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Insights into microbial ecosystems using a new computational approach.

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Technological advances in high throughput DNA sequencing have revolutionised microbiology and changed the concept of the pathogenic functions of microbes that persisted for almost two centuries, and was shaped by the germ theory established by Koch and Pasteur. The decreasing costs of next-generation sequencing have allowed more rigorous investigations and improved insight into diverse and complex microbial communities with increasing resolution, particularly through the use of culture-independent approaches. A burst in interest in the role of the microbiome in both health and disease, and the launch of the Human Microbiome Project (HMP) by NIH in 2008 (Human Microbiome Project, 2012), helped confirm that microbes not only cause disease, but also, play an important part in healthy human life. Micro-organisms have indeed co-existed with the host for a long time, resulting in a mutualistic and symbiotic relationship with the microbiome providing benefits to the host, for example, in terms of cell metabolism, host tissues and immune development, as well as protection from pathogen invasion (Costello et al., 2012, Dethlefsen et al., 2007). The human-associated microbial community is dynamic; it is largely composed of symbionts that are continuously adapting to their challenging environment, heavily influenced by the host diet, genetics, gender, lifestyle and health status. The microbial dynamics of this ecosystem also include synergy and competition between microbes, adding to its daunting complexity. Many competitive microbial strategies have evolved, based on ecological conditions and resources available. These also include mutation and transfer of genetic material within panmictic microbial communities, which may lead to the acquisition of beneficial phenotypes or other properties that increase the community's chances of surviving selective pressures (Ghoul & Mitri, 2016). However, these also have serious public health consequences due to, for example, increased antibiotic resistance and the potential emergence of pathogenicity. The hugely rich microbial population at each habitat is diverse and dynamic; it is shaped by the above factors and by strong interactions with the host. Several studies have reported large intra- and inter-individual variations in microbiome composition, and emphasise the importance of carrying out additional investigations on temporal variations observed within the same day in the same individuals (Flores et al., 2014, Gao et al., 2007). The different microbial assembly scenarios are the results of the individual's life experience and lifestyle choice, where every event or action taken generates a unique ecosystem.

The human microbiome is acquired from environmental exposure at birth (Dethlefsen et al., 2007), and develops through ecological succession (Costello et al., 2012). Although initially unstable, it reaches equilibrium after several months or years once the various microbial habitats in the body have diversified in composition and abundance. Potential long-term effects on health of antibiotic exposure and other perturbations have been reported (Yassour et al., 2016), with a decrease in microbial diversity and the development of a less mature microbiota as a consequence. The example of the difference in the gut microbiota determined by the mode of

delivery at birth is interesting. Vaginally-delivered children tend to inherit the mother's vaginal and faecal microbiota, in contrast to children delivered by caesarean section whose gut microbiota is initially colonised by skin microbiota and harbours reduced abundance of Bacteroides and Bifidobacteria (Costello et al., 2012, Yassour et al., 2016).

Once established, the human microbiome is inherently stable and resilient (Cho & Blaser, 2012, Yassour et al., 2016). However, some significant diseases are described as dysbioses, arising from perturbations in the resident microbial communities and/or host responses to them resulting in loss of host-microbe homeostasis. Microbial disturbances such as those caused by prolonged exposure to antibiotics noted above may have long-term impacts on an individual's health and certainly warrants further investigation. Similarly, little is known of the mechanisms by which attempts to restore diversity-depleted microbiomes, such as probiotics and faecal microbiota transplantation (FMT), change the host ecosystem. An understanding of the processes that govern the biology of microbial and host-microbe interactions is fundamental if efficient manipulations of the microbiome are to be designed and approved for maintaining health and resolving dysbiosis. To this effect, there has been great interest in the use of ecological theories to understand the ecological processes of the human-associated microbiome (Costello et al., 2012). A recent study by Bashan et al. (2016) explores such processes through the design of a new computational approach, which the authors applied to two large metagenomics datasets from the HMP and the student microbiome project (SMP) (Flores et al., 2014), to characterise the underlying microbial dynamics at various body sites. Studies by the HMP consortium have already reported on the high inter-individual variations of the human microbial profile in health. Are these differences due to host-specific factors such as lifestyle, physiology and genetics, or are they caused by the sets of colonizing species, which differ between individuals? The ability to answer this question holds great clinical significance and would help validate the use of generic microbiome manipulations if microbial dynamics are found to be universal (Bashan et al., 2016). The alternative would mean that development of highly personalised therapies is needed.

The underlying dynamics of a microbial community are complex; they are defined by growth rates and the intra- and inter-species networks of interactions, rendering the analysis and ecological interpretation of data difficult. In addition, numerous metagenomic studies have generated large amounts of sequencing data that have been made publicly available. However, there are methodological limitations on some of these data ranging from sample collection to the pipeline used for analyses. Some of these datasets vary in sequence quality and may not fully capture the demographics and circumstances of the individuals sampled that can be related to the microbial profiles. Others may not have been rigorously analysed, using tools that may fail to identify microbiome characteristics of clinical significance (Noecker et al., 2016). One approach to overcome these difficulties when studying microbial community dynamics is to define community

types (Costello et al., 2012). Several studies have described the classification of gut microbiota into enterotypes based on taxonomic similarities (Arumugam et al., 2011, Ding & Schloss, 2014). In the case of Bashan et al. (2016), three different scenarios of microbial dynamics were considered. The first described individual dynamics where ecological parameters are unique for each individual. The second described group dynamics where individuals are clustered based on certain host factors, with those belonging to the same group sharing similar sets of parameters. The last scenario portrayed universal dynamics, with all individuals sharing the same sets of parameters. In order to determine which scenario best embodies microbial ecosystems in health, the authors used an ingenious strategy that by-passes the overwhelming complexity of the microbial networks: instead of trying to identify the dynamics as individual, group or universal, they simplified the research question to whether the dynamics are universal or not. Indeed, inferring that from the complex system parameters using a population dynamic model is an impossible task due to the constraints of data currently available, which do not consistently contain high quality time series information.

In order to examine and compare the inter-individual variability between samples, two factors were identified: the difference in species composition, and the difference in the abundance profiles. The former was described as the overlap of species assemblage, and the latter as dissimilarity between renormalized abundance profiles (Bashan et al., 2016). These two contributors to microbiome variation can be methodically calculated for sample pairs from any given microbiome dataset, and the results for each pair can be displayed as a dot within a dissimilarity versus overlap graph. The overall collected data can then be used to carry out non-parametric regression and bootstrap sampling to obtain a dissimilarity-overlap curve (DOC) with its corresponding confidence interval. The authors used the interpretation of the DOC slope to infer the type of microbial dynamics occurring within the sample data. A flat curve would be best explained as the result of the lack of inter-species interactions, where the increase of species overlap does not affect the level of dissimilarity observed. A curve with a negative slope is evidence for universal dynamics and inter-species interactions, meaning that as species overlap increases, the abundance profiles become more similar between the given sample pairs. Synthetic and real data were used to test the DOC methodology. When applied to cross-sectional microbial samples from the HMP and SMP datasets at various body sites (gastrointestinal, oral, skin, urogenital and airways), DOC analysis revealed that the microbial dynamics of the gut and oral microbiomes could be defined as universal. The skin microbiomes of the palm and elbow seemed to show the least universality in their patterns. The authors confirmed these results with further analyses taking into account certain confounding factors, such as body mass index, age and diet, which might have contributed to the negative slope results in the gut and oral samples by having an effect as driving forces for microbial compositions. The authors acknowledge that

current datasets do not allow for the exclusion of other potentially important factors such as inflammation, genetics or drugs as drivers of community development.

Following from these interesting findings, the question of the presence of universality of dynamics in disturbed microbiomes was raised. Clostridium difficile infections (CDI) arise from such microbiomes, usually as a result of antibiotic exposure causing loss of microbial diversity in the gut. Continued use of antibiotics for CDI treatment contributes to persistent disruption of the gut microbiota, and can lead to increased risk of CDI recurrence by more than 60% after the second episode (Leffler & Lamont, 2015). There is, however, growing evidence for the successful treatment of recurrent CDI using FMT (Brandt & Reddy, 2011). Bashan et al. (2016) applied their DOC analysis to the microbiome data of 17 patients with recurrent CDI before and after FMT. Their analysis showed a flat DOC in the CDI samples before FMT, indicating the loss of universal dynamics in individuals with disrupted gut microbiota compared to healthy ones. The microbial dynamics seemed to return to universality after FMT in the same individuals, with DOC results revealing clear negative slopes. These results show the gut's capacity for adaptation and resilience, and indicate that the dynamics of our microbiome, in particular that in the gut and mouth, are governed by universal processes that are mostly independent of host factors. Dysbiosis, represented by the occurrence of CDI in this study, results from the loss of universality of the microbial interactions within the observed microbial community, and seems to be reversible.

The computational approach designed by Bashan et al. (2016) is a testimony to the significance of ecological theories to refine our study of the microbial world, to better understand the underlying ecological host-associated microbial dynamics. There is a need, not only, to define these microbial dynamics, but also, to decipher what microbial functions are most important and how microbes interact with the host, on a molecular level and at individual cellular levels. Further advances in technology will ensure that meta-omics approaches will provide improved quality data which will contribute to unbiased and rigorous characterisation of the human microbiome, and allow translation of ecological knowledge into clinical practice (Costello et al., 2012). The successful use of FMT for resolving CDI and CDI recurrence is a great example; however, further research is needed to optimise its methodologies to make it equally applicable to other diseases such as inflammatory bowel disease (Pigneur & Sokol, 2016). Currently, there are too many undefined variables and issues related to healthy donor's faecal samples, such as potential for diseases transmission, making FDA approval difficult to obtain. An alternative approach to FMT has been designed by Seres Therapeutics, which aims to use a selection of key organisms and spores within a better controlled system (Khanna et al., 2016). However, the recent failure of its new drug SER-109 at the second phase of clinical trial suggests that microbial ecology is

incompletely understood, and requires much further research to fully grasp the systems at play, in order to exploit them to our advantage.

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