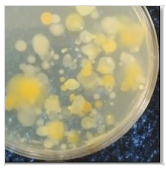

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Preparation of soil bacteria for FCM

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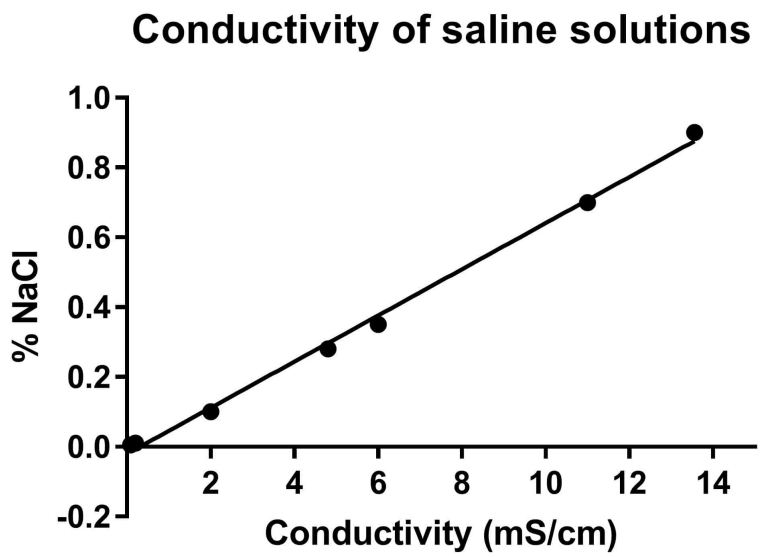
Extraction of soil bacteria

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.

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

Resuspend in 2 mL of solution N.

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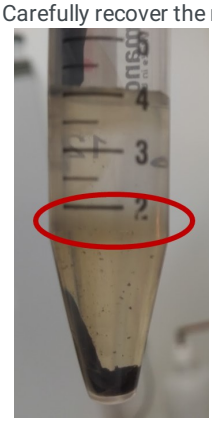
9 **Concentration of soil bacteria by density centrifugation:** Prepare solutions of [M]**60 Mass Percent w/v (1.3 g/mL)** Histodenz (Sigma-Aldrich). Autoclave. Take 2 mL from step 8 and carefully pour onto a Histodenz cushion of 2 mL.

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**).

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.

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**).

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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 (**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.

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