JOURNAL OF
EMERGING INVESTIGATORS

Article

Pediatric probiotic culture survival study in acidic pH using an *in vitro* model

Sana Dhillon[1], Simone Renwick[2], Brittany Berdy[3], Ashita Dhillon[1]

[1] Ursuline Academy, Dedham, Massachusetts, USA

[2] University of Guelph, Guelph, Ontario, Canada

[3] Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA

SUMMARY

***Lactobacillus* and *Bifidobacterium* are two genera of bacteria in probiotics that are known to possess significant immunomodulatory health-promoting properties. Probiotics are allowed to be used in foods and vitamins by the Food and Drug Administration (FDA) with little regulation as long as there are no claims to treat any disorder or condition. Regulatory agencies throughout the word classify and define probiotics differently with an uncertainty on the efficacy. The viability of bacterial strains influence probiotic stability and properties can be influenced by manufacturing and storage processes. It is imperative to also consider the viability of the probiotics after consumption. The aim of this study was to investigate the survival of the strains in the commercial probiotic Lovebug in acidic conditions modeling the human upper gastrointestinal (GI) tract *in vitro*. To test the ability of probiotics strains in the Lovebug probiotic to survive under acidic conditions, we incubated the probiotics in degassed acidified 0.8% sodium chloride at various pH levels for 2 h and measured the resulting colony forming units. Our study observed an overall survival of approximately 20–40 % after being incubated for 2 hours at pH 2–4. This supports that the bacterial genera of *Lactobacillus* and *Bifidobacterium* in the probiotic Lovebug would likely survive at a high enough rate in the human upper GI tract to provide benefit to the pediatric population.**

INTRODUCTION

Previous studies have reported that gut microbiota play a role in preventing pathogen colonization, stimulating the production of gastrointestinal (GI) hormones, regulating brain behavior through production of neuroactive substances, and shaping our immune system (1-3). During neonatal and childhood development, different sites in the human body get colonized by microbial communities and the community composition varies at different sites, as well as in a healthy versus diseased state. The gut microbiome plays an important role in influencing human health as well as disease development starting *in utero* and extending into adolescence (4). It is mainly body habitats (i.e., skin, mouth, and gut) that determine the community composition (5). The microbiome in the human gut is influenced by evolutionary selection forces acting both at microbial cell and at host level. Microbial diversity is based on gut colonization ecological selection pressure, which is alike for mutualist as well as pathogens (6). Throughout the human body several genera and species from the following bacterial phyla predominate the microbiome composition: Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria (7, 8). The normal intestinal microbiota of various mammals consists of *Bifidobacteria* and *Lactobacilli*, and these genera are the best characterized and most widely used in commercialized probiotics (9). *Bifidobacteria* and *Lactobacilli* are non-spore-forming, gram-positive, lactic acid-producing bacteria (LAPB). *Bifidobacteria* are important producers of short-chain fatty acids (SCFA), whereas *Lactobacilli* have limited biosynthetic abilities and ferment refined sugars, generating lactic acid as the major end product (10, 11). Despite the fact that *Lactobacilli* and *Bifidobacteria* have some common properties they belong to two taxonomically distinct groups: the genus *Lactobacillus* belongs to phylum Firmicutes and the genus *Bifidobacterium* to phylum Actinobacteria (12).

Firmicutes and Bacteroidetes usually dominate the adult intestinal microbiota, whereas Actinobacteria, Proteobacteria, and Verrucomicrobia are considerably less abundant (9, 13). Studies have shown that the most common species found in healthy infants are *Bifidobacterium infantis* and *Bifidobacterium breve* (14). Even though *Lactobacilli* and *Bifidobacteria* are less dominant in adulthood, they remain stable elements of the normal intestinal microbiota and play an important role in diseases such as inflammatory bowel disease, irritable bowel syndrome, obesity, and allergic disorders (15). The role probiotics play in the GI tract is suggested to enhance intestinal barrier functions, stimulate immunity, and modulate inflammatory diseases (9). Probiotics are known to have bactericidal effects on pathogenic bacteria by restoring gut homeostasis and inhibiting pathogen and toxin adhesion to the intestinal epithelium (3).

The addition of probiotics to foods for infants and children for the treatment of acute gastroenteritis and the prevention of antibiotic-associated diarrhea and gastroenteritis is supported by the American Academy of Pediatrics (16). Antibiotic use has been linked to disruption of the gut microbiota (dysbiosis), even leading to low species diversity and taxonomic richness (17–19). Antimicrobial agents can cause a reduction in microbial diversity of intestinal and oral microbiota; subsequently, complete recovery of the initial bacterial community composition is rarely achieved (20, 21). Antimicrobial treatment has additional risks associated with it, including the selection of antibiotic-resistant strains of bacteria and the development of *Clostridium difficile* associated diarrhea (22). Due to dysbiosis with antibiotic treatment, the presence and expression of microbial genes

are altered (23). The impact on microbial diversity has a detrimental impact and may lead to decreases in SCFA production, vitamin production, and glycolysis, which may impact protection against pathogens (24, 25). Compared to antibiotics that can result in dysbiosis, probiotics are defined as live microorganisms that, in adequate amounts, provide health benefits to the host: supporting a healthy digestive tract and a healthy immune system (26, 27). Normal intestinal microbiota of various mammals contains *Bifidobacteria* and *Lactobacilli,* which are well-characterized species and widely used in commercialized probiotics (9). For example, *Lactobacillus rhamnosus* GG (LGG; ATCC 53103) has shown to be effective in controlling erythromycin-induced diarrhea when administered in yogurt (28).

It is critical that probiotics are manufactured in a reproducible manner, not only in terms of delivery but protection technologies, too (29). The Food and Drug Association (FDA) and European Food Safety Authority (EFSA) have not approved probiotics for use in health claims. As the probiotic market is increasing across the world, there is a need further investigation for probiotic efficacy (3). There are risks of using probiotics without consulting a physician since there is no clinical evidence showing benefits in immunocompromised patients with gut issues (30). Serious infections with probiotic strains of *Lactobacillus* are very uncommon, though *Lactobacillus rhamnosus* bacteremia (presence in the blood) is an emerging clinical entity (30, 31). To be effective the probiotics strains should be able survive gastrointestinal digestive process. Probiotic study in healthy children has demonstrated presence and survival of *L. casei* for up to 3 days after consumption thus proving resistance from gastric juices, hydrolytic enzymes and bile salts (32). Administration of prebiotics (non-living, usually fibrous, compounds intended to "feed" the microbiota) can enhance beneficial effects by enhancing metabolic activity and growth of administered probiotics as well as the endogenous gut *Bifidobacteria* and *Lactobacilli* (33).

In our study, the viability and survival of the pediatric Lovebug probiotics with rich bacterial diversity containing LGG, *Bifidobacterium infantis, Bifidobacterium lactis, Lactobacillus reuteri, Bifidobacterium longum, Lactobacillus casei, Lactobacillus gasseri*, and *Lactobacillus paracasei* was investigated by mimicking the acid pH condition of the human upper GI tract. The study was initiated from a pediatric public health perspective to confirm the viability and survival through gut-like conditions to confer the benefits in toddlers as recommended by the manufacturers. Our study supported our hypothesis of survival with lower viability of the strains at acidic pH 2 than at pH 3-4.

RESULTS

To test the ability of probiotics strains in the Lovebug probiotic to survive under acidic conditions, we incubated the probiotics in degassed acidified 0.8% sodium chloride at various pH levels for 2 hours and measured the resulting Colony Forming Units (CFU) per 100 ml. These conditions simulated the transit time of food and probiotics through the infant gut. Sodium chloride was selected to prevent cell lysis, while the pH was to cover the acidic range of the stomach (pH 2–4) and a neutral pH of 7 was the control.

Probiotic survival was determined by counting the colonies after incubation. Two different colony morphologies were observed on the plates, as expected, from a mixed culture sample of *Bifidobacteria* and *Lactobacillus* species (**Figure 1**). Dilution plating on blood agar plates from 10^{-1} through 10^{-3} had too many colonies to count or referred to as too numerous to count (TNTC), but colony counts could be obtained on the more dilute plates at pH 7, 4, and 3 (**Table 1**). At pH 7, 4, and 3, the colony counts were taken from 10^{-4}–10^{-6}-fold dilution plates. Plates incubated at pH 2 had no live colonies after the 2 hr incubation on the 10^{-5} and 10^{-6} dilution plates. However, the colonies could be counted on the 10^{-3} and 10^{-4} dilution plates at pH 2 (**Table 1**). It was determined that the number of viable cells in the probiotic was $4.7x10^{10}$ CFU per dose, based on growth after incubation in pH 7 for 0 h, which was more than the manufacturer's count of $1.5x10^{10}$ CFU per dose. However, these figures are within the same order of magnitude and demonstrates that the manufacturer's claim that there are at least $1.5x10^{10}$ CFU per dose is accurate. Approximately 20–40% of the initial bacteria strains in the probiotic survived the 2-hr incubation at pH 7, 4, and 3 (**Figure 2**). We found the pH of the environment had a substantial influence on the survival rate of the bacteria in the probiotic, as can be seen by the less than 10% survival when incubated in pH of 2 for 2 hours (**Figure 2**).

DISCUSSION

Our study demonstrated probiotic survival was 20–40% at pH 3–4. Interestingly, incubating the probiotic at pH 7 also seemed to impact the survival of the bacteria strains with a little over 20% survival. Other studies have also reported to be around 20–40% for selected strains of *Bifidobacteria* and *Lactobacilli* (33). A pH 7 also seemed to impact the survival strains, which could be attributed to the fact that most *Lactobacillus* strains are acidophilic or aciduric in nature (34). *Bifidobacteria* sampling from the cecum in humans have shown that, when probiotics are given in fermented milk, they had a survival of 23.5% ±10.4% of the administered dose (35). While other studies have shown that *B. bifidum* and *L.*

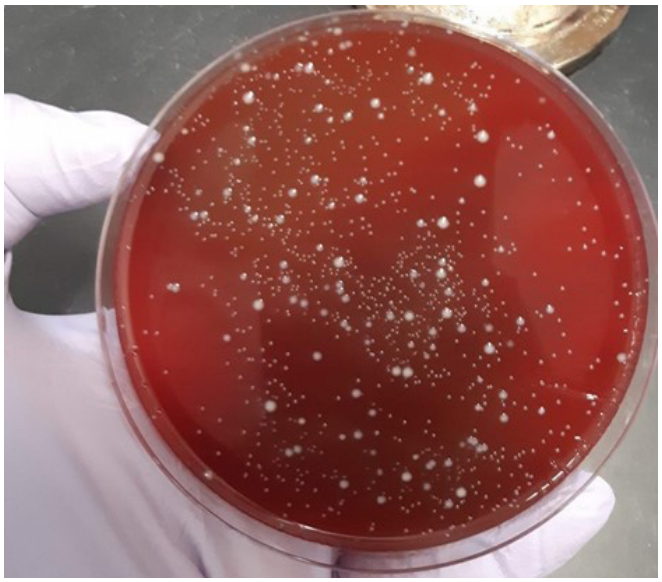

Figure 1: Representative FAA Plate. Colonies observed after Lovebug Toddler Probiotic was treated with 0.9% sodium chloride spread plated and incubated for 48 hrs at 37°C under anaerobic conditions are shown.

Incubation Time (h)	*pH*	*Colony count (CFU/mL) at respective dilutions*					
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
0	7	TNTC	TNTC	TNTC	6.4x10^7	1.4X10^8	1.2X10^9
2	7	TNTC	TNTC	TNTC	4.0X10^7	1.3X10^8	1.5X10^8
2	4	TNTC	TNTC	TNTC	1.4X10^7	9.1X10^7	5.5X10^8
2	3	TNTC	TNTC	TNTC	2.2X10^7	9.1X10^7	1.5X10^8
2	2	TNTC	TNTC	4.0X10^6	2.29X10^7	0	0

Table 1: CFU Counts at Different pHs. Lovebug Toddler Probiotic after incubation at various pH values under anaerobic conditions was plated and colonies counted. Control was a 0-hr incubation at pH 7. Following treatment, 1 mL of suspension was serially diluted in tryptic soy broth and plated on FAA supplemented with 5% sheep's blood. Colonies were counted after a 48-hr incubation at 37ºC under anaerobic conditions. TNTC: Too numerous to count.

acidophilus delivery to the cecum was 30% and 10% of the administered dose, respectively (36). This study supports our hypothesis that the viability of the bacteria strains is lower in acidic environments with a pH 2 than at neutral pH 7. The beneficial effects of probiotics in influencing intestinal ecosystems support their survival capabilities in the gut (1). Our results, along with the customer reviews for lovebug, also support the hypothesis that *Lactobacillus* and *Bifidobacterial* strains survived the gut environment after ingestion. The details on the species-level survival of LGG*, B. infantis, B. lactis, L. reuteri, B. longum, L. casei, L. gasseri,* and *L. paracasei* were beyond the scope of this study.

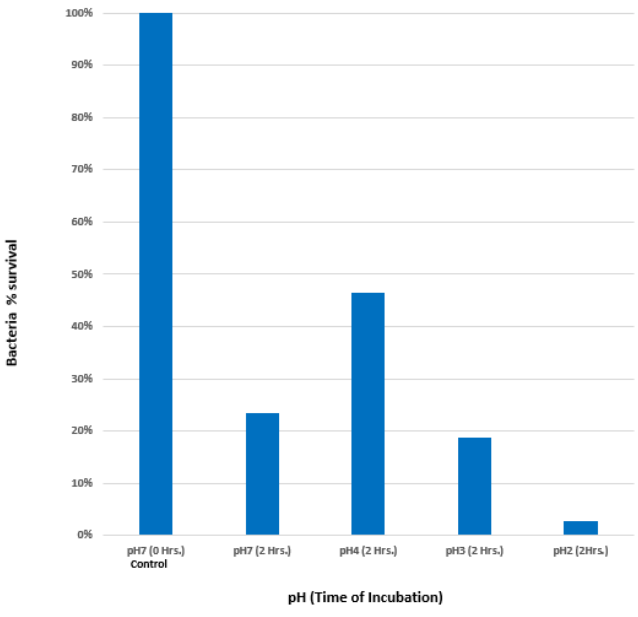

Figure 2: Incubation was at various pH values (2, 3, 4, 7) under anaerobic conditions for 2 hrs. Following treatment, 1 mL of suspension was serially diluted in tryptic soy broth and plated on FAA supplemented with 5% sheep's blood. Colonies were counted after a 48-hr incubation at 37ºC under anaerobic conditions, and the average CFU/100 mL of each suspension was calculated. All treatment values have been normalized to the control, which was a 0-hr incubation at pH 7.

The study demonstrates that bacteria in the Lovebug probiotic survive in acidic conditions like the gut environment, which could account for Lovebug probiotics' positive customer reviews about its desired effect (**Appendix 1**). While our experiment does show some of the bacteria survive acidic conditions, there is a reduction in percent survival that supports our hypothesis. The bacteria survival at pH 7 was also low, and this could be attributed to acidophilic nature of the bacteria instead of being neutrophilic. Static experiments have shown that *Bifidobacterium* spp. and *L. acidophilus* are more acid-resistant than are *L. bulgaricus* and *S. thermophilus* (37). This could be attributed to the fact that *Lactobacillus* strains, which constitute the majority of strains in the probiotic, are acidophilic or aciduric in nature and prefer acidic environment (34). Our study demonstrated total survival rates comparable with what has been observed previously. The experiment could be repeated at pH 5 and 6 to further support the observation that pH impacts bacterial survival. Additional time intervals could be added to the experiment to generate a more fine-scale timeline of survivability for the strains in this toddler probiotic. The main obstacle to survival of the strains is gastric acidity. Viability depended on the pH, length of the exposure to acid, and bacteria species and strain. Probiotic survival in the small intestine is impacted by presence of bile salts, which are known to cause cell lysis (33). However, for the purpose of this study we focused on pH. Additionally, testing different concentrations of bile acid could be done to assess the impact of bile acids on infant probiotics.

Our study took into consideration gastric passage time, and hence, a 2-hour incubation time was selected. In the absence of exposure to bile, our study aligns with the results of the low bile with 30–40% survival where delivery of *B. bifidum* and *L. acidophilus* to the cecum was 20% and 10%, respectively. However, it has been shown that in the presence of physiologic bile salt concentrations that can hydrolase bile the delivery percentages were 50% and 30%, respectively

(33, 36). *Lactobacillus* and *Bifidobacterium* long-term gut colonization potential have shown that some strains pass through, but others colonize the gut permanently. Studies have shown that strains that stably engraft in the gut exert beneficial effects on the host by increasing the efficiency of metabolic activity. *Lactobacillus* and *Bifidobacterium* species, are likely to stably colonize in the gut, based on their history *B. longum* appears to be an exemplary species with long-term colonization potential. More studies are needed to select or tailor probiotic strains with long-term gut colonization ability in a rational manner however *Lactobacillus* species (*L. rhamnosus)* have proven to stably flourish in the gut (38). Both *B. longum* and *L. rhamnosus* were in the Lovebug probiotic. Our study observed the presence and viability of *Lactobacillus* and *Bifidobacterium* in the Lovebug probiotic based on two different morphologies. However, based on the colony morphology on the plate the species survival cannot be determined. Studies to identify the survival of difference species could also be carried out using 16S rRNA sequencing. Using this technique Yang *et al*. have demonstrated the abundances of the phyla in the gut (13).

We demonstrated that pH reduced the percentage of surviving bacteria, however future studies should address methods to increase survivability. Strain selection has generally been based on *in vitro* tolerance of physiologically relevant stresses (e.g., low pH, elevated osmolarity and bile) (39). Physiologically stresses like low pH, elevated osmolarity, and bile have been used *in vitro* to select tolerant strains. Selection of optimal culture medium and cell protectants is also crucial to ensure the efficacy of the probiotic product. Microencapsulation can protect probiotic bacteria and has been proposed to improve the stability of the strains which can adapt to the GIT conditions (40). It is critical to ensure optimal culture medium and cell protectants for the efficacy of probiotic product. Microencapsulation also protects probiotic strains and can improve the stability in the gut environment

MATERIAL AND METHODS

Probiotic

Lovebug Toddler Probiotics for ages 12 months to 4 years containing 15 billion CFU of *L. rhamnosus* GG*, B. infantis, B. lactis, L. reuteri, B. longum, L. casei, L. gasseri,* and *L. paracasei* were purchased from Amazon (Appendix 1). Fastidious anaerobic agar (FAA) (Lansing, MI, USA) supplemented with 5% sheep's blood was used for cultivation. Degassed 0.9% sodium chloride was used for resuspension of the probiotic and pH was adjusted using HCl. Tryptic soy broth (Millipore Sigma, Burlington, MA, USA) was used for the serial dilutions.

Survival Assay and Colony Counting

In an anaerobic chamber (AS-580, Anaerobe Systems, Morgan Hill, CA, USA) under anaerobic conditions (10% H_2, 10% CO_2, balanced with N_2), one sachet (1.5 g) of Lovebug Toddler Probiotic was added to 100 mL of degassed acidified 0.9% sodium chloride at various pH values (2, 3, 4, 7). The suspension was incubated at 37ºC for 2 hrs, and the control was at pH 7 incubated for 0 hr. Serial dilutions from 10^{-1} to 10^{-10} were made in tryptic soy broth, and 0.1 mL of each dilution was spread/plated on FAA supplemented with 5% sheep's blood. FAA plates were then incubated at 37ºC for 48 hours until colonies were visible and conducive to counting. Plates with CFU counts between 30–300 colonies were counted. The plating was done in triplicates and averaged for the final count.

Data Analysis

Colony counts were used to calculate the CFU/mL present before and after treatment with acidified 0.9% sodium chloride at various pH values (2, 3, 4, 7). The recorded number of colonies was multiplied by the dilution factor and divided by the volume plated. The CFU/100 mL was calculated, then the average across a given treatment was calculated. Graph was plotted as percent survival with the Control pH 7, 0 hr as 100%.

APPENDIX

Source of Lovebug probiotic:
www.amazon.com/dp/B01HLSK5NA?ref=nb_sb_ss_w_as-reorder-t1_ypp_rep_k0_1_7&amp&crid=1LHWA1R2UTEAT&amp&sprefix=lovebug

ACKNOWLEDGMENTS

We would like to express our sincere gratitude to Dr. Emma Allen-Vercoe in the Department of Molecular and Cellular Biology at the University of Guelph, Ontario for graciously providing her lab to conduct the experiment. We also want to thank Mrs. Mathew and the Science club at Ursuline Academy in Dedham, MA who supported us during the MA Science Fair application process.

Received: April 24, 2022
Accepted: May 23, 2022
Published: December 29, 2022

REFERENCES

1. Hooper, Laura and Jeffrey Gordon. "Commensal Host Bacterial Relationships in the Gut". *Science*, vol. 11, no. 292(5519), 2011, pp.1115–8, doi: 10.1126/science.1058709.
2. Maslowski, Kendle M and Mackay, Charles. "Diet, Gut Microbiota and Immune Responses". *Nature Immunology*, vol.12 (1), 2011, pp. 5–9, doi:10.1038/ni0111-5.
3. Hage, Racha El, *et al*. "Emerging Trends in "Smart Probiotics"**:** Functional Consideration for the Development of Novel Health and Industrial Applications". *Frontiers in Microbiology*, vol. 8, 2017, Sep 29, pp.1889, doi:10.3389/fmicb.2017.01889
4. Ihekweazu, Faith. D and Versalovic, James. **"**Development of the Pediatric Gut Microbiome: Impact on Health and Disease". *The American. Journal of Medical Sciences*, vol. 356 (5), 2018, pp. 413–423, doi:10.1016/j.amjms.2018.08.005
5. Costello, Elizabeth K, *et al*. "Bacterial Community Variation in Human Body Habitats Across Space and Time". *Science*, vol. 326 (5960), 2009, pp. 1694–1697, doi: 10.1126/science.1177486.
6. Ley, Ruth E, *et al*. "Ecological and Evolutionary Forces Shaping Microbial Diversity in the Human Intestine". *Cell*, vol. 124(4), 2006, pp. 837–848, doi: 10.1016/j.cell.2006.02.017
7. Spor, Ayme, *et al*. "Unravelling the Effects of the Environment and Host Genotype on the Gut Microbiome". *Nature Reviews Microbiology*, vol. 9(4), 2011, pp. 279–

290, Doi: 10.1038/nrmicro2540
8. Johnson, Coreen. L and Versalovic, James, "The Human Microbiome and Its Potential Importance to Pediatrics". *Pediatrics*, vol. 129(5), 2012, pp. 950–960, doi:10.1542/peds.2011-2736
9. Vlasova, Anastasia, *et al*. "Comparison of probiotic Lactobacilli and Bifidobacteria Effects, Immune Responses and Rotavirus Vaccines and Infection in Different Host Species". *Veterinary Immunology and Immunopathology*, vol.172, 2016, pp.72–84, doi: 10.1016/j.vetimm.2016.01.003
10. Wells, Jerry M. "Immunomodulatory Mechanisms of Lactobacilli". *Microbial Cell Factories*, vol. 10 (Suppl 1), 2011, S17, doi: 10.1186/1475-2859-10-S1-S17.
11. Tojo, Rafael, *et al*. "Intestinal Microbiota in Health and Disease: Role of Bifidobacteria in Gut Homeostasis". *World Journal of Gastroenterology*, vol. 20, 2014, pp.15163–15176, doi:10.3748/wjg.v20.i41.15163
12. Rajilić-Stojanović, Mirjana and de Vo s, Willem. "The First 1000 Cultured Species of the Human Gastrointestinal Microbiota". *FEMS Microbiology Reviews*, vol. 38(5) 2014, pp. 996–1047, doi: 10.1111/1574-6976.12075.
13. Yang, Xing, *et al*. "More than 9,000,000 Unique Genes in Human Gut Bacterial Community: Estimating Gene Numbers Inside a Human Body". *PLoS ONE,* vol. 4, Issue 6, 2009, doi: 10.1371/journal.pone.0006074
14. He, Fang. "Comparison of Mucosal Adhesion and Species Identification of Bifidobacteria Isolated from Healthy and Allergic Infants". *FEMS Immunology and Medical Microbiology*, vol. 30, 2001, pp. 43–47, doi: 10.1111/j.1574-695X.2001.tb01548.x
15. Gerritsen, Jacoline, *et al*. "Intestinal Microbiota in Human Health and Disease: The Impact of Probiotics". *Genes and Nutrition*. vol. 6, 2011, pp. 209–240, doi: 10.1007/s12263-011-0229-7.
16. Shane, Andi L. "Applications of probiotics for neonatal enteric diseases". *Journal of Perinatal and Neonatal Nursing,* 22(3), 2008, pp. 238–243, doi:10.1097/01.JPN.0000333926.30328.26
17. Langdon, Amy, *et al*. "The Effects of Antibiotics on the Microbiome Throughout Development and Alternative Approaches for Therapeutic Modulation". *Genome Medicine*, vol. 8, 2016, pp. 39. doi:10.1186/s13073-016-0294-z
18. Dethlefsen, Les and Relman, David A. "Incomplete Recovery and Individualized Responses of the Human Distal Gut Microbiota to Repeated Antibiotic Perturbation". *Proceedings of the National Academy of Science of the U.S.A,* vol. 108 (Suppl.1), 16 Sep. 2010, pp. 4554–4561, doi:10.1073/pnas.1000087107
19. Francino, M. P. "Antibiotics and the Human Gut Microbiome: Dysbiosis and Accumulation of Resistances". *Frontiers in Microbiology*, vol. 6, 2016, pp. 1543, doi:10.3389/fmicb.2015. 01543
20. Jakobsson, Hedwig E, *et al*. "Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome". *PLoS ONE*, vol. 5 Issue 3, 2012, pp. 9836, doi: 10.1371/journal.pone.0009836
21. Jernberg, Cecilia, *et al*. "Long-term impacts of antibiotic exposure on the human intestinal microbiota". *Microbiology*, vol. 156 (11), 2010, pp. 3216–3223, doi:10.1099/mic.0.040618-0.
22. Chang, Ju Young, *et al*. "Decreased Diversity of the Fecal Microbiome in Recurrent *Clostridium difficile*-Associated Diarrhea." *The Journal of Infectious Diseases*, vol. 197, no. 3, 1 Feb. 2008, pp. 435–438, doi: 10.1086/525047
23. Reijnders, Dorien. *et a*l. "Effects of Gut Microbiota Manipulation by Antibiotics on Host Metabolism in Obese Humans: A Randomized Double-Blind Place Controlled Trial". *Cell Metabolism.*, vol. 24, 2016, pp. 63–74, doi: 10.1016/j.cmet.2016.06.016.
24. Guarner, Francisco, and Malagelada, Juan R. "Gut Flora in Health and Disease". *Lancet*, vol. 361, 2003, pp. 512–519, doi:10.1016/S0140-6736(03)12489-0
25. Francino, M. P. "Antibiotics and the Human Gut Microbiome: Dysbiosis and Accumulation of Resistances". *Frontiers in Microbiology*, vol. 6, 2016, pp. 1543, doi:10.3389/fmicb.2015. 01543
26. WHO/FAO (2006). Probiotics in Food: Health and Nutritional Properties and Guidelines for Evaluation. Rome: Food and Agriculture Organization of the United Nations.
27. Hill, Colin, *et al*. "The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotics". *Nature Reviews Gastroenterology and Hepatology,* vol. 11, 2014, pp. 506–514, doi: 10.1038/nrgastro.2014.66
28. Dunn, Stephen R, *et al*. "Effect of Oral Administration of Freeze-dried Lactobacillus acidophilus on Small Bowel Bacterial Overgrowth in Patients with End-stage Kidney Disease: Reducing Uremic Toxins and Improving Nutrition". *International Dairy Journal*, vol. 8, 1998, pp.545–53 doi**:** 10.1016/s0958-6946(98)00081-8
29. Sousa e Silva, J Paulo and Freitas, Ana Cristina. Probiotic Bacteria, 1st Edn. Singapore: Pan Stanford Publishing. 2014, doi:10.1201/b15676
30. Vahabnezhad, Elaheh, *et al*. "Lactobacillus Bacteremia Associated With Probiotic Use in a Pediatric Patient With Ulcerative Colitis". *Clinical Gastroenterology,* vol. 47(5), 2013, pp. 437-439, doi: 10.1097/MCG.0b013e318279abf0
31. Gouriet, F, *et al*. "L*actobacillus rhamnosus* Bacteremia: An Emerging Clinical Entity". *European Journal of Clinical Microbiology & Infectious Diseases,* vol. 31, 2012, pp. 2469–2480, doi:10.1007/s10096-012-1599-5
32. Radicioni, Milko, *et al*. "Survival of L. casei DG® (*Lactobacillus paracasei* CNCMI1572) in the Gastrointestinal Tract of a Healthy Pediatric Population". *European Journal of Nutrition,* vol. 58, 2019, pp. 3161–3170, doi:10.1007/s00394-018-1860-5.
33. Bezkorovainy, Anatoly. "Probiotics: Determinants of Survival and Growth in the Gut." *The American Journal of Clinical Nutrition*, vol. 73 (2), 2001, pp. 399–405, doi:10.1093/ajcn/73.2.399s.
34. Tannock, Gerald W. "Special Fondness for Lactobacilli". *Applied and Environmental Microbiology*, vol. 70, 2004, pp. 3189–3194, doi: 10.1128/AEM.70.6.3189–3194.2004
35. Pochart, P, *et al*. "Survival of Bifidobacteria Ingested via Fermented Milk during their Passage through the Human Small Intestine: an in Vivo Study Using Intestinal Perfusion". *The American Journal Clinical Nutrition,* vol. 55, 1992, pp.78–80, doi.org/ 10.1093/ajcn/55.1.78.
36. Marteau, P, *et al*. "Survival of Lactic Acid Bacteria in a Dynamic Model of the Stomach and Small Intestine: Validation and the Effects of Bile". *Journal of Dairy*

Science, vol. 80, 1997, pp. 1031–1037, doi: 10.3168/jds.S0022-0302(97)76027-2.
37. Lindwall, S., and Fonde'n, R. "Passage and survival of Lactobacillus acidophilus in the human gastrointestinal tract." *IDF Bulletin'* International. Dairy Federation, Brussels, Belgium, vol. 21. 1984, pp. 179.
38. Xiao, Yue, *et al*. "Mining Lactobacillus and Bifidobacterium for organisms withlong-term gut colonization potential". *Clinical Nutrition*, Vol. 39 (5) 2020, pp. 1315-1323, doi: 10.1099/mgen.0.000581
39. Tuomola, E, *et al*. "Quality Assurance Criteria for Probiotic Bacteria". American Journal of Clinical Nutrition. vol.73(2). 2001, 393S-398S, doi:10.1093/ajcn/73.2.393s
40. Heidebach, Thomas, *et al*. "Microencapsulation of Probiotic Cells for Food Applications". *Critical Reviews in Food Science Nutrition*, vol. 52, 2012, pp.291–311 doi/org 10.1080/10408398.2010.499801