

Lactobacillus fermentum as a Treatment for Intestinal Infection

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Abstract

Intestinal infections caused by pathogenic bacteria can present as especially virulent infections and can be difficult to treat. Pathogenic bacteria reproduce rapidly and when introduced into the intestines can overpopulate the intestinal flora leading to infection. Antibiotics have become standard treatment for infections so probiotics have not been researched as a possible way to prevent or reduce the severity of an intestinal bacterial infection. I researched this problem by comparing *in vitro* growth rates of *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis* with several commercial probiotic products. The pathogenic bacteria and probiotics were incubated and, starting from a standard concentration, growth rates were measured to establish a growth curve for each sample. The growth curves showed that the pathogenic bacterial strains grow faster than the probiotics with the exception of the probiotic that contains *Lactobacillus fermentum*. A one-sample t-test showed that the probiotic containing *L. fermentum* did not have a significantly different growth curve than the pathogenic bacteria tested. Pathogenic bacterial strains grow much quicker than most probiotics and healthy bacteria and this could explain why pathogenic bacteria cause such virulent intestinal infections and how they are able to cause such a disruption in the intestinal flora. However, the growth of *L. fermentum* is similar to pathogenic bacterial strains and could offer a natural combatant to bacterial infections in the intestine.

Keywords: Probiotics; Intestinal infection; Antibiotic; Microbiome; *L. fermentum*

Introduction

Bacterial infections in the intestines are caused by an overgrowth of pathogenic bacteria. These infections can be the result of antibiotic therapy or the introduction of pathogenic bacteria into the small intestine. The intestines are normally populated by good bacteria that are referred to as the gut flora or microbiome. Antibiotics are indiscriminate in their eradication of bacteria and can have a deleterious effect on the healthy bacteria in a person's body as the antibiotic works to eliminate the pathogenic bacteria. Antibiotic treatment can alter the composition or the function of the intestinal gut flora which results in overgrowth of pathogenic, toxigenic and antibiotic-resistant bacteria and reduction or possibly even complete loss of beneficial bacterial strains [1]. Antibiotics can kill the good bacteria in the gut, leaving the body more susceptible to harmful pathogens [2]. When a bacterium that is normally present in the large intestine is introduced into the small intestine, this can result in a condition known as small intestine bacterial overgrowth (SIBO). Any disruption in the microbiome can cause dysbiosis, or the beneficial bacteria being overgrown by pathogenic bacteria.

A bacterial infection of the intestines, regardless of its origin, is at the very least an unpleasant experience and at its worst, a potentially life-threatening diagnosis. Pathogenic bacteria multiply very quickly and if they can outcompete with healthy bacteria for attachment space on the intestinal wall, the infection will become much more difficult to treat [3]. The growth rates of several pathogenic bacterial strains known to cause intestinal infections will be compared with the growth rates of probiotic bacteria, which will serve as a sample set of healthy indigenous gut bacteria. This comparison will determine if the pathogenic bacteria multiply quicker than the healthy gut bacteria in standardized *in vitro* conditions. The pathogenic bacteria chosen for this study, *E. coli*, *S. aureus* and *E. faecalis* are known to cause intestinal infections. The five probiotic products chosen represent a variety of products from across a spectrum of brand, number of species per product and cost.

Methods

The pathogenic bacteria used in this study, *E. coli*, *S. aureus* and *E. faecalis* were obtained from Carolina Biological Supply Company, Burlington, NC, USA. Using the streak plate method, the *E. coli* and *S. aureus* were placed onto tryptic soy agar (TSA) plates and the *E. faecalis* was placed onto Eosin Methylene Blue (EMB) agar plates (both agars were manufactured by Carolina Biological Supply Company, Burlington, NC, USA). The plates were incubated at 37°C for 24 hours at which time colonies were isolated from each plate using a sterilized loop.

The probiotics used were: Basic Probiotic Gold Acidophilus, manufactured by Basic Drugs, Inc., Vandalia, OH, USA; Kroger 4X, manufactured by Perrigo, Allegan, MI, USA; Sundown Naturals Probiotic Balance, manufactured by Rexall Sundown, Inc., Boca Raton, FL, USA; Nature's Bounty Probiotic CD, manufactured by Nature's Bounty, Bohemia, NY, USA; and Megaflora Megafood Probiotic, manufactured by Megafood, Derry, NH, USA. A sample of 0.05 grams of each probiotic was measured out to be incubated for the study.

The bacterial colonies and probiotic samples were each added to a test tube containing 10 mL of tryptic soy broth (TSB) (manufactured by Carolina Biological Supply Company, Burlington, NC, USA) and mixed using a vortex. Each test tube was capped and placed in a shaking incubator for 24 hours at 37°C. The samples were removed from the incubator and each solution was diluted with TSB until an optical density (OD) reading of 0.05 was reached using a spectrophotometer (Thermo Scientific Spectronic 200 Educator Visible Spectrophotometer) set at

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600 nm. The initial OD₆₀₀ reading was recorded as 0.05 at 0 minutes for each sample. The samples were placed back into the shaking incubator and at 15 minute intervals the OD₆₀₀ was measured and recorded to establish a growth curve for each sample. The OD₆₀₀ measurements were stopped at 405 minutes for a total of 28 OD₆₀₀ readings for each sample.

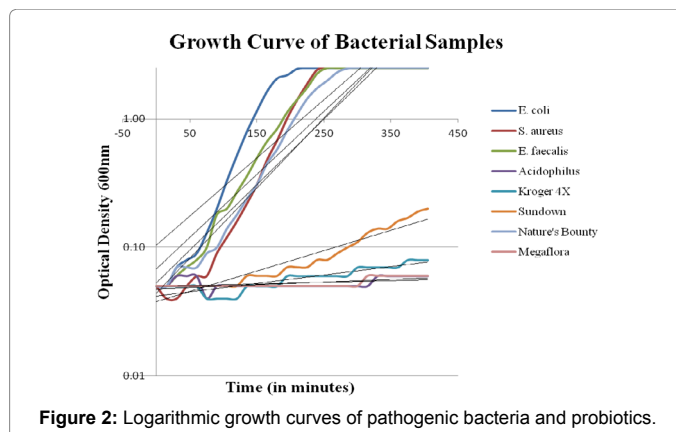
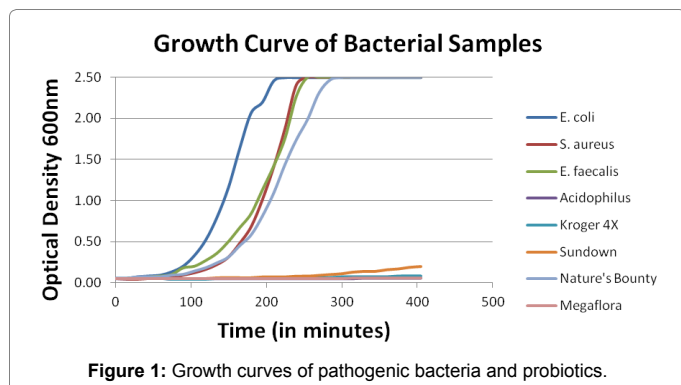
Results

The pathogenic bacteria had all reached an optical density (OD₆₀₀) of 2.50 at 255 minutes into the study. At the same time measurement, the Nature's Bounty probiotic measured an OD₆₀₀ of 1.99 and the remaining probiotics all measured an OD₆₀₀ of under 0.10. At 300 minutes the Nature's Bounty probiotic reached an OD₆₀₀ of 2.50. The Sundown Naturals probiotic measured an OD₆₀₀ of 0.09 at 270 minutes and began to steadily increase in concentration for the remainder of the study. The Basic Acidophilus, Kroger 4X and Megaflora probiotics showed a negligible increase in concentration measured by OD₆₀₀ throughout the study (Figure 1).

Formatting the growth curve to a logarithmic scale, the slope of the bacterial samples are as follows: *S. aureus*-0.0126, Nature's Bounty-0.0117, *E. faecalis*-0.0113, *E. coli*-0.0104, Sundown Naturals-0.0037, Kroger 4X-0.0015, Megaflora-0.0005, and Acidophilus-0.0003. The logarithmic graph is used to show the exponential growth pattern of bacteria. The pathogenic bacteria show similar growth rates, with the slopes ranging from 0.0126 to 0.0104; the Nature's Bounty probiotic showed a growth rate within that range of 0.0117. The other probiotic products did not fall within a similar slope range, those products' slopes ranged from 0.0037 to 0.0003. Using a one-sample t-test with a significance level of 0.05 to determine the statistical significance of the growth curve slopes, the average of the pathogenic bacteria slopes ($\mu=0.0114$) was used to calculate the p-value for each sample. The p-values were calculated as follows: *S. aureus*-0.2092, Nature's Bounty-0.7168, *E. faecalis*-0.8539, *E. coli*-0.2470, Sundown Naturals-0.0067, Kroger 4X-0.0041, Megaflora-0.0034, and Acidophilus-0.0033. This t-test showed that the Sundown Naturals, Kroger 4X, Megaflora and Acidophilus probiotics were all significantly different than the average growth of the pathogenic bacteria as their p-values were each below 0.05. The Nature's Bounty probiotic had a p-value of 0.7168, above the 0.05 significance level indicating that its growth was not significantly different than the average of the pathogenic bacteria studied. As shown below in Figure 2, the Nature's Bounty probiotic has a slope or growth pattern well within the range of the pathogenic bacteria.

Discussion

Pathogenic bacteria multiply very quickly which can lead to bacterial infections. When these infections occur in the intestine, treatment is



often in the form of antibiotics which further decimates the healthy gut bacteria. Probiotics are commercial products that contain species of healthy bacteria typically found in the digestive tract. There are various products available that contain a variety of bacterial species (Appendix A). Taking probiotics prophylactically may improve a person's overall health and reduce the risk of SIBO or other bacterial-related infections in the digestive tract. Maintaining a healthy microbiome may decrease the likelihood of developing an intestinal infection as the intestinal lining will be coated with healthy commensal bacteria which can decrease the rate at which pathogenic bacteria multiply and adhere to the intestinal wall [3,4].

As shown in Figure 1, the growth curve comparing the pathogenic bacteria to the probiotics, most of the probiotics grow at a substantially slower rate which means that when the pathogenic bacteria is present in the intestine, there is an increased risk of infection. When a person is diagnosed with an intestinal bacterial infection, probiotics may be considered as a treatment option. The probiotics are healthy bacteria that to date have not been shown to cause any adverse side effects and instead of killing both bad and good bacteria as an antibiotic will do, the probiotic will help replenish the good bacteria. Increasing the population of healthy bacteria which will crowd out the pathogenic bacteria could provide a more natural treatment without the possible negative side effects and complications from antibiotic treatment [5].

The Nature's Bounty probiotic grew much quicker than the other probiotics. As shown in Figure 2 and by the results of the t-test, its logarithmic growth rate is similar to the pathogenic bacterial strains tested. This could be due to the presence of *Lactobacillus fermentum*, which is the only bacterial species present in the Nature's Bounty product that is not present in any of the other products tested in this study (Appendix A). As there are numerous strains of *L. fermentum*, further testing needs to be performed using *L. fermentum* to determine which strains grow more quickly than other gut bacteria. *L. fermentum* could be used as a treatment for bacterial infections of the intestine or taken prophylactically if a patient is going to be at risk for developing an intestinal infection. Pathogenic bacteria have various mechanisms with which to attack the intestinal wall [3] and building up the population of commensal bacteria in a person who will be at risk to develop an infection would be a potential guard against life-threatening bacterial infections of the intestine.

The limitations on this study were that the bacteria were grown in TSB and *in vitro*. This study could be reproduced using other growth media under anaerobic conditions and if the results were viable, further

studies could be conducted in murine trials to observe efficacy of *L. fermentum in vivo*. Future studies could be conducted to determine that if strains of *L. fermentum* are shown to grow more quickly whether or not these specific strains of bacteria possess any growth factors that may affect other bacterial strains present in the gut. If there is a growth factor that causes the *L. fermentum* to grow quickly, this factor could possibly be extracted and introduced into other healthy bacteria to promote faster growth so that the growth of all probiotics could be sped up to match the growth rates of pathogenic bacteria. This could result in the use of probiotics to treat intestinal bacterial infections instead of antibiotics. One strain of *L. fermentum* has been shown to have “functional efficacy of the antimicrobial and antioxidative activity” against intestinal pathogens [6]. Further study of this strain as well as others could lead to development of probiotic treatment for intestinal infections which would provide a more natural treatment with less deleterious effects on a patient’s overall health.

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