Biota Vol. 14 (3): 150-155, Oktober 2009
ISSN 0853-8670

Construction of Soil Metagenomic Library to Obtain Recombinant Clones with an Indigenous Lipase Activity

Pembuatan Perpustakaan Metagenom Tanah untuk Mendapatkan Klon Rekombinan dengan Aktivitas Lipase *Indigenous*

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Abstrak

Penelitian ini bertujuan untuk mengonstruksi perpustakaan metagenom tanah dari sekitar lokasi industri pengolahan minyak kelapa di Kabupaten Banyumas, Jawa Tengah, dalam upaya mendapatkan klon rekombinan dengan aktivitas lipase *indigenuos*. Penelitian dilakukan di Laboratorium Genetika, Fakultas Biologi, Universitas Jenderal Soedirman mulai bulan Mei hingga Desember 2006. Secara umum tahapan penelitian terdiri atas ekstraksi dan pemurnian DNA tanah, isolasi pUC19, pemotongan DNA tanah, pemotongan dan defosforilasi pUC19, ligasi fragmen-fragmen DNA tanah ke dalam pUC19 untuk menghasilkan molekul DNA rekombinan, dan transformasi sel inang *E. coli* JM109 menggunakan molekul DNA rekombinan yang diperoleh untuk mengonstruksi perpustakaan metagenom tanah. Pemotongan DNA tanah menghasilkan fragmen-fragmen yang sebagian besar berukuran lebih kurang 5 kb sehingga gen lipase dimungkinkan untuk dibawa di dalamnya. Transformasi sel inang *E. coli* JM109 memperlihatkan efisiensi 1.7 x 10^5 cfu/µg plasmid. Di antara koloni-koloni yang diperoleh, terdapat 102 koloni berwarna putih, yang menunjukkan keberadaan molekul DNA rekombinan. Koloni putih ini dapat digunakan sebagai materi untuk skrining klon rekombinan dengan aktivitas lipase.

Kata kunci: perpustakaan metagenom tanah, pUC19, *E. coli* JM109, lipase *indigenous*

Diterima: 02 Februari 2009, disetujui: 03 Agustus 2009

Introduction

Lipases (E.C. 3.1.1.3) are enzymes catalyzing hydrolysis of lipid or triacylglycerol into glycerol and free fatty acids. These enzymes usually exhibit broad substrate specificity and degrade acyl p-nitrophenyl esters, Tweens and phospholipids, often with positional selectivity, stereoselectivity, and chain length selectivity. They have been recognized as very useful biocatalysts because of their wide-ranging versatility in industrial applications (Saxena *et al*., 2003; Gupta *et al*., 2004).

To obtain microbial lipases by means of molecular approach are commonly carried out by exploring one of a certain strain of microorganism. With this strategy, it will, however, result in a considerable loss of microorganisms having potential to produce lipases as not all of them could be cultivated using standard techniques known so far (Triana *et al*., 2007). Only 0.1% of approximately 10^7 cells in a gram of soil sample could be cultivated (Kellenberger, 2001).

Recently, a new approach of accessing genetic sources from environmental samples, particularly soil, without need of microorganism cultivation, has been developed. In the so-called as metagenomic approach, the whole genomes of all microorganisms presenting in a certain

habitat are directly cloned to provide a metagenomic library as source of materials to screen for genes of interest (Lorenz and Schleper, 2002).

Soils exposed to coconut oil effluent in a long period of time have potential to contain microorganisms which produce lipases, so that such types of soils can be used to explore lipase genes employing a metagenomic approach. Several coconut oil home industries exist in Banyumas regency, Central Java, from which soil samples could be taken as DNA sources in the construction of metagenomic library to obtain lipase genes. Following this way, indigenous microbial lipases will be expectantly acquired. This study was aimed to construct a soil metagenomic library in *Escherichia coli* JM109 cell hosts to provide recombinant clones which will be screened further for an indigenous lipase activity.

Materials and Methods

This study was conducted in the Laboratory of Genetics, Biology Faculty, Jenderal Soedirman University from May to December 2006 involving some consecutive steps, i.e. soil sampling, soil DNA extraction and purification, pUC19 plasmid isolation, partial digestion of soil DNA, digestion and dephosphorilation of pUC19, ligation of soil DNA fragments into linear pUC19 to produce recombinant DNA molecules, and transformation of *E. coli* JM109 cell hosts with the recombinant DNA molecules to construct a soil metagenomic library.

Soil sample of approximately 5 g was taken from around a location of coconut oil home industry in Banyumas regency, Central Java at 15 cm depth using sterile spatula after that it was put into a sterile plastic bag. This sample was then stored at 4°C until DNA extraction was carried out.

DNA was extracted and purified using Power Soil DNA Extraction Kit (MoBio, USA). On the other hands, pUC19 was isolated from *E. coli* JM109 using Wizard Miniprep Kit.

The purified soil DNA was partially digested with *Bam*HI in which the reaction was incubated at 37°C for 30 minutes. The enzyme was inactivated at 65°C for 15 minutes. At the same time pUC19 was also cut with *Bam*H I by incubating the reaction at 37°C for 60 minutes prior to inactivation at 65°C for 15 minutes. To remove the remaining *Bam*H I from both soil DNA and pUC19 reaction of digestion, purification was made using Wizard DNA Purification Kit. Then, the linear pUC19 was dephosphorilated with calf intestinal alkaline phosphatase (CIAP) provided by Promega. To remove the remaining CIAP from the dephosphorilation reaction, Wizard DNA Purification Kit was also used.

Ligation of soil DNA fragments into the linear pUC19 was conducted using T4 DNA ligase with overnight incubation at 4°C. This ligation mixture was purified from the residual T4 DNA ligase using Wizard DNA Purification Kit. The recombinant pUC19s generated was then used to transform *E. coli* JM109 competent cells.

To achieve the highest efficiency of transformation, *E. coli* JM109 competent cells were incubated at 4°C for 16 hours. As much as 200 µl competent cell cultures was added with 10 µl recombinant pUC19 after the heat shock was immediately applied at 42°C for 50 seconds. Transformation of *E. coli* JM109 using intact pUC19 was also given as control. The transformed colonies were plated onto L-agar medium containing ampicillin and X-gal/IPTG.

Both the number of all colonies formed and that of white colonies observed were counted. The efficiency of transformation was calculated as the ratio of *E. coli* JM109 colonies formed (cfu) to recombinant pUC19s added (µg).

Results and Discussion

Soil DNAs of about the same quality and concentration are resulted from twice extraction and purification as shown in Figure 1. The electrophoretic bands indicated that the DNAs obtained were relatively free from humic acids, which frequently contaminate the extracted soil DNA and may disturb the following enzymatic manipulation processes (Ogram *et al.*, 1987; Porteous and Armstrong,

1991; Endo *et al*., 1992; Jacobsen and Rasmussen, 1992; Picard *et al.*, 1992; Smalla *et al.*, 1993; Volossiouk *et al.*, 1995; Zhou *et al.*, 1996). On the other hands, post-extraction DNA purification to remove humic acids often results in fewer soil DNA as well as physical DNA degradation.

Comparing the fluorescence of soil DNA bands and that of *Hin*d III cut λ marker we estimated the soil DNA concentration was approximately 100 ng/µl. Because the entire DNAs from 0.25 g soil samples were eluted in a volume of 100 µl, it could be said that the extraction and purification yielded 40 µg DNA g^{-1} soil. This much exceeded the yield of soil DNA extraction and purification after Wechter *et al*., (2003), i.e. 5.22 µg DNA g^{-1} soil, with the same time length of processing (2 hours).

The DNA band in the respective lane showed that the DNA was not subject to physical fragmentation, or in other words, intact lipase gene might be carried in it and the soil DNA could be proceded to the next treatment, i.e. partial digestion using a restriction enzyme. The results of partial digestion using *Bam*HI could be seen in Figure 2 lane 4 and 5. Most DNA fragments generated seemed to be of approximately 5 kb, so that they could be expected to contain intact lipase genes. Henne *et al*., (2000) reported that recombinant clones showing lipase activity could be obtained by screening a soil metagenomic library having insert size of 5 to 8 kb.

The pUC19 isolated from *E. coli* JM109 was visualized in Figure 2 lane 1, while the results of digestion and dephosphorilation of this plasmid could be seen in Figure 2 lane 2. It was shown that pUC19 has been subject to complete digestion and could be used as cloning vector to bear the partially digested soil DNA fragments. Comparing the fluorescence of linear pUC19 and that of *Hin*d III cut λ marker we estimated the plasmid concentration of approximately 30 ng/µl.

The purification of digestion mixture, both of soil DNA and of pUC19, resulted in the fragments that were visualized in Figure 3. From the electrophoretic bands, it could be said that the recovery of both pUC19 dan soil DNA fragments was sufficiently high that they could used in the ligation process.

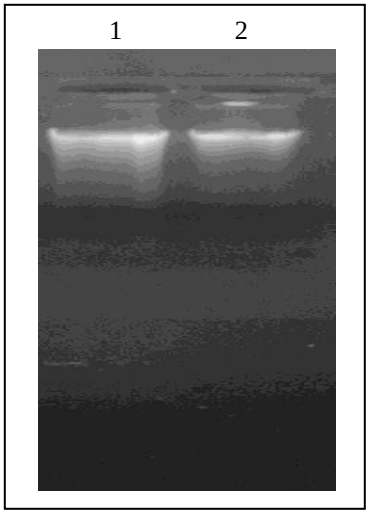

Figure 1. Electrophoregram of extracted and purified soil DNAs
Notes: 1. Soil DNA of first extraction
2. Soil DNA of second extraction

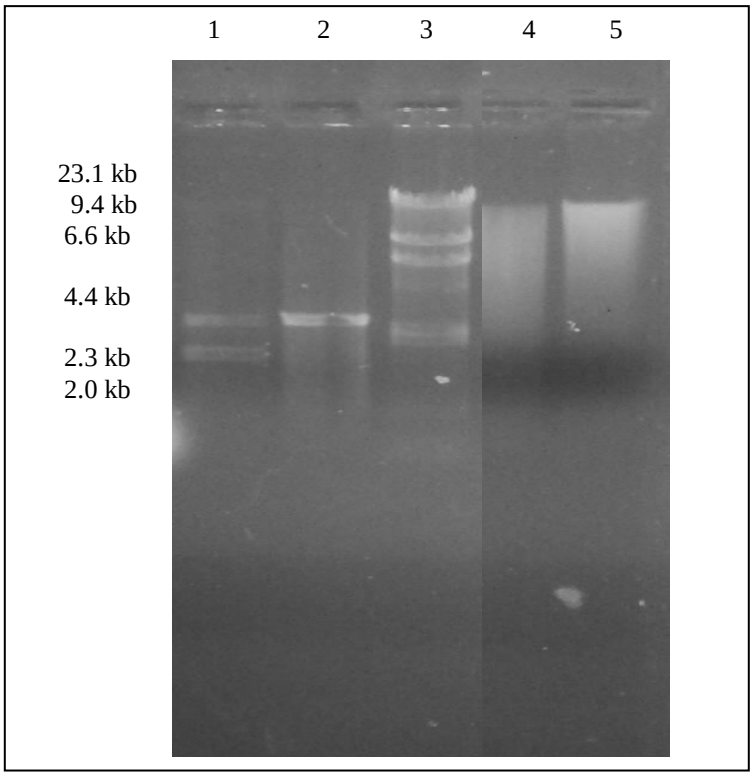

Figure 2. Electrophoregram of pUC19 and soil DNA digestion.
Notes: 1. Intact pUC 19
2. pUC 19 cut with *Bam*H I
3. *Hin*d III cut λ marker
4. First extracted soil DNA cut with *Bam*H I
5. Second extracted soil DNA cut with *Bam*H I

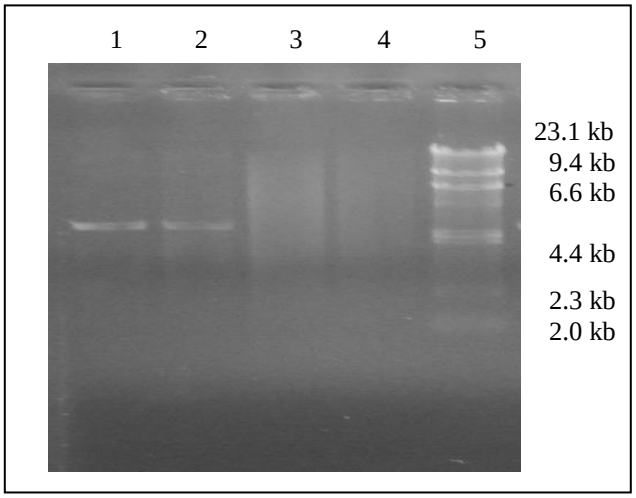

Figure 3. Electrophoregram of linier purification pUC19 and soil DNA digestion.
Notes: 1. First purified linear pUC19
2. Second purified linear pUC19
3. First purified soil DNA fragments
4. Second purified soil DNA fragments
5. *Hin*d III cut λ marker

The recombinant pUC19s as a result of ligation with soil DNA fragments were directly used to transform *E. coli* JM109 competent cells. In 20 µl ligation mixture, as much as 3 µl (90 ng) pUC19 was used resulting in the plasmid concentration of 4.5 ng/µl. From this mixture, 5 µl (22.5 x 10^{-3} µg) pUC19 was used to transform *E. coli* JM109 cells.

As many as 102 white colonies and 848 blue colonies were observed in L-agar medium containing ampicillin and X-gal/IPTG. On the other hands, the transformation control with intact pUC19 resulted in 2,540 blue colonies. Because the transformant culture plated was 250 µl of a total volume of 1 ml, the efficiency of transformation in the control was 2,540 x 4 cfu/22.5 x 10^{-3} µg = 4.5 x 10^5 cfu/µg, while using recombinant pUC19 was 950 x 4 cfu/22.5 x 10^{-3} µg = 1.7 x 10^5 cfu/µg. Transformation is said to be efficient when it reaches a value of 10^5 cfu/µg (Sambrook *et al*., 1989).

The pUC19s were isolated from both white and blue colonies as shown in Figure 4. The difference in the band pattern of pUC19s from white and blue colonies indicated that pUC19s from white colonies bear soil DNA fragments. Therefore, screening for lipase activity among the white colonies could be further employed.

Conclusion

It is concluded that metagenomic DNA could be isolated and purified from soil exposed to coconut oil effluent around a home industry location in Banyumas regency, Central Java. A metagenomic library from the soil DNA could then be constructed in *E. coli* JM109 cell hosts to provide recombinant clones which will be screened further for an indigenous lipase activity.

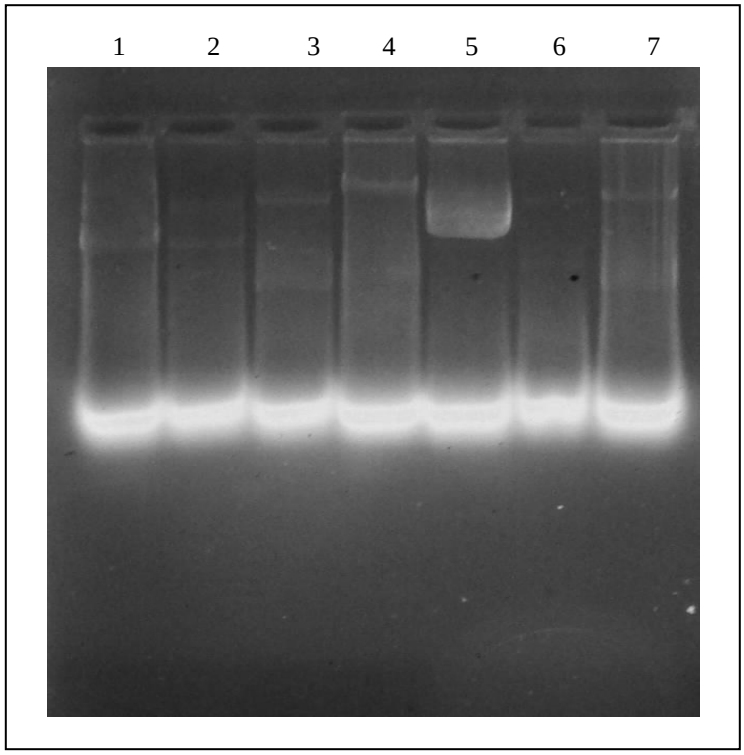

Figure 4. Electrophoregram of isolated pUC19s from *E. coli* JM109 transformants
Notes: 1 - 5 pUC19s from white colonies
6 - 7 pUC19s from blue colonies

Acknowledgement

We address our thanks to Hibah Bersaing Project XIV/I year 2006 for financial support provided.

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