**Vaginal TM7 host bacteria, and the mechanisms needed to absorb amino acids.**

**Introduction**

Bacterial Vaginosis is an abnormal condition, in which naturally occurring bacteria is depleted resulting in an unbalanced microbiome. It has been shown to affect up to 36% of women, with half of these cases being asymptomatic, where the other half reported a “fishy-smelling” discharge (Hay). Bacterial Vaginosis occurs when there is a depletion of Lactobacillus, and an overgrowth of anaerobes which results in a loss of acidity. Lactobacillus has a protective role over the microbiome, by lowering the environmental pH, and the disappearance of this key species and overgrowth of potentially harmful bacteria is the main problem with Bacterial Vaginosis (Ravel). One such microbes is the vaginal TM7 strand. TM7 is a division of the bacteria domain, which has been associated with other human mucosal inflammatory diseases, such as; inflammatory bowel disease and periodontitis (He).

TM7 is characterized by its environmental 16s ribosomal DNA sequence (Hugenholtz). TM7 is a unique division, because it has an atypical base substitution, which makes it streptomycin resistant. Streptomycin is a widely used antibiotic. TM7 has been found in many different environments, such as, peat bog, termite gut, and wastewater, as well as having multiple strands being found within the human body (Barton). One such strand is the Oral TM7 bacteria, denoted TM7x. Because vaginal TM7 is being studied in this experiment, TM7x will be a frame of reference as it in one of the few TM7 strands have already been sequenced.

Figure 1: Human oral TM7 attached to host HX001.

Adapted from He’s Fig.1

TM7x is an obligate epibiont, which means it is an organism that can only live on the surface of another organism, in this case another bacteria;Actinomyces odontolyticus (He)**.** Through this parasitic relationship, TM7x is able to absorb key substances it can’t naturally produce, of interest is its need to absorb amino acids. It’s been shown to have the genes for Type III signaling proteins, which are hollow tubes that span between the two cellular membranes (Fujii). This has led to the hypothesis that there has been potential lateral gene transfer, as TM7x is able to alter the immune response of the host, during the coevolution (He).

It can be assumed that vaginal Tm7 will be similar to oral TM7 because they both are able to exist in the human microbiome. They are not identical though, because the DNA sequence of both bacteria are different. Both sequences are small, both having a sequence length of about 700 kilobases. TM7x’s genome is lacking genes necessary for de novo synthesis of amino acids, which means it can’t create certain amino acids by itself. In He’s experiment it was shown that there is a large range of conserved genes, in comparison to the oral and two other non-human TM7 strands (**Figure 2**). This could indicate that the two human TM7’s would have an even larger range of conserved genes. Therefore it may be likely that Vaginal Tm7 and Oral Tm7 could have the same type of parasitic relationships, and have the same type of need for amino acid acquisition.

The first step is to test the correlation of TM7 and other bacteria, to see possibilities for potential host organisms. Due to the reduced genomic size, it’s very likely that TM7 must have some sort of parasitic relationship in order to survive. There are two different host options that TM7 can rely on; another bacteria species, or the human host. In both cases, TM7 will need to have a specific mechanism in order to absorb the needed amino acids from the host. It’s been shown that Oral TM7 only has genetic codes to create Arginine, but no other nucleotide (Barton). This would mean that the organism has to get other amino acids from an outside source, which would be the host cell in this case.

Figure : Comparative gene analysis of TM7x, to other non-human TM7 sequences.

 He, fig. 2.

**Experiment**

The purpose of this experiment is to determine what mechanisms TM7 needs in order to absorb the nucleotides that it cannot naturally produce. The first step in this process, it to determine what the host of Vaginal TM7 is. This is done by first identifying which bacteria is present in the samples collected from vaginal secretions. Based on this data, the correlation can be tested, between TM7 and any other bacteria. To determine this, the ratio between how much of the potential host is found, versus the expected amount, is constructed. The higher this ratio is, the more probable it is be the host for TM7.

Once the potential host has been determined, the sequences of both the host, and TM7 must be found. McLean’s (2015) experiment discusses the process of sequencing Actinomyces odontolyticus. The same method will be used for the potential Vaginal TM7 host cell sequence. The first step of the procedure begins by extracting the DNA, through methods such as the Epicentre MasterPure DNA purification kit. Next, using Illumina sequencing, the complete genomic sequence can be put together, to then be run against the Prokaryotic Genome Automatic Annotation Pipeline (PGAAP), provided by National Center for Biotechnology Information (NCBI). This will show if there are any known genes that are able to be easily identified. This experiment is more focused on data analysis, rather than working with, and altering, the species.

**Results**

 Idealistically, because other human and non-human TM7 genomes have been sequenced, even if not completely, the Vaginal TM7 will be able to have many hits for known genes. This could include comparing the genes of the two bacteria found in this experiment, with those found in He (2015) experiment. This could show the possible connections that would demonstrate similar properties between the two relationships and mechanics between the cells.

 There could be also no major matches, which would mean further investigation between the two bacteria would have to take place. Finding out what the host organism is, is the first step in figuring out what to do next after these results. Presently, vaginal TM7 is unable to be grown by itself, but knowing the host cell will enable researchers to grow them in a co-habitation. Once TM7 is able to be successfully grown, forced deletions in the unknown gene will show what each gene does (Alberts).

Overall this information will help in a few major ways. It can show a possible distinctive way of protein transfer, since TM7 has been shown to be such a unique collection of bacteria. It can help researchers better grow TM7, which allow them to have easier access to the bacteria, by learning how the TM7 gains its important genomic building blocks. Since TM7 is streptomycin resistant, learning more about it can lead to new ways to destroy the cells, without the need of this widely used antibiotic.

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