The Future of Astronaut Nutrition: Daily Production of Kefir Nutrient Packs in a Lunar Analog Mission

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HI-SEAS
Hawai'i Space Exploration Analog and Simulation

Abstract

Astronaut nutrition will need to be supplemented with fresh nutrients and probiotics for long duration space travel. Kefir is a fermented milk beverage that provides probiotic bacteria, protein, calcium, and vitamin K. It has been historically cultured for thousands of years and is a modern-day health food. During the BioNutrients-2 experiment, one generation of kefir was cultured by astronauts on the ISS in fluorinated ethylene propylene (FEP) bags. This follow-on experiment aimed to test the hypothesis that kefir can be produced at ambient temperatures and safely passaged to make a series of nutrient packs in an analog environment. The Hawaiian Space Exploration Analog and Simulation (HI-SEAS) is a semi-controlled habitat located on the Mauna Loa volcano on the island of Hawaii at 2,500 m elevation. The recent EMMIHS (EuroMoonMars, International MoonBase Alliance, HI-SEAS) missions comprised of interdisciplinary, international crews for mock lunar missions. During a six-day mission in March 2024, crew members tested continuous passaging of kefir cultures (C-FIR commercial strain). After approximately 24 hours of growth, a portion of culture was used to seed the next generation of culture bags for four passages. A color board and pH indicator allowed the crew to easily determine when the culture had reached optimal pH. Laboratory analysis at NASA Ames Research Center (ARC) showed that the pH met kefir standards (<5). The yeast and lactic acid bacteria were in normal range and no contamination was detected in the final passage. By successfully accomplishing this experiment the team has demonstrated the ability to safely produce daily cultures of kefir in a microgravity applicable growth system.

Image 1. HI-SEAS habitat on the Mauna Loa volcano. The dome contains living quarters, galley, workspace, and a laboratory.

Objectives

(1) Test continuous passaging and growth methods of BioNutrients-3 kefir cultures (commercial strain) in a lunar analog setting (austere environment with minimal resources); (2) Utilize sterilized potable water to hydrate the powdered growth medium and starter culture. Monitor the pH of the cultures with the internal pH indicator; (3) Return samples to ARC to conduct contamination checks, measure pH, and viability.

Hypotheses

1. Kefir can be safely produced in the lunar analog using minimal resources (ambient temperature produced kefir).
2. Kefir can be safely passaged to make a series of nutrient packs in the lunar analog.

BioNutrients Background

The BioNutrients Project by the Synthetic Biology team at NASA Ames Research Center is a series of three ISS missions to evaluate the on-demand production of nutrient packs containing genetically engineered organisms that produce vitamins or other essential nutrients that are lacking in food stored for long durations. Specifically yeast production packs were engineered to produce β-carotene. In BioNutrients-2 yogurt and kefir were introduced[1]. A yogurt bacterial strain producing green fluorescent protein (GFP) was cultured on the ISS as a proof of concept. During on-board operations one cycle of culture was conducted.

Image 2. NASA astronaut and Expedition 68 Flight Engineer Josh Cassada works in the International Space Station's Harmony module on the BioNutrients-2 investigation.

Results and Methods

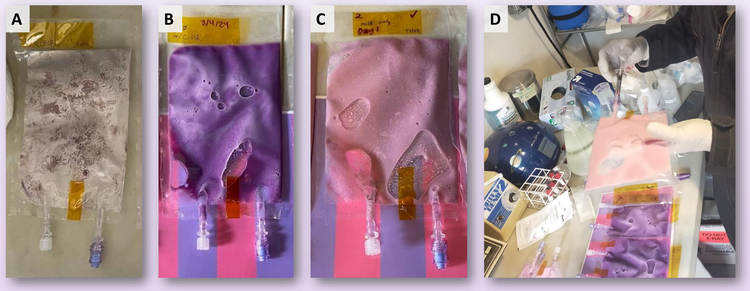

Figure 1. A) Kefir production packs. FEP bag pre-hydration containing 8.0 g Franklin nonfat dry milk, 0.4 g red cabbage powder (pH indicator), and 0.3 g kefir starter (C-FIR from Dairy Connection). **B)** Hydrated FEP bag with 72 mL of 0.22 um filtered potable water treated with 100 mg/L sodium thiosulfate to neutralize residual oxidants. **C)** Kefir culture after 24 h of growth at ~24°C, note the pH indicator has turned from purple to pink. A pH color board was used to aid analysis. **D)** Passaging 0.5 mL of grown kefir (pink sample) into hydrated bags only containing media (purple), each port was wiped with 70% isopropanol before passaging.

Table 1. Experimental timeline. The generation (Gen) of culture and the time of incubation cycle are listed.

Kefir Bags (n=3)	Incubation Time
Gen-0	24 h 40 min
Gen-1	24 h 30 min
Gen-2	31 h 5 min*
Gen-3	23 h 10 min
Gen-4	21 h 15 min

*Overnight temperature drop, incubated longer

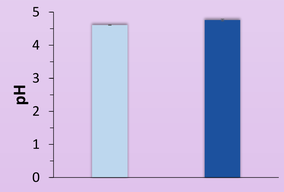

Figure 2. pH of first and last set of kefir bags. N=3. Error bars ± standard deviation.

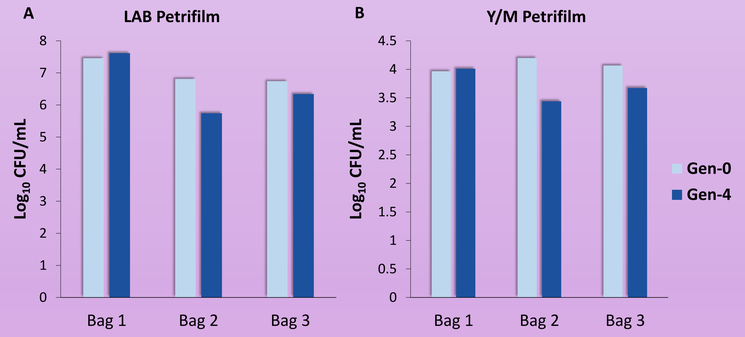

Figure 3. Viability counts of lactic acid bacteria (LAB) and yeast/mold (Y/M). 1 mL of diluted culture from 10^{-3} to 10^{-8} for LAB (a) and 10^{-3} to 10^{-5} for Y/M (b) was plated onto each petrifilm and incubated at 26 °C for 72-96 h.

Table 2. Gen-4 contamination checks with petrifilm. Kefir culture bags were thawed and plated onto petrifilm for aerobic count, coliform, *Salmonella*, *Staphylococcus*. 1 mL of the undilute culture was plated for CC, SALX, and STX. For AC a 10^{-6} dilution was cultured.

Gen-4 Bag	Aerobic Count (AC) CFU/ml *	Coliform Count CFU/ml	*Salmonella* (SALX) CFU/ml	*Staphylococcus* (STX) CFU/ml
1	1.20×10^6	<1	<1	<1
2	1.28×10^6	<1	<1	<1
3	6.10×10^5	<1	<1	<1

*AC count is expected. Proprietary mix of organisms includes some aerobes.

Figure 4. *Staphylococcus* petrifilm, Gen-4, dilution 10^{-2}. Absence of *Staphylococcus* confirmed.

Table 3. Summary of temperature and humidity data recorded by the HOBO logger during the shipment, the experiment, and post experiment with dry ice. SD= standard deviation.

	Pre-Experiment	During-Experiment	Post-Experiment*
Temperature ± SD (°C)	18.60 ± 2.95	24.23 ± 3.47	-12.64 ± 4.06
Relative Humidity ± SD (%)	56.62 ± 3.57	38.58 ± 9.28	76.62 ± 6.33
Duration (Days)	4.02	5.19	2.96

*HOBO battery dead or memory full at handoff to FedEx

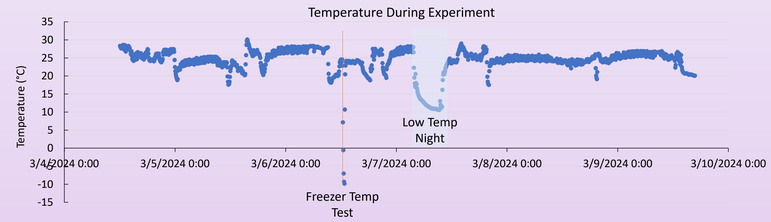

Figure 5. Temperature profile in the lunar analog habitat. The kefir cultures were placed on a shelf across from the heater in the galley. A HOBO logger was kept with the bags and moved into the lab for passaging events. The HOBO was placed in the lab freezer to check the temperature. The low temperature night with no heater due to a power outage is highlighted.

Conclusions

Image 3. HI-SEAS crew members conducting kefir passaging.

The team has demonstrated the ability to produce daily fresh cultures of kefir that may provide future astronauts valuable probiotic cultures and nutrients. Overall, the experiment was simple to execute with minimal resources and time. The pH indicator and color board allowed the crew to easily determine when the culture had reached the optimal pH. To keep the cultures at optimal growth temperature the cultures were placed in front of the habitat heater, requiring no extra incubator.

The crew experienced low temperatures during the third night due to a power outage. After troubleshooting the issue, the crew ultimately had to wait for the sun to recharge the batteries later that morning. Also, the blue luer lock ports on some of the bags were difficult to push through, requiring extreme force and two bags had to be switched out for spares. During return shipping, the plane was delayed in Honolulu one day and the bags were received thawed but still cold and immediately placed at -20 °C.

Post Analysis at Ames Research Center:

- Kefir bags arrived thawed but cold from HI-SEAS, then were frozen on arrival.
- No coliform, *Salmonella*, or *Staphylococcus* contamination observed in Gen-4 bags, suggesting no contamination of these organisms through the passaging process.
- Colony growth on Gen-4 AC are expected due to presence of aerobic organisms in proprietary mix.
- Gen-0 and Gen-4 LAB viability was lower than expected from pre-viability check.
- Different shade of purple/pink at HI-SEAS; sodium thiosulfate ruled out as cause.

Gen-4 bags were incubated 21 h vs 24 h and had an additional freeze-thaw due to a petrifilm growth temperature issue, these factors could have impacted:

- Slight elevation in pH in Gen-4 bags.
- Growth of LAB on Gen-4 Bags slower than growth of Gen-0 Bags.
- Slightly less viability of YM and LAB observed for Gen-4 petrifilms compared to Gen-0.

Image 4. Gen-4 kefir bags at 21 h of growth. Bags were then placed in the freezer and all bags were transported back to ARC.

Reference

[1] Ball, N., Hindupur, A., Kagawa, H., Kostakis, A., Gresser, A. L., Sims, K., Sharif, S., Villanueva, A. G., Donovan, F., Mark Settles, A., & Hogan, J. A. (2021). BioNutrients-2: Improvements to the BioNutrients-1 Nutrient Production System. *50th International Conference on Environmental Systems*.

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