Transformation of pTrcHis and pUC8.2-14 into *Escherichia coli* BL21 (DE3)

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Abstract— Recombinant microorganism is a genetically modified microorganism which specifically designed for the expression of target protein production or bioreaction process. As different expression host may contribute to superior results, the transformation of a plasmid carrying the gene of interest into the same strain of competent cells would assist in research assessment. This study focused on the transformation of recombinant lipase from two different sources into *Escherichia coli* competent cells, BL21 (DE3). The recombinant cells, pUC8.2-14 (American Type Culture Collection (ATCC)® 68046™) was harbouring *Rhizopus (delemar) oryzae* lipase and *E. coli* Top 10 pTrcHis was harbouring *Staphylococcus hyicus* lipase, respectively. The transformation is involved preparation of competent cells, polymerase chain reaction (PCR) and DNA sequencing. The DNA sequences obtained was inserted into Basic Local Alignment Search Tool (BLAST), under the databases of National Centre for Biotechnology Information (NCBI), USA. The findings show the respective plasmids were successfully carried by the *E. coli* BL21 (DE3) for further investigation.

Keywords— transformation, Escherichia coli, recombinant, lipase, plasmid

1. INTRODUCTION

In pharmacies and marketplaces, recombinant proteins are increasingly in demand due to the growth of population and lifestyle of the human being. Insulin and chymosin (also known as rennin) for an example are very common products, but not many know that some of these are from recombinant products. Many advantages of recombinant over natural produce were discussed in previous studies, [1, 2]. Due to high demands, low cost and need of high purity products, many recombinants studies and developments were investigated in molecular and bioprocesses facilities. In this research, the potential of recombinant lipase will be examined for laboratory scale production. Because of having different sources of a recombinant plasmid, the objective of this preliminary study is to transform the extracted plasmids of various sources into the same strain of self-prepared competent cells.

2. LITERATURE REVIEW

A. Lipase

Lipases or specifically, triacylglycerol acyl hydrolase (EC 3.1.1.3) can be found in all living organisms. Lipases act at the oil-water edge to transform water insoluble glycerides into smaller compounds which can be readily transported across the corresponding cell wall. Due to the high potential of lipases in the industry [3, 4], the demands of lipases are increasingly shown by many productions from wild-type [5, 6] or genetically modified organisms [7-9]. In a comparison of wild-type and recombinant lipase production, the wild-type was found capable of consuming a broader range of oil-based substrate compared to the recombinant type [10]. It was presumed to the natural behaviour of wild-type in adaptation to its resources. While for the recombinant, it was producing higher activity but only for the desired protein translated in the recombinant gene. Hence, any production of lipase should consider the aim of its respective condition. Besides production, the enzyme was also used in the microalgae cultivation to convert fatty acids into biodiesel [11]. By having microorganism to aid in the conversion, fewer chemicals will be involved in the biodiesel production and consequently supports green processes.

B. Escherichia coli BL21 (DE3) as Expression Host

Many expression hosts from *Escherichia coli* derivatives were used in harbouring recombinant protein. *E. coli* was known as well studied and fast growing bacterium among other species and potentially harvested up to 100 g of dry cell weight per L. One of common use as an expression host is *E. coli* BL21 (DE3). Many studies have been done in detail about BL21 (DE3) [12], the ancestors and relatives [13], and proven previous research in the production of protein expressions [14-16]. Rosano and Ceccarelli, 2014 [17] also suggested two strains of *E. coli*, BL21 (DE3) and some derivatives of K-12 lineage in the first screening. BL21 (DE3) was recorded to be missing of two genes which are contributing to the better performance of the protein expression, Lon protease [18] and OmpT [19]. By the result of thorough justification and previous studies, thus BL21 (DE3) was chosen in this study to be the expression host for the recombinant plasmids of lipase production.

3. MATERIALS AND METHODS

The recombinant bacterium, pUC8.2-14 (ATCC®68046™) was purchased from ATCC, USA harbouring *Rhizopus (delemar)oryzae* lipase and *E. coli* Top 10 pTrcHis harbouring *Staphylococcus hyicus* lipase was received from Universiti Putra Malaysia, respectively. All chemicals were of high purity and prepared accordingly to the respective concentrations.

A. Plasmids Extraction and Quantification

One millilitre of an overnight culture of *E. coli* Top 10 pTrcHis and *E. coli* ATCC®68046™ pUC8.2-14 suspension were added into 1.5 mL microcentrifuge tubes. The microcentrifuge tubes were centrifuged at 10, 000 rpm for 1 min. The supernatant was discarded and only the pellets were used in the following procedure using *EasyPure*® Plasmid MiniPrep Kit, TransGen Biotech, China. Two microliters of extracted plasmid were read against deionized water by NanoDrop spectrophotometer, Thermo Scientific. The nucleic acids concentration was measured at 260 nm while the purity was calculated using absorbance of 260/280 nm and 260/230 nm ratio. The isolated plasmids, pTrcHis and pUC8.2-14 were respectively stored at -20°C for future use.

B. Transformation of Plasmids

Competent cells were prepared by calcium chloride ($CaCl_2$) treatment of *E. coli* BL21 (DE3) [20]. A volume of 100 µL of the competent cells (CC) was transferred into 2 mL cryovial and kept at -80°C for the transformation process. Prior transformation, CC was thawed on ice and immediately 2.5 µL of extracted plasmid was added into each 100 µL of CC. The mixtures were incubated for 20 min on ice. After incubation, a heat-shock treatment was performed by incubating the tubes at 42°C for 90 seconds. After heat-shock, the tubes were incubated on ice for one min. A volume of 1 mL Luria Bertani Broth was added into the tube. The tube was incubated at 37°C, 200 rpm for about 1 h. After incubation, the tubes were centrifuged at 10, 000 rpm for one min and after centrifugation, 800 µL supernatant was discarded. A volume of 100 µL of the transformant was spread onto Luria Bertani Agar plates containing ampicillin, 100 µg/mL and 50 µg/mL for transformant carrying pTrcHis and pUC8.2-14, respectively. The plates were incubated for 16 h at 37°C. The grown transformants were subsequently followed the plasmid extraction and quantification as described in section A.

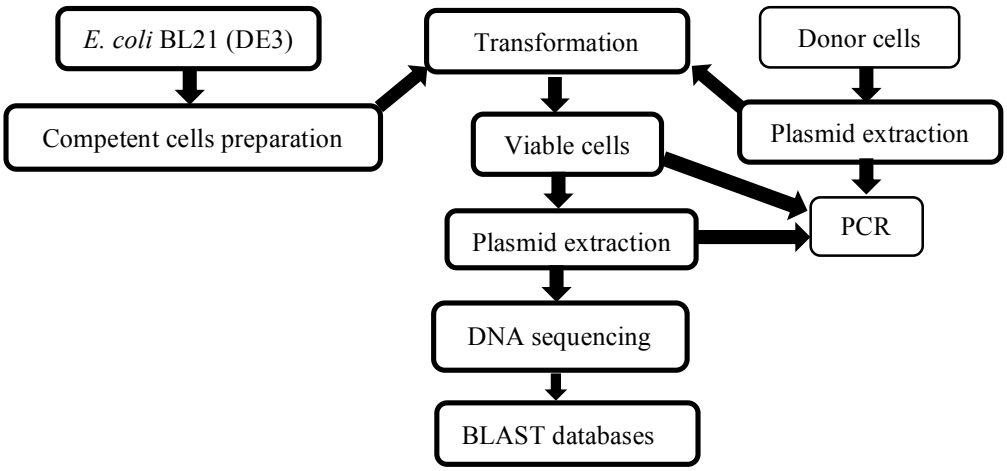

Figure 1: Flow chart of study.

C. Polymerase Chain Reaction

PCR was done respectively from the respective colony culture and extracted plasmids as shown in Fig. 1. A single colony of transformant BL21 pTrcHis was subjected to the colony PCR with primer forward (5' GTA AAA GCA GCA CCT GAA GCC 3') and primer reverse (5' TTA TGC GTT CTT TGT GCT TTC TGC 3') of *Staphylococcus hyicus* lipase. A single colony of transformant of BL21 pUC8.2-14 was using M13 forward pUC 40 (5' GTT TTC CCA GTC ACG AC 3') and M13 reverse pUC 26 (5' CAG GAA ACA GCT ATG AC 3'). The denaturation, annealing and extension reaction were repeated for 35 cycles. After the PCR had been completed, the PCR products were loaded into 1% (w/v) of 1x Tris-acetate-EDTA agarose gel. DNA marker, one kb was used as a standard marker. After the electrophoresis had been completed, gel imaging was captured by gel documentation machine.

D. DNA Sequencing

Both extracted DNAs from the respective transformants were sent to Bioneer Sequencing Service, Seoul, South Korea to obtain the sequence of plasmids, respectively. The Bioneer Service used DNA Analyser model ABI 3730XL in the process. The DNA sequences for forward and reverse of respective pTrcHis and pUC8.2-14 were inserted into BLAST on the website of NCBI, USA. By the database, the respective nucleotides were compared and probabilities of identification were generated by the search tool. For a better notion, Fig. 1 shows the flow of study from sources of plasmid donor up to the BLAST application.

4. RESULTS AND DISCUSSION

A. Concentration *and Plasmids Purity*

The extracted plasmids from *E. coli* Top 10 pTrcHis, *E. coli* ATCC®68046™ pUC8.2-14 and the respective transformants, *E. coli* BL21 (DE3) pTrcHis, and pUC8.2-14 were quantified to evaluate the plasmid concentration in the respective cultures. High concentration and ideal purity of plasmids were required to perform DNA sequencing. From three replicates of cultures measured, the concentration and purity of pTrcHis and pUC8.2-14 from different hosts were obtained as shown in Table 1.

From Table 1, the concentrations from donor plasmids were lower and less purity namely the *E. coli* ATCC®68046™ pUC8.2-14. Hence, for the transformants, high concentration and a better ratio of purity were gained by a collective of four tubes of extracted plasmids and repurification by GeneAll® Expin™ Combo GP, GeneAll, Korea. Later, both plasmids from the transformants showed high concentration and ideal ratio of purity as suggested by Held, 2001 [21]. The absorbances used from the spectrometry were 260nm for nucleic acids, 230nm for phenolic compounds and polysaccharides while 280 nm for protein contaminants. The ideal purity $A_{260/280}$ of any extracted DNA plasmid is the ratio between 1.8 to 2.0, and higher ratio indicating of RNA contamination. Ideal purity $A_{260/230}$ should be more than 2.0, representing higher DNA products over protein contaminants. Based on the high concentration and ideal purity of the respective ratios, both plasmids were accepted for observation on gel electrophoresis and DNA sequencing.

Table 1: Concentration and purity of pTrcHis and pUC8.2-14 from different hosts.

Host	***E. coli* Top 10**	***E. coli* ATCC® 68046™**	***E. coli* BL21 (DE3)**	
Plasmid	pTrcHis	pUC8.2-14	pTrcHis	pUC8.2-14
Concentration at 260 nm (ng/µL)	67.13	64.5	131.5	192.5
Ratio $A_{260/280}$	1.98	1.67	1.865	1.851
Ratio $A_{260/230}$	2.25	0.97	2.156	2.305

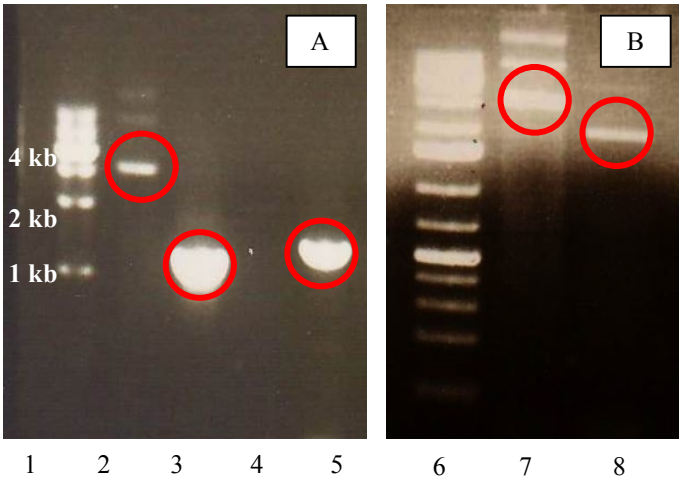

Figure 2: Plasmids and PCR products of respective pTrcHis and pUC8.2-14.
Lane 1-1 kb DNA marker, Lane 2- pTrcHis; Lane 3- pTrcHis PCR product; Lane 4- pUC8.2-14;
Lane 5- pUC8.2-14 PCR product; Lane 6 – 1kb plus DNA marker, Lane 7- pTrcHis and Lane 8- pUC8.2-14.
The red circles were showing the respective plasmids and PCR products.

B. PCR Products

From the 1% (w/v) agarose gel in Fig. 2A, the plasmid and PCR products of the respective plasmids were shown present, except for pUC8.2-14 in lane four. The absence was due to low concentration in loading the samples for plasmid which was 1 µL for each while 5 µL for PCR products. Loading was then increased to 5 µL for plasmid in Fig. 2B and thus the plasmids were exhibited at approximately 4 kb and 3 kb respectively for pTrcHis and pUC8.2-14 (indicated with the red circles at lane seven and eight). The PCR products of both lipase genes were obtained approximately at 1.2 to 1.3 kb. The pUC8.2-14 was a match expectedly as described by the previous researcher in the ATCC repository [22]. While for *Staphylococcus hyicus*, there were two groups of nucleotide registered on NCBI, *Staphylococcus hyicus ssp hyicus* [23] and *Staphylococcus hyicus* ATCC11249. Both groups were designated to lipase genes.

C. DNA Sequencing Analysis from NCBI Databases

DNA sequencing obtained from the sequencing service was uploaded into BLAST using the NCBI website. From the non-redundant databases, the respective plasmids were found to be 99% and 47% query cover for pTrcHis and pUC8.2-14 respectively. The pTrcHis alignment was confirmed as *Staphylococcus hyicus* strain ATCC11249 and lip gene for lipase [23]. In a recent study, ATCC11249 was also being analysed for exudative epidermis of swine [24]. Meanwhile, pUC8.2-14 alignment was found containing fragment from *Rhizopus delemar* carboxyl ester hydrolase mRNA, *Rhizopus niveus,* and *Rhizopus oryzae* gene for lipase [22].

5. CONCLUSIONS

This preliminary stage of research highlighted the transformation of two recombinant plasmids into expressing host, *Escherichia coli* BL21 (DE3). The transformations were successfully extracted, sequenced and confirmed by databases on the NCBI website. By having the same expression host, the further experimental comparison between the plasmids and protein expression would be consistent and fairly justified without concerning the effect of different strains as expression hosts. In engaging *E. coli* BL21 (DE3) as the expression host, the benefits described previously in the literature were also considered as an additional advantage in bioprocess monitoring.

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