Host Range Project: SEA-PHAGES 2016-2017

The Project

Test whether a panel of phages can infect a host other than *Mycobacterium smegmatis* mc^{2}155.

Explanation

We have collectively isolated and sequenced over 1000 phages on *Mycobacterium smegmatis* mc^{2}155. Though some testing has been done to determine whether these phages can infect hosts other than the one they were isolated on, we have yet to make a concerted, focused effort to characterize the host range of these phages. This goal of this project is to test a known panel of ~20 phages on a variety of hosts and thus collect meaningful and consistent host range information about these phages.

How it works

You select a host, within the Actinobacteria, to use for the project. The phylogenetic tree for the Actinobacteria is too large to display here, but you can evaluate possible host choices by reviewing phylogenies of the Actinobacteria at the Patric website. You will probably want a host that is readily available from either ATCC or ARS-NRRL, and that is reasonably closely related to *Mycobacterium smegmatis* mc^{2}155. You will order the host (or hosts) and grow them at your institution. We will coordinate the project to make sure we're not duplicating hosts at different institutions.

We will then send you 200 µl of each of twenty (20) mycobacteriophage lysates. You should use those aliquots to freeze and store the lysates at your institution AND test on the additional host(s) that you have. You may also want to amplify the phages that you receive. Remember to amplify from a single plaque.

Instructions

To participate, you must sign up and complete an MTA to receive the phages. The MTA must be completed by **July 25, 2016**. To sign up, send an email to djs@pitt.edu stating what host(s) you will use, your intentions to participate, AND update your seaphages.org contact information to include your institution's legal contact name and email AND make sure the shipping address is correct. We will then process the MTA. You and your legal contact will need to sign it for its completion, so look for its return to you. We intend to ship the lysates on **October 1, 2016**.

When you obtain your host, you will want to prepare aliquots to freeze for future culture seedings. You will also want to make sure you can make a smooth culture to plate a lawn.

Protocol

1. Plate your bacteria as per recommendations at PhagesDB.

1. Using PDG protocol 11.4, prepare plates for the two strains (mc^{2}155 and your host).

2. Prepare serial dilution of phages to be tested (sequenced or unsequenced), and spot them on both plates in rows as shown in PDG Figure 11.4.1, with the most concentrated spots on the left and most dilute on the right. You can spot six different phages on each set of plates.

3. Observe the plates for lysis. Photograph your plates, and post them on the Host Range Project forum at seaphages.org. Email djs@pitt.edu about any candidate phages that you find.

Findings
There are 3 predictable 'types' of results.

1. No infection is observed. Record and report findings.
2. Infection is observed with a plating efficiency (EOP) of 1. That means that the phage infects your host at the same frequency as it infects m. smegamtis mc^2155.
3. Infection is observed with a plating efficiency of less than 1. In particular, one that plates at an EOP of $<10^{-3}$.

Interpretations and Further Investigations:

If the phages do not infect your host, record and report your findings. No further investigation is needed. Photographs are required. But now that you have a new host you've grown, why not try finding some phages that can infect it?

If the phages infect the new host and M. smegmatis mc2155 with an EOP of 1, record and report your findings. Photographs are required. This finding demonstrates that the phage has a broader host range.

If the phages infect the new host with an EOP of less than 1 but $>10^{-3}$, you will want to investigate further. This can involve the following:

- A careful repeat of the initial screening
- Purification of the mutant that is infecting the new host
- Sequencing of the the mutant.

Before proceeding, please touch base with the Hatfull lab by emailing djs@pitt.edu.