Space Algae: Understanding the Genomic Impacts on Microalgae After Growth in the International Space Station

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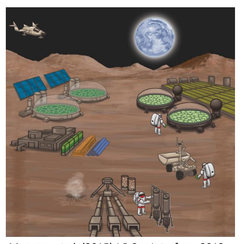
Abstract
Plants and microbes can be used for biological support of crewed space missions. The radiation and microgravity environment of spaceflight is expected to increase genetic mutation of all organisms. It is essential to understand how spaceflight impacts mutation rates in photosynthetic organisms to enable appropriate countermeasures and ensure productivity during long duration and deep space missions. The Space Algae flight experiments to the International Space Station (ISS) are studying the genomic stability of microalgae that could potentially be used in biological life support systems. Space Algae-1 grew ultraviolet light mutagenized *Chlamydomonas reinhardtii* in the VEGGIE plant growth chamber for approximately 40 mitotic generations over one month on the ISS. Whole genome sequencing from pooled cell samples every 10 generations revealed that spaceflight cultures had an ~50% increase in DNA polymorphisms relative to ground controls. These mutations had a novel base substitution signature and suggested a risk that microalgae may be unstable for long-term production in space. Space Algae-2 is focusing on the edible cyanobacterium *Arthrospira platensis*, commonly known as Spirulina. This experiment seeks to grow serial cultures to allow the organism to evolve in long-term spaceflight. Biological responses of the cells to spaceflight will be assessed with multi-omics analyses to determine mutation load, gene/protein expression, metabolic/nutritional composition, and cell morphology.

Introduction

Zhang et al. (2020) Front. Plant Sci. 11:631. doi: 10.3389/fpls.2020.00631, under CC BY 4.0

Research & Development


Menezes et al. (2015) J R Soc Interface. 2012: 20140715. doi:10.1098/rsif.2014.0715, under CC BY 4.0

Unicellular microalgae recycle carbon dioxide into oxygen and can generate biomass for use as human food, animal feed, or as a production organism for biofuels or organic feedstocks. Although long proposed for deep space missions, significant research and technology development is needed to implement algae production in deep space.

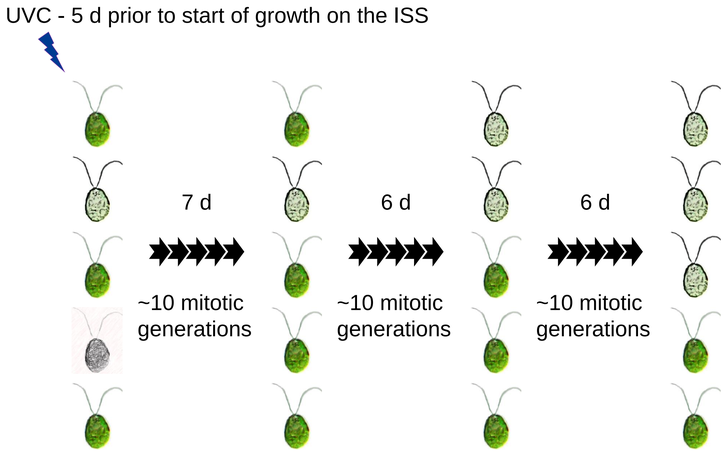
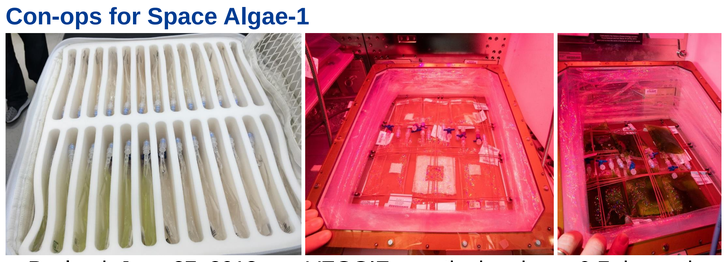
Space Algae-1: Genetic selection scheme


Figure 1. Genetic selection for growth rate. Two strains of haploid *Chlamydomonas reinhardtii* were mutagenized with UVC at doses that allowed 5-10% cell survival. UVC-mutagenesis results in random mutations that will predominantly reduce or knockout gene function. Mutations required for cell growth, depicted as a gray cell, will be lost or reduced in frequency. Mutations that increase cell growth rates, shown as a pale green cell, will increase in frequency in the cultured population.

Con-ops for Space Algae-1

Payload: June 27, 2018 — VEGGIE growth chamber July 3, 2018 — 6-7 d growth


Passage three biological replicates of two strains: wild-type (CC-5082) and *cw15* (CC-1883)

Figure 2. Concept of operations for Space Algae-1. Three biological replicates of each strain were inoculated in TAP media and launched in the SpaceX CRS-15 Dragon cargo capsule. The cells were grown mixotrophically in the VEGGIE plant growth chamber for 6-7 d growth periods. The stationary cultures were passaged into new media and the remaining culture was stored in ambient dark conditions until returned on the CRS-15 Dragon capsule.

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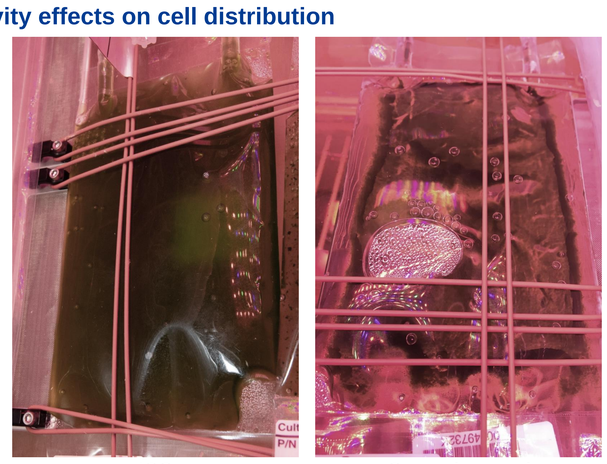
Gravity effects on cell distribution

ISS — Ground control

Figure 4. Algae cell distribution in microgravity versus 1 g. Microgravity on the ISS resulted in *Chlamydomonas reinhardtii* cells being uniformly distributed throughout the static cultures after 6-7 d. In ground control cultures, cells settled to the lower surface of the culture bags.

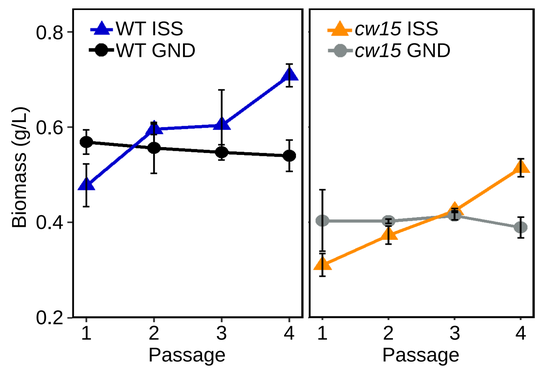
Biomass increased in spaceflight


Figure 5. Algal biomass. Biomass was measured at the end of the growth experiment. The grown cultures were stored in the dark at ambient temperature after each passage. Passage 1 was stored for 30 days prior to biomass measurements. Lack of convective air mixing around ISS stored cultures may have contributed to lower biomass after storage.

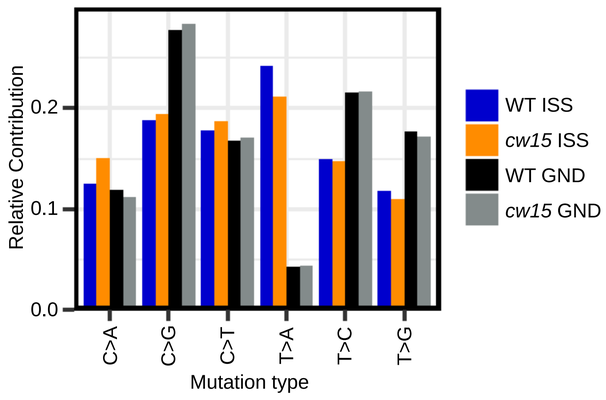
Spaceflight increased mutation rate


Figure 6. Spaceflight samples had more mutations that were enriched for T>A changes. Single Nucleotide Variants (SNVs) were identified from each culture using Whole Genome Sequencing and CRISP. Ground controls had 21-22 k mutations, while the ISS strains had 32-33 k mutations. The additional mutations were enriched for T>A changes.

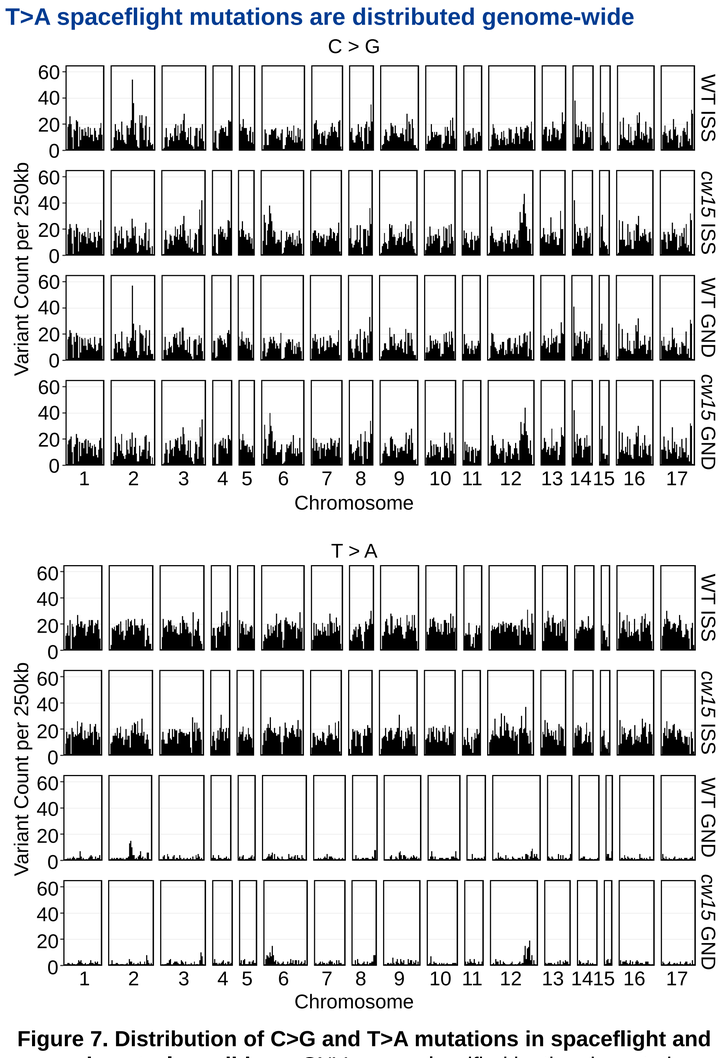
T>A spaceflight mutations are distributed genome-wide


Figure 7. Distribution of C>G and T>A mutations in spaceflight and ground control conditions. SNVs were classified by the six mutation types in Figure 6 and binned into 250 kb windows of the reference *Chlamydomonas reinhardtii* genome sequence. The C>G mutation pattern was enriched after UVC mutagenesis in both pre-flight and ground control experiments.

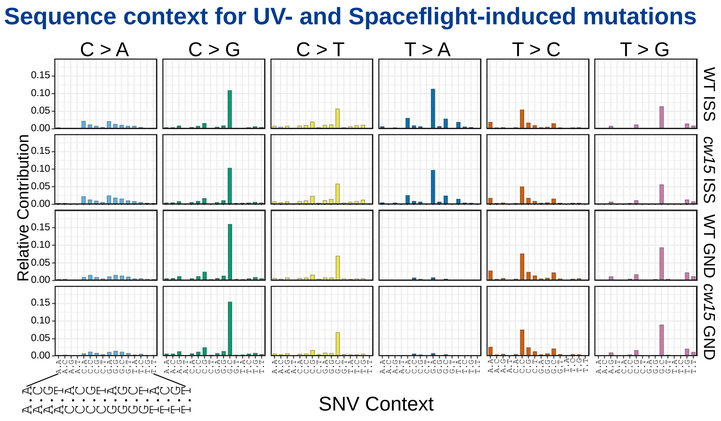
Sequence context for UV- and Spaceflight-induced mutations


Figure 8. Frequency of SNV in 3-base contexts. SNVs were categorized by the 5' and 3' base surrounding the base change. Spaceflight-induced T>A mutations were enriched for the sequence: 5'-GTA-3'.

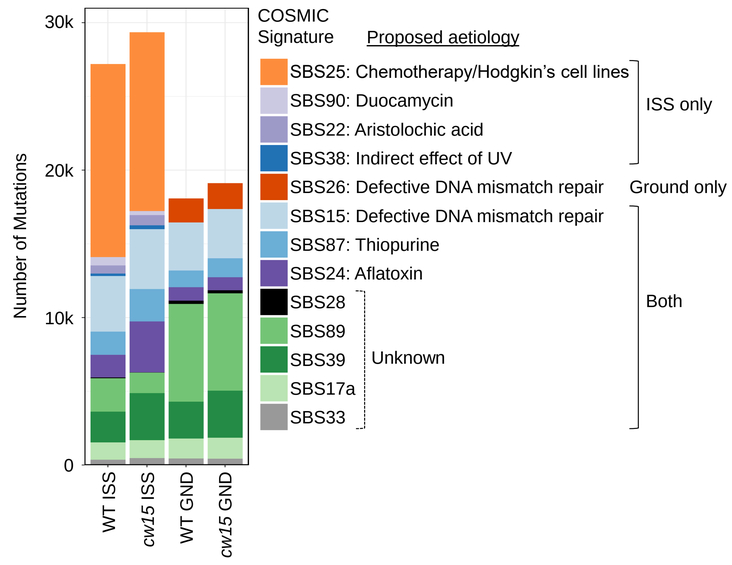
DNA damage signatures of spaceflight


Figure 9. COSMIC DNA damage signatures identified by SigProfiler. Eight DNA damage profiles that were found to be in common between spaceflight and ground controls were inferred to be UVC-induced. Four profiles were identified as unique to spaceflight.

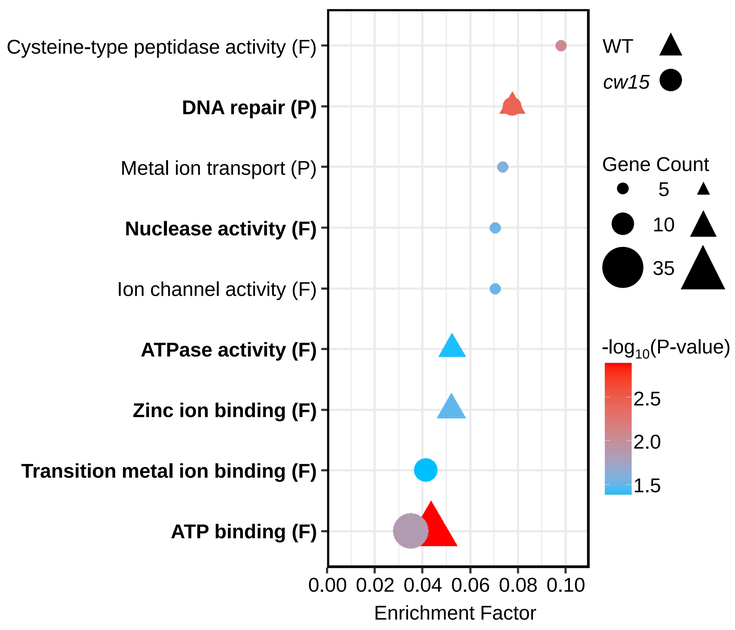
Genes selected in spaceflight


Figure 10. Gene ontology terms enriched in differential selected genes. SNPGenie was used to identify genes under differential selection between the ISS and Ground control conditions. AgriGO was used to identify GO terms that were enriched in spaceflight samples. These analyses found greater selection for functions involved in DNA metabolism and repair in the ISS samples (bold lettering on the y-axis).

Space Algae-2: Long-duration growth of *Arthrospira platensis*


Figure 11. Space Algae-2 will focus on Spirulina. *Arthrospira platensis* is a cyanobacteria that is used as a nutritional supplement. Space Algae-2 will complete multi-omics analysis of wild-type spirulina grown for 5-6 months on the ISS.

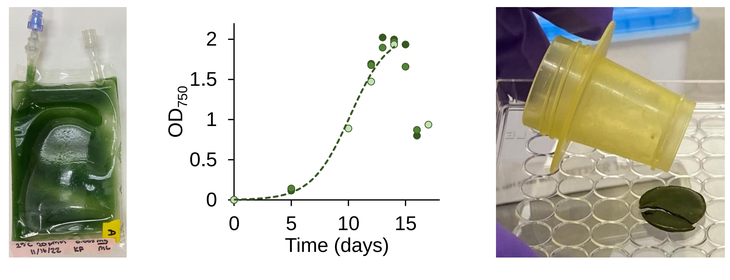


Figure 12. *Arthrospira platensis* cultures grow well in FEP plastic bioreactors used for Space Algae-1. Cultures take 13-15 days to grow to high density using 20 µmol/m^2/s PAR at 25.5°C. Doubling time is estimated to be 28 hours and a rapid culture decline is observed after 15 days. Algae biomass can be harvested with a filter for frozen sample return.