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Purification of Novel Lipase Lip4 Gene Cloned From Local Isolate of Bacillus Amyloliquefaciens in Escherichia Coli

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Annotation: Lipase, an enzyme that catalyzes the hydrolysis of triacylglycerol, is of significant interest in the field of biotechnology. Especially in modern food industries, instead of traditional chemical reactions. In this study, different lipase-producing isolates were obtained from different sources by two-step screening and identified as Bacillaceae. The highest producer of these isolates was found to belong to Bacillus amyloliquefaciens and named MMO (OR701819.1) with an enzyme activity determined at 59.6 U/ml. The lip4 gene responsible for lipase in this strain was studied in detail, and it was found to involve 600 bp of nucleotides (OR744906.1), encoding for 199 amino acids. This gene was linked with the expression pET-28a(+) plasmid and cloned into high-efficiency Escherichia coli DH5α, used as a host. Lipase was found to be produced from recombinant bacteria with an enzyme activity higher than was expressed in a wild strain, demonstrating that post-translational modification was achieved. The lipase activity achieved was 199.3 U/ml, three times more than in the wild strain. The

enzyme produced by the cloned cells was subjected to a purification process to homogeneity in three sequential phases. The results showed an increase in the enzyme activity, reaching 344.2 U/ml at the end of the final purification processes. The purification fold was found to be 11.2. The recovery rate of lipase during the purification process was determined to be 51.8%. Then it can be used in the medical field, such as drugs for the treatment of indigestion and stomach terrible, as well as in food technology.

Keywords: Fold, Plasmid, Bacillaceae, Expressed.

1. Introduction

Lipases are lipid hydrolysis enzymes that analyze the ester bonds of the carboxyl groups in triglycerides and produce di- and mono-fats, free fatty acids, and glycerol (enzyme commission number 3.1.1.3, triacylglycerol acyl hydrolase) [1,2]. It is considered an enzyme and one of the largest groups of industrial enzymes, such as in the food and pharmaceutical industries, the detergent and cosmetics industries, the production of biofuels, and the treatment of wastewater. In the food industry, lipases have been extensively utilized to improve flavor by producing esters of short-chain fatty acids with glycerol. They have been used to develop flavor in the manufacture of dairy products such as cheeses, especially mature ones, butter, margarine, and others. Lipases have also been used in the manufacture of low-fat meat and fish products, or what is known as lean meat. It is also used in preparing pastries because of its important role in improving the flavor, increasing the strength and stability of dough, and increasing the volume and rheological properties of dough [3,4,5,6]. According to surveys, the lipase market is estimated to reach $590.5 million by 2020 [7]. Lipases are produced naturally by many organisms, such as plants, animals, and microorganisms. Microorganisms are the best source for their production compared to plant and animal sources due to their ease of handling, the small space they occupy, the diversity of lipases produced from them, and the ease of genetic modifications to make them more productive of the enzyme and more suitable for various applications [8,9]. Bacterial lipases have gained great importance in the dairy industry due to their direct relationship with the appearance of rancid flavor in milk, which has been used in the manufacture of some types of ripened cheeses such as cheddar [10,11]. The lipases produced by *Bacillus* bacteria have properties that differ from those produced by other microorganisms, such as their high thermal stability and effectiveness in basic media [9]. The genus *Bacillus* is one of the most complex and ecologically diverse groups of bacteria on planet Earth. It is found in various environments, such as soil, fresh and salt water, and on plants and animals. It is capable of consuming a wide range of simple and complex organic compounds. This wide distribution indicates its high ability to adapt and perform many metabolic processes, which in turn is reflected in the diversity of enzymes capable of producing them [12,13]. It is characterized by producing many extracellular compounds such as polysaccharides, proteins, and external DNA, as well as being characterized by its abundant production of external

enzymes, the most important of which are amylases and lipases [14]. This study aimed to achieve the following: obtaining an isolate of *Bacillus* that has a high efficiency to produce lipase and identifying it at the level of species by different methods, including molecular ones. Then cloning the gene of lipase of this isolate into *E. coli* to get a highly effective enzyme and purify it for possible use in many industrial and food applications.

2. Materials and Methods

2.1. The Sample Compilation

Various samples potentially harboring the target bacteria, *Bacillus* spp., including soil, industrial water waste, and leftovers of lipid-rich foods, were collected from variety places in Kirkuk, Iraq. Ten grams of each sample were suspended in distilled water, adjusted to a pH of 7.0, and subjected for 10 min to heating at 80°C to eliminate vegetative cells of bacteria. After serial dilution, a 0.1 ml portion of the sample was evenly distributed on nutrient agar (HiMedia, India). The resulting pure isolates were then evaluated further to determine their lipase production capabilities in a subsequent culturing phase [15].

2.2. Lipase Producing

2.2.1. Primary Screening

The lipase-producing potential of the isolates was assessed by growing them on 1% tributyrin and 2% agar in distilled water, autoclaved at 121°C [16,17]. Following the incubation period at 37°C for 24 hours, the extent of lipase production by the bacterial isolates was quantified by estimating the analysis of the clear zone around the colonies. The isolates that showed the highest clear zone around the colonies were chosen and underwent secondary screening.

2.2.2. Secondary Screening

The isolates chosen from primary screening were exposed to secondary screening by constitutive determination of their lipase production by using a submerge culture medium containing 100 ml distilled water melted with 0.5 g peptone and beef extract and autoclaved at 121°C for 30 min; then lower the heat at 60°C for the purpose of adding 10 ml/L of olive oil as a source of tributyrin acids, and then the pH adjustment to 7.0. Every 100 ml of the medium was inoculated with 1×10^{8} of bacterial suspension cells selected from primary screening. Inoculated media were kept in shaker incubators at 37°C for 48 hours with a speed of 150 rpm; the supernatant was prepared by centrifugation at 150 rpm; it was assumed to be crude enzymes [18], and then lipase activity was assayed. The isolate that showed the highest activity was selected to complete the study.

2.2.2.1. Activity Assay

The activity assay of lipase was carried out according to [19]. So, the 1.0 ml of crude enzyme was added to a substrate composed of 10 ml of homogenized olive oil (10%), arabic gum (10%), and 2 ml of calcium chloride (0.6%), and the pH was adjusted to 7.0. After that, the reaction was solution incubated for 30 min at 37°C in a shaker incubator at 150 rpm, then appended 20 ml of the solvent mixture acetone-alcohol by a percentage of 1:1. The mixture solution was calibrated with a sodium hydroxide of concentration 0.1 mol/L with the detector phenolphthalein reagent and compared with the control sample. The blank sample was prepared with the same previous components, except that 1.0 ml of distilled water was added instead of the enzyme extract. Lipase activity was determined according to the following formula:

Activity=(volume of NaOH in ml× 39.997×1000×df)/1 ml (crude enzyme)×reaction time in min

The definition of lipase activity was 1.0 U of enzyme; that amount releases 1 mole of fatty acid/min under the conditions of the experiment.

2.2.2.2. Protein Assays

The concentration of the protein produced enzyme was measured using the Bradford method[20].

2.3. The Isolates Identification

All the isolates obtained at the beginning of this study were identified through morphological, physiological, and biological tests [21]. The *Bacillus* sp. that was found to be a high producer of lipase after secondary screening was further identified to species level based on biochemical characteristics using the Vitek2 compact system BCL card-Bacillaceae [22], and molecular identification via sequencing of 16S rRNA gene with primers designed according to [23]. Subsequently, this strain was registered as a novel entity in the NCBI database under Accession Number OR701819.1.

2.4. Preparation Steps for *lip4* Gene Amplification

2.4.1. Determine The *Bacillus amyloliquefaciens lip4* Gene

To find the sequence of the *lip4* gene in *B. amyloliquefaciens*, data from the NCBI were used. This data included different strains of *Bacillus amyloliquefaciens* (Table 1). The SnapGene 7.0.3 software was employed to compare the sequences of the lipase *lip4* gene (Figure 1). This process included the initial step of designing specific primers [15].

Table 1: Strains of *B. amyloliquefaciens* that were used to find the location of the lipase *lip4* gene

The strains in the NCBI database		Accession number
1	*B. amyloliquefaciens* MBLB 0692	CP115158.1
2	*B. amyloliquefaciens* TPS17	CP085282.1
3	*B. amyloliquefaciens* strain Nsic-8 lipase gene	KF040967.1
4	*B. amyloliquefaciens* strain PS-35 lipase *lip4* gene	KM225297.1

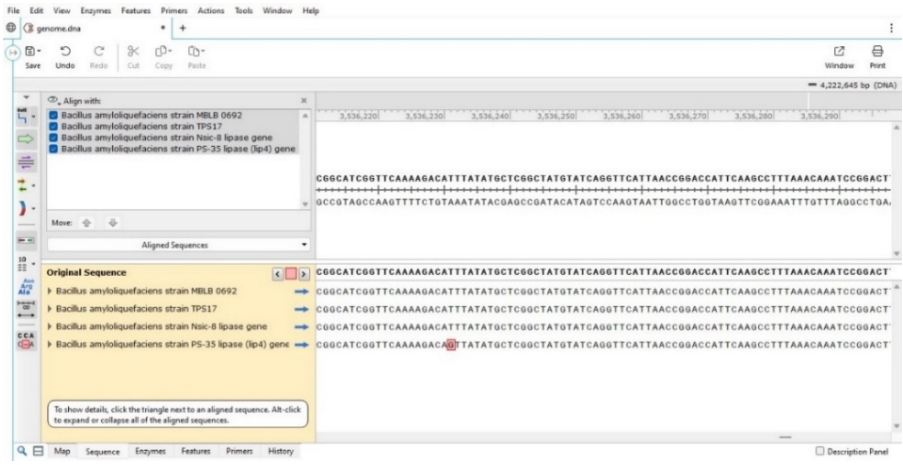

Figure 1: Find and compare the lipase *lip4* gene using Software SnapGene 7.0.3

2.4.2. Design Specific Primers for Lipase *lip4* Gene Amplification

Specific primers were crafted utilizing the Primer-BLAST tool from the NCBI, as shown in Figure 2. These primers, produced by Alpha DNA Company USA, comprised a forward and reverse primer aimed at amplifying a segment with an anticipated length of 600 bp.

forward (5′-ATCAAGAGCAAGATCATCGCCA-3′)

reverse (5′-CTTGATCAGGCCGTTCACCT-3′).

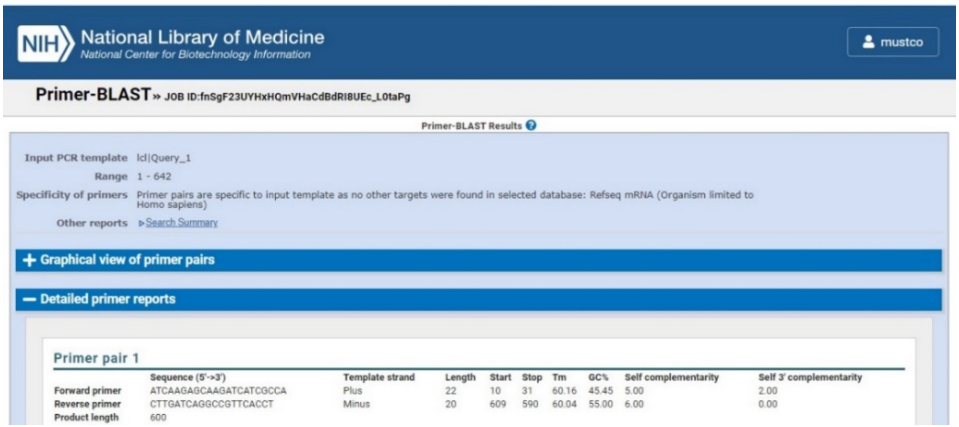

Figure 2: Primer-BLAST tool applying specific Primers designed to amplify the lipase *lip4* gene

2.4.3. Lipase *lip4* Gene Amplification

The PCR reaction mixture, comprising 2 µl PCR premix, 4 µl of DNA template, 2 µl primers, and a total volume of 20 µl nuclease-free water (Promega Co.), was utilized for gene amplification reactions using a PCR system from Bioneer Co. The PCR conditions details are mentioned in Table 2. After that, 2 µl of the PCR product and DNA ladder size 2000 bp (Promega Co.) were run on electrophoresis on a 1% agarose gel at 90 volts for 45 minutes. The amplified gene products were then sent to Korean Macrogen Co. for sequencing. The sequenced gene was registered in the NCBI under the number (OR744906.1).

Table 2: Cycling conditions of lipase *lip4* gene amplification

Steps	cycles	Time	Temperature °C
Denaturation	1	5:00	95
Denaturation	35	00:45	95
Annealing	35	00:45	55
Extension	35	00:45	72
Final extension	1	10	72
Cooling	1	∞	4

2.5. Cloning and Expression *lip4* Gene from *B. amyloliquefaciens* into *E. coli*

2.5.1. Cloning TA end Plasmid into Competent Cell *E. coli* DH5α

The initial step in cloning involved the *lip4* gene extraction from agarose and purification using (gel/PCR DNA extraction kit from Bioneer Co.). The *lip4* gene from the pure extract was joined to a vector pTG19-T plasmid by T4 DNA ligase (Table 3). The resulting pTG19-T-*lip4* was then inserted into an *E. coli* DH5α competent cell (TA Cloning Kit). The *E. coli* were then plated on (Luria Bertani-Agar, HiMedia, India), containing 50 µg/ml ampicillin, 20 mg/ml IPTG, and 80 µg X-Gal, then incubated for 24 hours at 37°C. The white colonies, which represented positive *E. coli* DH5α containing pTG19-T-*lip4,* were extracted, and the recombinant plasmid was extracted using a plasmid extraction kit obtained from (Bioneer Co.). The pTG19-T-*lip4* was *treated by the BamHI* enzyme, followed by purification through 1.0% agarose electrophoresis.

2.5.2. Expression *lip4* Gene into pET-28a(+) Plasmid

The purified DNA fragments of *lip4* were ligated by T4 DNA ligase with the pET-28a(+) expression vector. At the final step, pET-28a(+)-*lip4* was inserted into highly efficient expression *E. coli* BL21 (DE3), which was cultured on L.B. agar containing 50 µg/ml kanamycin antibiotics. After that, incubate the culture in a petri dish at 37°C for 24 hours. Grown colonies were taken with a swab and grown in LB-broth (HiMedia, India), incubated under the same conditions in a shaking at 220 rpm. Until the O.D. reached 0.6–0.8 at 600 nm. The *lip4* gene expression cells

were further incubated with 1.0 mM IPTG and 0.2 mM $CuSO_4$ for four hours at 37°C and then put in an ice bath. The cells were broken and sonicated for 10 min intermittently. After that, they were centrifuged at 12,000xg for 15 min, and the sediment was neglected; the supernatant was considered a crude enzyme [23].

Table 3: The mix of the ligation pTG19-T vector with the *lip4* gene

pTG19-T vector 25 ng/ul	2 µl
lip4 gene	2 µl
T4 DNA ligase 200 u/ul	2 µl
10X Buffer ligase	1 µl
Free nuclease water	Up to 10µl

2.6. Purification of Lipase Produced from Recombinant *E. coli*

All purification procedures were conducted at a temperature of 7°C. The production of enzyme from recombinant cells was achieved by incubation under the conditions mentioned in the secondary primer step (section 2.2.1), then harvested for the extraction of enzyme as stated in the same section. Crude enzyme was treated with ammonium sulfate to get a saturation level of 30–80%. Then further purified by removing impurities using dialysis bags with a molecular weight cutoff of 12–14 kDa against deionized water for 72 hours. The obtained enzyme was submitted to DEAE-cellulose (Whatman Co., England) at a rate of 40 ml/h in 3 ml tubes with 0.05M potassium phosphate buffer (pH 7.0). And then it was eluted by NaCl gradients from 0.1 to 0.5 M. The parts that showed lipase activity were concentrated on a Sephadex G-100 (Pharmacia Co., Sweden) in a column 60 x 1.5 cm at a rate of 45 ml/h in 3 ml tubes. Then the active fractions were concentrated using polyethylene glycol 6000. The volume of enzyme (ml), activity (U/mL), and protein concentration (mg/mL) were estimated [24]. The data were processed using the Microsoft Office, Excel application.

3. Results

3.1. The Novel Strain of *Bacillus sp.* Isolation and Identification

The study examined 25 *Bacillus* sp. isolated from diverse sources to assess their lipase production capabilities. The majority of investigated lipases originate from bacterial sources. *Bacillus* lipases have been extensively studied and, from an industrial standpoint, are more appealing due to their capacity to be obtained in high concentrations and their distinctive properties concerning thermal stability and substrate specificity and demonstrating stable activity at elevated temperatures across a wide pH range [25]. On this basis, these bacteria were chosen in this study. One isolate of 25 was found as the best producer of lipase, exhibiting an activity measured 59.6 U/ml. This isolate was termed MMO. The isolate was initially identified according to morphological and physiological characteristics. It was rod-shaped and gram-positive [21,26]. Other biochemical characteristics, which were achieved by the Vitek 2 compact system, indicated that the isolate belongs to *B. amyloliquefaciens* with a probability of 92%. *A molecular process was used to confirm the identification by amplifying the 16S rRNA gene and sequencing analysis.* The amplified results have shown only one band by electrophoresis (Figure 3), containing 1500 bp. The amplified gene was sequenced by the Korean company Macrogen. The sequence's result was found to have a similarity of 99.79 –100 % in comparison with data present in BLAST-NCBI (Table 4). In many of the studies a resemble results were found [27], for example, mentioned that the base pair of the 16S gene of an isolate of *B. amyloliquefaciens* is equal to 1500 bp. And another research found it is about 1438 bp [28]. The molecular techniques, including PCR and sequencing of the 16S rRNA gene, are powerful tools for specific genetic identification as elucidated by [29]. According to all these results, the isolate was classified as a *B. amyloliquefaciens* and named as MMO in this study, and will be mentioned by the same name in further studies.

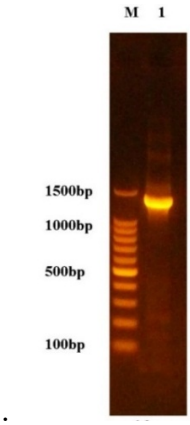

Figure 3: Electrophoresis results of amplified 16S rRNA gene of *Bacillus* sp. isolate, which shows high production of lipase among the others. M: ladder 100-1500bp, 1: 16S rRNA gene

Table 4: The identity percentage of the 16S rRNA gene sequence of *B. amyloliquefaciens* MMO compared with the data database in NCBI

The strains		Identity (%)	Accession
1	*B. amyloliquefaciens* strain RX7	100.00	KU301791.1
2	*B. amyloliquefaciens* strain PM415	99.79	CP130280.1
3	*B. amyloliquefaciens* strain ZJLMBA1908	99.79	OR060690.1
4	*B. amyloliquefaciens* strain CRRU18	99.79	OQ195988.1
5	*B. amyloliquefaciens* strain HBUAS69224	99.79	OP420623.1

3.2. Amplification and Sequence Analysis of the *lip4* Gene

The lipase gene of *B. amyloliquefaciens* MMO strain was subjected to amplification. A single band of approximately 600 base pairs was obtained through electrophoresis as depicted in Figure 4. The result of the analysis of the nitrogenous sequences based on the report received from the Macrogen company has also shown that it contained 600 bp. Comparative analysis of the *lip4* gene by BLAST-BLAST alignment revealed an identity sequence ranging from 97.25% to 100% (Accession number KM225297.1) and the lip4 gene (Accession number MN877411.1).

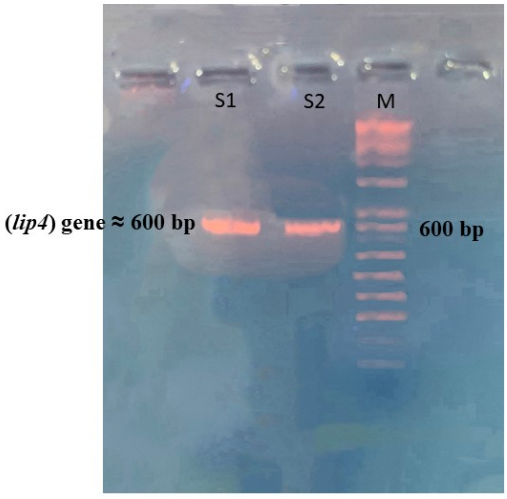

Figure 4: Amplification result of lipase *lip4* gene electrophoresis of *B. amyloliquefaciens* MMO strain. M: ladder 2000bp, S1,S2: lip4 gene

This result was compared after reviewing a number of investigations that studied the sequences of nitrogen bases of the *lip4* gene. A study of [30] revealed that a *lip4* gene from a strain of *B. subtilis* used for cloning in *E. coli* has a size of 639 bp. A similar result was found by [31], who obtained that the lipase gene, composed of 639 bp, translates to a signal peptide of 93 amino acids. They recorded this result in GenBank under accession number JX101459. While [32] identified that the same gene is composed of 650 bp from the *B. amyloliquefaciens* E1PA strain. Additionally, [33] reported that the *lip4* gene from the *B. amyloliquefaciens* G7 strain has a size of approximately 650 bp (Accession number MN877411).

3.3. Cloning and Expression *lip4* Gene from *B. amyloliquefaciens* into *E. coli*

3.3.1. Cloning of TA end Plasmid

The amplified *lip4* gene was successfully integrated into the vector pTG19-T plasmid, and the resulting construct was introduced into competent cells *E. coli* DH5α, which were identified on LB agar **through blue-white screening (Figure 5).** The growth medium contained X-gal, IPTG, and the antibiotic Ampicillin. After incubation at 37°C for 24 hours, the presence of the antibiotic in the medium inhibited the growth of all cells that did not incorporate or capture the vector plasmid. Meanwhile, cells transformed with vectors not hybridized with the *lip4* gene fragment formed blue colonies. This is because they carry the gene responsible for the breakdown of X-Gal, represented by the β-Galactosidase enzyme gene. This enzyme cleaves and degrades X-Gal into two fragments, one of which rapidly converts into a complex, insoluble, intensely blue-colored oxidized dimer, as X-Gal in its natural state is colorless. Therefore, the appearance of blue color is indicative of the colonies' capable to produce the β-Galactosidase enzyme. However, cells transformed with the hybrid plasmid, where the *lip4* gene was inserted at the site of the aforementioned enzyme gene, leading to the disruption of that gene, produced white colonies (Figure 5) due to their inability to degrade X-Gal [34].

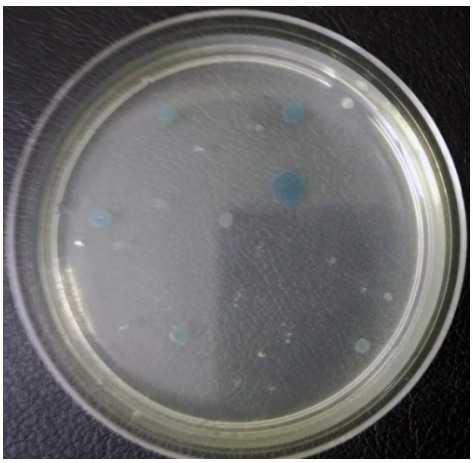

Figure 5: Results of the Blue-White test for competent cells. The white colonies have the recombinant plasmid pTG19-T- *lip4* gene.

Therefore, these colonies were chosen. Subsequently, the (pTG19-T-*lip4* gene) plasmid was extracted from the cells and subjected to *BamHI* restriction endonuclease enzyme digestion. The success of digestion was improved by electrophoresis on the agarose gel, where two bands appeared. One was approximately 2900 bp another 600 bp, which represented pTG19-T and the *lip4* gene, respectively. The size of the band was estimated in comparison with a 1 Kb ladder applied under the same conditions (Figure 6). These results provided conclusive evidence of the success of the process of linking the gene to the vector plasmid and then extracting it with the restriction enzyme to prepare it and insert it into the expression plasmid.

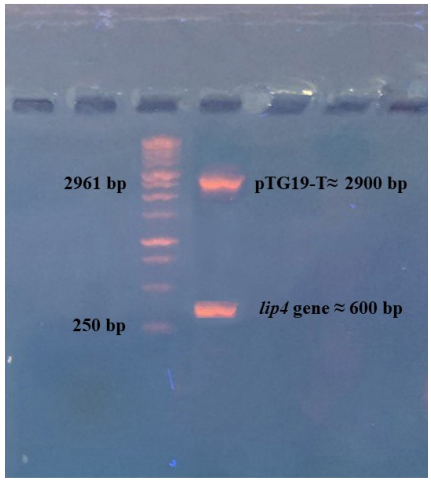

Figure 6: Results of electrophoresis of the *BamHI* restriction enzyme of recombinant plasmid pTG19-T-*lip4* gene

3.3.2. Expression *lip4* Gene

The *lip4* gene was successfully expressed by ligation with pET-28a(+) plasmid and inserted into high-efficiency *E. coli* BL21 (DE3) to express the recombinant lipase via growth in LB agar-kanamycin (Figure 7), providing evidence that the BL21 (DE3) strain has been resistant to the antibiotic kanamycin, and the resulting recombinant plasmid was named plasmid pET-28a(+)-*lip4*. The purpose of this final step of cloning is to produce the lipase from the high-efficiency cells with an unlimited high activity. The IPTG compound in the medium LB-broth acts to stimulate cells to produce the enzyme, as it is a lactose analog that binds to the repressor, releasing the promoter to bind with the polymerase enzyme, initiating the replication process within the mechanism of controlling gene expression [35,36]. The test of activity revealed that the lipase activity reached a level of 199.3 U/ml. Lipase was found to be produced from recombinant bacteria with an enzyme activity higher than was expressed in a wild strain, demonstrating that post-translational modification was achieved.

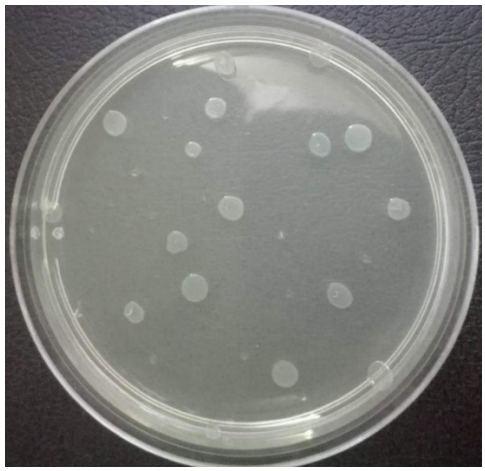

Figure 7: Transformed *E. coli* BL21 (DE3) contain pET28a(+)-*lip4* gene on L.B Kanamycin agar

The cloning of the lipase *lip4* gene has been extensively reported in various studies. The research successfully cloned and sequenced a lipase gene from *B. thermoleovorans* ID-1, encoding a 416 amino acid residue protein, including the conserved pentapeptide Ala-X-Ser-X-Gly, typical of *Bacillus* lipases [37]. In another study, [38] cloned, overexpressed lipase genes from *Geobacillus thermocatenulatus* BTL2 in *E. coli* BL21(DE3). Therefore, the thermophilic lipase was efficiently expressed with an enzyme activity of 39.50 U/mg. This study found that the recombinant lipase had an excellent biocatalytic ability to achieve mild hydrolysis of triglycerides to form LCFAs.

Another research focused on the cloning of a moderate heat-resistant lipase gene from *B. subtilis* FS1403, expressed in *E. coli* BL21, revealing a 212-amino-acid protein with the same conserved motif [30]. The research team reported the cloning of a lipase gene from *B. subtilis* strain I4 in *Escherichia coli* with a deduced amino acid sequence comprising a 31-amino-acid signal sequence and an 181-amino-acid mature part, leading to a molecular mass of approximately 19.33 kDa [31]. Kanmani [39] described the cloning and heterologous expression of a lipase gene from *B. amyloliquefaciens* PS35 in *E. coli* DH5a cells, showing up to 98% homology with other lipase sequences in the NCBI database. Similarly, [32] cloned and expressed an alkaline lipase gene from *Bacillus amyloliquefaciens* E1PA, achieving successful expression and secretion in a heterologous *E. coli* host.

3.4. Recombinant Lipase Purification

Purification of recombinant lipase was done through three steps (Table 5). The chromatogram found in the step of DEAE-cellulose (Figure 8) demonstrated the presence of four distinct protein peaks and a single lipase activity peak inside fraction numbers 51-56, which were subsequently collected and concentrated.

Table 5: Steps of purification of lipase produced from recombinant *E. coli*

Purification steps	Volume (ml)	Enzyme activity (U/ml)	Protein concentration (mg/ml)	Specific activity (U/mg)	Total activity (U)	Purification fold	Recovery (%)
Crude enzyme	50	199.3	0.395	504.6	9965	1	100
Precipitation 30-80%	15	498.7	0.587	849.6	7480	1.7	75.1
Dialysis	18	410.3	0.445	922.0	7385	1.8	74.1
DEAE-cellulose	18	365.5	0.134	2727.6	6579	5.4	66.0
Sephadex G-100	15	344.2	0.061	5642.6	5163	11.2	51.8

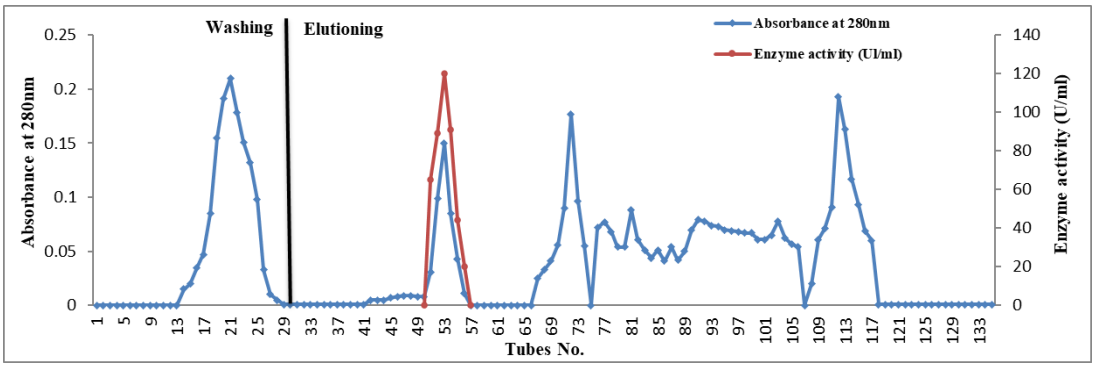

Figure 8: Chromatogram of DEAE-cellulose for the purification of recombinant lipase

The Sephadex G-100 column was used as the last stage of purification (Figure 9). Different peaks were observed, only one peak shows to have lipase activity when activity assy. The presence of enzymes in the recovery phase of DEAE-cellulose indicates that the protein is attached to the negative ion exchanger and that the enzyme carries negative charges under the separation conditions. During the last stage of purification, the protein yield was 11.2-fold, with a recovery rate of 51.8% and an enzyme activity of 344.2 U/ml. Table 5 provides a concise overview of the purification method for the enzyme. These results suggest a higher level of purification for the enzyme [40].

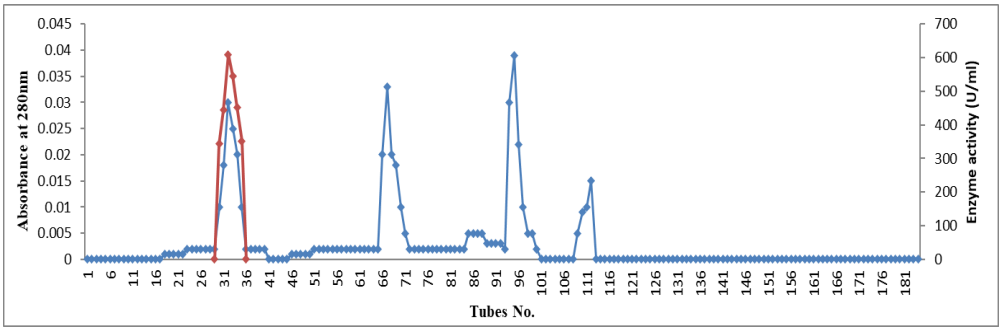

Figure 9: The chromatogram of Sephadex G-100 for the purification of recombinant lipase

The purification of lipase enzymes from recombinant *E. coli* has been successfully achieved in several studies. Brod [41] cloned and expressed the lipase gene from *Staphylococcus xylosus in E. coli* and purified the recombinant enzymes using affinity chromatography in an HPLC system on a Superdex 200 Prep Grade column lipases, and thus concluded the recombinant lipase *from S. xylosus* presented in that work to be useful in production esters of short-chain fatty acids. In another study, Gao [42] was able to clone, overexpress, and purify lipase genes from *Paenibacillus pasadenensis* CS0611 in *E. coli* BL21 (DE3). The recombinant lipase activity was approximately 1631-fold higher than the wild type; his-tagged recombinant lipase was purified rapidly and efficiently by using Ni-charged affinity chromatography with 63.5% recovery and a purification factor of 10.78. The purpose of purification is to isolate specific enzymes from a crude extract of cells containing many other unwanted components to obtain the maximum specific activity with the best possible recovery of the initial activity [43]. There are several procedures that are widely used for enzyme purification; the key to success for the enzyme purification process is the selection of the most appropriate treatment stage. The degree of purification of the enzymes depends on the purpose for which the enzymes will be used. There are several methods for the purification and isolation of enzymes produced on a large scale in industry [44]. The final yield of homogeneous product can be increased by using gel filtration as a final step. In order to attain the main objective of lipase production for industrial use, the techniques of lipase purification should be rapid, high-yielding, and should be inexpensive. The purification steps depend on the quality of the product for a specific requirement from an economic point of view. Just for example, in the pharmaceutical industry, the lipases need a further degree of purification for synthesis reactions [45].

4. Conclusion

In this study, an isolate of *Bacillus* sp. was found to have a high ability to produce lipase and identified as *B. amyloliquefaciens*. The *lip4* gene of this strain was cloned in an expression vector and introduced into *E. coli*, elevating the enzyme activity to 199.3 U/ml, more than the original strain by three times. Also, through this study, there is the possibility of purifying the enzyme in three main stages. Further structural and biochemical characterization is necessary for a full understanding of this enzyme and its reaction mechanism. The values of these characteristics have been identified in another study under publication. The result in this study represented the foundation of a potential lipase-producing strain by cloning. This strain can be used in the production of lipase as a constitutive enzyme with good purity. If full purity can be reached, then the enzyme could be useful in the medical field, such as drugs for the treatment of indigestion and stomach problems, as well as in food technology.

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