Rhodobacter capsulatus Gene Transfer Agent (RcGTA) Activity Bioassays

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[Abstract] RcGTA, a small phage-like particle produced by *Rhodobacter capsulatus*, was initially identified in culture filtrates as a DNase-resistant form of DNA transfer between *R. capsulatus* cells. This gene transfer assay has been used to identify RcGTA-producing strains and to help determine the roles of genes thought to be responsible for RcGTA production.

Materials and Reagents

1. GTA donor strain (see Notes 1)
2. Recipient strain (see Notes 1)
3. RCV broth (Beatty and Gest, 1981)
4. YPS broth (Wall *et al.*, 1975)
5. YPS agar (2 plates per recipient-donor combination, one plate per donor, and one plate per recipient)
6. 0.22 µm filtered GTA buffer (Solioz and Marrs, 1977)
7. 0.45 µm low protein binding (PVDF) syringe filters (*e.g.* EMD Millipore, catalog number: SLHV033RB)
8. Plate spreader (Including 95% ethanol + flame)
9. 1 ml syringes
10. Test tubes for aerobic culturing (*e.g.* Fisher brand, catalog number: 14-961-30) w/caps
11. Culture tubes for anaerobic/photosynthetic culturing (*e.g.* Fisher brand, catalog number: 14-959-37A w/screw caps)
12. Polypropylene sterile culture tubes (*e.g.* Simport, catalog number: T405-2A)
13. Optional (if transferring photosynthesis marker): Chambers/packs for anaerobic plate growth
14. RCV Medium (see Recipes)
15. YPS Medium (see Recipes)
16. GTA buffer (see Recipes)

Equipment

1. Shaking incubator
2. Microcentrifuge
3. Incandescent light-box or light incubator
4. 1 ml pipettes
5. 1.5 ml Microcentrifuge tubes

Procedure

A. Preparation:

1. 3 days prior to the assay, inoculate GTA donor strains in RCV broth and grow aerobically overnight at 35 °C (200-250 rpm).
2. 2 days prior, measure optical densities (OD) of overnight cultures of GTA donor strains and normalize them by dilution with RCV broth. It is simplest to dilute all ODs to match the lowest, as the actual OD is irrelevant so long as all cultures are at the same final density. Use a 1% v/v inoculum of normalized donor to inoculate YPS broth without antibiotics to grow anaerobically and photosynthetically over two days at 35 °C without shaking, mixing occasionally by inverting the culture tubes. These culture tubes should be filled to the brim with YPS broth and sealed tightly, to create anaerobic conditions, and placed equidistant from the light source. Incandescent bulbs are better than fluorescent bulbs, but the heat generated from the bulbs must be dissipated *(e.g.* by having the culture tubes in a water tank) in order to maintain culturing temperature.
3. 1 day prior, inoculate recipient strain in RCV broth and grow aerobically overnight at 35 °C (200-250 RPM).

B. Assay:

1. Pass donor strain cultures through a 0.45 µm filter, collecting the filtrates in polypropylene tubes.
2. Centrifuge 1 ml of recipient cultures, decant the supernatant and re-suspend in an equal volume of GTA buffer.
3. Mix the following in a polypropylene tube:
   -Filtrate Controls (for each Donor filtrate): 0.5 ml GTA buffer, 0.1 ml filtrate.
   -Recipient Controls (for each Recipient Strain): 0.5 ml GTA buffer, 0.1 ml recipient cells.
   -Experimental samples: 0.4 ml GTA buffer, 0.1 ml filtrate, 0.1 ml recipient cells.
4. Incubate tubes at 35 °C for 1 h with no shaking.
5. Add 0.9 ml RCV broth to each tube.

6. Incubate tubes at 35 °C for 3 h with shaking at ~200 rpm.
7. Transfer the 1.5 ml mixtures from the polypropylene tubes to individual 1.5 ml microcentrifuge tubes.
8. Plate 150 µl of each filtrate + recipient mix on plates to represent 10% of the total. This is not necessary for the recipient and filtrate controls.
9. Centrifuge all tubes, decant supernatant, and resuspend pellets in the small (~100 µl) volume that remains. Spread these resuspensions on plates to represent 90% and 100% for experimental and control assays, respectively. These plates should be selective for the transfer of the marker, or grown in conditions that select for a transferred marker.
10. Grow for 2-3 days at 30-35 °C.
11. Count colonies. Determine ratios of colonies on experimental plates over the number of colonies found on a positive control (*e.g.* a wild type strain).

Notes

1. One common bioassay employs monitoring the transfer of the *puhA* gene to *puhA* deletion mutant DW5 (Wong *et al.*, 1996), with selection for transfer of the *puhA* gene being the ability to grow photosynthetically. This bioassay shows no spontaneous mutation background. Another bioassay is the transfer of rifampicin resistance, which is a property of some strains of *R. capsulatus*, to rifampicin-sensitive strains such as the natural isolate strain B10 (Weaver *et al.*, 1975). This bioassay has a low but detectable rate of spontaneous mutation background that must be accounted for in the data analysis.
2. It is essential to compare transfer of the same marker, as marker sizes can affect bioassay results (Hynes *et al*., 2012), presumably affecting both packaging and recombination rates.
3. Bioassay absolute numbers can vary greatly depending on growth state of donor and recipient cells, batch of media (*e.g.* the batch of Yeast Extract was used for YPS), so it is essential to compare ratios within one bioassay, and perform multiple independent replicates. When performing bioassays for or into strains/mutants with impaired growth or viability, it can help to normalize transfer rate to the number of viable cells (either donors or recipients, whichever is impaired) by performing viable cell counts alongside the bioassay.

Recipes

1. RCV Medium
   7.5 mM $(NH_4)_2SO_4$

30 mM DL-malate, pH 6.8 with NaOH
54 µM EDTA
0.8 mM $MgSO_4$
0.51 mM $CaCl_2$
43 µM $FeSO_4$
3 µM Thiamine-HCl
9.5 µM $MnSO_4$
45 µM H_3BO_3
0.2 µM $Cu(NO_3)_2$
0.83 µM $ZnSO_4$
3 µM $NaMoO_4$
4.5 mM KH_2PO_4
5.1 mM K_2HPO_4
-pH to 6.8

2. YPS Medium
   3.0 g/L Yeast Extract
   3.0 g/L Peptone
   2 mM $MgSO_4$
   2 mM $CaCl_2$
   pH to 7.0 with NaOH or HCl, as needed
   Add 1.5% Agar for solid media
3. GTA buffer
   10mM Tris-HCl (pH 7.8) with NaOH
   1.0 mM $MgCl_2$
   1.0 mM $CaCl_2$
   1.0 mM NaCl
   500 µg/ml BSA (Fraction V)
   Filter sterilize with 0.22 µm filter.

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