Rapid Method for Measuring the Effect of Prebiotics on Probiotic Bacterial Growth

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Abstract

Prebiotics are used to stimulate probiotic bacterial growth in the gut to optimize their health benefits. A rapid method was developed to evaluate potential growth enhancement by prebiotics on probiotic bacteria using a programmable spectrophotometer, standard microtiter plates, and commercial media, with growth enhancement results ready in 12 hours. *Lactobacillus* strains were grown in MRS broth while *Bifidobacterium* strains were grown in MRS broth with L-cysteine. Each culture was back diluted to an OD_{600} of 0.1 with the appropriate MRS broth then inoculated into wells (48 well plate) containing individual prebiotics. Plates were placed in a Tecan Infinite M200 spectrophotometer and incubated at 37ºC with A_{600} readings taken for 12 h. Growth curves were done in triplicate with results compared to controls to determine extent of prebiotic growth enhancement. To optimize the method MRS concentrations of 20%, 35%, 50% and 100% were tested at selected pHs (7.0, 5.5, 5.0, 4.5, and 4.0) using 5 probiotic cultures. Addition of the bio-catalytic oxygen-reducing reagent oxyrase to test wells just prior to testing significantly enhanced growth of *Bifidobacterium* species and some lactobacilli such as *Lb. acidophilus*. Results indicated a 25% MRS broth at pH 5.0 with 2% oxyrase addition optimized prebiotic growth enhancement. Using this method, the stimulatory effect of added prebiotics (2% v/v) FOS, GOS, and XOS was determined for *Bifidobacterium infantis* M-63, *Bifidobacterium longum* BB536, and *Bifidobacterium lactis* BL-04, *Lactobacillus rhamnosus* LR-32 and *Lactobacillus acidophilus* NCFM. All three significantly improved growth of M-63, but only FOS increased growth of BL-04. For BB536, just GOS enhanced growth. GOS and FOS slightly improved growth of NCFM but no oligosaccharides enhanced growth of LR-32. The method allows rapid testing of various inoculum levels, prebiotic concentrations, media pHs, and prebiotic combinations for any probiotic strain including *Bifidobacterium.* With multiple samples run concurrently, comparisons can readily be made. In addition, the method can determine optimum enhancement of individual prebiotics or prebiotic combinations for any probiotic strain.

Introduction

The digestive tract of a human is composed of many different environments. There are low pH environments, such as the stomach, environments which have less oxygen, such as the intestines and environments with inadequate or abundant sources of nutrients. These environments can have an effect on the growth and type of bacteria which reside within the digestive tract, including the small and large intestines. The large intestines contain up to 10^{12} bacteria per gram of waste content (4). With this high concentration of bacteria, it is important to consider the type of products these bacteria produce, some can be beneficial while others can be harmful. Beneficial bacteria, known as probiotic bacteria, can produce products which the human body can utilize, or even prevent the establishment of harmful bacteria.

There are several types of probiotic bacteria, such as the *Lactobacillus* strains or *Bifidobacterium* strains (1), but considering they require different environments, it is reasonable to contemplate on their optimal environments within the setting of the human digestive tract. Once this environment is established (pH, oxygen and ambient nourishment) then additional food sources need to be considered (2). To enhance growth of these probiotic bacteria, specialized oligosaccharides, known as prebiotics, are used. Oligosaccharides, of Xylose (XOS), Fructose (FOS), and Galactose (GOS), were used as the primary food source in these experiments.

A method was developed to evaluate both environment and food source utilizing a spectrophotometer plate reader, mimicking the gut environment for the growth of five different bacteria; three *Bifidobacterium* strains and two *Lactobacillus* strains (3).

Materials and Methods

Media: MRS broth at varying concentrations and MRS broth with 0.05% L-cysteine were used.

Bacteria Preparation: *Bifidobacterium infantis, longum,* and *lactis* powdered cultures were transferred with a sterile swab into 9 mL MRS broth tubes containing 0.05 L-cysteine and placed into an incubator with a Gaspak to facilitate an anaerobic environment and incubated for 24 hours at 37ºC. *Lactobacillus rhamnosus* and *acidophilus* were placed into 9mL MRS broth tubes at 37ºC for 24 hours.

Media and Oxyrase Optimization: Diluted (0.1 OD_{600}) *Bifidobacterium longum, infantis, and lactis* were run on a separate plate than *Lactobacillus acidophilus* and *rhamnosus* due to space on the 48 well-plate. The volume added to each well was 1.2 mL of 20%, 35% and 50% MRS broth containing each *Bifidobacterium* (0.1 OD_{600}) or 1.2 mL of broth. Oxyrase was added to certain wells in 1%, 2%, or 4% concentrations.

To control for oxyrase effects, each *Bifidobacterium* was also grown without oxyrase. To ensure that the MRS broth and oxyrase was sterile, 20%, 35% and 50% MRS broth with and without oxyrase were added to the plate.

Diluted (0.1 OD_{600}) *Lactobacillus acidophilus* and *Lb. rhamnosus* in 20%, 35% and 50% MRS broth were transferred to a 48 well plate (1.2 mL in each specified well). Similar to the *Bifidobacterium* plate, specific *Lactobacillus acidophilus* wells contained 1%, 2%, or 4% oxyrase concentrations. This was done to ensure *Lactobacillus acidophilus* grew well during the test.

To control for oxyrase effects, each *Lactobacillus acidophilus* was grown without oxyrase. To ensure that the MRS broth and oxyrase was sterile, 20%, 35% and 50% MRS broth with and without oxyrase were added to the plate. Each well plate was run for 12 hours at 37 ºC with readings taken every 30 minutes in a spectrophotometer in triplicate.

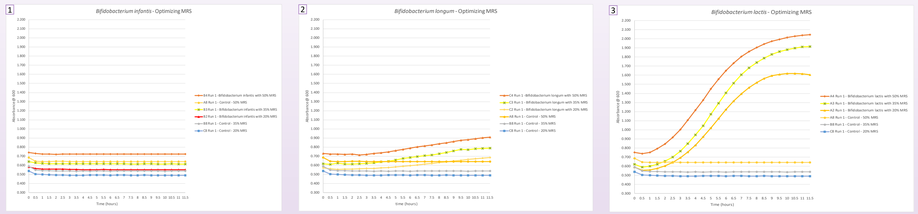
pH Optimization: *Bifidobacterium infantis, longum,* and *lactis* powdered cultures were transferred with a sterile swab into 9 mL MRS broth tubes containing 0.05 L-cysteine and placed into an incubator with a Gaspak to facilitate an anaerobic environment and incubated for 24 hours at 37ºC. *Lactobacillus rhamnosus* and *acidophilus* were placed into 9 mL MRS broth tubes at 37ºC for 24hrs.

After the incubation period, each culture was transferred to new tubes and back diluted to a OD_{600} of 0.1. Each culture was then transferred to 48 well plates. As indicated below, each culture was grown at specified pH levels (5.5, 5.0, 4.5, and 4.0) at 4% oxyrase, except for *Lactobacillus rhamnosus*. 1.2 mL of culture or media was added to each well indicated with the appropriate amount of oxyrase (48 µL of oxyrase to each indicated well). Each well plate was run for 12 hours at 37 ºC, each reading taken every 30 minutes in a spectrophotometer in triplicate.

Prebiotic (oligosaccharide) Tests: Lyophilized cultures of *Bifidobacterium infantis, B. longum,* and B. *lactis* were inoculated with a sterile swab into 9 mL MRS broth tubes containing 0.05 L-cysteine and placed into an incubator with a Gaspak to facilitate an anaerobic environment and incubated for 24 hours at 37ºC. *Lactobacillus rhamnosus* and *Lb. acidophilus* were placed into 9 mL MRS broth tubes at 37ºC for 24 hrs.

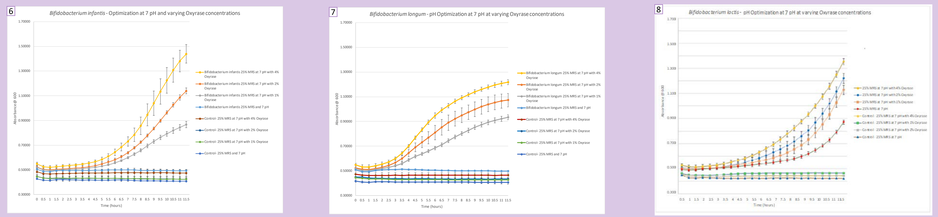
After the incubation period, diluted (0.1 OD_{600}) individual probiotic bacteria were transferred to 48 well-plates. Each well contained 2% of each oligosaccharide (XOS, FOS, GOS, and combination D), summing to 1.2 mL of dilute and oligosaccharide. *Bifidobacterium infantis, B. longum, B. lactis,* and *Lactobacillus acidophilus* contained 2% oxyrase within the 1.2 mL, as above, *Lactobacillus rhamnosus* was grown and tested without oxyrase. Each well plate was run for 12 hours at 37 ºC, each reading taken every 30 minutes in a spectrophotometer in triplicate.

Results – Media Optimization

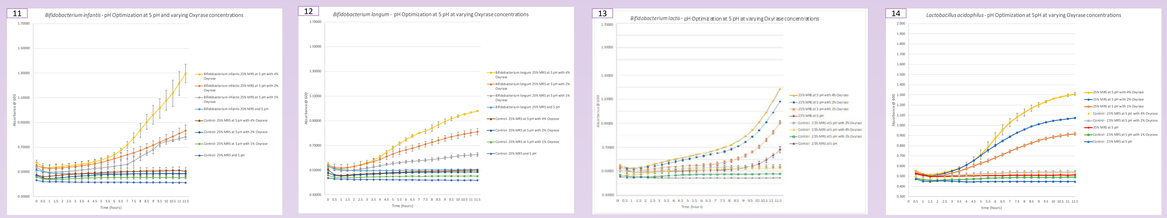


Figures 1-5. The effects of different MRS concentrations on each of the five probiotic bacteria over a 12 h period. Readings were taken every 30 minutes.

Results – Oxyrase and pH Optimization



Figures 6-10. The effects of 7 pH at varying oxyrase concentrations (1%, 2% and 4%) for four probiotic bacteria. This was done over a 12 h period, readings taken every 30 minutes.



Figures 11-14. The effects of 5 pHs at varying oxyrase concentrations (1%, 2% and 4%) for four probiotic bacteria. This was done over a 12 h period, readings taken every 30 minutes.

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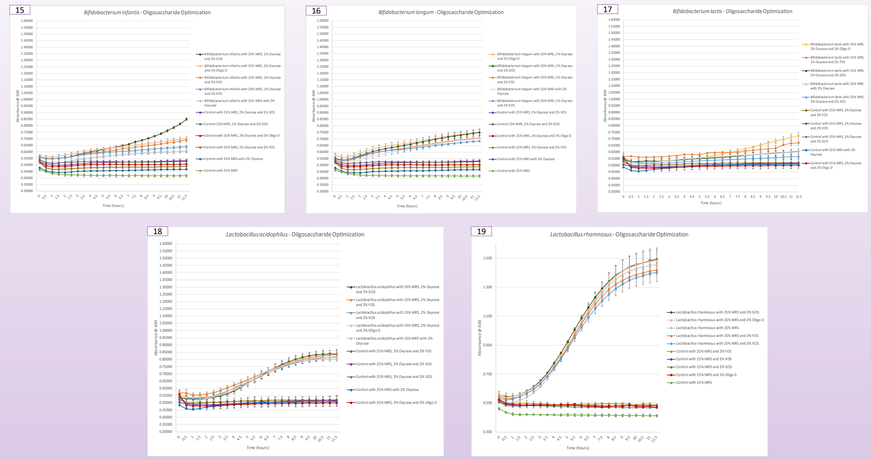
Results – Prebiotic Utilization



Figures 15-19. The effects of each oligosaccharide (XOS, FOS, GOS and oligosaccharide combination D) for each probiotic bacteria. The readings were taken every 30 min for 12 h.

Conclusions

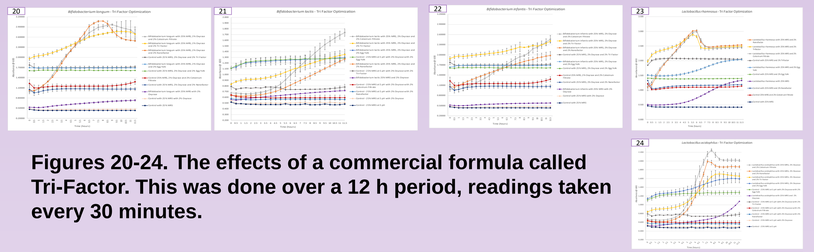
During the optimization phase of the project, the results indicate that as pH decreases, so does the growth of each bacteria. The growth curves of each bacteria mimicked one another, at both pH 7 and pH 5, except in the base of *Bifidobacterium infantis*, which seems to have been the result of little to no living bacteria during the experiment, since the growth curves above clearly indicate that this bacteria will grow in both pH 7 and 5.

The oxyrase concentration levels had a significant effect on each *Bifidobacterium* species and *Lactobacillus acidophilus*. Being an oxygen-reducing enzyme, it assisted in scavenging oxygen from the media environment, which in turn supported anaerobic processes. *Lactobacillus rhamnosus* did not need such enzymatic support in order to grow since it was able to establish itself with, or without, oxyrase.

With the optimization complete, insight was granted into how much MRS, oxyrase, and pH level was needed to facilitate an environment similar to the duodenum and onwards. This would have a higher pH than the stomach, but still less than a pH of 7. From this data, 25% MRS, a pH of 5 and an oxyrase concentration of 2% was chosen. If the concentration of MRS was higher, it could mask any growth enhancement by the oligosaccharides, which were a primary focus. Similar light of thought went into how the oxyrase was being utilized.

With each oligosaccharide implementation, the method used gave some clear indicators on which bacteria were affected significantly or similar to the other oligosaccharides. For both *Lb. rhamnosus* and *Lb. acidophilus*, the oligosaccharides displayed little difference between each other. Whereas the *Bifidobacterium* species showed varying effects between the oligosaccharides. *Bifidobacterium infantis* was significantly effected by the oligosaccharide GOS compared to the other treatments. *Bifidobacterium longum* showed no singular oligosaccharide treatment was significant. *Bifidobacterium lactis* was effected more by the oligosaccharide D blend compared to the next closest treatment, FOS. XOS had no significant impact on each probiotic's growth in comparison to the other oligosaccharides.

Tri-Factor Analysis (Method Application)



Figures 20-24. The effects of a commercial formula called Tri-Factor. This was done over a 12 h period, readings taken every 30 minutes.

References

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