JJBS

Volume 15, Number 2,June 2022
ISSN 1995-6673
Pages 199 – 203
https://doi.org/10.54319/jjbs/150206

Jordan Journal of Biological Sciences

Expression of Recombinant Lipase from *Serratia marcescens* LII61 in *Escherichia coli*

Sri Sumarsih[1,*], Fatimah[2, 3], Sofijan Hadi[1], Ni'matuzahroh[2, 3], Almando Geraldi[2, 3], Rizka Diah Fitri[2], Gilva Illavi[2]

[1] *Department of Chemistry, Universitas Airlangga, Surabaya, Indonesia;*[2] *Department of Biology, Universitas Airlangga, Surabaya, Indonesia;*[3]*University Center of Excellence Research Center for Bio-molecule Engineering, Universitas Airlangga, Surabaya, Indonesia*

Received: January 27, 2021; Revised: May 8, 2021; Accepted: June 15, 2021

Abstract

Recombinant lipase encoding gene of *Serratia marcescens* LII61 has been successfully expressed in *Escherichia coli*. The gene was amplified from genomic DNA of *S. marcescens* LII61 by PCR method using lipase specific primers. The amplified DNA was then subsequently inserted in pGEM®-T easy vector and transformed into *E. coli* JM109. The inserted DNA fragment in the plasmid was analyzed for studying the lipase gene sequence. The recombinant gene was subcloned in pET-28b(+) vector/ *E. coli* BL21(DE3) system. The positive clones were selected by growing *E. coli* cells in antibiotic medium and lipase-specific medium. The analysis of recombinant DNA showed that the lipase gene of *S. marcescens* LII61 was 1845 bp in size. The inserted gene in the pGEM-T easy vector was composed of 1842 bp lipase gene and flanked by several restriction enzyme as stated on the map vector. The recombinant *E. coli* BL21 (DE3) showed a fluorescent orange color on LB-IPTG-rhodamine B-olive oil agar plate, indicated that the recombinant bacteria were able to express the lipase gene from *S. marcescens* LII61. This report is the first endeavor on cloning and expression of lipase from Indonesian isolate of *S. marcescens*.

Keywords: Lipase, Recombinant Protein, *Serratia marcescens*, *Escherichia coli*,

1. Introduction

Micobial lipases have received special attention from biotechnology industries and been widely used in synthesis of organic compounds because the enzymes are selective, substrate-specific, stable to organic solvents, and due to regio/stereo-selectivity (Sharma et al., 2001; Hasan et al., 2006; Thakur, 2012; Andualema and Gessesse, 2012; Borrelli and Trono, 2015). Microbial lipase has been widely studied by researchers, both in terms of structure, characteristic, production, increasing productivity, development, and exploration of producing microorganisms as a new source for lipase production. Several bacteria and fungi isolated from various sources were potential lipase producers; for example, *Micrococcus luteus* L69 Microbial lipase has been widely studied by researchers, both in terms of structure, characteristic, production, increasing productivity, development, and exploration of producing microorganisms as a new source for lipase production. Several bacteria and fungi isolated from various sources were potential lipase producers; for example, *Micrococcus luteus* L69 isolated from POME-contaminated soil (Sumarsih *et al*., 2020), *Mycobacterium* sp. isolated from pulp and paper mill effluent (Tripathi *et al*., 2014), *Sporobolomyces salmonicolor* OVS8 from oil mill spillage (Thabet *et al*., 2012). Several genera of bacteria and fungi have been screened as sources of lipase in large-scale production, including *Bacillus, Achromobacter, Alcaligenes, Arthrobacter, Pseudomonomas, Penicillium, Fusarium, Aspergillus* (Chandra *et al*., 2020). Microbial enzymes are easy to produce in large quantities, easily manipulated genetically, have high activity in various environmental conditions and are very useful in a variety of industrial applications (Andualema and Gessesse, 2012). However, native microbial enzymes have many disadvantages, including non-reproducibility, low yield for certain bio-catalytic processes and requiring optimization of conditions. This disadvantage can be overcome by using molecular technology for recombinant proteins production, which allows the catalytic improvement, protein over-expression and production (Borrelli and Trono, 2015).

The Gram-negative enteric bacterium *Serratia marcescens* represent as one of best lipase producers, in particular Family I.3 lipase (Borrelli and Trono, 2015, Lee *et al*., 2007). Several strains *S. marcescens* had been explored for lipase production (Mohanasrinivasan *et al*., 2018; Nwachukwu *et al*., 2017). The extracellular lipases are widely used as biocatalyst in enantioselective hydrolysis and synthesis of many chiral drug precursors. *S. marcescens* ES-2 showed high enantioselectivity for (S)-flurbiprofen (Lee *et al*., 2007). Lipase from *S. marcescens* ECU1010 used in asymmetric synthesis of trans-3-(4-methoxyphenyl) glycidic acid methyl ester] (Shibatani *et al*., 2000; Zhao *et al*., 2010).

* Corresponding author e-mail: sri-sumarsih@fst.unair.ac.id.

Serratia marcescens strain LII61 was isolated from slaughterhouse waste in North Surabaya Indonesia, was a good lipase and biosurfactant producer (Ni'matuzahroh et al., 2017; Renjana et al., 2017). The bacterium was a potent bacterial for oil sludge cleaning agent, showed oil sludge dissolving activity by 86.38 ± 2.39% (Ni'matuzahroh *et al*., 2017). The bacteria isolate LII61 showed unique character, had variable gram characters, indicated by the change in color of the cell wall resulting from gram staining at the observed age of the culture (Fatimah *et al*., 2019). The isolate was similar (99%) to *S. marcescens* based on its 16S ribosomal RNA gene sequence (Renjana *et al*., 2017; Fatimah *et al*., 2019).

Serrattia marcescens is human opportunistic pathogen (Haddix and Shanks, 2017; Takayama and Kato, 2020). Therefore, a recombinant production in realtively safer baterial strain is needed for future mass production and industrial application, especially in foods, pharmaceutical, and cosmetic industries. In this first effort on constructing recombinant *E. coli* strain expressing lipase from Indonesia *S. marcescens* isolate, lipase-encoding gene was amplified from *S. marcescens* LII61 genome, then inserted in plasmid pGEM®-T easy and expressed in pET-28b(+)/ *Escherichia coli* system.

2. Materials and Methods

2.1. Bacteria, Plasmid and Media

The bacteria S. marcescens LII61 (GenBank accession number of 16S ribosomal RNA gene: MK702080.1) was used as a source of lipase gene, was grown on nutrient agar medium. The bacteria used as host cells in the cloning process, *E. coli* JM109 and *E. coli* BL21 (DE3), were grown in Luria Bertani (LB) medium (1% NaCl, 1% tripton and 0.5% yeast extract). The plasmids used as vectors for construction of recombinant DNAs were pGEM®-T Easy (Promega) and pET-28b(+) (Novagen). Medium used for selection of recombinant *E. coli* strain was LB agar supplemented with 50 mg/mL X-gal (5-bromo-4-chloro-3-indolyl-beta-D-galacto-pyranoside), 100mM IPTG (isopropyl-β-D-thiogalacto-pyranoside) and suitable antibiotics. The medium used in the lipase expression test was LB agar supplemented 100mM IPTG and 50 µg/mL kanamycin, olive oil and rhodamin-B.

2.2. Lipase gene amplification

Lipase gene was amplified from genomic DNA of *S. marcescens* LII61 using forward primer (lip-F) 5'-GGCCAGGCGGCATAATTC-3' and reverse primer (lip-R) 5'–GGCCAACACCACCTGATCG-3' (Lee *et al*., 2007). The components in the PCRmix consisted of Go Taq® Green Master Mix (Promega), primers, genomic DNA, Nuclease-Free Water. The thermal cycling process was initiated by pre-denaturation for 2 minutes at 95 °C. The PCR process was carried out in 35 cycles with the conditions of each cycle: 95 °C-denaturation for 30 seconds, 49.4 °C- annealing for 45 seconds, 72 °C-extension for 1 minute. The PCR product was then purified using GenElute™ Gel Extraction Kit (Sigma Aldrich) and sent to 1st Base Laboratories, Malaysia for sequencing.

2.3. Cloning and nucleotide sequence analysis of lipase gene

The purified PCR product was inserted into pGEM®-T easy vector, then transformed into *E. coli* JM109 host cells (Promega). The mixture containing recombinant DNA was spread on solid LB media supplemented with Ampicillin (100 µg/mL), IPTG (100 mM) and X-gal (20 µg/mL followed by incubation at 37 ^{0}C for overnight. The presence of recombinant pGEM®-T plasmid (pGEM-lip) in the white colonies were verified using colony PCR method (Woodman, 2008).

PGEM-lip were isolated from the positive clones using using The Wizard® Plus SV Minipreps DNA Purification System (Promega) and sent to 1st Base Laboratories, Malaysia for sequencing. The analysis of DNA sequences was performed using BioEdit (version 7.2.5) software. The translated amino acid sequence of the lipase gene of *S. marcescens* LII61 was then aligned with other sequences of *S. marcescens* strains lipase using BLASTp (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.4. Subcloning of recombinant lipase

The recombinant plasmid pGEM-lip was digested out using restriction enzyme *Eco*RI. The digested-lipase gene was ligated using T4 DNA ligase into *Eco*R1 digested-pET28b (+) plasmid, then transformed into *E. coli* BL21 (DE3). The transformant then was inoculated onto LB agar containing kanamycin (50 µg/ml) and incubated at 37ºC for 18 hours. The bacterial clones were picked and inoculated onto lipolytic specific medium, LB agar containing kanamycin (50 µg/ml), 100 mM IPTG, olive oil, and rhodamine B. Recombinant *E. coli* BL21 (DE3) that produced and excreted recombinant lipase showed a fluorescent orange color under UV light. This condition proved recombinant *E. coli* BL21 harboring recombinant plasmid with *S. marcescens* LII61 lipase (pET28b-lip.)

3. Results

3.1. Construction of recombinant gene

Lipase gene of *S. marcescens* LII61 was successfully amplified. Single band of approximately 1800 bp was observed in the electrophoregram shown in Figure 1.

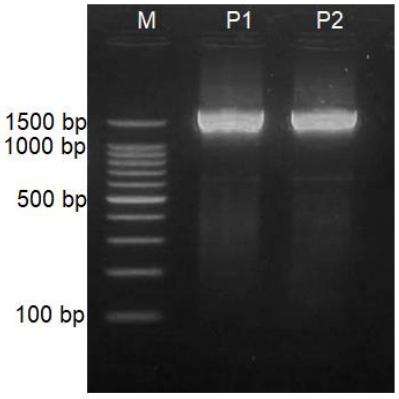

Figure 1. Agarose electropherogram of lipase gene from *S. marcescens* LII61 (P1 and P2).M: DNA ladder 100 bp

The analysis of PCR product, showed a coding sequence of 1845 bp has been amplified from *S. marcescens* LII61 genome. The sequence of the PCR product showed highly similarities to sequence of *S. marcescens* strain ECU1010 extracellular lipase lipA gene (99.73%) (GenBank Acc. No. DQ884880.1) and lipase lipB gene (99.46%) (GenBank Acc. No. HM440338.1).

The PCR product was successfully inserted into the pGEM®-T easy/ *E. coli* JM109 system, which was shown by the presence of white colonies on LB medium containing ampicillin, IPTG and X-gal after incubation for 20 hours. The presence of target gene in the recombinant plasmids was confirmed by colony PCR using the lipase-specific primers (Lee *et al*., 2007). The presence of PCR product of approximately 1800 bp indicated that the picked recombinant *E. coli* JM109 colonies carried the target lipase gene.

The sequencing of recombinant plasmid was performed using universal primers T7 and SP6, as well as a pair of lipase-specific primers, lip-F and lip-R, for the determining the entire nucleotide inserted in the recombinant pGEM®-T easy. Nucleotide sequence analysis revealed the cloned insert in the pGEM®-T easy plasmid was 1874 bp containing lipase gene starting at 32th nucleotide. The sequence of inserted gene was identical to the sequence gene encoding lipase of *S. marcescens* LII61. According to the pGEM-T easy vector map, the cloned insert is flanked by several restriction enzyme sequences, as shown in the schematic in Figure 2.

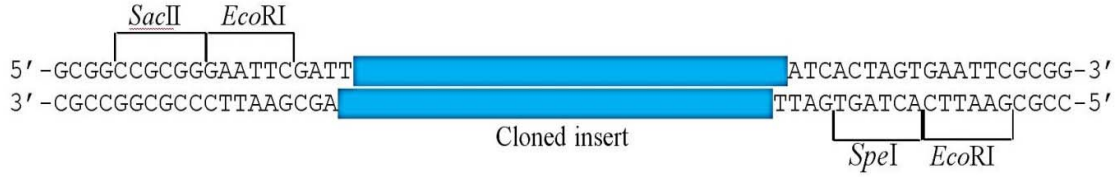

Figure 2. Scheme of cloned insert in pGEM®-T easy vector

Based on sequence analysis, the lipase gene inserted in the pGEM(R)-T plasmid consisted of 1842 bp nucleotides which encode a protein composed of 614 amino acids which was predicted to have a molecular weight of 64.8 KDa. Protein analysis using Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) revealed that the recombinant lipase have high similarity to extracellular lipase of *S. marcescens* GenBank Accession no. ABI83633.1 (99.67%) and ADI77082.1 (99.02%). Based on amino acids sequence, the relationships of recombinant lipase to the other deposited lipase in GenBank shown in Figure 3. The phylogenetic tree was constructed using MEGA X program.

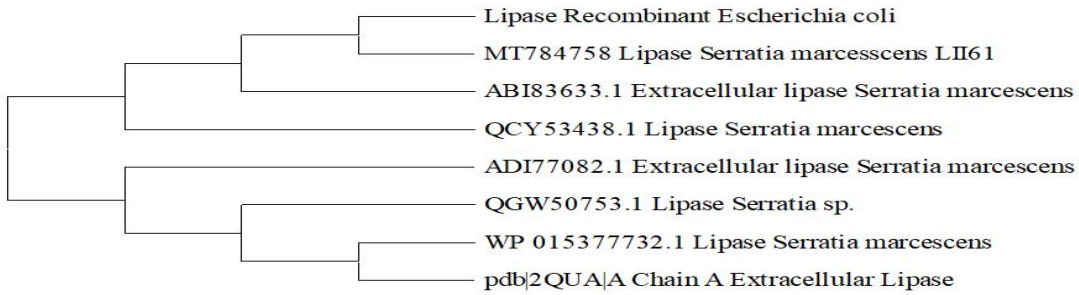

Figure 3. Relationships of recombinant lipase to the other deposited lipase in GenBank

3.2. Expression of lipase in E. coli BL21

The lipase gene constructed into *Eco*RI site in pET28b(+) vector. The constructed recombinant plasmid pET28b-lip is shown in Figure 4.

The recombinant plasmid pET28b-lip was transformed in competent cells of *E. coli* BL21(DE3). The positive cloned bacteria were collected from solid antibiotic-LB media. Lipase expression was detected by cultivating the recombinant *E. coli* cells in the lipolytic specific medium containing IPTG as an inducer. The presence of IPTG in the LB medium induced recombinant bacteria to express the lipase gene. The positive clone, harboring pET-28b-lip capable expressed the lipase gene, gives an orange fluorescent at bacterial culture.

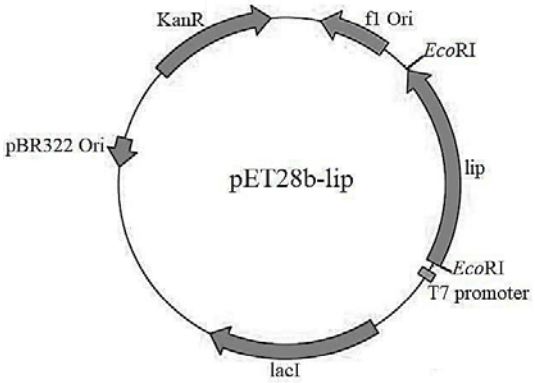

Figure 4. The map of recombinant plasmid pET28b-lip

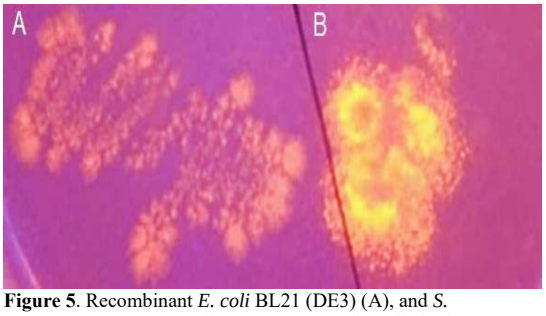

Figure 5. Recombinant *E. coli* BL21 (DE3) (A), and *S. marcescens* LII61 (B) cells grown on LB-olive oil-rhodamine B agar plate

Figure 5. showed recombinant *E. coli* BL21 (DE3) and *S. marcescens* LII61 cells appeared grown well on lipase-specific medium. The production of extracellular lipase is indicated by the presence of a fluorescent orange color around the colony that is visible under UV light. The cationic form of indicator dyes reacts with free fatty acids to form complex orange fluorescent compounds which excite at 350 nm (Lanka and Latha, 2015). The recombinant bacteria colonies showed a fluorescent orange color under UV light indicated that the lipase gene from *S. marcescens* LII61 produced extracellularly. Meanwhile, *S. marcescens* LII61 cells as the source of the lipase gene showed mixed color (yellow and orange), because bacteria the produce Prodigiosin which gives bacteria a red color, which appears yellow under UV light as shown at Figure 5.

4. Discussion

Here, the first endeavors to clone and express recombinant lipase from Indonesian isolate of *S. marcescens* (strain LII61) were presented. The lipase-encoding gene from *S. marcescens* LII61 was successfully constructed in pET-28(+) vector and transformed into *E. coli* BL21(DE3) for further expression and mass production studies. The amino acid sequence of the recombinant lipase from *S. marcescens* strain LII61 showed high similarity to extracellular lipase of *S. marcescens* ECU1010, which was utilized to produce anti-inflammatory drug (S)-ketoprofen (Long *et al*., 2007).

Furthermore, the lipase expression test using LB-IPTG-rhodamine B-olive oil agar plate indicated that the recombinant lipase produced extracellularly in *E. coli* BL21(DE3). This result is interesting due to the fact that recombinant proteins are usually expressed intracellularly in *E. coli* (Fakruddin *et al*., 2013; Ma *et al*., 2020). Moreover, in various studies, recombinant *S. marcesces* lipase in *E. coli* were reported to be expressed intracellularly (Su *et al*., 2014; Mohammadi *et al*., 2016; Chen *et al*., 2017; Yin *et al*., 2020)

As previously reported, *S. marcescens* LII61 lipase exhibited high activity (Ni'matuzahroh *et al*., 2017; Renjana *et al*., 2017). However, the industrial application of the lipase is hampered by the pathogenicity of *S. marcescens*. Recombinant production *S. marcescens* LII61 lipase in E. coli BL21(DE3) and other Generally Recognized as Safe (GRAS) microorganisms is critical for the development of the lipase as industrial enzyme. In the future study, the mass production, purification, kinetics study, and substrate conversion using recombinant *S. marcescens* LII61 lipase need to be conducted.

5. Conclusion

Lipase encoding gene from *S. marcescens* LII61, which was isolated from slaughterhouse waste in Indonesia, was successfully cloned and constructed into pET-28(a) plasmid. The extracellular expression of the lipase gene was observed based on assay on LB-IPTG-rhodamine B-olive oil agar plate.

Acknowledgment

The authors would like to thank Direktorat Riset dan Pengabdian kepada Masyarakat, Deputi Penguatan Riset dan Pengembangan, Kementerian Riset dan Teknologi / Badan Riset dan Inovasi Nasional Indonesia for funding this research.

References

Andualema B and Gessesse A. 2012. Microbial lipases and their industrial applications: review. *Biotechnology*., **11(3)**:100-118.

Borrelli GM and Trono D. 2015. Recombinant lipases and phospholipases and their use as biocatalysts for industrial applications. *Int. J. Mol. Sci*., **16**:20774-20840. doi:10.3390/ijms160920774.

Chandra P, Singh R, and Arora PK. 2020. Microbial lipases and their industrial applications: a comprehensive review. *Microb Cell Fact.*, **19**:169. doi:10.1186/s12934-020-01428-8.

Chen KC, Zheng MM, Pan J, Li CX, and Xu JH. 2017. Protein engineering and homologous expression of *Serratia marcescens* lipase for efficient synthesis of a pharmaceutically relevant chiral epoxyester. *Appl Biochem Biotechnol*., **183(2)**:543-54. doi:10.1007/s12010-017-2543-z.

Fakruddin M, Mohammad Mazumdar R, Bin Mannan KS, Chowdhury A, and Hossain M. 2013. Critical factors affecting the success of cloning, expression, and mass production of enzymes by recombinant *E. coli*. *Int Sch Res Notices*., **2013**:1-7. doi: https://doi.org/10.5402/2013/590587

Fatimah, Fitri RD, Illavi G, Renjana E, Pratiwi IA, Ni'matuzahroh, and Sumarsih S. 2019. Cell wall response of bacteria *Serratia marcescens* LII61-lipase and protease producer-in gram staining. *Ecol Environ Conserv.*, **25**(*Suppl. Issue*):S76-S80.

Haddix PL and Shanks RMQ. 2018. Prodigiosin pigment of *Serratia marcescens* is associated with increased biomass production. *Arch Microbiol*., **200**:989–999. doi:10.1007/s00203-018-1508-0.

Hasan F, Shah AA, and Hameed A. 2006. Industrial applications of microbial lipases. *Enzyme Microb Technol*., **39**:235–251. doi:10.1016/j.enzmictec.2005.10.016

Lanka S and Latha NL. 2015. A short review on various screening methods to isolate potential lipase producers: lipase the present and future enzymes of biotech industry. *Int J Biol Chem.,* **9(5)**:207-219. doi:10.3923/ijbc.2015.207.219.

Lee KW, Bae HA, and Lee YH. 2007. Molecular coning and functional expression of *esf* gene encoding enantioselective lipase from *Serratia marcescens* ES-2 for kinetic resolution of optically active (S)-flurbiprofen. *J Microbiol Biotechnol*., **17(1)**:74–80.

Long ZD, Xu JH, Zhao LL, Pan J, Yang S, and Hua L. 2007. Overexpression of *Serratia marcescens* lipase in *Escherichia coli* for efficient bioresolution of racemic ketoprofen. *J Mol Catal B Enzym*.,**47(3-4)**:105-10. doi:10.1016/j.molcatb.2007.04.004.

Ma Y, Lee CJ, and Park JS. 2020. Strategies for optimizing the production of proteins and peptides with multiple disulfide bonds. *Antibiotics*., **9(9)**:541. doi:10.3390/antibiotics9090541.

Mohammadi M, Sepehrizadeh Z, Ebrahim-Habibi A, Shahverdi AR, Faramarzi MA, and Setayesh N. 2016. Enhancing activity and thermostability of lipase A from *Serratia marcescens* by site-directed mutagenesis. *Enzyme Microb Technol*., **93**:18-28. doi: 10.1016/j.enzmictec.2016.07.006.

Mohanasrinivasan V, Devi CS, Jayasmita D, Subathra C, Selvarajan E, and Naine SJ. 2018. Purification and characterization of extracellular lipase from *Serratia marcescens* VITSD2. *Proc Natl Acad Sci India B.*, **88**:373–381.

Ni'matuzahroh, Pratiwi IA, Surtiningsih T, Fatimah, and Sumarsih S. 2017. The potency of *Micrococcus* sp. LII61 bacteria as oil sludge cleaning agent. *J Biol Res.*, **22(2)**:38-42.

Nwachukwu E, Ejike EN, Ejike BU, Onyeanula EO, Chikezie-Abba RO, Okorocha NA, and Onukaogu. 2017. Characterization and optimization of lipase production from soil microorganism (*Serratia marcescens*). *Int J Curr Microbio. App Sci.*, **6(12)**:1215-1231.

Renjana E, Helga L, Fatimah, Sumarsih S, and Ni'matuzahroh. 2017. Co-Utilization of molasse and glycerol as carbon sources on the production of biosurfactant by isolate bacteria LII61. *J Appl Environ Biol Sci.*, **7(11)**:174-180.

Sharma R, Christi Y, and Banerjee UC. 2001. Production, purification, characterization, and applications of lipases. *Biotechnol. Adv.*, **19(8)**:627-662. doi:10.1016/S0734-9750(01)00086-6.

Shibatani T, Omori K, Akatsuka H, Kawai E, and Matsumae H. 2000. Enzymatic resolution of diltiazem intermediate by *Serratia marcescens* lipase: molecular mechanism of lipase secretion and its industrial application. *J. Mol. Catal. B Enzym.*, **10(1)**:141-149. doi: 10.1016/S1381-1177(00)00122-3.

Su E, Xu J, and You P. 2014. Functional expression of *Serratia marcescens* H30 lipase in *Escherichia coli* for efficient kinetic resolution of racemic alcohols in organic solvents. *J Mol Catal B Enzym.*, **106**:11-6. doi:10.1016/j.molcatb.2014.04.012.

Sumarsih S, Fatimah, Hadi S, Adhiningsih RT, and Prasetyo FE. 2020.Characterization and lipase production of *Micrococcus* sp. L69 isolated from palm oil-contaminated. *Asian J. Water, Environ. Pollut.*, **17(3)**:77-80. doi:10.3233/AJW200040.

Takayama Y and Kato N. 2020. Inhibition of quorum sensing in opportunistic pathogen, Serratia marcescens, using cyclodextrin-immobilized, multiple parallel gel filaments fabricated with dynamic flow of polymer blend solution. *Mater Sci Eng C.* **107**:110331. doi:10.1016/j.msec.2019.110331.

Thabet HM , Pasha C, Ahmed MM, and Linga VR. 2012. Isolation of novel lipase producing *Sporobolomyces salmonicolor* OVS8 from oil mill spillage and enhancement of lipase production. *Jordan J Biol Sci.*, **5(4):**301-306.

Thakur S. 2012. Lipase, its sources, properties and applications: a review. *Int J Sci Eng Res*., **3(7)**:1-21.

Tripathi R, Singh J, Bharti RK, and Thakur IS. 2014. Isolation, purification and characterization of lipase from *Microbacterium* sp. and its application in biodiesel production. *Energy Procedia*., **54**:518 – 529. doi:10.1016/j.egypro.2014.07.293.

Woodman ME. 2008. Direct PCR of intact bacteria (colony PCR). *Curr Protoc Microbiol*., **9**: A.3D.1-A.3D.6. doi: 10.1002/9780471729259.mca03ds9.

Yin YC, Li HQ, and Wu XS. 2020. Refolding with simultaneous purification of recombinant *Serratia marcescens* lpase by one-step ultrasonication process. *Appl Biochem Biotechnol.*, **21**:1-4. doi:10.1007/s12010-019-03172-1.

Zhao LL, Pan J, and Xu JH. 2010. Efficient production of diltiazem chiral intermediate using immobilized lipase from *Serratia marcescens*. *Biotechnol Bioprocess Eng*., **15**:199-207. doi:10.1007/s12257-009-0173-1.