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Isolation of fungal strains potentially active in soil remediation

This method allows the isolation of fungal strains from samples of polluted soil, making possible the assessment of their potential role in the biotransformation of organopollutants.

1. Method

1.1. Sample preparation

The amount equivalent to 10 grams of dry weight is calculated by weighing 10 g of sample on an electronic scale and dry it in an oven for 24 hours at 60 ° C. Suddenly, it is re-weighed and, with the proper proportions, was originally the equivalent of 10 g dry weight. Then, an amount equivalent to 10 g dry weight (dw) of soil is suspended in 90 mL of sterile tetrasodium pyrophosphate solution to disperse organic colloids. The suspension has to be homogenised by agitation for 30 min. The further dilutions are performed by adding 3 mL of soil suspension in 27 mL sterile physiological solution up to obtaining the 1:10000 dilution.

1.2. Sample inoculum

An aliquot (1 mL) of the sample final dilution is plated in a Petri dish (15 mm diameter). The inoculum is performed in at least 20 replicates: 10 on generic culture medium (MEA), 10 in specific medium (PR478A), containing the dye Poly R478, whose degradation by ligninolytic enzymes is considered predictive of the polycyclic aromatic hydrocarbons biodegradation capabilities [1]. The still liquid culture medium (30 mL) is to added far from the sample inoculum, in order to avoid inactivation of fungi due to the hot temperature.
Immediately after the medium addition, the plates are agitated with circular movement, in order to homogenised the inoculum, until the medium solidifies.

1.3. Sample inoculum in spread plates

An aliquot (1 mL) of the final dilution is inoculated in Petri dishes (15 mm diameter) containing a AW medium (30 mL) on which 600 µL crude oil solution was previously spread, as sole carbon source for fungal growth (in alternative standard hydrocarbons, such as naphthalene or n-hexadecane added on a disc of sterilized filter paper placed in the plate lid, can be used). The sample inoculum is spread with a sterile bend glass Pasteur pipette. The inoculum is performed in at least 10 replicates for each sample.

1.4. Plates incubation and count of colony forming units

Plates are incubated in the dark at the same temperature of the sampling site. Regularly colonies are counted and to isolate and to track the progress of slowly growing fungi, for 4 weeks. The presence of decolourisation halos is recorded. The number of colonies forming unit per g of dry weight (CFU g^{-1} dw) are calculated both for the total mycoflora and for each species or morphotype.

1.5. Statistical analyses

The nonparametric Spearman test was run to assess the significance ($p \leq 0.05$) of the quantitative and qualitative differences between the two media (MEA and PR478A) and between general medium MEA and selective medium with crude oil.

2. Materials

2.1 Sterile tetrasodium pyrophosphate solution

Prepare a 0.1 w/v $Na_4P_2O_7 \cdot 10\ H_2O$ solution and sterilize it.

2.2 Sterile physiological solution

Prepare a solution with 9 g L^{-1} NaCl and sterilize it.

2.3 Sterile Petri dishes

Sterile Petri dishes of 15 cm diameter.

2.4 Culture media

The generic MEA contains: 20 g L^{-1} malt extract, 20 g L^{-1} glucose, 2 g L^{-1} peptone, 18 g L^{-1} agar. The PR478A contains: 20 g L^{-1} malt extract, 20 g L^{-1} glucose, 2 g L^{-1} peptone, 18 g L^{-1} agar, 0.2 g L^{-1} Poly R478. After sterilisation by means an autoclave, both media are supplemented with 15 mg L^{-1} streptomycin sulphate and 50 mg L^{-1} chloramphenicol to inhibit bacteria growth. AW medium contains 9 g L^{-1} NaCl.

2.5 Crude oil solution

The crude oil qualitative standard (60 µl) are solved in 600 µl petrolether..

3. References

Leung P., Pointing SP. 2002. Effect of different carbon and nitrogen regimes on Poly R decolorization by white-rot fungi. Mycol Res, 106:86-92.