I. Project Title
Impact of Probiotics on Gastrointestinal Pathogenic Growth and Host Tight Junction Integrity

II. Project Completion Date
June 7, 2019

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VI. Executive Summary
Over the past decade, interest in the use of probiotics, or “good bacteria”, for human health advancement and remediation has grown. Previous studies have shown that some health benefits conferred by probiotics are strain specific, therefore individual probiotic effects must be evaluated. This study evaluated some probiotic candidates on their ability to produce metabolites which inhibit the growth of foodborne pathogens. Probiotics are known to inhibit growth of other bacteria through competitive exclusion and production of bactericidins. In addition, previous studies have established that some probiotics promote the integrity of tight junctions, proteins that play a role in intestinal epithelial cell adherence and regulation of ion transport between intestinal cells and the lumen.

To investigate if and how some bacteria inhibit pathogens, candidates (*Pediococcus acidilactici, Pediococcus pentosaceus, Lactobacillus platarum*) were cross-streaked with pathogens (*Listeria monocytogenes* and *Vibrio cholerae 569B*) and the zone of inhibition was observed. No probiotic inhibition of either pathogen was observed in the cross-streak assays (Figure 1). To further probe this question, lawns of both pathogens were streaked onto individual agar plates and 10 µl of probiotic culture (from each probiotic candidate) was pipetted on the pathogen lawns. Using this method, zones of inhibition were present for both *L. monocytogenes* and *V. cholerae*, with the zones averaging 2-4 mm and 6-8 mm, respectfully (Figure 2). To investigate whether growth inhibition was dependent on the presence of the probiotic bacteria, lawn assays were repeated using 10 µl of sterile supernatant from the overnight probiotic cultures. The sterile supernatant of all three probiotic candidates inhibited the growth of *V. cholerae* but did not effect the growth of *L. monocytogenes* (Figure 2). Being that all three probiotic candidates are lactic acid bacteria and produce lactic acid as
a byproduct of their metabolism, I hypothesized that the growth inhibition of *V. cholerae* was due to the acidity of the sterile supernatant. In order to address this question, the sterile supernatant of all three candidate strains was pH adjusted to a final pH of ~6.8. No growth inhibition was observed after the application of the pH adjusted supernatant (Figure 2).

To further probe the potential growth inhibition of *V. cholerae* and *L. monocytogenes* by the probiotics, 1:1 liquid cultures of MRS/BHI broth with each pathogen/probiotic combination were cultured. Percent recovery of each pathogen were calculated by comparing colony forming units (CFUs) of pathogen culture to co-culture CFUs after the addition of the probiotic bacteria. Cultures adjusted to a pH of ~6.8 exhibited greater percentage of recovery, suggesting low pH was a major pathogenic growth inhibitory factor (Figure 3).

In order to assess the impact of these three probiotic strains on human intestinal tight junction integrity, a Caco-2 human intestinal epithelial cell culture system was used. Caco-2 cells were grown on transwell inserts and allowed to differentiate for 21 days, with media exchanges every 3 days. Probiotic bacteria were applied at a MOI of 100:1 for 24 hours. Then, tight junction integrity was assessed by measuring the transepithelial electrical resistance (TEER) of the intestinal cells. Addition of each probiotic strain alone and in combination did not cause significant changes in the average TEER of the Caco-2 cells (Figure 4). These data suggest that the probiotic co-culture with Caco-2 cells for 24 hours is not sufficient to modulate TEER and that these strains may not change TEER of Caco-2 cells. In addition, when *V. cholerae* was applied to this cell culture model, TEER decreased as expected. However, addition of the probiotic strains did not rescue the defect in TEER (Figure 5).

In conclusion, observed growth inhibition varies based on assay method. *L. monocytogenes* exhibited a 2-4 mm zone of inhibition for probiotic culture via lawn assay and *V. cholerae* exhibited a 6-8 mm zone of inhibition for probiotic culture and sterile probiotic supernatant via lawn assay. PH adjustment of the probiotic supernatant to ~6.8 decreased its inhibitory effects on both pathogens’ growth and increased pathogen recovery from liquid co-cultures. Therefore, the major growth inhibitory mechanism is likely related to the acidity of probiotic the supernatant. The average TEER of probiotic treated Caco-2 cells is the same or slightly greater than that of untreated Caco-2 cells, indicating that tight junction integrity stays relatively stable with probiotic treatment. Probiotic treatment does not appear to rescue TEER decrease in Caco-2 cells after *V. cholerae* application. These data bolster a plethora of studies that suggest that probiotic bacteria may indeed be beneficial for human health, through inhibition of pathogenic growth of *V. cholerae* and *L. monocytogenes* via secretion of acid; however further work must be done using extensive in vivo models to validate these findings.

**Figures:**

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Cross streaks of *Listeria monocytogenes* and *Vibrio cholerae* with (A) *Pediococcus pentosaceus* (A), *Pediococcus acidilactici* (B), and *Lactobacillus plantarum* (C). No inhibition was observed at the intersection of the pathogens and the probiotics.
Figure 2. V. cholerae lawn (A-D) with 10 µl of P. pentosaceus and P. acidilactici cultures (A), L. plantarum cultures and sterile P. pentosaceus supernatant (B), sterile supernatant of P. pentosaceus, P. acidilactici, and L. plantarum (C), and sterile supernatant (pH ~6.8) of P. pentosaceus, P. acidilactici, and L. plantarum (D). L. monocytogenes lawn (E-G) with 10 µl of P. pentosaceus, P. acidilactici, and L. plantarum cultures (E), sterile supernatant of P. pentosaceus, P. acidilactici, and L. plantarum (F), and sterile supernatant (pH ~6.8) of P. pentosaceus, P. acidilactici, and L. plantarum (G).

Figure 3. Average percent pathogen recovery for V. cholerae and L. monocytogenes after overnight growth with each of the following treatments. 1:1 liquid cultures of MRS/BHI broth was used. Percents were calculated by comparing CFUs of pathogen culture to co-culture CFUs after the addition of the probiotic bacteria.
Figure 4. Average TEER ($\Omega. \text{cm}^2$) of Caco-2 cells in the presence of probiotic bacteria. The values are shown as the percentages of untreated Caco-2 cell. Error bars represent standard deviation.

Figure 5. Average TEER ($\Omega. \text{cm}^2$) of Caco-2 cells in the presence of probiotic bacteria and *V. cholerae*. The values are shown as the percentages of initial (pre-treatment) value. Error bars represent standard deviation.

VII. Major Accomplishments

(1) Established a growth medium that supported the growth of both lactic acid bacteria and enteric pathogens

(2) Created three assays for determining growth inhibition

(3) Established a protocol for measuring TEER

(4) Presented poster at the Cal Poly College of Science and Math Student Research Conference and at the Cal Poly Honors Student Showcase

VIII. Expenditure of Funds

Funding was used to purchase the following materials: EVOM voltmeter, Caco-2 intestinal epithelial cells.

A voltmeter capable of measuring transepithelial electrical resistance was required in order to measure the TEER of the Caco-2 cultures to use as a proxy for tight junction integrity. In addition, new Caco-2 intestinal epithelial cells were purchased as the current frozen stock was not viable. Viable Caco-2 cells are required to perform the in vitro portion of the project.

IX. Impact on Student Learning

This project allowed me to learn new laboratory techniques, establish new protocols, and teach other students. I learned how to conduct three different types of bacterial growth assays and measure TEER. In addition, I established three new growth assay protocols in the Yeung lab, a new media formulation for the growth of lactic acid bacteria and enteric pathogens, and created a TEER protocol. Over the course of my project I taught two students how to conduct bacterial growth assays and three students how to culture mammalian cells in lab. After completing this project, I have gained confidence in my ability see a project through from start to finish and I look forward to pursing my graduate training in a related field.