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Juice-Based Living Probiotics Survive Stomach Acid Significantly Better Than Dry Powder Live Probiotics

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ABSTRACT: This *in vitro* study aimed to assess whether a pre-hydrated version of Doctor's Biome Signature Probiotic Blend (DBSPB), comprising five strains of *Bifidobacterium* and ten strains of *Lactobacillus* in a fruit and vegetable juice carrier, can survive stomach acidity better than the same blend in dry powdered form. We initially mixed DBSPB with a proprietary sterilized blend of organic green juices and observed that the probiotics thrived in the juice. When exposed to a highly acidic environment (HCl at pH 1.5), the probiotics in the juice survived and formed colonies, whereas those in dry powdered form did not. These results suggest that probiotics in juice have significantly greater resistance to stomach acid than their dry counterparts. The enhanced survivability of the hydrated probiotics may be attributed to the combined effects of cellular hydration, buffering properties of the juice, and the presence of glucose. These patent-pending results, supported by visual and enumeration observations, suggest that incorporating probiotics into a fruit and vegetable juice carrier can improve their resistance to stomach acid and thereby offer a more effective delivery system for probiotic dietary supplements and medical foods.

Keywords: Juice-Based Probiotics; Dry Powder Probiotics; Pre-hydrated Probiotics; Probiotic Survival in HCl solution; Probiotic Resistance in Stomach

INTRODUCTION

Probiotic are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host. The particular health benefits of probiotics include helping reduce the incidence of conditions such as antibiotic-associated diarrhea, digestive discomfort (including in irritable bowel syndrome), colic symptoms in breastfed babies, atopic issues such as eczema in infants, symptoms of lactose maldigestion, acute pediatric infectious diarrhea, upper respiratory tract infections (such as the common cold), and gut infections [1,2].

Nonetheless, there are investigators and organizations that remain skeptical about the benefits of probiotics. For example, the American Gastroenterological Association does not recommend the use of probiotics for most digestive conditions [3]. Some critics argue that the scientific evidence supporting probiotic benefits is not sufficiently robust, citing that many studies are small, short-term, or yield conflicting results. Additionally, probiotics vary in dosage and bacterial strains, and not all strains have the same effects.

Probiotic dietary supplements are typically consumed as dry powder encapsulated in capsules, and to a lesser extent in the form of tablets or gummies. Once the gelatin capsule or gummy is digested by stomach pepsin, the dry powder probiotics are exposed to stomach acid (HCl at a pH of ~1.5). Existing literature from clinical trials demonstrates either the ineffectiveness or limited effectiveness of various dry powder probiotics following ingestion.

Prantera et al. (2002), in a randomized controlled trial, reported ineffectiveness of probiotics (*Lactobacillus* GG) in preventing recurrence after curative resection for Crohn's disease [4].

Mastretta et al. (2002) found that *Lactobacillus* GG was ineffective in preventing nosocomial rotavirus infections, whereas breast-feeding was effective [5].

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Kuisma et al. (2003) reported that a single-strain probiotic bacterium supplement of *Lactobacillus* GG changed the ileal pouch intestinal bacterial flora, but did not evoke a clinical or endoscopic response and hence was ineffective as a primary therapy [6].

Banaszkiewics and Szajewska (2005), in a double-blind, placebo-controlled randomized trial, found *Lactobacillus* GG used as an adjunct to lactulose to be ineffective for the treatment of constipation in children [7].

Marteau et al. (2006), in a double-blind, placebo-controlled randomized trial, determined that *Lactobacillus johnsonii* LA1 is ineffective for prophylaxis of postoperative recurrence in Crohn's disease [8].

Hegar et al. (2015) conducted a double-blind randomized trial in Indonesian children and showed probiotics to be ineffective in acute diarrhea [9].

Little et al. (2017) found in a randomized controlled factorial trial that neither probiotics nor advice to chew xylitol-based chewing gum was effective for managing pharyngitis [10].

There is also abundant evidence indicating that the survivability of probiotics in the harsh acidic environment of the stomach is a critical determinant of their effectiveness.

Tompkins et al. (2011) studied meal impact on a probiotic during transit through a model of the human upper gastrointestinal tract and showed that bacterial survival was best when given either with a meal or 30 minutes before a meal [11].

Fredua-Agyeman and Gaisford (2015) explored the survival of commercial probiotic formulations in biorelevant gastric fluids, conducting real-time measurements using microcalorimetry. They found that, in general, liquid-based products tended to give superior survival results compared with freeze-dried products [12].

Corcoran et al. (2005) analyzed the effect of glucose on *Lactobacillus rhamnosus* GG survival after 90 minutes in simulated gastric juice at pH 2.They found that glucose concentrations from 1 to 19.4 mM enhanced bacterial survival, achieving final bacterial concentrations from 6.4 to 8 $\log_{10}$ CFU ml^{-} Subsequent experiments with dilute HCl confirmed that glucose was the sole component responsible, with survival in acidic conditions occurring only in the presence of sugars that the bacteria could metabolize efficiently. Specifically, they determined that provision of ATP to F_0F_1-ATPase via glycolysis enables proton exclusion and thereby enhances survival during gastric transit [13].

In current practice, dry powder probiotics are protected from direct exposure to stomach acid by means of acid-resistant, delayed-release enteric-coated capsules, which help ensure that the probiotics reach the less harsh environment of the small intestine and therefore realize optimal effectiveness. However, these enteric-coated capsules are often more expensive than regular capsules because the coating process incurs additional cost.

We hypothesized that pre-hydrating dry powder probiotics in a fruit and vegetable juice carrier would enhance their resistance to stomach acid on account of the combined effects of cellular hydration, buffering properties and sugar content of the juice.

The aim of this *in vitro* study was to determine whether a pre-hydrated Doctor's Biome Signature Probiotic Blend (DBSPB) in a fruit and vegetable juice carrier can better survive the stomach's acidic environment (HCl at pH 1.5) compared to the same probiotic blend in its dry powdered form.

MATERIALS and METHODS

Doctor's Biome Signature Probiotic Blend (DBSPB):

Bacteria of the genera *Bifidobacterium* and *Lactobacillus* are broadly recognized for their key roles in the human intestinal microflora. We designed a proprietary blend of five strains of *Bifidobacterium* and ten strains of *Lactobacillus* obtained from the reputable company Cultures Supporting Life (Table 1). Species identification of these probiotics has been performed by sequence analysis of the 16S rRNA gene, and strain identification by pulse field gel electrophoresis. These probiotics have been shown to be sensitive to antibiotics and have passed microbiological assays and heavy metal analyses. They are not genetically modified, are free from allergens, are considered safe with respect to bovine spongiform encephalopathy, and do not contain colorants.

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Table 1. Composition of Doctor's Biome Signature Probiotic Blend (DBSPB) used in this study.

Doctor's Biome Signature Probiotic Blend (DBSPB)		
Genus	Species	Strain
Bifidobacterium	*bifidum*	SP 9
Bifidobacterium	*breve*	BBR8
Bifidobacterium	*infantis*	SP 37
Bifidobacterium	*longum*	SP 54
Bifidobacterium	*animalis* subsp. *lactis*	BLC 1
Lactobacillus	*acidophilus*	LA1
Lactobacillus	*brevis*	SP 48
Lactobacillus	*bulgaricus*	LB2
Lactobacillus	*casei*	BGP 93
Lactobacillus	*gasseri*	LG050
Lactobacillus	*paracasei*	101/37
Lactobacillus	*plantarum*	14D
Lactobacillus	*reuteri*	LR92
Lactobacillus	*rhamnosus*	SP 1
Lactobacillus	*salivarius*	SP 2

Organic Green Juice (OGJ):

As an optimum liquid carrier, we chose a proprietary sterilized blend of 100% organic green fruit and vegetable juices, specifically mint, cucumber, apple, lettuce, kale, celery and lemon juice (pH 4.0).

Study Design:

Table 2 summarizes the evaluations used in this study and associated sample counts.

1) Evaluation of the PRE-HYDRATED probiotic blend
- Testing performed at room temperature
- 0.154 g probiotic blend added to 59 ml OGJ, mixed for 1 min
- Stored for 0 min, 30 min, and 60 min at room temperature

2) Evaluation of the DRY probiotic blend
Testing performed in a 37 °C water bath
0.154 g probiotic blend added to 59 ml HCl at pH 1.5, mixed for 1 min
Stored for 0 min, 30 min, and 60 min in a 37 °C water bath

3) Evaluation of the COMBINED pre-hydrated probiotic blend (in juice) & the dry probiotic blend
- Pre-hydrated probiotics in OGJ and dry probiotics in HCl prepared as above
- Combined OGJ preparation with HCl preparation in the 37 °C water bath
- Stored for 0 min, 30 min, and 60 min at 37 °C

Table 2. Summary of the study design.

Analysis	Products	Time Points	Replicates	Total Tests
Evaluation of the pre-hydrated probiotic blend				
Bifidobacteria Enumeration	1	3	3	9
Lactic Acid Bacteria Enumeration	1	3	3	9
Evaluation of the dry probiotic blend				
Bifidobacteria Enumeration	1	3	3	9
Lactic Acid Bacteria Enumeration	1	3	3	9
Evaluation of the combined pre-hydrated and dry probiotic blends				
Bifidobacteria Enumeration	1	3	3	9

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Lactic Acid Bacteria Enumeration	1	3	3	9
Baseline evaluation				
pH of the Organic Green Juice	1	1	3	3
pH during dilution of HCl	1	1	3	3

Probiotic Enumeration (Three Replicates)

Probiotic enumeration was performed following published reference methods [14,15]. This evaluation was performed three times.

Baseline Evaluation

The pH of the Organic Green Juice (three separate aliquots) was assessed (AOAC 981.12) at baseline, and that of the HCl throughout the titration to achieve a final working solution with pH 1.5.

RESULTS

Visual Observation:

As can be observed in the representative images of enumeration plates (Figure 1, 30 minutes storage at 37 °C), when mixed in OGJ ("baseline"), colonies of the probiotic *Bifidobacteria* (1B) and lactic acid bacteria (1L) happily survive and grow. However, when the corresponding dry powder formulations (2B and 2L) are mixed with HCl solution (pH 1.5), the bacteria do not survive to form colonies. Interestingly, when the probiotic bacteria pre-hydrated in OGJ are combined with the HCl solution (3B and 3L), a substantial number survive to form colonies—far more than with direct mixing of the dry powder probiotic and HCl solution.

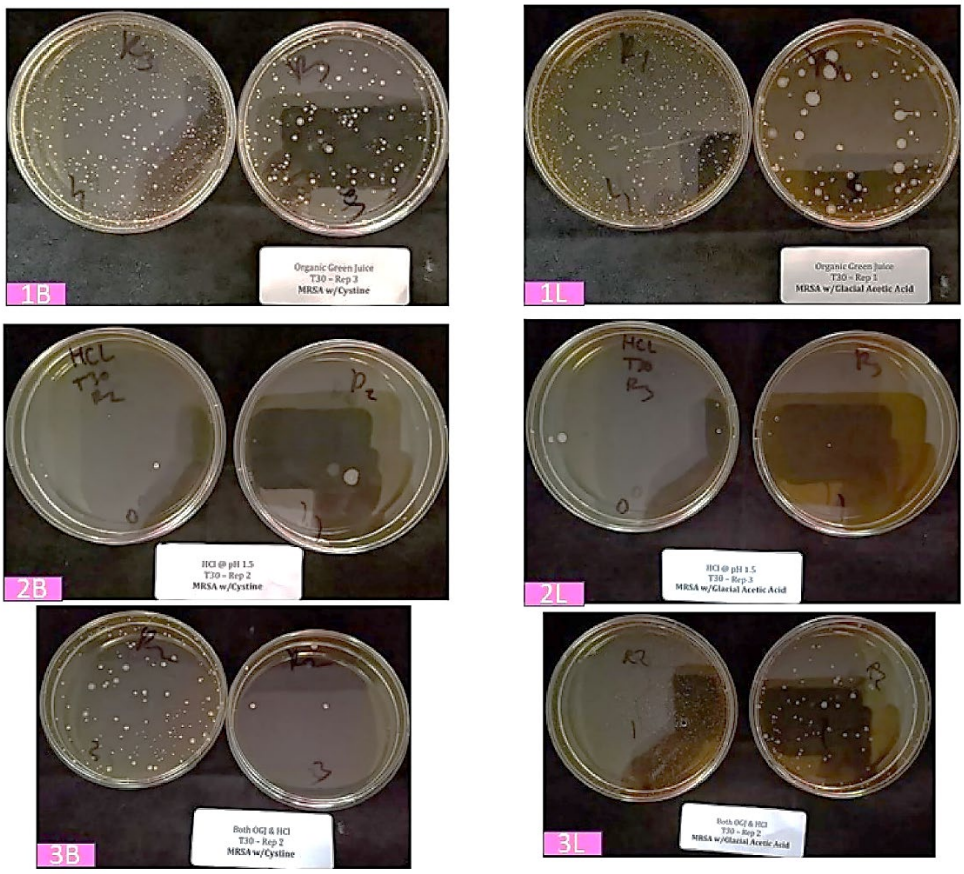

Figure 1. Pictures of Bacterial Culture Plates. **1B.** Organic Green Juice (OGJ), *Bifidobacteria*, **1L.** OGJ, lactic acid bacteria, **2B.** HCl, *Bifidobacteria*, **2L.** HCl, lactic acid bacteria, **3B.** OGJ + HCl, *Bifidobacteria*, **3L.** OGJ + HCl, lactic acid bacteria.

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Enumerative Observation:

Table 3 presents the enumerations of probiotic *Bifidobacteria*, lactic acid bacteria, and their total in OGJ, HCl @ pH 1.5, and the combined treatment.

For probiotics hydrated in OGJ, both individual and total bacterial numbers were high and relatively constant (36 million CFU/g to 28 million CFU/g) for up to 60 minutes. Let's consider this 36 million CFU/g as the baseline.

For the dry powder formulation, the total bacterial number was reduced to 64,000 CFU/g upon mixing with HCl (pH 1.5), declined further to 7 CFU/g after 30 minutes, and reached a low of 2 CFU/g after 60 minutes. For practical purposes, the bacteria were eliminated.

In contrast, when pre-hydrated probiotics were mixed with HCl (pH 1.5), the baseline abundance was immediately reduced to 3.2 million CFU/g, then to 25,900 CFU/g after 30 minutes, and finally to 5,100 CFU/g after 60 minutes. This represents impressive resistance to elimination.

Table 3. Survival of Juice-Based Living Probiotics and Dry Powder Live Probiotics Upon Exposure to Stomach Acid.

Organic Green Juice			
	Bifidobacteria	**Lactic Acid Bacteria**	**Total (*Bifidobacteria* + Lactic Acid Bacteria)**
Time (minutes)	**Average CFU/g**	**Average CFU/g**	**Average CFU/g**
0	21,000,000	15,000,000	36,000,000
30	22,000,000	14,000,000	36,000,000
60	17,000,000	11,000,000	28,000,000
HCl (pH 1.5)			
	Bifidobacteria	**Lactic Acid Bacteria**	**Total (*Bifidobacteria* + Lactic Acid Bacteria)**
Time (minutes)	**Average CFU/g**	**Average CFU/g**	**Average CFU/g**
0	29,000	35,000	64,000
30	5	2	7
60	2	0.33	2.33
Combination of Organic Green Juice + HCl (pH 1.5)			
	Bifidobacteria	**Lactic Acid Bacteria**	**Total (*Bifidobacteria* + Lactic Acid Bacteria)**
Time (minutes)	**Average CFU/g**	**Average CFU/g**	**Average CFU/g**
0	1,900,000	1,300,000	3,200,000
30	16,000	9,900	25,900
60	3,000	2,100	5,100

Graphical Observation:

Figure 2 presents the enumeration data in graphical form, vividly that illustrating the greatly superior survival of probiotics exposed to stomach acid (HCl, pH 1.5) when delivered in a juice carrier rather than as a dry powder.

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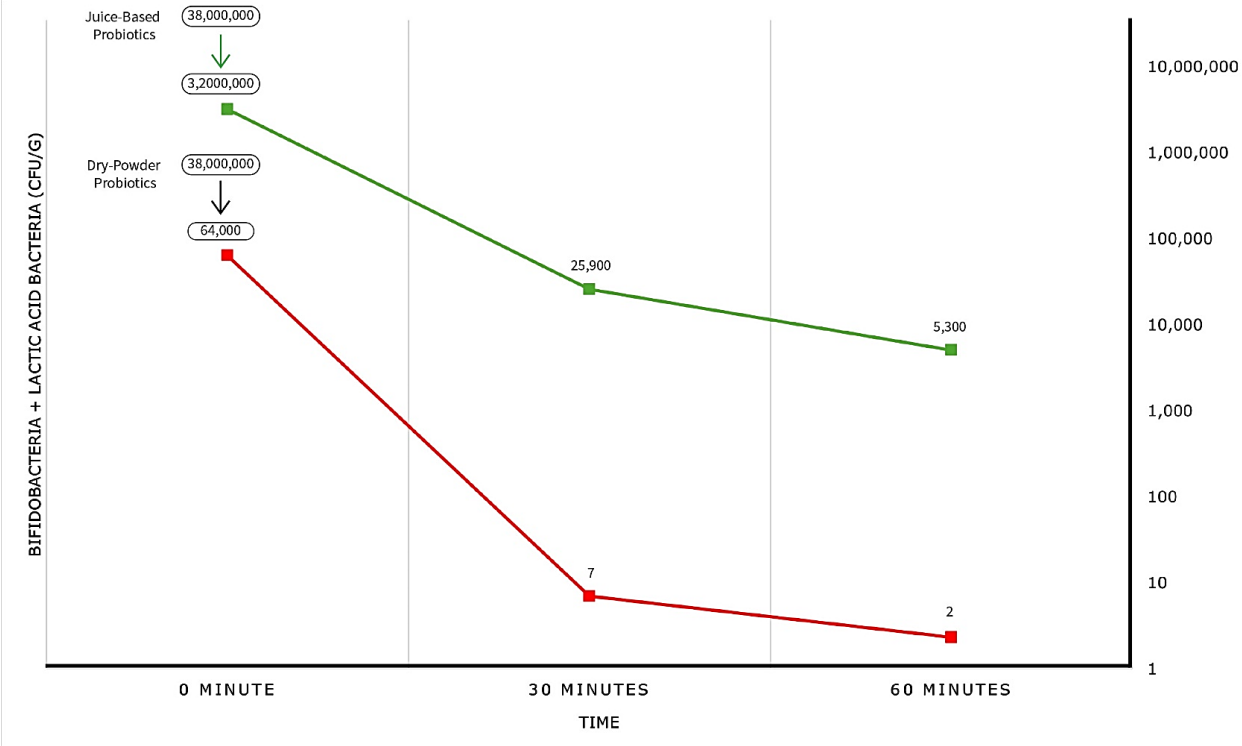

Figure 2. Survival of Juice-Based and Dry Powder Live Probiotics. Upon Exposure to Simulated Stomach Acid (HCl, pH 1.5).

DISCUSSION

From the averages of our enumerative observations, we can calculate the "Survival Ratio" at each timepoint of CFUs in HCl for OGJ pre-hydrated DBSPB and for dry DBSPB. Table 4 presents these values. In short, the pre-hydrated probiotics survived stomach acidity 50 times better at time zero, 3,700 times better after 30 minutes, and 2,188 times better after 60 minutes.

These results confirm our hypothesis that incorporating dry powder probiotics like DBSPB into a fruit and vegetable juice carrier enhances their resistance and survivability when exposed to stomach acid.

We believe this enhanced survivability is due to the combined effects of hydrating the probiotic cells, the buffering capacity of the juices, and the presence of glucose in the juices. In dry powder form, probiotic cells quickly absorb proton ions from HCl, leading to their inactivation, whereas in pre-hydrated form, the cells absorb proton ions more gradually. Additionally, the juices provide buffering capacity, meaning the solution can resist pH changes when acids or bases are introduced. Finally, glucose metabolism has been shown to improve probiotic survival during exposure to gastric acid.

In light of the above, it is appropriate to designate a blend of optimal probiotics (such as DBSPB) delivered in fruit and vegetable juices as a "Next Generation Probiotic."

Table 4. Survival Ratio of Juice-Based Living Probiotics Over Dry Powder Live Probiotics Upon Exposure to Stomach Acid.

SURVIVAL RATIO (after 0 min) Upon Addition & Gentle Mixing	Pre-hydrated DBSPB in OGJ into HCl at pH 1.5	3,200,000	50 times
	Dry DBSPB in HCl at pH 1.5	64,000	
Survival Ratio (after 30 min)	Pre-hydrated DBSPB in OGJ into HCl at pH 1.5	25,900	3,700 times
	Dry DBSPB in HCl at pH 1.5	7	

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Survival Ratio (after 60 min)	Pre-hydrated DBSPB in OGJ into HCl at pH 1.5	5,100	2,188 time
	Dry DBSPB in HCl at pH 1.5	2.33	

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Author Contributions: ARK proposed, designed and oversaw this study and prepared the first draft of the manuscript. HFR had observed the efficacy of juice-based probiotics in his patients, and reviewed the draft of the manuscript. EF assisted in the preparation of the samples, contractual arrangements with Certified Laboratories, conversion of tabulated values to graphs, and reviewed the draft of the manuscript.

Data Availability Statement: The dataset (final report from Certified Laboratories) used and/or analyzed during the current study is available from the corresponding author upon reasonable request.

Competing interests: There is no competing interest in this study.

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