Quantifying Growth Rates in Coral Reef Microbes to Guide Conservation Efforts

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Abstract

Understanding microbial growth dynamics in the ocean is crucial for environmental conservation. Growth rates are commonly assessed using culture dependent techniques which can prove challenging given that only 1% of microbes are culturable. Metagenomics provides an alternative route to predict microbial growth. In this study we utilized MEGAHIT to assemble metagenomic samples collected from coral reefs across the central Pacific Ocean representing a spectrum of bacterial growth rates. We estimated growth rates from assembled contigs using Codon Usage Bias, and three Peak-to-Trough Ratio approaches. We aim to use these tools to understand the relationship between coral reef degradation and bacterial growth rates given that degraded coral reefs are overgrown in bacteria. Doing so will allow us to have a better understanding of how we can target bacterial growth rate for conservation efforts and remediation of degraded coral reefs.

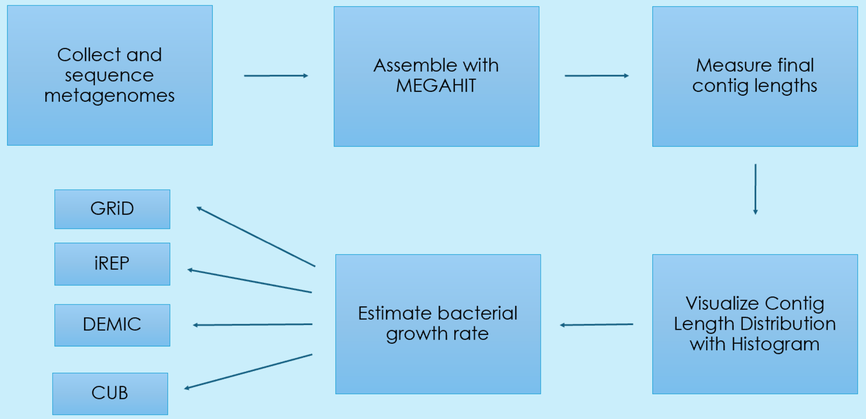
Background/Motivation

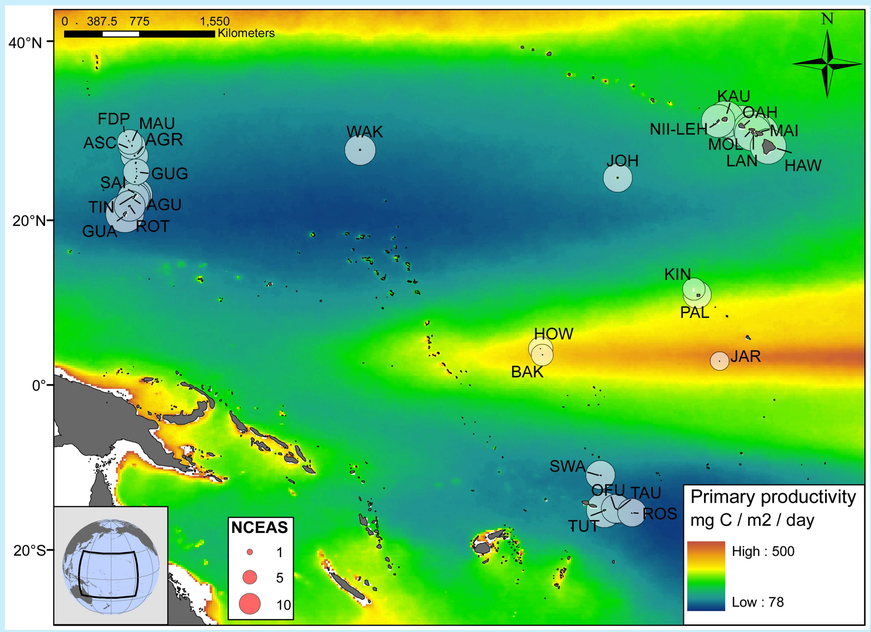
- Microbial populations are responsible for nutrient recycling in coral reefs and indicate environmental stressors such as pollution, presence of pathogens, climate change, and ocean acidification.
- DNA-based techniques like CUB and PTR can analyze a broader range of microbes directly from environmental samples without needing to culture them, allowing for high-throughput detection of all species regardless of cultivability or abundance.
- These methods are less labor-intensive compared to traditional growth rate analysis techniques.

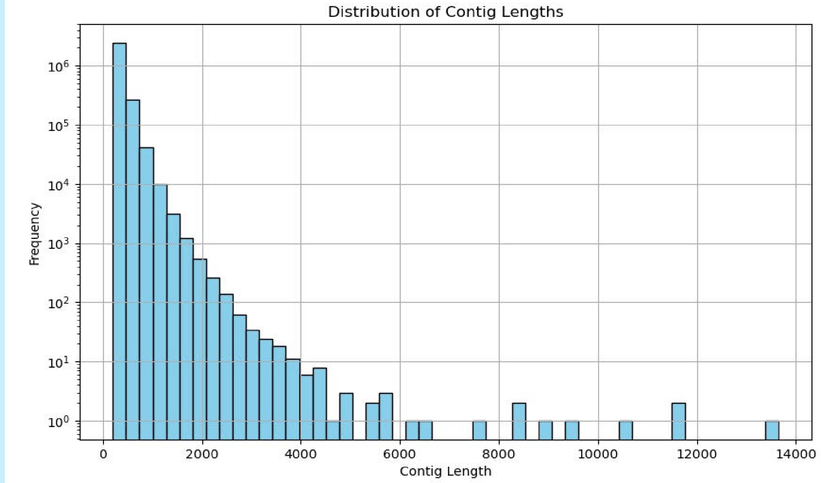
Methodology

The data samples were collected from various points around Hawaii and the Pacific islands and sequenced with Illumina MiSeq. The subsequent metagenomic analysis was performed with the Hoffman2 Cluster. To begin the analysis, we used MEGAHIT to assemble contigs into longer sequences to reconstruct sections of bacterial genomes. To understand more about the final contigs, we built a histogram to visualize the distribution of contig lengths.

To estimate growth rates, we will be employing both peak-to-trough ratio (PTR) and codon usage bias (CUB) analyses. The PTR method can be performed in multiple ways depending on the quality and type of sequences that are being used. We will be using iREP, GRiD, and DEMIC.







The histogram is right skewed, with a long tail extending into higher base pair contigs, demonstrating a majority of contigs were short, or less than 1,000 base pairs. This could be from repetitive sequence, poor assemblage, or fragmented assembly that could result from sequencing errors, high complexity regions, or low coverage.

Current Progress and Future Directions

Grace: I am currently in the process of working through the PTR analysis method developed by Brown et al, which relies on draft-quality (as opposed to finalized) genomes to map reads to.

Eleanor: I am currently running analyses on the PTR of the sampled genomes, using DEMIC, a method developed by Gao and Li. This method relies on the distance of contigs from the origin of replication, allowing us to compare growth rates between bacterial samples. This dataset will allow for comparisons to be drawn between sampled genomes and can further be parsed to give bacterial growth rates by location, size of genome, and identifiable genes.

Natalie: I have been using Growth Rate InDex (GRiD), a PTR method that calculates the growth rate of metagenomically assembled genomes with low coverage by sorting genome fragments according to their *dnaA* and *dif* genes.

Sarah: I'm using a CUB analysis which works by analyzing the preferential usage of certain codons over others, as organisms with faster growth rates often exhibit a higher frequency of optimal codons that enhance translational efficiency.

Future Directions: By employing these techniques, we aim to explore the impact of microbial growth rates on coral reef health and their viral communities. We will apply this framework of analysis to investigate other microbial ecosystems, such as the soil and human microbiome, to investigate how human activities may be accelerating pathogen growth rates.

Brown, C., Olm, M., Thomas, B. *et al.* Measurement of bacterial replication rates in microbial communities. *Nat Biotechnol* **34**, 1256–1263 (2016). https://doi.org/10.1038/nbt.3704
Gao Y, Li H. Quantifying and comparing bacterial growth dynamics in multiple metagenomic samples. Nat Methods. 2018 Dec;15(12):1041-1044. doi: 10.1038/s41592-018-0182-0. Epub 2018 Nov 12. PMID: 30420687; PMCID: PMC6289653.
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