Delving deeper into the metabolic potential of the ISS microbiome

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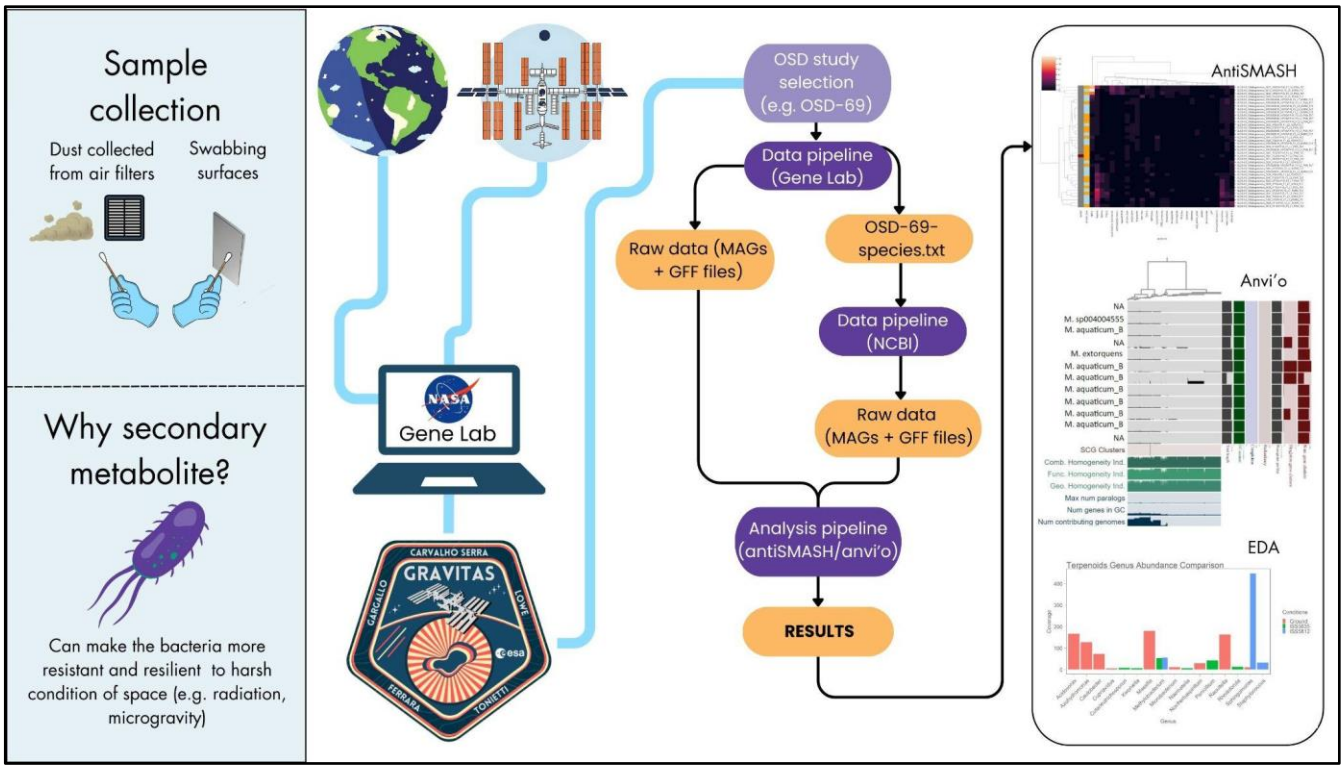
Introduction: The International Space Station (ISS) is a closed environment which harbors a unique ecosystem exposed to microgravity (µg), increased levels of radiation, and a continuous human presence. It hosts a core microbiome that remains stable over time, irrespective of module location and crew changes[1]. Through sampling various ISS surfaces[2-4], it is suggested that this extreme environment can induce changes in biofilm formation, increase antibiotic resistance, and enhance the virulence of certain species[5]. The impact of this extreme environment on secondary metabolites (SM), i.e., the molecular repertoire facilitating adaptation to the environment, remains to be thoroughly explored, especially regarding adaptations to radiation exposure.

Methods and Data: To expand on the SM potential of ISS microorganisms and characterize their adaptations to radiation, we selected metagenome-assembled genomes (MAGs) from NASA's GeneLab database (experiments OSD-69 and OSD-252), which share similar sampling methods across different surfaces in the ISS, and spanning multiple flights. The tool antiSMASH[7,8] was used to identify gene clusters related to SM function, from which heatmaps were generated to showcase trends in our datasets. For ISS core species, phylogenetic neighbors (terrestrial) were selected from NCBI, representative of non-µg conditions. Pangenomes were created for groups of interest using Anvi'o[9], identifying unique gene clusters across samples. All of these steps are incorporated in our open source pipeline[11]. Downstream analyses include KBase implementations of DRAM to annotate raw fasta files[6], and KEGG[10] to analyze molecular functions related to µg conditions.

Results: Heatmaps relating SM function between NCBI ground controls and ISS OSD-69 MAGs displayed the most notable increase in gene annotations of non-ribosomal peptide synthase[12], a major class of enzymes involved in SM, but only during one flight, indicating no stable adaptation of the core microbiome. On the contrary, an increased coverage of terpenes was found across all flights, especially by *Sphingomonas* sp. and *Methylobacterium* sp., conferring a possible adaptation to the ISS environment. Terpenes are interesting SM due to their role in oxidative and osmotic stress alleviation, maintenance of cell membrane integrity, photoprotection, host growth promotion, and defense[13]. Inspection of KEGG orthologs for *Sphingomonas* reveals an increase in biosynthetic pathways for a wide range of terpenes. Regarding *Methylobaterium*, a similar trend was found, but specific for osmotic stress response. These features may be associated with potential adaptations to radiation.

Conclusion: In this work we have demonstrated the effectiveness of our pipeline in utilizing open source databases to perform SM analysis for environmental samples. OSD-69 findings paved new venues for the potential discovery of space-induced alteration of metabolic routes, such as the one regarding terpene production.



Graphical abstract: analyzing secondary metabolism to understand adaptation of ISS microorganisms. Moving from left to right, descriptions of the rationale (left), methods and data (middle), and results (right).

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