Molecular Aspects of Medicine xxx (2017) 1–12

Contents lists available at ScienceDirect

Molecular Aspects of Medicine

journal homepage: www.elsevier.com/locate/mam

Personalized microbiome dynamics – Cytometric fingerprints for routine diagnostics

Christin Koch, Susann Müller*

UFZ – Helmholtz Centre for Environmental Research, Department of Environmental Microbiology, Permoserstr. 15, 04318 Leipzig, Germany

ARTICLE INFO

Article history:
Received 7 April 2017
Received in revised form
26 June 2017
Accepted 28 June 2017
Available online xxx

Keywords:
Microbiome
Microbial flow cytometry
Single cell analysis
Microbial ecology
Human gut microbiome

ABSTRACT

Microbiomes convoy human life in countless ways. They are an essential part of the human body and interact with its host in countless ways. Currently, extensive microbiome analyses assessing the microbiomes' composition and functions based on sequencing information are still far away from being routine analyses due to the complexity of applied techniques and data analysis, their time demand as well as high costs. With the growing demand for on-time community assessment and monitoring of its dynamic behavior with high resolution, alternative high-throughput methods such as microbial community flow cytometry come into focus. Our flow cytometric approach provides single-cell based high-dimensional data by using only three parameters but for every cell in a system which is enough to characterize whole communities' attributes with high acuity over time. To interpret such complex cytometric time-series data, novel concepts are required.

We provide a workflow which is applicable for easy-to-use handling and measurement of microbiomes. Drawing inspiration from macro-ecology, in which a rich set of concepts has been developed for describing population dynamics, we interpret huge sets of community single cell data in an intuitive and actionable way using a series of bioinformatics tools which we either developed or adapted from sequence based evaluation approaches for the interpretation of single cell data. The developed evaluation pipeline tests for e.g. ecological measures such as community assembly, functioning, and evolution. We also addressed the meta-community-concept which is a well acknowledged idea in macro-ecology on how interconnected communities perform. The last concept discusses stability which is a metrics of paramount importance. A fast quantification of stability properties may not only detect disturbances and their impact on the organisms but also allow for on-time microbiome treatment.

The workflow's immanent ability to support high temporal sample densities below bacterial generation times provides new insight into the ecology of microbiomes and may also provide access to community control for microbiome based health management. The future developments will facilitate cytometric fingerprinting for human routine diagnostics to be as simple and meaningful as a blood count today.

1. Introduction

The human meta-organism consists of eukaryotic human cells as well as a significant share of cells of microbial origin (Savage, 1977; Sender et al., 2016). These trillions of microbial cells represent our individual human microbiome and contain up to 300-fold more unique genes then the human genome (Yang et al., 2009; Blaser et al., 2013). The presence of our microbiome is vital for our wellbeing. The beneficial microbiota in the different habitats provided by our skin protect us against invasion of pathogenic microorganisms and modulate our innate as well as adaptive immune response (Grice and Segre, 2011). Up to 10^7 bacteria (number based on colony forming units, cfu) reside a cm^2 of our skin dependent on body site (Leyden et al., 1987) with up to 10^{12} cells in total on the skin of a single person (Wilson et al., 2002). And despite the exposure of the skin to the external environment, the skin microbiome is individual to location and person and relatively stable over time (Oh et al., 2016). In the same way our outer body is covered by microorganisms also our inner mucosal surfaces are populated by diverse microbiomes. The oral cavity contains up to

* Corresponding author.
E-mail address: suann.mueller@ufz.de (S. Müller).

http://dx.doi.org/10.1016/j.mam.2017.06.005
0098-2997/

10^7 cfu ml^{-1} saliva of *Lactobacillus* species (Dal Bello and Hertel, 2006) or up to 10^9 bacteria ml^{-1} saliva (Berg, 1996) and is the most variable body site regarding microbial community composition over time (Ding and Schloss, 2014). The highest cell density is reached in the colon with up to 10^{11}-10^{12} microorganisms per gram of colon content (Berg, 1996; O'Hara and Shanahan, 2006). The colonization of the human gastrointestinal tract starts directly after birth and following ecological succession a stable diverse gut microbiome develops within the first years of life (Lozupone et al., 2012). The gut microbiome not only determines the success of our daily digestion and hence the exploitation of nutrients from food and formation of micronutrients (Cassani et al., 2012; Crozier et al., 2010; Kau et al., 2011), it also severely impacts the health status of the human host and is associated to obesity-associated inflammation (Shen et al., 2013) and diabetes (Hartstra et al., 2015) and even a potential gut-brain axis has been identified (Benakis et al., 2016).

Altogether, our body constitutes numerous individual ecosystems with strong niche specialization (Human Microbiome Project, 2012). Thereby, the relationship between the human host and its microbiome is mainly symbiotic (Falk et al., 1998). However, temporal variations in the microbiome composition at different body sites can be found as a result of natural selection due to extrinsic (e.g. food, medication) and intrinsic factors (e.g. adaptive immune system) (Caporaso et al., 2011). The underlying mechanisms that shape our microbiome and the dependent effects on our health are not yet understood and require comprehensive studies that resolve the ongoing mechanisms.

The recent advances in DNA based culture independent methods have expanded our knowledge on the diversity of the human microbiome, its biogeography and its dynamics. Nevertheless, extensive microbiome analyses are until now only performed within research projects and still far away from being routine analyses due to the complexity of applied techniques. Although the development of sequencing based technologies regarding speed and costs are promising (Kuczynski et al., 2012; Goodwin et al., 2016), the main unresolved challenge is the automated data analysis and interpretation and especially the translation of the results into clinical actions and treatments (Cho and Blaser, 2012; Fournier et al., 2014). Further, one has to differentiate the clinical requirements. While the presence of a specific antibiotic marker or virulence is well targeted with sequencing based technologies (Fournier et al., 2014; Deurenberg et al., 2017), the demand for rapid and high-throughput microbiome monitoring can be perfectly met with microbial flow cytometry. Neither 16S rRNA gene affiliation (Bloom et al., 2011), gene association studies (Jostins et al., 2012), expression profiles of genes (Schulze et al., 2016) or metaproteomes (Schaubeck et al., 2016) are given by microbial flow cytometry. Instead, cellular characteristics such as morphology and physiology are recorded which describe the state of a microbial community being determined by its cytometric diversity and function. This review introduces the principle and analyzing routines of this single-cell based method for microbiomes (section 2) and illustrates its application and identification of underlying ecological patterns (section 3).

2. Cytometric characterization of microbiomes

Flow cytometry is a powerful technology that is widely applied in biomedical research and clinical routine diagnostics by now more than 30 years. It allows the characterization and discrimination of single human cells with high precision and in a high-throughput manner providing the classical foundation for disease monitoring from the standard blood count until advanced tumor biology and immune therapy (Muirhead et al., 1985; Perfetto et al., 2004).

A flow cytometer consists of a measurement chamber and the connected light source (one or several lasers of different wavelength) as well as the optical bank with the optical filters and photomultiplier that convert the optical into a digital signal. The cells are introduced in a fluid stream and pass the laser beam cell by cell. When the cells pass through the beam the light is scattered (forward and sideward scatter signal) and possible present fluorochromes are excited. In most cases, specific monoclonal fluorescently labeled antibodies for certain cell or cell-surface targets are applied. In this way, the specific optical characteristics of every individual cell are recorded and up to 10 000 cells s^{-1} can be easily measured. In addition to the high resolution and speed of analysis, cell sorting is a striking feature that allows to physically separate cells with a specific combination of optical characteristics into a test tube or on a microscopy slide facilitating further analysis. Recent advances in automated data analysis also enabled to work with more complex multi-parametric datasets (Bashashati and Brinkman, 2009; Lugli et al., 2010). In this way, flow cytometry represents an established powerful tool for monitoring states of health and disease in humans.

The application of flow cytometry is not restricted to human eukaryotic cells. Also microbial cells, which can be associated to the human body, can be measured. The general difference lies in the cellular characteristics of prokaryotic in contrast to human eukaryotic cells. Besides the cell size (0.3 to a few μm) and the cell volume (0.1–10 μm^3) being (an) order(s) of magnitude lower than the average eukaryotic cell, the high diversity of different microbial species results in a multitude of different cellular characteristics (e.g. differences in gram staining, presence of surface proteins, lectins, etc.) and impedes the establishment of general, antibody based markers for cellular surface characteristics. This challenge is further complicated regarding the development of protocols and the confirmation of their specificity of the chosen markers (e.g. membrane integrity, enzyme activity, presence of storage compounds; Müller and Nebe-von-Caron, 2010) as many microorganisms in environmental samples cannot be cultivated and thus protocols cannot not be verified. Despite their high diversity, the least common denominator in all microbial cells is the presence of DNA. Therefore, DNA labelling serves as a universal marker that can be applied to all microbial cells independent of their phylogeny, metabolic capacity or actual activity. Although being a universal marker, the DNA staining specifically mirrors the DNA content in the microbial cells being representative of their number of chromosome equivalents and their physiological state (Müller, 2007). In this way, also complex microbial communities can be characterized in a simple and rapid way (Koch et al., 2013c) and the state and dynamics of these communities interpreted as marker for healthy and disturbed ecosystems (Günther et al., 2016; Koch et al., 2013b; Zimmermann et al., 2016).

In the following sub-chapters the general procedure of microbial flow cytometry and cytometric fingerprinting is described, including routine laboratory as well as data analysis procedures which are also applicable to human associated microbial samples.

2.1. Microbial flow cytometry

Most microbial samples are suitable for cytometric characterization and an established step-by-step protocol with several variants for specific sample requirements (e.g. flocs and biofilms, anaerobic samples) is available (Koch et al., 2013c). First the samples are stabilized by fixation (e.g. 2% paraformaldehyde for 30 min at room temperature, followed by centrifugation at 3200*g*, 20 min, 15 °C and subsequent resuspension in 70% ethanol (1:8 volsample/volfixative) and storage at −20 °C (Günther et al., 2016)). Alternative fixation methods are described for different sample

Please cite this article in press as: Koch, C., Müller, S., Personalized microbiome dynamics – Cytometric fingerprints for routine diagnostics, Molecular Aspects of Medicine (2017), http://dx.doi.org/10.1016/j.mam.2017.06.005