Phages infecting *Gordonia terrae*

The ATCC strain 25594 is *G. terrae* C-6: a non-motile, gram-positive Actinobacterium (a phylum of gram-positive bacteria that can be either terrestrial or aquatic).

*G. terrae* part of the *Gordoniaceae* family that is an enzothiophen-desulfuring bacterium isolated from oil-contaminated soil in GuDao Oile Filed, China.

The typical morphology of *G. terrae* round, convex, slightly raised, slightly rough, dry and 1-3mm colonies.

The lawn of *G. terrae* typically reflect a pinkish-orange color and most colonies are orange, which deepens with time. *Gordonia* typically takes about 2 days for the lawn to grow at 37°C.

The plate to the right shows a typical plaque morphology for a *Gordonia* phage “S&P-500” isolated in Fall 2017. Plaques are clear and 3-4mm in diameter. It is our only phage discovered to infect *Gordonia* after two semesters, and more than 60 attempts.

Phages infecting *Microbacterium foliorum*

*Microbacterium foliorum* is a gram-positive rod-shaped bacterium that when grown in culture produces bacterial colonies that can be yellow or tan in color.

The bacteria can be isolated from a variety of locations such as soil, water and even cheese rinds. The strain of bacteria used in our lab is *M. foliorum* SEA B-24224 isolated from grass in Germany. In our lab, *M. foliorum* was grown on a peptone yeast calcium (PYCa) agar and incubated at 30°C overnight.

The image on the right shows a spot test serial dilution of a *M. foliorum* phage on a lawn of the bacteria. The original phage sample (10⁰) was diluted to 10⁻⁸. Approximately 10 microliters of each dilution was then spotted onto a bacterial lawn. The plate was incubated overnight at 30°C.

When observed the following day, phage plaques, clearings in the bacterial lawn, could be identified. The plaques reveal areas of the bacterial lawn successfully lysed by bacteriophage. The goal of this spot test was to estimate the virus titer for a medium titer lysate, and it was ~10⁷ pfu/ml.

**Phages infecting Bacillus thuringiensis strains**

*B. thuringiensis* is gram-positive. *Bacillus* species are typically aerobic however some are anaerobic. There are defining features of *Bacillus* such as the production of endospores (resistant to heat, UV light and some chemicals) and are typically non-pathogenic with the exception to *B. cereus* and *B. anthracis*.

*Bacillus* strains will grow and divide on many types of nutrient rich media. Growth can occur at various temperatures however the fastest noted overnight growth is calculated at 37 degrees Celsius. Our phages are grown with the bacteria at 30°C.

Colonies have an off-white and rough appearance.

*Bacillus* phage can play a large role in the survival and pathogenicity of *Bacillus* bacteria.

We use *B. thuringiensis* as a non-pathogenic (to humans) host bacteria.

**Bacillus thuringiensis kurstaki**

“BTK” has been tested for insect control (bio-insecticides), specifically for Lepidoptera and Diptera Species as well as moths, butterflies, skippers, cabbage worms and spruce budworms. It is approved for field use on crops such as berries, fruits, nuts melons, cucumber, squash, eggplant, tomato, broccoli, cabbage, kale, mustard, parsley, spinach, and tobacco because it is non-toxic to human exposure.

BTK produces cuboidal, spherical and bipyramid crystals that are toxic to insect larvae.

**Bacillus thuringiensis 350**

“Bt 350” is closely related to BTK. We find Bt 350 to sometimes be a better host to our phages in host range testing.

Panel a shows a spot test serial dilution of a phage on a lawn of BTK. This phage is still causing isolated plaques at 10⁻⁸ dilution, and our estimate of titer is >10⁹ pfu/ml. The plaques were clear in the center with turbid rings around the clear center.

Panel b shows typical plaque morphology of a phage infecting Bt 350. This phage is a confirmed mixed morphology, with 0.5-2 mm clear plaques.

Panel c shows a spot test serial dilution of a phage infecting Bt 350. This phage makes tiny, clear plaques, < 1mm in diameter. The spot tests showed isolated plaques at 10⁻⁸ and 10⁻⁹, suggesting an estimated titer of 10⁹ pfu/ml.