Bacterial abundance from soil or shrub leaf litter - Flow cytometry

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1 Works for me Share dx.doi.org/10.17504/protocols.io.bvqmn5u6

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ABSTRACT

Bacterial abundance is a fundamental metric for understanding the population dynamics of soil bacteria and their role in biogeochemical cycles. Despite its importance, methodological constraints hamper our ability to assess bacterial abundance in terrestrial environments. Here, we aimed to optimize the use of flow cytometry (FCM) to assay bacterial abundances in soil while providing a rigorous quantification of its limitations. Soil samples were spiked with *Escherichia coli* to evaluate the levels of recovery efficiency among three extraction approaches. The optimized method added a surfactant (a tetrasodium pyrophosphate [TSP] buffer) to 0.1 g of soil, applied an intermediate degree of agitation through shaking, and used a Nycodenz density gradient to separate the cells from background debris. This procedure resulted in a high (average, 89%) level of cell recovery. Recovery efficiencies did not differ significantly among sites across an elevation gradient but were positively correlated with percent carbon in the soil samples. Estimated abundances were also highly repeatable between technical replicates. The method was applied to samples from two field studies and, in both cases, was sensitive enough to detect treatment and site differences in bacterial abundances. We conclude that FCM offers a fast and sensitive method to assay soil bacterial abundance from relatively small amounts of soil. Further work is needed to assay differential biases of the method across a wider range of soil types.**IMPORTANCE**The ability to quantify bacterial abundance is important for understanding the contributions of microbial communities in soils, but such assays remain difficult and time-consuming. Flow cytometry offers a fast and direct way to count bacterial cells, but several concerns remain in applying the technique to soils. This study aimed to improve the efficiency of the method for soil while quantifying its limitations. We demonstrated that an optimized procedure was sensitive enough to capture differences in bacterial abundances among treatments and ecosystems in two field studies.

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MATERIALS TEXT

10 % Pi buffered GTA

0.4 g NaH2PO4

1.23 g Na2HPO4

Dissolve in **80 mL** distilled water

Filter sterilize

Add **20 mL** 50 % (wt/wt) Glutaraldehyde

1% Pi buffered GTA

1 part 10% Pi buffered GTA and 9 parts 0.9% Saline

Tetrasodium Pyrophosphate [M]250 Milimolar (mM) solution

11.15 g dissolve in 80 ml distilled water. Warm the solution to dissolve (DO NOT boil).

Let cool down before adjusting the pH**8.0** by adding HCl.

Fiter sterilize

Tetrasodium Pyrophosphate [M]50 Milimolar (mM) solution

Dilute the 250 mM solution of TSP in distilled water to **[M]50 Milimolar (mM)**

Filter sterilize

Tween 80 cat number P8074-100ml from Sigma Aldrich

Nycodenz 80% solution

12 g of Nycodenz dissolved in **8 mL** of **[M]50 Milimolar (mM)** TSP. Warm the solution while mixing.

Cool down solution and then fill up to **15 mL** .

Filter sterilize

200x Sybr Green

Dilute 10000x Sybr Green to 200x with TE pH 8.0

0.9% Saline
50 ml Falcon tubes
2 ml microcentrifuge tubes
microcentrifuge
vortexer
ultrasonic bath sonicator
Flow cytometer (Novocyte, Agilent)
0.2 um Syringe filters or bottle top filters (depending on the volume prepared)
Syringes
pipet tips 100-1000
2-10ul
5 ml

1 Add **5 mL** 1%Pi buffered GTA buffer to 0.1-0.2 g of soil or ground shrub leaf litter in a 50 ml tube. Store at **4 °C** in the dark up to 30 days.

2 When you are ready to extract bacteria from your samples add **1.2 mL** **[M]250 Milimolar (mM)** TSP solution and **31 µl** Tween 80 to a final concentration of **[M]0.5 % (v/v)**

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30m 30s

3 Vortex each sample **00:00:30** and transfer the samples into the ultrasonic bath sonicator. Sonicate samples for **00:30:00** at **4 °C**

3.1 In the meantime fill 2 ml microcentrifuge tubes with **0.5 mL** 80% Nycodenz solution

1m

4 Let the sample slurry stand for **00:01:00** after sonication.

5 ⚠

Carefully layer 2x **1.0 mL** aliquots of the slurry to two the tubes from step 4.

Slowly let the slurry run down the wall of the tube to layer on top of the Nycodenz

30m

6 Centrifuge tubes at **14000 x g** for **00:30:00**

6.1 While the samples are centrifuging fill an equal number of 2 ml microcentrifuge tubes with **1.0 mL** of **50 Milimolar (mM)** TSP buffer.

7 After centrifugation transfer the upper and middle cell-containing phases to the tubes with the TSP buffer from step 6.1. With environmental samples, the cell containing layer might be harder to recognize. Be consistent with your collection.

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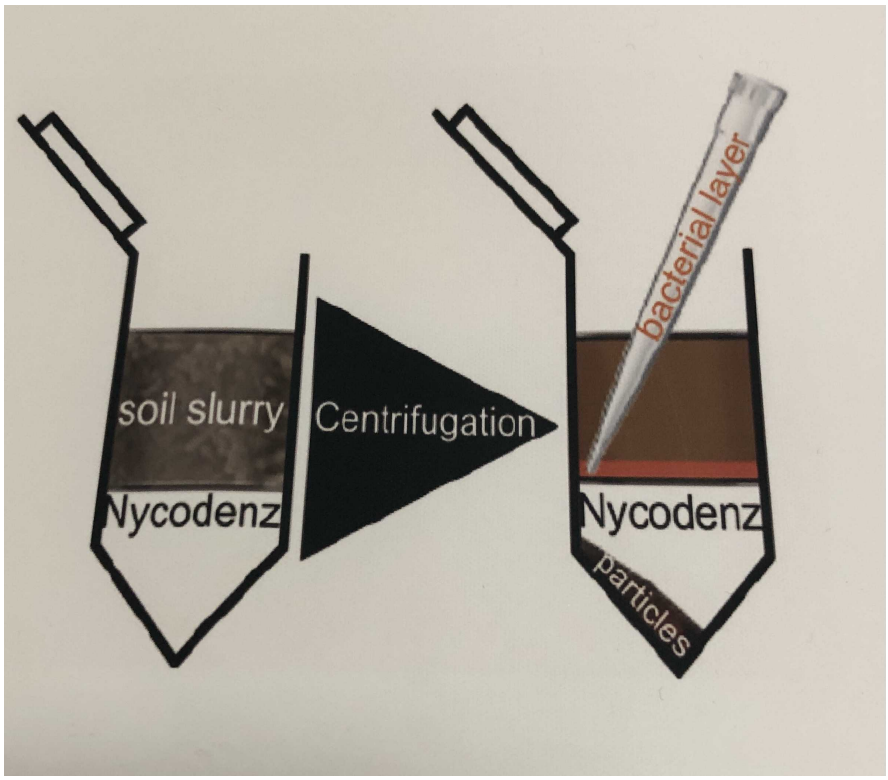

Schematic of result for step 5 through step 7

After samples are added to the TSP containing tubes mix those well.

8 Centrifuge tubes at **17000 x g** for **00:25:00** 25m

9 Discard the supernatant and resuspend the pellet in **0.8 mL** **50 Milimolar (mM)** TSP buffer. Cover the samples to protect them from light and store them at **4 °C**

10 On the Novocyte Flow cytometer use green fluorescence channel (FL1) and forward scatter (FSC-H) detectors to reduce the natural autofluorescence found in environmental samples. Vortex each sample before running on the Flow cytometer.
Each sample should be run at 40 ul / min for 1 min. Make sure that the events/sec stay between 100-1500 events/sec to get more reliable counts. Samples might need to be diluted differently if this number gets too high.
Run unstained Pi-buffered GTA as a control for autoflourescence of the buffer. And stained Pi-buffered GTA as a control that the buffer was not contaminated. Run a few unstained samples to account for autoflouresence from environmental conditions.

11 Take absolute counts (evnets/ul) from the gate and subtract the absolute counts within the gate from the background sample. Multiply this by the dilution factor and finally by 1000 to get counts/ml. To get counts / g dry weight litter make sure the calculations get adjusted accordingly.

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