Roseobacter Screening Of Surface Waters For Viruses

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Abstract

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Guidelines

Notes:

Goal initially is to screen several seawater samples on several roseobacter hosts to determine breadth of rosephage present at each location and then to focus on a single site.

Nomenclature for viruses should include a designation for the site of seawater collection followed by a number and the host it was isolated on.

Put information into an Excel worksheet. Create a separate Excel file for each source of virus. Use a different tab in each worksheet for different hosts.

Turbid plaques indicate possible lysogenic phage that may be of interest to Feng Chen's laboratory in Maryland. Keep the plaque plug at 4°C. Take 50-100 µl from a few and grow up small 1 ml cultures to cryopreserve (glycerol) them. Here trying to get the putative lysogenic cells (prophage containing confers resistance to colonies of cells within the plaque = turbid).

Protocol

Growth

Step 1.
Pull Roseobacter cultures from freeze and grow in 5 ml broth cultures

NOTES
VERVE Team 09 Jul 2015
Matt has 14 isolates from Wendy Ye in Mary Ann Moran's lab in Georgia. Two of them are from open ocean (CCS1 and CCS2) and they are grown at 20-22°C in 1/10 YTSS. All others are from coastal waters and are grown at 30°C in 1/2 YTSS. For virus screening, grow them to log phase (OD_{600} = 0.6 - 0.8). Some will grow quickly (overnight) while others will take 1-4 days to reach a log phase.

Screening

Step 2.
Perform virus screening in sterile 96 well flat-bottom tissue culture treated microtiter plates

NOTES

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Viruses will be screened from seawater collected from various sources.

Screening

Step 3.

Store seawater filtrates at 4°C

NOTES

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Ice crystal formation at -20°C **bad**.

Absorption

Step 4.

Absorb the viruses to the bacteria

Absorption

Step 5.

Place 15 µl of log phase cells and 15 µl of seawater filtrate (containing virus) into wells of a 96 well plate

NOTES

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Uninfected controls should surround infected wells on each plate in a checkerboard pattern.

Absorption

Step 6.

Allow the viruses to absorb to the bacteria for 1 hour

DURATION

01:00:00

Absorption

Step 7.

Add 200 µl growth media (1/2 or 1/10 YTSS) to the wells

Absorption

Step 8.

Seal the plate with parafilm and incubate with shaking

Absorption

Step 9.

Look for infected wells that show lysis with respect to uninoculated controls

Enrichment

Step 10.

Enrich for viruses by transferring from initial 96 well plate to another 96-well plate containing fresh liquid media (“frogging” into new wells)

NOTES

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Does not require fresh log-phase host cells, the cells come with the transfer. Ideally frog **before** cells into stationary phase.

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Enrichment for viruses may take several rounds of culturing because we're assuming initial concentrations of phages in the seawater are low.

Enrichment

Step 11.
Once enriched sufficiently, filter out cells with 0.2um filtration into 1.5ml microfuge tubes

Enrichment

Step 12.
Spin for 5 mins. at max to pellet cells

DURATION
00:05:00

Enrichment

Step 13.
Transfer supernatant to fresh tube for storage

Enrichment

Step 14.
Store at 4°C

Plaque Purification

Step 15.
Plaque-purify wells containing virus by growing cells plus virus on solid media

Plaque Purification

Step 16.
Prepare solid agar media and 0.5% overlay agar

Plaque Purification

Step 17.
Absorb virus to cells using as a starting point, 25 µl virus from 96 well plate enrichments and 200 µl log-phase host bacteria for 1hr

DURATION
01:00:00

NOTES
VERVE Team 12 Aug 2015
Try growing lawns first, maybe needs to be up to 1 ml cells.

Plaque Purification

Step 18.
Mix the virus-absorbed cells in the 5 ml overlay agar

Plaque Purification

Step 19.
Pour over the solid media

Plaque Purification

Step 20.
Incubate and look for plaque formation

NOTES
VERVE Team 12 Aug 2015
Will have several possible results: no plaques (not likely if using virus-enriched cultures from 95 well plates), too many plaques to purify them (in which case, a dilution of virus is made and plaque assay repeated), or well-resolved plaques. If well-resolved plaques, then cored from agar using Pasteur pipet and dispensed into 100µl YTSS broth in 1.5ml tubes.

Plaque Purification

Step 21.

Pick representatives of all plaque types present and record appearance of the plaques

NOTES

VERVE Team 12 Aug 2015

Clear, well-lysed plaques are of the most interest to us. Turbid plaques may indicate lysogenic phage and some should be picked, but these will be sent to another laboratory for further study (see guidelines).

Plaque Purification

Step 22.

Store the broth that contains the agar plugs at 4°C and allow the viruses to diffuse out of the agar into the broth

DNA Purification Determination

Step 23.

Once the viruses are plaque-purified they can be scaled-up in 5 ml broth cultures for DNA purification

DNA Purification Determination

Step 24.

Take 25 µl of the plaque-purified virus (agar plug in 100 µl broth) and absorb to 200 µl log-phase bacteria for 1 hr

DURATION

01:00:00

DNA Purification Determination

Step 25.

Add to 5 ml broth and grow with shaking

DNA Purification Determination

Step 26.

Centrifuge out cells and filter culture through 0.2 µm filter

DNA Purification Determination

Step 27.

SYBR stain a portion of the filtrate to determine how much virus is present

NOTES

VERVE Team 10 Jul 2015

If a lot of virus is present, then may be enough to purify DNA.

DNA Purification

Step 28.

Purify DNA using the Promega Wizard Lambda DNA kit

NOTES

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Goal is to obtain about 1 µg DNA for future work. If 5 ml does not yield that amount, will need to grow up more (e.g. 225 ml volume) and repeat DNA purification.