Calculating the internal bacterial load of Drosophila

Zita Santos, Patrícia Francisco, Ricardo Leitão-Gonçalves, Margarida Anjos, Célia Baltazar, Ana Paula Elias, Gabriela Tondolo Fioreze, Margarida Anjos, Célia Baltazar, Ana Paula Elias, Pavel M. Itskov, Matthew D. W. Piper, Carlos Ribeiro

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.

Citation: Zita Santos, Patrícia Francisco, Ricardo Leitão-Gonçalves, Margarida Anjos, Célia Baltazar, Ana Paula Elias, Gabriela Tondolo Fioreze, Margarida Anjos, Célia Baltazar, Ana Paula Elias, Pavel M. Itskov, Matthew D. W. Piper, Carlos Ribeiro Calculating the internal bacterial load of Drosophila. **protocols.io**

dx.doi.org/10.17504/protocols.io.heib3ce

Published: 25 Apr 2017

Before start

All reagents (1x PBS) and materials (pestles, beads) used in this protocol need to be sterile.

Protocol

Step 1.

Prepare enough MRS, mannitol and LB plates to perform at least 3 replicates of at least 2 dilutions per condition, according to the Guidelines in Growing Drosophila gut bacteria protocol.

Step 2.

Sample preparatio

Step 3.

Take these Eppendorf tubes to the flow hood and process all conditions in parallel.

The next steps are performed in a laminar flow hood and all reagents and material need to be sterile.

Step 4.

Add 70% ethanol to the Eppendorf tube and invert it multiple times during one minute to surface

sterilize the flies.

DURATION
00:01:00

Sample preparation - Washing flies
Step 5.

Remove the ethanol.

Sample preparation - Washing flies
Step 6.

Add sterile 1x PBS, mix well and remove.

Sample preparation - Washing flies
Step 7.

Repeat step 6 once.

Sample preparation - Homogenizingflies
Step 8.

Add 100 µl of 1x PBS to each Eppendorf tube (EppA) and homogenize using a sterile pestle until there are no intact flies and the sample looks homogeneous.

NOTES
Carlos Ribeiro 30 Mar 2017

We start the homogenization with a smaller volume so that the PBS does not spill out of the Eppendorf tube.

Sample preparation - Homogenizing flies
Step 9.

Add 400 µl of 1x PBS to each Eppendorf tube for a total volume of 500 µl of sample.

Sample preparation - Homogenizing flies
Step 10.

Wait that the debris sink. This can take up to 5-10 minutes. Use this time to process the next conditions.

DURATION
00:05:00

Sample preparation - Homogenizing flies
Step 11.

Prepare a new Eppendorf tube (Epp B) for each condition.

Sample preparation - Homogenizing flies

Step 12.

Pipette 250 µl of supernatant from the EppA solution into EppB and add 200 µl of 1x PBS to EppB. If you are not able to pipette 250 µl of the samples without taking a lot of debris, you should wait until the samples settle a bit more.

Sample preparation - Homogenizing flies

Step 13.

At this stage every EppB should contain the final volume of 450 µl.

Serial dilutions

Step 14.

Do serial dilutions (1:100 and 1:1000) for each condition as follows in the next steps.

Serial dilutions

Step 15.

To make a 1:100 dilution, pipette 100 µl from EppB tube to a 15 ml Falcon tube and add 9900 ul of 1x PBS.

Serial dilutions

Step 16.

To make a 1:1000 dilution, pipette 1 ml from the 1:100 dilution to a 15 ml Falcon tube and add 9 ml of 1x PBS.

Inoculation of the plates with bacteria

Step 17.

To inoculate the plates transfer 50 µl solution from the corresponding Falcon tube onto the plate making sure the liquid is distributed over its surface.

Inoculation of the plates with bacteria

Step 18.

Transfer sterile glass beads to the plates, stack 4-5 plates containing the beads and shake them vigorously in all horizontal directions until the sample covers the plate surface evenly.

Inoculation of the plates with bacteria

Step 19.

Remove the beads by just opening the lid the minimum amount necessary to avoid contamination.

Inoculation of the plates with bacteria

Step 20.

Repeat steps 17-19 in this section for all conditions/dilutions.

Inoculation of the plates with bacteria

Step 21.

Incubate MRS plates at 37ºC for 48h, LB plates at 37ºC for 24h, and Mannitol plates at 30º C for 24h. It is advisable to check the plates before the full time has elapsed to avoid an overgrowth of the culture due to a high amount of colonies which could preclude counting separate colonies.

NOTES

Carlos Ribeiro 10 Apr 2017

Much longer incubation times can lead to the appearance of unspecific colonies from other than the 5 bacteria being surveyed by this protocol. This is especially the case when using non gnotobiotic flies.

Calculation of bacterial CFU

Step 22.

Count the number of colonies in each plate making sure that the plate only contains separated, well defined single colonies. Calculate the CFU/fly for every plate using the following formulas:

- CFU/ml = ((total number of colonies on a plate) * (dilution factor (100 or 1000) * 1,8)) / (plated volume)
- CFU/fly = ((CFU/ml) * total volume in EppA)) / (number of flies per condition)