Genetic and Phenotypic Analysis of Gammaproteobacteria

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Introduction

Historically, studies of microbial organisms have been based on microbial biochemistry, investigating microbial phenotypic growth characteristics. More recently, with the advancement and lowered costs of genetic sequencing technology, microbial analyses have transitioned to gene-based. True scientific discovery lies in the fusion of microbial genetic and biochemical information to form new understandings of microbial processes and significance. The aim of this study is to identify strains of Gammaproteobacteria isolated from the similar environmental locations that are genetically similar, but display varying phenotypic profiles, and investigate the genetic source of functional variation.

Methods

- Over 650 bacterial samples isolated from the surface of Macrocystis pyrifera and the water column from three locations off the coast of southern California (Catalina Island, Point Loma, and La Jolla).
- Whole genome sequencing on the Personal Genome Machine (PGM) was executed for the model organism Vibrio cyclitrophicus to analyze genome variation. Genomes were then assembled (SPAdes and MIRA) and annotated via Rapid Annotation using Subsystem Technology (RAST).
- Isolated samples were tested for various individual substrate utilization in order to develop phenotypic profiles.
- Phylogenetic association was formulated using 16s ribosomal RNA sequence analysis, and was compared to averaged phenotypic profiles in order to identify genetically similar, but phenotypically varying strains.
- Differences in protein content were investigated further to describe the adaptations occurring selected organisms.

Results

Analysis of 57 protein-coding genes that are unique to the M. pyrifera tissue (ED144) found:
- 19% were involved with phage/prophage proteins, lysis modules, packaging machinery, replication, tail fibers, and tail proteins.
- Increased number of functional proteins in Type VI secretion system, including an uncharacterized protein encoded near VgrG-3.
- Functional genes for Chorismate synthesis.
- Functional genes for Threonine degradation.
- Functional genes for D-gluconate and ketogluconates metabolism.
- Increased number of genes for programmed cell death and toxin-antitoxin systems.
- Increased number of membrane transport genes, including Type II protein secretion system for Widespread colonization Island associated with ATFase and Fip plus assembly.

Analysis of 45 protein-coding genes that are unique to the water column (ED252) found:
- 24% were involved with unique capsular and extracellular polysaccharide synthesis/metabolism.
- 22% were associated with Vibrioferrin synthesis.
- Functional genes for Glycine and Methionine synthesis.
- Functional genes for Chitin and N-acetylglucosamine utilization.
- Unique membrane transport genes, including genes for Mannose-sensitive hemagglutinin type 4 pilus and choline transport.

Conclusions

Unique capsular and extracellular polysaccharide synthesis mediates direct interactions between bacteria and its environment.

ED144
- The finding of increased functional proteins in the Type VI secretion system supports the concept of VgrG/toxin genetic coupling, and is an indication of a bacterial defense system, as the VgrG subunit is specific to puncturing and releasing toxins into neighboring cells as a response to population density.
- The synthesis of Chorismate is helpful for survival as it is a key intermediate for the production of various necessary metabolites.
- The genes for Widespread Colonization Island assist in bacterial colonization of surfaces and potentially assist in biofilm formation.

ED252
- Vibrioferrin is a carboxylate class siderophore used in iron acquisition and metabolism.
- Chitin is an abundant source of carbon, nitrogen, and energy, and many marine Vibrio species are capable of using chitin as a sole carbon source.

It is expected for ED144 to not contain the same functional pathways for Glycine and Methionine synthesis as ED252, as ED144 can obtain said amino acids from interactions with M. pyrifera.

Further investigation of the genomic variation of microbes isolated from both the water column and the surface kelp in M. pyrifera beds will provide a better understanding of the ecological significance of microbial-kelp relationships, and potential anthropogenic impacts.

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