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2018 - Nu Skin: Anthocyanin Rich Extracts Effects on Lactobacillus acidophilus - Poster Presentation

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Nu Skin: Anthocyanin Rich Extracts Effects on *Lactobacillus acidophilus*

INNOVABIO INNOVATIONS IN CONTRACT RESEARCH AND SERVICES

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Background & Objectives

Anthocyanins are flavonoids found in plants. They give the red, blue and purple color to plants(ex: blueberry, raspberry, black rice...etc). Anthocyanins frequently interact with other phytochemicals to potentiate beneficial biological effects. We are interested in testing whether certain anthocyanins have the desired effect on two human gut bacteria; *Bifidobacterium longum infantis*, and *Lactobacillus acidophilus*. It is suggested that anthocyanins & Fructooligosacchride (FOS) have a growth promoting effect on these two strains.

InnovaBio® was contracted to test whether anthocyanin rich vegetable extracts will promote or inhibit the growth of these two bacterial strains. *Bifidobacterium* and *Lactobacillus* both grow under anaerobic conditions in the human gut. Our experiments were conducted in the absence of oxygen and under nitrogen and carbon dioxide gases using several methods, including the glove box(figure 1). The medium for each bacterium has specific reagents to support growth. To do the experiments we made both complex and basal growth medium. The basal medium has fewer nutrients and will be used to examine changes in growth in the presence of additives. We determined growth rates of *Lactobacillus* under anaerobic conditions in the complex growth medium. We are currently growing bacteria in basal medium with different anthocyanin rich plant extracts and FOS. We have basal as negative control, Glucose as positive control, and anthocyanin rich vegetables extracts and FOS as our test media. We used colony forming unit (CFU) test as our quantitative indicator of cell viability.

Project Overview

- Since we are testing the effects of anthocyanin rich extracts on *Lactobacillus acidophilus*, we needed to provide anaerobic conditions, because *Lactobacillus* grows in the human gut, where oxygen is absent.
- We used PLAS-LABS glove box(figure 1)to provide anaerobic conditions. We also used Hungate tubes (see materials & methods) to carry out the experiments outside the glove box.
- *Lactobacillus acidophilus* colonies are white & pearl shaped.(figure 2)

Figure 1

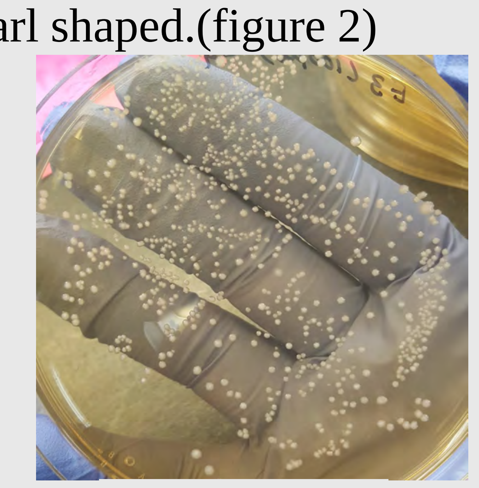


Figure 2

Variables

Controlled Variables	Independent Variable	Dependent Variable
• Basal as negative control • Glucose and FOS as positive control	• Anthocyanin rich extracts	• Serial dilutions & plating • Colony Forming Unit Method. (CFU)

Purpose

Testing whether Anthocyanin(figure3)rich extracts & FOS promote the growth of *Lactobacillus acidophilus* under anaerobic conditions.

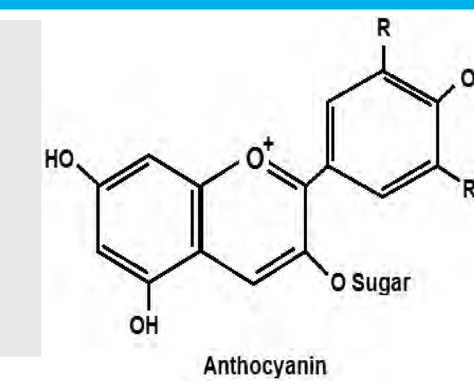
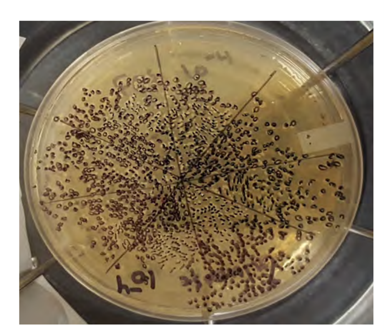


Figure 3

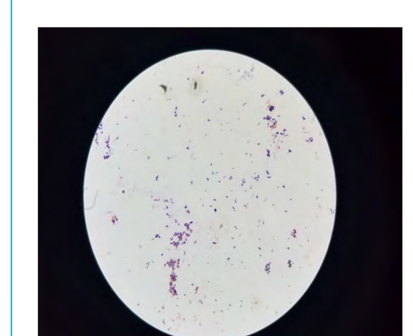
Methods & Materials

CFU



Colony Forming Unit method.

Gram Staining



Lactobacillus stains, gram positive.

Hungate Tubes

Keep the culture anaerobic.

Anaerobic Jar

Keeps the plates anaerobic.

CFU Determination

Cells grow anaerobically to stationary phase (in defined media & media supplemented with Glucose, FOS, or Anthocyanin rich extracts)

Serial dilutions & plating on agar complex medium (allow colony formation in anaerobic jar)

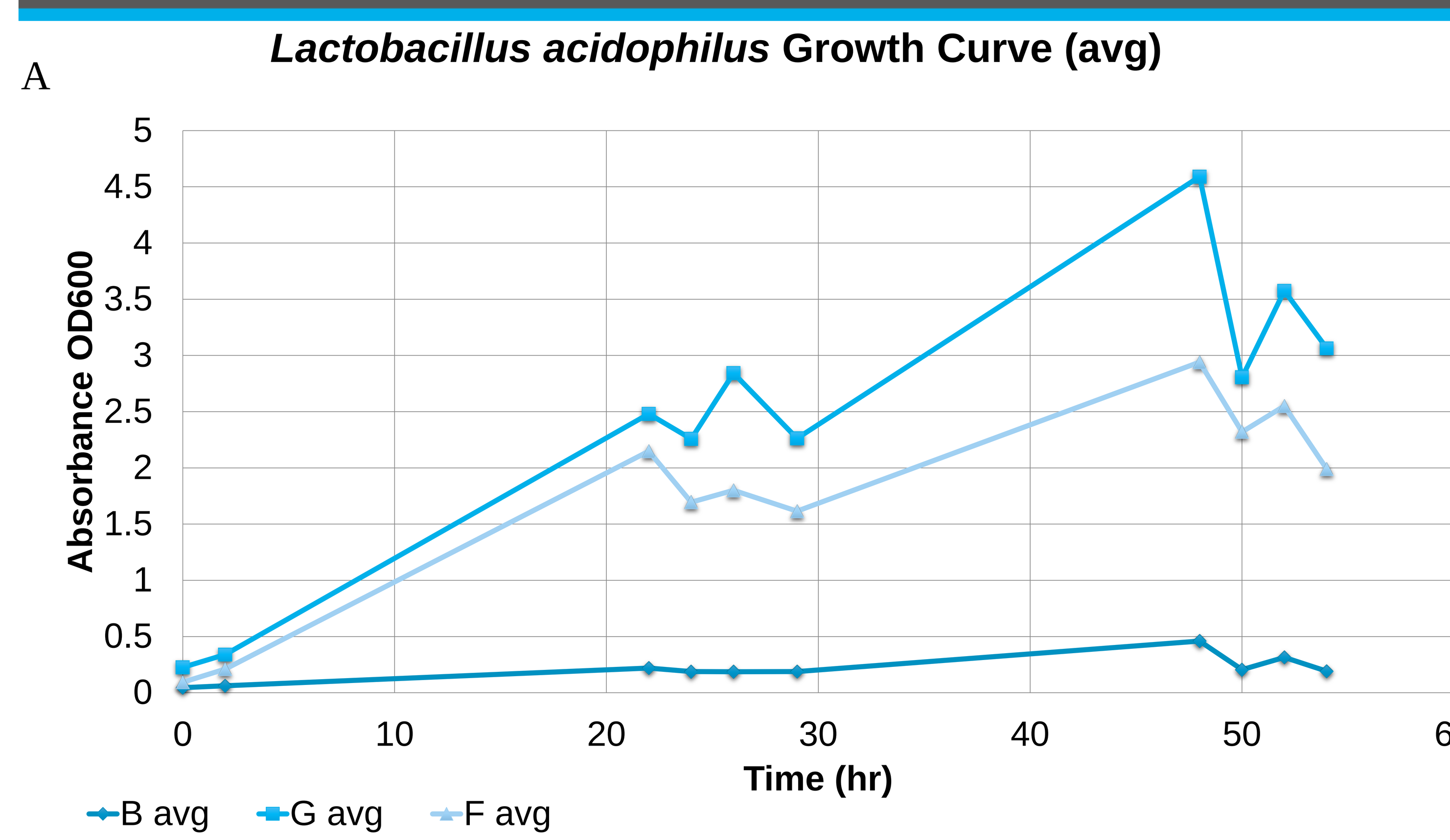
Colony count & calculation of CFU values

CFUs

Culture	10^-4	10^-5	10^-6	10^-7
Basal	93	175	112	79
Glucose	202	175	107	70
FOS	400	173	123	56
E3	280	163	114	5
E4	155	110	74	145

Lactobacillus acidophilus Growth

A



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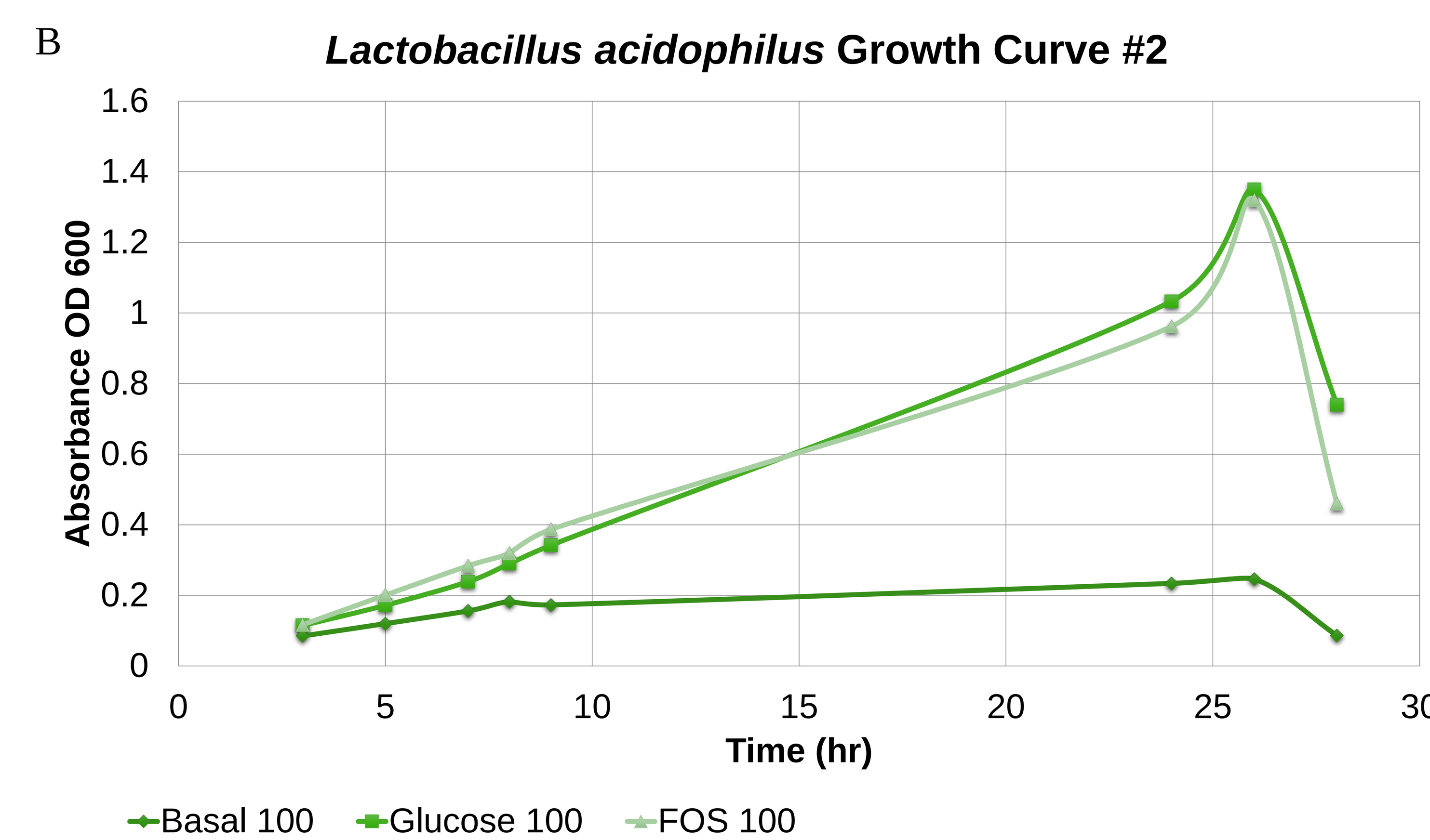

Figure 4-A&B

Growth analysis of *Lactobacillus acidophilus, to identify inoculation times.*

Conclusion

- Looking at the growth curve, we can see that glucose promotes the growth of *Lactobacillus acidophilus* at higher rates than FOS.
- Comparing the CFU values, we can see that FOS promoted the growth of the bacterium at higher rates than Glucose. We can also see that E3 promotes the growth of the bacterium, when comparing the values with the negative & positive controls.

References & Acknowledgments

Bingyong Mao, Dongyao Li, Jianxin Zhao, Xiaoming Liu, Zhennan Gu, Yong Q. Chen, Hao Zhang and Wei Chen. In vitro fermentation of fructooligosacchrides with human gut bacteria. *Food Funct.*, 2015, 6: 947-954.

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