



COMPARISON OF THREE DNA ISOLATION AND PURIFICATION METHODS OF BACTERIAL DNA

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ABSTRACT

The objective of this study was to compare the efficiency of three methods of nucleic acids extraction from gram-positive bacteria by evaluating the quantity and purity of DNA extracts. Nucleic acids extraction of gram-positive bacteria is normally hampered by a thick and resistant cell wall. Gram positive bacteria usually have a thick cell wall consisting mainly of many layers of peptidoglycan, which is not easily destroyed. This paper compares the different procedures based on mechanical and enzymatic cell breakage to extract DNA from *Rhodococcus pyridinovorans* using GES method, Ultraclean Microbial DNA isolation Kit, and Prepman Microbial DNA isolation kit. DNA extracts were analyzed by agarose gel electrophoresis and UV spectroscopy. Yield and quality of DNA obtained by the GES method were higher than the other methods. Nucleic acids extracts with the highest yield and purity were amplified by Polymerase Chain Reaction (PCR) using various primers targeted on gene encoding nitrilase gene such as BLITF and PNITR, α NH1 and α NH2, β NH1 and β NH2, Amd1 and Amd 2. The gene encoding for nitrilase were amplified which was confirmed by sequencing analyses. However, the targeted gene length from the primers was not obtained. Therefore further amplification optimization may be needed.

Key words: extraction, DNA isolation *Rhodococcus pyridinovorans*, nitrilase gene

INTRODUCTION

The isolation and purification of DNA is a key step for most protocols in molecular biology studied and all recombinant DNA techniques. In genetic test and analyses, extraction of DNA which is then used as a template is the first step that can have a decisive influence on the test result (Niemi *et al.*, 2001). The problem of these molecular biological approaches is extraction process of nucleic acids from Gram-positive bacteria. To extract nucleic acids from bacterial, bacterial cell are destroyed to release the nucleic acids contained within the cytoplasm and then the remaining protein should be removed (Fujimoto *et al.*, 2004). The cell walls of gram-positive bacteria can be efficiently broken by use of the peptidoglycan-degrading enzyme lysozyme and mutanolysin because gram-positive bacteria have a thick cell which is not easily destroyed (Kaisu & Jari, 2002).

Nucleic acids extracts with the highest yield were amplified using various primers to get the nitrilase gene. Bacterial nitrilase has great potential for the production of numerous industrial acid products which is suggested give more advantages over traditional chemical methods such as milder reaction conditions (O'Reilly C & Turner, 2003)

MATERIALS AND METHODS

Three DNA extraction methods were evaluated in this study. For protocol 1, 2 and 3, the single colony of *Rhodococcus pyridinovorans* was subjected to different extraction protocols.

DNA extraction by GES Method

DNA was isolated by GES Method (Pitcher *et al.*1989). One strain of the *R. pyridinovorans* TPIK grown in medium nutrient agar at 37°C overnight. The bacteria were suspended in 1 ml TE buffer (10mM Tris-HCl, 1 mM EDTA, pH 8). The mixture then centrifuged 1000 rpm for 15 min at 4°C . The pellet was added with 50 µL lysozyme solution and incubated at 37°C for 30 min. An amount of 250 µl of GES solution (prepared with 60g Guanidium Thiocyanate, 20 ml of 0,5 M EDTA at pH 8, 5 ml of 10% Sarkosyl and distilled water to 100 ml of the solution) was added to bacterial suspension and left at RT for 10 min then subsequently cooled in ice. After that, an aliquot of 125 µl of 7.5 M ammonium acetate was added and the resulting solution was cooled in ice for another 10 min. The mixture was added with 500µl chloroform, mixed and then centrifuged. The supernatant (the upper layer of fluid) was decanted in to a new tube, added and mixed with one half amount of isopropanol and DNA was recovered as a pellet by centrifugation. The DNA pellet was washed twice with 70% ethanol and then allowed to dissolve in 100µL sterile distilled water (Pitcher *et al.*, 1989).

DNA extraction by Prepman Microbial DNA isolation kit.

The bacterial culture were suspended in 25 mL Prepman's solution. The mixture was mixed well by vortex for 10 s. Mixture was heated at 100 °C for 10 min. Subsequently, the mixture was centrifuged at 12,000 rpm for 10 min at 25 °C. The resulting supernatant was collected.

DNA extraction by Ultraclean Microbial DNA isolation Kit

The extraction of DNA was done according to the manufacturer's instruction, with some modifications

DNA quantitation

For DNA quantitation, DNA sample was calculated from the optical density absorbance value measured by a spectrophotometer (ABI) on 260 and 280 nm

RESULT AND DISCUSSION

The quality of the extracted DNA was evaluated by the A_{260}/A_{280} ratio and values higher than 1.8 indicate a good DNA extract without protein contamination (Samuel *et al.*2003). Table 1 showed the result of the quality and yield of the extracted DNA using three different methods. Result for DNA for DNA quality showed that DNA extract using GES method gave the best result among others result indicating by the A_{260}/A_{280} ratio value which was higher than 1.8. The ultra clean method gave an OD A_{260}/A_{280} ratio less than 1.

A good DNA extraction method should not give only high DNA purity, but also high DNA yield. The findings of the current study showed that DNA extracted with GES method produced the highest yield compared to the other methods. GES method uses a peptidoglycan nicking enzymes such as lysozym which may can effectively break the gram-positive bacterial wall down. However the residues of these enzymes may affect PCR and therefore, the amount of enzyme should be minimized (Fujimoto *et al.*2004).

The quality of the extracted DNA was determined by agarose gel electrophoresis too. The good quality was indicated by the sharpness of DNA band was visualized without any denaturation signed. The results showed that DNA extracted using GES method gave a good result (Figure 1).

Table 1. DNA yields of *Rhodococcus pyridinovorans* TPIK bacteria extracted using different DNA extraction methods

Methods	A ₂₆₀	DNA (μL/mL)	A _{260/280}
GES Method	1,2	55.526	1,8954
Prepman	0,205	10.003	1.8048
Ultraclean	0,59	14,839	0,9581

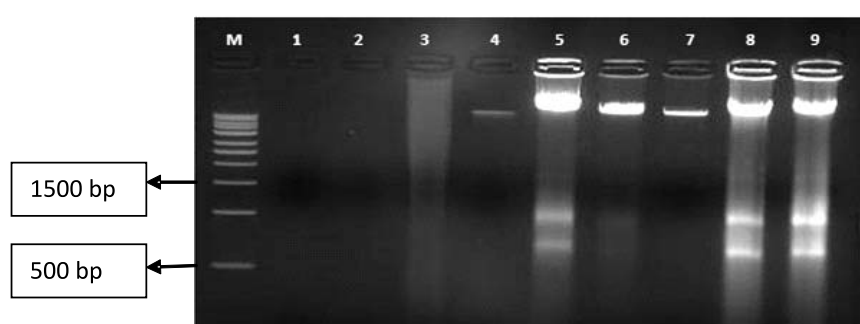


Figure 1. Agarose gel electrophoresis (1%) of the extracted DNA. M= marker 1 kb plus, line 1-3: DNA extract using Prepman method, line 4: DNA extract using ultraclean method, line 5-9: DNA extract using GES method

PCR detection of nitrilase genes

Nucleic acids extracts with the highest yield and purity were amplified by Polymerase Chain Reaction (PCR) using various primers targeted on gene encoding nitrilase such as BLITF and PNITR, α NH1 and α NH2, β NH1 and β NH2, Amd1 and Amd 2. The amplification product was checked using agarose gel electrophoresis. As a shown in Figure 2 a specific DNA fragment (about 400 bp) were observed. This PCR product size did not match with the correct size of the nitrilase gen (about 900 bp). However, the DNA fragments were then confirmed as the correct nitrilase gen by sequencing analyses. The result of sequencing analyses using primer β NH1 dan β NH2 *Bacillus* sp. LC5B2 was nitrile hydratase beta subunit (*nha2*) gene partial (Table 2). However, the targeted gene length from the primes was not obtained. It might need further amplification optimization

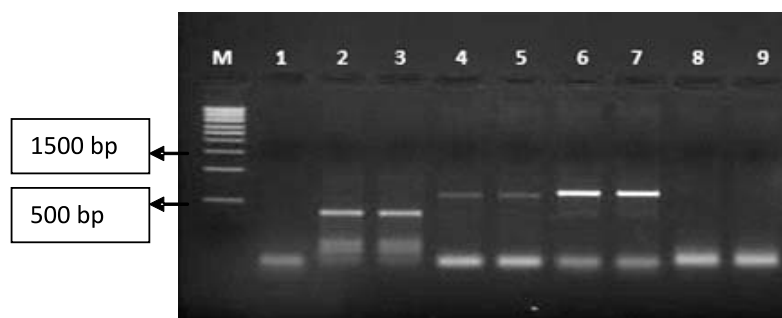


Figure 2. Agarose gel electrophoresis (1%) of the extract DNA. M= marker 1 kb plus, line 1:control negative, line 2-3 using primer BLTF dan PNITR, line 4-5: : primer α NH1 dan α NH2, 6-7: : primer β NH1 dan β NH2, line 8-9: primer Amd1 dan Amd2

Table 2. Sequencing analyses using primer β NH1 and β NH2

No	ID Isolate and primer	Sequencing (1 st-Base)	Homology(100 %)
1	TP6_ β NH1	<i>Bacillus</i> sp. LC5B2 nitrile hydratase beta subunit (nha2) gene, partial	99
2	TP6_ β NH2	<i>Paenibacillus</i> sp. NN34 nitrile hydratase beta sub unit (nha1) gen	99

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