Evaluation of Pre-Lysis Rinses To Improve DNA Yield and Purity

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Evaluation of Pre-lysis Rinses To Improve DNA Yield and Purity

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Abstract

The widespread sampling of environmental DNA from soils has led to a fundamental shift in our understanding of Earth’s microbiome by identifying previously unknown microbes that have never been grown in the laboratory. Many of the published protocols for the extraction of environmental DNA differ based on the starting material. These differences include changes to buffer composition, detergents, and enzymatic digest. To remove this bias, we sought a standardized protocol for samples from Wind Cave National Park, which includes both above ground prairie soil and paleo-fill from the deepest depths of the cave system. Pre-lysis rinsing samples with either of two solutions, 100 mM sodium phosphate pH 7.2 (NaPO₄) and 100 mM Tris pH 8.0, 5 mM EDTA, 200 mM sodium chloride (TEN), was compared and the resulting DNA was visualized using agarose gel electrophoresis. Incorporating rinses resulted in darker bands of the expected size (greater than 10 kilobases) and less DNA degradation, meaning the rinses helped increase yield while isolating more intact DNA. In the future, including a pre-lysis rinse will improve the limit of detection in cave samples that contain low microbial abundance, allowing us to better understand microbial species composition in isolated environments.

Background

DNA extraction kits are commonly used for soils and other environmental samples which contain large amounts of inhibitors against DNA-sequencing because of their relative speed and ease of use. However, even the best commercial kits lose 83% of the starting DNA and thus can only isolate about 17% of the available sample (Hershey, Kallmeyer, and Barton 2019). This decreases the limit of detection for commercial kits. Instead of commercial kits, some researchers have published a variety of protocols designed for their particular environments. For example, Zhou, Bruns, and Tedje (1996) compared the effect of CTAB and PVP to lysis on humic contamination, using 5 g of starting material in their extraction buffer. A study by Hopkin-Robinson et al. (2018) used 8 g of soil as the starting material and used a sodium phosphate buffer to rinse the soil before running a DNA extraction kit on the product. Starting with 50 or 200 mg of soil, Gurna et al. (2020) conducted a study comparing SDS to CTAB as detergents in a phosphate lysis buffer. Each of these studies used different amounts of starting material with different buffers for protocols specific to the samples they obtained.

Pre-lysis rinsing of soil samples was used in an early study by Tsa and Olson (1991). Their protocol included a sodium phosphate pre-lysis rinse as part of the DNA extraction protocol and yielded bright bands on their agarose gel. Later studies (Tarvistiukka et al. 2018; Yamaguchi et al. 2012; Rainer W. Eib and Irene Wagner-Döblin 1993) followed the same methods as Tsa and Olson. He, Zu, and Hughes (2005) tested the effect of including a pre-lysis rinse to their DNA extraction protocol. They found that including a phosphate rinse before lysis of cells decreased humic acids and increased DNA yield when compared to the absence of a pre-lysis rinse. These limited results suggest sodium phosphate is a useful buffer for pre-lysis rinsing of environmental samples.

In this study, we compared pre-lysis rinses to test if they increase DNA yield and purity from our environmental samples. In addition to a sodium phosphate rinse, we tested a TEN rinse since TEN is the base of our lysisbuffer.

Methods

Results

DNA extraction kits are commonly used for soils and other environmental samples which contain large amounts of inhibitors against DNA-sequencing because of their relative speed and ease of use. However, even the best commercial kits lose 83% of the starting DNA and thus can only isolate about 17% of the available sample (Hershey, Kallmeyer, and Barton 2019). This decreases the limit of detection for commercial kits. Instead of commercial kits, some researchers have published a variety of protocols designed for their particular environments. For example, Zhou, Bruns, and Tedje (1996) compared the effect of CTAB and PVP to lysis on humic contamination, using 5 g of starting material in their extraction buffer. A study by Hopkin-Robinson et al. (2018) used 8 g of soil as the starting material and used a sodium phosphate buffer to rinse the soil before running a DNA extraction kit on the product. Starting with 50 or 200 mg of soil, Gurna et al. (2020) conducted a study comparing SDS to CTAB as detergents in a phosphate lysis buffer. Each of these studies used different amounts of starting material with different buffers for protocols specific to the samples they obtained.

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Table 1. Experimental Design for Soil and Stream Sediment Pre-lysis Rinse Trials

<table>
<thead>
<tr>
<th>Tube</th>
<th>Sample</th>
<th>Pre-lysis Rinse</th>
<th>Detergent Buffer</th>
<th>Chelator</th>
<th>Concentration</th>
<th>Lysis Buffer</th>
<th>Precipitation Buffer</th>
<th>Precipitated</th>
<th>Precipitation Buffer</th>
<th>Precipitated</th>
<th>Final Buffer</th>
<th>Final Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Soil</td>
<td>No Rinse</td>
<td>0.2% SDS 100 mM</td>
<td>EDTA</td>
<td>0.5% NaCl</td>
<td>TEN</td>
<td>1.5 μL Tris</td>
<td>20 μl Tris</td>
<td>1.5 μL Tris</td>
<td>20 μl Tris</td>
<td>1.5 μL Tris</td>
<td>TEN</td>
</tr>
<tr>
<td>2</td>
<td>Soil</td>
<td>TEN</td>
<td>0.2% SDS 100 mM</td>
<td>EDTA</td>
<td>0.5% NaCl</td>
<td>TEN</td>
<td>1.5 μL Tris</td>
<td>20 μl Tris</td>
<td>1.5 μL Tris</td>
<td>20 μl Tris</td>
<td>1.5 μL Tris</td>
<td>TEN</td>
</tr>
<tr>
<td>3</td>
<td>Soil</td>
<td>TEN</td>
<td>0.2% SDS 100 mM</td>
<td>EDTA</td>
<td>0.5% NaCl</td>
<td>TEN</td>
<td>1.5 μL Tris</td>
<td>20 μl Tris</td>
<td>1.5 μL Tris</td>
<td>20 μl Tris</td>
<td>1.5 μL Tris</td>
<td>TEN</td>
</tr>
<tr>
<td>4</td>
<td>Soil</td>
<td>TEN</td>
<td>0.2% SDS 100 mM</td>
<td>EDTA</td>
<td>0.5% NaCl</td>
<td>TEN</td>
<td>1.5 μL Tris</td>
<td>20 μl Tris</td>
<td>1.5 μL Tris</td>
<td>20 μl Tris</td>
<td>1.5 μL Tris</td>
<td>TEN</td>
</tr>
</tbody>
</table>

Citations

The addition of pre-lysis rinses yielded more DNA with less degradation (Fig. 2). It is unclear whether rinse solutions of TEN or sodium phosphate perform better as variation was found between separate trials (Fig. 3). Pre-lysis rinses had inconsistent effects on the purity of DNA (Table 2). Now, with a protocol that consistently gives us higher DNA yields in both soil and stream sediment, we can use this method for further study of the soils within and around the caves at Wind Cave National Park.

Acknowledgements

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