RUSSIAN ACADEMY OF SCIENCES
G.K.SKRYABIN INSTITUTE OF BIOCHEMISTRY AND PHYSIOLOGY OF MICROORGANISMS
ALL-RUSSIAN COLLECTION OF MICROORGANISMS

SOP Isolation of actinorhizal plant root nodule bacteria

Protocol designed by Oleg Stupar (June 1987).

1. Method

1.1. Surface sterilization

The nodules from the root system of each plant are subjected to sterilization to remove the extraneous bacterial flora.

1. Put the druses of nodules in 250 ml flask with 50 ml of 1% solution of nontoxic detergent (7X, Serva) and shake for 30-60 sec. Divide the druses into individual nodules and repeat washing with detergent solution. Rinse nodules with tap water five times. Thereby, large impurities such as sand and soil residues are dislodged.
2. Transfer 5-10 nodules to tube containing 10 ml of 96% ethanol and leave for 3 min while shaking several times. Remove nodules from ethanol and air dry on a Petri dish.
3. Transfer nodules to tube containing 3 ml of 3%OsO_4 solution for 3 min. (**CAUTION**! Use gas mask during working with OsO_4 solution to protect your lungs and eyes!)
4. Pour off OsO_4 solution to waste tank, add 10 ml sterile tap water to tube and shake for 1-2 min. Starting from this stage use sterile instruments to work with nodules. Repeat rinsing with sterile tap water for 5 times.

1.2. Isolation

Transfer individual nodule to sterile Petri dish, slice for pieces of 0.5-1 mm thick with scalpel and put pieces to tubes (1 piece per tube) containing 10 ml of sterile liquid OS-1 medium. Incubate tubes at 29°C for up to 120 days. Control the growth regularly each week. Isolated cultures of endophyte are identified as Frankia sp. using phase contrast microscopy by its characteristic morphology - by the presence of sporangia with immobile spores on substrate mycelium. The cultures can be stored by periodic sub culturing in OS-1 medium (up to for 3 month) or frozen in cryovials (Nunk).

2. Materials

2.1. Sterile tap water

Fill some bottles (0.5 or 1 l) and glass tubes (20 ml) with tap water and sterilize.

2.2. OsO_4 3%

Sterilize a bottle with 97 ml distilled water. Add afterwards 3.0 g OsO_4. Store the solution in a tightly closed bottle.

2.3. Sterile instruments

Sterilize forceps and scalpels in drying oven (60 min at 170°C)

2.4. OS-1 medium

K_2HPO_4 0.15 g
NaH_2PO_4 x H_2O 0.1 g
KCl 0.1 g
$MgSO_4$ x $7H_2O$ 0.1 g
Peptone 2.5 g
Yeast extract 0.25 g
Sodium acetate 0.5 g
Tween-80 0.5 g
Trace elements solution 1 ml
Distilled water 1000 ml

Trace elements solution:
FeNa citrate 5 g
H_3BO_3 0.75 g
$MnSO_4$ x $5H_2O$ 0.4 g
$ZnSO_4$ x $7H_2O$ 0.3 g
$(NH_4)_6Mo_7O_{24}$ x $4H_2O$ 0.1 g
$CoSO_4$ x $7H_2O$ - 0.01 g
Distilled water 1000 ml

Medium is sterilized 30 min at 121 °C.

3. References

Gavristov A.V., Stupar O.S., Zhitskay E.A. Influence of nutrient medium concentration on growth of Frankia sp. strain NLR 010210// Applied Biochemistry and Microbiology, 1990, N3, 399-403 (in Russian)