“Aerobic anoxygenic phototrophic bacteria and their photosynthetic membranes : imaging from macro- to nanoscale” - My Master Thesis

Hello! My name is Ole Franz, I am a German biologist who has been working for the shared light project with some interruptions since early 2021 at the University of Jyväskylä. I also did my master degree project related to this project under the supervision of Janne. This article is meant to introduce and inform you about the experiments and results of this thesis. In case you are interested, the entire thesis is available for anyone to read on the webpage of JYU – I will copy the link below. I suppose many of you are well informed about the topic already. However, since I am not sure if everybody is at the same level, I may include some information which could be obvious for those more familiar with the project. In case details spark your interest, or you´d like to understand something better, you can contact me so I can try to help.

One can split the thesis into two main topics. The first one includes the identification and partially the characterization of anaerobic anoxygenic phototrophic (AAP) bacteria from environmental samples with different means. As of now, it is not very well known where, when and to which extend AAP bacteria grow - especially in terrestrial environments. Additionally, impact and role of these bacteria on the environment are poorly understood. AAP bacteria are heterogenous group (so not a taxonomic group) which are mainly united by their ability to perform photosynthesis under aerobic conditions without producing oxygen – just as the name suggests. The diversity of species capable of this particular phototrophy is far from understood. A simple and fast, but accurate identification of culturable AAP bacteria is of great help to address these questions.

The most familiar AAP detection device for you may be the 3D-printed imager which can detect if bacteria growing in colonies contain bacteriochlorophyll a. If this pigment is present in aerobically grown bacteria, it is very likely phototrophic and therefor belongs to the group of AAP bacteria. The imager utilizes UV induced near-infrared (NIR) fluorescence; A UV light excites the bacteriochlorophyll a which in turn fluoresces in the NIR region around ~880-900 nm. The 3D-printed imager uses a filter to only allow light in this wavelength to hit the camera. The camera can be very cheap to work well – the only requirement is that it cannot have a so-called hot mirror in front of the sensor which is the default option for most consumer cameras as it filters out unwanted infrared light, among others. In short, this imager works very well for initial identification, as it is fast and accurate. Drawbacks are that the acquired information stops at the pure identification. The bacteria are not further characterized spectroscopically, as the result is binary – AAP bacteria, yes or no (Fig. 1 A & B). This is also because intensity differences are influenced by the uneven lighting conditions.

It could be possible to gain more information with a similarly quick measurement (but longer result analysis) by using so-called hyperspectral cameras. These quite expensive cameras can record a spectrum for each pixel of the image. In practice, one can record an image of a petri dish and later select the pixels covering the area of a bacteria colony and average them. Depending on the position of the light source, absorbance or fluorescence spectra can be then analyzed for this area. In addition of a yes/no analysis, we are able to analyze the peak positions, shapes and relative heights which could help to characterize bacteria (Fig. 1). We could potentially identify bacteria with different sub-types of AAP machineries straight from the Petri dish. In my thesis I made initial tests with hyperspectral cameras and compared the results with the 3D-printed imager. For me it was possible to acquire accurate absorbance spectra from a Petri dish (Fig. 1 C), but I faced problems with the accuracy of the fluorescence spectra. However, all these were initial

tests and require further testing. Additionally, I included other spectroscopic measurements of isolated bacteria to obtain reference spectra of the bacteria.

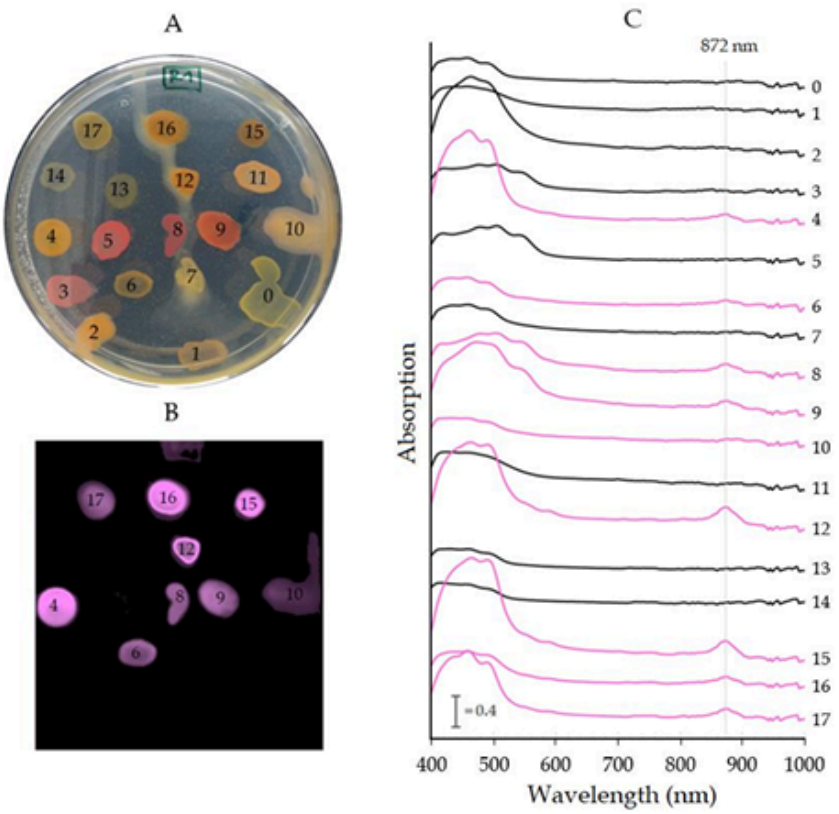

Figure 1 Comparison of bacteria analysis with **A)** white light, **B)** the fluorescence mode of the 3D-printed imager and **C)** results of the hyperspectral pocket camera Specim IQ. For the results of the hyperspectral camera, absorbance was calculated as -log of the transmittance. The shown spectra were averaged from 10x10 pixels manually placed on each colony. Numbers on the right of each spectrum correspond to numbers on the bacteria in A and B. All spectra of AAP positive bacteria detected with the 3D-printed imager in B have been colored pink in graph C for clarity.

The second main topic of my thesis was the extraction and imaging of the photosynthetic membranes present in the *Sphingomonas glacialis* strain S2U11, collected by Riitta from *Diapensia lapponica* (Uuvana). I was able to extract membranes analyze them spectroscopically and likely image the photosynthetic complex (light harvesting complex and reaction center including the NIR fluorescent bacteriochlorophyll a). The imaging was performed with an atomic force microscope, potentially capable of resolving structures in the low nanometer or even sub-nanometer scale. For more details on the imaging mechanism, methods and results, you can browse my thesis.

My personal goal was to include method development in my thesis, learn more about different imaging techniques and generally include a variety of processes. As a result, the thesis may not follow a strict and straight narrative. However, I am very happy with the number of skills I have acquired and the general outcome of the thesis.

Link to the thesis

https://jyx.jyu.fi/handle/123456789/84233