Design Brief

Biosynthetic production of vitamin C in space*

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Maintaining sufficient vitamin C intake is essential for astronauts' health, particularly during long-duration space missions where the risk of deficiency increases. As astronauts embark on multi-year expeditions, the vitamin C content in processed foods aboard spacecraft degrades over time. Without sufficient vitamin C, tissue growth and repair, wound healing, and immune functions are significantly debilitated. We identified two plasmids containing the vitamin C production pathway from Arabidopsis thaliana to transform into Escherichia coli. By engineering a cosmic radiation-resistant variant of E. coli isolated by a group of researchers with the vitamin C pathway genes, our project would create a sustainable source of vitamin C for astronauts. To improve protein expression, we would use CRISPR to knock out the OmpT and Lon proteases in the radiation-resistant E. coli chassis. E. coli exposed to microgravity environments displays increased growth and production of recombinant DNA. Moreover, the adaptability of this method could be extended to the production of other essential vitamins, such as vitamin A and vitamin D. We hope to provide astronauts with the necessary nutrients to support their health during periods of deeper space exploration.

Sufficient nutrients are essential for maintaining human health and quality of life. Manned spacecraft exploration is expanding from the Moon to Mars and our Solar system. As space exploration expands, consuming sufficient nutrients in space becomes crucial for astronauts. Astronauts spend extended time in space, sometimes for months, and even years. Without access to fresh produce, astronauts need to gain access to appropriate nutrients. One of these critical nutrients is vitamin C (L ascorbic acid) (see Table 1 below), usually found in fruits and vegetables. As fresh produce is not readily accessible in space, providing astronauts with ample vitamin C poses a challenge.

Historically, vitamin C deficiency has been a significant factor in the failure of terrestrial explorations. Scurvy was common among explorers who lacked access to fresh produce in the 15th to 18th centuries. Explorers from Magellan and da Gama to 20th-century polar explorers suffered from scurvy, which significantly hindered their progress. As vitamin C is now more readily available through supplements and fresh produce and can be delivered, scurvy is no longer a problem (NASA, 2023). However, as the scope of space exploration expands to environments that cannot maintain a fresh supply system, and span multiple-year

Table 1. Percent degradation of three common mixtures of vitamin C in 98% relative humidity after 12 weeks (Hiatt et al. 2011).

Formulation	Percent degradation in 98% relative humidity after 12 weeks
ascorbic acid	9.6 ± 4.8
sodium ascorbate	98.7 ± 1.8
calcium ascorbate	100.0 ± 0.2

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voyages, scurvy and other vitamin C deficiency-related diseases can pose a threat to astronauts.

Vitamin C degradation depends on how it is consumed. Half of it takes four weeks to degrade when in the form of fruit juice. Figure 1 shows the degradation of three common forms of vitamin C after 12 weeks in 98% relative humidity, highlighting the challenges in preserving vitamin C for space missions. Water-soluble vitamins have taken significantly longer to degrade, but this time should be extended when exploring more profound parts of space.

NASA's research indicates that simple vitamin C supplements will no longer be sufficient to provide astronauts with vitamin C for further exploration into deep space outside the solar system. With each trip taking multiple years, preserving vitamin C in supplemental capsules or any other form of food is impossible. Vitamin C in space-processed food degrades to lower levels in three years. (Tang, 2021) Supply missions are possible, but they incur a significant cost. The most efficient means of transporting resources to low Earth orbit costs $ 2,720 per kilogram. This figure would significantly increase for missions outside low Earth orbit. Therefore, the most efficient way is to produce vitamin C in space. (NASA, 2023)

The main synthetic biology approaches use *E. coli* to produce vitamin C. *E. coli* is a standard chassis in synthetic biology, and it utilizes different microbial carbon fixation systems. A group of scientists from Aboa integrated the iGEMINI optogenetic flavor system into their project.

In an iGEM study, researchers produced vitamins A, C, and E using *E. coli*. For vitamin C, they utilized a five-step pathway to convert from GDP-mannose, which is naturally present in *E. coli*.

However, another group of scientists found that *E. coli* does not produce enough GDP-mannose to direct it toward vitamin C production (Tian et al., 2022). In the Tian et al. (2022) study, *E. coli* was engineered with 10 genes separated into two plasmids: one for producing GDP-mannose from D-glucose and another for converting GDP-mannose into vitamin C (L-ascorbic acid).

The necessary genes from *A. thaliana* were codon-optimized and chemically synthesized using a two-step PCR-based DNA synthesis method. Then, the gene expression cassette was constructed from the T7 promoter and the T7 terminator.

The first five genes (*AtHXK1S, AtPGIS, AtDIN9S, AtPMMS, and AtVTC1S*) expression cassettes were re-amplified with primers containing restriction enzyme digestion sites. This led to the process of the last five genes (*AtGMES, AtVTC2S, AtVTC4S, AtGalDHS*, and *AtGLDHS*). Additionally, introns in the gene sequences were removed to enable functionality in bacterial cells. This two-step fermentation process is significantly more environmentally friendly than other processes.

To produce vitamin C efficiently during long-term space missions, we have selected *E. coli* as the chassis due to its versatile nature. Biological methods enable astronauts to produce vitamin C on demand, using fewer resources. Two primary environmental conditions in space differ significantly from those on Earth: microgravity (MG) and cosmic radiation. In a 2009 study, *E. coli* exposed to MG environments exhibited increased growth and production of recombinant DNA, indicating greater efficiency in producing vitamin C (Xiang et al., 2009). Our design addresses the effect of cosmic radiation on *E. coli* by engineering a particular strain of *E. coli*. Recently, a group of researchers subjected *E. coli* to 100 cycles of experimental selection under radiation dosages that killed 99% of the population. The *E. coli* that survived evolved a protective protein that confers IR resistance, similar to that of *Deinococcus radiodurans* (Bruckbauer et al., 2020). With this specific strain as the chassis, cosmic radiation, composed mainly of ionizing radiation, should pose less of a problem. We would use CRISPR to knock out the OmpT and Lon proteases in the chassis to improve protein expression. Previous research suggests that knocking out OmpT and Lon proteases prevents the breakdown of expressed proteins and consequently increases protein yield. (Waegman 2011) The *E. coli* cells would be stored on board in a dessicated form, where they would remain dormant until needed for use. This approach places less stress on the cells compared to methods such as freeze-

drying (Wolkers, W. F., & Oldenhof, H., 2021). With the demonstrated feasibility of the GDP-D-Mannose pathway, *E. coli* can provide astronauts with a reliable supply of vitamin C in space (Tian et al., 2022).

Systems level

Our system enables on-demand production of vitamin C in space using a genetically engineered strain of *E. coli,* addressing the challenge of maintaining astronauts' health during long-duration space missions. We designed a two-plasmid pathway, based on Tian et al. (2022), that expresses ten codon-optimized genes from *Arabidopsis thaliana.* The first plasmid converts D-glucose to GDP-mannose, an intermediate in the biosynthesis of vitamin C; while the second transforms GDP-mannose into L-ascorbic acid, the active form of vitamin C required for astronauts' health. All genes are under T7 control, with introns removed and sequences adapted for bacterial expression.

To improve resilience in space, we utilize the radiation-resistant *E. coli* strain developed through experimental evolution (Bruckbauer et al., 2020). We also employ CRISPR knockouts of OmpT and Lon proteases to enhance protein yield. These modifications were made to BL21, a common laboratory strain used for recombinant protein production. Microgravity enhances the growth and recombinant expression of *E. coli* (Xiang et al., 2009), further improving efficiency. For storage, cells are cryopreserved onboard, avoiding stress from freeze-drying (Wolkers & Oldenhof, 2021). On the spaceship, astronauts will be able to use a centrifuge to separate vitamin C from the cells, then refine the product into pills for human consumption. This modular, space-adapted system ensures a reliable, resource-efficient supply of vitamin C for astronauts on long-duration missions. (Holpuch 2018)

Device level

For our design to work, we must examine its key components—the radio-resistant strain IR9-100-2 *E. coli*. A radioresistant strain is suitable for microgravity environments, so

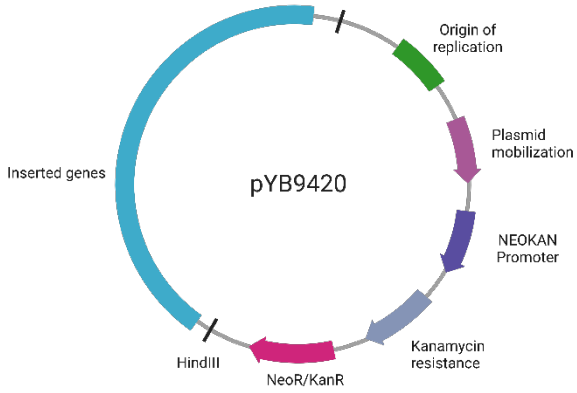

Figure 1. Plasmid PYB9420 was Created in BioRender. Collier, B. (2025) https://BioRender.com/pqq0fav.

finding a strain like IR9-100-2 as a chassis is a good starting point. Another crucial part of our design is to help our chassis better produce vitamin C. To achieve this, we will eliminate two enzymes in the chassis. We will use CRISPR to remove both Lon and OmpT proteases from IR9-100-2. This will ultimately improve our growth while the *E. coli* is in space. Lastly, we will insert a gene for a new enzyme called the T7 promoter. This will promote the development of vitamin C in *E. coli,* which is healthy for humans to consume.

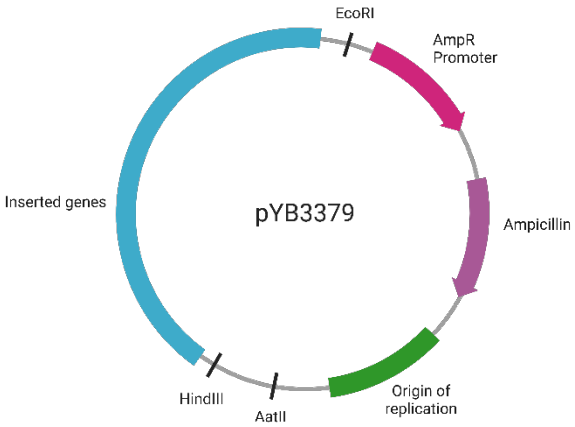

Figure 2. PYB3379 Created in BioRender. Collier, B. (2025) https://BioRender.com/z0b1k37.

Parts level

The 10-step biosynthetic pathway for vitamin C production is divided into two parts, each

consisting of five genes, which work together to convert simple sugars into the active form of vitamin C. The first five genes (*AtHXK1S, AtPGIS, AtDIN9S, AtPMMS,* and *AtVTC1S*) convert D-glucose to GDP-Mannose, which is responsible for not only vitamin C biosynthesis, but also cell wall generation and protein glycosylation. The second part (*AtGMES, AtVTC2S, AtVTC4S, AtGalDHS,* and *AtGLDHS*) produces ascorbic acid from GDP-Mannose. The S following the gene names indicates that they have been codon/optimized by the researchers who developed this synthetic pathway (Tian et al., 2022). Codon optimization is important because it enhances gene expression in heterologous hosts, leading to increased protein production. Both components of the pathway employ the T7 promoter and terminator, creating a gene expression cassette (Figures 3 & 4). They are located in the corresponding ORF of two plasmids.

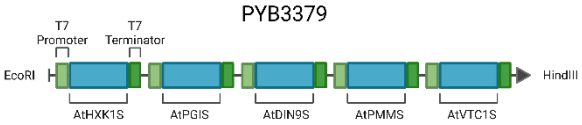

Figure 3. ORF of Plasmid PYB3379 Created in BioRender. Collier, B. (2025) https://BioRender.com/9vlhaz7.

The plasmid containing the first part is PYB9420 (Figure 1). This plasmid includes the NEOKAN promoter, which drives the expression of the selectable marker (kanamycin and neomycin resistance) downstream. It also has a section that enables the plasmid to move between bacteria via conjugation. The second plasmid, PYB3379 (Figure 2), uses the ampicillin resistance gene as the selectable marker. Both plasmids have the same restriction sites: EcoRI and HindIII (Genbank).

The two genes we are knocking out from our chassis to enhance recombinant protein production are Lon and OmpT proteases. We would outsource the specifics of the gene removal to New England Biolabs. Together, they are responsible for the quality control of proteins (Jeong, H. et al, 2015). Lon protease degrades misfolded or damaged proteins. Removing them with CRISPR prevents the unwanted degradation of recombinant proteins.

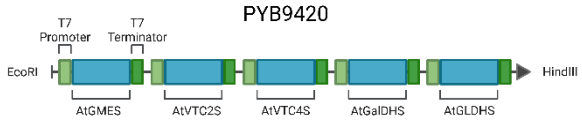

Figure 4. ORF of Plasmid PYB9420 Created in BioRender. Collier, B. (2025) https://BioRender.com/b5wgq2k.

Safety

According to the Centers for Disease Control and Prevention (CDC), *E. coli* is a potentially harmful bacterium that can cause severe gastrointestinal illness if ingested or mishandled. In laboratory research, *E. coli* is classified as a Biosafety Level 2 (BSL-2) organism, meaning it poses a moderate risk to personnel and the environment. BSL-2 laboratories are required to include safety measures such as handwashing and eye-washing stations, automatically closing doors, and equipment for waste decontamination, such as autoclaves and incinerators, If appropriate safety considerations are followed, there will be minimal risk of health issues.

E. coli infection could result in diarrhea, pneumonia, and meningitis. In space, in micro-gravity conditions, *E. coli* becomes more virulent and produces more biofilm (Chavez 2024), which enhances its immunity to antibiotics and host immune systems. This additional risk means astronauts have to be even more cautious when handling *E. coli.*

CRISPR-Cas9 gene-editing technology is highly precise but not without risk. Off-target genetic changes and unintended biological effects can occur, especially in complex organisms. Ethical and safety reviews, along with containment procedures, are essential when using CRISPR in both clinical and experimental settings.

Discussion

The IR9-100-2 *E. coli*, developed by Brauckbauer et al., has radio-resistant properties that allow it to be safely used in space. However, it lacks some of the enhanced protein production characteristics of the commonly used BL21 *E. coli.* The

BL21 *E. coli* has been engineered to produce the T7 promoter and is deficient in Lon and OmpT proteases. This leads to increased protein production, allowing it to be used as the primary strain of *E. coli* for recombinant protein production (Ratelade 2009).

Lon and OmpT proteases serve to degrade misfolded proteins and prevent specific proteins from accumulating. *E. coli* strains that are deficient in these proteases are more amenable to the production of certain proteins, including vitamin C. Furthermore, in Lon cells, SulA, a cell division inhibitor, accumulates and causes cells to become hypersensitive to DNA damage. This significantly inhibits the ability of *E. coli* to produce vitamin C in space.

By knocking out Lon and OmpT proteases from IR9-100-2, we can increase protein yield and reinforce the resistance of IR9 to microgravity and other space conditions.

The modified IR9-100-2 can be used to produce other essential vitamins. Previous research suggests that *E. coli* could be used to make vitamin C, vitamin E, and beta-carotene (Ponda et al. 2024). Vitamin E helps maintain healthy skin and eyes and strengthens the body's immune system. (NHS 2020) Beta-carotene acts as a precursor to vitamin A and is a powerful antioxidant.

Each product uses different genes to produce. As mentioned above, Vitamin C requires AtGMES, AtVTC2S, AtVTC4S, AtGALDHS, and AtGLDHS (Tian et al. 2022). Vitamin E requires hpd, CrtE, ggh, hpt, and cyc (Albermann et al. 2008). Beta-carotene requires CrtB, CrtI, and CrtY. By using different genes, the modified *E. coli* will be able to provide a sustainable source of nutrients in space, not only for vitamin C, but also for vitamin E and beta-carotene.

Next steps

When we want to begin constructing our design, we will contact the ABOA science group that made the anti-microgravity *E. coli*. Therefore, we will be able to start with our base chassis. Once we contact them, we will explain our project to the group in return for the *E. coli* IR9-100-2. We will then need to get the promoter enzymes for the IR9-100-2 *E. coli*. We will use the T7 Promoter found in the regular *E. coli.* This enzyme will help speed up the growth of the vitamin C inside, making it purer and healthier for astronauts. We will obtain the last piece of the puzzle once we alter the IR9-100-2 to make vitamin C. pYB3379, a recombinant plasmid that has the necessary genes to create vitamin C within the *E. coli.* This particular plasmid is derived from a different *E. coli* strain that was transformed by a group of scientists at BMC who have already done this, and I will contact them for insights on their work.

We will utilize New England BioLabs' EnGen Spy Cas9 HF1 system to knock out the OmpT and Lon proteases in the IR9-100-2 strain of *E. coli* using CRISPR. (New England Biolabs) This will be done by delivering RNP complexes along with donor DNA templates for precise genome editing.

We will transform the obtained plasmids into the altered IR9-100-2. After recovery, we will place the bacteria on a plate supplemented with ampicillin and kanamycin for selection. The final transformant will need to be tested for its efficiency in producing vitamin C, preferably under conditions simulating the interior of a spacecraft. This will help us determine if it's worth bringing *E. coli* to space, considering it will have to be maintained and there exists the serious risk of contamination. Then we will have to convert the vitamin C solution into a safe, consumable form. To achieve this, centrifugation or filtration is necessary to separate the cells from the solution, and freeze/drying is required to improve the purity of the Vitamin C (Lee 1992).

Author contributions

BF contributed by developing the initial idea, writing the parts-level content, and sorting the citations. HY contributed by writing the system-level safety and discussion sections. BC contributed by writing the device-level content, creating figures, editing, and producing the video. In addition, we all contributed to writing the introduction and abstract.

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