

Isolation of a Lipolytic and Proteolytic *Bacillus licheniformis* from Refinery Oily Sludge and Optimization of Culture Conditions for Production of the Enzymes

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With the increasing demand for enzymes in industrial applications there is a growing need to easily produce industrially important microbial enzymes. This study was carried out to screen the indigenous refinery bacterial isolates for their production of two industrially important enzymes i.e. lipase and protease. A total of 15 bacterial strains were isolated using Soil Extract Agar media from the oil-contaminated environment and one was shown to produce high quality lipase and protease enzymes. The culture conditions (culture duration, temperature, source of nitrogen, carbon, and pH) were optimized to produce the optimum amount of both the lipase ($37.6 \pm 0.2 \text{ Uml}^{-1}$) and the protease ($41 \pm 0.4 \text{ Uml}^{-1}$) from this isolate. Productivity of both enzymes was shown to be maximized at pH 7.5 in a medium containing yeast extract and peptone as nitrogen sources and sucrose and galactose as carbon sources when incubated at $35 \pm 1^\circ\text{C}$ for 48 h. Bacterial strain SAB06 was identified as *Bacillus licheniformis* (MT250345) based on biochemical, morphological, and molecular characteristics. Further studies are required to evaluate and optimize the purification and characterization of these enzymes before they can be recommended for industrial or environmental applications.

Keywords: *Bacillus licheniformis*, refinery sludge, enzymes, optimization, cultural conditions

Introduction

Microorganisms are ubiquitous and have been isolated from conditions ranging from the deepest ocean sediments to high atmospheric pressure conditions to extremely high and cold temperature niches besides highly polluted environment. They have a high degree of adaptability and consequently influence the ecological balance and are essential for the survival of various other organisms. Refineries generate large amounts of oily sludge as waste which are considered as hazardous pollutants [1]. Microorganisms that survive in harsh

environments utilize hydrocarbons as the source of carbon and energy by producing different extracellular enzymes with different catabolic activities. Some of these extracellular enzymes are excellent sources of economically important enzymes that have several practical and industrial applications. Microbial enzymes have gained importance over other sources of enzymes like plants and animals due to their unique properties such as wide availability, high yield, higher stability, and low cost of production [2]. Lipase and protease are the two most important hydrolytic enzymes which have a wide range of industrial and biotechnological applications. Lipase is produced by a wide range of animals, plants, bacteria, fungi, and yeasts [3]. However, bacteria are known as prolific producers of different enzymes as they show higher activities than the enzymes produced by

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fungi and yeast and show stability over a wide range of environmental conditions, are easy to cultivate, have higher yield [2, 4]. A large number of bacterial species belonging to genera like *Pseudomonas*, *Acinetobacter*, *Enterococcus*, *Flavobacterium*, and *Bacillus* have been reported as producers of lipase [4, 5]. Lipase producing bacteria have been isolated from different environments such as oil-contaminated soil, oil factories, agricultural and industrial wastes, and dairy industries [5, 6].

Lipases are triacylglycerol acyl hydrolase that catalyse various reactions at the interface of oil and water, and helps in esterification and hydrolyse water-insoluble long-chain triglycerols [7]. They show many potential uses in fat and oleochemical industries, help in degradation of oil, wastes and effluents, and used as biosensors and applied for sewage treatment. They are also used in oil processing, pulp and paper industries and are also used for the production of biodiesel [4, 8, 9]. The ability of lipase to perform very specific chemical transformations (biotransformation) has made this enzyme increasingly popular in the food, detergent, cosmetic, organic synthesis, and pharmaceutical industries [10]. Lipases have emerged as one of the leading biocatalysts with proven potential for contributing to the multibillion-dollar underexploited lipid bio-industry and have been used in *in-situ* lipid metabolism and *ex-situ* multifaceted industrial applications [11].

Proteases are hydrolytic enzymes that hydrolyse peptic bonds in aqueous environment and are another industrially important enzyme that represents about 60% of the total enzymatic sales in the world [12]. They have been used in industries such as pharmaceutical, food, and leather besides environmental applications [12, 13]. The production of microbial proteases from bacteria depends on factors like media composition, method of cultivation, growth of bacteria, pH of media, temperature, and duration of incubation [14]. Bacterial proteases are preferred due to ease in production, the rapid growth of the source organism, and their genetic manipulation for improved yield and biosynthesis of new enzymes [15]. Microbial proteases are one of the largest groups of protease produced mainly by different genera of *Bacillus* such as *Bacillus subtilis*, *B. amyloliquifaciens*, *B. coagulans*, *B. licheniformis* etc. [16]. Protease and lipase show higher catalytic properties due to their ability to act on a wide range of natural as well as artificial sub-

strates over broad environmental conditions such as high pH and temperature and solubility in organic solvents. The industrial demand for new sources of lipase and protease with different catalytic characteristics encourages work on the isolation and selection of new strains. Culture parameters are one of the crucial factors determining the yield and rate of enzyme production by microorganisms. Microbial lipases and proteases are mostly extracellular and their production is greatly influenced by medium composition, incubation time besides physicochemical factors such as temperature, pH, and dissolved oxygen [17]. The present study, therefore, was envisaged to screen potent bacterial strains having the ability to produce both protease and lipase and to optimize the culture condition for better yield of the enzymes.

Materials and Methods

Soil characteristics and isolation of bacteria

Soil samples were collected from soil mixed with oily sludge of refinery waste of Bongaigaon refinery, Assam, India (26°31'00.81"N; 90°31'53.85"E). Isolation of bacteria was carried out using Soil Extract Agar media which contained 500 ml/l of soil extract and 15 g/l agar [18]. Single colonies of bacteria were isolated and pure culture of the isolates was maintained on nutrient agar slants. Screening for lipase and protease activity was done following the methods of Lee *et al.* [19] and Suganthi *et al.* [20] respectively. Quantitative estimation of enzyme production was performed and potent isolate which produced both lipase and protease enzymes was selected. The culture conditions of the most potent isolate were optimized for maximum yield of both the enzymes.

Qualitative screening of enzyme assay

Lipase assay. The bacterial isolates were screened for the production of lipase by following the methods as described earlier by Bharathi *et al.* [5] with some modifications using the following methods:

Phenol Red Agar plate method. The phenol red agar plates were prepared by adding phenol red (0.01% w/v), olive oil (0.1% v/v), CaCl₂ (0.1% w/v), and agar (2% w/v). Phenol possesses endpoint at 7.3 to 7.4 pH. A slight

decrease in the pH will result in a change of colour of the media from red to yellow indicating lipase activity of the isolate.

Tributylin agar plate method. Tributyrin agar medium supplemented with 1.0% (v/v) tributyrin was prepared and sterilized at 15 lb/inch² pressure for 15 min. Isolated strains were streaked on the tributyrin agar plates and were incubated at 32°C for 24 h to observe the halo zone of clearance.

Protease assay. Preliminary screening of bacteria for proteolytic activity was carried out following the different plate assay methods:

Skimmed milk agar plates assay. It was performed using Luria-Bertani (LB) agar supplemented with 1% (w/v) skimmed milk and chloramphenicol (12.5 µg ml⁻¹). SMA (skimmed milk agar) and LB media were prepared separately. LB was sterilized at 121°C for 15 min, while 10% (w/v) milk powder solution was autoclaved at 115°C for 10 min. Exactly 10% (w/v) milk solution was mixed with LB agar to a final concentration of 1% (w/v). Plates were incubated for 72 h at 37°C. Zone of clearance indicated the presence of proteolytic activity.

Casein hydrolysis test. The bacterial strains were screened on agar plates supplemented with 5% NaCl and 1% casein (MNA) for the production of protease. The plates were incubated overnight at 37°C. The protease producing strains were selected based on the zone of clearance produced in the plates [21].

Identification of bacteria. The potent bacterial strains were identified on the basis of morphological and biochemical characteristics following the Bergey's Manual of determinative bacteriology [22]. 16S rRNA gene analysis was also carried out for confirmation of the identification. Bacterial genomic DNA was extracted from the bacterial culture using standard protocol [23]. PCR amplification of the genomic DNA was carried out using the universal primers 27F and 1492R having reaction conditions set at 94° for 3 min, followed by 35 cycles of 94° for 30 sec, 55° for 30 sec and 72° for 90 sec and then final extension at 72° for 5 min. Amplification products were analysed on 1.5% (w/v) agarose gels in TAE buffer.

16S rRNA amplicon from each strain was sequenced, and the data were searched using NCBI-BLAST search tool for identification of the strain type. Phylogenetic analysis was inferred with the help of Neighbor-joining (NJ) methods [24] conducted in Mega 7 [25].

Quantitative enzyme assay

Lipase. Quantitative estimation of lipase was carried out using colorimetric method as described by Winkler *et al.* [26] using basic medium containing glucose (1.0%), yeast extract (0.2%), KH₂PO₄ (0.5%), MgSO₄ (0.2%), CaCl₂ (0.1%), pH 7. Lipase activity was measured using p-nitrophenol palmitate (pNPP) as a substrate according to the methods of Kilcawley *et al.* [27]. One unit of lipase was expressed as µmole (s) of p-nitrophenol released min⁻¹ml⁻¹ of enzyme.

Protease production assay

Protease was assayed using casein as a substrate following the methods of Beg *et al.* [28]. Overnight cultured bacterial inoculum was inoculated in nutrient media with 1% casein as the substrate and incubated for 44–72 h at 37°C under 150 rpm shaking condition. The culture was centrifuged at 10,000 rpm and cell-free supernatant was used for enzyme assay. The reaction mixture contained 1% casein added to 1 ml of enzyme which was incubated for 10 min at 37°C and the reaction was stopped by adding 1 ml of 10% trichloroacetic acid (TCA) reagent. The reaction mixture was incubated for 30 min at room temperature and then centrifuged at 15000 rpm for 15 min. The filtrate (2 ml) was added to 3 ml of 500 mM sodium carbonate solution and absorbance was read using a spectrophotometer at 280 nm. One unit of protease is expressed as the amount of enzyme releasing 1 µg of tyrosine per min during the assay. The estimation was calculated with the help of a calibration graph with tyrosine as the standard.

Optimization of culture conditions

Production of extracellular enzymes by bacteria is dependent on the composition of nutrient media, incubation time, temperature, and pH. Different culture conditions such as temperature, pH, C and N sources were optimized for optimum production of the enzymes [5, 26]. Basal media containing glucose (1.0%), yeast extract (0.2%), KH₂PO₄ (0.5%), MgSO₄ (0.2%) and CaCl₂

(0.1%) was used for the enzyme assay. Basal media was supplemented with 1% olive oil and 1% casein for the assay for lipase and protease activity respectively. Optimization for different cultural parameters was performed keeping the remaining parameters constant. Bacterial strain was inoculated into the basal media and incubated at different temperatures ranging from 30–45°C. Effect of pH on the yield of bacterial lipase and protease was assayed by adjusting the pH of the basal medium from 7.5 to 9. The pH was adjusted by adding 0.1 N NaOH or 0.1N HCl in the basal medium. To study the effect of nitrogen and carbon sources on the production of lipase and protease different nitrogen sources like yeast extract, ammonium nitrate, and peptone were added to the basal medium at a final concentration of 1% (W/V). Similarly different carbon sources (1% W/V) such as sucrose, glucose, and galactose were used to determine the effect of carbon sources on enzymatic activity.

Statistical analysis. All experiments in the present study was repeated thrice and the results obtained are expressed as mean \pm SD ($n = 3$). Statistical significance of the values of different assays was determined using one-way analysis of Variance (ANOVA) at $p < 0.05$ followed by the least significant difference (LSD).

The Genbank accession number of the bacterial 16S rRNA sequence reported in this paper is MT250345.

Results

Isolation and Identification of Bacteria

A total of 15 bacterial strains were isolated using Soil Extract Agar media which were screened for the production of lipase and protease. Seven bacterial strains showed positive results in lipase and protease assay. The highest production of both lipase and protease by one potent bacterial strain, SAB06, amongst the seven strains was confirmed based on the qualitative screening plate assay and quantitative enzymatic assay (Fig. 1). The biochemical characterization of the isolate identified the bacteria as a species of the genus *Bacillus* (Table 1). The most efficient bacteria, SAB06, for lipase and protease production was further identified by 16S rRNA gene sequencing analysis following the standard method of DNA isolation of bacteria and sequence analysis. The 16S rRNA sequence was submitted to the GenBank under the accession number MT250345. The molecular evolutionary relationship was inferred by the phylogenetic tree constructed using the Neighbour-Joining method (Fig. 4). The 16S rRNA gene analysis confirmed the identification of the strain SAB06 as *Bacillus licheniformis* with 99% similarity with other 16S rRNA sequences of *B. licheniformis* strains available in the database. *B. licheniformis* showed the highest protease and lipase in quantitative assay which were further optimized using different culture conditions for

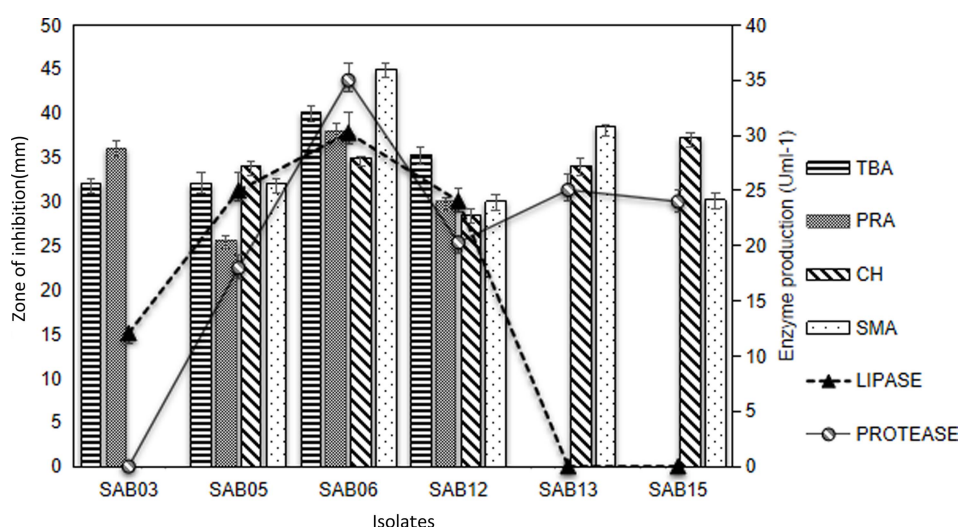


Fig. 1. Qualitative and quantitative screening of bacterial isolates for production of Lipase and Protease (TBA- Trybutyrin Agar plate assay; PRA- Phenol Red Agar plate assay; CH- Casein hydrolysis; SMA- Skim Milk Agar plate assay). Vertical bars represent \pm SD, Data average of three replicates

Table 1. Morphological and biochemical characteristics of the potent bacterial strain SAB06 isolated from refinery sludge.

Characteristic features	Observations
Colony morphology	Irregular, large, round and opaque colonies
Gram reaction	Gram positive rod
Endospore staining	Spore forming
Catalase	Positive
Oxidase	Negative
Starch hydrolysis	Positive
Gelatin hydrolysis	Positive
Citrate test	Negative
Nitrate test	Positive
Starch fermentation	Positive
Sucrose fermentation	Positive
Glucose fermentation	Positive
Lactose fermentation	Negative
Production of H ₂ S	Negative
Indole test	Negative
Methyl Red	Negative
Vogues Proskauer	Positive

optimum yield of enzymes.

Qualitative screening of the isolate for lipase and protease production

In the Qualitative screening assay, all the isolated bacterial strains were screened for the production of lipase, and protease in plate assay which was followed by quantitative assessment of enzymes production. Out of 15 isolates, seven bacterial isolates showed clear halo

Table 2. Halo zone (mm) produced by lipase and protease enzymes produced by the potent isolate, SAB06, in plate assay after 24 h and 48 h of incubation.

Enzymes	Plate assay method	Halo zone (mm)	
		24 h	48 h
Lipase	Tributyrin Agar method	28 ± 0.8	40 ± 0.06
	Phenol Red Agar plate method	20 ± 0.05	38 ± 0.02
Protease	Casein hydrolysis test	25 ± 0.5	35 ± 0.02
	Skim milk Agar plate assay	28 ± 0.06	45 ± 0.05

zone (mm) in the screening methods such as Tributyrin Agar plate assay, Phenol Red Agar plate assay for lipase and Casein hydrolysis and the Skim Milk Agar test for production of protease (Fig. 1). While screening for the efficient production of both lipase and protease, one potent isolate, SAB06 showed the highest zone of clearance (> 35 mm) in all the screening tests and also showed positive enzyme activity for both the enzymes (Fig. 1). The maximum halo zone(mm) for lipase assay (40 ± 0.06 mm) was observed in Tributyrin Agar Plate at 48 h of incubation. The isolate showed maximum zone of clearance (45 ± 0.05 mm) in Skim milk Agar plate after 48 h of incubation indicating the production of protease enzyme (Table 2). Isolate SAB06 was further optimized for the optimum production of lipase and protease enzymes using quantitative methods.

Optimization of culture conditions

Incubation time and shaking condition. Maximum production of both protease and lipase in the basal media was recorded after 48 h of incubation at a shaking condition of 160 rpm in the log phase. The highest growth of

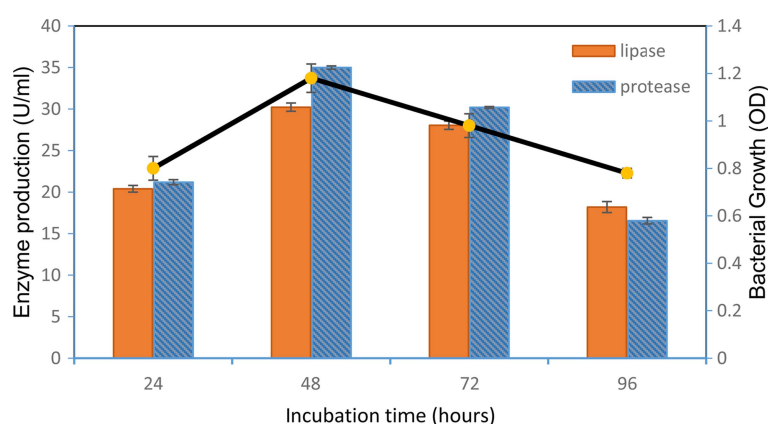


Fig. 2. Effect of incubation period on growth (OD₆₀₀) and production of enzymes (Uml⁻¹) by the bacterial strain SAB06. Vertical bars represent ± SD, Vertical bars represent ± SD, Data average of three replicates

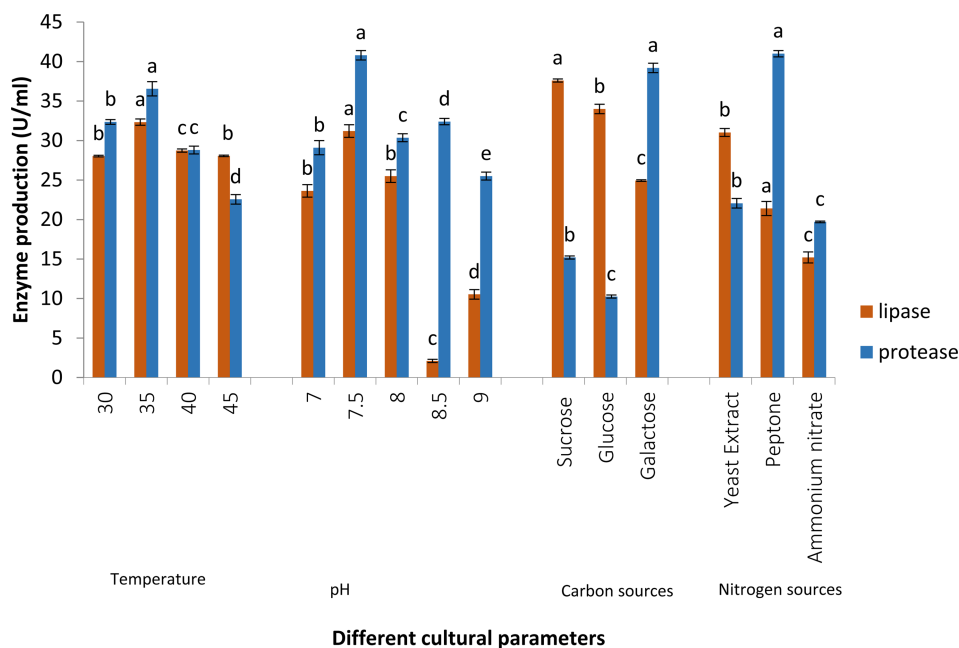


Fig. 3. Effect of different temperature and pH ranges and carbon and nitrogen sources on the production of bacterial lipase and protease by SAB06. Vertical bars represent \pm SD, Vertical bars represent \pm SD, Data average of three replicates. Different letters in the legends of the bars indicate mean with significant difference ($p < 0.05$).

bacteria ($OD_{600} = 1.5 \pm 0.06$) was observed at 48 h of incubation (Fig. 2). The isolate SAB06 required an optimum period of 48 h of incubation for the production of enzymes. It produced the highest amount of protease (35 ± 0.02 U/ml) and lipase (30.23 ± 0.5 U/ml) at 48 h of incubation. The amount of both the enzymes was recorded to decrease when the culture was incubated beyond 48 h (Fig. 2).

Effect of temperature

Temperature is an important factor required for growth and extracellular activities of bacteria. The effect of temperature on growth and enzyme production is shown in Fig. 3. The studied strain SAB06 showed maximum growth at 35°C . The production of lipase and protease were significantly higher at 35°C ($p < 0.05$).

Effect of pH

In the present study, the isolate SAB06 exhibited growth and produced enzymes at pH ranging from 7 to 9. The maximum production of protease (40.8 ± 0.6 U/ml) and lipase (31.2 ± 0.8 U/ml) was observed in basic condition at pH 7.5 (Fig. 2). The differences in enzyme production varied significantly ($p < 0.05$) at different pH

range. Production of protease by the strain SAB06 was observed to be stable and higher than lipase at pH 8.5 and 9 (Fig. 3).

Effect of carbon sources

Growth of the isolate using different carbon sources affected the production of bacterial lipase and protease ($p < 0.05$). Maximum production of lipase (37.6 ± 0.2 U/ml) was observed when sucrose was used as a carbon source rather than glucose (34 ± 0.6 Uml $^{-1}$) and galactose (24.93 ± 0.1 Uml $^{-1}$) (Fig. 3). Production of protease (39.2 ± 0.6 U/ml) however, was maximum when galactose was added as the carbon source in the basal media and minimum when glucose was the carbon source (Fig. 3).

Effect of nitrogen sources

The strain SAB06 produced the highest amount of lipase (31.02 ± 0.5 U/ml) when yeast extract was used as the nitrogen source followed by peptone (21.4 ± 0.9 Uml $^{-1}$) and ammonium nitrate (15.2 ± 0.7 Uml $^{-1}$) (Fig. 3). The use of peptone as the nitrogen source in the basal media influenced the high production of protease (41.0 ± 0.4 U/ml) by SAB06. Sources of nitrogen significantly influenced the production of lipase and protease enzymes ($p < 0.05$).

Discussion

With the increasing demands and application of microbial enzymes in industrial and biotechnological applications, interests in new sources of microbial enzymes have increased. Bacteria are one of the most intensely studied groups of microorganisms having the ability to produce a wide range of important hydrolytic enzymes. Earlier studies have shown that the production of lipase and protease was related to the presence of petroleum hydrocarbon pollutants in soil [13]. Lipases are one of the most important hydrolytic enzymes being used in different industries owing to their multifaceted properties. Soil lipases are regarded as an important indicator of oil biodegradation [29]. Microbial lipases have been extensively used in the management of the environment polluted with oil because of oil drilling, refining, etc. [30]. Besides lipase, protease has also played a great role in different industries e.g. pharmaceutical industries for the development of various therapeutic agents [13]. The present study incorporated lipase and protease because of their potential use in different food and pharmaceutical industries. Recently, microbial enzymes have gained much attention due to their unique properties such as stability over extreme envi-

ronmental conditions, low production cost, high yield in a short period, and due to the ease of the genetic manipulation for production of new enzymes with broad activity. The results of the present study showed petroleum hydrocarbon contaminated soil as a suitable environment for the isolation of lipase and protease producing bacteria. Lee *et al.* [19] isolated lipase and protease producing bacteria from wastewater indicating their ability to grow in different environmental conditions. In the present study maximum isolates were obtained when soil extract agar media was used for isolation of bacteria. This might be attributed to their adaptability and ability of indigenous bacteria to utilize organic pollutants present in soil extract agar media which was devoid of other external nutrients. Bacteria are reported as the most efficient producer of protease and the species of the genus *Bacillus* being the most prominent producer with significant proteolytic and lipolytic activities [31]. Hasan *et al.* [32] showed enhanced production of lipase and protease by *Bacillus* sp. and *Pseudomonas* sp. The study suggested the use of different plate assay for the detection of enzymatic activity of the isolated bacteria. The screening of bacteria for lipase and protease activity resulted in the detection of the potent strain *B. licheniformis* (MT 250345), an efficient producer of

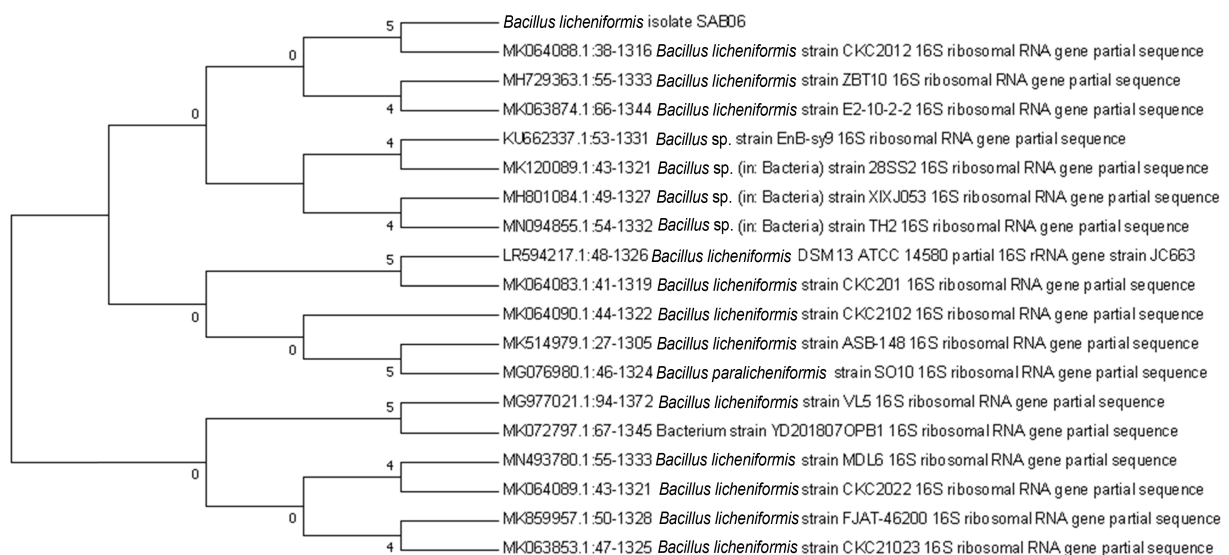


Fig. 4. Neighbor-joining phylogenetic tree showing the relation of *Bacillus licheniformis* strain SAB06 with other related taxa based on available 16S rRNA gene sequences. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. Evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site

lipase and protease. The isolate SAB06 (MT 250345) showed 99% similarity with the 16S rRNA gene sequence of *B. licheniformis* strain CKC2012 bearing accession number MK064088 (Fig. 4).

Optimization of different culture parameters showed the ability of the strain SAB06 to produce the highest amount of protease (39.2 ± 0.1 U/ml) and lipase (37.6 ± 0.2 U/ml) after incubation at 35°C for 48 h under shaking condition. Continuous shaking increased aeration which is essential for the production of different extra and intracellular enzymes [33]. Similar results were also observed by various other researchers who indicated the highest production of lipase and protease by *Bacillus licheniformis* after 48 h of incubation [34]. However, several other studies have shown 72 h of optimum incubation at 30°C for the highest production of enzymes by bacteria [35]. The optimum incubation period for the highest production of lipase and protease, however, ranged from 48 to 72 h for other bacterial species such as *B. cereus*, *E. aerogenes*, *E. intermedius* [36].

The present study reported the highest production of both lipase and protease at pH 7.5 which is in agreement with the observations made by Hasan *et al.* [4]. Abada [37] has reported the maximum yield of lipase by *Bacillus stearothermophilus* AB-1 at 35°C and pH 7.5. *Pseudomonas aeruginosa* showed the optimum production of lipase at 30° after 72 h of incubation at pH range of 7–9 [38]. Venugopal and Sharamma [31] showed that bacteria isolated from mangrove sediments though exhibited highest protease activity at 40°C and pH 8, but it was stable over a wide range of pH 7–11 and temperature (up to 50°C).

Optimization of culture conditions helps in increasing the productivity of bacterial enzymes under laboratory conditions. Production of microbial enzymes is dependent on the various physicochemical conditions such as pH, temperature, nutrient sources used in the culture media. Dalmau *et al.* [39] showed that lipase production is enhanced by the use of different carbon sources. Glucose was found as the effective carbon source for the production of extracellular lipase in culture media [40]. However, the highest production of lipase (37.6 ± 0.2 Uml⁻¹) in the present study was achieved using Sucrose as the carbon source which is similar to the results obtained by Bharathi *et al.* [5]. Galactose showed the highest production of protease (39.2 ± 0.6 Uml⁻¹) fol-

lowed by sucrose (15.2 ± 0.2 Uml⁻¹) and glucose (10.25 ± 0.2 Uml⁻¹). Various studies have shown increased yield of protease in the presence of xylose, lactose, maltose, and sucrose in the medium [20].

Nitrogen source showed a significant effect on the bacterial growth and production of extracellular enzymes. In the present study the highest production of lipase (31.05 ± 0.2 Uml⁻¹) was observed when yeast extract was used as the nitrogen source. This result is similar to the results of several other studies where yeast extract and peptone were used as the source of nitrogen for the production of lipase by different species of genus *Bacillus* such as *Bacillus alkalophilus*, *B. cereus*, *B. subtilis*, *B. licheniformis* and *B. coagulans* [41]. Use of peptone as the nitrogen source in the culture media enhanced the production of protease (41 ± 0.4 Uml⁻¹) by *Bacillus licheniformis* which corroborates the findings of Nadeem *et al.* [42]. Other studies reported the maximum yield of protease when beef extract, yeast extract, and a mixture of yeast extract and peptone were used in the culture media [16].

Cultural conditions determine the cost and yield of industrially important enzymes as 40% of the cost of enzyme application depends on the cost of growth medium used for the production of enzymes [43]. Optimization of culture conditions in the present study showed the highest production of extracellular lipase by the indigenous bacteria, *B. licheniformis*, at 35°C and pH 7.5 in a basal culture media containing yeast extract and sucrose as sources of nitrogen and carbon respectively. The sources of nitrogen and carbon for the optimum production of protease by the same species, however, was different, the other culture conditions remaining the same. The sources of nitrogen and carbon for protease were peptone and galactose respectively. The results of the present study showed the higher productivity of enzymes occurred when the cells were at log phase of bacterial growth which indicated that a high amount of industrially important enzymes could be produced by mass cultivation of bacteria under laboratory conditions owing to its stability under a wide range of temperature and pH.

The present study showed that bacteria indigenous to refinery sludge containing petroleum hydrocarbons could be a potential source of microbial enzymes. *B. licheniformis* (MT250345) was identified as the most

potent bacteria isolated from petroleum hydrocarbon contaminated soil of Assam for production of industrially important protease and lipase enzymes. Optimization of the culture conditions of the isolate resulted in a high yield of protease and lipase under laboratory conditions. Further studies on purification and characterization of these important enzymes are required besides possible genetic manipulation of the strain for enhancing the yield. Extensive screening of bacteria for the production of extracellular enzymes will accelerate the isolation and identification of potent bacterial strains to meet the rising demand for new sources of microbial enzymes.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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