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Actinobacteria collection, enrichment and isolation

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1 *Works for me* dx.doi.org/10.17504/protocols.io.brztm76n

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MATERIALS TEXT

Materials

Glass jars with screw caps of the capacity of 600 ml
Cool boxes
Ice bags
pH meter
GPS
Notebook
Polypropylene tubes of 15 mL
Polypropylene tubes of 30 mL
Beakers 50 mL
Absorbent paper
Tips of 10 µL, 200 µL, 1000 µL
Permanent marker for labeling
Gloves
Slides and coverslips
Glass flask de 50 and 100 mL

Reagents

Double Concentrated Sales Broth (50% of water from the collection area add 50% distilled water, add 0.6% Yeast Extract, 2% glucose with 1% chloramphenicol and 1% fluconazole at pH 7)
Agar R2A (Kasvi ref. K25-610129)
Gram staining reagents
Modified Agar nutrient (Glucose 10.0 g, meat extract 1 g**,** Yeast Extract 3.0 g, peptone 3 g, Agar-Agar 20.0 g, Saline Water from the collection area 50 mL, Distilled Water 950 mL, pH 7, 0.1% Fluconazole, and 0.1 % Chloramphenicol)

Solutions

Sterile deionized water
Chloramphenicol at 1%
Fluconazole at 1%
R2A broth (Difco ref. 2 34000)
Artificial Seawater (ASW) (0.1 g of (KBr) potassium bromide; 70 g of (NaCl) sodium chloride; 10.61 g of ($MgCl_2$-$6H_2O$) magnesium chloride; 1.47 g of ($CaCl_2$-$2H_2O$) calcium chloride; 0.66 g of (KCl) potassium chloride; 0.04 g of ($SrCl_2$ _ $6H_2O$) strontium chloride; 3.92 g of (Na_2SO_4) sodium sulfate; 0.19 g of ($NaHCO_3$) sodium bicarbonate; 0.03 g of (H_3BO_3) boric acid)
NaCl
Glycerol 20%

Other

Micropipette of 10 µL, 200 µL, 1000 µL
Analytical balance
Freezer
Cryogenic freezer
Centrifugate
Water bath
Microscopy
Stereoscope

DISCLAIMER:

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Collection

1 Collect samples aseptically in 600 ml screw-capped glass jar.

2 Pack the flasks in cool boxes with ice bags and transport them to the laboratory

3 Identify each sample by writing a code, sampling date, depth, sample color, pH, and sample location

Enrichment and pre-treatment of samples

4 Prepare 10 mL of Double Concentrated Sales Broth at pH 7, in a beaker of 50 mL per sample.

5 Autoclave the broth in a glass flask, at 121°C and 15 psi of pressure for 20 min.

6 Inoculate the broth with 10 mL of water and sediment from saline samples and take them to a water bath at 50 °C for 60 minutes to reduce bacterial contaminants load that is not of interest.

7 Incubate flasks at 28 °C for 7 days in aerobic conditions

8 Verify the growth of bacteria through microscopic observation.

9 Use plates with Modified Nutrient Agar at pH 7 for the isolation of actinomycetes

10 Culturing was carried out from the surface with apparent growth confirmed by microscopic observation

11 Incubate the plates at 28 °C for up to 30 days, evaluating the development of characteristic colonies and taking into account criteria such as macroscopic aspects of shape, size, elevation, consistency, and color of the colonies, also, microscopic features that characterize aerial filaments, substrate, as well as reproductive filaments and physicochemical characterization.

12 The isolates were preserved in 20% glycerol at -80 °C at the laboratory

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Reactivation isolates

13 Store the cryopreserved isolates in a refrigerated chamber at -20 °C

14 Culture the isolates in R2A broth supplemented with artificial seawater (ASW) with 7% NaCl.

15 Culture the isolates in Petri dishes containing Agar R2A with ASW supplemented with 7% NaCl and incubate the plates at 28 °C for 7 to 30 days.

16 After Gram staining, observe under a microscope and stereoscope for macromorphological characterization.

17 Preserve the isolates in 20% glycerol and keep them at -80 ° C for subsequent molecular identification studies.

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