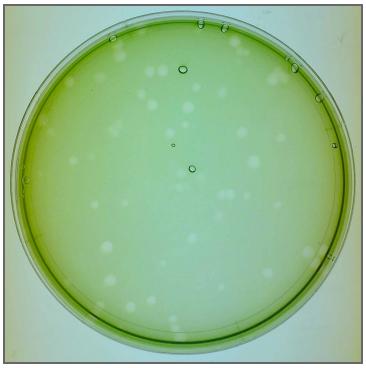

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Plaque Assay for Microcystis aeruginosa NIES-298 and phage Ma-LMM01

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ABSTRACT

This protocol describes a plaque assay for the enumeration of phage infecting *Microcystis aeruginosa* NIES-298. It is a single agarose layer assay and reproducibly quantifies infection by the *Microcystis*-infecting phage Ma-LMM01. This protocol has been adapted from work by Moore et al. (2007) as per Lindell (2014).

GUIDELINES

The protocol has been designed to work with cultures / lysates in a laboratory setting. If phage that will infect this host are present in natural samples, there is no reason the protocol should not detect them.

MATERIALS

Microcystis aeruginosa NIES 298 is available from the National Institute for Environmental Studies (Japan)
Ma-LMM01 was kindly provided by Professor Professor Takashi Yoshida (Kyoto University).

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Prepare CT Agarose

1 Make 0.56% agarose in CT medium base by adding 0.56 g low melting point agarose / 100 mL CT medium prior to autoclaving. You will need 12 mL of CT agarose per plate. Note: the final agarose concentration in plates will be ~ 0.45% once culture and lysate are added (see below). CT medium is modified from Watanabe and Hiroki, 1997 by adding phosphorus of equal concentrations using sodium phosphate in place of Na_2*beta-glycerophosphate.

2 After autoclaving, place in water bath at 34 °C. Add vitamins once agarose has cooled but not solidified. Make 12 mL aliquots and keep each in a water bath at 34 °C until ready to plate. Alternatively, CT agarose (without vitamins) can be prepared in advance and microwaved prior to the water bath step.

Concentrate Microcystis aeruginosa cells

3 Concentrate log-phase *M. aeruginosa* NIES-298 cells (culture should be no more than 3-4 days old and ~ 1-2 * 10^6 cells/mL) by centrifugation at 8000 xg for 10 min (x2). The first centrifugation step will collapse the gas vacuoles; the second will form the pellet. Remove excess medium.

4 Resuspend cells in fresh CT medium. Cells should be concentrated to ~2-3 * 10^7 cells/mL. You will need 2 mL of concentrated cells per plate.

Dilute Viral Lysate

5 Make a serial dilution of virus containing solution to be tested using CT media. Fresh Ma-LMM01 lysates generally contains ~ 2-6 * 10^7 pfu/mL, so 10^{-5} -10^{-7} dilutions will get plates in the countable range of ~ 20-

200 pfu (plaque forming units) per plate. PFUs outside this range are difficult to quantify accurately.

Plate

6 Add 1 mL of diluted sample to 2 mL of concentrated cells. For negative controls, add 1 mL of CT medium to 2 mL concentrated cells.

7 Pour the 12 mL aliquot of CT low melt agarose into the plate. Add the 3 mL combination of lysate and cells next to the agarose. Swirl gently to mix together to form one homogenous layer.

Incubate

8 Plates can be carefully moved to an incubator adjusted for *Microcystis* growth immediately after pouring, or once they are solidified. Note: plates will take 1-2 hours to solidify.

Important: unlike standard bacterial plaque assays **do not flip plates**. Plates will not be firm enough for flipping for at least one day.

9 Incubate at 26 °C and 30-40 µmols photons m^{-2} s^{-1} continuous light. Plates can be flipped on day 2. Plaques should start to become visible ~day 3, and can be counted through day 5.