BioNutrients: Microbial on-demand production of short shelf-life micronutrients in space

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Sustained human exploration of deep space will stress re-supply capacity and surpass current shelf life limitations for critical nutrients and therapeutics.

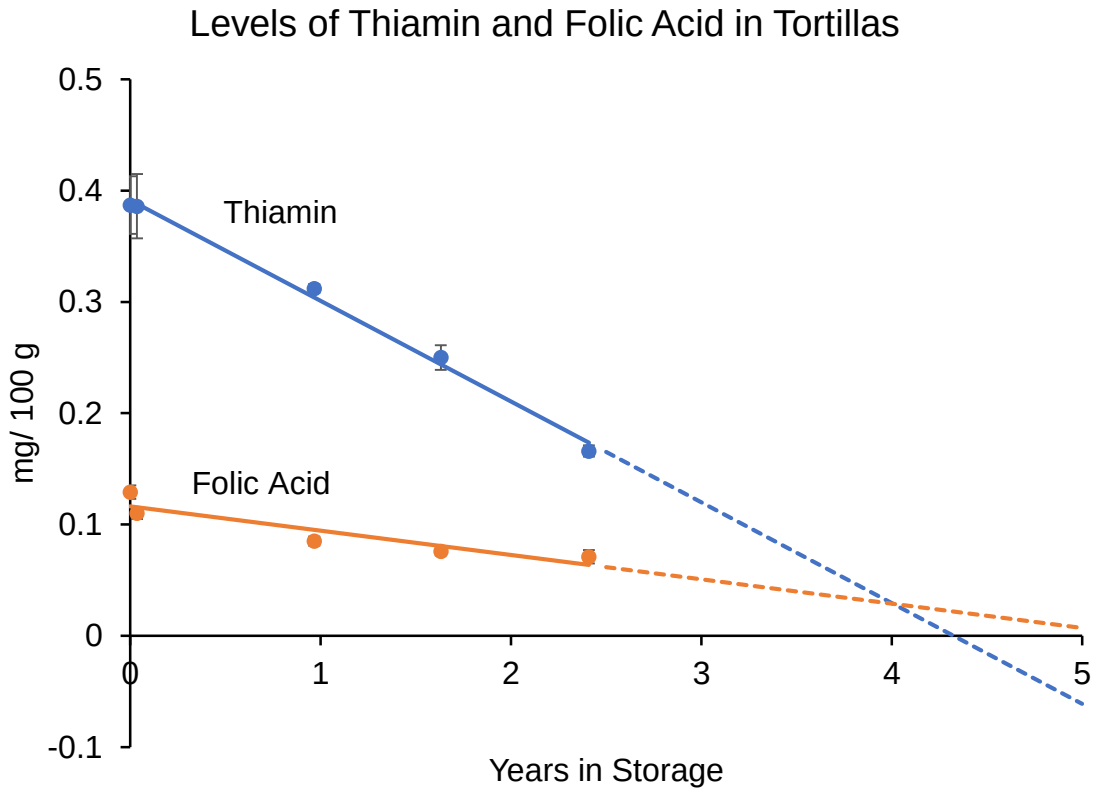
Credits: NASA

Nutrients are a critical supply risk: Essential nutrients degrade in long-duration storage

Studies of the NASA pre-packaged food system have found declines in:

- Folic acid/Folate
- Vitamin A
- Thiamin (Vitamin B_1)
- Riboflavin (Vitamin B_2)
- Vitamin B_6
- Vitamin B_{12}
- Vitamin C
- Vitamin E
- Vitamin K

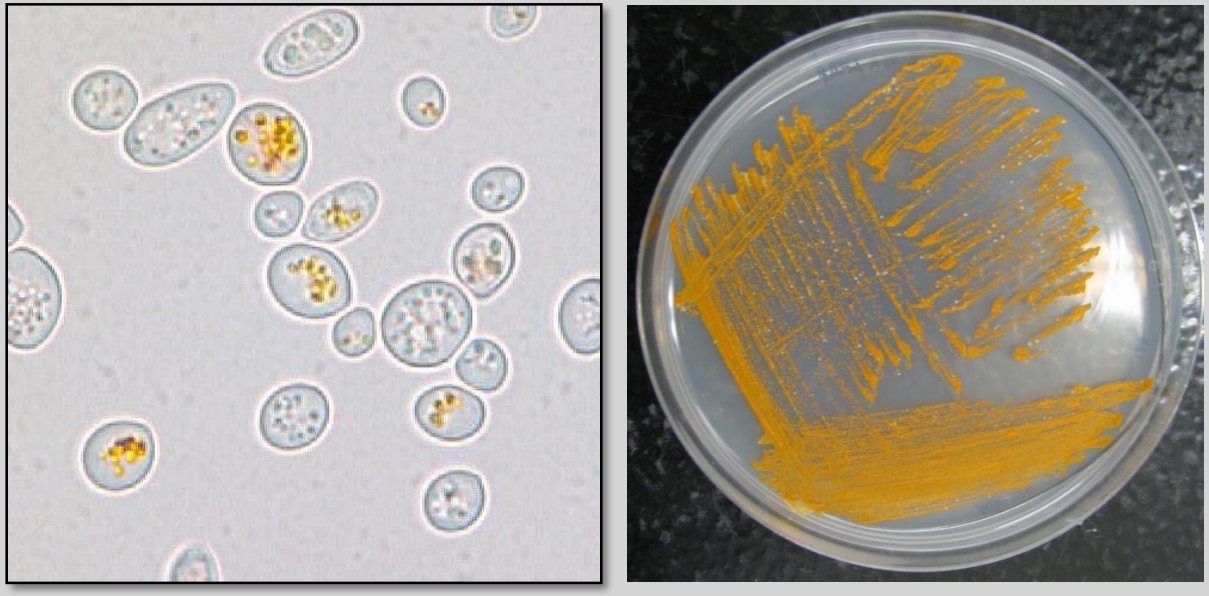
Need to produce some foods *in situ*



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

BioNutrients: In Mission production of nutrients as an alternate strategy

Microbially fermented foods engineered to produce essential nutrients or protein therapeutics, on-demand, surviving ambient storage conditions.



S. boulardii producing β-carotene

Rationale: Engineer yeast and/or bacteria to produce essential nutrients when grown for a short period of time in edible media.

- Rapid, on-demand production
- Room temperature storage of organisms
- Enriches pre-packaged foods with labile nutrients

Carotenoids selected as initial nutrient:

- Light sensitive and degrades in packaged foods
- Important to protect from macular degeneration
- Antioxidant activity to protect against DNA damage

Future implementation concept

Premixed dry media and microbes
Ambient temperature storage

Hydrate and dissolve media
Incubate to grow microbes

Deactivate microorganisms
Consume as a supplement

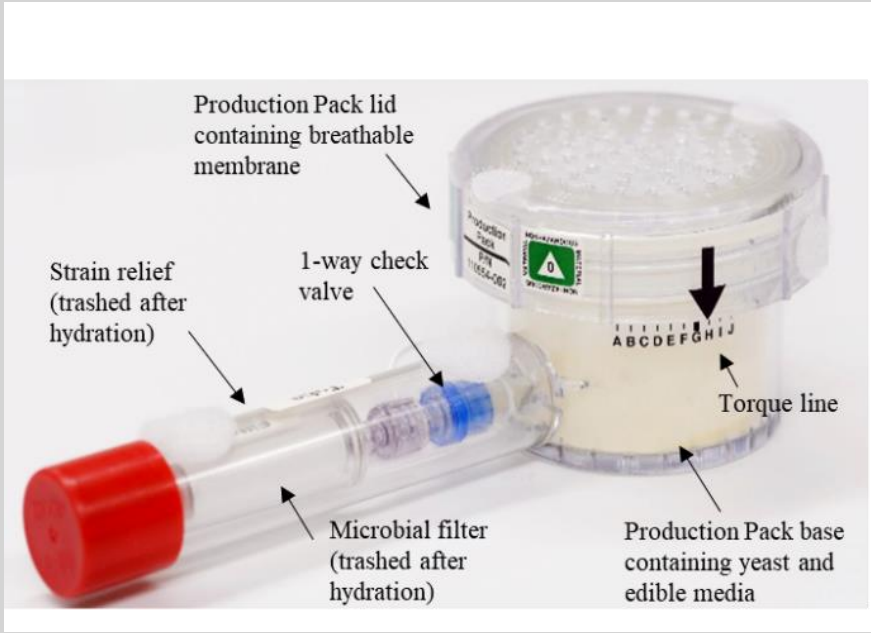
Image Credits: NASA

BioNutrients Development Plan

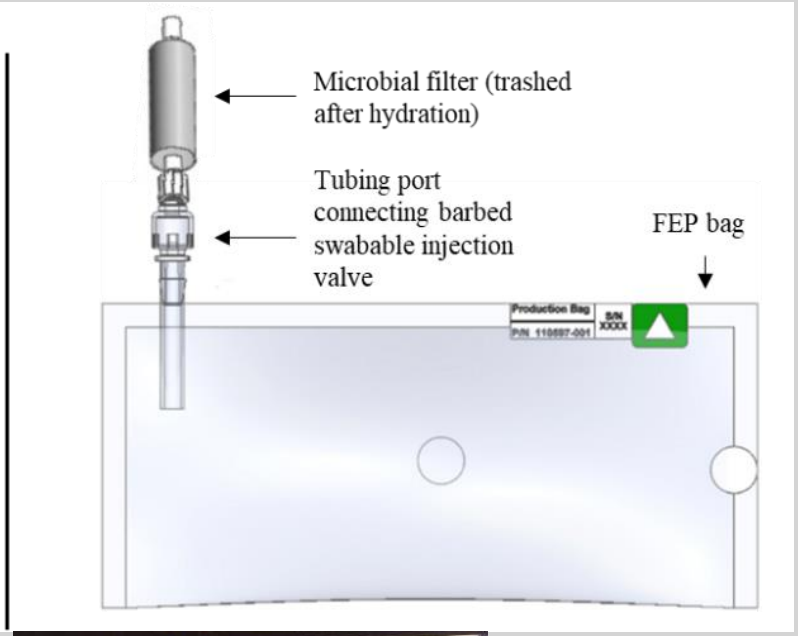
Mission	Objectives	Launch
BN-1	**5-year test of storage and production on the ISS** Engineer yeast strains to produce **carotenoids in edible media** Test media and related packaging Develop Gen-0 bioreactor **Test other microorganisms for long-duration storage**	✓ NG-11, April 2019
BN-2	<1-year flight test Expand products to **yogurt** and **kefir**, continue **carotenoid** testing Engineer medical countermeasure product (**follistatin**) Develop Gen-1 bioreactor to decrease system mass Develop HACCP food safety plan	Target NG-18, August 2022
BN-3	Engineer system to produce **multiple nutrients in low ESM package** Develop Gen-2 bioreactor Demonstrate **reliability of HACCP plan**	FY24

BioNutrients 1 hardware vs BioNutrients-2: Reduced Mass, Volume

BN-1: 117 g Bioreactor



BN-2: 10 g Bioreactor



BN-2: Change to a Fluorinated Ethylene Propylene (FEP) bag bioreactor – lower mass and volume, performance to be compared to BN-1 hard shell with gas permeable membrane.

FEP is gas permeable.

BN-1 hardshell bioreactor growing yeast expressing carotenoids in SABL on the ISS. BN-2 FEP bag growing yogurt with a pH indicator dye on during pre-flight testing

For more detail please see Ball et al. (2021) Proc. Int. Conf. Envir. Systems. ICES-2021-331. https://ttu-ir.tdl.org/handle/2346/87260

BioNutrients-1 Stasis Packs

Stasis packs seek to identify species and conditions that will provide 5-year shelf life in ambient temperature storage.

- 9 microbe species investigated
  - Baker's yeast and probiotics
  - Species for yogurt
  - Species that can metabolize diverse carbon sources
- Varied storage and pre-flight treatments
  - vary storage preparation (encapsulation, pellet forms, additives)
  - mutagenize for genetic screens
- 3 media formulations

➢ Compare ground control to ISS storage.
➢ Assay for viability at 3- to 6-month intervals over 5 years.

✓ Variation in viability observed by 2 years of storage.

Santa Maria et al (2020) found yeast to be stable for 2 years at room temperature storage.

Santa Maria et al (2020) Astrobiology. https://doi.org/10.1089/ast.2019.2073

Summary

Fermented foods provide an additional strategy to produce essential nutrients for extended shelf life.

- Microbes can be engineered to produce multiple nutrients or countermeasures.
- Media and microbes need to be shelf stable for 5 years (current production pack tested >2 years to date).
- Growth cycles are short, on the order of <1-3 days instead of weeks to months.

Ongoing development needed for full implementation

- More comprehensive microbe engineering, product development.
  - Critical products, testing of palatability, crew acceptance of nutrient supplementation via microbial production.
- Engineering food safety and process controls.
  - In mission: cooking or other methods to end microbial growth and assess safety of products.
  - HACCP planning and other ground based processes to ensure safety.

GCD Synthetic Biology Project Team

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