BioNutrients: Microbial production of on-demand nutrients on the International Space Station

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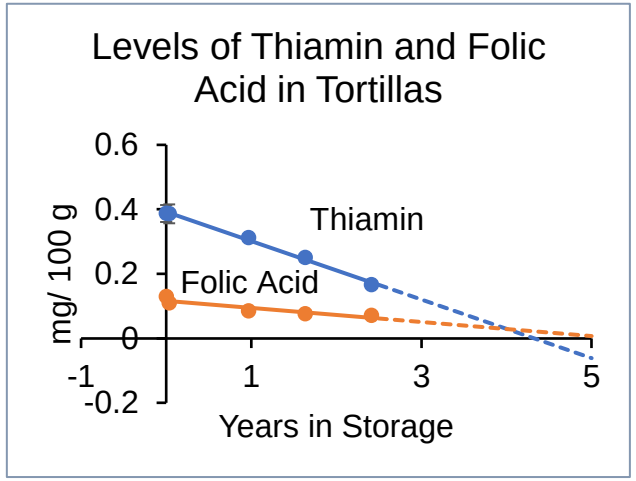
NASA Food System

- Nutrients degrade with time in food and supplements
  - Enhanced nutritional needs in space travel
  - Radiation and microgravity countermeasures
  - Disease-specific concerns
- Psychology of food/isolation
- **Need to produce some foods *in situ***



Adapted from Zwart et al (2009) *Food Science* 74(7):H209-H217. https://doi.org/10.1111/j.1750-3841.2009.01265.x

Credits: NASA

BioNutrients (BN) Projects

Objective: To develop an on-demand biological production system capable of rapid and safe delivery of fermented food products in single-use production packs for long-duration missions.

Description: *In-situ* production of essential nutrients and fermented food products for human consumption following long-term storage, revival, and growth. This technology will help to mitigate demonstrated losses in nutrition in stored food supplies.

Crew member David Saint-Jacques hydrating a BioNutrients-1 production pack.

Crew member Nicole “Duke” Aunapu Mann hydrating a BioNutrients-2 production bag.

BioNutrients Concept

- Flight-test microbial production platform evolvable for future surface missions – capable of producing many compounds (e.g., nutrients, medicines)

- Nutrients produced during fermentation period in a single use production pack

Future Mission Implementation Concept

Incubation

Safety Test

Engineering microbes to produce nutrients and medicines on-demand.

Crew member Josh Cassada working on BioNutrients-2 yogurt samples.

5-Year ISS Storage-Reactivation Demonstration – NG-11 (04/2019)

BN-1 five-year flight project currently on the International Space Station

- Testing the long-term storage of various microorganisms for the biomanufacturing of space-relevant compounds.
- Validating the performance of the first generation of production packs.

6-Month ISS Reactivation Demonstration – SpX-26 (11/2022)

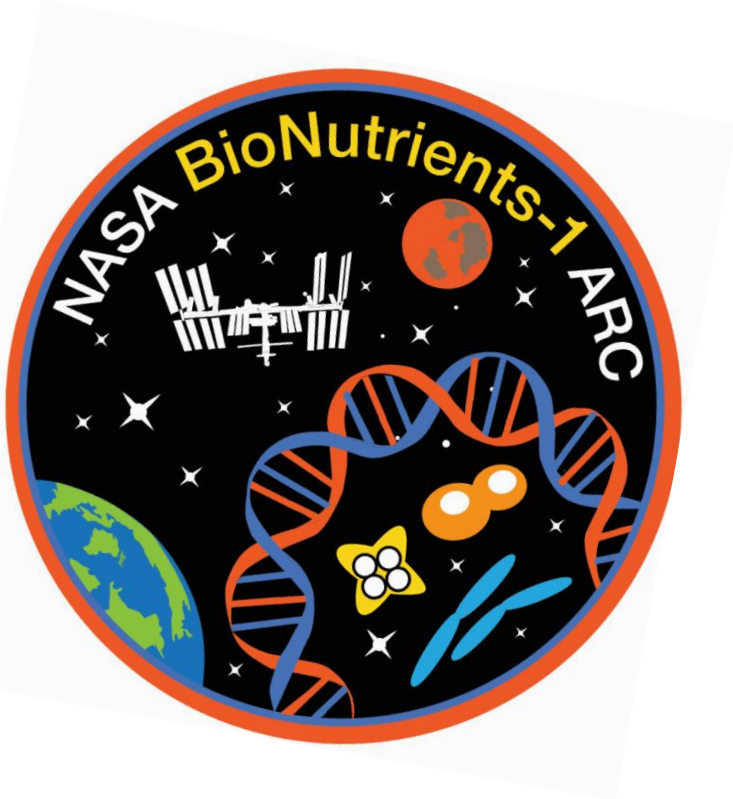
BN-2 expands the BN-1 flight project scope

- Introducing novel products.
- Broadening the range of microbial food sources.
- Improving upon production pack hardware.

Future flight project – Slated for flight in 2024

BN-3 expands on food safety and consumability

- Multi-nutrient production in a single bioreactor.
- HACCP plan development and food safety testing.

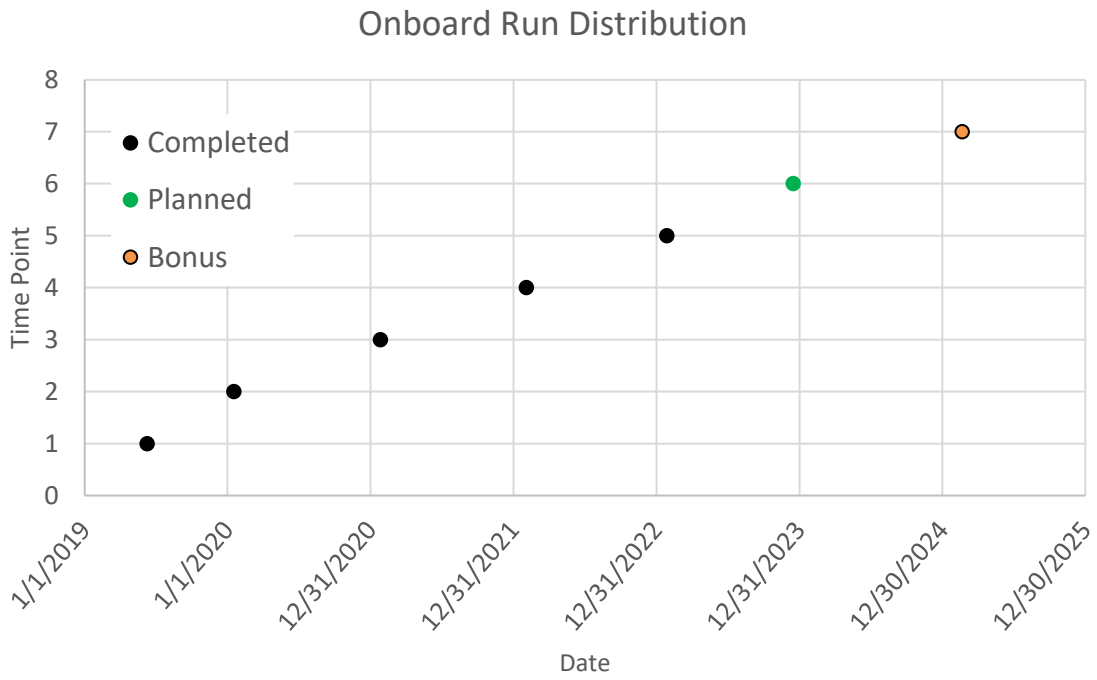


BioNutrients-1 Production Packs (5-year flight test)

- Biomanufacturing of the carotenoids, zeaxanthin and β-carotene in *Saccharomyces cerevisiae* and *Saccharomyces cerevisiae* var. *boulardii,* respectively

- To date, there have been 5 on-orbit operations

BioNutrients-1 production packs in the SABL incubator.



BioNutrients-1 Experimental Design

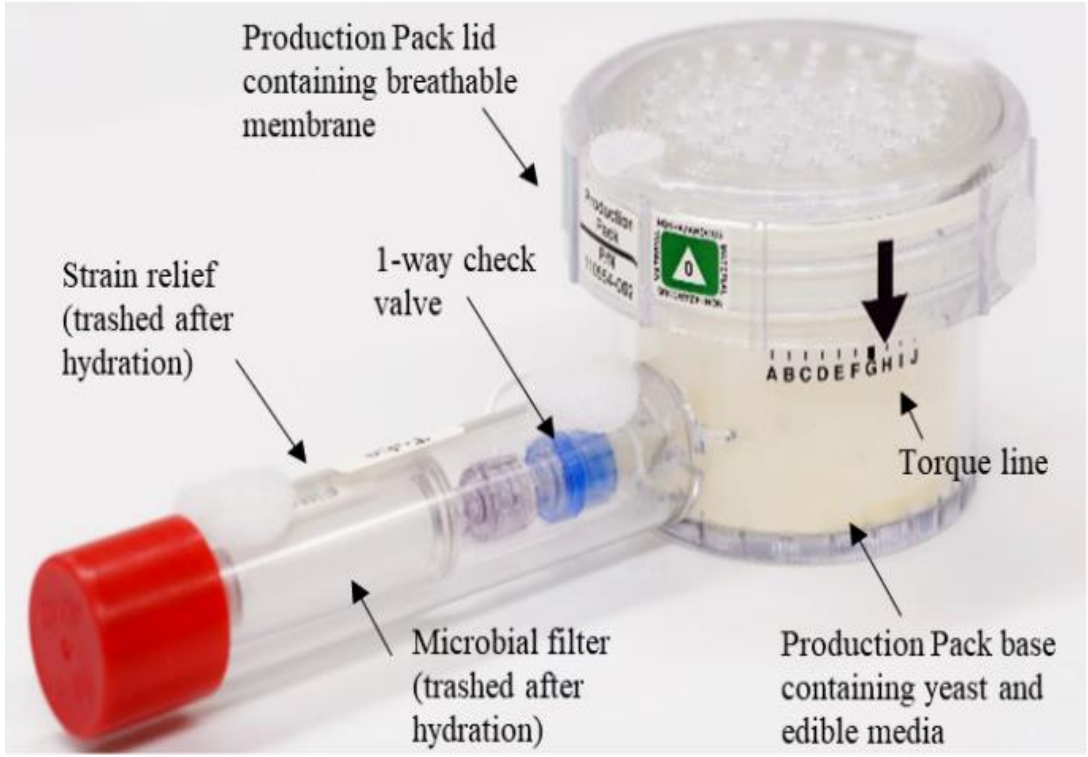
BN-1 Production Pack

- Hard-shell polycarbonate pack
- Polytetrafluoroethylene membrane for gas exchange
- Microbial filter for sterile hydration

Hydration: Sterile water

Incubation: 30 °C for 48-hours

Returned frozen for analysis



BioNutrients-1 Stasis Pack Overview (5-year flight test)

Research Questions:

Are there microbial species relevant for space biomanufacturing that are stable at ambient temperature storage for 5 years?

- Nine species tested for long-term viability

Does alginate encapsulation improve long term viability?

- Five species compared

Are there common genes required for long-term viability?

- Five species mutagenized for increased diversity

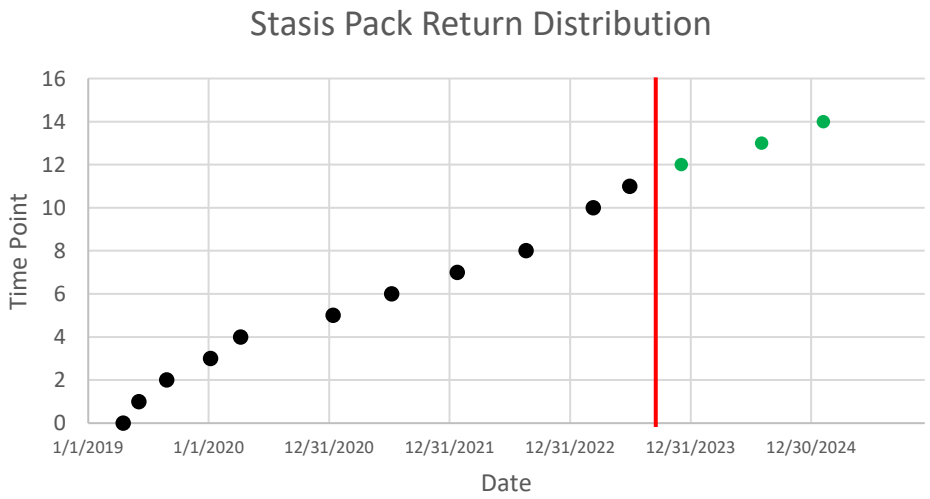
Will microbial media be stable for five years?

- Three media types stored

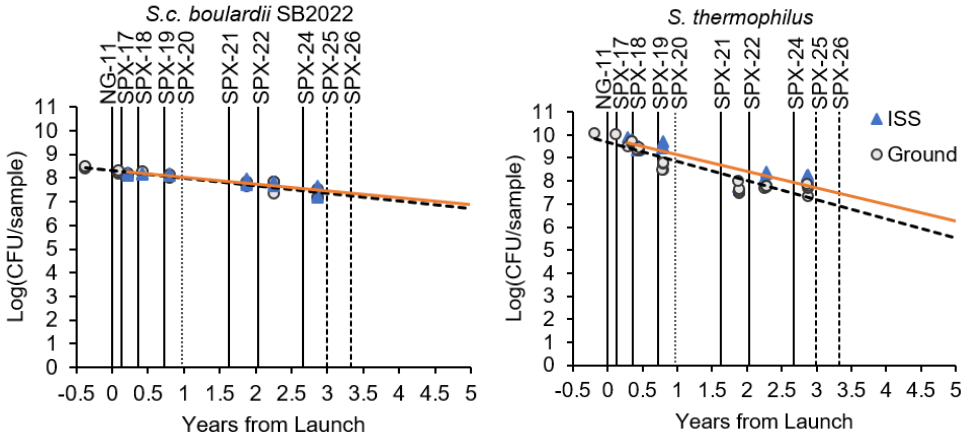
Species/Sample Description	Treatment variable	Viability after 4 years
Saccharomyces cerevisiae (ATCC BY4743) knockout lines	Heterozygous/ Homozygous	✓ All treatments
Saccharomyces cerevisiae subsp. boulardii (SB2022, β-carotene production)	Encapsulated	✓ Non-encapsulated
Saccharomyces cerevisiae subsp. boulardii (DT3032, β-carotene production, desiccation tolerance)		✓ All treatments
Saccharomyces cerevisiae (Y55-1034, zeaxanthin production)		Not tested
Kluyveromyces lactis (ATCC 8585)	UV treated	✓ All treatments
Komagataella phaffii Kurtzman (ATCC 20864)	UV treated	✓ All treatments
Streptococcus thermophilus (ATCC BAA-491) with GFP plasmid	Encapsulated	✓ Non-encapsulated
Lactobacillus delbruekkii subsp. bulgaricus (ATCC-BAA-365)	Encapsulated	Not viable
Bacillus subtilis (ATCC 6633)	Encapsulated	✓ All treatments
Bacillus coagulans (ATCC 7050)	Encapsulated	✓ Encapsulated
Methylobacterium extorquens AM1 (ATCC 14718)	UV treated	✓ All treatments
Cupriavidus necator (ATCC 17699)	UV treated	Not viable
Media A (Production Pack media)		
Media B (Vitamin K production media)		
Skim milk powder (Yogurt/Kefir media)		

BioNutrients-1 Stasis Packs Cont.

- A total of 11 sets of Stasis Packs have been returned from ISS to date
- Earth and ISS stasis packs have similar viability changes during storage
- 7 of the 9 species tested are still viable after 4 years



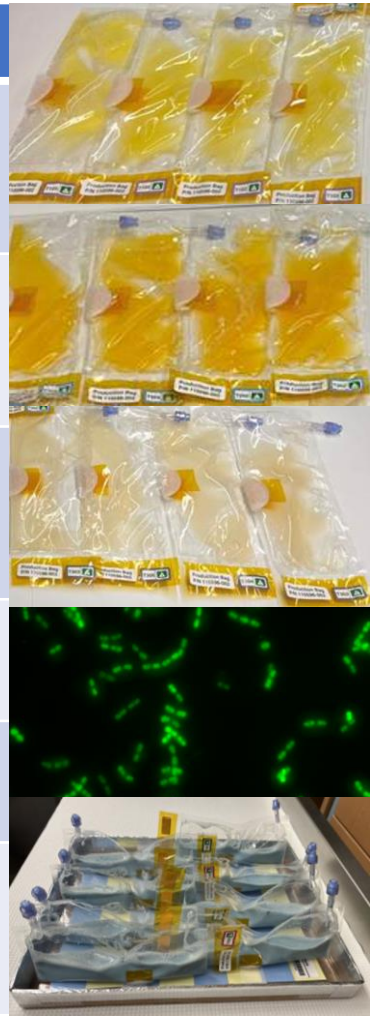
BN-1 Stasis Packs - (Left) Photo of Stasis Pack culture and media containers in storage format and (right) inside opened vacuum mylar storage bag.



Example time-course viability data for *S. c. boulardii* (left) and *S. thermophilus* (right).

BioNutrients-2: Expanding BN-1 Flight Scope

Strains for flight testing	Product	Purpose
Saccharomyces cerevisiae	Zeaxanthin	Aid in macular degeneration
Saccharomyces cerevisiae var. *boulardii*	β-carotene	Vitamin A precursor
Kluyveromyces lactis	Follistatin	Protein therapeutic to promote muscle formation
Streptococcus thermophilus	GFP	Demonstrate ability to engineer yogurt organism
Kefir-producing mixed organism culture	Kefir	Test production quality using a commercial kefir starter
Streptococcus thermophilus & Lactobacillus bulgaricus (Yogurt)	Yogurt	Test production quality using a commercial yogurt starter

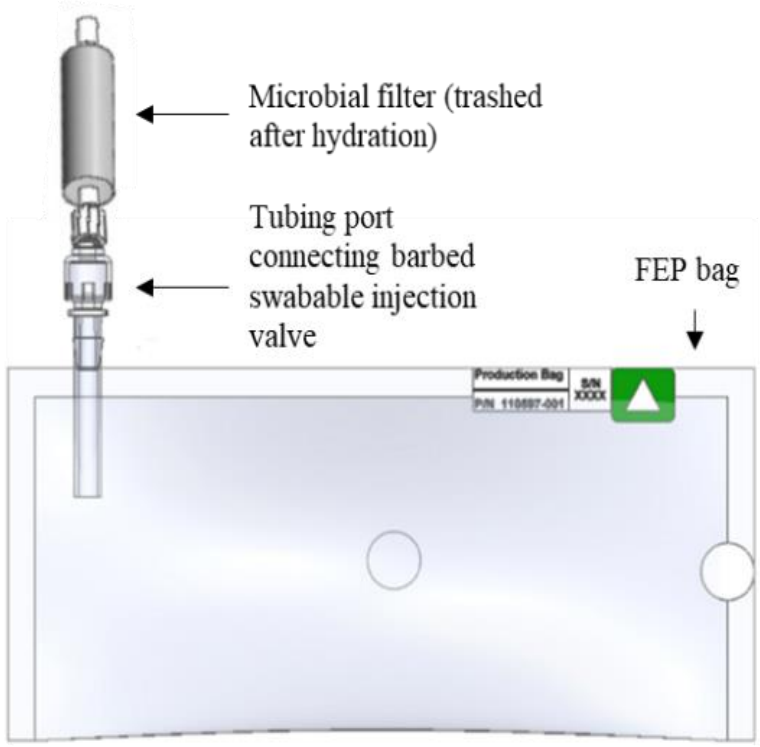


BioNutrients-2 Production Bag

- Fluorinated Ethylene Propylene (FEP)
- Gas permeable
- Supports growth and production of space relevant compounds while minimizing volume and mass compared to BN-1 hardshell pack

Hydration: Sterile water

Returned frozen for analysis



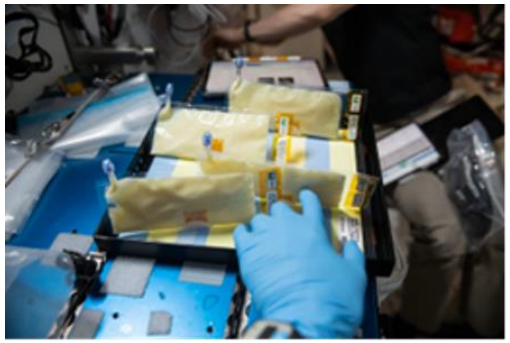
BioNutrients-2 FEP production bag

BioNutrients-2 Flight Operations

On-orbit operations were conducted in January and May with corresponding ground controls

BN-2 Run 1: 01/03 – 01/06/2023

BN-2 Run 2: 05/03 – 05/07/2023

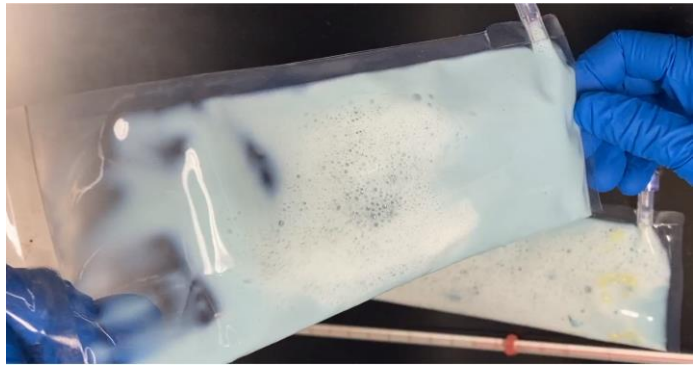


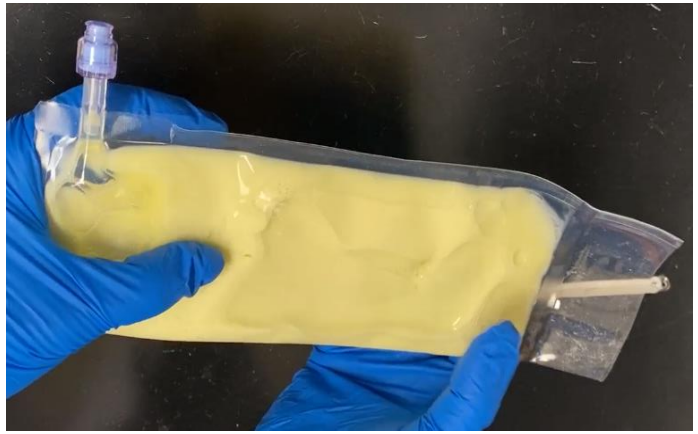
BN-2 Set 2 operations successfully performed May 3-7, 2023. Left to right: Kefir after 23 hours incubation in SALI. yogurt after 23 hours incubation in SABL. Yeast after 48 hours incubation in SABL where orange indicates beta carotene, yellow indicates zeaxanthin, and white indicates follistatin production.

BioNutrients-2 Yogurt/Kefir Flight Products

In situ production of fresh yogurt and kefir provides:

- Psychological benefits to crew members as a fresh and familiar food
- Can potentially contribute to microbiome health





Blue at hydration

Yellow after fermentation

pH indicator dye reported acidification of the yogurt or kefir on ISS.
Printed card stock SABL interface board has matching colors of pre- and post-product formation pH conditions.

BioNutrients-3

Planned flight experiment to demonstrate ability to make multiple nutrients within the same production pack, and to investigate food safety procedures.

- Yeast production microbes are being engineered to produce additional B vitamins in conjunction with previous carotenoids β-carotene and zeaxanthin
- Testing lower-temperature heat-kill techniques to allow microbial food safety compliance (pasteurization)
- Investigating E-Nose volatile organic chemical sensing technique to detect unwanted microbes and their products

ISS Food Warmer

Acknowledgements

This work is funded through the NASA Space Technology Mission Directorate's Game Changing Development program Synthetic Biology Project.

We thank Julie A. Levri, Paul G. Milazzo, Alan Noblitt, Daniel Varnum-Lowry, and Marilyn Murakami for technical assistance.