Letters in
General Microbiology

# The Effects of Prebiotic Supplementation on Growth and Survival of *Lactobacillus bulgaricus*

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## Abstract

Probiotic bacteria, which are present as both innate components of the gut microflora and dietary supplements, have numerous benefits to the host including: reduction of inflammatory response, improved nutrient absorption, aid in recovery from courses of antibiotics (Sonnenburg 2006). Though the metabolic mechanisms through which these effects are conveyed are not fully understood, it is believed that supplementation with prebiotic factors such as oligosaccharides and dietary fiber components enhance probiotic growth and survival by serving as a nutrient source (Gibson 1999). In this current study, I tested the effects of prebiotic supplementation with oligosaccharides on the growth of *Lactobacillus bulgaricus* under conditions of pH 6.0 and mimicking the pH environment of the digestive system at 3.0. Based upon the results, I was unable to conclude that prebiotic supplementation enhanced *L. bulgaricus* growth.

## Introduction

Probiotics are microbes that have a beneficial relationship with hosts that can be seen in the gastrointestinal (GI) system. Though the mechanisms of their actions aren't fully understood, there are many studied positive effects including mediation of inflammation and allergy response, improved nutrient absorption and enhancement of the natural gut flora, particularly after a course of antibiotic treatment (Sonnenburg 2006; Fernandez 2012). Probiotic bacteria can be found as live strains in fermented dairy food sources and can also be supplemented in the diet (Sonnenburg 2006). Species of the genus *Lactobacillus*, Gram-positive facultative anaerobes, are among the most studied probiotic bacteria.

Prebiotics are characterized as the dietary fibers and sugars that probiotic species require for nutrition and thus enhanced survival and proliferation (Anderson 2004). Supplementation with components such as oligofructose and inulin is thought to enhance gut microflora because probiotic species such as *Lactobacilli* and *Bifidobacteria* utilize oligosaccharides as a primary metabolic carbon source (Gibson 1999, Corcoran 2005; Guarner 2007; Ganzle 2012). Better understanding the factors required for successful probiotic growth and nutrition has important clinical implications in the treatment and management of GI inflammatory diseases (Sonnenburg 2006; Guarner 2007).

The purpose of this experiment is to evaluate the effects of supplementary prebiotic nutrients on the growth and survival of *Lactobacillus* in both a neutral pH and in an acidic pH to mimic digestive conditions. I hypothesized that under both pH conditions, the introduction of prebiotic nutrient supplements will result in increased concentration of probiotic strains in culture. The null hypotheses are: there is no difference in *Lactobacillus bulgaricus* growth between control conditions at pH 6.0 and conditions at pH 6.0 with prebiotic supplementation and there is no difference in *L. bulgaricus* growth in pH 3.0 and pH 3.0 with prebiotic supplementation.

## Materials & Methods

*Lactobacillus bulgaricus* strains were grown using the Natren yogurt starter kit and whole milk. The yogurt starter powder was not purely *Lactobacillus bulgaricus.* The contents of the kit are: *Lactobacillus bulgaricus* Super Strain LB-51, *Streptococcus thermophilus* Strain BC 122, nonfat milk solids, lactose and whey. The strains were cultured in plates containing de Man-Rogosa-Sharpe (MRS) agar that was made according to the Biokar protocol. The prebiotic dietary supplement, Prebiotin™, containing oligofructose enriched inulin was used. The pH conditions were measured at plating and adjusted using hydrochloric acid (HCl). Strains were cultured 72 hours in after which time the plates were serially diluted for counting. There were four different plating conditions: *L. bulgaricus* on MRS agar pH 6.0 (control), *L. bulgaricus* + prebiotic supplement, *L. bulgaricus* on MRS agar adjusted to pH 3.0, *L bulgaricus* on MRS agar pH 3.0 + prebiotic supplement. Each of the conditions was tested in the original concentration and in a series of titrations: 1/10, 1/25, 1/50, 1/100.

## Results

There are no observed trends in measured probiotic growth. Results were plotted as measured prebiotic growth in millimeters vs. the four *L. bulgaricus* probiotic growth conditions, respectively: *L. bulgaricus* (L.b) at pH 6.0, *L. bulgaricus* at pH 3.0 (HCl), *L. bulgaricus* at pH 6.0 plus prebiotic (prebiot.) supplementation, *L. bulgaricus* at pH 3.0 (HCl) plus prebiotic supplementation. Because the p values of 0.230 and 0.725, respectively, were not less than or equal to 0.05, I was unable to reject the null hypotheses that supplementation with prebiotic factors at pH of 6.0 and pH of 3.0 (Figure 1). Among the dilutions of *L. bulgaricus* grown in pH 3.0 there was an observed increase in growth in the $^1/_{10}$ concentration.

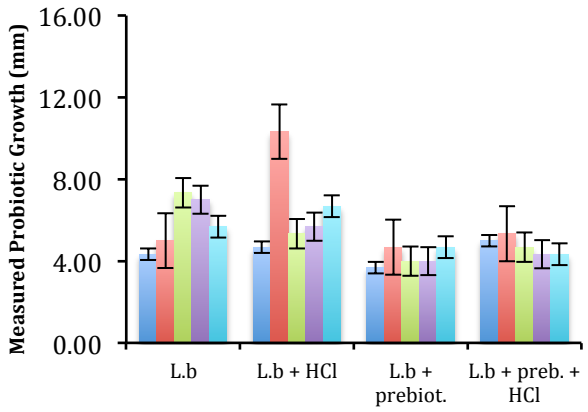


Figure 1. Measured *Lactobacillus bulgaricus* growth under four growth conditions at different titrations. (ttest value comparing L.b and L.b + prebiot.= 0.230; ttest value comparing L.b + HCl and L.b + prebiot. + HCl= 0.725). Colored bars indicate dilution: dark blue is full concentration, red is $^1/_{10}$, green is $^1/_{25}$, purple is $^1/_{50}$, light blue is $^1/_{100}$. Error bars represent standard deviation. (p=0.230 for L.b compared with L.b + prebiotic; p=0.725 for L.b + prebiotic compared with L.b + prebiotic + HCl)

## Discussion

The purpose of this study was to investigate the affects of prebiotic oligosaccharide supplementation on *L.* bulgaricus growth and survival. Based upon the results, I am unable to confirm the hypothesis that supplementation with the prebiotic factors, oligosaccharides and inulin, enhanced probiotic growth in acidic conditions. The only observable increase in probiotic growth occurred at the $^1/_{10}$ dilution of *L. bulgaricus* at pH 3.0 which measured an average of 10.33 mm versus 4.67mm, 5.33mm, 5.67mm and 6.67mm at the other 4 dilutions. There are a few factors to consider in investigating this finding in future studies. This growth increase could suggest that at this concentration under the acidic conditions mimicking the digestive system, the principle of quorum sensing is demonstrated and that prebiotic growth is maximized at this condition.

Another important factor to consider is that the prebiotic strain tested was not purely *Lactobacillus bulgaricus*. Because of cost limitation, a commercially available, powdered strain also containing *Streptococcus thermophilus* Strain BC 122, nonfat milk solids, lactose and whey was utilized. It is possible that each of these additional components also individually affected probiotic bacterial growth. The experiment should be completed with a pure bacterial strain in order to draw further conclusions. It would also be beneficial to recreate the experiment under different growth conditions utilizing a liquid broth medium under more optimal, controlled anaerobic conditions at 37°C in a $CO_2$ chamber.

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