### Title

Molecular pharming to support human life on the moon, mars, and beyond.




### Journal

Critical reviews in biotechnology, 41(6)

### ISSN

0738-8551



### Authors

McNulty, Matthew J
Xiong, Yongao Mary
Yates, Kevin
et al.



### Publication Date

2021-09-01

### DOI

10.1080/07388551.2021.1888070

Peer reviewed

Taylor & Francis
Taylor & Francis Group

Critical Reviews in Biotechnology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ibty20

# Molecular pharming to support human life on the moon, mars, and beyond

Matthew J. McNulty, Yongao (Mary) Xiong, Kevin Yates, Kalimuthu Karuppanan, Jacob M. Hilzinger, Aaron J. Berliner, Jesse Delzio, Adam P. Arkin, Nancy E. Lane, Somen Nandi & Karen A. McDonald

**To cite this article:** Matthew J. McNulty, Yongao (Mary) Xiong, Kevin Yates, Kalimuthu Karuppanan, Jacob M. Hilzinger, Aaron J. Berliner, Jesse Delzio, Adam P. Arkin, Nancy E. Lane, Somen Nandi & Karen A. McDonald (2021): Molecular pharming to support human life on the moon, mars, and beyond, Critical Reviews in Biotechnology, DOI: 10.1080/07388551.2021.1888070

**To link to this article:** https://doi.org/10.1080/07388551.2021.1888070

CRITICAL REVIEWS IN BIOTECHNOLOGY
https://doi.org/10.1080/07388551.2021.1888070

Taylor & Francis
Taylor & Francis Group

REVIEW ARTICLE

OPEN ACCESS Check for updates

# Molecular pharming to support human life on the moon, mars, and beyond

Matthew J. McNulty[a,b], Yongao (Mary) Xiong[a,b]*, Kevin Yates[a,b]*, Kalimuthu Karuppanan[a,c], Jacob M. Hilzinger[a,d], Aaron J. Berliner[a,d], Jesse Delzio[a,b], Adam P. Arkin[a,d], Nancy E. Lane[e], Somen Nandi[a,b,f] and Karen A. McDonald[a,b,f]

[a]Center for the Utilization of Biological Engineering in Space (CUBES), Berkeley, CA, USA; [b]Department of Chemical Engineering, University of California, Davis, CA, USA; [c]Radcliffe Department of Medicine, Oxford University, Oxford, UK; [d]Department of Bioengineering, University of California, Berkeley, CA, USA; [e]Center for Musculoskeletal Health, School of Medicine, University of California, Davis, CA, USA; [f]Global HealthShare® Initiative, University of California, Davis, CA, USA

**ABSTRACT**

Space missions have always assumed that the risk of spacecraft malfunction far outweighs the risk of human system failure. This assumption breaks down for longer duration exploration missions and exposes vulnerabilities in space medical systems. Space agencies can no longer reduce the majority of the human health and performance risks through crew members selection process and emergency re-supply or evacuation. No mature medical solutions exist to address this risk. With recent advances in biotechnology, there is promise for lessening this risk by augmenting a space pharmacy with a biologically-based space foundry for the on-demand manufacturing of high-value medical products. Here we review the challenges and opportunities of molecular pharming, the production of pharmaceuticals in plants, as the basis of a space medical foundry to close the risk gap in current space medical systems. Plants have long been considered to be an important life support object in space and can now also be viewed as programmable factories in space. Advances in molecular pharming-based space foundries will have widespread applications in promoting simple and accessible pharmaceutical manufacturing on Earth.

**ARTICLE HISTORY**
Received 29 September 2020
Revised 27 November 2020
Accepted 19 December 2020

**KEYWORDS**
Plant molecular pharming; synthetic biology; biomanufacturing; space medicine; medical foundry; medical countermeasure; oxygenic photoautotroph; space exploration

## Re-thinking human health for deep space missions

Humanity has collectively returned its gaze to the stars as space agencies and companies around the world work to develop new strategies to extend human presence farther into the universe. To arrive there, we need to transition from Earth-reliant to Earth-independent mission architecture. Agencies like the National Aeronautics and Space Administration (NASA) and European Space Agency (ESA) have developed exceptional life support systems for Earth-reliant human missions into space [1]. Carefully planned medicine, food, and environmental control re-supply shuttles working in concert with on-board environmental control and life support systems maintain a habitable environment for astronauts in the International Space Station (ISS) [2].

However, as space missions become longer and they probe deeper into the solar system – to the Moon, to Mars, and beyond – frequent re-supplies for life support systems will become increasingly burdensome. Current exploration medical capabilities are particularly vulnerable to a lower rate of resupply and longer missions. The list of necessary supplies to address persistent exposures of space travel adds up quickly, including: countermeasures for increased radiation [3,4], bone loss [5,6], kidney stones [7,8], vision impairment [9], and adverse behavioral conditions [10] to name a few. The list of supplies begins to look unmanageable when you add in intermittent, or even unanticipated, exposures such as microbial infection [11–13], and spaceflight-induced genome instability and metabolic changes [14]. As mission duration increases, the risk of a low probability medical condition is amplified. When an astronaut is on Mars and the closest hospital or medical re-supply is at least 200 days of interplanetary travel away [15], it is critical that astronauts are medically self-sufficient.

CONTACT Karen A. McDonald kamcdonald@ucdavis.edu Department of Chemical Engineering, University of California, Davis, CA, USA
*These authors contributed equally for this manuscript

Supplementary data for this article can be accessed here.

Furthermore, the recent literature highlights systemic vulnerabilities in space-flown pharmaceutical life support in the biased and underreported historical data of in-flight pharmaceutical use and efficacy, limited fidelity of current ground-analog models, and the in-flight instability of drug formulations [16]. Of the small molecule solid formulations tested thus far, three-quarters will have degraded by the end of the proposed first Mars mission duration [15]. There has been no spaceflight testing of biologics, a critical category of pharmaceuticals known to be less stable than small molecule drugs [17,18]. These issues highlight the need to develop platform technologies for the on-demand production of medicines.

## Defining a medical foundry for space exploration

The medical systems of future space exploration will need to be reconfigured to guarantee astronaut health. The contemporary standard is the NASA-provided ISS crew health care system (CHeCS) consisting of three sub-systems: (1) the countermeasures system (CMS) composed of exercise hardware and monitoring devices, (2) the environmental health system (EHS) composed of hardware for environmental monitoring, and (3) the health maintenance system (HMS) composed of a medical kit for supporting routine minor medical needs for up to 180 days [19]. Earth-reliant medical systems like CHeCS will need to be augmented with medical foundries for self-sufficiency in Earth-independent space mission architectures. A space medical foundry will expand mission capabilities to include high-value medical product manufacturing, of which pharmaceuticals will be a critical product class. This is particularly important for extended duration exploration, and settlement, of extraterrestrial bodies such as the Moon and Mars.

A space foundry, of which a medical foundry is a subset, must be capable of utilizing a limited set of inputs (ideally *in situ* resources with minimal flown resources) to generate a wide spectrum of outputs and must be able to do so in a simple, closed loop. Recent literature has detailed a compelling narrative for the use of biotechnology to answer these challenges [15,20,21]. The Center for Utilization of Biological Engineering in Space (https://cubes.space) is a multi-university effort to realize the inherent mass, power, and volume advantages of space biotechnology and advance the practicality of a nearly closed loop, photoautotrophic factory for production of food, pharmaceuticals, and materials on a Mars mission.

An alternative method for pharmaceutical production is chemical synthesis. In producing small molecule pharmaceuticals, chemical synthesis is often advantageous on Earth. However, as stereochemical complexity and size of the target pharmaceutical increases, chemical synthesis often becomes dramatically less feasible and attractive. For perspective, there are examples of chemical synthesis used commercially to produce pharmaceuticals as large as peptides (5–50 amino acids) [22,23], but antibodies, an example class of life-saving pharmaceuticals produced only in biological systems (termed "biologics"), are two orders of magnitude larger (~1,400 amino acids) than that. Chemical synthesis of pharmaceuticals can also be contrasted with biological production as having highly reaction-specific inputs and complex synthesis steps, often requiring the use of organic solvents and generating substantial waste by-products, all of which are undesirable attributes for space applications. Chemical synthesis may be necessary for a robust medical foundry for space, indeed it will likely be required to synthesize nucleic acids to mobilize biological production in space [24], but it will not be sufficient to produce all countermeasures.

Space biotechnology has primarily focused on microbes [15,20], fungi [25], and plants [20,26]. From this perspective, we review the potential utility of plants as a molecular medical foundry for the production of pharmaceuticals in deep space and contrast this with the capabilities of alternative biological organisms.

## Plants in space

Plants are an established facet of space mission architecture, with research dating back to the 1950s [27]. Most recently, a study on red romaine lettuce grown in the International Space Station (ISS) using the Vegetable Production System (Veggie) has reported that leafy vegetable crops can be grown and consumed safely in the ISS as a dietary supplement [28].

Resource flexibility is essential in the confined environments of a space mission, and researchers have shown that plants serve as versatile assets in a space mission life support system. Up to this point, studies have focused on the value of plants to harness solar energy and provide nutrients, and for water treatment, air treatment, and behavioral health [27–31]. Accordingly, research into advancing the capabilities of plants for space has primarily focused on those key areas. What has not been captured in published research is the potential of plants to

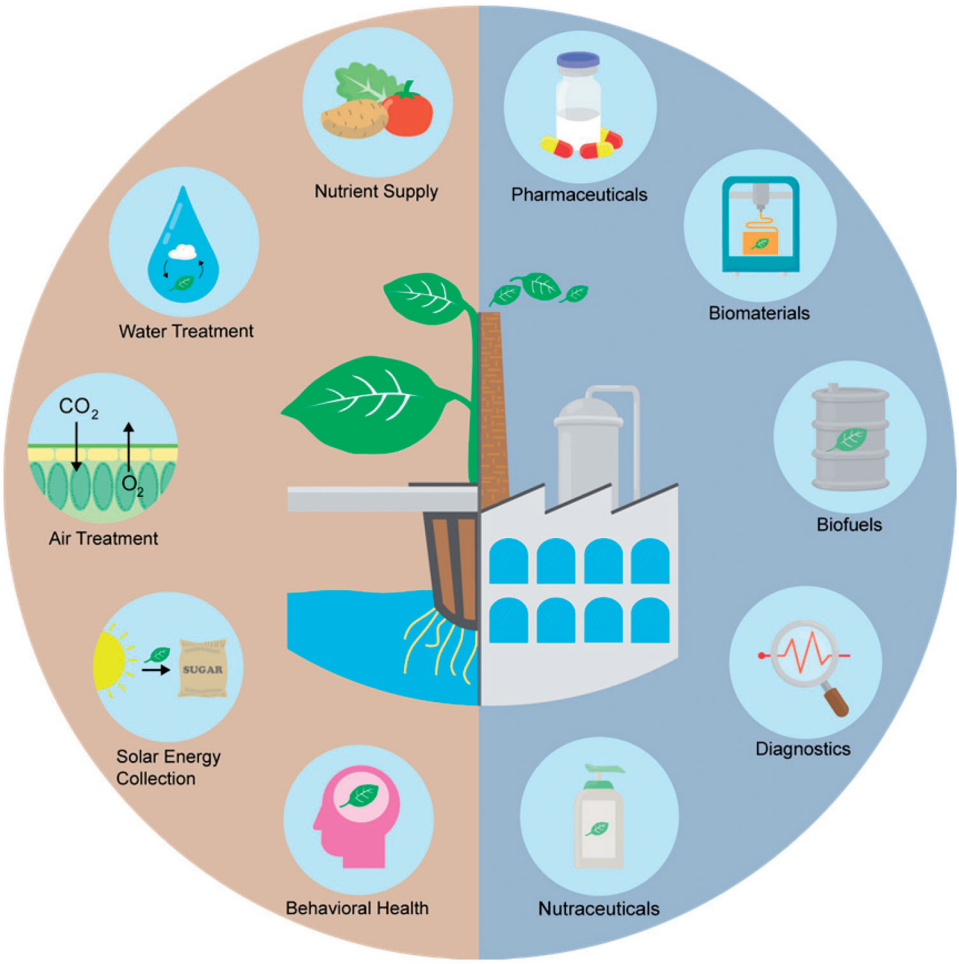


**Figure 1.** Molecular pharming embodies the perspective that plants are chemical factories. Viewing plants as factories vastly expands the bioregenerative life support capabilities of plants in space. Here we focus on molecular pharming of pharmaceuticals.

provide astronauts with pharmaceuticals and other high value products, which is formally known as molecular pharming (Figure 1) [32].

## Supporting life with molecular pharming

Humans have looked to plants as a source of healing for thousands of years [33]. To date, there are over 120 commercially available drugs consisting of distinct chemical substances that have been derived from plants [34]. This list includes widely used medications such as aspirin [35], the most commonly used drug in the world, paclitaxel [36], which is used to treat various forms of cancer, and artemisinin [37], an antimalarial compound.

The breadth of therapeutically-relevant molecules that we can now produce in a plant to support human life has exploded with recombinant DNA technology. Plants have been used to produce a wide variety of complex products for supporting human life – ranging from products as diverse as diagnostic reagents and therapeutic proteins, to biomaterials and biofuels. Pioneering work in the past twenty years on plant-based production systems has positioned molecular pharming competitively for commercial applications of these diverse products on Earth [38–43]. Continuing those advances, we focus on producing pharmaceuticals as a high-priority application of molecular pharming to mitigate human health risks in extended deep space exploration.

The first commercial therapeutic protein to be produced recombinantly in plant cells (Elelyso®) was approved for enzyme replacement therapy in 2012 [44,45]. While this product is produced in plant cell culture, it has established a regulatory pathway for addressing concerns with plant-based production in general. There is currently a wide range of whole plant-produced pharmaceuticals in commercial pipelines. Perhaps most notably, Medicago's clinical program

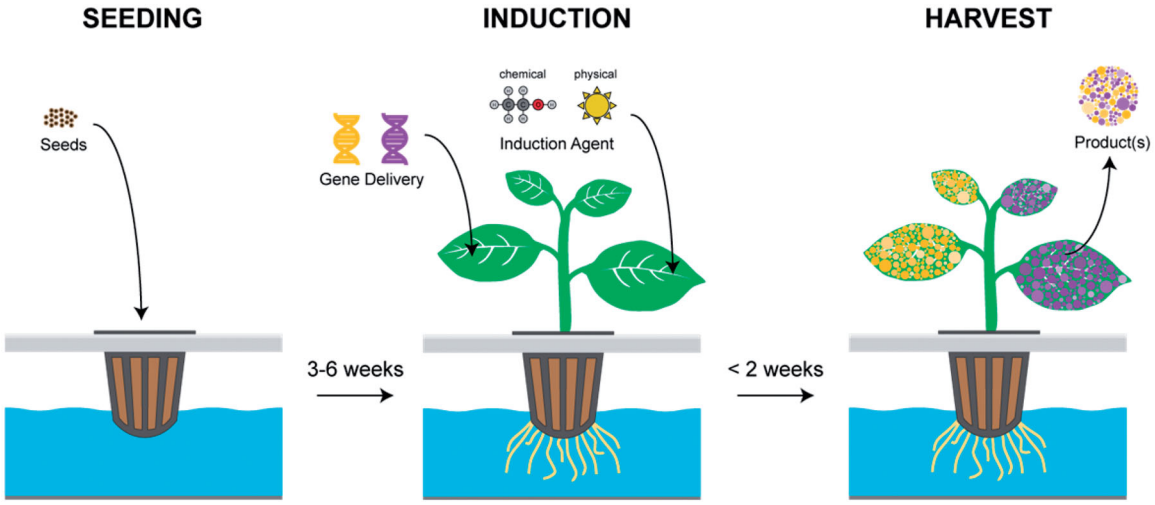


**Figure 2.** The simplicity of molecular pharming, illustrated. Producing recombinant products can be induced *via* gene delivery (transient production) or an induction agent (inducible transgenic production). Recombinant products accumulate in constitutive transgenic production without an induction step.

consists of an influenza vaccine in Phase 3 trials (ClinicalTrials.gov Identifier: NCT03739112) and several other vaccine candidates in earlier stages. Molecular pharming has also found commercial success in other application areas. For example, in diagnostic reagents, with avidin produced in maize [46], veterinary medicine, with canine interferon-alpha produced in strawberry [47], nutraceuticals, with human growth factors produced in barley [48], and commodity chemicals, with cell culture media components produced in rice [49].

### *Pairing production strategies with disease states*

Molecular pharming with whole plants can be performed by using one of two strategies: transient production using gene delivery systems to introduce genes for the plant to temporarily transcribe and translate on-demand, or transgenic production using plants with recombinant genes inserted into the genome for stable translation (Figure 2). Either strategy can be executed to produce recombinant products using a simple process flow.

Transient production is a strategy that can provide on-demand transformation of food into a medical, or some other high-value product, resource. This enables a rapid response in which initiation of production is linked to the exceeding of some risk threshold, be it triggered by the emergence of a diagnosed disease state or an increased probability of occurrence. This allows stockpiles to be minimized for low frequency disease states, and perhaps, most importantly, builds capability to respond to unanticipated disease states. Key parameters of transient production to meet these capabilities are the production lead time (how fast can a dose of medicine be produced), the specific productivity (how much biomass is needed for a dose of medicine), and the manufacturing resources (which equipment and materials are needed for production). There are a variety of established transient production systems that employ both biotic and abiotic methods as shown in Figure 3. Table 1 summarizes key process differences in these transient production systems. Selecting the most effective transient production system depends on the disease state (e.g. a small time-to-treatment window) and the exploration mission architecture (e.g. available resources).

Transgenic production is the simplest form of molecular pharming. Pharmaceutical production capability is hardwired into the genome of the plant through either nuclear or plastid engineering [50]. No additional manufacturing resources beyond those used for plants as a traditional bioregenerative life support object are needed, except an induction agent (e.g. heat, ethanol) for inducible promoter-controlled transgenics [51]. This allows for the simple and sustained production of pre-determined molecular target(s) for which a consistent demand is anticipated. Transgenic plants for medical countermeasure production will most likely be distinct resources from food crops unless strategies such as inducible promoters or tissue-specific expression (e.g. pharmaceuticals produced only in inedible biomass) are employed.

Combined, transient and transgenic production systems have the potential to cover the breadth of pharmaceutical production needs for deep space missions. Anticipated human health-impacting exposures in deep space missions include intermittent and

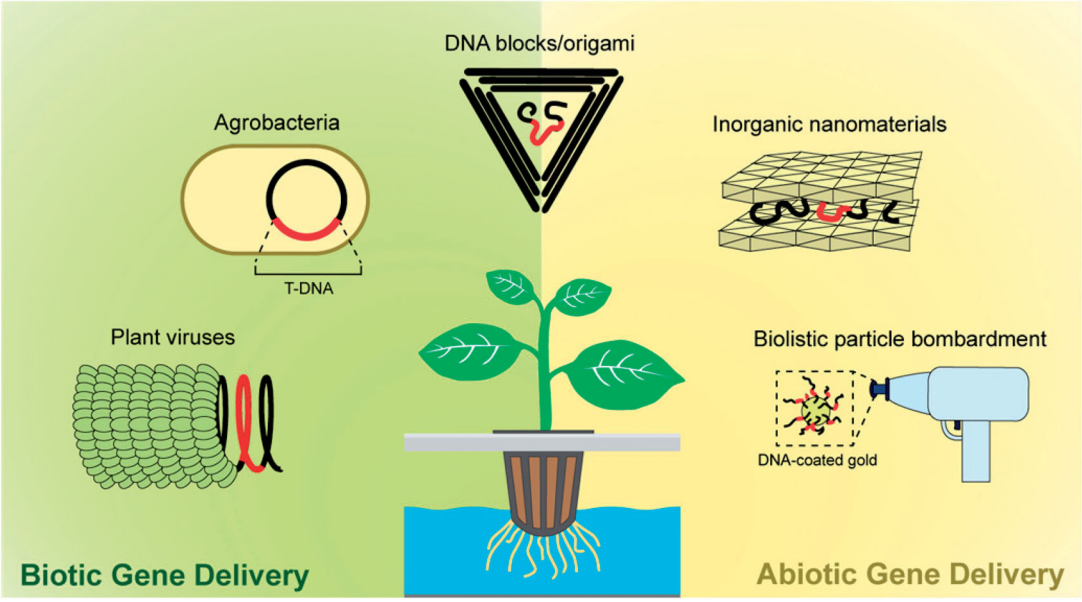


**Figure 3.** A look at previously established biotic and abiotic methods (also referred to as indirect and direct methods, respectively) for transient expression of recombinant products in plant systems.

**Table 1.** A comparison of key attributes between molecular pharming-based transient production methods.

| | Plant viruses [103] | Agrobacteria [104] | DNA blocks/origami [105] | Inorganic nanomaterials [106] | Biolistic particle bombardment [107] |
|---|---|---|---|---|---|
| Mode of administration | Mechanical transmission | Vacuum, syringe, spray | Vacuum, syringe | Root drenching | Gas-pressured gene gun |
| Vehicle size | 10–500 nm | 1000–3000 nm | 100–500 nm | 1–60 nm | 500–1600 nm |
| Host range | Virus-specific by plant family (can be more; TMV infects 11 families) | Dicot and certain monocot species | Unrestricted[a] | Unrestricted[a] | Unrestricted[a] |
| Insert size | <10 kbp | <150 kbp | Unrestricted[a] | Unrestricted[a] | <25 kbp[a] |
| Level of expertise | Low | Medium | Medium | Low | Low |
| Equipment requirements | Low | Medium | Low | Low | Medium |

Level of expertise and equipment requirement rankings were determined using working process knowledge and the cited reference material.
[a]Based on limited research data available; potential limitations may be uncovered with further investigation.

persistent modes, within which both acute and chronic disease states are possible.

Chronic disease states needing a constant supply of medical countermeasures are most likely best addressed by using the simpler manufacturing of transgenic production. Transient production is also a viable strategy for meeting the medical needs of chronic disease states; it often yields higher specific productivity of a product per biomass basis [52]. However, the higher resource demand of production and concerns of long-term pharmaceutical stability (if stockpiles were generated using transient production) raise potential disadvantages in transient production for chronic disease states, such as microgravity-induced osteopenia.

For acute disease states above a certain risk level, defined by both likelihood of occurrence and severity of mission impact, it may be valuable to generate transgenic plants to produce countermeasures. On the other hand, transient production may be a more cost-effective strategy for reducing mission risk associated with lower risk, and unanticipated, disease states. Here we reiterate that providing medical countermeasures for unanticipated disease states should not be underestimated.

The delineation of best use cases for transgenic and transient production system selection depends on mission architecture and the specific resource availability. The decision tree shown in Figure 4 provides a foundational logic framework for evaluating and selecting an appropriate molecular pharming production system on a situational basis.

## *A test case for molecular pharming in space*

Consider a deep space exploration mission in which plants are grown for their previously established

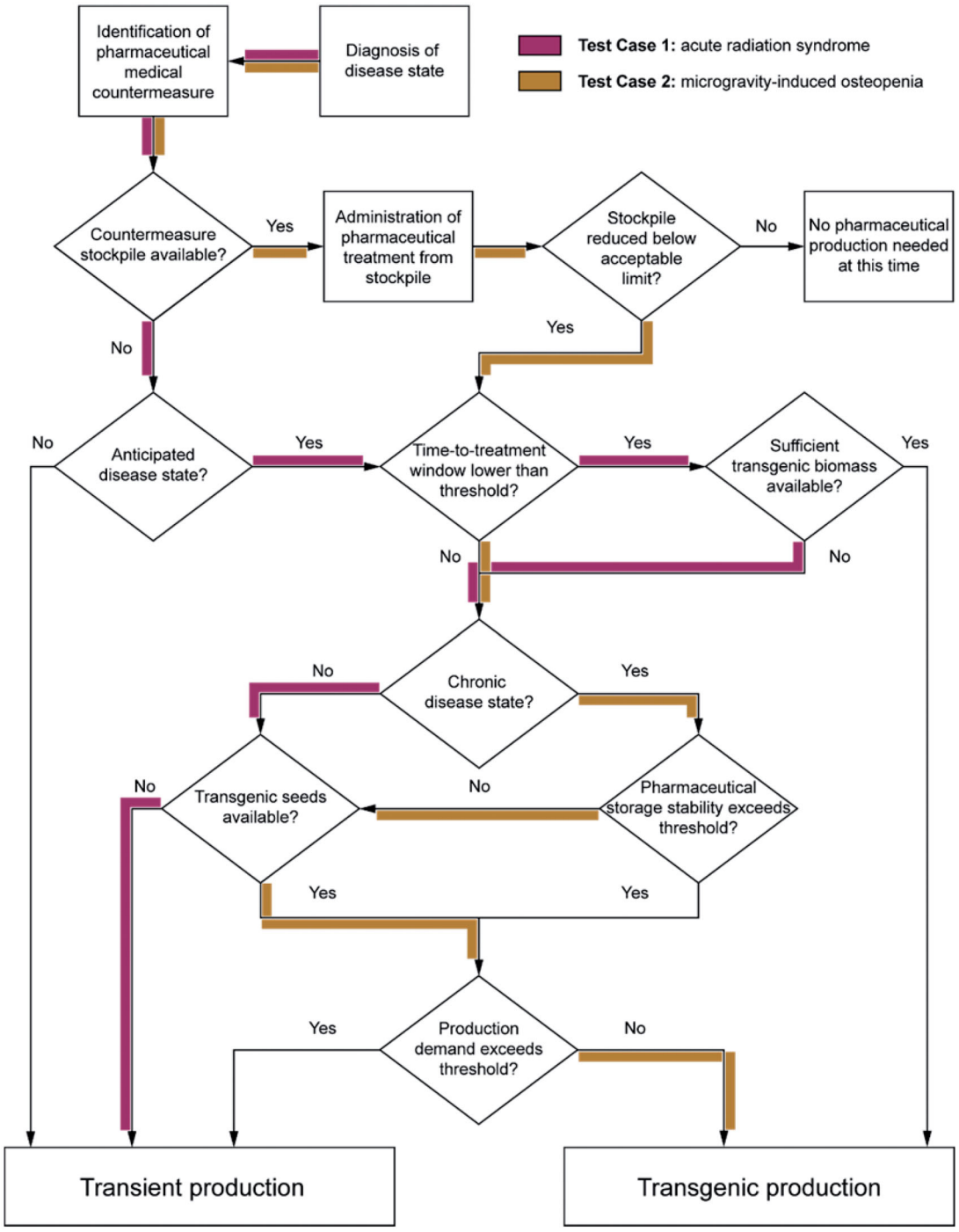


**Figure 4.** A decision tree for selecting a molecular pharming production strategy. This assumes that transient production is a more cost-efficient strategy above some threshold pharmaceutical demand, which is driven by the notion that transient production tends to yield higher product accumulation. This threshold depends on mission architecture, available resources, and the impact of the disease state to mission outcome. Two test cases of hypothetical disease state diagnoses are included; supporting information for the test cases are included in Supplementary Information.

utilities and a crew of six astronauts subsists on a diet supplemented with a single serving, 100 g fresh weight (FW), of lettuce or potato per crew member per day. The primary purpose for growing this single serving of plant-based food per day on an extended space mission is to meet the Food and Nutrition Board of the Institute of Medicine's Recommended Dietary Allowance (RDA) of nutrients. The current stated shelf life of prepackaged space food is only 18 months [53], and the degradation of key nutrients such as thiamine (vitamin B1) is well documented [54–56]. Just as when sailors suffered the effects of missing vitamin C on long

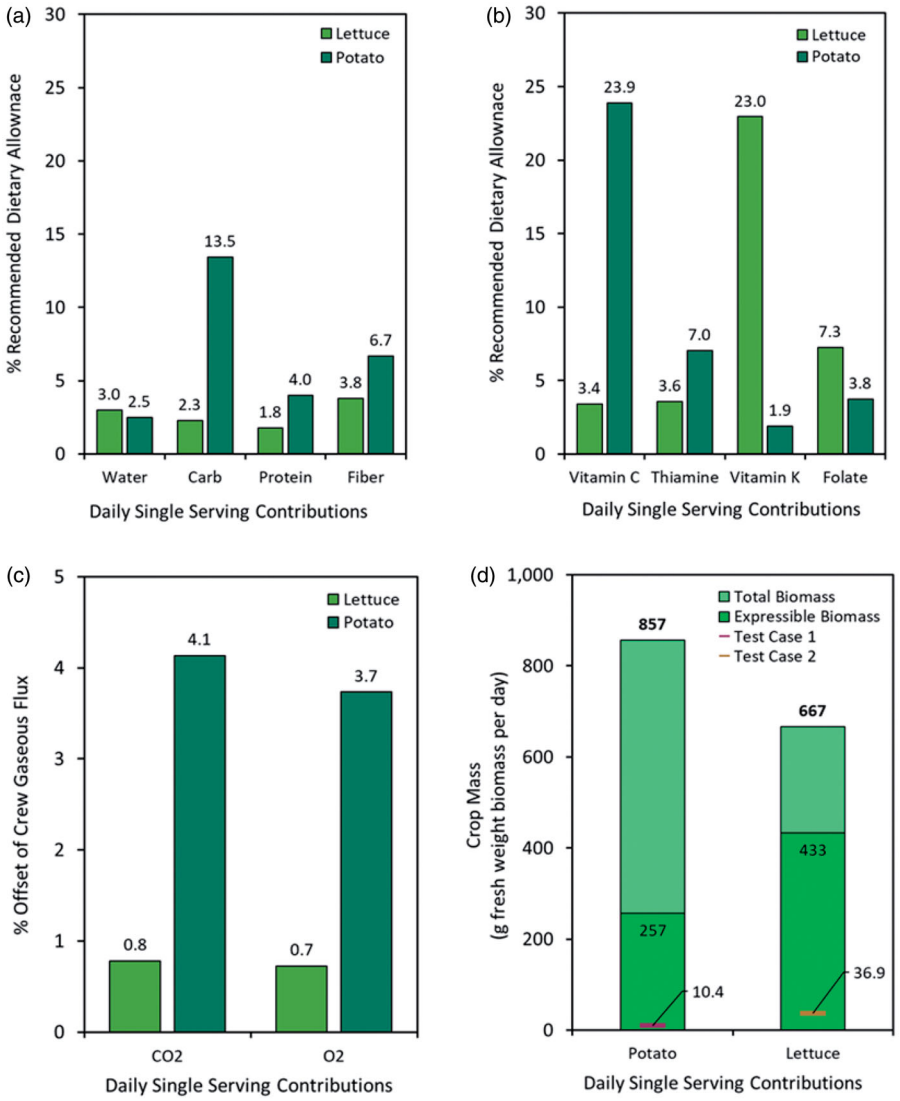


**Figure 5.** Cultivating a daily single serving (100 g fresh weight) of crop, either lettuce or potato, to supplement an astronaut's diet. Contributions of the daily single serving to Recommended Dietary Allowance are shown for select (a) macronutrients and (b) labile vitamins. (c) The biomass for a daily single serving supports air revitalization by partially offsetting human gaseous metabolic flux. (d) The biomass for a daily single serving could be more than sufficient for molecular pharming production of pharmaceutical-based medical countermeasures, as illustrated by crop mass requirements of Test Case 1 (granulocyte stimulating colony factor, 1 dose, produced in potato) and Test Case 2 (parathyroid hormone residue 1-34, 6 doses, produced in lettuce).

sea voyages, it will be critical to avoid vitamin deficiencies as we explore deep space. Figure 5(a,b) shows the macronutrient and labile vitamin contributions of the daily single serving of lettuce or potato as a percentage of recommended dietary allowance. A supplement selected from a variety of food crops would be most effective to meet the RDA, as well as to minimize menu fatigue [57].

Growing a daily single serving supplement of plant-based food is estimated to occupy 4.6 and 5.7 $m^2$ of cultivation area for lettuce and potato, respectively. This considers the plant inventory needed for sustained production of a single serving per day. The actual cultivation footprint is expected to be significantly smaller than the cultivation area, as hydroponic cultivation is typically conducted with multi-layered growth stages. The plant cultivation calculations were performed according to values listed in NASA's Baseline Values and Assumptions Document [58]. Supporting information for the assumptions and calculations can be found in the Supplementary Information (S2. Supplementary Tables).

In addition to supplying nutrients, this single serving will assist with other aspects of life support. Plant growth assists in air revitalization, offsetting crew carbon dioxide and oxygen flux by ~1% (lettuce) and ~4% (potato) (Figure 5(c)). It also serves to revitalize water as 9.7 (lettuce) and 22.8 (potato) liters per day of

**Table 2.** A list of assumptions used in the molecular pharming test case calculations.

| | Test Case 1 | Test Case 2 |
|---|---|---|
| Disease state | Acute radiation syndrome (acute disease) | Microgravity-induced osteopenia (chronic disease) |
| Countermeasure | Granulocyte stimulating factor | Parathyroid hormone residue 1-34 |
| FDA-approved product | NEUPOGEN® (filgrastim) BLA: 103353 | FORTEO® (teriparatide) NDA: 021318 |
| Medication demand | 300 μg (1 doses; 300 μg/dose) | 120 μg (6 doses; 20 μg/dose) |
| Production method | Transient production in potato leaves | Transgenic production in lettuce leaves |
| Expression level[a] | 250 μg drug/g potato leaf fresh weight | 10 μg drug/g lettuce leaf fresh weight |
| Expressible biomass | 23% total biomass fresh weight | 65% total biomass fresh weight |
| Drug delivery | Intravenous injection (50% drug loss in purification processes) | Intravenous injection (50% drug loss in purification processes) |

BLA: biologics license application; NDA: new drug application.
[a]Conservative estimates based on molecular pharming expression levels widely reported in literature. The estimates also reflect the general trend of lower expression levels in transgenic production as compared to transient expression.

clean water are released in gaseous form *via* transpiration, most of which can be recycled for crop cultivation unless needed in other operations, such as for pharmaceutical formulations. These simple calculations highlight the auxiliary value of plants for bioregenerative life support.

The contributions to all aspects of life support depend highly on the crop species and cultivation environment. For example, a previous study using a closed human/plant system has shown experimentally that 11.2 $m^2$ of wheat grown at high light intensity (1,500 μmol $m^{-2}$ $s^{-1}$) supplies sufficient oxygen for one person [59]. Wheat is one of the most productive crops for oxygen production, which is amplified by the high light intensity used and its tolerance of a 24-h light cycle. This crop cultivation strategy has been demonstrated to provide ∼13 times more oxygen than lettuce and ∼3 times more than potato. In addition to traditional life support metrics like oxygen productivity, crop selection for molecular pharming must also take into account factors such as the efficiency of transformation (e.g. wheat is difficult to transform and generally yields low product accumulation [60]) and characteristics of the host cell protein compared to the product target.

Now we look at two test cases in which the biomass generated for this daily single serving can be applied to molecular pharming for manufacturing of pharmaceutical countermeasures:

Test Case 1: Transient production of one dose of the granulocyte stimulating factor (G-CSF) from 42 g FW of potato leaves per crew member (this is equivalent to leaf mass concomitant with a single serving of potato tuber) purified as an injectable countermeasure to acute radiation syndrome, representative of an acute disease state.

Test Case 2: Transgenic production of a single dose per crew member (six doses total) of parathyroid hormone residue 1–34 (PTH) from 100 g FW lettuce leaves per crew member (this is equivalent to leaf mass less than a single serving of lettuce) purified as an injectable countermeasure to microgravity-induced osteopenia, representative of a chronic disease state.

Table 2 summarizes the key assumptions that were built into the two test cases. The logic for selection of the production method is shown in Figure 4 and further described in the Supplementary Information (S1.1 Decision Tree Walkthrough).

From the perspective of molecular pharming, lettuce [61,62] serves as a fast-growing crop with a small cultivation footprint in which the edible biomass is also the expressible biomass capable of producing pharmaceuticals. Potato [63,64] represents a slow-growing crop that has the advantage of distinct edible biomass (tubers) and expressible biomass (leaves); molecular pharming would not significantly impact the total available food resource. Leaves detached from the intact plant are capable of providing comparable pharmaceutical yields to those from the intact plant [62,65,66]. Production of pharmaceuticals in inedible biomass is one way to create physical separation of the food and pharmaceutical streams while maintaining resource flexibility. However, there are situations in which it may be advantageous for merged food and pharmaceutical streams; there are reports in literature on oral delivery of pharmaceuticals in both lettuce and potato tubers [67–69]. While promising, this technology is still in the early stages of development.

As shown in Figure 5(d), only 10.4 g FW (1.2% of the total crop biomass FW, 4.0% expressible biomass FW, 1.7% food resource biomass FW) is needed for the Test Case 1 acute disease state countermeasure in potato, while 36.9 g FW (5.5% of total crop biomass FW, 8.5% expressible biomass FW, 5.8% food resource biomass FW) is needed for the Test Case 2 chronic disease state countermeasure in lettuce. While these test cases are

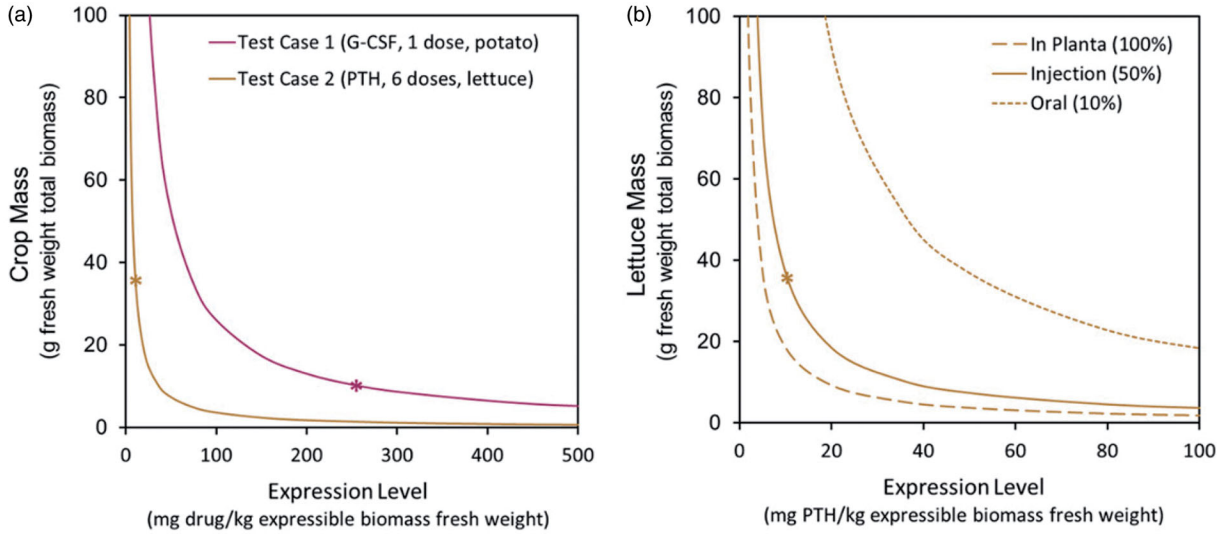


**Figure 6.** Plant cultivation requirements for molecular pharming are largely controlled by the product expression level, medication dosage, and medication delivery modality. (a) Test Case 1 and Test Case 2 sensitivity of crop mass demands to variation in pharmaceutical expression level. (b) Test Case 2 sensitivity of lettuce mass demands to selection of drug delivery modality, which we are representing as an approximated % availability of the produced drug. *signifies the expression level assumed in the test cases. G-CSF, granulocyte colony stimulating factor; PTH, parathyroid hormone residue 1-34.

**Table 3.** A comparison of key characteristics for space exploration of biological platforms for pharmaceutical production.

| System | In-Situ Resource Utilization | Just-In-Time Response | Operational Simplicity | Product Range | Crew & Planetary Safety |
|---|---|---|---|---|---|
| Bioreactor systems | | | | | |
| Insect cell | ★☆☆☆☆ | ★★☆☆☆ | ★★☆☆☆ | ★★★☆☆ | ★★☆☆☆ |
| Mammalian cell | ★☆☆☆☆ | ★★☆☆☆ | ★★☆☆☆ | ★★★★★ | ★★☆☆☆ |
| Plant cell | ★★★☆☆ | ★★☆☆☆ | ★★☆☆☆ | ★★★★☆ | ★★★★☆ |
| Bacteria, autotrophic | ★★★★☆ | ★★★☆☆ | ★★★☆☆ | ★☆☆☆☆ | ★★★☆☆ |
| Bacteria, heterotrophic | ★★☆☆☆ | ★★★☆☆ | ★★☆☆☆ | ★☆☆☆☆ | ★★☆☆☆ |
| Yeast | ★☆☆☆☆ | ★★★☆☆ | ★★☆☆☆ | ★★★☆☆ | ★★★★☆ |
| Cell-free expression | ★☆☆☆☆ | ★★★★☆ | ★★★☆☆ | ★★★☆☆ | ★★★☆☆ |
| Non-bioreactor systems | | | | | |
| Transgenic animals | ★☆☆☆☆ | ★☆☆☆☆ | ★☆☆☆☆ | ★★★★★ | ★★★☆☆ |
| Transgenic plants | ★★★★★ | ★★☆☆☆ | ★★★★★ | ★★★★☆ | ★★★★★ |
| Transient plants | ★★★★☆ | ★★★★☆ | ★★★★☆ | ★★★★☆ | ★★★★☆ |

driven by conservative assumptions of performance well-established in literature, it is important to note that biomass requirements are highly dependent on the rate of pharmaceutical accumulation (i.e. expression level), medication dose size, and drug delivery modality. Figure 6(a) illustrates how the total crop biomass demand differs between the two test cases based on the medication demand (dose size and number of doses) and over a range of conservatively estimated molecular pharming expression levels, while Figure 6(b) shows how the biomass requirements depend on drug delivery modality.

## Comparing molecular medical foundries for space

Since the founding of modern biotechnology with Cohen and Boyer's discovery of recombinant DNA technology in 1973 [70], biological organisms have risen to prominence as the primary means for producing high-value pharmaceutical proteins and other products, most of which are too complex to be economically and sustainably produced using current chemical synthesis approaches. In the half-century since inception of recombinant DNA technology, a plethora of biological platforms have been engineered as factories of recombinant products – microbial culture, eukaryotic (mammalian, insect, yeast, plant) cell culture, live animals, cell lysates, and whole plants. Table 3 shows a comparison of current pharmaceutical production platforms based on attributes relevant to their deployment for human health in space. Details of the category definition and system rankings are included in Supplementary Information (S1.2 Supporting Production Platform Comparisons). There are also new platforms on the horizon for production (e.g. microbiome engineering [71], gene therapy [72]) and drug delivery (e.g. microneedle-based transdermal [73]).

Commercial biopharmaceutical manufacturing on Earth is dominated by microbial fermentation and mammalian cell culture. Spread across over 1,700 production facilities globally, there is a commercial production capacity of 4.8 million liters for microbial fermentation and 15.0 million liters mammalian cell culture (online database; http://top1000bio.com/). Regulatory pathways have been well established, decades of intensive research have seen orders of magnitude increase in productivity, and billions of dollars have been invested into developing a culture-based system infrastructure.

However, this established dominance of culture-based systems does not easily translate into the implementation of human health in space for several reasons. The most glaring difficulty is with cell culture behavior, both with the cell biology [74] and fluid dynamics [75], in altered gravity; operation will need to be compatible with microgravity for in-flight production and reduced gravity for a Moon or Mars mission. There is a growing body of literature on the development of bioreactors with alternative containment and mixing for microgravity [76–78]. The main existing technical difficulties of culture-based systems in limited resource environments are the expensive and complex equipment requirements and the need for the aseptic operation for growing production host cells. Microbial fermenters and cell culture bioreactors are made of glass and/or a special grade (316 L) stainless steel for durability and corrosion resistance [79]. Bioreactors are generally designed with a suite of capabilities, including: culture agitation, aeration, sampling, in-line sensing, feedback control systems (for pH, temperature, dissolved oxygen, foaming), cleaning, and sterilization. This complex process equipment lowers general accessibility and increases the workforce specialization of operators, which in turn forms another barrier to application in limited resource environments.

The equipment burden of culture-based systems is largely a result of the need to maintain a sterile cultivation environment during operation. Without adequate environmental protection, cultures are susceptible to contamination by undesired organisms. Compromised sterility of processing can lead to significant product and patient impact [80–82].

In addition to complexity, stainless steel bioreactors impose significant mass and volume penalties that might prohibit adoption in a space mission. For example, a typical glass and stainless steel stirred tank reactor for 1 L working volume (HyPerforma Glass Bioreactors, ThermoFisher Scientific) of culture weighs 3.7 kg, not including liquid culture mass and auxiliary reactor components (e.g. probes, spargers, agitator, heating jacket).

A growing trend in culture-based systems is to employ single-use technology for cost-savings in cleaning validation, capital costs, and time [83]. Single-use technology for culture-based systems typically consists of a multi-layered plastic bag used in lieu of, or with support of, a stainless steel vessel. Of specific importance to space missions, these savings could also translate into significant reductions in mass and volume requirements. However, as the name “single-use” states, these plastic bioreactor housings are only used once, introducing significant consumable and waste streams to the pharmaceutical foundry. Therefore, single-use technology may introduce reliance on a stable supply chain for consumables that could strain feasibility in a limited resource environment. The use of recyclable materials (e.g. biopolymers) for single-use technology has not been commercially implemented but would serve to alleviate these concerns. The hindrance of consumable waste is offset by reduced cleaning requirements and should be evaluated within a mission architecture. For example, if pharmaceutical production is projected to be below a threshold capacity, then the extra consumables required to be flown may be acceptable.

Exceptional to the typical culture-based system vulnerabilities, microbial, oxygenic photoautotrophic cultures (i.e. microalgae [84,85] and cyanobacteria [86,87]) represent a promising subset of culture-based systems that may be better equipped for supporting human life in space. They share many of the same benefits of molecular pharming; these organisms are able to use available *in situ* resources (i.e. light and $CO_2$) as feedstocks, and some have been shown to be quite tolerant to a range of water qualities (e.g. polluted water) [88]. Additionally, some of these species have unique advantageous characteristics. They can serve as a food resource, grow under conditions that minimize the probability of contamination, and even be used as biofertilizer to improve soil quality and crop productivity [89–91].

A subset of these organisms, including the microalgal species *Chlamydomonas reinhardtii* and *Chlorella vulgaris*, and the cyanobacterial species *Arthrospira platensis* (commonly sold under the name spirulina), is categorized by the U.S. Food and Drug Administration as being Generally Recognized as Safe (GRAS), whereby these organisms are considered edible and are sold commercially as food and nutritional supplements [91,92]. The edible nature of these organisms presents a potential advantage to pharmaceutical foundries in

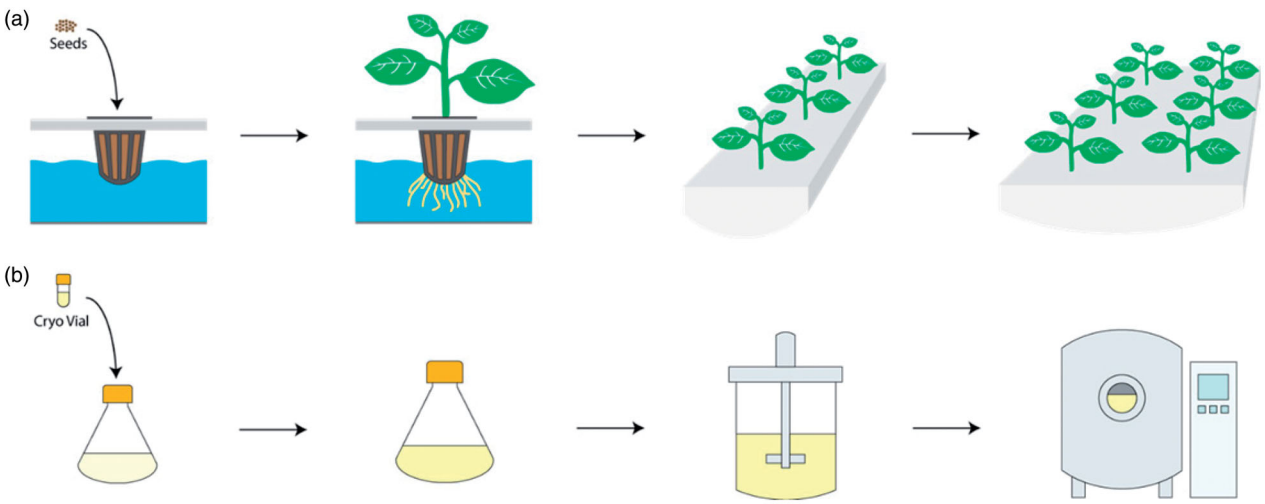


**Figure 7.** An illustration of the upstream manufacturing processes required for (a) whole plant molecular pharming, and (b) cell culture or fermentation-based biopharmaceutical production. Whole plant molecular pharming uses hydroponic or soil-based plant growth receptacles that scale linearly with demand, whereas cell culture and fermentation-based manufacturing use a series of bioreactors whose geometries are dependent on scale.

space in that if the target production molecules are bioavailable through simply eating the wet or dry biomass of the production host, no downstream purification is needed.

The microbial nature of these organisms provides potential advantages to plant systems. First, microalgae (in particular *C. reinhardtii*) and cyanobacteria have genetic tools that are typically more advanced than those of plants [92–94]. Although tools for engineering *A. platensis* have been reported [95], engineering this organism has remained a challenge in the field. To this end, we have recently developed a genetic toolkit for creating stable mutants of *A. platensis* (Hilzinger, Arkin, et al. Unpublished) that will help unlock this organism for metabolic engineering goals. Second, these organisms have faster growth rates than plants, which enables shorter times to reach the biomass necessary for molecular harvesting. Third, the larger metabolic diversity of microalgae and cyanobacteria compared to plants could help to metabolically engineer target molecules that are difficult or impossible to produce in plants using current technologies [92].

Therefore, these organisms may be well suited for pharmaceutical production, or for enhancing the nutritional load through vitamin supplementation. Thus, GRAS-status microbial oxygenic photoautotrophs are poised to become edible molecular pharming hosts for space missions. As these technologies continue to mature, a detailed techno-economic comparison between plants, microalgae, and cyanobacteria will be needed.

It may be that a robust pharmaceutical foundry for space ends up being less about selecting one system and more about selecting a network of systems. It is important that interconnectivity and synergy of different platforms be considered for biological-based production of pharmaceuticals and other high-value products to support human life (e.g. biomaterials).

A main distinguishing feature of whole plants as a pharmaceutical production platform is the freedom from complex equipment housing during operation; the supracellular structure of a plant serves as its own natural "bioreactor" for operational control (e.g. nutrient distribution) and protection against contamination. This effectively means that molecular pharming can be employed with lower complexity process control systems and equipment. Figure 7(a,b) illustrates the simplicity and linear scalability of producing pharmaceuticals in whole plants as compared to culture-based systems. However, an equivalent system mass (ESM) comparison of molecular pharming and culture-based systems for spaceflight is needed to rigorously evaluate the perceived advantage of molecular pharming simplicity.

This self-regulating behavior also suggests that plants may serve as a more robust production platform with higher tolerance to input quality variation for given output product quality. In the literature, the strength of molecular pharming production tolerance as compared to culture-based systems is as yet unproven, but would be a valuable avenue of research to directly investigate.

## The future of plant-based foundries in space

For decades, plants have been identified as important life support objects for human health in space. Here we

have presented the need for an Earth-independent pharmaceutical life support system and identified molecular pharming as a strategy to tap into the power of plants to serve as a pharmaceutical (and other high-value product) foundry to meet that need. Molecular pharming in space has the potential to provide manufacturing capacity to respond to both acute and chronic disease states in space with a relatively small amount of plant biomass. Selecting the set of the most appropriate molecular pharming-based production strategies should be carried out within a reference mission architecture, which considers key attributes that we have laid out here.

There are many ways to envision pharmaceutical foundries for interplanetary use. Chemical synthesis is limited in production targets and in reagent supply but it may be necessary when biology is not sufficient or capable (e.g. nucleic acid synthesis). Translating culture-based systems from Earth to space utility faces the challenges of cell biology, fluid dynamics, feedstock sustainability, mass/volume penalties, and crew training. Their relatively high productivity may position them as an effective platform for settlement missions to sustain larger populations. Autotrophic cultures are exceptional solutions to several challenges of traditional culture-based systems and have more potential as a near-term platform.

More thorough investigation is needed to select an appropriate set of pharmaceutical foundries. Process mass intensity (PMI) is a metric recently adopted by the biopharmaceutical industry to measure the environmental footprint of production [96]. PMI is defined as the total mass in kg of raw material and consumable inputs to produce 1 kg of active pharmaceutical ingredient. PMI can serve as a useful reference point when performing ESM analyses of pharmaceutical foundries in space. ESM analyses are typically performed to evaluate and optimize space mission payloads to minimize launch costs (or mission objective success) as a function of mass, volume, power, cooling, and crew time needs [97]. Pharmaceutical foundry considerations will also need to include medical risk and patient outcomes. NASA's exploration medical system trade study tools, which includes a systems engineering model and a medical risk analysis model, have the potential to serve as a foundation for this analysis [98].

There are many obstacles ahead before making pharmaceutical foundries in space a reality.

What has not been thoroughly discussed in this review is the downstream processing of a molecular medical foundry, which will depend on the purity needed for the pharmaceutical formulation, delivery method, production host, etc. Downstream processing, the purification of the target molecule from the production host, is a resource-intensive aspect of biopharmaceutical production across all platforms. There is a lack of downstream processing technology that translates well from Earth-based constraints to those of space, as they often require a high quantity of consumables, raw materials, equipment, and cleaning. This bottleneck will need to be addressed for pharmaceutical foundries in space to succeed. One approach is to conduct research on novel drug delivery modalities (e.g. plant-encapsulated oral administration [99]) to reduce the need for downstream processing, and another is to diminish the resource demands of the processing itself (e.g. bioregenerative and recyclable processing reagents). A growing emphasis on distributed and just-in-time pharmaceutical production for healthcare on Earth is already driving solutions to these downstream challenges [100].

The other major hurdle is in regulatory compliance. Production and administration of pharmaceuticals in space will require extensive quality control; manufacturing a small molecule might have 50 critical tests, while manufacturing a biologic may have over 250 tests [101]. Here, the advent of personalized medicine on Earth will illuminate a path forward. The shift from mass produced to individualized patient-specific medicine hinges on re-structuring the path to regulatory approval and quality control [102].

While there are many challenges ahead that need to be addressed to pave the way for Earth-independent life support, the rewards of this pursuit will include great insights into supporting life on Earth and beyond. Understanding this value, we aim to highlight the critical importance (and long lead time) of developing Earth-independent systems in the future of human exploration. We illustrate that molecular pharming provides a diverse production toolset that could be used to establish a robust molecular medical foundry subsisting on a small fraction of food crop needs. In addition to advocating for molecular pharming as a synergistic asset of space life support systems, we focus on the need for multi-faceted utilization of resources in limited environments such as space and extraterrestrial bodies.

## Disclosure statement

The authors report no conflicts of interest.

## Funding

This material is based upon work supported by NASA under grant or cooperative agreement award number NNX17AJ31G. This work was also supported by a NASA

Space Technology Research Fellowship (NASA grant number 80NSSC18K1157). KAM and SN would also like to acknowledge support by the Translational Research Institute through NASA NNX16AO69A. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Aeronautics and Space Administration (NASA) or the Translational Research Institute for Space Health (TRISH).

## ORCID

Matthew J. McNulty http://orcid.org/0000-0001-9025-102X
Kevin Yates http://orcid.org/0000-0001-8509-8251
Kalimuthu Karuppanan http://orcid.org/0000-0002-2696-8437
Jacob M. Hilzinger http://orcid.org/0000-0002-1876-1313
Aaron J. Berliner http://orcid.org/0000-0002-4817-3926
Adam P. Arkin http://orcid.org/0000-0002-4999-2931
Nancy E. Lane http://orcid.org/0000-0002-0177-2198
Somen Nandi http://orcid.org/0000-0001-5240-198X
Karen A. McDonald http://orcid.org/0000-0002-5145-9968

## References

[1] Meyer CE, Schneider WF. NASA Advanced Exploration Systems: 2018 Advancements in Life Support Systems. 2018. [cited 2021 Feb 9]. Available from: https://ntrs.nasa.gov/search.jsp?R=20180006596.
[2] Jackson S. Life support systems. 2016. [cited 2017 Oct 30]. Available from: https://www.nasa.gov/content/life-support-systems.
[3] Hu S, Barzilla JE, Semones E. Acute radiation risk assessment and mitigation strategies in near future exploration spaceflights. Life Sci Space Res (Amst). 2020;24:25–33.
[4] Chancellor JC, Blue RS, Cengel KA, et al. Limitations in predicting the space radiation health risk for exploration astronauts. Npj Microgravity. 2018;4:8–11.
[5] Gambacurta A, Merlini G, Ruggiero C, et al. Human osteogenic differentiation in Space: Proteomic and epigenetic clues to better understand osteoporosis. Sci Rep. 2019;9(1):1–10.
[6] Sibonga JD. Spaceflight-induced bone loss: is there an osteoporosis risk? Curr Osteoporos Rep. 2013;11(2):92–98.
[7] Kassemi M, Thompson D. Prediction of renal crystalline size distributions in space using a PBE analytic model. 1. Effect of microgravity-induced biochemical alterations. Am J Physiol Ren Physiol. 2016;311:520–530.
[8] Ciftçioglu N, Haddad RS, Golden DC, et al. A potential cause for kidney stone formation during space flights: enhanced growth of nanobacteria in microgravity. Kidney Int. 2005;67(2):483–491.
[9] Zhang LF, Hargens AR. Spaceflight-induced intracranial hypertension and visual impairment: pathophysiology and countermeasures. Physiol Rev. 2018;98(1):59–87.
[10] Jandial R, Hoshide R, Waters JD, et al. Space-brain: the negative effects of space exposure on the central nervous system. Surg Neurol Int. 2018; 9. Article Number: 9.
[11] Taylor PW. Impact of space flight on bacterial virulence and antibiotic susceptibility. Infect Drug Resist. 2015;8:249–262.
[12] Trudel G, Shafer J, Laneuville O, et al. Characterizing the effect of exposure to microgravity on anemia: more space is worse. Am J Hematol. 2020;95(3):267–273.
[13] Sobisch LY, Rogowski KM, Fuchs J, et al. Biofilm forming antibiotic resistant gram-positive pathogens isolated from surfaces on the international space station. Front Microbiol. 2019;10:543.
[14] Garrett-Bakelman FE, Darshi M, Green SJ, et al. The NASA twins study: a multidimensional analysis of a year-long human spaceflight. Science. 2019;364:eaau8650.
[15] Menezes AA, Cumbers J, Hogan JA, et al. Towards synthetic biological approaches to resource utilization on space missions. J R Soc Interface. 2015;12(102):20140715.
[16] Blue RS, Bayuse TM, Daniels VR, et al. Supplying a pharmacy for NASA exploration spaceflight: challenges and current understanding. Npj Microgravity. 2019;5:14.
[17] Mazzeo A, Carpenter P. Stability studies for biologics. In: Kim Huynh-Ba, editor. Handbook of stability testing in pharmaceutical development. New York: Springer; 2009. p. 353–369.
[18] Reynolds T, de Zafra C, Kim A, et al. Overview of biopharmaceuticals and comparison with small-molecule drug development. New York, NY: Elsevier Inc.; 2013. p. 3–33. (Nonclinical development of novel biologics, biosimilars, vaccines specialty biology).
[19] Barratt MR, Pool SL. Principles of clinical medicine for space flight. New York: Springer; 2008. (Principles of clinical medicine for space flight).
[20] Menezes AA, Montague MG, Cumbers J, et al. Grand challenges in space synthetic biology. J R Soc Interface. 2015;12(113):20150803.
[21] Nangle SN, Wolfson MY, Hartsough L, et al. The case for biotech on Mars. Nat Biotechnol. 2020;38(4):401–407.
[22] Vlieghe P, Lisowski V, Martinez J, et al. Synthetic therapeutic peptides: science and market. Drug Discov. 2010;15(1-2):40–56.
[23] Uhlig T, Kyprianou T, Martinelli FG, et al. The emergence of peptides in the pharmaceutical business: from exploration to exploitation. EuPA Open Proteomics. 2014;4:58–69.
[24] Hao M, Qiao J, Qi H. Current and emerging methods for the synthesis of single-stranded DNA. Genes (Basel). 2020;11(2):116.
[25] Cortesão M, Schütze T, Marx R, et al. Fungal biotechnology in space: why and how? In: Nevalainen H, editor. Grand challenges in fungal biotechnology. Cham, Switzerland: Springer; 2020. p. 501–535.
[26] Llorente B, Williams TC, Goold HD. The multiplanetary future of plant synthetic biology. Genes (Basel). 2018;9(7):348.

[27] Wheeler RM. Agriculture for space: people and places paving the way. Open Agric. 2017;2:14–32.
[28] Khodadad CLM, Hummerick ME, Spencer LE, et al. Microbiological and nutritional analysis of lettuce crops grown on the international space station. Front Plant Sci. 2020;11:199.
[29] Wolff S, Coelho L, Karoliussen I, et al. Effects of the extraterrestrial environment on plants: recommendations for future space experiments for the MELiSSA higher plant compartment. Life (Basel). 2014;4(2): 189–204.
[30] Nelson M, Pechurkin NS, Allen JP, et al. Closed ecological systems, space life support and biospherics. New York, NY: Humana Press; 2010. p. 517–565. (Environment biotechnology).
[31] Odeh R, Guy CL. Gardening for therapeutic people-plant interactions during long-duration space missions. Open Agric. 2017;2(1):1–13.
[32] Lomonossoff GP, DAoust M-A. Plant-produced biopharmaceuticals: a case of technical developments driving clinical deployment. Science. 2016;353(6305): 1237–1240.
[33] Veeresham C. Natural products derived from plants as a source of drugs. J Adv Pharm Tech Res. 2012; 3(4):200–201.
[34] Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ Health Perspect. 2001;109:69–75.
[35] Desborough MJR, Keeling DM. The aspirin story – from willow to wonder drug. Br J Haematol. 2017; 177(5):674–683.
[36] Zhu L, Chen L. Progress in research on paclitaxel and tumor immunotherapy. Cell Mol Biol Lett. 2019;24(1): Article number: 40.
[37] Su XZ, Miller LH. The discovery of artemisinin and the Nobel Prize in Physiology or Medicine. Sci China Life Sci. 2015;58(11):1175–1179.
[38] Hiatt A, Cafferkey R, Bowdish K. Production of antibodies in transgenic plants. Nature. 1989;342(6245): 76–78.
[39] Haq TA, Mason HS, Clements JD, et al. Oral immunization with a recombinant bacterial antigen produced in transgenic plants. Science. 1995;268(5211): 714–716.
[40] Pogue GP, Lindbo JA, Garger SJ, et al. Making an ally from an enemy: plant virology and the new agriculture. Annu Rev Phytopathol. 2002;40:45–74.
[41] Marillonnet S, Thoeringer C, Kandzia R, et al. Systemic *Agrobacterium tumefaciens*-mediated transfection of viral replicons for efficient transient expression in plants. Nat Biotechnol. 2005;23(6): 718–723.
[42] Werner S, Breus O, Symonenko Y, et al. High-level recombinant protein expression in transgenic plants by using a double-inducible viral vector. Proc Natl Acad Sci U S A. 2011;108(34):14061–14066.
[43] Ma JKC, Drossard J, Lewis D, et al. Regulatory approval and a first-in-human phase I clinical trial of a monoclonal antibody produced in transgenic tobacco plants. Plant Biotechnol J. 2015;13(8): 1106–1120.
[44] Mor TS. Molecular pharming's foot in the FDA's door: Protalix's trailblazing story. Biotechnol Lett. 2015; 37(11):2147–2150.
[45] Tekoah Y, Shulman A, Kizhner T, et al. Large-scale production of pharmaceutical proteins in plant cell culture-the protalix experience. Plant Biotechnol J. 2015;13(8):1199–1208.
[46] Hood EE, Witcher DR, Maddock S, et al. Commercial production of avidin from transgenic maizecharacterization of transformant, production, processing, extraction and purification. Mol Breed. 1997;3(4): 291–306.
[47] Sack M, Hofbauer A, Fischer R, et al. The increasing value of plant-made proteins. Curr Opin Biotechnol. 2015;32:163–170.
[48] Aldag C, Teixeira DN, Leventhal PS. Skin rejuvenation using cosmetic products containing growth factors, cytokines, and matrikines: a review of the literature. Clin Cosmet Investig Dermatol. 2016;9:411–419.
[49] Zhang D, Nandi S, Bryan P, et al. Expression, purification, and characterization of recombinant human transferrin from rice (*Oryza sativa* L.). Protein Expr Purif. 2010;74(1):69–79.
[50] Meyers B, Zaltsman A, Lacroix B, et al. Nuclear and plastid genetic engineering of plants: comparison of opportunities and challenges. Biotechnol Adv. 2010; 28(6):747–756.
[51] Borghi L. Inducible gene expression systems for plants. Methods Mol Biol. 2010;655:65–75.
[52] Thomas DR, Penney CA, Majumder A, et al. Evolution of plant-made pharmaceuticals. Int J Mol Sci. 2011; 12(5):3220–3236.
[53] Cooper M, Douglas G, Perchonok M. Developing the NASA food system for long-duration missions. J Food Sci. 2011;76(2):R40–R48.
[54] Zwart SR, Kloeris VL, Perchonok MH, et al. Assessment of nutrient stability in foods from the space food system after long-duration spaceflight on the ISS. J Food Sci. 2009;74(7):H209–H217.
[55] Goulette TR, Zhou J, Dixon WR, et al. Kinetic parameters of thiamine degradation in NASA spaceflight foods determined by the endpoints method for long-term storage. Food Chem. 2020;302:125365.
[56] Cooper M, Perchonok M, Douglas GL. Initial assessment of the nutritional quality of the space food system over three years of ambient storage. Npj Microgravity. 2017;3:17.
[57] Smith SM, Rice BL, Dlouhy H, et al. Assessment of nutritional intake during space flight and space flight analogs. Procedia Food Sci. 2013;2:27–34.
[58] Anderson MS, Ewert MK, Keener JF. Life support baseline values and assumptions document. 2018. Report number: NASA/TP-2015–218570/REV1. Hanover, MD: National Aeronautics and Space Administration
[59] Edeen MA, Dominick JS, Barta DJ, et al. Control of air revitalization using plants: results of the early human testing initiative phase I test. In 26th International Conference on Environmental Systems, Conference, Monterey, CA, United States. SAE Tech Pap. Report number: 961522. Warrendale, PA: SAE International; 1996.

[60] Ramessar K, Capell T, Christou P. Molecular pharming in cereal crops. Phytochem Rev. 2008;7(3):579–592.
[61] Chen Q, Dent M, Hurtado J, et al. Transient protein expression by agroinfiltration in lettuce. Methods Mol Biol. 2016;1385:55–67.
[62] Joh LD, Wroblewski T, Ewing NN, et al. High-level transient expression of recombinant protein in lettuce. Biotechnol Bioeng. 2005;91(7):861–871.
[63] Castañón S, Marín MS, Martín-Alonso JM, et al. Immunization with potato plants expressing VP60 protein protects against rabbit hemorrhagic disease virus. J Virol. 1999;73(5):4452–4455.
[64] Zhang Y, Chen M, Siemiatkowska B, et al. A highly efficient agrobacterium-mediated method for transient gene expression and functional studies in multiple plant species. Plant Commun. 2020;1(5):100028.
[65] Fujiuchi N, Matsuda R, Matoba N, et al. Removal of bacterial suspension water occupying the intercellular space of detached leaves after agroinfiltration improves the yield of recombinant hemagglutinin in a Nicotiana benthamiana transient gene expression system. Biotechnol Bioeng. 2016;113(4):901–906.
[66] Plesha MA, Huang T-K, Dandekar AM, et al. High-level transient production of a heterologous protein in plants by optimizing induction of a chemically inducible viral amplicon expression system. Biotechnol Prog. 2007;23(6):1277–1285.
[67] Lamson NG, Fein KC, Gleeson JP, et al. From farm to pharmacy: strawberry-enabled oral delivery of protein drugs. bioRxiv. 2020. DOI: 2020.03.11.987461.
[68] Kapusta J, Modelska A, Figlerowicz M, et al. A plant-derived edible vaccine against hepatitis B virus. Faseb J. 1999;13(13):1796–1799.
[69] Thanavala Y, Mahoney M, Pal S, et al. Immunogenicity in humans of an edible vaccine for hepatitis B. Proc Natl Acad Sci USA. 2005;102(9):3378–3382.
[70] Cohen SN, Chang ACY, Boyer HW, et al. Construction of biologically functional bacterial plasmids in vitro. Proc Natl Acad Sci U S A. 1973;70(11):3240–3244.
[71] Isabella VM, Ha BN, Castillo MJ, et al. Development of a synthetic live bacterial therapeutic for the human metabolic disease phenylketonuria. Nat Biotechnol. 2018;36(9):857–867.
[72] Anguela XM, High KA. Entering the modern era of gene therapy. Annu Rev Med. 2019;70:273–288.
[73] Li C, Wang J, Wang Y, et al. Recent progress in drug delivery. Acta Pharm Sin B. 2019;9(6):1145–1162.
[74] Feng M, Peng J, Song C, et al. Mammalian cell cultivation in space. Microgravity Sci Technol. 1994;7(2):207–210.
[75] Sani RL, Koster JN, editors. Low-gravity fluid dynamics and transport phenomena. Washington DC: American Institute of Aeronautics and Astronautics; 1990.
[76] Adam JA, Gulati S, Hirsa AH, et al. Growth of microorganisms in an interfacially driven space bioreactor analog. Npj Microgravity. 2020;6(1):1–7.
[77] Walther I. Space bioreactors and their applications. Adv Space Biol Med. 2002;8:197–213.
[78] Gòdia F, Albiol J, Montesinos JL, et al. MELISSA: a loop of interconnected bioreactors to develop life support in Space. J Biotechnol. 2002;99(3):319–330.
[79] Lydersen BK, D'Elia N, Nelson KL. Bioprocess engineering: systems, equipment and facilities. New York, USA: Wiley; 1994.
[80] Lotfipour F, Hallaj-Nezhadi S. Microbial quality concerns for biopharmaceuticals. London, UK: IntechOpen; 2012. (Latest research into quality control).
[81] Merten OW. Virus contaminations of cell cultures – a biotechnological view. Cytotechnology. 2002;39:91–116.
[82] Aggarwal S. What's fueling the biotech engine-2008. Nat Biotechnol. 2009;27(11):987–993.
[83] Jacquemart R, Vandersluis M, Zhao M, et al. A single-use strategy to enable manufacturing of affordable biologics. Comput Struct Biotechnol J. 2016;14:309–318.
[84] Zhang J, Müller BSF, Tyre KN, et al. Competitive growth assay of mutagenized *Chlamydomonas reinhardtii* compatible with the international space station veggie plant growth chamber. Front Plant Sci. 2020;11:631
[85] Matula EE, Nabity JA. Failure modes, causes, and effects of algal photobioreactors used to control a spacecraft environment. Life Sci Sp Res. 2019;20:35–52.
[86] Olsson-Francis K, Cockell CS. Use of cyanobacteria for in-situ resource use in space applications. Planet Space Sci. 2010;58(10):1279–1285.
[87] Billi D, Baqué M, Verseux C, et al. Desert cyanobacteria: potential for space and earth applications. Adaption of microbial life to environmental extremes: novel research results and application. 2nd ed. Cham, Switzerland: Springer International Publishing; 2017. p. 133–146. (Novel research results applied).
[88] Sorkhoh N, Al-Hasan R, Radwan S, et al. Self-cleaning of the Gulf. Nature. 1992;359(6391):109–109.
[89] Singh JS, Kumar A, Rai AN, et al. Cyanobacteria: a precious bio-resource in agriculture, ecosystem, and environmental sustainability. Front Microbiol. 2016;7 Article number: 529.
[90] Stewart JJ, Adams WW, Escobar CM, et al. Growth and essential carotenoid micronutrients in *Lemna gibba* as a function of growth light intensity. Front Plant Sci. 2020;11:480
[91] Furmaniak MA, Misztak AE, Franczuk MD, et al. Edible cyanobacterial genus Arthrospira: actual state of the art in cultivation methods, genetics, and application in medicine. Front Microbiol. 2017; 8:2541.
[92] Torres-Tiji Y, Fields FJ, Mayfield SP. Microalgae as a future food source. Biotechnol Adv. 2020;41:107536.
[93] Carroll AL, Case AE, Zhang A, et al. Metabolic engineering tools in model cyanobacteria. Metab Eng. 2018;50:47–56.
[94] Gordon GC, Pfleger BF. Regulatory tools for controlling gene expression in cyanobacteria. Adv Exp Med Biol. 2018;1080:281–315.
[95] Takeuchi R, Roberts J. Targeted mutagenesis in *Spirulina*. USA; 2017. US Patent Number: US 2017/

0298319 A1, Issuing By: U.S. Patent and Trademark Office.

[96] Budzinski K, Blewis M, Dahlin P, et al. Introduction of a process mass intensity metric for biologics. N Biotechnol. 2019;49:37–42.

[97] Levri JA, Fisher JW, Jones HW, et al. Advanced life support equivalent system mass guidelines document. Report number: NASA/TM-2003-212278. Moffett Field, CA: National Aeronautics and Space Administration.

[98] Amador JR, Thompson WK, Mindock JA. Enabling space exploration medical system development using a tool ecosystem. 2020 IEEE Aerospace Conference, Big Sky, MT, USA; 2020. p. 1–16.

[99] Kwon K-C, Daniell H. Oral delivery of protein drugs bioencapsulated in plant cells. Mol Ther. 2016;24(8): 1342–1350.

[100] Gomez-Marquez J, Hamad-Schifferli K. Distributed biological foundries for global health. Adv Healthcare Mater. 2019;8(18):1900184.

[101] Morrow T, Felcone LH. Defining the difference: what Makes Biologics Unique. Biotechnol Healthc. 2004; 1(4):24–29.

[102] Knowles L, Luth W, Bubela T. Paving the road to personalized medicine: recommendations on regulatory, intellectual property and reimbursement challenges. J Law Biosci. 2017;4(3):453–506.

[103] Gergerich RC, Dolja VV. Introduction to plant viruses, the invisible foe. Plant Heal Instr. 2006;. DOI:10.1094/PHI-I-2006-0414-01

[104] Gelvin SB. Agrobacterium-mediated plant transformation: the biology behind the "gene-jockeying" tool. Microbiol Mol Biol Rev. 2003;67(1):16–37, table of contents.

[105] Lakshmanan M, Kodama Y, Yoshizumi T, et al. Rapid and efficient gene delivery into plant cells using designed peptide carriers. Biomacromolecules. 2013; 14(1):10–16.

[106] Bao W, Wang J, Wang Q, et al. Layered double hydroxide nanotransporter for molecule delivery to intact plant cells. Sci Rep. 2016;6:26738.

[107] Gunadi A, Dean EA, Finer JJ. Transient transformation using particle bombardment for gene expression analysis. New York, NY: Humana Press Inc.; 2019. p. 67–79. (Methods of molecular biology).