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Conference Abstract

Characterization of DNA Degrading Microorganisms from Dewar Creek Hot Springs in Western Canada

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Abstract

Historically, discovery and subsequent characterization of microbial species relied on pure cultures. Some challenges associated with creating pure cultures have been overcome with advances in culture independent and DNA-based molecular methods such as single-cell genomics, metagenomics, or large scale amplicon sequencing. With these advances, the rate of discovery of new species from genomic data has quickly outpaced the number of organisms with cultured representatives. As description and characterization still rely on cultures, our understanding of yet uncultured species is greatly lacking. Major lineages in the bacterial domain equivalent to phyla that lack any cultured representatives are termed “candidate phyla.” Candidate phyla are found across the bacterial tree of life, and many uncultured organisms are found to be dominant in understudied environments. Extreme environments such as thermal springs are an example of understudied environments, making them excellent environments for studying novel microbial lineages. The objective of this research is to characterize uncultured bacterial lineages from Dewar Creek hot spring in Western Canada, with a focus on DNA and protein metabolizing bacteria. Based on previous genomic data from organisms in this hot spring, we hypothesize that the candidate phylum S2R-29, with extremely low GC content, metabolizes extracellular DNA or protein

Dewar Creek is a thermal spring located in the Purcell Wilderness Conservancy in British Colombia, Western Canada. It is one of Canada’s hottest springs, reaching temperatures

of up to 83˚C. Samples of DNA extracted from the Dewar Creek hot spring were PCR-amplified with primers for the v3v4 region of the 16S rRNA gene to detect S2R-29, and then sequenced on an Illumina Miseq. S2R-29 was found in samples ranging in temperature from 60˚C to 77 ˚C. In addition to samples from Dewar Creek, samples from other thermal springs in Canada, as well as samples from springs in New Zealand are also being sequenced with the same primers to determine the prevalence of this candidate phylum in other similar environments. This will give a better idea of the growth conditions and range of this organism.

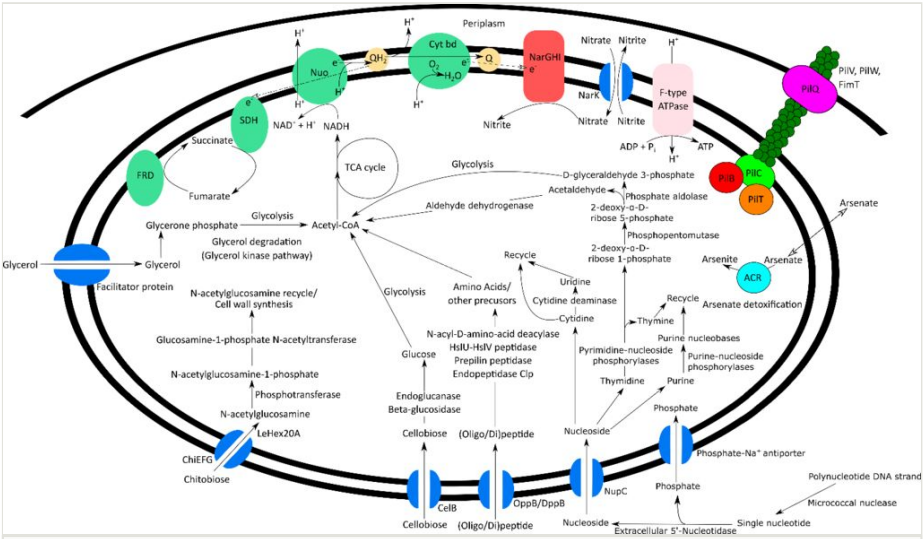

Figure 1. doi

Combined genomic analyses and metabolic prediction of S2R-29 SAGs. Pathways and enzymes annotation were obtained from JGI-IMG. Enzyme annotation was verified using closest BLAST hits with NCBI database as well as COGs databases. Pathways were cross-referenced using KEGG database, MetaCyc and literature searches. Transporters were shown in blue. Only potential carbon metabolism pathways were shown in detail within the cell. Electron transport chain was shown in green at the top left corner of the figure. Pilus-related proteins were shown in red, orange, green and pink at the top right corner of the figure.

Previously, S2R-29 single amplified genomes (SAGs) were generated from Dewar Creek samples. Analysis of these SAGs suggests that S2R-29 has the potential to use peptides and DNA as carbon sources (Fig. 1). To test the potential to metabolize DNA and protein, enrichments of samples with 13C labelled dNTPs or 13C labelled protein have been started. These enrichments will be used for stable isotope probing (SIP) to determine if any organisms in Dewar Creek are metabolizing dNTPs or protein. In addition to SIP, primers specific for S2R-29 for quantitative PCR (qPCR) have been designed and will be run on the enrichments to determine if there are any changes in the abundance of S2R-29 over time in these enrichments, further testing the metabolic potential of these organisms. Finally, probes for fluorescence in situ hybridization (FISH) will be designed for S2R-29. These will be used to perform FISH with samples from Dewar Creek in order to visualize

this candidate phylum. With this research, we hope to characterize this novel phylum, and possibly discover other novel lineages of DNA- and protein- metabolizing bacteria in Dewar Creek. This research will help better understand these processes in understudied environments as well as aid our understanding of the roles that bacterial metabolisms might play in biogeochemical cycles such as biodegradation of organic matter. In all, we hope that this research gives insight into the kinds of lifestyles bacteria have evolved in order to thrive in high temperature environments.

Keywords

candidate phylum, S2R-29, DNA metabolism, Bacteria, thermophile, extremophile

Presenting author

Sabina Pang

Presented at

ISEB-ISSM 2023, I would like to present this research as a poster under the theme of Emerging Tools and Areas of Scientific Inquiry

Conflicts of interest

The authors have declared that no competing interests exist.