SINGLE-CELL TECHNOLOGIES FOR MICROBIAL COMMUNITY CHARACTERIZATION

KANTIEN, KANODREEF 1, 9000, GENT

- MORNING SESSION -

Novel Insights and Method Developments

8h45 Registration & Coffee

9h00 – 9h30

CAN MICROBIAL COMMUNITIES BE CONTROLLED IN MANAGED SYSTEMS?

Prof. Dr. Susann Müller
(Helmholtz Centre for Environmental Research - UFZ)

The principal aim of engineers in process control of bioreactors is to construct a niche with boundaries to strengthen and sustain an initially manufactured community. In order to probe, monitor, and eventually control natural communities in managed systems, fast detection and evaluation methods are required. We apply community flow cytometry, which provides single cell-based high-dimensional data. To interpret the resulting time series data, we draw inspiration from macro-ecology, in which a rich set of concepts has been developed for describing population dynamics. First, we focused on the stability paradigm and present a rapid workflow to monitor and compute the stability properties resistance, resilience, displacement speed, and elasticity. In addition, the principal roles of neutral vs. niche differentiation mechanisms in insular environments such as bioreactors have been studied. In completely insular microbial communities, the evolution of a community structure cannot be shaped by the immigration of new community members, and if run in steady-state, the influence of environmental factors on community assembly is reduced. Yet, in parallel setups variability does occur. Our findings suggest that complex microbial communities in insular steady-state environments can be difficult to synchronize or maintained in their original or desired structure.

9h45 – 10h15

HIGH-RESOLUTION FLOW CYTOMETRY OF THE INTESTINAL MICROBIOTA

Dr. Hyun-Dong Chang

(German Rheumatism Research Centre)

The intestinal microbiota is a complex ecosystem of 10^{13} bacteria of several hundred bacterial species. Besides nutrient metabolism, the microbiota is vital for the proper development and functioning of the mammalian immune system. Pathogenic changes to the microbiota, known

as dysbiosis, have been implied in immune-mediated diseases like inflammatory bowel disease (IBD), arthritis, and diabetes. However, microbiota analysis for the diagnosis and prognosis of diseases is still rare, largely due to the lack of fast and efficient preparative methods to dissect the heterogeneity of the microbiota. Currently, most studies rely on next-generation sequencing of the bacterial metagenome. This is rather slow, expensive, population-based, restricted to genome and transcriptome, and does not allow to isolate defined bacteria for further analysis.

Here we present a flow-cytometric approach to unravel the complexity of the commensal microbiota. High-resolution determination of the bacterial forward scatter and DNA content allows for the discrimination of different bacterial subpopulations per sample from formaldehyde-fixed murine stool samples. Using this method, we tracked the changes of the microbiota in the course of several murine models for IBD and confirmed by 16s rDNA sequencing of sorted sub-populations that the composition of cytometrically defined populations is phylogenetically homogenous. We are currently establishing additional dyes and are developing monoclonal antibodies to increase the dimensionality of microbiota cytometry with the goal to increase the resolution and to allow the detection and isolation of specific bacteria.

10h30 – 10h45

Coffee break

Emerging Technologies

10h45 – 11h15

APPLICATION OF RAMAN MICRO-SPECTROSCOPY TO SINGLE CELL BIOLOGY

Prof. Dr. Wei Huang

(University of Oxford)

Single cell Raman spectra (SCRS) provide label-free biochemical profiles of individual cells, and such intrinsic 'fingerprints' can be regarded as single cell phenotype, reflecting gene expression and metabolic functions, and revealing spatial and temporal relationships of cells. Sorting cells based on SCRS would link phenotypes to genomics and transcriptomics and proteomics at the single cell level.

We developed Raman activated cell sorting (RACS) based on microfluidic device and Raman activated cell ejection (RACE) according to SCRS. RACS is able to sort cells from cell suspension and RACE to sort cells directly from the surface of microscopic slides. We have demonstrated that RACS and RACE were applied to sort single cells. The sorted cells were used to cell cultivation and single cell genomics analysis. The RACS and RACE technology will be useful to dissect single cell functions and sort cells of interest in various biological samples (e.g. clinic and environmental samples).

11h30 – 12h00

PHENOTYPIC HETEROGENEITY IN BIOPROCESS MONITORING

Prof. Dr. Frank Delvigne

(University of Liège)

Bioprocess deviations are likely to occur at different operating scales, leading in most of the case to substrate deviation from main metabolic routes and impact product synthesis. Correlating q_S and q_P is of outmost importance for bioprocess observability and control and can be modeled actually by advanced metabolic flux models. However, if most of these models are able to make prediction about metabolic switches, they still do not incorporate deviation due to biological noise, i.e. phenotypic and genotypic heterogeneity. These limitations impair observability and thus the use of fundamental knowledge about biological network for practical application, i.e. metabolic engineering or bioprocess scale-up. The fundamental question that will be addressed here is to what extent information gathered at a single cell resolution can help improving global observability of bioprocesses. This fact will be illustrated by two case studies involving cell-environment and cell-cell interactions.

12h15 – 13h45

Lunch break

- AFTERNOON SESSION -

Emerging Technologies: applications

13h45 – 14h00

(BIG DATA)^2: ON-LINE/REAL-TIME FLOW CYTOMETRY FOR MICROBIAL MONITORING

Dr. Michael Besmer

(OnCyt; Swiss Federal Institute of Aquatic Science and Technology)

With bacteria being an integral part of natural and engineered aquatic ecosystems, it is crucial to closely monitor their number and composition. This allows for better understanding and management of bacteria and their impacts. One critical aspect in this is the tracking of microbial dynamics at short timescales (seconds to days) given that those ecosystems are usually highly variable rather than stable over time. However, tracking of these dynamics requires sampling and analysis at very short intervals and ideally in real-time allowing for immediate interpretation/reaction. This can only be achieved through full automation of in-situ sampling, sample processing, and detection. For decades this was impossible with conventional cultivation-based methods but also with advanced molecular methods, which are still too labor intensive, time consuming, and costly for such applications. However, in recent years much progress was made on automated monitoring. One promising approach was the automation of flow cytometry. Given the richness in data collected for every single cell in a single flow cytometric measurement, the possibility of highly resolved time-series multiplies the

possibilities for in-depth analysis to maximize information gain. Hence, automated flow cytometry can strongly complement equally data-rich next generation sequencing approaches but additionally has specific advantages (e.g., rapidness, sensitivity, reproducibility, accuracy in quantification, differentiation of total and intact cells). By now, fully automated flow cytometry technology is commercially available and is rapidly becoming a standard tool in fundamental and applied research but also various industries. Hence, here we present the scientific bases of the method that were established in the last five years. Specifically, we show experimental data on (1) growth behavior of natural bacteria in cell numbers and community fingerprints, (2) short-term detachment dynamics in full-scale water treatment and distribution linked to operational practices, (3) effects of water pollution and disinfection through oxidation on cell numbers and viability.

14h00 – 14h30

RAPID DETECTION OF LEGIONELLA BY IMMUNOMAGNETIC SEPARATION AND FLOW CYTOMETRY

Dr. Björn Biedermann

(rqmicro)

Rqmicro's separation and detection technology for microbiological tests in the water and food industry delivers reliable, quantitative and cost-effective results in less than 2 hours. Our expertise lies in isolating and purifying target cells from complex water samples while eliminating more than 95% of the competing flora. Benefits from the rqmicro product list and service offers to mitigate the risk of Legionella remaining unnoticed. This presentation will discuss:

- Introduction to rqmicro and the technology
- Validation of the assay and comparison to ISO 11731
- Detection of the total fraction of Legionella in the sample, including VBNC
- Examples of contamination: drinking water, cooling tower, process water (e.g. from paper industry)

14h45 – 15h30

SET-UP AND DESIGN CONSIDERATIONS FOR ROUTINE FLOW CYTOMETRIC MONITORING OF MICROBIAL POPULATIONS

M.Sc. Cristina García Timermans

(Ghent University)

15h30 – 16h00 Closing statements