Determination of the *Dictyostelium discoideum* plaque-forming dose 50% (PFD_{50})

Nathan Fisher

July 5, 2014

Abstract

Your abstract.

1 Introduction

This protocol is designed to quantitate the relative susceptibility of one or more test species of bacteria to predation by the bacterivorous amoeba *Dictyostelium discoideum*. The PFD_{50} is a measure of the number of amoeba cells required to form a visual plaque on a lawn of the test bacteria 50% of the time. It is analagous to the lethal dose 50% calculation that many scientists are familiar with.

2 Required Materials

- Fresh experimental and control bacteria strains grown on agar plates (DH5a, *E. coli B/r*, and *K. aerogenes* are typical negative controls).
- Sterile broth media (usually LB or SM/2).
- Sterile 15-ml conical tubes, one per bacteria strain.
- Mature plate of *D. discoideum* with obvious fruiting bodies.
- SM/2 agar plates (with any required additives), 3 per strain/condition to be tested.
- Sterile eppindorf tubes.
- Sterile PCR-tube strip (8) for dilutions.
- Parafilm.
- Sterile ddH_2O.
- Sterile plastic or glass spreaders.
- Sterile plastic or metal inoculation loops.
- P10 or P20, P200, and P1000 pipetters and sterile tips.

- Multichannel P10 or P20 pipetter.
- Microcentrifuge.
- Spectrophotometer and cuvettes.
- Form XXX (excel spreadsheet).

3 Procedure

Day 1

1. For each bacteria to be tested, add 3-ml sterile broth media (usually LB or SM/2) to a 15-ml conical tube.
2. Using aseptic technique, inoculate each tube with a single bacterial colony from an agar plate.
3. Incubate overnight at an appropriate temperature, shaking at approx. 250-rpm.

Day 2

4. For each bacteria to be tested, add 3-ml sterile broth media (usually LB or SM/2) to a new sterile 15-ml conical tube.
5. Asceptically transfer 300-μl of the overnight to the new tube.
6. Incubate at an appropriate temperature, shaking at approx. 250-rpm, for 6-8 hours or until the culture reaches early stationary phase.
7. Transfer 1-ml of each culture to a sterile eppindorf tube.
8. Pellet cells in a microcentrifuge (Max speed for 30-sec).
9. Remove supernatant and suspend pellet in 1-ml sterile ddH_2O.
10. Transfer 100-μl to a clean cuvette with 900-μl ddH_2o. Mix well.
11. Read the OD_{600} of the 1:10 dilution and enter it into Form XXX.
12. Follow directions in Form XXX to adjust the OD_{600} of the bacterial suspension to 1.0.
13. Transfer three, 100=μl aliquots to three SM/2 agar plates with any necessary additives. (Typically, you'll need three plates per bacteria per condition tested).
14. Spread evenly over the surface of the agar and allow to dry for 1-hour.

    Note: If plates remain moist after 1-hour, dry under the laminar flow hood for 15-min with the lid slightly ajar.
15. While plates dry, fill seven sterile eppendorf tubes with 900-μl of ddH_2O, cap and set aside.

16. Using a sterile inoculating loop, harvest 50-100 large fruiting bodies from the *D. discoideum* plate taking care to avoid contact with the agar and bacterial lawn.

    Note: this procedure generates enough amoeba spores to test 38 strains and/or conditions, including controls.

17. Suspend fruiting bodies in 1-ml sterile ddH_2o in an eppindorf tube.

18. Ensure cap is tight and wrap with parafilm. Vortex on high setting for 2-min.

19. Pipette up-and-down at least 10 times with a P200 set to 200-μl to further disrupt fruiting bodies and release spores.

20. Using eppindorf tubes prepared in step 15, prepare 1:10 serial dilutions of amoeba spores.

    Need to include spore titer.

21. Using the multichannel P10 or P20 pipetter, spot 4 rows of 2-μl of each amoeba spore dilution...

    Metal transfer tool worked better (5 dilituions). This makes it challenging to calculate dose given. Could adjust titer in PFD50 worksheet such that it forces the Ec or Ka PFD50 to be 0.5 spores. OR could calculate spores/ml from hemocytometer. Maybe this is the best way, as long as there is an Ec or Ka control group.

3.1 How to Leave Comments

Comments can be added to the margins of the document using the todo command, as shown in the example on the right. You can also add inline comments:

This is an inline comment.

Here's a comment in the margin!

3.2 How to Include Figures

First you have to upload the image file (JPEG, PNG or PDF) from your computer to writeLaTeX using the upload link the project menu. Then use the includegraphics command to include it in your document. Use the figure environment and the caption command to add a number and a caption to your figure. See the code for Figure 1 in this section for an example.

3.3 How to Make Tables

Use the table and tabular commands for basic tables — see Table 1, for example.

Figure 1: This frog was uploaded to writeLaTeX via the project menu.

Item	Quantity
Widgets	42
Gadgets	13

Table 1: An example table.

3.4 How to Write Mathematics

LaTeX is great at typesetting mathematics. Let $X_1, X_2, \ldots, X_n$ be a sequence of independent and identically distributed random variables with $\mathrm{E}[X_i] = \mu$ and $\mathrm{Var}[X_i] = \sigma^2 < \infty$, and let

$$S_n = \frac{X_1 + X_2 + \cdots + X_n}{n} = \frac{1}{n}\sum_{i}^{n} X_i$$

denote their mean. Then as n approaches infinity, the random variables $\sqrt{n}(S_n - \mu)$ converge in distribution to a normal $\mathcal{N}(0, \sigma^2)$.

3.5 How to Make Sections and Subsections

Use section and subsection commands to organize your document. LaTeX handles all the formatting and numbering automatically. Use ref and label commands for cross-references.

3.6 How to Make Lists

You can make lists with automatic numbering ...

1. Like this,
2. and like this.

... or bullet points ...

- Like this,
- and like this.

... or with words and descriptions ...

Word Definition

Concept Explanation

Idea Text

We hope you find writeLaTeX useful, and please let us know if you have any feedback using the help menu above.