

JoVE: Science Education

Community DNA Extraction from Bacterial Colonies

--Manuscript Draft--

Manuscript Number:	10218
Full Title:	Community DNA Extraction from Bacterial Colonies
Article Type:	Manuscript
Section/Category:	Manuscript Submission
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Table 1. Comparison of bacterial fractionation and *in situ* lysis methodologies for the recovery of DNA from soil.

Issue	Bacterial Fractionation	<i>In Situ</i> Lysis
Yield of DNA	1-5 μ g/g	1-20 μ g/g
Representative of community	Less representative because of cell sorption	More representative, unaffected cell sorption
Source of DNA recovered	Only bacteria	Mostly bacteria but also fungi and protozoa
Degree of DNA shearing	Less shearing	More shearing
Average size of DNA fragments	50 kb	25 kb
Degree of humic contamination	Less contaminated	More contaminated
Ease of methodology	Low, laborious	Faster, less labor-intensive

Table 2. Spectroscopic analysis of DNA.

<p>The amount of DNA is estimated from the 260 nm reading. An absorbance reading of 1.0 is equivalent to 50 µg of DNA per ml of solution.</p> <p>The purity of DNA is estimated from the ratio of the reading at 260 nm to that at 280 nm. A value > 1.7 indicates relatively pure DNA. The maximum theoretical value is 2.0.</p>
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JoVE Science Education Series: Environmental

Title: Community DNA Extraction from Bacterial Colonies

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Overview:

Traditional methods of analysis for microbial communities within soils have usually involved either cultural assays utilizing dilution and plating methodology on selective and differential media or direct count assays. Direct counts offer information about the total number of bacteria present, but give no information about the number or diversity of populations present within the community. Plate counts allow enumeration of total cultural or selected cultural populations, and hence provide information on the different populations present. However, since less than 1% of soil bacteria are readily culturable, cultural information offers only a piece of the picture. The actual fraction of the community that can be cultured depends on the medium chosen for cultural counts. Any single medium will select for the populations that are best suited to that particular medium.

In recent years, the advantages of studying community DNA extracted from soil samples have become apparent. This nonculture-based approach is thought to be more representative of the actual community present than culture-based approaches. In addition to providing information about the types of populations present, this approach can also provide information about their genetic potential. As with any technique, there are limitations to the data that can be obtained with DNA extraction. Therefore, many researchers now use DNA extraction in conjunction with direct and cultural counts to maximize the data obtained from an environmental sample.

Principles:

Initially, two approaches were developed for isolation of bacterial DNA from soil samples. The first was based on fractionation of bacteria from soil followed by cell lysis and DNA extraction. The second method involved *in situ* lysis of bacteria within the soil matrix with subsequent extraction of the DNA released from cells (**Table 1**). Subsequent to the development of these two approaches, *in situ* lysis has become the commonly used extraction procedure primarily because it is easier and faster, it yields more representative DNA, and because commercial kits have made it easier to purify the DNA.

Once DNA has been extracted from bacterial communities within soil, the DNA can be subjected to molecular analysis such as next generation sequencing or other

Commented [ASW1]: Since the procedure covers the fractionation technique as opposed to the *in situ* technique, provide a similar write-up of the principles for the fractionation.

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“omic” analyses to determine “who” was within the community and what “functions” they undertook.

The ***in situ* lysis** method involves lysing the bacterial cells within the soil and releasing their DNA prior to extraction of DNA from the sample. Lysis methodology has usually involved a combination of physical and chemical treatments. For bacteria, physical treatments have involved freeze–thaw cycles and/or sonication or bead beating, and chemical treatments have often utilized a detergent such as sodium dodecyl sulfate (SDS) and/or an enzyme such as lysozyme or proteinase. Following lysis, cell debris and soil particles are removed by precipitation and centrifugation, and the DNA in the supernatant is precipitated with ethanol. The DNA can be further purified by sorption onto homemade or commercial columns packed with ion-exchange resins or gels that can subsequently be rinsed for removal of humic materials that can inhibit DNA analysis. Further purification can be achieved with phenol–chloroform/isoamyl alcohol extractions, followed once more by ethanol precipitations. Pure samples of DNA are necessary to allow subsequent molecular analyses such as with the polymerase chain reaction (PCR). However, regardless of what purification methodology is employed, each step in the purification process causes loss of DNA. Thus, purified DNA is obtained only at the expense of DNA yield.

Once a sample of purified DNA is obtained from the soil sample, it can be quantified by ultraviolet (UV) spectroscopy or fluorometry. Normally, UV readings are made at wavelengths of 260 and 280 nm, from which the purity and quantity of DNA can be estimated (**Table 2**).

Commercial kits are now available for processing soils for community DNA extraction, but there are still problems related to the procedures. One problem is the sorption of DNA from the lysed cells by clay or humic colloids. In addition, some kits will lyse all soil microorganisms including fungi and protozoa.

In this video, community DNA extraction by lysing cells obtained from dilution and plating of soil on R2A media is illustrated. This can be followed by molecular analyses to identify all culturable isolates that were originally on the plate.

Procedure

1. Conduct a dilution and plating experiment to obtain culturable heterotrophic colonies on R2A media.
2. Select a plate with between 30 and 200 distinct bacterial colonies.
3. Gently rinse the plate with molecular grade water so that all visible portions of the bacterial colonies are contained within a water suspension. Capture water in 15 mL conical tube.

3.1 Colonies that do not rinse easily (actinomycetes) can be scrapped with a sterile loop and suspended in water by swirling the loop.

4. Vortex the suspension for 10_s to separate individual cells within the colony fragments.

5. Centrifuge for 1-2_min at >12,000_g_rpm to form a pellet at the bottom of the conical tube. Note: If cells do not grow well on the plate, or cell density is less than 10^7 , a pellet might not be visible after centrifugation.

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6. Aspirate water in conical tube down to ~500_μL to remove excess supernatant.

7. Resuspend pellet the water that remains by vortexing conical tube for 10_s. Cell concentration is now denser, since excess supernatant was removed.

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8. Transfer the 500_μL suspension into LoBind Eppendorf tube. Ensure cap is secure.

9. Place Eppendorf tube into floating device and then place it into bath containing boiling water for 10_min. This disrupts cell walls, releasing nucleic acids into supernatant.

10. Shear DNA by vortexing for 1-2_min.

11. Pellet cell debris by centrifuging for 5_min at >12,000 rpm in table-top centrifuge. Nucleic acids remain suspended in supernatant.

12. Transfer the top 100_μL of supernatant into a sterile LoBind Eppendorf tube.

12.13. Conduct spectroscopic readings on the DNA at 260/280 nm to determine the quantity and purity of the DNA.

13.1 If nucleic acid concentration is too high for accurate readings, dilute supernatant to 1:10 or 1:100.

Applications

1) Omic approaches for diversity estimates.

Community DNA from cultured colonies or extracted from soil can be subjected to bioinformatics and “omic” approaches that allow for characterization of the original bacteria within the sample. The omic approaches include:

- Metagenomics – determination of “who” is within the community via 16s rRNA sequencing. This gives an estimate of the diversity within the community.

2) Calculation of the number of bacterial cells within the soil sample.

Community DNA is extracted from a soil and quantified by spectroscopic analyses. The estimated quantity of DNA measured as μg DNA per mL of solution is related back to the total volume of DNA extracted in solution to give a total amount of DNA per g of soil. By knowing the theoretical value of DNA per cell, the total number of cells per g of soil can be calculated.

Example

A soil has 0.12 μg DNA per g of soil

If each cell has 4 femtograms of DNA

$$\text{Number of cells} = \frac{0.12 \times 10^{-6} \text{ g DNA/g soil}}{4 \times 10^{-15} \text{ g DNA/cell}}$$

$$= 0.03 \times 10^9 \text{ cells/g soil}$$

$$= 3 \times 10^7 \text{ cells/g soil}$$

3) Identification of specific bacteria within a community via PCR.

The extracted community DNA can be subjected to PCR analysis using specific primers to determine if a particular species is present within the community. Examples include specific bacterial pathogens such as *Clostridium perfringens* or *Bacillus anthracis*.