Influence of Culture Conditions on the Production of Extracellular Esterase from Bacillus licheniformis and Its Characterization

Kamal Kumar Bhardwaj, Akshita Mehta, Lalit Thakur, and Reena Gupta*

Department of Biotechnology, Himachal Pradesh University, Summer Hill Shimla-171005, INDIA

Abstract: Esterases catalyze the hydrolysis of ester bonds in fatty acid esters with short-chain acyl groups. In the present study, thirty-seven bacterial isolates were isolated from soil contaminated with waste cooking oil, dairy waste etc. from Shimla and Solan district of H.P. Out of 37 isolates, the isolate RL-1, which gave maximum activity, was identified as Bacillus licheniformis MH061919. The optimization of various production parameters resulted in maximum activity at inoculum age of 24 h and inoculum size of 1.5% (v/v). Esterase gave considerable activity in production medium containing sodium chloride (0.5% w/v), galactose (1%, w/v), coconut oil (2.0%, v/v) and beef extract (0.3%, w/v) at a temperature of 45°C and pH 8.5. The enzyme production was enhanced by 3-fold after optimization of production parameters. Further, on optimizing reaction conditions, enzyme gave maximum activity at a temperature of 45°C and pH 8.5. The para-nitrophenyl acetate (p-NPA) was found to be optimum substrate and metal ions and detergents have inhibitory effect on esterase activity.

Key words: esterase, coconut oil, p-NPA, Bacillus licheniformis, metal ions

1 Introduction

Microorganisms inhabit all possible locations from those offering ideal conditions for growth and reproduction to those representing extreme environments. Microbial lipolytic enzymes are more useful than those of other origins because of their high yields and ease of genetic manipulation. Compared with chemical catalysis, enzyme-catalyzed reactions are generally more selective and run under environmental friendly conditions with lower energy cost. Microbial esterases are resistant to organic solvents, which makes them important catalysts in organic syntheses and especially for enantioselective or stereoselective hydrolysis of esters. Lipolytic enzymes including lipases and esterases are one of the most important groups of biocatalysts applied in many industries such as food, detergent, chemicals, cosmetic, biodiesel and pharmaceuticals.

Esterases (E.C. 3.1.1.1) belong to hydrolases class of enzymes which catalyze the cleavage and synthesis of ester bonds. Esterases have broad substrate specificity, high chemo-, regio-, enantio-selectivity and non-aqueous catalytic properties. Lipases and esterases can be distinguished on the basis of their substrate specificity, as esterases catalyze the hydrolysis of carboxylic ester bonds of short chain fatty acids (<10 carbon atoms), with tributyrin as the standard substrate. However, true lipases have marked preference for long chain fatty acids (>10 carbon atoms) as substrates. Esterases can catalyze esterification, interesterification and transesterification reactions in non-aqueous media without using cofactors. They can also catalyze the ester synthesis and trans-esterification in water free or water-restricted medium.

Esterases have a wide range of industrial applications. Microbial esterases perform some useful reactions like the synthesis of short-chain esters, alcohol, lactones, and phenolic compounds, which contribute to the typical flavour of food. Esterases hydrolyse ester bonds and act on a wide variety of natural and xenobiotic compounds which makes them quite useful enzymes in bioremediation. Marine esterases play an important role in marine organic carbon degradation and cycling.

There is a great urge to explore novel esterases from diverse microbial sources for potential industrial applications. However, the growing demand for esterases can be satisfied by developing the suitable production strategies. The present paper focused on isolation of esterase producing microorganism and optimization of enzyme production.
and reaction parameters for maximal enzyme activity from *Bacillus licheniformis*.

### 2 Materials and Methods

#### 2.1 Chemicals

All the chemicals used in the present investigation were procured from Hi-Media and Merck, Mumbai, India. The substrate *p*-nitrophenyl acetate (*p*-NPA) for esterase assay was procured from Sigma, Aldrich (U.S.A.).

#### 2.2 Sample collection

The esterase producing bacteria were isolated from the common garbage site (comprising wastes of kitchens, restaurants, dairies, plastic wastes, sewerage and decaying plant parts) of Shimla and Solan districts of Himachal Pradesh, India. The collected samples were brought to the laboratory in sealed polythene bags or plastic vials for further studies.

#### 2.3 Screening for esterase producing isolate

The isolation was done through enrichment method. Soil samples were serially diluted and plated onto nutrient agar plate containing tributyrin (0.5%) by spread plate method and incubated at 37°C for 24 h. The plates were then observed for halo zone formation. The isolates for further (secondary) screening were selected on the basis of ratio of halo diameter to colony size. Each bacterial isolate was aseptically transferred to nutrient broth to produce seed culture. It was incubated at 37°C with continuous shaking at 151 rpm for 24 h. Seed culture (1%) of each of the bacterial isolates was separately inoculated in 50 mL of the sterile production medium containing 1% (v/v) coconut oil as a sole source of carbon in a flask (250 mL). The flasks were incubated at 37°C with continuous shaking at 151 rpm for 48 h. The esterase activity was assayed for each isolate in the supernatant. The isolate secreting higher amount of esterase enzyme was selected for further study.

#### 2.4 Enzyme assay

The activity of esterase was assayed using method of Immanuel et al.\(^{18}\) by incubating 2.9 mL of Tris-HCl buffer (0.1 M, pH 8.0) with 60 µL of the substrate (*p*-nitrophenyl acetate, 20 mM) and incubated at 40°C in water bath for 10 min thereafter 40 µL of enzyme was added. The reaction mixture was again incubated at 40°C in water bath for 10 min. The reaction was then stopped by chilling at \(-20\)°C. The amount of *p*-nitrophenol released was measured at 410 nm (Perkin Elmer UV/VIS Spectrophotometer lambda 12). The corresponding concentration of *p*-nitrophenol released was determined from reference curve prepared from *p*-nitrophenol.

One unit of esterase activity was defined as amount of enzyme required to release one micromole of *p*-nitrophenol from the substrate (*p*-nitrophenyl acetate) per minute under standard assay conditions.

#### 2.5 Identification of esterase producing bacterial isolate

The isolated strain with high esterase activity was identified by 16S rRNA gene sequencing from Xceleris Labs Ltd., Ahmedabad. DNA was isolated from the bacterial strain RL-1 using GeneliPureTM Bacterial DNA Purification Kit and was analyzed by 1.2% agarose gel electrophoresis. Isolated DNA was amplified with 16S rRNA Specific Primer, PCR amplicon was enzymatically purified and further subjected to Sanger Sequencing. The sequences of closely related strains were retrieved for multiple nucleotide sequence analysis from the National Center for Biotechnology Information (NCBI) server BLAST and aligned using a MEGA version 5.0 software\(^{20}\). The phylogenetic tree was constructed using the Neighbor-Joining method\(^{21}\) with bootstrap consensus tree inferred from 1,000 replicates\(^{22}\). The evolutionary distances were computed using the Kimura 2-parameter method\(^{23}\).

#### 2.6 Growth profile of *Bacillus licheniformis*

Growth profile of *B. licheniformis* was studied by evaluating biomass growth of selected bacterial isolate in seed medium till 66 h. After every 6 h of incubation, optical density of culture broth was taken at 660 nm. The culture broth was then centrifuged at 20,000 rpm for 10 min and supernatant was used to assay enzyme activity.

#### 2.7 Optimization of physicochemical conditions for extracellular production of esterase by selected bacterial isolate

##### 2.7.1 Optimization of inoculum size

The inoculum size for the production of extracellular esterase by the selected bacterial isolate was determined by inoculating production medium varying size of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% (v/v).

##### 2.7.2 Optimization of different production media

The selected bacterial isolate was grown in six different production media (PM1-PM6) with pH 7.5, at 40°C reported by various workers (Table 1).

##### 2.7.3 Optimization of carbon (lipid) and nitrogen source

Different carbon sources (1%, v/v) olive oil, Tween 80, peppermint oil, linseed oil, castor oil and coconut oil were supplemented in the production medium.

Each of the various nitrogen sources peptone + yeast extract + beef extract, peptone, yeast extract, beef extract, gelatin, potassium nitrate were individually added to the production medium at a concentration of 0.1% (w/v) and enzyme activity was assayed. A control without carbon and nitrogen source was also run.

##### 2.7.4 Optimization of production temperature and pH

To study the effect of temperature, production medium
was incubated at different temperatures such as 25, 30, 35, 40, 45, 50, 55, 60 and 65°C under optimized conditions. For optimization of pH, production medium at different pH values such as 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5 and 11.0 was inoculated with bacterial culture under previously optimized production conditions and esterase activity was determined.

2.8 Biochemical characterization of crude esterase from *Bacillus licheniformis*

The culture broth was centrifuged at 10,000 rpm for 10 min at 4°C. The esterase activity was assayed in the super-

| Medium | Components | Concentration (g/L) | Reference |
|--------|------------|---------------------|-----------|
| PM1    | Peptone    | 5.0                 |           |
|        | Yeast extract | 3.0               |           |
|        | Tributyrin | 1%                  | 24)       |
|        | Tween 80   | 2%                  |           |
|        | Tryptone   | 10.0                |           |
|        | Yeast extract | 5.0               |           |
| PM2    | NaCl       | 10.0                |           |
|        | Tributyrin | 1%                  |           |
|        | Glucose    | 10.0                | 25)       |
|        | Sodium acetate | 10.0             |           |
|        | K2HPO4     | 1.0                 |           |
| PM3    | MgSO4      | 0.1                 |           |
|        | NaCl       | 10.0                |           |
|        | Yeast Extract | 3.0               |           |
|        | Tributyrin | 10%                 | 26)       |
|        | Peptone    | 2.0                 |           |
|        | Yeast extract | 2.0               |           |
|        | L-Cystine.HCl | 0.5              |           |
|        | NaCl       | 0.1                 |           |
| PM4    | NaHCO3     | 2.0                 |           |
|        | K2HPO4     | 0.04                |           |
|        | MgSO4.7H2O | 0.01                |           |
|        | CaCl2.6H2O | 0.01                | 27)       |
|        | Tween 80   | 2%                  |           |
|        | Peptone    | 5.0                 |           |
|        | Beef extract | 3.0               |           |
| PM5    | NaCl       | 5.0                 |           |
|        | Yeast extract | 5.0               |           |
|        | Dextrose   | 1.0                 | 28)       |
|        | Beef extract | 10.0              |           |
|        | Yeast extract | 2.0               |           |
|        | NaCl       | 5.0                 |           |
| PM6    | Peptone    | 10.0                |           |
|        | Gum acacia | 5.0                 | –         |
|        | Coconut oil | 1%                |           |
2.8.1 Effect of incubation time and temperature on the activity of crude esterase from Bacillus licheniformis

To determine the optimum incubation time, the reaction mixture was incubated for different time intervals 5, 10, 15 and 20 min at 40°C and enzyme activity was measured under standard assay conditions. To evaluate the effect of reaction temperature on the esterase activity, the reaction was assayed at various temperatures (35, 40, 45, 50 and 55°C). Enzyme activity was determined by standard assay method.

2.8.2 Effect of different buffers and pH on the activity of esterase from Bacillus licheniformis

To study the effect of different types of buffers on the esterase activity, buffers (0.1 M) of different pH range (Tris-HCl buffer (pH 8.0), Sodium phosphate buffer (pH 7.5), Potassium phosphate buffer (pH 7.0), Sodium citrate buffer (pH 6.0)) were used separately in reaction mixture and the esterase activity was assayed in each case by standard assay method.

The optimized buffer (0.1 M) of pH 7.0, 7.5, 8.0, 8.5 and 9.0 was used to determine enzyme activity by standard assay method.

2.8.3 Effect of different substrates on the activity of esterase from Bacillus licheniformis

To study the substrate specificity of the enzyme, different substrates, para-nitrophenyl acetate (p-NPA), para-nitrophenyl benzoate (p-NPBenz), para-nitrophenylformate (p-NPF) and para-nitrophenylpalmitate (p-NPP) were used. The substrate concentration used was 20 mM and the enzyme activity was measured.

2.8.4 Effect of different metal ions and detergents on the activity of crude esterase from Bacillus licheniformis

The effect of various metal ions Fe³⁺, Mg²⁺, Hg²⁺ and Na⁺ on esterase activity was evaluated. Each of the metal ion was separately incubated in the reaction mixture at a final concentration of 1 mM. The reaction mixture was incubated at 45°C for 10 min and the esterase activity was measured. The esterase activity in each case was determined with respect to control (without any metal ion).

Effect of different detergents was studied by adding different detergents (1 mM) in the reaction mixture. Detergents used were SDS, EDTA, Tween 20, Tween 60 and Tween 80. A control without detergent was also assayed for esterase activity simultaneously.

2.9 Statistical analysis

Standard Deviation (S.D.) was calculated from the data obtained for three replicates of the parameters studied and the Student’s test was applied.

### Table 2. Bacterial isolates showing esterase activity.

| S. No. | Bacterial isolate | Activity (U/mL) |
|--------|-------------------|-----------------|
| 1      | RL-1              | 0.27 ± 0.011    |
| 2      | RL-2              | 0.21 ± 0.03     |
| 3      | RL-3              | 0.16 ± 0.006    |
| 4      | RL-4              | 0.19 ± 0.016    |
| 5      | RL-5              | 0.17 ± 0.003    |
| 6      | RL-6              | 0.15 ± 0.04     |
| 7      | RL-7              | 0.14 ± 0.015    |
| 8      | RL-8              | 0.18 ± 0.012    |
| 9      | RL-9              | 0.22 ± 0.02     |
| 10     | RL-10             | 0.2 ± 0.018     |
| 11     | RL-11             | 0.17 ± 0.05     |

Values are mean ± Standard Deviation (S.D.) of three observations

Fig. 1 Tributyrin agar plate showing zone of hydrolysis by Bacillus licheniformis.

3 Result and Discussion

3.1 Screening for esterase producing bacterial isolate

Out of the thirty seven bacterial isolates obtained, eleven showed the esterase activity higher than 0.14 U/mL. Of these, RL-1, which showed the highest activity (0.27 U/mL) was selected for further studies (Table 2). The zone of hydrolysis produced by the isolate RL-1 is shown in Fig. 1. Recently, tributyrin assay method was ascertained to be the most favourable method of screening for lipolytic activity.

3.2 Identification of strain

Bacterial isolate RL-1 was characterized by partial sequencing of 16S rRNA gene and was identified as Bacillus licheniformis (NCBI Accession No.: MH061919). Sequence analysis of obtained sequence revealed a homology of 99% with isolate Bacillus licheniformis strain ZBM5 (Fig. 2).

The evolutionary tree displayed a cluster for Bacillus licheniformis with isolate strain ZBM5.
cheniformis strain of the present study along with Bacillus licheniformis strain ZBM5, which is further distinguished from other strains (Fig. 2). Morphologically, Bacillus licheniformis was shown to form rough creamish white colonies on nutrient agar medium and was characterized as rod shaped Gram positive bacilli by Gram staining. Biochemically, Bacillus licheniformis was found to be positive for catalase, nitrate reductase and glucose.

3.3 Growth profile of Bacillus licheniformis

In the present study, the esterase production from Bacillus licheniformis was observed to increase with increase in inoculum age. Its maximum activity was 0.16 U/mL at 24 h in seed medium (Fig. 3). The organism produced maximum enzyme during early hours of growth within short fermentation time and it started declining after 24 h.

3.4 Optimization of physicochemical conditions for extracellular production of esterase by selected bacterial isolate

3.4.1 Optimization of inoculum size

As maximum enzyme production occurs in log phase of culture, an inoculum size of 1.5% (v/v) was able to provide higher levels of enzyme activity at 24 h of inoculum age (Fig. 4). In another study, maximum esterase activity from organism Lysinibacillus fusiformis was found to be 158.5 U/mL at 4.6% (v/v) inoculum size\(^{30}\).
3.4.2 Optimization of different production media

Among different production media used for esterase production by *Bacillus licheniformis*, the maximum esterase activity of 0.41 ± 0.003 U/mL was observed in the originally used production medium i.e. PM6 ([Table 3](#table3)). In a different study, esterase enzyme from *Bacillus* sp. DVL2 gave maximum activity i.e. 0.0092 U/mL in medium PM2 containing g/L peptone 5.0, yeast extract 3.0, tributyrin 1.0 and Tween 20 2.0. In another study, medium PM2 containing g/L peptone 3.0, yeast extract 2.0, tributyrin 1.0 mL and Tween 80 1.0 mL was chosen as the best medium for maximum esterase production from *Bacillus* sp. after 24 h incubation time [31].

3.4.3 Optimization of carbon (lipid) and nitrogen source

The coconut oil, found to be optimized carbon source, gave maximum activity of 0.41 ± 0.002 U/mL for production of esterase from *Bacillus licheniformis* ([Fig. 5a](#fig5a)). Tween 80 was found to be the least suitable carbon source with activity of 0.13 ± 0.014 U/mL. Although carbon sources are required for microbial growth and production of biochemical materials, their efficiencies for production of any target material can differ among microbial species and under different fermentation conditions. Earlier, castor oil from *V. fischeri* had the maximum influence on esterase production and activity was found to be 33.8 U/mL [32].

3.4.4 Optimization of temperature and pH

Most favourable temperature for esterase activity in production medium by *Bacillus licheniformis* was found to be 45°C with maximum enzyme activity of 0.78 ± 0.018 U/mL. As the temperature rose from 25°C to 45°C, there was an increase in esterase production ([Fig. 6a](#fig6a)). In another study, optimum temperature for esterase production from *Bacillus* sp. was found to be 37°C and from *Vibrio fischeri* 30°C [32].

Esterase activity was maximum 0.82 ± 0.017 U/mL in a production medium at a pH of 8.5. There was an increase in esterase production as the pH rose from 6.5 to 8.5 and thereafter a decrease in activity was noticed ([Fig. 6b](#fig6b)). The maximum extracellular esterase activity from *Lantinus ti-

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**Table 3** Effect of medium on production of esterase from *Bacillus licheniformis*.

| Production medium | Enzyme activity (U/mL) |
|-------------------|------------------------|
| PM1               | 0.23 ± 0.002           |
| PM2               | 0.25 ± 0.04            |
| PM3               | 0.13 ± 0.01            |
| PM4               | 0.33 ± 0.014           |
| PM5               | 0.18 ± 0.02            |
| PM6               | 0.41 ± 0.003           |

Values are mean ± Standard Deviation (S.D.) of three observations

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**Fig. 4** Effect of inoculum size on esterase production from *Bacillus licheniformis*.

**Fig. 5a** Effect of different carbon sources on esterase production from *Bacillus licheniformis*.

**Fig. 5b** Effect of different nitrogen sources on esterase production from *Bacillus licheniformis*.

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The maximum esterase activity of 0.631 ± 0.004 U/mL was observed when beef extract was used as a nitrogen source ([Fig. 5b](#fig5b)). In a different study, esterase from *Bacillus circulan* showed maximum activity of 50.3 U/mL when supplemented with ammonium hydrogen carbonate [19]. Earlier, soyabean meal from *Trichophyton ajelloi* observed the maximum esterase activity of 121.42 U/mL at 0.1 w/v nitrogen source concentration [33].
grinus was found to be at pH 9.036. In general, enzyme production by bacteria is strongly influenced by the pH of the growth medium. pH plays a significant role in enzyme stability as it maintains the necessary three-dimensional structure required for biological activity of enzyme.

3.5 Biochemical characterization of crude esterase from Bacillus licheniformis

3.5.1 Effect of incubation time and temperature on the activity of crude esterase from Bacillus licheniformis

In the present study, the maximum enzyme activity (0.82 U/mL) was observed at incubation time of 10 min (Fig. 7a). The decrease in enzyme activity might be due to the denaturation of enzyme on prolonged incubation or due to product inhibition. In a different study, incubation time of 10 min gave maximum activity of esterase from Bacillus sp.36. Also in another study, esterase EstOF4 isolated from Geobacillus stearothermophilus showed the maximum enzyme activity at incubation time of 15 min36.

The optimum temperature for the esterase was found to be 45°C with 0.91 U/mL activity (Fig. 7b). In another study, the enzyme purified from Salimicrobium sp. LY19 displayed optimal activity at 50°C and it retained 48% activity at 80°C37. The optimal temperature of feruloyl esterase purified from Lactobacillus sp. ranged from 45 to 50°C38.

3.5.2 Effect of different buffers and pH on esterase activity from Bacillus licheniformis

The maximum enzyme activity (0.97 U/mL) was observed in Tris-HCl buffer (Table 4). In a different study, the maximum enzyme activity was found with Tris-HCl buffer (pH 7.5). At this pH, esterase purified from thermophilic microorganism, Bacillus thermoleovorans ID-1 showed maximum activity39.

The maximum enzyme activity (1.17 U/mL) of esterase

Table 4 Effect of different buffers on the activity of esterase from Bacillus licheniformis.

| Buffer system (0.1 M)       | Enzyme activity (U/mL) |
|-----------------------------|------------------------|
| Tris-HCl pH (8.0)           | 0.97 ± 0.019           |
| Sodium phosphate (pH 7.5)   | 0.78 ± 0.03            |
| Potassium phosphate (pH 7.0) | 0.54 ± 0.012           |
| Sodium citrate (pH 6.0)     | 0.745 ± 0.15           |

Values are mean ± Standard Deviation (S.D.) of three observations.
from *Bacillus licheniformis* was observed in 0.1M Tris-HCl buffer at pH 8.5 (Fig. 8). Earlier, esterase purified from *Geodermatophilus obscurus* showed high stability in alkaline conditions i.e. Tris-HCl buffer pH range between 7.5 and 9.5\(^{40}\). Also furoyl esterase purified from lactic acid bacteria showed maximum specific activity in 100 mM phosphate buffer having pH 7.0\(^{38}\). Enzymes are most active at their optimum pH, as their active sites have maximum interaction with the substrate. Any drastic change in the pH of a medium leads to denaturation of the enzyme resulting in the loss of its activity\(^ {41}\).

### 3.5.3 Effect of different substrates on the activity of esterase from *Bacillus licheniformis*

The maximum esterase activity was observed with the \(p\)-NPA (1.17 U/mL) and minimum with \(p\)-NPP (0.493 U/mL) as shown in Fig. 9. In another study, the activity of purified esterase was determined using \(p\)-NP esters having different carbon chain lengths from C-2 to C-12. The highest hydrolytic activity was found towards \(p\)-NPA\(^ {42}\). Enzymes are usually very specific as to which reactions they catalyze and the substrates that are involved in these reactions. Complementary shape, charge and hydrophilic/hydrophobic characteristics of enzymes and substrates are responsible for this specificity.

### 3.5.4 Effect of different metal ions and detergents on the activity of esterase from *Bacillus licheniformis*

The metal ions can either have stimulatory or inhibitory effect on the esterase activities. All metal ions inhibited the esterase activity as compared to control (Table 5). The effect of metal ions on esterase activity is complex to interpret because it probably results both from a change in solubility behaviour of ionized fatty acid at interface and from the change in catalytic property of enzyme itself\(^ {43}\). In a recent study on esterase purified from *Geobacillus* sp. enzyme activity was enhanced by Mn\(^ {2}\) and Ni\(^ {2}\), but was inhibited by Hg\(^ {2}\) and Cu\(^ {2}\), and was unaffected by Ca\(^ {2}\), Mg\(^ {2}\), and Co\(^ {2}\)\(^ {44}\).

### Table 5: Effect of different metal ions and detergents on the activity of esterase from *Bacillus licheniformis*.

| Metal ion and detergents (1mM) | Enzyme activity (U/mg) |
|-----------------------------|------------------------|
| Control                     | 1.17 ± 0.285           |
| Fe\(^ {3}\)                 | 0.71 ± 0.02**          |
| Mg\(^ {2}\)                 | 0.52 ± 0.196**         |
| Hg\(^ {2}\)                 | 0.32 ± 0.137***        |
| Na\(^ {+}\)                 | 0.31 ± 0.108***        |
| SDS                         | 0.88 ± 0.13*           |
| EDTA                        | 0.76 ± 0.11**          |
| Tween 20                    | 0.68 ± 0.09**          |
| Tween 60                    | 0.61 ± 0.07**          |
| Tween 80                    | 0.52 ± 0.08**          |

Values are mean ± Standard Deviation (S.D.) of three observations ***\(p<0.001\), **\(p<0.01\) and *\(p<0.05\) as compared to control.
In the present study, out of the various detergents used, all of them decreased enzyme activity (Table 5). Minimum loss of activity was observed with SDS while maximum loss of activity was with Tween 80. Detergents, in general, denature enzyme through disruption of the tertiary structure. Inhibition of esterase by specific binding of detergent like SDS could be attributed to unfavourable electrostatic interactions that may cause unfolding of esterase. Non-ionic detergents like Tween 20, Tween 80 mildly bind to the enzyme but at higher temperature, the enzyme attains more flexibility that makes the enzyme more prone to unfolding, resulting in denaturation and hence protein inactivation. Recently, the esterase isolated from Bacillus sp. showed suppressed activity in detergents like TritonX-100, Tween 20, Tween 80 and sodium dodecyl sulfate.

4 Conclusion
The present study showed unique properties of an extracellular alkaline esterase of Bacillus licheniformis with optimum reaction temperature 45°C and pH 8.5. Moreover, because of its relatively high specificity towards lower C-chain length substrates, its potential application in the synthesis of esters as flavour and fragrance compounds and degradation of xenobiotic compounds will be further explored in organic media by performing esterification and trans-esterification reactions.

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Conflicts of interests
The authors declare that there is no conflict of interests regarding the publication of this article.

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