# Exploitation of pre-, pro- and postbiotics for organic fruit productions: hopes and reality

E. Malusà<sup>1,2</sup> L. Sas<sup>1</sup> and N. Vassiliev<sup>3</sup>

# Abstract

Plant beneficial microorganisms (PBM) have been designed and thought to improve crop productivity in view of sustainable agriculture and, currently, also as a tool to mitigate increasing environmental and climate concerns. The emphasis of the scientific activity in the field of PBM is now on developing environmentally friendly and efficient microbial formulations taking into consideration also how introduced microorganisms affect the microbial communities, the specific plant-microorganisms interactions as well as the overall impact on biodiversity. However, it is now becoming clearer that an approach combining pro-, pre- and postbiotics should be applied to better exploit both the characteristics of PBM and those of the existing beneficial soil and plant microorganisms, particularly in organic productions. Using prebiotics, i.e. products that can improve microbial diversity by promoting the growth of microorganisms already present within the soil-plant system, together with probiotics, i.e. PBM which present health promoting and nutrient mobilizing properties, can be a strategy that could overcome some of the bottlenecks and limitations currently hampering a wider use of microbial-based products. Moreover, an integrated approach could also foresee the application of postbiotics, metabolic derivatives of PBM which can avoid the risks associated with the application of "alien" microbial cells to the crops. Different issues related to the use of these kinds of plant and soil biostimulants, the interactions with the plant and crop management methods, as well as some regulatory aspects concerning the marketing and field application in organic farming of these products are presented.

Keywords: beneficial microorganisms, biostimulants, biofertilisers, biopesticides.

# Introduction

Microorganisms and their communities (microbiomes) play critical roles in diversification and functioning of all other living organisms (Berg et al. 2020). Many of them play a major role in ecosystem functioning determining soil fertility and provide plant growth promotion and disease suppression (Maron et al., 2018). Plant-microbiome association is known to influence the capacity of a plant to cope with abiotic and biotic stresses and have biological (e.g. on physiology or metabolism) and ecological (e.g. in plant-pest interactions) implications for agricultural production (Berg et al., 2017).

A large number of microorganisms have been isolated, characterized and tested as biofertilizers and biocontrol agents under field conditions in the past 20-30 years. However, the role of soil as a microbiome reservoir opens new perspectives to exploit the microorganisms' potential to address several agronomical and environmental challenges (Blaser et al., 2016). Practical methods for this exploitation would include the application of effective single or multiple microbial inoculants or managing the existing microbiome, as tested by the EXCALIBUR project ("Exploiting the multifunctional potential of belowground biodiversity in the horticultural farming" - <u>www.excaliburproject.eu</u>).

<sup>&</sup>lt;sup>1</sup> National Research Institute of Horticulture, Skierniewice, Poland; <sup>2</sup> CREA-Center for Viticulture and Enology,

Conegliano, Italy; <sup>3</sup> Department of Chemical Engineering, Institute of Biotechnology, University of Granada, Granada, Spain

# Strategies for an effective microbiome management in organic orchards

Strategies for microbial management of soil-plant microbiome can be selected based on the use of prebiotics, probiotics, and postbiotics. Prebiotics are products which improve microbial diversity and functions by promoting the growth of microorganisms already present within the soil-plant system. Organic fertilisers, including those derived from agro-wastes processing (e.g. compost, humus, animal manure, biochar and chitin-based products), ameliorating the soil structure, chemical and biochemical activity can be considered prebiotics due to their positive impact on microbial populations and their diversity (Malusà et al., 2014). Compost and animal manure can be considered synbiotics, as they contain microorganisms that can have beneficial properties or can be additionally inoculated with PBM.

Selected strains of beneficial microorganisms act as prebiotics. They exert plant health promotion and soil nutrients mobilization thriving in the rhizosphere or as endophytes inside the plant or on its phyllosphere (Hardoim et al., 2015). Probiotics must develop a sufficient population size in the soil or plant to exert their beneficial traits. However, this process is highly dependent on the soil characteristics, the environmental conditions and plant genotype. Frequently, a single microorganism cannot reach this critical size, but formulation technologies, either for liquid or solid products, should contribute to high colonizing effectiveness and competitiveness (Malusa and Vassilev, 2014). The application method would define which formulation is the most suitable for a specific use: (i) solid (granular) products should be favoured for mixing with plant growing media (e.g. in nursery production), (ii) liquid or wettable powder formulations are best suited for spray application or through fertigation systems, (iii) "sticky" powders can be used for bare roots treatment (Malusà et al. 2012).

Postbiotics are metabolic derivatives of PBM, which provide growth promotion and/or biocontrol effects on plants avoiding the risks associated with applying microorganisms wrongly formulated or under stressing environmental conditions or the level of plant needs and capacity to attract and feed beneficial microorganism, which can provoke inconsistent field results. However, the application of specific metabolites in soil should be assessed carefully, considering that a great variety of microorganism, involved in a wide number of interrelated cooperative or antagonistic actions, are present in the rhizosphere (Besset-Manzoni et al., 2018).

An example of an integrated strategy and its results on nursery tree growth are presented in the following table.

Table 1. Effect of different prebiotics (manure, humus and plant extracts), probiotics (microbial consortium) and postbiotics (stillage) alone or in associations on 1-year old maiden apple plants cv. Topaz grown in organic nursery.

	Trunk diameter	Tree height	Number of	Total shoots
	(mm)	(cm)	lateral shoots#	length (cm)
Control (not treated)	10.5 a	97 a	3.2 a	17 a
Dry manure	12.9 c	113 b	4.3 b	58 c
Microbial consortium (MC)	12.4 c	106 ab	4.0 b	15 a
Dry manure + MC	14.3 d	118 b	5.5 c	49 b
Humus extract	13.1 d	121 b	3.4 a	61 c
Humus extract + MC	11.4 b	122 b	5.3 c	58 c
Plant extract	11.0 ab	114 b	3.1 a	41 b
Plant extract + MC	10.8 a	111 ab	6.0 c	48 b
Stillage	12.2 c	117 b	3.9 ab	47 b
Stillage + MC	13.9 d	130 c	5.6 c	48 b

#shoots longer than 3 cm; letters in each column indicate differences according to Tukey's test at P ≤0.05

The application of soil microbial inoculants as biofertilizers and biopesticides in agriculture is still limited, but their world-wide market was expected to be worth about 15 Billion USD by 2025 (Kowalska et al. 2020). The multifunctional use of microbial inocula could be exploited to support the marketing and application of microbial products. However, the current legal framework in the European Union on marketing of these products poses serious challenges to such multiple use. Legislation on registration procedures is distinguishing between "biofertilizers" and "biopesticides" effects. The classification has been useful to rank the potential risks for human health associated with the use of these products, clearly higher for biopesticides than for biofertilizers. However, a microorganism cannot be classified for having biofertilizer or biopesticide properties since both can be expressed by the same organisms under different conditions. The genus Bacillus is a typical example of the multifaceted capacity of a microorganism. Therefore, the current classification of microbial products either as biostimulants or as biopesticides is contrasting with the biological reality, and can hamper the development and use of multifunctional microbial products. Even though a revision of procedures is ongoing, the EU registration process for microbial-based pesticides is similar to that of chemical pesticides (Regulation EC 1107/2009), not taking into consideration the characteristics of the biopesticide mechanisms of action (Chandler et al., 2011). Similarly, the new EU Regulation 2019/1009 on fertilising products includes the category microbial biostimulants, but currently foresees only four groups of microorganisms (Azotobacter spp., mycorrhizal fungi, Rhizobium spp., Azospirillum spp.), in contrast with the various genera and species that are recognized as plant growth promoters.

#### **Concluding remarks**

Even though microbial products have been successfully applied in agriculture since the beginning of the last century (the first N-fixing product was marketed in the 1920s), failures still occur under field conditions. There is still a limited knowledge about the impact on their efficacy from pedo-climatic conditions, application methods and dosage, crop management practices and the interactions of the bioinoculant with the native soil microbiota. The knowledge gap requires to be addressed with a vision that considers the complexity of soil and plant microbiomes. To this aim, a parallelism between the human gut microbiome and plant microbiome could be drawn (Adam et al., 2016). Following the human gut microbiome example, the new strategies for exploitation of PBM should be based on an integration of prebiotics, probiotics, synbiotics and postbiotics. Such fully integrated strategy would foster the "One Health-One Environment" approach that is at the heart of both EU Green Deal strategy and UN's Sustainable Development Goals.

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