Forum

Synthetic plant microbiota challenges in nonmodel species

Francesca Vaccaro,[1]
Lisa Cangioli,[1]
Alessio Mengoni ,[1,*] and
Camilla Fagorzi[1]

Plant-associated microbiota are becoming central in the development of ways to improve plant productivity and health. However, most research has focussed mainly on a few model plant species. It is essential to translate discoveries to the many nonmodel crops, allowing the design and application of effective synthetic microbiota.

Opportunities from synthetic microbial communities in sustainable agriculture

Recent studies estimate that the world population will reach 9.8 billion people by 2050. To meet the increasing demand for food, feed, and bioenergy, agricultural production should increase by 70% but the use of artificial fertilizers, such as synthetic nitrogen, is energy-intensive and is not environmentally sustainable [1]. A very innovative and ecofriendly strategy to face the agricultural challenges is represented by the naturally occurring microbial systems [2]. Microbial communities are ubiquitous in the environment and they have a pivotal role in the production of foods, the recycling of micronutrients, and in maintaining the health of humans, animals, and plants. Plant-associated microbes represent an enormous potential because of their plant-growth-promoting (PGP) traits. Thanks to the recent advances in 'omics technologies, and to the innovative computational tools, it has been possible to systematically explore and discover novel gene functions in microbiomes and identify and characterize biosynthetic gene clusters to produce secondary metabolites [3]. The exploration of plant microbiomes is expected to increase the exploitation of these gene functions. Biocontrol, biofertilization, and biostimulation can now be engineered [2].

Conventional inoculants cannot establish durable and efficient associations with plants under field conditions, leading to unsatisfactory results [4]. A number of strategies, from seed coating to using carriers, are on the market, but in many cases they still rely on empirical evidence [5]. The assessment of synthetic microbial communities (i.e., phytomicrobiome engineering, when talking about crop productivity) allows us to overcome this limitation by allocating functional components between two or more subpopulations of cells by means of the rational engineering of living organisms. Additionally, such synthetic communities are also a tool for discovering plant–microbe interactions and to predict the host–plant phenotype.

Prioritizing nonmodel plants

Moving from laboratory-scale evidence of synthetic microbial communities to field application is one of the greatest challenges of microbiome studies, on both the animal (human) and plant levels [6]. Concerning the plant microbiome, model plant species have been deeply studied and an understanding of the functions and interaction between the plant and the microbes is becoming more and more clear [7]. However, some model species, such as *Arabidopsis*, can have limitations, due for instance to the short life span (i.e., which does not extend to an entire year or several years, possibly limiting the knowledge about seasonal dynamics of the microbiota) or to the lack of interactions with a relevant portion of the soil microbiota (e.g., arbuscular mycorrhizal fungi, with which Brassicaceae do not form symbiosis [8]). The work on cereals – such as maize, rice, wheat, and barley – has shown the impact of domestication on the root-associated microbial communities [9], indicating that varieties and wild nonmodel relatives offer the opportunity to investigate the evolutionary interactions between microbes and plants.

It is becoming clear that we still do not know much about the peculiarities of the plant microbiota with respect to plant species, especially those of relevant use in agriculture, but still neglected as models in laboratory research. In a tentative list of plant species, we may include crops, such as several forage and grain legumes (e.g., alfalfa, chickpea, lentil), landraces of cereals (e.g., rice, maize, and wheat), which can have relatives as model species (such as *Medicago truncatula* for alfalfa) but which hold their own specific features (such as peculiar structure and physiology). Relevant examples emerged a few years ago from landraces of maize [10], and from experiments with field varieties and nonlaboratory bacterial strains in forage legumes (*Medicago sativa*, alfalfa) [11] (Box 1). These examples reflect the relevance and opportunities for investigation of nonmodel species in plant–microbe interaction studies in terms of microbial biodiversity discovery and importance of metabolic and signaling complexity for plant colonization. Moreover, tetraploid alfalfa and local wheat germplasm may also offer the opportunity to investigate basic biology phenomena, such as the effect that polyploidization and hybridization have on the recruitment of plant-associated microbiota and on the plant holobiont phenotypic plasticity. Such knowledge can be key for plant-breeding programs, focused on novel varieties with improved yield, higher resilience, and less nutritional requirement than current ones.

Ideally, research priorities should be given to considering plant germplasm for which close relatives, such as model species, exist, in order to allow an easier translation

> **Box 1. Examples of microbiological studies on nonmodel plants**
>
> The extensive investigations on legume–rhizobia and cereal model systems have paved the way to the development of laboratory and computational methods for establishing mechanistic relationships between plant and microbiota. Such methods, once translated to nonmodel plant species, will allow us to reduce the gap between the laboratory and the field. Examples are studies on the model legume *M. truncatula*, easily translated to the crop alfalfa (*M. sativa*). Here, the use of local varieties shed light on the existence of genotypic interactions that influence the phenotypic traits of the symbiosis [11]. In particular, a large fraction of microbial gene expression in the early symbiotic steps was influenced by the genotype of the variety of host plant tested (16%), by the strain genotype (35%), and by strain-by-host plant genotype interactions (29%), indicating that alfalfa can largely benefit from breeding programs taking into account the selectivity of the symbiotic rhizobia recruitment.
>
> Shifting from the single-strain interaction model to microbiomes, comparison studies between rhizospheric microbiomes of modern model crops, wild relatives and landraces of barley (*Hordeum vulgare*) represent an opportunity to investigate the structure and the evolution of the microbiota and are ultimately critical to design synthetic communities [9].
>
> Because of their economic interest, diazotrophic microbial associations with cereals arouse great interest. In the case of indigenous maize landraces, biological nitrogen fixation is supported by a microbiota found in the mucilage secreted by an aerial root system. This example highlights the importance of research on nonmodel systems [10]. In this case, the fact that other cereal crops produce these secretions from underground and aerial roots could allow the transfer of this trait by breeding.

of methods and knowledge gained from such model species to the nonmodel germplasm. However, in a world increasingly more subjected to climate change, reliance on a limited number of crops raises questions on food and nutritional security. Therefore, the knowledge on a few model plants and crops needs to be translated to several nonmodel crops, which are generally more resilient to environmental stresses but which suffer from the lack of information on associated microbes, which can assist and promote yield, resistance, and nutrition in a sustainable agriculture scenario. Underutilized and climate-resilient crops – such as the Chenopodiaceae *Amaranthus* spp., and *Chenopodium quinoa* (quinoa) – but also cereals such as *Eragrostis tef* (teff) and *Setaria italica* (foxtail millet), are local sources of food, characterized by high biological value protein content (https://cropsforthefutureuk.org/), which can largely benefit from nitrogen-fixing synthetic microbiota applications to increase their yield and nutritional value with low-input agricultural management. Prioritizing microbiological investigations on such nonmodels, using the methods and knowledge learned from model species, is urgently needed for securing future food security.

Translating from models to nonmodels

Systems biology has emerged as an effective top-down approach for designing, predicting, and manipulating the behavior of microbial populations. This approach allows us to rationally assemble microbial consortia and gives the opportunity to provide an initial framework to predict how the application of synthetic microbial communities can affect nonmodel crop health and yield. Complex biological phenomena, such as the behavior of microbial consortia, can be simulated by (i) dynamic computational models that can predict how the community will respond, given a parameter, (ii) stoichiometric models, such as the flux balance analysis (FBA) that allows us to simulate metabolic networks, based on genome-scale reconstructions (GENRE) and stoichiometric coefficients of every single cellular reaction, or (iii) agent-based models to simulate the behaviors of consortia over time [12].

Bacterial genome-wide association analysis (GWAS) is another tool to define genetic determinants related to phenotypic traits of interest, and a model of epistasis

can be applied to define higher order interactions among strains and between strains and host plant [13].

Once a hypothetical community is assembled, these modeling approaches are able to predict growth capability, intracellular reactions, metabolic phenotypes, competition or synergism, based on molecular crosstalk [4]. Moreover, not only is it possible to model community metabolic networks, but also to develop a holistic *in silico* representation of the integrated metabolism of the holobiont as proposed few years ago for *Medicago truncatula–Sinorhizobium meliloti* symbiosis [14]. Finally, the ideal synthetic microbial community, once inoculated in the soil, should grow and persist in the presence of the indigenous microbiome and local environmental conditions. As any community, it will be influenced by four high-level processes, namely (i) selection, (ii) dispersal, (iii) drift, and (iv) speciation. Integrating community ecology theory with systems biology into plant microbiome research is needed to develop a more holistic and mechanistic synthesis in plant microbiome research [15].

Such technical approaches and interpretative models are very relevant for translating knowledge gained on controlled (and often simplified) plant model systems to nonmodel species (Figure 1). Years ago research priorities were identified [16] for addressing key questions related to the improvement of yield and stress resistance in crops. The identification of core microbiota and the recognition of the cultivar-specific response to microbiota are key to the bioinoculant preparation, as well as a precise understanding of strain dynamics, compatibility, and persistence in the synthetic community. Here, a possible limiting factor can be found in the culturability of strains of the microbiota, which consequently affect their investigation and use. Strategies of culturomics, by growing microbes on plant-derived substrates, but also by growing as consortia 'per se'
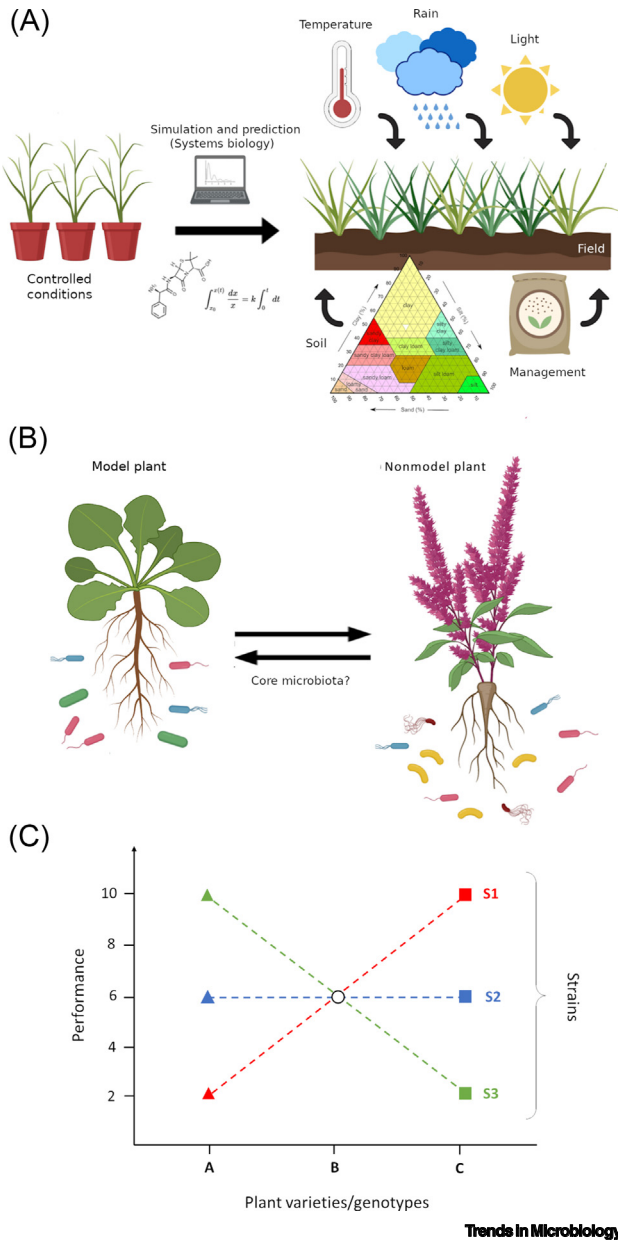
Figure 1. Key points and questions on translating knowledge of microbiota from model to nonmodel plants. (A) In the translation of acquired knowledge on the microbiota of model plants to nonmodel plants it is necessary to take into account the impact and synergy, over the bioinoculant, of environmental variables (a classical from-lab-to-the-field problem), including climatic conditions, soil composition, and crop agronomical management. Mathematical modeling and simulations based on systems biology analyses using multi-omics and synthetic microbiota data offer the opportunity to shorten the gap between laboratory studies on model plants to the assessment of microbial inoculant efficacy in the field. (B) To facilitate systems biology predictions, and allow to propose to the market a microbial inoculant with efficacy on a broad panel of plant species, the identification of a core plant microbiota, that is, a number of microbial taxa shared among multiple crops and between model and nonmodel species, needs a special effort. Moreover, studies on model plants have shown that plant performance is dependent on both additive and nonadditive interactions between plant genotype and microbial genotype. Studies deciphering such interactions (C), identifying strains which have a differential impact on plant performance in a genotype-dependent way, are compulsory. An example of genotype × genotype interaction is reported (S1, S3, plant genotype-dependent effect; S2, plant genotype-independent effect).

Declaration of interests

No interests are declared.

[1]Department of Biology, University of Florence, Florence, Italy

*Correspondence:
alessio.mengoni@unifi.it (A. Mengoni).

References

1. Le Mouël, C. and Forslund, A. (2017) How can we feed the world in 2050? A review of the responses from global scenario studies. *Eur. Rev. Agric. Econ.* 44, 541–591
2. Ke, J. *et al.* (2021) Microbiome engineering: synthetic biology of plant-associated microbiomes in sustainable agriculture. *Trends Biotechnol.* 39, 244–261
3. Levy, A. *et al.* (2018) Genomic features of bacterial adaptation to plants. *Nat. Genet.* 50, 138–150
4. de Souza, R.S.C. *et al.* (2020) From microbiome to traits: designing synthetic microbial communities for improved crop resiliency. *Front. Plant Sci.* 11, 1179
5. Vassilev, N. *et al.* (2020) Formulation of microbial inoculants by encapsulation in natural polysaccharides: focus on beneficial properties of carrier additives and derivatives. *Front. Plant Sci.* 11, 1–9 270
6. Sessitsch, A. *et al.* (2019) Microbiome applications from lab to field: facing complexity. *Trends Plant Sci.* 24, 194–198
7. Hou, S. *et al.* (2021) A microbiota–root–shoot circuit favours Arabidopsis growth over defence under suboptimal light. *Nat. Plants* 7, 1078–1092
8. Cosme, M. *et al.* (2018) Non-mycorrhizal plants: the exceptions that prove the rule. *Trends Plant Sci.* 23, 577–587
9. Bulgarelli, D. *et al.* (2015) Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell Host Microbe* 17, 392–403
10. Van Deynze, A. *et al.* (2018) Nitrogen fixation in a landrace of maize is supported by a mucilage-associated diazotrophic microbiota. *PLoS Biol.* 16, e2006352
11. Fagorzi, C. *et al.* (2021) Nonadditive transcriptomic signatures of genotype-by-genotype interactions during the initiation of plant–rhizobium symbiosis. *mSystems* 6, e00974-20
12. McCarty, N.S. and Ledesma-Amaro, R. (2019) Synthetic biology tools to engineer microbial communities for biotechnology. *Trends Biotechnol.* 37, 181–197
13. Ludington, W.B. (2022) Higher-order microbiome interactions and how to find them. *Trends Microbiol.* 30, 618–621
14. diCenzo, G.C. *et al.* (2020) Genome-scale metabolic reconstruction of the symbiosis between a leguminous plant and a nitrogen-fixing bacterium. *Nat. Commun.* 11, 2574
15. Dini-Andreote, F. and Raaijmakers, J.M. (2018) Embracing community ecology in plant microbiome research. *Trends Plant Sci.* 23, 467–469
16. Busby, P.E. *et al.* (2017) Research priorities for harnessing plant microbiomes in sustainable agriculture. *PLoS Biol.* 15, e2001793

and not as pure cultures only, should be implemented to increase the culturable fraction of the microbiota. Finally, the effect of management and environments on plant–microbiome interactions still deserve increased attention. Here, the abovementioned systems biology methods can help translating from the finding on model species (where controlled experiments are available) to nonmodel species and field applications, where statistical methods allow a reduction in number and size of the field trials and may result in a faster delivery to the market.