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Microbial food safety in space production systems

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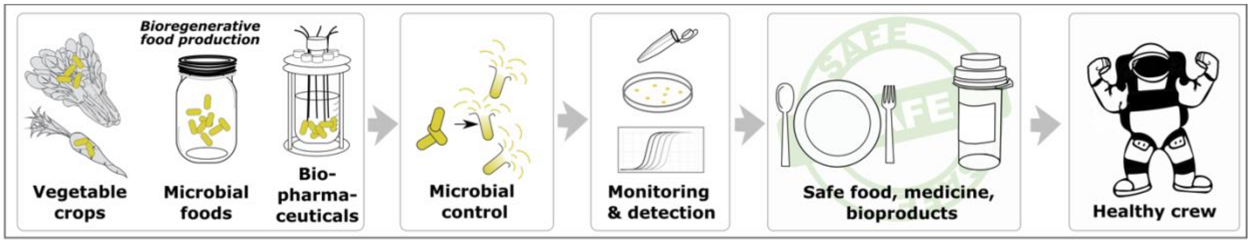
While traveling to deep space is difficult for many reasons, food is a crucial one. Round-trip Mars mission scenarios last 3 years, demanding food with a shelf-life of 5 years [1]; this means that feeding human crew sustainably for long-duration missions beyond low Earth orbit (LEO) will ultimately lead to a paradigm shift away from the current Earth-based food production system, which depends upon storing and transporting prepackaged foods, and toward bio-regenerative production of food in space [1,2]. Pharmaceuticals and nutritional supplements face similar challenges [2,3]. Moreover, the methods we currently use to detect dangerous microbes in food require sample return to Earth, a situation not viable for deep-space missions. While the science behind generating foods and bioproducts is covered by other white papers, in this paper we discuss a crucial gap uniting all of them: how to ensure that such products are free of unwanted microbial contamination and safe for crew to consume. Because Earth-based food safety systems cannot be directly applied in space, safety assurance is currently a critical bottleneck in the space production of food and other bioproducts. ***Future sustainable deep-space missions will require NASA to devote more resources in the coming decade to understanding the biological and physical science principles underlying microbial food safety in space, and to developing efficient, reliable methods in this area.***



BACKGROUND

NASA's current space food system is Earth-dependent and mostly sterile. The food system currently feeding the ISS crew consists of a rotating menu with ~200 items prepared on Earth, described in depth elsewhere [1,4,5]. All foods must be stable at room temperature, as the ISS has no dedicated cold storage for food. Pre-packaged food types include thermostabilized (retort process) dishes, irradiated meat items, dried and freeze-dried foods, extended shelf-life bread products, dry beverage mixes, and natural-form foods, supplemented by a few fresh foods as preference items and, only recently, some space-grown crops. Food safety in processed foods relies on safe production methods: the Hazard Analysis and Critical Control Point (HACCP) system and Good Manufacturing Practices (GMPs). Foods that are not commercially sterile must meet standards for microbial tolerances (2x10^4 CFU/g total aerobic count; 10^3 CFU/g yeasts and molds; 10^2 CFU/g coliform or coagulase-positive *Staphylococci*; no *Salmonella*) [6]. Crew are trained to discard uneaten food within 2 hours of preparation, and are not permitted to consume fermented foods, probiotics, or other products with live microorganisms. No cases of foodborne illness have ever been reported [5]. Thus, from a food safety perspective, the current system has been considered sufficiently reliable for a future mission to Mars. But where the system is *not* sufficient is in the areas of nutritional value and acceptability. And because solving the nutrition and acceptability problems will likely entail the development of novel production methods, a novel safety assurance system will also be required.

Sustainable exploration beyond LEO will ultimately require bio-regenerative food production in space. Initial missions to Mars would require foods with a minimum shelf life of 5 years at room temperature, to allow for transit time, potential prepositioning of food, and contingencies [1]. Currently, NASA's pre-packaged foods have a stated shelf life of 2 years [5], due not to safety concerns but to declines in acceptability and nutrient content. According to one study, Vitamins C and B1 degrade sufficiently quickly that within three years, the standard ISS diet would not provide adequate levels for crew health; vitamins A, B6, and B12 also showed some degradation [7]. Deep-space radiation may accelerate food degradation further, though data are lacking [5]. Space explorers thus face one of the same challenges that faced explorers of Earth centuries ago, when vitamin C deficiency (scurvy) incurred disastrous losses on historic voyages such as Anson's global circumnavigation [8].

In addition to maintaining essential nutrition, numerous other reasons exist for deep-space explorers to consume fresh food. Several compounds typically found in plants could serve as dietary radioprotective agents [9]. Including probiotics in the diet may potentially counteract the microbiome shifts observed in astronauts [10,11]. In situ resource utilization (ISRU) through bio-regenerative food production could ultimately save space and mass in food transport; for instance, through fixation of waste CO_2 and by minimizing food packaging, which comprises 15-17% of the current food system [5]. Finally, the psychological benefits of caring for plants [12], eating diverse foods, and preparing food may sustain crew mental health on long-duration missions [8,13]. Thus, while initial missions to Mars will likely depend primarily on the current system of pre-packaged foods, a sustainable human presence in deep space will ultimately require bio-regenerative food production [1,2].

The landscape of microbial food safety risks is fundamentally different in space. Microbial food safety assurance entails 1) assessing the risk of foodborne illness to consumers and quantifying acceptable levels of risk; 2) establishing a method of production that minimizes risk; and 3) testing to ensure that food products meet standards. Risk assessments are context-dependent; ***crucially, many of the factors that play into food safety risk assessments are different in the space environment, but we do not have sufficient data to recalculate risks accordingly.*** These include:

- **Constrained resources and dramatically different supply chains.** Unique environments and novel production systems require new solutions for monitoring, processing, and storage.
- **Reduced medical care availability**. On Earth, we accept that 7-20% of Americans will suffer from foodborne illness and mitigate the consequences with medical treatment [14], but in space, the scope of medical care is limited, which strongly encourages prevention rather than treatment.
- **Compromised immune response in human crew.** Spaceflight-induced immune system [15] and gut microbiome stress [10,11] may require stricter microbial contamination control [16].
- **Potential for increased microbial virulence**. Safety methods must account for unpredictable changes in virulence and biofilm formation of both pathogens and non-pathogens [17,18].
- **Microorganisms will likely feature prominently in space-produced foods and biopharmaceuticals**. Without animal husbandry, a sustainable food system will require engineered microorganisms to produce essential micronutrients for the near future, and microbial bioreactors will likely precede agriculture as the first food production systems on Mars [2].

Space-based foods currently in development have limited microbial management processes. Here is a summary of the status of vegetable crops, microbial foods, and recycled water on the ISS.

***A) Horticultural crops*.** Fresh produce (leafy greens, radishes, peppers, and soon tomatoes) has been grown periodically on the ISS for crew consumption since 2015 [19,20], but production is still limited and far from standardized. In contrast with pre-packaged foods, microbial safety standards for on-orbit produce are assessed on a case-by-case basis. Initial approval for crew consumption of crops by flight surgeons and safety boards came primarily from ground studies. HACCP plans have now been developed for space-grown produce; in addition to considerable preflight precautions, crew procedures include wearing gloves when handling plants, and precautionary sanitizing. Crops are grown from sterilized seeds and have been found, by microbial analyses of frozen samples returned to Earth (e.g., [21]), to be colonized during growth by microbes from the surroundings.

Microbial levels on fresh produce grown on ISS tend to be higher than terrestrial ground controls, and microorganisms that may be associated with health risks have occasionally been found on produce, but only at levels well below levels of possible concern for human health. Sanitization of crops to enable consumption currently entails gently pressing leaves of leafy produce between layers of Pro-San® sanitizing wipes, or wiping non-leafy produce, for 30 seconds [19]. This approach is labor-intensive and has the potential to miss microbial contaminants or damage produce.

B) Microbially produced foods and supplements. The BioNutrients project is a series of ISS experiments using microbes to produce micronutrients with short shelf-life in pre-packaged foods. BioNutrients-1 demonstrates the growth of yeast engineered to produce zeaxanthin and beta-carotene, two carotenoid compounds important for photoprotection, vision, and oxidative stress mitigation, which are also sensitive to ionizing radiation and heat. The cultures were flown to the ISS in dehydrated form in April 2019 and so far have demonstrated three on-orbit operations spanning two years of storage in space [22]. BioNutrients-2, to fly in 2022, improves the bioreactor to a lower-mass FEP bag, and expands the microbial species and products: included are the fermented foods yogurt and kefir, and microbes engineered to produce follistatin, a muscle-enhancing protein and potential reduced gravity countermeasure for astronauts [23]. As demonstrated by BioNutrients, microbial cultures may be used to produce not only familiar fermented foods but also essential nutritional supplements and pharmaceuticals for crew health. Other related future applications of cell culture (microbial and eukaryotic) may include probiotic supplements and lab-grown meat, all of which will require novel food safety protocols. Although the BioNutrients products use microbial strains commonly used in food production, they are returned to Earth without being consumed, as there is currently no method for inactivating the microbes, and ISS food systems policy does not allow crew to consume high abundances of any live microorganisms.

C) Water recycling. One of the longest-lived examples of microbial management in the space food system is the Water Recovery System (WRS), launched in 2008 to generate potable water from urine distillate, humidity condensate, and Sabatier product water, as well as the occasional off-loading of ground, supplied water [24]. This system recycles >90% of ISS water, and its products are used for drinking, washing, and rehydrating food. The WRS consists of the Urine Processor Assembly (UPA) and the Water Processor Assembly (WPA). The reservoir for these products, the wastewater tank, does not have a means of microbial control and, a year into operation, saw decreased outflow due to an obstructing biofilm [24]. The issue has been remedied: while the wastewater tank likely contains a thriving microbial community, catalytic oxidation, filtration, and the addition of a biocide (iodine, I_2) during processing serve to reduce microbial contamination in the potable water substantially. The ISS water is held to stringent acceptability limits (50 CFU / mL total bacteria, assessed monthly; no detectable coliforms per 100 mL, quarterly [25]). There has never been a true positive coliform detected, and, apart from the early checkout process [26], the levels of culturable bacteria are well below the requirement. This system has proven a reliable source of safe water and will likely serve as an example for crewed missions and deep space exploration. However, microbial monitoring for the WRS entails crew culturing bacteria and subsequent return to Earth for DNA sequencing. The present methods have significant limitations, including the length of time between sample collection on-orbit and subsequent analysis and possible degradation during transport from ISS back to the JSC Microbiology Laboratory on Earth.

Space-based monitoring and detection of microbial communities are in the early stages of development (Table 1). Nucleic acid technologies have seen the most progress, including spaceflight technology demonstrations, due to their diverse applications. However, none have yet been tested on food samples. Table 1 highlights selected other methods used by the the food industry that have potential for space food applications but need development.

Table 1. Current and future technologies that could support in-flight pathogen detection.

Method	**Non-flight technology**	**Tested in flight**	**Gaps in implementation**
Nucleic acid amplification and sequencing	• Real-time PCR • Genomic sequencing • LAMP-BART [27] SHERLOCK (CRISPR-Cas) [28] • CRISPR-chip [29]	• WetLab-2: sample extraction (custom platform; SimplePrep X8 being validated) & qPCR (Cepheid SmartCycler) [30,31] • BEST: Biomolecule Extraction and Sequencing Technology (miniPCR extraction, MinION sequencing). First space DNA sequencing; on-orbit data analysis demonstrated [32–34] • Razor EX: qPCR platform [35] • µTitan: custom automated nucleic acid extraction for diverse low-biomass samples (tested in parabolic flight) [36]	• Time-intensive • Requires sample prep • Reagents have short shelf-life (lyophilization is potential fix) • Data usually downlinked to ground for analysis • Does not determine live/dead (viability PCR is potential fix) • PCR detects only specified pathogens
Chemical sensors	• E-nose [37,38] • E-tongue [39] • surface-enhanced Raman spectroscopy [40,41]	• E-nose	• Detection limits for VOCs • Sensor drift • May require culturing of food item to detect • Raman requires sample adherence to metal nanoparticles; error-prone due to food particles
Antigen or protein	• RAPID testing [42,43] • lateral flow assays [44]	n/a	• Detects only specified pathogen • Requires sample prep • Reagent storage
Culture-based	• PetriFilm plates [45]	n/a	• Plates have ~1 yr shelf life • Plating in lowered gravity unknown • Amplifying potential pathogens

GAPS AND RECOMMENDATIONS

A. Risk assessment and policy revision. The first step in establishing a coherent food safety assurance plan is to quantify the risks of microbial contamination and establish standards for control and monitoring, as the standards for pre-packaged foods are not appropriate for space-produced bio-regenerative foods. Recent reviews on space-produced food conspicuously lack safety discussions, and one recent review dedicated to safety acknowledged the dearth of data [4]. There has been one report of a Quantitative Microbial Risk Assessment for exposure of ISS crew to *Salmonella* spp., *Staphylococcus aureus*, and *Pseudomonas* spp. through consumption of lettuce and radishes grown in space [46], which found the annual risk of infection to be as high as 10^{-1} for some scenarios. However, due to the paucity of data, it used numerous assumptions and presented a worst-case scenario for a conservative estimate. More data are needed to constrain risk estimates and generate targets and guidelines for safe food. Given the potential future role of microbial foods in space, developing sustainable safety practices for bio-regenerative space foods may entail a paradigm shift away from a philosophy of "the only good bugs are dead bugs," toward a focus on healthy microbiomes.

B. Developing space-specific food production pipelines with safety in mind. The development of microbial space bioproducts should consider the downstream steps required for food safety assurance: for instance, design of bioreactors that facilitate microbial monitoring and product extraction; and methods for maintaining quality in recycled water (i.e., biocides) that do not interfere with the growth of crops or microbial cultures for which the water is used. Horticultural crops can themselves be improved through the identification of new cultivars with rapid flowering, high yield, and extended shelf life and nutrient retention [47], or by engineering such traits using CRISPR/Cas [48]. Hydroponic farming and machine learning technologies for growth monitoring can also aid in producing crops with delayed senescence [49,50]. While all ISS crops are currently grown from sterile seeds, inoculation of crops with probiotic or beneficial microbiota might improve crop health and lower the risk from pathogens [51]; however, extensive research is required. Finally, HACCP plans are the current standard for minimizing safety risk through the entire food production pipeline [52], and will need to be developed for diverse production platforms, both microbial and agricultural. Process-driven protection practices should be developed with input from flight crew.

C. Food storage and quality monitoring. Improved plans for post-harvest and post-production storage, such as refrigeration, freezing, and dehydration, can help to prevent spoilage and food waste and increase food supply flexibility. Methods for processing and storage at microgravity, and packaging with necessary barrier and sustainability properties, require development. Passive refrigeration/ freezing utilizing the low temperatures of space should be explored. Furthermore, the development of non-destructive and rapid methods for monitoring senescence and deterioration in vegetables and fruits, such as state-of-the-art food shelf-life modeling, imaging-based sensors, and machine learning approaches, will aid in enabling the storage of produce to meet shelf-life goals [53].

D. Microbial control. Food system process controls are standardized methods to reduce the chance of contamination. These procedures reduce the microbe population by inhibiting growth or killing live microbes. The only process control developed for space-produced foods is the use of sanitizing wipes on space-grown vegetables. Other methods need to be developed, such as:

- **Heat-based microbial inactivation**, such as cooking or pasteurization. As evidenced by the Zero G Space Oven [54], reduced heat transfer due to the altered convective flows in reduced gravity [55] can make the simplest of Earth food processing methods complicated in space. Both basic research and development of heating techniques are required in this area.
- **Irradiation methods**, such as gamma or UV irradiation [56]
- **Novel approaches** such as exposing food to cold plasma or other sterilizing technologies [5,57]

E. Monitoring microbes and detecting pathogens. Standard terrestrial methods for pathogen detection in food systems by culturing pathogens from samples are slow, require significant material resources, and risk contaminating the crew habitat with pathogens. As shown in Table 1, a suite of diverse microbial monitoring technologies awaits development for application in space food safety. In addition, non-invasive technologies, such as hyperspectral imaging [53,58], could be employed to detect biotic stress in horticultural crops during growth as a means of assessing contamination risk.

CONCLUSION

The 2011 Decadal Survey listed "Food, nutrition, and energy balance in astronauts" as a cross-cutting issue of highest priority, stating, "It is critical that NASA: Address nutrient stability over time as part of planning for long-duration exploratory missions, and; Develop a food system that can support such a mission" [59]. Many specific goals are described in NASA's Human Research Roadmap Risk of Performance Decrement and Crew Illness Due to Inadequate Food and Nutrition [60]. **While progress in the past decade has been made toward *producing* plant and microbial foods, the development of *food safety assurance* lags substantially behind.** Closing the gaps described above in the coming decade will require both technology development and fundamental research in a wide range of disciplines in the biological and physical sciences such as microbial physiology and ecology, host-microbe interactions, human immunology, microbial detection, synthetic biology, nutritional biochemistry, and heat and fluid dynamics in reduced gravity.

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