Development of Spaceflight Foods with High Microbial Concentrations

Short: High Micro Food
(MTL 678)

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EXECUTIVE SUMMARY

The use of bioregenerative food systems has been proposed for long duration spaceflight missions, though the impact of this type of system on microbial contamination and crew health is greatly understudied. Current spaceflight food requirements are inadequate for microbial monitoring associated with crop production, the use of bulk foods, and subsequent processing and handling, as well as foods with inherently high microbial concentrations, such as probiotics, which may be beneficial for crew health. This review provides an initial investigation into (a) the potential of microbial load from bioregenerative produce, (b) the current microbial strains and uses of live cultures in shelf stable foods, (c) a comparison of levels of microbial load in these foods/supplements to current requirements and an assessment of additional risk, and (d) an investigation of current literature and commercial operations to provide guidance in establishing microbiological requirements and testing methods for foods with high and diversified microbial concentrations.

The evidence from this review indicates a need for further research to determine the procedures necessary for growing and processing regenerative produce and development of specific cleaning methods to mitigate microbiological risks. In addition, data mining performed during this review identified potential benefits for the use of food products with high microbial concentrations. Specifically, this review has performed an extensive market survey to generate a comprehensive list of potential probiotic products, which should be evaluated in future research to determine preferred probiotic formulations for spaceflight. Future research would also determine the specific microbial testing procedures for those formulations and assess the risk of adding the formulation to the food system.

INTRODUCTION

In adherence to NASA exploration mission requirements, the space food system must provide the necessary nutrients to sustain the health and performance of the crew throughout the specified mission. For the very short Mercury missions, this requirement was translated to a food system composed of pureed foods in tubes and bite-sized cubes. As missions extended to days and weeks during subsequent programs, the food system became more complex – meal and snack items are produced in single-serving packages, packed into flight containers, and then sent with the crew on the mission vehicle. With the exception of Skylab, no refrigerator or freezer dedicated to food storage has flown on any United States space vehicle. Consequently, the food is provided in a shelf-stable format for storage at ambient temperature. To achieve stability, the food may undergo a variety of microbial mitigation techniques during ground processing. Although processing the packaged foods to commercial sterility provides a safe food system, this level of processing can reduce the quality of the food, including nutritional content and acceptability.

The different forms in which space food is provided include the following:

1. Thermostabilized – This process, also known as the retort process, heats food to a temperature that renders it free of pathogens, spoilage microorganisms and enzyme activity. NASA thermostabilized products include pouched soups, sides, desserts, puddings, and entrees.
2. Irradiated – Irradiation is not typically used to process foods to commercial sterility. However, NASA has received special dispensation from the Food and Drug Administration (FDA) to prepare nine irradiated meat items to commercial sterility (FDA 2009).
3. Rehydratable – Both commercial and internally processed freeze-dried foods are included in the NASA food provisions and then rehydrated during the mission using the potable water supply. Rehydratable foods are typically side dishes, like spicy green beans and cornbread dressing, or cereals.

Ambient and hot water are available to the crew for rehydration of these items.

4. Natural form – Natural-form foods are commercially available, shelf-stable foods. The moisture of the foods may range from low moisture (such as almonds and peanuts) to intermediate moisture (such as brownies and dried fruit), but all have reduced water activity, thus inhibiting microbial growth. These foods help to round out the menu by providing very familiar menu options, additional menu variety, and foods requiring no preparation time.
5. Extended-shelf-life bread products – Items such as scones, waffles, tortillas, and dinner rolls can be formulated and packaged to give them a shelf life of up to 18 months. Like the natural-form foods, breads add to menu variety and address crewmembers' desire for familiarity.
6. Fresh Food – Foods such as fresh fruits and vegetables, which have a short shelf life, are provided on a limited basis, more for psychological support than as a means to meet dietary requirements.
7. Beverages – The beverages currently used on the International Space Station (ISS) and the Space Shuttle are either freeze-dried beverage mixes (such as coffee or tea) or flavored drinks (such as lemonade or orange drink). The drink mixes are weighed and then vacuum sealed inside a beverage pouch. In the case of coffee or tea, sugar or powdered cream can be added to the pouch before sealing. Empty beverage pouches are also provided for drinking water.

While the above system has proven adequate for Space Shuttle and International Space Station missions, the food system has deficiencies for extended missions. NASA has a goal to achieve a manned mission to Mars, a mission lasting approximately 3 years for crew members. For such a mission to be successful, the food system must provide sufficient nutrition while ensuring safety and maintaining palatability. Currently, the spaceflight food system has an average shelf life of 2 years; after that time, nutrient and sensory quality degradation renders the food system inadequate to support mission success. A space habitat food system that involves growing and processing fresh produce *in situ* (bioregenerative foods) and launching payloads of unprocessed grain, legumes, and seeds (bulk foods) is being explored as an avenue to higher quality food. However, these foods could contain a high concentration of microorganisms with respect to current microbiological standards for microbial load of spaceflight foods.

The extended mission length requires mission planners to also consider avenues to proactively support health beyond preparatory training, exercise during the mission, the standard diet, and vitamin D supplements. With mounting evidence of a link between the consumption of probiotic microorganisms and health benefits, including increased immune function and improved gastrointestinal function, the inclusion of probiotics in the spaceflight food system is also being considered. The potential to impact crew member health through functional foods is yet unexplored but highly relevant to risks associated with space travel. As with extraterrestrial food processing, probiotic foods and supplements fall outside of the current microbiological standards for spaceflight.

This review seeks to leverage current research to assess the feasibility of developing a microbially-safe, spaceflight food system that includes extraterrestrial processing and/or probiotics. If deemed feasible, guidance for standards of microbial acceptability and appropriate testing methods for foods/supplements will be developed during the next phase. For simplicity of evaluation, the review examines microbial introduction from fresh food and processing separately from the purposeful addition of probiotics but does conclude with an overall recommendation on high microbial foods in the spaceflight system. Specifically, the review includes:

- An assessment of the expected microbial load from bioregenerative produce and developed cleaning procedures
- An assessment of the current strains and uses of live cultures in shelf stable foods
- A comparison of levels of microbial load in these foods/supplements to current requirements and an assessment of additional risk, if necessary
- An investigation of current literature and commercial operations to provide guidance in establishing microbiological requirements and testing methods for foods with high and diversified microbial concentration

BIOREGENERATIVE FOOD SYSTEM

Crops produced *in situ* and food processing in space have been often discussed in the realm of crew self-sufficiency and long duration space missions. NASA initiated the Controlled Ecological Life-Support System (CELSS) program in 1978 to develop a bioregenerative life support model which could provide basic and continuous habitability requirements such as food, drinking water, and breathable atmosphere by using plants and microbes as the central recycling components. In a bioregenerative food system, vegetables and fruit would be grown on the lunar or Mars surfaces and baseline crops such as soybeans, wheat, rice, peanuts, and dried beans would be grown or launched in bulk from Earth. The baseline crops would then be processed into edible ingredients. The edible ingredients and freshly grown fruits and vegetables would be used to prepare meals in the galley.

A bioregenerative food system for long duration missions is advantageous to crew and mission planners for a plethora of reasons – better food nutrient quality, fresher tasting food, lessened risk of menu fatigue, reduced payload, reduced trash production, ability to recycle atmosphere and water through plant growth, and psychological therapy via farming (Fu and Nelson 1994; Zasypkin and Lee 1999; Monje 2003; Hayashi 2008). However, the bioregenerative system is not without risk. Food insecurity becomes a prominent concern when the diet is dependent upon a specified crop harvest; crops can fail due to inadequate light, air flow, and water conditions, microbial attack, and the inherent challenges of growth in a microgravity or hypogravity environment (Schuerger 1998; Leach 2001; Stutte 2006). An adverse psychological impact to the crew occurs when the plants die prematurely or become infested (Bates 2011). The change from a commercially sterile, prepackaged food supply to a more dynamic stream food supply introduces the risk of microbial contamination of the food supply as well. Because of the chances of microbial interaction with crops and of microbial contamination of the food supply in storage and cooking, the bioregenerative food system is considered a high microbial food system.

Crop Contamination

While limited research has been conducted to determine which microbes have the greatest probability of impacting the food system in space, knowledge from analogs and terrestrial data can be applied to draw conclusions on the potential microbial impact. More than 80% of terrestrial greenhouse epidemics are due to the fungal genera Phytophthora, Pythium and Fusarium, which have been found in life support system test-beds (Fjällman and Hall 2005). Determining the risk to crop production is difficult because pathogenic relationships in space do not necessarily correspond to known Earth relationships. One instance is recorded from Shuttle experimentation where a fungus, *Neotyphodium* endophyte, destroyed wheat plants even though the fungus normally does not host on wheat (Bishop 1997). The microbes noted in the closed International Space Station environment – bacteria belonging to *Staphylococcus* sp. genus and fungi of the *Aspergillus* sp. and *Penicillium* sp. genera – would likely be the same to survive in a closed planetary surface habitat and influence plant growth and food preparation surfaces (Novikova 2006).

Pathogen presence on the crops within an extraterrestrial growth chamber could have significant impact to crop yield, resources required for produce sanitation, and the instances of foodborne illness among the crew. Although some of the common risks of contamination of produce on Earth are eliminated in the controlled environment of space, some risks are magnified. The greatest driver in risk difference is that hydroponic farming involves a closed environment absent of soil, as pictured in Figure 1. Table 1 lists the identified sources of contamination for fruits and vegetables in a space hydroponic system as adapted from Beuchat and Ryu (1997). For instance, wild and domestic animal pathogens are a terrestrial risk of plant growth, but eliminated for space missions because such animals will not be present at the space habitat. On the other hand, insects may be transferred inadvertently to space with some of the other bulk food items, thereby continuing the threat of insect contamination. The air handling system is of greater import since it is a closed environment. Despite these possibilities, the most likely sources of contamination in the bioregenerative food system are contamination of the nutrient solution of the hydroponic growth chambers and human handling. The pathogenic risk of the nutrient solution is especially high if the solution is not adequately controlled due to proposed accumulation of many recycle streams into the nutrient solution. In the NASA experimental profile for NASA/MIR - Greenhouse 1, the bioregenerative system used a microbial reactor that processed urine, feces, inedible plant mass, and grey water for recycle back to the biomass growth (1995). This system is diagrammed for review in Figure 2.

Photo by: FAO/ J.Izquierdo

Photo by: USDA/Scott Bauer

Figure 1. Environmental Differences of Hydroponic Farming and Soil Farming

Table 1. Sources of pathogenic microorganisms on fresh fruits and vegetables (Adapted: Beuchat and Ryu 1997)

Preharvest	Irrigation water	Air (dust)
	Water used to apply fungicides, insecticides	Insects
	Human handling	
Postharvest	Human handling (workers, consumers)	Sorting, packing, cutting, and further processing equipment
	Transport containers	Ice
	Insects	Improper storage (temperature, physical environment)
	Air (dust)	Improper packaging (includes new packaging technologies)
	Wash and rinse water	Cross-contamination (other food in storage, preparation, and display areas)

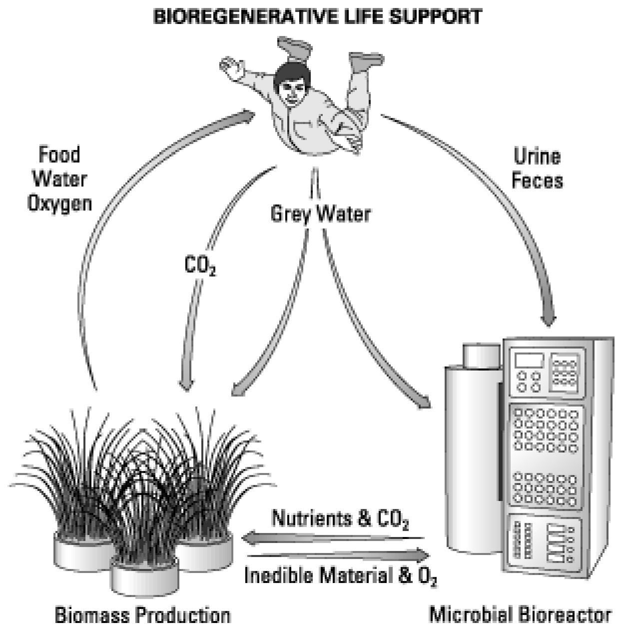

Figure 2. Bioregenerative life support systems represent a likely method for recycling waste and providing food, water, and oxygen on long-duration space flights. (From: NASA 1995)

The probability of contaminated hydroponic crops due to the presence of pathogens in the nutrient supply has been well-documented by researchers attempting to clarify mechanisms by which foodborne illness outbreaks may have occurred. Guo (2002) reported that salmonellae could be transported from an inoculated nutrient solution to the hypocotyls-cotyledons, stems, and leaves of young tomato plants in a hydroponic growth system. Additionally, hydroponically grown radish sprouts have been thought to serve as a vehicle in the transmission of *Escherichia coli* O157:H7 infection of over 6000 schoolchildren in Japan (Hara-Kudo 1997; World Health Organization 1996). The Hepatitis A virus has been shown capable of contaminating green onions grown hydroponically and in soil; research was spurred by a hepatitis A outbreak in November 2003 stemming from green onions (Chancellor 2006). Pathogens have been noted to survive internally in lettuce, spinach, and strawberries as well (Yu 2001; Niemira 2008;

Sharma 2009). Once the pathogens reside within the plants, surface treatments are unable to remove the pathogen.

Bulk Food Contamination

The next consideration of microbial impact to the bioregenerative system is bacterial contamination that could be introduced along with the stowed food from Earth. The foods under consideration for bulk commodity shipment to an extraterrestrial habitat are rice, wheat, soybeans, peanuts, and dried beans. Additionally, a host of dried spices and other dry goods, such as sugar, yeast, and spray-dried, pasteurized eggs, are also planned for stowage to complement a bioregenerative food system. Due to the low water activity of most of these foods, most microorganisms cannot effectively utilize these substrates for growth and survival. Molds are the primary microorganism of concern for the dry goods because molds can grow at reduced water activity levels. Molds tend to change the flavor and aroma of foods so sensory detection prior to ingestion is often possible. Of greater concern is the potential production of mycotoxins, and specifically aflatoxin, by fungi within the food which is not detected prior to consumption.

The baseline crops, if launched from Earth, would be minimally processed prior to shipment, except for any irradiative treatment or specialized packaging to add to the food stability. Environmentally-controlled storage may also be required to ensure the quality of some of the stowed ingredients throughout the five years. Table 2 shows the proven stabilization conditions – processing and/or storage - that ensure functional and organoleptic quality for many of the proposed baseline crops. Most of the stabilization conditions do not incorporate a microbial kill step. Consequently, control of pathogen levels becomes paramount to maintaining the food supply. Similar to current NASA food safety procedures, bulk foods can be tested for microbial levels prior to final packaging and storage. Larger food quantities will drive a more complex sampling plan but the risk of bacterial contamination from the ground is minimized with these controls. Alternatively, technologies that maintain food functionality and stability while allowing for elimination of bacteria should be investigated.

Table 2. Methods to Ensure Long-Term Storage of Bulk Food Ingredients

Bulk Commodity	***Stabilization Conditions***	***Aim of Mitigation***	***Shelf Life***	***Reference***
Rice	Sealed hermetically in #10 cans, flushed with N_2.	Oxidation, infestation	30 y	Coons and others (2004)
Wheat Berries	~ 10-12% moisture and dry, unheated storage	Wheat enzyme activities and microbiological, mainly mold, spoilage	25 y	Fellers and Bean (1977)
Wheat Flour	Irradiate w/ 1 kGy	Insect infestation	2 y	Pixton and others (1976) Lorenz and Miller (1975)
Whole Wheat Flour	Irradiate w/ 1 kGy	Insect infestation	6 mo	Marathe and others (2002)
Dried Beans/ Lentils	Refrigerated hypobaric conditions (4.5°C, 50-60% RH, pressure of 125 mm Hg)	Hard-to-Cook (HTC) phenomena	2 y	Berrios and others (1999)
Dried Soybeans	< 12% moisture, stored below 15°C and 50% RH	Oxidation,	5 y	Shurtleff and Aoyagi (1979)
Peanuts (in-shell)	Dry to 10% moisture, store at 0°C in moisture barrier packaging	Mold development, insect infestation, oxidation	24 mo	Shewfelt and Young (1977)
Seeds (most species)	None	N/A	4 y	Priestly (1986)
	Storage at low temperature and humidity	Deteriorative chemical reactions	50 -100 y	Roos and Davidson (1992)

Food processing and human handling

Most terrestrial foodborne illnesses result from mistakes at the processing facility or in the preparers' kitchen, not with the ingredients themselves. McCabe-Sellers and Beattie reported that the top five contributing factors of recognized foodborne illness in the United States from 1993 – 1997, listed high frequency to low frequency, were improper holding of hot or cold food, poor personal hygiene, cross contamination, inadequate cooking, and unsafe food source (2004). The translation of these factors to a space habitat is primarily affected by the absence of raw animal products and constraints on water and power usage.

The absence of raw meat and unpasteurized egg and dairy products in postulated space habitat food systems decreases the risk that cross contamination or inadequate cooking will result in foodborne illness of the crew. Likewise, the most likely impact of improper holding temperature of prepared foods is sensory unacceptability, not microbial growth. The limited raw food supply is advantageous to the crew in this regard.

The resource constraints of a space habitat (water, power, living volume) increase the risk that foodborne illness will result from the ingredient processing, food preparation, or human handling interactions. For example, Calcivirus, the viral family that includes Norovirus, is the leading cause of gastroenteritis in the United States is spread by the fecal-oral route of transmission and has demonstrated remarkable virulence on cruise ships due to the contained environment (McCabe-Sellers and Beattie 2004). Calcivirus can be transmitted via contaminated surfaces, hands, people, food, and water supply. Limited resources impact the ability to proactively control microbial populations in closed environments.

- Water will likely be the most demanded consumable resource during a habitat mission. Without adequate hot water for hygiene, dish washing, and food preparation, the probability of spreading Calcivirus and other viruses increases. In the FMARS2007 study – an Antarctica-based Mars analog at the Flashline Mars Arctic Research Station, crew members showered only once per week and used hand sanitizer in lieu of hand washing to conserve water (Bamsey 2009). The water conservation methods seen during FMARS2007 could prove dangerous to a real crew, as studies have shown alcohol-based sanitizer to be ineffective against Calcivirus populations (Liu 2010).
- Power is required to keep hot foods hot, to keep cold foods cold, and to create hot water. In a bioregenerative system, where multiple menu items are being prepared in a small galley area, the need to warm foods for an hour or more while a complementary dish is being prepared is probable. The food warming time is extended even longer if the crew eats at staggered meal times due to mission responsibilities. Maintaining the correct food temperature will require power inputs. Hot water and, in some cases, steam, are required for food processing equipment and dish sanitation; hot water is required for hand sanitation. If power usage becomes a concern during the mission, power used during food preparation may be limited, thereby increasing risk of foodborne illness.

Testing Procedures

The current evidence indicates a clear potential for microbial contamination associated with crop production, the use of bulk foods, and their processing and handling. However, to fully elucidate these microbial risks, the contamination potential must be put in context with established methods to mitigate the risk. Thus, the final consideration of microbial impact to the bioregenerative system is the process to ensure the safety of the food system of a remote habitat. A variety of methodologies and practices are utilized to promote and verify the microbial safety of terrestrial food systems or food production facilities from farm to table. Notermans (1997) identified the conventional and rapid methods for microbial evaluation of food systems as shown in Table 3 below. A similar safety program would need to be implemented for an extraterrestrial bioregenerative food system.

Table 3. Conventional and Rapid Microbiological Methods in Safe Food Production

Use of microbiological methods	Relative Importance [a]	Most Suited Methods	
		Conventional [b]	Rapid and Automated [c]
Safe food production			
Monitoring and surveillance			
Detection of pathogens	-	-	++
Detection of indicator organisms	+	+	-
Detection of bacterial toxins	-	-	++
Storage Tests	++	+	+
MCT (microbial challenge testing)	++	+	+
Predictive models			
Performance testing	+	+	+
Mathematical models	-	-	-
Management of safe food production			
GMP [d]	+	++	++
HAACP [e]			
Hazard analysis	+	+	-
Identification of critical control points	±	+	-
Monitoring	-	-	-
Verification	-	-	-
Failure analysis	+	++	-
Food borne disorders			
Testing of reported outbreaks	+	++	-
Sentinel studies	++	-	++
Risk assessment studies	++	++	+

[a] Necessity and convenience of a microbiological technique for obtaining reliable and/or applicable results

[b] Methods based on enumeration of organisms, such as determination of colony-forming particles, and methods allowing organisms to be obtained in a pure state for a further characterization. Conventional methods for detecting bacterial toxins are those using animal models.
[c] Methods that detect organisms on the basis of production of metabolic products or compounds. Such methods for bacterial toxins use a direct test system for the toxin itself.
[d] GMP (Good Manufacturing Practice)
[e] HAACP (Hazard Analysis Critical Control Point) a systematic approach to food safety that identifies and mitigates the biological, chemical, and physical process hazards of a given production cycle.

PROBIOTICS

Probiotics are defined as live organisms that, when ingested in adequate amounts, confer a benefit to the host (FAO and WHO 2002). Probiotics have a long history that dates back to ancient times, particularly the consumption of fermented milk and lactic acid bacteria for health. Medical historians cite both a Persian version of the Old Testament that describes Abraham consuming sour milk, and Roman historian Plinius' recommendation of fermented milk for gastroenteritis as early as 76 BC (Schrezenmeir and de Vrese 2001). As interest in alternative medical therapies has grown, interest in probiotics in general has also grown; Americans' spending on probiotic supplements, for example, nearly tripled from 1994 to 2003 (NCCAM 2008).

The class of microorganisms recognized as probiotics in humans is relatively small. Almost exclusively, the bacteria come from two groups, *Lactobacillus* or *Bifidobacterium*, though *Saccharomyces boulardii*, a yeast, is also considered a probiotic (NCCAM 2008). The criteria for a classification as a probiotic, according to the Food and Agricultural Organization (FAO) and World Health Organization (WHO) (2002), is as follows:

1. The genus and species of the strain must be identifiable.
2. The strain must have proven health efficacy *in vivo.*
3. The strain must be safe for consumption and without contamination in its delivery form.

Research on the benefits of probiotics has increased dramatically over the decade, especially in the last 5 years. There is a wide-range of benefits that have been identified and are currently being validated in double-blind placebo trials. As research progresses, it seems that the benefits are dependent on the specific strains of probiotic bacteria used. A short list of validated human health benefits from specific probiotic formulations is shown below (Gill 2001; Clancy 2005; NCCAM 2008; Baron 2009; Johannes 2009; Fitzpatrick 2010):

- **Immune stimulatory effects:**
  - **Increase immune response during viral challenge**
  - **Preliminary indications of reversal of decreased T-cell cytokine (IFNγ) secretion during stress/fatigue**
  - **Enhanced Natural Killer Cell Activity**
- Prevention and/or reduction of duration and symptoms of rotavirus-induced diarrhea
- Prevention and treatment of antibiotic-associated diarrhea
- Normalization of stool consistency and frequency in subjects suffering from irritable colon
- Reduction of the concentration of cancer-promoting enzymes and/or putrefactive (bacterial) metabolites in the gut
- Reduction in recurrence of bladder cancer
- Prevention and treatment of infections of the urinary tract or female genital tract
- Prevention of respiratory tract infections (common cold, influenza) and other infectious diseases
- Prevention and alleviation of unspecified problems of the gastrointestinal tract

The immune stimulatory effects are of particular importance to space-flight, since there has been documented evidence of viral reactivation and decreased T-cell activity during spaceflight (Cohrs 2008; Crucian 2008; Pierson 2005).

Probiotics are available for human consumption as a dietary supplement, within foods as a naturally occurring component, and within foods as a supplemental component. However, probiotics cannot be added arbitrarily to food. For probiotic foods, the choice of delivery vehicle has significant impact to functionality, inducing changes in the cell composition and physiological status of the probiotics and providing complementary physiologically active agents such as fiber or fermentation end-products (Sanders and Marco 2010). Additionally, the food substrate has to support the proliferation of the microbes at a prescribed level for the duration of the food shelf life and the microbes must be able to survive and withstand the processing and storage conditions. Figure 3, from Sanders and Marco (2010), summarizes the three realms of consideration to sustaining and influencing probiotics physiology.

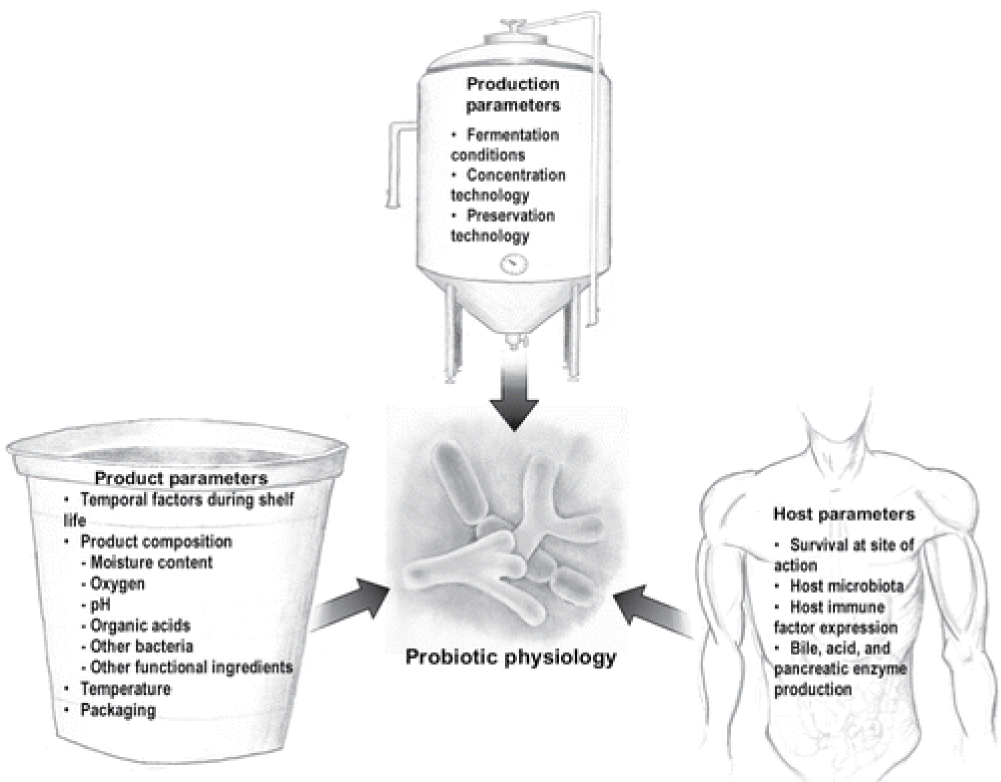

Figure 3. Factors that influence probiotic physiology (Sanders and Marco 2010).

The current options for probiotic carriers are limited. Dairy substrates, such as yogurt, milk, and cheese, are the most common probiotics carriers. This position is largely due to consumer acceptance of dairy products as healthy and natural carriers of living bacteria but bovine milk does meet the standards of good probiotics substrates by offering complete nutrition via macronutrients and micronutrients along with inherent bioactive compounds (Sanders and Marco 2010). The milk products mainly require refrigerated storage and have a limited shelf life as compared to commercially sterile items. Other carriers with documented efficacy are Nestle Good Start Protect PLUS infant formula and Attune candy bars. The Attune candy bars still require refrigeration but the infant formula is shelf stable. Kashi Vive Probiotic Digestive Wellness Cereal included lactic acid probiotic bacteria in a shelf-stable blend of graham twigs, whole grain flakes, and vanilla-dipped rice crisps. Ganedan Labs has extended probiotics into shelf stable formats with their marketing of a spore-forming bacteria GanedanBC[30], or *Bacillus coagulans* GBI-30, 6086. Because *B. coagulans* is resistant to heat, drying, and freezing processes, it has been successfully incorporated into baked goods, granola bars, and even tea pouches (Durkee 2010). Appendix A shows the range of products that commercial manufacturers are utilizing for probiotics delivery and captures both capsules and supplemented foods.

The Codex Alimentarius Standard for yogurt developed by the FAO/WHO states that the microorganism for which the product is labeled for must have a minimum of at least 1 million CFU per gram to be considered yogurt (FAO/WHO 2003). Using this amount of probiotic organism as the standard to develop non-yogurt products, such as the shelf-stable products listed in Appendix A, it is apparent that this amount of a probiotic microorganism does exceed the microbiology acceptability limits for spaceflight food as described in table 4, even though it may yield a beneficial effect. Spaceflight food requirements would have to be tailored to allow for probiotic organisms if such food were to be deemed feasible.

Table 4: Microbiology Acceptability Limits for Spaceflight Food

(JSC 16888, Microbiology Operations Plan for Space Flight)

Factor	***Limits***
Total aerobic count	20,000 CFU/g for any single sample (or if any two samples from a lot exceed 10,000 CFU/g)
Coliform	100 CFU/g for any single sample (or if any two samples from a lot exceed 10 CFU/g)
Coagulase positive *Staphylococci*	100 CFU/g for any single sample (or if any two samples from a lot exceed 10 CFU/g)

Salmonella	0 CFU/g for any single sample
Yeasts and molds	1000 CFU/g for any single sample (or if any two samples from a lot exceed 100 CFU/g or if any two samples from a lot exceed 10 CFU/g *Aspergillis flavus*)

Probiotic Safety and Risk

The Joint FAO of the United Nations and WHO working group (2002) defined a probiotic to be 'live microorganisms which when administered in adequate amounts confer a health benefit on the host'. Several microorganisms that are used as probiotics or in probiotic formulations are on the Food and Drug Administration (FDA) Generally Recognized as Safe (GRAS) list. (Table 5)

Table 5: Common probiotic microorganisms listed on the U.S. Food and Drug Administration's Generally Recognized as Safe (GRAS) list

Probiotic Microorganism	**GRAS list number**	**Intended Use**
Bacillus coagulans	GRN 378 (Pending)	Antimicrobial in food
Bifidobacterium animalis subsp. *lactis* strain Bf-6	GRN 377	Ingredient in food
Bifidobacterium lactis Bb-12	GRN No. 49	Ingredient in food
Bifidobacterium longum strain BB536	GRN 268	Probiotic in food
Lactobacillus acidophilus	GRN 378 (Pending)	Antimicrobial in food
Lactobacillus acidophilus NCFM	GRN No. 357	Ingredient in food
Lactobacillus bulgaricus	GRN 378 (Pending)	Antimicrobial in food
Lactobacillus paracasei subsp. *paracasei*	GRN 378 (Pending)	Antimicrobial in food
Lactobacillus plantarum	GRN 378 (Pending)	Antimicrobial in food
Lactobacillus reuteri DSM 17938	GRN 254	Probiotic in food
Lactobacillus rhamnosus HN001 (DR20)	GRN 281	Probiotic in food
Lactobacillus rhamnosus GG (LGG)	GRN No. 231	Ingredient in food
Proprionibacterium freundenreichii subsp. *shermanii*	GRN 378 (Pending)	Antimicrobial in food
Streptococcus themophilus strain Th4	GRN No. 49	Ingredient in food

The FAO and WHO developed guidelines for assessing probiotic safety and identified 4 potential areas of risk: systemic infections, deleterious metabolic activities, excessive immune stimulation in susceptible individuals, and gene transfer (FAO/WHO 2002). There has been some documentation of health risks from the continued use of probiotics in special populations. Probiotics have been linked to a risk of sepsis, both in animal studies and humans (Wagner 1997, Land 2005). An overview of documented isolated cases are provided by Boyle (Boyle 2006) presented in Table 6 below. These risks will need to be considered when assessing the safety of using probiotics in-flight since the scientific community is still determining the long-term effect of microgravity on the human immune system.

Table 6. Human Cases of Sepsis Related to Probiotics Consumption
(Boyle and others 2006)

Cases of bacterial sepsis temporally related to probiotic use in humans[1]

Study	Age	Risk factors	Probiotic	Method of identification[2]	Form of sepsis
Rautio et al (24)	74 y	Diabetes mellitus	LGG	API 50 CH, PFGE of DNA restriction fragments	Liver abscess
Mackay et al (25)	67 y	Mitral regurgitation, dental extraction	*Lactobacillus rhamnosus,* 3 x10^9 CFU/d	API 50 CH, pyrolysis mass spectrometry	Endocarditis
Kunz et al (26)	3 mo	Prematurity, short-gut syndrome	LGG	No confirmatory typing	Bacteremia
	10 wk	Prematurity, inflamed intestine, short-gut syndrome	LGG	PFGE of DNA restriction fragments	Bacteremia
De Groote et al (27)	11 mo	Prematurity, gastrostomy, short-gut syndrome, CVC, parenteral nutrition, rotavirus diarrhea	LGG, 1/4 capsule/d	rRNA sequencing	Bacteremia
Land et al (28)	4 mo	Cardiac surgery, antibiotic diarrhea	LGG, 10^{10} CFU/d	Repetitive element sequence-based PCR DNA fingerprinting	Endocarditis
	6 y	Cerebral palsy, jejunostomy feeding, CVC, antibiotic-associated diarrhea	LGG, 10^{10} CFU/d	Repetitive element sequence-based PCR DNA fingerprinting	Bacteremia
Richard et al (29)	47 y	Not stated	*Bacillus subtilis,* 8 x10^9 spores/d	Antibiotic susceptibility	Bacteremia
	25 y	Not stated	*B. subtilis,* 8 x10^9 spores/d	Antibiotic susceptibility	Bacteremia
	63 y	Neoplastic disease	*B. subtilis,* 8 x10^9 spores/d	Antibiotic susceptibility	Bacteremia
	79 y	Not stated	*B. subtilis,* 8 x10^9 spores/d	Antibiotic susceptibility	Bacteremia
Oggioni et al (30, 31)[3]	73 y	Chronic lymphocytic leukemia	*B. subtilis,* 10^9 spores/d	16S rRNA sequencing	Bacteremia

[1] Where no dose is given, there was no precise dose described in the original publication. CVC, central venous catheter; rRNA,

ribosomal RNA; PFGE, pulsed-field gel electrophoresis; PCR, polymerase chain reaction; LGG, *Lactobacillus rhamnosus* GG; CFU, colony forming units.
[2] API 50 CH; BioMerieux, Hazelwood, MI.
[3] Fatal outcome not clearly related to probiotic sepsis.

In addition to validating the benefits of probiotics, several studies are being performed to evaluate toxicity of commercial probiotic strains. Several animal studies have been performed evaluating their continued use (90 days and 1 year) and bacterial concentration (Enders 2009, Enders 2011). Future studies should be evaluated to determine the probiotic formulation that has the potential greatest benefit with minimal side effects.

Probiotic Detection

If probiotics are added to the space food system, a method of verification will be required to ensure that only the declared probiotics are added to the substrate and that the microbial load corresponds to the intended level. Bacterial verification will include both culture based methods and genetic identification to ensure accurate bacterial concentration and species are added to the food. The exact methods will be determined once the ideal probiotic formulation(s) have been selected. Appendix B gives additional details about probiotic formulation and detection.

CONCLUSION

This review indicates current strategies for bioregenerative food systems could result in high microbial concentrations associated with crop production, the use of bulk foods, and subsequent processing and handling. While preventive measures commonly address this concern in terrestrial food systems, the specific requirements for the unique systems and limitations of spaceflight food systems on long duration human spaceflight missions has not been sufficiently studied. The evidence from this review indicate a need for further research to determine the procedures necessary for growing and processing produce in space and development of specific cleaning methods to mitigate microbiological risks. In addition, data mining during this review identified potential benefits for the use of food products with high concentrations of beneficial microorganisms. In particular, the documented benefits to the immune system could be advantageous for spaceflight and further research is recommended. This review has performed an extensive market survey to generate a comprehensive list of potential probiotic products (Appendix A), which should be evaluated in future research to determine preferred probiotic formulations for spaceflight. Future work would also determine the specific microbial testing procedures for those formulations and assess the risk of adding the formulation to the food system.

Based upon the evidence of this data mining research, immediate forward work for this study would include (1) the investigation of the survival of supplemented microorganism populations after freeze drying carrier foods, after thermostabilization of carrier foods, and after addition to shelf-stable, microbe-friendly carriers (such as chocolate) and (2) the determination of the corresponding stability of "beneficial" or "benign" microorganisms over time. Additional future research topics may include:

- Bioregenerative Life Support System Testing:
  - Determine microbial safety procedures for hydroponic produce
    - Identify nondestructive method of internal flesh and surface microbial load assessment
    - Determine nutrient solution requirements for feed into hydroponic system
    - Develop produce treatment procedures which reduce microbial load while being compatible with other components of the life support system (i.e. bound enzymes, digesters, etc.)

- Probiotic Product Testing:
  - Determine the viability and/or shelf-life of probiotic products
    - Flight experiment with ground-based controls
    - Shelf-life extension to determine the long-term viability of probiotic products
  - Perform a survey of shelf-stable products for characterization and enumeration of probiotic bacterial components
  - Cell culture based assays to measure immune function *in vitro*

  - Modeled GI tract microbial consortium response to the addition of probiotic cultures

- Interdisciplinary studies evaluating effects of probiotics

- Probiotic Human-Based Tasks
  - Investigation of the impact of probiotics and/or hydroponically grown foods on the intestinal contents of bed rest participants (bacterial diversity and respective concentrations)
  - Perform a crew survey to identify probiotic candidates for further investigation as to their potential to be spaceflight foods
  - Evaluate probiotic effects on immune function during spaceflight

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Appendix A: Commercial Survey of Probiotics as of March 2011

PRODUCT NAME	BRAND/MFR	PROBIOTIC BACTERIA	BACTERIAL COUNT	FORM
ACIDOPHILUS PEARLS	Enzymatic Therapy	*Lactobacillus acidophilus and Bifidobacterium longum(6 "pearl" types to choose from, some have additional bacteria)*		capsule-like "pearl"
ACTIMEL/DANACTIVE	Dannon /Danone	*Lactobacillus casei* Defensis	10 billion	food - needs refrigeration
ACTIMINT	Ecobrands, Ltd	*Lactobacillus acidophilus* L10	150 million live	food - mint, shelf stable
ACTIV8 PROBIOTIC ORGANIC CRUNCH BAR	Cascade Fresh		5.5 CFU	food - bar, needs refrigeration
ACTIVE PROBIOTIC CEREAL	HEB	*limited info on website - may be discontinued*		food - cereal, shelf stable
ACTIVIA	Dannon USA	*website down*		
ALIGN	P&G	*Bifidobacterium infantis* 35624	1 billion	capsule
ALIXIR REGULARIS BISCOTTO CON PROBIOTICI VIVI	Barilla Italia	*Lactobacillus acidophilus, Bifidobacterium longum*	10 billion CFU	food - shelf stable
ATTUNE BARS	Attune	*Lactobacillus casei* Lc-11, *Lactobacillus acidophilus* NCFM, *Lactobacillus fermentum, Bifidobacterium lactis* HN019	Live Active	food - needs refrigeration
BIO BEADS ACIDOPHILUS COMPLEX	Natrol	*Lactobacillus acidophilus blend*	2.5 billion	capsule-like "BioBeads"
BIOGAIA PROBIOTIC DROPS	BioGaia	*Lactobacillus reuteri* protectis	100 million	supplement - drops - needs refrigeration
BIOGAIA PROBIOTIC STRAWS	BioGaia	*Lactobacillus reuteri* protectis	100 million	straw - shelf stable
BIOGAIA PROBIOTIC TABLETS	BioGaia	*Lactobacillus reuteri* protectis	100 million	capsule - chewable - shelf stable
BOOST KID ESSENTIALS	Nestle Nutrition	*Lactobacillus reuteri* protectis	100 million/straw	straw
CHEWABLE PROBIOTIC TABLET	American Health	*Lactobacillus acidophilus, Lactobacillus bifidus*	1 billion @ time of manufacture	capsule - shelf stable
CULTURELLE FOR KIDS	Amerifit	*Lactobacillus rhamnosus* GG	1 billion cells	supplement - beverage powder, shelf stable
CULTURELLE W LACTOBACILLUS GG	Kirkman Labs	*Lactobacillus rhamnosus* GG		capsule

PRODUCT NAME	BRAND/MFR	PROBIOTIC BACTERIA	BACTERIAL COUNT	FORM
DANACTIVE	Dannon USA	*Lactobacillus bulgaricus, Lactobacillus casei* Immunitas		food - needs refrigeration
DHA AND PROBIOTIC BABY CEREAL - OATMEAL and RICE	Gerber/Nestle	*Bifidobacterium lactis,* BIFIDUS BL (TM)		food - instant cereal for babies, to be mixed with milk/water, shelf stable
DIELAC PEDIA	Vinamilk	*Bifidobacterium* BB-12, *Lactobacillus rhamnosus* GG		food - beverage, shelf stable
FIVELAC	Global Health Trax	*Bacillus coagulans, Bacillus subtilis,Enterococcus faecalis, Lactobacillus acidophilus, Bifidobacterium longum*	2 billion	packet - put into water
FREEZE DRIED KEFIR STARTER	Lifeway	*Lactobacillius lactis , Lactobacillus rhamnosus Streptococcus diacetylactis, Lactobacillus plantarm, Lactobacillius casei, Saccharomyces florentinus, Leuconostoc cremoris, Bifidobacterium longum, Bifidobacterium breve Lactobacillus acidophilus, Bifidobacterium lactis* HN019, *Lactobacillus acidophilus* NCFM	7-10 billion CFU, live and active	food - freeze dried powder, to be combined with milk, no refrigeration needed
FREEZE DRIED YOGURT STARTER	YoGourmet	*Lactobacillus bulgaricus, , Lactobacillus acidophilus*		food - freeze dried powder, to be combined with milk, no refrigeration needed
GLUTAPAK REUTERI	Victus	*Lactobacillus reuteri* Protectis	100 million	food - powdered beverage, shelf stable
GOODBELLY TOGO, PROBIOTIC JUICE DRINK POWDER	GoodBelly	*Lactobacillus plantarum* 299v, *Lactobacillus acidophilus, Bifidobacterium lactis* Bi-07	20 billion at time of manufacture	food - powdered beverage or supplement, shelf stable
GRADUATES YOGURT MELTS	Gerber/Nestle	*Emailed company for info (Bifidobacterium lactis)*	Live Active	food - freeze dried, shelf stable
GUM PERIOBALANCE	Sunstar	*Lactobacillus reuteri* Protectis		food - chewing gum, shelf stable
HAPPYMELTS	HappyBaby	*Lactobacillus bulgaricus, Lactobacillus acidophilus, Bifidobacterium lactis, Lactobacillus paracasei, Lactobacillus rhamnosus*		food - shelf stable

PRODUCT NAME	BRAND/MFR	PROBIOTIC BACTERIA	BACTERIAL COUNT	FORM
HIGH CULTURE FROZEN YOGURT	YOCREAM	*Lactobacillus acidophilus* NCFM, *S thermophilus, Lactobacillus bulgaricus, Lactobacillus lactis and Lactobacillus acidophilus*	1 billion	food - frozen yogurt, stable at frozen temps
HSO PROBIOTIC POWDER	Garden of Life	*Lactobacillus plantarum,* Bifidobacterium *lactis,* Bifidobacterium *bifidum, Lactobacillus rhamnosus,* Bifidobacterium *breve, Lactobacillus casei, Bacillus subtilis, Lactobacillus brevis, Lactobacillus. salivarius, Lactobacillus acidophilus, Lactobacillus paracasei,* Bifidobacterium *longum*		supplement - beverage powder, shelf stable
IFLORA MULTI PROBIOTIC CAPSULES	Sedona Labs	*Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium lactis* (Infantis), *Bifidobacterium lactis* HN019, *Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus gasseri, Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus salivarius, Lactococcus lactis*	20 billion	Capsule, contains fructooligosaccharides
JARRO-DOPHILUS	Jarrow Formulas	*Lactobacillus rhamnosus* R0011, *Pediococcus acidilactici* R1001, *Bifidobacterium longum* BB536 (Morinaga strain), *Bifidobacterium breve* R0070, *Lactobacillus acidophilus* R0052, *Lactobacillus casei* R0215, *Lactobacillus plantarum* R1012, *Lactococcus*	5 billion	capsule - shelf stable
KEFIR BAR	Lifeway	*Lactobacillius lactis , Lactobacillus rhamnosus, Streptococcus diacetylactis, Lactobacillus plantarm, Lactobacillius casei, Saccharomyces florentinus, Leuconostoc cremoris, Bifidobacterium longum, Bifidobacterium breve, Lactobacillus acidophilus, Bifidobacterium lactis* HN019, *Lactobacillus acidophilus* NCFM	7-10 billion, live active?	food - bar, shelf stable

PRODUCT NAME	BRAND/MFR	PROBIOTIC BACTERIA	BACTERIAL COUNT	FORM
LATERO-FLORA PROBIOTIC GOOD BACKTERIA COLON HEALTH	O'Donnell Formulas, INC	*Bacillus laterosporus*		capsule
LEMON GINGER HERB TEA PLUS PROBIOTIC	Bigelow	GanedenBC30™ *(Bacillus coagulans* GBI-30, 6086),		food - tea bag, shelf stable
LIVING FOODS BAR	Garden of Life	*Lactobacillus acidophilus, Lactobacillus rhamnosus* (LGG), *Bifidobacterium longum*	Live Active?	food - bar, shelf stable, with oat beta glucan
MAX CRUNCH BAR	Max Muscle Sports Nutrition	*Bacillus coagulans* GBI-30 6086	250 million	food - shelf stable
MARAMOR PREMIUM DARK AND MILK CHOCOLATE	MARAMOR CHOCALATES	*Lactobacillus helveticus R0052 Bifidobacterium longum R0175*	1 billion	Chocolate bar – shelf stable
MOJOMILK	Abunda Functional Foods	GanedenBC30™ (*Bacillus coagulans* GBI-30, 6086)	2 billion	food - beverage powder, shelf stable
NATREN HEALTHY TRINITY	Natren	*Lactobacillus acidophilus Bifidobacterium bifidum*, and *Lactobacillus bulgaricus*	30 billion	capsule
NATURE'S SECRET DIGESTIVE BLISS PROBIOTIC	Nature's Secret	*Lactobacillus acidophilus, Bifidobacterium bifidum, Lactobacillus rhamnosus, Lactobacillus salivarius, Lactobacillus helveticus, Bifidobacterium infantis, Bifidobacterium lactis, Bifidobacterium longum, Enterococcus faecium, Lactobacillus brevis, Lactobacillus casei, Lactobacillus fermentum Lactobacillus keferi Lactobacillus paracasei, Streptococcus thermophilus, Lactobacillus reuteri, Lactococcus lactis sp. lactis*	4 billion CFU, patented capsule delivery system	Capsule, *Contains FOS (Fructooligosaccharides)*
NEVELLA PRO	Heartland Sweeteners	*Bacillus coagulans GBI-30 6086*	62.5 million CFU/tsp	food - no calorie sweetener, shelf stable
ORGANIC BABY CEREAL - OATMEAL	HappyBellies	*Lactobacillus acidophilus, Bifidobacterium lactis, Lactobacillus salivarus, Lactobacillus plantarum*		food - instant cereal for babies, to be mixed with milk/water, shelf stable
ORGANIC BABY CEREAL -BROWN RICE	HappyBellies	*Lactobacillus acidophilus, Bifidobacterium lactis, Lactobacillus salivarus, Lactobacillus plantarum*		food - instant cereal for babies, to be mixed with milk/water, shelf stable

PRODUCT NAME	BRAND/MFR	PROBIOTIC BACTERIA	BACTERIAL COUNT	FORM
ORGANIC BABY CEREAL -MULTI GRAIN	HappyBellies	*Lactobacillus acidophilus, Bifidobacterium lactis, Lactobacillus bulgaricus, Lactobacillus casei, Streptococcus thermophilus, and Lactobacillus rhamnosus*		food - instant cereal for babies, to be mixed with milk/water, shelf stable
ORGANIC YOGURT SNACKS	HappyBellies	*Lactobacillus bulgaricus, Lactobacillus acidophilus, Bifidobacterium lactis, Lactobacillus paracasei, Lactobacillus rhamnosus*		food - freeze dried yogurt and fruit snacks, shelf stable
PHILLIPS COLON HEALTH	Bayer	*Lactobacillus gasseri, Bifidobacterium bifidum, Bifidobacterium longum*	1.5 billion cells	tablet
PIZZA CRUST, BREAD STICKS	Naked Pizza Co	GanedenBC30™ *(Bacillus coagulans* GBI-30, 6086),		
PROBIOKID	Institute Rosell	*Lactobacillus helveticus* Rosell-52, *Bifidobacterium longum,* Rosell-71, *Bifidobacterium* Rosell-33		supplement - beverage powder, appears to be shelf stable
PROBIO'STICK	Institute Rosell	*Bifidobacterium longum* Rosell-175 & *Lactobacillus helveticus* Rosell-52		food - bar, appears to be shelf stable, microorganisms microencapsulated with Probiocap® technology
PROBIOTIC ACIDOPHILUS	Accuflora	*Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, Lactobacillus salivarius,* and *Streptococcus thermophilus*	500 million	capsule - enteric coated
PROBIOTIC DEFENSE POWDER	NOW (nutrition for optimal wellness)	*Lactobacillus acidophilus. Bifidobacterium lactis, Lactobacillus brevis, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus salivarius, Lactobacillus paracasei, Lactobacillus bulgaricus, Bifidobacterium bifidum, Bifidobacterium brevis, Bifidobacterium longum, and Saccharomyces boulardil*	1 billion	supplement - beverage powder, shelf stable
PROBIOTIC GRANOLA MUNCH	Attune Foods	*Lactobacillus acidophilus (HOWARU™ blend)*		food- likely needs refrigeration
PROBIOTIC TORTILLAS	El Lago			
PROBIOTICS FOR KIDS	Garden of Life	*Lactobacillus gasseri, Lactobacillus plantarum, Bifidobacterium lactis,*	5 billion CFU @ end of Best Use	supplement - beverage powder, shelf stable

PRODUCT NAME	BRAND/MFR	PROBIOTIC BACTERIA	BACTERIAL COUNT	FORM
		Lactobacillus casei, Lactobacillus acidophilus	Date	
PROBUGS KEFIR	Lifeway	*Lactobacillius lactis , Lactobacillus rhamnosus Streptococcus diacetylactis, Lactobacillus plantarm, Lactobacillius casei, Saccharomyces florentinus, Leuconostoc cremoris, Bifidobacterium longum, Bifidobacterium breve Lactobacillus acidophilus, Bifidobacterium lactis* HN019, *Lactobacillus acidophilus* NCFM	7-10 billion CFU, live and active	food - beverage, needs refrigeration
PROTECT PLUS PROBIOTIC BABY FORMULA	Gerber/Nestle	*Bifidobacterium lactis,* BIFIDUS BL (TM)		food - instant formula mix for babies, shelf stable
PROVIVA SHOT!	ProViva AB	*Lactobacillus plantarum* 299v	250 million/ml	food - beverage, needs refrigeration
RAW MEAL REPLACEMENT	Garden of Life	*Bacillus coagulans, Saccharomyces cerevisiae, Lactobacillus bulgaricus*	35 billion CFU	supplement - beverage powder, shelf stable
RAW PROTEIN SUPPLEMENT	Garden of Life	*Bacillus subtilis, Saccharomyces cerevisiae, Lactobacillus bulgaricus*		supplement - beverage powder, shelf stable
SHAKTI SYNBIOTIC	Shakti/Ubernatural	*Complete Lactobacillus Cultures - Lactobacillus acidophilus, Lactobacillus delbruekii, Lactobacillus caseii, Lactobacillus bulgaricus, Lactobacillus causasicus, Lactobacillus fermenti, Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus helveticus, Lactobacillus lactis, Bifidabacterium bifidum, Lactobacillus leichmannii. Beneficial Yeasts - Saccharomyces Cerevisiae and Saccharomyces Boullardii*		food - powdered beverage or supplement, shelf stable
SUSTENEX W GANEDEN BC 30 PROBIOTIC		*Bacillus coagulans*	2 billion viable cells with enteric coating	capsule

PRODUCT NAME	BRAND/MFR	PROBIOTIC BACTERIA	BACTERIAL COUNT	FORM
ULTIMATE PROBIOTIC FORMULA	Lee Swanson	*Lactobacillus acidophilus, Bifidobacterium lactis, Bifidobacterium longum, Bifidobacterium bifidum, Lactobacillus rhamnosus, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus salivarius, Lactobacillus bulgaricus, Lactobacillus sporogenes*	66 billion	capsule
YAKULT	Yakult	*Lactobacillus casei* Shirota	100 million CFU/ml	food - needs refrigeration
YOMI PROBIOTIC CHOCOLATE YOGURT VITAMINS	Anlit	*Lactobacillus acidophilus and Bifidobacterium bifidum*	400 million CFU	supplement - chocolates, shelf stable
YO-PLUS YOGURT	Yoplait	*Lactobacillus bulgaricus, Streptococcus thermophilus, Bifidobacterium* Bb-12		food- requires refrigeration

Appendix B: Additional detail on probiotic formulation and detection.

There are various specific methods available for the production of probiotic foods. Generally, dairy foods such as yogurt are cultured in a two-step process using and initial starter culture to produce the desired effect (acidification, texture, flavor, etc.) that may or may not be a designated probiotic organism. After the initial fermentation of the food product, probiotic cultures, such as lactobacilli or bifidobacteria, are added. There are many aspects of the food product, such as pH, oxygen content, which affect the multiplication of the organism in the product itself that the specific culture method should be tailored to the organism at the strain level. (Champagne 2005)

Bacterial verification will include both culture based methods and genetic identification to ensure accurate bacterial concentration and species are added to the food. Probiotic bacteria can be cultured non-selectively on many different types of media, but the selective enumeration method would be specific to the organism. For example, enumeration of *B. coagulans*, the organism in Ganaden BC30®, could be enumerated using the manufacturer enumeration protocol or on Dextrose Tryptone Agar in aerobic conditions. The non-selective enumeration of lactobacilli and bifidobacteria can occur on many types of media, such as de Man Rogosa Sharpe (MRS) media in an anaerobic environment, but if the probiotic food product is a mixture of organisms such as *S. thermophilus*, lactobacilli, and bifidobacteria, the bifidobacteria should be selectively enumerated on Arroya, Martin and Cotton (AMC) agar (Roy 2003). Tharmaraj found that selective enumeration of *Lactobacillus rhamnosus* and *Lactobacillus casei* are possible in anaerobic conditions on MRS-vancomycine at 37^{o}C and 43^{o}C respectively, while *Lactobacillus acidophilus* can be enumerated on Basal agar-sorbitol at 37^{o}C in an anaerobic environment (Tharmaraj 2003).

Genetic identification of *Bacillus coagulans*, bifidobacteria or lactobacilli could be performed by the standard 16S rDNA sequencing for the identification of environmental samples (Castro). The MicroSeq 16S rDNA system developed by Applied Biosystems recognizes *B. coagulans*, 15 species of bifidobacteria, and 45 species of lactobacilli. The

Vitek system by Biomérieux can identify *B. coagulans, L. acidophilus, Lactobacillus gasseri, and Bifidobacteria* ssp. by biochemical methods.

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