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The Effect of Probiotics, Prebiotics, and Synbiotics on Indicators of Lactose Intolerance: A Systematic Review

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THE EFFECT OF PROBIOTICS, PREBIOTICS, AND SYNBIOTICS ON INDICATORS OF LACTOSE INTOLERANCE: A SYSTEMATIC REVIEW

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by

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ABSTRACT

THE EFFECT OF PROBIOTICS, PREBIOTICS, AND SYNBIOTICS ON INDICATORS OF LACTOSE INTOLERANCE: A SYSTEMATIC REVIEW

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Abstract

Lactose intolerance disproportionately affects racial minority groups in the United States, increasing the incidence of calcium deficiency and low bone mineral density in these populations. The nutritional quality of lactose-containing food products incentivizes the investigation of long-term treatment options for lactose intolerance. Modifying the gut microbiome to increase the quantity of lactose-hydrolyzing bacteria in the intestines is a promising avenue of treatment that merits investigation. Such modification is typically achieved via consumption of probiotics, prebiotics, or synbiotics in various forms. This systematic review examined 25 studies measuring outcomes of lactose intolerance in subjects given probiotic, prebiotic, or synbiotic treatments. Bacterial strains with the greatest degree of evidence for the reduction of undesirable outcomes of lactose intolerance were *Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus delbrueckii* subspecies *bulgaricus,* and/or *Streptococcus thermophilus*. Inoculated dairy products also showed strong evidence for the attenuation of lactose intolerance outcomes.

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CHAPTER I
INTRODUCTION

Lactose intolerance is a universal human concern that disproportionately affects several minority groups in the United States, adversely impacting the incidence of calcium deficiency and low bone mineral density in these populations. The high nutritional quality of many lactose-containing food products incentivizes the investigation of effective long-term treatment options for lactose intolerance. Modifying the gut microbiome to increase the quantity of lactose-fermenting bacteria in the intestines is a promising avenue of treatment that merits investigation.

Probiotics and modifications to the gut microbiome have gained support in contemporary research due to the specificity, longevity, and associated health benefits of the microbial treatment options available. The emerging field of synbiotics investigates the effectiveness of pairing probiotic bacteria with supplements that are intended to encourage proliferation of these lactose-hydrolyzing bacteria in the intestines. Immense variation is possible in the particular probiotic/prebiotic combination, time-course of treatment, strain, colony-forming units (CFUs), and method of probiotic encapsulation used in these studies. Emerging evidence suggests that probiotics, prebiotics, and synbiotic combinations may be effective at alleviating the symptoms of lactose intolerance, but a thorough statistical analysis of available evidence is necessary to determine whether these treatments might be effective enough to influence contemporary dietetic practices or recommendations.

Purpose Statement: The purpose of this systematic review is to generate a summative evaluation of studies that examine the association between probiotic, prebiotic, and synbiotic treatments and a reduction in lactose intolerance indicators, with an emphasis on species and strain.

CHAPTER II
LITERATURE REVIEW

Lactose Intolerance

Summary and Health Implications: Lactose intolerance is the inability or, more commonly, the reduced ability to digest the milk sugar lactose. Ideally, lactose is either hydrolyzed by the endogenous lactase enzyme that is produced in the brush border of the small intestine or it is digested by bacteria in the gut microbiome that produce similar beta-galactosidase enzymes.[1] When lactose is not hydrolyzed by the consumer nor processed by bacteria in the small intestine, common symptoms are flatulence, diarrhea, abdominal pain, cramping, bloating, and nausea.[1,2,3] Interestingly, symptoms unconnected to the digestive tract have also been observed, including eczema, fatigue, headaches, sinusitis, and cardiac arrhythmia.[1]

These symptoms are understandably upsetting for the consumer, and they can promote avoidance of lactose-containing products. This moratorium can be difficult to maintain given the ubiquity and popularity of milk-based beverages, coffee creamers, chocolates, cheeses, butter, ice cream, and even lactose-based sweeteners used in food manufacturing. Successful avoidance of such products by lactose-intolerant individuals is associated with increased rates of osteoporosis, osteopenia, and low bone mineral density.[2,3] These effects are ostensibly due to insufficient calcium consumption. This illustrates the connection between lactose intolerance and critical measures of human health.

History and Demographics: Lactase enzyme production into adulthood, also called lactase persistence, is an evolutionarily new development. Soon after agrarian and pastoral Neolithic societies began to flourish in Europe between 8500 and 6000 years ago, a beneficial mutation called C/T-13910 emerged in these populations, promoting increased rates of lactase persistence

in Northern Europeans.[4] This genetic bias toward lactase persistence in adulthood still exists today, as evidenced by lactose intolerance prevalence that is comparatively low (14-28%) among central and western Europeans and extremely high in Asian Americans (approximately 80-90%), African-Americans (75-90%), and indigenous Americans (nearly 100%).[3,5] Regional prevalence of lactase persistence is another consideration, as some endogamous or otherwise genetically homogenous populations can have very consistent and extreme rates of lactase persistence. In 2005, Paul Sherman and his research team[5] discovered a 2% prevalence of lactose intolerance in a sample of Denmark citizens and a 100% prevalence of lactose intolerance in a sample taken from Zambia. Providing an effective means of promoting lactose digestion in populations lacking lactase persistence may increase dairy product consumption, attenuate calcium deficiency, and improve bone mineral density. This is especially critical for traditionally marginalized populations who, in addition to having greater prevalence of lactose intolerance, also may have reduced access to affordable healthcare or may lack health insurance entirely.[6]

Available Treatments: The most common treatment for lactose intolerance that does not require dietary restriction is ingestion of exogenous lactase enzyme. Lactase enzyme products contain beta-galactosidases in concentrations that will vary between manufacturers but tend to have between 3000 and 6000 IU of active lactase enzyme. These pills, capsules, or tablets are intended to be consumed immediately before a meal and are regarded as effective at hydrolyzing consumed lactose.[7] In key studies of the effectiveness of exogenous lactase enzyme consumption, both gastrointestinal distress symptoms and breath hydrogen values were significantly reduced in treatment groups of various ages following a 25g lactose bolus.[7,8] Similar commercial lactase products, whether in pill, capsule, or chewable form, were not as effective at hydrolyzing a larger bolus of 50g lactose,[8] though it is unlikely for a typical person to

consume such a high dose of lactose in one sitting. Limitations of lactase enzyme consumption include the narrow timeframe in which the product will function[6,7] and the requirement that the lactase be consumed before every lactose-containing meal. Attenuating lactose intolerance via adjustments to the gut microbiome is another potentially viable treatment option. While much is known about the requisite microbiology, contemporary studies attempting to investigate this avenue of treatment tend to have mixed results, and no robust meta-analyses of contemporary data have been performed. Consequently, there is not yet sufficient evidence as to whether a specific probiotic, prebiotic, or synbiotic (see Table 1) treatment is effective in reducing lactose intolerance symptoms.

Table 1. Glossary of Terms

Probiotics	Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host[9]
Prebiotic	A substrate that is selectively utilized by host microorganisms conferring a health benefit[9]
Synbiotics	A mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host[10]
Microbiome	A characteristic microbial community occupying a reasonable well-defined habitat which has distinct physio-chemical properties. This community is integrated in macro-ecosystems including eukaryotic hosts, and it can be crucial for the host's functioning and health[11]
Microbiota	The assemblage of living microorganisms present in a defined environment[11]
Dysbiosis	An imbalance in the composition and functional capacity of the host microbiota[12]
Metagenome	The collection of genomes and genes from the members of (in this context) the host microbiota[13]

The Gut Microbiome as a Target of Lactose Intolerance Intervention

Measuring Alterations in the Gut Microbiome: Experimentally researching the gut microbiome is a laborious and imprecise endeavor. To establish precise causal relationships between bacteria

and human health, pure axenic cultures of the bacteria must be made, their effects on a host must be observed, and their biological roles in the human body must be ascertained from this isolated data. These data are still limited in their external validity because each bacterium lives amid millions of immediate bacterial neighbors in the small intestine, and each study participant's gut microbiome composition will be different. The metabolism of any given bacterium will also vary based on the host's nutrition, stool consistency, drug treatments, hydration status, stress level, and other factors.[14]

The goal of a probiotic and/or prebiotic intervention, then, is to promote a change in the gut microbiome of a sufficient magnitude to result in an observable change in the host via stool samples, breath composition tests, lactose tolerance tests, or perceived symptoms of gastrointestinal distress over time.[14,15] Methods to quantify and describe gut microbiome composition via stool samples or biopsied small intestine samples include metagenomics and the complementary approach of culturomics.[16] Metagenomics is the practice or field of study concerned with the analysis and categorization of a microbial metagenome via extraction of nucleic acids from microbial samples, either via shotgun sequencing of the whole DNA sample or via polymerase chain reaction and subsequent 16s rRNA sequencing.[16,17,18] The results are then compared to an expanding database of 16s rRNA gene sequences and more broad genomic profiles intended for shotgun sequencing such as those catalogued by NCBI's Gene Expression Omnibus (GEO),[19] GenBank,[20] and the Genomics OnLine Database (GOLD).[21] There is some contention as to the terminology used in this field; some researchers consider 16s rRNA to be a type of targeted metagenomics and shotgun sequencing to be shotgun metagenomics,[17] while others do not consider 16s rRNA sequencing to be an application of metagenomics at all.[18] In either case, analyzing the genomic profile of a subject's intestinal bacteria can provide

information about probiotic treatment outcomes, at least in terms of colonization. There are drawbacks to this approach, however. A sufficient quantity of high-quality nucleic acid samples are required for metagenomic analysis, and the quality of samples may vary between extraction methods.[17,18] There is also some difficulty achieving sufficient sequence coverage for minority bacterial populations, so metagenomic analyses are not always sensitive enough to detect bacteria that are present in small quantities due to this "depth bias".[17,22] Sorting genes of interest into orthologous groups during a metagenomic analysis can help researchers avoid some of these issues and glean useful data about the subject's gut microbiome composition, as demonstrated in 2011 by M. Arumugam et al.[23] Still, depth bias presents identification issues, as does the amount of "microbial dark matter", or unassigned gene sequences, yet to be described and identified by metagenomics.[16] This highlights the benefit of performing a mixed-methods analysis of fecal samples. Such mixed-methods analyses may include elements of both metagenomics and culturomics.

Culturomics is the field of study concerned with culturing microorganisms from a bacterial or fungal sample in order to collect data about the physical, chemical, and metabolic properties of an organism or of the organisms in a microbiome. Methods of bacterial identification and description intrinsic to culturomics include liquid and solid culture media, gram staining, fluorescent molecule staining, electron microscopy, carbohydrate utilization tests, enzyme function tests, and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS).[16,17,22,24] MALDI-TOF MS is a method of bacterial identification valued for its speed and cost-effectiveness[15] and it is sometimes preferred over identification via 16S rRNA sequencing.[25] Through culturomics, researchers have been able to expand knowledge of the human gut microbiome, reduce the breadth of microbial dark matter,

and gradually refine and optimize ideal culture conditions for the growth of previously uncultured species.[16,22,26]

The efficacy of the synthesis of metagenomics and culturomics has been demonstrated in a study of dysbiosis in *Clostridium difficile* infection in which the researchers cultured 112 new bacterial species[27] and observed little overlap between species detected by metagenomics and those detected by culturomics. A study examining fungal populations in the human gut mycobiome (fungal microbiome) observed the same small overlap between species detected by metagenomic methods as compared to culturomics,[28] reinforcing the notion that a mixed-methods approach provides more detail about the gut microbiome. Studies investigating the effect of probiotics and prebiotics may obtain broader outcome data by examining subjects' stool samples before and after treatment using both metagenomics and culturomics-based approaches. Researchers may also benefit from utilizing these approaches with samples taken from the small intestine, where lactase production and carbohydrate hydrolysis ordinarily take place. In this review, the methodology by which researchers analyze and describe the outcomes of probiotic and prebiotic treatment will be considered, with an emphasis on conclusions derived from mixed-methods analyses.

Hydrogen breath tests, lactose tolerance tests, and subjective measures of gastrointestinal symptoms of maldigestion are more consistent measuring tools for bacterial activity than stool samples; measurements can be taken repeatedly over a span of multiple hours and can be used to ascertain both baseline and endpoint values.[29] These outcome variables, in contrast to metagenomic and culturomic data, are frequently available in the existing literature and were additional outcomes of interest in this review.

Mechanisms of Action of Probiotic Bacteria

Overview: The wide genotypic and phenotypic diversity of the human gut microbiome gives probiotics manufacturers a rich bevy of candidate species to choose from when developing products, and each species or combination of species may have different avenues through which they improve human health. The exact number of species present in the gut microbiome is unknown and is the subject of contemporary research. In early 2019, a research team from the Wellcome Genome Campus and Centre for Innate Immunity and Infectious Diseases identified 2,505 total intestinal microbial species, including 1,952 uncultured species, using shotgun sequencing and nucleotide frequency analysis data from 75 different studies to construct metagenome-assembled genomes (MAGs).[30] While this gives some idea of the breadth of species in the gut microbiome, it might be more practical to look at species that are shared within-groups to identify a core representative human gut microbiome composition. In the fall of 2019, a research team based out of George Washington University published an analysis of 98 fecal samples from healthy subjects, identifying 155 different bacterial organisms and a contingent of 84 species that were present in all samples.[31] The majority (79.7%) of the 155 species identified belonged to the phyla Firmicutes, Actinobacteria, and Bacteroidetes. This finding is consistent with the taxa of common probiotic candidates, including various species of the genus *Bifidobacterium* from the phylum Actinobacteria and various species of the recently-reclassified clade of *Lactobacillus* from the phylum Firmicutes.[32,33] The diversity of probiotic species is reflective of the variety of benefits that these probiotics are intended to have for their hosts. Benefits of probiotics that pertain to attenuation of lactose intolerance outcomes include competitive exclusion of potentially pathogenic bacteria, immune modulation, and the production of beta-galactosidase enzyme.[34,35]

Competitive Exclusion and Growth Inhibition of Pathogenic Bacteria: Organisms of different species that occupy the same ecological niche tend to compete for the limited resources within that niche, and gut microbes are no exception. Through hydrophobic interactions with the host's intestinal lining, acidification of the luminal environment, and the production of specialized proteins, probiotic bacteria are able to adhere to the gastrointestinal tract and prevent the adhesion of pathogenic bacteria.[35] In this way, probiotic bacteria have been shown to inhibit the intestinal cell adhesion and colonization of *Salmonella typhimurium*, *Clostridium sporogenes*, and *Enterococcus faecalis.*[36] *Lactobacillus* and *Bifidobacterium* species are particularly good candidates for promoting competitive exclusion due to the mucin-binding proteins and fibronectin-binding proteins that are characteristic of these species, a prime example of which is *Lactobacillus rhamnosus*, which has a mucin-binding protein on the tips of its pili.[37] Recently, it has also been proposed that probiotic bacteria may interfere with a type of communication called quorum sensing among pathogenic bacteria by inhibiting and degrading signaling molecules in the human gastrointestinal tract.[38] This mechanism of pathogenic inhibition has shown efficacy in reducing the population of pathogenic *Aeromonas hydrophila* in the intestines of goldfish.[39]

Aside from adhering to the intestinal wall and facilitating the interruption of quorum sensing, some probiotic bacteria also practice competitive exclusion by producing antimicrobial proteins called bacteriocins.[34,36] A prime example is the production of plantaricin by *Lactobacillus plantarum*; plantaricin is deadly to many bacteria associated with foodborne illness, including multiple species of the genera *Staphylococcus* and *Listeria.*[40] Similarly, Lactococcus lactis produces the bacteriocin nisin, which has been shown to be antagonistic to the gram-positive bacteria *Bacillus cereus* and *Staphylococcus aureus*, as well as the gram-negative bacterium *Salmonella thyphimurium.*[41] This is of particular interest because gram-negative

bacteria are typically resistant to the effects of bacteriocins.[42] Probiotic bacteria have a wide variety of avenues through which they might practice competitive exclusion. Contemporary research of these antimicrobial and anti-pathogenic properties of probiotics make them a promising treatment candidate for lactose intolerance, as they may confer some benefit against foodborne illness in addition to improving the digestibility of lactose-containing products.

Immune Modulation: In addition to the competitive exclusion of potentially pathogenic bacteria, some probiotic bacteria have been shown to affect the host's immune system. The same mechanism of adhesion to intestinal epithelial cells described above allows probiotic bacteria to remain adjacent to the glycocalyces of intestinal epithelial cells, where microbial metabolites, signaling proteins, and cell-surface molecular patterns exert an influence on specialized pattern recognition receptors (PRRs), including toll-like receptors.[43] Responses of the intestinal epithelium and the immune cells of the lamina propria include the reinforcement of tight junctions, the stimulation of dendritic cells, and the production of interleukins and cyclooxygenases. This innate immune response has been shown to prime adaptive immune responses by modulating CD4 T-cell generation and activity.[44] For example, a dose of 5E9 CFUs of *Lactobacillus casei* fed to Lewis rats 3 times per weeks for several months resulted in a statistically significant reduction in $CD4^+$ T-cells responsible for producing tumor necrosis factor alpha (TNF-α) and a statistically significant increase in $CD4^+$ T-cells responsible for producing the anti-inflammatory cytokine interleukin-10.[45] The rats in this treatment group experienced significantly fewer clinical signs of collagen-induced arthritis, including paw swelling, which indicates a systemic effect on inflammatory response that was not localized to the intestines.

A later study investigated the effect of a combined probiotic containing *Bifidobacterium bifidum*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus reuteri*, and *Streptococcus*

thermophilus on intestinal inflammation in BALB/c mice. Numerous in vivo and ex vivo experiments were performed, and the research team discovered that administration of 5E8 CFUs/day of the combined probiotic over 20 days induced $CD11c^+$ regulatory dendritic cells, which generated $CD4^+Fox3^+$ regulatory T-cells that had an anti-inflammatory effect on the intestinal cells of mice with inflammatory bowel disease.[46] This research is promising in that it shows a pronounced effect on immune and inflammatory responses from a relatively short course of probiotic treatment, and it reinforces the fact that probiotics have varied and diverse effects on the gastrointestinal tract.

The wide variety of these effects necessitates caution when interpreting the results of lactose intolerance interventions. In 2009, an exceptionally thorough systematic review revealed that probiotics can have an effect on lactose intolerance symptoms such as diarrhea and colitis in either the presence or absence of lactose intolerance.[47] In short, some probiotics which are hypothesized to alleviate lactose intolerance symptoms may do so through mechanisms that have little, if anything, to do with improving carbohydrate digestion. This may help to explain why some probiotic treatments result in a reduction in lactose intolerance symptoms with no significant changes in hydrogen breath test values.[29]

Lactase Enzyme Production: Improving carbohydrate digestion is typically the primary aim of lactose intolerance interventions, unless the chosen intervention is the avoidance of lactose-containing foods. In addition to competitive exclusion of potentially pathogenic bacteria and the modulation of the host's immune system, many probiotic bacteria that are intended to alleviate lactose intolerance symptoms are selected because they produce beta-galactosidase, or lactase enzyme, which hydrolyzes lactose into glucose and galactose. This sounds like a straightforward and logical solution to lactose intolerance, particularly in alleviating the osmotic load presented

to the colon by undigested lactose, but this mechanism is complicated by the observation that gases produced by colonic bacteria from fermented carbohydrates also contribute to lactose intolerance symptoms such as bloating, flatulence, and borborygmi.[48] Also, a 28-subject study conducted in 2005 investigated the microbial compositions of subjects' fecal samples and discovered that there was no significant difference in the prevalence of beta-galactosidase producing bacteria in the samples of lactose-intolerant subjects as compared to lactose-tolerant subjects.[49] Interestingly, there was also no correlation between the prevalence of beta-galactosidase producing bacteria and beta-galactosidase activity. Again, this hints at the complexity of the interplay between probiotic bacteria and lactose intolerance outcomes; simply providing the host with probiotic bacteria that can produce lactase enzyme may not reliably and significantly alleviate lactose intolerance symptoms.

Still, lactase production is a desirable quality in probiotic species intended for lactose intolerant individuals. Lactase producing bacteria that are generally recognized as safe include *Bacillus licheniformis*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subspecies *bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus reuteri*, *Streptococcus thermophilus*, and many others.[50,51,52] Some molds such as *Aspergillus phoenicis* have also been shown to produce lactase enzyme,[53] but they are typically just used for the production of commercially available exogenous lactase products and are less common as probiotic candidates. Normally, the host's endogenous lactase production occurs in the jejunum and, to a lesser extent, the ileum, and this is where the hydrolysis of lactose and absorption of the resultant glucose and galactose occur.[54] Though bacteria populate the colon significantly more densely than the small intestine, the ideal site of colonization for a probiotic intervention that produces lactase enzyme would be the small intestine,[55] as both unabsorbed

carbohydrates the reach the colon and the gaseous products produced by colonic bacteria contribute to lactose intolerance symptoms.[48] In order to assess whether the effect of a probiotic or prebiotic intervention on lactose intolerance outcomes was due to lactase enzyme activity, the lactose tolerance test is preferred to the hydrogen breath test,[56] as the lactose tolerance test measures a change in blood glucose in response to a bolus of lactase. An increase in exogenous lactase production by probiotic bacteria in the small intestine may allow the host to absorb the products of lactose hydrolysis and thus will impact blood glucose. This is preferable to the fermentation of carbohydrates in the colon, which does not allow the host to absorb glucose or galactose.

Mechanisms of Action of Prebiotics and Synbiotics

As a reminder, prebiotics are substrates that are selectively utilized by the host's microbiota to confer a health benefit upon the host,[9] while synbiotics are combinations of probiotic and prebiotic treatments that confer a health benefit upon the host.[10] Prebiotics are intended to be digested by gut bacteria, so common candidates for research are polysaccharides and oligosaccharides that humans cannot digest such as inulin,[57] fructo-oligosaccharides, galacto-oligosaccharides, trans-galacto-oligosaccharides,[58] raffinose, stachyose,[59] and fucosyl-oligosaccharides.[60] The host's gut microbiome composition changes in response to the diet, but prebiotics allow the host to make deliberate and targeted modifications. When the gastrointestinal tract is consistently exposed to the same carbohydrate sources, bacteria that favor these carbohydrate sources tend to flourish in a process that is, in this context, called adaptation.[61,62] Adaptation of the gut microbiome in response to prebiotics may then exert the same mechanisms of action (competitive exclusion, immune response modulation, and enzyme production) described above.

Of particular interest is the administration of specific formulations and doses of prebiotics to elicit a reliable change in the prevalence of particular species of bacteria, but adaptations in the gut microbiome are reported more broadly as genus-wide changes. This is especially evident in the "bifidogenic effect" of fructo-oligosaccharides, and galacto-oligosaccharides.[63] As early as 1990, it was shown that consuming 10g of raffinose and stachyose daily for 3 weeks increased participants' fecal concentrations of bifidobacteria, but no specific species were evaluated in vitro.[64] In vivo, however, this same raffinose and stachyose prebiotic was shown to be fermented as effectively as glucose by *Bifidobacterium longum*, *Bifidobacterium breve*, and *Bifidobacterium infantis*, but less effectively by *Bifidobacterium bifidum*. Pathogenic bacteria including *Clostridium perfringens*, *Escherichia coli*, and *Clostridium difficile* did not ferment the prebiotic, indicating the benefit of selective substrate utilization in microbial dynamics such as competitive exclusion.[64] More recently, a research team from the Department of Microbiology and Immunology in Leuven, Belgium, observed a modest increase in populations of *Bifidobacterium* and *Anaerostipes* species in the gastrointestinal tracts of subject who were fed inulin.[65] It may be the case that species-level adaptations are much more easily achieved via probiotic use, whereas prebiotics tend to have a significantly less targeted effect.

Synbiotics seek to combine probiotic bacteria and the prebiotics that are best-suited to those bacteria. There is some evidence that inulin and/or fructo-oligosaccharides may help support probiotic species such as *Lactobacillus paracasei* and *Bifidobacterium bifidum*,[66] but few studies examine alterations of the gut microbiome in response to synbiotic treatment. An in vitro study conducted in 2016 investigated the fermentative capacity of five *Lactobacillus* species and 2 Lactococcus species and discovered that, of the xylo-oligosaccharides, xylobiose was fermented the most effectively, particularly by *Lactobacillus acidophilus*.[67] Though this research

may not generalize well to the complicated bacterial milieu of the gut microbiome, analyzing the effectiveness with which probiotic bacteria ferment particular oligosaccharides may give some insight as to which prebiotics will pair best with which probiotics. Ostensibly, this synergy may improve probiotic colonization and the capacity for competitive exclusion of pathogens.

Contemporary Research

Recent systematic reviews of the topic have either revealed inconclusive[68] or overall positive[69,70,71] results, but these investigations tended to be limited by their heterogeneity of outcome variables, small quantity of included studies, small sample sizes of included studies, methodological heterogeneity, and lack robust statistical models examining the available data.

A 2005 systematic review examining the effect of probiotic supplementation on participants' hydrogen breath test values or lactose intolerance symptom scores reported inconclusive results.[68] The researchers searched AMED and Medline databases for relevant studies, and they called and emailed researchers and *Lactobacillus* probiotic manufacturers to locate additional studies. A lack of standardized data prevented a pooled statistical analysis of the 10 included studies, and the small sample sizes of the studies (from 5 to 20 participants) may have promoted an increased incidence of type II errors among the study results. Of the 9 studies measuring hydrogen breath test values, an equal number reported positive, negative, and mixed outcomes. Of the 7 studies measuring symptom outcomes, one returned positive results, five returned negative results, and one returned mixed results.[68] The researchers concluded that strain, concentration, and duration of wash-out period might affect the measured outcomes. A robust statistical analysis should ideally stratify the data by these variables to examine their effect on the outcomes of interest.

The 2012 textbook *Probiotics* contains a chapter specifically addressing the use of probiotics to alleviate lactose intolerance symptoms. This chapter describes bacterial species known to increase β-galactosidase activity in the feces, including *Bifidobacterium breve*, *Bifidobacterium longum*, and *Lactobacillus casei.*[69] Antibiotics are included as a potential confounding factor in the effectiveness of probiotic treatments, which indicates that recent and ongoing antibiotic use would be a sensible exclusion criterion for probiotic trials. The authors concluded that probiotics provide a promising avenue of treatment for lactose intolerance symptoms, and there is evidence that some strains of bacteria are effective, but further research is needed.

A 2018 systematic review examined studies that investigated the effects of probiotic supplementation on a variety of outcomes, particularly outcomes pertaining to symptoms of lactose intolerance. Databases and catalogues used included PubMed, Google Scholar, Ovid MEDLINE, Web of Science, Food Science and Technology Abstracts, Cochrane Central Register of Controlled Trials, Current Contents Connect, and Clinicaltrials.gov.[70] Of the 94 unique full-text clinical trials assessed, 15 were included in the review, and a total of 8 bacterial species were investigated in the selected studies. The researchers reported mixed results but an overall positive trend of reduction in the incidence and severity of lactose intolerance symptoms, though these results varied between species. Bacteria displaying moderately strong evidence for relief of one or more lactose intolerance symptoms included *Bifidobacterium longum*, *Bifidobacterium animalis*, and *Lactobacillus rhamnosus*, with the caveat that consumption of *Lactobacillus rhamnosus* probiotics may have adverse side effects for populations with poor immune function.[70] By contrast, the researchers concluded that there is insufficient evidence to support probiotic supplementation using *Lactobacillus bulgaricus*, *Lactobacillus reuteri*,

Lactobacillus acidophilus, *Saccharomyces boulardii*, and *Streptococcus thermophilus* to alleviate lactose intolerance symptoms.[70] In the case of *Lactobacillus reuteri*, the two clinical trials that were examined yielded positive results, but more studies are necessary to provided definitive evidence of its effectiveness. Many of these studies were limited in sample size and duration, and no statistical analysis was performed. Similar to previous systematic reviews, this research highlights the need for a robust statistical analysis that pools available data and stratifies outcome variables by bacterial species to offer a clearer measure of the effectiveness of a given probiotic treatment.

More recently, a systematic review published in 2020 examined 9 studies investigating the effects of prebiotic and/or probiotic supplementation on a reduction in lactose intolerance indicators and found an overall positive trend. The researchers drew the studies from PubMed and SCOPUS databases, then they manually searched the bibliographies of these studies for additional resources.[71] Statistically significant reductions in hydrogen breath test values and/or lactose intolerance symptom scores were reported in studies of probiotics that contained *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, and *Streptococcus thermophilus*, while the study examining the effect of *Lactobacillus plantarum* and *Bifidobacterium animalis* did not show significant results.[71] Of the 9 studies included, only two were discussed in the aforementioned 2018 systematic review, and the findings concerning the effectiveness of *L. acidophilus*, *B. animalis*, and *L. reuteri* differed between the two reviews.[70,71] This may be due to the differences in database search methodologies and outcome variables measured in these reviews, and it indicates that a more inclusive literature search could be performed.

CHAPTER III
METHODS

Protocol and Registration

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist,[72] and the study rationale and methodology were prospectively submitted to PROSPERO.

Inclusion Criteria

A study was eligible for inclusion in the systematic review if it met the following criteria: (1) Human participants in a treatment group received probiotic and/or prebiotic supplementation of a specified dosage. Genus and species of the probiotic(s) and/or chemical name(s) of the prebiotic(s) were identified. (2) Human participants in a control group received a placebo that was unlikely to substantially affect gut microbiome composition or participants' pre-test and post-test results were evaluated. (3) The outcomes of interest included any indicator of lactose maldigestion or lactose intolerance. (4) The study excluded participants with gastrointestinal diseases, cancer/chemotherapy, antibiotics, milk protein allergy, and/or those who were taking medications that may affect gastrointestinal motility or microbiome. One exception to this criterion is IBS with concomitant lactose intolerance, as this is a clinically relevant subgroup of lactose intolerant individuals and does not preclude their participation in lactose intolerance research. (5) The study was peer-reviewed, and its full text was accessible.

Literature Search

Search databases included SCOPUS, MEDLINE, WORLDCAT, EMBASE, Web of Science, Cochrane Library, NIH-HMP studies, and Central Washington University's OneSearch. The initial search string used was (Probiotic* OR prebiotic* OR synbiotic* OR "beneficial bacteria") AND ("Lactose intolerance" OR "lactase nonpersistence" OR "lactase deficiency")

AND ("Lactose intolerance symptoms" OR bloating OR gas OR flatulence OR diarrhea OR "gastric distress" OR indigestion) NOT (rat* OR mice OR mouse). An additional search was performed using the terms *Lactobacillus*, *Bifidobacterium*, probiotic*, prebiotic*, synbiotic*, "lactose intolerance", *Saccharomyces boulardii*, and *Streptococcus thermophilus*.

Review papers and reference lists were also examined for suitable studies. Searches were not limited by language. Studies written in non-English languages were translated to English via the Linguee database, and any unclear translations were cross-referenced with SDL Trados Studio translation software.

Study Selection

Eligible studies were sorted to eliminate duplicates, then the titles and abstracts of the eligible studies were examined to determine whether they fulfilled all of the above inclusion criteria. In the event of ambiguity or significant threats to internal validity, suitability for a study's inclusion in the review was determined by committee to minimize selection bias. Selected studies were read in full and further evaluated for inclusion, again based on the above criteria.

Quality Assessment and Data Gathering

Data gathering was conducted according to the Cochrane training manual[73] methodology. A study quality and risk of bias assessment was performed according to the Cochrane Revised Tool to Evaluate Risk of Bias in Randomized Trails,[74] also known as the RoB 2, while cohort and case-control trials were to be assessed via the 9-point Newcastle-Ottawa Scale.[75]

Statistical Analysis

Due to significant methodological heterogeneity in the included studies, statistical analysis was planned but not performed. Cochrane Library's RevMan 5.4.1 software was to be

used for all stages of statistical analysis. Potential publication bias was to be assessed via Begg's funnel plots, and Egger's linear regression test was to be used to evaluate heterogeneity. Additional assessments of heterogeneity would include an I^2 calculation and the Q statistic at a level of significance of $P < 0.10$. A pooled Chi-square analysis was to be performed on the lactose intolerance symptom score data, and a comparison of pooled means was to be used to evaluate the hydrogen breath test data, adjusted for age, sex, and dietary intake. A random-effects model was to be used.

CHAPTER IV
RESULTS

The search string yielded a total of 1,411 search results comprised of 1,046 items from Central Washington University's OneSearch database, 75 from PubMed, 257 from WorldCat, 33 from Cochrane Library, and 0 from the NIH Human Microbiome Project. Of these items, 135 duplicates were eliminated. When duplicates were eliminated, only the most recently-updated item was retained so that revised papers would be preferentially spared. In an abstract review of the remaining 1,276 results, 978 were eliminated for not matching the relevant study parameters, including 922 studies investigating unrelated topics and 56 studies without interventions or treatments. The studies investigating unrelated topics primarily featured investigations of other GI disorders causing similar symptoms, so when this systematic review is updated or replicated it may be useful to update the search string to dis-include terms such as "irritable bowel syndrome", "*Clostridium difficile* infection", "radiotherapy", "chemotherapy", and "small intestinal bacterial overgrowth". Of the remaining results, 275 were somewhat relevant and were considered for citation mining and for potential inclusion in a review of relevant literature, and 23 were read in full and considered for inclusion in the review after an evaluation of their methodologies (see Figure 1). Citation mining uncovered 2 additional studies, so 25 studies were included in the review.[76-100]

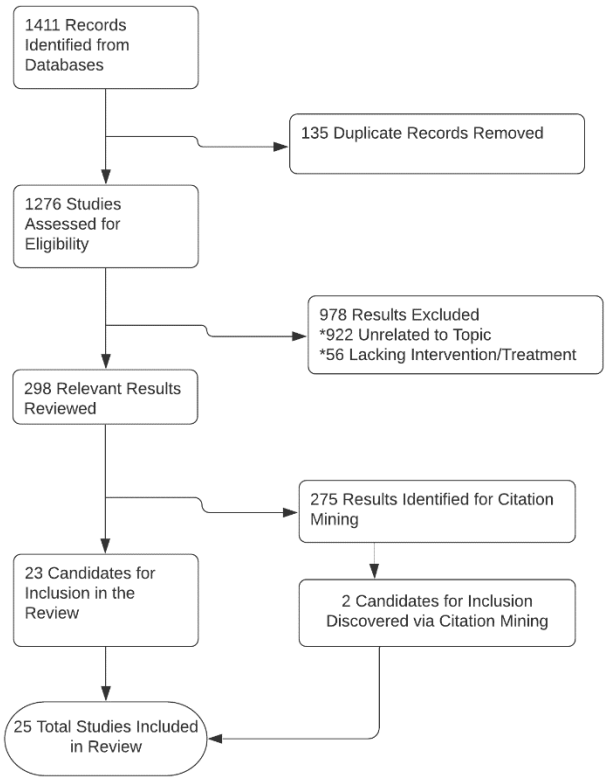

Figure 1: Flow diagram of study selection process including records identification, eligibility assessment, citation mining, and total number of studies included (n = 25)

Cochrane Risk-of-Bias Assessment

The RoB 2 is a risk-of-bias assessment tool that evaluates studies for their degree of bias along the domains of selection, adherence, outcomes, measurements, and reporting. Possible evaluations for each domain are either "low risk of bias," "some concerns," or "high risk of bias."[74] Of the 25 studies included in the systematic review, 9 featured some concerns in the selection domain, primarily due to unspecified or nebulous methodology regarding recruitment of subjects.[78,81,84,86,88,89,91,92,96] The adherence domain evaluates both adherence to study protocols and blinding. Only 5 studies showed some concerns due to a lack of blinding,[76,82,84,90,95] while one study showed high risk of bias in this domain due to a failed attempt at blinding, wherein the majority of participants could differentiate between cow's milk and soy milk.[97] Several studies

featured unclear methodology regarding dissemination of outcome data,[76,78,91] but risk of bias was assessed as low. In the measurement domain, one study showed high risk of bias due to a likely carryover effect,[88] while another study showed a carryover effect but adjusted for it.[92] Other causes for concern were the use of a 50g lactose bolus[82] and the allowance of caffeine during testing.[84] All but one study showed low risk of bias in the reporting domain. This study relied on unpublished research to evaluate the effect of a probiotic treatment,[78] so risk of bias was considered high for this domain. For any study and domain not referenced above, risk of bias for that domain was assessed as low. No study had a high overall risk of bias.

CHAPTER V
SYSTEMATIC REVIEW

The treatments utilized for each study and the primary study results and conclusions are described in Table 2 below, then this is followed by a narrative synthesis and systematic review of the included studies.

Table 2. Key Characteristics and Conclusions of Probiotic Studies

Research Team	Year	Sample	Treatment(s)	Findings
Almeida et al	2012	27	2E7-2E9 CFUs *L. casei* Shirota and 5E7 to 5E9 CFUs *Bifidobacterium breve* Yakult 3x/d for 4 weeks	*Total lactose intolerance symptom scores were reduced in the treatment group compared to baseline ($p < .01$). *Total breath hydrogen scores were reduced in the treatment group compared to baseline ($p = .04$). *Total symptom scores and breath hydrogen scores were still significantly reduced upon 3-month follow-up ($p < .05$)
Cano-Contreras et al	2020	48	Probiotic i3.1: 3E9 CFUs *Pediococcus acidilactici* CECT7483 and *Lactobacillus plantarum* CECT7484 and CECT7485 daily for 8 weeks	*Total lactose intolerance symptom scores were reduced in the treatment group compared to baseline ($p < .001$). *46.4% of subjects in treatment group and 0% of subjects in placebo group achieved 50% or greater reduction in total symptoms relative to baseline.
Gingold-Belfer et al	2019	8	Bio-25 probiotic containing 11 bacterial strains (see below) daily for 6 months	*Significant reductions in symptom frequency ($p <.05$) for bloating, flatulence, abdominal discomfort, and constipation, but not diarrhea. *Breath hydrogen scores trended toward a reduction over study duration (no significance reported).

Table 2 (Continued)

Research Team	Year	Sample	Treatment(s)	Findings
He et al	2008	11	125g yogurt containing *L. bulgaricus, S. thermophilus*, and appx 1E8 CFUs *B. animalis* per gram 3x/d, as well as 2E8 CFUs *B. longum* in capsule 3x/d for 2 weeks	*Beta-galactosidase activity in feces was significantly increased after treatment ($p < .01$), but not after 8-day follow-up. *Total symptom scores were significantly lower upon follow-up than at baseline ($p < .02$).
Kim et al	1983	24	5mL/kg body weight of whole milk containing either 0, 2.5E6, 2.5E7, or 2.5E8 CFUs/mL of *L. acidophilus* daily for one week	*Significant reductions in breath hydrogen were observed in the 2.5E6 CFUs/mL ($p < .025$) and 2.5E8 CFUs/mL ($p < .01$) groups, but not the 2.5E7 CFUs/mL ($p > .35$) group.
Labayen et al	2001	22	25g lactose from appx 500mL yogurt containing 1E8 CFUs/g of both *L. bulgaricus* and *S. thermophilus* or 500mL pasteurized yogurt containing < 100 CFUs/g of the same daily for 15 days	*Significant reductions in total GI symptoms were observed for lactose malabsorbers consuming non-pasteurized yogurt as compared to pasteurized yogurt ($p = .037$). *No significant differences in fecal weight nor frequency. *Overall shorter orocecal transit time for pasteurized yogurt (10.5 hr ± .6) compared to non-pasteurized (12.1 hr ± .5)

Table 2 (Continued)

Research Team	Year	Sample	Treatment(s)	Findings
Lin et al	1998	20	*Lactobacillus acidophilus* B or *Lactobacillus bulgaricus* 449, each at 1E8 and 1E9 CFUs/mL in 400mL 2% nonfermented milk consumed once	*Both L. bulgaricus-containing milks resulted in significant reductions in hydrogen breath values ($p < .05$) and symptom scores ($p < .05$) compared to control milk. *L. acidophilus milk containing 1E9 CFUs/mL resulted in significant reduction in symptoms scores ($p < .05$) but no significant reduction in hydrogen breath values.
Montes et al	1995	20	250mL milk with 1E10 CFUs *L. acidophilus* or containing 1E8 CFUs *L. lactis* and 1E10 CFUs *S. thermophilus*	*Both L. acidophilus and L. lactis/S. thermophilus milks resulted in significantly lower mean symptom scores ($p < .001$ and $p < .05$, respectively) than un-inoculated milk. *Significantly lower breath hydrogen scores were seen with L. lactis/S. thermophilus milk ($p < .001$).
Mummah et al	2014	16	Raw milk, pasteurized milk, or soy milk, each consumed in doses of 16oz days 1 and 8. Consumption was 4oz on day 2, increasing by 4oz/d to 24oz on day 7.	*No significant difference observed between raw and pasteurized milk in terms of symptom scores. *Day 1 peak and AUC breath hydrogen scores were significantly higher for subjects consuming raw milk than for pasteurized milk ($p = .01$). *Day 8 AUC breath hydrogen for subjects consuming raw milk were significantly lower than day 1 scores ($p = .05$), indicating potential microbial adaptation.
Ojetti et al	2010	60	9,000 standard units of tilactase 15 minutes before hydrogen breath test (HBT) or 4E8 CFUs *L. reuteri* pill 2x/d for 10 days	*Significant reduction in hydrogen breath test scores was recorded for both L. reuteri ($p < .01$) and tilactase ($p < .001$) compared to placebo. *Significant reduction in clinical symptom scores was observed for both L. reuteri ($p < .0001$ for both) compared to placebo.

Table 2 (Continued)

Research Team	Year	Sample	Treatment(s)	Findings
Pakdaman et al	2016	38	1E10 CFUs *L. acidophilus* DDS-1 daily for 4 weeks	*Significant reductions in overall symptom scores were observed in the treatment group ($p = .037$) as compared to the control. Specifically, there were reductions in abdominal cramping ($p = .012$), diarrhea ($p = .033$), and vomiting ($p = .002$).
Rampengan et al	2010	79	4E9 CFUs *L. rhamnosus* Rosell-11 and *L. helveticus* Rosell-52 ("Live probiotic") daily for 2 weeks or Dialac ("Heat-killed probiotic")	*Both live and killed L. rhamnosus and L. helveticus probiotic treatment resulted in significantly lower hydrogen breath test scores ($p < .001$). * Hydrogen breath test scores of subjects taking live probiotics were not significantly different from those taking heat-killed probiotics ($p = .453$). *At baseline, 13.9% of subjects were asymptomatic. After treatment with either probiotic 58.2% of subjects were asymptomatic, but no statistical significance was calculated or reported.
Rizkalla et al	2000	24	500g/d fresh yogurt containing > 1E7 CFUs/g of *L. bulgaricus* and *S. thermophilus* or pasteurized yogurt containing < 100 CFUs/g of the same daily for 15 days	*Breath hydrogen AUC was significantly reduced after both fresh and heated yogurt consumption ($p < .001$ for both) as compared to baseline. *Fresh yogurt had a treatment effect on baseline HBT values that carried beyond the washout period, indicating potential adaptation of the gut microbiome. *Significantly greater increases in plasma butyrate concentrations were observed for fresh yogurt as compared to pasteurized yogurt ($p < .03$).

Table 2 (Continued)

Research Team	Year	Sample	Treatment(s)	Findings
Roškar et al	2017	44	5E9 CFUs *L. plantarum* MP2026 and 5E9 CFUs *B. animalis* IM386 daily for 6 weeks	*Significant reductions in the incidence of diarrhea ($p < .05$) and severity of flatulence were observed for the treatment group. *Significant reductions in abdominal pain ($p < .01$), flatulence ($p < .01$), and boborygmi ($p < .05$) were observed for the placebo group. *While symptom scores generally trended toward improvement, no statistically significant difference was found between the probiotic and placebo groups, indicating a strong placebo effect.
Ruchkina et al	2013	60	1E7 CFUs *B. longum* and 1E7 CFUs *E. faecium* 3x/d in addition to standard mesim forte therapy daily for 2 weeks	*Significant reduction in HBT scores compared to baseline ($p < .01$) in the treatment group. *Significant reduction in bacterial overgrowth syndrome in treatment group ($p < .01$) and 70.8% of participants experienced a return to eubiosis.
Saltzman et al	1999	18	1E10 CFUs *L. acidophilus* BG2FO4 2x/d for 7 days, with or without 40mg daily omeprazole	*No significant difference in HBT AUC from baseline values for either omeprazole or non-omeprazole group. *No significant differences in total or individual symptom scores from baseline values for either group.

Table 2 (Continued)

Research Team	Year		Treatment(s)	Findings
Savaiano et al	1991	10	8 total treatments: 400mL of unfermented 2% milk containing either 1E7 or 1E8 CFUs/mL of *S. thermophilus* and *L. bulgaricus*, or 400mL of 2% unfermented milk containing any of 3 *L. acidophilus* strains at either 1E7 CFUs/mL or 1E8 CFUs/mL	*Significant reduction in HBT values observed in subjects taking L. acidophilus LA-1 milk at 1E8 CFUs/mL ($p < .05$) and S. thermophilus/L. bulgaricus milk at 1E8 CFUs/mL ($p < .05$) as compared to control. * S. thermophilus/L. bulgaricus milk at 1E8 CFUs/mL resulted in a greater reduction in symptoms than L. acidophilus milk of the same concentration.
Savaiano et al	1996	15	400mL milk with $5*10^8$ CFUs/mL *B. longum* B6 grown using lactose, 400mL milk with $5*10^8$ CFUs/mL *B. longum* B6 grown using lactose and glucose, or 400mL milk with $5*10^8$ CFUs/mL *B. longum* ATCC 15708 grown using lactose	*Total breath hydrogen excretion was reduced for milk containing lactose-grown B. longum ($p = .0001$) and milk containing B. longum ATCC ($p = .0128$) as compared to control. *Milk containing lactose-grown B. longum also resulted in lower hydrogen excretion than that of B. longum grown with both lactose and glucose ($p = .0014$).

Table 2 (Continued)

Research Team	Year	Sample	Treatment(s)	Findings
Savaiano et al	1997	6	0.6g lactose/kg body wt/d, increasing to 0.8g/kg day 3 and 1g/kg day 5, then 1g/kg/d was consumed until day 11	*Fecal samples taken after lactose feeding showed appx 33% the rate of hydrogen gas production as fecal samples taken after the feeding of a dextrose control, and this difference was significant (p = .006).
Savaiano et al	2013	85	RP-G28 galacto-oligosaccharide in escalating doses for 5 weeks, beginning at 1.5g/d and ending at 15g/d	*Both mean and median HBT values consistently decreased with RP-G28 treatment, but the difference was not significant. *Incidence of abdominal pain decreased significantly compared to placebo (p = .0288) and the number of subjects reporting no abdominal pain was significantly higher than placebo (p = .019). *Incidence of bloating and cramping was reduced as compared to baseline values in the treatment group, but not significantly more than placebo.
Savaiano et al	2020	377	5g RP-G28 galacto-oligosaccharide 2x/d for 10d increasing to 7.5g 2x/d for 20d, or 7.5g 2x/d for 10d increasing to 10g 2x/d for 20d	*There was a significantly greater incidence of reduction in total symptom scores with both the lower dose (p = .043) and the higher dose (p = .029) than the placebo. *In both treatment groups, there was a significantly greater likelihood of elimination of all GI symptoms (p = .004) than in the placebo group. *Treatment groups showed significantly higher milk consumption upon 30-day follow-up (p = .008) than placebo group. *Marked bifidogenic effect: Significant (p < .05) increases in relative abundance of *Bifidobacterium longum, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium catenulatum, Bifidobacterium angulatum,* and *Bifidobacterium gallicum.*

Table 2 (Continued)

Research Team	Year	Sample	Treatment(s)	Findings
Turck et al	2020	68	1.8E12 CFUs *B. animalis* subspecies *lactis* Bi-07	*Significant reduction in AUC for hydrogen breath values in two separate trials ($p = .0012$ and $p = .0156$) as compared to placebo. *In one trial, significantly higher odds ratio of severe nausea was observed for probiotic as compared to the placebo (OR 4.31) and lactase (6.98). *No significant reduction in incidence or severity of GI symptoms observed for probiotic treatment.
Vitellio et al	2019	23	4E9 CFUs *B. longum* BB536 and 1E9 *L. rhamnosus* HN001 with 1.4mg vitamin B6 daily for 30 days	*Significant reduction in bloating noted in treatment group ($p = .028$) as compared to placebo. *87.5% of subjects initially experiencing constipation in treatment group achieved normal Bristol scores. *No statistically significant differences in abdominal pain between groups. *Significant increases ($p < .05$) in relative abundance of *Slakia*, *Thricoccus*, *Bifidobacterium*, and *Enterococcus* genera and significant decreases in *Klesbiella*, *Serratia*, and *Enterobacter* genera were observed in the treatment group as compared to placebo.
Yesovitch et al	2004	10	VSL3 probiotic containing either 4.5E11 or 1.8E12 CFUs of *L. casei*, *L. plantarum*, *L. acidophilus*, *L. bulgaricus*, *B. longum*, *B. breve*, *B. infantis*, and *S. salivarius* daily for 17 days	*No statistically significant reduction in HBT values nor symptom scores noted in either treatment group as compared to baseline.

Table 2 (Continued)

Research Team	Year	Sample	Treatment(s)	Findings
Zhong et al	2006	11	125g yogurt containing *L. bulgaricus*, *S. thermophilus*, and appx 1E8 CFUs *B. animalis* per gram 3x/d, as well as 2E8 CFUs *B. longum* in capsule 3x/d for 2 weeks	*Beta-galactosidase activity in feces was significantly increased after treatment ($p < .01$), but not after 8-day follow-up. *Total symptom scores were significantly lower upon follow-up than at baseline ($p < .02$).

Probiotics

Probiotics are live microorganisms that, when administered in sufficient amounts, promote health benefits. For the purpose of this review, synbiotic treatments that include a probiotic component are discussed in both this section and the synbiotics section. This allows for a more comprehensive assessment of the strength of evidence that a given probiotic species may affect outcomes of lactose intolerance.

Bifidobacterium animalis

Bifidobacterium animalis describes *Bifidobacterium animalis* subspecies *animalis* and *Bifidobacterium animalis* subspecies *lactis*, once believed to be two separate species. It, like many of the bacterial species in this review, is a Gram-positive anaerobe. Though both subspecies have been studied for their probiotic properties, *Bifidobacterium animalis* subspecies *lactis* has been shown to grow easily in milk and milk-based media.[101]

Four studies in this review examined the effects of *Bifidobacterium animalis* on outcomes of lactose intolerance. Two studies showed a significant reduction in lactose intolerance symptom scores and improvement in other measures[76,79] while two had mixed results.[77,78] The studies showing a

significant reduction in symptom scores were closely related and both featured a treatment of *Bifidobacterium longum* capsules combined with 125g yogurt containing *Lactobacillus delbrueckii* subspecies *bulgaricus, Streptococcus thermophilus* and *Bifidobacterium animalis* each day for 2 weeks. Both research teams noted a Bifidogenic effect of the treatment and a significant reduction in the incidence of diarrhea as compared to the baseline values.[76,79] In one study, the fecal beta-galactosidase activity of participants was significantly increased following treatment.[76] A 6-week study investigating the effect of *Bifidobacterium animalis* subspecies *animalis* IM386 combined with *Lactobacillus plantarum* MP2026 elucidated a significant reduction in the incidence of diarrhea and flatulence in both the probiotic and control groups as compared to baseline.[77] This indicates a strong placebo effect. The researchers also did not find any significant differences in hydrogen breath test (HBT) values when compared to baseline values, and there was no correlation between HBT scores and symptoms. Effects of *Bifidobacterium animalis* subspecies *lactis* Bi-07 on lactose digestion were formally evaluated by the European Food Safety Authority based on the results of two unpublished studies provided by the health claim applicant. In these studies, the researchers noted significant reductions in HBT scores in the treatment group as compared to placebo, but no significant differences in attenuation of lactose intolerance symptoms.[78] There was also a carryover effect observed between groups, so the results may have been marred by the adaptation of participants' gut microbiomes to the probiotic treatment. The European Food Safety Authority ruled against the applicant's health claim that the Bi-07 strain promoted a clear beneficial physiologic effect.[78]

Taken together, these findings provide little evidence that *Bifidobacterium animalis* supplementation is beneficial to individuals with lactose intolerance, and there may be strain-specific considerations when conducting research into the potential of either subspecies of this bacterium to affect outcomes of lactose intolerance. Additionally, the majority of these studies investigated the

effect of *Bifidobacterium animalis* combined with other probiotic species or prebiotic treatments, so it would be problematic to attribute the outcomes of those treatments to *Bifidobacterium animalis* alone.

Bifidobacterium breve

Bifidobacterium breve is a bacterial species commonly used in probiotics for infants and in IBS research.[102] Three studies in this review examined the effect of *Bifidobacterium breve* on outcomes of lactose intolerance. In 2012, the effect of a 4-week supplementation period of *Lactobacillus casei* Shirota and *Bifidobacterium breve* Yakult on lactose intolerance symptoms and hydrogen breath test values was investigated. The research team observed a significant reduction in both breath hydrogen and symptom scores compared to baseline values in the treatment group, and the reduction in total symptom scores was comparable to that observed for lactase enzyme.[80] Upon 3-month follow-up, both lactose intolerance symptom scores and breath hydrogen scores remained significantly lower than baseline values, potentially indicating a lasting adaptation of the gut microbiome. A longer-term experiment investigated the effect of a 6-month regimen of the Bio-25 probiotic formula, which contains the following 11 bacteria: *Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus casei, Lactococcus lactis, Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus rhamnosus,* and *Streptococcus thermophilus*. Each participant reported a reduction in baseline symptoms, but this was only statistically significant for bloating and flatulence, but not for diarrhea and abdominal pain.[81] Two participants showed reduced HBT scores such that they were no longer diagnosed as lactose intolerant after only two months, whereas the other participants saw some non-significant reduction in breath hydrogen. Though the results of this study are promising, it was a pilot study and was limited by its small sample size of 8 subjects. Another pilot study measured hydrogen breath test values and symptom scores in 10 participants after a 17-day treatment with a VSL3 multi-probiotic containing

Bifidobacterium breve, Bifidobacterium longum subspecies *infantis, Bifidobacterium longum* subspecies *longum, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus plantarum,* and *Streptococcus thermophilus*. There were no significant reductions in lactose intolerance symptom scores nor HBT scores, though the study duration was fairly short for such a large (50g) lactose challenge.[82]

These studies provide a small degree of evidence that *Bifidobacterium breve,* when in combination with other probiotic species, may help attenuate outcomes of lactose intolerance. As with any study investigating a treatment that is comprised of multiple bacterial species, it is difficult to determine to what extent *Bifidobacterium breve* may have affected the outcome and to what extent it was facilitated by the other bacteria.

Bifidobacterium bifidum

Bifidobacterium bifidum is a key microorganism in neonatal development, and its growth and prevalence in the gut microbiome are stimulated by human milk oligosaccharides early in life. It has the capacity to digest oligosaccharides found in mucin, which may allow this bacterium to more easily colonize the gastrointestinal tract.[103] It has also been hypothesized that this digestion of intestinal mucin may promote increased mucin production by the host and contribute to the epithelial barrier of the intestines.[104]

Only one study included in this review investigated the effects of *Bifidobacterium bifidum* probiotic consumption on outcomes of lactose intolerance. The treatment was the aforementioned 6-month regimen of the multi-probiotic Bio-25, which contains 11 probiotic species. While results trended toward a reduction in bloating and flatulence, and HBT scores were reduced, it would be unwise to attribute these effects solely to *Bifidobacterium bifidum.*[81] This was also a pilot study with an exceptionally low sample size and exclusively female participants, so its results are not very

generalizable. Substantially more research is needed to determine the effectiveness of this bacterium at attenuating lactose intolerance outcomes.

Bifidobacterium longum

Bifidobacterium longum is another Gram-positive, rod-shaped anaerobe colonizing the human GI tract, and taxonomically it is a combination of what were formerly three separate biotypes of Bifidobacteria: *Bifidobacterium infantis*, *Bifidobacterium longum*, and *Bifidobacterium suis.*[105] It has the ability to digest a diverse range of carbohydrates in the intestines and is a common candidate for probiotic research.[106] *Bifidobacterium longum* was present in 4 of the aforementioned studies included in this review. In combination with yogurt containing *Bifidobacterium animalis* and *Lactobacillus delbrueckii* subspecies *bulgaricus*, daily consumption of a probiotic capsule containing *Bifidobacterium longum* subspecies *longum* for 2 weeks was shown to alleviate diarrhea and promote a bifidogenic effect,[76,79] and to increase fecal beta-galactosidase activity as compared to the control.[76] It was also present in the Bio-25 and VSL3 multi-probiotic treatments described previously,[81,82] the former of which was associated with a reduction in lactose intolerance symptoms scores and a trend toward reduction in HBT values, while the latter showed no reduction in HBT values nor symptom scores.

Additionally, subjects receiving a 30-day treatment of 4E9 CFUs of *Bifidobacterium longum* subspecies *longum* BB536, 1E9 CFUs of *Lactobacillus rhamnosus* HN001, and 1.4mg vitamin B6 per day were observed to have significantly less bloating and a notable bifidogenic effect as compared to those receiving a maltodextrin and corn starch control.[83] The researchers also observed a negative correlation between the relative abundance of *Bifidobacterium* in subjects' feces and the outcomes of bloating and abdominal pain. Interestingly, the ability of *Bifidobacterium longum* to attenuate outcomes of lactose intolerance may also depend on its growth conditions. In another study,

researchers recorded the effects on lactose intolerance outcomes of one-time consumption of 400mL lowfat milk either alone as a control, containing 5E8 CFUs/mL *Bifidobacterium longum* subspecies *longum* B6 grown using lactose, 5E8 CFUs/mL *Bifidobacterium longum* subspecies *longum* B6 grown using both lactose and glucose, or 5E8 CFUs/mL *Bifidobacterium longum* subspecies *longum* ATCC 15708 grown using lactose. In this context, "B6" refers to the strain of bacterium, not vitamin B6 or pyridoxal as was the case in the previous study. Participants consuming the milk containing *Bifidobacterium longum* subspecies *longum* B6 that was grown using exclusively lactose experienced significantly less flatulence and lower HBT scores than the control group,[84] and a reduction in HBT scores was also noted in the group consuming milk inoculated with *Bifidobacterium longum* subspecies *longum* ATCC 15708 grown with lactose. Lastly, researchers investigating the effects of 2 weeks of daily supplementation with *Bifidobacterium longum* subspecies *longum* 107 and *Enterococcus faecium* 107 in patients with both severe lactase deficiency and dysbiosis discovered significant differences in the treatment group as compared to the control group. The treatment group showed both significantly lower HBT scores and a significantly greater percentage of subjects (70.8%) who were able to establish small bowel eubiosis by the end of the treatment period.[85]

Of the 7 studies in this review, 6 provided some evidence that *Bifidobacterium longum*, either alone or in combination with other probiotic species, may attenuate at least one outcome of lactose intolerance, while only one showed no significant results. Taken together, these studies provide strong evidence that *Bifidobacterium longum* probiotics may be a viable treatment option for lactose intolerance, but there is substantial heterogeneity in the taxa of *Bifidobacterium longum* that have been investigated, so it is as of yet unclear which subspecies or strain may be the best candidate for further research.

Enterococcus faecium

Enterococcus faecium is a commensal bacterium that is potentially pathogenic if it over-proliferates. It is a common cause of wound infections and urogenital infections, and it can be especially problematic if it develops vancomycin resistance. *Enterococcus faecium* is somewhat similar to *Enterococcus faecalis*, though the former can't ferment mannitol.[107]

As described previously, a study investigating the effect of *Bifidobacterium longum* subspecies *longum* 107 and *Enterococcus faecium* 107 on HBT scores and re-establishment of eubiosis elucidated significant differences between the treatment group and control group[85]. It may be necessary to screen this bacterium for vancomycin resistance prior to its use as a probiotic agent, and there is still much room for research as to whether it is of any use in reducing undesirable outcomes of lactose intolerance.

Lactobacillus acidophilus

Lactobacillus acidophilus is a Gram-positive microaerophilic bacterium that has been studied extensively for its probiotic properties. As early as 1977, it was demonstrated that *Lactobacillus acidophilus* NCFM inhibits the growth of pathogenic *Clostridium perfringens*, *Salmonella enterica* serovar *Typhimurium* (formerly *Salmonella typhimurium*), and *Staphylococcus aureus*, while *Lactobacillus acidophilus* 4962 inhibits the growth of *Escherichia coli*, *Salmonella enterica* serovar *Typhimurium*, and *Staphylococcus aureus*[108]. Yogurt is a common vehicle for administering *Lactobacillus acidophilus* as a probiotic, though care should be taken not to administer a hypercaloric diet because this has been associated with weight gain in subjects receiving yogurt-based *Lactobacillus acidophilus* treatment[109]. This is not always the case, however. Researchers may opt for yogurt-based, powdered, or pill-form probiotics, strain-specific research is often performed, and

durations of treatment vary greatly, each of which contributes to the diversity and heterogeneity of the available literature.

Of the 8 studies in this review that investigated the effects of *Lactobacillus acidophilus,* 2 have previously been described. The Bio-25 probiotic showed a reduction in lactose intolerance symptoms and a trend toward HBT score reduction[81], while the VSL3 probiotic showed no significant reduction in either of these measures[82]. Of the 6 remaining studies, 5 showed some reduction in lactose intolerance symptoms or HBT scores associated with *Lactobacillus acidophilus* probiotic treatment, while one did not.

A crossover study investigating the effect of four treatments 400mL of milk combined with either *Lactobacillus acidophilus* B or *Lactobacillus bulgaricus* 449, each at concentrations of either 1E8 CFUs/mL or 1E9 CFUs/mL, discovered a dose-dependent relationship. The one-time administration of *Lactobacillus acidophilus* milk containing 1E8 CFUs/mL failed to significantly reduce HBT scores or attenuate lactose intolerance symptoms, while milk with a concentration of 1E9 CFUs was associated with a significant reduction in mean lactose intolerance symptom scores but not HBT scores.[86] The subjects were allowed to consume black coffee during the treatment period, which has been shown to increase the survival rate of intestinal *Lactobacillus acidophilus* and, particularly in the case of dark roast coffee, may inhibit the growth of *Escherichia coli* and *Staphylococcus aureus.*[110] In another crossover study, supplementation of 1E10 CFUs of *Lactobacillus acidophilus* for 4 weeks in a maltodextrin capsule was shown to significantly reduce diarrhea, abdominal cramping, vomiting, and mean symptom scores upon 25g lactose challenge as compared to the control, although flatulence scores were somewhat elevated at the end of the treatment period.[87] There were no significant differences in Bristol stool scale or HBT scores.

In a study of 20 children with lactose maldigestion, subjects received 250mL of milk containing a total dose of 1E10 CFUs *Lactobacillus acidophilus* NCFM, the same volume of milk containing 1E10 CFUs *Streptococcus thermophilus* and 1E8 CFUs *Lactobacillus delbrueckii* subspecies *lactis*, and 250mL of an uninoculated control milk, each one day apart. Only 8 subjects were able to consume the required amount of each treatment, so these were the only subjects included in the final analysis. Despite this, mean symptom scores were significantly lower upon consumption of the *Lactobacillus acidophilus* milk than the control, while HBT scores were somewhat, but not significantly, higher.[88] In addition to poor rates of completion of the treatment by participants, this study was limited by the exceptionally short washout period between treatments, such that there is a high risk of a carryover effect.

Another study of the effect of milks inoculated with *Lactobacillus acidophilus* on HBT scores revealed a reduction for 2 of the 3 doses tested. Subjects were fed 10mL/kg body weight per day of either an uninoculated control milk or milk inoculated with 1E6 CFUs/mL, 1E7 CFUs/mL, or 1E8 CFUs/mL *Lactobacillus acidophilus* for one week. For subjects of a typical weight, this would amount to several servings of milk per day. The subjects consuming milk containing 1E6 and 1E8 CFUs of *Lactobacillus acidophilus* had significantly lower HBT scores than the control, but this effect was not observed for the dose of 1E7 CFUs/mL.[89] The researchers did not detect any significant differences in lactose intolerance symptom scores, and the study was also limited by the small sample size of 6 subjects in each group.

Similarly, subjects receiving 2E10 CFUs of *Lactobacillus acidophilus* BG2FO4 for one week were observed to have no significant reduction in individual or total symptom scores, and the researchers conducting this study also noted no significant changes in baseline HBT values.[90]

Interestingly, participants in this study self-reported their lactose intolerance, but approximately half of the subjects were found to be lactose digesters.

Finally, a study investigating the effect of milks inoculated with 2 different doses of 3 strains of *Lactobacillus acidophilus* and of yogurt starter culture revealed a strain-dependent effect on HBT scores. Subjects received 400mL of milk containing *Lactobacillus acidophilus* LA-1, *Lactobacillus acidophilus* LA-2, *Lactobacillus acidophilus* NCFM, or both *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subspecies *bulgaricus*, and each probiotic treatment was administered in concentrations of either 1E7 or 1E8 CFUs/mL. Significant reductions in HBT scores were observed for milk containing 1E8 CFUs/mL of *Lactobacillus acidophilus* and milk containing the same concentrations of *Lactobacillus delbrueckii* subspecies *bulgaricus* and *Streptococcus thermophilus.*[91] There was also a reduction in overall symptom scores in the latter group.

There is a moderate degree of evidence that some strains and doses of *Lactobacillus acidophilus* may reduce undesirable outcomes of lactose intolerance. Of the 8 studies in this review, 6 showed a significant reduction in either HBT scores or lactose intolerance symptoms, and 5 showed a significant reduction in symptoms. Common limitations of these studies included short washout periods between trials and small sample sizes. Future research could benefit from more homogeneity in study duration, strain selection, and dosage to determine the most effective treatment modality.

Lactobacillus delbrueckii* subspecies *bulgaricus

Lactobacillus delbrueckii subspecies *bulgaricus* is, like many of the bacteria in this review, a Gram-positive, non-motile, non-spore forming bacterium. It is commonly used in the production of fermented dairy products, particularly yogurt,[111] and this use of *Lactobacillus delbrueckii* was briefly featured in episode 6 of the animated science fiction series *Love, Death, and Robots.*[112] Qualities of great interest for the probiotic capacity of this bacterium are its constitutive expression of the beta-

galactosidase producing gene and its inability to digest the galactose monosaccharide upon the cleavage of lactose.[113] Rather than digesting galactose, *Lactobacillus delbrueckii* subspecies *bulgaricus* releases it into its external environment. This is nutritionally relevant in that galactose exuded into the small intestine by probiotic bacteria may be absorbed by the host and thus may potentially reduce bacterial gas production.

Of the 6 studies examining the effects of *Lactobacillus delbrueckii* subspecies *bulgaricus* on outcomes of lactose intolerance, 5 have previously been described. Daily consumption of a probiotic capsule containing *Bifidobacterium longum* subspecies *longum* for 2 weeks along with 125g yogurt fermented using *Lactobacillus delbrueckii* subspecies *bulgaricus* and fortified with *Bifidobacterium animalis* was shown to alleviate diarrhea and promote a bifidogenic effect,[76,79] and to increase fecal beta-galactosidase activity as compared to the control.[76] By contrast, a 17-day study investigating the effect of the VSL3 multi-probiotic containing *Lactobacillus delbrueckii* subspecies *bulgaricus* discovered no significant difference in HBT scores nor symptom scores as compared to baseline,[82] though the 50g lactose challenge may have been a limitation. A single dose of nonfermented milk containing either 1E8 or 1E9 CFUs of this probiotic bacterium significantly reduced both HBT scores and total symptom scores as compared to baseline at either bacterial concentration.[86] This research team also noted a moderate degree of bile sensitivity and ease of cell wall lysis in *Lactobacillus delbrueckii* subspecies *bulgaricus*, which likely contributes to host lactose digestion via the release of endogenously-produced bacterial lactase upon lysis. This contextualizes the increase in fecal beta-galactosidase observed in a previous study.[76] Also, one-time consumption of 400mL of milk containing *Lactobacillus delbrueckii* subspecies *bulgaricus* and *Streptococcus thermophilus* in concentrations of either 1E7 or 1E8 CFUs/mL was shown to reduce both incidence of lactose intolerance symptoms and HBT scores at the latter concentration.[91] The researchers also noted a high

lactase enzyme concentration (3 standard units/mL) in the milk inoculated with *Lactobacillus delbrueckii* subspecies *bulgaricus* prior to its consumption. Another yogurt study investigated the effect of consumption of 500g/day for 15 days of either unpasteurized yogurt containing greater than or equal to 1E7 CFUs of *Lactobacillus delbrueckii* subspecies *bulgaricus* and *Streptococcus thermophilus* or pasteurized but otherwise identical yogurt. Outcomes measured included blood glucose, serum lipids, serum short-chain fatty acids, and HBT scores. In lactose maldigesters, HBT scores were significantly lower, both initially and also after 15 days of fresh yogurt consumption, as compared to baseline values, but this effect was not observed for pasteurized yogurt.[92] Lactose-intolerant subjects also had significantly higher plasma propionate concentrations after the treatment period than at baseline, but no other findings were significant for this group.

Taken together, these studies provide moderately strong evidence that *Lactobacillus delbrueckii* subspecies *bulgaricus* probiotics, particularly as a component of either yogurt or probiotic-fortified dairy products, may be effective at reducing deleterious outcomes of lactose intolerance. In future research, follow-up symptom score assessments may be useful to determine if these effects persist beyond the 8-hour window for which outcomes were commonly recorded in the above studies. Due to its high degree of beta-galactosidase production and activity and its tendency to exude rather than ferment galactose, this bacterium is an interesting and promising candidate for potential probiotic treatment of lactose intolerance.

Lactobacillus casei

Lactobacillus casei is a probiotic bacterium that is closely related to *Lactobacillus paracasei* and *Lactobacillus rhamnosus*. It has been studied extensively for its probiotic potential, and it has been shown to have a high degree of acid-resistance and potential bile salt resistance,[114] which may help protect the bacterium as it travels through the host's gastrointestinal tract.

Studies investigating the effects of *Lactobacillus casei* by itself are rare, as it's commonly included in multi-probiotic treatments. In the aforementioned study investigating the effect of daily consumption of a probiotic containing *Lactobacillus casei* Shirota and *Bifidobacterium breve* Yakult for 4 weeks, the treatment resulted in a reduction in baseline lactose intolerance symptom scores and HBT values upon completion of the treatment regimen, and these effects persisted in a 3-month follow-up.[80] The Bio-25 pilot study also showed a significant reduction in lactose intolerance symptom scores after a 6-month treatment with a multi-probiotic containing *Lactobacillus casei.*[81] By contrast, subjects consuming the VSL3 multi-probiotic containing *Lactobacillus casei* were not observed to have any reduction in symptom scores nor HBT values.[82]

There is a small degree of evidence that *Lactobacillus casei* may, when combined with other probiotics, have some effect on outcomes of lactose intolerance, but more research is needed. In particular, research that investigates the effect of solely *Lactobacillus casei* on these outcomes in lactose intolerant individuals would contribute greatly to the literature.

Lactobacillus helveticus

Lactobacillus helveticus is a potentially probiotic bacterium that is closely related to *Lactobacillus acidophilus*. While it has not, as of yet, been as extensively researched as many other probiotics, it has been shown to adhere well to the host's mucosal layer and to competitively exclude the pathogenic bacteria *Escherichia coli* 0157:H7 and *Campylobacter jejuni*.[115] Only one study[93] in this review investigated the effects of *Lactobacillus helveticus* on outcomes of lactose intolerance in lactose maldigesters, and its results are not especially generalizable to a larger population. A 2010 study investigated the effects of daily consumption of 4E9 CFUs of *Lactobacillus rhamnosus* Rosell-11 and *Lactobacillus helveticus* Rosell-52 for 2 weeks on symptom scores and HBT values in children (age 10-12) with lactose maldigestion as compared to the effect of a heat-killed probiotic. The

researchers noted a significant decrease in HBT scores and the number of asymptomatic subjects, the latter of which improved from 13.9% of subjects at baseline to 58.2% of subjects following treatment of the live probiotic.[93] As with *Lactobacillus casei*, it would be useful to investigate the effect of *Lactobacillus helveticus* alone rather than in combination with another probiotic species, and this study population is not representative of typical lactose maldigesters, so there is not sufficient evidence to determine the effect of *Lactobacillus helveticus* on outcomes of lactose intolerance in typical lactose-intolerant individuals.

Lactobacillus delbrueckii* subspecies *lactis

Lactobacillus delbrueckii subspecies *lactis* is similar to *Lactobacillus delbrueckii* subspecies *bulgaricus* in that it's commonly used in the production of fermented dairy products, especially yogurt. In the literature, this bacterium should be clearly differentiated from *Lactococcus lactis*, as *Lactobacillus delbrueckii* subspecies *lactis* was formerly considered the separate species *Lactobacillus lactis*, thus "*L. lactis*" is somewhat ambiguous. In contrast to the *bulgaricus* subspecies, the *lactis* subspecies has retained more ancestral genes pertaining to carbohydrate digestion, and it is specialized toward absorbing lactose via a phosphotransferase system that excels in lower concentrations of lactose than the *bulgaricus* subspecies.[113] Despite this method of lactose transport, it may prove to be a similarly effective probiotic in attenuating undesirable lactose intolerance outcomes.

This bacterium was present in the Bio-25 probiotic, which has shown some ability to alleviate lactose intolerance symptoms when taken daily for 6 months,[81] and *Lactobacillus delbrueckii* subspecies *bulgaricus* was also used, as part of a yogurt starter culture, to inoculate milk that was administered to lactose-maldigesting children in 250mL doses. Researchers in the latter study observed a significant reduction in total lactose intolerance symptoms and HBT scores compared to

baseline in the lactose-maldigesting children who completed the treatment,[88] but full compliance was only achieved by 8 of 20 children. While results were generally positive for both treatments, these studies do not provide strong evidence that *Lactobacillus delbrueckii* subspecies *lactis* has a reliable effect on outcomes of lactose intolerance, and more research is needed. It may be useful to include the bacterium as a component of yogurt or inoculated milk, as is often done with the *bulgaricus* subspecies.

Lactobacillus paracasei

Lactobacillus paracasei is, as the name may imply, closely related to *Lactobacillus casei*, so much so that they share many of the same properties and have historically been difficult to differentiate from one another. Like its relatives, this bacterium is commonly used to ferment dairy products and is a viable probiotic candidate due to its acid resistance and inhibition of the proliferation of *Helicobacter pylori*.[114] In this review, only the Bio-25 multi-probiotic study investigated a treatment containing *Lactobacillus paracasei*,[81] so there is little evidence to support its use as a probiotic to attenuate lactose intolerance outcomes.

Lactobacillus plantarum

Lactobacillus plantarum is a Gram-positive, genetically diverse, aerotolerant bacterium commonly found in animal products and fermented vegetable foods such as kimchi.[116] Due to its ability to digest lactose and its acid tolerance, it is a candidate bacterium for the digestion of silage and waste products such as whey that are produced in dairy manufacturing.[117] These qualities also make *Lactobacillus plantarum* a suitable candidate for lactose intolerance research.

Several studies described above included *Lactobacillus plantarum* as part of a multi-probiotic treatment, including the Bio-25 pilot study[81] and VSL3 pilot study.[82] Additionally, daily consumption of a probiotic containing 5E9 CFUs of *Bifidobacterium animalis* subspecies animalis IM386 and

Lactobacillus plantarum MP2026 resulted in significant reduction in the incidence of diarrhea and flatulence compared to baseline, but no other symptoms, and HBT scores were unaffected.[77] In a 2020 randomized controlled trial, the daily consumption of 3E9 CFUs of *Pedicoccus acidilactici* and *Lactobacillus plantarum* strains CECT7484 and CECT7485 for 8 weeks was associated with a significant reduction in total symptom incidence and severity scores in the treatment group.[94] Nearly half (46.4%) of the treatment group experienced greater than or equal to 50% reduction in symptoms, while none of the control group experienced this.

Together, these studies provide a moderately low degree of evidence that *Lactobacillus plantarum*, in combination with other probiotic bacteria, may attenuate undesirable lactose intolerance outcomes. More research is needed, but this bacterium is a promising candidate for probiotic interventions in lactose intolerant populations.

Lactobacillus reuteri

Lactobacillus reuteri is a commensal bacterium normally present in the human gut that has been shown to produce antimicrobial peptides and perform competitive exclusion of pathogenic bacteria. It is adapted to a wide range of conditions and hosts, and it colonizes the proximal GI tract as well as the colon,[118] which sets it apart from many other probiotic bacteria and makes it a promising research candidate. A 2010 study involving 60 lactose intolerant patients investigated the effects of either 9000 units of lactase enzyme immediately before a lactose challenge or daily consumption of 8E8 CFUs of *Lactobacillus reuteri* in pill form 10 days before a lactose challenge. Both treatment groups experienced statistically significant reductions in total symptom scores, each individual symptom score, and HBT scores.[95] The group receiving lactase enzyme showed significantly lower scores than the probiotic group, so more research is needed to determine whether *Lactobacillus reuteri* potentially represents an equally viable lactose intolerance treatment option.

Lactobacillus rhamnosus

As mentioned above, *Lactobacillus rhamnosus* is genetically and functionally similar to both *Lactobacillus casei* and *Lactobacillus paracasei*. One characteristic that makes *Lactobacillus rhamnosus* an interesting probiotic candidate is the presence of mucous-binding proteins on the tips of its pili.[37] This bacterium, particularly the GG strain, is commonly used in food manufacturing and has been studied for its probiotic potential,[119] but the interventions included in this review either do not specify a strain or involve non-GG strains.

The Bio-25 multi-probiotic, which again was associated with some significant symptom reduction, contained *Lactobacillus rhamnosus.*[81] A synbiotic study investigated the effect of 4E9 CFUs of *Bifidobacterium longum* subspecies *longum* BB536, 1E9 CFUs of *Lactobacillus rhamnosus* HN001, and 1.4mg vitamin B6 per day on lactose intolerance symptoms and gut microbiome composition. The researchers observed a negative correlation between the relative abundance of *Bifidobacterium* in subjects' feces and the outcomes of bloating and abdominal pain, a significant reduction in bloating and constipation, and significant decreases in *Klesbiella*, *Serratia*, and *Enterobacter* genera in the treatment group.[83] Also, as discussed above, daily consumption of 4E9 CFUs of *Lactobacillus rhamnosus* Rosell-11 and *Lactobacillus helveticus* Rosell-52 in children from 10 to 12 years of age was associated with a significant decrease in HBT scores and in the number of asymptomatic subjects.[93]

Taken together, these studies provide a small degree of evidence that *Lactobacillus rhamnosus* probiotics, when in combination with other probiotic species, may attenuate undesirable outcomes of lactose intolerance. Further research will be necessary to determine which strains and what dosages are the most effective. It may also be useful to see *Lactobacillus rhamnosus* studied alone rather than as a component of multi-probiotic treatments.

Pediococcus acidilactici

Pediococcus acidilactici is a Gram-positive, sphere-shaped, comparatively resilient bacterium that colonizes the entire human gastrointestinal tract. This makes it a promising probiotic candidate, and it has been studied for the potential anti-tumor, immunomodulatory, and antioxidant properties of its metabolites.[120] There is little evidence that *Pediococcus acidilactici* has an effect on outcomes of lactose intolerance, however. As mentioned above, an 8-week study investigated the effects of daily supplementation of *Pediococcus acidilactici* CECT7483 and *Lactobacillus plantarum* strains CECT7484 and CECT7485 on outcomes of lactose intolerance and found a significant reduction in total symptom scores and symptom severity in the treatment group,[94] but substantially more research is needed to determine to what extent this effect was promoted by *Pediococcus acidilactici* specifically.

Streptococcus thermophilus

Streptococcus thermophilus is another Gram-positive, non-spore forming, non-motile anaerobe, and it is used alongside *Lactobacillus delbrueckii* subspecies *bulgaricus* to ferment dairy products. The two species are mutualistic as they supply one another with amino acids, formic acid, folic acid, and carbon dioxide for essential metabolic processes.[121] It has been studied extensively for its probiotic properties and for its production of metabolically useful metabolites, including the ability of the APC151 strain to produce GABA in yogurt.[122]

As it is commonly found alongside other bacteria, it may be difficult to separate the beneficial effects of *Streptococcus thermophilus* from the effects of other probiotics commonly included in yogurt or fortified into probiotic milks. Consequently, each *Streptococcus thermophilus* study included in this review has been described above. Daily consumption of 125g yogurt containing *Bifidobacterium animalis*, *Streptococcus thermophilus*, and *Lactobacillus delbrueckii* subspecies

bulgaricus alongside a probiotic capsule containing *Bifidobacterium longum* subspecies *longum* for 2 weeks was associated with a reduction in diarrhea, promotion of a bifidogenic effect,[76,79] and an increase in fecal beta-galactosidase activity.[76] The Bio-25 multi-probiotic included *Streptococcus thermophilus* and was associated with alleviation of some lactose intolerance symptoms.[81] Children with lactose maldigestion who were given 400mL of milk inoculated with 10E8 CFUs *Lactobacillus delbrueckii* subspecies *lactis* and 1E11 CFUs *Streptococcus thermophilus* showed significant reductions in total symptom scores and HBT values compared to baseline, although compliance was poor (40%) for this subgroup.[88] A dose-dependent relationship may also exist for this bacterium. A study investigating the effects of one-time consumption of 400mL of milk inoculated with 1E7 CFUs/mL or 1E8 CFUs/mL of a yogurt starter culture or of various strains of *Lactobacillus acidophilus* concluded that higher doses (1E8 CFUs/mL) of the yogurt starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subspecies *lactis* effectively reduced lactose intolerance symptoms and HBT values.[91] Also, fresh yogurt containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subspecies *bulgaricus* was shown to reduce HBT scores more than pasteurized yogurt.[92]

All 6 studies investigating the effects of *Streptococcus thermophilus* probiotics, yogurts, or inoculated milks found significant reductions in at least one outcome of lactose intolerance. This bacterium shows a strong degree of evidence for attenuation of undesirable lactose intolerance outcomes, with caveat that this effect may be due to any of the other bacteria in the multi-probiotic treatments that were administered. This is not necessarily problematic, as studying *Streptococcus thermophilus* alone, as an axenic probiotic, might remove the mutualistic benefit offered by *Lactobacillus delbrueckii*.

Prebiotics

Prebiotics, as a reminder, are substrates that are selectively utilized by the host's microbes to confer a health benefit upon the host, in this context by promoting adaptive changes in the composition of the gut microbiome.[9] These are commonly indigestible carbohydrates, or even poorly-digested carbohydrates such as raffinose and stachyose. In the case of lactose intolerant individuals, lactose is an indigestible or poorly-digested carbohydrate, so it potentially fits the definition of a prebiotic, and studies investigating the prebiotic potential of lactose were included in this review. Studies that investigated the effects of bacterial inoculations, supplementation, or fortification in combination with lactose or lactose-containing foods were considered synbiotic rather than prebiotic studies for the purpose of this review. This includes studies in which yogurt was the treatment.

Lactose

There are logistical and potentially even ethical issues with using lactose as a treatment in lactose-intolerant individuals. Researchers should be mindful of the discomfort, pain, and undesirable outcomes that will likely be experienced by subjects when this treatment is applied, and substantial attrition should be expected. Two studies included in this review investigated the effect of either lactose or milk administered to lactose-intolerant subjects. The first investigated the effect of increasing doses of lactose on hydrogen gas production and consumption over 10 days. The initial dose was 0.6g/kg body weight on day 1, which increased to 0.8g/kg day 3 and 1g/kg day 5, which is equivalent to more than 4 glasses of milk for most subjects. Interestingly, fecal hydrogen production by each fecal bolus was measured for 24 hours after defecation, and fecal hydrogen production was significantly lower in the lactose group at 3 hours and 24 hours.[96] No breath hydrogen scores were recorded, nor were symptom scores, but this study does provide some evidence of potential microbial adaptation to lactose as a prebiotic.

The second study investigated the effect of raw milk consumption, as compared to pasteurized milk or soy milk consumption, on breath hydrogen production and lactose intolerance symptom scores. In a crossover trial, subjects consumed raw milk in doses that began at 16 ounces on day 1, dropped to 4 ounces on day 2, increased by 4 ounces per day until day 7, then returned to a baseline dose of 16 ounces on day 8. Hydrogen breath tests were performed in response to the 16-ounce bolus of milk on days 1 and 8. There was a borderline significant reduction in total and peak hydrogen gas production by subjects consuming raw milk,[97] and these values were comparable to those observed for consumption of pasteurized milk on day 8. This may indicate some colonic microbial adaptation to raw milk, but not in excess of that observed for pasteurized milk. No differences in symptom scores were observed. No evidence was found to support the use of lactose as a prebiotic to attenuate undesirable outcomes of lactose intolerance.

RP-G28 galacto-oligosaccharide

Two studies included in this review investigated the effect of a novel glacto-oligosaccharide on outcomes of lactose intolerance. The first investigated the effects of two levels of RP-G28 galacto-oligosaccharide on symptom scores, number of responders (those with a reduction in symptom composite score of 4 or greater or those reporting a symptom score of 0), quality of life, gut microbiome composition, and lactose consumption upon follow-up. In total, 377 subjects were included and randomized into three groups. One received a corn starch based placebo, one received 5g of the treatment 2 times per day for 10 days then 7.5g of the treatment 2 times per day for 20 days, and the third group received 7.5g of the treatment 2 times per day for 10 days then 10g of the treatment 2 times per day for 20 days. There was a 40% response rate to RP-28 treatment and a 26% response rate to the placebo, and there were significant reductions in abdominal pain, abdominal cramping, bloating, and gas in the treatment groups as compared to the placebo group.[98] Subjects in

each treatment group also reported consuming significantly more milk per day than they had previously, and this increase was greater than that seen in the placebo group, potentially indicating an increase in milk tolerability.[98]

The second study investigated the effects of the same prebiotic in escalating doses of 1.5g/day to a maximum of 15g/day over the course of 35 days on HBT scores and lactose intolerance symptoms. Self-reported dairy consumption and an additional HBT were recorded during a 30-day follow-up. Compared to the corn syrup placebo, subjects consuming the prebiotic experienced generally, but not significantly, lower HBT scores.[99] Lactose intolerance symptom scores trended toward reduction, with 72% of the treatment group reporting a reduction in symptoms as compared to 28% of the placebo group. The number of subjects reporting no abdominal pain by the end of the treatment period was significantly higher for the treatment group than the placebo group. Subjects in the treatment group were also significantly more likely to report tolerance of dairy upon follow-up. Symptom scores, interestingly, were lower upon follow-up than they were at the end of the treatment period.[99] Taken together, these findings indicate some promise for RP-G28's potential to alleviate deleterious outcomes of lactose intolerance. Of note is that there was a substantial overlap in the membership of the research teams conducting these two studies. In the interest of replication, it would be helpful to see a similar study conducted by an unrelated research team.

Synbiotics

Synbiotics are mixtures of probiotics and substrates selectively utilized by those probiotics that confer a health benefit upon the host. For the purpose of this review, yogurt was considered a synbiotic treatment, as was the treatment composed of *Bifidobacterium longum* subspecies *longum*, *Lactobacillus rhamnosus*, and vitamin B6. Although, strictly speaking, vitamin B6 is ostensibly not selectively utilized by these bacteria any more than it is by the host, the nature of synbiotic treatment

generally involves any bacterium combined with a micronutrient or macronutrient intended to support the probiotic potential of the bacterium, so a more inclusive interpretation of synbiotics will be applied in this case.

Inoculated dairy products

Inoculated dairy products contain both lactose-hydrolyzing bacteria as well as the nutrient-rich media in which these bacteria might propagate, both prior to digestion and when they are introduced to the bacterial milieu of the gastrointestinal tract. In total, 9 studies included in this systematic review investigated the effect of inoculated dairy products on outcomes of lactose intolerance. Within these studies, treatments that were associated with reductions in both symptom scores and HBT values were 400mL of milk inoculated with 5E8 CFUs/mL of lactose-fed *Bifidobacterium longum* B6,[84] 400mL of milk inoculated with 1E8 or 1E9 CFUs/mL of *Lactobacillus delbrueckii* subspecies *bulgaricus*,[86] 250mL of milk inoculated with 1E8 CFUs/mL *Lactobacillus delbrueckii* subspecies *lactis* and 1E10 CFUs/mL *Streptococcus thermophilus* administered to children with lactose intolerance,[88] and 400mL of milk inoculated with 1E8 CFUs/mL of *Lactobacillus delbrueckii* subspecies *bulgaricus* and *Streptococcus thermophilus*.[91] Treatments that were associated with symptom score reduction but not HBT score reduction were 125g yogurt containing *Lactobacillus delbrueckii* subspecies *bulgaricus*, *Streptococcus thermophilus* and *Bifidobacterium animalis* combined with a probiotic capsule containing 2E8 CFUs of *Bifidobacterium longum*,[76,79] as well as a treatment of 25g of lactose from an unspecified quantity of unpasteurized yogurt containing 1E8 CFUs/g *Lactobacillus delbrueckii* subspecies *bulgaricus* and *Streptococcus thermophilus*.[100] Treatments associated with a reduction in HBT scores but not symptom scores were 500g/day fresh yogurt containing greater than or equal to 1E7 CFUs/g of *Lactobacillus delbrueckii* subspecies *bulgaricus* and *Streptococcus thermophilus*,[92] as well as 10mL/kg body weight of milk inoculated with 1E6 and 1E8 CFUs/mL of *Lactobacillus*

acidophilus.[89] Taken together, these studies provide moderately strong evidence that inoculated dairy products containing at least 1E8 CFUs/mL of *Lactobacillus delbrueckii*, particularly subspecies *bulgaricus*, and *Streptococcus thermophilus* may attenuate undesirable outcomes of lactose intolerance.

Probiotics combined with vitamin B6

One synbiotic study included in this review investigated the effects of 4E9 CFUs of *Bifidobacterium longum* subspecies *longum* BB536, 1E9 CFUs of Lactobacillus rhamnosus HN001, and 1.4mg vitamin B6 per day on outcomes of lactose intolerance, including symptom scores and gut microbiome composition. The treatment was associated with a notable Bifidogenic effect and significantly less bloating as compared to the control.[83] This study does not provide conclusive evidence that vitamin B6 has a synergistic effect on *Bifidobacterium longum* and *Lactobacillus rhamnosus* in vivo, and this treatment option merits further study.

CHAPTER VI
DISCUSSION

In this systematic review of 25 studies investigating the effects of probiotic, prebiotic, and synbiotic interventions on outcomes of lactose intolerance, there was a generally positive effect of nearly all treatments. Only 2 probiotic studies and 2 milk/lactose prebiotic studies showed a complete lack of significant improvement in any of the outcomes assessed for each included study. One of these probiotic studies utilized a large bolus of 50g lactose for the lactose challenge after a relatively short treatment duration,[82] and half of the participants in the other probiotic study were found to be lactose digesters upon closer examination,[90] so the lack of significant differences may be explained by the methodology employed in these studies. Moderately strong evidence for attenuation of lactose intolerance outcomes was observed for *Bifidobacterium longum*, *Lactobacillus delbrueckii* subspecies *bulgaricus*, *Lactobacillus acidophilus*, and *Streptococcus thermophilus*. A 2018 systematic review assessing the same outcomes from among 15 probiotic and prebiotic studies also concluded that *Bifidobacterium longum* probiotic supplementation showed a moderately strong degree of evidence of its effectiveness,[70] and a similar conclusion was reached in a 9-study 2020 systematic review,[71] so this bacterium may be an especially promising candidate for future lactose intolerance research.

The beneficial effects of the ingestion of lactic acid bacteria used as yogurt starter cultures, e.g. *Lactobacillus delbrueckii* subspecies *bulgaricus* and *Streptococcus thermophilus*, are likely due to the lysis of bacterial cells in the consumer's stomach and the release of bacterial beta-galactosidase. This is noteworthy because traditional probiotic benefits are often presumed to come from bacterial colonization. *Lactobacillus delbrueckii* subspecies *bulgaricus* and *Streptococcus thermophilus* do not generally survive passage through the digestive tract, particularly if the product containing the bacteria is heat-treated.[123] Lysis of these bacteria in the stomach and failure to colonize the gastrointestinal tract would cause the beneficial effects of these bacteria to be short-lived. It would be

fruitful to investigate the duration of improvement of lactose intolerance outcomes upon ingestion of inoculated dairy products as compared to ingestion of lactase enzyme pills or capsules. *Bifidobacterium longum* probiotics, by contrast, may have a longer-term effect on lactose maldigestion due to the ability of probiotic capsules to remain intact in the stomach and release their contents in the intestine.[124] In either case, further research is necessary to determine the duration of the beneficial effects of these probiotic bacteria and the precise mechanisms of action that promote the observed benefits.

Although a meta-analysis was originally planned, the substantial methodological heterogeneity between studies included in this review prevented pooling or statistical comparison of results. Studies varied in treatment dosage, method or schedule of treatment administration, dietary restriction imposed upon subjects, contents of multi-probiotics administered, duration, follow-up protocols, mass of the lactose bolus used for the HBT, and scale used to measure or report lactose intolerance symptom scores. Future studies may benefit from consistency, or even homogeneity, in these parameters, though few validated methods and agreed-upon values exist for each. The quantity of lactose for assessing HBT scores is a minor exception. Formerly, this test used a 50g bolus but has shown approximately equal sensitivity with a 25g bolus of lactose[125] and researchers are beginning to recognize the problematic nature of assessing lactose intolerance using a full 50g bolus of an indigestible carbohydrate.[126] It may be more practically useful to simply assess lactose intolerance symptoms, as HBT scores are not always closely associated with lactose intolerance symptom scores.

Gut microbiome composition may be a fruitful treatment outcome to investigate. Some studies noted a Bifidogenic effect of probiotic treatment and a reduction in lactose intolerance symptom scores, so it may be useful to assess how long this colonic adaptation persists via a follow-up assessment. This would help solidify the connection between probiotic treatment, intestinal

colonization, and long-term attenuation of undesirable lactose intolerance outcomes. The duration for which a probiotic treatment persists will depend on the resilience of the microbiota. In microbiome research, resilience is the ability of the microbiome to return to its baseline composition following a perturbation or alteration.[127] Such alterations may be caused by foodborne illness, dietary changes, a regimen of antibiotics, a regimen of probiotics, or other factors. In the context of a probiotic regimen, maintaining a diet that selectively supports the probiotic bacteria could favorably reduce resilience and lengthen the duration of treatment.[128]

Another noteworthy consideration is the safety of probiotic treatments. Each probiotic bacterium may have its own considerations or risks. As mentioned above, *Enterococcus faecium* is potentially pathogenic, so it should not be taken as a probiotic by those with any significant immunosuppression, nor if the strain is found to have vancomycin resistance.[107] Otherwise, no overtly pathogenic bacteria are known to be common probiotics. In a study[129] investigating the effect of a multispecies probiotic 2 times per day for 4 weeks on fecal and gastrointestinal outcomes of 298 patients with severe acute pancreatitis, 9 subjects within the treatment group (n = 153) developed bowel ischemia and 8 of these subjects died. The researchers offered the hypothesis that the probiotic treatment may have increased local intestinal oxygen demand and promoted bowel ischemia,[129] so risk of bowel ischemia may be a contraindication of probiotics in the acute care setting. Other sensible contraindications may include the presence or history of bowel perforation, which risks bacterial translocation into the host's systemic circulation. Otherwise, probiotics that are applied to food, in addition to a variety of probiotic metabolites,[130] are generally recognized as safe by the FDA.

CHAPTER VII
CONCLUSION

A systematic review of 25 studies revealed a moderately strong degree of evidence that *Bifidobacterium longum, Lactobacillus delbrueckii* subspecies *bulgaricus,* and *Streptococcus thermophilus* probiotics can alleviate undesirable outcomes of lactose intolerance. There was strong evidence that inoculated dairy products containing *Lactobacillus acidophilus, Lactobacillus delbrueckii* subspecies *bulgaricus,* and/or *Streptococcus thermophilus* can also alleviate undesirable outcomes of lactose intolerance.

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