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Process optimization for obtaining a maximum yield of alkaline thermostable lipase from *Bacillus stratosphericus*-MK788130

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Abstract

Since last few decades, continuous efforts are being made to screen the lipase producing bacterial strains from the environment because of its diverse commercial applications. In this study, *Bacillus stratosphericus* MK-788130 was isolated from a frying oil contaminated soil although it was previously thought to be the atmospheric bacterium. It showed lipolytic zones of 0.44 mm, 0.51 mm and 0.37 mm on peptone yeast agar, olive oil hydrolysis agar and chromogenic plate agar respectively. It produced an extracellular lipase 42.7 µM/ml. This bacterium preferred acidic pH 5 for growing optimally at 45 °C when the medium was supplemented with 1% olive oil. The olive oil induced its growth up to 9 hours. The protein content of the purified lipase was 85 mg/ml as compared to its crude form i.e. 220 mg/ml. The purified lipase was found to be alkaline thermostable as its optimum activity was observed at pH 9 and 90 °C. The factors that optimized its activity included 0.1mM Na ions (0.06 U/ml), 0.1 mM fructose (0.1 U/ml), 1% olive oil (0.7 U/ml), 1% corncob (0.1 U/ml), 1% yeast extract (0.05 U/ml), 1% casein (0.05 U/ml) and 0.1 mM commercially available detergents (0.06-0.07 U/ml). It was observed stable with metal ions (Na, Fe and Ca), induced by Tween 80, Tween 20 but inhibited by Triton X-100 and SDS. The purified lipase showed a polypeptide of 14 kDa on SDS-PAGE. Its property as a biosurfactant as well as in oil bioremediation has broadened its application in the biotechnological industry.

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Introduction

The concept of enzymes as catalytic agents is as old as the human history (Guerrand, 2017; Shamim *et al*., 2019). Out of 4000 enzymes reported so far, only 200 are in commercial use. Out of them, 75% are of microbial origin (Javed *et al*., 2018). Microbial enzymes are preferred over synthetic enzymes because they are environmental friendly, cheaper, highly specific and easier to produce (Shamim *et al*., 2018). Lipases (triacyglycerol acylhydrolases, EC 3.1.1.3) are serine hydrolases that convert triacylglycerides into glycerides (diglycerides or monoglycerides) by acting on carboxylic ester bond (Daroonpunt *et al*., 2018). They are reported in plants, animals and microorganisms (Iqbal and Rehman, 2015; Javed *et al*., 2018). More than 160 microbial lipase producing strains are reported among which *Pseudomonas*, *Bacillus* and *Streptomyces* are found to given highest yield (Habibollahi and Salehzadeh, 2017). Bacterial lipase production depends on strains, species and culture conditions (Ghaima *et al*., 2014). Common bacterial lipase producers (Habibollahi and Salehzadeh, 2018) are *S. aureus*, *B. coagulans*, *B. cereus* (Kim *et al*., 2015), *P. aeruginosa*, *P. fluorescens*, (Ghaima *et al*., 2014), *P. fragi*, *Burkholderia glumae* (Sangeetha *et al*., 2014; Iqbal and Rehman, 2015), etc. Mostly lipases are extracellular inducible enzyme. It is induced by fatty acids, glycerol, oils, hydrolysable esters, etc. (Zheng, 2017). The rise in practical application of enzymology at industrial scale is reported by Pliego *et al*. (2015). The versatility of the lipases can be judged from the fact that it has wide range of industrial applications in industries like leather, food, dairy, flavor, pharmaceuticals, detergent, cosmetics, chemicals, biofuels, etc. In order to be used in industries, the physicochemical properties of purified lipase must be taken into consideration (Niyonzima and More, 2014a). The temperature and pH are among those physicochemical factors that affect the production of lipase (Zheng, 2017). Following production, its purification involves various steps of ammonium sulfate precipitation, chromatographic techniques including hydrophobic interaction and ion exchange (Lailaja and Chandrasekaran, 2013). Thermostable microbial enzyme is desired because higher temperature improves their performance by enhancing properties like stability, reaction rate, solubility and lesser substrate viscosity as well as contamination (Hasan *et al*., 2006; Niyonzima and More, 2014a). The improvement in lipolytic activity in the presence of metal ions like Ca has already been reported in *Bacillus* species (Annamalai *et al*., 2011; Niyonzima and More, 2014a). In this study, an attempt had been made to unveil the presence of extracellular lipase producing bacteria from fry-oil contaminated soil of food stalls. The isolation, purification and optimization of the lipase produced as well as its potential applications were studied.

Materials and methods

Sample collection, isolation and purification of bacterial isolates

The soil samples were collected from food stalls where oil was spilled during deep fry. Luria Bertani (LB) medium was used to isolate and purify bacterial colonies using standard microbiological techniques. After sterilization of the growth medium, it was supplemented with 1% olive oil. The cultural, morphological and biochemical characterization of the colonies obtained were determined (Cheesbrough, 2001).

Screening and selection of lipase producing bacteria

The screening and selection of lipase producing bacteria were done on the basis of results of the following experiments.

Olive oil hydrolysis assay

In this method, the zone of lipolysis wasobserved on LB agar plates (tryptone 10 g, yeast extract 5 g, NaCl 5 g, agar 15 g) after incubation of 24 h at 37 °C (Iqbal and Rehman, 2015).

Peptone yeast agar assay

The method of Iqbal and Rehman, (2015) was followed. The bacterial colonies obtained were streaked on the peptone yeast agar medium (in one liter: peptone 10 g, yeast extract 10 g, tween 80 3 ml,

olive oil 10 ml and agar 15 g) followed by incubation of 24 h at 37 °C. The plates were observed for the zone of lipolysis.

Chromogenic plate assay

The plates containing chromogenic substrate was prepared as follows: 0.01% phenol red, 4% tween 80, 20 mM $CaCl_2$ and 2% olive oil and pH 7. All the ingredients were mixed thoroughly in the form of a homogenized paste. In it, 4% hot melted agar was added which made its appearance as orange-red color medium, followed by immediate pouring in the Petri plates and solidification at room temperature. By the help of Pasteur pipette, wells were made in the plates and culture (overnight grown) supernatants were added in the wells.

The plates were incubated at 37 °C for 30 min. The change in color from orange-red to yellow was the indication of lipase activity of the bacterial strains (Amara *et al.*, 2009).

Selection of efficient lipase producing bacteria

Depending on the zone of lipolysis obtained in different experiments performed above, one bacterial strain was selected. It was designated as ML-4.

Characterization of ML-4

The cultural, morphological and biochemical characterization of ML-4 was performed (Cheesbrough, 2001). The molecular characterization was requested from Macrogen®.

Optimization of growth conditions

The optimization of growth conditions including temperature, pH and carbon source was done as follow:

Optimum temperature

The selected strain was checked for its optimum growth at different temperatures (25, 37, 50, 70 90 °C). In 5 ml sterilized LB broth, a loopful inoculum was given. The tubes were incubated at respective temperatures for 24 h. Next day optical density was read at 580 nm.

Optimum pH

Different pH (4, 5, 6, 7, 8, 9) were selected to check the optimum growth of the selected strain. After preparing the LB broth, it was divided in different beakers. The pH of each beaker containing medium was adjusted with HCl or NaOH. After adjusting the pH, the medium was poured in properly labeled flasks and autoclaved under the standard conditions of temperature and pressure (121 °C, at 15 psi and 15 min). After autoclaving, 1% fresh culture was added and incubated at 37 °C. After 24 h, the growth of ML-4 in all flasks was noted by reading their OD at 580 nm.

Optimum carbon source

In 5 ml LB broth, 1% different oils (sunflower, canola, olive, ghee, almond) were added as carbon sources followed by 1% inoculum. The test tubes were incubated at 37 °C for 24 h. Next day optical density was observed at 580 nm.

Growth curve analysis

After selection of the optimum growth conditions of ML-4, the effect of oil on its growth was checked. For this, the LB growth medium was supplemented with 1% olive oil and 1% culture. In control, olive oil was not added. The OD was read at 580 nm after every hour up to 9 hours (Iqbal and Rehman, 2015).

Quantitative analysis of lipase

The method of Bussamara *et al.* (2010) was followed with slight modifications. The fresh culture grown in 1% olive oil overnight was harvested at 1980 x *g* for 15 min. The supernatant (100 µl) was shifted to an eppendorf. In it, 900 µl substrate solution (1 ml solution A: 1 ml isopropanol containing 3 mg *p*-nitrophenol palmitate (pNPP) + 9 ml solution B: 200 ml 50 mM Tris-Cl pH 8.0, 0.2 ml Tween 80, 0.8 ml Triton X-100) was added. It was mixed properly and incubated at 37 °C for 30 min. The increase in absorbance was read at 410 nm. For control, culture grown without olive oil was used. The following formula was used to determine the activity units. One unit of lipase is the amount of enzyme liberating 1 µmol of para-nitrophenol per ml per minute under

standard assay conditions (Winkler and Stuckmann, 1979).

Lipase activity (U/ml) = $\Delta A \cdot V / \varepsilon \cdot t \cdot v$
ΔA = Change in optical density
V = Total volume in ml
ε = Extinction coefficient (ε_{410} for *p*-nitrophenol = 15,000 cm^2/mg)
t = Incubation time in minutes
v = Volume of enzyme

Isolation and purification of lipase

Preparation of phosphate buffer 1M (pH 7.0)

It was prepared by mixing 30 ml 0.5 M Na_2HPO_4 (7.098 g in 80 ml distilled water, pH 7.0) and 80 ml 0.5 M NaH_2PO_4 (5.99 g in 80 ml distilled water, pH 7.0). The final volume was made up to 1000 ml by distilled water (csh protocol, 2016).

Salt precipitation

The fresh culture (1 ml) was harvested at maximum speed for 20 min. It was shifted to a new eppendorf and 0.6 g ammonium sulfate salt was added to ensure 90% purification. The solution was made homogenized by proper mixing. It was placed in refrigerator overnight. After 24 h, it was centrifuged at 1968 x *g* for 30 min. The supernatant of jelly-like consistency was shifted to a dialysis membrane. Before shifting, Bradford assay was performed (Bradford, 1970).

Dialysis

The dialysis membrane (5 cm) was prepared for this step by folding its one end and closing it with clamp. The supernatant obtained (above step) was pipetted in it. The other end of the membrane was immediately closed in same manner by folding its end and putting clamp on it. It was suspended in a glass beaker containing 0.1 M phosphate buffer pH 7.0 (prepared above) overnight. The beaker was placed in a refrigerator. Next day, solution inside dialysis tubing was shifted to an eppendorf and centrifuged at following conditions; 1968 x *g*, 10 min, 4 °C. After harvesting, the supernatant was shifted to a new eppendorf, Bradford assay was performed. The sample at this stage was ready for column chromatography.

Colum chromatography

In a clean column, a piece of cotton was placed. It was filled with Sephadex G-100 (5 g) followed by 2 ml 0.1 M phosphate buffer (pH 7.0). As Sephadex particles got settled inside the column, 100 µl sample was injected in it. The sample was allowed to pass through the column and collected in collection tubes. All the collected fractions were subjected to Bradford assay. Those showing higher values were collected in one vessel. At this stage, the sample was ready for ion exchange chromatography.

Ion exchange chromatography

Here a clean column was filled with DEAE G-100. The sample collected in above step was allowed to pass through it. Similarly the fractions of the sample showing high protein content were collected in one vessel. The Bradford assay was performed. The collected sample was lyophilized and stored at -20 °C. All experiments (optimization, SDS-PAGE and industrial applications) were performed using this purified lipase.

Optimization of purified lipase

The optimization experiments were performed by using 100 µl purified lipase and performing quantitative assay as mentioned above (Bussamara *et al.*, 2010).

Temperature

Different temperatures (25, 37, 45, 50, 60, 70, 80 and 90 °C) were selected to optimize the purified lipase.

pH To determine the optimum pH of purified lipase, following pH were used; 5, 6, 7, 8 and 9.

Metal ions

The selected metals were 0.1 mM Na, K, NH_4, Zn, Cr, Ca, Fe, Mg and Mn.

Carbon sources

This experiment was performed by using 0.1 mM

glucose, fructose, maltose, lactose and galactose.

Oils

The 1% olive, sunflower, canola and mustard oils were used.

Agro-wastes

The wastes of banana, corn, wheat, sugarcane and pea peels were used as 1%.

Nitrogen sources

They included 1% peptone, yeast extract, casein and tryptone.

Detergents

Here 0.1 mM sodium dodecyl sulfate (SDS) was used in addition to some commercially available detergents (Ariel®, Bonus®, Bright® and Surf Excel®).

Additives

The additives (1%) used were; tween 20, tween 80 and triton X-100.

Applications

The potential application of the purified assay was performed as follow (Iqbal and Rehman, 2015).

Hydrocarbon degradation

The petrol was added in fresh culture supernatant in equal quantity. The mixture was thoroughly mixed for 15 min and allowed to settle down. The tube was observed for formation of any layer.

Alkane hydroxylase assay

For this LB containing 1% kerosene oil and 1% petrol was prepared. It was incubated with the bacterial isolate and incubated at 37 °C for 48 hrs at 1968 x *g*.

Detergent assay

In order to perform this assay, three beakers were taken. The first beaker was filled with 50 ml water, second with 50 ml purified lipase and third with 50 ml detergent of registered brand. Two clean pieces of white cloth were taken, one was marked with blue ink and ketchup was thrown on the other piece. Both pieces were allowed to dry at room temperature.

They were cut in three equal parts and dipped in three beakers. They were placed in beakers for 1 hour followed by washing with tap water. After washing, they were air dried and compared with each other.

Bio-surfactant assay

A drop of olive oil was placed on a clean glass slide. About 10 µl sample of purified lipase was placed in the center of the oil drop. The change in the shape of oil drop was observed up till one minute.

Results

From eight frying oil contaminated soil samples, six bacterial isolates were obtained. The colonies were purified for the screening of lipase producer. All six bacterial colonies were screened on the PYA, olive oil hydrolysis and chromogenic plate. The zones of lipolysis were observed (Table 1).

Table 1. The zone of lipolysis of different bacterial strains as obtained in screening methods.

Sr. No.	Bacterial strains	Zone of lipolysis		
		PYA assay	Olive oil hydrolysis agar assay	Chromogenic plate assay
1.	ML-1	0.23 mm	0.14 mm	0.11 mm
2.	ML-2	0.19 mm	0.22 mm	0.19 mm
3.	ML-3	0.31 mm	0.19 mm	0.27 mm
4.	ML-4	0.44 mm	0.51 mm	0.37 mm
5.	ML-5	0.21 mm	0.34 mm	0.18 mm
6.	ML-6	0.11 mm	0.47 mm	0.16 mm

The quantity of lipase present in all six bacterial isolates is given in Table 2. On the basis of screening methods and quantification of lipase, ML-4 was selected as a potential lipase producing bacterium. Biochemical characterization showed ML-4 a *Bacillus* sp. It was found to be *B. stratosphericus* on the basis of 16S rRNA sequencing. It got registered in NCBI GenBank under accession number MK-788130. *B. stratosphericus* MK-788130 showed optimum growth in the presence of 1% olive oil at 45 °C and pH 5.

Table 2. Quantification of lipase of six bacterial isolates using *p*-NPP as substrate.

Bacterial isolates	Quantification (OD)	Lipase concentration (µmoles/ml)
M1	0.375	33.1
M2	0.328	31.4
M3	0.354	32.7
M4	0.454	42.7
M5	0.402	36.8
M6	0.322	31.6

The olive oil was found to be the inducer of *B. stratosphericus* MK-788130. The cells continued to grow even after 9 h (Figure 1). The protein content at different steps of enzyme purification is given in Table 3. Purified lipase showed optimum activity at 90 °C, pH 9 in the presence of Na ions. Fructose was inducer while sucrose was inhibitor for it. Additionally, 1% of olive oil, corncob extract, yeast extract, casein, all commercially available detergents and tween 20 enhanced its activity (Figures 2-4). The purified lipase was found to be thermostable and alkalophilic. Furthermore, the results in Figure (5) showed its potential application as a biosurfactant (Table 4). According to SDS-PAGE results, the purified lipase polypeptide has a molecular weight of 14 kDa (Figure 6).

Table 3. The protein content (mg/ml) of *B. stratosphericus*-MK788130 at different steps of lipase purification.

Enzyme purification steps	Protein content (mg/ml)
Supernatent (crude)	220
After ammonium sulphate precipitation	114
After dialysis	107
After column chromatography	91
After ion-exchange chromatography	85

Discussion

Lipases breakdown triglycerides to diacylglycerides, monoglycerides and fatty acids. Their commercial significance made them an important enzyme at industrial scale (Daroonpunt *et al.*, 2018). For employing them in industries, their various properties like growth conditions, enzyme properties, etc. should be studied in detail. Among various microorganisms studied for lipase isolation and characterization, *Bacillus* holds special importance as they are non-pathogenic and easy to culture.

In this study, *Bacillus stratosphericus* was isolated from soil contaminated by frying cooking oils. Cooper *et al.* (2010) identified seventy three microorganisms from such cooking oil contaminated soil samples.

Table 4. The results of various assays to check the potential industrial application of isolated lipase of *B. stratosphericus*-MK788130 (Table 4 is added in the "Results" section).

Sr. No.	Assays	Results
1.	Biosurfactant assay	Positive
2.	Tube emulsification test	Negative
3.	Alkane hydroxylase assay	Negative
4.	Detergent assay	Negative

They included *Alcaligenes*, *Micrococcus*, *Proteus*, *Flavobacterium*, *Pseudomonas* and *Bacillus* along with some fungal microbial flora (Popoola and Onilude, 2017). Shivaji *et al.* (2006) isolated *B. stratosphericus* from altitude of 24-41 km. *B. stratosphericus* is a Gram positive, catalase positive (Lai *et al.*, 2014), oxidase negative, citrate positive, indole negative, and endospore forming bacterium

that belongs to phylum Firmicutes. Its diverse habitat can be estimated from the fact, that it is present at 24 km altitude in stratosphere as well as at the depth of 5,500 m (Shivaji *et al*., 2006; Lima *et al*., 2013; Lata *et al*., 2017). Liu *et al.* (2013) reported its presence in the marine environment. The lipase from *B. stratosphericus* L1 was reported by Gricajeva *et al.* (2016). Different statistical softwares were used for the enhancement of its extracellular lipase (Ismail *et al*., 2018).

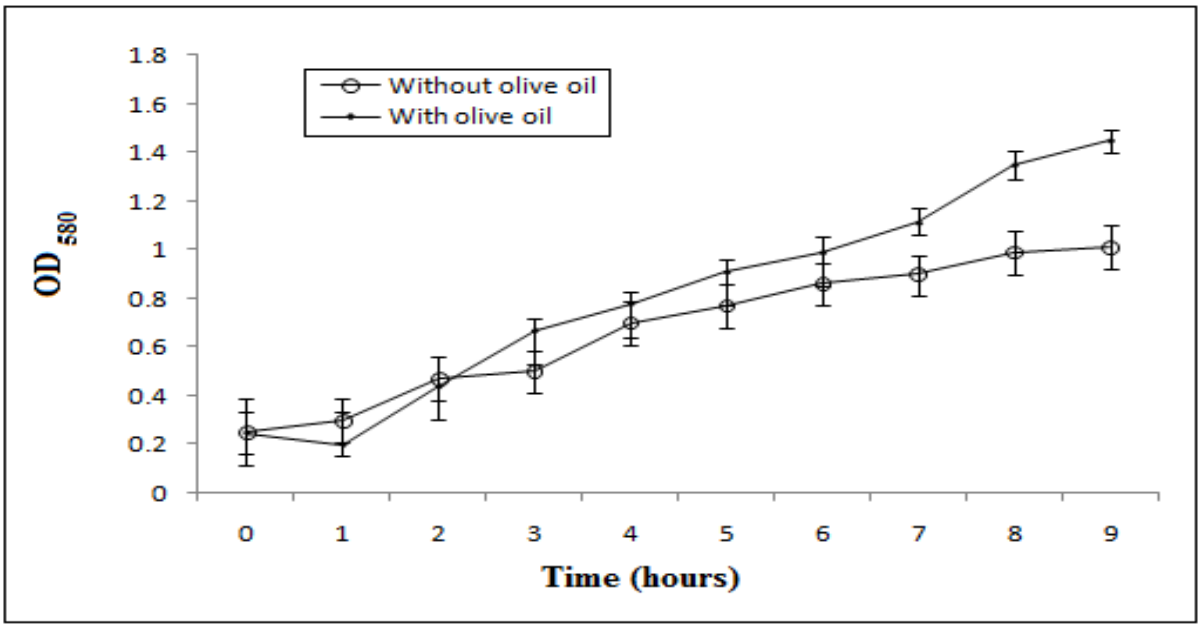

Fig. 1. Growth curve study in the presence and absence of 1% olive oil revealing olive oil as an inducer for *B. stratosphericus*-MK788130 cells.

The use of coconut dregs as a substratum for its lipase production was reported by Zin *et al.* (2017). According to Odisi *et al.* (2012), it did not need any inducer for induction of its lipases and cellulases. The published literature reported *B. stratosphericus* in different contexts e.g. with respect to its alkaline protease (Bindu *et al*., 2013), degradation ability of azo dyes (Akansha *et al*., 2019), its cytochrome 450 enzyme (Hazarika *et al*., 2018), involvement in the synthesis of nanoparticles (Hosseini-Abari *et al*., 2014), as a PGPR (Susilowati *et al*., 2015), as a potent probiotic (Mukherjee *et al*., 2016), in asparaginase production (Pola *et al*., 2018), etc. Its activity against bacterial phyto-pathogens for controlling plant diseases is also reported (Durairaj *et al*., 2017). Its statistical optimization of β-glucosidase was studied by Dutta *et al.* (2017). In this study, lipase was screened by different methods (Table 1). Among six lipase producing bacterial strains, one strain ML-4 was selected on the basis of quantification (Table 2) of lipase i.e. 42.7 µmoles/ml. The cultural, morphological and molecular characterization showed it *B. stratosphericus*-MK788130. It showed optimum growth in the presence of 1% olive oil, pH 5 and 45 °C. Our results are in contradiction with the studies of Shivaji *et al.* (2006) and Ismail *et al.* (2018) who reported its optimum growth conditions as 8-37 °C, pH 6-10 and 34.8 °C, pH 6.98 respectively. The possible explanation of this contradiction in temperature and pH is due to difference in sampling site and sample type. The temperature at altitude is lesser as compared to the heating temperature of frying oil sites. Here slight acidic (pH 5) corresponds to the fact that frying items mostly contain vinegar or other sauces as a part of their species, organisms growing in such acidic pH got adapted to it. In our study, olive oil induced the growth of *B. stratosphericus*-MK788130 cells up to 9 hours (Figure 1). Our results are in contradiction with Iqbal and Rehman, (2015) who observed sluggish growth of *B. subtilis* I-4 cells in the presence of olive oil. Adentunji and Olaniran, (2018) reported induction of

B. aryabhattai SE3-PB lipase by using sunflower oil in the growth medium. The induction in the presence of oil gives a clue of its potential application in oil bioremediation (Lee *et al.*, 2015). Before heading towards the optimization of the bacterial lipase under study, there is a dire need to purify them from unwanted impurities which hinder its catalytic sites as well as its efficiency (Kornberg, 2009).

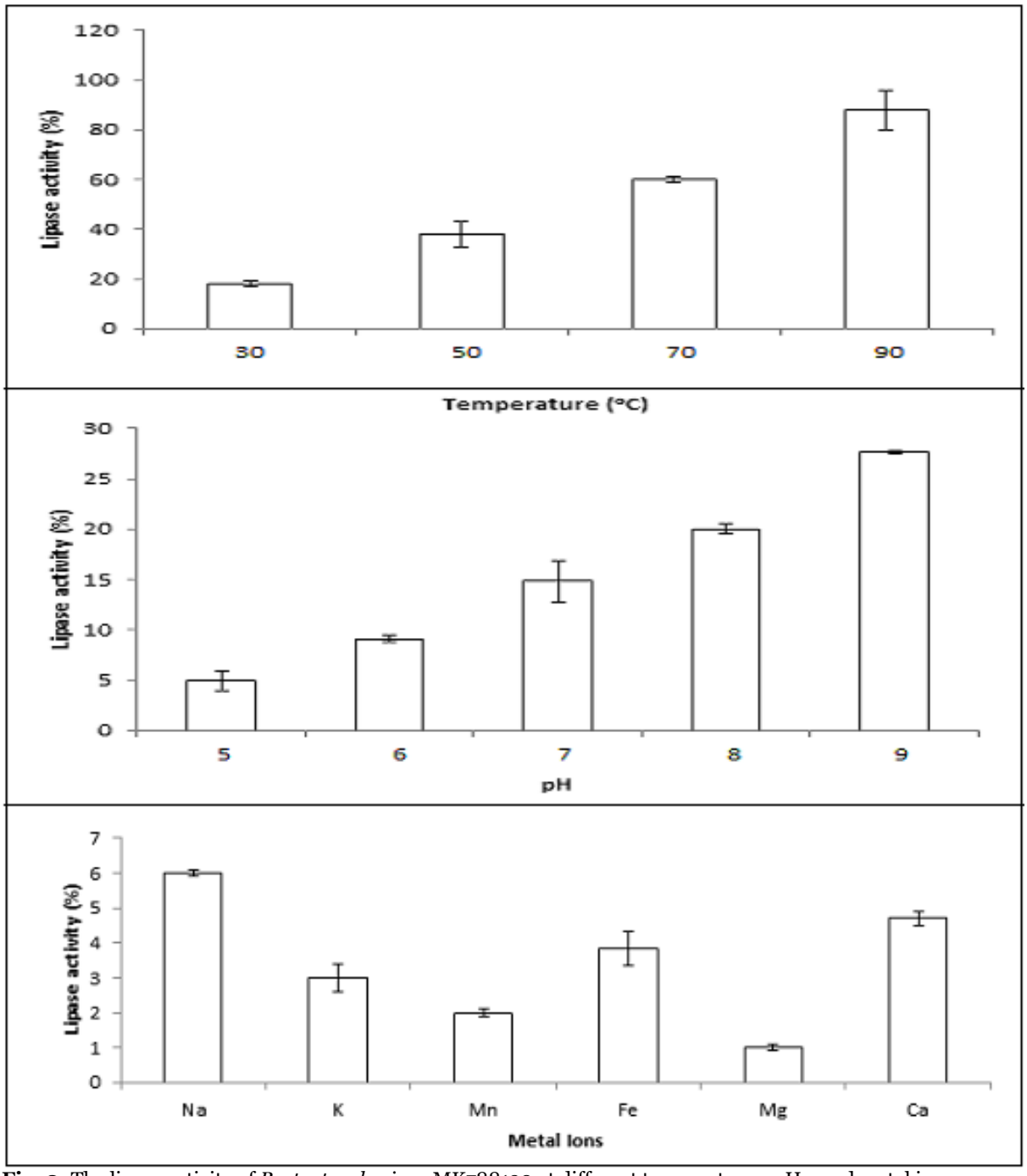

Fig. 2. The lipase activity of *B. stratosphericus*-MK788130 at different temperatures, pHs, and metal ions.

The purification of bacterial lipases is previously reported (Andualema and Gessesse, 2012; Dey *et al.*, 2014; Rabbani *et al.*, 2015; Bhosale *et al.*, 2016; Sharma *et al.*, 2017). In this study, the purification of *B. stratosphericus*-MK788130 lipase resulted in decrease in protein content from 220 ml/ml to 85 mg/ml (Table 3) which is in agreement with Borkar *et al.* (2009) who reported same decrease in protein concentration from 884 mg to 0.68 mg for *P. aeruginosa* SRT9 lipase purification.

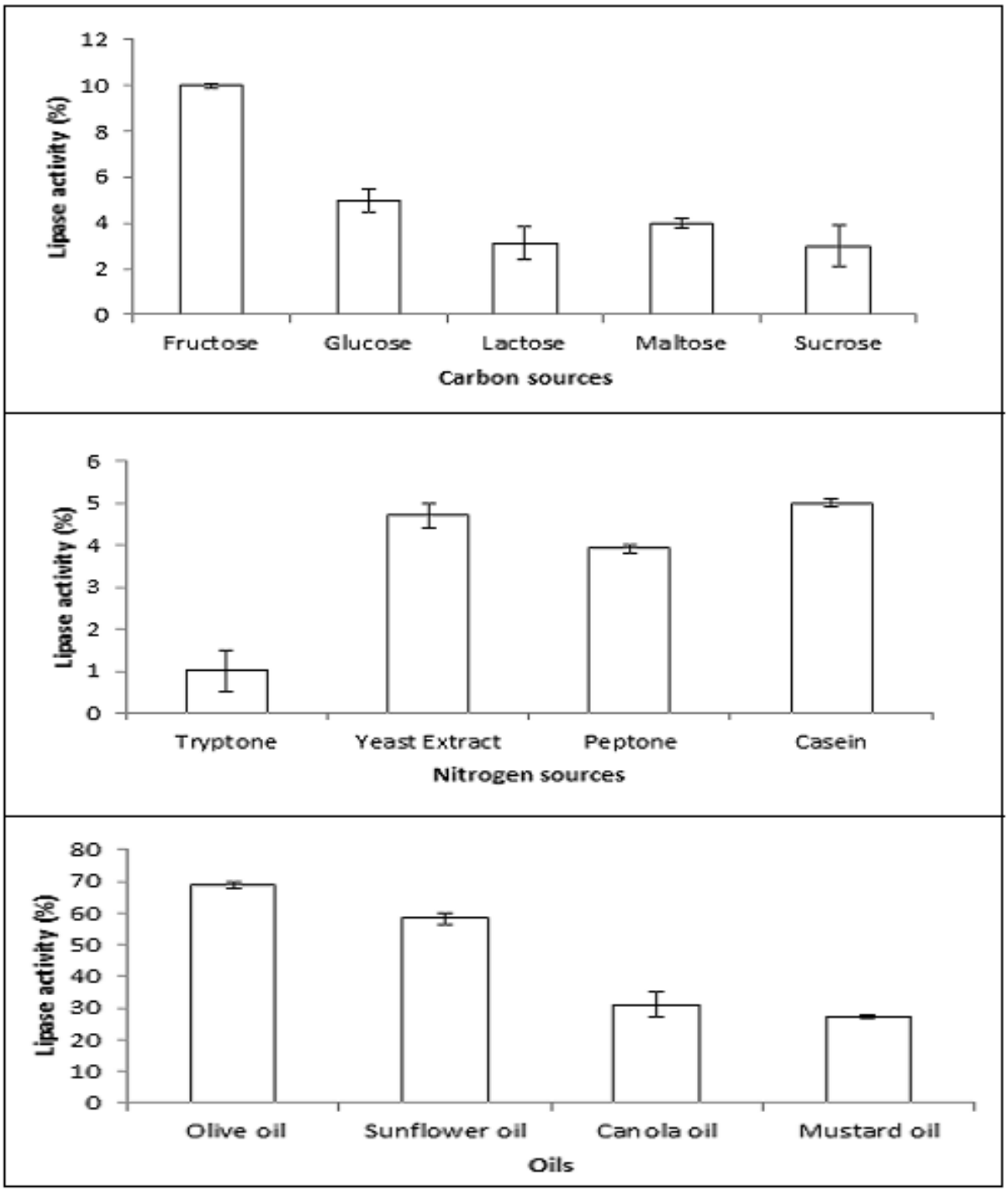

Fig. 3. The lipase activity of *B. stratosphericus*-MK788130 at different carbon sources, nitrogen sources and oils.

The purified lipase of *B. stratosphericus*-MK788130 was found to be thermostable as it showed optimum activity at 90 °C, pH 9 and in the presence of Na ions (Figure 2). Lipase usually performs lipolysis between pH 4-11 and its optimum temperature range between 30-60 °C (Xiao *et al.*, 2017). According to Lomthaisong *et al.* (2012), the lipase of *P. xinjiangensis* CFS14 showed maximum activity at 37 °C, pH 8 and in the presence of Mg ions (Lomthaisong *et al.*, 2012). *Bacillus* sp. W130-35 lipase, isolated from tidal mud flat showed its maximal activity at pH 9, 60 °C and Ca ions (Kim *et al.*, 2015). Tambekar *et al.* (2017) reported alkaline lipase production at pH 9 and 60 °C by *B. flexus*. In another study (Niyonzima and More, 2014b), the lipase of *B. flexus* was found functioning optimum at pH 10 and 70 °C. It was furthermore studied by the same group that this lipase was stable in Tween 80

and Triton X-100 which partially agrees with our findings (Figure 4) as here maximum lipase activity was observed with Tween 80 and minimum with Triton X-100. *B. flexus* lipase also showed stability in the presence of commercially available detergents which strongly agree with our investigations (Niyonzima and More, 2014b). For *B. subtilis* I-4, the optimum enzyme activity conditions were; 50 °C, pH 7.0, Ca ions, olive oil and Tween 80 (Iqbal and Rehman, 2015).

Habibollahi and Salehzadeh, (2017) presented their investigations about lipase of *Pseudomonas* sp. KY 288051 as follows: 37 °C, pH 7, peptone and olive oil.

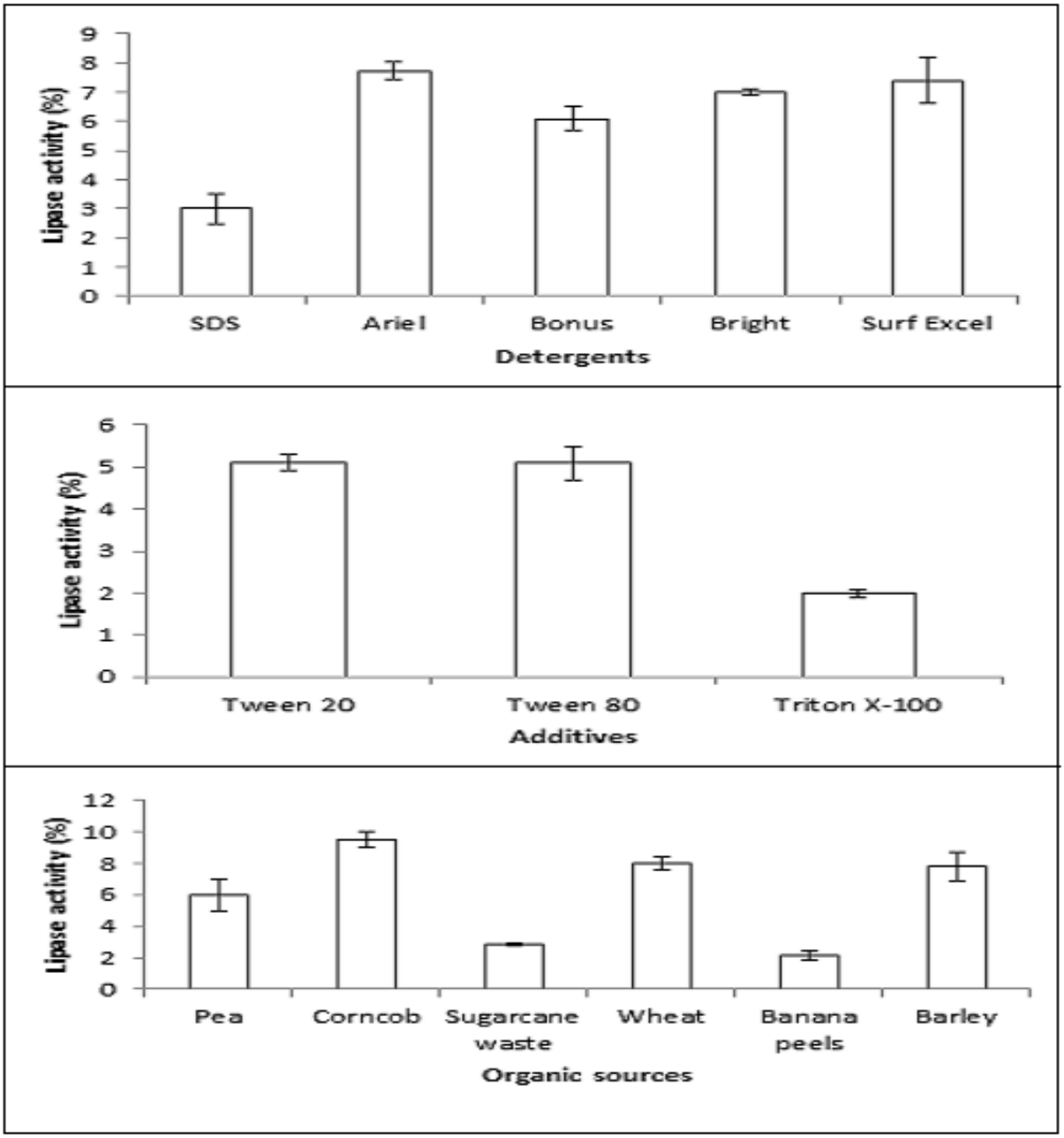

Fig. 4. The lipase activity of *B. stratosphericus*-MK788130 at different detergents, additives and organic sources.

The lipase of *Staphylococcus aureus* worked optimum (15.8 U/ml) at 37 °C and pH 7 and peptone (Sirisha *et al.*, 2010). In their study, olive oil induced the expression of lipase (12.5 U/ml) which is in agreement with our results. Previous literatures also supported our results (Muralidhar *et al.*, 2001; Fadiloglu and Erkman, 2002). The lipase obtained from *B. cereus* showed maximum activity at pH 8

(60.2 U/ml), 35 °C (55.25 U/ml), maltose (66 U/ml) and peptone (66 U/ml) (Ghaima *et al.*, 2014). The lipase of *B. sonorensis* 4R had highest activity at 80 °C, pH 9, Mg and Ca ions (Bhosale *et al.*, 2016). The lipase of a psychrophilic strain of *Pseudomonas* sp. LSK25 was obtained at 10 °C and pH 7 (Salwoom *et al.*, 2019).

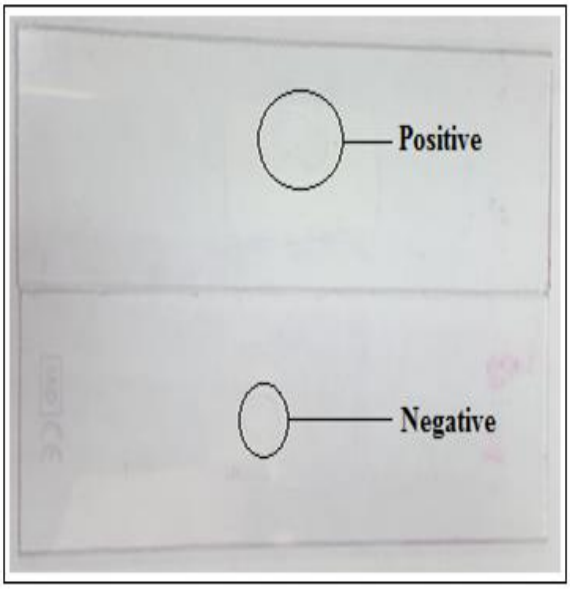

Fig. 5. A drop of oil was dispersed by adding purified lipase showing positive results for its feature as a biosurfactant. On the other hand, an intact oil droplet showed negative results.

The purified enzymes isolated from *Pseudomonas reinekei* was found stable over a wide range of pH 5-9 and at 40 °C (Priyanka *et al.*, 2019; Ryan *et al.*, 2019). According to Zarinviarsagh *et al.* (2017), the yield of *Ochrobactrum intermedium* MZV101 lipase was 69 % which was found stable at pH 10-13 and 70-90 °C. The fructose was found to be the best carbon source for optimal functioning of lipase (0.1 U/ml) which contradicts with the findings of Sooch and Kauldhar, (2013) where it induced 53.2 IU/ml lipase activity as compared to glucose (77.2 IU/ml). In another study, 1.5% glucose was reported as best carbon source for lipase activity (1590 u/mg) followed by 1-2% fructose (1595 u/mg) for *Bacillus* sp. ZR-5 (Soleymani *et al.*, 2017). According to Mazhar *et al.* (2016), maltose made maximum lipase expression (28.91 u/ml) of *B. cereus* PCSIR NL-37 possible followed by fructose (27.2 u/ml) (Mazhar *et al.*, 2018). Here polypeptide of the purified lipase of *B. stratosphericus*-MK788130 was of 14 kDa (Figure 6). The lipase of *O. intermedium* MZV101 was of 99.42 kDa as studied by Zarinviarsagh *et al.* (2017). Rabbani *et al.* (2015) reported 31 kDa lipase from *B. subtilis*. Gururaj *et al.* (2016) observed lipase of 45 kDa in *Acinetobacter* sp. AU07. Similarly previous literatures published lipase of different molecular weights as 13.9 kDa, 31.3 kDa, 43 kDa, 50 kDa, in psychrotrophic *Pseudomonas* ADT3 (Dey *et al.*, 2014), *Enterobacter* sp. Bn12 (Farrokh *et al.*, 2014), *Staphylococcus* SDMlip (Tipre *et al.*, 2014), *Leuconostoc mesenteroides* subsp. *mesenteroides* ATCC 8293 (Sukohidayat *et al.*, 2018) and *Pseudomonas reinekei* (Priyanka *et al.*, 2019) respectively. In this study, the purified lipase showed the property of a biosurfactant (Figure 5) which is in agreement with the findings of Iqbal and Rehman, (2015), who also observed similar property of *B. subtilis* I-4.

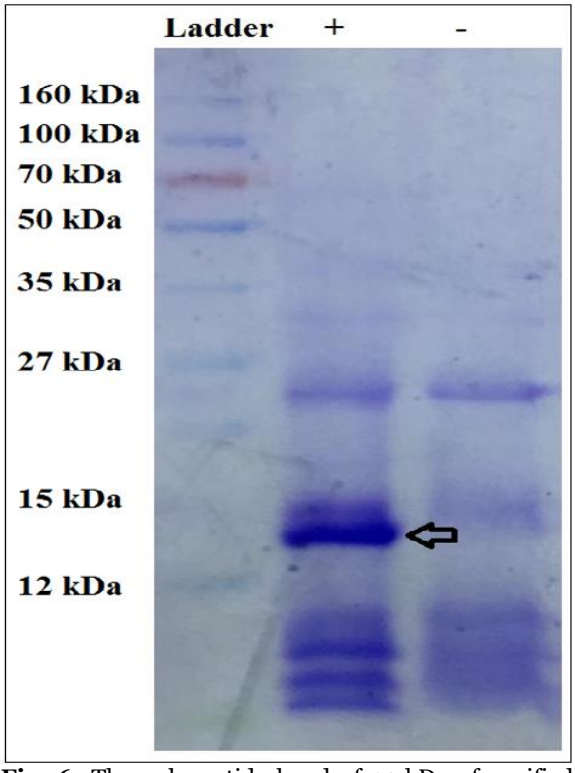

Fig. 6. The polypeptide band of 14 kDa of purified lipase as shown by SDS-PAGE. In the figure, "+" sign shows the presence of 1% olive oil and "-"sign shows the absence of olive oil.

The biosurfactants are microbial metabolites that are surface-active molecules meant to reduce the surface tension (Santos *et al.*, 2016). They have wide range of industrial applications e.g. agrochemicals, foods,

pharmaceuticals, cosmetics, fertilizers, petrochemicals, etc. (Vijayakumar and Saravanan, 2015). Here in this study, the *B. stratosphericus* MK-788130 was found to split the oil droplet by reducing its surface tension (Figure 4), thus indicating its potential industrial application. Our results are in agreement with previous literature where biosurfactant producing bacterial strains are already reported (Pacwa-Plociniczak *et al*., 2011; Reis *et al*., 2013; Zarinviarsagh *et al*., 2017; Lajis, 2018).

Conclusion

This study reports about the isolation, purification and optimization of *B. stratosphericus* MK-788130 lipase isolated from fry-oil contaminated soil although it was previously thought to be a bacterium of stratosphere; a layer of atmosphere. The properties of alkaline thermostable lipase of *B. stratosphericus* MK-788130 can be further explored and studied. Its potential application in the field of oil bioremediation as well as biosurfactant has made it a suitable candidate for biotechnology industry. (Repetition of "Conclusion" and an extra heading of "References" deleted from here).

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