Ecology, growth rates, and rRNA operons: a marine view

Plus, hunting for *nanoarchaea*

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SUMMARY:

Abstract:

The uneven distribution of nutrients and available resources in the coastal marine water column suggests that different substrates will provide niches for different ecological growth strategies, known as r and K-selection. Here I test the hypothesis that particulate matter in the water column (“marine snow”) will be associated with early colony forming bacteria as compared to free-living bacteria. Furthermore, fresh decaying matter such as a dead fish will contain even more fast-growing bacteria. Secondly, I inconclusively test whether these “fast-growers” have more copies of the rRNA operon, thus correlating their ecological strategy with genome evolution. Finally, I present a mini-project using Nanoarchaeae-specific PCR primers on a variety of environmental DNA extracts to look for this novel phylum in the mesophilic marine environment.

Objectives:

1. Use the coastal marine environment to assess slow vs. fast growing colony-forming bacteria
2. Correlate the abundance of fast/slow growers with presumed nutrient availability as indicated by “marine snow” (particulate matter)
3. Correlate growth differences with rRNA operon copy number for fast-growers
4. Use Nanoarchaeae PCR primers to look for mesophilic, marine Nanoarchaeae

Methods:

1. Seawater filtering, plating onto low-nutrient agar plates
2. Identify rRNA operon copy number (rrn) with an rrn-specific homing endonuclease digestion and subsequent pulse field gel electrophoresis (PFGE)
3. Environmental PCR with nanoarchaeae specific primers

Results:

1. Colony-forming curves for the 2 different filters indicate that more fast-growing bacteria are associated with particulate and that even more fast-growers are associated with dead, decaying flesh.
2. rRNA operon copy number can be assessed by restriction digest with Iceu1 and PFGE, although conditions need to be optimized.
3. No nanoarchaeae species were found, although sequences clustered into a small, uncultured euryarchaeae clade.
**Background:**

The number of rRNA operons per bacterial genome can vary from 1-15. The selective pressure that has caused and maintained this variety is not well understood. Originally, scientists hypothesized that operon number simply indicated growth capacity. However, recent studies have disproven this idea by demonstrating high growth rates with single operons.

Klappenbach et al. demonstrated that rRNA operon copy number correlates with growth rates of soil bacteria (Klappenbach *et al.*, 2000). Namely, redundancy of the rRNA operon appeared to favor rapid colony formation, whereas a single operon copy was associated with slower colony formation. The authors ultimately conclude that operon number often correlates with the ecological “strategy” of the species and its ability to respond to available resources. This study was the first to implicate an ecological role for redundant rRNA operon copy number. This correlation could broaden our understanding of the genetic pressures at work in the ecology of microbial communities. However, in order to validate and understand these experiments in a broader sense, we must test and expand this hypothesis in different, diverse microbial communities.

Marine environments provide just such a distinctly different and readily available microbial habitat. In particular, previous work by Delong et al. has shown that “marine snow” (fine, suspended particulate matter) and the surrounding water, provide two phylogenetically distinct microenvironments (Delong *et al.*, 1993). This dichotomous environment provides an optimal place to study r- vs. K-selection strategies presented in the original Klappenbach et al paper. Because the particulates presumably contain a nutrient-rich environment with plenty of readily available organics, we presume that attached species will need to “hurry up and eat” and should therefore be K-selected; they should colonize and grow fast to take advantage of this fleeting, rich energy source. Conversely, the free-living bacteria suspended in the adjacent water, depend on a slow, steady stream of soluble nutrients and might appear to be more oligotrophic. This
community is presumed to contain more r-strategists that slowly, but efficiently, consume the low levels of constantly available nutrients in the water.

Finally, the *Nanoarchaeae* project was inspired by Karl Stetter’s symposium presentation that briefly discussed the discovery of the archaeal symbiont, *Nanoarchaeae equitans*. Since this phylum’s identification, several more uncultured isolates have been discovered in hyperthermophilic environments somewhat similar to the initial discovery’s environment (Huber *et al.*, 2002; Waters *et al.*, 2003). However, given that archaeal diversity is not entirely understood, it is conceivable that novel, mesophilic *Nanoarchaeal* symbionts exist in a mesophilic environment.

**Methods:**

Water sample collections were taken at a depth of approximately 3 meters with a van Dorn water sample. The samples contained fine particulate matter and, obviously, surrounding water. Initially, I filtered 2 samples of 100 mL of water onto 2 different size filters of 3.0 uM and 0.2 uM. Each filter was subsequently scraped gently twice and resuspended in 5 mL. This resuspension was then serial diluted and plated on 1/10 diluted Difco marine agar supplemented with 3% washed agar. Once a day for 10 days I counted and marked with a different color marker new colonies that appeared.

At the conclusion of the first sets of plating, it seemed that my experimental design needed improvement because the 0.2 uM filter also contained particulate matter. To resolve this problem, I took the flow-through from the 3.0 uM filter and filtered it on the 0.2 uM filter attempting to separate the microbial community attached to particulate and those that are free-living in the water column. Additionally, I vigorously vortexed the resuspension to prevent regional lawns on my plates that I believe resulted from clumps of material not separated during the resuspension of the filter matter.

I additionally wanted to correlate the growth curve analysis with rRNA operon copy number assessment. Because of the short duration of the independent project, I selected 10 fast-growing colonies from different plates and different samples. Through colony PCR, I was able to sequence 5 isolates (Fig.3) and compare the surrounding
phylogenetic relatives of known rRNA operon number using rrndb (Klappenbach et al., 2001) in order to give a rough indication of rrn number.

In a more precise attempt to identify the number of rRNA operons, I suspended 10 fast-growers in liquid media, spun them down, and did whole-cell agarose-embedded cell lysis and restriction digest with Iceu1, a homing endonuclease that specifically cuts at the rRNA operon. The restriction digest products were then run on a 1% agarose pulse field gel electrophoresis (PFGE) for 24 hours.

As a side project, I used 4 sets of specific 16S rRNA PCR primers, previously published by Stetter’s group that were used to identify the recently discovered phylum *Nanoarchaeota*, on DNA extractions from a wide variety of local marine, freshwater, and terrestrial mesophilic aerobic and anaerobic environments. 25 different DNA environmental extractions were tested. These environmental DNA samples were generously donated by different class members and were chosen because of availability. Given more time, I would have tested more anaerobic environments known for higher archaeal abundance.

Results:

Ultimately, it seems that the growth curves resulting from the past 3 weeks are inconclusive and simply require more replication, precision, and optimization. My original method described above (Fig.1) did not result in a significant difference between the 2 filters. Interestingly, a comparison between figures 1 and 2, shows that the amount of early colony-formers is distinctly lower in the first method compared to the second. It is unclear what the cause of this discrepancy might be.

Once I began using the flow-through from the 3.0 uM filter on the 0.2uM filter, I began to see a difference between the two samples and assumedly the two different “micro-environments.” In fact, it does appear that the CFU curves resulting from 7-21-05 (Fig.2) support the hypothesis that particulates will contain more fast-growers. Unfortunately, however, the data from 7-18-05 are inconsistent with the hypothesis, mainly because of the egregious standard deviation.
The most striking result from the growth curves was a last-minute decision to look at the bacterial community associated with decay. During a trip to collect water, I was able to sample a decaying fish head taken from a lobster trap. Two grams of the tissue was homogenized and serially diluted for plating. As Figure 2 indicates, more than 80% of the colonies from this sample appeared within the first day of counting.

The phylogenetic tree of the five fast-growing isolates (Fig.3) indicates that isolates are clustering with relatively high rrn number species in between 4-13 for the group. These results are very loose correlations and are only intended as an initial indication. The PFGE initial results (Fig.4) indicate that this technique, although it requires further optimization could be useful in determining rrn number. However, 4 bands were the most bands clearly resolved for any species, seen in lane 10. Based on the smears of DNA in many lanes, it does seem that other nucleases have contaminated the reactions, most likely during lysis or during the wash series.

On the side, the allegedly Nanoarchaea-specific PCR primers produced 4 promising products of the correct molecular weight (Figure 5). One of these products yielded a successful clone library that was sequenced. Figure 6 shows the phylogenetic results of these sequences.

Discussion:

The growth curve results suggest a possible model for fast and slow-growing bacteria in coastal marine water. Namely, the most nutrient-rich environments like dead fish will provide a niche for fast-growing bacteria. Mid-nutrient environments like particulate contain slightly less, but a significant proportion of fast as well as slow growers and free-living bacteria in the water column are less likely to be fast-growers, but must still be able to successfully metabolize the freely available resources in the water column within a few days. This model, although inferred from some tenuous data makes intuitive sense and is worth investigating more closely over a broader range of substrates.
The PFGE and phylogenetic analysis of rRNA operon number are still particularly preliminary, but are somewhat correlative with previous studies in soil. This correlation between rrn and ecological niche is intriguing because it attempts to localize a phenotypic ecological effect with genome evolution.

Finally, the *Nanoarchaea* results remind the molecular biologist that, in the absence of the “real gene” PCR can frequently give non-specific results. However, the clade where the products clustered into is an uncultured, poorly understood group previously only found in Antarctica. These results suggest that this group might be more widely dispersed and abundant than previously realized.

References:


Figure 1: Growth curves of 0.2 and 3.0 uM filtering of 2 separate samples of 100 ml each. Flow-through was not used here.

Figure 2: Growth curves of colonies formed after 3.0 uM filtering of 100 mL of seawater, followed by collection of flow-through and subsequent filtering on 0.2 uM filter.
Figure 3: Phylogenetic tree of 16S rRNA from 5 isolates obtained from day 1-forming colonies ("fast-growers") from 7-14-05 and 7-18-05. Associated rrn was obtained from the rrndb at Michigan State University.
Figure 4: PFGE (Pulse Field Gel Electrophoresis) of 10 fast-growing colonies digested with Iceu1, to detect rRNA operon copy number.
Figure 5: *Nananoarchaea* primer design and positive PCR results. Note, primer set 2 and 3 did not give non-specific products.

Possible PCR products:
- 1500 bp
- 500 bp
- 1000 bp
- 500 bp

Primer set used: 1 1 4 4 4

Non-specific

Figure 6: Phylogenetic tree of the resulting clone library from PCR products above and coincident archaeal clone library.