Lycopene-Enhanced Algae for Space Exploration

Gabriella Castro-Rovira, Rohini Josh, Ishaani Sharma, Katherine Song, Jenny Yu

BioBuilder Club, Andover High School, Andover, Massachusetts, USA

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For much of the last decade, the possibility of an expedition to Mars has kindled interest. However, scientists are still experimenting with agriculture in space in order to sustain human deep space exploration. Thus, we propose a food source in the form of a microorganism that can survive with few resources and provide sustenance for space exploration. Our research focuses on a microalgae called *Klebsormidium flaccidum,* a species of green algae. We found that *K. flaccidum* has a high baseline nutritional value and is well suited to dry environments with scarce resources, making it ideal for space agriculture. Taking advantage of algae as a food source would allow us to optimize the use of limited space and resources and reduce the costs of food/nutrient transportation.

To enhance the nutritional value of the algae, we plan to modify *K. flaccidum* with the beta-carotene pathway to produce astaxanthin. Astaxanthin is an antioxidant that counteracts many of the adverse effects of low-gravity environments, such as bone loss, effects of radiation exposure, and eye damage from bodily fluid pressure. It is also found in shrimp and salmon. First, we intend to engineer the beta-carotene pathway to stop at lycopene, as it is less complex and easier to execute. If this succeeds, we will complete the pathway to reach astaxanthin. Both lycopene and astaxanthin are a bright red color, and can therefore act as reporters for themselves.

Keywords: Algae, space, food, lycopene, astaxanthin, beta-carotene

Mentors: Jason Boock, Lindsey L'Ecuyer

Authors are listed in alphabetical order.
Please direct all correspondence to: lindsey.lecuyer@andoverma.us

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Background

For years now, there has been much interest in widening the bounds of human space exploration. We have not yet found a reliable method of growing food in space, which is necessary if humanity plans to conduct space expeditions for extended periods of time. Although scientists are developing methods to grow plants in space, they take up essential time, space, and expense.

While researching this problem, we found that algae is increasingly being recognized as a valuable source of protein and other nutrients; the algae biomass sector in the EU is valued at €1.69bn (Spicer and Molnar 2018). Algae are able to grow with only water, light, and a few inorganic compounds. Furthermore, algae are already being considered as a food source in space (Beall 2019). In particular, green microalgae have a nutritional content that is high in protein.

Narrowing our search, we discovered the microalgae *Klebsormidium flaccidum*, a species that is desiccation tolerant and cold acclimatable (Kondo et al. 2016; Nagao et al. 2008). The dried biomass of *K. flaccidum* var. ZIVO was found to contain ≥40% protein by mass, ≥150 µg/100 g of vitamin K_1, ≥1.5 mg/100 g niacin, and ≥300 mg/100 g polyphenols (Brickel et al. 2018). Therefore, *K. flaccidum* bypasses many of the challenges of growing plants, such as soil and space constraints, and could be a useful source of protein that other plants cannot provide. This would greatly reduce the costs of transportation and resources used for current food being sent to astronauts in space.

Our plan is to use CRISPR to modify our chassis, *K. flaccidum,* to produce lycopene. Although CRISPR has not yet been used with *K. flaccidum*, this should be an attainable approach because it has been applied successfully in several other algal species (Spicer and Molnar 2018). One of these is *Chlamydomonas reinhardtii,* which shares crucial commonalities with *K. flaccidum*, including similar cell wall materials (alkanes and triacylglycerols) and a nuclear genome size of approximately 120Mb (Kondo et al 2016; Baba et al. 2013). Furthermore, *Klebsormidium* is a predecessor of *Oryza sativa*, which has already been engineered with the carotenoid synthesis pathway (Team SCAU-China 2016; Hori et al. 2014). All of this corroborates successful gene editing in *K. flaccidum*.

While researching, we also found a bright red compound called astaxanthin. Astaxanthin is an antioxidant that can counteract many adverse effects of low-gravity environments, such as bone loss, effects of radiation exposure, and eye damage from bodily fluid pressure (Baker and Cortez 2019; Pilinska et al. 2016; Ambati et al. 2014). Using astaxanthin, we can also determine whether or not the transcription and translation of the gene sequence were successful by the appearance of a bright red color.

Lycopene is a precursor to astaxanthin along the beta-carotene pathway (Figure 1). It is bright red and is found in tomatoes and other fruits and vegetables. It has similar health benefits as astaxanthin, counteracting effects of radiation exposure and eye damage and benefiting bone health (Pirayesh Islamian and Mehral 2015; Selvan et al. 2011). Reported values of daily intake of lycopene range from 0.7 to 25.2 mg, while it has been found that daily doses of 5-10 mg significantly increased serum lycopene levels and significantly reduced lipid and protein oxidation (Selvan et al. 2011). However, the dosage of lycopene required for the aforementioned effects to occur has yet to be determined through experimentation.

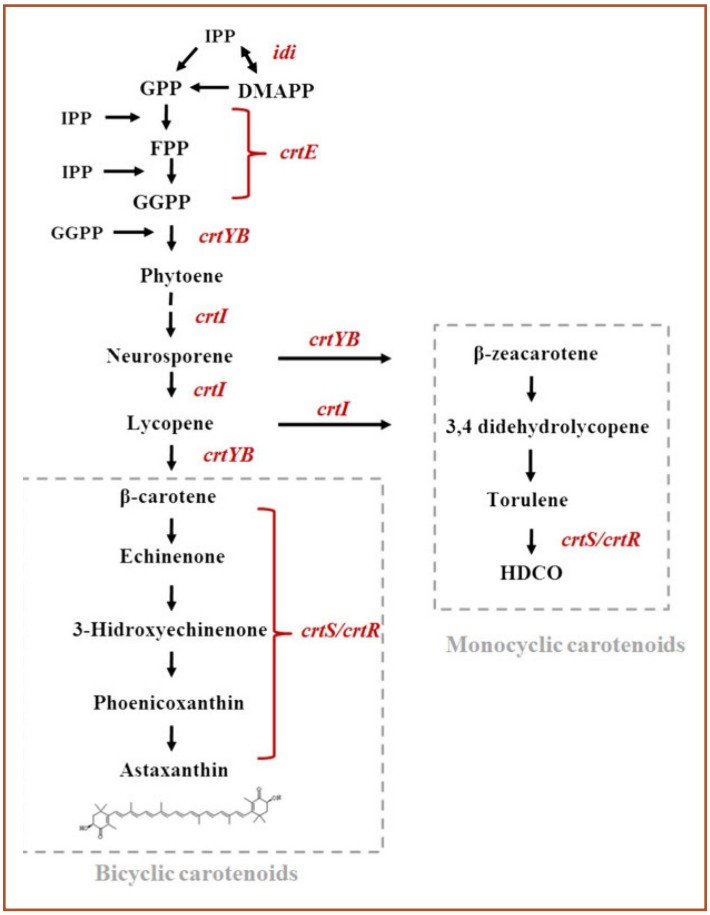

Figure 1. *Depiction of the beta-carotene biosynthesis pathway (Source: Contreras et al. 2013).*

Systems Level

Our proposed system is a food source that can be grown in space and will counteract many of the health-related issues that occur in space, as previously stated. The system consists of the microalgae chassis *Klebsormidium flaccidum* transformed to produce astaxanthin (Figure 2). Astaxanthin acts as both an antioxidant and a reporter by which we will determine whether or not the transcription and translation of the gene sequence are successful. At the systems level, we have identified light and nutrients, which are necessary for the algae to survive, as input, and astaxanthin as the output. The device-level description will take a closer look at the mechanism behind this system.

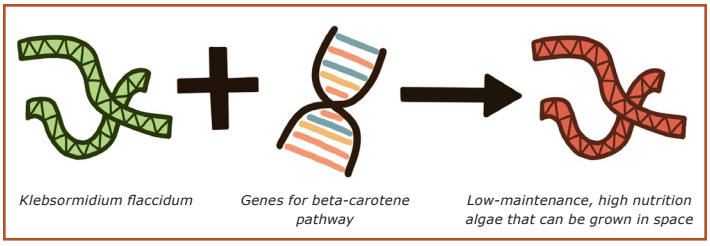

Figure 2. *Depiction of the systems level.*

Device Level

The chassis of our device is *Klebsormidium flaccidum.* The pathway that produces astaxanthin is the beta-carotene biosynthesis pathway. As depicted in Figure 1, the enzymes required to synthesize astaxanthin from farnesyl pyrophosphate are encoded by the genes crtE, crtYB, crtI, and crtS/crtR. To synthesize lycopene, only crtE, crtYB, and crtI are needed. Farnesyl pyrophosphate (farnesyl diphosphate, or FPP) is a molecule already produced by green algae and primitive plant species and is already produced by *K. flaccidum* (Baba et al. 2013; BioCyc 2019). Experimentation is required to determine the ideal strength of expression for lycopene and astaxanthin biosynthesis, as strong expression may inhibit the cells' ability to survive. Since the astaxanthin pathway is not normally utilized by *K. flaccidum*, it will be imperative to ensure that the chassis expresses the gene but is not overstressed.

Parts Level

We have identified the parts necessary for our design, all of which can be found in the iGEM registry; see Fig. 3 for a depiction of the parts level, along with the part identities in the iGEM registry.

The first part is a heat and light inducible promoter from *Chlamydomonas reinhardtii.* This part allows the coding sequence to run only in the presence of heat or light (McCall et al. 2018). This will prevent the *K. flaccidum* from expending too much energy while being stored or in transit. Since the health of the chassis, K. *flaccidum*, is central to the design's function, it is necessary to ensure that it is not overexerted by the new genes. Thus, we added the inducible promoter so that *K. flaccidum* will only express the genes in a suitable environment.

The next part comprises the ribosome binding site (RBS) and open reading frame (ORF), or coding sequence, for crtE from *Pantoea ananatis* (Zhang and Wang 2015). This encodes geranylgeranyl pyrophosphate synthase. This enzyme will allow the algae to convert farnesyl diphosphate, FPP, into geranylgeranyl pyrophosphate, or GGPP. As previously stated, FPP is already produced by *K. flaccidum*.

The next part is an ORF for crtYB, also from *P. ananatis*. This encodes phytoene synthase, an enzyme which allows the cell to manufacture phytoene from GGPP (Contreras et al. 2013).

The last ORF encodes crtI, also from *P. ananatis*l. This encodes phytoene dehydrogenase, an enzyme that catalyzes the conversion of phytoene to lycopene in a one-step reaction (BioCyc 2019). This part has been used previously in *Oryza sativa* for astaxanthin biosynthesis.

The final part is a double terminator. Having a double terminator increases the reliability of the part as opposed to a single terminator.

All of these parts will be compiled into a plasmid. The plasmid will be copied using PCR and will then be inserted into *K. flaccidum*. All of the parts are specific to algae or cyanobacteria, so they should be compatible with *K. flaccidum*.

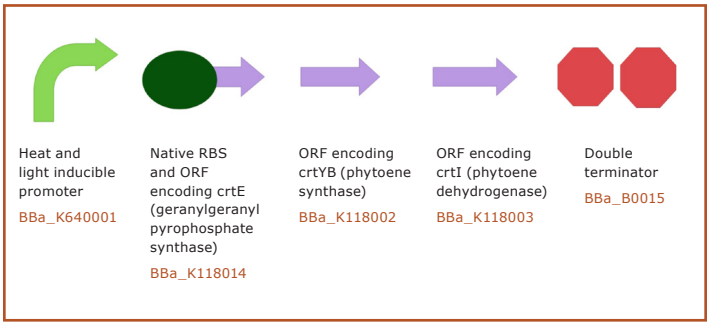

Figure 3. *Depiction of parts and BioBrick identities.*

Safety

None of the materials used and produced through our design are toxic to animals. The production of lycopene-enhanced *K. flaccidum* will be done in a controlled lab environment. *K. flaccidum* was already tested in an experiment in 2018, which was conducted using KALGAE™, a dried form of *K. flaccidum* harvested by ZIVO Biosciences, Inc. For a period of 90 days, rats were fed KALGAE™ in varying amounts, and no negative effects were observed in them (Brickel et al. 2018). The lycopene-enhanced *K. flaccidum* is anticipated to yield the same results as *K. flaccidum* var. ZIVO. These data can be used to reasonably predict the safety of lycopene-enhanced microalgae. However, before being cleared for use as a food source or made available commercially, the lycopene-enhanced microalgae will be thoroughly tested. Through extensive testing, we will be able to accurately determine the safety of ingesting the engineered space algae.

Discussions

As *K. flaccidum* has not yet been bioengineered, this experiment will also serve as a proof of concept. If it is found to be impractical to use *K. flaccidum* for this design, then we can attempt the experiment with another algae chassis. (*Chlamydomonas reinhardtii* could be a promising candidate for future research.)

We do not know how *K. flaccidum* will grow in space as the species has never been sent to space before. Thus, we must first observe any changes in the algae as it adapts to the microgravity environment; this would need to be done on the International Space Station (ISS). The microgravity environment may also stimulate a stress response that could affect the nutritional value of the algae (Baker and Cortez 2019).

Desiccation resistance is an asset when transporting algae because less water (and therefore less weight) is needed to sustain its dormant state. *K. flaccidum* reproduction slows under arid conditions, allowing it to withstand short periods without water. This ability would reduce the weight and resources needed to transport the algae. Similarly, cold tolerance is of use in the logistics of transportation into space. The method of transport for the lycopene-enhanced *K. flaccidum* will be similar to the transportation of other materials into space.

Aboard a space station or ship, maintaining a suitable atmosphere for astronauts is crucial to future space expeditions. To this end, algae will be useful in filtering carbon dioxide and producing oxygen through photosynthesis. The algae will be cultivated in space using gas-permeable cell culture bags, allowing the algae to use the carbon dioxide in the air to function and produce oxygen (Settles 2019). We have yet to determine the best method of harvest and consumption, but this process would be similar to methods already being employed on Earth and in space. We do not expect water to be a problem, as advanced closed systems are already in place on the ISS regarding water conservation. Sufficient artificial lighting must be provided in order for the algae to photosynthesize and grow.

This design would provide an insight into lycopene and astaxanthin production and biosynthesis on Earth to improve terrestrial manufacturing and storage methods. It may also help us better understand the extent of the nutritional benefits of lycopene and astaxanthin.

The product could also be repurposed for humanitarian purposes to provide food to areas that have been affected by famine or drought. In addition, it could be made accessible to the general public as a nutritional supplement, and it has been shown to have cancer preventing effects (McCall et al 2018; Zhang and Wang 2015).

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