Prevention of *Escherichia coli* Biofilm Formation Using SLpA Protein Extracted From *Lactobacillus helveticus*

Scarlet M. Renner[1,2], Kayla Zepeda[1], Ileene R. Guillen[1], Dr. Frank E. Cappuccio[1], Dr. Jenny A. Cappuccio[1]

[1] CHEM 435L - Biochemistry II Lab - Department of Chemistry, Humboldt State University, Arcata, CA, USA
[2]Department of Biological Sciences, Humboldt State University, Arcata, CA, USA

Department of Chemistry, Biochemistry and Biological Sciences

Abstract

Dental health in pets often goes neglected, despite the large amounts of bacteria that cultivate in their mouths often leading to painful gingivitus and periodontitis. Certain probiotic *Lactobacillus* strains have shown to help inhibit the growth of oral pathogenic bacteria, which could be beneficial in the development of more cost effective and efficient preventative measures against dental disease. More specifically, it's thought that the surface layer proteins in these probiotics prevent biofilm formation of pathogenic bacteria by occupying binding sites. This experiment explored the use of the probiotic *Lactobacillus helveticus* and its Surface Layer Protein A (SLpA) in their ability to prevent biofilm formation in mCherry containing *Escherichia coli.* We approached this by culturing the whole organisms, followed by extraction of the SLpA using lithium chloride and centrifugation. The *E. coli* was induced to provide fluorescence and mixed with samples containing both whole *L. helvetius* and the SLpA protein. Fluorescence values were collected using spectrofluorometry with a black 96 well plate which contained a sodium alginate and calcium chloride gel to allow adherence and compared to a blank. Our results indicate that *L. helvetius* and the SLpA protein both inhibit biofilm formation of *E. coli*. Further research should be done using specific strains of pathogenic bacteria.

Introduction

The mouths of pets have long been known to harbor vast amounts of bacteria.

Probiotics, especially that of which belong to the genus *Lactobacillus*, have been studied for their ability to maintain a healthy gut microbiome in both humans and pets alike.

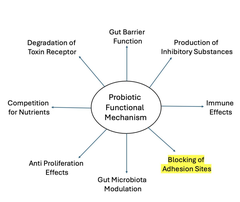

Figure 1. Probiotic Functional Mechanism Flow chart for experiment background. SLpA is thought to block adhesion sites of pathogenic bacteria.

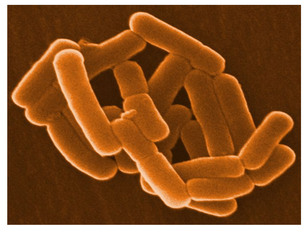

Figure 3. *Lactobacillus helveticus* scan (1 µm) from journal article *The Biological Role of the S-Layer Produced by Lactobacillus helveticus 34.9 in Cell Protection and Its Probiotic Properties.*

Roughly 80% of dogs will contract periodontal disease in their lifetime.

We will investigate an avenue of deterance for pathogenic bacterial growth overtime.

Samples to test:

- *Lactobacillus helveticus* probiotic and SLpA protein
- E. *coli* as 'pathogenic' bacteria
- Biofilm as medium for growth

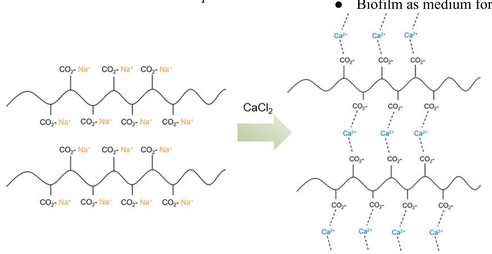

Figure 4. Sodium alginate formation with calcium chloride from journal article *Polymers, Giant Molecules with Properties: An Entertaining Activity Introducing Polymers to Young Students* in the Journal of Chemical Education.

Methods

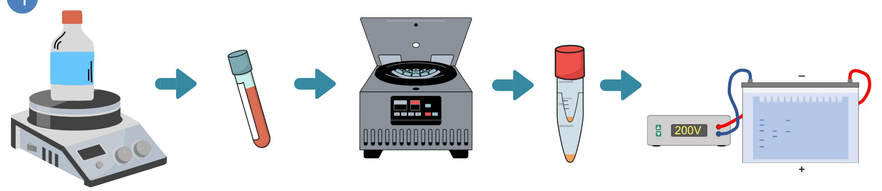

Figure 5. The above figure shows the steps taken in a flow chart. The steps are categorized in three parts labeled 1, 2, and 3. Part 1 shows the preparation of MRS Media and protein extraction from *L. helveticus*. Part 2 shows the preparation of the Biofilm. Part 3 shows the E. coli formation snd preparation.

Results

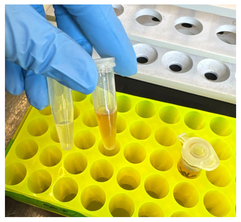

Figure 6. SLpA protein exctrations following treatment with Lithium Chloride and centrifugation. The samples were purified via ultrafiltration buffer exchange using MRS media.

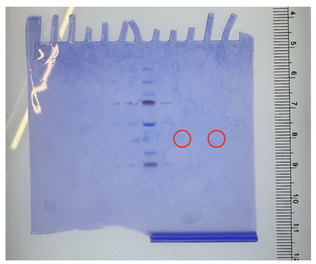

Figure 7. SDS-PAGE was ran in order to determine purity of our product. Unfortunately results were inconclusive, although given our fluorescence data we are fairly confident we were successful in extracting the protein. Lack of sufficient protein in the samples is the most likely error as an estimate was taken at 280 nm rather than testing against a set of standards due to time constraints. Our protein weighs about 44 kDa and should've been seen in the circled areas.

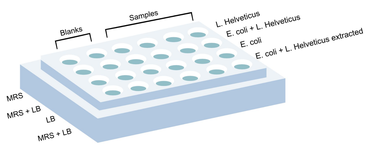

Figure 8. Fluorescence spectroscopy well setup. MRS for de Man, Rogosa, and Sharpe broth, LB for Luria-Bertani broth, *L. helveticus* for *Lactobacillus helveticus*. Each sample aside from blanks were 1/10 dilutions.

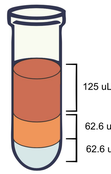

Figure 9. Fluorescent spectroscopy individual well components.

Results

Table 1. Fluorescence data ($x10^5$) of our samples. Blank fro *L. helveticus* was MRS, LB for E. *coli*, and a MRS & LB mixture for our mixed samples.

	Blank	Blank	1	2	3	4
L. helveticus	5.97	5.30	5.18	4.2	4.01	4.44
E.coli + L.helveticus untreated	4.82	5.08	39.2	39.7	42.8	44.3
E.coli	3.12	3.21	61.4	44.4	21.0	49.3
E.coli + SLpA	4.69	4.28	38.7	40.4	36.2	8.91

Table 2. Fluorescence data averages ($x10^5$) of our samples, and calculated percent decrease in fluorescence when whole probiotic organism and protein are added.

	Blank average	Sample Average	Average difference	Percent Decrease
E.coli + L.helveticus untreated	4.95	41.5	36.5	10.51%
E.coli	3.17	44.0	40.8	-
E.coli + SLpA	4.49	31.0	26.5	34.99%

Fluorescent Changes in E.coli with and without L. Helveticus probiotic

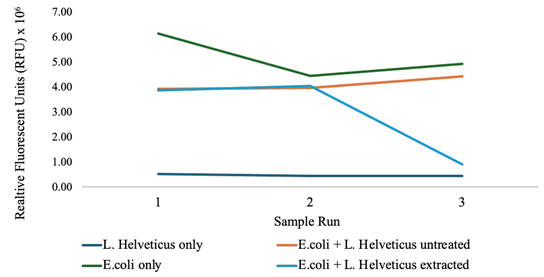

Figure 10. Fluorescent changes can be seen from E. *coli* to solutions of E.coli and L. helveticus combined. The data exhibits a decrease in E.*coli* growth once the *L. helveticus* is added. There is a higher increase in inhibition with the extracted protein at 34.99% decreased growth.

Conclusions

- S-layer protein of *Lactobacillus helveticus* demonstrated inhibition of E. *coli* bacteria adhering onto a synthetic biofilm surface. Further research on pet teeth as an experimental surface will provide a more accurate determination of the inhibition by *L. Helveticus* bacteria of harmful plaquing bacteria often found in oral cavities of pets.
- *L. helveticus* can be often found in healthy gut microbiomes of pets. Efforts to deliver the bacteia safely to the oral cavities of pets can reduce innterferences in the pet's body, and directly introduce the bacteria to the target site.
- It is imperative that treatment can be delivered safely, efficiently and easily as it will aid pet owners to maintain their pet's oral health that could be heavily neglected. An easy and efficient method will make pet owner more likley to take steps to maintain their pets health.

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Department of Biochemistry

Department of Biological Sciences

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