Comparison of methods for genomic DNA isolation of rhizospheric microorganisms

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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 analyzed based on three parameters: (1) the yield 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 isolation, agarose gel plug method gave the best result compared to other methods.

Keywords: genomic DNA - rhizospheric microorganisms

Introduction

Rhizospheric microorganisms are 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 (Klopper, 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 microorganisms 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 and used for isolation of DNA from prokaryotic cells.

Materials and Methods

Microorganisms. Three isolates of rhizospheric microorganisms, designated as AU-19, M7, and A-82, were used.

Methods for isolation of genomic DNA.

Methods for isolation of 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 lysosome solution (containing 0.1 ml lysosome (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 2 ml proteinase-K solution (containing 30 ml 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 at 55°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 pellet was subsequently collected using Pasteur pipette and resuspended in 1 ml TE. RNase (10 µg/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 lysosome solution containing 125 µl of 10 mg/ml lysosome and 212.75 µl cold STE (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 NaCl, 0.5 ml EDTA 5 M, and 1.25 ml of 20% SDS and incubated at 55°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, 1991). The fourth method (agarose gel plug / Gel Plug Kit), based upon method for DNA preparation by Pulsed Field Gel Electrophoresis (Birnboim and Divers, 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
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>
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<th>Method of isolation</th>
<th>Method of purification</th>
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<td>SQK-S</td>
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<td></td>
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The level of purity of DNA was assayed by spectrophotometric determination at 260 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 attributed 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](image1.png)
Figure 1. 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 proteinase K, with Wizard clean-up kit, Pro-K, with proteinase K treatment.

![Figure 2](image2.png)
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 proteinase K, with Wizard clean-up kit, Pro-K, with proteinase K treatment.

![Figure 3](image3.png)
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 proteinase K, with Wizard clean-up kit, Pro-K, with proteinase K treatment.

![Figure 4](image4.png)
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 proteinase K, 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 A82, 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.
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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References


