Protocol for selective growth of Acetobacter pomorum (Ap) and Lactobacillus plantarum (Lp)

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PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

Based on the protocol used in François Leulier's lab for selective growth of Ap and Lp *in vitro*.

Each step in this protocol corresponds to a day in the general procedure: step 1 corresponds to day 1, step 2 corresponds to day 2 (with everything that should be done on that day), and so on.

SAFETY WARNINGS

All the steps must be performed in laminar flow hood or by flame!

BEFORE STARTING

Plate Ap and Lp in fresh mannitol and MRS plates, respectively, from -80 ºC stocks. Don't use plates more than one month old. Incubate Ap at 30 ºC and Lp at 37 ºC for approximately 48 hours in the static incubators.

1 - Inoculate fresh mannitol (200 ml) and MRS (10 ml) media (less than a week old) with a single colony of Ap and Lp, respectively. Incubate Ap at 30 ºC, 180 rpm; and Lp at 37 ºC, without shaking.

2 - Dilute 250 µl Lp culture in fresh 10 ml MRS media.

- Ask for MRS, MRS + Kanamycin (50 µg/ml) and MRS + Ampicillin (10 µg/ml) plates. The plates should be as fresh as possible - don't use plates more than one week old.

3 - Measure OD (600 nm) of each culture.

- Dilute the highest OD (should be Lp) to the same value of the lowest OD in liquid media.
  - For example, if Lp has an OD of 4.6 and Ap, 0.61, dilute Lp in 1 ml MRS by mixing 132 µl Lp culture and 868 µl MRS to obtain cultures with equivalent numbers of Lp and Ap cells.

- Prepare adequate Ap and Lp dilutions in PBS 1X and inoculate 50 µl of each culture (Ap and Lp) in each plate:
  - Two MRS plates
  - One MRS + Kan plate
  - One MRS + Amp plate

- Use sterile glass beads to spread the cells and incubate the plates (for 48 hours) as follows:
  - One MRS plate at 37ºC (optimal growth of Lp)
  - One MRS plate at 30ºC (optimal growth of Ap)
  - MRS + Kan plate at 37ºC
  - MRS + Amp plate at 30ºC

4 - Verify if Ap and Lp have grown in the following conditions:
  - Ap should only grow in MRS (either 37ºC or 30ºC) and MRS + Amp
  - Lp should only grow in MRS (either 37ºC or 30ºC) and MRS + Kan

5 For separate plating of Ap and Lp, the same steps are performed, but 50 µl of each culture are plated in each plate of MRS, MRS + Kan and MRS + Amp, separately. The total number of plates will double.