ABSTRACT 2034

Improving the Quality and Quantity of Total Genomic DNA Isolated from Difficult Plant and Soil Samples

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Abstract

Direct lysis techniques have proven extremely useful for studying the nucleic acids of microbial communities of soils and plants because they avoid the traditional and time-consuming culture-based microbiological methods. However the major drawback with direct lysis techniques is that large amounts of PCR-inhibitory substances are extracted along with the DNA. These inhibitors interfere with the cell lysis step, inactivate the thermostable DNA polymerase and interfere with nucleic acids. A number of different techniques are used to remove PCR inhibitory substances, and the most common is the incorporation of PCR facilitators directly in the PCR reaction. However, a more efficient method to deal with the inhibitors would be to exclude their co-purification during nucleic acid extraction. Thus, in this study we tested and compared a number of different nucleic acid extraction methods for isolating DNA from soil and plant samples, and we found that there is a significant difference between these methods for removing PCR inhibitors.

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Introduction

- Detection of specific DNA sequences related to target microorganisms found in soil and plant samples is a useful tool to improve our understanding of microbial communities. However, current DNA isolation methods and kits often result in DNA samples that do not perform well in downstream applications such as PCR, due to the co-purification of PCR inhibitors that exist in soil or higher plant species. Humic acids and phenolic compounds are some of these known PCR inhibitors. Extensive purification steps are required in order to improve the quality of the isolated DNA, however these processes are often labour intensive and time consuming, and require the use of hazardous chemicals. The ideal method for DNA isolation from soil and plants has to provide high quality inhibitor-free DNA with rapid, safe and easy sample processing.

- Most of the current commercially available kits for the isolation of DNA from environmental samples isolate a good quantity of DNA, however the DNA is often contaminated with PCR inhibitors, rendering it a poor quality. Moreover, the quality and quantity of the purified DNA is often plant and soil specific, as some plant and soil samples are known to contain more PCR inhibitors and therefore are more difficult to extract high quality total DNA from. In this study, we have chosen the most difficult plant and soil samples to test, including pine needles, grapes, strawberries and top soil. Norgen's Plant/Fungi DNA Isolation Kit and Norgen's Soil DNA Isolation Kit were used here as examples of commercially available column-based kits for the isolation of DNA from environmental samples, and instead of using the provided Lysis Buffer five different lysis buffer conditions were tested to evaluate their effects on quantity and quality of the purified DNA.

Methods

- Norgen's Plant/Fungi DNA Isolation Kit and Norgen's Soil DNA Isolation Kit were used as examples of commercially available column-based kits for the isolation of total DNA from plant and soil samples. The kits were used as per the manufacturer's instructions, however instead of using the supplied Lysis Buffer five different Lysis Buffers of varying compositions were tested for their effect on the quantity and quality of DNA that could be isolated from the environmental samples. For the isolation of total plant DNA 500 µL of each lysis buffer tested was added during the lysis step, and for the isolation of soil DNA 750 µL of each lysis buffer tested was added during the lysis step. Total DNA was isolated from 50 mg samples of grape, peach, strawberry, apple, pear and pine needle using the modified Plant/Fungi DNA Isolation Kit. Total DNA was also isolated from 250 mg samples of top soil, clay and potting soil using the modified Soil DNA Isolation Kit.

- DNA quantity was visually analyzed by running 10 µL aliquots of each elution on 1.5% agarose gels.

- Plant DNA quality was evaluated by using the isolated DNA as the template in real-time PCR reactions (SYBR Green) using plant universal 18s rRNA primers. For each real-time PCR reaction, 2 µL of the purified DNA was used as a template in 20 µL of PCR reaction volume. Soil DNA quality was evaluated by end-point PCR using prokaryotic specific primers. For each end-point PCR reaction, 1 µL of DNA template was used in 20 µL of PCR reaction volume, and 10 µL of each PCR product was run on a 1.5%

Results

A. Quantity of Genomic DNA Isolated from Various Plant Samples

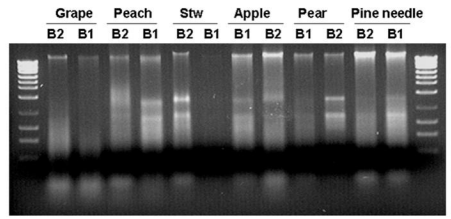

Figure 1. Comparison of total DNA yield from different plant samples when processed with Lysis Buffer 1 (B1) or Lysis Buffer 2 (B2). Ten µL of each 100 µL DNA elution was loaded on a 1X TAE, 1.5% agarose DNA gel without RNase treatment. The quantity of DNA isolated from grape, strawberry and pear leaf samples was significantly greater when Lysis Buffer 2 was used, while the quantity of DNA isolated from peach, apple and pine needle samples did not vary greatly when either Lysis Buffer 1 or Lysis Buffer 2 were used.

B. Quality of Genomic DNA Isolated from Various Plant Samples

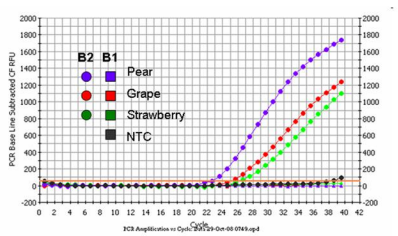

Figure 2. Comparison of DNA quality from different plant samples when processed with Lysis Buffer 1 (B1) or Lysis Buffer 2 (B2). Quality was tested by using the purified DNA as a template in real-time PCR (SYBR Green) with universal plant 18s rDNA primers. The data clearly showed that DNA prepared using Lysis Buffer 2 (B2) (coloured circles) was a much higher quality, and allowed for the detection of 18s rDNA in the real-time PCR system, while Lysis Buffer 1 (B1) (coloured squares) resulted in DNA that failed to amplify in the real-time PCR. The purple signal corrresponds to pear DNA, the red signal corresponds to grape DNA, and the green signal corresponds to strawberry DNA. The black signal is the non-template control.

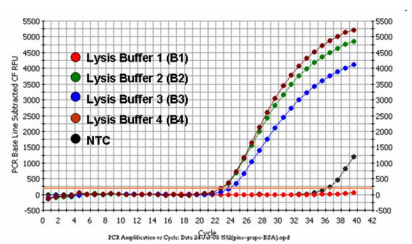

Figure 3. Comparison of DNA quality isolated from grape leaves when processed with Lysis Buffer 1 (B1), Lysis Buffer 3 (B3), Lysis Buffer 4 (B4) or Lysis Buffer 5 (B5). Quality was tested by using the purified DNA as a template in real-time PCR (SYBR Green) with universal plant 18s rDNA primers. The data clearly showed that DNA isolated from grapes using Lysis Buffer 3 (green circles), Lysis Buffer 4 (blue circles) and Lysis Buffer 5 (dark red circles) are of a high quality, and allow for the detection of 18s rDNA in the real-time system. Grape DNA isolated using Lysis Buffer 1 (red circles) failed to amplify, indicating that this Lysis Buffer does not alow for the isolation of high quality DNA. Similar results were seen in Figure 2 above using Lysis Buffer 1.

C. Quantity of Genomic DNA Isolated from Various Soil Samples

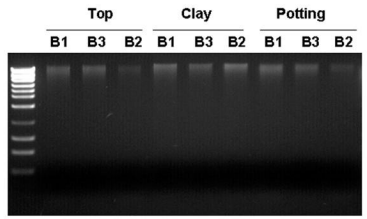

Figure 4. Comparison of total DNA yield from different soil samples when processed with Lysis Buffer 1 (B1), Lysis Buffer 2 (B2) or Lysis Buffer 3 (L3). DNA was isolated from 250 mg samples of top soil, clay and potting soil. Ten µL of each 100 µL DNA elution was loaded on a 1X TAE, 1.5% agarose DNA gel. There was very little difference observed in terms of DNA yield between Lysis Buffer 1 (B1), Lysis Buffer 2 (B2) and Lysis Buffer 3 (L3).

D. Quality of Genomic DNA Isolated from Various Soil Samples

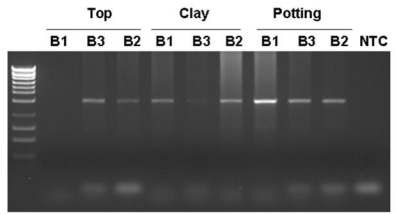

Figure 5. Comparison of DNA quality from different soil samples when processed with Lysis Buffer 1 (B1), Lysis Buffer 2 (B2) or Lysis Buffer 3 (L3). Quality was tested by using the purified DNA as a template in end-point PCR using prokaryotic specific primers. The data showed that DNA prepared using Lysis Buffer 2 (B2) and Lysis Buffer 3 (B3) was of the highest quality, as DNA isolated from all 3 soil types using these buffers could be successfully amplified using end-point PCR. DNA isolated using Lysis Buffer 1 could be amplified for 2 soil types (clay and potting soil).

Conclusions

- The use of different lysis buffers during the preparation of plant and soil DNA has an effect on the quantity and quality of DNA isolated.

- In terms of DNA quantity, it was found that in plants the yield of DNA was greatest when Lysis Buffer 2 was used. For the soil samples there did not appear to be very significant differences in yield when comparing Lysis Buffer 1, Lysis Buffer 2 and Lysis Buffer 3.

- In terms of plant DNA quality, it was found that DNA isolated using Lysis Buffer 1 showed the lowest quality, as the DNA failed to be amplified in all the real-time PCR reactions. DNA isolated using the other 4 buffers was of a high quality and could be amplified in the real-time PCR reactions.

- In terms of soil DNA quality, it was found that Lysis Buffer 2 and Lysis Buffer 3 resulted in the highest quality of DNA, as all the soil DNA samples could be successfully amplified in the end-point PCR reaction

- Overall Lysis Buffer 2 is the best buffer to allow for the isolation of high yields of plant and soil DNA that are also of a high quality and can be used successfully in sensitive downstream applications, therefore indicating that the DNA is free of inhibitors.

- Lysis Buffer 2 has been incorporated into Norgen's Plant/Fungi DNA Isolation Kit and Norgen's Soil DNA Isolation Kit, therefore these kits allow for the purification of high yields of DNA that are of the highest quality and can be used in sensitive downstream applications.

References

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