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Cloning and Expression of Gene Encoding Lipase from Local Isolate *Bacillus cereus* Isolated from Compost Jambangan Indonesia

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Abstract

Bacillus cereus from Jambangan compost showed lipolytic activity and produce the thermostable lipase. Cloning and expression of lipase gene from the local bacteria was done to increase the enzyme production to support its use in many fields. In this research, the lipase gene was isolated from *Bacillus cereus* by PCR method using a pair of F-Lip and R-Lip primers, then cloned in *E. coli* using the pGemT vector and expressed with pCold II DNA vector. The amplification of lip gene by PCR could produce DNA fragments measuring 0.9 kb. The DNA fragment was then inserted into the pGemT cloning vector and resulted in pGemT-*lip*recombinant at 3.9 kb. The DNA fragment 3.9 kb represented a combination of pGemT size (3.0 kb) with lip gene (0.9 kb). The expression of the lipase gene in the *E. coli* BL21 (DE3) host was carried out with the pCold II DNA expression vector resulting in recombinant DNA at 5.2 kb. The 4.3 kb DNA fragment corresponds to the empty pCod II-DNA plasmid DNA, while the 0.9 kb fragment corresponds to lip gene. The production of recombinant lipase was carried out by cold shock technique at 15 °C when the culture reached OD 600 0.4-0.5 and was followed by induction of 0.1 mM IPTG. The results of SDS-PAGE analysis showed the presence of protein band 30 kDa at SDS PAGE electroforegram. The enzyme showed specific activity of as46.03 U/mg. The results indicated the lip gene encoding lipase from*Bacillus cereus* could be expressed well in the host of *E. coli* BL21 (DE3).

Keyword : Lipase, Bacillus cereus, cloning, gene expression

1. Introduction

Enzymes have been used for decades in various chemical industrial processes (Pliego et al., 2015). In general, enzymes are proteins that catalyze chemical reactions. The lipase enzyme (triacylglycerol acylhydrolase, E.C.3.1.1.3) has a characteristic structure consisting of α/β hydrolase folding which is commonly found in hydrolase enzymes (Kapoor & Gupta, 2012). Lipase has eight parallel folds except in the ß2 region. The β3-β8 folds are connected byα helical bonds arranged on both sides of the fold. The active site of α/β hydrolase has a part called the catalytic triad. Lipase can be used in several reactions such as transesterification, alcoholysis, interesterification, esterification, and aminolysis (Choudhary, 2017). Research on lipase is developed for biodiesel production because it is able to catalyze the hydrolysis reaction of triacylglycerol into glycerol, diacylglycerol, monoglycerol, and free fatty acids (Treich et al., 2010). In biodiesel, the lipase enzyme is used as an enzyme that has high thermostability. The ability of thermostability makes lipase more stable at high temperatures, thermostable enzymes are generally produced from thermophilic microbial strains which are basically resistant to heat (Hama et al., 2018, Purkan et al., 2017).

Thermostable lipase from compost bacteria has been reported to be able to work at temperature of 60-75°C (Maruwka et al., 2009). Shaini and Jayasree, (2016) reported the presence of 24 strains of lipase-producing bacteria from compost. The existence of this bacterial strain can be used for recombinant DNA technology. The use of recombinant DNA technology can be used as effort to provide enzymes quickly, easily and in large quantities and about 90% of industrial enzymes are recombinant products (Adrio & Demain, 2014). Purkan, et al. (2018) have screened lipolytic bacteria producing lipase enzymes from compost and found *Bacillus cereus* bacteria capable to produce thermophilic lipases that are resistant to temperatures of 60-70°C. In an effort to overexpress the enzyme that can support the provision of abundant and superior lipase, cloning and expression of the gene encoding the lipase enzyme from local isolates of *Bacillus cereus* was carried out. Several stages that were carried out in this study include genomic DNA isolation, primer design, PCR, lipase gene cloning, analysis with restriction enzymes, sequencing, gene expression and enzyme activity tests.

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2. Materials and Methods

2.1. Bacteria sample and chemical

Bacillus cereus was obtained from the research results of Purkan et al., 2018. *E. coli* Top10, *E. coli* BL21 (DE3), pGemTand pCold II DNA were obtained from the Biochemistry Laboratory, FST Unair. Ampicillin, T4 DNA ligase, Taq DNA polymerase, *Nde*I, *Xba*I, and IPTG. PCR primers were synthesized at Macrogen, Singapore. The primers used were F-Lip and R-Lip which were designed based on the lipase gene *Bacillus cereus* ATCC 14579 (NC-004722) as a reference taken from Genbank (www.ncbi.nlm.nih.gov/). Other chemicals used are reagents commonly used in chemistry and molecular biology laboratories such as materials forcompetent cell manufacture,electrophoresis, lipase enzyme activity testing and SDS-PAGE.

Table 1 Primers designed based on lipase gene*Bacillus cereus* ATCC 14579(NC-004722)

No	Primer Name	Primer Sequence (5'→ 3')
1	F-Lip	TCGCTCCATATGAAGCGATTTAGC TATTTTATGG
2	R-Lip	TAGGTCTCTAGATTATTTTTCAA CTTTGATCGGATTTG

Liquid LB medium was made from a mixture of 0.5% (w/v) yeast extract, 1% (w/v) NaCl, and 1% (w/v) triptone. Solid LB medium has the same composition as liquid LB medium, but contains 2% (w/v) Bacto agar. Preparation of LB-ampicillin medium was carried out by adding 100 µg/mL into sterile LB medium.

2.2. Cloning of lip geneinto pGemT vector

Thegenomic DNA of*Bacillus cereus* was extracted by using Wizard Genomic DNA Purification kit Promega. The product then was used as template for amplification of lip gene with PCR technique using F-Lip and R-Lip primers. The PCR process was carried out on a DNA-thermal cycler machine for 30 cycles. The conditions for each cycle consisted of a predenaturation step at 94 ºC for 7 minutes and the denaturation of the PCR process at 94 ºC for 1 minute. Annealing of primers in the PCR process was set on 55-65 ºC for 1 minute, extension at 72 ºC for 1.5 minutes, then final extension process at 72 ºC for 5 minutes. The PCR products were checked on agarose gel (1%) by electrophoresis. Purification of PCR product DNA from agarose gel was carried out using a Geneiad PCR/Gel purification kit column. The purified PCR results were ligated using T4 DNA ligase according to the Promega procedure into pGemT plasmids and transformed into *E. coli* Top10 competent cells. The results of the transformation were then checked using the blue-white screening method with solid LB media containing ampicillin/X-Gal/IPTG. Isolation of pGemT-lip recombinant plasmid from positive clones was performed with the QIAprep Spin Miniprep Kit (Qiagen). The recombinant plasmids were cut using restriction enzymes (*Nde*I and *Xba*I) and examined on agarose gel (1%) by electrophoresis. Subsequently, DNA lip sequences were performed in the pGemT-lip recombinant plasmid using the dideoxy-Sanger method with an automatic sequencer (ABI PRISM) in Macrogen, Singapore.

2.3. Sequence Analysis

The nucleotide alignment of the sequencing resultsingsequence of lipase *Bacillus cereus* ATCC 14579NC-004722 as referencethan were performed using the SeqMan programDNA Star Software (Lasergen). The results of in-silico translation of lip gene nucleotides were carried out using the EditSeq Software DNA Star program between the *Bacillus cereus* reference lipase and local strain lipase from Jambangan Surabaya.

2.4. Insertion of lip gen to pCold II DNAvector

DNA lipase fragments were cut from the pGemT-lip recombinant plasmid using restriction enzymes (*Nde*I and *Xba*I) and examined on agarose gel (1%) by electrophoresis. Then it was purified using the Geneiad PCR/Gel purification kit column. The DNA fragments were then ligated using T4 DNA ligase according to the Promega procedure into pCold II DNA plasmids and transformed into competent *E. coli* BL21 (DE3) cells. Isolation of the pCold-lip recombinant plasmid from a positive clone of *E. coli* BL21 (DE3)-lip was carried out with the QIAprep Spin Miniprep Kit (Qiagen). The recombinant plasmids were then cut using restriction enzymes (*Nde*I and *Xba*I) and examined on agarose gel (1%) by electrophoresis.

2.5. Lipase expression

E. coli BL21 (DE30 [pCold II-lip] was grown in 25 mL of liquid LB medium containing 100 µg/mL ampicillin, then incubated with 150 rpm shaking at 37ºC for 5-6 hours, to obtain OD_{600} 0,4-0,5. The culture was then cooled by means of incubation at 15 ºC for 30 minutes without shaking. To express protein lipase, culture was induced with the addition of 0,1 mM IPTG, then incubated again at 15 ºC with 150 rpm shaking for 24 hours. Subsequently, the culture was centrifuged at 5000 rpm for 10 minutes at 4ºC to obtain cell pellets, the lysed by untrasonicator waves. The isolated protein was then analyzed by SDS-PAGE to determine its molecular weight focusing the lipase protein.

2.6. Lipase activity assay

The lipase activity test was carried out in several stages, including making standard curves of p-NP with concentrations of 20, 40, 60, and 80 µM and measuring the absorbance value using a UV-Vis spectrophotometer at a maximum wavelength (λmax) of 410 nm.

Determination of lipase activity was performed by using p-nitrophenylpalmitate (p-NPP) as a substrate. Substrate solution was prepared by adding 1800 µL of substrate mixture and 100 µL of enzyme solution and 100 µL of Tris-HCl buffer. The solution was incubated at 45 °C for 15 min. Then 250 µL Na2CO3 (0,1 M) was added to the solution. The absorbance was determined after 15 minutes at λ= 410 nm using a spectrophotometer.

One unit of activity (U) was expressed as the amount of enzyme required to produce 1µM of the p-nitrophenol (p-NP) product released every minute under assay conditions (Ertugul,et al., 2007). The unit of enzyme activity is calculated using the

$$\text{Unit activity (U/mL)} = \frac{[\text{p-NP}]\ (\mu\text{M}) \times 1000 \times \text{Fp}}{\text{Time}}$$

The p-NP, time, the number of 1000 and Fp in the formula shows respectively to the p-nitrophenol, time for

enzyme reaction, unit conversion from mM to uM, and the dilution factor of enzyme.

Determination of protein levels in this research was detected by Bradford method with a standard solution using Bovine Serum Albumin (BSA). Detection of absorbance was carried out at 595 nm.

3. Results and Discussion

3.1. Insertion of the PCR-Resulted Lipase gene into the pGemT Vector

The purified DNA lip from the PCR was inserted into the pGemT plasmid. The splicing of the two DNA fragments can take place properly through the insertion mechanism as shown in Fig 1.

Transformation of *E. coli* Top10 bacteria with the product of ligation process resulted 2 white colonies and 7 blue colonies were obtained. The white colonies obtained were thought to carry recombian plasmid, so that they were further characterized. While the blue colonies are transformant cells that have no recombinant DNA. The results of the transformation can be seen in Fig 2.

The data in Fige 2.C as a negative control in LB-ampicillin media showed that no colonies grew, so it can be ascertained that the media was positive for ampicillin which was active in killing wild type microbes. In Figure 2.D, the *E. coli* Top10 competent cells grow well on LB media without ampicillin, this reflects that the cells performance for transformation process is good. Inserted DNA as a positive control in LB-ampicillin media obtained many colonies of *E. coli* Top 10 (Figure 2.B) with blue and white color, this indicates that *E. coli* Top10 has inserted with empty pGemT plasmid or pGemT-DNA insert positive control. Therefore, these bacteria can grow on media containing ampicillin. Fig 2A showed the presence of blue colonies and white colonies; this indicates that there are colonies containing empty pGemT and recombinant pGemT.

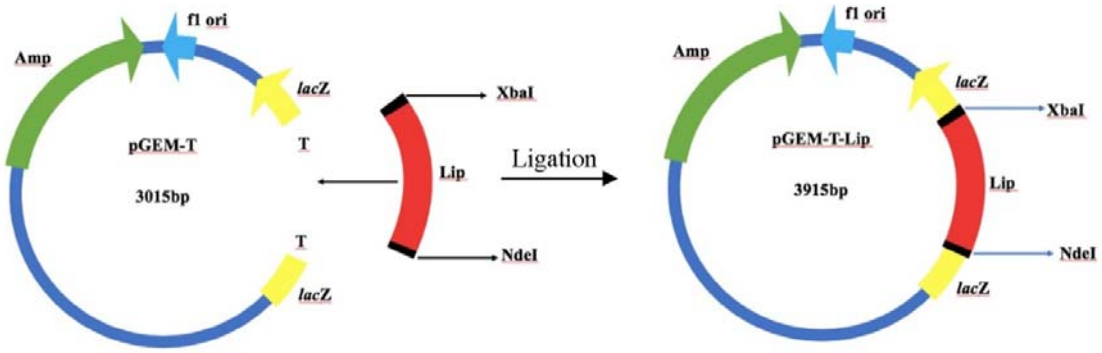

Figure 1. The process of ligation of the lipase gene into pGemTvector

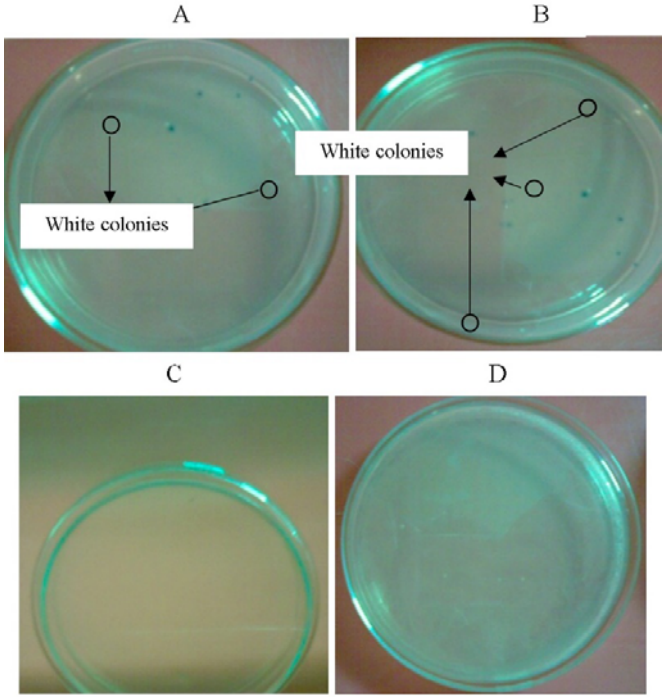

Figure 2. Plating results of *E. coli* cells of Top 10 transformants on LB amphicilin media. A) transformed with pGemT-lip, produced blue and white colonies; B) the result of the transformation with positive control insertion DNA, resulted in blue and white colonies; C) competent cells in LB media with ampicillin; D) competent cells in media LB without ampicillin.

The isolated recombinant plasmids were detected by agorase gel electrophoresis, and the results were shown in Fig 3. The isolated recombinant plasmids were characterized by cutting with restriction enzymes *Nde*I and *Xba*I, and the results can be seen in Fig 4. Cutting of pGemT plasmids with restriction enzyme EcoRv resulted in one band at 3 kb (Fig 4 lane 2). This restriction enzyme has one truncation site on the pGemT plasmid map. The size of the 3 kb band that was cut with the EcoRv enzyme was in accordance with the size of the pGemT plasmid DNA. Cutting of the recombinant pGemT-lip plasmid with *Nde*I and *Xba*I enzymes each produced one DNA band of 3.9 kb in size (Lane 4 and 5). The pGemT vector has *Nde*I and *Xba*I enzyme cleavage sites. The appearance of this 3.9 kb band represents a combination of the size of pGemT (3.0 kb) with DNA lip (0.9 kb). With these results, it can be stated that the DNA lip has been inserted properly in the pGemT vector. Further characterization of DNA lip was carried out by sequencing technique.

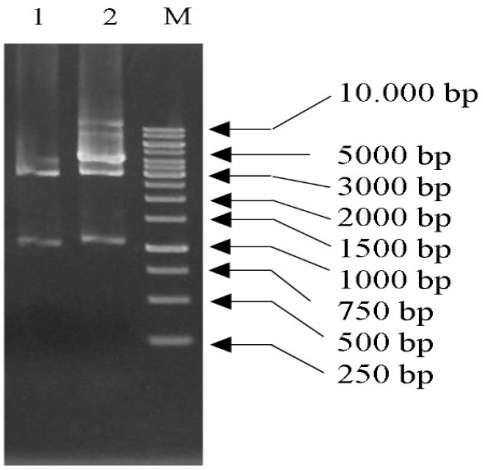

Figure 3. Electrophoregram of isolated recombinant plasmids from two white colonies. Lane 1, the results of the isolation plasmid from colony one, and lane 2, from colony two.

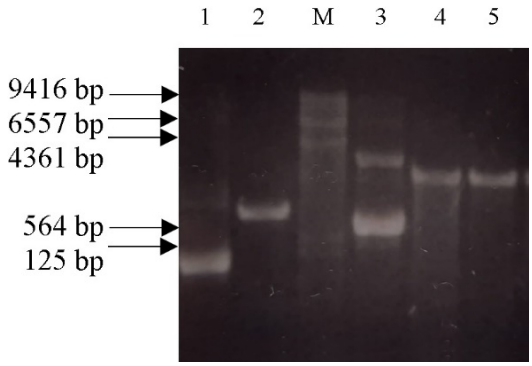

Figure 4. Electrophoregram of pGemT-lip recombinant DNA cutting with several restriction enzymes. Lane1, pGemT uncut; lane 2, pGemT/EcoRv (3 kb size); M, marker /*Hind*IIII; lane 3, pGemT-lip uncut; lane 4, pGemT-lip/*Nde*I (3.9 kb size); lane 5, pGemT-lip/*Xba*I (3.9 kb size)

3.2. Ligation lipasegene into pCold II-DNA expression vector

The lipase gene contained in the pGemT-lip recombinant plasmid was subcloned in the pCold II-DNA expression vector (Fig 5). Lip DNA was removed from the recombinant pGemT by cutting with two restriction enzymes *Nde*I and *Xba*I than it purified by PCR/Gel Purification kit Geneaid and ligated into the pCold II DNA expression vector. Prior to insertion, the pCold II DNA vector was cut with restriction enzymes *Nde*I and *Xba*I.

In the transformation of pCold-lip ligation mixture into competent cells of *E. coli* BL21(DE3) bacteria, 5 white bacterial colonies were obtained (Figure 6.A). Meanwhile, pCold II DNA emptyplasmid was transformed*into E. coli* BL21(DE3) which was cut with *Nde*I and *Xba*I enzymes did not produce cell colonies (Fig 6.E). This indicates that the restriction of the pCold II DNA plasmid by the two enzymes has been carried out perfectly. From the comparison of two data (Fig 6.A and E), it can be assumed that the cell colonies in Figure 6.A are *E. coli* carrying the recombinant pCold II-lip plasmid. Further characterization of this recombinant plasmid was carried out by plasmid isolation and cutting with restriction enzymes. The negative control on LB-ampicillin media (Fig 6.D) had no colonies grew, it could be ascertained that the media containing ampicillin kills the wild type bacteria. In Fig 6.C, the *E. coli* BL21 (DE3) grew well in the medium without ampicillin, this represent that the condition of competent cells for transformation step was health. The positive control in LB-ampicillin media was overgrown with recombinant *E. coli* BL21(DE3); the bacteria cells showed resistant to the antibiotic. Each white colony shown in Figure 6.A was then grown in LB-ampicillin media for plating duplication and then plasmid isolation and cutting with restriction enzymes were performed.

Digestion of the pCold II-lip recombinant plasmid with restriction enzymes was performed to characterize the recombinant plasmid. In addition, it was also used to confirm the successful process of ligation between DNA lip with the pCold II-DNA vector. Cutting the recombinant pCold II-lip plasmid (5.2 kb) using *Nde*I and *Xba*I enzymes could produce two DNA bands measuring 4.3 kb and 0.9 kb, respectively (Fig 7.C). The 4.3 kb DNA fragment corresponded to the pCod II-DNA (Fig. 7.A), while the 0.9 kb DNA fragment corresponded to the lip size (Fig. 7.B). The appearance of 2 bands from this double-cut result indicates that the insertion of the lip gene into the pCold II DNA vector was successful.

3.3. Sequencingof lip gene

Determination of lip gene nucleotideswere carried out by using the dideoxy-Sanger method with F-Lip and R-Lip primers. The entire ORF sequence of the lipase gene was sequenced. The results of the analysis of nucleotide alignment results from sequencing with reference lipases carried out with the SeqMan program on DNA Star Software (Lasergen) showed that the sample lipase gene had an identity (percent match) of 87-89% against the reference lipase *Bacillus cereus* ATCC 14579 (NC-004722). The electrophoregram of the nucleotide alignment results from this sequence is shown in Fig 8. The in silico translation of lipase gene nucleotides resulted in 295 amino acid residues. Alignment results between amino acid residues of protein lipase samples with reference are listed in Table 2. The analysis of the alignment results showed that there were 13 amino acid variants between the reference *Bacillus cereus* lipase and local strain lipase from Jambangan Surabaya. The 3D structure image of the recombinant lipase protein shown in

Fig 9 was predicated using the Swiss Model online software.The structural model constructed by using 2qtx.pdb template from *Bacillus subtilis* lipase that revealed an identity score of 36.1%. The model exhibited the Ramachandran favoured as 94.94% with structural α-helix and ß-sheet appeared dominantly (Fig 9).

Table 2. CLUTAL 2.1 multiple alignment of lipase amino acid residues

LipF	MKRFSYFMVGCLTLTLLVGCSAAEKPVEQVKQVKNTLTAKLATEEKMIEIDGQTIYFKKI
LipaseR	MKRFSYFMVGCLTLTLLVGCSAAEKPVEQVKQVTNTLTAKLATEEKMIEIDGQTIYFKKI
Reference	MKRFSYFMVGCLTLTLLVGCSAAEKPVEQVKQVKNTITAKLATEEKMVEIDGQTIYFKKI
LipF	GNEKPPLLMIHGFGGSSDGFRKIYSDLAKDHTIISVDALGFGRSSKPMDFYYSFPTHANL
LipaseR	GNEKPPLLMIHGFGGSSDGFRKIYSDLAKDHTIISVDALGFGRSSKPMDFYYSFPTHANL
Reference	GNEKPPLLMIHGFGGSSDGFQKIYSDLAKDHTIISVDALGFGRSSKPMDFYYSFPTHANL
LipF	YYKLMKKLGYDSFAILGHSMGGEISLNLTYLYPEAVTHLILTDATGGAHTLVNKQGSPKP
LipaseR	YYKLMKKLGYDSFAILGHSMGGEISLNLTYLYPEAVTHLILTDATGGAHTLVNKQGSPKP
Reference	YYKLMKKLGYDSFAILGHSMGGEISLNLTYLYPEAVTHLILTDATGGPHTFVTKQGSPKP
LipF	QLSTDLHTVSAIADYDESKVKFKRNDEEHYNKMKLWPRRLQINANEIQQPTLIIWGRNDS
LipaseR	QLSTDLHTVSAIADYDESKVKFKRNDEEHYNKMKLWPRRLQINANEIQQPTLIIWGRNDS
Reference	QLSTDLNAVSSITDYDESKVKFKRNDEEHYNKMKLWPRRLKINANEIKQPTLIIWGRNDS
LipF	SVSWKEGETYHQFLKNSTFHIIEKGYHAPFRQEPQEFVGYVKDFFKNNPIKVEK
LipaseR	SVSWKEGETYHQFLKNSTFHIIEKGYHAPFRQEPQEFVGYVKDFFKNNPIKVEK
Reference	SVSWKEGETYHQFLKNSTLHIIEKGYHAPFRQEPQEFVGYVKDFFKNNPIKVEK

Table 3. Amino acid residues variance between reference lipase (Genbank) and sample

Residual number	Amino acids		
	Lipase GenBank	Lipase F	Lipase R
37	I	L	L
48	V	I	I
81	Q	R	R
168	P	A	A
171	F	L	L
173	T	N	N
187	N	H	H
188	A	T	T
191	S	A	A
193	T	A	A
221	K	Q	Q
228	K	Q	Q
260	L	F	F

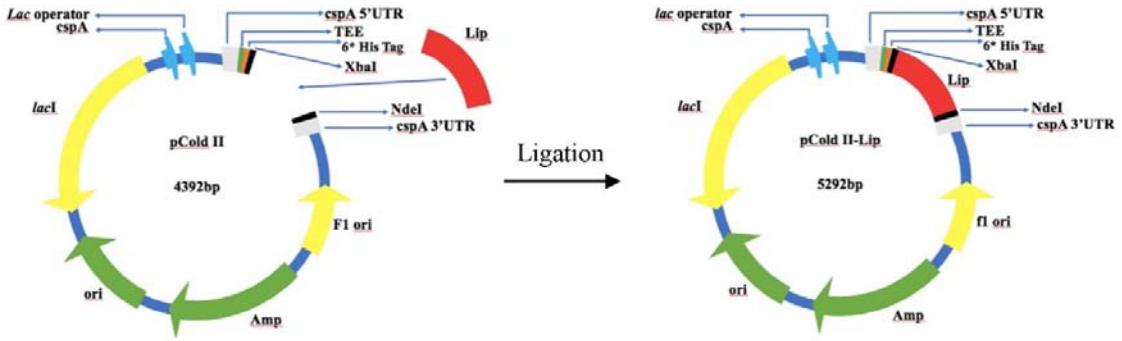

Figure 5. The process of ligation of the lipase gene to the pCold II-DNA vector.

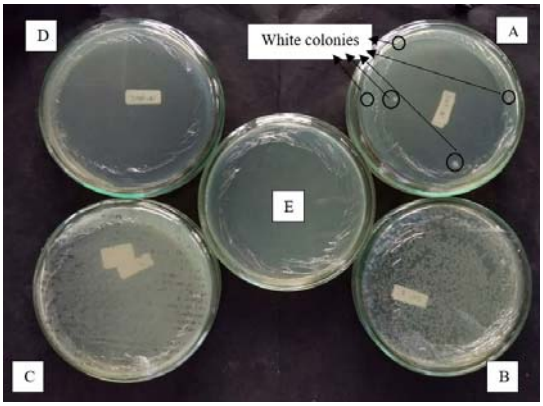

Figure 6. Product of transforman cells when *E. coli* BL21 (DE3) transformed with pCold II-lip. A. transformants with mixed pCold-lip ligation on LB-Ampicillin media (some colonies); B. transformants with pCold in LB-Ampicillin media (many colonies); C. Competent cells in LB media without ampicillin (many calonies); D. Competent cells in LB-ampicillin media (no colonies); E. transformants with pCold II/*Nde*I/*Xba*Iplasmid (no clonies)

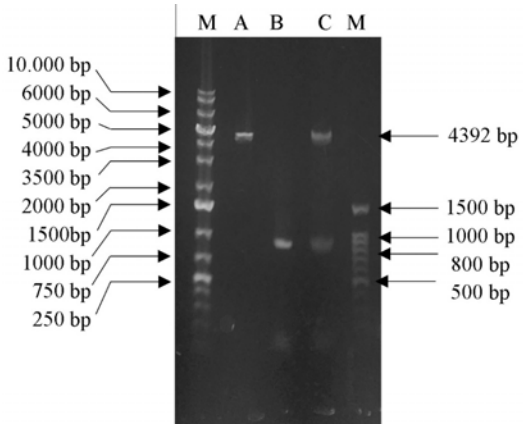

Figure 7. Electrophorogram of pCold-lip recombinant DNA cutting with restriction enzymes. M Lane, Marker; Lane A, cut pCold II with *Nde*I and *Xba*I enzymes (4392 bp); column B, insert, DNA lip (0.9 kb); lane C, cut pCold-lip with *Nde*I and *Xba*I enzymes, resulted in 2 bands measuring 4.392 kb and 0.9 kb

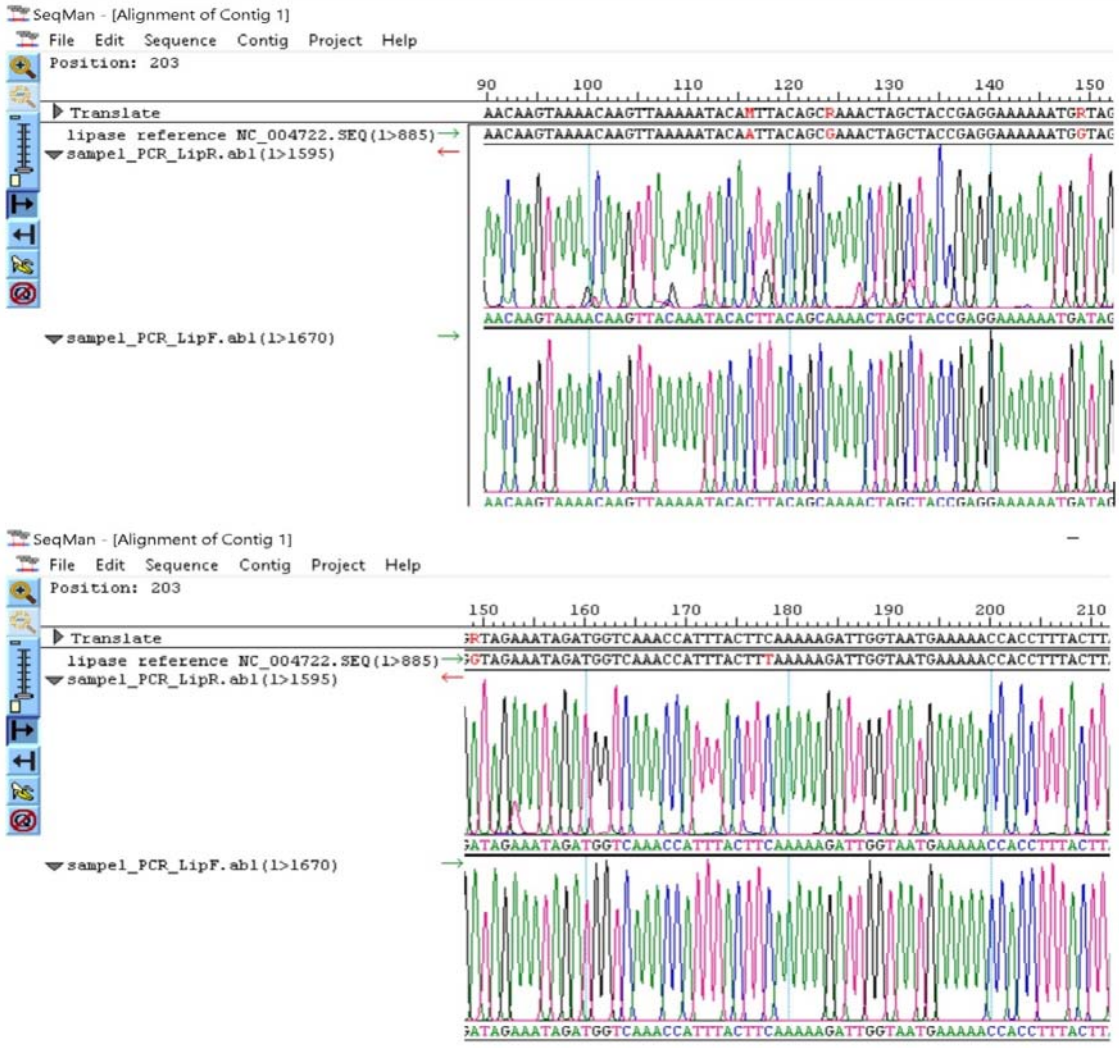

Figure 8. The results of this alignment of DNA lip nucleotides against the reference lipase from GenBank.

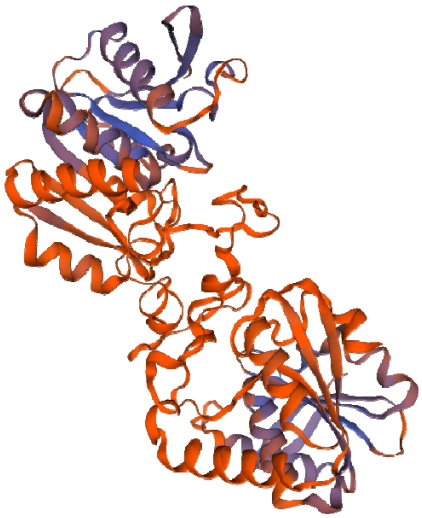

Figure 9. The model structure drawing of recombinant lipase of *Baccillus cereus* local strain of Jambangan origin

3.4. Lipase Gene Expression

Gene expression is an important step in protein production. *E. coli* cell is widely used as host for gene expression because they are easy to treat and the cost of culture is relatively cheap. Gene expression in *E. coli* host cells often results in insoluble proteins. This constraint can be minimized by using a low-temperature expression system.

Plasmid pCold II DNA is a cold shock expression vector; therefore, the expression of the lipase gene coding region was carried out at low temperatures. In the pCold II DNA vector, the expression of the inserted gene was controlled by the promoter derived from the cold shock gene (cspA) derived from *E. coli*. This can be advantageous in the process of lipase expression because the promoter can be well recognized by the host cell. The presence of a lac operator in the pCold II DNA vector that is inserted downstream of the cspA promoter allows the insertion gene to be expressed precisely. Another consideration designed with the expression system in the pCold II DNA vector is obtaining the lipase protein in a soluble state.

Expression of the lipase gene in the pCold II DNA vector was done to look at whether the protein lipase can be produced by *E. coli* BL21 (DE3)[pCold II-lip]. Extracts obtained from *E. coli* BL21 (DE3)[pCold II-lip] was analyzed by SDS-PAGE. The results of SDS-PAGE protein extract showed that *E. coli* carrying pCold II-lip and induced with IPTG, produced protein bands with a molecular mass of 30 kDa (Fig. 10, lane 1 and 2). The band was not appearfrom extract of *E. coli* that had nopCold II-lip (Fig 10,lane 3). The result showed that the lipase protein was expressed well.

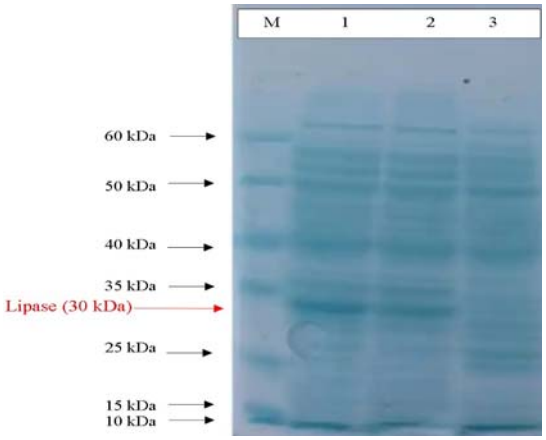

Figure 10. Results of SDS-PAGE protein expression results. M lane, marker; lane 1, sonicated protein; lane 2, protein extracted with SDS; lane 3, protein BL21 (DE3) without plasmid as a negative control

3.5. Lipase activity

Lipase activity test was carried out using p-nitrophenyl palmitate (pNPP) as a substrate. Lipase activity was measured based on changes in absorbance at a wavelength of 410 nm which indicated the release of p-nitrophenol compounds (pNP) from the substrate. Based on the results of the enzym activity test, it was obtained that was 0.083 U/mL with a total activity of 0.83 U.The specific activity of the lipase produced was 46.03 U/mg.The lipase activity of non recombinant E. *coli* BL21(*DE3*) in spectrophotometry was almost not detected; the absorbance was very low, so far from Beer-Lambert Law.

A number of studies have also reported the activity of recombinant lipases, including *Pyrococcus furiosus* which has an activity of 8.9 U/mg with a molecular weight of 48.8 kDa (Alqueres at al., 2011) and recombinant lipase from isolate S4-01 which has relatives with *Bacillus amyloliquefacies*has an activity of 3.91 U/mg with a molecular weight of 53.2 kDa (Sembiring at al., 2015).

The production of recombinant proteins is growing every year, leading to the emergence of new host cells, vectors, and cloning and expression techniques that are more diverse and of higher quality. Various recombinant lipase enzymes have been produced and studied by a number of researchers. Lipase is an enzyme that has a molecular weight in the range of 19–60 kDa, belonging to the α/β hydrolase family. This enzyme has an active site formed by the catalytic triad Ser, Asp/Glu and His. Protein Lipases have a unique sequence consensus, namely GXSXG in their primary structure, where X is an amino acid residue (Balan A et al., 2012). Lipase properties from various sources have been reported in several publications. The expression of the lipase gene from *Bacillus subtilis* strain IFFI10210 using plasmid vector pBSR2 that had a strong lipase promoter in the host cell *Bacillus subtilis* A.S.1.1655 was able to produce lipase with 100 times higher activity than the original source. The obtained lipase has a molecular weight of 24 kDa (Yapin L et al., 2010, Jisheng Ma et al., 2006). The lipase production has also been reported to increase after the yeast of *Sporobolomyces salmonicolor*was developed by mutagenesis and molecular expression. The research could provide the mutant OVS8 yeast with capacity on lipase production 3.2 time greater than its wild typestrain (Thabit

et al, 2012).*Geobacillus thermodenitrificans* IBRL-nra has also been reported to produce thermostable lipases capable of being active at 65ºC. The resulting lipase has a BM of 30 kDa (Balan A et al., 2012).

In this study, the lipase gene expression of *Bacillus cereus* was carried out in *E. coli* BL21(DE3) cells. The results of the study obtained protein lipase with a molecular weight of 30 kDa and had good enzyme activity. The recombinant lipase showed a high lipolytic activity, therefore in the future it is necessary to develop its production on a large scale to support its further application for many purposes.

4. Conclusion

The conclusions of this research were that the construction results of pGemT-lip and pCold-lip recombinant plasmids respectively could produce 3.9 kb and 5.2 kb recombinant DNA. The 3.9 kb recombinant DNA band represents the combination of pGemT (3 kb) with DNA lip (0.9 kb). Meanwhile, 5.2 kb recombinant DNA showed a combination of pCold II DNA vector (4.3 kb) with DNA lip (0.9 kb).The lipase gene had an identity percent (percent match) of 87-89% against the lipase reference *Bacillus cereus* ATCC 14579 (NC-004722). Alignment analysis showed that there were 13 amino acid variants between the *Bacillus cereus* reference lipase and local strain lipase from Jambangan Surabaya.The result of lip gene expression in *E. coli* BL21 (DE3) host cells was able to produce 30 kDa protein on SDS PAGE electrophoregram. The protein showed a specificlipase activity of 46.03 U/mg protein.Referring to the high activity, the scale up production on the recombinant enzyme is important, developed to support its further application for many purposes.

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