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Comparison of methods for genomic DNA isolation of rhizospheric microorganisms

Triwibowo Yuwono[1,2,*], Ali Ikhwan[3], and Joedoro Soedarsono[1,2]

1) Laboratory of Microbiology, Inter University Center for Biotechnology
2) Faculty of Agriculture, Gadjah Mada University, Yogyakarta, Indonesia
3) Muhammadiyah University, Malang, Indonesia

Abstract

A comparative study for the isolation of genomic DNA of rhizospheric microorganisms is described. The methods tested were based upon the methods of: (1) standard glass rod, (2) boiling glass rod, (3) Promega's Wizard genomic DNA isolation method, and (4) agarose gel plug, with some modifications of each method. The efficiency and suitability of the method for genomic DNA isolation were analysed based on three parameters : (1) the level of DNA purity, (2) the concentration of DNA. and (3) the intactness of the DNA. It was observed that the standard glass-rod method gave the best results in terms of DNA purity and the DNA concentration obtained. However, in terms of DNA intactness, agarose gel plug method gave the best result compared to other methods.

Keywords: genomic DNA - rhizospheric microorganisms

Introduction

Rhizospheric microorganisms is a group of bacteria which colonise the plant roots and develop specific interaction with the plants resulting in either deleterious or beneficial effects to the plants (Kloepper, 1993). The molecular study on interaction between rhizospheric microorganisms and plants is of paramount importance as it would broaden the perspective of physiological processes underlying plant growth and development. Such molecular study, however, necessitates the availability of suitable protocol for isolation of genomic DNA, either from the plant or from microbial cells which interact with the plant. Several methods have been developed for isolation of genomic DNA from prokaryotic and eukaryotic cells (Sambrook *et al.*, 1989; Roe *et al.*, 1996).

Rhizospheric microorganisms which colonise plant roots often consist of a group of microorganims of different genera. Therefore, within this group, cell structural differences are often quite wide, which in turn impose a problem in adapting a protocol, developed for specific organism, to different organism. The available methods for isolation of genomic DNA are in general developed for standard laboratory species, such as *Escherichia coli*, and *Bacillus subtilis*. Certain group of microorganisms, such as actinomycetes, has a rather different cellular structure to that of standard laboratory species. Therefore, modifications of the protocol for genomic DNA isolation are often performed in order to get the most efficient results. In this paper we describe the use of several protocols for genomic DNA isolation from the cells of rhizospheric microorganisms. The paper also compares the suitability of each method and suggests improvement of the available method. The microorganisms used were isolates of osmotolerant rhizospheric microorganisms obtained from different rhizosphere of plants grew on dry region of Yogyakarta Province, Indonesia.

*) Corresponding author. Mailing address: Laboratory of Microbiology, Inter University Center for Biotechnology, Gadjah Mada University, Jalan Teknika Utara, Barek, Yogyakarta 55281, Indonesia.
e-mail: tribowo@iuc-biotec.ugm.ac.id. Facsimile: (0274) 520842

Materials and Methods

Microorganisms. Three isolates of osmotolerant rhizospheric microorganisms, designated as Al-19, M7b, and A-82, were used.

Methods for isolation of genomic DNA. Methods for isolation od genomic DNA were based upon four different methods with modification of each method, i.e. : (1) standard spooling glass-rod method, (2) boiling glass-rod method, (3) Promega's Wizard genomic DNA isolation kit, and (4) agarose gel plug.

Standard spooling glass-rod (GR-S) method for DNA isolation is as follows: cells were pelleted and washed with 5 ml 50mM EDTA. The cells were then centrifuged at 8000 g for 15 min. The cell pellet was then resuspended in 2 ml lysozyme solution (containing 0.1 ml lysozyme (10 mg/ml), 1.7 ml of 25 % sucrose, and 0.2 ml of 0.5 M EDTA) and incubated at 37°C for 1 hour, followed by the addition of 3 ml proteinase-K solution (containing 50 µl of 10 mg/ml proteinase-K, 1.0 ml of 5 M NaCl, 0.5 ml of 0.5 M EDTA, and 1.25 ml of 20% SDS). The mixture was then incubated 50°C for 1 hour, followed by the addition of chloroform and mixed gently. Following centrifugation at 8000 g for 10 min, the uppermost layer was gently pipetted and mixed gently with 2 volumes of absolute ethanol. The DNA spool was subsequently collected using pasteur pipette and resuspended in 1.0 ml TE. RNase (10 (g/ml) was added to the DNA suspension and incubated at 37°C for 1 hour.

The boiling glass-rod (BGR) method for DNA isolation is as follows: cell pellet was washed with 5 ml cold STE (0.1 M NaCl, 10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0) followed by centrifugation at 8000 g for 15 min. The pellet was resuspended in lysozyme solution containing 112.5 µl of 10 mg/ml lysozyme and 2127.5 µl cold STET (STE plus 5% Triton X-100). The suspension was transferred to a glass tube and then boiled on a bunsen lamp. Afterwards, the suspension was placed in boiled water for 40 sec and then transferred to ice for 5 - 10 min. The suspension was then mixed with a solution containing 1.0 ml 5 m NaCl, 0.5 ml EDTA 0.5 M, and 1.25 ml of 20% SDS and incubated at 50°C for 1 hour. Afterwards, chloroform was added to the suspension and mixed gently. The next steps were the same as in the standard GR-S method.

The Promega's method for genomic DNA isolation used was essentially as described in the kit supplied by the manufacturer (Anonim *a*, 1995). The fourth method (agarose gel plug/Gel-Plug method), based upon method for DNA preparation for Pulsed Field Gel Electrophoresis (Birren and Lai, 1993), is as follows: cell pellet was resuspended in 1 ml 0.5 M EDTA, then centrifuged at 10,000 g for 15 min. The pellet was then resuspended in 0.6 ml 100 mM

EDTA and 0.4 ml *Low Melting Agarose*, followed by vortexing for 10 - 20 sec. Agarose plug containing the mixture was then made with the volume of 0.2 ml each. The plug was then suspended in lysis solution (6 mM Tris-HCl pH 7.6, 1 m NaCl, 0.1 M EDTA, 0.5 mg/ml lysozyme, and RNase µg/ml) at a ratio of 1 : 5, followed by incubation at 37°C overnight. The plug was then suspended in 1 ml proteinase-K (100 (g/ml) and incubated at 50°C for 2 x 24 hours. The plug was subsequently suspended in 1 ml TE (pH 8,0) and incubated at 50°C for 30 min followed by incubation at room temperature for 2 hours. The DNA in the plug was isolated by melting the agarose plug at 70°C, then centrifuged at 10,000 g for 10 min. The supernatant was collected and concentrated by adding 0.1 volume of 3 M sodium acetate and 2 volumes of absolute ethanol. The mixture was then incubated at -20°C overnight, followed by centrifugation at 10,000 g for 10 min. The DNA pellet was then washed with 70% ethanol and recentrifuged at 10,000 g for 10 min. The DNA pellet was finally resuspended in TE. Another alternative of this method (designated as Gel-melt method) was by using melted agarose (the agarose was not plugged), while other steps were the same as described above.

Modification of each method was carried out by further purification of the DNA obtained by: (1) adding proteinase-K, (2) phenol/chloroform purification, (3) using Promega's Wizard DNA Clean-up Kit (Anonim *b*, 1994).

Analysis of purity level, concentration, and the intactness of DNA. The purity and concentration of the DNA obtained was analysed by spectrophotometric method at 260 and 280 nm (Sambrook *et al.*, 1989). The intactness of DNA was analysed electrophoretically.

Results and Discussion

The purity and concentration of genomic DNA isolated using different protocols. The protocols for genomic isolation are summarised in Table 1.

Table 1. Summary of methods for genomic DNA isolation

Method of isolation	Method of purification	Note
1. Standard glass-rod	a. proteinase-K b. phenol/chloroform c. Wizard clean-up kit	SGR-S SGR-P GR-WC
2. Boiling glass-rod	a. proteinase-K b. phenol/chloroform	BGR-S BGR-P
3. Wizard genomic DNA kit	a. standard b. proteinase-K c. phenol/chloroform	WG-S WG-Pro-K WG-P
4. Agarose gel plug	a. proteinase-K b. proteinase-K	Gel-Plug Gel-melt

The level of purity of DNA was assayed by spectrophotometric determination at 260 nm and 280 nm. It is known that the ratio of optical density at 260 nm and 280 nm (OD 260/280) gives the estimate of the purity of DNA. The pure DNA preparation has the OD 260/280 of 1.8 (Sambrook *et al.*, 1989). The results of genomic DNA isolation from different isolates of rhizospheric microorganisms using different protocols are presented in Figure 1, 2, and 3. In general, it was observed that standard glass-rod method gave the best results, in terms of DNA purity, for the three isolates. Subsequent purification using Wizard DNA Clean-up kit (GR-W) did not improve the purity. In the case of Al-19 isolate, subsequent purification with Wizard DNA clean-up kit has made spectrophotometric determination rather odd (Figure 1, GR-W). This deviation may be at-

tributed by the fact that the kit contains special matrix which may interfere with the spectrophotometric determination. It was also observed that suitability of a method for genomic DNA isolation depended upon the species used. A ready-to-use genomic DNA isolation kit (Figure 2 and 3, WG-S) was found less suitable, in terms of DNA purity, for isolation of genomic DNA from isolate A82 and M7b.

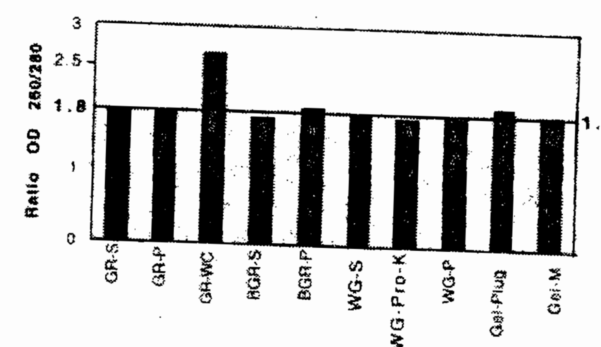


Figure 1. The level of DNA purity isolated from Al-19 isolate using different methods. GR: glass rod method, BGR: boiling glass rod method, WG: Wizard genomic DNA isolation kit, Gel-Plug: agarose gel plug, Gel-M: melted agarose gel, S: standard method, P: with phenol purification, WC: with Wizard clean-up kit, Pro-K; with proteinase K treatment

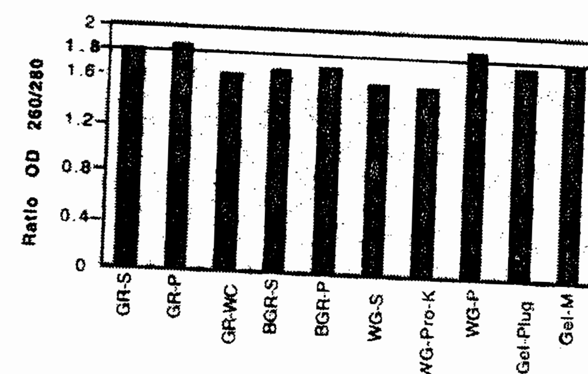


Figure 2. The level of DNA purity isolated from A82 isolate using different methods. GR: glass rod method, BGR: boiling glass rod method, WG: Wizard genomic DNA isolation kit, Gel-Plug: agarose gel plug, Gel-M: melted agarose gel, S: standard method, P: with phenol purification, WC: with Wizard clean-up kit, Pro-K; with proteinase K treatment

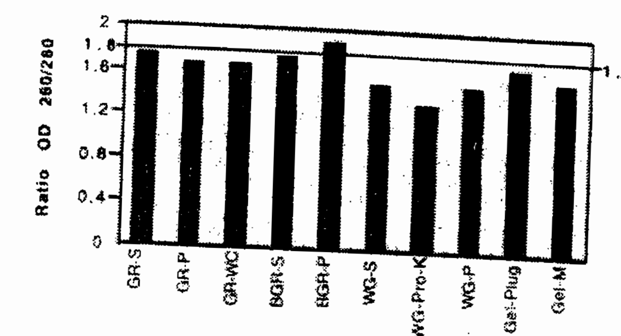


Figure 3. The level of DNA purity isolated from M7b isolate using different methods. GR: glass rod method, BGR: boiling glass rod method, WG: Wizard genomic DNA isolation kit, Gel-Plug: agarose gel plug, Gel-M: melted agarose gel, S: standard method, P: with phenol purification, WC: with Wizard clean-up kit, Pro-K; with proteinase K treatment

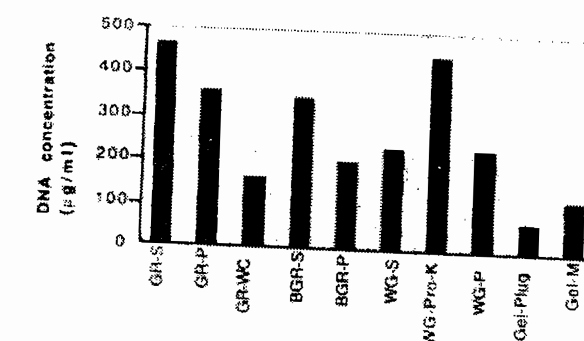


Figure 4. Concentration of genomic DNA isolated from Al-19 isolate using different methods. GR: glass rod method, BGR: boiling glass rod method, WG: Wizard genomic DNA isolation kit, Gel-Plug: agarose gel plug, Gel-M: melted agarose gel, S: standard method, P: with phenol purification, WC: with Wizard clean-up kit, Pro-K; with proteinase K treatment

In terms of DNA concentration, the standard glass-rod method gave the best results for isolate Al-19 and A-82, whereas for isolate M7b this method was less suitable. It was also observed that genomic DNA isolation kit with an extra purification step using proteinase-K (Figure 1, WG-Pro-K) gave a comparable result to the standard glass-rod method for Al-19. The same protocol, however, was found much less suitable for A82

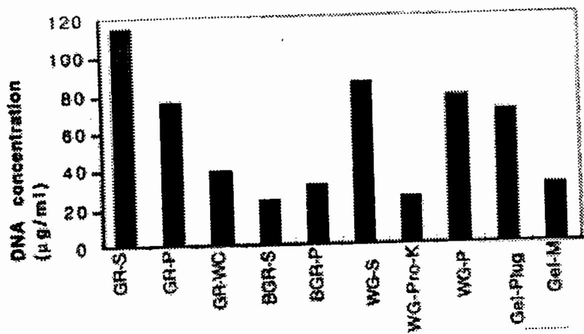


Figure 5. Concentration of genomic DNA isolated from A82 isolate using different methods. GR: glass rod method, BGR: boiling glass rod method, WG: Wizard genomic DNA isolation kit, Gel-Plug: agarose gel plug, Gel-M: melted agarose gel, S: standard method, P: with phenol purification, WC: with Wizard clean-up kit, Pro-K; with proteinase K treatment

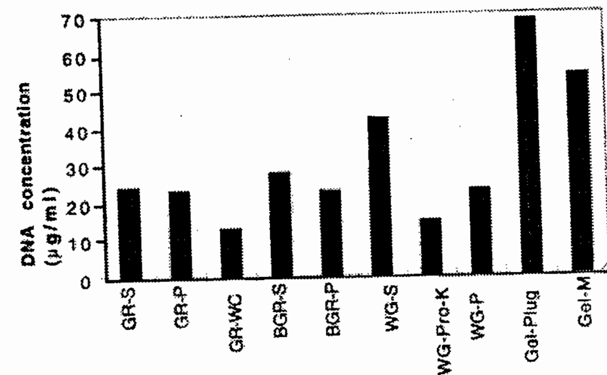
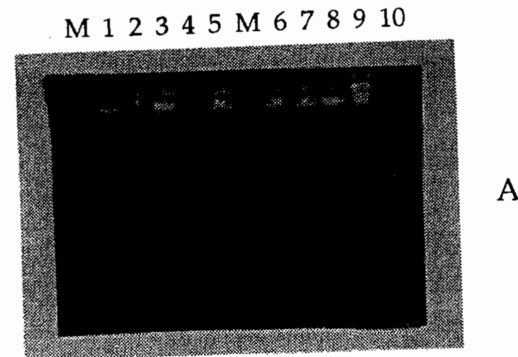
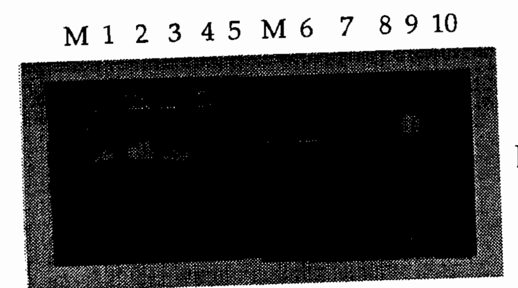


Figure 6. Concentration of genomic DNA isolated from M7b isolate using different methods. GR: glass rod method, BGR: boiling glass rod method, WG: Wizard genomic DNA isolation kit, Gel-Plug: agarose gel plug, Gel-M: melted agarose gel, S: standard method, P: with phenol purification, WC: with Wizard clean-up kit, Pro-K; with proteinase K treatment

(Figure 2, WG-Pro-K). Interestingly, for isolate M7b, the best result was obtained when genomic DNA isolation was carried out by using agarose gel plug method (Figure 3, Gel-Plug). These results thus confirmed that suitability of method for genomic DNA isolation was species-specific.

The intactness of DNA. The intactness of genomic DNA obtained was analysed by electrophoresis on agarose gel. In general, it





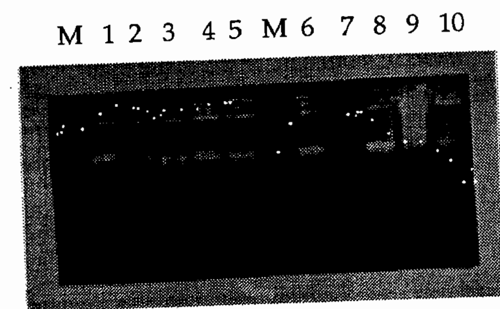


Figure 7. Electrophoretic profile of genomic DNA isolated from Al-19, A-82, and M7b isolates using different protocols. A: isolate Al-19, B: isolate A-82, C: isolate M7b.
M: marker, λDNA digested with *EcoR I* and *Hind* III, 1: standard glass rod method, 2: glass rod method with phenol purification, 3: glass rod method with Wizard clean-up kit, 4: boiling glass rod method, 5: boiling glass rod method with phenol purification, 6: Wizard genomic DNA isolation kit method, 7: Wizard genomic DNA isolation kit with proteinase-K treatment, 8: Wizard genomic DNA isolation kit with phenol purification, 9: agarose gel plug method, 10: melted agarose gel method.

was observed that genomic DNA isolation using agarose gel plug gave the best results for the three isolates (Figure 7A, 7B, 7C, lane 9). This was evidenced by the appearance of DNA band which had a bigger molecular weight than DNA obtained using other protocols. This result was understandable as agarose gel plug method practically did not involve any vortexing thus protect the DNA from shearing.

Conclusion

The results described above clearly show that protocol for genomic DNA isolation depends largely on the species being used. In terms of DNA purity and concentration, the standard glass-rod method gave the best results, except for isolate M7b. If the intactness of DNA is the critical point, then the agarose gel plug method is the method of choice. The agarose gel plug method, however, is rather time-consuming as it involves overnight incubation to lyse the cell. The standard glass rod method, on the other hand, is relatively simple and cheap but should be performed with a great care to prevent DNA breakage.

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