



Undergraduate

Research Symposium

ADVANCING RESEARCH AND STEM FIELD ENGAGEMENT



PROJECT

45

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Caldwell University, Class of 2021

Major: **Biology**

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Advisor: Department of Natural Sciences

*Effects of Carrot Root on the Growth of Probiotic GI Bacterium *Bacillus coagulans* and Pathogenic Bacterium *Salmonella enterica**

The human gastrointestinal (GI) tract maintains a densely populated community of diverse microbiota that is linked with overall metabolic, immunologic, and physiologic human health. The upheaval of the microbiome balance may result in significant health problems such as gastrointestinal and systemic diseases, neurological disorders, and colorectal cancer. A major factor in disrupting the microbiota health is the consumption of antibiotics. Used for destruction of pathogenic microorganisms in the body, antibiotics not only target pathogens, but also the beneficial host-associated gut microbiomes. The regeneration of healthy biome is possible through consumption of dietary fibers, probiotics, and prebiotics. Carrot is a rich prebiotic source due to its high level of dietary fibers. In our study, carrot's effect on bacterial growth is analyzed using *Bacillus coagulans*, a probiotic bacterium, and *Salmonella enterica*, a foodborne pathogen that can grow in the GI tract. For each bacterium, three different agar media were prepared. The first medium consisted of nutrient broth (NBA), the second of a mixture of nutrient broth and carrot pulp (NBCA), and the last one was supplied with only carrot pulp (CA). Three trials were performed where each bacterium was applied on the three types of agar media, incubated, and analyzed for growth. The densities of bacteria were measured using OD at 600 nm wavelength. Samples used for OD were collected from 1cm^2 of each petri plate using a cotton swab. The average densities for *B. coagulans* on NBA, NBCA, and CA were 0.172 g/mL, 0.601 g/mL, and 0.525 g/mL, respectively. While, the resulting average densities for *S. enterica* on NBA, NBCA, and CA were 0.156 g/mL, 0.608 g/mL, and 0.475 g/mL, respectively. Obtained results show that the densities of both *B. coagulans* and *S. enterica* were significantly higher in CA than in NBA (p-values of 0.00849 and 0.00248, respectively), as well as in NBCA compared to NBA (p-values of 0.00007 and 0.01348, respectively). The difference between density for both bacteria was not significant between CA and NBCA (p-values of 0.21711 and 0.13792). These results suggest that carrot significantly improves bacterial growth and acts as an effective prebiotic. However, as of the current circumstances of our study, whether carrot acts differently with probiotic and pathogenic bacteria cannot be affirmed.



Effects of Carrot Root on the Growth of Probiotic Bacterium *Bacillus coagulans* and Pathogenic GI Bacterium *Salmonella enterica*



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INTRODUCTION:

Human Gastrointestinal (GI) Microbiota: Linked with overall metabolic, immunologic, and physiologic human health

Carrots: High levels of dietary fiber

***Bacillus coagulans*:** Probiotic bacteria residing in the GI tract

***Salmonella enterica*:** Foodborne pathogen located in the GI tract

PURPOSE: To determine the efficacy of Carrot as a prebiotic using GI bacteria *Bacillus coagulans* and *Salmonella enterica*.

MATERIAL & METHODS:

- Preparation of 3 types of agar: Nutrient Broth Agar (NBA), Nutrient Broth and Carrot Agar (NBCA), and Carrot Agar (CA)
- Establishing the bacterial lawns
- Incubation
- Measuring bacterial growth using Vernier Spectrophotometer at OD 600

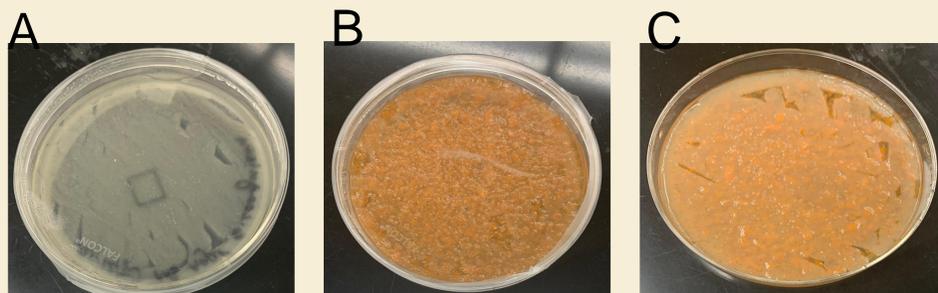


Figure 5. *B. coagulans* growth in Trial 1. (A) *B. coagulans* growth in NBA. (B) *B. coagulans* growth in CA. (C) *B. coagulans* growth in NBCA

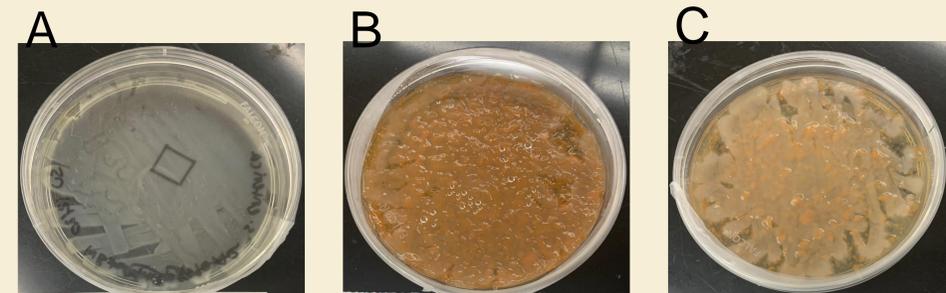


Figure 6. *S. enterica* growth in Trial 1. (A) *S. enterica* growth in NBA. (B) *S. enterica* growth in CA. (C) *S. enterica* growth in NBCA

RESULTS:

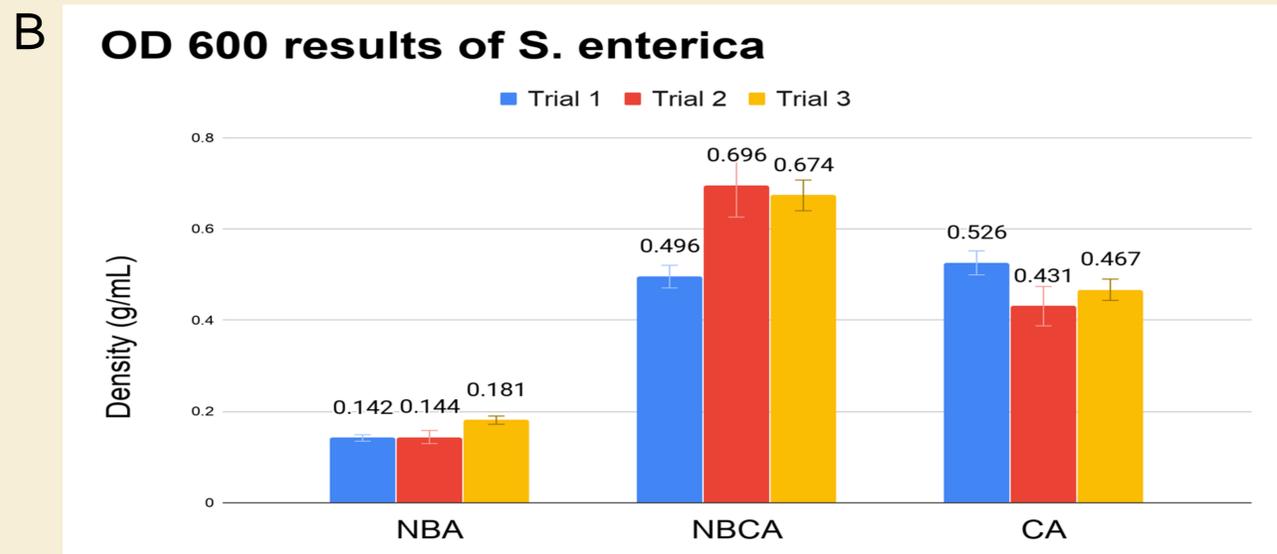
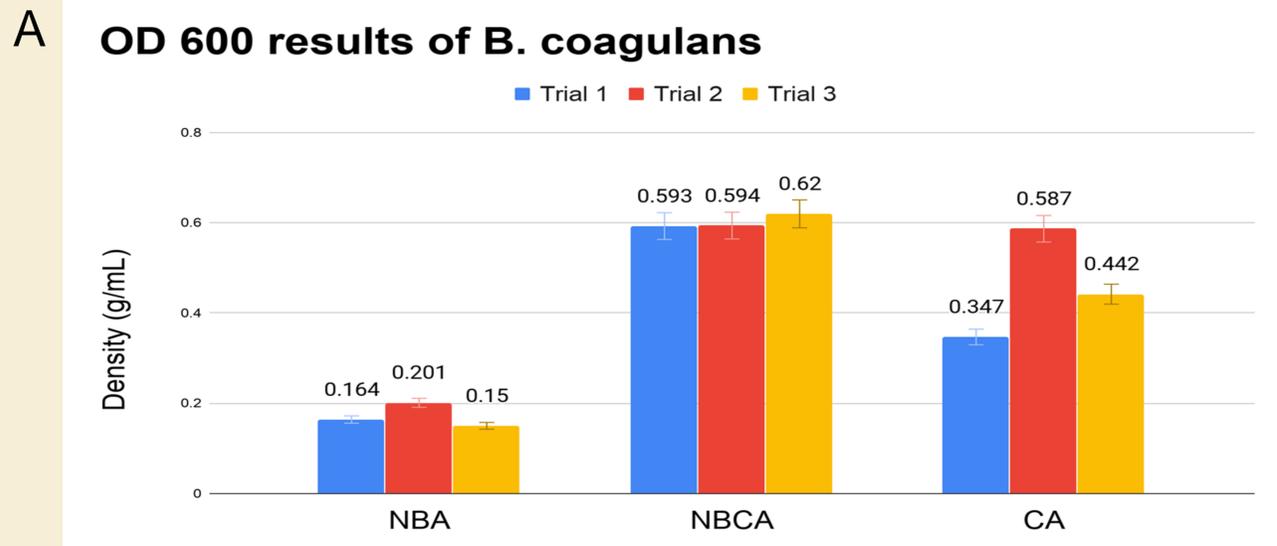


Figure 4. Density Measurements of Bacterial Growth in 1ml of 3 Types of Agar. (A) Densities of *B. coagulans* growth on NBA (Nutrient Broth Agar), NBCA (Nutrient Broth and Carrot Agar), and CA (Carrot Agar) of three separate trials. (B) Densities of *S. enterica* growth on NBA, NBCA, and CA of three separate trials

	NBA	CA	NBCA
<i>S. enterica</i> Average Growth (g/mL)	0.156	0.475	0.608
<i>B. coagulans</i> Average Growth (g/mL)	0.172	0.525	0.601

Table 1. Average density of bacterial growth of *S. enterica* and *B. coagulans*. The average of densities from three trials measured from 1ml of the media NBA (Nutrient Broth Agar), NBCA (Nutrient Broth and Carrot Agar), and CA (Carrot Agar)

<i>Bacillus coagulans</i>	
Independent T-test variables	p-value
NBA vs CA	0.0025
NBA vs NBCA	0.0135
CA vs NBCA	0.1379
<i>Salmonella enterica</i>	
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Table 2. Independent T-test results between each media of *Bacillus coagulans* and *S. enterica* growth. CA and NBCA have significantly greater efficacy in both *B. coagulans* and *S. enterica* growth compared to NBA. There is no significant difference of bacterial growth between CA and NBA for both *B. coagulans* and *S. enterica*

CONCLUSION: Carrot significantly improves growth of *B. coagulans* and *S. enterica* and acts as an effective prebiotic



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INTRODUCTION

Human Gastrointestinal (GI) Microbiota

- Linked with overall metabolic, immunologic, and physiologic human health
- Disruption may result in significant health problems (e.g. gastrointestinal and systemic diseases, and colorectal cancer)
- Antibiotics pose as a threat to the healthy gut balance
- The regeneration is possible through consumption of dietary fibers, probiotics, and prebiotics.

Carrots

- High levels of dietary fiber (1.2-6.44% dietary fiber composition, 80.94% of which is cellulose)
- Rich in carotenoid

Bacillus coagulans

- Probiotic bacteria residing in the GI tract
- Specializes in producing lactic acid and helping with the digestion of carbohydrates and proteins

Salmonella enterica

- Foodborne pathogen located in the GI tract

PURPOSE

To determine the efficacy of Carrot as a prebiotic using GI bacteria *Bacillus coagulans* and *Salmonella enterica*.

MATERIALS

- *Salmonella enterica* (*S. enterica*)
- *Bacillus coagulans* (*B. coagulans*)
- Nutrient Broth
- Agar
- Carrots
- Petri plates
- Cotton Swabs
- Bunsen burner
- Serological pipettes and pipette pump
- Test tubes
- Incubator oven
- Autoclave
- Vernier Spectrophotometer
- Three Agar Media :
 - a. Nutrient Broth Agar (NBA)
 - b. Carrot Agar (CA)
 - c. Nutrient Broth and Carrot Agar (NBCA)



Figure 1. Mini-carrots



Figure 2. Autoclave



Figure 3. Vernier spectrophotometer

RESULTS

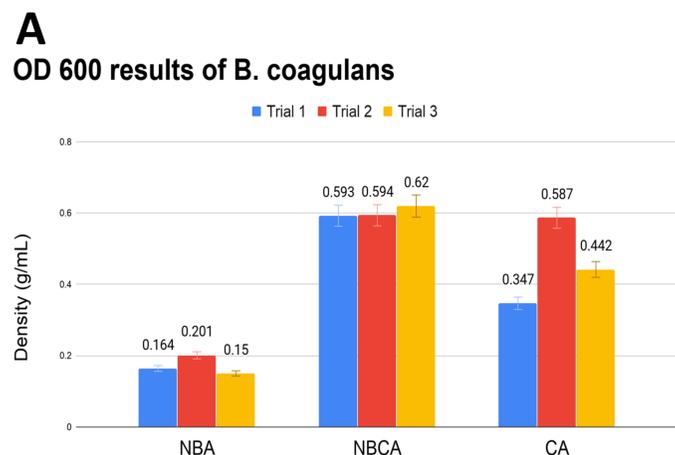
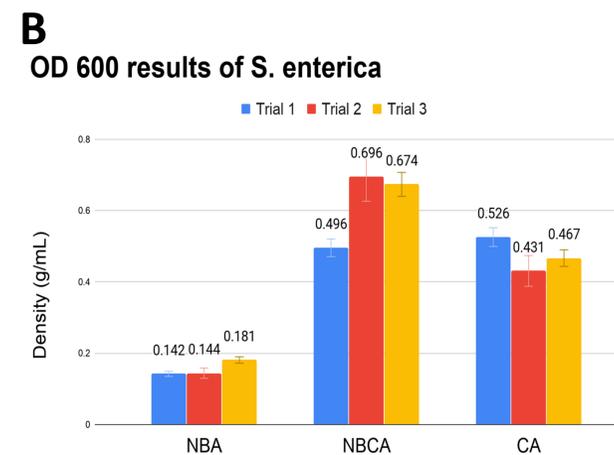


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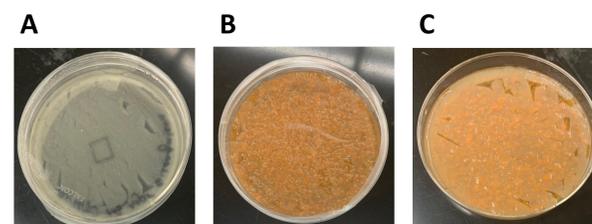


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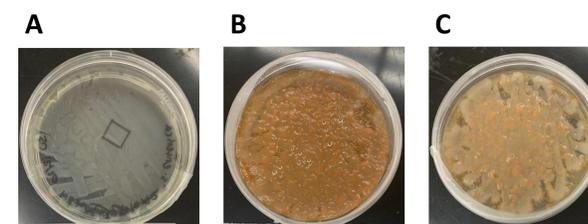


Figure 6. *S. enterica* growth in Trial 1. (A) *S. enterica* growth in NBA. (B) *S. enterica* growth in CA. (C) *S. enterica* growth in NBCA

METHODS

- **Preparation of agar media**
 - Nutrient Broth Agar: 3.75 g of agar, 2 g of nutrient broth, and 250 mL of water
 - Carrot Agar: 3.75 g of agar, 110 g of carrot pulp, and 250 mL of water
 - Nutrient Broth and Carrot Agar: 3.75 g of agar, 1 g of nutrient broth, 55 g of carrot pulp, and 250 mL of water
- **Establishing the bacterial growth:** 300 μ L of each bacterium was spread on plates of the three types of media
- **Incubation:** Plates were placed in a 37°C incubator for at least 48 hours
- **Measuring bacterial growth:**
 - From each plate, bacteria within 1cm² were collected using cotton swabs and dissolved in test tubes containing water
 - OD 600 densities of each test tube containing the bacterial solution was measured using Vernier Spectrophotometer

CONCLUSIONS

Carrot significantly improves growth of *B. coagulans* and *S. enterica* and acts as an effective prebiotic

FURTHER RESEARCH

- Test the effect of other prebiotic food on the growth of *B. coagulans* and *S. enterica*
- Test the effect of carrot using other pathogenic and probiotic bacteria
- Imitate the gut environment and test the effect of carrot under such conditions
- Test the effect of carrots coupled with antibiotics against beneficial gut bacteria

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