European Plant Science Organisation
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***Report and recommendations***

# EPSO Workshop on Plants and Microbiomes Vienna, 23.2.2017

*Brussels, 23.3.2017*

## 1. Background

Globally biomass production faces numerous challenges such as the increasing human population requiring 50-75% increased food production by the year 2050. Furthermore, global climate change, increasing temperature and increasing drought scenarios will increasingly challenge agricultural production. At the same time high quality land resources allowing crop production decrease in many areas worldwide. Sufficient food quantities are required, but also the production of food of high nutritional quality with minimal or no chemical, allergen or toxin concentrations has to be feasible. Overall, this requires sustainable agricultural production with minimal use of chemical inputs.

In the last century food production has substantially improved through breeding of plant lines with desirable traits such as high yields, nutritional quality, pest or pathogen resistance and improved tolerance to abiotic stress. However, although modern breeding has dramatically improved intensive crop production, global crop production still relies heavily on external inputs such as pesticides or inorganic fertilizers. Crop production faces yet unsolved challenges such as extreme weather events including reduced water availability or emerging pests or pathogens. Globally, there is the ambition to introduce more sustainable agricultural practices to ensure human and environmental safety and to protect important resources needed to ensure food security on the long term.

Similar to the human gut plants are tightly associated with complex microbiota, which play an important role in plant health and nutrition. Few examples of beneficial plant–microbe interactions are well investigated and explored with regard to their importance in agricultural systems. These include biological nitrogen fixation by rhizobia, which establish a symbiosis with legumes and represent the basis of crop rotations including legumes contributing to the maintenance of soil fertility. Furthermore, about 80% of land plant species are internally colonized by arbuscular mycorrhizal fungi. In this symbiosis, arbuscules and vesicles are formed from the hyphae being particularly important for plant nutrient acquisition. In addition, the more specialized symbiotic defensive mutualism between Pööideae grasses and endophytic fungi of the Epichloë is well explored and important for pasture production. Apart from these well-known mutualistic plant–microbe interactions, plant microbiota have been hardly considered in crop production strategies. However, considering the demonstrated functional importance of the plant microbiome, the effects that can be observed upon the inoculation of selected microorganisms and the fact that plants and microorganisms carry genetic determinants needed for their interaction, it can be predicted that plant microbiome functions have the potential to enable new approaches in tomorrow's crop production.

In February, 2017, the newly established EPSO Working Group 'Plants and Microbiomes' launched a workshop with the participation of 38 experts from academia and industry from Austria, Belgium, Denmark, Finland, France, Germany, Israel, Italy, Spain and The Netherlands. The aims of the workshop were to: i) elaborate the R&D needs additional to those defined in previous EU workshops; ii) discuss suitable reference plant systems for Europe and microbiome aspects to be addressed; iii) elaborate ways to provide innovations for the industry and to promote sustainable crop production; iv) elaborate technological

challenges to be addressed to advance microbiome understanding; and v) to discuss governance and regulatory issues and international cooperation. Generally, the high potential of the plant microbiome for new innovations in improving crop production was acknowledged. Due to the high potential major ag-companies as well as many SMEs, particularly in the US but also increasingly in Europe, have started to invest in plant microbiome R&D. However, as plant microbiome research is a rather young and highly complex field of research, the overall aim of this workshop was to provide recommendations advancing the understanding and in particular the implementation in applicable solutions.

## 2. Recommendations

***Main message:***
***1-Diverse crops with diverse microbiomes for diverse diets for human and animal health and resilient production systems***

***2-The term plant microbiome / plant microbiota comprises all microorganisms being associated with the plant including human/animal/plant pathogens***
There is some controversy how to interpret the term plant microbiome. The participants clearly recommend to include all microorganisms (fungi, bacteria, archaea, viruses, protists) living at least part of their time in a sphere, which is influenced by the plant (i.e. rhizosphere, endosphere, phyllosphere etc.), whereby different plant compartment and tissues host distinct microbiota. Beyond this, the insects should be conserved as well which form together with the plant microbiome the plant holobiome.

***3-Moving from correlation to causation under lab, greenhouse and field conditions***
We currently base our understanding mostly on correlations (e.g. microbiome diversity with certain plant traits or functions), and need additional understanding on causality. Therefore we need to develop and utilize a 4 component system (synthetic communities + gnotobiotic plants + defined cultivation matrix + defined growth conditions) for the main model crops in Europe to change only one component and trace its impact.
This has to be applied stepwise at laboratory, greenhouse and open field conditions.
Genetics and thus the use of genetic modified organisms in research are important to increase mechanistic understanding. Performing field experiments with such GMOs for solely experimental purposes would support efforts to advance our understanding of plant-microbe interactions under non-artificial conditions.
In particular, the following topics need further understanding: i) functions of the microbiome and interactions among microbiota members including aspects such as signal exchange, ii) plants responses to microbiota, iii) functional aspects, including plant nutrition N, P, of the microbiome and iv) linking between the microbiome and plant traits. Two additional aspects are becoming more important to investigate: The role of epigenetics within the plant microbiome and in plant-associated pathogens/beneficials, and the mobile elements of microbes such as plasmids and transposons (called the mobilome), which spread resistance and are of special interest for plant and human health.

***4-More understanding on the complexity of the ecosystem-plant-microbiome system is needed***
Finally, we have to take the ecosystem complexity into consideration. Ultimately, microbiome understanding will lead to new aspects to be integrated in precision farming.
Multidisciplinary research is needed to understand the relevance of plant microbiomes in an ecosystem context. (Plant) Microbiomes are highly complex and we have to understand ecology and functioning at a multi-trophic level. This includes also a holobiont approach considering all organisms interacting as one biont and aspects such as parallel evolution, adaptation and transmission routes are to be considered.
The topics for understanding equal those at lab and field approach (see previous point).

***5-Plant mechanisms to interact with microbiota require understanding***

Limited understanding exists on how plants respond to beneficial microorganisms besides few well investigated examples such as rhizobia or mycorrhizae. The identification of plant (genetic) markers correlating with a beneficial plant response or being responsible for the interaction with specific microorganisms could open new avenues for plant breeding.

***6-Proposed reference plants for Europe include barley, potato, tomato, pea and strawberry***

As model crops barley (for cereals, monocots), potato (dicots), tomato (for vegetables), pea (for legumes) and strawberry (for fruits) are proposed as they all are agronomic relevant in Europe. Tomato and barley are model crops, which are already in use in plant research. It should be considered that particularly for the understanding of mechanisms it is important that mutants and breeding lines are available and that research with well-established model plants such as *Arabidopsis* and *Medicago* should be further pursued.

***7-Precompetitive research should address the identification of microbiome-based plant health and resilience indicators and microbiome understanding needed by the industry***

The industry is interested to provide solutions in form of microbial products for sustainable crop production. However, the question remains if such solutions can be provided at local or global scales. Since abiotic factors, edaphic factors and crop species can greatly influence the microbiome, it is important to define a 'healthy microbiome', which necessitates the identification of plant health indicators. It is also important to identify if a 'core microbiome' or 'key species in the microbiome' is associated with healthy plants. This requires in depth investigation of crop-plant microbiomes as it is done in the human microbiome project. This type of research will lead to the identification of diverse sets of microbiomes as resilience indicators. This will contribute to the goal of diverse crops with diverse microbiomes.

***8-More understanding is needed to understand the interaction of plants (e.g. secondary metabolites) with the animal and human microbiomes, which is likely to affect animal/human health***

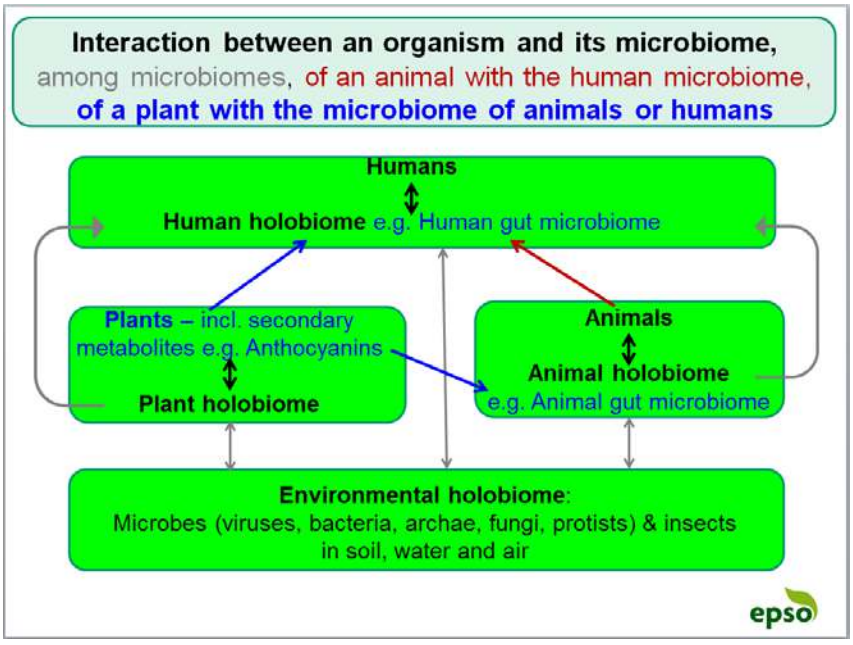


*Figure 1: Various interactions between organisms and microbiomes and among microbiomes*

In addition to the direct effects between the plant microbiome (e.g. endophytic microorganism) on the human gut microbiome, there are crucial indirect effects through the plants: plant metabolites (bioactives produced by the plant and /or by microbial endophytes living within the plant), and effects deriving from the interaction between plants and microbes, influence animal/human microbiomes and thereby impact human/animal health. To better understand the interaction of plant metabolites with the human microbiome we will have to consider two perspectives: (i) Identification of derived colonic metabolites with potential

health benefits and (ii) The role of plant metabolites in animal / human microbiota modulation as a possible mechanism by which they may exert their effect. These are crucial to support the application of plant metabolites in the human diet and will be a component of clinical trials to further assess their bio-kinetics.
In addition, we can apply concepts, basic understanding and methodologies to different microbiomes.

***9-Plants may host human/animal pathogens, which should be considered in applications as well as in food safety assessment***
Microorganisms belonging to pathogenic phyla or being at least related to human pathogens are commonly found in plant environments. A better understanding on the ecology of such pathogens in agricultural systems is needed to warrant food safety from plant produce.

***10-International (beyond Europe) cooperation is highly recommended***
International cooperation avoids research duplication and increases impact from funding coming from different countries, thereby allowing effective use of public funding. Sharing of databases and culture collections (comprising not only single strains but also consortia) as well as experiments, protocols, standardized procedures, testing environments would help make results more widely understandable and usable.
In addition, research collaborations across countries would allow testing and exchange of microbiomes across geo-climatic zones, leading to diverse global agricultural systems. Similarly to sharing plant genetic resources, for Australian crop microbiomes may benefit southern European systems by providing crop-microbiome adaptations.

***11-Early and wide communication of plant and microbiome science and applications is recommended***
To avoid experiences made e.g. with the public perception of GM plants or synthetic biology, it is recommended to address stakeholders at the local level and communicate clear and balanced messages, possibly formed into stories and illustrated by examples. Local stakeholders should include the public, NGOs, farming communities including organic and conservation farmers, retailers like SMEs and supermarkets, wholesale buyers, schools and regulators. Communication should also allow the public to see and experience plants and microbiomes, e.g. by including microbes in activities of the EPSO Fascination of Plants Day or in natural sciences museums. It is important to educate the public that most (plant) microbiome members are beneficial and very important for ecosystems and our well-being, but that some microorganisms can be / are pathogenic.

***12-The industry needs personnel trained in classical microbiology and modern microbiome skills***
To improve current university education programmes, we propose to organize an international, interdisciplinary Master programme on plant microbiomes including classical microbiology, soil science, plant physiology, plant molecular biology, microbial ecology and bioinformatics under the Erasmus Plus scheme.

***13-Regulation of microbial products requires improvement to support European bioeconomy and make best use of the plant microbiomes potential***
Current regulatory demands are prohibitive for SMEs to bring microbial products to the market. Regarding biocontrol products (against pests and pathogens), the regulatory process is too long. We recommend focusing the registration on safety and efficacy and introducing a “fast-track” procedure for organisms, which are not registered pathogens. This would support SMEs as often such products have only a limited market potential.
Regarding biofertilisers, currently different regulation requirements exist in different European countries. This I sunder revision and we suggest aiming for one regulation across Europe.
In addition, the regulation for bi-functional microbiome products with biocontrol as well as biofertiliser effects currently requires adhering to regulations for both uses. This prevents such products or they are only marketed for one of the two uses they actually have.

SMEs in the microbiome product development suggest introducing a voluntary code of conduct among companies to state the active ingredient(s) and their proven effects. Currently several poorly defined microbial products are on the market which could cause a negative effect for the entire sector.
Microbiome applications might have various specifics from a regulatory point of view, which should be discussed between scientists and (EU) regulatory bodies. In addition, IP issues such as patenting of genes, which are likely to massively derive from microbiome sequencing, should be discussed as well.

***14-Public programmes should focus on the lower TRLs, leaving the differentiation to companies themselves***

Companies are best to take existing / proven knowledge and technologies closer to the market themselves to differentiate their products from their competitors. Therefore industry suggests that public funding supports development of basic proven knowledge and technologies towards solutions and may join such collaborative projects in the early stages of the research and innovation cycle (lower TRLs). Companies could be partner or member of a user group in such projects to facilitate guidance and early up-take by the industry. Companies will then undertake themselves up-scaling and validation in an economy-based context.
It will be important to apply the holistic and multidisciplinary approach - considering the complex interactions between soil, plant, environment and the microbiome - in the early as well as later stages of R&D to result in microbiome-based discoveries and innovations.
Industry is interested in a short time to market, increasing yield and thereby profit, while academia aims at open access and publication. This has to be discussed to support both sectors at the highest level. Model consortium agreements will be an important tool.

***15-Open access databases integrating (plant) microbiome and meta-data are required***

There is currently a severe lack of capacity in sequence data storage and processing in Europe. This applies mainly to metagenome and other -omics data and related analyses. As research is moving from amplicon-based analyses to metagenomics-based research, the situation is likely to worsen in (the near) future. This includes a current tremendous dependency on US-based open access databases, which are largely overloaded, as well as limited available storage space for the deposition of raw sequence data needed for publication. A solution would be the establishment of a European (plant) microbiome database, enabling data deposition as well as data analyses. Ideally, the database is combined with other microbiome database resources in Europe, enabling utilization of e.g. annotation data from human, animal or environmental metagenomes. This database should be curated, manned and maintained by European resources. Metagenomic (DNA/RNA) based data should be accompanied with continued (accelerated) accumulation of genomic data from well characterized isolates from plant associated bacteria, but also from archaea, fungi, viruses and protists, which are currently underrepresented in the genomic databases. This is important for better and more accurate annotation of meta-omic data in future.

***16-Standards - best practices in plant microbiome research need to be implemented***

Current lack of standards in sequence data (processing/deposition) makes meta-analyses comparing data from different experiments/research groups difficult and inaccurate. There is a need for introducing standards for minimum number of biological replicates, sampling procedures, sample treatment, recording of metadata, analytical pipelines and bioinformatic analysis.

***17-European infrastructure recommended for plant microbiome research***

Infrastructure related to database development and maintenance was considered highly important. The establishment of a European Microbiome Competence Center (similar to the Joint Genome Institute in the US) would highly strengthen European Microbiome science. Such a center, not necessarily limited to plant microbiomes, should unify all aspects related to microbiomics. Such a center would act as a platform to unify and integrate all types of microbiome data. It is recommended to use already existing infrastructures in Europe to build

a true collaborative infrastructural network. Existing plant phenotyping facilities in EU can be well integrated in and are highly useful for plant microbiome research.

The report was written by Angela Sessitsch, Karin Metzlaff, Corné Pieterse and Stijn Spaepen with input from the EPSO workshop participants.

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