Dairy and Non-Dairy Based Probiotic Drinks

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‘Probiotics’ is defined by the Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) as “live microorganisms which when administered in adequate amounts confer a health benefit on the host”. Two of the most widely used probiotics are from the genera *Lactobacillus* and *Bifidobacterium*, as both constitute most of the normal intestinal microbiota in various mammalian species. Right after birth, maternal microbiota will colonize the gastrointestinal tract (GIT) in which the first colonizers are facultative anaerobes such as *Lactobaciili*, *Enterococci*, and *Enterobacterium*. Anaerobic bacteria such as *Bifidobacterium*, *Bacteroides*, and *Clostridium* will colonize the GIT with an increase in age and compete with the facultative anaerobes, setting the colon environment to favor anaerobe growth over time.

lactic acid bacteria | probiotication | plant-based juices | functional and nutraceutical food

1. Probiotics

More than 400 bacterial species can be found in a normal adult human gastrointestinal tract (GIT). Surprisingly, 50% of the wet weight of human feces is contributed to by bacterial biomass. The intestinal microflora maintain their population density by preventing opportunistic colonization by pathogenic bacteria with a mechanism referred to as “colonization resistance” or “barrier effect” [1][2]. Therefore, probiotics are beneficial bacterial supplements, usually in a dried form or as live culture, and, when ingested as food, can assist the preventive mechanism of the host’s gut microbiota against bad microorganisms.

1.1. Beneficial Claims of Probiotics

Research demonstrating the beneficial effects of probiotics to human health has been widely published. Probiotics must grow and/or perform therapeutic activity to benefit the host [3][4]. Although the mutualistic effect of probiotics is yet to be elucidated, their possible inhibitive mechanism may include the production of antimicrobial compounds, the competitive exclusion of pathogen binding, competition for nutrients and space, and the modulation of the host’s immune system. As shown in **Table 1**, the beneficial claims of probiotics include the reduction in infectious diarrhea and allergic reaction, alleviation of gastritis, alternative cancer remedy, modulation of immune cells, and bacterial vaginosis treatment.

Park et al. [5] reported that children with infectious diarrhea caused by rotavirus have shown a reduced frequency of diarrhea and vomiting after three days of oral administration of s powdered probiotic formula containing 20 × 10^9 CFU/g of *Bifidobacterium longum* BORI and 2 × 10^9 CFU/g of *Lactobacillus acidophilus* AD031 compared to the placebo treatment (probiotic-free skim milk). However, the underlying therapeutic mechanism is yet to be investigated. According to an in vitro study conducted by Wang et al. [6], it was revealed that yoghurt supplemented with *Bifidobacterium lactis* Bb12 at a minimal viable cell count of 9 × 10^8 CFU/mL suppresses the growth of *Helicobacter pylori* and decreased the C-urea breath test (UBT) value, suggesting the decreased urease activity of *H. pylori*. This finding is supported by Midolo et al. [7], whereby lactic acid produced by *Lactobacillus* sp. strongly inhibited the growth of *H. pylori* NCTC 11637 in vitro in a pH- and concentration-dependent manner. Coconnier et al. [8] found a decrease in urease activity and cell viability of *H. pylori* with no gastric histopathological lesions in human muco-secreting HT29–MTX cells after the administration of the *Lactobacillus acidophilus* strain LB spent culture supernatant (LB–SCS).

Ingestion of a minimum dosage of probiotics is recommended to bestow health benefits on the host. As such, manufacturers should indicate a minimum daily dosage and expiry date for each strain of their probiotic products, supported by strong scientific evidence and sales approval [9]. The dosage, however, varies in opinion among scientists. According to Sanders [10], a range of doses between 10^9 and 10^{11} CFU/mL of probiotics is the effective daily dose. Furthermore, Martins et al. [11] and

Shori [12] agreed that 10^6–10^7 CFU/mL should be the minimum number of viable probiotic cells to be ingested daily. Recently, Gangwar et al. [13] suggested that a probiotic product with 10^6–10^8 CFU/mL or g of cells can exert therapeutic effects and meet the daily requirement of probiotics.

Moreover, a person with a gastrointestinal disease such as acute diarrhea should ingest a larger dose of probiotics daily. Basu et al. [14] stated that ingesting 10^{10} to 10^{12} CFU of *Lactobacillus rhamnosus* GG daily was effective in treating acute diarrhea compared to 10^7 CFU/day. A minimum dose of 10^8–10^9 CFU/day showed a reduce rotavirus concentration in patient fecal matter [15][16]. In contrast with the positive findings, a supplementation of 10^8 CFU/day of *S. thermophilus* and 10^9 CFU/day of *B. lactis*, however, did not reduce the duration of rotavirus diarrhea [17]. This suggests that there is no definite minimum daily dose of probiotics required for daily ingestion, as varying factors such as probiotic strain, type of causative pathogen, severity of symptoms, age, and race can render the therapeutic effects of probiotics ineffective.

Probiotic administration can regulate the neuropsychological functions of the central nervous system. The bidirectional gut–brain relationship can be influenced by the population of gut microflora. A study by Liu et al. [18] revealed that mice supplemented with probiotics have reduced depression compared to non-probioticated mice. Under stress conditions, one group of mice is supplemented with fluoxetine hydrochloride and another group of mice is given multi-strains of probiotic daily for 8 weeks. The mice subjected to the probiotics showed less depressive-like behavior due to the lowered corticosterone levels in their blood serum. The probiotic-treated mice showed a higher number of fecal microbiotas, suggesting that the probiotics may have released certain compounds capable of lowering the mice's blood corticosterone levels [18].

Administration of *Lactobacillus* sp. decreases self-injurious behavior (SIB) in primates. SIB is a complex phenotype that occurs in 10–12% of non-human primates and 7–34% of humans. The condition is caused by abnormally elevated hypothalamic-pituitary-adrenocortical (HPA) axis activity, which causes primates to self-harm such as biting fingers or other body parts during sleeping. The injuries sustained may cause infection in the wild. According to research by McGinn [19], the rhesus macaque monkeys with SIB supplemented with *Lactobacillus reuteri* containing an average 200 million CFU/tablet modestly decreased biting behavior. Therefore, the sleeping quality of the monkeys in the SIB group is improved.

1.2. Probiotic Attributes

To exert their beneficial effects on the host, probiotic strains need to pass through the harsh conditions of the GIT. Gastric-acid and bile-acid resistance are two main criteria measured in selecting viable probiotic strains. Food travels along the digestive tract starting from the mouth, esophagus, stomach, small intestines, and lastly the large intestine [20]. The stomach is considered hostile to most bacteria. The parietal cells release gastric juice in response to a histamine released by enterochromaffin-like cells in the presence of gastrin [21]. Gastric juice contains hydrochloric acid (HCl) which causes the pH of normal adult stomach fluid to be acidic (1.5 to 3.5) (Halperin, n.d.). The acidic condition is optimal for pepsin activity. Within 15 min, most bacteria die in the presence of HCl and pepsin at pH levels lower than 3.0 [22].

Depending on the species and strain, acid sensitivity was observed in most microorganisms at pH levels below 3.0 [23][24]. Tennant et al. [25] revealed that the Gram-negative pathogenic bacteria, *Y. enterocolitica* 8081u^{-b} (mutant), dramatically reduced in viability from 114.9 at pH 7.0 to 12.3 at pH 3.5, respectively, with all cells wiped out below pH 3.0. In contrast, the wild-type *Y. enterocolitica* 8081^{b} also experienced reduced cell viability but was still present even at pH 2.0. The acid resistance of the wild type contributed to its ability to produce urease, while the urease mutant cannot synthesize urease, causing it to be susceptible to acid [25]. While stomach acid is an effective evolutionary mechanism to inhibit the growth of pathogens, beneficial microbes can be killed as well.

Food typically stays in the stomach for around 2 to 4 h before being emptied out during gastric digestion [26]. *Lactobacillus* species, one of the main inhabitants of the colonic compartment, are essentially resilient to stomach acid. The human-derived strain *L. rhamnosus* GG is a commercial probiotic strain that has been shown to survive passage through the highly acidic stomach with a tolerance to acid as low as 2.5 for 4 h [27]. It was thought that Gram-positive bacteria express a multiple-subunit membrane-bound ATP synthases called F_0F_1-ATPase as a shielding mechanism against acidic conditions [28]. The

F_0F_1-ATPase consists of two protein portions of F_1 and F_0. The F_1 portion consists of α, β, γ, δ, and ε subunits responsible for the catalyzation of ATP hydrolysis. The F_0 is the integral membrane portion that contains a, b, and c subunits which form membranous channels for proton translocation [29]. The F_0F_1-ATPase is expressed at low pH levels and generates a proton motive force via portion expulsion, which reduces cytoplasmic H^+ concentration, leading to an increased intracellular pH at low extracellular pH [30]. Cocoran et al. [31] proved that fermentable sugars in food can assist the survival of *L. rhamnosus* GG in an acidic environment as the probiotic metabolize the sugar through glycolysis to provide sufficient ATP reserves for F_0F_1-ATPase function in pH homeostasis.

Bile acid is produced by hepatocytes through daily cholesterol degradation at approximately 350 mg [32] to remove harmful metabolic waste substances such as bile salts, bilirubin phospholipid, cholesterol, heavy metals, and toxins [33]. Bile salts form a major part of this complex aqueous secretion and function to emulsify fats from food for easier absorption. In addition, bile salts also act as antimicrobial agents to control the population of intestinal microbiota. Bile inhibits the growth of bacteria by disrupting their cellular membranes [34], inducing DNA damage [35], misfolding proteins [36], and chelating iron and calcium [37][38].

Table 1. Beneficial claims of probiotics.

Beneficial Claims	Probiotic Treatment	Main Findings	Reference
Prevention of infectious diarrhea	*Bifidobacterium longum* BORI and *Lactobacillus acidophilus* AD301	Reduced duration of rotavirus diarrhea in young Korean children.	Park et al. [5]
Alleviate symptoms of type B gastritis and peptic ulcers and prevention of gastric cancer	*Bifidobacterium lactis* Bb12	Growth inhibition of *Helicobacter pylori* leading to a decrease in urease activity, a key enzyme essential for survival of the pathogen in the stomach acid after 6 weeks of therapy.	Wang et al. [6]
Relieve inflammatory bowel disease syndromes	*L. casei*, *L. plantarum*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *B. longum*, *B. breve*, *B. infantis*, and *Streptococcus salivarius* subsp. *thermophilus*	Restoration of microbial flora to normal level through observation of increased lactobacilli, bifidobacterial, and *Streptococcus salivarius* in patient's fecal matter, leading to a reduced inflammation and symptoms of chronic pouchitis.	Gionchetti et al. [39]
	Lactobacillus GG	Significant reduction in Crohn's disease activity and increased intestinal permeability after 4 weeks medication of *Lactobacillus* GG enterocoated tablets containing 10^{10} CFU/g.	Gupta et al. [40]
Alternative prevention for cancer	*Lactobacillus rhamnosus* strain GG and LC-705	Decrease in carcinogenic aflatoxin level in the chicken lumen after daily ingestion probiotic strains.	El-Nezami et al. [41]
Modulate host's immunity	*L. acidophilus*, *L. casei*, *L. reuteri*, *Bifidobacterium bifidum*, and *Streptococcus thermophilus*	Induced hyporesponsiveness of T- and B-cells, non-apoptotic downregulation of T helper (Th)1, Th2, and Th17 cytokines, and generation and increased suppressor activity of $CD4^+CD25^+$Tregs.	Kwon et al. [42]
Allergy prevention and treatment	*B. longum* NCC 3001 and *Lactobacillus paracasei* NCC 2461	Downregulation of allergen-specific immune responses contributing to airway inflammation in mucosal lining of polysensitize mouse.	Schabussova et al. [43]
	Bifidobacterium lactis Bb-12 and *Lactobacillus* strain GG	Improved skin condition in infants suffering atopic eczema after 2 months supplementation of the probiotic formulas.	Isolauri et al. [44]

Beneficial Claims	Probiotic Treatment	Main Findings	Reference
Bacterial vaginosis treatment	Yoghurt (containing mostly *Lactobacillus* sp.)	Cured bacterial vaginosis after 1 to 2 months of intra-vaginal treatment through the increased lactobacilli flora and vaginal pH correction.	Neri et al. [45]

To counteract the detergent activity of bile salts, enteric bacteria develop bile resistance through the enhancement of the cell membrane by: (1) expression of long O-antigen chains in the lipopolysaccharides [46]; (2) expression of AcrAB-TolC efflux system where the TolC protein channel, AcrB transporter, and AcrA periplasmic protein act together to pump out bile salts from the cytoplasm [47][48]; and (3) performance of DNA repair mechanisms such as SOS—associated DNA repair, recombinational repair by the RecBCD enzyme, and base excision repair (BER) in response to bile-induced DNA damage [49]. Nevertheless, tolerance to bile salts is also species- and strain-dependent, as reported by Davati et al. [50] who discovered that *P. pentosaceus* has more than a 90% survival rate compared to *L. mesenteroides* (less than 40%) after 6 to 24 h of incubation with 0.4% (*w*/*v*) bile salts.

1.3. The Market of Probiotic Drinks

Probiotication is a process of inoculating beneficial microorganisms (mostly lactic acid bacteria) into a liquid substrate to manufacture functional beverages, which subsequently adds market value due to the various health benefits of probiotics [51]. Changes in pH, sugar content, acidity, and viable cell count are observable on various types of raw materials when applying probiotics as the fermenter [13][52][53][54]. Basically, probiotic drinks can be made up of dairy- and non-dairy-based ingredients. In 2019, the global market size of probiotic drinks was worth USD 13.65 billion, and its compound annual growth rate (CAGR) is expected to increase by 6.1% from 2020 to 2027. Nonetheless, the market of probiotic drinks is still dominated by dairy-based drinks, which held more than 55% of the revenue share in 2019 [55].

On the other hand, the fastest market growth was seen in the plant-based product segment over the forecasted period. There is a significant increase in the demand for plant-derived drinks, including probiotic fruit and vegetable juices [56]. The driving force of the shift towards a non-dairy-based product are lactose-intolerant, vegetarians, and animal-lover consumers [55]. On top of that, the increasing awareness of the detrimental effects of milk products such as high cholesterol and fat content and the presence of allergens may discourage a lot of milk-lovers [57].

2. Dairy and Non-Dairy Based Probiotic Drinks

A dairy-based probiotic drink is a milk supplemented with probiotics [58]. The milk is mostly sourced from cows, but other animal sources such as goats, sheep, and water buffalo milks can also be used [59]. A typical production procedure involves pasteurization, where the milk is heated to 71.7 °C for 15 to 25 s, followed by a brief and immediate cooling below 3 °C to extend the shelf-life by inactivating the spoilage microorganism and its enzymes to preserve the nutritional value, before being aseptically inoculated with the probiotic strain for fermentation. A non-dairy based probiotic drink uses non-milk substrates such as fruits, vegetables, and oatmeal [60]. Similar to milk processing, the non-dairy substrate needs to be sterilized by autoclaving at 121 °C at 15 psi for 15 min [13] prior to the fermentation process.

Milk is a nutrient-dense substrate which provides a sufficient supply of carbon (lactose), nitrogen (casein and whey), and mineral sources (calcium, phosphorus, sodium, and potassium) [59][61] for the probiotics to grow. In contrast, non-dairy substrates made up of fruits and vegetables possess a wide range of nutritional composition, depending on the maturation stage of the fruit and the parts, species, and variety of the fruit and vegetables themselves. Lim et al. [62] compared the nutrient composition of cempedak fruit, *Artocarpus champeden,* and its hybrid *Nanchem* and found that the flesh of *A. champeden* contained higher total carbohydrates (16.2–28.3 g/100 g) compared to *Nanchem* (7.5–30.0 g/100 g). The crude protein, fat, and ash contents of *A. champeden* flesh were also relatively higher than *Nanchem*. In terms of the maturation stage, the unripe *A. champeden* fruit contained a higher percentage of ash (4.6–5.0%), crude fiber (12.9–23.9%), crude protein (7.3–15.9%), and crude fat (3.9–6.4%) than the ripe flesh. However, the ripe *A. champeden* fruit stored higher total carbohydrates (16.2–28.3 g/100 g) than the unripe flesh (2.4–5.1 g/100 g).

2.1. Cholesterol and Fat Content

Eating a diet with high fat and cholesterol greatly increases the risk of developing cardiovascular diseases [63]. The World Health Organization stated that the main cause of global death is cardiovascular diseases. Annually, around 17.9 million people die due to heart-related complications [64]. The complex dairy fat is made up of 400 types of fatty acid species, where 65–70% of it is saturated fatty acids (SFA) [65]. Faye et al. [66] discovered that fresh cow's milk contains relatively higher cholesterol levels (8.51 mg/100 g) compared to camel's milk (5.64 mg/100 g). On top of that, the fat content of cow's milk is also higher (4.52 g/100 g) than camel's milk (2.69 g/100 g). Cholesterols are only synthesized in animals, including humans [67]. Therefore, cholesterol is only sourced from animal and dairy-based products, whereas plant-based foods such as fruit, vegetables, nuts, and grains are free of cholesterol (Heart UK, n. d.). The University of California San Francisco (UCSF) Health [68] recommends an intake of not more than 300 mg of cholesterol per day.

Cholesterol is important for retaining the fluid mosaic model of mammalian cellular membrane, as it assists the lipid bilayer in terms of permeability, hydrophobicity, and fluidity [69]. However, an excessive intake of saturated fats and cholesterol causes an increase in low-density lipoprotein (LDL) in the blood plasma, and the accumulation of these bad cholesterol leads to atherosclerosis, a condition where the artery experiences a blockage due to plaque formation, and the supply of oxygen to the heart is cut off [70]. This will result in the occurrence of heart attacks and, even worse, can lead to death. In developing countries, atherosclerosis is reported as the major cause of mortality [71]. Published data by WHO in 2017 revealed that 22.13% of total deaths in Malaysia were caused by heart attacks, and the rate increased to 24.69% in 2018 [72].

Excessive fatty food consumption along with a sedentary lifestyle and genetics contribute to overweightness and obesity [73]. The Cleveland Clinic recommends an adult only consume fats around 20% to 35% of their daily total calories. Furthermore, to reduce detrimental health issues, it is suggested to only consume 15% to 20% monosaturated fats, 5% to 10% polysaturated fats, <10% saturated fats, and zero trans-fat out of the recommended daily intake in fat percentage [74]. Overweightness and obesity are associated with various medical complications such as fatigue, diabetes, high blood pressure, heart disease, and several types of cancer [75]. In all plant-based products, the fats are predominantly unsaturated fats with a low percentage of saturated fats [76], making it a healthier alternative for functional beverages.

2.2. Allergens

Generally, two major milk proteins responsible for sparking an allergic response in humans are casein and whey. The acidification of the cow milk results in two fractions: Fraction 1 appears as solid coagulum (coagulated proteins) called casein contributes to 80% of the total milk protein, and Fraction 2 appears with a liquid consistency (lactoserum) called whey contributes to 20% of the total milk protein [77][78][79][80]. Allergens in the casein fraction comprised 32% αS1-casein, 28% β-casein, 10% αS2-casein, and 10% K-casein [81]. Meanwhile, the whey fraction contains allergens such as α-lactalbumin (5%) and β-lactoglobulin (10%) [79][82], as well as traces of immunoglobulins, bovine serum albumin, and lactoferrin [83].

The allergens in milk exhibit immunoglobulin E (IgE) epitope clusters [84]. The IgE epitopes cluster in the allergen aids in the cross-linking between IgE antibodies and effector cells such as mast cells [85]. Then, the mast cells secrete histamine, an inflammatory mediator commonly associated with allergic responses, and promote vasodilation (dilating of blood vessel to deliver immune cells to the affected site) and tissue damage [86]. Gupta et al. [87] reported the 2.4% prevalence of a cow's milk allergy (CMA) in consumers aged 18 to 29 years old in the U.S. Thus, non-dairy beverages are an excellent alternative for consumers with CMA. On another note, the use of soybeans to replace cow's milk has also shown allergic reactions in consumers (allergic to soy) due to the soy protein [88]. Hence, fruits and vegetables are the best alternatives to dairy [60].

2.3. Consumers Preference on Non-Dairy Products

The preference of non-dairy- over dairy-based products is more prevalent among lactose-intolerant and vegetarian consumers. Animal abuse, environmental damage, and the search for new taste profiles drive consumers to resort to non-dairy products. Milk contains lactose ($C_{12}H_{22}O_{11}$), a disaccharide made up of glucose and galactose subunits. Lactose makes up 2% to 8% of milk by weight [89]. To metabolize lactose, mammals, including humans, secrete a lactase enzyme called β-d-galactosidase which cleaves the glycosidic bonds between the glucose and galactose. However, the expression of lactase decreases with age as humans consume less milk throughout adulthood [90]. A meta-genomic analysis from Storhaug et al. [91] estimated that 68% of the world's population is lactose-intolerant in which individuals from Africa and Asia form the major proportion. Meanwhile, approximately 36% of the United States (U.S.) population has lactose malabsorption problems, whereas ethnic and racial groups including African Americans, American Indians, Asian Americans, and Hispanics possess lactose-digesting problems [92].

Furthermore, awareness of animal cruelty in the dairy industry has also increased over the years due to the emerging animal rights movement and advancements in mass media [93]. The People for The Ethical Treatment of Animals (PETA) [94], stated that cows are kept in confined compartments and treated as machines that exclusively produce milk without meeting their most basic desires. Female cows are impregnated forcefully through artificial insemination right after delivering their infants [95], while calves are taken away from their mothers within 24 h of birth [96], and the milk intended to be consumed by the calves is harvested and sold as functional beverages [61]. On top of that, a cow that has been confined for 2 to 3 months generally develops mastitis, a severe dermal inflammation caused by the overgrowth of *E. coli* on the mammary glands [97].

Factory-farmed animals, including those in dairy farms, produce manure, which is extremely detrimental to the environment. The U.S. Department of Agriculture reported 1.5 billion metric tons of animal waste produced each year by the U. S. meat and dairy industries [98]. Eventually, these waste materials end up into waterways, polluting downstream rivers and lakes [99].

In addition to the above, commercialized fermented fruit and vegetable juices provide a wide range of taste profiles for all age groups of consumers compared to probiotic milk [60]. Not only are fruit and vegetable juices nutritious, but they are also highly refreshing and thus are suitable candidates in manufacturing a healthy functional drink [56].

2.4. Recent Development of Non-Dairy Probiotic Drinks

The global market for dairy alternatives is growing annually. In 2020, the global market for non-dairy-based products was USD 12,270 million, and this is forecasted to increase in CAGR from 2021 to 2026 by 11.0% [100]. The global food and beverage industry shows an increase in demand for dairy substitutes. Therefore, many companies have emerged to become the key players in the industry for non-dairy-based beverages. Lifeway, a company selling cultured milk kefir, expanded their market into vegan-based products through the invention of Plantiful, a probioticated pea juice [101]. **Table 2** shows a list of commercialized non-dairy probiotic products corresponding to their manufacturer, probiotic strain, and the raw materials used.

Table 2. List of non-dairy probiotic products sold commercially.

Product Name	Manufacturer	Probiotic Strain (s)	Non-Dairy Substrate
Biomel	Biomel, UK	*B. bifidum*, *B. coagulans*, and *L. plantarum*	Coconut milk and grape extract
Califia Farms	Califia Farms, California	*Bifidobacterium* BB-2, *S. thermophilus*, and *L. bulgaricus*	Almond milk, coconut cream, and oat fiber
GT's Organic Kombucha	GT, Los Angeles	Lactobacillus bacterium (species not specified) and *Bacillus coagulans* GBI-30 6086	Black and green tea (to make Kombucha) and kiwi juice
KeVita Apple Cider Vinegar Tonics	KeVita, California	Water kefir (starter culture) and *Bacillus coagulans* GBI-30 6086	Apple juice (to make apple cider), apple juice, and lemon extract.

Product Name	Manufacturer	Probiotic Strain (s)	Non-Dairy Substrate
Plantiful	Lifeway Foods Inc., Illinois, U.S.	*L. casei*, *L. plantarum*, *B. bifidum*, *B. animalis* subsp. *lactis*, *B. longum* subsp. *longum*, *L. acidophilus*, *L. paracasei*, *L. rhamnosus*, *L. lactis* subsp. *lactis*, and *S. thermophilus*.	Non-GMO pea protein
GoodBelly JuiceDrink	NextFoods, Boulder, Colorado	*Lactobacillus plantarum* LP299V	Mango: pear juice, mango puree, banana puree, oat flour, barley malt Cranberry watermelon: grape juice, pear juice, cranberry juice, strawberry juice, oat flour, watermelon juice, barley malt, vegetable juice
Harmless Harvest Dairy-Free Yogurt	Harmless Harvest, Thailand	*L. acidophilus*, *B. lactis, S. thermophilus*, *L. casei*, *L. bulgaricus*, *B. bifidum*, *L. rhamnosus*, and *Bifidobacterium lactis* HN019	Young Thai coconut milk and water
Tropicana Essentials Probiotics® Pineapple Mango	PepsiCo, U.S.	*Bifidobacterium lactis*	Mango puree, pineapple, banana puree, and vegetable juice
VitaCup Immunity Coffee Pods	VitaCup, San Diego	*Bacillus coagulans*	Coffee, Inulin
Gut Shot®	Farmhouse Culture	Naturally occurring bacteria in the cabbage (Not specified)	Sauerkraut brine (fermented cabbage) and apple
Dee-V Drinks	Dates Valley, Malaysia	Not specified	Khal dates cider with four optional flavors (honey, berry, ginger, lemon)
Gut Kulture	Steve's PaleoGoods, New Jersey	Naturally occurring probiotics culture (Not specified)	Beet, carrot, sarsaparilla, turmeric, ginger, burdock root, kudzu root, astragalus root, shatavari root, dandelion root, white ginseng, ashwaganda, rhodiola root

Many manufacturers use a combination of multiplex probiotic strains, while some only use a single probiotic strain, e.g., NextFoods utilized only *Lactobacillus plantarum* LP299V to produce three distinct types of GoodBelly Juice Drinks [102]. The use of a single probiotic strain reduces interspecific competition for limited nutrients and space [103]. Nonetheless, multiple bacterial strains could coexist in the same media, as shown by Lifeway Foods—their Plantiful vegan-based drink utilized ten types of probiotics [104]. Griffin and Silliman [104] stated that multiple organisms can coexist through resource partitioning, where different forms of limited resources are slightly consumed, or the same limited resource is consumed at different times and locations to reduce interspecific competition. In addition to this, gut microflora coexist and perform competitive exclusion, a mutualistic mechanism to prevent the growth of invading microorganisms [105].

Melo-Bolivar et al. [106] discovered that bacterial gut microbiomes from a Nile tilapia inhibited the growth of the pathogenic *Streptococcus agalactiae*, and notable changes were observed in the dominant species in the culture (*Lactococcus* spp. replaced *Cetobacterium* and become the dominant species in the community). The ten probiotics in Plantiful belong to the genera *Lactobacillus*, *Bifidobacterium*, and *Streptococcus*, all of which comprised the normal intestinal microbiota in humans. Therefore, these probiotics can grow in parallel to one another in the same medium, similar to what complex indigenous microbiome performs in the human intestine [107].

Biomel, GT's Organic Kombucha, KeVita Apple Cider Vinegar Tonics, and VitaCup Immunity Coffee Pods contain *Bacillus coagulans* [108][109][110][111], which can reproduce by spore formation. The use of spores is beneficial in the manufacturing and storage process of probiotic drinks, as the *B. coagulans* spores are resistant to heat, cold temperatures, and stomach acid and remain dormant in the juice but germinate once it passes through the gastric compartment [112]. While most companies utilized commercial probiotics, some used naturally growing bacteria from a fermented starter culture. GT's Organic Kombucha, KeVita Apple Cider Vinegar Tonics, and Gut Shot® applied starter cultures from kombucha (fermented tea), apple cider (fermented apple juice), and sauerkraut brine (fermented cabbage), respectively. The use of fermented substrates already enriched with microbial populations effectively shortens the fermentation period for the next batch of fresh substrate [113].

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