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RESEARCH INTERNSHIP AT KNOWLES LAB

THE GUT MICROBIOME, WHAT IS IT? WHAT DOES IT DO?

Gut microbiome research has exploded in popularity over the past 15 years, and some of that popularity has entered into mainstream popular science in the past few years, with “gut friendly” drinks and foods becoming hitting the shelves and many more people becoming aware of its importance. In essence, the gut microbiome (GM) is a community of microbes (mainly bacteria, but also fungi, archaea and viruses) which live within the gastrointestinal tract of animals. It is in a way an organism within an organism, as the average human GM weighs 2 kilograms, and contains 150 times more genes than humans! It is to no surprise then that such a community has a profound effect on its host. The GM has been found to modulate all kinds of aspects of the organism, such as immune function, nutrient absorption and quite possibly even mood and behaviour. It seems critical that we are able to learn more about this community, so that we are able to answer questions such as “How does it do that?”, “How is it formed?” and “How can we improve it?”.

The research I was assisting in is striving to help provide answers for the second question – “how is it formed?”. As the GM exists within the gut, it is obvious and well-studied that the composition of the GM will be affected by food. For example people consuming high fibre diets harbour a higher a microbial community structure which appears to be beneficial. However, diet is not the only predictor of the gut microbiome – these microbes must initially come from somewhere. Prior to birth, the gut is sterile, and only becomes colonised by microbes at birth, which then grow in the gut and establish a microbiome. This means that the only source through which microbes can colonise the gut is obviously the environment. My supervisor Aura Raulo is interested in how microbiomes can be transmitted between individuals through behaviour. She believes that anaerobic microbes (ones which cannot survive with oxygen) are more likely to be transmitted from individual to individual directly through social interactions, such as grooming or biting in mice. This is because anaerobic microbes cannot survive outside the host, as oxygen present in the air can kill them.

This insight leads to a curious possible conclusion. Since anaerobic microbes are transmitted through close social interaction, does this lead to some anaerobic microbes forming an association with their hosts over evolutionary timescales. If this is the case, it is possible that mice and some of their microbes in their GM may have evolved together, which would be a fascinating finding, implying that some microbes have become specialised to their hosts over a timescale of thousands of years.

HOW DO WE STUDY THIS?

Fortunately, there is a simple way to study the gut microbiome. It is non-invasive and relatively easy. Unfortunately, it involves faecal samples. These samples have been collected from mice on Skokholm island in the previous years and stored at a temperature of -80 degrees C until now. My job during this internship was to use those samples and extract the microbial DNA present in them so that in the future those samples can be sent off to a laboratory for sequencing which will tell us which specific bacteria were present in which mouse.

Skokholm island – place of sample collection

DESCRIPTION OF METHODS

The methods I used in order to accomplish these goals were relatively straightforward. In order to achieve to goal of extracting DNA from faecal samples, two things need to happen.

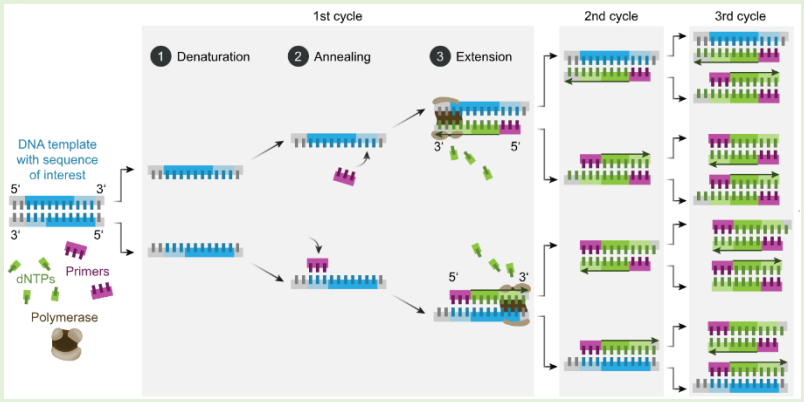
1. Break open the bacterial cells within the sample in order to access their DNA
2. Get rid of all other matter present in the sample other than the DNA

Now these steps sound simple on the surface, however in reality it's a multi-step process which utilises many intriguing techniques.

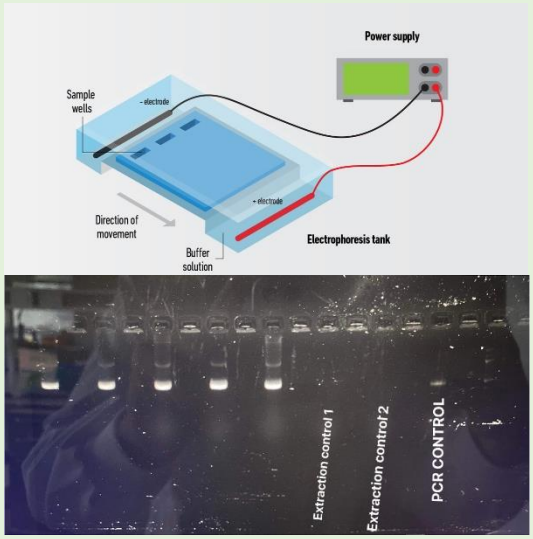
The process of breaking open the membrane of a cell is called lysis. In order to lyse the cells I placed each sample into a tube filled with silica like beads, and added a lysis solution. Once the resulting substance was shaken for enough time, using a centrifuge I was able to get rid of most solid matter from the sample, with the DNA hopefully being suspended in liquid. That liquid was then filtered three times, with the last filter being so dense as to only let through DNA.

After the DNA was extracted, the next crucial step involves making sure it's all been done correctly. Keep in mind that if any stray bacteria went into the samples as I was preparing them, or if a little bit of DNA from one sample got into another one, the data would be useless. To make sure such contamination didn't happen, I had to check each batch after extraction. This was accomplished by using something known as Polymerase Chain Reaction, which is a fascinating technology that we hijacked from living organisms and repurposed as a now essential tool in microbiology.

Simply put, when cells divide, they must divide their DNA as well. In order to create copies of DNA, cells use proteins known as enzymes. These enzymes work like little robots, splitting the DNA strand into two, and then attaching complimentary nucleotides to each strand, creating two strands. This works as each nucleotide can only bind to its complimentary nucleotide, with Adenine binding to Thymine, and Cytosine binding to Guanine. We are able to use these enzymes in order to create massive numbers of DNA copies in a very short amount of time, as new strands are created exponentially.



PCR schematic depicting DNA replication



Top: Gel electrophoresis diagram
Bottom: Picture of gel under UV light with bright bands indicating DNA

Once my sample was ready to be checked, a little bit of each sample including the (hopefully DNA-free) control sample was amplified using PCR. Once these new, concentrated samples were ready, I was able to add a specific dye, which binds to DNA and fluoresces under UV light. These dyed samples were then placed into a block of gel through which current was passed. Because DNA is a highly polar molecule, the current causes it to be dragged through the gel. This gel contains many small pores through which the DNA squeezes through. The shorter the DNA strand is, the faster it can squeeze through, resulting in the DNA from the sample becoming separated on the gel by size, which can then be visualised beautifully under UV light.

If a batch was done correctly, I should expect to see no bright DNA bands at the control, and every other sample should have bright bands at the DNA length correspondent to bacterial DNA.

EXPERIENCE GAINED

I believe I gained lots of experience during this wonderful opportunity that I was given. Working with such delicate samples taught me a lot about avoiding contamination and how to take proper precautions to avoid that. Additionally, DNA extraction, PCR and gel electrophoresis are all very popular technologies which I now have under my belt and can use for various microbiology applications – not only microbiome research. Most importantly though, I got a taste for living and working within a lab group - a crucial skill for any budding researcher. Going to lab meetings, working in the department, and spending time with my lab mates was a very developing experience, something that I did not experience much during my undergraduate studies. I am extremely grateful for the help, guidance, and welcoming atmosphere that I was given by the entirety of the Knowles lab, which reassured me in my decision to continue my master's degree with them. I am also of course most thankful to the Rokos award, without which none of this would have happened.