Trends in Microbiology

CellPress

Forum

Microbiome-on-a-Chip: New Frontiers in Plant–Microbiota Research

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An enigmatic concoction of interactions between microbes and hosts takes place below ground, yet the function(s) of the individual components in this complex playground are far from understood. This Forum article highlights how microfluidic – or 'Microbiome-on-a-Chip' – technology could help to shed light on such relationships, opening new frontiers in plant–microbiota research.

Challenging the 'Black Box' Approach

Almost all higher organisms, including plants, insects, and mammals, are colonised by complex microbial communities and harbour a microbiome. One of the most exciting and important scientific discoveries of the last decade is that members of a microbiome can influence host processes, including behaviour, appetite, and health, in animals and contribute to nutrition and health of plants [1]. However, characterisation of the plant microbiome is predominantly carried out on the macroscale, using a 'black box' approach, where a snapshot of microbiome composition is acquired at a singular point in time with a limited appreciation of what is taking place at the cellular level and of how different microbiome members interact. This has been difficult to investigate to date as it is challenging to visualise these dynamic and minuscule interactions.

In recent years it has been demonstrated that so-called microfluidic or 'Lab-on-a-Chip' technology offers several new opportunities to study whole (living) organisms and their interactions [2]. We therefore provide a short introduction to microfluidic technology to raise awareness of this method to the microbiology community and highlight the major advantages associated with the technique for probing plant microbiome dynamics, microbe–microbe and microbe–host interactions. Finally, a number of directions in which Microbiome-on-a-Chip technology could aid research in plant–microbe interactions and microbial ecology are discussed (Figure 1).

Zooming into the Microscale

Microfluidics is the study of systems that manipulate and control small fluid volumes, having internal dimensions most easily measured in micrometers (Box 1) [3]. This technique has a great potential to provide a unique view of biological events at the level of single organisms and cells (i.e., microbe–microbe interactions). Herein, we highlight four major advantages afforded by microfluidic technology that we believe will significantly impact future studies on the plant microbiome. First, precise *environmental control* enables a microbe's environment to be manipulated in a user-defined way. For example, the physical and chemical properties within microchannels can be tuned by using controlled fluid flows and velocity gradients, chemical surface treatments, and defined chemical gradients. The latter feature is especially important in enhancing our understanding of microbial navigation strategies, allowing chemotaxis studies to be performed at the single-cell level and individual bacterial trajectories recorded in response to chemical stimuli (e.g., root exudates and nutrients) [4]. As chemotactic processes play an essential role in defining plant-microbiome community dynamics, for example in the attraction of and communication with beneficial microbes and the defence against pathogens, microfluidic technology therefore offers a means to begin to unravel these dynamics with high spatiotemporal control.

Owing to the confinement of microorganisms within *micro*channels and the optical properties of transparent microfluidic devices made from the elastomeric polymer poly(dimethylsiloxane) (PDMS), *high-resolution dynamic imaging* studies can be conducted. Importantly, this means that single-organ, single-cell and subcellular studies can be performed using a range of optical microscopies. Time-lapse studies, cell-tracking and automated image analysis, for example, allow the dynamic responses of microorganisms to be followed with high spatiotemporal resolution. Such multidimensional image acquisition also means that *quantitative information* can be extracted, allowing the experimenter to assess phenotypes and begin to unravel the nature of the interactions between microorganisms, hosts, and one other [5]. This can, for example, be used to link microbiota dynamics with plant immune response and plant nutrition [6].

The soil environment is extremely complex, being highly heterogeneous and dynamic in nature in terms of its physical, chemical, and biological properties. *Simulating environmental complexity and diversity* under laboratory conditions has therefore presented many challenges. Microbiome-on-a-Chip technology will enable experiments to be conducted in increasingly complex environments, facilitating multiplexed treatments, the application of localised stimuli (e.g., root exudates), confinement of organisms to specific regions, and more accurate representations of the three-dimensional soil structure for example. There remain many challenges ahead, including difficulties associated with studying thicker roots and the possibility that specialised bacteria are unable to grow under the controlled conditions present in these devices. However, a bottom-up

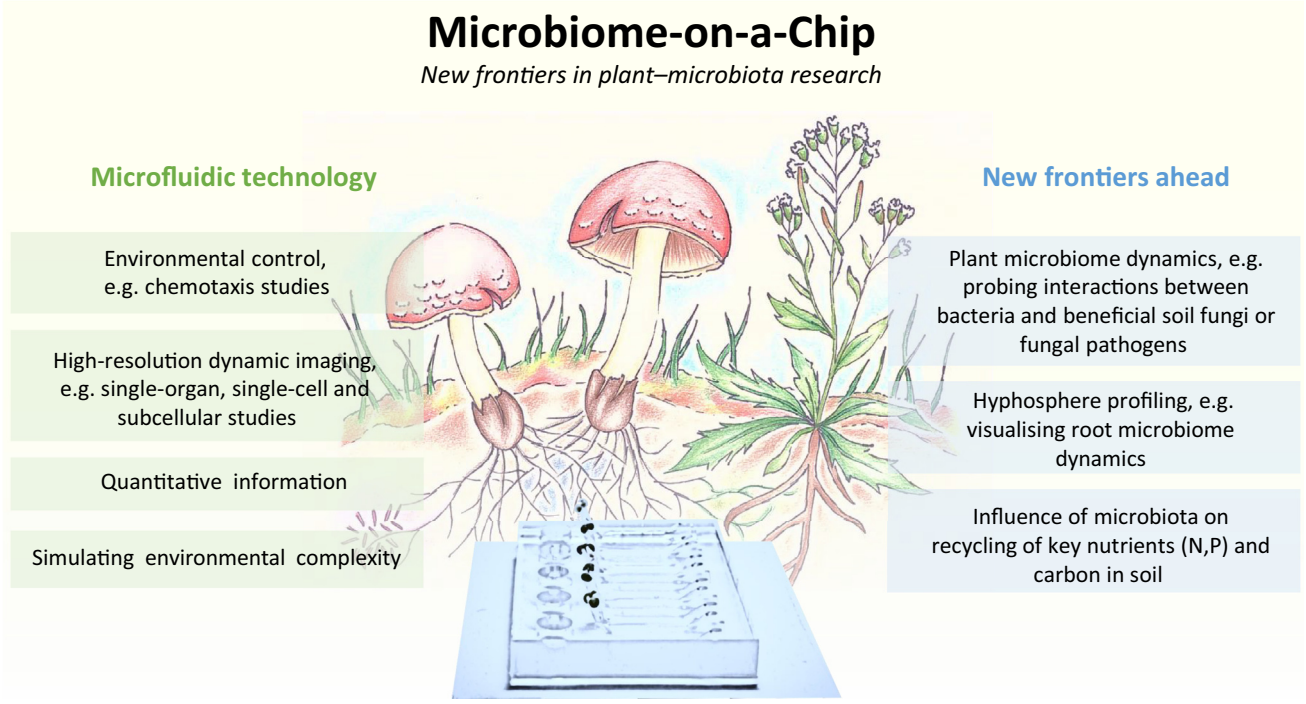

Figure 1. Microbiome-on-a-Chip. The central cartoon illustrates the principle of 'Microbiome-on-a-Chip', the use of microfluidic technology to probe plant microbiome dynamics and interactions. The cartoon is intended purely for illustrative purposes. *Left side*. Microfluidic technology. Four major advantages associated with the application of microfluidic technology for the study of plant–microbiota interactions (i.e., microbe–microbe and microbe–host interactions) have been highlighted. Precise *environmental control*, that is, by tuning the physical and chemical properties within a microchannel, provides a variety of desirable features that can be exploited. For example, defined chemical gradients can be created and used to perform chemotaxis studies at the single-cell level [4]. The confinement of microorganisms within micrometer-sized channels allows *high-resolution dynamic imaging* studies. This allows single-organ, single-cell and subcellular studies to be conducted with ease using a range of optical microscopies. *Quantitative information* can be easily extracted and used to assess phenotypes. For example, the bacterial–fungal interaction device enables growth rates of individual hyphae to be extracted and assessed, whereas traditional bacterial–fungal interaction assays on plates afford only qualitative information [5]. *Simulating environmental complexity* using *in vitro* systems presents many challenges. Microbiome-on-a-Chip technology will enable experiments to be conducted in increasingly complex environments, thus better mimicking the natural environment and allowing future studies to decipher the influence of environmental biodiversity on key biological processes. *Right side*. New frontiers ahead. It is envisaged that the application of microfluidic technology to this field will greatly impact the study of *plant microbiome dynamics*, allowing interactions between bacteria and beneficial soil fungi to be visualised and the underlying mechanisms investigated at the cellular level. Such interactions are critical in helping plants to acquire limited resources [12]. We envisage that microfluidic technology will aid *hyphosphere profiling* to visualise the spatial distribution and activities of different types of bacteria on fungal hyphae. Finally, there exists a great potential to use such tools for investigating the *influence of microbiota on recycling of key nutrients (N,P) and carbon in soil*, for example in deciphering whether bacteria associated with hyphae are responsible for the beneficial effects of mycorrhizal fungi on plants.

approach will yield important information on how environmental diversity influences physiological and developmental processes and complement current methods [7].

New Frontiers Ahead

Plant roots form symbiotic associations with mycorrhizal fungi, soil fungi that aid key processes in plant nutrition and participate in one of the most agriculturally important symbioses [8]. Arbuscular mycorrhizal (AM) fungi extend into the soil volume, providing opportunities for further interaction with bacteria and other fungi. A new frontier is to use microfluidic technology to investigate *plant–mycorrhiza–microbiome dynamics*, allowing interactions between bacteria and beneficial soil fungi to be visualised and the underlying mechanisms to be investigated at the cellular level. We know largely what is contained within the 'black box' under specific experimental conditions, but how these microorganisms perceive, interact with, and respond to one another, as well as the function of these individual components, is not fully understood. *RootChip* technology (Box 1) [9] provides a means to cultivate plant roots within microchannels and uses an on-chip perfusion system for supplying pulsed treatments to the roots. It is envisaged that such a device could be adapted to investigate tripartite systems involving plant roots, fungi, and bacteria, enabling microbes to be cocultured and localised within the vicinity of plant roots, as well as to facilitate localised stimulation of plant roots and microorganisms, simulation of environmental complexity, and cultivation of plants other than Arabidopsis. The