

## Single-cell analysis of attached microbes in sediment and biofilm

Advancement in biological sciences enabled researchers to detect the genetic material of microbes. Based on the specific sequence of the small subunit of ribosome, scientists categorized all living things into three groups called eukaryotes, prokaryotes and archaea. This has led to the development of methods to study microbial communities in environmental samples. Even microbes of the same species show variation as a result of their age and environmental stimuli. For example Alphaproteobacteria show different shapes at different ages. Moreover, studies of microbial communities give high probability to detect the dominant microbes while others may remain unknown. Therefore, so-called single-cell studies (studies of genomes of individual cells) were devised to investigate microbial communities.

In nature, microbes may exist independently as single cells or in aggregates such as free swimming, biofilms, consortia or microbial mats. Single cell microbes are capable to attach to each other and form microbial aggregates when they are triggered by environmental signals such as starvation or chemicals. Attachment is mediated by extracellular polymers secreted by the cells. This polymer is a mixture of carbohydrate, protein, genetic material, lipids and humic substances. Thousands of microbial cells may attach to each other and to particles or surfaces using the humic substance as glue. This makes cells inaccessible which is a big challenge in single-cell studies. Consequently, methods that detach or remove cells from surfaces and each other are needed.

Our project aimed to develop detaching and purifying techniques for cells from sediment and sludge collected from Uppsala Stadsskogen pond, Lake Erken, Lake Plåten and from the Uppsala waste water treatment plant. Chemical as well as mechanical detachment and the combination of both were used. The detached microbes were purified with suitable techniques from which clean cells could be recovered in abundance. These methods are called centrifugation and density gradient centrifugation. The purified cells were collected on filter paper with tiny pores, stained with dyes that integrate into the genetic material and finally visualized under fluorescence microscope. The dyes that emit strong light when fluorescent light falls on them are called fluorochromes. Additionally, the purified microbial cells were analysed with a method called *in situ* hybridization in which a piece of the genetic material is attached to a fluorochrome and allowed to form hybrids with specific part of the endogenous genetic material within the cell. Then excitation of the fluorochrome by fluorescence light reveals an image of the individual cells under microscope.

The detached and purified cells from sediment and sludge were sorted by an instrument called “Fluorescence-activated cell sorting” (FACS). This method sorts stained individual cells in a very small volume aided by laser light. The laser light excites the fluorochrome within each stained cells and then the cell falls in the sorting tubes. Each cell was lysed with chemicals and their genetic material amplified at the SciLifeLab facility Microbial Single Cell Genomic. The genetic material was used for analysis of specific genes.

The chemical detachment followed by centrifugation resulted in that many cells could be visualized under the microscope. Microscopic and genetic analysis showed the presence of bacteria, Archeae and Eukarya in all samples except Lake Plåten.