Contribution ID: **257** Type: **Poster**

Genomic signatures of cyanobacterial endurance under space conditions

Introduction

The pursuit of life beyond our planet goes through the research of biosignatures of a past or present life from potentially habitable planets in the Solar system or around other stars.
The bedrock of this assumption lies in the capability of some microorganisms to thrive on Earth in different extreme conditions that are lethal to the majority of life forms since they pose a particular threat to nucleic acids' integrity, causing cell death.
To unravel the limit of life as we know it, astrobiological experiments have been conducted by exposing extremophiles to non-Earth conditions taking advantage of space and planetary laboratory-simulations and space facilities.
Desert strains of the cyanobacterium Chroococcidiopsis are extremely resistant to desiccation and radiation and were included in a selection of extremophiles exposed to space and Mars-like conditions by using the ESA facility EXPOSE-R2 installed outside the International Space Station.
Moreover, during the EXPOSE-R2 space mission these cyanobacteria were part of the BIOMEX (BIOlogy and Mars Experiment) and BOSS (Biofilm Organisms Surfing Space).
Throughout these space missions, when exposed in the dried state and rehydrated once retrieved back to Earth, Chroococcidiopsis strains resulted to be more resistant than other cyanobacteria suggesting the presence of crucial genetic components related to space-survival, like mechanisms to limit and/or repair DNA damage.
In particular, Chroococcidiopsis sp. CCMEE 029, CCMEE 057 and CCMEE 064, together with Nostoc sp. CC-Cryo 231-06, showed a remarkable degree of radiation and desiccation resistance, whereas Nostoc sp. PCC7524 and Anabaena variabilis SAG1403-4b resulted to be sensitive or low tolerant to space radiation.

Aim of the study

Our work aims to elucidate the genetic core among these species that provides the means for space-related survival by performing a bioinformatic pangenome-based comparative analysis of the space sensitive and space resistant strains of the aforementioned cyanobacteria. Hence the bioinformatics analysis was performed on three space-resistant Chroococcidiopsis strains and Nostoc sp. CCCryo 231-06, on the space-sensitive, but desiccation-resistant strains Nostoc sp. PCC7524 and Anabaena variabilis SAG1403-4b and on the desiccation and radiation-sensitive strain Synechocystis sp. PCC 6803.

Materials and Methods and Results

Previously sequenced Illumina and Oxford Nanopore libraries of the three Chroococcidiopsis strains were first cleaned and filtered for low quality reads and potential contaminants, for them to be assembled into a single circular chromosome, respectively of 5.7Mbp for Chroococcidiopsis sp. CCMEE 029, Chroococcidiopsis sp. CCMEE 057 of 5.7Mbp and Chroococcidiopsis sp. CCMEE 064 of 5.08Mbp.
We retrieved the already assembled genomes of Nostoc sp. CCCryo 231-06, Nostoc sp. PCC7524, Anabaena variabilis SAG1403-4b and Synechocystis sp. PCC 6803 from the NCBI database (Accessions: GCA_023522315.1, GCA_000316645.1, GCA_003991931.1, GCA_018845095.1) and we provided all of the assembled genomes to the RIBAP pangenomic pipeline.
This software makes use of robust annotation tools such as PROKKA, producing an in-silico proteome per input genome, which get successively compared through an all-vs-all alignment computed by MMseqs2 whose result gets combined with the sequence homology information from Roary with smart pairwise ILP calculations.

We then manually curated the annotation results using reliable proteic evidence from the UniProt database, EggNog, InterProScan and BlastKoala, together with SignalP6 and Phobius softwares and literature evidence.
Finally, we examined the resulting gene clusters from RIBAP and we computed statistics.

From our analyses, we find a total of 13,414 clusters, of which 1,883 build a core gene set.
In particular, 122 genes are exclusively found among the Chroococcidiopsis strains and Nostoc sp. CCCryo 231-06. Interestingly, Nostoc sp. CCCryo 231-06 possesses a remarkable number of unique genes: 1,936, greater than the shared core genome, while Chroococcidiopsis sp. CCMEE 029, CCMEE 057 and CCMEE 064 harbor 1,107, 687 and 406 unique genes respectively and share 407 additional genes among them.
Moreover, a total of 565 genes are found in every investigated organism except for Synechocystis sp. PCC6803.

Future perspectives

It is yet to be defined whether variation in the presence/absence or in the copy number of these genes is the key to decipher what confers space-survival capabilities, i.e., the exposure in the dried state to space conditions using the EXPOSE-R2 facility, and under laboratory conditions after retrieval back to Earth. With this in mind, we will be focusing on this objective in order to get a deeper understanding of the genetics of these cyanobacteria and expand our current knowledge about space-survival mechanisms. This will also be accomplished through future RNA-sequencing experiments on cyanobacteria exposed to space conditions in the hydrated form, specifically on upcoming space platforms such as the Space Rider.

Thanks to these studies, we expect to be able to elucidate the genetic biosignatures accountable for space-survival by analyzing both the nature of gene class transcription and its levels compared to the control RNA levels. Moreover, we will be thoroughly analyzing both the shared and unique genes of these microorganisms under a functional point of view. In fact, through nucleotide and protein alignments we will be determining the presence of functional protein domains that could be ubiquitously found and thus confer the means for resistance, as well as the SNPs' impact on protein length and composition.

Primary author: RIGANO, Gabriele (Department of Biology, University of Rome Tor Vergata, Rome, Italy)

Co-authors: Dr DONATI, Claudio (Research and Innovation Center, Fondazione Edmund Mach, San Michele all'Adige, Italy); Dr BILLI, Daniela (Department of Biology, University of Rome Tor Vergata, Rome Italy)