Delivery of Probiotics in the Space Food System

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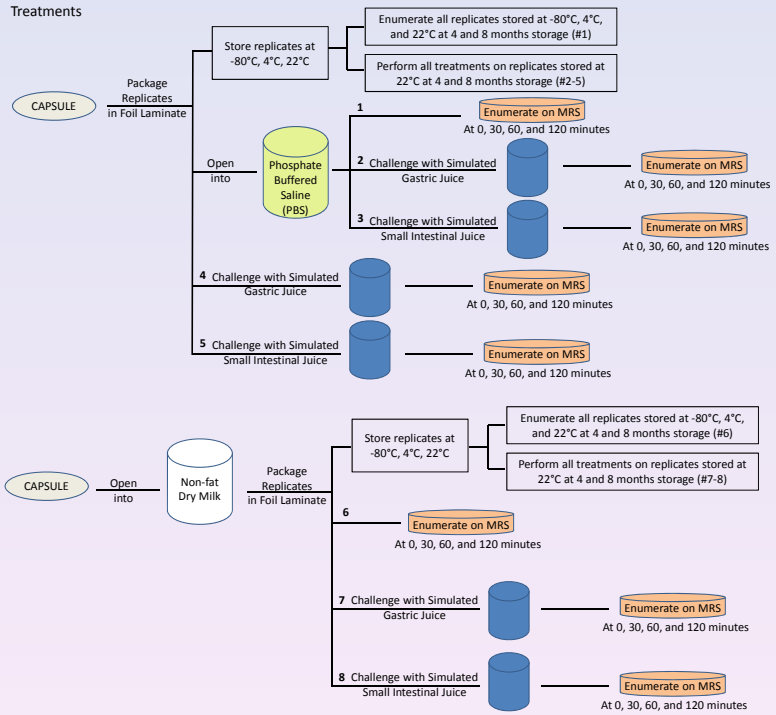
ABSTRACT

The addition of probiotic bacteria to the space food system is expected to confer immunostimulatory benefits on crewmembers during spaceflight, counteracting the immune dysregulation that has been documented in spaceflight [1]. Specifically, the probiotic *Lactobacillus acidophilus* has been shown to promote health benefits including antagonism towards and inhibition of virulence related gene expression in pathogens, mucosal stimulation of immune cells, and a reduction in the occurrence and duration of cold and flu-like symptoms [2-5]. The optimum delivery system for probiotics has not been determined for spaceflight, where the food system is shelf stable and the lack of refrigeration prevents the use of traditional dairy delivery methods. This work proposes to determine whether *L. acidophilus* is more viable, and therefore more likely to confer immune benefit, when delivered in a capsule form or when delivered in nonfat dry milk powder with a resuscitation opportunity upon rehydration, following 0, 4, and 8 months of storage at -80°C, 4°C, and 22°C, and both prior to and after challenge with simulated gastric and intestinal juices. We hypothesize that the low moisture neutral dairy matrix provided by the nonfat dry milk, and the rehydration step prior to consumption, will extend probiotic viability and stress tolerance compared to a capsule during potential storage conditions in spaceflight and in simulated digestion conditions.

OBJECTIVES

Determine the viability of *L. acidophilus* that is added to non-fat dry milk and rehydrated prior to consumption or provided in a capsule through eight months of storage at -80°C, 4°C, and 22°C and exposure to simulated digestion conditions.

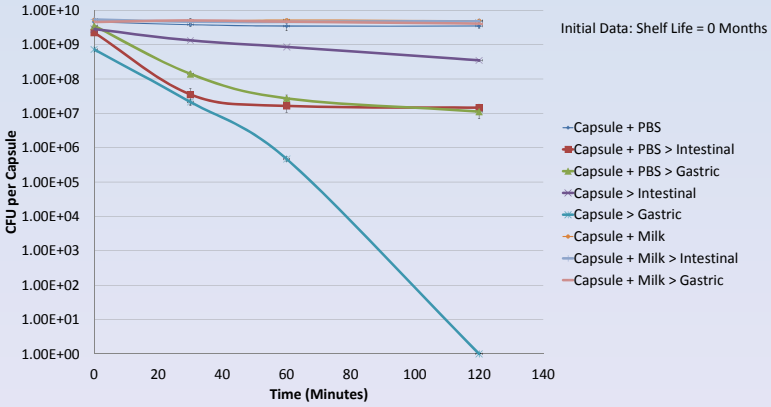
METHODS



Treatments: 1 – Capsule contents dispersed into phosphate buffered saline (PBS) and enumerated. **2 –** Capsule + PBS challenged with simulated gastric juice (0.5% wt/vol NaCl containing 3 g/L pepsin, pH 2.0) [6] and enumerated over 2 hours. **3 –** Capsule + PBS challenged with simulated small intestinal juice ((0.5% NaCl containing 1 g/L pancreatin and 3 g/L oxgall, pH 8.0) [6] and enumerated over 2 hours. **4 –** Capsule dispersed directly into simulated gastric juice and enumerated over 2 hours. **5 –** Capsule dispersed directly into simulated small intestinal juice and enumerated over 2 hours. **6 –** Capsule contents dispersed into milk and enumerated. **7 –** Capsule + milk challenged with simulated gastric juice. **8 –** Capsule + milk challenged with simulated small intestinal juice. Replicates of each treatment are being stored at -80°C, 4°C, and 22°C at 4 and 8 months. All samples are enumerated at each time point and temperature. Samples stored at 22°C will additionally be challenged with simulated gastric and small intestinal juice at each time point. Samples are enumerated on de Man, Ragosa, and Sharpe (MRS) agar in biological triplicates and technical replicates are performed for each treatment. All probiotic capsules (Acidophilus NOW 2Billion) were from the same lot.

RESULTS

Currently, only the initial analysis (shelf life Time 0) has been completed. All remaining samples are currently in controlled storage under described conditions.



- **Capsule + PBS:** The viable cell count was stable over time when rehydrated in PBS. The viable cell count declined over time when challenged with simulated gastric and small intestinal juice, but appeared to stabilize after a 2 log decrease.
- **Capsule:** Viable cells were not obtained after a two hour direct challenge with simulated gastric juice. The viable cell count was greater following direct challenge with simulated small intestinal juice than when rehydrated first with PBS, however, the viable cell count declined by 1 log after two hours and do not appear to have stabilized.
- **Capsule + milk:** Cells remained viable at original levels when rehydrated in milk prior to a two hour challenge with simulated gastric and small intestinal juice.
- The results shown here are the average of three independent assays +/- one standard deviation.

DISCUSSION

- The capsule material disintegrated in both simulated small intestinal and gastric juice in less than 20 minutes with manual agitation(performed in duplicate), exposing the injured dry probiotic cells directly to the extreme pH conditions and enzymes of each environment. Gastric exposure could be several hours in length [7], and cells are required to make it through digestion and thrive in the small intestine where they are expected to interact with the microbiome and human cells to provide their beneficial effects.
- Survival of *L. acidophilus* in milk through challenge with simulated gastric and small intestinal juice supports the hypothesis that the dairy matrix provides protection to the cells.
- Rehydration in PBS indicated that rehydration alone did not improve *L. acidophilus* survival in simulated small intestinal conditions, but did provide protection in simulated gastric conditions. It is suggested that the proteins in milk provided more efficient buffering capacity in all conditions compared to PBS.
- Current results indicate that probiotics will be more effectively administered in an appropriate food matrix than in a capsule.

Future Work

- This work will be repeated on replicate samples following 4 and 8 months of storage at 22°C.
- Additionally, replicates will be enumerated following 4 and 8 months of storage at -80°C and 4°C to indicate the benefit of colder temperatures to probiotic provisioning for long duration missions.
- This data will inform provisioning strategies for probiotics, and inform mission planners of storage requirements to probiotic survival trade-offs.

References

[1] Gueguinou N. et al. (2009) J. Leukoc. Biol. 86, 1027. [2] Medellin-Pena M.J. et al. (2007) Appl. Environ. Microbiol. 73, 4259. [3] Leyer G.J. et al. (2009) Pediatrics 124, e172. [4] Chen C.C. et al. (2005) Pediatr. Res. 58, 1185. [5] Bernet M.F. et al. (1994) Gut 35, 483. [6] Goh. Y.J. and Klaenhammer T.R. (2010) Appl. Environ. Microbiol. 76, 5005. [7] Camilleri et al. (1989) Am J Physiol Gastrointest Liver Physiol. 257, 284.