Predicted regulon of the regulatory RNA ryhB in Vibrio fischeri (Iron regulation in Vibrio project)

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Project
» Iron limitation, carbon metabolism and siderophore production in marine bacteria - a systems biology approach (Iron regulation in Vibrio)

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Dataset Description

An RNA-Seq experiment was performed in order to define the regulon of the small, regulatory RNA RyhB in Vibrio fischeri ES114. This was done by comparing the global gene expression in wildtype and ryhB mutant strains of V. fischeri. Since RyhB is de-repressed only under iron limiting conditions, this comparison must be performed when the level of intracellular iron is low.

Methods & Sampling

Wildtype and mutant ryhB strains of ES114 were grown in Aquil media with glucose and NH4 as carbon and nitrogen sources, respectively. Parallel cultures of each strain were resuspended in media containing low iron and followed until the growth rates decreased. At this point, samples for RNA were collected by filtration, stored in RNAlater and later extracted using standard methods. rRNA depleted cDNA libraries were submitted to UGA’s Georgia Genomics Center for sequencing on an Illumina MiSeq instrument.

Data Processing Description

Base quality and trimming of reads was assessed using the FastX-Toolkit v0.0.13.2, only reads above a Q30 value were retained. Reads were mapped back to the Vibrio fischeri reference genome using TopHat v1.2.0. Expressed genes and transcripts were assembled for each replicate culture separately using Cufflinks v2.1.1. Cuffmerge v.2.1.1 was then utilized to merge all assemblies from the wildtype and ryhB mutant cultures, respectively. Finally, differentially expressed genes were identified using Cuffdiff v2.1.1.

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Data Files

File
Vibrio_RNASeq5.csv (Comma Separated Values (.csv), 23.20 KB) MD5:f5342a6bd8280dc915e34124d7731542 Primary data file for dataset ID 471914

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Parameters

Parameter	Description	Units
gene_function	gene function: specific category	unitless
gene_name	gene name	unitless
gene_identifier	The VF_#### code includes a code for the particular organism (VF=Vibrio fischeri) and the gene's location (e.g. VF_0619 and VF_0620 will be adjacent genes)	unitless
predict_bind	Genes marked with a "P" have a predicted RyhB binding site identified by the programs sTarPicker or TargetRNA	unitless
FPKM_RyhB	RyhB mutant fragments per kilobase of gene per million fragments mapped	fragments/kilobase/million fragments mapped
FPKM_WT	wild type mutant fragments per kilobase of gene per million fragments mapped	fragments/kilobase/million fragments mapped
FPKM_RyhB_WT	ratio of RyhB to wild type	unitless
Log2_FPKM_RyhB_WT	log base 2 of ratio	unitless
p_value	probability of significance	unitless

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Instruments

Dataset-specific Instrument Name	Automated Sequencer
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Illumina MiSeq
Generic Instrument Description	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

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Project Information

Iron limitation, carbon metabolism and siderophore production in marine bacteria - a systems biology approach (Iron regulation in Vibrio)

This award is funded under the American Recovery and Reinvestment Act of 2009 (Public Law 111-5).

Iron limitation of heterotrophic bacteria has substantial biogeochemical implications, including lower assimilation efficiencies and reduced incorporation of CO2 into biomass. Marine bacterioplankton also have a large impact on iron speciation in seawater through their production of siderophores, ligands with a high affinity for iron. Over 99.9% of the dissolved iron in seawater is strongly bound to organic ligands, which are likely to include siderophores since they have functional groups and conditional stability constants for iron that are similar to chelators detected in situ. Iron limitation and siderophore synthesis are linked since these ligands are produced specifically in response to low intracellular iron concentrations. Neither process is completely understood in marine bacteria. For instance, the production of iron binding ligands in seawater unexpectedly increases, not decreases when iron is added to HNLC regions. One possible explanation is the role of carbon, which complicates studies of both iron limitation and siderophore synthesis. The extent to which low iron concentrations reduce bacterioplankton growth efficiencies or productivity in situ is not well-quantified because of the rapid stimulation of primary production that occurs after iron limitation of phytoplankton is relieved. The resulting increase in available carbon makes it is difficult to distinguish between direct and indirect iron (i.e. carbon) limitation of marine bacteria. Carbon source and availability may also play a secondary but important role in regulating siderophore production, which can be either stimulated or repressed by the addition of glucose.

The objective of this project is to model the interacting gene regulatory networks that control iron acquisition and carbon metabolism in gamma -proteobacteria, specifically Vibrio fischeri. Iron and carbon regulatory pathways are tightly linked in a complex web of relationships mediated by global transcriptional regulators (Fur and CRP) and the small RNA RyhB. In order to construct a gene regulatory network model, the PIs will use an integrated systems biology approach that combines computational, bioinformatics based research and laboratory experimentation. Predictions of gene expression, siderophore production and the flux of iron with changing environmental conditions will be validated by quantifying gene expression using qRT-PCR and global transcriptome analyses, as well as determining iron quotas and iron uptake rates.

Intellectual Merit: This study will contribute to the general understanding of iron speciation in the upper ocean by elucidating the interactions between iron and carbon limitation, carbon source and siderophore production. The gene regulatory network model will be capable of identifying environmental variables critical to siderophore production and evaluating potential biomarkers for iron limitation. This project will facilitate the discovery of new genes and control mechanisms involved in iron metabolism and shed light on other processes such as virulence and luminescence, which utilize the same or similar interactions and transcription factors. Finally, this approach also provides a framework for synthesizing information on the genetic level and using it to make predictions about processes such as siderophore production that are ecologically important.

Broader Impacts: This project will enhance infrastructure for scientific research and education by mentoring young scientists and supporting both outreach programs and course development at several educational levels. Support is requested for graduate students to participate in the proposed research and all three PIs will recruit undergraduates for summer internships, often targeting underrepresented groups. Research Experiences for Teachers supplements are requested to allow two high school science educators to participate in our research at the Skidaway Institute of Oceanography and design a laboratory exercise that will illustrate how microbes respond to nutrient limitation. The aim is to provide a research experience that will strengthen collaborations among educators at both the local and national levels.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0929203

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