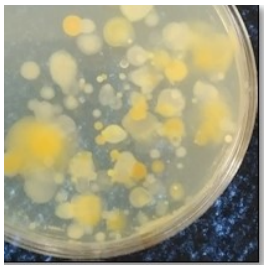
Jul 07, 2020

Preparation of soil bacteria for FCM



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Protocol status: Working
We use this protocol and it's working

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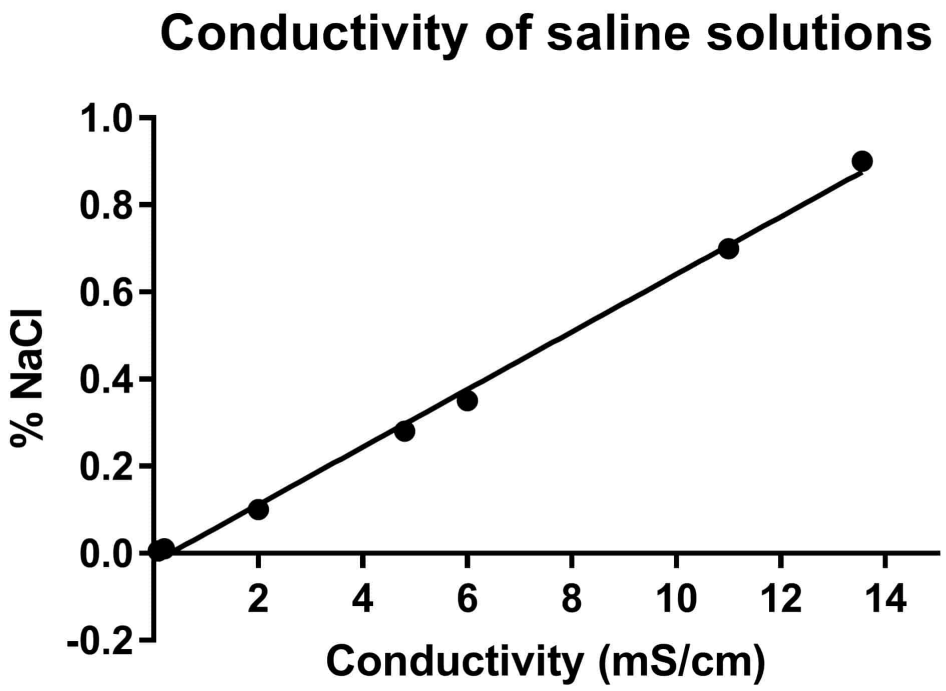
Extraction of soil bacteria

1 Measure the conductivity of the soil sample with a conductivimeter.

2 **Prepare solution N:** Add the needed amount of NaCl to 100 mL of distilled water to prepare a saline solution with the same conductivity that of the soil (see figure below). Autoclave.



Correlation between the concentration of NaCl in NaCl dilutions and their conductivity values

3 **Prepare the soil slurry:** Weight 20 g of soil and add 40 mL of sterile solution N.

4 Vortex the soil slurry for 00:20:00 .

5 Let the soil slurry settle for 00:05:00 .

6 **Remove big particles from soil slurry:** Prepare a sterile vacuum filtration system with a hydrophilic membrane of 12 µm of pore size. Filter the soil slurry and retain the pass-through.

7 **Wash the soil particles:** Prepare a sterile vacuum filtration system with a hydrophilic membrane of 0.1 µm of pore size. Filter the pass-through from step 6. Vortex the filter in a sterile standard tube with 5 mL of solution N for 00:02:00 .

8 **Concentration of the soil particles:** Centrifuge the suspension from step 7 (6000 x g, 20°C, 00:05:00). Remove supernatant.

Expected result

Pellet of approximately 10^9 particles between the sizes of 0.1 and 12 µm.

Resuspend in 2 mL of solution N.

9 **Concentration of soil bacteria by density centrifugation:** Prepare solutions of [M] 60 Mass Percent w/v (1.3 g/mL) Histodenz (Sigma-Aldrich). Autoclave. Take 2 mL from step 8 and carefully pour onto a Histodenz cushion of 2 mL.
Centrifuge (7155 x g, 20°C, 00:30:00).
Carefully recover the microbial fraction (shown in picture below inside the red circle).

Centrifuge the recovered fraction (6000 x g, 20°C, 00:05:00).

Expected result

Pellet of approximately 10^9 microorganisms (mostly bacteria).

Extraction of soil bacteria after cultivation on agar

10 Resuspend the pellet from step 9 in solution N. Prepare serial dilutions in solution N and plate onto R2A agar plates.

11 Incubate the plates at 17 °C for 96:00:00 .

12 Resuspend the grown biomass on agar plates from the previous step in solution N in several microcentrifuge tubes. Centrifuge them (6000 x g, 20°C, 00:05:00) and remove supernatant.

Expected result

Pellet of approximately 10^9 readily culturable microorganisms (mostly bacteria).

Staining procedure

13 **Prepare staining solution**:
To a solution of Potassium phosphate buffer pH 7.0 at [M] 0.1 Molarity (M) , add: Propidium iodide ([M] 80 micromolar (µM) in water), 5(6)-carboxyfluorescein diacetate ([M] 10 micromolar (µM) in water) and EDTA ([M] 60 micromolar (µM) in water).

14 Add 1 mL of staining solution to each pellet from steps 9 and/or 12. Mix by vortexing.

15 Incubate samples from step 14 in the dark at 30 °C for 00:30:00 .

16 Prepare FCM tubes with 1 mL of PBS added with bovine serum albumin ([M] 0.8 Mass / % volume and refrigerate them for 01:00:00 .

17 Add 10 µL of a sample from step 15 to a tube from step 16 for FCM analysis. Filter all the content of the tube through a hydrophilic membrane of 0.2 µm of pore size. Proceed to FCM analysis.