggregated lipase was mixed with 2-propanol (70%, v/v) and disaggregated lipase was obtained as a single activity peak in Sephacryl S200 gel filtration chromatography corresponding to 82 kDa molecular mass. This monomeric species was less active than aggregated purified preparation. Hence, further characterization and studies were carried out on purified active aggregate lipase. It was stable in a wide range of organic solvents at high concentration up to 24 h. The organic solvent stability was further investigated by Far UV CD spectroscopy and fluorescence spectroscopy.

Conclusion: Due to the fact that *Marinobacter* sp. EMB5 lipase remained stable in organic solvents for prolonged incubation periods, it has potential to be used in industrial organic synthesis.

Keywords: Halophilic lipase; Solvent tolerant lipase; *Marinobacter* lipase; Active lipase aggregates; Haloalkaliphilic lipase

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Introduction

Halophilic lipases are interesting for biotechnological applications as they retain their functionality even under high salt and low-water conditions. Low-water conditions predominate within

the halophilic cell, due to excessive accumulation of salts within the cytoplasm as an adaptation mechanism. In the industry, organic synthesis reactions carried out under solvent-rich conditions mimic such low-water conditions. Since the activity

of halophilic lipases is not compromised even in presence of very high concentration of organic solvents, they are very promising candidates for carrying out lipase-catalyzed organic biotransformation [1-3].

Although a large number of halophilic lipases have been detected and listed on the NCBI's protein database (<http://www.ncbi.nlm.nih.gov/protein>), very few have been purified and characterized in detail. These include *Oceanobacillus rekensis* PT-11 lipase [4], *Haloarcula* sp. G41 lipase [5], *Idiomarina* sp. W33 lipase [2] and LipBL from *Marinobacter lipolyticus* SM19 [6]. Moreover, not much is known about the correlation of their solvent stability with structural features. Structural studies further require purified form of lipase.

Purification of halophilic lipase, in general, poses problem for two reasons, (i) Requirement of incorporating the salt (necessary to maintain native structure of halophilic proteins) throughout the purification process. Otherwise, protein is denatured leading to loss of activity [7]. But the addition of salt disrupts the chromatographic procedure. (ii) Propensity of aggregation in lipase causes formation of aggregates during purification, making purification quite unpredictable.

Most halophilic lipases are also organic solvent tolerant owing to a similarity in the micro-environment created by the presence of organic solvents and high salt concentration. LipBL from *Marinobacter lipolyticus* displayed remarkable stability in presence of both hydrophobic as well as hydrophilic solvents, with maximum stability in presence of methanol [6]. The *Idiomarina* sp. W33 and *Haloarcula* sp. G41 lipases were stable in presence of hydrophobic organic solvents [2,5]. *Oceanobacillus rekensis* PT-11 lipase was stable in presence of long-chain alcohols [4]. Lipase from *Idiomarina* sp. W33 and *Haloarcula* sp. G41 have been successfully used for biodiesel production. Protein structural studies to support organic solvent tolerant stability in halophilic lipases, are however not available. The present study addresses this gap.

Halophilic *Marinobacter* sp. EMB5 showing extracellular lipolytic activity was reported from our lab previously which showed significant levels of lipase secretion [8]. The present work describes the purification and characterization of the naturally aggregated lipase of this strain that showed remarkable organic solvent stability. The organic solvent stability of the lipase was further investigated by secondary and tertiary structure studies in presence of a stabilizing as well as destabilizing organic solvent.

Materials and Methods

Materials

Sephacryl S200 and p-nitrophenyl esters were a product of the Sigma Chemical Company (St. Louis, MO, USA). Polyacrylamide and bis-acrylamide were procured from GE Healthcare Life Sciences (Bangalore, India). Sodium dodecyl sulphate (SDS) and Bovine serum albumin (BSA) was purchased from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). Other media components were purchased from Hi-Media Laboratories (Mumbai, India). All other chemicals used were of analytical grade. All the solvents used in the present study were treated with molecular sieve (4 Å) prior to use.

Methods

Strain isolation, identification and lipase production: *Marinobacter* sp. EMB5 was isolated from the sea coast of Kozhikode (Kerala, 11°25'N 75°77'E) by salt enrichment. It was found to be related to *Marinobacter* sp. by the 16S rDNA sequence analysis which was submitted to GenBank, NCBI, USA with accession number FJ525429 [8]. Lipase was produced in a medium containing (g/l): peptone 5.0; yeast extract 5.0; NaCl 50.0; gum arabic 1.0 and olive oil 5.0 ml/l. The pH of the medium was maintained at 9.0. The production medium was seeded with 4% (v/v) inoculum raised overnight in nutrient broth with 5% (w/v) NaCl ($A_{660} \sim 1.0$) and incubated at 30°C and 160 rpm for 48 h. Cell-free supernatant (CFS), containing the extracellular lipase was obtained by pelleting down the cells by centrifugation of the culture broth at 8,000 rpm for 15 min.

Lipase assay: Lipase was assayed following the method of Kilcawley et al. [9] using p-nitrophenyl octanoate as the substrate. Briefly, 1.8 ml of Tris-HCl (50 mM, pH 8.5) containing 0.5% (v/v) Triton X-100 and 200 µL of diluted enzyme was pre-incubated for 10 min following which 20 µL of 50 mM p-nitrophenyl octanoate (PNPO) dissolved in acetonitrile was added to the reaction mixture. PNPO hydrolysis was carried out for 20 minutes at 50°C. The p-nitrophenol released by enzyme action was measured at 400 nm. One unit (U) of lipase activity was defined as the amount of enzyme releasing 1 nmol of p-nitrophenol equivalent per minute from the ester under standard assay conditions.

Lipase purification and characterization: The crude lipase was subjected to ultrafiltration using 10 MWCO and retentate, so obtained was subjected to size-exclusion chromatography by loading onto a Sephacryl S200 column (1.5 cm × 50 cm) equilibrated with Tris-HCl buffer (50 mM, pH 8.5, containing 2% (w/v) NaCl) and eluted at a flow rate of 24.0 ml/h. Fractions of 2 mL each were collected using the fraction collector (BioRad Model 2110, California, USA). Each fraction was assayed for lipase activity. The active fractions were pooled and characterized.

Disaggregation of EMB5 lipase: The *Marinobacter* sp. EMB5 lipase aggregates were treated with 70% 2-propanol, which was included in the SEC elution buffer as per the method of Dunhaupt et al. [10] (Table 2).

Protein determination, electrophoresis and zymogram analyses: Protein content was determined using the Bio-Rad Bradford Assay Kit (BioRad, California, USA) with bovine serum albumin as the standard. In order to determine the molecular mass of the lipase, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Laemmli [11]. A 12.5% SDS-PAGE gel was used on a BioRad Model Mini Protean gel electrophoresis unit (BioRad, California, United States). Silver staining was used to visualize protein bands on gel. Relative molecular mass (M_r) was estimated by comparison with molecular mass standard (14-97.4 kDa) (Bangalore Genie, Bangalore, India). Zymography was done by detection on lipolytic bands on the SDS-PAGE gel using tributyrin as substrate [12].

Characterization of EMB5 Lipase

Effect of temperature and pH on lipase activity and stability

The optimum temperature for enzyme activity was determined by assaying at various temperatures ranging between 30°C and 60°C.

Table 1 Purification of *Marinobacter* sp. EMB5 lipase.

Purification step	Total activity	Total protein	Yield	Specific activity	Fold purification
	(U)	(mg)	(%)	(U/mg)	
Crude	115874	200	100	579.37	1.0
Ultrafiltration(10000 MWCO)	88418	20	76.3	4420.9	7.6
Sephacryl S200	6525	0.4	5.6	16312.5	28.2

Table 2 Effect of various additives on lipase aggregation.

Source organism for lipase	Additive and its concentration	Molecular Mass of monomer (kDa)	Reference
<i>Pseudomonas cepacia</i>	70% (v/v) 2-propanol	33	[9]
<i>Bacillus thermocatenuatus</i>	1% (w/v) Sodium cholate	40	[24]
<i>Bacillus thermocatenuatus</i>	5% (v/v) 2-propanol and 2 mM LDAO	40	[14]
<i>Thermosyntropha lipolytica</i>	1% (w/v) SDS	56	[25]
<i>Marinobacter</i> sp. EMB5	70% 2-propanol	10*	Present study

*Molecular mass of monomer, as determined by gel filtration chromatography.

Thermal stability was determined by pre-incubating the enzyme at various temperatures (50°C-70°C) for different time periods and then measuring the residual lipase activity. Effect of pH on lipase activity was determined by assaying in buffers (50 mM) of varying pH viz. sodium acetate (pH 4.0-5.0), sodium phosphate (pH 6.0-7.0), Tris-HCl (pH 8.0-10.0) and Borax-NaOH buffer (pH 10.0-12.0). The effect of pH on stability was investigated by pre-incubating the lipase at various pH (ranging from 4.0 to 12.0) for 12 h at 25°C. The residual activity was determined under standard assay conditions.

Determination of kinetic constants

K_m and V_{max} values of the purified lipase were determined by assaying at different p-nitrophenyl octanoate (PNPO) concentrations. Kinetic constants were calculated using the Line weaver-Burk plot.

Effect of metal ions and additives

Lipase activity was studied in presence of 1 and 5 mM of metal salts viz. Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Zn^{2+} , Cu^{2+} , Hg^{2+} and additives phenylmethylsulfonyl fluoride (PMSF), p-chloromercuribenzoic acid (PCMB), Dithiothreitol (DTT), β -mercaptoethanol and Ethylene diamine tetra acetate (EDTA).

Surfactant stability

The effect of detergents, Sodium dodecyl sulfate (SDS), Cetyl trimethylammonium bromide (CTAB), Tween 80, Triton X-100 and Brij 35 at 1% (v/v) concentration, was examined by incubating the enzyme with these detergents at 30°C for 5 h. The residual activity was then measured by standard assay procedure.

Substrate specificity

Substrate specificity of lipase was checked against 50 mM of p-nitrophenyl esters of acetate (C2), butyrate (C4), caprylate (C8), caprate (C10), laurate (C12), myristate (C14), palmitate (C16) and stearate (C18) under the standard assay conditions.

Organic solvent stability

One milliliter of lipase was mixed with equal volume of organic solvents (ethanol, isopropanol, butanol, dichloromethane,

benzene, toluene, cyclohexane, hexane and decane) in screw capped tubes. The mixture was incubated at 30°C with constant shaking at 200 rpm for 24 h. Samples were withdrawn from aqueous phase and the residual enzyme activity estimated. Enzyme incubated without solvent was treated as control and its activity was taken as 100%.

Far UV CD spectroscopy

In order to study the effect of solvents on the secondary structure, the far UV CD spectrum of the control, cyclohexane and 2-propanol treated enzyme samples was recorded on a JASCO J-815 spectrometer (Jasco Corporation, Tokyo, Japan) using a cell with a path length of 0.1 cm and a scanning rate of 50 nm min⁻¹ with a 2 nm bandwidth over the wavelength range 200-250 nm for far-UV with five scans averaged for each spectrum using a protein concentration of 0.5 mg/ml in the respective buffers. All CD spectra were corrected for buffer contributions.

Fluorescence spectroscopy

The effect of cyclohexane and 2-propanol on the protein tertiary structure was studied from the fluorescence spectra recorded at 25°C on a Perkin Elmer LS50 spectrofluorimeter (PerkinElmer, MA, USA). The fluorescence emission spectra were recorded from 300 to 400 nm upon excitation at 295 nm in presence of cyclohexane and 2-propanol. These organic solvents were chosen based on the organic solvent stability of the lipase, which was highest in cyclohexane and least in 2-propanol. The excitation and emission slit widths were kept at 2.5 nm and 5 nm, respectively. All fluorescence spectra were normalized and corrected for buffer contributions. All the experiments were done in triplicate and the variation was within \pm 5%.

Results and Discussion

Halophiles have been perceived as a potential source of industrially useful enzymes endowed with exceptional stabilities in high salt, alkaline pH and organic solvents [13,14]. The isolation of the halophile *Marinobacter* sp. EMB5 strain, from Kozhikode sea water, India, has been previously reported by us [8]. The isolate was Gram-negative rod and grew well at high salt concentrations (1-20%, w/v) and pH range of 6.0-9.0. Identification of the isolate by 16S rDNA analysis

related it to genus *Marinobacter*. The sequence has been submitted in GenBank, NCBI, USA with accession number FJ525429.

The isolate was found to be a potent lipase producer. Lipase production was growth dependent. The Cell-free Supernatant (CFS) collected at 48 h was used for all purification and characterization studies. The crude was quite stable in cyclohexane and therefore an interesting system to understand the structural basis of this phenomenon in halophiles.

Lipase purification

Since very few lipases are purified from halophiles, *Marinobacter* sp. EMB5 lipase was first attempted to be purified by conventional gel chromatography using Sephacryl S200 (Table 1). The purification chromatogram is shown in Figure 1. The lipase activity was eluted close to the void volume, indicating possibility of high molecular weight lipase aggregates. Aggregation in non-halophilic lipases is well known and has been widely studied [15,16]. In the extreme halophile *Haloarcula marismortui*, an esterase with a tendency to aggregate in presence of high salt concentration has been previously described by Rao et al. [17]. Although aggregation is less expected in halophiles because the presence of high salt concentration in the cytoplasm may prevent aggregation of proteins. The *Marinobacter* sp. EMB5 lipase also shows the same phenomenon wherein despite presence of 2% (w/v) NaCl in the CFS, lipase aggregation is observed. These lipase aggregates are soluble, active and have a high molecular mass of 180 kDa (Figure 2).

Previous studies on disaggregation of other lipases have shown that certain additives such as 2-propanol, sodium cholate, lauryldimethylamine-N-Oxide (LDAO) and SDS successfully dissociate them into monomeric forms (Table 2). The *Marinobacter* sp. EMB5 lipase aggregates were disaggregated by the inclusion of 70% 2-propanol in the elution buffer of Sephacryl S200 gel chromatography. The lipase peak fractions obtained from SEC were pooled, concentrated against sucrose and subjected to SDS-PAGE. A single protein band confirmed the homogeneous purification of lipase with a molecular mass of approx. 82 kDa on the SDS PAGE gel (Figure 3a). Similar molecular weight lipases have been reported from non-halophiles, *Vibrio* and *Burkholderia* species and strains of *Pseudomonas fluorescens* (<https://www.ncbi.nlm.nih.gov/protein/?term=LIPASE>). The zymography also revealed the presence of hydrolysis zone in the tributyrin agar plate confirmed it to be a lipase (Figure 3b).

Thus, our study confirms the aggregation phenomenon in *Marinobacter* lipase despite the presence of 2% NaCl. The ultrafiltration (10,000 MWCO) followed by Sephacryl S200 gel exclusion chromatography in presence of 70% (v/v) 2-propanol led to disaggregation of lipase aggregates. Various combinations of chromatographic procedures have been employed for purification of halophilic lipases in the past. Two steps led to 2.5 fold purification with 15% yield in halophilic lipase from *Oceanobacillus* strain PT-11 [4]. Ammonium sulphate precipitation using 75% (w/v) ammonium sulphate followed by DEAE- Sepharose anion exchange and Sephacryl S-200 gel filtration chromatography resulted in 7.4 purification fold with 24.6% yield in case of *Idiomarina* sp. W33, extracellular lipase [2]. Thus, our strategy to purify monomer (disaggregated form) by incorporating 2-propanol and to purify aggregated form by omitting 2-propanol worked well.

Characterization of purified lipase

The purified aggregated lipase showed 16312.5 U/mg specific activity while the disaggregated showed 208.28 U/mg. Considering that (i) lipase is more active in aggregated form, as observed in

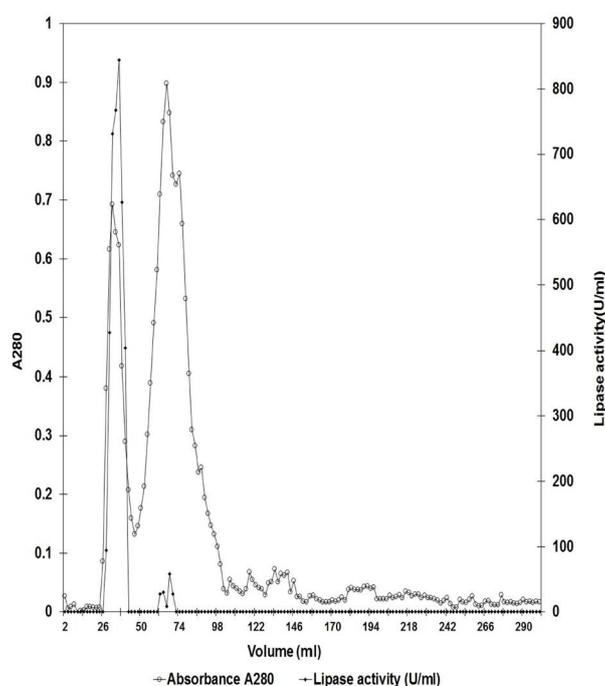


Figure 1 The first step of CE-mediated hydrolysis involves the nucleophilic attack of Ser –OH group on the –CO carbon of the ester bond thereby releasing an alcohol moiety and an acylated enzyme. The second step works on nucleophilicity which increases affinity to water and releases acid moiety and the enzyme [5].

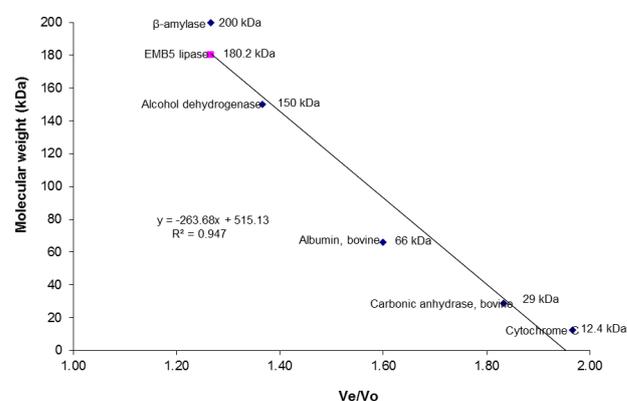


Figure 2 Molecular weights were determined from standard molecular weight markers blue dextran (2000 kDa), β -amylase (200 kDa), alcohol dehydrogenase (150 kDa), bovine albumin (66 kDa), carbonic anhydrase (29 kDa) and cytochrome C (12.4 kDa).

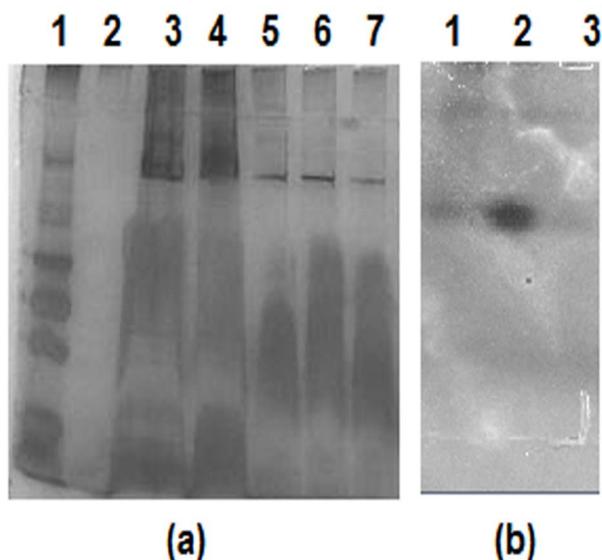


Figure 3 Gel electrophoresis of *Marinobacter* sp. EMB5 lipase. (a) SDS-PAGE followed by silver staining. Lane 1, molecular mass markers; lane 3-4, crude lipase; lanes 5-7, purified lipase; (b) SDS PAGE followed by zymography - Lane 2 tributyrin hydrolysis by lipase seen on agarose gel.

case of other lipases too [18] and (ii) that from the application point of view, the purified active lipase was characterized.

Enzymatic properties: The enzymatic characteristics of *Marinobacter* have not been investigated in details before. The enzyme exhibited broad substrate specificity with maximum activity towards C8 and C10 substrates (**Figure 4**). Similar substrate specificity has been reported in case of *Salicola* sp. IC10 [19] and LipBL from *Marinobacter lipolyticus* lipa [6] lipases. It indicates that *Marinobacter* sp. lipase in the present case has better affinity for medium chain length fatty acids.

The K_m and V_{max} of the lipase towards p-nitrophenyl octanoate (PNPO) were 1.43 μ M and 1.43 μ moles/ml/min respectively. Among other enzymatic properties, the EMB5 lipase had temperature optima at 50°C and pH optima at 9.0. The lipase was stable in the pH range 8.0-10.0 up to 12 h. Thus the *Marinobacter* lipase was alkaline in nature. These are in agreement with other halophilic lipases. Lipase LipBL from *Marinobacter lipolyticus* was stable over the pH range of 7.0-10.0 [6] but that from *Oceanobacillus* was stable only in a narrow range between pH 8.0-9.0 [4].

As a very novel feature, the *Marinobacter* lipase was significantly heat stable as compared to other reported halophilic lipases. It had a half-life of 90 minutes at 60°C and 30 min at 70°C. This property makes it quite attractive for applications at elevated temperatures. Among the lipases which have their temperature optimum at 50°C, *Aspergillus terreus* lipase is stable at 60°C for more than 12 h [20].

Effect of additives and metal ions: The effect of metal ions and additives (oxidizing, reducing and chelating agents) was tested at 1 and 5 mM in 0.1 M Tris-HCl buffer at pH 8.5. Lipase was activated by Ca^{2+} ions and was inhibited by the presence of K^+ , Cu^{2+} , Hg^{2+}

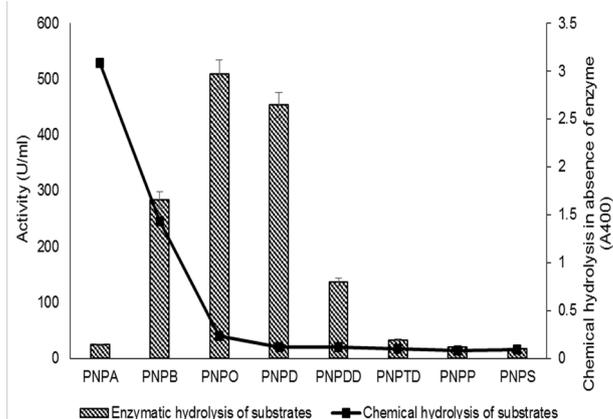


Figure 4 Substrate specificity of purified lipase.

and Ba^{2+} . The activation in presence of Ca^{2+} ions was even more pronounced at 5 mM concentration. Lipases in general are known to be activated by Ca^{2+} [13]. The lipase was less responsive to PMSF, indicating the non-involvement of serine, unlike other lipases. However, PCMB caused a decrease in activity, indicating involvement of cysteine residue.

Organic solvent and surfactant stability: Solvent stability is a generic feature of all halophilic proteins [13,21]. This is attributed to the fact that they work at high salt surroundings which is akin to low water medium. Lipase in the present study was stable in 50% (v/v) concentration of cyclohexane (**Table 3**). Polar solvents are known to strip off essential water of activity from active site universally causing loss in activity, so was in the present case. Among the halophilic lipases, solvent stability has been reported from *Haloarcula*, *Idiomarina*, *Alkalibacillus salilacus* [2,3,5].

Surfactant stability: The lipase was found to be extremely stable in presence of CTAB, Triton X100, Tween 80 and Brij 35 except anionic SDS which is a highly desirable property for lipase from the viewpoint of detergent applications (**Table 4**). The halophilic lipases have hydrophobic amino acid residues in the lid that covers the lipase active site. The organic solvent molecules interact with these residues and are likely to maintain the enzyme in the open conformation. This may contribute mainly to their stability in presence of organic solvents and surfactant stability [22]. This is the first study which reports both these properties for *Marinobacter* lipase, making it quite attractive for applications.

Structural Studies

Far UV CD spectroscopy

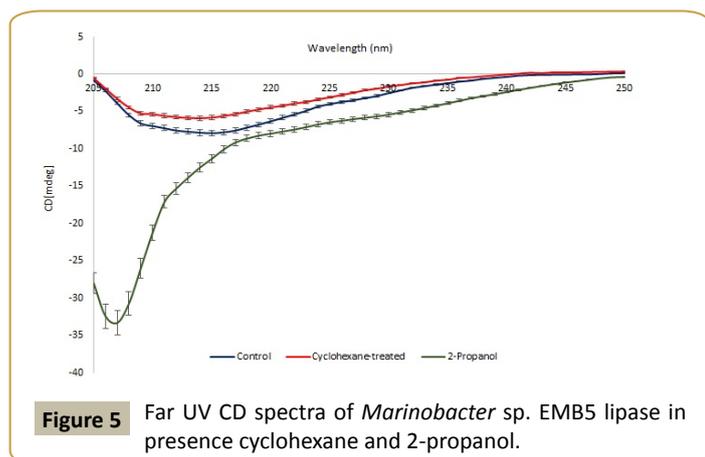
In order to understand solvent stability, the CD spectra of purified lipase were recorded in presence and absence of cyclohexane at 25°C and pH 8.5 (**Figure 5**). *Marinobacter* lipase was extremely stable in presence of cyclohexane up to 24 h. The results show changes in the peak position of negative ellipticity between 222 and 225 nm. This region corresponds to the spectral profile of α -helix [23]. In presence of cyclohexane, there was only a minor decrease in negative ellipticity as compared to the control. Thus, the CD spectra have given us an insight into the

Table 3 Stability of *Marinobacter* sp. lipase in organic solvents.

Solvent	Log P value	Relative activity in 50% (v/v) after 24 h (%)
Control	-	100
Ethanol	-0.235	29
Isopropanol	0.05	23
Butanol	0.8	44
Cyclohexane	3.2	116
Hexane	3.9	77
Decane	6.0	16

Table 4 Stability of *Marinobacter* sp. lipase in surfactants.

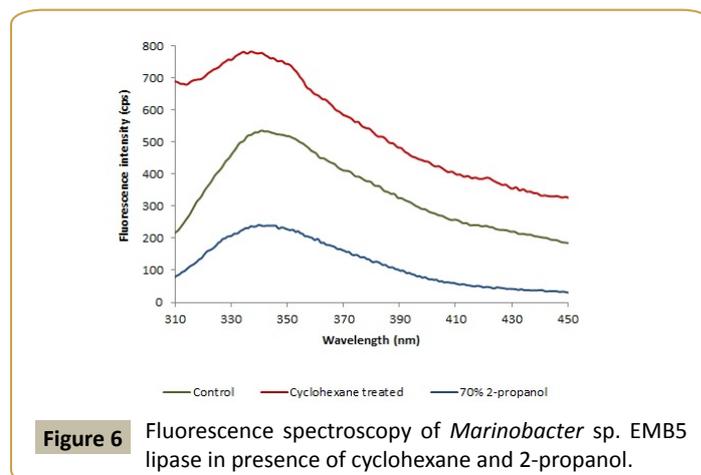
Surfactant	Relative activity after 5 h at 1% (v/v) (%)
Control	100
CTAB	190
Triton X100	131
Tween 80	153
Brij 35	141
SDS	64

**Figure 5** Far UV CD spectra of *Marinobacter* sp. EMB5 lipase in presence cyclohexane and 2-propanol.

secondary structure stability in presence of an organic solvent in which the lipase displays enormous stability. Despite a very high concentration of the organic solvent and a prolonged exposure to solvent, there were no distinct changes in the secondary structure.

Fluorescence spectroscopy

The effect of cyclohexane on the tryptophan fluorescence spectrum of lipase was studied by exciting the samples at 295 nm (**Figure 6**). The solvated tryptophan residues in aqueous solutions have fluorescence maxima at 350 nm. Under conditions that promote protein folding, the tryptophan residues are firmly embedded in the non-polar protein core causing a characteristic blue shift of its fluorescence spectrum. Denaturing solvents, on the other hand cause an increase in fluorescence intensity with a characteristic red shift of tryptophan fluorescence in the 328 to 348 nm wavelength range. Incubation of EMB5 lipase with cyclohexane caused a red shift from 344.5 nm to 346.5 nm. This suggested a slight increase of the accessibility of these residues to the solvent, i.e., increase of the protein molecule hydrodynamic volume, evidently fluorescence quenching in presence of cyclohexane. Similar phenomenon has been previously reported

**Figure 6** Fluorescence spectroscopy of *Marinobacter* sp. EMB5 lipase in presence of cyclohexane and 2-propanol.

by us in case of halophilic protease in presence of n-heptane and n-decane [7]. Presence of a biphasic solvent system of water and cyclohexane causes conspicuous changes in the immediate microenvironment of the lipase. However, due to the presence of salt we expect these changes to have minimal effect on the interactions that stabilize the protein structure. In fact, decreasing fluorescence intensity is suggestive of tryptophan residues still buried in the EMB5 lipase hydrophobic core which is part of the enzyme active site. This explains the stability of the halophilic enzyme even in presence of a high cyclohexane concentration. Most probably the interactions responsible for the functional tertiary structure are more or less intact and there is very little if any loss of structure. The results are in agreement with secondary structure data to suggest that the structural changes whatsoever are minimal and do not result in compromising enzyme function.

Conclusions

Halophilic isolate *Marinobacter* sp. EMB5 produced a lipase with interesting surfactant and organic solvent stability. The lipase is prone to aggregation. It was purified 28.2 fold by size-exclusion chromatography on Sephacryl S200 column. The active form of lipase occurred in aggregated form. The active form lipase was stable in a wide range of organic solvents at 50% (v/v) concentration even up to 24 h. This property was well correlated to its retaining its secondary and tertiary features. It was haloalkaline in nature, having an optimum pH at 9.0 and salt 2% (w/v), respectively. It was stable across a wide pH range of 5.0 to 10.0 and temperature up to 60°C. Thus, the *Marinobacter* sp. EMB5 lipase emerges as a novel halo-alkaline lipase which could be successfully purified by Sephacryl S200.

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Conflict of Interest

The authors RH and SKK declare that they have no conflict of interest.

Ethics Statement

This article does not contain any studies with human participants or animals performed by any of the authors.

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