Isolation of microorganisms from soil and sediment samples

General considerations

- Soil and sediment samples are collected following the *Sample collection procedure.*
- Each sample are analyzed in triplicate.
- Selective media is used in order to identify a variety of microbial communities and the culture media used will correspond to the environments characteristics.
- Soils typically contain 10^9 to 10^{10} microorganisms per gram (dry weight), which may represent more than a million bacterial species

A. Soil samples

1. Isolation of bacteria by plating method

- Soil samples collected are analyzed following a sequential enrichment protocol to select the microorganisms existing in soil samples.
- 1 g of initial soil microbiota is subjected to serial 10-fold dilutions and further inoculated on solid media for isolation (Figure 1).

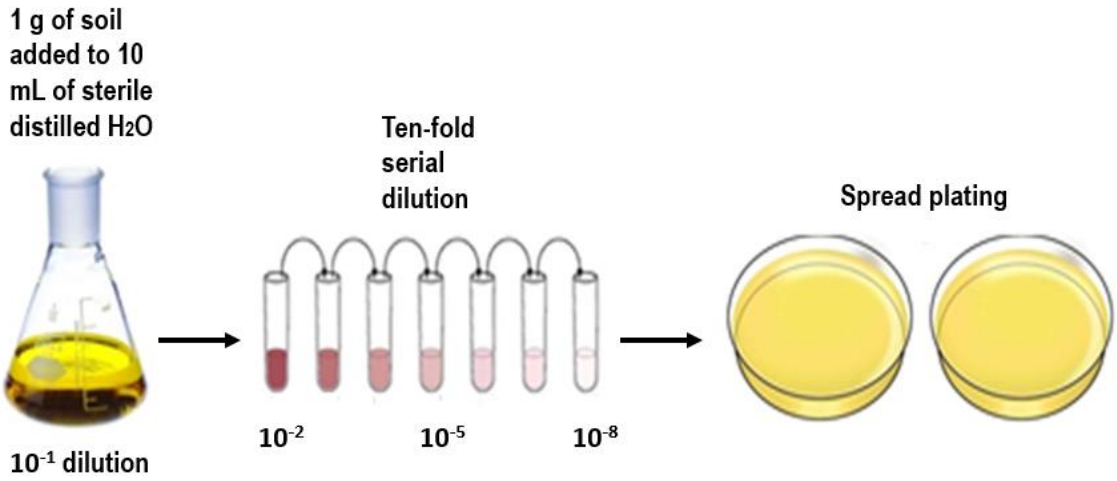

Figure 1. Soil dilution technique

- 1 g of soil is added to 10 mL of distilled sterile H_2O, resulting in a dilution of the sample by 1/10.
- The sample is incubated, under shaking, at room temperature for 30 minutes.
- Afterwards, before the soil settles, ten-fold serial dilutions are made by pipetting 1 mL of suspension from 1/10 dilution into 9 mL distilled sterile H_2O.
- Further, 1 mL of sample is transferred through successive 9 mL of distilled sterile H_2O in order to reach 10^{-5} dilution.

- 0.1 mL of each dilution is transferred aseptically to a Petri plate containing solid growth media and plate is inoculated by spread plating method.
    - For culturing fungi and actinomycetes, 1 mL of sample should be used.
- The plates are incubated in appropriated conditions (37°C for 24-48 h in case of bacteria, and 5-10 days for fungi and actinomycetes).
- The colonies are counted, characterized, and recorded.
- The results are expressed as the number of CFU per gram of soil.
- Isolated colonies with different morphologies are transferred in liquid media and after three consecutive propagations, single colonies, grown on solid media, are selected for further evaluation.
- The next steps, purification of colonies and the morphological characterization of microbial cultures, are described in the isolation of microorganisms from water sample, and will be applied as well for the soil and sediment samples (see *Isolation of microorganisms from water samples*).

2. Isolation of soil bacteria by membrane filtration method

- 1 g of soil is homogenized using 100 mL of peptone water in order to revive the bacterial species present in soil.
- The suspension is incubated at 30°C for 2 hours, under linear agitation.
- Serial dilutions are made due to the high amount of bacteria present in soil.
- The dilutions of 10^6 and 10^7 are filtered through 0.45 µm filter membrane and the filters are inoculated at the surface of the Tryptic Soy Agar plates and incubated at 30°C.

3. Direct isolation of heavy metal resistant strain by plating

- 2 g of soil are suspended in 20 mL of distilled sterile water, to obtain soil bacterial supernatant.
- The suspension are incubated at 30°C, with linear agitation of 150 rpm, for 30 minutes.
- Solid media (yeast tryptophan peptone glucose – YTPG) supplemented with heavy metals ($CuSO_{0.1}$) of different concentrations is used to isolate heavy metal tolerant bacteria.
- Some antifungals are added to the culture media to avoid interference of fungi in bacterial growth.
- 0.1 mL of the liquid bacterial supernatant is plated on each Petri plate and incubated at 30°C for 3-7 days.
- The formed colonies are transferred to plates containing higher heavy metal concentrations to screen highly tolerant strains
- In order to obtain highly resistant bacterial strains, the strains are transferred to liquid media (YTPG media) containing heavy metal concentration.
- The liquid cultures are incubated at 30°C, with linear agitation of 150 rpm.
- The isolation and obtaining of heavy metal resistant strains is necessary for future bioremediation processes.
- Some physiological and biochemical parameters, including temperature and pH range for growth, tolerance of different NaCl concentrations and resistance to antibiotics are tested.

B. Sediment samples

Studies on the soil/sediment highlight the impact of metal contamination on the groups of bacteria involved in the nitrogen cycle.

- Sediment samples were collected to approximately 1 cm depth using a sterile scoop.
- 1 g (wet weight) of sediment is dispersed into 9 mL of sterile saline
- The mixture is incubated at 37°C for 60 min with shaking at 200 rpm.
- The resulting slurry was then serially diluted with sterilized saline and 0.1 mL spread onto Petri plates containing solid media
- The Petri dishes are incubated at 37°C for one to six weeks.
- For the purification and morphological characterization of resulted colonies, the protocol described for water samples will be applied

References

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