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Recovery of Viable Bacteria from Probiotic Products that Target Oral Health

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Abstract

Probiotic therapy has predominantly been directed toward promoting and maintaining intestinal health. In recent years, however, probiotic regimens that target oral health have appeared on the market. These regimens are often delivered in the form of lozenges. Despite the oral health claims made by the manufacturers of these products, there is little independent evidence in the literature to support such claims. In theory, probiotic organisms can be beneficial by several different means including direct inhibition of pathogens and boosting of the host immune response, with the underlying assumption that these mechanisms require a critical number of viable organisms. In this study, five brands of probiotics marketed for oral health were tested for the recovery of viable bacteria. For only one brand could viable bacteria be recovered within one log of the manufacturer's stated starting amount of bacteria. Nearly a billion viable bacteria could be recovered from a lozenge of this brand. The other brands claimed similar starting amounts of bacteria at the time of manufacture but at least a three-log drop off was observed in the amount of viable bacteria recovered from those products. Refrigeration of the probiotics significantly improved the recovery for one brand, but recoveries for all but one brand remained below the recommended daily dosage for probiotic regimens. It is concluded that probiotic brands differ significantly in the quantities of bacteria that remain viable with most failing to meet recommended dosage targets.

INTRODUCTION

Health-conscious consumers have been targeted by an increasing array of products that contain bacteria designated as probiotic species. The majority of these products are designed to improve or maintain intestinal health. However, several manufacturers now market products that are claimed to promote oral health. Although there is evidence for the utility of certain intestinal probiotic applications (see recent reviews by Andrews and Tan [1], Rupa and Mine [2], and Balakrishnan and Floch [3]), broadly speaking, the current science of probiotics leans as much, or more, on theory and inference as on clinical data demonstrating efficacy. This is true as well for the oral health probiotic regimens. Product claims include plaque reduction and protection against cavities, promotion of gingival and periodontal health, and protection against sore throat, ear aches, and bad breath. Some products include additives that themselves may promote oral health, including calcium or vitamins. Xylitol is commonly added as a sweetener but is unlikely to be at amounts known to be protective against dental caries. Promising results following probiotic administration have been obtained when measuring surrogate markers of periodontal disease and caries such as reductions in pro-inflammatory cytokines, matrix metalloproteinases, and disease-associated bacterial species (see recent reviews by Haukioja [4], Anilkumar and Monisha [5], and

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Bizzini et al. [6]). A relatively small number of studies have included clinical outcomes as endpoints. Some of these studies report statistically significant improvements associated with probiotic consumption, though the magnitude of the improvements may be modest or only involve a subset of participants. Collectively, the body of evidence reveals both a need for larger random clinical trials and an encouraging rationale for undertaking them.

Similar to intestinal probiotic regimens, probiotics marketed for oral health include species of *Lactobacillus* and *Bifidobacterium*. The role of these genera dates back to some of the earliest work with probiotics [5]. Even though species from these genera have been implicated in dental caries, the probiotic species are distinct and a number of studies have shown that they can reduce oral levels of the cariogenic species *Streptococcus mutans* [4–6]. Probiotics targeting oral health also rely on species of *Streptococcus*. *Streptococcus salivarius* K12 produces bacteriocins that are lethal for pathogens such as *Streptococcus pyogenes* and *Streptococcus pneumonia*, and in probiotic usage has been shown to prevent recurrent pharyngitis, tonsillitis, and otitis media [7]. *Streptococcus salivarius* M18 is reportedly inhibitory towards *S. mutans*, and may also help prevent dental caries by virtue of dextranase and urease enzymatic activities [8]. In a double-blind, placebo-controlled trial probiotic administration this strain was associated with lower plaque scores and with lower levels of *S. mutans* in a subset of subjects colonized with M18 [9]. Species such as *Streptococcus uberis* and *Streptococcus oralis* are found in inverse proportions to periodontal pathogens [10]. *Streptococcus rattus* JH145 is missing lactate dehydrogenase activity, diminishing its own acidogenic potential, and has been shown to displace wild-type *S. mutans* in an animal model [10–11]. Inhibition of pathogens by probiotic species is a common theme and, not surprisingly, a basis for screening for probiotic strains along with other desirable traits such as an ability to adhere to targeted sites [12]. Consequently, in order to potentially exhibit the full range of beneficial properties ascribed to probiotic strains, they must remain viable. Although dosages vary from study to study, recommendations for daily consumption are generally in the range of 10^9 to 10^{10} colony forming units (CFU) [2]. With that in mind, the objective of this study was to enumerate the recovery of viable bacteria from five different probiotic regimens marketed for oral health.

MATERIALS and METHODS

Probiotic Products

Five brands were chosen based on availability over the internet and manufacturer claims directed at oral health: Udo's Choice Super 5 Probiotic Lozenges (Flora, Inc., Lynden, WA), Life Extension Advanced Oral Hygiene (Quality Supplements and Vitamins, Inc., Ft. Lauderdale, FL), Nature's Plus Adult's Dental Care Probiotic Lozenges (Natural Organics Laboratories, Inc., Amityville, NY), TheraBreath Multi Symptom Probiotics (Dr. Harold Katz, LLC, Los Angeles, CA), and Garden of Life Probiotic Smile (Garden of Life, LLC, West Palm Beach, FL). Two independent purchases were made. The first shipment was stored at room temperature upon receipt and the second shipment was stored at 4°C. The one exception was Udo's Choice; room temperature storage was not tested since its label instructed that it should be refrigerated. It was the only brand that was shipped with a cold pack.

Recovery of Viable Bacteria

Four of the five brands consisted of tablet lozenges designed to be sucked by the consumer to allow extended release and exposure to the probiotic organisms. The fifth brand, TheraBreath, consisted of a crystal powder to be dissolved in water and used as a mouthrinse. To test the recovery of viable bacteria, a single dose of the probiotic was added to 50ml of phosphate-buffered saline (PBS) and stirred continuously at 37°C until dissolved.

Dilutions were made as necessary in PBS and inoculated onto Trypticase Soy Agar with 5% sheep's blood (Remel, Thermo Fisher Scientific, Lenexa, KS) and MRS agar plates (Anaerobe Systems; Morgan Hill, CA). The blood agar was expected to support the growth of all probiotic species; the MRS agar is formulated for optimal recovery of lactobacilli. Each probiotic brand had a unique combination of bacterial strains all of which belonged to the genera *Streptococcus* and *Lactobacillus* with one exception. Advanced Oral Hygiene included a *Bacillus* species (Table 1). Plates were incubated in a CO2 incubator at 37°C for up to 48 hours. Colonies were counted to determine the colony forming units (CFU) per ml of inoculum and then multiplied by 50 to obtain the total number of viable bacteria recovered from a single lozenge or powder packet. No advice was sought from the suppliers for recommended methods of optimal recovery. The authors have no conflict of interest with any of the brands tested.

RESULTS

The manufacturers' claims for the numbers of viable bacteria per lozenge at the time of manufacture ranged from roughly 300 million to 4 billion (Table 2). The recovery of viable bacteria from the different brands varied greatly. Udo's Choice stood out from the rest numerically and statistically when all brands were stored in the refrigerator. Udo's Choice was the only brand for which the label directed refrigerated storage. Therefore, it was not tested at room temperature. Of the remaining brands, only Nature's Plus demonstrated a statistically higher recovery of viable bacteria with refrigeration compared to room temperature storage. There were no statistical differences in the recoveries of bacteria on the two media. No viable bacteria could be recovered from Garden of Life lozenges. The limits of detection were 1000 bacteria. Advanced Oral Hygiene, Nature's Plus and TheraBreath each displayed a minimum of a three-log reduction in viable bacteria between manufacture and use. The reduction for Udo's Choice was less than one log. Furthermore, Udo's Choice was the only brand for which the number of viable bacteria recovered met the recommendations for daily dosage of a probiotic regimen [2]. There did not appear to be contaminating species in any of the probiotic preparations based on expected colony morphologies.

DISCUSSION

There is still much to investigate with respect to the efficacy of probiotics for various health concerns. At a more fundamental level, however, there is virtually no chance that a probiotic regimen will be of benefit if the numbers of viable bacteria consumed are below a critical threshold. Although critical concentrations will likely vary for each species and targeted health benefit, general recommendations for daily dosages are in the range of 10^9 to 10^{10} CFU [2]. In our experience, these dosages are easily obtained by consumption of yogurt with active cultures or fermented milk products. We were interested in determining the extent to which dehydrated forms of probiotic strains retain viability.

Since only a single oral health-related probiotic brand was available in local health food stores, the five products tested were purchased via the internet. We cannot rule out exposure to temperature extremes during shipping. Two batches of each probiotic product were obtained through independent shipments and tested within two weeks and within the stated expiration date limits; one set was placed at room temperature while the other set was refrigerated. Udo's Choice was shipped cold. Nature's Plus instructed users to refrigerate after opening and it was the only brand for which a statistically higher recovery of viable organisms was obtained from the refrigerated sample. Other brands stated that the product should be stored in a cool, dry place.

The bacterial species represented in the five products were all from the genera *Lactobacillus* and *Streptococcus*. One brand included the species *Bacillus coagulans* which holds promise as an intestinal probiotic to prevent *Clostridium difficile* colitis [13] and for its immunomodulatory effects [14]. All were expected to grow on the blood agar. The MRS agar was included as a precaution since it is optimized for the recovery of lactobacilli. Recoveries on the two media did not differ statistically for any of the brands. Advanced Oral Hygiene, for which recoveries were numerically but not statistically higher on MRS agar, did not contain any species of lactobacilli. Garden of Life was tested using both aerobic and anaerobic incubation atmospheres but no bacteria were recovered under either condition. Although the protocol employed should provide a reasonable estimate of the viable bacteria that can be recovered from the tested products, the authors acknowledge that the efficiency of laboratory recovery may differ from what occurs *in vivo*.

This investigation did not address whether any of the tested brands could accomplish the health claims made by the manufacturers. Consequently, we cannot say definitively that the relative yields of viable bacteria are a determinative variable in the efficacy of the product. Still, we anticipate that the probability of a beneficial effect is at least somewhat proportional to the availability of viable bacteria, being the basis for recommended daily dosages of a billion to 10 billion organisms. Based on this criterion, only Udo's Choice meets the dosage recommendation. It is possible that effective dosages will be less for oral applications than intestinal applications since the bacteria do not have to survive the low pH, bile acids and other anti-microbial components in the alimentary canal. However, the rapidity with which dehydrated forms of the bacteria revive within the oral cavity is uncertain and may influence the effective dose as well. For the oral health studies included in the cited review articles [4–6], most utilize at least 10^8 organisms. Those studies employed various probiotic species and targeted oral health both on mucosal surfaces and teeth. Variations in effective dosages will almost certainly occur based on the intended application and the properties of individual probiotic strains, but the current state of knowledge dictates that efficacy be established using dosages and deliveries that are balanced between ensuring at least temporary retention at the target site and avoiding an immune suppressive effect. The results of this study highlight the importance of accurate determinations of viable bacteria in studies investigating the efficacy of probiotic products. Additionally, consumers and health professionals should be aware that dehydrated forms of probiotics may not deliver the expected dosages and may be highly sensitive to temperature fluctuations that can occur during shipment, personal transit or storage.

LITERATURE CITED

1. Andrews JM, Tan M. Probiotics in luminal gastroenterology: the current state of play. Int Med J. 2012; 42:1287–1291.
2. Rupa P, Mine Y. Recent advances in the role of probiotics in human inflammation and gut health. J Agric Food Chem. 2012; 60:8249–8256.
3. Balakrishnan M, Floch MH. Prebiotics, probiotics and digestive health. Curr Opin Clin Nutr Metab Care. 2012; 15:580–585. [PubMed: 23037903]
4. Haukioja A. Probiotics and oral health. Eur J Dent. 2010; 4:348–355. [PubMed: 20613927]
5. Anilkumar K, Monisha ALS. Role of friendly bacteria in oral health – a short review. Oral Health Prev Dent. 2012; 10:3–8. [PubMed: 22908082]
6. Bizzini B, Pizzo G, Scapagnini G, Nuzzo D, Vasto S. Probiotics and oral health. Curr Pharm Design. 2012; 18:5522–5531.
7. Di Pierro F, Donato G, Fomia F, Adami T, Careddu D, Cassandro C, Albera R. Preliminary pediatric clinical evaluation of the oral probiotic *Streptococcus salivarius* K12 in preventing recurrent pharyngitis and/or tonsillitis caused by *Streptococcus pyogenes* and recurrent acute otitis media. Int J Gen Med. 2012; 5:991–997. [PubMed: 23233809]

8. Heng NC, Haji-Ishak NS, Kalyan A, Wong AY, Lovric M, Bridson JM, Artamonova J, Stanton JA, Wescombe PA, et al. Genome sequence of the bacteriocin-producing oral probiotic *Streptococcus salivarius* strain M18. J Bacteriol. 2011; 193:6402–6403. [PubMed: 22038965]

9. Burton JP, Drummond BK, Chilcott CN, Tagg JR, Thomson WM, Hale JDF, Wescombe PA. The influence of the probiotic *Streptococcus salivarius* M18, on indices of dental health in children: a randomized double-blind placebo-controlled trial. J Med Microbiol. 2013 [E-pub ahead of print;

10. Hillman JD, McDonell E, Hillman CH, Zahradnik RT, Soni MG. Safety assessment of ProBiora3, a probiotic mouthwash: subchronic toxicity study in rats. Int J Toxicol. 2009; 28:357–367. [PubMed: 19815843]

11. Johnson KP, Hillman JD. Competitive properties of lactate dehydrogenase mutants of the oral bacterium *Streptococcus mutans* in the rat. Arch Oral Biol. 1980; 27:513–516. [PubMed: 6956265]

12. Samot J, Badet C. Antibacterial activity of probiotic candidates for oral health. Anaerobe. 2013; 19:34–38. [PubMed: 23211763]

13. Fitzpatrick LR, Small JS, Green WH, Karpa KD, Farmer S, Keller D. *Bacillus coagulans* GBI-30, 6086 limits the recurrence of *Clostridium difficile*-induced colitis following vancomycin withdrawal from mice. Gut Path. 2012; 4:13–21.

14. Benson KF, Redman KA, Carter SG, Keller D, Farmer S, Endres JR, Jensen GS. Probiotic metabolites from *Bacillus coagulans* GanedenBC30™ support maturation of antigen-presenting cells *in vitro*. World J Gastroenterol. 2012; 18:1875–1883. [PubMed: 22563167]

TABLE 1

Properties and Ingredients of Five Probiotic Products That Target Oral Health

BRAND	Form	Probiotic Species	Other Notable Ingredients*
Udo's Choice	Lozenge	*Bifidobacterium bifidum* HA-132 *Lactobacillus acidophilus* HA-122 *Lactobacillus casei* HA-108 *Lactobacillus rhamnosus* HA-111 *Lactobacillus salivarius* HA-118	Dried Sugar Cane Juice, Milk, Soy
Advanced Oral Hygiene	Lozenge	*Bacillus coagulans* GB-30 6086 *Streptococcus salivarius* K12	Xylitol, Mannitol, Milk, Corn, Yeast
Nature's Plus	Lozenge	*Streptococcus salivarius* M18 *Lactobacillus acidophilus*	Xylitol, Vitamin D3, Calcium, CoEnzyme Q
TheraBreath	Crystal Powder	*Streptococcus salivarius* M18 *Streptococcus salivarius* K12	Xylitol, Sorbitol
Garden of Life	Lozenge	*Streptococcus oralis* KJ3 *Streptococcus uberis* KJ2 *Streptococcus rattus* JH145	Xylitol

*Each product contained flavors and various extracts.

TABLE 2

Recoveries of Viable Bacteria from Five Probiotic Products That Target Oral Health

BRAND	Minimum Log_{10} CFU Upon Manufacture*	Medium	Room Temperature Storage[a] Log_{10} CFU Recovery (Mean ± Std. Dev.)	Refrigeration Storage Log_{10} CFU Recovery (Mean ± Std. Dev.)	Room Temp. vs. Refrigeration p Value
Udo's Choice	9.30	MRS	------	8.90[b] ± 0.31	------
		Blood Agar	------	8.96[b] ± 0.26	------
Advanced Oral Hygiene	8.76	MRS	6.00 ± 0.31	5.77 ± 0.09	0.273
		Blood Agar	4.71 ± 0.30	4.40 ± 0.23	0.226
Nature's Plus	9.60	MRS	4.71 ± 0.57	6.19 ± 0.42	0.023[c]
		Blood Agar	5.37 ± 0.08	6.22 ± 0.34	0.045[c]
TheraBreath	9.00	MRS	5.29 ± 0.82	5.54 ± 1.16	0.826
		Blood Agar	6.45 ± 0.06	5.37 ± 0.91	0.235
Garden of Life	8.48	MRS	Undetected[d]	Undetected[d]	------
		Blood Agar	Undetected[d]	Undetected[d]	------

*Based on manufacturer's claims.

[a]Udo's Choice was not tested at room temperature because its label indicated that it should be stored refrigerated.

[b]Recovered CFU from Udo's Choice was statistically higher than from any of the other probiotic brands for both media tested based on ANOVA, $p < 0.01$.

[c]Recovered CFU from room temperature stored product is statistically different than recovered CFU from refrigerated product based on a student's t test comparing means.

[d]Limit of detection was 3.00.