High Frequency of Horizontal Gene Transfer in the Oceans

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Microbes rely on mutation and the processes of horizontal gene transfer (HGT; conjugation, transformation, and transduction) to acquire new traits. Gene transfer agents (GTAs) discovered in the purple nonsulfur bacterium *Rhodobacter capsulatus* (formerly *Rhodopseudomonas capsulata*) are host-encoded viruslike elements that package random fragments of the host chromosome and are found in the genome of almost every sequenced member of the α-Proteobacteria order Rhodobacterales (*1*). To test whether GTAs are natural vectors of gene transfer, we grew nine strains of marine α-proteobacteria containing putative GTA cassettes (table S1) and screened them for the production of GTA-like particles.

Both *Roseovarius nubinhibens* ISM and the isolate *Reugeria mobilis* 45A6 reproducibly produced putative GTA particles during stationary phase growth. We then generated genetically marked donor strains of *R. nubinhibens* and *R. mobilis* containing the transposon Tn5. GTA production in these marked donor strains was equivalent to that of the wild-type strains. To document gene transfer frequencies, we subjected wild-type strains or natural communities from a range of environments to treatment with donor strain GTAs and documented the rates of GTA-mediated gene transfer of kanamycin resistance (fig. S1). In the coral reef environment, spontaneous kanamycin resistance was 4.6×10^{-4}, whereas the GTA-mediated frequency was significantly higher at 2.5×10^{-2} ($P = 0.028$, Student's t test).

For this experiment, both spontaneous mutants and GTA treatments were examined for the presence of the Tn5 streptomycin kinase gene. A total of 47% of the GTA-treated viable colonies but none of the spontaneous revertants contained the gene. That 53% of the putative transductants did not contain the gene is not surprising because these may have contained only the kanamycin resistance gene (*npt*II) and not the flanking streptomycin kinase gene.

The recovery of the streptomycin kinase sequence, which is ~1000 base pairs (1 kbp) from the active site of the kanamycin resistance gene, suggested that up to 1 kbp of the central region of Tn5 was transferred. This is consistent with extracted DNA from the GTAs, which ranged from about 500 to 1000 bp in length (fig. S3). No spontaneous double antibiotic (kanamycin and streptomycin) resistance was detected, and the GTA-mediated frequency of 1.06×10^{-4} was significantly higher ($P = 0.023$). The Tn5 streptomycin kinase sequence was recovered in 1 in 10 viable double antibiotic–resistant strains, suggesting that modifications, truncations, or rearrangements may have occurred, as in natural transformation (*2*).

Similar frequencies of transfer were observed among differing environments (Table 1), demonstrating that cultivated GTAs transduce natural communities of marine bacteria. The 16*S* ribosomal RNA sequences examined showed that the majority of natural GTA recipients were most similar to marine *Flavobacterium* or *Flexibacter* strains (table S2), consistent with the prior reports of abundant *Flavobacterium* in marine systems (*3*).

R. nubinhibens contains both a GTA and an inducible prophage (*4*). Transmission electron microscopy (TEM) demonstrated that *R. nubinhibens*–induced prophage preparations contained tailed phage (*4*), whereas GTA particles were nontailed (fig. S2A), resembling the GTA of *Silicibacter pomeroyi* (*5*). In contrast to the GTA particles, the purified prophages of *R. nubinhibens* had no gene transfer activity. Additionally, maximal expression of the *R. nubinhibens* GTA terminase gene cooccurred with maximal GTA production (fig. S4). TEM of GTAs of *R. mobilis* revealed tailed viral particles (fig. S2B).

GTA dose, or multiplicity of infection (MOI), was linearly correlated with increased resistance to antibiotics (MOI range from 0.01 to 10, $R^2 = 0.9593$), which enabled extrapolations of gene transfer frequencies to natural systems (*6*).

GTAs from *R. nubinhibens* ISM show a wide host range and interspecific gene transfer under ecologically relevant conditions. Environmental gene transfer frequencies ranging from 6.7×10^{-3} to 4.7×10^{-1} (Table 1) are 1900 to 459 million times the frequency for transformation (*2*) and 650,000 to 31 million times the frequency of transduction previously measured in the marine environment (*7*). These results suggest a genomic flexibility in marine microbial populations that facilitates their adaptation to changing environmental conditions.

Table 1. Frequencies of transfer of marker genes to both cultured and natural communities. N/A indicates not applicable; BDL, below detection limit.

Environment	Avg. spontaneous frequency	Range	Avg. GTA-mediated rate	Range	Number of trials
		Roseovarius nubinhibens GTA filter matings			
Culture	6×10^{-7}	5.2×10^{-8} – 2.0×10^{-6}	1.7×10^{-5}	7.5×10^{-8} – 7.9×10^{-5}	$n = 5$
Estuary	1.6×10^{-4}	2.8×10^{-5} – 3.0×10^{-4}	8.9×10^{-4}	6.2×10^{-5} – 1.1×10^{-3}	$n = 3$
		Roseovarius nubinhibens GTA liquid matings			
Estuary	1.2×10^{-3}	N/A	3.1×10^{-2}	1.2×10^{-2} – 5.0×10^{-2}	$n = 2$
Coastal	4.3×10^{-2}	N/A	2.8×10^{-1}	N/A	$n = 2$
Open ocean	2.5×10^{-2}	6.7×10^{-3} – 4.3×10^{-2}	3.9×10^{-1}	2.8×10^{-1} – 4.0×10^{-1}	$n = 3$
Reef	4.6×10^{-4}	N/A	2.5×10^{-2}	N/A	$n = 1$
Reef (double antibiotic)	BDL ($<10^{-6}$)	N/A	1.06×10^{-4}	N/A	$n = 1$
		Reugeria mobilis (45A6) GTA liquid matings			
Estuary	1.2×10^{-3}	0–2.1×10^{-2}	2.4×10^{-2}	4.2×10^{-2} – 1.1×10^{0}	$n = 1$
Coastal	4.3×10^{-2}	3.0×10^{-2} – 5.6×10^{-2}	2.8×10^{-1}	1.6×10^{-1} – 6.4×10^{-1}	$n = 1$
Open ocean	3.3×10^{-3}	$0 - 1 \times 10^{-2}$	4.7×10^{-1}	$0 - 3.6 \times 10^{0}$	$n = 2$
Reef	4.6×10^{-4}	N/A	1.1×10^{-1}	N/A	$n = 1$

References and Notes

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Supporting Online Material

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Materials and Methods
Figs. S1 to S4
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References

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