Proposal bachelor or master thesis with Jurgen Haanstra and Rosanne Hertzberger.

**Variability of glycogen metabolic abilities amongst *Lactobacillus crispatus* vaginal isolates.**

Proposed start date: September 2018

**Supervision**

oversight experiments, daily questions, basic skills, introduction to lab facilities: Jurgen Haanstra.

Supervision, writing, background, scientific progress: Rosanne Hertzberger (biweekly).

**Note**: This project will follow “Open Kitchen Science” principles, which means that we will aim to publish all outcomes, including methods, data, posters, positive and negative results, on a blog (www.reblab.org) and on various other open science platforms (Zenodo, FigShare etc). This does not preclude publishing at a later stage in most traditional academic literature, such as the ASM journals.

**Background:** The bacterial communities colonizing the vagina of reproductive-age women have a remarkable low level of diversity. In most cohorts studied so far, a majority of women harbor bacterial communities dominated by lactobacilli. The most frequently encountered species of lactobacilli are *Lactobacillus crispatus* and *L. iners*. These lactobacilli produce high levels of lactate (up to 100 mM) and acidify the vagina (pH<4). The absence of *Lactobacillus* species is frequently accompanied by an overgrowth of various gram-negative and –variable species, commonly referred to as Bacterial Vaginosis (BV). This common vaginal microbial state is often asymptomatic, but in some cases leads to abnormal odor and secretions and is generally associated with a higher risk of acquiring sexually transmitted infection and premature birth.

Little is known about the carbon sources that fuel colonization and acidification by vaginal lactobacilli. One of the possible vaginal carbohydrates available to microbes is glycogen, which is present in high levels in the epithelial layers of the uterus, exocervix and vagina of reproductive-age women. Previous experiments suggest that various BV-associated microbes (*Gardnerella* and *Prevotella*) as well as *Lactobacillus iners* and some *Lactobacillus crispatus* isolates can utilize glycogen as a carbon and energy source.

Recently we found remarkable variability in glycogen metabolism amongst a group of ~20 vaginal isolates of *Lactobacillus crispatus*. This ability to use external glycogen for growth and acidification was found to correspond to a type 1 pullulanase gene variant. The strains that are able to grow using glycogen as their sole carbon and energy source have an intact N-terminal signal peptide in their type 1 pullulanase gene. We hypothesize that this signal peptide may be involved in allocating the enzyme to the outermost S-layer which is probably important to access and breakdown the large molecules of extracellular glycogen in smaller units that can be transported over the cell membrane. The subset of *Lactobacillus crispatus* strains that are unable to grow using glycogen as a carbon source have various mutations disrupting the signal peptide in this area of the type 1 pullulanase gene.

Lastly, expression of the activity was only detected during growth of *L. crispatus* on glycogen and not on glucose. This indicates either (1) carbon catabolite repression: repression of the expression of some or all genes involved in glycogen metabolism when extracellular glucose is detected or (2) induction of genes involved in glycogen metabolism in the presence of glycogen or one of the breakdown product or (3) regulation on enzymatic level (feed-back inhibition?).

Understanding these basic aspects of vaginal microbial metabolism may lead to developing better treatment for these very common vaginal symptoms, thereby increasing reproductive health.

**Hypothesis:** An intact S-layer signal peptide of the *Lactobacillus crispatus* type 1 pullulanase is essential for allocating the pullulanase that is responsible for catalysis of the first step of glycogen metabolism to the outermost membrane layer.

**Experimental plan**: Currently, the evidence supporting the hypothesis consists of a link between glycogen breakdown phenotype and type 1 pullulanase genotype in a set of ~20 *Lactobacillus crispatus* strains (genotype-fenotype). In this project we aim to gather further evidence for the hypothesis by:

-identification of the pullulanase with various substrates (glycogen, starch, amylose) and studying production of breakdown products (maltotriose, maltopentaose, maltose, glucose) using Thin Layer Chromatography. (The enzymatic activity has been detected so far only with a basic starch-iodine assay.)

-studying cellular location of the activity by comparing supernatants and pellets before and after disruption of the cells.

-studying the regulation of expression: tracking enzymatic activity during growth experiments on glycogen and/or glucose in various concentrations and in growth experiments perturbed with glucose, glycogen or glycogen breakdown products.

**Techniques to be used:** anaerobic culture, enzymatic assay, medium preparation, sterile working, optical density measurements, inoculation and plating, Thin Layer Chromatography