Gene Transfer Agents in the Reef Environment

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Today

• What are Gene Transfer Agents?
• What are they doing in the oceans?
• What might they be doing in the reef environment?
Gene Transfer Agents (GTAs)

• First discovered in 1974 (*Rhodobacter capsulatus*)
• Type of bacterial “sex”
• Package random chunks of DNA from the host—led to high rates of gene-swapping
• “Little Genetic Escape Pods”
GTAs and Alpha Proteobacteria

• Most Bacteria of this type (Alphas) contain them
• Alphas commonly Associated with Reef Environments and *Symbiodinium* endosymbionts
• What are GTAs doing?

GTA gene cassette
GTAs and Alpha Proteobacteria

- Obtained Several Strains with Known GTAs from Culture Collections
- Isolated Several Strains From *Symbiodinium* endosymbionts
- Screened them for GTA Production (*Roseovarius nubinhibens, Ruegeria mobilis 45A6*)

Culturable Bacteria Associated with *Symbiodinium*

Marine Agar GASWA
Can GTAs transfer Genes to Ambient Community?

- *R. mobilis* and *R. nubinhibens* were genetically “marked” with antibiotic resistance genes.
- GTAs purified from marked strains were added to reef associated natural seawater community.
- Incubated overnight over Looe Key Reef.
GTA-mediated Transfer in Coral Mucus

Spontaneous KanR

KanR Resistant Cells/ml

Series 1

MOI 0.1

MOI 0.01

KanR cells/ml

Looe Key MOI study

Treatment

Spontaneous KanR

MOI 1
GTA-mediated transfer of marker genes documented to both cultured and natural populations.

McDaniel et al. (2010). Science 330, 50
What else can GTAs Do?

• *Roseobacters* and *Marinobacters* have been shown to increase growth rate of *Symbiodinium* (Ritchie, in prep)

• Since GTAs are from these kinds of bacteria, could GTAs alone have an effect on *Symbiodinium*?

• Maybe on the corals themselves?
Coral Reef Settlement Hypothesis

Ho: GTAs have no effect on coral larval settlement.
Ha: GTAs have an effect on coral larval settlement.

Methods Overview

1) Coral larvae collected and counted.
2) Larvae incubated with GTAs and settling surface.
3) Settling surface scored for settling and metamorphosis.
• **Porites astreoides** - Brooding Coral

• **Montastraea faveolata** - Broadcast Spawner
Experimental Set-up
Each treatment replicated 10 times with 20 larvae/plate, plus 1 biofilm/plate, (provided on glass slides cured in the natural reef environment for 3 weeks), plus 20 ml sterile seawater/plate.

Treatments: (Varied per Experiment)
- Media Controls
- GTAs from *Reugeria 45A6*
- Inactivated GTAs from *Reugeria 45A6*
- Non-specific viruses
- chloramphenicol/GTAs (bacterial protein synthesis inhibitor)
- DCMU/ GTAs (photosystem II metabolic inhibitor)
- *Reugeria mobilis* bacterial strain
GTA treatment resulted in statistically significantly higher settlement than any other treatment.
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What’s going on here?

• Increased settlement requires procaryotic protein synthesis and photosynthetic energy
• Controls indicate that facilitated settlement is peculiar to GTAs and not viruses in general
Theories

GTAs transfer genes from *Reugeria mobilis*:

- to α-proteo’s associated with the coral larvae.
- to α-proteo’s associated with *Symbiodinium*.
- to α-proteo’s already on the surface of the biofilm.
- directly to the *Symbiodinium*.
- directly to the larvae.
Applications to Protecting the Reef Environment:

- Can GTAs be a sort of “Gene Therapy” for the coral reef environment???
- A possible way to deliver beneficial genes to the coral holobiont.
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