Growing Drosophila gut bacteria

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Abstract

This protocol is part of the manuscript: Gonçalves et al. Commensal bacteria and essential amino acids control food choice behavior and reproduction. Plos Biology. 2017 Apr 18.

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Guidelines

The following bacterial species and strains (kindly provided by François Leulier, IGFL, France and Won-Jae Lee, SNU, South Korea) were used in this study: *Lactobacillus plantarum*^{WJL} [1], *Lactobacillus brevis*^{EW} [1], *Acetobacter pomorum* [1]*, Commensalibacter intestini*^{A911T} [1] and *Enterococcus faecalis* [2].

All bacterial media and bacterial manipulations are performed in a laminar flow hood.

Solid media are prepared as follows:

- MRS - Mix 51 g/l of MRS (Sigma-Aldrich, #69966), 4 ml/l of Tween 20 (Sigma-Aldrich, #P9416) and 15 g/l agar (Difco, #214530) with milliQ filtered water. Autoclave the medium for 15 minutes at 121ºC and pour into Petri dishes.
- Mannitol medium - Mix 3 g/l Bacto peptone (Difco, #0118-17), 5 g/l yeast extract (Difco, #212750), 25 g/l D-Mannitol (Sigma-Aldrich, #M1902) and 15 g/l agar (Difco, # 214530) with milliQ filtered water. Autoclave the medium for 15 minutes at 121ºC and pour into Petri dishes.
- LB - Mix 35 g/l of LB Broth with Agar (lennox) (Sigma Aldrich #L2897) with milliQ filtered water.

Autoclave the medium for 15 minutes at 121ºC and pour into Petri dishes.

Liquid media are prepared as follows:

- MRS - Mix 51 g/l of MRS (Sigma-Aldrich, #69966) and 4 ml/l of Tween 20 (Sigma-Aldrich, #P9416) with milliQ filtered water. Autoclave the medium for 15 minutes at 121ºC.
- Mannitol medium - Mix 3 g/l Bacto peptone (Difco, #0118-17), 5 g/l yeast extract (Difco, #212750), and 25 g/l D-Mannitol (Sigma-Aldrich, #M1902) with milliQ filtered water. Autoclave the medium for 15 minutes at 121ºC.
- LB - Mix 20 g/l of LB Broth (Sigma-Aldrich #L3022) with milliQ filtered water. Autoclave the medium for 15 minutes at 121ºC.

References

1. Ryu J-H, Kim S-H, Lee H-Y, Bai JY, Nam Y-D, Bae J-W, et al. Innate Immune Homeostasis by the Homeobox Gene Caudal and Commensal-Gut Mutualism in Drosophila. Science. 2008;319: 777–782. doi:10.1126/science.1149357

2. Cox CR, Gilmore MS. Native Microbial Colonization of Drosophila melanogaster and Its Use as a Model of Enterococcus faecalis Pathogenesis. Infect Immun. 2007;75: 1565–1576. doi:10.1128/IAI.01496-06

Before start

All bacterial media and bacterial manipulations are performed in a laminar flow hood.

Protocol

Start bacterial cultures on plates

Step 1.

Prepare MRS, Mannitol and LB plates according to the Guidelines.

All steps should be performed in a laminar flow hood and all reagents and material should be sterile.

Start bacterial cultures on plates

Step 2.

Start the bacterial cultures by streaking out *Lactobacilli* in MRS agar plates, *A. pomorum* and *C. intestini* in Mannitol agar plates, and *E. faecalis* in LB agar plates starting from bacterial stocks that have been kept at -80ºC in 50% glycerol using a 1 µl sterile loop.

Start bacterial cultures on plates

Step 3.

Incubate the MRS plates (*Lactobacilli)* at 37°C, for 48 h; the Mannitol plates (*C. intestini*^{A911T} and *A. pomorum)* at 30°C, for 24-48 h; and the LB plates (*E. faecalis)* at 37°C for 24 h.

DURATION
48:00:00

NOTES
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These plates can be kept at 4ºC if not used immediatly.

Liquid bacterial cultures

Step 4.

Prepare liquid MRS, Mannitol and LB media according to the Guidelines.

Liquid bacterial cultures

Step 5.

Pick a single colony to start liquid culture using a 1 µl sterile loop. Wash the loop with the bacteria in the different liquid media as follows:

- Culture *Lactobacilli* in 10 ml of liquid MRS medium using 14 ml culture tubes (Thermo Scientific,#150268), at 37°C, for 24 h without agitation.
- For *C. intestini* use 20 ml of liquid mannitol medium in 50 ml tubes (Falcon). For *A. pomorum* use 200 ml of medium in 500 ml flasks (Sigma Aldrich #CLS4985500). Culture *C. intestini* and *A. pomorum* at 30°C, for 48h using 170 rpm agitation.
- Culture *E. faecalis* in 200 ml of liquid LB medium in 500 ml flasks (Sigma Aldrich #CLS4985500), at 37°C for 24 h using 220 rpm agitation.

NOTES
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Remember to include all the corresponding media without any bacterial inoculation as controls for possible media contaminations.

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We found these bacteria to have very reproducible growth rates, having ODs close to the

references that we describe in Calculating the internal bacterial load of flies protocol. If these ODs are much higher than the reference values, it likely means that a contamination occured. If they are much lower, it is likely to mean that the bacteria are no longer healthy. In these cases we advise that you start a new solid culture from the frozen bacterial stock.

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Inspect the morphology of the colonies on the plates to ensure the purity and health of the bacterial culture.

Step 6.

If you are doing consecutive experiments start a new solid culture when preparing the liquid cultures. Pick a colony from the cultures on the plates and proceed as described in Step 2.