EXECUTIVE SUMMARY

In this project, we will be measuring the forces of the binding of bacteria. We will be working with *Lactobacillus acidophilus* NCFM, *Lactobacillus gallinarum* 33199, *Lactobacillus ruteri* 23272, and *Bifidobacterium*. These bacteria are lactic acid bacteria and are used as probiotics. We also reviewed literature on the bacterial cell wall constituents and the properties of adhesion applicable to these organisms.

INTRODUCTION

The main goal of the project is to measure the force of bacterial adhesion to milk fat globule membranes. This information can help us gain insight into the probiotic properties, or health benefits, of certain bacteria. This work can also aid in understanding biofilm formation properties. The main technique of this project is the application of optical tweezers to biological subjects. Optical tweezers basically consist of an infrared laser beam focused through a high-powered microscope objective lens; the laser can then be used to “trap” particles, or in our case bacteria, and after calibration, use this to determine the force exerted by the trap.

Our optical tweezers system is composed of a 1064 nm laser and a Zeiss Axiovert microscope utilizing an NA1.25 oil immersion objective lens.
BACKGROUND

Optical traps, also known as optical tweezers, were invented about 25 years ago by Arthur Ashkin, the pioneer of laser-based optical trapping. To trap an object, a laser beam must be focused through an oil immersion microscope objective. The tweezers have been used to manipulate and measure objects since their invention, however their use on biological subjects is relatively recent. The full potential of the optical tweezers technology has yet to be reached, resulting in much scientific excitement.

The concept of optical tweezers is based on the principles of optical refraction and momentum conservation. The optical trap of the tweezers is a focused laser beam that is projected onto a particle. As light hits the particle, the refractive properties of matter forces the light to bend inward around the particle. Since the light has momentum and has changed direction, there is a force exerted on the particle. It is this force which pinches the particle and holds it in place. The strength of the trap is determined by the intensity of the light. The goal of the aforementioned calibration is to map a light intensity to the corresponding force exerted on the particle (which is usually measured in piconewtons).

To conduct experiments on bacterial adhesion, a basic understanding of adhesion mechanisms is essential. The bacteria we are using are *Lactobacillus acidophilus NCFM*, *L. gallinarum 33199*, *L. reuteri 23272*, and *Bifidobacterium spp*. Studies have shown that bacterial adhesion to host cell surfaces is facilitated by hydrophobic/hydrophilic, Lewis acid/base, electrostatic, and van der Waals interactions. Other bacterial adhesion factors (adhesions) that are specifically present in *Lactobacillus spp.* and *Bifidobacterium spp.* are the S-layer and the lipoteichoic and teichoic acids.

DESIGN

Figure 1 shows a schematic diagram of the tweezers system while Figure 2 is a photograph of the laboratory set-up. The lab is located in Building 52 in the Dynamical Systems Imaging Laboratory.
EXPERIMENTS

Bacteria

Unfortunately, we have not been able to conduct any experiments on bacteria because we have not received bacterial samples or the Milk Fat Globule Membrane coated spheres from the Dairy Science Department.
**Calibration**

Using a moving piezoelectric stage, we measured the trap strength by measuring the drag force (also called Stokes drag) on the trapped particle due to moving fluid. By adjusting the stage velocity, we can vary the drag force until the particle just detaches from the trap. The trap force can be calculated from the following equation:

\[
\text{Force} = 6\pi \eta v_{\text{max}} r
\]

Where \(\eta\) is the fluid viscosity (= 0.001 Pa s for water at 22°C), \(r\) is the particle radius (we used 0.5 \(\mu\)m radius polystyrene beads), and \(v_{\text{max}}\) is the maximum velocity of the stage at which the particle detaches from the optical trap. We explored the trapping strength as a function of the laser power, noting that trap strengths of order 10 pN can be obtained. We also measured the trap strength as a function of distance from the coverslip, showing that strong trapping can only be effective from about 5 to 30 \(\mu\)m from the coverslip. The trap is most effective at a level of 10 \(\mu\)m above the coverslip. This is discussed further under the calibration section.

**RESULTS**

**Research**

Due to a lack of samples to work with, I have been focusing on researching the bacteria we plan to use. Topics of research include properties of lactic acid bacteria, probiotics, the gram positive cell wall constituents, and bacterial adhesion.

**Lactic acid bacteria**

Lactic acid bacteria are phylogenetically diverse, gram positive bacteria that produce lactic acid as a sole or main end product of sugar fermentation. Lactic acid bacteria are non-sporing and generally non-motile. They lack cytochromes and obtain energy by substrate level phosphorylation, rather than by electron transport, ATP synthase, and oxidative phosphorylation. *Lactobacillus spp.* are widely used by the food industry in the production of fermented vegetable products, beverages, sourdough bread, and several meat and dairy products, however some species can also be associated with food spoilage.
Species of the genera *Lactobacillus* are generally recognized as safe (GRAS) due to non-pathogenicity and their long history in the food industry.

**Probiotics**

The large intestine of humans and animals contains a complex, but balanced microbiota, which normally prevent infection and have a positive affect on nutrition. This microbial balance can be upset by stress, antibiotic therapy, or an abrupt change in diet, leading to host susceptibility to disease and a decrease in food use efficiency. Probiotics are the oral administration of living microorganisms and have the potential to reestablish the natural balance and return the host to normal health and nutrition. Probiotic organisms have been proven to provide health benefits to animals such as sheep and cattle, however there is evidence that certain probiotic microorganisms may also offer considerable benefits to humans as well. Some of these potential benefits include anticarcinogenic activity, control of intestinal pathogens, reduction of serum cholesterol concentration, and the improvement of lactose use in individuals who are lactose intolerant. The development of probiotics for humans is still in the early stages, but exhibits a promising future.

**Cell wall constituents**

The gram positive cell wall consists of a single peptidoglycan, also called murein, layer that is 20 to 80 nm thick and lies outside of the plasma membrane. Peptidoglycan is a polymer containing two sugar derivatives and several different amino acids. Usually, gram positive cell walls also contain large amounts of teichoic acids (not present in gram negative bacteria), which are polymers of phosphate, glycerol or ribitol, and a side chain R, which represents glucose, an amino acid, or some other molecule. The teichoic acids can either be covalently bonded to the peptidoglycan, or to the plasma membrane lipids, in which case they are called lipoteichoic acids. Teichoic acids extend out towards the surface of the peptidoglycan and because they have a negative charge, contribute to the negative charge of the gram positive cell wall.

Many gram negative and gram positive bacteria, including all species we are studying, possess an S-layer on their surface. The S-layer is formed by an intrinsic self-assembly process and is a regularly-structured, lattice pattern of
protein, glycoprotein, or both, exhibiting oblique, square, or hexagonal symmetry. S-layers are highly porous, with pores of identical size and morphology that cover up to 70% of the S-layer surface area.

Bacterial adhesion

Studies have shown that bacterial adhesion to host cell surfaces is facilitated by hydrophobic/hydrophilic, Lewis acid/base, electrostatic, and van der Waals interactions. Bacterial adhesion factors (adhesions) that are present in Lactobacillus spp. and Bifidobacterium spp. are the S-layer and the lipoteichoic and teichoic acids.

Both hydrophobicity and electric charge are consequences of the chemical properties of the bacterial cell surface. The S-Layer proteins convey hydrophobicity to the Lactobacillus spp. cell surface, while the teichoic and lipoteichoic acids give the surface a negative charge. A specific feature of Lactobacillus spp. S-layers is the higher content of hydrophobic amino acids and amino acid residues with hydroxyl groups, than S-layers of other organisms.

The exact function of the S-layer is unknown, however it does play a role in bacterial adhesion. It is hypothesized that the method of mediating adhesion is through hydrophobicity. Removal of the S-layer proteins by LiCl extraction significantly reduces the adhesiveness of the bacteria. Also, experimental approaches to apply the binding receptor sites of the S-layer protein to other non S-layer possessing bacteria can be demonstrated. Non-adhesive lactic acid bacteria can be turned into adhesive by inducing the expression of a different species’ S-Layer protein or its receptor binding region.

Previously, the tweezers were applied directly to the bacterium. To perform a much more exact and reliable force calibration, a “handle” needs to be attached to one end of the bacterium. A possible handle would consist of a small polystyrene sphere coated with streptavidin and adhered to biotinylated bacteria. Also, the milk fat globule membrane-coated spheres will be adhered to the coverslip. These two adjustments will allow the measurements obtained to be more accurate. However, the Dairy Science Dept. is having trouble functionalizing the bacteria to adhere by any method.
Calibration

Using the Stokes Drag method described above, I measured the force exerted on the trapped particle as it relates to laser power and found they had a linear relationship, as shown in Figure 3.

![Force Exerted on Particle vs. Laser Power](image)

Figure 3: Measured force on a trapped 1 µm diameter particle as a function of laser power.

I measured the trap strength as a function of the distance above the coverslip and found that trapping can only be achieved at a distance of about 5 to 30 µm above the coverslip, with maximum strength at 10µm above the coverslip.

![Trap Strength vs Distance above Coverslip](image)

Figure 4: Trap strength as a function of distance above coverslip.
CONCLUSION

The research conducted on the bacteria did not produce the exact results desired. I was really hoping to find information about the exact binding mechanisms of the bacteria we were studying, but had to settle on a general approach due to lack of experimentation and research conducted on our specific bacteria. Using Stokes Drag equation, we can calculate the force exerted on a trapped particle and therefore measure the force of bacterial adhesion. All trapping will need to be conducted at a level of 10µm above the coverslip to achieve maximum force. When we finally receive the bacterial cultures that have been functionalized to attach to a “handle” and the milk fat globule membrane-coated spheres, we can begin to collect data on the bacterial force of adhesion to the milk fat globule membrane.

There is one other issue that Dr. Sharpe and I recently discovered. When a small particle in the laser trap, such as the bacteria we want to use, is advanced toward a large particle, like the 10µm spheres we planned on coating with the MFGM membrane, there are fluctuations in the trap strength. Two possible solutions would be to: 1) calculate the fluctuations and consider them in our adhesion measurements, or 2) we may be able to avoid the fluctuation phenomena by using 1µm or maybe even 5µm beads to coat with the MFGM membrane.

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REFERENCES


