Metabolomics data for two strains of Pelagibateriales

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Project
» Collaborative Research: EAGER: Salinity-based selection between sister clades of abundant coastal bacterioplankton (CoastalSAR11)

Abstract
In an abundant aquatic groups of microorganisms, SAR11, the transition between salt- and freshwater environments has happened only once: all freshwater SAR11 belong to subclade IIIb/LD12, which has also been found to inhabit coastal environments where salinity varies widely. The first reported isolates of the SAR11 freshwater clade LD12 and a member of the sister clade IIIa from the same region are now available. This project quantified concentrations of select, known intracellular metabolites within two strains of SAR11: LSUCC0261 and LSUCC0530. Large-volume cultures with cellular abundances of > 1.5 x 10^10 cells were required to obtain quantifiable levels of metabolites. The metabolites were extracted from the cellular biomass using established protocols and analyzed on a triple quadrupole mass spectrometer. The resulting data analysis provides the molar concentrations of metabolites that participate in central carbon metabolism.

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Methods & Sampling

Sample Collection

Two strains for members of the Alphaproteobacteria order Pelagibacteriales (SAR11) were cultured in the laboratory. LSUCC0261 is an isolate from the SAR11 subclade IIIa and was grown in JW2 media[Henson et al. 2016]. LSUCC0530 is a freshwater strain in the LD12 subclade, and was grown in JW5 media [Henson et al. 2018]. Cells were harvested at late exponential phase by filtration onto 0.1 um Omnipore (Millipore) filters. The filters were stored frozen at -80C until extraction in the laboratory.

In the metadata, 'volume' is the volume filtered for each sample. For each sample, 'cells' is the number of cells per milliliter.

Extraction

A protocol adapted from [Rabinowitz et al. 2007] was used for extraction of intracellular metabolites. Filters were removed from the cryovials and cut into small pieces with methanol-rinsed scissors on combusted aluminum foil. The pieces of the filter were placed in an 8 ml combusted amber glass vial and 1 ml of -20 °C 40:40:20 acetonitrile:methanol:water + 0.1 M formic acid was added to each vial. 25 µl of 1 µg/ml deuterated standard mix (d3-glutamic acid, d4-4-hydroxybenzoic acid, and d5-taurocholate) were added to act as extraction recovery standards. The vials were vortexed to shake apart filter pieces and fully expose them to the ice-cold solvent. They were sonicated for 10 minutes and the extract was transferred to micro-centrifuge tubes using Pasteur pipettes. The filter pieces were rinsed with three times with 500 µl of extraction solvent and the rinse was added to the eppendorf tubes. The extracts were centrifuged at 20,000 × g for 5 minutes. The supernatant was transferred to new 8 ml amber glass vials leaving behind any scraps of filter or pieces of cellular detritus. The extracts were neutralized with 51.2 µl of 6 M ammonium hydroxide and dried down in a vacufuge. The samples were reconstituted in 100 µl labeled injection mix (95:5 water:acetonitrile containing D2 biotin, D6 succinic acid, D4 cholic acid, and D7 indole 3 acetic acid). The solution (100 µl) was placed in a glass insert in an autosampler vial for targeted metabolomic analysis.

Data Processing Description

Data transformation

The resulting data files were converted to mzML files using the msConvert tool[Chambers et al. 2012] and processed with El-MAVEN[Agrawal et al. 2019]. Data are given as ng per ml in the extract. The extract volume for all samples is 100 ul.

Metabolite identification

The targeted metabolomics compound identifications were based on measurements of authentic standards on the same mass spectrometer. Identifications were 'MSI Level 1' identifications, unless otherwise noted, based on the established criteria[Sumner et al. 2007]. The method in use cannot separate alanine and sacrosine, the concentrations are reported as 'alanine/sarcosine'. The method also cannot separate threonine and homoserine, the concentrations are reported as 'threonine / homoserine'.

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Parameters

Parameters for this dataset have not yet been identified

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Project Information

Collaborative Research: EAGER: Salinity-based selection between sister clades of abundant coastal bacterioplankton (CoastalSAR11)

Coverage: Coastal Louisiana, northern Gulf of Mexico

NSF award abstract:
Adaptation to new environments is a fundamental challenge for organisms, including microbes, in expanding their habitat range. It is important to investigate the cellular mechanisms underlying salinity tolerance in coastal bacterioplankton and their different responses to salinity in nature because (i) it will provide fundamental understanding for how microorganisms evolve to inhabit environments with different salinities, and (ii) alterations in coastal salinity are connected to climate change, so the way these alterations affect abundant coastal microorganisms also alters the biogeochemical cycling of, e.g., carbon. The project will examine microbial adaptations to salinity and determine how changes in salinity affect microbial metabolism using two closely related groups of abundant coastal bacterioplankton as model taxa. In addition, the research will continue and expand microbiology Course-based Undergraduate Research Experiences (mCUREs) in high-throughput cultivation and microbial characterization at the Lousiana State University. Sections of freshman biology laboratories will learn how to isolate, characterize, and molecularly identify microorganisms from local aquatic systems. mCURE sections will lead to newly isolated strains, genome sequences, and physiological data, these results will be published with the contributing students as co-authors. The relative success of mCURE sections will be assessed compared to traditional freshman biology sections. mCURE sections will offer unique opportunities for LSU students by creating excitement about research through discovery of new organisms and generating knowledge of the coastal habitats that are essential to the livelihood of the Gulf Coast.

The evolutionary transition between salt- and freshwater environments occurs rarely in microorganisms. In one of the most abundant aquatic groups, SAR11, the transition between salt- and freshwater environments has happened only once: all freshwater SAR11 belong to subclade IIIb/LD12, which has also been found to inhabit coastal environments where salinity varies widely. The first reported isolates of the SAR11 freshwater clade LD12 and a member of the sister clade IIIa from the same region are now available. These pure culture representatives provide a powerful model for experimentally investigating adaptations to new environments in microorganisms, specifically (i) the genomic pathway and regulatory distinctions that arise during the

evolutionary transition from marine to freshwater environments, and (ii) the physiological mechanisms that underlie the ecological restrictions imposed on microorganisms by ionic strength in coastal and freshwater environments. Furthermore, because these organisms have distinct differences in metabolic potential, the isolates facilitate testing (iii) the effects of changing coastal salinity on microbial contributions to other biogeochemical cycles, such as that for carbon. The project will test the hypothesis that the relative ionic strength tolerances between the sister lineages (LD12, IIIa) result from fundamental differences in metabolic flexibility at a genomic and regulatory level. To do so it will assess transcriptional and metabolic responses to varied ionic strength for both taxa and measure the distribution and activity of both groups in nature to translate laboratory findings to the field. The research will provide new understanding of LD12 habitat range and insights into how the "freshwater" lineage evolved from a SAR11 common ancestor. The project will also more generally provide important information on microbial responses to salinity changes in coastal systems and the evolutionary paths separating freshwater and marine microorganisms.

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Funding

Funding Source	Award
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