International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Immobilization of Extremophile *Bacillus Licheniformis* Lipase on Silica & Celite Matrices & its Characterization

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Running Title: *Characterization of immobilized lipase of Bacillus licheniformis MTCC-10498*

Abstract: ***A purified alkaline thermotolerant lipase from Bacillus licheniformis MTCC-10498 was immobilized on Silica and Celite-545 matrices. The swelling capacity of silica and celite was recorded as 1.04 and 1.6 times respectively. The purified lipase (2.0 U/ml; protein content 0.06 mg and specific activity 32 U/mg) was used for immobilization by adsorption on silica and celite. The silica and celite showed 85.7% and 80%binding/ retention of purified lipase. The immobilized enzyme possessed a specific activity of 28 U/mg and 26.8 U/mg respectively. The optimal activity of purified lipase, silica bound, celite-bound was seen at pH 8.0 and at temperature of 55 ºC (2.0 ± 0.01; 1.211 ± 0.01; 1.216 ± 0.02).The hydrolytic activity of purified lipase and immobilized lipase was maximum for p-NPP. The hydrolytic activity of purified lipase, silica bound and celite bound lipase preparation reduced to approx. 50%, in 3 h. The presence of metal salts and EDTA inhibited lipase activity of purified, silica bound and celite bound lipase. Presence of PMSF (20mM) in the reaction mixture severely decreased the hydrolytic activities.***

Keywords: *Bacillus licheniformis* MTCC-10498, hydrolytic activities, immobilized lipase, PMSF

1. Introduction

Immobilized enzymes are used in the manufacture of many commercialized products for higher yields. Lipases have emerged as key enzymes in various industries like food, chemical, pharmaceutical, cosmetic and detergent production and leather processing. Lipases of microbial origin have been explored to catalyze the synthesis of variety of esters of sugars and carbohydrates. The fatty acid/carbohydrate esters are of immense use as neutraceuticals and additives in cosmetics. They also appear to possess surfactant properties that must be explored. Lipase (s) catalyzes both esterification as well as transestrerification reactions in organic free / restricted media. The use of immobilized matrices facilitates easy separation of biocatalyst and product (s) being formed.

A variety of carriers/matrices have been reported for immobilization of microbial enzymes. The immobilization of lipase on strong and well-defined supports activated with hydrophobic groups at low ionic strength proceeds by a mechanism completely different to the conventional hydrophobic adsorption of protein on hydrophobic supports (Malcata et al., 1990; Bastida et al., 1998, Kumar et al., 2006; Kanwar et al., 2006; Kanwar et al., 2007; Kanwar et al., 2008, Sharma et al., 2011). The enzyme when immobilized appears to become more stabilized and more active. The synthetic potential of lipase(s) has been widely recognized in water restricted organic solvents (Ghosh et al., 1996; Kanwar et al., 2006; Kanwar et al., 2007; Kanwar et al., 2008; Sharma and Kanwar, 2011; Sharma and Kanwar, 2012). The catalytic site of most lipases resembles that of serine proteases (Winkler et al., 1990). The catalytic site is a triad of serine, aspartate (or glutamate) and histidine but serine is a highly conserved amino acid with consensus sequences G-XI-S-X-G, as the catalytic moiety where S= Serine, XI= Histidine, G= Glycine, X= Glutamic or Aspartic acid. Metal-ions act as important cofactor or accessory indispensable moieties required for maintaining/restoring enzyme activity. Interestingly, both divalent ions (such as Ca^{2+}, Ba^{2+}, Mg^{2+}, Mn^{2+}, and Hg^{2+}) as well as mono-valent cations (N^{a+} and K^{+}) are strong modulators of lipase activity (Kojima et al., 1994; Sidhu et al., 1998; Hiol et al., 1999).

2. Materials & Methods

2.1 Chemicals

$NaNO_3$, K_2HPO_4, KCl, $MgSO_4$, $FeSO_4.{}_7H_2O$, $(NH_4)_2SO_4$ Celite-545 (S. D. Fine –Chem. Ltd., Hyderabad, India); yeast extract and gum acacia (Hi-Media Laboratory, Ltd., Mumbai, India); sucrose, KCl, KI, KNO_3, isopropanol, ammonium persulphate, 2-mercaptoethanol, HCl, p-nitrophenyl formate (p-NPF), p-nitrophenyl acetate (p-NPA), p-nitrophenyl benzoate (p-NPB) p-nitrophenyl caprylate (p-NPC), p-nitrophenyl laurate(p-NPL), p-nitrophenyl palmitate (p-NPP) from Alpha-aesar, Heysham, England. n-Hexane, Silica gel (0.040-0.063 mm, 230-400 mesh) acetic acid and Triton–X100, Tween-20, 40 and 80 (Qualigens Chemicals, Mumbai, India); phenyl methylsulphonyl fluoride (PMSF), sodium dodecyl sulphate (SDS), sodioum lauryl sarcosine (SLS), acrylamide, bisacryl amide (N,N-methylenebisacrylamide) glycerol, glycine and Tris (2-hyroxymethyl-2-methyl-1,3-propanediol) (Sigma Chemicals Co., USA). All chemicals were of analytical grade and were used as received.

2.2 Colorimetric assay method

The lipase activity was assayed using p-nitrophenyl palmitate (p-NPP), a chromomeric substrate. Lipase activity

of crude lipase, purified or matrix bound lipase was assayed employing a modified colorimetric method (Winkler and Stuckmann, 1979). The stock solution (20 mM) of p-NPP was prepared in HPLC grade isopropanol. The reaction mixture contained 80 µl of p-NPP stock-solution, 20 µl of lipase and Tris buffer (0.05 M, pH 8.5) to make final volume 3 ml. The reaction mixture was incubated at 55 °C for 10 min in a water bath (Banglore Genei Pvt. Ltd., Banglore). Further lipase reaction was stopped by chilling at -20 °C for 5 min. Control containing heat inactivated (5 min in boiling water bath) enzyme was also incubated with each assay. The absorbance (A_{410}) of heat inactivated lipase was subtracted from the absorbance of the corresponding test sample. The absorbance A_{410} of the p-nitrophenol released was measured and expressed in millimoles (mM). The unknown concentration of p-nitrophenol released was determined from a refrence curve of p-nitrophenol (2-20 µg/ml in 0.05 M Tris buffer pH 8.0) (Appendix 1). Each of the assays was performed in triplicate unless otherwise stated and mean values ± S.D. were presented. Stock solutions of various p-nitrophenyl esters p-NPF, p-NPA, p-NPC and p-NPL were also prepared for use in the some of the experiments.

2.3 Protein Estimation

A standard quantitative assay for determining the protein content in a solution was used (Lowry et al., 1951).

2.4 Purification of *B. licheniformis* MTCC-10498 lipase

The purification of bacterial lipase was performed using techniques of ammonium sulphate salting out, dialysis and hydrophobic interaction chromatography (Octyl sepharose) respectively.

2.5 Immobilization of *B. licheniformis* MTCC-10498 lipase on various matrices

2.5.1 Swelling capacity

The swelling capacity (Sw) of matrix in distilled water was found as follows:
Sw = W2-W1/W1
W1: wt. of dry matrix in g
W2: wt. of wet matrix in g (*i.e.*, net wt. of matrix after suspending it in excess volume of water for 1 h at 55 ºC.

2.5.2 Silica matrix

Immobilization of lipases is frequently performed by adsorption through hydrophobic interactions between the enzyme and the silica as a support (Soares et al., 2005). Inorganic matrices such as silica have been successfully used for the immobilization of many enzymes. Inorganic matrices such as silica have been successfully used for the immobilization of enzymes. Silica is an amorphous inorganic polymer composed of siloxane groups (Si-O-Si) in the inward region and silanol groups (Si-OH) distributed on its surface.

2.5.3 Celite matrix

Immobilization by adsorption using inorganic matrices like celite has been most widely used for immobilization of various enzymes. Diatomaceous earth (celite) is naturally occurring, soft, chalk-like sedimentary rock that is easily crumbled into a fine white to off-white powder. This powder has an abrasive feel, similar to pumice powder and is very light, due to its porosity. The use of a porous support material is desirable/ recommended for immobilization of lipase so that suitable amounts of lipase may be spreaded on a surface area without conformational changes.

2.6 Characterization of silica and celite immobilized lipase

2.6.1 Hydrolytic properties of silica and celite-bound lipase

The matrix bound lipasewas evaluated to study the effect of pH, temperature, thermo-stability, and specificity toward the hydrolysis of p-nitrophenyl esters of varying C-chain lengths as well as synthesis of methyl cinnamate and butyl butyrate in organic synthesis.

2.6.2 Effect of reaction buffer pH

Effect of reaction buffer pH on catalytic potential of silica, celite- bound lipase was assayed by incubating immobilized lipase in Tris buffer (0.05 M), with 0.1% gum acaia w/v; Triton X-100; 0.05%) adjusted pH at 7.0, 7.5, 8.0, 8.5, 9.0,10.0 and 11.0. The lipase activity was assayed at 55 ºC after 10 min of incubation.

2.6.3 Effect of reaction temperature on silica and celite bound lipase

Activity of bound lipase was assayed separately by incubating the reaction mixture taken in glass-tube at temperature ranging from 40-70 ºC. The lipase activity was assayed at 55 ºC after 10 min of incubation.

2.6.4 Effect of reaction time on lipase activity

To determine the optimum reaction time, the reaction mixture pretreated at 55 ºC was incubated for varying time range of 5, 10, 15 and 20 minutes and then lipase assay was performed.

2.6.5 Determination of substrate specificity of lipase production

The substrate specificity was determined by using a variety of p–nitrophenyl esters (p–NP formate, p-NP acetate, p–NP benzoate, p–NP myristate, p–NP caprylate, p-NP laurate and p-NP palmitate (prepared as 20 mM concentration isopropanol). The amount of p–nitrophenol released was assayed by spectrophotometer at A_{410}.

2.6.6 Thermo-stability of lipase

To examine the effect of the variation of temperature on stability of the matrix bound enzyme (0.2 ml) was kept separately in eppendorf tubes for 30 h at 55 ºC. At intervals (30 min) 20 µl of purified lipase was sampled and assayed for residual lipase activity. The activity measured immediately before incubation was defined as 100% of hydrolytic activity.

2.6.7 Effect of Salt ions on lipase activity

To evaluate the effect of various metal- ions on lipase activity, an attempt to was made to study the effect of Li^+, Na^+, K^+, Fe^{3+}, Zn^{2+},Mn^{2+}, Co^{2+}, Hg^{2+}, Mg^{2+}, Cu^{2+} as $LiCl_3$, NaCl, KCl, $FeCl_3$, $ZnSO_4$, $MnCl_2$, $CoCl_2$, $HgCl_2$, $MgCl_2$, $CuSO_4$ respectively) on lipase activity. Each of the salt ions

was separately, included in the reaction mixture at a final concentration of 1 mM. The lipase activity was assayed after 10 min of incubation at 55 ºC.

2.6.8 Effect of EDTA (chelating agent) and surfactants

EDTA in the concentration of 50 mM, 100 mM, 150 mM and 200 mM was separately added in the each of the reaction mixture tube and lipase activity was checked. Various detergents both ionic as well as non-ionic have the property to denature proteins. To study the effect of such detergents on *B. licheniformis* lipase, each detergent (Tween-20, Tween-60, Tween-80, Triton X-100, Sodium dodecyl Sulphate, Sodium lauryl sarcosine; 0.05%, v/v) was included separately in reaction mixture. A lipase activity was recorded at A_{410}.

3. Results

3.1 Charactrerization of *B. licheniformis* MTCC 10498 lipase

Purified lipase of *B. licheniformis* MTCC 10498 was characterized for various physico-chemical parameters with appropriate optimal enzyme content.

3.2 Swelling capacity, immobilization and binding efficiency of silica and celite matrices

The swelling capacity of silica and celite was recorded as 1.04 and 1.6 times respectively. The purified lipase (2.0 U/ml; protein content 0.06 mg and specific activity 32.7 U/mg) was used for immobilization by adsorption on silica and celite. The silica showed 85.7% binding/ retention of purified lipase. The silica-immobilized enzyme possessed a specific activity of 28 U/mg. The purified lipase of *B. licheniformis* MTCC-10498 was optimally bound to celite that retained 80% specific activity 26.8 U/mg.

3.3 Effect of pH on purified and immobilized lipase activity

Actual inorganic state of lipase was essential for its optimal activity. The optimal activity of *B. licheniformis* MTCC-10498 purified lipase, silica bound, celite-bound was seen at pH 8.0 (2.0 ± 0.01; 1.211 ± 0.01; 1.216 ± 0.02). The lipase activity decreased more sharply at acidic pH (Table 1).

Table 1: Effect of pH on purified and immobilized lipase activity

pH	Purified lipase (U/ml)	Silica bound (U/g)	Celite bound (U/g)
4.0	**0.150 ± 0.01**	**0.102 ± 0.04**	**0.216 ± 0.01**
4.5	0.236 ± 0.03	0.294 ± 0.03	0.413 ± 0.05
5.0	0.642 ± 0.01	0.418 ± 0.01	0.623 ± 0.02
5.5	0.705 ± 0.02	0.462 ± 0.02	0.656 ± 0.01
6.0	0.866 ± 0.02	0.506 ± 0.01	0.702 ± 0.02
6.5	0.978 ± 0.01	0.712 ± 0.02	0.778 ± 0.01
7.0	1.162 ± 0.03	0.821 ± 0.01	0.821 ± 0.01
7.5	1.734 ± 0.02	0.964 ± 0.01	1.018 ± 0.02
8.0	**2.000 ± 000**	**1.211 ± 0.01**	**1.216 ± 0.02**
8.5	1.779 ± 0.01	1.171 ± 0.02	1.191 ± 0.01
9.0	1.762 ± 0.02	1.089 ± 0.01	1.004 ± 0.04
9.5	1.552 ± 0.02	0.972 ± 0.01	0.978 ± 0.02
10.0	1.168 ± 0.04	0.814 ± 0.02	0.956 ± 0.02
10.5	0.814 ± 0.02	0.719 ± 0.01	0.866 ± 0.01
11.0	0.315 ± 0.02	0.456 ± 0.01	0.576 ± 0.02

3.5 Effect of temperature on purified and immobilized lipase activity

The purified as well as silica and celite bound lipase exhibited maximum lipase activity at pH 8.0 *viz.* 2.0 ± 0.01; 1.21 ± 0.01; 1.21 ± 0.02 at 40-70 ºC respectively (Table 2).

Table 2: Effect of temperature on lipase activity

Temperature	Purified lipase (U/ml)	Silica bound(U/g)	Celite bound(U/g)
40	0.864 ± 0.01	1.086 ± 0.01	0.994 ± 0.02
45	1.369 ± 0.01	1.102 ± 0.04	1.023 ± 0.03
50	1.804 ± 0.02	1.161 ± 0.03	1.154 ± 0.05
55	**2.000 ± 0.01**	**1.211 ± 0.01**	**1.216 ± 0.02**
60	0.988 ± 0.02	1.081 ± 0.01	1.164 ± 0.01
65	.612 ± 0.01	0.881 ± 0.01	1.009 ± 0.02
70	0.468 ± 0.05	0.578 ± 0.01	0.679 ± 0.03

3.6 Substrate specificity of purified and immobilized lipase

The purified as well as bound enzyme exhibited highest affinity towards the p-NPP followed by p-NPM. The purified, silica bound and celite bound lipase was specific toward p-NPP but shows variable specificities towards other esters. The hydrolytic activity of purified lipase for hydrolysis of p-NPM was approximately lower than p-NPP. Hydrolytic activity for p-NPF for purified, silica bound and celite bound lipase was 14.6%, 32%, 43.5% lower than for p-NPP respectively (Table 3). The bacterial lipase thus showed a preferentially higher specificity and hydrolytic activity towards the longer C-chain esters. However, it appeared that p-NPP was hydrolyzed most efficiently than the other selected acyl esters

Table 3: Substrate specificity of purified and immobilized lipase

pNP-Substrate (20 mM)	Purified lipase (U/ml)	Silica bound (U/g)	Celite bound (U/g)
Formate (C1)	0.292 ± 0.01	0.389 ± 0.01	0.529 ± 0.01
Acetate (C2)	0.362 ± 0.01	0.421 ± 0.01	0.646 ± 000
Benzoate (C6)	0.582 ± 0.01	0.654 ± 0.05	0.790 ± 0.01
Caprylate (C8)	0.982 ± 0.04	0.865 ± 0.04	0.898 ± 0.01
Laurate (C12)	1.672 ± 0.02	1.009 ± 000	0.915 ± 0.01
Myristate (C14)	1.718 ± 0.01	1.020 ±0.002	1.068 ± 0.03
Palmitate (C16)	**2.000 ± 0.030**	**1.211 ± 0.01**	**1.216 ± 0.02**

3.7 Thermostability of purified, silica and celite immobilized lipase

The hydrolytic activity of purified lipase (0.69 ± 0.01), silica bound (0.5 ± 0.07) and celite bound (0.65 ± 0.01) lipase preparation reduced to approx. 50%, in 3 h. After 5 h lipase activity reduced to 22% in case of purified lipase (Table 4).

Table 4: Thermostability of lipase

Time (h)	Purified lipase (U/ml)	Silica bound (U/g)	Celite bound (U/g)
0.5	1.872 ± 0.05	1.009 ± 0.03	1.100 ± 0.04
1.0	1.603 ± 0.04	0.866 ± 0.02	0.980 ± 0.04
2.0	1.291 ± 0.06	0.675 ± 0.01	0.870 ± 0.04
3.0	0.923 ± 0.05	0.566 ± 0.04	0.724 ± 0.04
4.0	0.692 ± 0.01	0.515 ± 0.07	0.704 ± 0.04
5.0	0.413 ± 0.07	0.495 ± 0.07	0.652 ± 0.01
Control	**2.000 ± 0.03**	**1.211 ± 0.01**	**1.216 ± 0.02**

3.8 Effect salts on lipase activity of purified and bound lipase

The presence of metal salts of Li^{3+}, Na^{+}, K^{+}, Fe^{3+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Hg^{2+}, $Mg^{2+}$and Cu^{2+} inhibited lipase activity of purified, silica bound and celite bound lipase (Table 5).

Table 5: Effect of salt ions on lipase activity

Salts (1 mM)	Purified lipase (U/ml)	Silica bound (U/g)	Celite bound (U/g)
Control	2.000 ± 0.03	1.211 ± 0.01	1.216 ± 0.02
$LiCl_3$	1.500 ± 0.05	1.174 ± 0.01	1.104 ± 0.01
NaCl	1.090 ± 0.04	1.203 ± 0.07	1.080 ± 0.02
KCl	1.167 ± 0.04	1.199 ± 0.02	0.944 ± 0.03
$FeCl_3$	1.876 ± 0.02	1.088 ± 0.09	0.968 ± 0.04
$ZnSO_4$	1.416 ± 0.03	0.743 ± 0.02	0.818 ± 0.02
$MnCl_2$	1.576 ± 0.02	0.842 ± 0.01	0.804 ± 0.01
$CoCl_2$	1.236 ± 0.01	0.769 ± 0.02	0.817 ± 0.03
$HgCl_2$	1.161 ± 0.04	0.812 ± 0.01	0.850 ± 0.02
$MgCl_2$	1.067 ± 0.03	0.887 ± 0.05	0.898 ± 0.03
$CuSO_4$	1.678 ± 0.02	1.023 ± 0.04	0.964 ± 0.02

3.9 Effect of EDTA (chelating agent) and surfactants

It was observed that as the concentration of EDTA increased activity decreased drastically. The free enzyme, silica bound and celite bound has left residual activity of 23%, 27% and 47% respectively (Table 6).

Table 6: Effect of EDTA (chelating agent)

EDTA (mM)	Purified lipase (U/ml)	Silica bound (U/g)	Celite bound (U/g)
50	1.567 ± 0.03	0.714 ± 0.04	0.904 ± 0.02
100	0.962 ± 0.04	0.504 ± 0.01	0.817 ± 0.01
150	0.812 ± 0.02	0.415 ± 0.03	0.652 ± 0.01
200	0.468 ± 0.04	0.330 ± 0.04	0.573 ± 0.01
Control	2.0 ± 0.03	1.211 ± 0.01	1.216 ± 0.02

Except for Tween-80 (0.05% v/v) all other detergents reduced the lipase activity of purified, silica bound and celite bound lipase. Lipase activity of purified lipase remains same with the treatment of Tween-80 (Table 7). However, there is minor increase in the activity of silica bound (4%) and celite bound lipase (5%).

Table 7: Effect of detergents on lipase activity

Surfactant (0.05%, v/v)	Purified lipase (U/ml)	Silica bound (U/g)	Celite bound (U/g)
Control	2.000 ± 0.03	1.211 ± 0.01	1.216 ± 0.02
Tween-20	1.800 ± 0.03	1.115 ± 0.02	1.104 ± 0.01
Tween-60	1.868 ± 0.04	1.150 ± 0.03	1.143 ± 0.02
Tween-80	**2.000 ± 0.02**	**1.263 ± 0.04**	**1.287 ± 0.01**
Triton-X100	0.996 ± 0.02	0.968 ± 0.01	0.998 ± 0.02
SDS	0.918 ± 0.03	0.612 ± 0.03	0.636 ± 0.01
SLS*	0.816 ± 0.04	0.595 ± 0.04	0.612 ± 0.02

*Sodium lauryl sarcosine

Table 8: Effect of tween-80 concentration

Surfactant Tween-80 (v/v)	Purified lipase (U/ml)	Silica bound (U/g)	Celite bound (U/g)
Control	2.000 ± 0.03	1.211 ± 0.01	1.216 ± 0.02
0.04	1.842 ± 0.01	1.091 ± 0.05	1.004 ± 0.02
0.05	**2.000 ± 0.02**	**1.263 ± 0.04**	**1.287 ± 0.01**
0.06	1.749 ± 0.01	1.115 ± 0.02	1.176 ± 0.01
0.07	1.612 ± 0.02	0.875 ± 0.04	0.942 ± 0.01
0.08	0.816 ± 0.04	0.815 ± 0.01	0.886 ± 0.02

3.10 Effect of PMSF on lipase activity of purified, silica bound and free lipase

Inhibition kinetics of *B. licheniformis* MTCC 10498 lipase was directly proportional to PMSF concentration (i.e. at 1, 5, 10 and 20 mM). The activity of lipase declined. Presence of PMSF (20mM) in the reaction mixture severely decreased the hydrolytic activities (Table 9).

Table 9: Effect of PMSF on lipase activity

PMSF (Concentration)	5 min	10 min	15 min
None	1.203 ± 0.02	2.000 ± 0.03	1.976 ± 0.02
5 mM	1.612 ± 0.01	0.892 ± 0.04	0.814 ± 0.01
10 mM	0.868 ± 0.02	0.742 ± 0.03	0.256 ± 0.05
15 mM	0.420 ± 0.03	0.202 ± 0.03	0.256 ± 0.05
20 mM	0.030 ± 0.001	0.040 ± 0.002	0.070 ± 0.001

4. Discussion

The purified lipase was subsequently used to perform reaction in both aqueous and organic media, and repetitive use of lipase immobilized onto silica and celite matrices was also studied to economize the catalytic processes in aqueous and organic media. Lipases of microbial origins were influenced by the presence of metal ions albeit at low concentrations(s). In the present study, lipase activity of *B. licheniformis* MTCC-10498 lipase was inhibited by most of the metal/salt ions in concentration of 5 mM. However, in 1 mM concentration the lipase activity was enhanced by Fe^{3+} ion. Recently, inhibition of lipase activity of thermophillic *B. cereus* lipase MTCC 8372 strain by Al^{3+}, Co^{2+}, Mn^{2+}, Zn^{2+} has been reported (Verma and Kanwar, 2008). The presence of metal ions has been previously, reported to influence the hydrolytic activites of microbial lipases of bacterial as well as fungal origin. PMSF and EDTA did not affect the lipase of *Mucor* sp. (Hiol et al., 1999) as well as a lipase of non-microbial origin (Arreguin-Espinosa et al., 2000). The probable mechanism of retention and or enhancement of catalytic activity of this lipase either at an alkaline pH during a short incubation time or at slightly acidic pH during a prolonged incubation time appeared to be a charge rearrangement at the catalytic site of enzyme. A strain of *B. cereus* MTCC-8372 (Verma and Kanwar, 2008) and a thermoalkaliphilic lipase showed a preferential specificity towards longer chain lengths substrates. A lipase from psychotropic *Pseudomonas* sp. has been reported to display the highest activity towards, C-10 acyl groups of para-nitrophenyl esters (Rashid et al., 2001). A purified lipase from *Pseudomonascepacia* immobilized on a commercially available micro-porous polypropylene support showed a higher yield with p-nitro phenyl acetate and very low yield with p-NPP (Pancreac'h et al., 1997). The lipase of *B. licheniformis* MTCC-10498 was effectively inhibited by a serine protease inhibitor (PMSF), which indicate that this lipase is a typical lipase with serine protease activity at the catalytic site unlike a pancreatic lipase. The hydrolytic activity of a pancreatic lipase remains unaffected in the presence of PMSF and thus did appear to be regulated by a serine residue at the catalytic site. (Gupta et al., 2004; Kanwar et al., 2004). The para-nitrophenyl esters of various chain lengths–fatty acids were used as substrate and release of para-nitrophenol has been measured spectrophotometrically at 410 nm (Winkler and Stuckman, 1979; Pancreac'h and Baratti, 1996). The short chain esters being water soluble and their enzymatic hydrolysis provided a measure of esterase rather than lipase activity. However, p-nitrophenyl palmitate being water insoluble has been most commonly used to measure lipase activity (Gupta et al., 2004). Anionic detergents SDS and SLS inhibited the catalytic activity of *B. licheniformis* lipase in the presence of chromogenic substrate.

The present study showed that, *B. licheniformis* MTCC-10498 lipase possessed an extra cellular alkaline thermophillic lipase was unique in many properties. The catalytic activities of this lipase was inhibited by most of the metal / salt ions except $Fe^{3+}$and completely inhibited by serine protease inhibitors (PMSF). Moreover, it was highly hydrolytic towards higher C-chain length (C 16).

The stability and catalytic activities of lipases tend to increase when it is immobilized on to a suitable matrix (Reetz et al., 1998; Kanwar et.al., 2004, Kanwar et.al., 2005; Kanwar et.al., 2008; Verma et.al., 2008; Sharma et.al., 2011). The most popular methods of lipase immobilization are based upon their adsorption on non well –defined supports such as inorganic materials like silica, celite etc., as well as on more or less hydrophobic supports (Reetz et al., 1998; Basri et al., 2001). Enzymes immobilization is the inherent advantage to isolate the biocatalyst from the reaction product and reuse it in order to increase the process productivity (Zou et al., 2010; Gao et al., 2010; Itoh et al., 2010). Immobilization by adsorption has been most widely used for immobilization of various enzymes (Chaubey et al., 2009; Yilmaz et al., 2001). Highly porous inorganic matrices such as silica aerogels with differing balances of hydrophobic and hydrophilic functionalities have been successfully used for the immobilization of enzymes. Silica aerogels can be considered as "solid" solvent for the enzymes that are able to provide hydrophobic/hydrophilic characteristics differing from those prevailing in the liquid surrounding the aerogels (Zaiden et al., 2001). Previously, the highest esterification activity that was 6.7 folds higher than the free enzymes was observed for lipase of *P. fluorescens* immobilized to celite (Ivanov and Schneider, 1997). The modulation of catalytic / hydrolytic activity of an immobilized lipase in the presence of a strongly anionic denaturing agent i.e. SDS has not been documented in literature.

5. Conclusions

The present study establish that immobilization of the lipase obtained from *B. licheniformis* MTCC-10498 on Silica/ Celite was quite stable as the Silica and Celite retained hydrolytic activity towards the p-NPP.

6. Future Perspectives

The efficacy of this lipase in the bipoprocess development at large scale in organic solvent/water restricted/ water-controlled media are still required to be explored in future study.

Acknowledgements

The author thankfully acknowledges the UGC, New Delhi for providing meritorious fellowship & contingency to author (2008-2012). I am especially thankful to my mentor Prof. S.S. Kanwar for the esteem guidance, innovative ideas & contagious enthusiasm toward scientific research for welfare of mankind. I also thank Department of Biotechnology, GoI, for providing infrastructural grants to Himachal Pradesh University, Shimla.

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