A new method for extracting DNA from the grape berry surface, beginning in the vineyard

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1. Introduction

Metagenomic analysis is now crucial to the study of microbial diversity, but its application in natural environments is problematic due to the need for high quality DNA obtained from less-than-ideal environmental situations. Isolating DNA from the surface of a grape berry involves aggressive and disruptive actions, due to tight adhesion of microbes to the thick berry skin and cuticle, making it difficult to wash microbes off the surface using most commercial kits, with some exceptions. More commonly, researchers have used culture-based methods or sampled grape must to conduct microbial ecology studies.

A plethora of DNA extraction methods exists already, ranging from DNA extraction kits to traditional methods, such as CTAB extractions. The efficacy and cost of these techniques vary. Many published methods are not useful for complex plant tissues which often contain inhibitory compounds, or for samples like grape berries that have a challenging surface from which to extract microbes. Furthermore, the process of DNA extraction typically begins in the laboratory after samples have been collected from the field, which increases the likelihood that microbial communities can be altered or disrupted from the time they are collected until the time they are processed. For example, published methods relying upon harsh chemicals, such as phenol or chloroform, require that samples be brought into the lab before the extraction procedure can begin, and additionally raise worker safety concerns. For these reasons, we developed a DNA extraction procedure that starts in the field, efficiently extracts microbial DNA from the surface of the grape, is cost effective, and can be made from commonly available laboratory chemicals with low toxicity.

2. Materials & methods

A grape berry of Vitis vinifera cv. Red Globe was excised directly from a cluster into a 50 mL Falcon tube containing 5 mL of a TE buffer (10 mM Tris-HCl + 1 mM EDTA, pH 8.0) solution containing 10% NaCl. 500 μL of 10% SDS was added to the Falcon tube containing the TE-NaCl solution, vortexed for 5 seconds and left at room temperature for 15 min. A freeze-thaw sequence consisting of 30 min in a -80 °C freezer and five min in 60 °C water bath was repeated three times to lyse the fungal and bacterial cells. The solution was then centrifuged at 15000 g, 4 °C for 10 mins to
remove debris and salt-SDS reaction flakes. The supernatant was carefully collected and a 750 μl aliquot was transferred to a 2 mL centrifuge tube, along with 750 μl ice-cold isopropanol and vortexed for approximately 30 s. The solution was centrifuged for 10 min at 4 C, 17000 g (13000 rpm). The supernatant was carefully removed from the tube and the pellet was washed twice with 500 μl of ice-cold 70% ethanol at 17000 g (13000 rpm) for 2 m. The pellet was re-suspended in 30 μl TE buffer after it was dried in 50 C heat block for 5–10 m.

For comparison to commonly used protocols, we used a CTAB extraction and the MoBio PowerSoil commercial kit, both with the TE + NaCl and SDS solution. For the CTAB extraction, a 500 μl aliquot of the supernatant was mixed well with 670 μl of CTAB buffer in a 2 mL centrifuge tube and heated at 65 °C for 30 m. After cooling down to room temperature, it was mixed with 750 μl of phenol:-

To understand how this DNA extraction procedure may be impacting the physical surface of the grape berry, we conducted three extractions of supermarket-purchased Vitis vinifera cv. Flame Seedless grape berries using a negative control protocol (just TE buffer, no NaCl or SDS) and three with the previously described extraction buffer, and used a scanning electron microscope (SEM) to observe the changes in the grape surface. The samples were vortexed for 5 s in their respective buffers, then the skin of the berries was removed and cut into 0.5 cm-diameter fragments. To prepare samples for the SEM, we fixed the fragments in 3% glutaraldehyde, then placed in a buffered phosphate solution, and then in a post-fixation of 2% osmium. We then conducted two rinses in the phosphate buffer. We immersed the samples for one hour in each of 25%, 45%, 70%, 95% and 100% ethanol, followed by critical point drying and sputter coating.

3. Results

3.1. PCR results

The 150-bp bacterial amplicon and the 280-bp fungal amplicons were observed in all replicates using each of the DNA extraction techniques (Fig. 1), meaning that each of the methods were successful in amplifying DNA off the grape berry surface. The amount of DNA retrieved similar in our method was not significantly different than that of the MoBio Inc., Hercules, CA). DNA analysis was performed on the QIAxcel Advanced system (Version: 9001421, QIAGEN, Germany) using the OM400 method described in the QIAxcel DNA Handbook. The results were displayed as a gel image using QIAxcel system software.

Comparison of three DNA extraction techniques in the amplification of bacterial and fungal DNA. (M) is the marker; (BA) is the positive bacterial control using Acetobacter acti et and extraction method (A); (BC) is the positive fungal control using Botrytis cinerea and extraction method (A); (A) is our method of DNA extraction from a grape berry; (B) is the extraction from a grape berry using a standard CTAB extraction; (C) is the extraction from a grape berry using the MoBio PowerSoil commercial kit. All 16S primers generated 150-bp amplicons while the ITS primer generated a 280-bp amplicons.
which the TE buffer was used alone (Fig. 3A), whereas there is a
extraction took between 2.5 and 3 hours.

50
extraction (Fig. 2). Per 10 samples, our method took approximately
PowerSoil Kit commercial kit and both were higher than that of the CTAB
were then
berry skin was removed and cut into 0.5 cm-diameter pieces. The skin fragments
Tris-EDTA buffer and vortexed for 5 s at room temperature, after which the

Fig. 3. Scanning Electron Microscope photos showing two 0.5 cm-diameter
pieces of grape berry skin from supermarket-purchased Vitis vinifera cv. Flame
Seedless grape berries. (A) A grape berry was placed in a 50 mL Falcon tube with
Tris-EDTA buffer and vortexed for 5 s at room temperature, after which the
berry skin was removed and cut into 0.5 cm-diameter pieces. The skin fragments
were then fixed in 3% glutaraldehyde and then observed with the SEM. (B) A
grape berry was placed in a 50 mL Falcon tube with Tris-EDTA–NaCl buffer
solution plus 10% SDS and vortexed for 5 s at room temperature, after which the
berry skin was removed and cut into 0.5 cm-diameter pieces. The skin fragments
were then fixed in 3% glutaraldehyde and then observed with the SEM.

PowerSoil Kit commercial kit and both were higher than that of the CTAB
extraction (Fig. 2). Per 10 samples, our method took approximately
50–60 minutes, while the MoBio PowerSoil Kit took 30–40 and the CTAB
extraction took between 2.5 and 3 hours.

3.2. Microscopy results

The SEM photos reveal an intact waxy cuticle on those fragments in
which the TE buffer was used alone (Fig. 3A), whereas there is a
disruption in the waxy cuticle in those in which the NaCl and SDS was
used in the extraction (Fig. 3B).

4. Discussion

This protocol was used in both Sanger sequencing and Illumina
sequencing studies [13], yielding fungal and bacterial data. In one study,
microbes were isolated from the surface of pea-sized grape berries, an
difficult to remove from the surface, it also works well for DNA
extractions of pure isolates in the lab [13].

Due to the increasing prevalence of microbiome studies, it is impor-
tant to develop new techniques that address the challenges of certain
matrices, like the grape berry surface. Techniques such as this one, which
not only successfully extract the microbes from the grape surface, but
does so in a safe, inexpensive, high-yielding and expeditious fashion
could allow for increased accessibility of microbial studies on many
different plant surfaces that were previously determined to have limited
microbial populations.

Table 1 Comparison of toxicity levels of reagents used in our method of DNA extraction,

|                  | Phenol | Chloroform | Ethanol | Isopropanol |
|------------------|--------|------------|---------|-------------|
| LD50 Dermal      | 630    | >20000     | 16000   | 12800       |
| LD50 Oral        | 317    | 695        | 7060    | 5045        |
| LC50 Inhalation  | 8h 900 mg/m³ | 47702 mg/m³ | 4h 117-125 | 4h 37.5 mg/m³ |
| Our method       | x      | x          |         | x           |
| MoBio            | x      | x          |         |             |
| PowerSoil Kit    |        |            |         |             |
| CTAB             | x      | x          | x       | x           |

sequencing, we continued using the protocol. While there may be options
for DNA extraction that provide higher-quality DNA, our method of
extraction is advantageous in three respects. (i) The reduced number of
steps should translate into increased yield, important for small biomass
samples, though admittedly at the cost of quality; (ii) The DNA extraction
begins in the field, by cutting the berries directly into a tube containing
the extraction buffer, and the agitation that it undergoes during transport
back to the lab aids in removing microbes from the berry surface; (iii) the
cost of materials required for the extraction procedure is very low in
comparison to the cost of commercial kits; and (iv), all of the solutions
required for the procedure have low toxicity, unlike the phenyl-
chloroform extraction in which part of the extraction procedure takes
place in a fume hood (Table 1). While we have used this technique solely
on grape berries, these three components allow this DNA extraction
method to be widely applicable in applied scientific research that in-
volves field sampling. Instead of risking the manipulation of surface
microbial communities by placing the sample in a bag and transporting it
to the lab, during which the sample is subjected to temperature and/or
humidity differences, as well as variable incubation periods, this tech-
nique allows the extraction to start as soon as the sample is removed from
the plant. Moreover, it is not only useful for samples in which the mi-
crobes are difficult to remove from the surface, it also works well for DNA
extractions of pure isolates in the lab [13].

$\begin{array}{cccc}
\text{mg/kg} & \text{Phenol} & \text{Chloroform} & \text{Ethanol} & \text{Isopropanol} \\
\text{LD50 Dermal} & 630 & >20000 & 16000 & 12800 \\
\text{LD50 Oral} & 317 & 695 & 7060 & 5045 \\
\text{LC50 Inhalation} & 8h 900 mg/m³ & 47702 mg/m³ & 4h 117-125 & 4h 37.5 mg/m³ \\
\text{Our method} & x & x & x & x \\
\text{MoBio} & x & x & & \\
\text{PowerSoil Kit} & & & & \\
\text{CTAB} & x & x & x & x \\
\end{array}$

Declarations

Author contribution statement

M. E. Hall: Conceived and designed the experiments; Performed the
experiments; Analyzed and interpreted the data; Contributed reagents,
materials, analysis tools or data; Wrote the paper.
L. Cadle-Davidson: Conceived and designed the experiments.
W. F. Wilcox: Conceived and designed the experiments.
Contributed reagents, materials, analysis tools or data. Z. Fang: Per-
formed the experiments.

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Fund.

Table 1
Comparison of toxicity levels of reagents used in our method of DNA extraction,
the MoBio PowerSoil Kit and with a CTAB extraction. All toxicity levels were
acquired from Safety Data Sheets (SDS). LD50 Dermal toxicity levels are reported
on rabbits and LD50 Oral and Inhalation toxicity levels are reported on rats.
Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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