Chuck Berry - An approach in 3D imaging

Thiele, S.
Department of Molecular Ecology, Max-Planck-Institute for Marine Microbiology, Bremen, Germany
Contact: sthiele@mpi-bremen.de

Microbial Diversity Course 2012

Abstract

Here I describe an approach to investigate a pink berry aggregate in its natural structure using a 3D approach. I used catalyzed reporter deposition fluorescence in situ hybridization (CARD FISH) and confocal laser scanning microscopy (LSM) to generate 2 videos of different sections of a pink berry. The functionality of this approach was shown, while further improvement in the CARD FISH staining needs to be done.

Introduction

In Little Sippewisset Salt Marsh microbial consortia of mainly purple sulfur bacteria, sulfate reducing bacteria and members of the *Bacteroidetes* phylum, accompanied by diatoms, were frequently found in shallow ponds. These consortia form aggregates of various sizes (um to cm scale) embedded in a stable matrix. This matrix is problematic for nondestructive analyses of the bacterial community within the berry. Therefore, I used CARD FISH and confocal microscopy to investigate the bacterial distribution in these berries in their original structure and to produce 3D images of a so called pink berry.

Methods

Several pink berries, microbial consortia consisting of purple sulfur bacteria, sulfate reducing bacteria and *Bacteroidetes*, were collected during a field trip to Little Sippewisset Salt Marsh on the 12th of June 2012. The berries were fixed with 2% formaldehyde final conc. for 2 hours, washed and stored in PBS until further processing. CARD FISH was done using two probes, CF319a (35% FA) and SRPiBe213 (45% FA) in liquid after a standard protocol (Pernthaler et al 2002, Thiele et al., 2011). Both hybridization steps were done over 12 h. Amplification for CF319a was done using ALEXA488, while ALEXA633 was used for SR PiBe213. After the CARD FISH procedure the berries were DAPI stained and kept in PBS in the dark until microsocpic analyses. Microscopy was done using a 40x apochromatic water objective on the Zeiss LSM710 microscope (inverted) with a 2 photon system (Zeiss, Jena, Germany) and the ZEN2011 sofware (Zeiss, Jena, Germany). As sample holder I used a small petri dish with a cover slip covered hole in the bottom. This petri dish allows the analyses of a specimen in a liquid environment. Two Z-stacks of areas of the berry were taken and further rendered to a movie using the ZEN2011 software. No post-processing was used during the generation of the movies.

Results

Due to limitations of the 2 photon system only DAPI, ALEXA488 and the autofluorescence of the purple sulfur bacteria were analyzed. From the resulting Z-stacks, videos were produced, showing an aggregation of purple sulfur bacteria (Video: PSB) and a deep section of 132 um through the berry

(Video: Chuck Berry). Due to overnight storage in the petri dish, the sample dried out. For the second analyses it had to be rewetted but some part of the 3D structure might have been lost.

Discussion

First trial were done also with a Zeiss LSM710 in normal mode, but large dark areas were seen. The 2 photon system combined with a Zeiss LSM710 proved to be able to penetrate deep into the berry matrix and thus analyses resulted in two videos. Both videos show good resolution and very long working distance within the examined specimen. Nevertheless, there are drawbacks in the method. In Vid. 2 dark spots can be seen within dense bacterial aggregates. Here the laser power of the 2 photon laser was also not sufficient to penetrate the aggregate, resulting in “blind spots”. Furthermore, the CARD FISH probes used in this approach seem to bind to all cells, resulting in good signals but no taxonomic specificity. Thus further improvements are needed. Especially for the CARD FISH procedure and the specificity of the probes, further research is needed.
Still confocal laser scanning microscopy proved to be a good tool to investigate the microbial community within pink berries and the 3 dimensional distribution of the microbes.

Acknowledgement

This goes out to the mighty Chuck Pepe-Ranney for all his help during the course. Dude, the berry is yours, besides it is a funny name. And thanks to Ryan and the Zeiss guys for your patience.

References

Pernthaler A, Pernthaler J, Amann R. 2002. Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. Appl. Environ. Microbiol. 68:3094–3101

Thiele S, Fuchs BM, Amann R. 2011. Identification of microorganisms using the ribosomal RNA approach and fluorescence in situ hybridization. In Wilderer, P. (ed), Treatise on Water Science, vol. 3., Elsevier, Oxford: Academic Press, pp. 171–189

Videos

PSB : DAPI (blue) and ALEXA488 (green) overlay of stained purple sulfur bacteria in a pink berry.

Chuck Berry: DAPI (blue), ALEXA488 (green) and autofluorescence (red) overlay of multiple layers of stained cells in a pink berry.