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Multitargeted Analyses are Instrumental for Microbial Ecology Studies

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Editorial

The current understanding of the roles of microorganisms in nature, in health, and in disease is still based mainly on studies with pure cultures. However, this area of research is much more complex, since most bacteria are unculturable under laboratory conditions, leading us to the philosophical question of how to uncover microbial functions if we do not even know the growth requirements of a large portion of the naturally occurring microbiota. The recovery of microorganisms from complex environments (i.e., soil, sputum, food matrices) largely depends on the knowledge of abiotic factors such as moisture, water activity, pH, salinity, composition of the atmosphere, temperature, and nutrient sources[1,2]. Moreover, in complex matrices, the microbial interactions (i.e., competition, antagonism) are also of key importance. Thus, the most robust models for microbial ecology studies should consider both abiotic and biotic factors to better mimic the autochthonous communities.

The advancement of omics-based technologies such as metataxonomics, metagenomics, metatranscriptomics, metametabolomics, and single-cell gene sequencing of microbial communities have provided a more in-depth description of the metabolic and taxonomic traits of microbial ecosystems, generating data on culturable and non-culturable microbiota[1,3]. Besides, these novel techniques also shed light on the complex relationships among microorganisms from food, human, animal, soil, and vegetal microbiomes. In parallel, another interesting paradigm in microbial ecology is culturomics, a term introduced by Lagier et al.[4], which represents the recovery of previously overlooked bacterial species from the human gut using high-throughput culturing methods and MALDI-TOF mass spectrometry taxonomic identification[4,5]. Basically, with this approach, the samples are partitioned and processed into different culture media and incubated under different conditions of temperature, pressure, or other abiotic factors in order to augment the probability of isolating bacteria from complex samples[5]. Overall, omics-based methods and culturomics allow for accessing the information regarding the abiotic and biotic factors needed by bacteria in order to mimic their original ecosystems in laboratory conditions.

In this methods collection, contributions are presented from different research groups of diverse countries reporting

on the advancement of microbial ecology studies related to omics-based methods and culturomics. Studies on the use of metataxonomics (also known as metabarcoding) are fundamental to monitor complex environments and to assemble culturable and unculturable microbial profiles in diverse ecological niches. Yarimizu et al.[6] provide a robust methodology to evaluate algal blooms in marine ecosystems and address an important matter in microbial ecology by describing adequate sampling and a strategy for data analysis to make the studies reproducible. Allying microscopy and physicochemical techniques, the authors validate a method to screen seawater samples to detect potential algae species involved with economical losses in the aquaculture of commercial fish products.

Another fundamental concern in ecology is the compartmentalization of microbes (i.e., their assemblage distribution). Pioli et al.[7] present a microfluidic platform geometrically built on the basis of a polymeric matrix polydimethylsiloxane (PDMS) to trap bacteria, using *Escherichia coli* as an example. The authors show that the spatial dynamics in controlled conditions aid in the understanding of microbial interactions and single-microorganism physiology. Besides, Masters-Clark et al.[8] also show the application of a microfluidics platform based on PDMS to study fungal-fungal and fungal-bacterial interactions in a controlled environment. The main differences between the protocols reside in the design of the channels to accommodate the microorganisms, considering that to reproduce soil geometry and to avoid extreme fungal growth in the matrix, the channels must be carefully evaluated in the protocol of Master-Clark et al.[6] , while in the protocol by Pioli et al.[5], the use of colloidal patterning is proposed to calibrate and generate complex multimaterial patterns in the microfluidic matrix design. These studies provide elegant protocols to engineer experiments to model the effective and fundamental ecological niches, which mean the viability of an organism in contact with other organisms in real-life situations and the biotic potential of an organism under ideal conditions to grow, respectively[9].

Another way to characterize the microbial functions of microbiomes, as well as to mimic microbial complexes in laboratory conditions, is by the development of synthetic matrices. In this context, Vieira et al.[10] propose a novel method to simulate the sputa of cystic fibrosis patients, aiming to predict the effects of oxygenation on the microbial composition and clinical outcomes of the affected patients.

Finally, the paper by Berni et al.[15] shows that the application of dedicated image-processing software with specific scripts may be useful to evaluate bacterial and eukaryotic cell-to-cell interactions[11,12,13,14]. Those authors describe a protocol based on a *Salmonella* strain carrying a fluorescence reporter plasmid for describing the bacterial pathogenic behavior in contact with epithelial cells and to show how it accumulates and hyper-replicates in the cytosolic environment.

As guest editors, we are pleased that, through this collection, we have shared very successful protocols to explore the current paradigms and needs of microbial ecology, reminding us that a single method is not enough to access the multitude of information in the microbial world and that it is necessary to use as many protocols as possible to answer ecological questions.

Disclosures

The authors have nothing to disclose.

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