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Selection of endophytic bacterial communities, genes and secondary metabolites for the sustainable cultivation of tomatoes in greenhouse

PhD Thesis

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Abstract

This thesis project aimed to acquire the bases for biotechnological interventions on the tomato microbiome in order to improve growing conditions of the crop its resistance and resilience to stresses and the characteristics that lead to the formation of quality itself in the perspective of sustainable agriculture focused to the protection of the environment and the consumer. Tomato belongs to the Solanaceae family and is the second most important fruit or vegetable crop next to potato. The intensive management required to mitigate serious economic losses, has encouraged the search of alternative approaches for the control of tomato diseases, including the use of biological control agents. In our model study we used different approaches to study microbial communities of tomato root compartments in a real-word environment represented by an intensive tomato cultivation area characterized by extra-seasonal productions in the greenhouse. A metagenomics approach by amplicon sequencing was used for a comprehensive and systematic evaluation of the community structure and composition related to the formation and composition of the bacterial and fungal tomato communities in commercial conditions from nursery production materials to greenhouse commercial materials. The analysis revealed substantial differences in the composition and the assembly of microbial communities in tomatoes grown in agricultural soil or in cultivation outside soil using a coconut fibre substrate, even if they come from the same batch of seeds and seedlings nursery here also analysed. In particular, microbial communities of soilless grown plants appeared to be affected by bacterial communities formed in the nursery, while plants grown in agricultural soil in a short time have been affected by it. Although in recent years the advances in next-generation omics technologies have led to the possibility of revealing plant-associated microbiomes, culture-dependent methods are still necessary to bioprospect natural diversity as a source of new tools for sustainable agriculture. The cultivation approach was therefore applied to explore and compare the natural biodiversity of tomato root-associated bacterial communities in farms with plants grown in agricultural soil and form the endosphere from seeds and roots of plants at different stage of growth and grown in different cultivation systems (i.e. the samples analysed with the metagenomic approach). In both experiment a phenotyping scheme based on the analysis of traits correlated Plant Growth Promotion and Biocontrol activity was applied. More than 500 characterized bacterial isolates were analysed and constitute a biobank for further work. More than 100 strains were assigned to a known taxon by 16S rRNA gene (rDNA) sequencing. *In planta* activity against phytopathogenic bacteria of a subset of *Bacillus* and *Pseudomonas* isolates was also assessed. The draft genome sequences of two *Pseudomonas* strains were also obtained.

Keywords and Abbreviations

Key words: Microbiome, PGPR, BCAs, Tomato, Endophytes.

List of Abbreviations

PGPR Plant Growth Promoting Rhizobacteria

BCAs Biological control agents

PCR Polymerase Chain Reaction

qPCR Quantitative Polymerase Chain Reaction

BLAST Basic Local Alignment Search Tool

BLASTN Nucleotide BLAST

bp Base pairs

HCN Hydrogen cyanide

ACC deaminase 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase

DI Disease index

dpi days post inoculation

OTUs Operation taxonomic units

Cmm Clavibacter michiganensis subsp. *michiganensis*

Pco Pseudomonas corrugata

Pto Pseudomonas syringae pv. *tomato*

Xep Xanthomonas euvesicatoria pv. *perforans*

Fol Fusarium oxysporum f. sp. *lycopersici*

Bot Botrytis cinerea

NGS Next Generation Sequencing

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1. Introduction and Aim of the Study

The quality and safety of food and raw materials has received increasing attention from consumers and producers over the past decade as they play an important role in preventing disease and promoting well-being of the population. According to the study 'Tomorrow's healthy society research priorities for foods and diets' account, promoted by the European Commission to identify the priority research areas in the Horizon 2020 programme, this attention will increasingly play a role socially and economically crucial to the impact on health systems, the ageing of the population and the high individual and economic costs of disease.

At the same time, the awareness of environmental protection and the use of sustainable production methods have grown. This involved a review of the technical means for production (fertilizers, agro-pharmaceuticals), a development of biological cultivation methods and a re-orientation of production lines towards formulations based on the properties of microorganisms such as biostimulants, biofertilizers and bioagropharmaceuticals.

It is therefore necessary to "redesign" innovative production methods, based on the new knowledge that biology and molecular technologies offer. Research in this field has had a rapid rise thanks to the overcoming of the technological limits of classical microbiology through the next generation sequencing platforms that allow the simultaneous study of communities of microorganisms (metagenomics) high-resolution. Enriching the plant, or the environment where it lives, of "good" bacteria through bioinocula is an idea that is becoming increasingly important in the scientific and agricultural community. Not for nothing is a now common thought that "Understanding the correlation between the structure of microbial communities and their function is the great challenge of the new decade".

This thesis project, funded by the Italian Ministry of University and Research under the call *PON* FSE-FESR R&I 2014-20 '*Dottorati innovativi con caratterizzazione industriale*', aimed to acquire the bases for biotechnological interventions on the microbiome of tomato in order to improve growing conditions of the crop, its resistance and resilience to stresses, and the characteristics that lead to the formation of quality itself in the perspective of sustainable agriculture focused to the protection of the environment and the consumer.

Tomato crop in protected cultivation was selected as: *i)* it occupies an important position for the economy of the country and Sicily; *ii)* presents a high degree of intensification of production processes which frequently undergoes stress conditions; *iii)* the crop is the subject of numerous molecular researches and many useful data are available to complete the research in a relatively short time.

The project is in accordance with the *National Strategy of Intelligent Specialization* (SNISI), in the area *Health, Nutrition, Quality of Life*. In particular, with the objectives of agriculture of the future that emphasize the issues relating to organic production due to the lesser impact they have on the ecosystem. Objectives pursued through the development and application of biotechnologies, key enabling technology (KET) of an intelligent specialization.

The High-Throughput Sequencing Technologies (Next Generation Sequencing) have changed the 'feeling' of the plant from an independent entity to a holobiont or "super organism" integrated with the associated microorganisms (microbiota) and their genetic information (microbiome). In fact, it is possible to study the microbiome of plants and detect its fluctuation in relation to endogenous or exogenous factors, helping to provide the basis for understanding the more complex interactions (Hardoim *et al.* 2015; Vandenkoornhuyse *et al.*, 2015). Microorganisms, in particular endophytic ones, are seen as a reservoir of additional genes and functions for their hosts and as an opportunity to be investigated and exploited for the beneficial effects they can provide (Berg and Smalla, 2009; Gaiero *et al.,* 2013). The scientific community, operators, and producers of technical means for agriculture show a growing interest in their use to promote plant growth, contain plant parasites and pathogens, mitigate damage from abiotic stress and in bioremediation (Mercado-Blanco and Lugtenberg, 2014)

The specific objectives of the project refer to the selection of bacteria capable of endophytically colonizing tomato plants, which act as biostimulants, biofertilisers and/or biological control agents and the development of a model system for the NGS analysis of the traceability and profile of tomato plant microbiome, from seed to cultivation in soil and soilless.

The project includes workpackages (WPs) that were implemented with minor changes in relation to interim results and the COVID-19 pandemic.

Results presented in this dissertation are organized in 4 different chapters:

- State of the art on microbiome studies with special emphasis to studies on tomato microbiome;

- *Bioprospecting of beneficial bacteria traits associated with tomato root in greenhouse environment reveals that sampling sites impact more than the root compartment.* (Published: 13 April 2021, in *Frontiers Plant Science* 12, doi: 10.3389/fpls.2021.637582) this manuscript includes results dealing with WP2 and 3.
- *Assembly and evolution of microbial community on soil and soilless grown tomato, from the nursery to the greenhouse* accomplishing WP1 and 3 (results will be submitted for publication);
- *Draft genome sequences of Pseudomonas citronellolis f1 and Pseudomonas sp. 172 isolated from tomato* planned in WP4 (results will be published as genome announcement to make public the data obtained).

1.1. The plant microbiome

Decades of research have demonstrated that cooperative microbial symbionts play an important role in their host's life and fitness (Kiers and Van Der Heijden, 2006), as shown by the difficulty of culturing transplants of different species in the absence of bacteria and fungi (Hardoim *et al.*, 2008) and the role of microbial communities in plant development and health throughout the entire life cycle (Mendes *et al.*, 2011; Philippot *et al.*, 2013).

The microbial community associated with the plants (inside the tissues, on the surface of the organs or adjacent to them), is very wide and extends from prokaryotes to eukaryotes which, together with viruses and viroids, may have beneficial, neutral or adverse effects on the plant (Hardoim *et al.*, 2008). All the microorganisms (fungi, bacteria), and smaller biological entities (e.g. viruses), that live or can live on the plant, in its immediate surroundings or within it, constitute the microbiome, while the set of insects, mites, nematodes, and other invertebrates or parasites that live in association or at the expense of the host represents the macrobiome (Lorito and Scala, 2012).

The microbiome is involved in multiple plant functions, from nutrition to resistance to biotic and abiotic factors. So, the global performance of a plant is the direct consequence of its genetic makeup associated with its microbiota, or the set of microorganisms as taxonomic units (Vandenkoornhuyse *et al.*, 2015). The study of the plant microorganism interactions and the several factors in the assembly of the microbial community led to

understanding of the plant as a meta-organism, that benefit from their "microbial partners" (Vorholt, 2012). This has modified the "feeling" of the plant as an independent entity in the most recent description classification of the “holobiont”, or a "super organism" integrated with its associated microorganisms (microbiota) and their genetic information (often referred to as microbiome) (Vandenkoornhuyse *et al.*, 2015). The concept of “holobiont”, also called plant microbiota, involves a collective view of the functions and interactions occurring between the host macroorganism and its associated microorganisms (i.e., a single dynamic entity). While ‘*the genomic reflection of the complex network of symbiotic interactions that link an individual of a given taxon with its associated microbiome*’ is named holobiome (Guerrero *et al.*, 2013; Vandenkoornhuyse *et al.*, 2015).

This revolutionary advance in biological sciences has redirected plant research towards a more holistic view (Berg *et al.*, 2016), based on the symbiotic relationships that microorganisms and plants have developed to adapt to environmental changes. Microorganisms promote plant growth by enhancing nutrient bioavailability, suppressing plant pathogens, and increasing plant tolerance to abiotic stress factors such as drought and salinity (Yang *et al.*, 2009; Berendsen *et al.*, 2012). In return, plants provide carbon sources, including a wide variety of sugars, amino acids, and secondary metabolites, via root exudates and tissue debris (Bais *et al.*, 2004; Philippot *et al.*, 2013). Understanding the intricate relationship between microbiota and host plants will improve our ability to harness these activities for increased crop productivity.

1.1.1. Microbial niches in plants

Three main areas of plant-microbiome interaction could be differentiated: the phytosphere, the rhizosphere, and the endosphere (Turner *et al.*, 2013; Andreote *et al.*, 2014) (Figure 1).

The aerial portions of the plant, in its generality named phytosphere, are commonly colonized by a multitude of microorganisms widely different. The microorganisms living on them, called "epiphytes" are distributed on leaves, fruits, and flowers. Therefore, the specific areas they colonize are further classified in: phyllosphere, carposphere and antosphere. Although some investigations have been carried out on the epiphytes of buds and flowers, most of the phytosphere studies focus on the leaves, therefore on the phyllosphere. The leaf surface is considered an unsuitable habitat for many

microorganisms. This is related not only to poverty in terms of concentration and composition of nutrients, that sustain microbial growth, but also to the dynamism of the abiotic conditions to which it is exposed, namely temperature, humidity, radiations, which together make the leaf an environment that is not suitable for the growth (Lindow and Brandl, 2003). The microbial communities that inhabit plant leaves are mainly characterized by bacteria, followed by filamentous fungi and yeasts, without excluding algae and, in rare cases, also protozoa (Lindow and Brandl, 2003). Observing the distribution of microbial communities on the leaf, it is possible to see that the surface irregularities of many plants often caused by epidermal cells with consequent production of swellings, undulations, hairs, determine the discontinuity of this distribution with the creation of micro-areas. The microbial communities, however are not distributed randomly over areas, but rather situated in "ecological niches" called "microsites" where they in turn can give rise to microfilm and microaggregates (Lindow and Brandl, 2003; Vorholt, 2012; Peñuelas and Terradas, 2014).

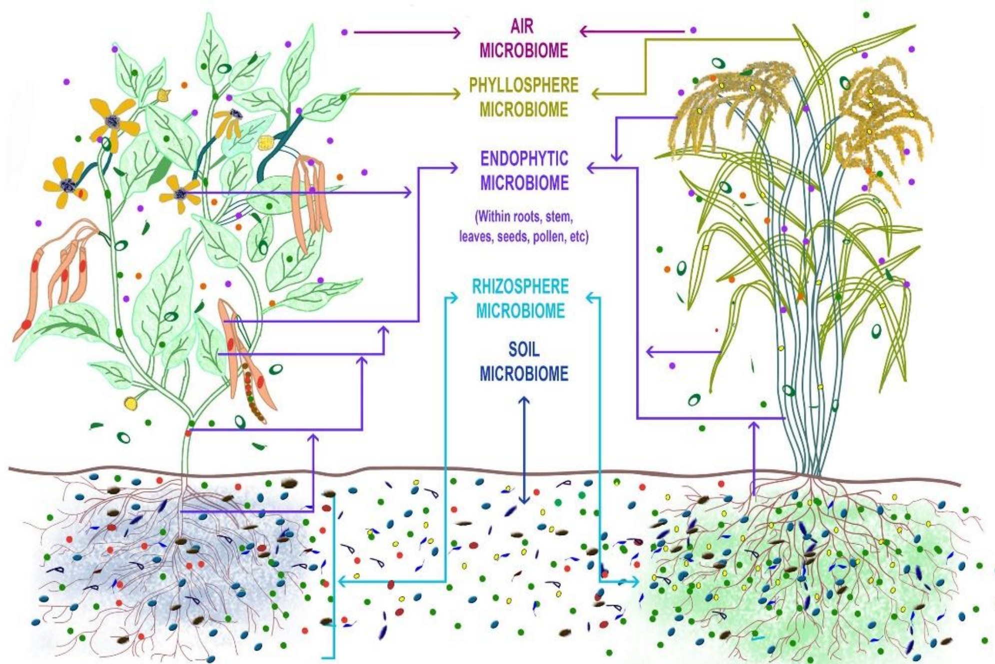

Figure 1: The 'Plant Microbiome' can be described as the sum total of the genomic contribution made by the diverse microbial communities that inhabit the surface and internal tissues of the plant parts. The rhizosphere, endosphere, phyllosphere constitute the major compartments in which the microbial communities reside in the plant. The soil microbiome is the main source from which the plant selects and builds its microbiome profile. The plant genotype, its root exudates, the soil types and properties, and the environmental factors influence the plant microbiome makeup (From Gopal and Gupta, 2016) https://creativecommons.org/licenses/by/4.0/.

The term rhizosphere was first used by Lorenz Hiltner in 1904 to describe the influence of leguminous roots on nitrogen-fixing bacteria, that is, that portion of soil in which the roots were able to influence the activity of microorganisms. Today this term has a broader application to indicate the portion of soil that is around the root and in which complex processes of a biological and ecological nature take place.

The endosphere consists of the inner plant tissues, inhabited by microorganisms intimately interacting with the host (Hardoim *et al.*, 2008; Berg *et al.*, 2014). This compartment is composed of the internal root tissue (endorhizosphere), the internal shoot and leaf tissue (endophyllosphere), the internal plant reproductive tissue, and the internal seed tissue (Compant *et al.*, 2005; Hardoim *et al.*, 2012; Truyens *et al.*, 2015). The rhizosphere and the endosphere microbiome will be described in deep in paragraphs 1.2., and 1.3.

1.1.2. Methods for characterization of plant microbiome composition and function

The methods applied for the study of the microbiome in the agro-environmental field are many and allow to obtain a wide range of information on the diversity of the microorganisms that make it up. These techniques can be distinguished into:

- Culture-dependent methods: used to study microbial communities. This approach allows identifying only 1-3% of the microorganisms present in natural samples, losing 97-99% of the information, because of their particular growing conditions. These mainly aim at selecting plant growth promoting rhizobacteria and biocontrol agents.
- Culture-independent methods: used to identify the microbiome, understanding where they come from and the main driving conditions that modify the microbiota, but not their actual role. This approach contributed to the understanding of the complex network established between the plants and its microbiota.

The evolution from dependent to independent culture methods has made possible to broaden knowledge about the microbiota of the plant, also allowing the study of a larger fraction of those microorganisms whose isolation and cultivation in purity was impossible

for various reasons, thus opening new horizons in the study of the microbial communities of the plant.

With the advances in High-Throughput Sequencing Techniques (HTS) and the increasing number of microbial culture libraries that characterized the last decade, it is now possible to map the microbial community of interest in a fast and cost-effective way (Mendes *et al.*, 2011; Bulgarelli *et al.*, 2012; Berg *et al.*, 2016; Hugerth and Andersson, 2017) (Figure 2). These advances are enabling us to assess the community composition, function, and activity of both culturable and nonculturable organisms in the phytobiome.

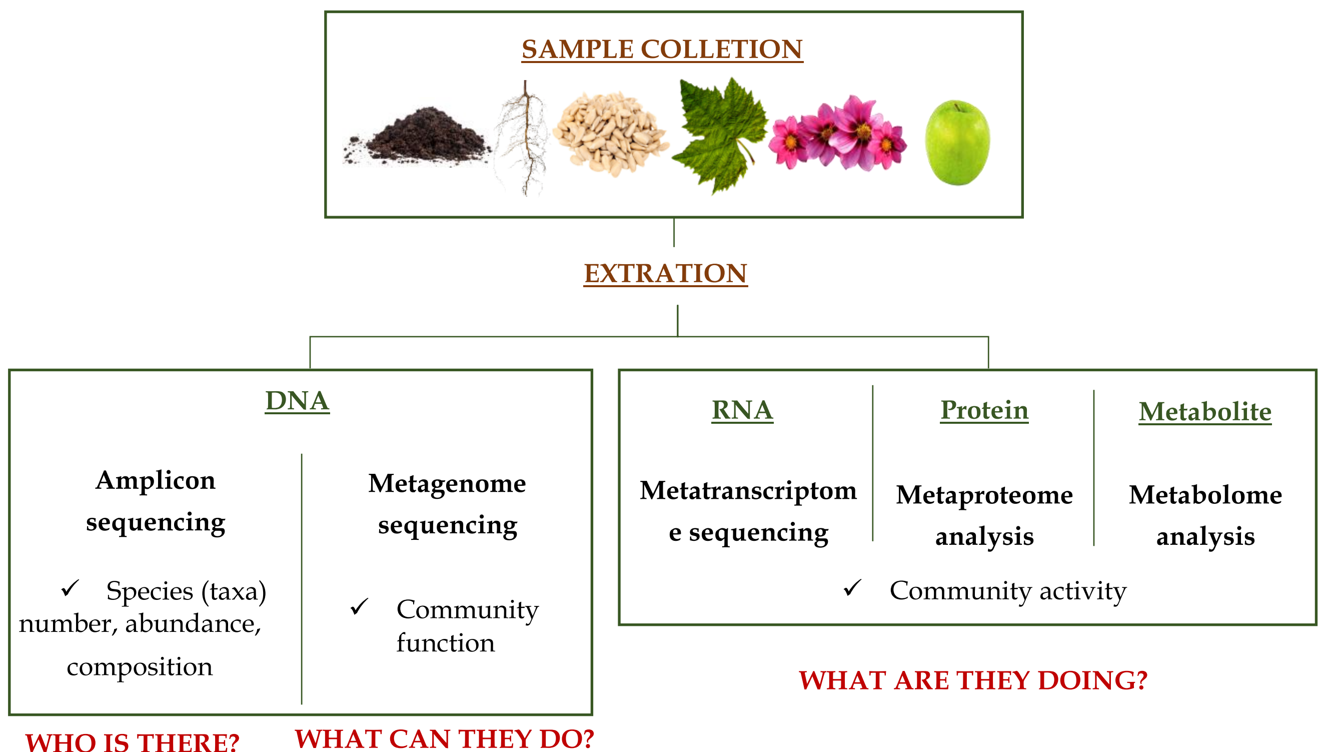

Figure 2: Schematic representation of the study of microbial communities through metagenomics (Modified from http://www.phytobiomes.org/).

As of today, the most employed applications for the investigation of plant microbial communities are DNA metabarcoding and shotgun metagenomics. DNA metabarcoding relies on a combined mass PCR amplification and sequencing of a single marker gene (DNA barcode) (Figure 3). These applications represent powerful tools for the in-depth characterisation of microbial communities and for the detection of indicator species and community shifts. On the other hand, shotgun metagenomics relies on the random fragmentation and sequencing of genomic DNA (shotgun metagenomics).

By reconstructing the set of genes of the microbial community, metagenomics-based applications allow study of both composition and functional capabilities. While the

advantages connected with these applications enabled our understanding of the composition and dynamics of plant associated microbiomes, it is also important to underline the limitations that these technologies have. The reliance on in-silico data alone represents the main disadvantage for NGS-based methodologies since they can only infer phenotypical characteristics. For this reason, an approach that combines both cultivation dependent and independent techniques is indispensable for a complete understanding of the ecological role of these microorganisms and, most of all, for their biotechnological applicability.

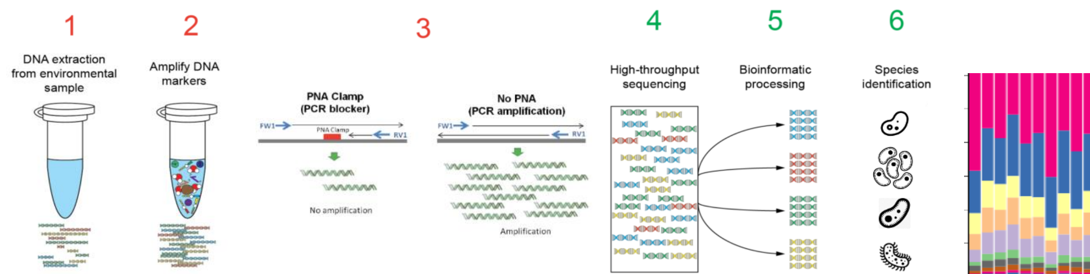

Figure 3: Overview of the DNA metabarcoding procedure.

1.2. Interactions in the rhizosphere

The rhizosphere is a hot spot of microbial interactions as exudates released by plant roots are a main food source for microorganisms and a driving force of their population density and activities. The rhizosphere harbours many organisms that have a neutral effect on the plant, but also attracts organisms that exert deleterious or beneficial effects (Raaijmakers *et al.*, 2009) (Figura 4). In the rhizosphere, there is a complex and dynamic interactive network of both biological and chemical nature between plant roots, its microbiome, and soil characteristics (Hartmann *et al.*, 2008).

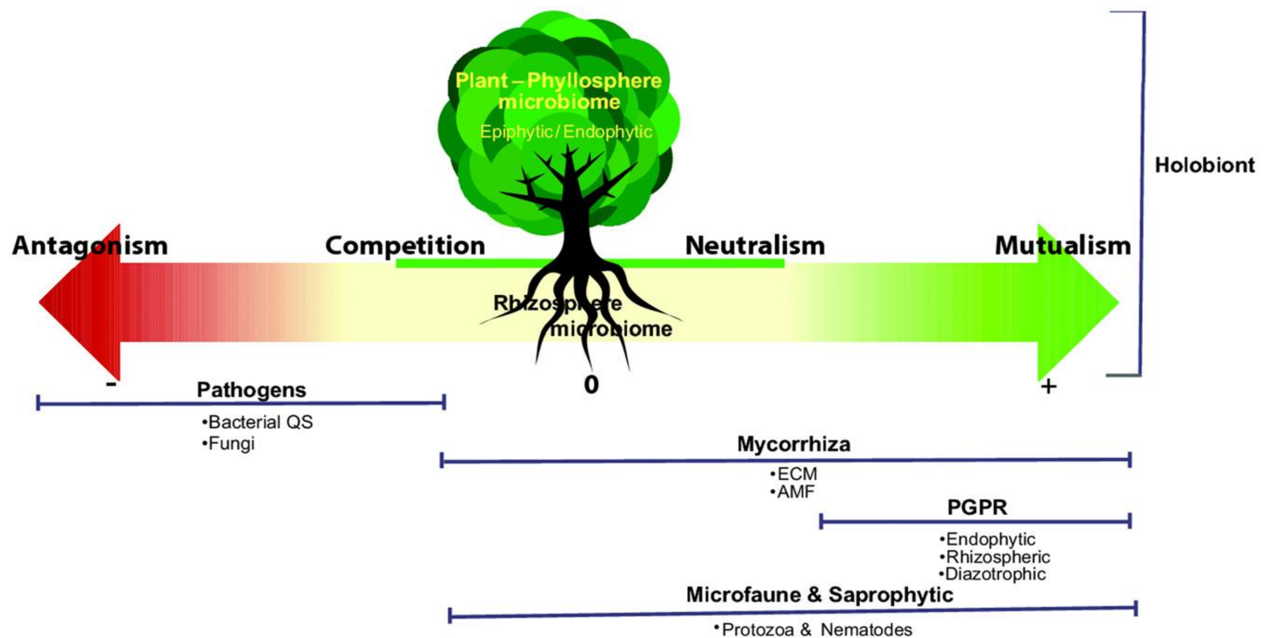

Figure 4: The different interaction staking place with in the plant–microbiome meta-organism. A vast spectrum of microorganisms is involved in these interactions: ectomycorrhiza (ECM), arbuscular mycorrhizal fungi (AMF), plant growth promoting rhizobacteria (PGPR), phosphate-solubilizing organisms (PSOs), endophytes, epiphytes, and microfaunal organisms. (From: Quiza *et al.*, 2015) https://creativecommons.org/licenses/by/4.0/.

1.2.1.The rhizosphere composition

Three separate rhizosphere fractions are considered (McNear Jr., 2013): the endorhizosphere includes portions of the cortex and endodermis wheremicrobes and cations can occupy the "free space" between cells (apoplastic space); the rhizoplaneis the medial zone directly adjacent to the root including the root epidermis and mucilage; the ectorhizosphere (the outermost zone) which extends from the rhizoplane out into the bulk soil. This conceptually simple subdivision reveals its complexity when it is necessary to study the different regions that compose it.

The rhizosphere is also influenced by the plant, in fact the interaction between microorganisms and the plant's root system is regulated by multiple and complex mechanisms. More specifically regarding the plant, the rhizosphere is characterized by its

investment in creating an efficient radical architecture, which in turn depends on the specific conditions of the ecosystem, the species and the phenological phase (Hartmann *et al.*, 2008). Among such mechanisms, the secretion of radical exudates occupies a prominent position. The secretion of exudates by the roots triggers a continuous and intense exchange of signals and nutrients between the different components of the rhizosphere, and the plant itself (Brimecombe *et al.*, 2001; Wei *et al.*, 2015).

This component is strongly influenced by the metabolism of the plant, through the root respiratory and secretion processes, not only of highly complex organic substances, but also of antibiotics released both from the roots of the plants and originating from the lysis of the radical cells, these substances take the name of "exudates", compounds of significant scientific interest (Vandenkoornhuyse *et al.*, 2015).

Thanks precisely to the multi-functionality of a wide range of compounds secreted by the plant at the same time in the rhizosphere, the microbial populations have a wide spectrum of organic compounds available, therefore the "rhizodeposition", understood as the quantity of radical exudates emitted actively or not by the plant, plays a pivotal role in regulating the percentages of carbon fixed by the microorganisms present in it to promote its growth processes.

1.2.2.The rhizosphere effect

The influence of the plant on the microorganisms of the rhizosphere can be quantified with "the rhizosphere effect" that expresses the ratio between the number or degree of activity of the microorganisms present in the rhizosphere and in free soil. In fact, values higher than one will indicate a certain degree of promotion of the microbial populations of the rhizosphere, while values equal to or less than one will indicate no effect, or an inhibitory effect by the plant on the populations, respectively (Bulgarelli *et al.*, 2013).

Soil microbial communities represent the greatest reservoir of biological diversity known in the world so far (Berg *et al.*, 2005; Mendes *et al.*, 2013; Vandenkoornhuyse *et al.*, 2015). The rhizosphere, which is the narrow zone of soil that is influenced by root secretions, can contain up to 10^{11} microbial cells per gram of roots and more than 30,000 prokaryotic species. The collective genome of this microbial community is much larger than that of the plant and is also referred to as the plant’s second genome. An increasing body of evidence also signifies the importance of this root microbiome, which consists of

the entire complex of rhizosphere-associated microbes, their genetic elements, and their interactions, in determining plant health (Berendsen *et al.*, 2012). During the growth processes, the plants establish a dense network of interaction with the multiple microorganisms that populate the soil and the microbiotic component of the rhizosphere. The microorganisms that populate the rhizosphere are numerous, dynamic, and widely diversified in relation to a multitude of factors of both biotic and abiotic nature. These microorganisms may have a neutral, pathogenic, or beneficial interaction with their host plant (Raaijmakers *et al.*, 2009).

1.2.3.Plant Growth Promoting Rhizobacteria

Plant Growth Promoting Rhizobacteria (PGPR) are bacteria that carry out a promotional action for the plant's growth processes, due to at least two of the following characteristics they must meet: competitively colonize the roots; stimulate plant growth; reduce the incidence of diseases.

The beneficial microorganisms of plants are numerous, and they can diversely interact with the plant:

- Direct, with the production of phytohormones and the supply of nutrients.
- Indirect, with a complex of mechanisms that compete with the negative effect of plant pathogens mainly land by means, competition for space and nutrients, production of antibiotic substances or, through the induction of mechanisms of resistance in the same plants.

Direct mechanisms, lead to supporting the decomposition and mineralization of organic residues, facilitating the absorption of nutrients, carrying out nitrogen-fixation and solubilization of phosphate, producing siderophores, cyanidric acid (HCN), phytohormones and 1-aminocyclopropane-1-carboxylate (ACC) deaminase. While indirect mechanisms involve the activation of different biochemical mechanisms to block the growth and development inhibition of plants by phytopathogenic microorganims. A process based on their “biostimulation” and “biofertilization” capability.

“Biostimulation” (or phytostimulation) is understood as the direct promotion of plant growth driven by microorganisms through the production of phytohormones (Bloemberg and Lugtenberg, 2001). In plants, phytohormones contribute to the coordination of various processes, both physiological and of resistance to abiotic factors, also acting as suppressors or inducers of gene expression and synthesis of metabolites, enzymatic complexes and pigments. The phytohormones produced by the beneficial microorganisms of the soil have a great impact on root morphogenesis and ion absorption, enhancing the growth and development process of the plant, and altering its balance. One of these modes of action is the Tryptophan-Dependent Synthesis of Indole-3-Acetic Acid (IAA). Inactivation of genes involved in tryptophan biosynthesis and in a putative tryptophan-dependent IAA biosynthesis pathway led to reduction of both IAA levels and plant growth promoting activity in the respective mutant of *Bacillus amyloliquefaciens* strains FZB42 (Idris *et al.*, 2007). For example, seed treatment with FZB42 increased root production, an indicator of auxin production, but significantly repressed root phosphate (P) uptake at low environmental P concentrations (Talboys *et al.*, 2014).

Another mechanism is the production of volatiles, such as 2,3-Butanediol and Acetoin, released by rhizospheric bacteria, enhancing plant growth. To synthesize 2,3-butanediol, pyruvate is converted to acetolactate by acetolactate synthase, which is subsequently converted to acetoin by acetolactate decarboxylase (Ryu *et al*., 2003).

"Biofertilization" is the activity of microorganisms that facilitate the availability or the increase the supply of nutrients to the plant (Bashan, 1998). Among the most studied forms of biofertilization there is nitrogen fixation and the conversion of atmospheric nitrogen into ammoniacal nitrogen (Bloemberg and Lugtenberg, 2001).

Another important element is phosphorus, which is very abundant in soil, and one of the major limiting factors for plant production. It is present in the soil in two forms: mineral phosphates (calcium phosphates, inorganic phosphates of soil minerals, hydroxyapatite) and organic phosphorus (phytates, inositol phosphate and phosphoesters). The bioavailability of these two forms is rather limited as it is strictly linked to their solubility in water (Khan and Weber, 2006; Kruse *et al.*, 2015). The lack of bio-availability of this element is responsible of the "immobilization of phosphorus, caused by its presence in the form of insoluble calcium phosphates in basic pH soils, while in acid soils is present

in the form of iron and aluminium phosphates which are poorly soluble. Therefore, in the great majority of soils the concentration of soluble phosphorus does not exceed micromolar values. In contrast, the solubilization of phosphorus by promoting root growth is proposed as a mechanism to make phosphorus bioavailable. A conversion is based on the "production of organic acids", which acidify the intercellular spaces causing the release of phosphate ions through the protonic substitution of the Ca^{++} ion or through specific enzymatic mechanisms, such as the mineralization of the organic phosphorus of the soil (Sharma *et al.*, 2013).

1.2.4.Biocontrol agents

“Biological control” of plant diseases is the containment of populations of plant pathogens by living organisms (Heimpel and Mills, 2017), referred to as microbial biological control agents (BCAs). BCAs are applied to crops to reduce primarily the density of inoculum or the pathogenic capacities of a parasite, in its active or dormant state (Gabriel and Cook, 1990). They act by inducing resistance or priming plant defences without requiring any direct interaction with the targeted pathogen or can act indirectly, modifying the growth conditions of the pathogen (e.g., siderophore production) or competing for nutrients (reviewed in Köhl *et al.*, 2019). In particular, the siderophores, small peptide molecules that have side chains and functional groups to which ferric ions can bind (Goswami *et al.*, 2016), produced by some microbes can prevent or lessen pathogen proliferation by reducing the amount of iron that is available to a pathogen (Shen *et al.*, 2013). They bind tightly to most of the Fe^{3+} that is present in the rhizosphere of the host plant and take up into either the plant growth promotion bacteria (PGPB) or the host plant. In such a way, pathogens in the host plant rhizosphere, where the biocontrol PGPB is bound, have less iron for their growth and, being unable to proliferate, lose the ability to act as pathogens. The effectiveness of this method of biocontrol is based on the fact PGPB siderophores have higher affinity for iron (Kloepper *et al.*, 1980).

Other mechanisms may induce of a wide range of host defences as the production of antimicrobial plant metabolites, reactive oxygen species, phytoalexins, phenolic compounds, or pathogenesis-related proteins to the formation of physical barriers, like modifications of cell walls and cuticles (Wiesel *et al.*, 2014), while priming of defence

allows plants to face a subsequent biotic stress quickly and vigorously, limiting energetic costs (Mauch-Mani *et al.*, 2017). Both strategies implicate a cascade of events between the BCA and its host and, depending on plant endogenous factors, the subsequent release of specific inducers, their interaction with host-specific receptors and finally the activation of the host plant pathways resulting in the onset of defence reactions, through different low molecular weight signalling compounds (e.g., cell wall components), or priming of host defences (Romanazzi *et al.*, 2016).

Another way of acting of BCAs is through the production of lytic enzymes, volatile compounds, and antimicrobial metabolites. The organic, low-molecular weight secondary metabolites are produced *in situ* and affect the growth or metabolic activities of other microorganisms (Thomashow *et al.*, 1997). When they are present into the environment in sufficiently high quantities, confer to the microorganisms advantages over competitors in environments with limited resources (Raaijmakers and Mazzola, 2012) or, unlike antibiosis, at low concentration act for signalling or nutrient mobilization functions.

Some antimicrobial metabolites have shown broad spectrum activity against several plant pathogens (e.g., selected *Pseudomonas* spp. DAPG-producing isolates) and variable effect within pathogen populations (Köhl *et al.*, 2019). Production of antimicrobial metabolites, mostly with broad-spectrum activity, has been reported for biocontrol bacteria belonging to *Agrobacterium*, *Bacillus*, *Pantoea*, *Pseudomonas*, *Serratia*, *Stenotrophomonas*, *Streptomyces*, and many other genera (Lodewyckx *et al.*, 2002; Aktuganov *et al.*, 2008; Liu *et al.*, 2010).

In the case of soil-borne pathogens, which live as saprophytes in the rhizosphere, a successful infection is related to their capability to reach the infection site or to increase population on their host before starting the infection process. In fact, natural favourable soils can contain a pathogen infection to a certain level, according to a phenomenon known as general disease suppressiveness, attributed to the total microbial activity, enhanced by factors such as the cultural practices (e.g., the use of organic amendments) (Hoitink and Boehm, 1999). A more effective suppression activity is represented by 'specific suppression', involving specific microorganisms for the suppression of soil-borne diseases (Weller *et al.*, 2002; Garbeva *et al.*, 2004; Raaijmakers *et al.*, 2009).

Disease suppressiveness in soils has been reported for various diseases (Berendsen *et al.*, 2012) and is significant for single crop management, subject to the outbreak of specialized plant pathogens (Bennett *et al.*, 2012). Strains of *Bacillus*, *Pseudomonas*, *Glomus*, and others have been commercialized. The use of bacterial taxa in plant production has been reviewed previously for *Bacillus* (Borriss, 2011), *Pseudomonas* (Santoyo *et al.*, 2012; Sivasakthi *et al.*, 2014), *Actinobacteria* (Shivlata and Satyanarayana, 2017), and

Lactobacillus (Lamont *et al.*, 2017). In addition, *Acetobacter*, *Azospirillum*, *Paenibacillus*, *Serratia*, *Burkholderia*, *Herbaspirillum*, and *Rhodococcus* have also been shown to enhance crop production (Babalola, 2010)

Bacteria in the genus *Pseudomonas* are Gram-negative bacteria characterized by a high content of C+G genomic (59-68%), a versatile metabolism, aerobic respiration and equipped with polar flagella of variable number. This genus comprises multiple species and strains that suppress plant pathogens, promote plant growth, induce systemic resistance, in plants. Members of the genus *Pseudomonas sensu stricto* show remarkable metabolic and physiologic versatility, enabling the colonization of diverse habitats and of great interest because of their importance in plant and human diseases, and their growing potential in biotechnological applications (Silby *et al.*, 2011).

Bacteria in the genus *Bacillus* are a ubiquitous and highly adaptable (as well as related genera, *Geobacillus*, *Paenibacillus*) they are characterized by the production of conservation spores essential for survival in adverse environments (Borriss, 2011; 2015). For example, *B. subtilis*, adapted to an environment poor in proteins such as soil, but rich in carbohydrates of plant origin, possesses a wide spectrum of genes for carbohydrate metabolism. These bacteria play a key role in completing biogeochemical cycles and promoting root growth, so their presence is essential for maintaining soil fertility. In turn, the bacteria belonging to the genus *Bacillus* are also able to interact with other microorganisms, inactivating toxic components or producing attractive substances (e.g., certain *Paenibacillus* strains with *P. fluorescens* strains) improving their rhizosphere colonization capacity (Vacheron *et al.*, 2013). *Paenibacillus polymixa*, *Bacillus subtilis* and *B. thuringiensis* are involved in root nodulation, benefiting the activity of the rhizobes, and interacting with other promoter microorganisms. *Paenibacillus* with the species *P. azotofixans*, *P. peoniae*, *P. boeralis*, *P. graminis*, *P. odorifer*, *P. polymixa*, *P. macerans*, and *P. brasilensis* have a role in soil fertilization, making different nutrients available to the root (Fan *et al.*, 2017). Moreover, some aerobic sporogenes can produce different phytohormones, such as auxins and gibberellins, and are implicated in the regulation of production of ethylene through the synthesis of enzymes such as ACC-deaminase. In this context, for example, *Bacillus* strains have been shown to be active *B. pumilus* and *B. licheniformis*, with biostimulating activity through the production of gibberellins (Kudoyarova *et al.*, 2019). Several species of *Bacillus*, such as *B. amyloliquefaciens*, *B. cereus*, *B. subtilis* and B. *pumilus*, show antagonistic activity against pathogenic microorganisms (Borriss *et al.*, 2011). In particular, the antagonistic species dominating the rhizosphere are *B. subtilis*, *B. mycoides* and *B. cereus*, these produce numerous antibiotics including polymyxin, difficidin, subtilin, mycobacillin, zwittermicin A, which are active both against bacteria and fungi (Caulier *et al.*, 2019). It

has been observed that various species of Bacilli have antagonistic activity among them thanks to the production of compounds such as the "autolysins" also active against individuals of different species or genera, and the "bacteriocins" that demonstrate bactericidal activity only against individuals phylogenetically close to the producer (Subramanian and Smith, 2015). Among the species of the *B. cereus* group, turicin and waxes produced by *B. thuringiensis* and *B. cereus* have been identified, as well as the subtilin produced by *B. subtilis* (Gillor *et al.*, 2005). Instead, the "kinases" produced by *B. thuringiensis* and β-1,3-glucanases produced by *B. subtilis*, *B. clausii*, *B. circulans,* and *B. amyloliquefaciens* are implicated in antifungal activity.

1.3. Endophytes

The endosphere is populated by microorganisms that manage to invade and penetrate inside the plants, called "endophytes" (Yandigeri *et al.*, 2012). Initially they were defined as commensal or beneficial microorganisms, which could be isolated after a disinfection of the plant surface (Coombs and Franco, 2003). Now intended as microorganisms that can asymptomatically reside in the internal tissues of living plants without causing immediate obvious negative effects, for at least a part of their life cycle (Hardoim *et al.*, 2015).

They are ubiquitous with a rich biodiversity and are potential sources of new natural products for exploitation in medicine, agriculture, and industry. Interestingly, of the approximately 300,000 plant species existing on earth, each individual plant houses one or more endophytes (Strobel and Daisy, 2003). Endophytism is an interaction different from symbiosis, saprophytism and biotrophy; precisely indicates the interaction of a plant with a microorganism that lives inside it. Therefore, the endosphere is a sort of "secret world" within plants (Hardoim *et al.*, 2015).

Endophytes, as well as human intestinal bacteria, cause asymptomatic infections and often have positive effects on plants: they counteract pathogenic microorganisms and herbivores, mobilize nutrients, promote their absorption, increase tolerance to adverse environments (e.g., drought and salinity), and synthesize substances that promote plant growth.

Endophytes colonize plant apoplast, including the intercellular spaces of the cell walls and xylem vessels of plant roots, stems, and leaves, and are also found in tissues of

flowers (Compant *et al.*, 2011), fruits (de Pereira *et al.*, 2012), and seeds (Trognitz *et al.*, 2015).

Generally, endophyte population density is higher in plant roots and other underground tissues compared to the above ground ones (Bulgarelli *et al.*, 2013).

Endophytes are able to synthesize inside the plants bioactive compounds (antibiotics, antibiotics, anticancer agents, biological control agents, and other bioactive compounds) which plants use to defend against pathogens, some of which have proven useful for the discovery of new drugs (Owen and Hundley, 2004).

1.3.1.Classification of endophytes

Depending on the relationships established with the host plant, endophytic microorganisms can be classified into three different groups (Hardoim *et al.*, 2008):

- Obligatory endophytes: they are distinguished since, due to the strong relationships they establish with the plants, they cannot survive outside them. Therefore, this fraction is probably seed-transmitted through sexual reproduction, and then transmitted vertically to the progeny of the plant.
- Optional endophytes: they are the largest group, characterized by the ability to survive outside the plant, but in suitable conditions they can colonize the host through a process of coordinated infection in the internal tissues of the plant.
- Passive endophytes: they are the microorganisms that do not actively colonize the plant, but when proper conditions occur, such as opening wounds along the root hairs, they can colonize the host tissues passively. This characteristic makes them less competitive and therefore difficult to apply compared to the previous ones that boast of active colonization mechanisms.

1.3.2. Horizontal and vertical transmission of bacterial endophytes

Transmission mode is an important feature of endophytic bacteria. In general, the endophytic microbiome colonizes the host horizontally via the environment and soil, or vertically through seed-transmission from parent to progeny (Bright *et al.*, 2010; Edwards *et al.*, 2015; Truyens *et al.*, 2015). It is possible that some bacterial endophytes are transmitted both vertically and horizontally (mixed mode transmission) (Bright *et al.*, 2010)

Commonly it is considered that endophytic bacteria are predominantly assembled via horizontal transmission (recruitment of microorganisms from the surrounding soil) (Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012). In fact, the diversity of bacteria in seeds and seedlings grown under sterile conditions is typically lower than in plants grown in soil (Hardoim *et al.*, 2012), suggesting that most endophytes are acquired from the environment. On the other hand, bacterial endophytes are often generalist, as their beneficial properties, or they can typically move from one plant to another even if in very distant families (Compant *et al.*, 2005; Ma *et al.*, 2011; Khan *et al.*, 2012). However, recent studies have confirmed the existence of vertical (seed-based) transmission of endophytes in plant species such as rice, maize, tobacco, coffee, quinoa, common bean, grapevine, barley and pumpkin, but also in wild plants (reviewed in Frank *et al.*, 2017).

1.3.2.1. Vertical Transmission

Vertical transmission by seed is attracting increasing interest and has been the subject of several studies (Truyens *et al.*, 2015; Rodríguez-Leal *et al.*, 2017; Shade *et al.*, 2017; Bergna *et al.*, 2018; Nelson, 2018; Taffner *et al.*, 2020a). Bacteria have been detected in sterilized seed surfaces of different crops, such as rice, maize, tobacco, coffee, quinoa, common bean, grapevine, barley and pumpkin, but also in wild plants (reviewed in Frank *et al.*, 2017). Truyens and co-authors (2015) examined studies on seed endophytes, and observed the presence of specific genera, in particular *Bacillus* and *Pseudomonas*, but also *Paenibacillus*, *Micrococcus*, *Staphylococcus*, *Pantoea* and *Acinetobacter*. It has been shown that some seed endophytes may promote plant growth to releasing hormones, to supplying inorganic nutrients, to protecting by pathogens (Puente *et al.*, 2009; Rout and Southworth, 2013; Goggin *et al.*, 2015; Díaz Herrera *et al.*, 2016). A study based on a metagenomic approach has shown the seed microbiome community structure and

diversity depend on various factors such as host genotype, soil characteristics, and geography (Barret *et al.*, 2015).

Truyens and co-authors (2016) investigated the effect of different growth substrates (sand and. sand/soil mix) on the assembly of the endophytic community in *Arabidopsis thaliana*, and found that seed and root communities were similar to each other, but not to substrate communities, suggesting a selection by the plant. However, only a minor fraction of the seed communities was found in the leaves, while the remaining seemed to results from the non-soil environment, likely the atmosphere or the nutrient solution. While the results from these studies do not rule out vertical transfer of endophytes via seeds, they suggest that most seed endophytes colonize the seed horizontally. However, it is possible that some seed endophytes are occasionally transferred to the next generation; for example, a study of *A. thaliana* suggest that the plant may select seed endophytes based on environmental stressors and pass them into the next generation (Truyens *et al.*, 2013).

1.3.2.2. Horizontal transmission

Soil and areas surrounding the roots of the plant are considered the major drive of inoculum for endophytes, serving as a reservoir for both below- and above-ground plant microbiome (Hardoim *et al.*, 2008; Turner *et al.*, 2013). The plant genotype, as well the soil type, shape the composition of the rhizosphere community (Haichar *et al.*, 2008; Berg and Smalla, 2009), so that the “rhizosphere effect” mostly differs among plant species (Bulgarelli *et al.*, 2013), even if in some cases the action of soil type has a prevalent role by shaping bacterial communities (Lundberg *et al.*, 2012; Bulgarelli *et al.*, 2012). Plants use these selection capabilities at the time of establishment, throughout the course of their life, and in response to biotic stress (by other organisms), and/or abiotic stress (climate variables, soil microbial composition), recruiting specific microorganisms that facilitate adaptation to stress (Dennis *et al.*, 2010; Philippot *et al.*, 2013). For example, plants attacked by pathogens can recruit bacteria that enhance microbial activity to suppress pathogens in the rhizosphere (Berendsen *et al.*, 2012). The mechanisms behind plant-mediated modulation of the rhizo- and endorhizosphere microbiomes are currently being unraveled; it has been shown that defense hormone signaling selects specific bacterial families for colonization from the available microbial communities and shapes the root microbiome during plant nutrient stress (reviewed in Frank *et al.*, 2017).

1.3.2.3. Entry into Aerial Tissues

In addition to penetrating the roots through the soil and moving through the xylematic vessels, endophytes can enter the internal tissues through the stem, leaves, flowers (anthosphere), and fruits (carposphere). Many phyllosphere bacteria are probably deposited via bioaerosol, which are tiny particles that include bacteria, fungi, viruses or pollen, released into the atmosphere from different environments (Fröhlich-Nowoisky *et al.*, 2016).

Studies on bacterial pathogens suggest that in order to colonize the surface of leaves epiphytic microorganisms may use openings in the plant epidermis, including the stomata (which allows and control gas exchange and water transpiration), lenticels (raised pores in the stem of woody plants that also allow gas exchange), and hydathodes (water-secreting pores usually present near the leaf margin) (reviewed in Frank *et al.*, 2017).

1.4. Tomato as case study for microbiome analysis

1.4.1.Main characteristics of tomato crop

Tomato (*Solanum lycopersicum* L.) is widely grown and constitutes a major agricultural industry worldwide. It is one of the most consumed vegetables in the world, after potatoes and before onions. With a world production that exceeds 180 million tons in 2019. The tomato is the seventh most important species after corn, rice, wheat, potatoes, soy, and cassava. Whereas 20 years ago, Europe and the Americas represented the most important producers, today Asia dominates the tomato market with China ranking first, followed by USA, India, Turkey, Egypt, Italy, Iran, Spain, Brazil, and Mexico. The national production of tomatoes in open air and from Italian industry was 5.383 approximately million tons (data 2019), while the greenhouse tomato production was around 578 thousand tons in 2019.

From the botanical point of view, tomato is a fruit berry, and not a vegetable. Tomato belongs to the large *Solanaceae* family, which contains over 3000 species with important plants including potatoes, aubergines, petunias, tobacco, peppers and physalis. *Solanum* is probably the most economically important genus comprising between 1250 and 1700,

containing crop species and many other species that produce poisonous or medicinal compounds (Weese and Bohs, 2007).

Wild tomato species originate in western South America along the coast and the high Andes from central Ecuador, via Peru, northern Chile, and the Galapagos Islands. Wild tomato species are often limited to narrow and isolated valleys where they have adapted to climatic and soil types.

Tomato is grown in open fields, and greenhouses. Open-field production may be for the fresh market or the processing industry, while greenhouse production is only for the fresh market. Nurseries are the key to success for the tomato industry, providing a constant part of the planting materials in the sector (instead of farmers who raise their seedlings) and helping to promote new varieties. The production capacity of the tomato crop is influenced by the way the seedlings are raised. With the introduction of plastic in agriculture, the nursery business, firstly conducted in greenhouses covered with polyvinyl chloride (PVC) film and then with polyethylene (PE), has become increasingly detached from standard production activity. The commercial production of seedlings has got advantages such as the safety of the cultivar, the availability of the material in the correct period to carry out the cultivation planning, the uniformity of size and, considering the health certifications, lower risks of setting up the crop with infected plants (Antón *et al.*, 2005; Dorais *et al.*, 2010).

Tomato is the most important vegetable grown in agriculture soil and in soilless cultivation systems in greenhouses. Greenhouse cultivation of vegetables is not an intensive activity and implies perfect planning with numerous operational steps for its success. A greenhouse is defined as a covered structure capable of providing plants with optimally controlled microclimate growth conditions. In cold climates, there is a great advantage in having a controlled environment, while for moderate and tropical regions, it provides an extension of the production season and a protection against pests and diseases (Shamshiri *et al.*, 2018).

The tomato plant growth in agriculture soil to allows to increase content of organic matter, to improve soil texture and related characteristics (e.g., chemical properties and cation exchange capacity), to manage salinity and/or alkalinity and soil-borne pathogens, to supply adequate and balanced nutrients.

Soilless culture can be defined as “any method of growing plants without the use of soil as a rooting medium, in which the inorganic nutrients absorbed by the roots are supplied via the irrigation water” (Savvas *et al.*, 2013). The fertilizers containing the nutrients to be supplied to the crop are dissolved in the appropriate concentration in the irrigation water and the resultant solution is referred to as “nutrient solution”. In soilless crops, the

plant roots may grow either in porous media (substrates), which are frequently irrigated with nutrient solutions, or directly in nutrient solution without any solid phase. In recent decades, supplying nutrient solution to plants to optimize crop nutrition (fertigation or liquid fertilization) has become routine cultural practice, not only in soilless culture but also in soil grown greenhouse crops. Hence, the drastically restricted volume of the rooting medium and its uniformity are the only characteristics of soilless cultivated crops differentiating them from crops grown in the soil (Savvas *et al.*, 2013).

In addition to its economical relevance and to its relative ease of use under laboratory conditions, the susceptibility of this plant to phytopathogens has contributed to its extensive employment in studies focusing on disease resistance. For this reason, the tomato plant is one of the main model plants for the study of the biological control of these diseases (The Tomato Genome Consortium, 2012; Kwak et al., 2018).

Diseases are one of the main problems of the tomato industry worldwide, the major factor that significantly lowers the yields of this crop is represented by microbial pathogens as *Fusarium* sp., *Rhizoctonia* sp., and *Verticillium* sp. (Oerke, 2006). The biological control agents have emerged as an alternative approach for the control of tomato diseases. Characterization of bacterial communities associated with tomato plants will contribute to not only exploring the mechanisms of selectivity in bacterial colonization in different compartments of plants but also identifying potential candidates for biologic control (Singh *et al.*, 2017).

1.4.2.The baseline of tomato microbiome

The development of culture-independent, high-throughput sequencing-based metagenomic and genomic studies have been greatly contributing to the understanding of the establishment of the complex network between tomato rhizosphere and its microbiome. The information gained to date refers mainly to assessing ‘who is there?’, from where they come from and what are the main driving conditions for shaping the microbiome.

A combination of next-generation sequencing-based methods and bioinformatic analyses was used to visualize functional networks and identify key players in the microbiome of plants. The reconstruction of the bacterial and fungal assemblages associated with different tomato plant systems/compartments by 16S rRNA gene and ITS region metabarcoding revealed the structure of the tomato plant microbial community.

Research on tomato microbiome on and within plant organs (leaves, stems, roots, flowers, and fruits) has been going on since 2013 (Ottesen *et al.*, 2013).

The first study on tomato microbiome was aimed to define the baseline of the anatomical microbial ecology of *Solanum lycopersicum* (Ottesen *et al.*, 2013) (Figure 5). To describe "native" bacterial and fungal microflora of tomato organs (leaves, stems, roots, flowers and fruits) the DNA was amplified for targeted 16S and 18S rRNA genes and sheared for shotgun metagenomic sequencing. Ottesen and co-authors (2013) observed that several operational taxonomic units (OTUs) were unique to the combined fruit and flower datasets including: *Microvirga*, Microbacteriaceae, *Sphingomonas*, *Brachybacterium*, Rhizobiales, *Paracoccus*, *Chryseomonas* and *Microbacterium*. There were also unique OTUs in root samples, such as *Chryseobacterium*, *Leifsonia*, *Pandoraea*, *Dokdonella*, *Microbacterium*, *Arthrobacter*, *Phyllobacterium*, *Tetrasphaera*, *Burkholderia*, and unclassified Intrasporangiaceae. A few bacterial taxa were shared across all samples, including: *Curtobacterium*, *Methylobacterium*, *Sphingomonas*, and *Pseudomonas*. They also observed the fungal elements in tomato microbial ecology.

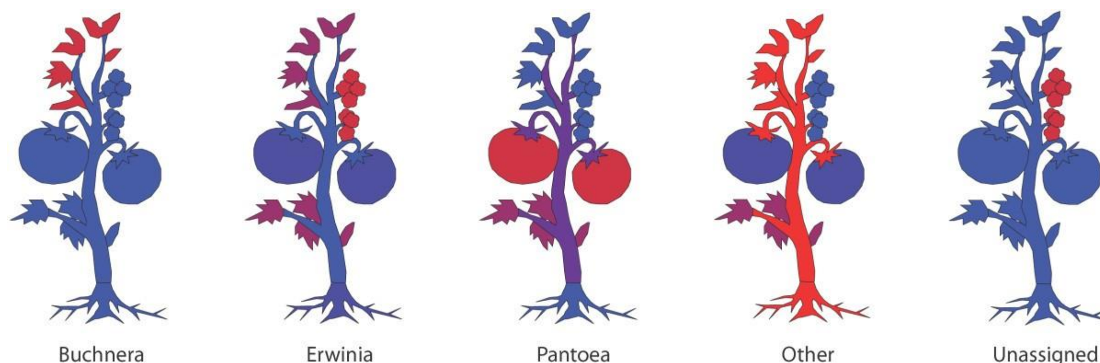

Figure 5: Taxonomic distribution of representative genera on the tomato plant using 16S with SitePainter. Images display the geographical location of observed genera Buchnera, Erwinia, Pantoea, Other and Unassigned, on tomato plants. The sites are colored by abundance, where red represents high abundance, blue represents low abundance and purple represents medium range (From: Ottesen *et al.*, 2013) https://creativecommons.org/licenses/by/4.0/.

Their results showed that fungal phyla included: Ascomycota, Basidiomycota, Chytridimycota, Glomeromycota, Zygomycota (unclassified) and Mucoromycotina. Dominant fungal genera that could be identified in aerial surfaces were *Hypocrea*, *Aureobasidium*, and *Cryptococcus*. They observed that distinct groupings and taxa could be ascribed to specific tomato plant organs, while at the same time, a gradient of compositional similarity was correlated to the distance of each plant part from the soil.

A number of recent studies have investigated the tomato microbiome in relation to soilborne pathogen infection (Li *et al.*, 2014b; Tian *et al.*, 2015; Larousse *et al.*, 2017; Kwak *et al.*, 2018), soil properties (Poli *et al.*, 2016; Dong *et al.*, 2019), tomato genotypes (Bergna *et al.*, 2018; French *et al.*, 2020; Manzotti *et al.*, 2020; Taffner *et al.*, 2020a), crop management, such as fertilization (Allard *et al.*, 2016), rootstocks and/or grafting (Poudel *et al.*, 2019; Toju *et al.*, 2019).

A recent transcriptomic and proteomic study showed the effect the overall characteristics of the substrate contribute more than plant genotype to shaping the molecular responses in tomato roots, and that only few genes respond differently in tomato plants grown in the two different native soils (Chialva *et al.*, 2018).

1.4.3.Influence of cultivation practices on tomato rhizobiome

A few studies suggested a correlation between the amended nutrient content in soils and changes in bacterial community structure in the rhizosphere, and in the phyllosphere of tomato plants (Allard *et al.*, 2016; Cai *et al.*, 2017). In particular, Allard and co-authors (2016) investigated whether the use of three organic field amendments (fresh poultry litter, sterilized poultry litter pellets, and vermicompost) prior to planting induce changes in tomato microbiome above and belowground at the harvest time. The analysis carried out by Illumina-based 16S rRNA gene sequencing showed that field location and soil characteristics had a stronger influence than poultry litter or vermicompost fertilizer on tomato bacterial communities of the rhizosphere, blossom and fruit. At the phylum level, the largest difference between above- and belowground bacterial communities was observed in the Proteobacteria, which were much more dominant on fruit and blossoms compared to bulk soil and rhizosphere. Field studies were conducted in two years, 2013 and 2014. The first year blossom and fruit surfaces were dominated by Pseudomonadaceae and Enterobacteriaceae. Whereas the rhizobacterial communities were dominated by Bacillaceae and Pseudomonadaceae, both highly enriched compared to the surrounding bulk soil. One year later, Pseudomonadaceae were elevated in blossoms compared to roots, however they were 9% less prevalent than in 2013. Instead, Xanthamonadaceae dominated on blossoms, and fruit supported a high relative abundance of Rhizobiaceae, mostly belonging to the genus *Agrobacterium*. Since both the Pseudomonadaceae and Xanthamonadaceae families contain pathogens that can infect tomato, it is possible that these pathogens occurred in the field. However, sequencing

resolution was not high enough to differentiate between pathogenic and non-pathogenic members of this taxa. The data indicated a potentially weak influence of poultry litter amendment on tomato blossom and rhizosphere bacterial communities, but not on fruit. Bacterial communities profiled from bulk soil did not respond to soil amendment, but the row location in the field appeared to influence the structure of soil bacterial community.

1.4.4. Influence of genotype on tomato rhizobiome

Recent studies have suggested that host plant genotypes control, at least partly, plant-associated microbiome compositions. However, the knowledge of how microbiome structures are determined in/on grafted crop plants, whose genotypes above-ground (scion) and below-ground (rootstock) are different are still limited (Toju *et al.*, 2019; Poudel *et al.*, 2019).

Poudel and co-authors (2019) investigated the effects of rootstock genotypes and grafting on endosphere and rhizosphere microbiomes of tomato by sequencing 16S rRNA in comparison with the microbiomes of ungrafted plants. Their study evaluated three rootstocks (BHN589, RST-04-106, and Maxifort) in four treatments: 1) nongrafted BHN589 plants; 2) self-grafted BHN589 plants (plants grafted to their own rootstock); 3) BHN589 grafted to RST-04-106 (hybrid rootstocks); and 4) BHN589 grafted to Maxifort (hybrid rootstocks). The results showed that rootstocks affected both microbial diversity and community composition. Whereas, grafting itself did not affect bacterial diversity; the self-graft and ungraft diversity was similar. In each treatment, bacterial diversity was higher in the rhizosphere than in the endosphere (Poudel *et al.*, 2019). Among the rootstocks, the self-graft had the highest percentage of Proteobacteria, Actinobacteria and Firmicutes were the other dominant phyla observed in the overall community. Firmicutes and Planctomycetes were enriched in the hybrid rootstocks compared to in the nongraft and self-graft, whereas the Bacteroidetes were depleted in the hybrid rootstocks. Comparison of community profiles of bacteria between the endosphere and rhizosphere showed that Proteobacteria, Actinobacteria, and Bacteroidetes species were more abundant in the endosphere than in the rhizosphere, whereas Planctomycetes, Firmicutes, and TM7 bacteria were more abundant in the rhizosphere (Poudel *et al.*, 2019).

Similar studies were performed analyzing the bacterial and fungal communities in tomato plants (Toju *et al.*, 2019) obtained by grafting in eight tomato rootstocks (“Chibikko”,

“Ganbarune”, “M82”, “Micro-Tom”, “Regina”, “Spike”, “Triper”, and “Momotaro-Haruka”) with “Momotaro-Haruka” scions. The results showed that microbial community structures did not significantly differ among tomato plants with different rootstock genotypes: rather, sampling positions in the farmland contributed to microbiome variation in a major way. The bacterial community of the tomato leaves, registered Sphingomonadales and Rhizobiales were dominant, and Pseudomonadales were frequently observed in all treatments. Meanwhile, bacteria in the order Deinococcales were abundant only in the ungrafted tomato individuals. At the genus-level, the genera *Sphingomonas*, *Methylobacterium*, and *Pseudomonas* were frequently observed across the rootstock varieties examined. In the leaf-associated fungal community, ascomycete fungi in the orders Capnodiales and Plesporales and the basidiomycete fungi in the orders Tremellales and Ustiaginales were abundant. At the genus level, *Cladosporium*, *Dioszegia*, *Moesziomyces* (anamorph = *Pseudozyma*), and *Hannaella* were frequently observed.

Other studies aimed to investigate the composition of tomato rhizo- and endorhizosphere microbial communities in tomato genotypes (French *et al.*, 2020; Manzotti *et al.*, 2020; Taffner *et al.*, 2020a), have identified the plant genotype as main factor that influence abundance and diversity of bacterial, fungal and archeal community. By analyzing the root bacterial communities of six domesticated tomatoes, and two wild tomato accessions they found that tomato accession genotypes significantly affected microbial community diversity in both the root endosphere and rhizosphere. Overall, genotype accounted for 10% of the variation in root microbiota. Two bacterial families, Bacillaceae and Rhizobiaceae, were enriched in the root endosphere in at least six of the eight tomato genotypes and varied quantitatively in abundance among a set of RILs (recombinant inbred lines). Inoculation of 16 RILs and their parents with an isolate in the same family as the high-frequency colonizers revealed that this isolate promoted both root and shoot growth in a genotype-dependent manner. Together, these data suggest a genetic under-pinning to tomato selection and responses to root microbiota and that microbiome strategies to improve tomato production should consider the specific tomato cultivar utilized (French *et al.*, 2020).

Plant genotype plays a major role also in shaping the fungal endophytic communities of tomato roots. This conclusion obtained by a recent study (Manzotti *et al.*, 2020) that investigated the influence of host genotype and phytohormones on the structure of the fungal endophytic communities of tomato roots in four different cultivar (Castlemart, *defenceless1* (*def1*), UC82B and *8338*) by amplicon sequencing of the ITS1 region and the isolation and functional characterization of the isolates. A significant effect of the host

genotype on the dominant fungal species was found by comparing the cultivars "Castlemart" and "UC82B" and, surprisingly, root pathogens were among the most abundant taxa. In contrast, smaller changes in the relative abundance of the dominant species were found in mutants impaired in jasmonic acid biosynthesis (*def1*) and ethylene biosynthesis (*8338*) compared to the respective wild types. However, *def1* showed significantly higher species richness compared to the wild type. Analysis of the phytohormone profiles of these genotypes indicates that changes in the phytohormone balance may contribute to the difference in species richness. Assessing the lifestyle of isolated fungi on tomato seedlings revealed the presence of both beneficial endophytes and latent pathogens in roots of asymptomatic plants, suggesting that the interactions between members of the microbiome maintain the equilibrium in the community preventing pathogens from causing disease (Manzotti *et al.*, 2020).

Archaeal communities were analysed by Taffner and co-authors (2020) in the rhizosphere of tomato plants (cv. Moneymaker and Hildares F1) grown in two different soil types (loamy and sandy). In addition, as complementary experiments, archaeal communities were assessed in two generations of tomato seeds. Overall, the archaeal community in tomato was dominated by *Thaumarchaeota* and *Euryarchaeota*. The core community consisted of species assigned to the soil crenarchaeotic group (*Thaumarchaeota*), *Methanosarcina* (*Euryarchaeota*), *Methanoculleus* (*Euryarchaeota*), and unassigned archaeal species. Their results suggested differences in abundance, diversity, and composition between cultivars were so distinctive to mask any effect determined by the type of soil. In seeds, archaeal abundance and diversity was comparably low and the composition showed random patterns; no indication of a plant-mediated vertical transmission was found. The plant genotype was identified as the main factor influencing abundance and diversity, while the soil type did not notably affect archaeal communities. Recent studies demonstrated transmission of archaea from the parent to the offspring plant, but there is no indication for a targeted selection as shown for bacteria (Bergna *et al.*, 2018).

Very few studies addressed vertical transmission of microbial communities. Investigating the endophytic microbial communities of seeds across two generations a continuous turnover of the seed bacterial and archaeal assemblage has been observed (Bergna *et al.*, 2018; Taffner *et al.*, 2020). By a continuous turnover of the seed bacterial assemblage through seed bacterial community analysis across a generation, Bergna and co-authors (2018) have demonstrated seeds act as a vehicle of beneficial bacteria over seed generations.

In addition, they observed that the plant seeds were a primary vehicle for transmission of PGPB. This previously undescribed vertical transmission of PGPB represents a strategy to maintain plant beneficial bacteria over generations and has an impact for the design of seed treatments.

1.4.5. Influence of soil and growing substrates on tomato rhizobiome

Different studies on the effect of soil in the tomato microbiome (Bergna *et al.*, 2018; Cheng *et al.*, 2020) have shown the soil bacterial community composition has a high impact on the bacterial community of below ground compartments (rhizosphere, root endosphere) in two tomato cultivars (cv. Moneymaker and Hildares F1) grown in two different soil (sandy and loamy) (Bergna *et al.*, 2018). However, the effect was progressively reduced from the rhizosphere to the root endosphere and finally to the seeds. Overall, soil, rhizosphere, and root endosphere were the microhabitats with the highest diversity, while seeds hosted more selective communities in both generations. The impact of the soil microbiota in the rhizosphere was high, comparable to other studies (De Ridder-Duine *et al.*, 2005; Inceoğlu *et al.*, 2012), and even if less evident, this signature was still visible in root endosphere. Interestingly, no instance of this phenomenon was evident in the bacterial community of second-generation seeds grown in different soil types Proteobacteria were predominant in all microhabitats. Other representative phyla were Firmicutes, Actinobacteria, and Bacteroidetes. Chloroflexi, Acidobacteria, and Planctomycetes, mostly found in soil samples. Pseudomonadaceae and Comamonadaceae were the most occurring bacterial families among rhizo-endosphere and seeds (both generations). Considering both cultivars, the seeds of the first generation were characterized by a selective bacterial community, where only few taxa were dominant (Burkholderiaceae, Pseudomonadaceae, and Comamonadaceae). Whereas the soil was characterized by the high abundance of Anaerolineaceae and Planctomycetaceae. The rhizosphere showed most abundant families (Comamonadaceae, Pseudomonadaceae, and Flavobacteriaceae), while the root endosphere was dominated by the family Pseudomonadaceae, and lesser abundance of Comamonadaceae, Bacillaceae, and Rhizobiaceae. Significant differences were attributable to taxa that dominated the community of seeds: the family Burkholderiaceae dominated the first generation and drastically decreased in the second generation, with Pseudomonadaceae

also decreasing. Conversely, families Comamonadaceae, Rhizobiaceae, and Oxalobacteraceae dominated the bacterial community of the second generation. Different plant compartments hosted distinctive bacterial communities depending on the cultivar or soil employed (Bergna *et al.*, 2018).

The variation, assembly, and composition of rhizobacterial communities were systematically investigated in 11 tomato cultivars, combined with one cultivar in seven different sources of soil and growth substrate (five natural field soils and two artificial commercial nutrient soils) (Cheng *et al.*, 2020).

The tomato rhizosphere microbiota was dominated by bacteria from the phyla Proteobacteria, Bacteroidetes, Acidobacteria, followed by Verrucomicrobia, Planctomycetes, Actinobacteria and Gemmatimonadetes. Proteobacteria was the predominant phylum with abundant Rhizobiales, Xanthomonadales, Burkholderiales, Nitrosomonadales, Myxococcales, Sphingobacteriales, Cytophagales and Acidobacteria. The results showed that the assembly process of rhizosphere bacterial communities was influenced by soil, including the available bacterial sources and biochemical properties of the rhizosphere soils, and plant genotype.

In addition, the tomato plants grown in different soils harbored rhizobacterial communities that varied significantly in structure and composition. They varied significantly between the five natural field soils and two artificial commercial nutrient soils. In general, similar community compositions of rhizosphere microbiotas were demonstrated in tomato cultivars, whereas the soil had the prevalent role in shaping the assembly and composition of the rhizosphere microbiome (Cheng *et al.*, 2020).

Dong and co-authors (2019) provided comprehensive insight into the bacterial communities associated with tomato plants analyzing the rhizosphere, phyllosphere and endosphere of roots, stems, leaves, fruits and seeds. The bacterial communities from the rootzone soil and rhizosphere showed the highest richness and diversity. The lowest bacterial diversity was observed in the phyllospheric samples, while the lowest richness occurred in the endosphere. Among the endophytic samples, both bacterial diversity and richness varied in different tissues, with the highest values in roots. In general, the richness decreased from root zone soil to rhizosphere to phyllosphere to endosphere, while the diversity decreased in an altered order: root zone soil > rhizosphere > endosphere > phyllosphere. Proteobacteria was the most abundant phylum in the tomato-associated community (except for the seeds and jelly, where Firmicutes were also dominant). At the genus level, the sequences of *Pseudomonas* and *Acinetobacter* were prevalent in the rhizosphere, and in the phyllosphere. For the endophytes, *Acinetobacter*, *Enterobacter*, and *Pseudomonas* were the abundant genera in the roots, stems and leaves.

Whereas, in the fruits, the bacterial endophytes varied in different compartments, with *Enterobacter* being enriched in the pericarp and seeds, *Acinetobacter* in the placenta, and *Weissella* in the jelly. Comparison of the bacterial communities associated with tomato plants reveals both ubiquitous and specific members in different sample types. Proteobacteria, Actinobacteria, Chloroflexi, Firmicutes, Acidobacteria and Gemmatimonadetes were the abundant phyla in the root zone soil, while in the rhizosphere, only Proteobacteria were enriched, and of this phylum, *Pseudomonas*, *Acinetobacter*, *Enterobacter* and *Rhizobium* were the abundant genera, confirming the results from other studies on the tomato rhizosphere (Ottesen *et al.*, 2013; Lee *et al.*, 2016). In the phyllosphere of tomato plants, only *Acinetobacter* was abundant, and the epiphytic bacterial communities from stems and leaves showed high similarity. In roots, stems, and leaves, the bacterial genera *Acinetobacter*, *Enterobacter*, *Pseudomonas* and *Pantoea* were abundantly present. Of them, only *Acinetobacter* overlapped with a previous report performed on the tomato leaf endosphere (Romero *et al.*, 2016), while the genera *Enterobacter*, *Pseudomonas* and *Pantoea* were also identified as endophytes in other plant hosts (Rosenblueth and Martínez-Romero, 2006; Afzal *et al.*, 2019).

Another recent study conducted a preliminary investigation of three kingdoms (bacteria, fungi, and archaea) in the rhizosphere, endosphere, and bulk soil samples of tomato plants (Lee *et al.*, 2019) (Figure 6). Distinct microbial communities were identified, according to tomato rhizo-compartments, regardless of differences in soil characteristics, and examined for species diversity, effects of edaphic factors, representative taxa, microbial network topology, and predictive functional gene profiles (Lee *et al.*, 2019). The results showed that the bacterial and fungal communities in the bulk soil and rhizosphere were correlated with soil physicochemical properties (pH, electrical conductivity, and exchangeable cation levels), while this trend was not evident in the endosphere samples. A small number of core bacterial operational taxonomic units (OTUs) were present in all samples from the rhizosphere and endosphere. Among these core microbes, OTUs belonging to the genera *Acidovorax*, *Enterobacter*, *Pseudomonas*, *Rhizobium*, *Streptomyces*, and *Variovorax*, members of which are known to have beneficial effects on plant growth, were relatively more abundant in the endosphere samples. Proteobacteria was the first most abundant phylum. At the class level, the relative abundances of Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria were similar in the bulk soil. In the rhizosphere, the relative abundance of Alphaproteobacteria was the highest, while the relative abundances of Betaproteobacteria and Gammaproteobacteria were higher than that of Alphaproteobacteria in the endosphere. Actinobacteria was the second most abundant phylum. The relative abundances of Bacteroidetes and Firmicutes in the endosphere were lower than those in the bulk soil and rhizosphere. The archaeal

and fungal communities were predominated by a few dominant phyla. The most dominant phylum was Thaumarchaeota, followed by Euryarchaeota in archaeal communities, whereas Ascomycota was the most abundant phylum in fungal communities. However, they occupied exclusively the endophytic fungal communities, while the bulk soil and rhizosphere harbored significant proportions of Basidiomycota and Zygomycota (Lee *et al.*, 2019).

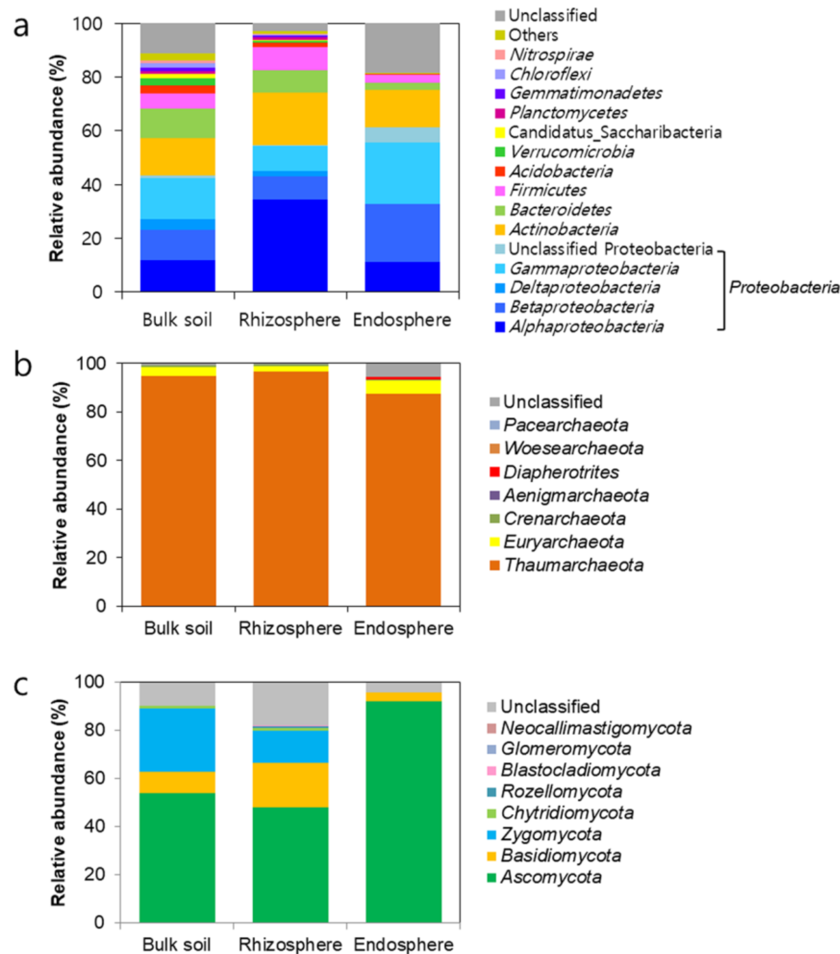

Figure 6: Comparison of taxonomic distributions between rhizocompartments. The average relative abundances of bacterial (**a**), archaeal (**b**), and fungal (**c**) phyla are represented according to rhizocompartment. The phyla with relative abundances less than 1% were classified into "Others" (From Lee *et al.,* 2019) https://creativecommons.org/licenses/by/4.0/.

1.4.6.Effect of plant diseases on tomato microbiome

There are only a few studies dealing with the influence of diseases on tomato microbiome. Two papers dealt with the structure and functional potential of communities in tomato plants infected by *Ralstonia solanacearum* (Li *et al.*, 2014b; Kwak *et al.*, 2018). Li and

co-authors (2014) investigated healthy and diseased tomato rhizospheres by 454 pyrosequencing to evaluate the changes in the bacterial communities inhabiting the rhizospheric soil and roots of tomato plants. They observed a significant shift in the microbial composition of diseased samples compared with healthy samples, which had the highest bacterial diversity. In terms of microbial activity, functional diversity was suppressed in diseased soil samples. The predominant phylum was Proteobacteria in all samples. The class Gammaproteobacteria was more abundant in healthy than in diseased samples, while the Alphaproteobacteria and Betaproteobacteria were more abundant in diseased samples. They observed the presence of Bacteroidetes and Actinobacteria in disease-free samples was promoted in both samples, suggesting the rapid propagation of these microorganisms in the presence of soil-borne pathogens. This is interesting because these phyla are typical bulk-soil inhabitants and represent stable components of the microbial ecosystem. Actinobacteria are known antibiotic producers, which might play a fundamental role in the maintenance of soil ecosystems through the production of antibiotics to counteract *R. solanacearum* infection. The proportions of the bacterial populations showed a similar trend both in rhizosphere soil and plant roots in diseased versus healthy samples.

Kwak *et al.* (2018) analysed the rhizosphere microbiomes of two different tomato varieties, the Hawaii 7996 (resistant to the soil-borne pathogen) and the Moneymaker (susceptible). They observed that the tailoring of rhizosphere microbiota from the soil milieu was plant genotype-specific driven by selection and coevolution of soil-borne strains. As if disease-resistant tomatoes may recruit bacterial allies to protect themselves from infection. In general, the results showed that species richness indices indicated that the number of OTUs in the plant rhizosphere was reduced compared with bulk soil samples. In all samples, species richness was reduced in the first sampling compared with the second sampling. At the first stage of growth the rhizosphere of Hawaii 7996 showed ≥1%outgrowth of Bacteroidetes among the phyla. At family levels Flavobacteriaceae, Sphingomonadaceae, and Pseudomonadaceae, appeared to be more abundant in Hawaii 7996 among those with populations higher in the rhizosphere than in bulk soil. Whereas, unclassified Alphaproteobacteria (more abundant in Moneymaker), unclassified Proteobacteria, Comamonadaceae and Oxalobacteraceae, the latter two belonging to Betaproteobacteria, seemed predominant in Moneymaker.

Another study investigated the rhizomicrobiome of tomato (cv. Jiabao, a tomato cultivar susceptible to *Meloidogyne incognita*) in association with infection by root knot nematodes (caused by *Meloidogyne incognita*), to observe the responses of bacterial communities during nematode pathogenesis (Tian *et al.*, 2015). Endophytes in the nematode-infected root were dominated by Streptomycetales and Micromonosporales, followed by Rhizobiales, Sphingomonadales, Burkholdales, and Pseudomonadales. Comparative community analysis of rhizospheric and endophytic bacteria in healthy and nematode-infected tomato roots showed that nematode pathogenesis revealed a decreased abundance of the predominant endophytic groups Streptomycetaceae and Pseudomonadales, both of which were known to produce a considerable diversity of active compounds against plant pathogens. The results indicated that infection by root-knot nematodes reassembled microbial communities of the root microbiome in diseased tomato host, especially in the specialized root galls formed by nematode infection (Figure 7).

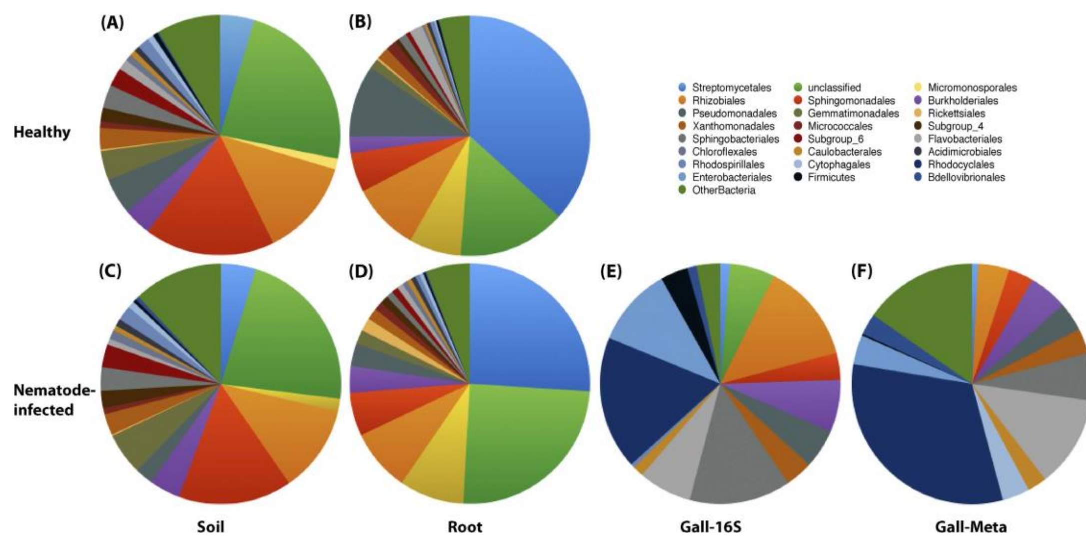

Figure 7. The composition and relative abundance of major bacterial taxa of the root-associated microbiome in healthy and nematode-infected tomato. (**A**) Soil rhizobacteria around the healthy tomato root (HRS); (**B**) Endophytes inhabiting the healthy tomato root (HRC); (**C**) Soil rhizobacteria around the nematode-infected tomato root (IRS); (**D**) Endophytes inhabiting the nematode-infected tomato root (IRC); (**E**) Community composition of the root gall-associated microbiome in the nematode-infected tomato based on 16S rRNA gene-based amplicon (Gall-16S); (**F**) Community composition of the root gall-associated microbiome in the nematode-infected tomato using the taxa-based extracted reads from sequenced shot-gun metagenome (Gall-Meta) (From Tian *et al.,* 2017) https://creativecommons.org/licenses/by/4.0/.

According to metagenomic analysis of the root gall-associated microbiome the nematode associated bacterial groups seemed to be involved in several key infection processes during nematode pathogenesis in tomato root, including reinforcing plant cell walls

destruction at the nematode feeding sites, or allowing a mutualistic relationship involving provision of nutrients.

Larousse and co-authors (2017) investigated the interactions between pathogenic oomycete (*Phytophthora parasitica*) and microbiota residing on the surface of the tomato root. They analysed the composition of the microbiome of tomato roots either free of or partly covered with *P. parasitica* biofilm. Their results suggest a *P. parasitica*-associated shift involving a Bacteroidetes/Proteobacteria transition in microbiota composition at the root surface. The Bacteroidetes dominate colonization of tomato roots inoculated with pathogen. The infection of a host plant by *P. parasitica* relies on the secretion of plant cell wall-degrading enzymes that leads to successful penetration of the host and to subsequent acquisition of nutrients (Blackman *et al.*, 2014). The ability of the oomycetes to efficiently depolymerize polysaccharides could contribute to the observed enrichment of Bacteroidetes within *P. parasitica*-associated microbiota.

An interesting study by Chialva *et al.* (2018) describes the responses of two tomato genotypes (susceptible or resistant to *Fusarium oxysporum* f. sp. *lycopersici*) grown on an artificial growth substrate and two native soils (conducive and suppressive to *Fusarium*) using transcriptomics, proteomics, and biochemistry. Their experiments, on two tomato genotypes growing in two native soils with different physicochemical and biological properties, have revealed some novel plant responses, which help to understand how crops respond to the stimuli that originate from the biotic and abiotic components of soils. Transcriptomics and proteomics demonstrated that the overall characteristics of the substrate contribute more than plant genotype to shaping the molecular responses in tomato roots, and that only few genes respond differently in tomato plants grown in the two different native soils. This means that, notwithstanding the significant abiotic and biotic differences of the soils, tomato roots seem to display a broadly similar expression profile when grown in native soils, as compared with roots grown in the control substrate.

1.4.7. Recent results on culturable microbial communities

Culture-dependent methods have been used to study microbial communities of the tomato root environment, mainly aimed at selecting plant growth promoting rhizobacteria and biocontrol agents, and a high number of papers reports on the isolation of biocontrol agents from tomato plants. However, only very few studies, recently published, deal with

the isolation from different compartments of the plant or even different compartments of the rhizosphere. In this paragraph we report on recent studies based on the use of a DNA barcode marker to identify bacterial isolates.

PGPR and BCA bacteria from the rhizosphere and endosphere have been searched in different tomato cultivars (Abbamondi *et al.*, 2016). The authors selected 23 bacterial strains some of which tested positive for the following PGP traits: 73% were able to produce organic acids, 89% indole acetic acid, 83% 1-aminocyclopropane-1-carboxylate deaminase, and 87% siderophores. However, the most interesting result was the remarkable increase in the formation of root hairs as observed in *Arabidopsis thaliana* seedlings inoculated with the isolated endophytes, and for the 50% of the seedlings inoculated with the rhizospheric strains. Taxonomic identification by 16S rDNA of a subset of bacterial strains showed they belonged to the genera *Agrobacterium* (5), *Microbacterium* (2), *Bacillus* (2), *Rhizobium* (2), *Ensifer* (1), *Chryseobacterium* (1), *Pseudomonas* (1), and *Rhodococcus* (1).

An increasing interest has been devoted to the isolation and role of the beneficial bacteria isolated from the endorhizosphere. Two recent papers are clearly explicative of the approach of this kind of study. Tian *et al.* (2017) attempted to identify plant growth-promoting endophytes within the bacterial groups identified as part of the core tomato root microbiome. They selected 49 bacterial endophytic strains, identified as members of the phyla of Firmicutes and Proteobacteria based on 16S rRNA gene sequences. The results indicated that the isolates clustered into groups of the orders Pseudomonadales, Enterobacteriales, Rhizobiales, Burkholderiales, and Xanthomonadales, representing the majority of the Proteobacterial groups of tomato root endophytes, in addition to the phylum Firmicutes. *In vitro* bioassays showed that most strains (31 of the 49 isolated endophytic strains) showed antagonistic activity against some microbial targets. However, the microorganisms tested were more bio indicators for the antagonistic activity than tomato pathogens (*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Aspergillus niger*). In particular, the authors observed that the endophytic isolates with antimicrobial activities were from the genera *Bacillus* and *Pseudomonas*. Almost all the isolated tomato root endophytic strains demonstrated an ability to promote plant growth. Their results suggested that in tomato root endophytes, most of the tested plant host-benefiting traits were derived from bacteria of three genera, *Pseudomonas*, *Bacillus*, and *Rhizobium*. The majority of *Pseudomonas* spp. showed the most promising potential in promoting plant growth, while other species demonstrated their ability as biological control agents, e.g. *Bacillus* spp., especially for the fungal targets.

Similarly, high temperature resistant bacterial isolates were searched in tomato root endosphere (Singh *et al.*, 2019) to select bacterial isolates able to survive at 45–47°C. They further selected five isolates identified as *Rhizobium pusense* (MS-1), *Bacillus flexus* (MS-2), *B. cereus* (MS-3), *Methylophilus flavus* (MS-4) and *Pseudomonas aeruginosa* (MS-5). These strains showed plant growth promoting traits such as phosphate solubilization, production of IAA, siderophore, ammonia, and nitrate reductase.

A large collection of 200 bacteria from the rhizosphere of healthy tomato plants grown in fields with a history of severe soil-borne diseases and mainly crown and root rots was screened for antagonistic activity *in vitro* against *Sclerotinia sclerotiorum* and *Rhizoctonia solani* (Ben Abdeljalil and Vallance, 2016). A subset of 25 most effective isolates, leading to suppression of both fungi by more than 45% over control was composed by bacteria, as assessed by sequencing of 16S rRNA and *rpo*B genes of four genera, namely *Bacillus*, *Chryseobacterium*, *Enterobacter*, and *Klebsiella* being the most frequent species *B. amyloliquefaciens*, *B. thuringiensis*, *B. megaterium*, *B. subtilis*, *E. cloacae, C. jejuense*, and *K. pneumoniae.* A high number of these strains showed plant growth-promoting properties (i.e. siderophore and indole-3-acetic acid (IAA) production, phosphate solubilization) and a large metabolic activity as they were able to use a wide range of carbon sources. In addition, the presence of genes encoding fengycin A and bacillomycin D biosynthesis was assessed by PCR amplification in 18 and 16 isolates, respectively. The 25 tomato-associated rhizobacterial isolates were assessed for their ability to utilize carbon sources using Biolog™ Ecoplates system. Average well-color development (AWCD) values were found to be positively correlated with the Shannon diversity index. Their results indicate that these native tomato-associated rhizobacteria displayed a large metabolic activity and they were able to use a wide range of carbon sources.

An interesting approach to biocontrol of phytopathogens aiming in defining the possible role of repressive analyzing the cultivable microorganisms was followed by Poli *et al.*, (2016) which had examined the effect of plant genotype, of soil, and of *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) on the cultivable component of rhizosphere and root-associated mycobiota of tomato. The structure of the mycobiota was significantly affected by the soil type in the rhizosphere as well as by the plant genotype within the roots. In addition, upon inoculation of *Fol* a change in the community structure was observed, particularly in soil A, where *Penicillium* spp. and *Fusarium* spp. were the dominant responding fungi. In the overall they observed that the soil is the major driving force in shaping the cultivable mycobiota, where *Fusarium*, *Penicillium*, *Sarocladium*, and

Trichoderma genera resulted dominant. In terms of quantitative evaluation, a significantly higher fungal load for the susceptible cultivar was measured in the rhizosphere of one of the soils.

All small sets of bacterial isolates from the tomato rhizosphere were analysed and selected based on the ability to show at least one of the five PGPB activity tested, being ammonia production the most common one (Sunera *et al.*, 2020). The authors did not show a clear relationship between the source of an isolate (rhizosphere or endosphere) and its PGPB activity, although a large proportion of endophytic isolates (50%) produced IAA, greater than rhizospheric isolates (10%). Two selected strains identified as *Bacillus cereus* isolated from the rhizosphere, and *Klebsiella variicola* isolated from root endosphere using 16S rRNA sequences were further used for *in planta* assays showed a higher ability in boosting plant growth and higher yield. However, in tomato, only one growth variable (shoot length) was increased over the control by one of the selected bacterial strains (*B. cereus*), whereas in mung bean, inoculation with either strain *B. cereus* or *K. variicola* increased shoot length and dry weight.

Analyzing three independent tomato rhizospheres Attia *et al.* (2020) selected 40 bacterial isolates with different colonies' morphology. Three strains were characterized as being able to effectively suppress *Alternaria solani* causing early blight, a disease of the epigean plant part. Molecular characterization by 16S rRNA gene allowed them to assign them to the species *Bacillus subtilis*, *Lysinibacillus fusiformis* and *Achromobacter xylosoxidans*. *In vivo* efficacy reduced the disease severity by 13.0% and recorded highly-protection percent (84.3%) when compared to non-treated plants. The infected plants pre-treated with PGPR for one week before *A. solani* infection, showed the most powerful effect in terms of the length of shoots and roots and the number of leaflets per plant. *A. solani* conidia showed remarkable morphological modifications after the treatment with PGPR strains.

Complexity and abundance of endophytic communities variability, according to the site of origin, was observed by Chaouachi *et al.* (2021) which isolated 50 bacterial strains from different organs of tomato plants sampled from six localities in Cape Bon region (Tunisia). The *in vitro* dual culture assays showed that 36% of the endophytic bacterial strains produce antifungal volatile organic compounds (VOCs) against *Botrytis cinerea*. By using 16S rRNA gene sequences 18 antagonistic endophytic bacteria strains selected based on antagonistic activity *in vitro* were identified as (2) *Bacillus amyloliquefaciens*, (5) *B. velezensis, B. vallismortis, B. pseudomycoides,* (2) *B. subtilis, B. toyonensis*, (2) *B. thuringensis*, *B. proteolyticus*, *B. nakamurai*, and *Enterobacter asburiae*, and *E. cloacae*. No statistical association between the endophytic bacterial species and their antifungal

effect on S2 or S5 B. Further studies on VOCs produced by five selected strain supported the results that they could be used to reduce postharvest decay of *B. cinerea*, and that the VOC 3-Methylbutan-1-ol is a promising antifungal volatile for postharvest commercialization of tomato fruit.

Tomato fungal endophytes and the implication on PGP and BC activity has been recently published (Sinno *et al.*, 2020). Several fungal endophytes species are good versatile BCA, controlling both pests and pathogens as demonstrated in the case of *Botrytis bassiana*, *Fusarium oxysporum*, *Niger solani,* and *Thicoderma harzianum,* which are amenable candidates as plant beneficial microbes, also considering their additional properties as plant biostimulants. Nonetheless, a few surveyed papers considered the possibility to use fungal endophytes species as a multi-use biocontrol agent, evaluating the simultaneous biocontrol of both pests and pathogens in tomato. Another interesting, potential application that has been poorly explored, is the possibility to use different fungal endophytes species in a consortium and/or with other beneficial microbes (reviewed in Sinno *et al.*, 2020).

2. Bioprospecting of Beneficial Bacteria Traits Associated with Tomato Root in Greenhouse Environment Reveals that Sampling Sites Impact more than the Root Compartment

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Abstract

Tomato is subject to several diseases that affect both field- and greenhouse-grown crops. To select cost-effective potential biocontrol agents, we used laboratory throughput screening to identify bacterial strains with versatile characteristics suitable for multipurpose uses. The natural diversity of tomato root-associated bacterial communities was bioprospected under a real-word environment represented by an intensive tomato cultivation area characterized by extra-seasonal productions in the greenhouse. Approximately 400 tomato root-associated bacterial isolates, in majority Gram-negative bacteria, were isolated from three compartments: the soil close to the root surface (rhizosphere, R), the root surface (rhizoplane, RP), and the root interior (endorhizosphere, E). A total of 33% of the isolates produced siderophores, and were able to solubilise phosphates and grow on NA with 8% NaCl. A total of 30% of the root-associated bacteria showed antagonistic activity against all the tomato pathogens tested, i.e. *Clavibacter michiganesis* pv. *michiganensis*, *Pseudomonas syringae* pv. *tomato*, *P. corrugata* and *Xanthomonas euvesicatoria* pv. *perforans* and *Fusarium oxysporum* f. sp. *lycopersici*. We found that the sampling site rather than the root compartment of isolation influenced bacterial composition in terms of analysed phenotype. This was demonstrated through a diversity analysis including general characteristics and PGPR traits, as well as biocontrol activity in vitro. Analysis of 16S rRNA gene (rDNA) sequencing of 77 culturable endophityc bacteria that shared multiple beneficial activity revealed a predominance of bacteria in Bacillales, Enterobacteriales, and Pseudomonadales. Their *in vitro* antagonistic activity showed that *Bacillus* spp. were significantly more active than the isolates in the other taxonomic group. *In planta* activity against phytopathogenic bacteria of a subset of *Bacillus* and *Pseudomonas* isolates was also assessed.

Keywords: Microbiome, Tomato, PGPR, BCA, Endorhizosphere.

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2.1. Introduction

Tomato is one of the most widely grown vegetables and represents a major agricultural industry, with a global production of over 180 million tons in 2018 (http://faostat.fao.org). It is one of the vegetables that is most consumed in the world (second to potatoes), and is also one with the most beneficial effects on human health (He *et al.*, 2006). Plant diseases seriously impact tomatoes in several geographical areas worldwide. At least 140 viral species have been reported, some of which have emerged in greenhouse grown tomato plants (Moriones and Verdin, 2020). Several bacterial species were described causing leaf spots, vascular diseases and roots (Catara and Bella, 2020). In addition, intensive greenhouse cropping systems have greatly facilitated the development of fungal and fungal-like diseases (Bardin and Gullino, 2020).

The intensive management required to mitigate serious economic losses, has encouraged the search of alternative approaches for the control of tomato diseases, including the use of biological control agents (Singh *et al.*, 2017).

Instead of an independent entity, according to the most recent definition, the plant is regarded as a holobiont or "super organism" that is integrated with the microorganisms associated with it (microbiota), and their genetic information (often referred to as the microbiome) (Vandenkoornhuyse *et al.*, 2015). The microbiome is involved in multiple plant functions, ranging from nutrition to resistance to biotic and abiotic factors (Hardoim *et al.*, 2008; Mendes *et al.*, 2011). The productivity, vigor and resistance of the plant is therefore not only the direct consequence of the genetic makeup of the plant itself, but also of its microbiome or set of microorganisms (Philippot *et al.*, 2013; Berg *et al.*, 2016).

There is a relatively large body of information on the tomato microbiome as many studies have explored the mechanisms of microorganism selection in different compartments of the plants, also identifying beneficial microorganisms and potential candidates for biological control. Metagenomic studies based on amplicon sequencing have identified the microbial communities associated with different tomato plant organs (Ottesen *et al.*, 2013). An interesting gradient with regard to the distance of each plant part from the soil has been observed as microbial diversity decreases as the distance from the soil increases (Ottesen *et al.*, 2013; Dong *et al.*, 2019). The most attention has been paid to the rhizosphere where there is a highly active microbial interaction as exudates released by plant roots are the main food source for microorganisms and a driving force for their population density and activities (Raaijmakers *et al.*, 2009; Bulgarelli *et al.*, 2013). A subset of rhizospheric microorganisms penetrates the plant roots and colonizes the

endosphere (horizontal transmission) (Compant *et al.*, 2010). Vertical transmission of bacterial endophytes via-seeds has been also reported in different crops (Truyens *et al.*, 2015; Cavazos *et al.*, 2018; Rezki *et al.*, 2018). These endophytes reside within plants with no obvious negative effects on the host, contributing to their growth and development and the ability to adapt to adverse conditions (Vandenkoornhuyse *et al.*, 2015; Sinno *et al.*, 2020). Tomato rhizo- and endorhizosphere microbial communities have been investigated according to soil characteristics (Poli *et al.*, 2016), genotypes (French *et al.*, 2020; Taffner *et al.*, 2020b), crop management (Allard *et al.*, 2016), rootstocks (Poudel *et al.*, 2019), and soilborne pathogen infections (Li *et al.*, 2014b; Tian *et al.*, 2015; Larousse *et al.*, 2017). Overall, the results suggest that the tomato endophytic microbiome is mainly horizontally transferred from the soil environment (Poli *et al.*, 2016; Chialva *et al.*, 2018), but also vertically transmitted via seeds from where it can be transmitted to the subsequent plant generation (Bergna *et al.*, 2018). Culture-dependent methods have been used to study microbial communities of the tomato root environment, mainly aimed at selecting plant growth promoting rhizobacteria and biocontrol agents (Abbamondi *et al.*, 2016; Ben Abdeljalil and Vallance, 2016; Tian *et al.*, 2017; Attia *et al.*, 2020; Sunera *et al.*, 2020). Microorganisms may have a neutral, pathogenic, or beneficial interaction with their host plant, and together with plant pathogens, beneficial microorganisms in the plants can interact in different ways with the plant (Raaijmakers *et al.*, 2009). The main roles of beneficial microorganisms are biostimulation (or phytostimulation), i.e. the direct promotion of plant growth by the production of phytohormones (Bloemberg and Lugtenberg, 2001); biofertilization (Bashan, 1998), i.e. the promotion of plant growth generated by the microorganisms that facilitate accessibility to essential nutrients or increase the supply of nutrients to the plant; and biocontrol activity, i.e. the ability to control plant pathogens (BCAs) through the competition for space and nutrients, the production of antibiotic substances or the induction of resistance mechanisms (Bloemberg and Lugtenberg, 2001; Heimpel G. E and Mills N.J, 2017). Bacteria that share at least two of these mechanisms of action are known as Plant Growth Promoting Rhizobacteria (PGPR) (Glick, 1995). The use of microorganisms, alone or combined in consortia, is foreseen as a method to positively modify the plant microbiome in order to improve the quantity and quality of agricultural crops. It has shown great potential as a low-environmental-impact alternative to agrochemicals and fertilizers (Ciancio *et al.*, 2016; Woo and Pepe, 2018; Compant *et al.*, 2019).

Microbiome studies based on metagenomics have greatly contributed to the understanding of the complex network established between the tomato rhizosphere and its microbiota. However, the information gained to date mainly refers to identify the

microbiota, understanding where they come from and what the main driving conditions are that modify the microbiota but not what their actual role is. To date, cultivation-dependent methods have been used to isolate and characterize bacterial isolates from tomato plants exhibiting appreciable PGPR and BCA capabilities (Enya *et al.*, 2007; Amaresan *et al.*, 2012; Xu *et al.*, 2014; Abbamondi *et al.*, 2016; Romero *et al.*, 2016; Tian *et al.*, 2017; Attia *et al.*, 2020; Sunera *et al.*, 2020). None of the studies on tomatoes have used a systematic sampling method with a high number of bacterial isolates in order for them to be compared with the origin of the isolation. On the other hand, these kinds of studies have been conducted for other crops (Vacheron *et al.*, 2016; Besset-Manzoni *et al.*, 2019).

Within the framework of a project on tomato microbiota aimed at the selection of bacterial isolates to be used in microbial consortia for seed or plantlet bacterization in the nursery, we investigated the diversity of the cultivable bacterial population associated with the tomato root environment. We particularly focused on bacterial endophytes in terms of being beneficial biocontrol agents. Samples were collected from farms from a restricted area specialized in the intensive cultivation of tomato under a greenhouse environment in Ragusa province (Sicily). This is the principal production area in Italy that uses greenhouses covered by plastic films, with more than half of the national tomato production. This area is characterized by sandy soil, high salinity conditions and favorable climatic conditions that permit extra-seasonal productions (up to two cycles a year), above all of cherry tomato typologies.

The main findings of our work were: *i)* cultivable bacterial population sizes in the root is higher in the rhizosphere and in the rhizoplane than in the endosphere compartment *ii)* the site of isolation (i.e. farm and agricultural conditions) rather than the root compartment drives the phenotypic characteristic of bacterial populations; *iii*) efficient cultivable bacteria from tomato endorhizosphere belong to Bacillales, Pseudomonadales, and Enterobacteriales order; *iv*) *Bacillus* spp. are significantly more effective in inhibiting tomato plant pathogens *in vitro*; *v*) preliminary *in vivo* results showed some *Pseudomonas* and *Bacillus* isolates from the endorhizosphere may protect tomato plants against plant pathogenic bacteria and thus deserve further investigation.

2.2. Materials and Methods

2.2.1. Sampling of tomato root-associated bacteria

Tomato plants (*Solanum lycopersicum* L.) were grown in unheated greenhouses on four farms located in an area devoted to greenhouse vegetable production in Ragusa province (Sicily, Italy). The positions, soil properties, and genotypes are shown in Table 1. Plants were grown in agricultural soil and watered by drip-irrigation, following standard agronomic practices. Five healthy plants from each farm, were randomly selected from the central rows of each greenhouse, and their associated root material was collected at the fruiting stage, in March 2018. Plant stems were cut 30 centimeters above the root collar, and the five root systems were placed in a plastic bag and immediately transferred to the laboratory in a cooler. The samples were preserved at 4°C and processed within 24 h.

Tomato root-associated bacteria were isolated from three compartments: the soil close to the root surface (rhizosphere, R), the root surface (rhizoplane, RP), and the root interior (endorhizosphere, E). Samples were processed according to the protocol described by Normander and Prosser (2000) and Wieland *et al.*, (2001), with some modifications, as follows:

- Rhizosphere (R): roots were shaken carefully to remove non-adhering soil. Five grams of soil adhering to the roots were manually collected and transferred in sterile 50 mL centrifuge tubes containing 20 mL of sterile saline buffer (0.85% NaCl), and then mixed thoroughly by vortex for 2 min.
- Rhizoplane (RP): roots (approx. 5 g), from which the rhizospheric soil had been dislodged, were soaked in 20 mL of sterile saline buffer (0.85% NaCl) and mixed thoroughly by vortex for 5 min.
- Endorhizosphere (E): after treatment for rhizoplane bacteria extraction, roots (approx. 5 g) were sterilized with 75% ethanol (2 min), 50% sodium hypochlorite solution (2 min) and ethanol 75% (1 min), and rinsed five times in sterile distilled water (SDW). Sterility was assessed by placing the sterilized roots on Potato Dextrose Agar (PDA, Oxoid, Milan, Italy) at 27°C for 4-7 days. A lack of bacterial growth ensured the sterility of the root surfaces. The roots were then homogenized with a sterile pestle and mortar in 20 mL of sterile saline buffer (0.85% NaCl).

Table 1: Data on sampling sites and number of bacterial isolates from the tomato root environment.

	Farm 1	**Farm 2**	**Farm 3**	**Farm 4**
Position				
Locality	Ispica (RG, Italy)	Ispica (RG, Italy)	Ragusa (Italy)	Vittoria (RG, Italy)
Geographic coordinates	36°42'35.62"N 14°57'36.13"E	36°42'59.08"N 14°58'59.98"E	36°51'3.24"N 14°27'41.40"E	36°56'40.49"N 14°23'42.37"E
Soil properties				
Soil texture	Sandy clay calcareous loamy	Sandy calcareous	Sandy calcareous	Sandy calcareous loamy
Organic matter (%)	1.93	2.1	1.07	2.5
pH	7.57	7.72	7.71	7.7
Electrical conductivity (mmhos cm^{-1})	2.85	8.45	2.13	3.52
P (mg kg^{-1})	102	655	135	155
Zn (mg kg^{-1})	1.9	11.6	6.9	5.9
Mn (mg kg^{-1})	22.8	32.4	14.4	13.2
Cu (mg kg^{-1})	6.1	13.2	4.8	14.4
Fe (mg kg^{-1})	12.2	49.2	15.6	4.6
K (mg kg^{-1})	391	507	96	747
Mg (mg kg^{-1})	254	327	203	529
Na (mg kg^{-1})	158	340	156	290
Ca (mg kg^{-1})	221	925	202	290
Tomato genotype				
Typology	Cherry	Mini plum	Cherry	Mini plum
Genotype	Casarino F1	Dulcemiel F1	Creativo F1	Miele F1
Number of isolates	70	132	85	136

2.2.2.Culturable bacterial population sizes

Serial ten-fold dilutions in sterile saline buffer (0.85% NaCl) were prepared from each extract (R, RP and E), and 0.1 mL of each dilution was plated onto the following media: Plate Count Agar (PCA; Lickson, Palermo, Italy), supplemented with cycloheximide (100 mg·mL^{-1}) to isolate and quantify the cultivable fast-growing bacteria; King's medium B agar (KB), supplemented with cycloheximide (100 mg·mL^{-1}) to count the fluorescent pseudomonads (King *et al.*,1954). In order to isolate spore-forming bacteria, each extract was heat-treated (90°C) for 10 min and mixed by vortex for 1 min (Janštová and Lukášová, 2001; Manzum and Mamun, 2019), and after serial ten-dilutions, 0.1 mL of

suspensions were plated onto Nutrient Agar (NA; Oxoid, Milan, Italy) with cycloheximide (100 $mg{\cdot}mL^{-1}$). For each compartment, dilution, and medium, three replicates were performed. The inoculated plates were incubated at 27°C for 48-72 h and the number of bacterial colony-forming units (CFUs) was then counted by visual observation and selected colonies were isolated in pure culture. The culturable population of tomato-associated bacteria was expressed as the log of the number of CFUs per gram of soil (rhizosphere) or of roots (rhizoplane, and endorhizosphere). The root-associated bacteria were selected from plates containing 30-300 colonies, i.e., typically 10^{-2} endorhizosphere (1:100) and 10^{-5} rhizosphere and rhizoplane dilutions (1:100000). Bacterial colonies were selected according to their macro morphological diversity (size, colour, and morphology of the colony), streaked twice on PDA medium and checked for purity. After 24 h of incubation, single colonies of the selected isolates were picked off and individually inoculated with a sterile toothpick in 96 microwell cell culture plates (Nunc™ MicroWell™ 96-Well, Collagen Type I-Treated, Flat-Bottom Microplate, Thermo Fisher Scientific) containing Luria-Bertani (LB) broth. After overnight incubation, the wells were supplemented with 15% glycerol and stored at -80°C. For routine growth, isolated bacteria were picked off from stock cell cultures using an 8x6 replica plater (Sigma).

2.2.3.Phenotypic characterization of bacterial isolates

2.2.3.1. General and PGPR traits

Colonies of bacterial isolates were preliminarily characterized in terms of color, shape, opacity, size, and morphology. The Gram reaction was performed using the 3% KOH test (Schaad *et al.*, 2001). The following features were assessed: siderophore production, salt tolerance, phosphate solubilization. Bacterial isolates from 24 h old cultures on PDA were plated using the replica-plate device (48 isolates per plate) in the respective media, and results were recorded for up to three days of incubation at 28°C. All strains were tested in three independent replicates.

To detect the phosphate solubilizing bacteria, bacterial isolates were streaked onto Pikovskaya's agar medium (Pikovskaya, 1948). Strains that induced a clear zone around the colonies were considered as positive. Siderophore production was determined on chrome-azurol S (CAS) medium (Schwyn and Neilands, 1987). The formation of orange

to yellow halos around the colonies confirmed the production of siderophores. The salt tolerance was evaluated by inoculating the isolates on three NA plates containing 0%, 2%, and 8% NaCl. Bacterial isolates were classified based on their growth at different NaCl concentrations in the medium.

2.2.3.2. Antimicrobial activity against tomato pathogens

To phenotype the biocontrol activity potential of the tomato root-associated bacteria, these bacteria were screened for their antimicrobial activity against a set of tomato plant pathogens usually occurring in the area: the Gram-positive bacterium, *Clavibacter michiganensis* subsp. *michiganensis* strain PVCT156.1.1 (*Cmm*), and the Gram-negative bacteria, *Pseudomonas corrugata* strains CFBP5454 (*Pco*), *P. syringae* pv. *tomato* strains PVCT28.3.1 (*Pto*), *Xanthomonas euvesicatoria* pv. *perforans* strain NCPPB4321 (*Xep*), and *Fusarium oxysporum* f. sp. *lycopersici* strain Saitama ly2 (*Fol*) (Table 2).

The antagonistic activity against plant pathogenic bacteria was tested on large PDA plates (Ø 20 cm). Bacterial suspensions in SDW (OD600 = 0.01) were obtained from overnight cultures of the plant pathogenic bacteria in Nutrient Broth (NB). A sterile swab was dipped into the inoculum tube and used to inoculate the plates by streaking the swab three times over the entire agar surface, and then rotating the plate approximately 60 degrees each time, as in the Kirby-Bauer antibiotic resistance test (Hudzicki, 2009). After drying, the plates were spot-inoculated with bacterial isolates for testing using sterile toothpicks. Forty-eight bacteria were inoculated on each plate and incubated at 28°C for 1-5 days. The antagonistic activity was expressed as the width (mm) of the growth inhibition area of phytopathogenic bacterium around the bacterial colonies. The experiments were performed in three independent replicates.

To test the antagonistic activity against *Fol,* bacterial isolates were spot inoculated near the border of small PDA plates (Ø 6 cm, four bacteria per plate). After 24 h of incubation at 28°C, a mycelial plug (0.5 cm x 0.5 cm) from a 4-day old culture of *Fol* was placed in the centre of each plate. Plates inoculated only with the fungal plug served as the control. All strains were tested in three independent replicates. The antifungal activity was expressed as the percentage of growth inhibition (PGI) according to Vincent (1947): PGI (%) = 100·(GC-GT)/GC where GC represents the mean value of the fungus radius in the absence of the bacteria (control) and GT represents the mean value of the fungus radius in the presence of antagonistic bacteria (treatment). Antagonist activity was recorded after incubation at 28°C for up to 5-7 days. The comparison of the antagonistic activity of the bacterial strains, was based on two arbitrary 0-3 scales. The antibacterial activity was

scored based on the growth inhibition area size as: 0, no antagonism; 1, < 3 mm; 2, ≥ 3 and <10 mm; 3, >10 mm. Antifungal activity was scored based on the PGI against *Fol* as follows: 0, no inhibition, 1, PGI <30%; 2, PGI 30- 60%; 3, PGI >60%.

Table 2: Tomato pathogens, bacteria and fungi, used in this study.

Species	Strain*	Origin	Disease	Reference
Pseudomonas corrugata (*Pco*)	CFBP 5454	Italy	Pith necrosis	(Trantas *et al.*, 2015)
P. syringae pv. *tomato* (*Pto*)	PVCT 28.3.1	Italy	Bacterial speck	(Bella and Catara , 1998)
Clavibacter michiganensis subsp. *michiganensis* (*Cmm*)	PVCT 156.1.1	Italy	Bacterial wilt and canker	(Ialacci *et al.*, 2016)
Xanthomonas euvesicatoria pv. *perforans* (*Xep*)	NCPPB 4321^T	USA	Bacterial spot	(Constantin *et al.*, 2016)
Fusarium oxysporum f. sp. *lycopersici* (*Fol*)	Saitama ly2	Japan	Fusarium wilt	(Hirano and Arie, 2006)

*CFBP, International Center for Microbial Resources, French Collection for Plant-associated Bacteria, INRA, Angers, France; NCPPB, National Collection of Plant Pathogenic Bacteria, Fera, York, U.K; PVCT, Patologia Vegetale, University of Catania, Catania, Italy.

2.2.4.Molecular identification of the bacterial endophytes

The 16S rRNA gene region was amplified and sequenced for taxonomic identification. Bacterial DNA targets for colony PCR were prepared by thermal lysis (10 min at 100°C) of cell suspensions (OD_{600}= 0.01) in 200 µL of SDW. PCR amplicons were generated using the universal 16S rRNA primer pair, 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) primer set (Edwards *et al.*, 1989; Lane, 1991) Master mixtures included 1 x Taq&Go G2 Hot Start colorless PCR Master Mix (Promega), 0.5 µM of each primer, and 1 µL of template in a total volume of 15 µL. Reactions were performed in a thermal cycler GeneAmp® PCR system 9700, with the following thermal protocol: DNA denaturation for 5 min at 95°C, amplification (35 cycles) at 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min, and ended with 10 min extension at 72°C. The 1400-bp PCR products were analysed by agarose gel

electrophoresis (1.0% (w/v) agarose, 90 V, 50 min). The DNA amplicons were quantified and sequenced by BMR Genomics (Padova, Italy).

2.2.5.Sequence analysis and construction of a phylogenetic tree

The sequences were searched against the nucleotide collection database at the National Center for Biotechnology Information (NCBI) nucleotide database using Basic Local Alignment Search Tool BLASTN (http://www.ncbi.nlm.nih.gov). Taxonomy information was assigned by the NCBI Taxonomy database according to the highest score sequence. Highly homologous sequences were aligned using Clustal-W algorithm within MEGA X the regions of ambiguous alignment were edited manually and a neighbour-joining tree was generated (Kumar *et al.*, 2018). Sequences were aligned by Clustal W within MEGA X. A phylogenetic tree was built including type strains of the bacterial species identified by BLAST search.

2.2.6.In vivo biocontrol activity assays

2.2.6.1. Bacterial pathogens and antagonists' inoculum preparation

Of the 77 endophytes belonging to the genera *Pseudomonas* and *Bacillus*, ten were selected to evaluate their biocontrol activity *in vivo* on tomato plants against *Cmm*, and *Xep*. The endophytes were selected on the basis of their taxonomy, i.e. representativeness of the species and the results of *in vitro* test (Supplementary Table S1). The strains selected were *B. velezensis* strain 263; *B. megaterium* strain 268; *B. amyloliquefaciens* strain 306; *B. pumilus* strain 265 and *B. mojavensis* strain 261; *P. putida* strain 171, *P. entomophila* strain 172, *P. citronellolis* strain f1, *P. monteilii* strain f53, and *P. plecoglossicida* strain f56. The inoculum of both pathogens and putative biocontrol agents was prepared from bacterial cells grown for 48 h on NDA. Single colonies were transferred into Luria-Bertani (LB) broth and incubated at 27 ± 1°C for 24 h in an orbital shaker at 150 rpm. The bacterial cultures were centrifuged at 7500 rpm for 15 min. The pellets were resuspended in sterile tap water, and the density adjusted to $2 \cdot 10^{8}$ CFU·mL^{-1} (OD_{600}= 0.1).

2.2.6.2. Plant material and inoculation of bacterial endophytes

Plantlets of tomato SIR ELYAN F1 three weeks after germination were obtained from a local nursery and transplanted into square pots (8 cm-side) containing nursery peat. In each trial, the pots were arranged in a completely randomized design, with 15 replicates per treatment. Independent trials were set up to assess the effect of the 10 endophytic strains on: *i)* PGP activity; *ii)* biocontrol of bacterial canker; and *iii)* biocontrol of bacterial spot. Plants were maintained in a growth chamber at 24±2°C, 68–80% RH, with 16 h of light and 8 h of darkness daily. They were watered as required with the same amount of tap water per pot. All experiments were conducted in duplicate. In all trials bacterial endophytes were inoculated by soil drenching with 20 mL inoculum. In the PGP trial thirty days after soil treatment, tomato seedlings were harvested. Height, fresh and dry weight of roots and shoots and shoot/root ratio were measured. To determine the dry weight, the samples were dried at 105 °C for 24 h. These parameters were compared to mock control plants drenched with tap water.

2.2.6.3. Plant challenge with bacterial pathogens

Tomato seedlings were inoculated with *Cmm*, bacterial suspension seven days after treatment with the putative BCAs or water (negative control). Aliquots of 20 mL of *Cmm* were poured into the soil near the stem crown. The roots were then damaged in order to facilitate bacterial penetration by inserting a scalpel at three points located 2 cm from the stem. Bacterial canker symptoms were recorded weekly for one month using a 0-5 disease scale developed for root inoculations, where 0 = no symptoms; 1 = chlorosis and loss of turgor; 2= wilt in 1 or 2 leaves, and / or cankers <0.5; 3 = wilt in 3 or more leaves, and / or cankers >0.5; 4 = fully withered plants; and 5 = dead plants (Bella *et al.*, 2012).

The area under disease progress curve (AUDPC) was calculated using weekly recorded data, as described by Madden and Campbell (1990). Using hand-trigger sprayers three days after the soil treatment with the putative BCAs or water (negative control), tomato seedlings were spray inoculated with *Xep* onto the abaxial and adaxial leaf surfaces of four replicate tomato plants until runoff. The inoculated plants were pre- and post-incubated for one day under transparent polyethylene sheets to increase the RH near to 100% to promote bacterial penetration. Ten tomato leaflets per plant were sampled randomly ten days after pathogen inoculation. Lesions on individual leaflets were counted and leaflet area determined; disease severity was quantified as number of lesions/cm^2 leaflet area (Ji *et al.*, 2006). The leaflet area was obtained by image processing and analysis in Java (ImageJ software). Disease severity data were log transformed and

subjected to analysis of variance. Percentage reduction in disease severity compared to the pathogen-only control was calculated according to Ji *et al.*, (2006).

2.2.7.Statistical analysis

The results of the screening indices were used to perform a principal component analysis (PCA) to detect patterns of similarity among the tomato root-associated bacteria. The PCA was calculated on binary data (0, isolate negative to the test; 1 isolate positive to test) using the 'prcomp' function of the 'stat' R package (Team, 2013). PCA biplot and loading projections were visualized through the 'factoextra' R package (Kassambara A, 2016). Mosaic plots were drawn using the 'stat' R package, the same package was also used to compute ANOVA and the post-hoc Tukey-Kramer test. Data of biocontrol assays were analysed by ANOVA using STATGRAPHICS Plus 5. Mean values were compared using the Student–Newman–Keuls test.

2.3. Results

2.3.1.Bacterial population size in tomato root environment

Cultivable population sizes of total, fluorescent and spore forming bacteria in the rhizospheric soil (R) of the four farms ranged from 6.8 to 8.8 , from 3.8 to 4.5 , and from 3.3 to 6.4 log CFU$\cdot$g^{-1}, respectively (Figure 1 A-C). On each farm, the populations were higher in the rhizosphere than in the endorhizosphere (E) (ANOVA; $p < 0.05$) (Figure 1 A-C). Population sizes in the rhizoplane (RP) and in the endorhizosphere ranged from 6.8 to 8.1, and from 3.7 to 6.4 log CFU$\cdot$g^{-1} for total bacteria, from 3.8 to 4.6, and from 2.3 to 3.5 log CFU$\cdot$g^{-1} for fluorescent bacteria, in the two root compartments, respectively (Figure 1 A-B). The population sizes of spore forming bacteria ranged from 3.6 to 6.4, and from 3.5 to 4.8 log CFU$\cdot$g^{-1}, for the two root compartments, respectively (Figure 1C).

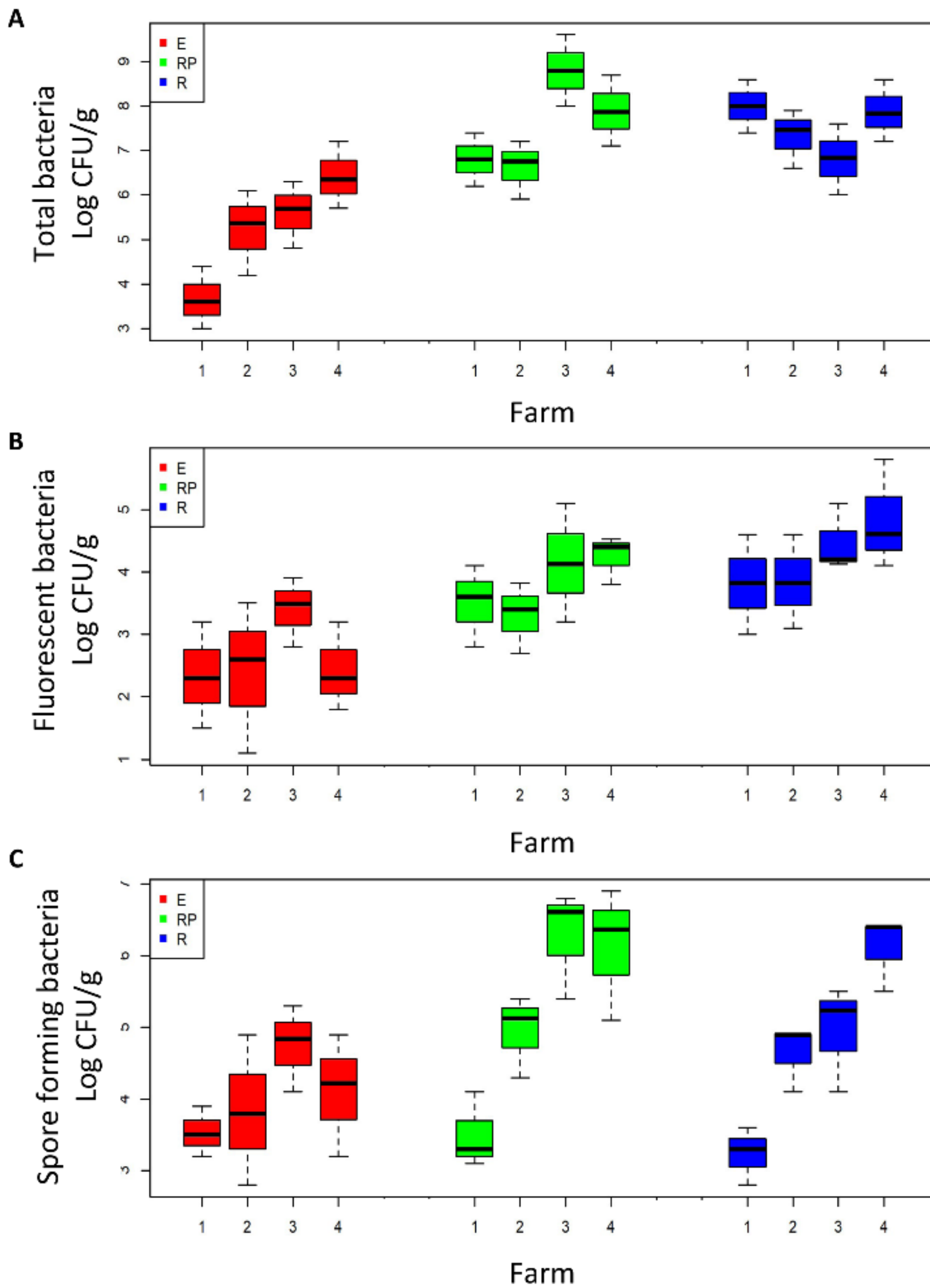

Figure 1: Boxplot of the total **(A)**, fluorescent **(B)**, and spore forming **(C)** cultivable bacteria in the three root-compartments: rhizosphere (R, in red), rhizoplane (RP, in green), and endorhizosphere (E, in blue). Bacteria are grouped according to the farm on which they were collected (X axis).

2.3.2. Beneficial phenotypes of bacteria from the root environment of tomato grown in agricultural soil

A total of 424 culturable bacterial strains were obtained in pure culture from the isolation plates of the four farms (70, 132, 85, 136 isolates for farms 1-4, respectively). The percentage of Gram-negative bacteria in the three compartments was 61, 86, and 78% for R, RP, and E, respectively. Among these, fluorescent pseudomonas represented approximately 18.2, 38.6 and 43.2% of the isolates obtained from the R, RP, and E respectively (Supplementary Table S1).

A total of 83.5, 86, and 89% of bacterial isolates from the R, RP, and E respectively were able to grow in up to the 8% NaCl (Supplementary Table S1). The production of siderophores on CAS agar was found in a similar relative frequency in the three rhizoplanes (33, 34, and 30% in R, RP and E) (Figure2A; Supplementary Table S1). A total of 64% of the endophytic isolates showed an ability to solubilize insoluble organic phosphate, while the number of isolates showing the same characteristic was 46.5 and 29.5% in R and RP, respectively (Figure 2B; Supplementary Table S1). All the isolates exhibited at least one of the three PGP traits tested (siderophore production, phosphate solubilisation, and tolerance to salinity), and most of the strains tested positive for at least two of the three traits with 139 out of the 424 isolates tested showing the three positive features: tolerance to salinity, siderophore production and phosphate solubilisation (Supplementary Table S1).

The tomato root-associated bacterial collection was further screened for the antagonistic ability to inhibit *in vitro* the growth of five detrimental tomato phytopathogens (Table 2; Figure 2C-E). All isolates were therefore tested against the Gram-positive *Clavibacter michiganensis* subsp. *michiganensis* strain PVCT156.1.1 (*Cmm*), *Pseudomonas corrugata* strain CFBP5454 (*Pco*), *Pseudomonas syringae* pv. *tomato* strain PVTC28.3.1 (*Pto*), *Xanthomonas euvesicatoria* pv. *perforans* strain NCPPB4321 (*Xep*), and the fungus *Fusarium oxysporum* f. sp. *lycopersici* strain Saitama ly2 (*Fol*). Approximately 30% of the tomato root-associated bacteria (127 out 423 isolates) showed antagonistic activity against all the tested bacterial phytopathogens and *Fol* (Figure 3; Supplementary Table S1). Of these, 42, 26 and 31% were isolated from R, RP and E compartments, respectively. The highest activity in terms of the number of antagonistic strains but also effectiveness in terms of inhibition zone was observed against *Cmm* (88% of the isolates) (Supplementary Figure S1). Among this group, 98% were ranked within class 3 (inhibition halo >10 mm). The lowest number of antagonistic bacteria was detected

against *Pco* (40%), and the antagonistic activity was ranked with 1 in the scale of activity (< 3mm). An intermediate behaviour was observed against the other two plant pathogenic bacteria (Supplementary Figure S1).The *in vitro* inhibition of *Fol* was observed, although to different extents, by all but three tomato root-associated bacterial isolates (Supplementary Figure S1). The percentage of bacterial isolates with antifungal activity was the highest for RP (33%), followed by R (32%), and E (21%). PGI values of the fungal colonies ranged from 8% to 100% after incubation for 6 days at 24°C (when the colonies on control plates reached the margin). Based on growth inhibition scores (0-3) exhibited towards *Fol,* 214 isolates were ranked in class 2, indicating that their relative percentages of growth inhibition were less than 30% (Supplementary Figure S1). Interestingly, 60 isolates led to more than 60% inhibition of pathogen growth and were, thus, ranked in class 3 (Supplementary Figure S1).

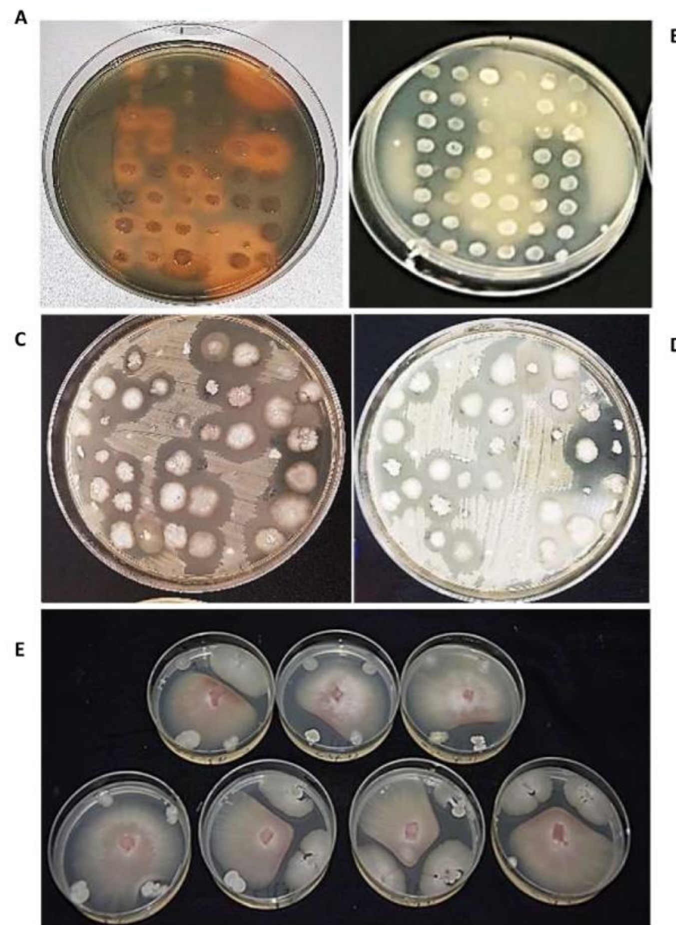

Figure 2: Bacterial isolates, tested for **(A)** siderophore production, orange halos indicates siderophore positive results; **(B)** phosphate solubilisation, cleared haloes indicates phosphate solubilization positive results; antagonistic activity against **(C)** *Xanthomonas euvesicatoria* pv. *perforans* (*Xep*), **(D)** *Pseudomonas syringae* pv. *tomato* (*Pto*), and **(E)** *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*).

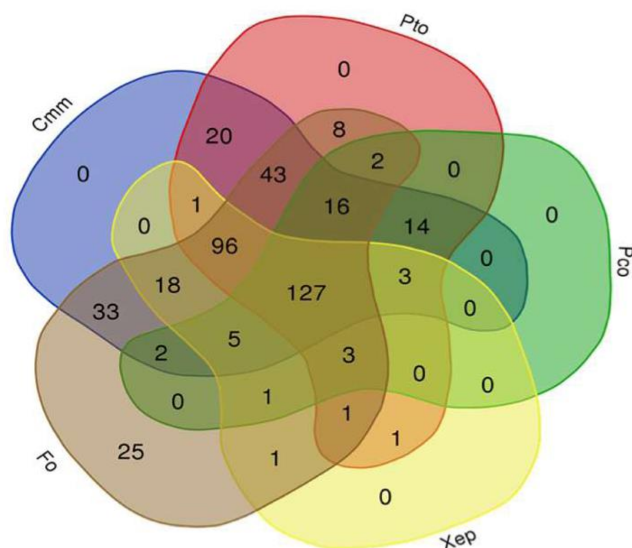

Figure 3: Venn diagram showing the antagonist activity of a collection of 424 bacterial isolates obtained from tomato root-environment against the tomato phytopathogenic bacteria *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*), *Pseudomonas corrugata* (*Pco*), *P. syringaea* pv. *tomato* (*Pto*), and *Xanthomonas euvesicatoria* pv. *perforans* (*Xep*), and the fungus *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*). (http://bioinformatics.psb.ugent.be/webtools/Venn/).

2.3.3.Source of isolation drives beneficial traits of bacterial isolates

Principal Component Analysis (PCA) (Figure 4A-B) was used to visualize the relationships between the ten phenotypic traits analysed (Gram reaction, fluorescence production, siderophore production, phosphate solubilisation, salt tolerance, antagonist activity against *Cmm*, *Pco*, *Pto*, *Xep*, and *Fol*) of all bacterial isolates and the source of isolation (farm; root compartment). The first two principal components (PCs) explained 41% of the total phenotypic variability (PC1 = 25.3%, PC2 = 15.7%, Figure 4A-B). Results enabled the bacteria to be clearly separated according to the farm in which they were isolated (Figure 4A), but not to the root compartment (data not shown): bacteria collected on Farm 1 were mainly separated according to PC2; while bacteria from Farms 2 and 3 clustered mainly in the upper-right PCA quadrant (PC1 > 0, PC2 > 0); and bacteria collected from Farm 4 were mainly plotted in the lower-right (PC1 < 0, PC2 > 0) and lower-left (PC1 and PC2 < 0) PCA quadrants (Supplementary Table S2). The variables greatly influencing the bacteria disposition along the first two PCs were the antagonistic

activity against *Pto*, *Pco* and *Xep* and siderophore production**.** The antagonistic activity against *Pto* and the siderophore production highlighted opposite directions in the PCA biplot since they were oriented toward the upper-right quadrant and lower-left quadrants, respectively. On the other hand, the antagonist activity against *Xep* and *Pco* was oriented toward the lower-right PCA quadrant.

Overall, the 10 traits employed in the PCA showed a pairwise correlation ranging from -0.45 (p value < 2.2-16) for siderophore production and antagonist activity against *Pto* to 0.34 (p value < 1.1-12) for antagonist activity against *Xep* and *Pto* (Figure 4C). An ANOVA test using the collection farm and the 10 traits as categorical variables showed p values that exceeded the significance threshold (p value < 0.05) for all traits tested. The traits showing the highest significance (p value < 0.0001) were the Gram reaction, siderophore production and the antagonist activity against *Cmm*, *Pto, Pco* and ***Fol*.**

The bacteria distribution among the four farms was consistent for siderophore production, and antagonist activity against *Cmm* and *Pto* (Figure 5A-L*)*. Isolates collected from Farm 2 and Farm 3 were characterized by a substantial absence of siderophore production (Figure 5A-B) and positive antagonist activity against *Cmm* and *Pto* in all the samples (Figures 5C-F), while a more admixed configuration was registered for Farm 1 and Farm 4 (Figures 5C-F).

The *Pco* antagonistic activity showed statistical differences among all the four farms analysed (Figure 5G-H), with bacteria collected on Farm 3 and Farm 1 showing the highest and lowest number of *Pco* antagonistic activity, respectively (Figure 5G). The antagonist activity against *Fol* was detected on all farms (Figure 5I-L).

2.3.4.Bioprospecting of tomato endophytic bacteria

Out of the 100 total tomato root bacterial endophytes in the working collection, 77 were selected based on their phenotype and representativeness of the PGP and biological control agent (BCA) traits, with at least two and/ or three PGP traits and antagonistic activity to at least three microorganisms. Partial sequences of the 16S rRNA genes of the 77 isolates obtained from the E were analysed. According to BLASTN similarity matches, isolates were identified by partial sequencing of their 16S rRNA gene, which enabled the isolates to be classified into three orders, namely: Bacillales, with all the bacterial isolates belong to the genus *Bacillus*; Pseudomonadales, with bacterial isolates

in the genera *Pseudomonas*, and *Acinetobacter*; Enterobacteriales with isolates in the genera *Enterobacter, Ewingella, Pantoea, Providencia,* and *Lelliottia*. Putative single isolate taxon is shown in Supplementary Table S3.

Four different *Bacillus* species were identified, three strains with 100% similarity to *B. subtilis* (GenBank acc. no CP051860.1, MT081484.1, KU729674.1); two strains with 100% similarity to *B. amyloliquefaciens* (GenBank acc. no MK501609.1); eleven strains with 99-100% similarity to *B. velenzensis* (GenBank acc. no MN559711.1, CP051463.1, KY927398.1, MT365117.1, MN654121.1, and CP024922.1) (all species of the *B. subtilis* clade, Fan *et al.*, 2017); one strain with 99% similarity to *B. megaterium* (GenBank acc. no KT883839.1). Two strains were only identified at the genus level as *Bacillus* sp. (100% similarity to GenBank acc. no. CP040881.1).

For isolates among the Enterobacteriales, the best hits were observed with the following species: ten strains with 97% similarity to *Enterobacter cancerogenus* (GenBank acc. no. FJ976582.1); one strain with 97% similarity to *E. tabaci* (GenBank acc. no. MF682952.1); one strain with 97% similarity to *E. mori* (GenBank acc. no. KJ589489.1); ten strains were only identified at the genus level as *Lelliottia* (96-97% similarity to strain GenBank acc. no. JN853247.1); three strains with 98-100% similarity to *Ewingella americana* (GenBank acc. no. MT101745.1, and KY126991.1). Three strains with 99% similarity to *Providencia vermicola* (GenBank acc. no. KX394623.1, and MK942706.1). Four strains were only identified at the genus level as *Pantoea* sp. (97% similarity to strains GenBank acc. no. MK229045.1, and MH884045.1).

Different species were found in the genus *Pseudomonas* all within the *P. putida* group within the *P. fluorescens* lineage (Mulet *et al.,* 2010): fourteen strains with 100% similarity to *P. plecoglossicida* (GenBank acc. no. MT367715.1); one strain with 100% similarity to *P. citronellolis* (GenkBank acc. no. KM210226.1); one strain with 100% similarity to *P. monteilii* (GenkBank acc. no. MH603875.1), four strains with 100% similarity to *P. putida* (GenkBank acc. no. LN866622.1), and CP026115.2). All the isolates in the genus *Acinetobacter* showed the highest similarity to *A. baumannii* (99-100% similarity to GenBank acc. no. MT256198.1, and CP050388.1).

In the dendrogram showing the phylogenetic relationships of the endophytic strains in which type strains of the putative bacterial species and some reference species were included the taxonomic position was confirmed although some isolates clustered with appropriate the taxonomic clade (e.g. *B. subtilis* or *P. putida* clade and not with the type strain of the bacterial species resulted from the BLAST similarity analysis. For this reason, sequences of the isolates were deposited at GenBank with the genus and strain

name under accession numbers from MW130753 to MW130829 (Figure 6; Supplementary Tables S3 and S4).

The Principal Component Analysis (PCA) calculated on the 77 endophytic bacteria showing antagonist activity to at least one pathogen is shown in Figure 7 A. The first two PCs accounted for 58.8% of the total phenotypic variability, with PC1 accounting for 33.1% and PC2 for 25.7%. Bacillales were mainly plotted in the upper-right quadrant of the PCA biplot (PC1 and PC2 > 0), while both Enterobacteriales and Pseudomonadales were mainly characterized by PC1 negative values (resulting in a high prevalence of bacteria plotted in the upper-left and lower-left PCA quadrants). The high effectiveness of PC1 in distinguishing between the Bacillales compared to the other 2 families was confirmed by the ANOVA test which showed a p value = 0.00003 (Figure 7B-D).

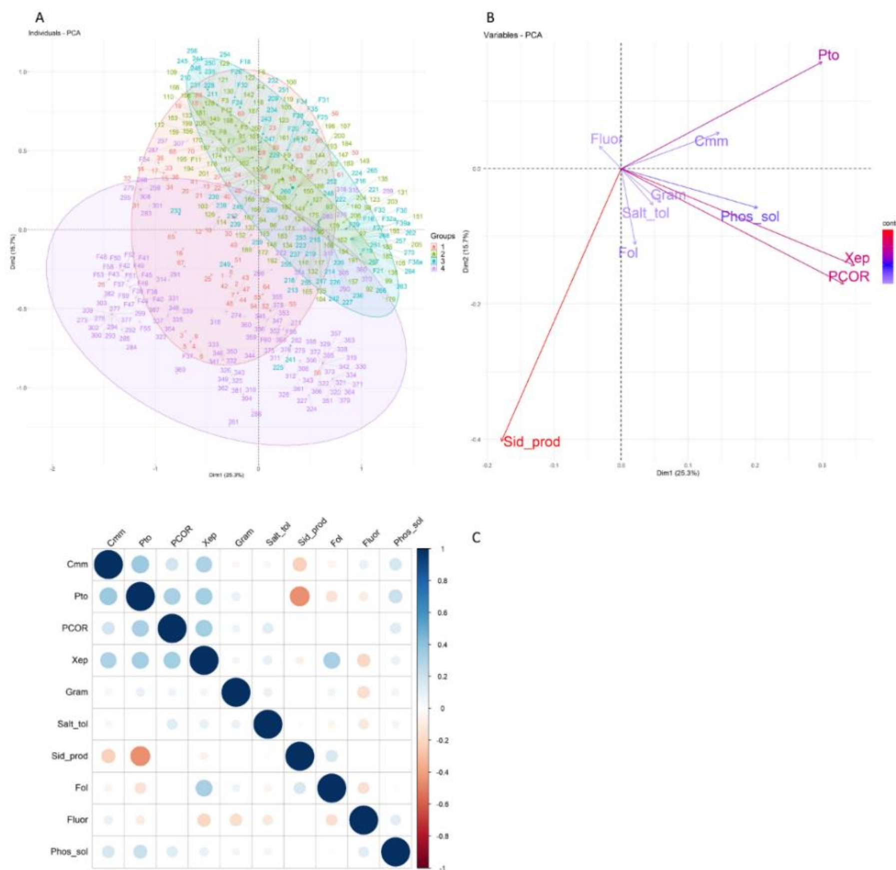

Figure 4: Principal Component Analysis (PCA) of the characteristics related to the collection of 424 bacterial isolates. **(A)** The first two principal components are shown in a biplot and bacterial isolates are colored according to the farm on which they were isolated. **(B)** Loading plot of the ten traits used to compute the PCA, namely: antagonist activity against *Cmm*, *Pco*, *Pto*, *Xep*, and *Fol* (*Cmm*, *Pco*, *Pto*, *Xep*, and *Fol*), fluorescence on KB (Fluor), Gram reaction (Gram), phosphate solubilisation (Phos_sol), salt tolerance (Salt_tol), siderophore production (Sid_prod). **(C)** Heatmap of the pairwise correlations between the traits analysed.

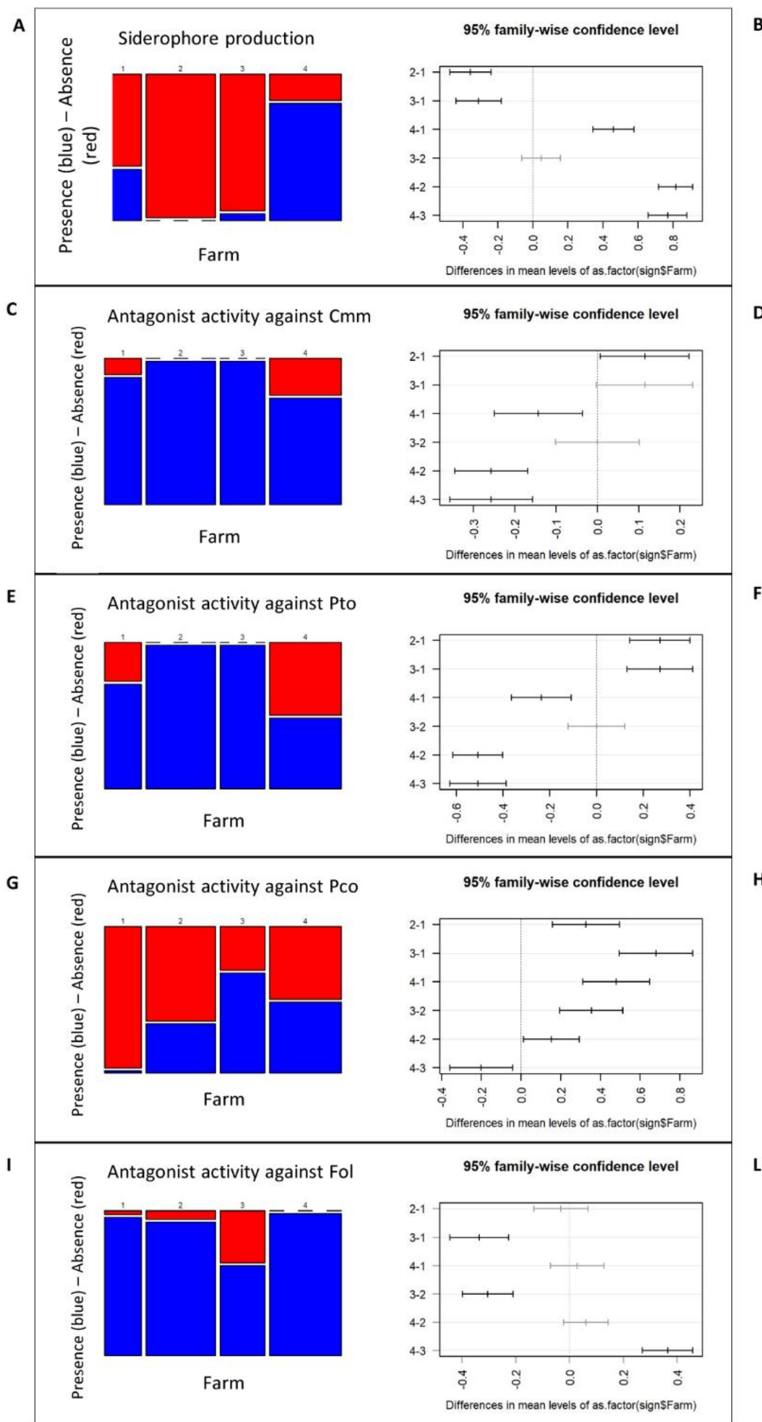

Figure 5: Mosaic plot and Tuckey plot of the traits showing the highest differentiation among farms (ANOVA test, p value < 0.0001), namely: siderophore production **(A)**, antagonistic activity against *Cmm* **(C)**, *Pto* **(E)**, *Pco* **(G)**, and *Fol* **(I)**. Mosaic plots (left of the panels **(B)**, **(D)**, **(F)**, **(H)**, **(L)**) show the relative frequency of the presence (blue) or absence (red) of a trait (y axis) given the farm (x axis); the width of the columns is proportional to the numerosity of the accessions isolated on each farm. On the right of the panels, the confidence intervals are shown of each pairwise comparison after the Tukey post-hoc test. Pairwise comparisons that were not statistically different are shown in grey.

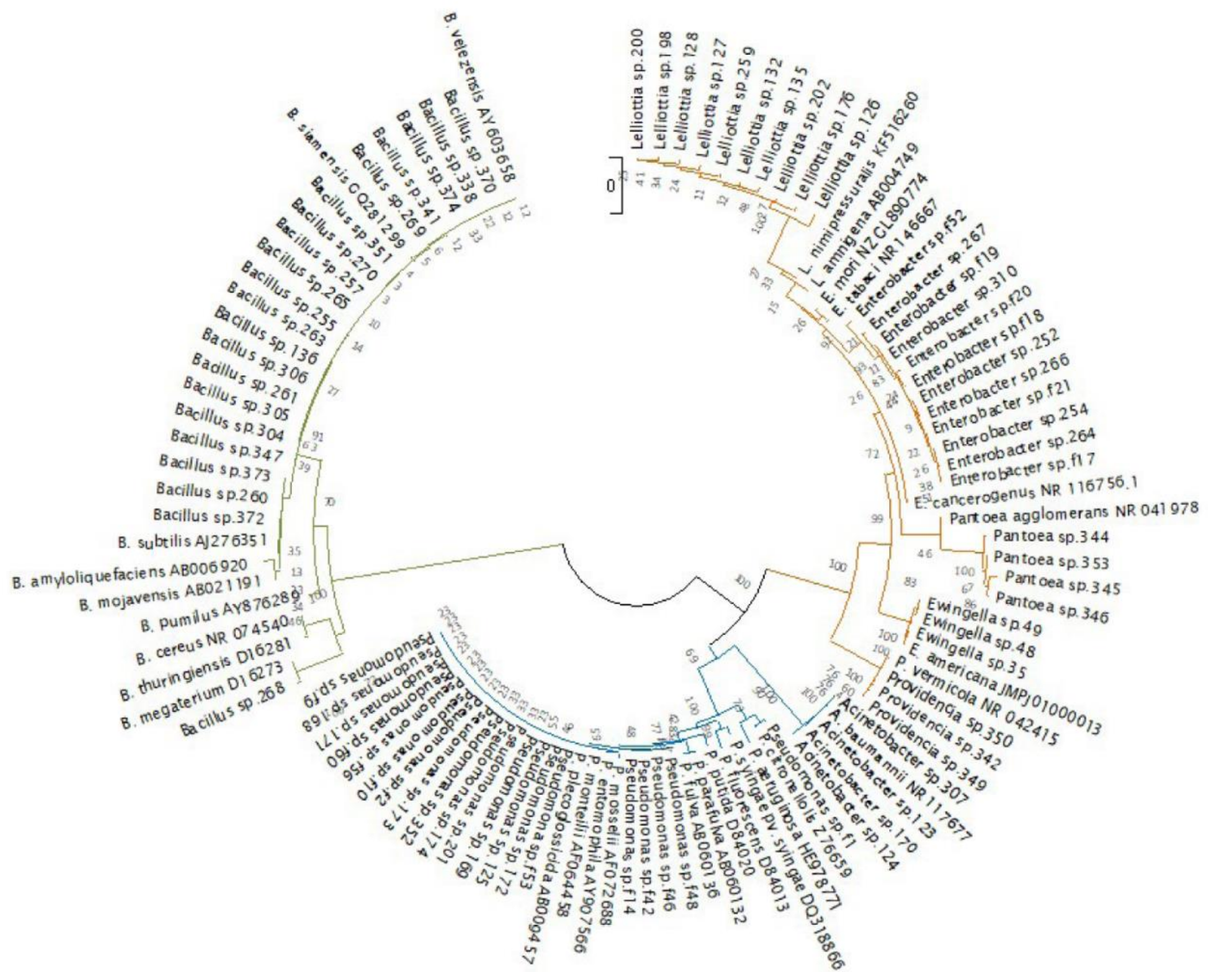

Figure 6: Phylogenetic tree of the 77 endophytic strains isolated in this study and 29 bacterial type strains. (*Bacillales* in green, *Pseudomonadales* in blue, and *Enterobacteriales* in orange). The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980). There were a total of 824 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018).

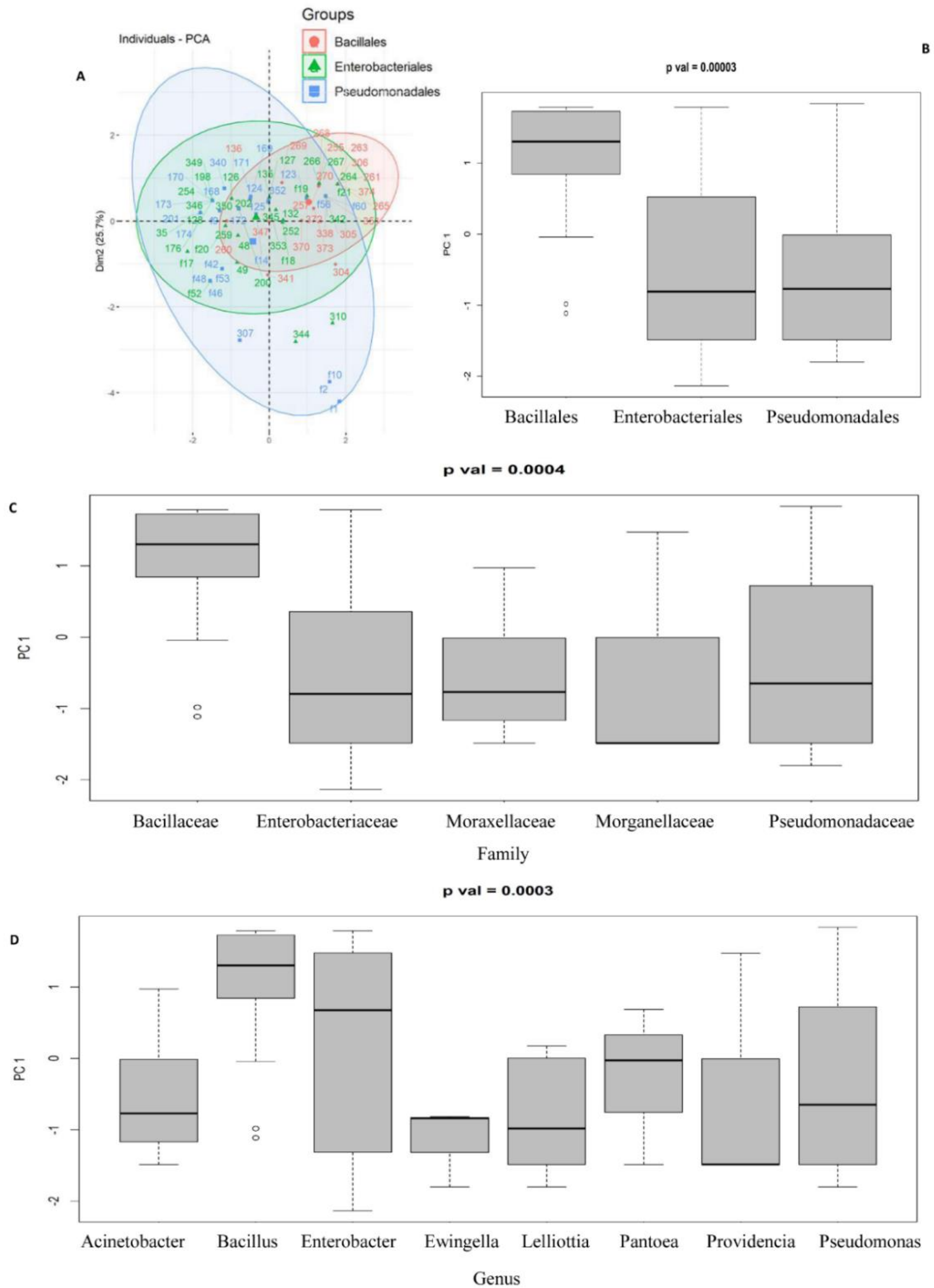

Figure 7: **(A)** PCA of the antagonist activity of the endophytic bacteria against *Cmm*, *Pco*, *Pto*, *Xep*, and *Fol*, boxplot of the distribution of the first principal component given the order **(B)**, family **(C)**, and genus **(D).**

2.3.5.In planta bioassays

Tomato seedlings treated by soil drenching with 10 bacterial strains belonging to the genus *Pseudomonas* and *Bacillus,* selected from the endorhizosphere isolated bacteria data set, showed an increase in plant height as compared to water treated control seedlings. Thirty days after the treatment with the bacterial strains tomato seedlings were from 1.3% to 22% higher than the control plants. *Pseudomonas* strains f56 and f1, and *Bacillus* strains 306 and 261 significantly promoted plant height compared to untreated controls ($p < 0.05$) (Supplementary Table S5). Variable results were recorded for the other growth parameters that didn't show a clear effect on plant weight (fresh and dry), dry matter percentage (Supplementary Table S5).

Symptoms of bacterial canker, caused by *Cmm*, were first observed in the control plants 14 days post inoculation (dpi). They consisted in the unilateral wilting of one or more leaflets. Generalised wilting symptoms started at 21 dpi. Thirty days post inoculation of *Cmm* the disease indexes (DI) of the plants treated with *P. citronellolis* strain f1 and *B. velenzensis* strain 265 was significantly lower ($p<0.05$) than that of control plants treated with a water. Both antagonistic isolates also reduced the percentage of dead plants, these were for the *P. citronellolis* strain f1, *B. velenzensis* strain 265 and control plants 0, 14.30 and 75%, respectively (Table 3). The values of the AUDPC, which records the progression of the disease, although were not significant statistically but were also lower (Table 3). The effect of the soil treatments with the tomato bacterial endophytes was also evaluated on the tomato leaf pathogen *Xep.* The occurrence of lesions on leaves was observed on positive control plants treated with water s starting from 6 dpi. In fact, there were minute chlorotic spots that turned necrotic and expanded by 10 dpi when the data were recorded. Significant differences were observed between the endophytes treated plants that showed fewer spots than control plants although differences were observed between bacterial strains. A reduction of the diseases ranging from 30 to 80% was observed (Table 3).

Table 3: Results of the *in vivo* assays of the biocontrol activity of bacterial endophytes against the tomato bacterial pathogens *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) and *Xanthomonas euvesicatoria* pv. *perforans* (*Xep*)

	Cmm			***Xep***	
Bacterial strains	DI 30 dpi[a]	AUDPC[b]	% of dead plants	n. spot/cm^2 leaf area[c]	% reduction[d]
P. plecoglossicida _171	4.71cd	51.78 d	84.70%	4.02 bc	43.50 +6.96
P. plecoglossicida _172	4.00 abc	35.29 abc	57.14%	1.46 a	79.50 +1.79
*P. citronellolis*_f1	2.28 a	15.86 a	0.00%	4.88 c	31.40 +14.76
*P. monteilii*_f53	4.14 abc	40.43 abc	57.14%	2.99 abc	57.91 +1.14
P. *plecoglossicida*_f56	4.71 cd	33.00 abc	84.70%	4.88 c	31.31 +12.39
B. velezenzis _261	3.28 abc	24.86 abc	14.30%	2.00 ab	71.83 +4.40
*B. velezenzis*_263	5.00 c	47.64 bc	100%	2.16 ab	69.61 +6.53
B. velezenzis _265	2.71 ab	30.35 abc	14.30%	1.30 a	81.70 +3.20
*B. megaterium*_268	3.00 abc	22.71 ab	28.57%	2.70 ab	61.96 +5.67
B. velezenzis _306	3.00 abc	32.93 abc	14.30%	2.06 ab	70.97 +6.82
Positive control	4.71 cd	36.92 abc	71.42%	7.11 d	/

[a]DI 30 dpi: disease index based on a 0-5 disease scale 30 days post *Cmm*-inoculation.

[b]AUDPC, area under the disease progress curve (AUDPC).

[c]DS: disease severity recorded as number of spot/cm2 leaf area assessed 10 days post-Xep inoculation

[d]Percentage reduction in lesion numbers per unit leaf area compared to the pathogen-only control + Standard error

Means in a column followed by the same letter are not significantly different according to Student–Newman–Keuls test at $P \leq 0.05$.

2.4. Discussion

The main aim of this study was to establish a collection of culturable tomato root-associated bacteria, as well as to bioprospect the natural diversity of root-associated bacterial communities under a real-word environment represented here by an intensive tomato cultivation area characterized by extra-seasonal greenhouse production. Although in recent years the advances in next-generation omic technologies have led to the possibility of revealing plant-associated microbiomes, culture-dependent methods are still necessary to bioprospect natural diversity as a source of new tools for sustainable agriculture (Quiza *et al.*, 2015; Lee *et al.*, 2016; Müller *et al.*, 2016).

In this study, bacterial population sizes of the total numbers of fluorescent and spore forming bacteria associated with the root environment of greenhouse tomato plants grown

in agricultural soils from four different farms varied according to the compartment of isolation (i.e.: rhizosphere, rhizoplane, endosphere) and in some cases the farms. The characterization of a collection of 424 bacterial isolates targeted at phenotypic traits (Plant Growth Promotion and/or Biocontrol of detrimental plant pathogens) did not show any clear relationships between the compartments of root isolation. In contrast, the isolates clustered according to the four isolation farms.

The four farms from where the samples were selected shared some common features of the cultivation area. Sicily is the principal tomato greenhouse production area in Italy and more than half of the tomato production comes from the Province of Ragusa where the four farms were located. This area is characterized by sandy soil and climatic conditions that facilitate out-of-season production. Tomatoes are grown for one or two cycles within the year in greenhouses covered with a plastic film. In the four greenhouses, four different genotypes of tomato were cultivated which differed according to the farm management (irrigation, fertilization, and pesticide use, and agronomic operations).

Overall, the phenotyping of 424 bacterial isolates from the tomato root environment revealed that this community was more represented by Gram-negative than Gram-positive bacteria, and that they possessed interesting PGP bacterial traits. In fact, 139 out of the 424 root-associated bacteria isolates were able to produce siderophores, solubilize phosphates and grow on a saline medium.

These characteristics could be of great interest in developing bioinoculant with also biofertilizer abilities that could also promote plant growth and yield. Phosphate-solubilizing microorganisms play an important role in supplementing phosphorus to the plants, allowing a sustainable use of phosphate fertilizers. P-solubilizing activity is related to the microbial production of organic acids, which chelate the cation bound to phosphate, thereby converting it to a soluble form (Sagoe *et al.*, 1998; Rashid *et al.*, 2004; Lugtenberg and Kamilova, 2009). The ability to produce secondary metabolites such as siderophore and antimicrobial peptides has been evaluated in many studies on PGPRs. The ability to produce siderophore and metabolites contributing to antibiosis has been the focus of many studies on PGPR (Sayyed *et al.*, 2005; Maksimov *et al.*, 2011).

The high number of salinity-tolerant bacterial isolates suggest that a selection may have occurred as salinity is one of the typical characteristics of the area (i.e. soils of all the farms showed an EC>2.0 mmhos cm^{-1} and high Na content). Tomato is moderately sensitive to salinity saline water that is used in greenhouse cultivations, however high salinity may affect plant physiology (Leonardi and Martorana, 2005). Several reports have shown that halotolerant PGPRs improve the growth of various agricultural crops

under salinity stress. Inoculating crops with halotolerant PGPRs isolated from halophytes has been successful in improving crop growth and tolerance under salt stress conditions (Shukla *et al.*, 2012; Khan *et al.*, 2016).

More importantly, approximately 30% (129 strains) of the root-associated bacterial isolates showed antagonistic activity against all the five tested phytopathogens, although to different extents. Their antagonistic activity as assessed *in vitro* suggests the production of secondary metabolites with inhibitory activity against fungi, Gram-positive and Gram-negative plant pathogenic bacteria. Some of these harmful pathogens are seed and/ or soil transmitted (Bardin and Gullino, 2020; Catara and Bella, 2020).

A large number of studies have shown that tomato bacterial communities, resolved by metagenomics based on amplicon sequencing, are influenced by different factors. Data however refer to the taxonomic operational units, and the PGP and BC activities can only be inferred. Amongst the biotic and abiotic factors, soil is considered the primary force driving plant–microbiota diversity (Jeanbille *et al.*, 2016). Different studies have demonstrated that the influence of the soil plays a stronger role on plant–microbiota diversity than the plant genotype (Poli *et al.*, 2016; Dong *et al.*, 2019). In addition, transcriptomics and proteomics have demonstrated that the overall characteristics of the substrate contribute more than plant genotype to shaping the molecular responses in tomato roots (Chialva *et al.*, 2018).

Our research also focused on bacterial endophytes which are good candidates for beneficial inoculants aimed at reducing the chemical inputs in conventional agricultural practices and increasing nutrient uptake and stress resilience in plant species (Ryan *et al.*, 2008; Gupta *et al.*, 2014). In fact, the endophytes interact more closely with their host than rhizospheric bacteria because they are located within the plant tissues (Hallmann *et al.*, 1997; Weyens *et al.*, 2013). In addition, as they live in the apoplast (or in the xylem), endophytes do not need to compete for nutrition and/or niche in the soil as bacteria do in the rhizosphere (Reiter *et al.*, 2002), and they or their metabolites can easily reach the pathogens within the plants (Gupta *et al.*, 2014).

A subset of bacterial endophytes isolated from tomato endorhizosphere (77 isolates), identified by partial sequencing of their 16S rRNA gene, belonged to two phila (Firmicutes and Proteobacteria) and to three orders, namely: Bacillales (27.3%), with all the isolates in the genus *Bacillus*; Pseudomonadales (31.2%), with isolates in the genera *Pseudomonas* and *Acinetobacter*; and Enterobacteriales (41.6%), with isolates in the genera *Enterobacter*, *Ewingella*, *Pantoea*, *Providencia*, and *Lelliottia*. Similarly, some of these genera (*Bacillus*, *Pseudomonas*, *Acinetobacter, Enterobacter*) have been isolated

from tomato endorhizosphere in studies on beneficial bacteria (Tian *et al.*, 2017; Singh *et al.*, 2019). Bacterial strains in the genera, *Rhizobium* and *Ralstonia* have also been isolated from the endorhizophere of tomato plants (Abbamondi *et al.*, 2016). Bergna and co-authors (2018) isolated *Ralstonia*, *Stenotrophomonas,* and *Bacillus* strains both from tomato root and seed endosphere. The high-throughput screening (HTS) and cultivable approach suggested that beneficial bacteria are seed transmitted (Bergna *et al.*, 2018).

Recent studies demonstrated that tomato bacterial communities of the root zone and of the rhizosphere exhibited the highest richness and diversity in comparison to those of the endorhizosphere (Lee *et al.*, 2019; Dong *et al.*, 2019). In general, the richness decreased from the root zone soil to rhizosphere to phyllosphere to endosphere, while the diversity decreased in a different order: root zone soil > rhizosphere > endosphere > phyllophere (Ottesen *et al.*, 2013; Dong *et al.*, 2019). Our results however, suggest that beneficial activities are commonly spread in each root compartment. However, the richness of the bacterial community is the lowest in the endorhizosphere. In this study, when the relationship between the bacterial families and the antagonistic activity of the tomato endophytes was investigated, *Bacillus* isolates were significantly more antagonistic *in vitro* against tomato plant pathogens than bacterial isolates belonging to Pseudomonadales and Enterobacteriales.

Almost 40% of the endophytic bacteria characterized here belong to the Enterobacteriales, more specifically to the Enterobacteriaceae family and as many as five different genera were recorded. Many studies have confirmed that Enterobacteriaceae are indigenous components of the plant microbiome in different species (Brandl, 2006; Teplitski *et al.*, 2011; Erlacher *et al.*, 2014, 2015; Tian *et al.*, 2017). Data on rocket salad suggested that the soil probably provides the largest reservoir from which enterics become established and spread within the whole plant (Cernava *et al.*, 2019). Enterobacteriaceae have been successfully evaluated as biocontrol agents in tomato (Xue *et al.*, 2009), however there are still some concerns regarding the use of these antagonistic bacteria since some species are human pathogens (Erlacher *et al.*, 2015; Cernava *et al.*, 2019).

Some *Pseudomonas* species show considerable potential for the suppression of plant pathogens, in promoting plant growth, inducing systemic resistance in plants and are widely used as biocontrol agents (Mercado-Blanco and Bakker, 2007). These bacteria produce several diffusible and/or volatile secondary metabolites with antibiotic properties such as diacetylphloroglucinol, pyrrolnitrin, cyclic lipopeptides phenazine (Haas and Keel, 2003; Raaijmakers and Mazzola, 2012).

Most endophytic *Bacillus* isolates identified here with the 16S rRNA gene sequence belong to the *B. amyloliquefaciens* and *B. subtilis* group. Members of the *B. subtilis* species complex, which includes at present more than 20 closely related species such as *B. subtilis*, *B. amyloliquefaciens*, and *B. pumilus*, and, to a lesser extent, the genus *Paenibacillu*s spp. have been proven to be efficient at plant growth promotion and biocontrol against plant pathogens such as viruses, bacteria, fungi and nematodes in the vicinity of plant roots (Vacheron *et al.*, 2013; Fan *et al.*, 2017). To date, bacilli are the most widely used bacteria on the biopesticide market (Borriss 2011; 2015). This is mainly due to their ability to produce durable endospores, which enable stable bioformulations to be prepared with a long shelf-life. These antagonistic strains produce numerous antibiotics including polymyxin, difficidin, subtilin, mycobacillin, zwittermicin A, which are active against plant pathogenic bacteria and fungi (Borriss, 2015; Caulier *et al.*, 2019).

Biological control of a set of *Bacillus* and *Pseudomonas* isolates from tomato endorhizosphere was tested in a growth chamber in two separate experiments. Two important bacterial pathogens that are common in the area of sampling and that represent important seed-transmitted pathogens were chosen: *i)* the vascular pathogenic bacterium *C. michiganensis* pv. *michiganensis* which causes tomato bacterial canker, and *ii)* one of the *Xanthomonas* species that causes bacterial spot of tomato, *X. euvesicatoria* pv. *perforans* (Bella *et al.*, 2012; Aiello *et al.*, 2013; Catara and Bella, 2020).

Biocontrol of bacterial pathogens reduces the impact of copper compounds. Our results were encouraging as in the growth chamber *Cmm* spread very quickly inside the plantlets. In fact, one month after inoculation *Cmm* led to the death of 100% of the plants in the control. Two isolates, *Pseudomonas* sp. f1 and *Bacillus* sp. 265, out of the ten bacterial isolates tested *in vivo* significantly reduced bacterial canker by delaying disease progress and reducing the number of dead plants at the end of the trial compared to the control. Several studies have shown that *Pseudomonas* or *Bacillus* strains inoculated in the soil or in the seeds can reduce the incidence and severity of bacterial canker, and in some cases an induction of systemic resistance (ISR) has been suggested (Boudyach *et al.*, 2004; Nandi *et al.*, 2018; Abo-Elyousr *et al.*, 2019). In our pathogenicity tests we used two different pathosystems. In the biocontrol trial on bacterial canker both the pathogenic bacterium and the biocontrol agent have been inoculated in the soil where the two microorganisms may have interacted by competition and/or antibiotic phenomena. All biocontrol bacteria tested were able to reduce the symptoms of bacterial spot. Since the phytopathogenic bacterium in this case was inoculated on the leaves, the spatial distance between the two suggests that the mechanism of action also involves induction of systemic resistance. Phage therapy is currently considered the most effective *Xep*

biological control method (Obradovic *et al.*, 2005). However, the effect of foliar biocontrol bacteria and plant growth promoting rhizobacteria (PGPRs) and *B. pumilis* in reducing bacterial spot in greenhouse and some field trials has been already demonstrated (Byrne *et al.*, 2005; Ji *et al.*, 2006).

The microbial collection generated in this study could provide the basis for the future development of bio-inoculants using single strains or synthetic microbial communities. The bacterial isolates were obtained from the same niche of pathogens, thus it is conceivable that they could colonize tomato roots, although endophytic colonization is still to be demonstrated. The use of microbial consortia has recently emerged as an approach to combine microorganisms with different traits, effects or mechanisms of action (Compant *et al.*, 2019). Future *in vivo* studies will demonstrate how successful this bottom-up approach is and whether the isolates could be used to inoculate plantlets in the nursery, thus providing intensive tomato cultivation areas with protected plants.

3. Microbial Community Assembly and Evolution from the Nursery to the Greenhouse on Soil and Soilless Grown Tomato[1]

Abstract

Microbial communities play a crucial role in plant health and productivity. The present study provides a holistic perspective of the composition, diversity and influential factors shaping the microbial communities in commercial tomato plants. In particular, we evaluated the formation and composition of the bacterial and fungal tomato communities in commercial conditions from the nursery production materials to greenhouse grown plants. In nursery, seeds (cv Proxy), virgin substrate (peat), and plantlets ready for transplantation where sampled. In the greenhouse, agricultural soil, substrate (coconut fiber), rhizosphere and endorhizosphere of tomato plants at flowering and early fruit stage, were sampled. At the phylum level the bacterial and fungal communities were mainly constituted in all microhabitats by Proteobacteria and Ascomycota, respectively. The microbial alpha-diversity as estimated by the Chao1 richness and the Shannon's diversity indexes, was highest among bacterial communities, followed by fungal communities. The taxonomic diversity in the bacterial communities of the endorhizosphere (both seed and root) was significantly lower than that of those in the rhizosphere, agricultural soil and growing substrates (peat and coconut fiber). All samples showed a high diversity of fungal communities, with the exception of those grown in coconut fiber substrate and seeds. A high dispersion within and btween the samples was detected in the fungal communities of all samples. The analysis of the bacterial communities showed that the rhizosphere of the plants grown in coconut fiber may have the influence of the bacterial communities of the nursery, while the plants grown in agricultural soil in a short time were influenced by it. In the endorhizosfere of plants grown in agricultural soil and in coconut fiber, representative genera were *Pseudomonas*, *Streptomyces* and *Bacillus*, and *Flavobacterium*, *Bacillus*, and *Rhodococcus*, respectively. The results are discussed in relation to the beneficial potential of taxa. In addition, some potentially beneficial bacterial strains have been isolated. These bacteria were identified by DNA barcoding, and their plant growth promotion activity was analysed. These efforts will provide an important data resource for further application of the beneficial bacteria in tomato production.

Keywords: tomato, nursery, greenhouse, bacterial community, fungal community**.**

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3.1. Introduction

Tomato, with a total estimated production and cultivated area of 180 Mt and 5.03 Mha, respectively, is the most important vegetable crop on a worldwide basis (FAOSTAT, 2019). In relation to the large consumption and to the content of health-promoting compounds tomato is considered a very important component of modern diet (Dorais *et al.*, 2010). Italy, Spain and Turkey are the largest producers in Mediterranean reagion both for processed produce and fresh consumed tomatoes. The former is cultivated during hot seasons, whereas the latter is produced all year round, mainly, in mild winter climate areas inside greenhouses.

The intensive management required to mitigate serious economic losses, has encouraged the search of alternative approaches for the control of tomato diseases, including the use of biological control agents (Singh *et al.*, 2017).
Soilless cultivation of tomato crops in greenhouses has increased considerably in recent years; this is because these systems have several advantages, such as the reduction of problems related to soil-borne diseases, the optimization of plant nutrition, etc. (Savvas *et al.* 2013). Many of the soilless cultivation systems are based on the use of solid rooting media for growing plants which are often called 'growing media' or 'substrates' (Gruda *et al.,* 2013).

The choice of growing media is considered a very important aspect in soilless cultivation. On the other hand, different materials can be used and they have wide differences in terms of physical and chemical characteristics, etc. As far as microbial content is concerned some substrates are virtually free of life since they derive from sieved volcanic minerals heated at temperatures of 700-1000°C (e.g.: rockwool, perlite) (Savvas *et al.*, 2013). Even when hydroponic substrates have a pretty reduced microbial contamination (e.g. peat or coconut fiber), after starting of a soilless culture microbial titre rapidly increases by introducing the plant and the irrigation water (Postma, 2010). The density and diversity of this microflora are affected by the type of substrate (organic or inorganic), the nutrients in the solutions and the age and cultivar of the plant species (Vallance *et al.*, 2011). Although controlling soil-borne diseases was one of the most important reasons for developing soilless culture some diseases have been still occurring there (Vallance *et al.*, 2011). In addition, opportunistic pathogen minor infections, sometimes not reported elsewhere have been described. Disease outbreaks sometimes unique or more severe than in soil have been described (Stanghellini and Rasmussen, 1994; Dimartino *et al.*, 2011; Vallance *et al.*, 2011; Aiello *et al.*, 2013; Caruso *et al.*, 2016).

Microbial communities have central roles in plant health and productivity throughout the entire life cycle (Mendes *et al.*, 2011; Philippot *et al.*, 2013). Its composition and structure varies according to plant organ and compartment (e.g. rhizosphere, ectorhizosphere, phyllosphere, and endosphere), which are specific habitats for microbial colonization (Bulgarelli *et al.,* 2013; Philippot *et al.,* 2013; Berg *et al.,* 2016; Yan *et al.,* 2017). The most attention has been paid to the rhizosphere where there is a highly active microbial interaction as exudates released by plant roots are the main food source for microorganisms and a driving force for their population density and activities (Raaijmakers *et al.*, 2009; Bulgarelli *et al.*, 2013). These communities establish a dense network of neutral, pathogenic or beneficial interactions with the plants (Raaijmakers *et al.*, 2009; Vandenkoornhuyse *et al.*, 2015). A subset of them from the rhizosphere penetrate the plant roots and become endophytes, terms that generally refers to those microorganisms that can asymptomatically reside within plant tissues (endosphere), without causing diseases (Hardoim *et al.*, 2015; Collinge *et al.*, 2019). Together with this horizontal transmission deriving from the soil environment vertical transmission *via* seeds has also been demonstrated, although to a lesser extent (Truyens *et al.*, 2015; Cavazos *et al.*, 2018; Rezki *et al.*, 2018; Bergna *et al.*, 2018). The endophytic microbiome is complex and asymptomatic plant tissues harbour both beneficial (mutualistic), neutral (commensal) and potentially harmful (pathogenic) microorganisms (Hardoim *et al.*, 2015; Brader *et al.*, 2017; Collinge *et al.*, 2019). The latter could include both plant pathogens and human pathogenic that are deleterious to plant growth and health (Berg *et al.*, 2005; Raaijmakers *et al.*, 2009; Mendes *et al.*, 2013).

Metagenomics studies have also improved the understanding of the formation of the tomato microbiome in the root environment. Studies have been investigated the composition of tomato rhizo- and endorhizosphere microbial communities in different soils (Poli *et al.,* 2016; Dong *et al.*, 2019), tomato genotypes (Taffner *et al.*, 2020; French *et al.*, 2020; Manzotti *et al.*, 2020), rootstocks (Poudel *et al.*, 2019) as well as in relation to seed transmission (Bergna *et al.*, 2018), crop management (Allard *et al.*, 2016; Cai *et al.*, 2017; Usero *et al.*, 2021), and soilborne diseases (Li *et al.*, 2014a; Tian *et al.*, 2015; Larousse *et al.*, 2017; Colagiero *et al.*, 2020).

Tomato microbial communities of the rhizosphere (mainly rhizosphere and rhizoplane) have been observed to exhibit the highest richness and diversity in comparison to those of the endorhizosphere (Ottesen *et al.*, 2013; Lee *et al.*, 2019; Dong *et al.*, 2019). Overall, the results suggest that the tomato endophytic microbiome is mainly horizontally transferred from the soil environment. Soil structure and composition also affect the microbial community of the rhizosphere and to a lower extent of the endorhizosphere

(Bergna *et al.* 2018; Taffner *et al.*, 2020). Rhizosphere bacterial communities varied significantly also between native and agricultural soils and commercial substrates (Cheng *et al.*, 2020). Chialva and co-authors (2018) have been demonstrated that native soil components elicit an alert status in the plant by enhancing the induction of genes involved in defense responses, as compared with plants grown in a disinfected substrate shaping the molecular responses in tomato roots. Anyway vertical transmission has been also demonstrated as investigating the endophytic microbial communities of seeds across two generations a continuous turnover of the seed bacterial and archaeal assemblage has been observed (Bergna *et al.*, 2018; Taffner *et al.*, 2020). Seeds have been demonstrated to act as a vehicle of beneficial bacteria over seed generations (Bergna *et al.*, 2018).

Despite almost ten years of research there are no studies on the formation and evolution of the tomato microbiome in commercial conditions from seed to the stage of production of transplant seedlings and the stage of cultivation in greenhouses. Tomato transplants are produced in seed nurseries produced and treated in such a way as to reduce the risk of transmission of plant pathogens via seed. The seedling grown on virgin substrates and/or containers is transplanted under different cultivation conditions that may vary from the common agricultural soil or, in the most recent cultivation techniques, in soilless conditions in containers of inert substrate where the nutritional component is supplied through nutrient solutions. The aim of this activity is to analyse the evolution of the endophytic bacterial communities of tomato roots during the production process of the plants and their formation under different cultivation conditions. Our hypothesis is that in the tomato cultivation chain several components (i.e.: biotic and abiotic) may play a significant role in the determinism of crop microbial communities that also could influence productivity and plant health. Therefore, we investigated by metagenomic approach the microbial communities at propagule level (i.e.: the seed), at seedling level (i.e.: the plantlet and the nursery substrate) and the crop either grown on soil and soilless (i.e.: the plant and the soil/substrate). A large collection of bacterial endophytes was also selected during the growing chain to test beneficial activities and that could be further exploited in tomato microbiome engineering in nursery.

3.2. Materials and Methods

3.2.1.Experimental design

The tomato transplants (*Solanum lycopersicum* L. cv "Proxy") were produced in standard conditions in a nursery in Ragusa, Italy. Seeds were sawn in trays filled with a nursery substrate (peat substrate). Seedlings were transplanted in a commercial greenhouse located in the same province in two different cultivation conditions: agricultural soil and "soilless" in coconut fiber substrate bags. Microbiome formation-related analyses were performed analysing samples at (Figure 1 and Table 1):

a) Nursery stage T0 and T1: seeds of tomato plants cv. Proxy (Seed_T0) and peat substrate (Peat_T0) used for sowing in trays; T1 root rhizosphere (Plant_T1_Rhizo) and endosphere (Plant_T1_Endo) of tomato transplants ready for sale;

   Samples of peat substrate were directly picked from three virgin bags with a sterile spoon and transferred in sterile tubes. Seeds were directly transferred from the producer bag in a sterile tube. Samples were transported to the laboratory in a thermal bag. Tomato transplants (sowing the 19th of January 2019) were transported in their growing trays to the laboratory for the analysis and to the greenhouse for transplanting (25th February 2019).

b) Greenhouse growing stage T2: rhizosphere and rhizoendosphere of tomato plants at flowering and fruit set after transplanting in two different growing condition in agricultural soil (Plant_T2_Soil _Rhizo; Plant_T2_Soil _Endo) and soilless in coconut fiber bags (Plant_T2_CF_Rhizo; Plant_T2_CF _Endo) were sampled. The agricultural soil (soil_T2); and the coconut fiber were tested before transplanting (CF_T2);

   Samples of agricultural soil and virgin coconut fiber substrate were sampled just before transplanting in the greenhouse of a farm which commercially produces tomatoes and transported to the laboratory in a thermal bag. Tomato plantlets were transplanted in two adjacent rows consisting of 20 plants directly in the agricultural soil and 20 in 10 coconut fiber bags (two plants per bag). Plants were

drip-irrigated. Five bulk samples of the roots from four plants for each condition (soil and soilless) were sampled in plastic bags and transported to the laboratory in a thermal bag (23rd of April 2019).

Five biological replicates, for each sample were sampled: 20 seeds; four plantlets or plants arranged in a randomized design, and four substrate and soil samples collected from different zones of soil.

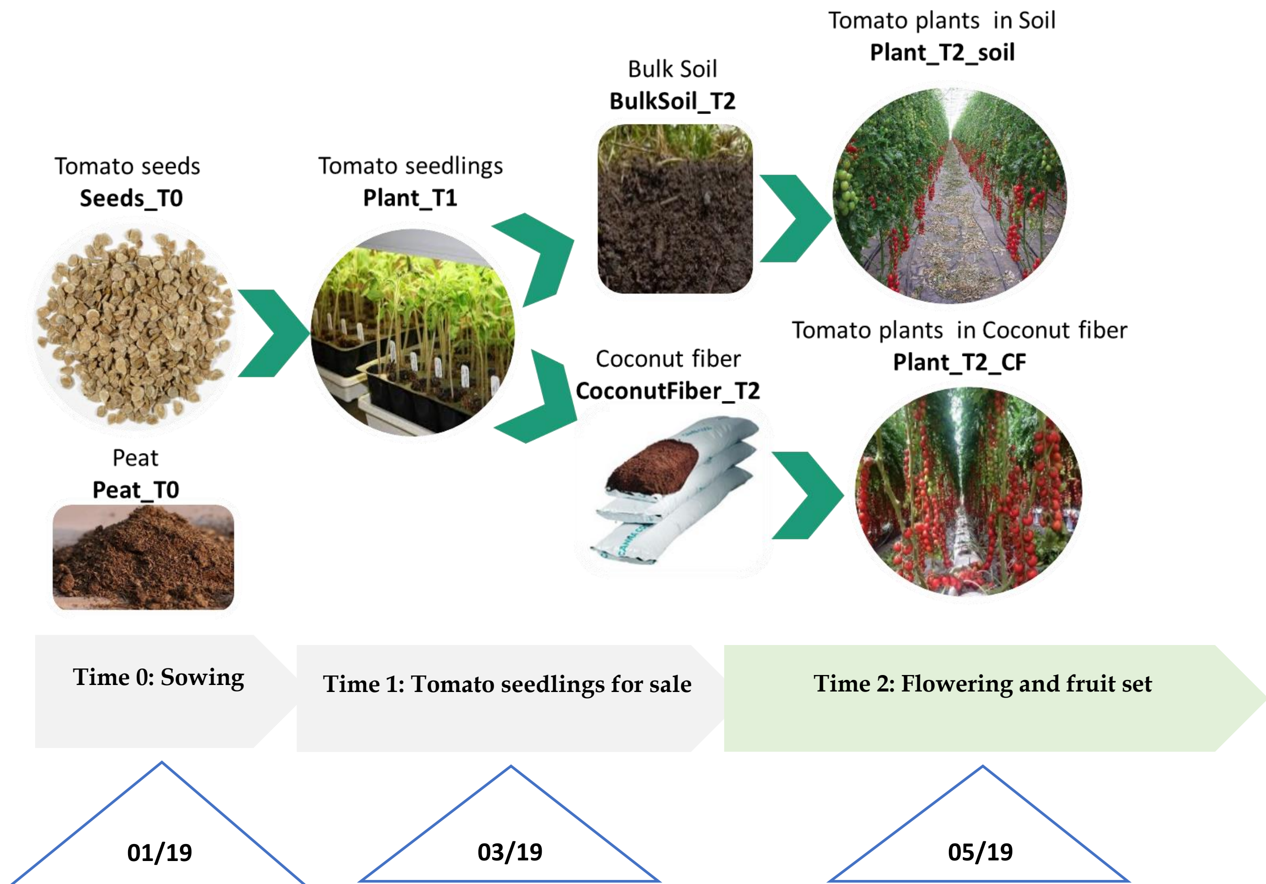


Figure 1: Experimental design of sampling.

Table 1: Source of samples and acronyms

	Nursery			Greenhouse				
	Phenological phase	Samples	ID_Samples	Phenological phase	Samples	ID_Samples	Samples	ID_Samples
SEEDS	T0	Seeds	Seeds_T0	T2				
		Seeds	Seeds_T0					
		Seeds	Seeds_T0					
		Seeds	Seeds_T0					
		Seeds	Seeds_T0					
SUBSTRATE	T0	Peat substrate	Peat_T0		Agricultural soil	Soil_T2	Cocunut Fiber substrate	CF_T2
		Peat substrate	Peat_T0		Agricultural soil	Soil_T2	Cocunut Fiber substrate	CF_T2
		Peat substrate	Peat_T0		Agricultural soil	Soil_T2	Cocunut Fiber substrate	CF_T2
		Peat substrate	Peat_T0		Agricultural soil	Soil_T2	Cocunut Fiber substrate	CF_T2
		Peat substrate	Peat_T0		Agricultural soil	Soil_T2	Cocunut Fiber substrate	CF_T2
RHIZOSPHERE	T1	Roots and soil	Plant_T1_Rhizo		Roots and soil	Plant_T2_Soil_Rhizo	Roots and soil	Plant_T2_CF_Rhizo
		Roots and soil	Plant_T1_Rhizo		Roots and soil	Plant_T2_Soil_Rhizo	Roots and soil	Plant_T2_CF_Rhizo
		Roots and soil	Plant_T1_Rhizo		Roots and soil	Plant_T2_Soil_Rhizo	Roots and soil	Plant_T2_CF_Rhizo
		Roots and soil	Plant_T1_Rhizo		Roots and soil	Plant_T2_Soil_Rhizo	Roots and soil	Plant_T2_CF_Rhizo
		Roots and soil	Plant_T1_Rhizo		Roots and soil	Plant_T2_Soil_Rhizo	Roots and soil	Plant_T2_CF_Rhizo
ENDOSPHERE	T1	Root endosphere	Plant_T1_Endo		Root endosphere	Plant_T2_Soil_Endo	Root endosphere	Plant_T2_CF_Endo
		Root endosphere	Plant_T1_Endo		Root endosphere	Plant_T2_Soil_Endo	Root endosphere	Plant_T2_CF_Endo
		Root endosphere	Plant_T1_Endo		Root endosphere	Plant_T2_Soil_Endo	Root endosphere	Plant_T2_CF_Endo
		Root endosphere	Plant_T1_Endo		Root endosphere	Plant_T2_Soil_Endo	Root endosphere	Plant_T2_CF_Endo
		Root endosphere	Plant_T1_Endo		Root endosphere	Plant_T2_Soil_Endo	Root endosphere	Plant_T2_CF_Endo

3.2.2.Sample preparation

To extract the microbial community, each replicate was processed as follow (Figure 2):

- Growing substrates and agricultural soil: five g aliquots were suspended in 20 mL sterile saline buffer (0.85% NaCl, SSD) in sterile tubes and shaken for 1 min by vortex.
- Rhizosphere (R): the tomato roots were vigorously shaken by hand to remove adherent soil particles. Five g of roots with firmly attached soil were collected and were suspended in 20 mL of SSD in sterile tubes and shaken for 5 min by vortex.

- Seeds (S) and root endosphere (E) samples were collected in sterile tubes with 20 mL SSD. Samples were then washed several times with sterile distilled water. Seeds and Root material was surface sterilized and processed according to the protocol described by Bragina *et al.* (2012). Sterility was assessed by placing the surface-sterilized seeds and roots on Potato Dextrose Agar (PDA, Oxoid, Milan, Italy) plates at 27°C for 4 days. All samples (roots and seeds) were homogenized with mortar and pestle and suspended in 20 mL of sterile saline buffer (0.85% NaCl).

Four replicates per sample of bacteria-containing pellets from both seeds and roots specimens were collected by centrifugation (20 minutes at 16.750 g) two replicates for DNA extraction were stored at -80 °C for DNA and two were immediately processed for bacterial isolation (Bergna *et al.*, 2018).

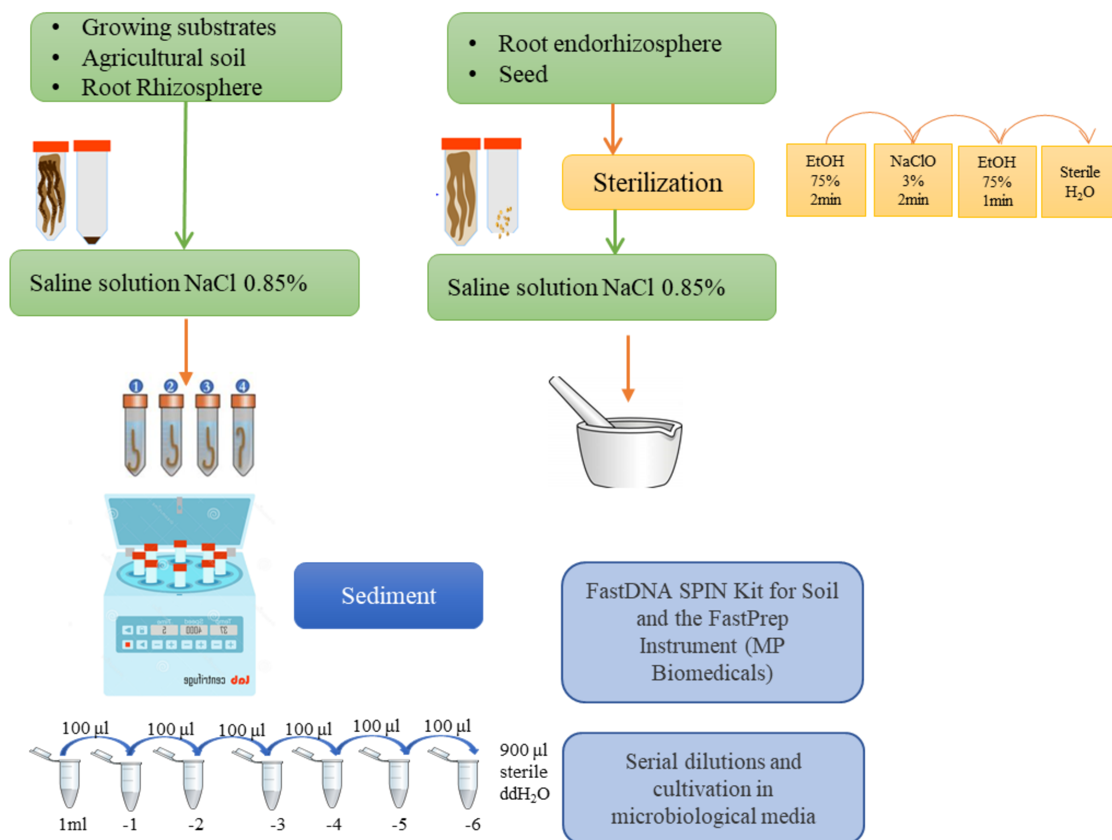


Figure 2: Protocol for isolation and analysis with molecular and culturable methods of microbial communities from tomato samples.

3.2.3.Metagenomics analysis

3.2.3.1. DNA isolation, PCR amplification and sequencing

The aforementioned pellets were used for the total community DNA isolations. DNA was isolated with the FastDNA® SPIN Kit for Soil and the FastPrep® Instrument (MP Biomedicals, Santa Ana, CA) protocol according to the manufacturer's instructions.

Three technical replicates per sample were subjected to PCR for 16S rRNA gene and fungal ITS region amplification (thermal cycler by Biometra GmbH, Jena, Germany) using Taq-&GO Ready-to-use PCR Mix (MP Biomedicals, Santa Ana, CA, USA) according to Bergna *et al.* (2018) and Wasserman *et al.* (2019), respectively. Two different barcoded primers 515f-806r targeting the 16S rDNA hypervariable region 4 (Caporaso *et al.*, 2010), and ITS1f-ITS2r to amplify part the ITS1 region of the fungal rRNA operon (White *et al.*, 1990). Barcode sequences for multiplexing of the data were used as provided by the earth microbiome project (earthmicrobiome.org/). In addition, peptide nucleic acid PCR clamps (PNAs) were used to block the amplification of plastid and mitochondrial 16S rRNA gene sequences of plants during the PCR amplification (Lundberg *et al.*, 2013). Technical replicates were combined and purified by using the Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, US), and the extracted DNA was quantified using both the Qubit dsDNA BR Assay Kits (Invitrogen, USA) and the Nanodrop 2000 (Thermo Scientific, Wilmington, DE, USA). A total of 126 barcoded samples were pooled equimolarly and sent for Illumina MiSeq sequencing (Eurofins Genomics Europe Sequencing GmbH, Germany).

3.2.3.2. Data analysis of 16S rDNA and ITS amplicon for determination of microbial community structure

The data analysis of microbial amplicons were performed according to Bergna *et al.* (2018) and Wasserman *et al.* (2019). Acquired 16S rRNA gene and ITS region sequences went through an initial quality check. Raw sequence data preparation and data analysis was performed using QIIME 2 (Quantitative Insights into Microbial Ecology, version 2019.10; Caporaso *et al.*, 2010; Bolyen *et al.*, 2019). Demulitplexing followed by quality filtering with QIIME 2 default parameters (Bokulich *et al.*, 2013) was conducted for the whole dataset. High quality reads were dereplicated and clustered with a similarity threshold of 97% via vsearch (version 2.7.1). After creating a set of representative

sequences, chimeras were filtered via both de novo reference based approaches while mapping high quality sequences (vsearch; Rognes *et al.*, 2016).

The taxonomical assignment was obtained by employing QIIME 2 environment RDP (default parameters) in combination with the SILVA 16S database (release 138; Quast *et al.*, 2013) for bacterial 16S rRNA and BLAST in combination with UNITE ITS database for fungal ITS region (version 8.1; Urmas Kõljalg *et al.*, 2013). Unassigned OTUs and non-bacterial contaminants were filtered from the resulting OTU table. OTUs abundances have been rarefied via subsampling in the QIIME 2 environment to allow comparisons between samples. A consensus-table was obtained by averaging the subsampled tables. The description of the bacterial community structure was performed using a QIIME 2 summarized table at phylum and family levels with samples belonging to the same microhabitat merged together.

Alpha diversity was calculated and rendered at OTU level in the R (R Core Team, 2013) with the Phyloseq package (McMurdie and Holmes, 2013) using Chao 1 and Shannon indexes. The PCoA plot was also generated with Phyloseq on an OTU table summarmuletized at genus level in QIIME 2. Selected OTUs were studied at more resolved taxonomic levels with the online nucleotide BLAST tool (blast.ncbi.nlm.nih.gov).

3.2.3.3. Quantitative real-time PCR

For quantifying gene copy numbers of bacteria, and fungi within tomato samples, a quantitative real-time PCR (qPCR) was performed using the following primer pairs: 515f–806r for bacteria (10 µM), and ITS1f–ITS2r for fungi (10 µM). The reaction mix contained 5 µL KAPA SYBR Green, 0.5 µL of each primer, 3 µL PCR- grade water, and 1µL of DNA template (diluted 1:10 in PCR grade water). Fluorescence intensities were detected in a Rotor-Gene 6000 real-time rotary analyzer (Corbett Research, Sydney, Australia). The cycling conditions were performed as described previously by Wassermann *et al.* (Wassermann *et al.*, 2019). Three individual qPCR runs were conducted for each replicate.

3.2.4. Molecular and phenotypic characterization of representative bacterial endophytes

3.2.4.1. Cultivable bacteria isolation

Serial ten-fold dilutions were prepared from the extract of different tomato plant samples. They were plated onto semi-selective culture media to enumerate the viable bacteria cells. Cultivable population sizes of total, fluorescent and spore forming bacteria were performed according to Anzalone *et al.* (2021). The result of cultivable bacterial population sizes were expressed as log CFU per gram of soils (growing substrates, and agricultural soil), roots (R, and E), and seeds (S).

The selection of bacterial isolated was carried out with a systematically randomized approach: solid media plates were divided in six equal parts and colonies of one of the six parts were collected according to Bergna *et al.* (2018). The colonies collected were purified, and preserved in 96 microwell cell culture plates.

3.2.4.2. Molecular identification of representative bacterial endophytes

The 16S rRNA gene region was amplified and sequenced for taxonomic identification. Bacterial DNA targets for colony PCR were prepared by thermal lysis (10 min at 100°C) of cell suspensions (OD_{600}= 0.01) in 200 µL of sterile distilled water. PCR amplicons were generated using the universal 16S rRNA primer pair, 27F/1492R primer set (Edwards *et al.*, 1989; Lane, 1991). Master mixtures included 1 x Taq&Go G2 Hot Start colorless PCR Master Mix (Promega), 0.5 µM of each primer, and 1 µL of template in a total volume of 15 µL. Reactions were performed in a thermal cycler GeneAmp® PCR system 9700, with the thermal protocol according to Anzalone *et al.* (2021). The DNA amplicons were quantified and sequenced by BMR Genomics (Padova, Italy).

3.2.4.3. Sequence analysis and construction of a phylogenetic tree

The sequences were searched against the nucleotide collection database at the National Center for Biotechnology Information (NCBI) nucleotide database using Basic Local Alignment Search Tool BLASTN (http://www.ncbi.nlm.nih.gov). Taxonomy information was assigned by the NCBI Taxonomy database according to the highest score

sequence. Highly homologous sequences were aligned using Clustal-W algorithm within MEGA X the regions of ambiguous alignment were edited manually and a neighbour-joining tree was generated (Kumar *et al.*, 2018). Sequences were aligned by Clustal W within MEGA X. A phylogenetic tree was built including all 16S rRNA gene sequences identified by BLAST.

3.2.4.4. Phenotypic characterization of representative bacterial endophytes

To evaluate *in vitro* plant growth promotion (PGP) activity of culturable bacteria we selected 94 endophytes in order to select potentially plant-beneficial bacterial endophytes. Siderophore production, phosphate solubilization, and resistance to salinity were assessed *in vitro* according to Anzalone *et al.* (2021). To evaluate the production of hydrogen cyanide (HCN) and of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase were performed by using the method of Strano *et al.* (2017), and Penrose and Glick (2003), respectively. All experiments were performed in three independent replicates.

3.2.4.5. Antimicrobial activity of representative bacterial endophytes

Bacterial endophytes were also tested for the antagonistic activity according to Anzalone *et al.* (2021). The following microorganisms were used: the bacteria *Clavibacter michiganensis* subsp. *michiganensis* strain PVCT156.1.1 (*Cmm*), *Pseudomonas corrugata* strain CFBP5454 (*Pco*), *P. siryngae* pv. *tomato* strain PVCT28.3.1 (*Pto*), *Xanthomonas euvesicatoria* pv. *perforans* strain NCPPB4321 (*Xep*) and the fungi *Fusarium oxysporum* f. sp. *lycopersici* strain Saitama ly2 (*Fol*), and *Botrytis cinerea* (*Bot*). All strains were tested in three independent replicates. Inhibition of bacterial pathogens was defined as the distance between the challenge bacterium and the marginal growth of endophytic bacteria colonies and was confirmed in all cases by using a fresh lawn of bacterial targets as controls. The antifungal activity was expressed as a Percentage of Growth Inhibition (PGI) according to Vincent (1947).

3.2.5.Statistical analysis

Phenotyping results were used to perform a principal component analysis (PCA) to detect patterns of similarity amongst tomato seed and root endosphere. The PCA was calculated on binary data (0, isolate negative to the test; 1 isolate positive to test) using the 'prcomp' function of the 'stat' R package (R Core Team, 2013). PCA biplot and loading projections were visualized through the 'factoextra' R package (Kassambara A, 2016). Mosaic plots were drawn using the 'stat' R package, the same package was also used to compute ANOVA and the post-hoc Tukey-Kramer test. Data of biocontrol assays were analysed by ANOVA using STATGRAPHICS Plus 5. Mean values were compared using the Student–Newman–Keuls test.

3.3. Results

3.3.1.General structure of the microbiome in the tomato growing chain

The DNA sequencing of marker genes from bacterial and fungal communities of seeds, growing substrates, agricultural soil, and tomato roots (rhizosphere and endosphere), resulted in a total of 11,096,000 reads. After discarding chimeras, singletons, chloroplast and non-bacterial and non-fungal contaminants, 581,129 reads remained. The bioinformatic reconstruction of the bacterial and fungal community identified a total of 7599 and 4886 distinct features, respectively. The bacterial communities were predominated by Proteobacteria phylum in all microhabitats (Figure 3A). Overall, in the seeds (Seeds_T0), endorhizosphere, and rhizosphere of plant grown on coconut fiber (Plant_T2_CF) and peat substrates (Plant_T1) they represent tha 45, 52, 55, 70, and 42% respectively. In the seeds (Seeds_T0), the heavily sequenced phyla included Proteobacteria (45%), Actinobacteria (30%), Firmicutes (13%), Bacteroidetes (10%) (Figure 3A). The Proteobacteria and Bacteroidetes phyla were enriched in the rhizospheric, and endophytic samples. The bacterial community of the rhizosphere of the

plants grown in coconut fiber substrate (Plant_T2_CF_Rhizo) is constituted by the 30% each of both Proteobacteria and Bacteroidetes. The soil and the growing substrates showed a profound different composition. Representative phyla were Actinobacteria (up to 60% in the coconut fiber substrate, CF_T2), Firmicutes, and Bacteroidetes. Verrucomicrobia, Chloroflexi, and Planctomycetes were mostly found in all samples although at different concentrations. The fungal communities were dominated by a few dominant phyla. Ascomycota was the most representative phylum in all samples (Figure 3B). The fungal community of the rhizo and endorhizosphere of the plants grown in soil (Plant_T2_Soil) and coconut fiber substrates (Plant_T2_CF) is constituted by the 7 and 11% of Mortierellomycota, respectively. The phylum Basidiomycota was present in a low percentage, only in the rhizosphere of plantlets grown in nursery (Plant_T1_Rhizo) is constituted by the 12% (Figure 3B).

3.3.1.1. Taxonomic distributions of bacterial communities

The taxonomy of the sequences was examined at the genus level (Figure 4 and Supplementary Figure S1). All growing substrates (Peat_T0, CF_T2) were characterised by deep differences within their bacterial communities. Seeds (Seeds_T0), agricultural soil (Soil_T2), and coconut fiber substrate (CF_T2) showed a dominant bacterial taxa *Rhodococcus* (18.3%), *Bacillus* (17.4%), and *Pseudonocardiaceae* (49.8%), respectively. The bacterial communities of the rhizosphere environment were characterized by bacteria of the genus *Flavobacterium* (9, 20, 15%, in rhizosphere of plants grown in peat substrate, Plant_T1_Rhizo; in agricultural soil, Plant_T2_Soil_Rhizo; and in coconut fiber substrate, Plant_T2_CF_Rhizo; respectively). The bacterial communities of the endorhizosphere of the plantlets grown in nursery (Plant_T1_Endo) were dominated by the genus *Enterobacter*. The endorhizophere of plants grown in agricultural soil (Plant_T2_Soil_Endo) were dominated by *Pseudomonas* (25.4%) and *Streptomyces* (11%) genera, whereases those of plants grown in coconut fiber substrate (Plant_T2_CF_Endo) were characterized by the dominant genera *Flavobacterium* (13.8%), and *Bacillus* (7%). Compared to the rhizospheric environment, the compositions of bacterial endophytes were quite different, and their distributions also varied in different growing substrates (Figure 4 and Supplementary Figure S1).

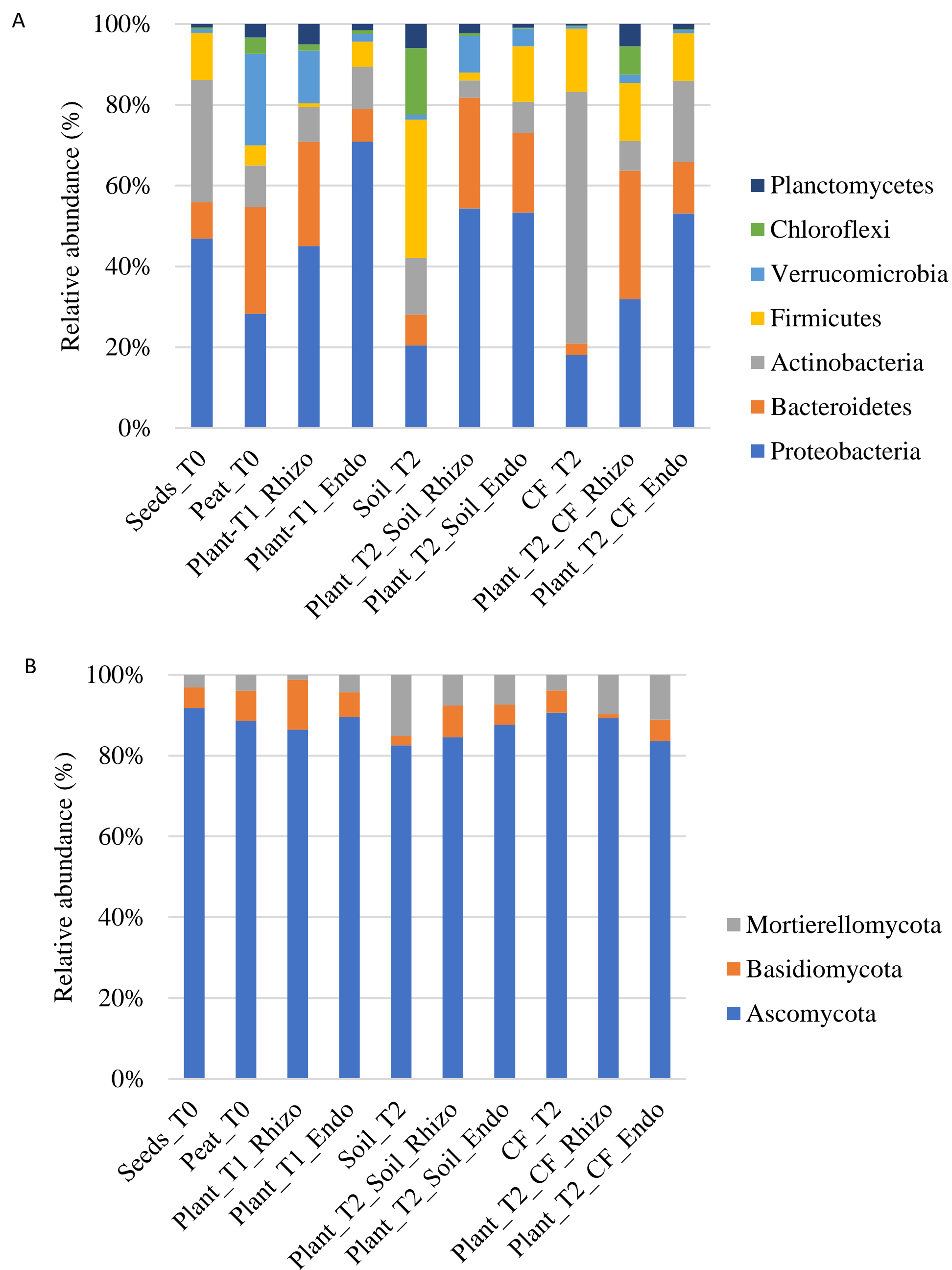


Figure 3: Comparison of taxonomic distribution in the samples of the tomato growing chain. The average relative abundances of bacterial **(A)**, and fungal **(B)** phyla are represented according to the samples.

3.3.1.2. Taxonomic distributions of fungal communities

The taxonomy of the sequences was examined at the family level (Figure 5 and Supplementary Figure S2). The two growing substrates (Peat_T0, and CF_T2) were found to be dominated by Trichocomaceae family (approximately 43%). Whereas, the

agricultural soil (Soil_T2) showed an increased abundance of members of the families Mortierellaceae and Nectriaceae (14.5, and 12.4%, respectively).

The rhizosphere fungal communities in the nursery (Plant_T1_Rhizo) is characterised by a high abundance of Pseudeurotiaceae family (45.4%). The rhizosphere of plants grown in coconut fiber substrate (Plant_T2_CF_Rhizo) showed Nectriaceae, Sordariomycetes, Plectosphaerellaceae, Mortierellaceae, Cladosporiaceae as most abundant taxa. Differently, those of plants grown in agricultural soil (Plant_T2_Soil_Rhizo) were characterised by a higher abundance of Trichocomaceae and a lower abundance of Sordariomycetes (Figure 5 and Supplementary Figure S2).

No substantial modifications in the diversity of the fungal communities was observed among the endorhizosphere of nursery and greenhouse plants. At the same time the endorhizosphere revealed a high abundance of the family Trichocomaceae that represents the taxon with the highest abundance across all the cultivation systems. In the nursery production materials (Peat_T0, and Seeds_T0) highly abundant fungal endophytes taxa

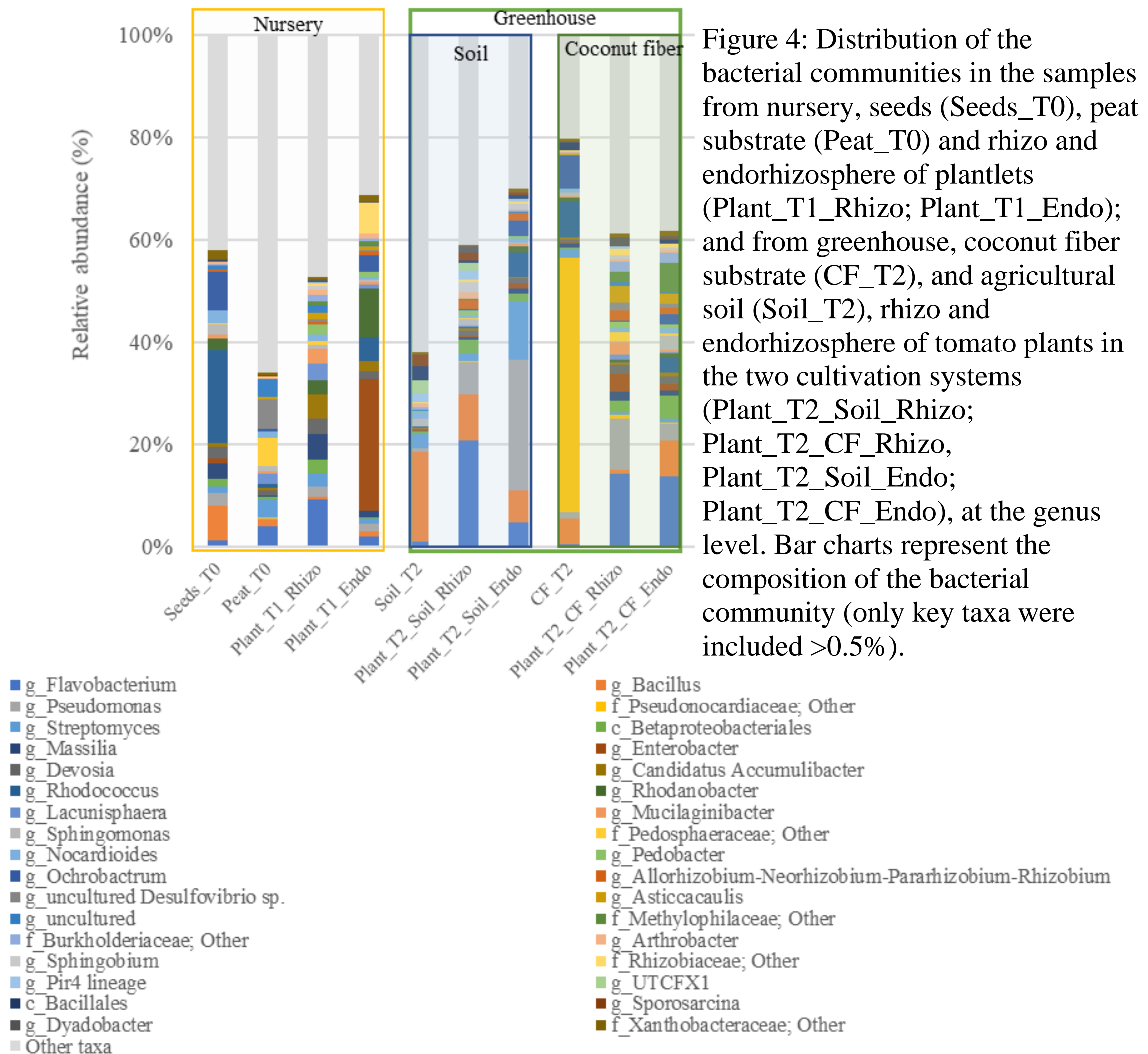


Figure 4: Distribution of the bacterial communities in the samples from nursery, seeds (Seeds_T0), peat substrate (Peat_T0) and rhizo and endorhizosphere of plantlets (Plant_T1_Rhizo; Plant_T1_Endo); and from greenhouse, coconut fiber substrate (CF_T2), and agricultural soil (Soil_T2), rhizo and endorhizosphere of tomato plants in the two cultivation systems (Plant_T2_Soil_Rhizo; Plant_T2_CF_Rhizo, Plant_T2_Soil_Endo; Plant_T2_CF_Endo), at the genus level. Bar charts represent the composition of the bacterial community (only key taxa were included >0.5%).

were Trichocomaceae, Mortierellaceae, Cladosporiaceae, Sordariomycetes, Nectriaceae (Figure 5 and Supplementary Figure S2).

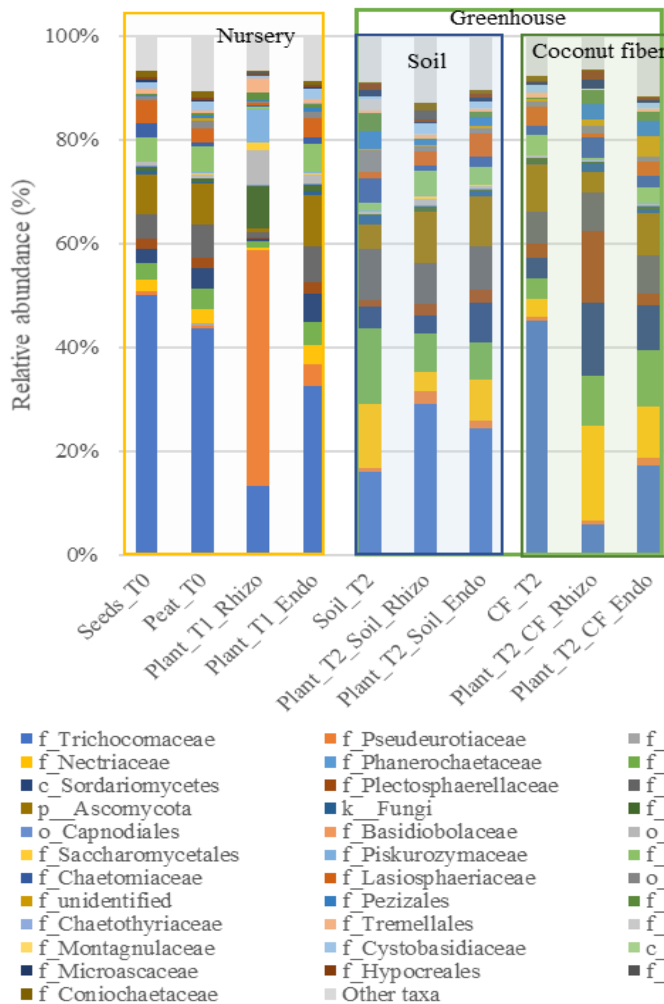


Figure 5: Distribution of the fungal communities in the samples from nursery, seeds (Seeds_T0), peat substrate (Peat_T0) and rhizo and endorhizosphere of plantlets (Plant_T1_Rhizo; Plant_T1_Endo); and from greenhouse, coconut fiber substrate (CF_T2), and agricultural soil (Soil_T2), rhizo and endorhizosphere of tomato plants in the two cultivation systems (Plant_T2_Soil_Rhizo; Plant_T2_CF_Rhizo, Plant_T2_Soil_Endo; Plant_T2_CF_Endo), at the family level. Bar charts represent the composition of the fungal community (only key taxa were included >0.5%).

3.3.2. Richness and diversity of microbial communities in the tomato growing chain

3.3.2.1. Richness and diversity of microbial communities

The microbial alpha-diversity as estimated by the Chao1 richness and the Shannon's diversity indexes, was highest among bacterial communities, followed by fungal communities (Figure 6A-D and Supplementary Table S1, and S2). Among the samples,

taxonomic diversity of the endorhizosphere (either of seeds and roots) was considerably lower than that of the rhizosphere, agricultural soil, and growing substrates. In particular, based on the Chao1 index was applied to measure the richness of the bacterial communities. The bacteria in peat substrate (Peat_T0) and agricultural soil (Soil_T2) showed the highest richness among all of the tested samples, followed by the bacteria in the rhizosphere. The lowest richness was observed in the seeds (Seeds_T0) and in the endorhizospheres (Figure 6A). The Shannon index was analysed to represent the diversity of the bacterial species. Similar to the richness analysis, the bacteria in peat substrate (Peat_T0) and in agricultural soil (Soil_T2) showed the highest diversity. The bacteria from the rhizosphere of nursery plantlets (Plant_T1_Rhizo) were more diverse than those from the rhizosphere of plants in the greenhouse in two cultivation systems (Plant_T2_Rhizo). However, differently to the richness, the diversity of endophytic bacteria was higher (Figure 6B). The fungal communities of the rhizosphere of the nursery substrate (Plant_T1_Rhizo) and agricultural soil (Plant_T2_Soil_Rhizo) showed the highest richness, whereas the lowest index was observed for samples obtained from the rhizosphere of plants grown in coconut fiber substrate (Plant_T2_CF_Rhizo) and in the endorhizosphere of plants grown in agricultural soil (Plant_T2_Soil_Endo). All samples showed a high diversity, except for the fungal communities from the coconut fiber substrate (CF_T2) and the seeds (Seeds_T0) (Figure 6C-D and Supplementary Table S1, and S2).

3.3.2.2. Evaluation of microbial communities

PCoA plotting of the beta-diversity (pairwise sample dissimilarity; Figure 7A-B and Supplementary Figure S3) showed dissimilarities among analysed samples. PCoA analysis was performed to test the effect of either the source of isolation (seeds, soil, growing substrates, rhizosphere, and endorhizophere) as well as the different stages during tomato growing chain from the nursery to the greenhouse commercial production on the microbial compositions. Axis1 and Axis2 accounted for 14.4% and 12.4% and for 22.5% and 15.7% of the total changes, for bacterial and fungal communities, respectively. The PCoA analysis of the combined data in the tomato growing chain grouped the samples based on the soil and growing substrates (Peat_T0, Soil_T2, CF_T2) but not based on nursery and greenhouse environments (T1, and T2). Despite the different soil types that the plants were grown in, the microbial communities were separated by rhizocompartments.

In the nursery condition, the bacterial communities of the seeds (Seeds_T0), the growing substrate (Peat_T0), the rhizosphere (Plant_T1_Rhizo), and the endorhizosphere (Plant_T1_Endo) were separated (Figure 7A). In greenhouse conditions, the agricultural soil (Soil_T2) and the coconut fiber substrate (CF_T2) in which the plantlets were transplanted exhibited a different community signature. The bacterial community analysis of the plants at the flowering and fruit set up phenological phase (i. e. two months after transplanting) highlighted drastic differences between the rhizosphere communities (Plant_T2_Soil_Rhizo, Plant_T2_CF_Rhizo).

The bacterial communities of the rhizosphere of plants grown in agricultural soil (Plant_T2_Soil_Rhizo) were clustered with their soil (Soil_T2), although they were distinct, and separately from all other samples. The most similar bacterial composition of the rhizosphere of the plants grown in coconut fiber substrate (Plant_T2_CF_Rhizo) was that of the nursery plantlets. Also in this case both samples clustered separately from the other samples. While the two substrates and agricultural soil, as well as their plant related rhizospheres, were clearly separated, the endorhizospheres were not easily distinguishable (Figure 7A and Supplementary Figure S3).

Complementary to the alpha-diversity analysis, the beta-diversity analysis highlighted the diversity of fungal communities that showed a high dispersion in all samples. In the

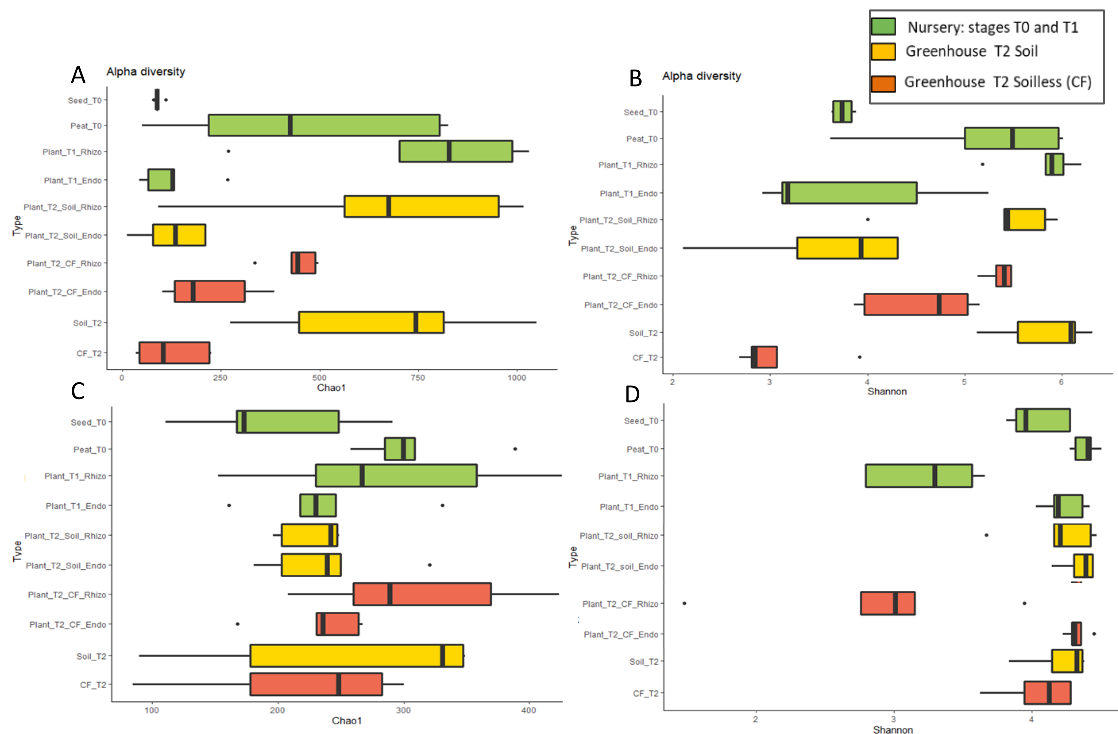


Figure 6: Estimation of the alpha diversity of the microbiome bacterial community **(A-B)**; and fungal community **(C-D)** in the different samples of the tomato growing chain based on amplicon sequencing data. The observed Chao1 and Shannon indexes were used in the analysis of the alpha diversity.

nursery condition (T0, and T1), only the fungal communities of the rhizosphere (Plant_T1_Rhizo) were distinct from the other samples (Figure 7B and Supplementary Figure S3).

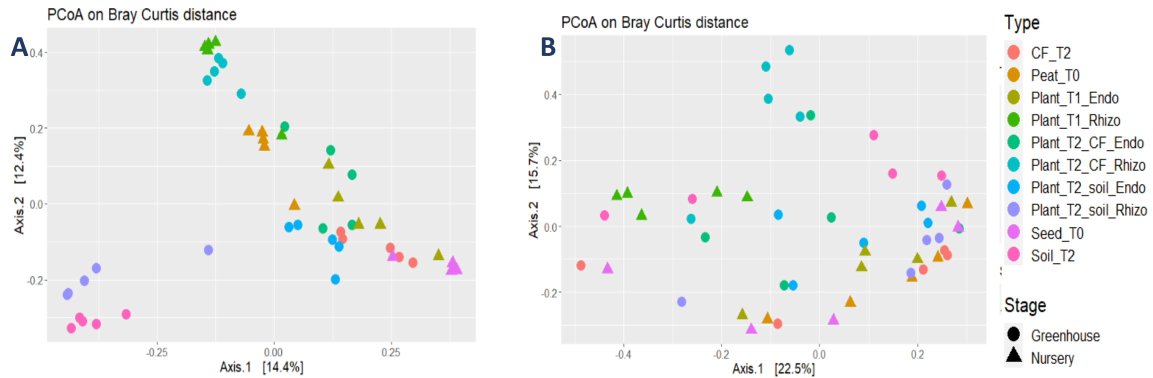


Figure 7: PCoA of bacterial **(A)**; and fungal communities **(B)** based on amplicon sequencing data. The sample clustering was based on the Bray–Curtis dissimilarity matrix. Each dot in the plot corresponds to a single sample (biological replication).

The analysis of the rhizoendosphere of plants in nursery and in greenhouse (Plant_T1, Plant_T2_Soil, and Plant_T2_CF) revealed differences in relative abundance of some bacterial OTUs. In Figure 8 the different pairwise comparison are shown. From this comparison is evident that some OTU are enriched or depleted between the endospheres of plants grown in nursery and in greenhouses (Figure 8). The bacterial communities of the endosphere of plants grown in coconut fiber substrate compared to those of plants grown in nursery showed 9 and 16 OTUs decreased and enriched in abundance, respectively. Most enriched OTUs belong to poorly studied species of Gram positive bacteria associated with plants other than *Bacillus*. The analysis between the samples of the plants cultivated in soil shows instead the enrichment of 8 OTUs (only one diminished), among these we find families with species known as PGPR and biological control agents (Pseudomonadaceae, Bacillaceae, Paenibacillaceae and Streptomytaceae).

Greenhouse plants grown earth in agricultural soil or coconut fiber substrate showed significant differences in bacterial composition athough plants originated from the same seed and transplant lot (Fig. 8C).

3.3.3.Abundance of microbial communities

The quantification via qPCR resulted in a high microbial abundances in all samples. Although differences in microbial abundance between the bacterial and fungal communities were observed (Figure 9). Analysis of bacteria, and fungi resulted in 8.7 and 8.0, mean LOG_{10} gene copy number of per mL of seed extract, respectively, whereas resulted in 8.4 to 9.7 per gram of soil and substrates for bacteria, whereas the range for fungi showed a minor gene copy numbers in soil and substrates (Figure 9). The rhizosphere compartments showed a higher value of gene copy number (both fungi and bacteria) comparing abundance in endorhizospheric compartments. The resulted showed a significant different (ANOVA $p<0.05$) for bacterial gene copy number in the soils samples (Soil_T2), and for fungi in the endorhizosphere of plants grown in coconut fiber substrate (Plant_T2_CF_Endo) (Figure 9).

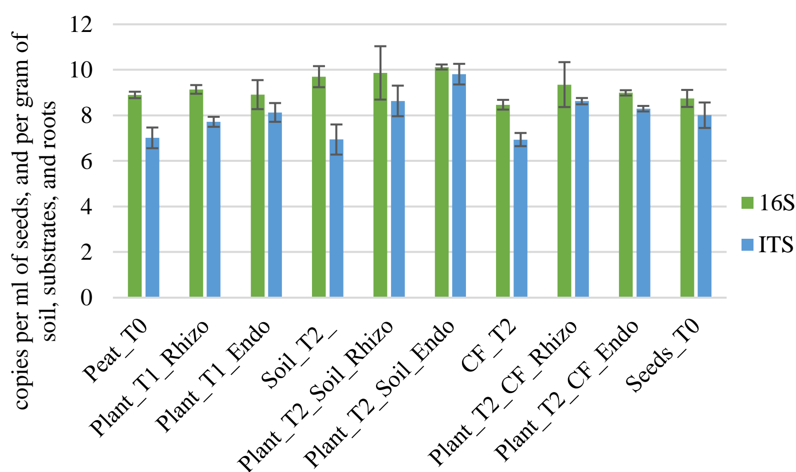


Figure 9: Microbial gene copy numbers in samples as by qPCR. Values are given by primers targeting bacterial 16S rRNA gene and fungal ITS region in the samples. Gene copy numbers are calculated per ml seed extract, per gram substrates, and roots used for the microbiome analysis.

3.3.4.Beneficial bacteria in the tomato endosphere

3.3.4.1. Large scale isolation of culturable bacteria

The diversity and the abundance of the culturable bacterial population size in the tomato growing chain were also investigated. The total and fluorescence population sizes in the seeds (Seeds_T0) ranged from 1.3 to 1.7, from 1.7 to 2.2 log CFU per gram of seed,

respectively. The agricultural soil (Soil_T2) showed a higher bacterial population size compared to those isolated from peat (Peat_T0) and coconut fiber substrates (CF_T2) (Figure 10). Plantlets in nursery showed a lower bacterial concentration than adult plants in the greenhouse when grown in agricultural soil and coconut fiber substrate that in turn showed a similar bacterial titre. Overall, total, spore forming, and fluorescent population sizes were significant differences (ANOVA $p<0.05$) in the rhizosphere samples grown in greenhouse (Plant_T2_Soil_Rhizo, and Plant_T2_CF_Rhizo) (Figure 10). Ninety-four representative bacteria from the endosphere compartments (Seeds_T0, Plant_T1_Endo, Plant_T2_Soil_Endo, and Plant_T2_CF_Endo) out of a collection of 2000 bacteria from entire experiments were selected for further investigation.

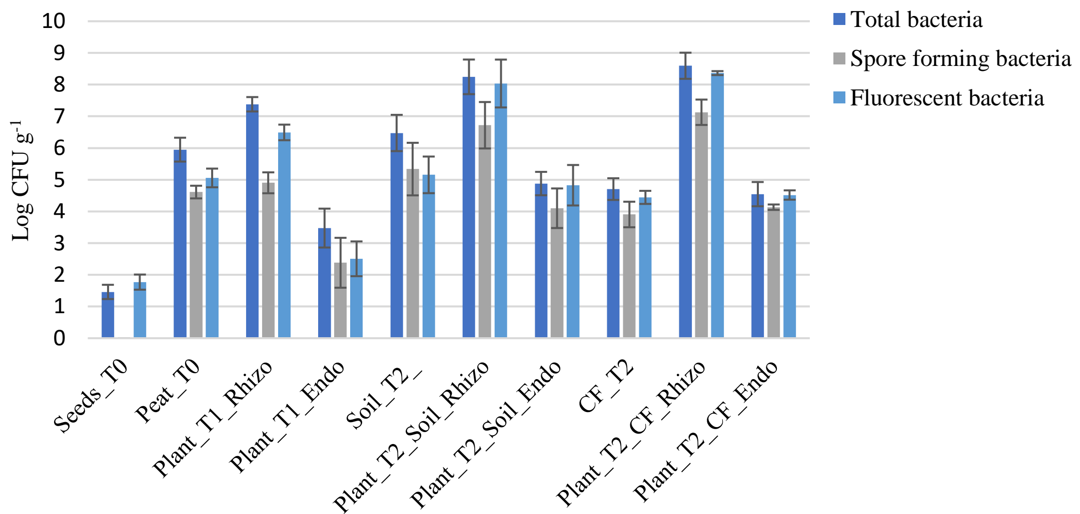
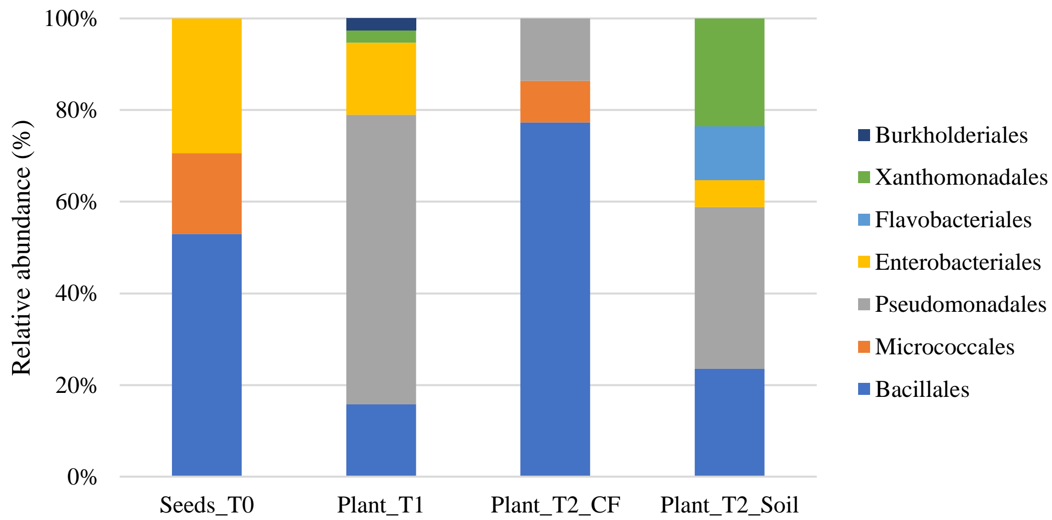


Figure 10: Bar charts of the total, fluorescent, and spore forming cultivable bacteria in different samples, the seed (Seeds_T0), peat substrate (Peat_T0) and rhizo and endorhizosphere of plantlets (Plant_T1_Rhizo; Plant_T1_Endo); and from greenhouse, coconut fiber substrate (CF_T2), and agricultural soil (Soil_T2), rhizo and endorhizosphere of tomato plants in the two cultivation systems (Plant_T2_Soil_Rhizo; Plant_T2_CF_Rhizo, Plant_T2_Soil_Endo; Plant_T2_CF_Endo).

3.3.4.2. Taxonomic identification of tomato endophytic bacteria

Partial sequences of the 16S rRNA genes of the 94 isolates obtained from the seeds and endorhizosphere of tomato plants were analysed. According to BLASTN similarity matches, isolates belonged to genera of seven orders: the Gram-positive Bacillales, and Micrococcales, and Gram-negative Pseudomonadales, Enterobacteriales, Flavobacteriales, Burkholderiales, and Xanthomonadales. More in detail the different bacterial orders include: Bacillales, with bacterial isolates belonging to the genera *Bacillus*, *Paenibacillus*, *Staphylococcus*, and *Priestia*; Micrococcales, with isolates in the genera *Glutamicibacter*, *Microbacterium*, *Curtobacterium*, *Paenarthrobacter*, and *Arthrobacter*; Pseudomonadales, with bacterial isolates in the genera *Pseudomonas*, and *Acinetobacter*; Enterobacteriales, with isolates in the genera *Enterobacter, Ewingella,* and *Serratia*; Flavobacteriales, with all bacterial isolates in the genus. *Flavobacterium*; Burkholderiales, with only one bacteria isolate in the genus *Delftia*; Xanthomonadales, with all bacterial isolates in the genus *Stenotrophomonas*. The results of BLASTN analysis and the assignment to a specific taxon are shown in Supplementary Table 3. In addition, sequences of the isolates were deposited at GenBank with the genus and strain name under accession numbers from MZ066824 to MZ066917 (Table S3).

Relative abundance of the cultivable bacteria at the order level in the different endosphere showed that the most part of the isolates belong to the order Bacillales and Pseudomonadales. From all the endospheres Bacillales were isolated, while no Pseudomonadales were found among the isolates selected from the seeds (Figure 11A). A dendrogram showing the phylogenetic relationships of the endophytic strains in this study is shown in Figure 11B.



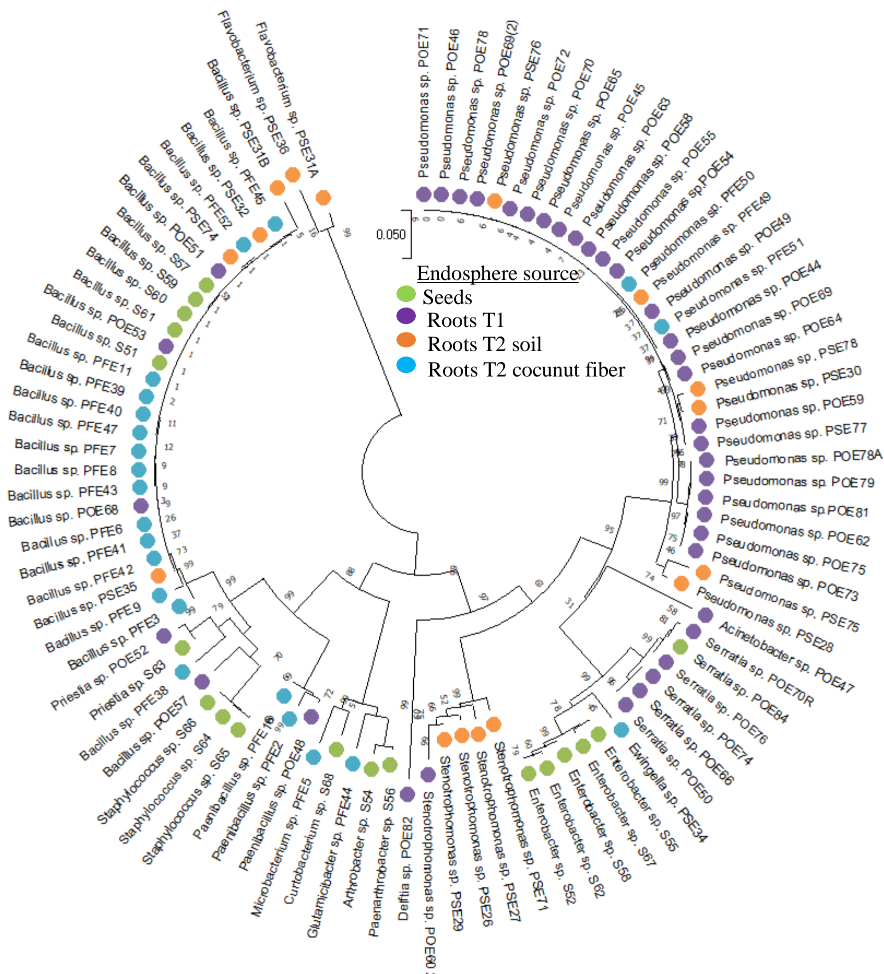


Figure 11: **(A)** Distribution of cultivable bacterial communities in the endosphere samples (Seeds_T0, Plant_T1, Plant_T2_CF, and Plant_T2_Soil) at the order level; **(B)** Phylogenetic tree of the 94 endophytic strains isolated in this study. The evolutionary history was inferred using the Neighbor-Joining method. The evolutionary distances were computed using the Kimura 2-parameter method. There were a total of 778 positions in the final dataset.

3.3.4.3. Phenotyping of beneficial traits

Isolates were characterized and selected for PGP properties and antagonistic activity against phytopathogens of tomato plants. Traits tested included siderophore production, hydrogen cyanide production (HCN), phosphate solubilisation, aminocyclopropane-1-carboxylate (ACC) deaminase activity, salt tolerance, antifungal and antibacterial activities as these are known to be important to promote plant health. Results indicate a high percentage of strains showing PGP traits; in particular, approximately 87% of bacterial isolates from the tomato endosphere compartments were able to grow in 8% NaCl. A total of 51% of endophytic bacteria were able to produce siderophores on CAS agar, and showed an ability to solubilize insoluble organic phosphate. Whereas, only 2 and 21% of isolates produced hydrogen cyanide, and aminocyclopropane-1-carboxylate (ACC) deaminase, respectively (Supplementary Table S4). Approximately 32% of the tomato endophytic bacteria (30 out 94 isolates) showed antagonistic activity against all the tomato phytopathogenic bacteria and fungi (Supplementary Table S5).

The highest activity in terms of the number of antagonistic strains but also effectiveness in terms of inhibition zone was observed against *Clavibacter michiganensis* subsp. *michiganensis*, followed by *Pseudomonas syringae* pv. *tomato*. The 56% of endophytic strains showed antagonistic activity against both the fungal targets *Fusarium oxysporum* f. sp *lycopersici* (*Fol*) and *Botrytis cinerea* (*Bot*) based on the percentage of growth inhibition (PGI) of fungal colony as compared to a non-challenged colony. Based on growth inhibition scores (0-3) 26, and 12% of isolates were ranked in class 3, indicating that their relative percentages of growth inhibition were more than 60%, against *Fol* and *Bot*, respectively (Figure 12, and Supplementary Table S6).

Principal Component Analysis (PCA) (Figure 12) was used to visualize the relationships between the eleven phenotypic traits analysed (Siderophore production, phosphate solubilisation, salt tolerance, ACC deaminase production, HCN production, antagonist activity against *Cmm*, *Pco*, *Pto*, *Xep*, *Fol*, and *Bot*) of all endophytic bacterial isolates and their identification at the order level. The first two principal components (PCs) explained 41.8% of the total phenotypic variability (PC1 = 24.7%, PC2 = 17.1%, Figure 13). Results enabled the bacteria to be clearly separated according to the order in which they were identified (Figure 13). The variables greatly influencing the bacteria disposition along the first two PCs were the antagonistic activity against *Pco*, and *Xep*, siderophore production, and phosphate solubilization (Supplementary Figure S4A-B-C-D).

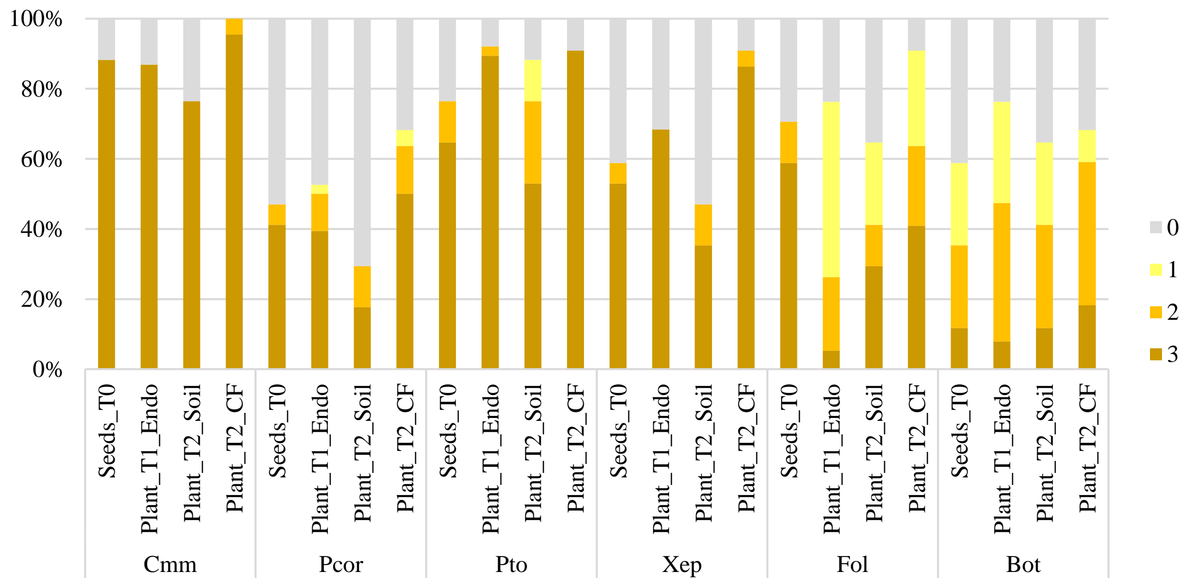


Figure 12: Categorization of the bacterial isolates from endosphere samples according to classes of biocontrol activity. Bacterial inhibition area: 0 no antagonism; 1 small area around the bacterial growth (1-3 mm); 2 large inhibition area (3-10 mm); 3 inhibition growth pathogens. Fungal Percentage of Growth Inhibition (PGI): 0, no antagonism; 1, PGI <30%; 2, PGI > 30 <60%; 3, PGI>60%.

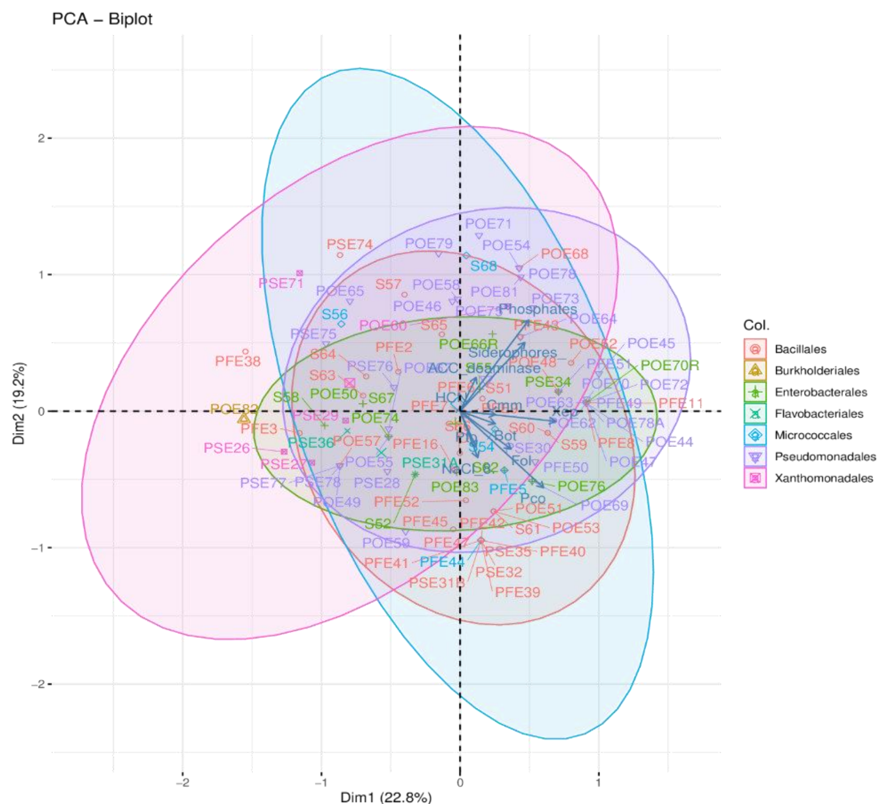


Figure 13: Principal Component Analysis (PCA) of the characteristics related to the bacterial isolates. The first two principal components are shown in a biplot and bacterial isolates are colored according to the order. In the PCA observed the eleven traits used to compute the PCA, namely: antagonist activity against *Cmm, Pco, Pto, Xep, Fol* and *Bot* (*Cmm, Pco, Pto, Xep, Fol,* and *Bot*), phosphate solubilisation (phosphate), salt tolerance (NaCl8%), production of siderophores (siderofore), HCN (HCN), ACC-deaminase (ACC).

3.4. Discussion

In the present study, by Illumina MiSeq amplicon sequencing, we studied the formation and evolution of bacterial and fungal tomato communities, and the factors driving their shifts. In particular, our experiment aimed to study the evolution of tomato microbiome starting from the nursery production materials, seeds (genotype) and a growth substrate (peat). From these materials tomato plantlets were obtained and two months after sowing, corresponding to the commercialization stage were sampled. During this period the microbial communities of plantlets may have been affected by the nursery environment and cultivation systems (e.g., irrigation, fertilization). The tomato plants were sampled again two months after transplanting in a greenhouse in two cultivation systems, agricultural soil, and soilless, by using bags of coconut fiber as a substrate. Bacterial and fungal communities from the endosphere of seeds, from rhizo and endorhizosphere of tomato plants both in nursery and in greenhouse and all the growing substrates and agricultural soil were analysed.

In particular, we analysed their correlation with seeds, soil and growing substrates. Most of the previous literature has involved field-grown tomato plants (Romero *et al.*, 2016; Toju *et al.*, 2019). Little information is available about the bacterial community structure of tomato cultivated in a greenhouse environment, which has been widely used in tomato production (Dong *et al.*, 2019; Cheng *et al.*, 2020). Overall, this study provides comprehensive insight into the microbial communities associated with tomato cultivated in a nursery and in a greenhouse agro-ecosystem, and provides useful information for their evolution. Our results showed that *i)* the microbiome is mainly transmitted horizontally by the soil, *ii)* the soil and growing substrates influenced the diversity and quality of tomato microbiome, *iii)* the agricultural soil in which there is a continuous cultivation of tomato has a microbial communities influenced by the culture itself; *iv)* the endophytic isolates obtained in culture were good candidates for further studies on seedling microbiological enrichment in nursery in agriculture.

The comparison of rhizosphere samples showed that the phylum Proteobacteria was predominant in all habitats analysed. In particular, the rhizosphere of the plantlets in the nursery hosted bacterial communities different from that of the nursery production materials (seeds and peat). The bacterial communities in the rhizosphere of plants grown in agricultural soil were enriched of phylum Proteobacteria. However, the bacterial

community of the rhizosphere of the plants grown in coconut fiber substrate is constituted by the 30% each of both Proteobacteria and Bacteroidetes. Other representative phyla were Actinobacteria, and Firmicutes (up to 13% in the rhizosphere of plants grown in coconut fiber substrate). Verrucomicrobia, Chloroflexi, and Planctomycetes were mostly found in all samples although at different concentrations. The same phyla with a prevalence of the Proteobacteria were reported in studies on tomato rhizosphere microbiome (Bergna *et al.*, 2018; Lee *et al.*, 2019; Poudel *et al.*, 2019; Cheng *et al.*, 2020). These phyla were also present in the rhizosphere of barley (Bulgarelli *et al.*, 2015), Arabidopsis (Bulgarelli *et al.*, 2012; Lebeis *et al.*, 2015), maize (Peiffer *et al.*, 2013; Fitzpatrick *et al.*, 2018).

Analyzing the communities at the genus level it is clear that the genus *Flavobacterium* was present in all rhizosphere environments in different percentages. In the rhizosphere of plants grown in nursery other representative genera were *Massilia* and *Candidatus Accumulibacter*, whereas in rhizosphere of plants grown in soil conditions representative genera were *Pseudomonas*, and *Sphingobium*; in those grown in coconut fiber substrate were *Pseudomonas*, and *Enterobacter*. Other studies on the tomato rhizosphere observed that the abundant genera in tomato rhizosphere were *Flavobacterium*, *Pseudomonas*, *Bacillus*, *Enterobacter* and *Streptomyces* (Ottesen *et al.*, 2013; Lee *et al.*, 2016). The genera *Pseudomonas*, *Acinetobacter*, and *Enterobacter* could further colonize roots (Dong *et al.*, 2019). However, some bacterial genera identified in the tomato roots at the fruiting stage grown in the fields, such as *Chryseobacterium*, *Leifsonia*, *Pandoraea*, and *Dokdonella* (Ottesen *et al.*, 2013), were not detected in our study. The microbial alpha-diversity showed the highest richness and diversity among rhizosphere samples, with the exception of those grown in coconut fiber substrate. This trend is also visible for the respective soils in which the plants have been cultivated.

PCoA plotting of the beta-diversity showed that the bacterial communities of the rhizosphere of plants grown in agricultural soil clustered with their soil, although they were distinct, and separate from all other samples. Instead, the rhizosphere of the plants grown in coconut fiber substrate clustered near that of the nursery plantlets although separately from the other samples. It is worth mentioning that both substrates, peat and coconut fiber, were never used before at the sampling time and use. Therefore, the results suggest that the nursery materials and cropping practices in the nursery shape bacterial community composition and that soilless cultivation in the greenhouse has a little influence at least in our conditions two month after transplanting. On the opposite, the agricultural soil exerted a higher influence on the tomato rhizosphere. The greenhouse in which the experiment was carried out, as well as the others in the area dedicated to

intensive cultivation, for years has been growing tomatoes, this suggests that the soil is already enriched with those communities that the tomato naturally selects during its cultivation.

Distinct tomato rhizosphere microbiota in terms of assembly and core community were also observed in commercial nutrient soils, peat and coconut fiber substrates, compared with natural field soils (Cheng *et al.*, 2020). Different studies have demonstrated that the influence of the soil plays a stronger role on plant–microbiota diversity than the plant genotype (Dong *et al.*, 2019, Cheng *et al.*, 2020). Loamy and sandy soil also affect the microbial community of the rhizosphere and to a lower extent of the endorhizosphere (Bergna *et al.* 2018; Taffner *et al.*, 2020). Bergna and co-authors (2018) have been shown that in the loamy soil bacterial communities were characterized by a higher number of rare OTUs and lower of dominant OTUs when compared with sandy soil. Conversely, the rhizosphere and root endosphere hosted a comparable number of rare OTUs, but a lower number of dominant OTUs in plants grown on sandy soil. However the same has not been observed for archeal communities as differences were observed in sandy and loamy bulk soils, but significant soil type-related effect on the abundance of the archaeal population in the rhizosphere has been observed (Taffner *et al.*, 2020). Rhizosphere bacterial communities varied significantly in natural field soils and artificial commercial soils. A higher species richness and species evenness than the peat and coconut bran based samples have been observed, suggesting that the bacterial species in the soils were more diverse and evenly distributed than those in the peat and coconut substrates (Cheng *et al.*, 2020). In addition, transcriptomics and proteomics have demonstrated that the overall characteristics of the substrate contribute more than plant genotype to shaping the molecular responses in tomato roots (Chialva *et al.*, 2018).

In the endorhizosphere samples it is possible to observe that the Proteobacteria phylum was predominant. In particular, this phylum has greater abundance in plantlets grown in nursery, whereas the Bacteroidetes and Firmicutes phyla were enriched in the endorhizosphere of the plants grown in the greenhouse. The Actinobacteria phylum was enriched only in the endorhizosphere of the plants grown in coconut fiber substrate. Same phyla were observed in the bacterial communities of seeds and root endosphere in a study by Bergna *et al.* (2018).

At the genus level the bacterial communities of the endorhizosphere of the plantlets grown in the nursery were dominated by the *Enterobacter* genus. The endorhizosphere of plants grown in agricultural soil were dominated by *Pseudomonas* and *Streptomyces* genera, whereases those of plants grown in coconut fiber substrate were characterized by the

dominant genera *Flavobacterium*, *Bacillus*, and *Rhodococcus*. Tian and co-authors (2015) observed that the abundance of the genera *Pseudomonas* and *Streptomyces* decreased in bacterial communities of the endorhizosphere of nematode-infected tomato roots. In fact, these genera were known to produce a vast diversity of active compounds against plant pathogens (Tian *et al.*, 2007; Vurukonda *et al.*, 2018). Taxonomic diversity, as estimated by the Chao1 richness and the Shannon's diversity index, of the endorhizosphere was considerably lower than that of the rhizosphere, agricultural soil, and growing substrates. However, differently to the Chao1 index, the Shannon index of endophytic bacteria was higher. Instead, the PCoA analysis has shown that the bacterial communities in the endorhizosphere samples, although distinct, were very close to each other.

Overall, soil, growing substrates, and rhizosphere were the habitats with the highest bacterial diversity, while endorhizosphere and seed hosted more selective communities. Similar trends were observed in other studies (Bergna *et al.*, 2018; Dong *et al.*, 2019). The Proteobacteria and Actinobacteria were dominant in the bacterial communities of seeds. Of them, only Proteobacteria overlapped with a previous report performed on the tomato seed (Bergna *et al.*, 2018; Dong *et al.*, 2019).

In the case of fungal communities, these results are the most variable under the conditions studied, plants in nursery peat and in greenhouse under the two growing conditions soil and soilless cultivation. The predominant phylum was Ascomycota in all habitats. The phylum which enriched in the rhizospheres in the greenhouse is Mortierrellomicota, whilst the Basidiomycota, although present in the rhizosphere, were reduced remarkably. The rhizosphere fungal community in the nursery was characterised by a high abundance of Pseudeurotiaceae family, followed by Trichocomaceae and Helotiales. Such a high abundance of these taxa is then connected with a low alpha diversity compared to the others. This peculiar fungal community setup makes these samples the most diverging from the rest of the samples (as visible in the PCoA). The rhizosphere fungal community of plants in coconut fiber and agricultural soil was more diverse. The rhizosphere of plants grown in coconut fiber substrate showed Nectriaceae, Sordariomycetes, Plectosphaerellaceae, Mortierellaceae, Cladosporiaceae as most abundant taxa. Differently, the fungal communities of the rhizosphere of plants grown in agricultural soil were characterised by a higher abundance of Trichocomaceae and a lower abundance of Sordariomycetes. No substantial modifications in the diversity of the fungal community was observed among the endosphere of nursery plants and greenhouse plants. The endorhizosphere revealed a high abundance of the family Trichocomaceae that represents the taxon with the highest abundance across all the endorhizosphere samples and

constitutes the highest abundant taxa in nursery production materials (both peat and seeds). Other highly abundant fungal taxa in this habitat were Mortierellaceae, Cladosporiaceae, Sordariomycetes, Nectriaceae.

Little is known about the influence of host genotype, and soil, on the composition of fungal communities of tomato plants. A few studies analysed the fungal community in tomato plants (Ottesen *et al.*, 2013; Lee *et al.*, 2019; Manzotti *et al.*, 2020). showed that fungal phyla Ascomycota, Basidiomycota, Chytridimycota, Glomeromycota, Zygomycota (unclassified)and Mucoromycotina (Ottesen *et al.*, 2013). Ascomycota exclusively occupied the endophytic fungal communities, the bulk soil and rhizosphere harboured significant proportions of Basidiomycota and Zygomycota (Lee *et al.*, 2019). In a study on fungal communities Manzotti *et al.* (2020) observed that the genotype plays a major role in shaping the fungal endophytic communities of tomato roots.

In our experiments we also investigated culturable bacteria associated with the endosphere environments (seeds and endorhizosphere of tomato plants grown in the nursery and the greenhouse). The bacterial colonies were selected from extracts plated in semi-selective media without choosing for color, shape, opacity, size, and morphology but with a systematically randomized approach. Molecular identification of these endophytic bacteria showed that the isolates belonged to genera of seven orders: the Gram-positive Bacillales, and Micrococcales, and Gram-negative Pseudomonadales, Enterobacteriales, Flavobacteriales, Burkholderiales, and Xanthomonadales. Similar results were obtained in a study on the endosphere tomato microbiome (Tian *et al.*, 2017). The analysis of the sequences of 16S rRNA gene has allowed us to identify among the 94 strains isolated most abundant OTUs in the metagenomic analysis.

Bacterial strains in the genera, *Rhizobium* and *Ralstonia* were isolated from the endorhizophere of tomato plants (Abbamondi *et al.*, 2016). Bergna and co-authors (2018) isolated *Ralstonia*, *Stenotrophomonas,* and *Bacillus* strains both from tomato root and seed endosphere. In our study these genera were not identified after cultivation except for *Bacillus* strains. However, bacteria from taxa corresponding to rare OTUs with metabarcoding approach have been cultured (e.g. *Priestia*, *Staphylococcus*, *Delftia*, *Glutamicibacter*). The most represented genera, as in metagenomic analysis, were *Pseudomonas* and *Bacillus*. From all endosphere environments Bacillales were isolated, while no Pseudomonadales were found among the isolates selected from the seeds The bacterial isolates showed a high ability to inhibit fungal and bacterial pathogens of tomato. In particular, among these, 15 out the 28 isolates belonging to the Bacillus species, and 12 out the 32 isolates belonging to Pseudomonas species showed antagonistic

activity against all pathogenic microorganisms. Overall, the phenotyping of 94 bacterial isolates from the tomato endosphere environments revealed that this community was more represented by Gram-negative than Gram-positive bacteria, and that they possessed interesting PGP bacterial traits. In fact, 48 out of the 94 root-associated bacteria isolates were able to produce siderophores, and solubilize phosphates, whereas 82 grow on a saline medium. In addition, only 21 and 2% were able to produce ACC-deaminase, and HCN, respectively. There is no evidence of correlation between source of isolation or taxonomy and beneficial phenotypic characters as probably all the isolates came from the endosphere. This is in harmony with the results of a previous study in which bacterial isolates from the tomato root compartments (rhizosphere, rhizoplane and endoshpere) phenotyped for their beneficial characters clustered on the basis of the farm of isolation and not for the root compartment (Anzalone *et al.*, 2021). Also in this study a large number of bacteria isolated from the tomato root endorhizosphere belonged to *Pseudomonas* and *Bacillus* although a high number of Enterobacteriaceae were also isolated. The use of microbial consortia has recently emerged as an approach to combine microorganisms with different traits, effects or mechanisms of action (Compant *et al.*, 2019). Therefore, isolates in this study could be part of an integrated approach aimed at reinforcing seedlings' microbiomes in the nursery that can positively assist them also during the following phase after transplanting.

In conclusion, the present study provides a holistic perspective of the composition, diversity and influential factors shaping the rhizospheric, endophytic bacterial and fungal communities from nursery production material to greenhouse grown tomato plants. Some potentially beneficial bacterial strains have been isolated in our laboratories, and their exact functions in tomato growth and health will be studied in the near future. These efforts will provide an important data resource for further application of the beneficial bacteria in tomato production.

4. Draft Genome Sequence of two Biocontrol Bacteria, *Pseudomonas citronellolis* strain f1 and *Pseudomonas* sp. strain 172 Isolated from Tomato Endorhizosphere[1]

Abstract

Here, we present the draft genome sequences of two *Pseudomonas* strains isolated from tomato endorhizosphere that showed *in vitro* and *in vivo* promising biocontrol activity. Results suggested that strain f1 belongs to the species *P. citronellolis*, within the *P. aeruginosa* genomic group, whereas strain 172 belongs to the *P. putida* genomic group ANIb suggests that strain 172 belongs to a new species.

Keywords: *Pseudomonas*; plant endophytes; biocontrol; draft genome sequencing.

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4.1. Introduction

The genus *Pseudomonas* comprises multiple species and strains that suppress plant pathogens, promote plant growth, induce systemic resistance, in plants. These strains defined as biocontrol agents produce several diffusible and/or volatile secondary metabolites with antibiotic properties (Haas and Keel, 2003; Raaijmakers and Mazzola, 2012). Members of the genus *Pseudomonas sensu stricto* show remarkable metabolic and physiologic versatility, enabling the colonization of diverse terrestrial and aquatic habitats and of great interest because their importance in plant and human diseases, and their growing potential in biotechnological applications (Silby *et al.*, 2011). *Pseudomonas* associated with plants, as other bacterial species, occupy different niches and vary for their effects on plants and coexisting microorganisms.

The genus *Pseudomonas* (*sensu stricto*) is currently restricted to those species related to the type species *P. aeruginosa* within rRNA similarity group I (Palleroni, 1984) or the fluorescens rRNA branch that belong to the gamma subclass of Proteobacteria (De Vos *et al.*, 1985). Phylogenetically the genus *Pseudomonas* is divided into three lineages and at least 19 groups and subgroups of species (Mulet *et al.*, 2010; Lalucat *et al.*, 2020). Comparative genomic studies also highlighted that several named species are synonymous and have to be reorganized in a single genomic species wherease many strains assigned to known species needs to be proposed as new genomic (Lalucat *et al.*, 2020).

Pseudomonas isolates strains f1 and 172 were obtained from the endorhizosphere of tomato plants grown in agricultural soil (Anzalone *et al.*, 2021). In this study the bacteria other beneficial traits associated with tomato roots in the greenhouse environment were bioprospected. Approximately 400 tomato root-associated bacteria were isolated and tested for different activity *in vitro*. Seventy-seven endophytic bacteria that showed multiple beneficial activity were identified by 16S rRNA gene (rDNA) sequencing. Strains f1 and 172 were able to grow up to the 8% NaCl, to solubilize of phosphates, to produce VOCs and siderophores. In addition, they showed antagonistic activity against tomato pathogenic bacteria and fungi (Anzalone *et al.*, 2021).

Results obtained *in vivo* in tomato showed that soil drenching with *Pseudomonas* strain f1 significantly promoted plant height compared to untreated controls and reduced significantly the wilting symptoms caused by *Clavibacter michiganensis* subsp. *michiganensis* as well as the percentage of dead plants and reduced the occurrence of leaf bacterial spot lesions in plants inoculated with *Xanthomonas euvesicatoria* pv. *perforans* (Anzalone *et al*., 2021). In bacterial spot biocontrol trials *Pseudomonas* strain 172 resulted in a remarkable reduction of symptoms.

The two isolates based on 16S rRNA were presumptively identified after BLASTn searches against the NCBI database as *P. citronellolis* (strain f1), and *P. plecoglossicida* (strain 172) (Anzalone *et al.*, 2021). A neighbour-joining tree was constructed within MEGA X (Kumar *et al.*, 2018) including type strains of these species supported the *Pseudomonas sensu strictu* lineage assignment, i.e. the *P. putida* and the *P. aeruginosa* lineages, respectively.

We therefore present here a summary of the draft genome sequence and annotation aiming in improving their taxonomic placement and in the future in dissecting their mechanism of action as promising BCA.

4.2. Material and Methods

4.2.1.Bacterial cultivation and DNA extraction

Pseudomonas sp. strains f1 and 172 were both isolated from the endosphere of tomato roots, collected from two different greenhouses located in Ragusa province (Sicily, Italy) (Anzalone *et al.*, 2021). The root samples were sterilized according to Bragina *et al.* (2012). The roots were then homogenized with a sterile pestle and mortar in 20 mL of sterile saline buffer (0.85% NaCl). Serial ten-fold dilutions in sterile saline buffer (0.85% NaCl) were prepared from endorhizosphere extract and 0.1 mL of each dilution was plated onto King's medium B agar (KB), supplemented with cycloheximide (100 $mg \cdot mL^{-1}$) to count the fluorescent pseudomonads (King *et al.*, 1954). Fluorescent bacterial colonies were selected and streaked twice on KB medium and checked for purity. Each isolate was grown under shaking in nutrient broth for 24 h at 25°C and then bacterial cells harvested by centrifugation for DNA extraction using the Gentra Puregene bacterial DNA extraction kit (Qiagen), following the manufacturer's specifications.

4.2.2.Library construction

A total amount of 1µg DNA per sample was used as input material for the DNA sample preparations. Sequencing libraries were generated using NEBNext® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) following manufacturer's recommendations and index codes were added to attribute sequences to each sample.

4.2.3.Sequencing and assembly

The whole genomes of *Pseudomonas* sp. strains f1 and 172 were sequenced using Illumina NovaSeq PE150 at the Beijing Novogene Bioinformatics Technology Co., Ltd. The raw data were filtered to obtain high quality reads and assembled using: SOAPdenovo software (version 2.04; Li *et al.*, 2008) with different K-mers (the default were 95, 107, 119) was selected for assembly and according to the project type, the optimal K-mer, further adjusting other parameters (-d -u -R - F, etc.) and the least scaffolds were chosen as the preliminary assembly result; SPAdes software (Bankevich *et al.*, 2012) with 2 Different K-mers (the default were 99 and 127) were selected for assembly. According to the project type, the assembly result was obtained with the optimal kmer and the least scaffolds. Assembled with Abyss software (Simpson *et al.*, 2009). K-mer 64 was selected for assembly and the assembly result was obtained. The assembly results of the three softwares were integrated with CISA software (Lin and Liao, 2013) and the assembly result with the least scaffolds was selected.

4.2.4.Genome Component prediction

Genome component prediction included the prediction of the coding gene, repetitive sequences, and non-coding RNA. GeneMarkS (Besemer *et al.*, 2001) program to retrieve the related coding gene. The interspersed repetitive sequences were predicted using the RepeatMasker (http://www.repeatmasker.org/; Saha *et al.*, 2008). The tandem Repeats were analysed by the TRF (Tandem repeats finder; Benson, 1999). Transfer RNA (tRNA) genes were predicted by the tRNAscan-SE (Lowe and Eddy, 1996; Lagesen *et al.*, 2007). Ribosome RNA (rRNA) genes were analysed by the rRNAmmer Smallnuclear RNAs (snRNA) were predicted by BLAST against the Rfam database (Gardner *et al.*, 2009; Nawrocki *et al.*, 2009).

4.2.5.Gene function

Six databases were used to predict gene functions. They were respectively GO (Gene Ontology; Ashburner *et al.*, 2000), KEGG (Kyoto Encyclopedia of Genes and Genomes; Kanehisa *et al.*, 2004), COG (Clusters of Orthologous Groups; Galperin *et al.*, 2015), NR (Non-Redundant Protein Database; (Li *et al.*, 2002), Pfam (Saier *et al.*, 2014) and Swiss-Prot (Bairoch and Apweiler, 2000). A whole genome Blast search (E-value less than 1e-5, minimal alignment length percentage larger than 40%) was performed against above six databases. We analysed the secondary metabolism gene clusters by the antiSMASH (Blin *et al.*, 2019).

4.2.6.Taxonomy

Gene-specific phylogenetic analysis of the following genes was undertaken: 16S rRNA gene, *gyrB* (b subunit of DNA gyrase), *rpoB* (beta subunit of RNA polymerase), and *rpoD* (sigma factor of RNA polymerase). Sequences were extracted from the assemblies of each strain. Sequences for reference strains were retrieved from GenBank (ncbi.nlm.nih.gov accessed on 14/04/201).

The evolutionary history was inferred by using the Maximum Likelihood method and General Time Reversible model (Nei, Masatoshi & Kumar, 2000). The tree with the highest log likelihood (-16838.20) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. Evolutionary analyses were conducted in MEGA X (Kumar *et al.* 2018).

The average nucleotide identity based on BLAST (ANIb) values between the genome sequence of strains f1 and 172 and the genome sequences of the type strains of the closest

related species were estimated by using PYANI (Python module for average nucleotide identity analyses) software (v0.2.10) (Pritchard *et al.*, 2020).

4.3. Results and discussion

4.3.1. General Genome Features of *Pseudomonas* sp. strains 172 and f1

The draft genome of *Pseudomonas* sp. strain f1 consists of 36 scaffolds, which are composed of 37 contigs. The N50 length is 699 kb and the largest contig approximately 1081 kb. The quality score of assembled sequences, rRNAs, tRNAs, and essential genes are 0.87, 0.9, 0.9, and 0.99, respectively. The final quality score of the draft genome is 0.929. The genome is composed of a circular chromosome without any extrachromosomal elements. The genome size was approximately 7,150,459 bp with a G + C content of 67.69 % (Table 1). A total of 6,145 protein coding genes were predicted, 81 are RNA genes. According to the COG assignment, 5,039 protein coding genes were assigned to a putative function with the 934 remaining annotated as hypothetical proteins or proteins of unknown functions.

The draft genome of *Pseudomonas* sp. strain 172 consists of 54 scaffolds, which are composed of 62 contigs. The N50 length is 178 kb and the largest contig approximately 378 kb. The quality score of assembled sequences, rRNAs, tRNAs, and essential genes are 0.87, 0.9, 0.9, and 0.99, respectively. The final quality score of the draft genome is 0.929. The genome is composed of a circular chromosome without any extrachromosomal elements. The genome size was approximately 5,750,132 bp with a G + C content of 63.94% (Table 1). A total of 5,183 protein coding genes were predicted and 91 are non coding RNA genes. According to the COG assignment, 4,125 protein

coding genes were assigned to a putative function with the 605 remaining annotated as hypothetical proteins or proteins of unknown functions.

Table 1: Genomic features, gene prediction, and annotation summary.

	***Pseudomonas* sp. 172**	***Pseudomonas* sp. f1**
Raw Reads (Mb)	2,316	1,857
Clean Reads (Mb)	1,72	1,452
DNA contigs (>500bp)	62	37
DNA, total number of bases in contigs	5,750,052	7,150,459
DNA scaffolds (>500bp)	54	36
DNA, total number of bases in scaffolds	5,750,132	7,150,459
DNA GC%	63.11	67.69
N90 Length (bp)	46,702	103,787
N50 Length (bp)	177,901	698,686
Total genes	5,183	6,145
Genes with function prediction	5,084	6,058
Protein coding genes assigned to SwissProt	2,476	2,932
RNA genes	25	24
tRNA genes	66	57
Tandem repeats (%genome)	0.69	1.16
Genes with function prediction	5,084	6,058
Genes assigned to COGs	4,069	5,067
COG's genes with unknown function	262	331
COG's protein coding genes for secondary metabolites biosynthesis, transport and catabolism	125	233
Genes assigned to KEGGs	5,018	6,004
Genes with Pfam domains	3,636	4,438
Genes assigned to GOs	3,636	4,438
GO's protein coding genes with transporter activity	317	365

4.3.2.Functional Annotation and Analysis

Using COG function assignment, 5973 of protein coding genes of strain f1 could be classified into 25 COG categories. The properties and the statistics of the genome are summarized in Table 2. The most abundant category of metabolism, information storage

and processing, and cellular processes and signaling are related to amino acid transport and metabolism (612, 10.24%), transcription (533, 8.92%), and signal transduction mechanisms (430, 7.19%).

Using COG function assignment, 4730 of protein coding genes strain 172 could be classified into 24 COG categories. The properties and the statistics of the genome are summarized in Table 2. The most abundant category of metabolism, information storage and processing, and cellular processes and signaling are related to amino acid transport and metabolism (512, 10,82 %), transcription (423, 8.94 %), and signal transduction mechanisms (343, 7.25 %). Taxonomic Classification Based on MLST (and Whole-Genome and Core Gene Analyses).

GO (Gene Ontology) (Supplementary Table S1), KEGG (Kyoto Encyclopedia of Genes and Genomes), COG (Clusters of Orthologous Groups), NR (Non-Redundant Protein Database), Pfam and Swiss-Prot prediction outputs are reported in supplementary materials (Supplementary Figure S1, S2, and Supplementary Table S1).

4.3.3.Secondary metabolites

Genome mining by antiSMASH 6 beta resulted in the prediction of 10 gene clusters in 9 regions associated with secondary metabolite biosynthesis in both *Pseudomonas* strains f1 and 172 (Supplementary Table S2). Some of the biosynthetic gene clusters encoded non-ribosomal peptide synthetase (NRPS) regions in f1 (13.1; 20.1) and in 172 (1.1, 1.2, 38.1, 41.1, 48.1) Some of them were putatively involved pyoverdine biosynthesis although they were split in different regions (Supplementary Table S2).

Pyoverdines are the fluorescent pigments produced by *Pseudomonas* species and their primary siderophore (Cornelis, 2010). Siderophores and antibiotics have both been shown to be involved in antagonistic activities against plant pathogens and plant promoting activities (Haas and Défago, 2005). Typically, a given strain produces from two to five pyoverdines, differing only in the small dicarboxylic acid side chain. Genes (pvd) responsible for the biosynthesis of pyoverdines are present in a single locus in some *Pseudomonads*, such as *P. syringae*, or up to five different loci in the genome of other species, such as *P. fluorescens* (Gross and Loper, 2009).

Table 2: Number of CDSs associated with COG functional categories.

		Pseudomonas sp. 172		*Pseudomonas* sp. f1	
Functional_class	**Class_description**	**No Gene**	**%**	**No Gene**	**%**
A	RNA processing and modification	1	0.02	1	0.02
B	Chromatin structure and dynamics	3	0.06	5	0.11
C	Energy production and conversion	290	6.13	394	8.33
D	Cell cycle control. cell division. chromosome partitioning	50	1.06	47	0.99
E	Amino acid transport and metabolism	512	10.82	612	12.94
F	Nucleotide transport and metabolism	94	1.99	109	2.30
G	Carbohydrate transport and metabolism	236	4.99	262	5.54
H	Coenzyme transport and metabolism	223	4.71	269	5.69
I	Lipid transport and metabolism	224	4.74	371	7.84
J	Translation. ribosomal structure and biogenesis	259	5.48	267	5.64
K	Transcription	423	8.94	533	11.27
L	Replication. recombination and repair	122	2.58	126	2.66
M	Cell wall/membrane/envelope biogenesis	273	5.77	286	6.05
N	Cell motility	117	2.47	159	3.36
O	Posttranslational modification. protein turnover. chaperones	169	3.57	202	4.27
P	Inorganic ion transport and metabolism	284	6.00	417	8.82
Q	Secondary metabolites biosynthesis. transport and catabolism	125	2.64	233	4.93
R	General function prediction only	460	9.73	603	12.75
S	Function unknown	262	5.54	331	7.00
T	Signal transduction mechanisms	343	7.25	430	9.09
U	Intracellular trafficking. secretion. and vesicular transport	87	1.84	82	1.73
V	Defense mechanisms	122	2.58	142	3.00
W	Extracellular structures	31	0.66	52	1.10
X	Mobilome: prophages. transposons	20	0.42	39	0.82
Z	Cytoskeleton	0	0.00	1	0.02
	TOTAL	4730		5973	

Both strains presented additional gene clusters for a number of secondary metabolite groups which have a potential role in antibiosis and deserve further analysis. In particular, the following clusters were reported (Supplementary Table S2): ribosomally synthesised and post-translationally modified peptide product (RiPP), RRE-element containing cluster, redox-cofactors, ranthipeptide. A thiopeptide cluster was found in strain f1. Since both bacterial strains were able to grow in 8% NaCl and were able to solubilise phosphates it is worth mentioning that they showed clusters for the production of the dipeptide N-acetylglutaminylglutamine amide (NAAG) and a redox cofactor similar to

pyrroloquinoline-quinone (PPQ). NAGGN production was discovered in the bacterium *Sinorhizobium meliloti* grown at high osmolarity, and subsequently shown to be synthesized and accumulated by a few osmotically challenged bacteria. NAGGN production and role in osmotolerance in *P. aeruginosa* and *P. syringae* was demonstrated (D'Souza-Ault *et al.*, 1993; Kurz *et al.*, 2010). Moreover, plant growth promoting bacteria that use glucose-dehydrogenase-PQQ holoenzyme for solubilization of both inorganic and /or organic phosphates in soil were studied (Sashidhar and Podile, 2010).

4.3.4.Taxonomic Classification

Phylogenetic analysis of the 16S rRNA gene of strains f1 and 172 allowed to establish that the two strains belong to the *P. aeruginosa* and *P. putida* genetic groups, respectively (Anzalone *et al.*, 2021). According to the four gene based scheme of Mulet *et al.* (2010) we performed a phylogenetic analysis using individual and concatenated phylogenetic trees from 16S rRNA, *gyrB*, *rpoB*, and *rpoD* partial gene alignments (Figure 1, and Supplementary Figure S3). The analysis included 8 *Pseudomonas* species type strains of the *P. aeruginosa* group and all the 7 type strains of the valid species of the *P. putida* group. The phylogenetic analysis of the concatenated series showed that strain f1 clustered close to *P. citronellolis* DSM 50332^{T} whereas strain 172 clustered separately but close to *P. montelii* DSM 17497^{T} and *P. putida* NBRC 14164^{T} which group together.

According to the MLSA results we chose the genome sequences of type strains within the the *Pseudomonas sensu stricto* lineages of *P. aeruginosa*, for strain f1, and of *P. putida* for strain 172 to calculate the Average Nucleotide Identity (ANI) from pairwise comparisons of all sequences shared between any two strains. These are measures of genetic relatedness based on sequences conserved among compared genomes and have gained acceptance as a method for defining bacterial species (Chan *et al.*, 2012).

The ANI (ANIb) value above the threshold range (95 to 96%) of species delineation (Richeter and Rossello-Mora, 2009) with the genome of *P. citronellolis* DSM 50332^{T} (98.38% ANI) indicates that strain f1 belongs to the same species (Figure 2).

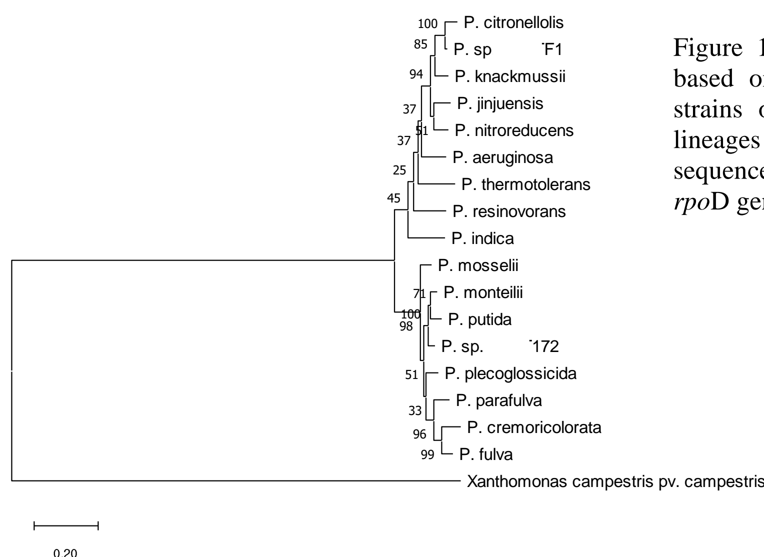

Figure 1. Comparison of strains f1 and 172 based on a MLSA-scheme to *Pseudomonas* strains of the *P. aeruginosa* and *P. putida* lineages with four concatenated partial sequences of genes 16S rRNA, *gyr*B, *rpo*B, *rpo*D genes.

The size of the genome of strain f1 was within the expected range based on the genome sequence of *P. citronellolis* strains deposited in GenBank at NCBI (ncbi.nlm.nih.gov accessed on 14/04/201)

Strain 172 showed the closest ANI values 89.69 and 89.57 % with the genomes of the type strains of the species *P. montelii* DSM 17497^T and *P. putida* NBRC 14164 ^{T}, respectively (Figure 3). Since the sequences of the type strains of all the 15 recognised species within the *P. putida* lineage of the *Pseudomonas sensu strict*u were included it could be argued that the new genome belongs to a new species.

The *P. putida* group of species is one of the most versatile and best studied. Comparative genomics showed that as well as 15 species with validly published names at least 36 genomic species can be delineated within the *P. putida* phylogenetic group of species (Peña *et al.,* 2019). Regarding their interactions with plants some strains of these species promote plant growth or act as plant pathogens. Their genome sizes are among the largest in the group, ranging from 5.3 to 6.3 Mbp (Peña *et al.*, 2019). The genome, GC content and total genes and genes with function prediction of strain f1 are within the range of the group according to Peña *et al.* (2019).

In a previous study on biodiversity of tomato bacterial endophytes of roots 18 *Pseudomonas* out of the 77 bacterial endophytes were accounted. All but one (strain f1) of these strains based on the 16S rRNA gene phylogeny clustered with *Pseudomonas* type strains of the *P. putida* group among them strain 172. This strain in particular cluster with other 13 strains isolated from two different farms. We therefore built a new phylogenetic tree including the16S rRNA genes of the type strains of the valid species of the other 7

Pseudomonads in the *P. putida* group and strains from the study of Anzalone *et al.*, (2021).

The 13 strains clustered tight to strain 172 (Supplementary Figure S4). These results suggest that this putative new bacterial species is common in tomato root environmentsand will deserve further studies to shed light on the taxonomy and the role as BCA.

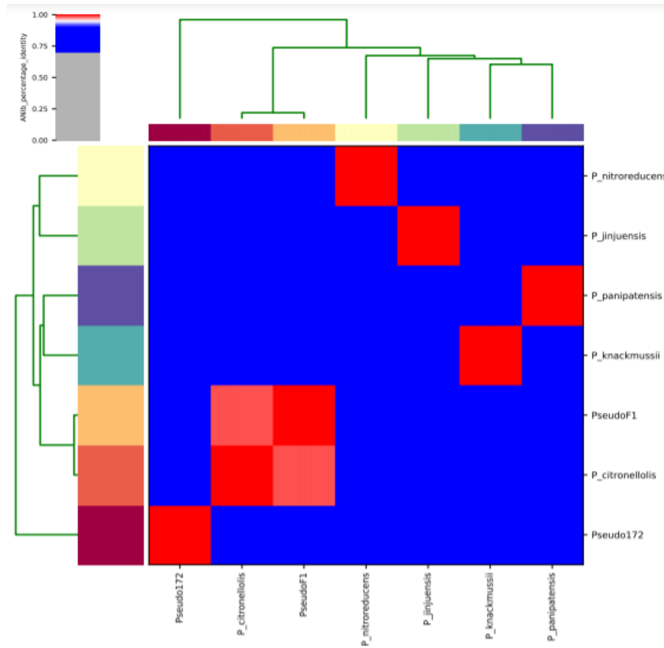

Figure 2: ANIb of *Pseudomonas* strain f1 and type strains fo species within the *P. aeruginosa* group.

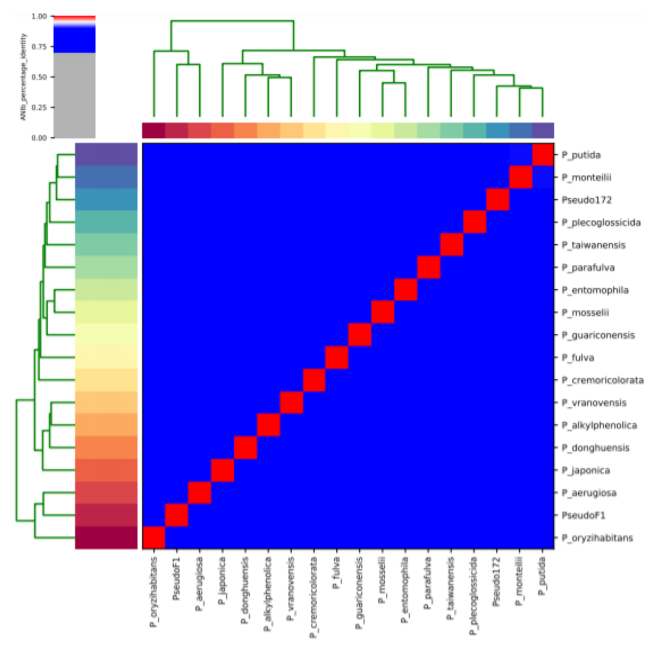

Figure 3: ANIb of *Pseudomonas* strain 172 and type strains of the 15 valide species within the *P. putida* group.

5. General discussion and Conclusions

The specific objectives of the PhD thesis project referred to the selection of endophytic bacteria of tomato equipped to act as biostimulants, biofertilizers and/or biological control agents, and to the development of a model system for the HTS analysis of traceability and profile of tomato bacterial and fungal communities: from nursery to greenhouse, in soil and in soilless.

In the last few years, the consumers' interest in healthy, food safety and environmental pollution has been increasing, stimulating a rise in the demand for organic food products whose regulations prohibit or greatly limit the conventional crop protection strategies aimed to increase the yields based on an abnormal use of chemicals to force the plant growth and to control of plant pathogens, responsible of progressive soil and water pollution. Nowadays, recent microbiological studies have rediscovered the importance of microbiome associated with plants , its role in the defense and growth of host plants, and have shown alternative solutions based on the use of selected microorganisms as biofertilizers, biostimulants and antagonists of plant pathogens (Berg *et al*., 2017).

They can have beneficial effects on host plants through a variety of mechanisms, promoting the plant growth, suppressing pathogens; increasing the competition for space, nutrients, in the ecological niches; producing antimicrobial substances and biostimulants, such as phytohormones and peptides, withoutnegative effects on the user, the consumer or the environment (Bulgarelli *et al.*, 2013; Gaiero *et al.*, 2013).

In this context we investigated the potential of the exogenous introduction of "beneficial" microorganisms to avoid or overcome some stress conditions. Then we developed a model system for the NGS analysis of the tomato microbiome associated to the system of tomato production and cultivation in protected culture, since its formation from the nursery material (seed, growth substrate, and plantlets) up to the greenhouse in two different cultivation systems, in agricultural soil and in coconut fiber substrate (soilless).

The results will help future analyses to investigate the beneficial extent of the introduction of “selected good microorganisms” in the nursery process to improve the production and health of the plantlets before transplantation in greenhouses.

During the experimentation more than 500 bacteria were isolated and characterized from the three compartments of the tomato roots (rhizosphere, rhizoplane, and

endorhizosphere) and from the endosphere of the seeds (activity in Chapter 2 and 3). This collection is now kept at the Di3A - Unict, for later studies and applications.

The phenotypic analysis of cultivable root-associated bacterial communities of tomato plants grown in the greenhouses showed that, beside the antagonistic activity *in vitro*, other “beneficial” traits were common to bacteria of the rhizosphere, rhizoplane and endorhizosphere increasing their for potential use as bioinoculants.

Most of the bacteria isolates were from farms of an intensive area of tomato cultivation in greenhouses that had a history of repeated cultivation; it istherefore conceivable that over the years bacterial communities with specific attitudes were selected.

Overall, the phenotyping of bacterial isolates from the tomato root environment revealed that the Gram-negative bacteria community was more represented than that of Gram-positive, and that they possessed interesting PGP bacterial traits. In fact, 139 out of the 424 isolates were able to produce siderophores, solubilize phosphates and grow on a saline medium, and approximately 30% of the 424 showed antagonistic activity against the five tested phytopathogens. These characteristics are of great interest in developing bioinoculants with biocontrol and biofertilizer abilities to promote plant growth and yield. In addition, results suggest that beneficial activities are commonly spread in each root compartment.

The bioprospecting allowed us to establish a collection of 424 phenotyped isolates and a subcollection of 77 endophytes whose taxonomic identification was performed by sequencing the 16S rRNA gene.

The investigation of the relationship between the bacterial families and the *in vitro* antagonistic activity of the tomato endophytes, showed that *Bacillus* isolates were significantly more active than isolates belonging to Pseudomonadales and Enterobacteriales to antagonize selected tomato pathogens. Almost the 40% of the endophytic bacteria characterized belong to the Enterobacteriales and include five different genera (including eleven strains of *Enterobacter*) of Enterobacteriaceae, reported as indigenous component of the plant microbiome in different plant species (Brandl, 2006; Teplitski *et al.*, 2011; Erlacher *et al.*, 2014; Erlacher *et al.*, 2015; Tian *et al.*, 2017).

Despite this, the use of bacteria of the genus *Enterobacter* is not encouraged because some species are associated with human diseases (Rock and Donnenberg, 2014). Unless future in-depth genomic analyses would ascertain if the communities identified in the endosphere of the plants have affinities with those found in humans or if the accessory genome has differentiated according to the endophytical niche they live in.

For this reason, the *in vivo* assays were performed with bacteria belonging to the genus *Pseudomonas* and *Bacillus* that are universally recognized as BCA and PGPR and used in microbial consortia as biofertilizers and biostimulants or as active substance in some biopesticide.

Some *Pseudomonas* species are widely used as biocontrol agents in (Mercado-Blanco and Bakker, 2007) thanks to the production of several diffusible and/or volatile secondary metabolites with antibiotic properties such as diacetylphloroglucinol, pyrrolnitrin, cyclic lipopeptides phenazine (Haas and Keel 2003; Raaijmakers and Mazzola 2012).

Most endophytic *Bacillus* isolates belong to the *B. amyloliquefaciens* and *B. subtilis* group and, to a lesser extent, the genus *Paenibacillu*s spp. have been proven to be efficient at plant growth promotion and biocontrol against plant pathogens such as viruses, bacteria, fungi and plant roots nematodes (Vacheron *et al.*, 2013; Fan *et al.*, 2017). They produce numerous antibiotics including polymyxin, difficidin, subtilin, mycobacillin, zwittermicin A, which are active against plant pathogenic bacteria and fungi (Borriss, 2015; Caulier *et al.*, 2019). Due to their ability to produce durable endospores, which enable stable bioformulations with a long shelf-life, they are the most widely used on the biopesticide market (Borriss 2011; 2015).

The bacteria used had been selected either for a wide range of antagonistic activity *in vitro* and for the intensity of this activity, and for representativeness with respect to their taxonomic position. As often verified in the literature, the highest *in vitro* capacity was not confirmed as the best active *in planta* assay (Berg *et al.,* 2002; Long *et al.,* 2008; Abbamondi *et al.,* 2016). In fact, complex interaction mechanisms can contribute to the success of the colonization of a bacterium ranging (e.g. motility, competition with other microorganisms, soils pH, MAMP recognition by the plant) (Lundberg *et al.*, 2012; Bulgarelli *et al.*, 2013).

The formation and evolution of bacterial and fungal tomato communities, and the factors driving their shifts, were studied by Illumina MiSeq amplicon sequencing, starting from the nursery production materials, seeds and a growing substrate (peat). Tomato plantlets obtained two months after sowing, corresponding to the commercialization stage, were compared with sister plants sampled two months after transplanting in agricultural soil and in bags of coconut fiber as a substrate. Bacterial and fungal communities from the endosphere of seeds, from rhizo and endorhizosphere of tomato plants both in the nursery and in the greenhouse and all the growing substrates and agricultural soil were analysed.

Our results showed that *i)* the microbiome was transmitted horizontally by soil, *ii)* the soil and growing substrates influenced the diversity and quality of tomato microbiome, *iii)*

the agricultural soil in which there is a continuous cultivation of tomato has developed a microbial communities influenced by the culture itself; *iv)* the endophytic isolates obtained in culture were good candidates for further studies on seedling microbiological enrichment in nursery in agriculture.

The rhizosphere of the plantlets in the nursery hosted bacterial communities distinguishable from that of the seeds and peat. The bacterial communities in the rhizosphere of plants grown in agricultural soil were enriched of phylum Proteobacteria. However, the bacterial community of the rhizosphere of the plants grown in coconut fiber substrate is constituted by the 30% each of both Proteobacteria and Bacteroidetes.

In the endorhizosphere samples the Proteobacteria phylum was predominant. In particular, this phylum has greater abundance in plantlets grown in the nursery, whereas the Bacteroidetes and Firmicutes phyla were enriched in the endorhizosphere of the plants grown in the greenhouse. The Actinobacteria phylum was enriched only in the endorhizosphere of the plants grown in coconut fiber substrate.Overall, soil, growing substrates, and rhizosphere were the habitats with the highest bacterial diversity, while endorhizosphere and seed hosted more selective communities.

In the case of fungal communities, these results are the most variable under the conditions studied, plants in nursery peat and in greenhouse under soil and soilless cultivation. The predominant phylum was Ascomycota in all habitats.

In conclusion, this study provides a holistic perspective of the composition, diversity and influential factors shaping the rhizospheric, endophytic bacterial and fungal communities from the nursery production material to the greenhouse grown tomato plants. Some potentially beneficial bacterial strains have been isolated in our laboratories, and will be helpful for future studies of their multiple functions in tomato growth and health. These efforts will provide an important data resource for further application of the beneficial bacteria in tomato seedlings in nursery.

The genomes of two of the *Pseudomonas* isolates used *in planta* biocontrol assay have been sequenced and the characteristics described. Using bioinformatic pipelines able to predict the clusters of secondary metabolites, we investigated their potential in relation to and in support of biological activities. The results obtained by comparative genomics allowed one of the bacterial strains to be assigned to the species *P. citronellolis* while the other strain though belonging to the lineage of *P. putida* within the *Pseudomonas sensu stricto* showed ANI percentages very low compared with lineage type strains, supporting the hypothesis of belonging to a new bacterial specie.

This information is of particular interest because, based on 16S rRNA gene (in Chapter 2), as many as 14 isolates were similar to the sequence. In addition, several metabolic clusters are common to other *Pseudomonas* interesting for the development of biological control and sustainable agriculture or useful to support the registration of bioinoculants products under the current legislation for biofertilizers and biopesticide.

To reach a comprehensive understanding of the dynamics that occur between plants and their rhizosphere microbiome the model organism *Solanum lycopersicum* and its associated rhizosphere microbial community was studied to provide a framework of the mechanisms and complexities that occur in this niche. The microbial collection generated could provide the basis for the future development of bio-inoculants using single strains or synthetic microbial communities. The bacterial isolates were obtained from the same niche of pathogens, thus it is conceivable that they could colonize tomato roots, although endophytic colonization is still to be demonstrated. The use of microbial consortia has recently emerged as an approach to combine microorganisms with different traits, effects or mechanisms of action (Compant *et al.*, 2019). Future *in vivo* studies will demonstrate how successful this bottom-up approach is and whether the isolates could be used to inoculate plantlets in the nursery, thus providing intensive tomato cultivation areas with protected plants.

6. References

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7. Supplementary material

7.1. Supplementary Material: chapter 2

Bioprospecting of beneficial bacteria traits associated with tomato root in greenhouse environment reveals that sampling sites impact more than the root compartment

Supplementary Figures

Figure S1: Heat map obtained using two arbitrary scales to quantitative evaluate the antagonistic potential bacterial collected according to the farms and root compartment of isolation (E, endorhizosphere, RP, rhizoplane and, R, rhizosphere). For a quantitative evaluation of the bacterial strains antagonistic activity two arbitrary 0-3 scales were used. The antibacterial activity was scored based on the growth inhibition area size as: 0, no antagonism; 1, < 3 mm; 2, ≥ 3, <10 mm; 3, >10 mm. Antifungal activity was scored based on the percentage of growth inhibition against *Fol* (PGI) as follows: 0, no inhibition, 1, PGI <30%; 2, PGI 30- 60%; 3, PGI >60%.

Supplementary Tables

Table S1: Phenotypic qualitative evaluation of the presence (1) or absence (0) of beneficial traits of bacteria isolated from rhizosphere (R), rhizoplane (RP), and endorhizosphere (E) .

Table S2: Principal Component Analysis (PCA) table of the full dataset on 424 bacteria, showing relationships between phenotypic traits (Gram reaction, fluorescence production, siderophore production, phosphate solubilisation, salt tolerance, antagonist activity against *Cmm*, *Pco*, *Pto*, *Xep*, and *Fol*) and bacterial isolates.

Table S3: Molecular identification of the endophytic bacteria based on the 16S rRNA gene sequence.

Table S4: Accession numbers of the sequences of the type strains used as references in the dendrogram in figure 6 in this study.

Table S5: Effect of the treatments by soil drenching of tomato plantlets grown in pots using bacterial endophytes belonging to the genus *Pseudomonas* and *Bacillus*. Plant height, fresh and dry weight of roots and shoots, dry matter and root/shoot ratio was measured 30 days after the treatment.

Figure S1: Heat map obtained using two arbitrary scales to quantitative evaluate the antagonistic potential bacteria collected according to the farms and root compartment of isolation (E, endorhizosphere, RP, rhizoplane and, R, rhizosphere). For a quantitative evaluation of the bacterial strains antagonistic activity two arbitrary 0-3 scales were used. The antibacterial activity was scored based on the growth inhibition area size as: 0, no antagonism; 1, < 3 mm; 2, ≥ 3, <10 mm; 3, >10 mm. Antifungal activity was scored based on the percentage of growth inhibition against *Fol* (PGI) as follows: 0, no inhibition, 1, PGI $<30\%$; 2, PGI 30- 60%; 3, PGI $>60\%$.

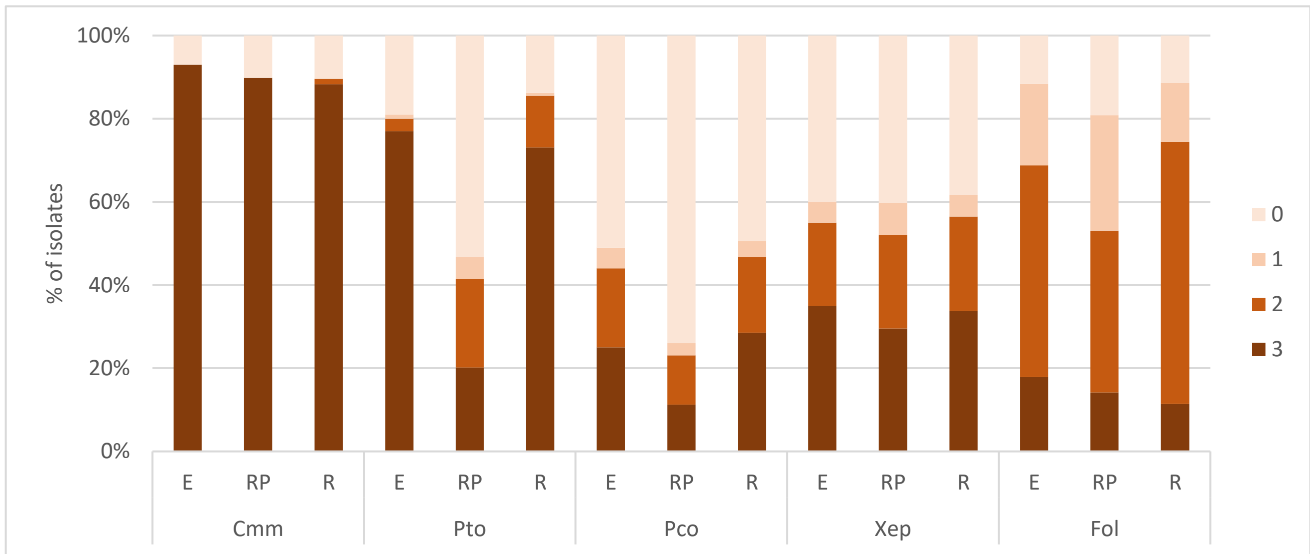

Table S1: Phenotypic qualitative evaluation of the presence (1) or absence (0) of beneficial traits of bacteria isolated from rhizosphere (R), rhizoplane (RP), and endorhizosphere (E).

Farm	Root compartment	Name ID	Gram	Fluorescence	PGP activity			Antagonistic activity				
					Siderophore production	Phosphate solubilization	Salt tolerance (NaCl 8%)	*Cmm*	*Pto*	*Pco*	*Xep*	*Fol*
1	RP	**1**	0	0	1	0	0	1	1	0	1	1
1	RP	**2**	0	0	1	0	1	1	1	0	1	1
1	RP	**3**	0	1	1	0	1	1	0	0	1	1
1	RP	**4**	0	0	1	0	1	1	0	0	1	1
1	RP	**5**	0	0	1	0	1	1	0	0	1	1
1	RP	**6**	0	0	1	0	1	1	0	0	1	1
1	RP	**7**	0	0	1	0	1	1	1	0	1	1
1	RP	**8**	0	0	1	0	0	1	1	0	1	1
1	RP	**9**	0	0	1	0	1	1	0	0	1	1
1	RP	**10**	0	0	0	0	1	1	0	0	1	1
1	RP	**11**	0	0	0	0	1	1	1	0	1	1
1	RP	**12**	0	0	0	0	1	1	0	0	1	1
1	RP	**13**	0	0	0	0	1	1	1	0	1	1
1	RP	**14**	0	0	0	0	1	1	0	0	0	1
1	RP	**15**	0	0	0	0	1	1	0	0	0	1
1	RP	**16**	0	0	1	0	1	1	1	0	0	1
1	RP	**17**	0	0	0	0	1	1	0	0	0	1
1	RP	**18**	0	0	0	0	1	1	1	0	0	1
1	R	**19**	0	0	0	0	1	1	1	0	0	1
1	RP	**20**	0	0	0	0	1	1	1	0	1	1
1	RP	**21**	0	0	0	0	1	1	1	0	1	1
1	RP	**22**	0	0	0	0	1	1	1	0	1	1
1	RP	**23**	0	0	0	0	1	1	1	0	1	1
1	RP	**24**	0	0	0	0	1	1	1	0	1	1
1	RP	**25**	0	0	1	0	1	0	1	0	1	0
1	RP	**26**	0	0	1	0	1	0	0	0	0	0
1	RP	**27**	0	0	0	0	1	1	1	0	1	1
1	RP	**28**	0	1	0	0	1	1	1	0	1	1
1	RP	**29**	0	0	0	0	1	1	1	0	1	1
1	RP	**30**	0	0	0	0	1	1	1	0	1	1
1	RP	**31**	0	0	0	0	1	0	0	0	0	1
1	RP	**32**	0	0	0	0	1	0	0	0	0	1
1	RP	**33**	0	0	0	0	1	1	0	0	0	1
1	RP	**34**	0	0	0	0	1	1	0	0	0	1
1	RP	**35**	0	1	0	0	1	1	0	0	0	1
1	RP	**36**	0	0	0	0	1	1	1	0	1	1
1	RP	**37**	0	1	0	0	1	1	1	0	1	1
1	RP	**38**	0	0	0	0	1	1	0	0	0	1
1	RP	**39**	0	0	0	0	1	1	1	0	1	1
1	RP	**40**	0	0	0	0	1	0	1	0	1	1
1	RP	**41**	0	0	0	0	1	1	1	0	1	1
1	RP	**42**	0	0	1	0	1	1	1	0	1	1

Farm	**Root compartment**	**Name ID**	**Gram**	**Fluorescence**	**PGP activity**			**Antagonistic activity**				
					Siderophore production	**Phosphate solubilization**	**Salt tolerance (NaCl 8%)**	***Cmm***	***Pto***	***Pco***	***Xep***	***Fol***
1	RP	**43**	0	0	1	0	1	1	1	0	1	1
1	RP	**44**	0	0	1	0	1	1	1	0	1	1
1	RP	**45**	0	0	0	0	1	1	1	0	1	1
1	RP	**46**	0	0	0	0	1	1	1	0	1	1
1	RP	**47**	0	0	1	0	1	1	1	0	1	1
1	E	**48**	0	0	1	0	1	1	1	0	1	1
1	E	**49**	0	0	0	1	1	1	0	0	1	1
1	R	**50**	0	0	0	1	1	1	1	0	1	1
1	R	**51**	0	0	0	1	1	1	0	0	1	1
1	R	**52**	0	0	1	1	1	1	1	0	1	1
1	R	**53**	0	0	1	1	1	1	1	0	1	1
1	R	**54**	0	0	1	1	1	1	1	0	1	1
1	R	**55**	0	0	1	1	1	1	1	0	1	1
1	R	**56**	0	0	1	1	1	1	1	1	1	1
1	R	**57**	0	0	0	1	1	1	1	0	1	1
1	R	**58**	0	0	0	1	1	1	1	0	1	1
1	R	**59**	0	0	0	1	0	1	1	0	1	1
1	R	**60**	0	0	0	1	0	1	1	0	1	1
1	R	**61**	0	0	0	1	1	1	1	0	1	1
1	R	**62**	0	0	0	1	0	1	1	0	1	1
1	RP	**63**	0	0	0	1	0	1	1	0	1	1
1	RP	**64**	0	0	1	1	0	1	1	0	1	1
1	RP	**65**	0	0	1	0	0	1	1	0	0	1
1	RP	**66**	0	0	0	0	0	1	1	0	0	1
1	R	**67**	0	0	1	1	0	0	1	0	0	1
1	R	**68**	0	0	0	1	0	0	1	0	0	1
1	R	**69**	0	0	0	1	0	1	1	0	0	1
1	R	**70**	0	0	0	1	0	0	1	0	0	1
2	R	**91**	0	0	0	1	1	1	1	0	0	1
2	R	**92**	0	0	0	1	1	1	1	1	1	1
2	R	**93**	0	0	0	0	1	1	1	1	1	1
2	R	**94**	1	0	0	0	1	1	1	1	0	1
2	R	**95**	0	0	0	0	1	1	1	1	1	1
2	R	**96**	0	0	0	1	1	1	1	1	0	1
2	R	**97**	1	0	0	1	0	1	1	1	1	1
2	R	**98**	0	0	0	1	0	1	1	1	1	1
2	R	**99**	1	0	0	1	1	1	1	1	1	1
2	R	**100**	0	0	0	0	1	1	1	0	1	1
2	R	**101**	0	0	0	0	0	1	1	0	1	1
2	R	**102**	0	0	0	0	0	1	1	1	0	1
2	R	**103**	0	0	0	0	1	1	1	0	1	1
2	R	**104**	0	0	0	0	1	1	1	1	1	1
2	R	**105**	0	0	0	1	1	1	1	1	1	1
2	R	**106**	0	0	0	0	0	1	1	0	0	1
2	RP	**107**	0	0	0	1	1	1	1	0	1	1
2	RP	**108**	0	0	0	0	0	1	1	0	1	1
2	RP	**109**	0	0	0	0	0	1	1	0	0	1

Farm	Root compartment	Name ID	Gram	Fluorescence	PGP activity			Antagonistic activity				
					Siderophore production	Phosphate solubilization	Salt tolerance (NaCl 8%)	*Cmm*	*Pto*	*Pco*	*Xep*	*Fol*
2	RP	**110**	0	0	0	0	1	1	1	0	0	1
2	RP	**111**	0	0	0	0	1	1	1	0	1	1
2	RP	**112**	1	0	0	0	1	1	1	0	0	1
2	RP	**113**	0	0	0	0	1	1	1	0	1	1
2	R	**114**	0	0	0	0	1	1	1	0	1	1
2	RP	**115**	0	0	0	1	1	1	1	1	0	1
2	RP	**116**	0	0	0	0	1	1	1	1	1	1
2	RP	**117**	0	0	0	0	1	1	1	1	1	1
2	RP	**118**	0	0	0	0	0	1	1	0	1	1
2	RP	**119**	0	0	0	0	1	1	1	0	1	1
2	RP	**120**	0	0	0	0	1	1	1	0	1	1
2	RP	**121**	1	0	0	0	1	1	1	0	0	0
2	RP	**122**	0	0	0	1	1	1	1	0	0	0
2	E	**123**	0	0	0	1	0	1	1	1	1	1
2	E	**124**	0	0	0	0	1	1	1	1	1	1
2	E	**125**	0	0	0	0	1	1	1	1	1	1
2	E	**126**	0	0	0	0	1	1	1	0	1	1
2	E	**127**	0	0	0	0	0	1	1	1	1	1
2	E	**128**	0	0	0	0	0	1	1	0	0	1
2	E	**129**	0	0	0	0	1	1	1	0	0	0
2	E	**130**	1	0	0	0	1	1	1	0	0	0
2	E	**131**	0	0	0	1	0	1	1	1	1	1
2	E	**132**	0	0	0	0	1	1	1	1	1	1
2	E	**133**	0	0	0	0	1	1	1	0	0	1
2	E	**134**	0	0	0	0	1	1	1	1	1	1
2	E	**135**	1	0	0	0	0	1	1	1	1	1
2	E	**136**	1	0	0	0	1	1	1	0	1	1
2	R	**137**	1	0	0	0	1	1	1	0	1	1
2	R	**138**	0	0	0	1	1	1	1	1	1	1
2	R	**139**	1	0	0	0	1	1	1	1	1	1
2	R	**140**	0	0	0	0	1	1	1	1	1	1
2	R	**141**	1	0	0	1	1	1	1	0	1	1
2	R	**142**	0	0	0	1	0	1	1	0	0	1
2	R	**143**	1	0	0	1	1	1	1	0	1	1
2	R	**144**	0	0	0	0	0	1	1	1	1	0
2	R	**145**	1	0	0	0	0	1	1	0	0	1
2	R	**146**	0	0	0	0	0	1	1	0	1	1
2	R	**147**	0	0	0	1	1	1	1	0	1	1
2	R	**148**	1	0	0	1	1	1	1	0	1	1
2	R	**149**	0	0	0	1	1	1	1	0	1	1
2	R	**150**	1	0	0	1	1	1	1	0	1	1
2	R	**151**	1	0	0	1	0	1	1	1	1	1
2	R	**152**	1	0	0	0	1	1	1	0	1	1
2	R	**153**	1	0	0	0	1	1	1	0	0	1
2	R	**154**	1	0	0	0	1	1	1	1	1	1
2	R	**155**	0	0	0	1	1	1	1	1	1	1
2	R	**156**	1	0	0	0	1	1	1	0	0	1

Farm	Root compartment	Name ID	Gram	Fluorescence	PGP activity			Antagonistic activity				
					Siderophore production	Phosphate solubilization	Salt tolerance (NaCl 8%)	*Cmm*	*Pto*	*Pco*	*Xep*	*Fol*
2	R	**157**	1	0	0	0	1	1	1	1	1	1
2	R	**158**	1	0	0	0	1	1	1	1	0	1
2	R	**159**	1	0	0	0	1	1	1	0	0	1
2	RP	**160**	1	0	0	0	1	1	1	0	1	1
2	RP	**161**	1	0	0	0	0	1	1	0	1	1
2	RP	**162**	1	0	0	0	1	1	1	1	1	1
2	RP	**163**	1	0	0	1	1	1	1	0	1	1
2	RP	**164**	1	0	0	1	1	1	1	0	0	1
2	RP	**165**	0	0	0	0	1	1	1	1	1	1
2	RP	**166**	1	0	0	0	1	1	1	1	1	1
2	RP	**167**	1	0	0	0	1	1	1	0	1	1
2	E	**168**	0	0	0	0	1	1	1	0	0	1
2	E	**169**	0	0	0	0	1	1	1	0	1	1
2	E	**170**	1	0	0	0	1	1	1	0	0	1
2	E	**171**	1	0	0	1	1	1	1	0	1	1
2	E	**172**	1	0	0	1	1	1	1	0	1	1
2	E	**173**	1	0	0	0	1	1	1	0	0	1
2	E	**174**	1	0	0	1	1	1	1	0	0	1
2	E	**175**	1	0	0	1	1	1	1	0	1	1
2	E	**176**	0	0	0	1	1	1	1	0	0	1
2	R	**177**	1	0	0	1	1	1	1	0	0	1
2	R	**178**	1	0	0	1	1	1	1	0	0	1
2	R	**179**	1	0	0	1	1	1	1	1	1	1
2	R	**180**	0	0	0	1	1	1	1	0	1	1
2	R	**181**	0	0	0	0	1	1	1	0	0	1
2	R	**182**	0	0	0	1	1	1	1	1	0	1
2	RP	**183**	0	0	0	1	1	1	1	0	1	1
2	RP	**184**	0	0	0	1	1	1	1	0	1	1
2	RP	**185**	0	0	0	1	1	1	1	1	1	1
2	RP	**186**	0	0	0	1	1	1	1	1	1	1
2	RP	**187**	1	0	0	1	1	1	1	0	0	0
2	RP	**188**	0	0	0	1	1	1	1	0	1	1
2	RP	**189**	0	0	0	1	1	1	1	0	1	1
2	RP	**190**	0	0	0	1	1	1	1	0	1	1
2	RP	**191**	0	0	0	1	1	1	1	1	1	1
2	RP	**192**	1	0	0	1	1	1	1	0	1	1
2	RP	**193**	0	0	0	1	1	1	1	0	1	1
2	RP	**194**	1	0	0	1	1	1	1	0	0	1
2	RP	**195**	0	0	0	1	1	1	1	0	0	1
2	E	**196**	0	0	0	1	1	1	1	0	1	1
2	E	**197**	0	0	0	1	1	1	1	0	0	1
2	E	**198**	0	0	0	1	1	1	1	0	0	1
2	E	**199**	0	0	0	1	1	1	1	0	0	1
2	E	**200**	0	0	0	1	1	1	1	0	1	1
2	E	**201**	1	0	0	1	1	1	1	0	0	1
2	E	**202**	0	0	0	1	1	1	1	0	1	1
2	E	**203**	1	0	0	1	1	1	1	1	1	0

Farm	Root compartment	Name ID	Gram	Fluorescence	PGP activity			Antagonistic activity				
					Siderophore production	Phosphate solubilization	Salt tolerance (NaCl 8%)	*Cmm*	*Pto*	*Pco*	*Xep*	*Fol*
2	E	**204**	0	0	0	1	1	1	1	1	1	0
2	E	**205**	0	0	0	1	1	1	1	1	1	1
2	E	**206**	0	0	0	1	1	1	1	0	0	1
2	E	**207**	1	0	0	1	0	1	1	0	0	1
2	E	**208**	0	0	0	1	0	1	1	1	1	1
2	RP	**F11**	0	0	0	1	1	1	1	0	0	1
2	R	**F13**	0	0	0	1	1	1	1	0	1	1
2	E	**F1**	0	1	0	1	1	1	1	1	0	1
2	E	**F2**	0	1	0	1	1	1	1	0	1	1
2	RP	**F3**	0	1	0	1	0	1	1	0	0	1
2	RP	**F4**	0	1	0	1	1	1	1	0	0	1
2	RP	**F5**	0	1	0	1	1	1	1	0	0	1
2	RP	**F6**	0	1	0	1	1	1	1	0	0	1
2	R	**F7**	0	0	0	1	1	1	1	0	0	1
2	R	**F8**	0	0	0	1	1	1	1	0	0	1
2	E	**F9**	0	1	0	1	0	1	1	0	1	1
2	E	**F10**	0	1	0	1	0	1	1	0	1	1
2	RP	**F12**	0	1	0	1	1	1	1	0	0	1
2	E	**F14**	0	1	0	1	0	1	1	1	0	1
3	RP	**209**	0	0	0	0	1	1	1	0	1	0
3	RP	**210**	0	0	0	0	1	1	1	0	0	0
3	RP	**211**	1	0	0	0	1	1	1	0	0	0
3	RP	**212**	0	0	0	0	1	1	1	0	1	1
3	RP	**213**	0	0	0	0	1	1	1	1	1	1
3	RP	**214**	0	0	0	0	1	1	1	1	1	1
3	RP	**215**	0	0	0	0	1	1	1	1	1	1
3	RP	**216**	0	0	0	0	1	1	1	1	1	1
3	RP	**217**	0	0	0	0	1	1	1	1	1	1
3	RP	**218**	0	0	0	0	1	1	1	1	1	1
3	RP	**219**	0	0	0	0	1	1	1	1	1	1
3	RP	**220**	1	0	0	0	1	1	1	1	1	1
3	RP	**221**	0	0	0	0	1	1	1	1	1	1
3	RP	**222**	0	0	0	0	1	1	1	1	1	1
3	RP	**223**	0	0	0	0	1	1	1	1	1	1
3	RP	**224**	0	0	0	0	1	1	1	1	1	1
3	RP	**225**	0	0	1	0	1	1	1	1	1	1
3	RP	**226**	1	0	0	0	1	1	1	1	1	1
3	RP	**227**	1	0	0	0	1	1	1	1	1	1
3	RP	**228**	0	0	0	0	1	1	1	0	0	0
3	RP	**229**	1	0	0	0	0	1	1	0	1	1
3	R	**230**	0	0	0	0	1	1	1	0	1	1
3	R	**231**	0	0	0	0	1	1	1	0	0	0
3	R	**232**	0	0	0	0	1	1	1	1	0	0
3	R	**233**	0	0	1	0	1	1	1	0	0	0
3	R	**234**	0	0	0	0	1	1	1	1	0	0
3	R	**235**	0	0	0	0	1	1	1	0	0	0
3	R	**236**	1	0	0	0	1	1	1	1	1	1

Farm	Root compartment	Name ID	Gram	Fluorescence	PGP activity			Antagonistic activity				
					Siderophore production	Phosphate solubilization	Salt tolerance (NaCl 8%)	*Cmm*	*Pto*	*Pco*	*Xep*	*Fol*
3	R	**237**	0	0	0	0	1	1	1	1	1	1
3	R	**238**	0	0	0	0	1	1	1	0	1	1
3	R	**239**	0	0	0	0	1	1	1	0	1	1
3	R	**240**	0	0	0	0	1	1	1	0	1	1
3	R	**241**	0	0	1	0	1	1	1	1	1	1
3	R	**242**	0	0	0	0	1	1	1	1	1	1
3	R	**243**	0	0	0	0	1	1	1	1	0	0
3	R	**244**	0	0	0	0	1	1	1	0	0	0
3	R	**245**	0	0	0	0	1	1	1	0	0	0
3	R	**246**	0	0	0	0	0	1	1	0	0	0
3	R	**247**	0	0	0	0	0	1	1	0	1	1
3	R	**248**	0	0	0	0	0	1	1	1	1	1
3	R	**249**	0	0	1	0	1	1	1	1	0	0
3	R	**250**	0	0	0	0	1	1	1	0	0	0
3	E	**251**	0	0	0	0	1	1	1	1	0	0
3	E	**252**	0	0	0	0	0	1	1	1	1	1
3	E	**253**	0	0	0	0	1	1	1	1	1	1
3	E	**254**	0	0	0	0	1	1	1	0	0	0
3	E	**255**	0	0	0	0	1	1	1	1	1	1
3	E	**256**	0	0	0	0	0	1	1	0	0	0
3	E	**257**	0	0	0	1	1	1	1	1	1	1
3	E	**258**	0	0	0	0	1	1	1	1	1	1
3	E	**259**	0	0	0	1	1	1	1	1	0	1
3	E	**260**	0	0	0	1	1	1	1	0	1	1
3	E	**261**	0	0	0	1	1	1	1	1	1	1
3	E	**262**	0	0	0	1	1	1	1	1	1	1
3	E	**263**	0	0	0	1	1	1	1	1	1	1
3	E	**264**	0	0	0	1	1	1	1	1	1	1
3	E	**265**	0	0	0	0	1	1	1	1	1	1
3	E	**266**	1	0	0	1	1	1	1	1	1	1
3	E	**267**	0	0	0	1	1	1	1	1	1	1
3	E	**268**	0	0	0	1	1	1	1	1	1	1
3	E	**269**	0	0	0	1	1	1	1	0	1	1
3	E	**270**	0	0	0	1	1	1	1	1	1	1
3	E	**F19**	0	0	0	1	1	1	1	1	1	1
3	E	**F21**	0	0	0	1	1	1	1	1	1	1
3	E	**F22**	0	0	0	1	1	1	1	1	0	0
3	E	**F24**	0	0	0	1	1	1	1	0	0	0
3	R	**F32**	0	0	0	1	1	1	1	0	0	0
3	R	**F38**	0	0	0	1	1	1	1	1	1	1
3	R	**F39**	0	0	0	1	1	1	1	1	1	1
3	E	**F16**	0	1	0	1	1	1	1	1	1	1
3	E	**F17**	0	1	0	1	1	1	1	1	0	0
3	E	**F18**	0	1	0	1	1	1	1	0	0	0
3	E	**F20**	0	1	0	1	1	1	1	1	0	0
3	RP	**F25**	0	1	0	1	1	1	1	1	0	0
3	RP	**F26**	0	1	0	1	1	1	1	0	0	0

Farm	Root compartment	Name ID	Gram	Fluorescence	PGP activity			Antagonistic activity				
					Siderophore production	Phosphate solubilization	Salt tolerance (NaCl 8%)	*Cmm*	*Pto*	*Pco*	*Xep*	*Fol*
3	RP	**F27**	0	1	0	1	1	1	1	1	1	1
3	RP	**F28**	0	1	0	1	1	1	1	1	0	0
3	RP	**F29**	0	1	0	1	1	1	1	1	1	1
3	RP	**F30**	0	1	0	1	1	1	1	1	0	0
3	RP	**F31**	0	1	0	1	1	1	1	1	0	0
3	R	**F32**	0	1	0	1	1	1	1	1	1	1
3	R	**F33**	0	1	0	1	1	1	1	1	1	1
3	R	**F34**	0	1	0	1	1	1	1	1	0	0
3	R	**F35**	0	1	0	1	1	1	1	1	0	0
3	R	**F36**	0	1	0	1	1	1	1	1	1	1
4	R	**271**	0	0	1	0		1	1	1	1	1
4	R	**272**	0	0	0	0	1	1	1	1	1	1
4	R	**273**	0	0	1	0	1	0	0	0	0	1
4	R	**274**	1	0	1	0	1	1	1	1	0	1
4	R	**275**	0	0	1	0	1	1	1	1	1	1
4	R	**276**	0	0	0	0	1	1	1	1	1	1
4	R	**277**	0	0	1	0	1	0	0	0	0	1
4	R	**278**	0	0	1	0	1	0	0	0	0	1
4	R	**279**	0	0	0	0	1	0	0	0	0	1
4	R	**280**	0	0	0	0	1	1	1	1	1	1
4	R	**281**	1	0	1	0	1	0	0	1	1	1
4	R	**282**	0	0	1	0	1	1	1	1	1	1
4	R	**283**	1	0	0	0	1	0	0	0	0	1
4	R	**284**	1	0	1	0	1	0	0	0	0	1
4	R	**285**	1	0	1	0	1	0	0	0	0	1
4	R	**286**	1	0	1	0	1	1	0	1	1	1
4	R	**287**	0	0	0	0	1	0	1	0	0	1
4	R	**288**	0	0	0	0	1	0	0	0	0	1
4	R	**289**	0	0	0	0	1	1	1	1	1	1
4	R	**290**	1	0	0	0	1	1	1	1	1	1
4	R	**291**	1	0	1	0	1	0	1	0	0	1
4	RP	**292**	0	0	1	0	1	0	0	0	0	1
4	RP	**293**	0	0	1	0	1	0	0	0	0	1
4	RP	**294**	0	0	1	0	1	0	0	0	0	1
4	RP	**295**	0	0	0	0	1	0	0	0	0	1
4	RP	**296**	0	0	0	0	1	1	0	1	1	1
4	RP	**297**	0	0	0	0	1	0	1	0	0	1
4	RP	**298**	0	0	0	0	1	0	0	0	0	1
4	RP	**299**	0	0	1	0	1	0	0	0	0	1
4	RP	**300**	0	0	1	0	1	0	0	0	0	1
4	RP	**301**	1	0	0	0	1	0	0	0	0	1
4	RP	**302**	0	0	1	0	1	0	0	0	0	1
4	E	**303**	0	0	1	0	1	0	0	0	0	1
4	E	**304**	0	0	1	0	1	1	0	1	1	1
4	E	**305**	1	0	0	0	1	1	1	1	1	1
4	E	**306**	1	0	1	0	1	1	1	1	1	1
4	E	**307**	0	0	0	0	1	0	1	0	0	1

Farm	**Root compartment**	**Name ID**	**Gram**	**Fluorescence**	**PGP activity**			**Antagonistic activity**				
					Siderophore production	**Phosphate solubilization**	**Salt tolerance (NaCl 8%)**	***Cmm***	***Pto***	***Pco***	***Xep***	***Fol***
4	E	**308**	0	0	0	0	1	0	0	0	0	1
4	E	**309**	0	0	1	0	1	0	0	0	0	1
4	E	**310**	0	0	1	0	1	1	0	1	1	1
4	R	**311**	0	0	1	0	1	1	1	1	1	1
4	R	**312**	1	0	1	0	1	1	1	1	1	1
4	R	**313**	0	0	0	0	1	1	1	1	1	1
4	R	**314**	1	0	1	0	1	0	1	0	0	1
4	R	**315**	0	0	0	0	1	1	1	1	1	1
4	R	**316**	0	0	0	0	1	1	1	1	1	1
4	R	**317**	1	0	0	0	1	1	1	1	1	1
4	R	**318**	0	0	1	0	1	0	1	1	0	1
4	R	**319**	0	0	1	1	1	1	1	1	1	1
4	R	**320**	1	0	1	1	1	1	1	1	1	1
4	R	**321**	1	0	1	1	1	1	1	1	1	1
4	R	**322**	1	0	1	1	1	1	1	1	1	1
4	R	**323**	0	0	1	1	1	1	0	0	0	1
4	R	**324**	1	0	1	1	1	0	1	1	1	1
4	R	**325**	1	0	1	1	1	1	0	0	1	1
4	R	**326**	1	0	1	1	1	1	0	0	1	1
4	R	**327**	1	0	1	1	1	0	1	1	1	1
4	R	**328**	0	0	1	1	1	0	1	1	1	1
4	R	**329**	0	0	1	1	1	1	1	1	1	1
4	R	**330**	1	0	1	1	1	1	1	1	1	1
4	RP	**331**	0	0	1	1	1	1	0	0	0	1
4	RP	**332**	0	0	1	1	1	1	0	0	1	1
4	RP	**333**	0	0	1	1	1	1	0	0	1	1
4	RP	**334**	1	0	1	1	1	1	1	1	1	1
4	RP	**335**	0	0	1	1	1	1	0	0	0	1
4	RP	**336**	0	0	1	1	1	1	1	1	0	1
4	RP	**337**	0	0	1	1	1	1	0	0	0	1
4	E	**338**	0	0	1	1	1	1	1	1	1	1
4	E	**339**	0	0	1	1	1	1	0	0	0	1
4	E	**340**	1	0	1	1	1	1	0	0	1	1
4	E	**341**	0	0	1	1	1	1	0	0	1	1
4	E	**342**	0	0	1	1	1	1	1	1	1	1
4	E	**343**	0	0	1	1	1	1	1	1	1	1
4	E	**344**	1	0	1	1	1	0	1	1		1
4	E	**345**	0	0	1	1	1	1	1	1	0	1
4	E	**346**	0	0	1	1	1	1	0	0	1	1
4	E	**347**	1	0	1	1	1	1	1	0	1	1
4	E	**348**	0	0	1	1	1	1	0	0	0	1
4	E	**349**	1	0	1	1	1	1	0	0	1	1
4	E	**350**	0	0	1	1	1	1	0	0	1	1
4	E	**351**	1	0	1	1	1	1	1	1	1	1
4	E	**352**	1	0	1	1	1	1	1	1	0	1
4	E	**353**	0	0	1	1	1	1	1	1	0	1
4	E	**354**	0	0	1	1	1	1	0	0	0	1

Farm	Root compartment	Name ID	Gram	Fluorescence	PGP activity			Antagonistic activity				
					Siderophore production	Phosphate solubilization	Salt tolerance (NaCl 8%)	*Cmm*	*Pto*	*Pco*	*Xep*	*Fol*
4	RP	**355**	0	0	1	1	1	1	1	0	1	1
4	RP	**356**	0	0	1	1	1	1	1	1	1	1
4	RP	**357**	0	0	1	1	1	1	1	1	1	1
4	RP	**358**	0	0	1	1	1	1	1	1	1	1
4	R	**359**	0	0	1	1	1	1	1	1	0	1
4	R	**360**	0	0	1	1	1	1	1	1	0	1
4	R	**361**	0	0	1	1	1	1	1	1	1	1
4	R	**362**	1	0	1	1	1	1	0	1	0	1
4	R	**363**	0	0	1	1	1	1	1	1	1	1
4	R	**364**	1	0	1	1	1	1	1	1	1	1
4	R	**365**	0	0	1	1	1	1	1	1	1	1
4	R	**366**	0	0	1	1	1	1	1	1	1	1
4	R	**367**	0	0	1	0	1	1	0	0	0	1
4	E	**368**	0	0	1	0	1	1	1	1	1	1
4	E	**369**	1	0	1	0	1	0	0	0	1	1
4	E	**370**	1	0	0	1	1	1	1	1	1	1
4	E	**371**	1	0	1	1	1	1	1	1	1	1
4	E	**372**	0	0	1	1	1	1	1	1	1	1
4	E	**373**	0	0	1	1	1	1	1	1	1	1
4	RP	**374**	0	0	0	0	1	1	1	1	1	1
4	RP	**375**	0	0	1	0	1	1	1	1	1	1
4	RP	**376**	0	0	1	0	1	1	1	1	1	1
4	RP	**377**	0	0	1	0	1	0	0	0	0	1
4	RP	**378**	0	0	1	1	1	1	1	0	1	1
4	RP	**379**	1	0	1	1	1	1	1	1	1	1
4	RP	**380**	0	0	0	1	1	1	1	1	1	1
4	RP	**381**	0	0	1	0	1	1	0	1	1	1
4	RP	**382**	0	0	1	0	0	0	0	0	0	1
4	RP	**F37**	0	0	1	0	1	1	0	1	0	1
4	RP	**F38**	0	0	1	0	1	1	0	0	0	1
4	RP	**F39**	0	0	1	0	1	1	0	0	0	1
4	RP	**F40**	0	0	1	0	1	1	0	0	0	1
4	RP	**F41**	0	1	1	0	0	1	0	0	0	1
4	E	**F42**	0	1	1	0	0	1	0	0	0	1
4	RP	**F43**	0	0	1	0	0	1	0	0	0	1
4	RP	**F44**	0	0	1	0	1	1	0	0	0	1
4	RP	**F45**	0	0	1	0	0	1	0	0	0	1
4	E	**F46**	0	1	1	0	1	1	0	0	0	1
4	RP	**F47**	0	0	1	0	1	1	0	0	0	1
4	E	**F48**	0	1	1	0	0	1	0	0	0	1
4	RP	**F49**	0	0	1	0	0	1	0	0	0	1
4	E	**F50**	0	1	1	0	0	1	0	0	0	1
4	RP	**F51**	0	0	1	0	0	1	0	0	0	1
4	E	**F52**	0	1	1	0	0	1	0	0	0	1
4	E	**F53**	0	1	1	0	0	1	0	0	0	1
4	E	**F54**	0	1	0	0	1	1	0	0	0	1
4	R	**F55**	0	1	1	1	1	0	0	0	0	1

Farm	**Root compartment**	**Name ID**	**Gram**	**Fluorescence**	**PGP activity**			**Antagonistic activity**				
					Siderophore production	**Phosphate solubilization**	**Salt tolerance (NaCl 8%)**	***Cmm***	***Pto***	***Pco***	***Xep***	***Fol***
4	E	**F56**	0	1	1	0	1	1	1	1	1	1
4	R	**F57**	0	1	1	0	1	1	0	0	0	1
4	E	**F58**	0	1	1	0	0	1	0	0	0	1
4	R	**F59**	0	1	1	0	1	1	0	0	0	1
4	E	**F60**	0	1	1	0	1	1	1	1	1	1

Table S2: Principal Component Analysis (PCA) table of the full dataset on 424 bacteria, showing relationships between phenotypic traits (Gram reaction, fluorescence production, siderophore production, phosphate solubilisation, salt tolerance, antagonist activity against *Cmm*, *Pco*, *Pto*, *Xep*, and *Fol*) and bacterial isolates.

Bacteria ID	PC1	PC2
1	-0.272	-0.295
2	-0.199	-0.4
3	-0.709	-0.643
4	-0.659	-0.708
5	-0.659	-0.708
6	-0.659	-0.708
7	-0.199	-0.4
8	-0.272	-0.295
9	-0.659	-0.708
10	-0.384	0.08
11	0.076	0.388
12	-0.384	0.08
13	0.076	0.388
14	-0.92	0.359
15	-0.92	0.359
16	-0.734	-0.121
17	-0.92	0.359
18	-0.459	0.667
19	-0.459	0.667
20	0.076	0.388
21	0.076	0.388
22	0.076	0.388
23	0.076	0.388
24	0.076	0.388
25	-0.456	-0.285
26	-1.451	-0.313
27	0.076	0.388
28	0.026	0.452
29	0.076	0.388
30	0.076	0.388
31	-1.144	0.255
32	-1.144	0.255
33	-0.92	0.359
34	-0.92	0.359
35	-0.97	0.424
36	0.076	0.388
37	0.026	0.452
38	-0.92	0.359
39	0.076	0.388
40	-0.149	0.284
41	0.076	0.388
42	-0.199	-0.4
43	-0.199	-0.4
44	-0.199	-0.4
45	0.076	0.388
46	0.076	0.388
47	-0.199	-0.4

Bacteria ID	PC1	PC2
48	-0.199	-0.4
49	-0.072	-0.033
50	0.389	0.275
51	-0.072	-0.033
52	0.114	-0.513
53	0.114	-0.513
54	0.114	-0.513
55	0.114	-0.513
56	0.624	-0.846
57	0.389	0.275
58	0.389	0.275
59	0.316	0.379
60	0.316	0.379
61	0.389	0.275
62	0.316	0.379
63	0.316	0.379
64	0.041	-0.409
65	-0.807	-0.016
66	-0.532	0.771
67	-0.719	-0.233
68	-0.444	0.554
69	-0.219	0.658
70	-0.444	0.554
91	-0.146	0.554
92	0.899	-0.058
93	0.586	0.055
94	0.142	0.237
95	0.586	0.055
96	0.364	0.221
97	0.917	-0.051
98	0.826	0.046
99	0.99	-0.155
100	0.076	0.388
101	0.003	0.492
102	-0.022	0.438
103	0.076	0.388
104	0.586	0.055
105	0.899	-0.058
106	-0.532	0.771
107	0.389	0.275
108	0.003	0.492
109	-0.532	0.771
110	-0.459	0.667
111	0.076	0.388
112	-0.368	0.57
113	0.076	0.388
114	0.076	0.388
115	0.364	0.221
116	0.586	0.055
117	0.586	0.055
118	0.003	0.492
119	0.076	0.388
120	0.076	0.388
121	-0.401	0.789

Bacteria ID	PC1	PC2
122	-0.179	0.773
123	0.826	0.046
124	0.586	0.055
125	0.586	0.055
126	0.076	0.388
127	0.514	0.159
128	-0.532	0.771
129	-0.491	0.886
130	-0.401	0.789
131	0.826	0.046
132	0.586	0.055
133	-0.459	0.667
134	0.586	0.055
135	0.604	0.063
136	0.167	0.291
137	0.167	0.291
138	0.899	-0.058
139	0.677	-0.042
140	0.586	0.055
141	0.48	0.178
142	-0.219	0.658
143	0.48	0.178
144	0.481	0.378
145	-0.441	0.675
146	0.003	0.492
147	0.389	0.275
148	0.48	0.178
149	0.389	0.275
150	0.48	0.178
151	0.917	-0.051
152	0.167	0.291
153	-0.368	0.57
154	0.677	-0.042
155	0.899	-0.058
156	-0.368	0.57
157	0.677	-0.042
158	0.142	0.237
159	-0.368	0.57
160	0.167	0.291
161	0.094	0.396
162	0.677	-0.042
163	0.48	0.178
164	-0.056	0.457
165	0.586	0.055
166	0.677	-0.042
167	0.167	0.291
168	-0.459	0.667
169	0.076	0.388
170	-0.368	0.57
171	0.48	0.178
172	0.48	0.178
173	-0.368	0.57
174	-0.056	0.457
175	0.48	0.178

Bacteria ID	PC1	PC2
176	-0.146	0.554
177	-0.056	0.457
178	-0.056	0.457
179	0.99	-0.155
180	0.389	0.275
181	-0.459	0.667
182	0.364	0.221
183	0.389	0.275
184	0.389	0.275
185	0.899	-0.058
186	0.899	-0.058
187	-0.088	0.676
188	0.389	0.275
189	0.389	0.275
190	0.389	0.275
191	0.899	-0.058
192	0.48	0.178
193	0.389	0.275
194	-0.056	0.457
195	-0.146	0.554
196	0.389	0.275
197	-0.146	0.554
198	-0.146	0.554
199	-0.146	0.554
200	0.389	0.275
201	-0.056	0.457
202	0.389	0.275
203	0.958	0.064
204	0.867	0.16
205	0.899	-0.058
206	-0.146	0.554
207	-0.129	0.562
208	0.826	0.046
F11	-0.146	0.554
F13	0.389	0.275
F1	0.314	0.285
F2	0.339	0.339
F3	-0.269	0.723
F4	-0.196	0.619
F5	-0.196	0.619
F6	-0.196	0.619
F7	-0.146	0.554
F8	-0.146	0.554
F9	0.266	0.444
F10	0.266	0.444
F12	-0.196	0.619
F14	0.241	0.39
209	0.044	0.607
210	-0.491	0.886
211	-0.401	0.789
212	0.076	0.388
213	0.586	0.055
214	0.586	0.055
215	0.586	0.055

Bacteria ID	PC1	PC2
216	0.586	0.055
217	0.586	0.055
218	0.586	0.055
219	0.586	0.055
220	0.677	-0.042
221	0.586	0.055
222	0.586	0.055
223	0.586	0.055
224	0.586	0.055
225	0.312	-0.733
226	0.677	-0.042
227	0.677	-0.042
228	-0.491	0.886
229	0.094	0.396
230	0.076	0.388
231	-0.491	0.886
232	0.019	0.553
233	-0.766	0.098
234	0.019	0.553
235	-0.491	0.886
236	0.677	-0.042
237	0.586	0.055
238	0.076	0.388
239	0.076	0.388
240	0.076	0.388
241	0.312	-0.733
242	0.586	0.055
243	0.019	0.553
244	-0.491	0.886
245	-0.491	0.886
246	-0.564	0.99
247	0.003	0.492
248	0.514	0.159
249	-0.256	-0.235
250	-0.491	0.886
251	0.019	0.553
252	0.514	0.159
253	0.586	0.055
254	-0.491	0.886
255	0.586	0.055
256	-0.564	0.99
257	0.899	-0.058
258	0.586	0.055
259	0.364	0.221
260	0.389	0.275
261	0.899	-0.058
262	0.899	-0.058
263	0.899	-0.058
264	0.899	-0.058
265	0.586	0.055
266	0.99	-0.155
267	0.899	-0.058
268	0.899	-0.058
269	0.389	0.275

Bacteria ID	PC1	PC2
270	0.899	-0.058
F19	0.899	-0.058
F21	0.899	-0.058
F22	0.332	0.44
F24	-0.179	0.773
F32	-0.179	0.773
F38	0.899	-0.058
F39	0.899	-0.058
F16	0.849	0.006
F17	0.282	0.504
F18	-0.229	0.837
F20	0.282	0.504
F25	0.282	0.504
F26	-0.229	0.837
F27	0.849	0.006
F28	0.282	0.504
F29	0.849	0.006
F30	0.282	0.504
F31	0.282	0.504
F32	0.849	0.006
F33	0.849	0.006
F34	0.282	0.504
F35	0.282	0.504
F36	0.849	0.006
271	0.239	-0.628
272	0.586	0.055
273	-1.419	-0.532
274	-0.133	-0.55
275	0.312	-0.733
276	0.586	0.055
277	-1.419	-0.532
278	-1.419	-0.532
279	-1.144	0.255
280	0.586	0.055
281	-0.283	-1.241
282	0.312	-0.733
283	-1.054	0.159
284	-1.328	-0.629
285	-1.328	-0.629
286	-0.058	-1.137
287	-0.684	0.563
288	-1.144	0.255
289	0.586	0.055
290	0.677	-0.042
291	-0.868	-0.321
292	-1.419	-0.532
293	-1.419	-0.532
294	-1.419	-0.532
295	-1.144	0.255
296	0.126	-0.253
297	-0.684	0.563
298	-1.144	0.255
299	-1.419	-0.532
300	-1.419	-0.532

Bacteria ID	PC1	PC2
301	-1.054	0.159
302	-1.419	-0.532
303	-1.419	-0.532
304	-0.149	-1.041
305	0.677	-0.042
306	0.402	-0.829
307	-0.684	0.563
308	-1.144	0.255
309	-1.419	-0.532
310	-0.149	-1.041
311	0.312	-0.733
312	0.402	-0.829
313	0.586	0.055
314	-0.868	-0.321
315	0.586	0.055
316	0.586	0.055
317	0.677	-0.042
318	-0.448	-0.557
319	0.624	-0.846
320	0.715	-0.943
321	0.715	-0.943
322	0.715	-0.943
323	-0.882	-0.542
324	0.491	-1.046
325	-0.256	-0.917
326	-0.256	-0.917
327	0.491	-1.046
328	0.4	-0.95
329	0.624	-0.846
330	0.715	-0.943
331	-0.882	-0.542
332	-0.346	-0.821
333	-0.346	-0.821
334	0.715	-0.943
335	-0.882	-0.542
336	0.089	-0.567
337	-0.882	-0.542
338	0.624	-0.846
339	-0.882	-0.542
340	-0.256	-0.917
341	-0.346	-0.821
342	0.624	-0.846
343	0.624	-0.846
344	-0.045	-0.767
345	0.089	-0.567
346	-0.346	-0.821
347	0.205	-0.61
348	-0.882	-0.542
349	-0.256	-0.917
350	-0.346	-0.821
351	0.715	-0.943
352	0.18	-0.663
353	0.089	-0.567
354	-0.882	-0.542

Bacteria ID	**PC1**	**PC2**
355	0.114	-0.513
356	0.624	-0.846
357	0.624	-0.846
358	0.624	-0.846
359	0.089	-0.567
360	0.089	-0.567
361	0.624	-0.846
362	-0.281	-0.971
363	0.624	-0.846
364	0.715	-0.943
365	0.624	-0.846
366	0.624	-0.846
367	-1.194	-0.428
368	0.312	-0.733
369	-0.793	-0.908
370	0.99	-0.155
371	0.715	-0.943
372	0.624	-0.846
373	0.624	-0.846
374	0.586	0.055
375	0.312	-0.733
376	0.312	-0.733
377	-1.419	-0.532
378	0.114	-0.513
379	0.715	-0.943
380	0.899	-0.058
381	-0.149	-1.041
382	-1.492	-0.428
F37	-0.684	-0.762
F38	-1.194	-0.428
F39	-1.194	-0.428
F40	-1.194	-0.428
F41	-1.317	-0.259
F42	-1.317	-0.259
F43	-1.267	-0.324
F44	-1.194	-0.428
F45	-1.267	-0.324
F46	-1.244	-0.364
F47	-1.194	-0.428
F48	-1.317	-0.259
F49	-1.267	-0.324
F50	-1.317	-0.259
F51	-1.267	-0.324
F52	-1.317	-0.259
F53	-1.317	-0.259
F54	-0.97	0.424
F55	-1.156	-0.581
F56	0.262	-0.668
F57	-1.244	-0.364
F58	-1.317	-0.259
F59	-1.244	-0.364
F60	0.262	-0.668

Table S3: Molecular identification of the endophytic bacteria based on the 16S rRNA gene sequence.

	Best hit (ref_seq)						This study GenBank accession no.
Strain ID	**Order**	**Family**	**Genus**	**Species**	**Ident. %**	**NCBI Acc. No.**	
124	Pseudomonadales	Moraxellaceae	*Acinetobacter*	*Acinetobacter baumannii*	100	CP050388.1	MW130753
170	Pseudomonadales	Moraxellaceae	*Acinetobacter*	*Acinetobacter baumannii*	100	CP050388.1	MW130754
123	Pseudomonadales	Moraxellaceae	*Acinetobacter*	*Acinetobacter baumannii*	100	MT256198.1	MW130755
307	Pseudomonadales	Moraxellaceae	*Acinetobacter*	*Acinetobacter baumannii*	100	MT256198.1	MW130756
263	Bacillales	Bacillaceae	*Bacillus*	*Bacillus velezensis*	100	MN559711.1	MW130757
373	Bacillales	Bacillaceae	*Bacillus*	*Bacillus subtilis*	100	CP051860.1	MW130758
306	Bacillales	Bacillaceae	*Bacillus*	*Bacillus velezensis*	100	CP051463.1	MW130759
341	Bacillales	Bacillaceae	*Bacillus*	*Bacillus velezensis*	100	KY927398.1	MW130760
268	Bacillales	Bacillaceae	*Bacillus*	*Bacillus megaterium*	99	KT883839.1	MW130761
261	Bacillales	Bacillaceae	*Bacillus*	*Bacillus velezensis*	99	CP051463.1	MW130762
304	Bacillales	Bacillaceae	*Bacillus*	*Bacillus velezensis*	100	CP051463.1	MW130763
305	Bacillales	Bacillaceae	*Bacillus*	*Bacillus velezensis*	100	CP051463.1	MW130764
255	Bacillales	Bacillaceae	*Bacillus*	*Bacillus velezensis*	100	MT365117.1	MW130765
265	Bacillales	Bacillaceae	*Bacillus*	*Bacillus velezensis*	100	MT365117.1	MW130766
351	Bacillales	Bacillaceae	*Bacillus*	*Bacillus amyloliquefaciens*	100	MK501609.1	MW130767
347	Bacillales	Bacillaceae	*Bacillus*	*Bacillus amyloliquefaciens*	100	MK501609.1	MW130768
374	Bacillales	Bacillaceae	*Bacillus*	*Bacillus velezensis*	100	KY927398.1	MW130769
257	Bacillales	Bacillaceae	*Bacillus*	*Bacillus amiloliquefaciens*	100	MK501609.1	MW130770
136	Bacillales	Bacillaceae	*Bacillus*	*Bacillus velezensis*	99	MN654121.1	MW130771
270	Bacillales	Bacillaceae	*Bacillus*	*Bacillus amiloliquefaciens*	100	MK501609.1	MW130772
372	Bacillales	Bacillaceae	*Bacillus*	*Bacillus subtilis*	100	MT081484.1	MW130773
260	Bacillales	Bacillaceae	*Bacillus*	*Bacillus subtilis*	100	KU729674.1	MW130774
338	Bacillales	Bacillaceae	*Bacillus*	*Bacillus sp.*	100	CP040881.1	MW130775
370	Bacillales	Bacillaceae	*Bacillus*	*Bacillus sp.*	100	CP040881.1	MW130776
269	Bacillales	Bacillaceae	*Bacillus*	*Bacilus velenzensis*	100	CP024922.1	MW130777
132	Enterobacteriales	Enterobacteriaceae	*Lelliottia*	*Lelliottia sp.*	97	JN853247.1	MW130778
127	Enterobacteriales	Enterobacteriaceae	*Lelliottia*	*Lelliottia sp.*	97	JN853247.1	MW130779
198	Enterobacteriales	Enterobacteriaceae	*Lelliottia*	*Lelliottia sp.*	97	JN853247.1	MW130780
135	Enterobacteriales	Enterobacteriaceae	*Lelliottia*	*Lelliottia sp.*	97	JN853247.1	MW130781
267	Enterobacteriales	Enterobacteriaceae	*Enterobacter*	*Enterobacter cancerogenus*	97	FJ976582.1	MW130782
f20	Enterobacteriales	Enterobacteriaceae	*Enterobacter*	*Enterobacter cancerogenus*	97	FJ976582.1	MW130783
f21	Enterobacteriales	Enterobacteriaceae	*Enterobacter*	*Enterobacter cancerogenus*	97	FJ976582.1	MW130784
f18	Enterobacteriales	Enterobacteriaceae	*Enterobacter*	*Enterobacter cancerogenus*	97	FJ976582.1	MW130785
252	Enterobacteriales	Enterobacteriaceae	*Enterobacter*	*Enterobacter cancerogenus*	97	FJ976582.1	MW130786
f17	Enterobacteriales	Enterobacteriaceae	*Enterobacter*	*Enterobacter mori*	97	KJ589489.1	MW130787
f19	Enterobacteriales	Enterobacteriaceae	*Enterobacter*	*Enterobacter cancerogenus*	97	FJ976582.1	MW130788
254	Enterobacteriales	Enterobacteriaceae	*Enterobacter*	*Enterobacter cancerogenus*	97	FJ976582.1	MW130789
266	Enterobacteriales	Enterobacteriaceae	*Enterobacter*	*Enterobacter cancerogenus*	97	FJ976582.1	MW130790

Strain ID	Best hit (ref_seq)						This study GenBank accession no.
	Order	Family	Genus	Species	Ident. %	NCBI Acc. No.	
128	Enterobacteriales	Enterobacteriaceae	*Lelliottia*	*Lelliottia sp.*	97	JN853247.1	MW130791
f52	Enterobacteriales	Enterobacteriaceae	*Enterobacter*	*Enterobacter tabaci*	97	MF682952.1	MW130792
126	Enterobacteriales	Enterobacteriaceae	*Lelliottia*	*Lelliottia sp.*	97	JN853247.1	MW130793
200	Enterobacteriales	Enterobacteriaceae	*Lelliottia*	*Lelliottia sp.*	96	JN853247.1	MW130794
202	Enterobacteriales	Enterobacteriaceae	*Lelliottia*	*Lelliottia sp.*	97	JN853247.1	MW130795
259	Enterobacteriales	Enterobacteriaceae	*Lelliottia*	*Lelliottia sp.*	97	JN853247.1	MW130796
176	Enterobacteriales	Enterobacteriaceae	*Lelliottia*	*Lelliottia sp.*	97	JN853247.1	MW130797
310	Enterobacteriales	Enterobacteriaceae	*Enterobacter*	*Enterobacter cancerogenus*	97	FJ976582.1	MW130798
49	Enterobacteriales	Enterobacteriaceae	*Ewingella*	*Ewingella americana*	100	MT101745.1	MW130799
48	Enterobacteriales	Enterobacteriaceae	*Ewingella*	*Ewingella americana*	99	MT101745.1	MW130800
35	Enterobacteriales	Enterobacteriaceae	*Ewingella*	*Ewingella americana*	99	KY126991.1	MW130801
346	Enterobacteriales	Enterobacteriaceae	*Pantoea*	*Pantoea sp.*	97	MK229045.1	MW130802
353	Enterobacteriales	Enterobacteriaceae	*Pantoea*	*Pantoea sp.*	97	MH884045.1	MW130803
264	Enterobacteriales	Enterobacteriaceae	*Enterobacter*	*Enterobacter cancerogenus*	97	FJ976582.1	MW130804
344	Enterobacteriales	Enterobacteriaceae	*Pantoea*	*Pantoea sp.*	97	MK229045.1	MW130805
345	Enterobacteriales	Enterobacteriaceae	*Pantoea*	*Pantoea sp.*	97	MK229045.1	MW130806
342	Enterobacteriales	Morganellaceaea	*Providencia*	*Providencia vermicola*	99	KX394623.1	MW130807
350	Enterobacteriales	Morganellaceaea	*Providencia*	*Providencia vermicola*	99	MK942706.1	MW130808
349	Enterobacteriales	Morganellaceaea	*Providencia*	*Providencia vermicola*	99	MK942706.1	MW130809
f1	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas citronellolis*	100	KM210226.1	MW130810
172	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas plecoglossicida*	100	MT367715.1	MW130811
f14	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas putida*	100	LN866622.1	MW130812
f53	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas monteilii*	100	MH603875.1	MW130813
125	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas plecoglossicida*	100	MT367715.1	MW130814
201	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas plecoglossicida*	100	MT367715.1	MW130815
168	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas plecoglossicida*	100	KJ819579.1	MW130816
169	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas plecoglossicida*	100	MT367715.1	MW130817
174	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas plecoglossicida*	100	MT367715.1	MW130818
352	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas plecoglossicida*	100	MT367715.1	MW130819
173	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas plecoglossicida*	100	MT367715.1	MW130820
f2	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas plecoglossicida*	100	MT367715.1	MW130821
f10	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas plecoglossicida*	100	MT367715.1	MW130822
f56	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas plecoglossicida*	100	MT367715.1	MW130823
f60	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas plecoglossicida*	100	MT367715.1	MW130824
f42	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas putida*	100	CP026115.2	MW130825
f48	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas putida*	100	CP026115.2	MW130826
171	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas plecoglossicida*	100	MT367715.1	MW130827

Strain ID	Best hit (ref_seq)						This study GenBank accession no.
	Order	Family	Genus	Species	Ident. %	NCBI Acc. No.	
f9	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas plecoglossicida*	100	MT367715.1	MW130828
f46	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas putida*	100	LN866622.1	MW130829

Table S4: Accession numbers of the sequences of the type strains used as references in the dendrogram in figure 6 in this study.

Species	Type Strain	Sequence accession no. (16S rRNA gene)
Bacillus subtilis	ATCC 6051^T	AJ276351
B. pumilus	ATCC 7061^T	AY876289
B. megaterium	ATCC 14581^T	D16273
B. siamensis	KCTC 13613^T	GQ281299
B. mojavensis	ATCC 51516^T	AB021191
B. amyloliquefaciens	ATCC 23350^T	AB006920
B. velezensis	CCUG 50740^T	AY603658
B. thuringiensis	ATCC 10792^T	D16281
B. cereus	ATCC 14579^T	AE016877
Acinetobacter baumannii	DSM 30007^T	NR_117677
Pseudomonas citronellolis	ATCC 13674^T	Z76659
P. plecoglossicida	ATCC 700383^T	AB009457
P. putida	ATCC 12633^T	D84020
P. monteilii	ATCC 700476^T	AF064458
P. mosselii	CIP 105259^T	AF072688
P. entomophila	CCUG 61470^T	AY907566
P. parafulva	DSM 17004^T	AB060132
P. fulva	DSM 17717^T	AB060136
P. syingae pv. syingae	NCPPB 281^T	DQ318866
P. fluorescens	DSM 50090^T	D84013
P. aeruginosa	DSM 50071^T	HE978771
Enterobacter cancerogenus	LMG 2693^T	NR 116756.1
E. mori	LMG 25706^T	NZ_GL890774
E. tabaci	KACC 17832^T	NR_146667
Ewingella americana	ATCC 33852^T	JMPJ01000013
Providencia vermicola	DSM 17385^T	NR_042415
Lelliottia amnigena	ATCC 33731^T	AB004749
L. nimipressuralis	DSM 18955^T	KF516260
Pantoea agglomerans	DSM 3493^T	NR_041978

Table S5: Effect of the treatments by soil drenching of tomato plantlets grown in pots using bacterial endophytes belonging to the genus *Pseudomonas* and *Bacillus*. Plant height, fresh and dry weight of roots and shoots, dry matter and root/shoot ratio was measured 30 days after the treatment.

Bacterial strains	Plant height		Root			Shoot			R/S
	T30	Gain (%)	fw	dw	DM	fw	dw	DM	
P. plecoglossicida _171	29.33±1.15 abc	11.36	0.70 ab	0.57 a	83.41 c	4.20 a	1.84 a	44.06 a	0.31 ab
P. plecoglossicida _172	29.83±1.89 abc	12.85	0.90 ab	0.53 a	60.14 ab	5.10 a	2.23 b	43.71 a	0.24 ab
*P. citronellolis*_f1	31.33±3.06 bc	17.02	1.13 b	0.63 a	56.00 a	5.13 a	2.23 b	44.62 a	0.29 ab
*P. monteilii*_f53	29.00±1.00 abc	10.4	0.93 ab	0.59 a	62.81 ab	4.63 a	1.73 a	37.82 a	0.34 b
*P. plecoglossicida*_f56	32.33±1.15 bc	19.59	0.66 a	0.58 a	87.22 c	5.36 a	2.26 b	42.41 a	0.25 ab
B. velezensis _261	31.67±2.52 bc	17.89	1.08 ab	0.54 a	54.42 a	5.03 a	2.22 b	46.18 a	0.24 ab
*B. velezensis*_263	30.33±0.58 abc	14.29	0.63 a	0.49 a	76.98 bc	4.90 a	2.19 b	44.76 a	0.22 a
B. velezensis _265	27.67±1.53 ab	6.02	1.13 b	0.65 a	57.77 ab	5.03 a	1.91 ab	37.98 a	0.35 b
*B. megaterium*_268	26.33±1.53 a	1.27	0.90 ab	0.62 a	70.00 abc	4.80 a	2.26 b	47.47 a	0.28 ab
B. velezensis _306	33.33±1.15 bc	22.00	1.00 ab	0.54 a	53.94 a	4.20 a	1.84 a	45.01 a	0.25 ab
Control	26.00±1.73 a	/	0.80 ab	0.53 a	62.91 ab	5.10 a	2.23 b	52.11 a	0.24 ab

fw, fresh weight, dw, dry weight; DM dry matter; R/S, root to shoot dry weight ratio.

Gain%: the effect of bacterial treatments on plant height was assessed as relative percentage change in comparison to the control. Means in a column followed by the same letter are not significantly different according to Student–Newman–Keuls test (P≤ 0.05)

7.2. Supplementary Material: chapter 3

Microbial community assembly and evolution from the seed to the field of on soil and soilless grown tomato

Supplementary Figures

Figure S1: Bacterial community composition at family level. Growing substrates: peat in the nursery; either soil or coconut fiber (soilless) in the greenhouse **(A)**. Rhizosphere of tomato plantlets in the nursery (T1) and after transplanting at flowering and fruit set in two different growing conditions, in soil (T2) and soilless (T2) **(B)**. Endorhizosphere in plantlets in the nursery (T1) and after transplanting at flowering and fruit set in two different growing conditions in soil (T2) and soilless (T2) **(C)**. Bar charts represent the composition of the bacterial community (only key taxa were included, >0.5%).

Figure S2: Fungal community composition at family level. Growing substrates: peat in the nursery; either soil or coconut fiber (soilless) in the greenhouse **(A)**. Rhizosphere of tomato plantlets in the nursery (T1) and after transplanting at flowering and fruit set in two different growing conditions, in soil (T2) and soilless (T2) **(B)**. Endorhizosphere of tomato in plantlets in the nursery (T1) and after transplanting at flowering and fruit set in two different growing conditions in soil (T2) and soilless (T2) **(C)**. Bar charts represent the composition of the bacterial community (only key taxa were included, >0.5%).

Figure S3: PCoA of bacterial **(A)** and fungal **(C)** communities with nursery production materials as a constraining factor; and PCoA of bacterial **(B)** and fungal **(D)** communities with greenhouse commercial materials as a as a constraining factor. The sample clustering was based on the Bray–Curtis dissimilarity matrix. Each dot in the plot corresponds to a single sample (biological replication).

Figure S4: Mosaic plot of the traits showing the highest differentiation among endosphere samples, namely: siderophore production **(A)**, phosphate solubilitation **(B)**, antagonistic activity against *Xep* **(C)**, and *Pco* **(D)**. Mosaic plots show the relative frequency of the presence (blue) or absence (red) of a trait (y axis) given the endosphere samples (x axis); the width of the columns is proportional to the numerosity of the accessions isolated on each environments.

Supplementary Table

Table S1: Summary alpha diversity indices. The table illustrates the diversity indices in bacterial communities calculated for each sample.

Table S2: Summary alpha diversity indices. The table illustrates the diversity indices in fungal communities calculated for each sample.

Table S3: Molecular identification of the endophytic bacteria based on the 16S rRNA gene sequence

Table S4: Identification and phenotypic qualitative evaluation of the presence (1) or absence (0) of beneficial traits of bacteria isolated

Table S5: Antagonistic activity of bacterial endophytes

Table S6: Evaluation of antagonistic activity of bacterial endophytes against phytopatogenic bacteria (inhibition halo - cm) and the fungi (Percentage of growth reduction – PGI).

Figure S1: Bacterial community composition at family level. Growing substrates: peat in the nursery; either soil or coconut fiber (soilless) in the greenhouse **(A)**. Rhizosphere of tomato plantlets in the nursery (T1) and after transplanting at flowering and fruit set in two different growing conditions in soil (T2) and soilless (T2) **(B)**. Endorhizosphere in plantlets in the nursery (T1) and after transplanting at flowering and fruit set in two different growing conditions in soil (T2) and soilless (T2) **(C)**. Bar charts represent the composition of the bacterial community (only key taxa were included, >0.5%).

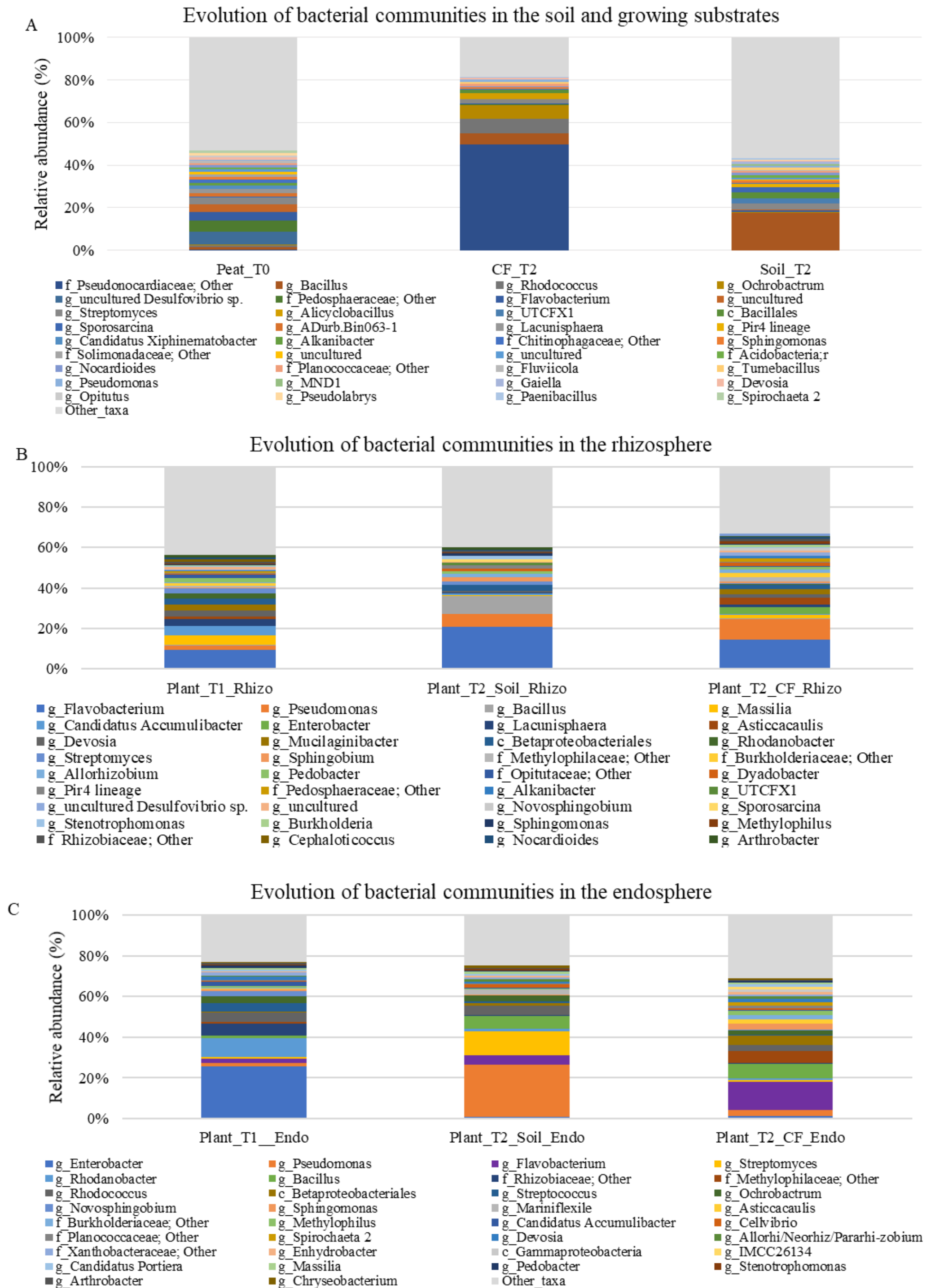

Figure S2: Fungal community composition at family level. Growing substrates: peat in the nursery; either soil or coconut fiber (soilless) in the greenhouse **(A)**. Rhizosphere of tomato plantlets in the nursery (T1) and after transplanting at flowering and fruit set in two different growing conditions, in soil (T2) and soilless (T2) **(B)**. Endorhizosphere of tomato in plantlets in the nursery (T1) and after transplanting at flowering and fruit set in two different growing conditions, in soil (T2) and soilless (T2) **(C)**. Bar charts represent the composition of the bacterial community (only key taxa were included, >0.5%).

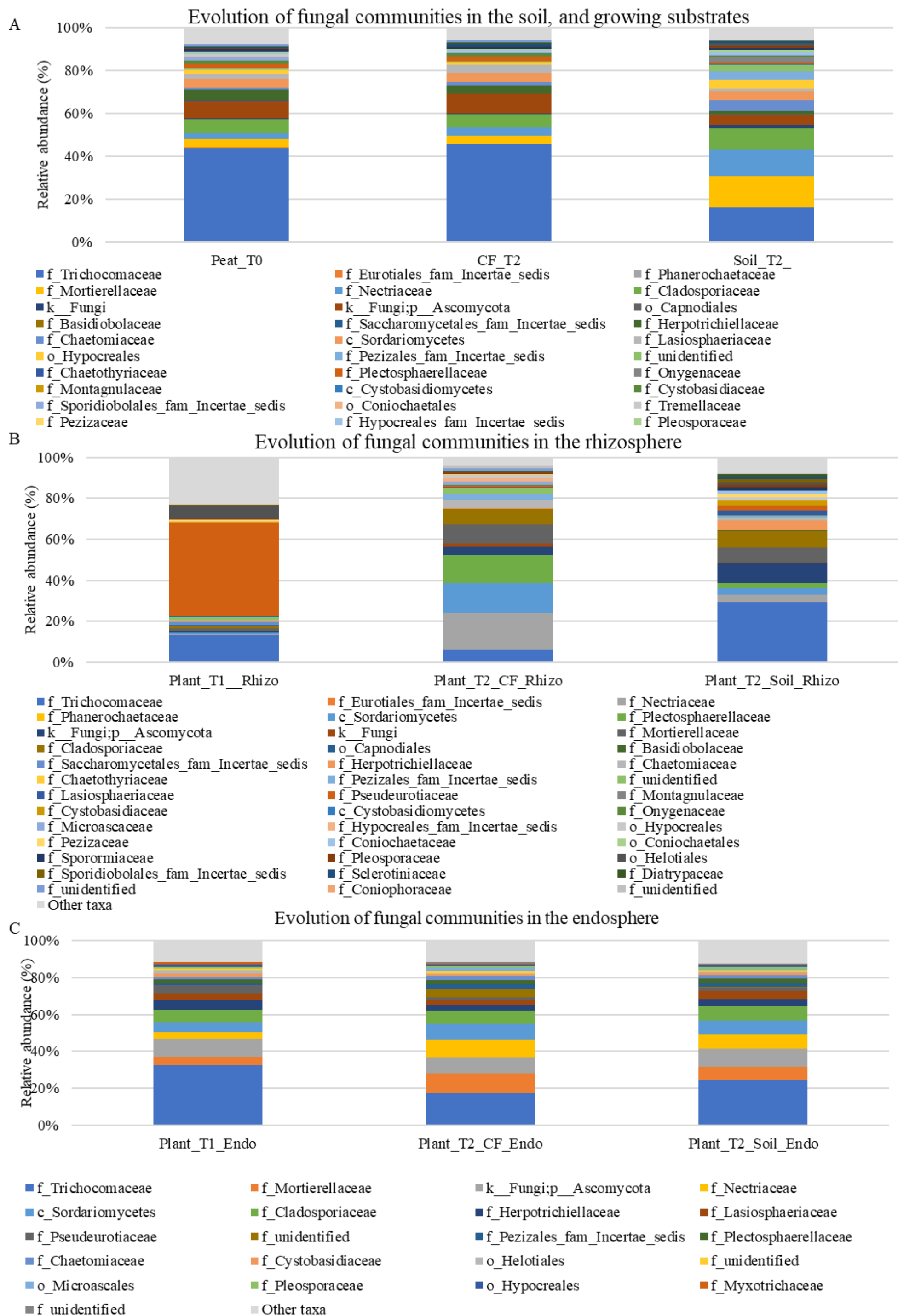

Figure S3: PCoA of bacterial **(A)** and fungal **(C)** communities in the nursery production materials as a constraining factor; and PCoA of bacterial **(B)** and fungal **(D)** communities with greenhouse commercial materials as a as a constraining factor. The sample clustering was based on the Bray–Curtis dissimilarity matrix. Each dot in the plot corresponds to a single sample (biological replication).

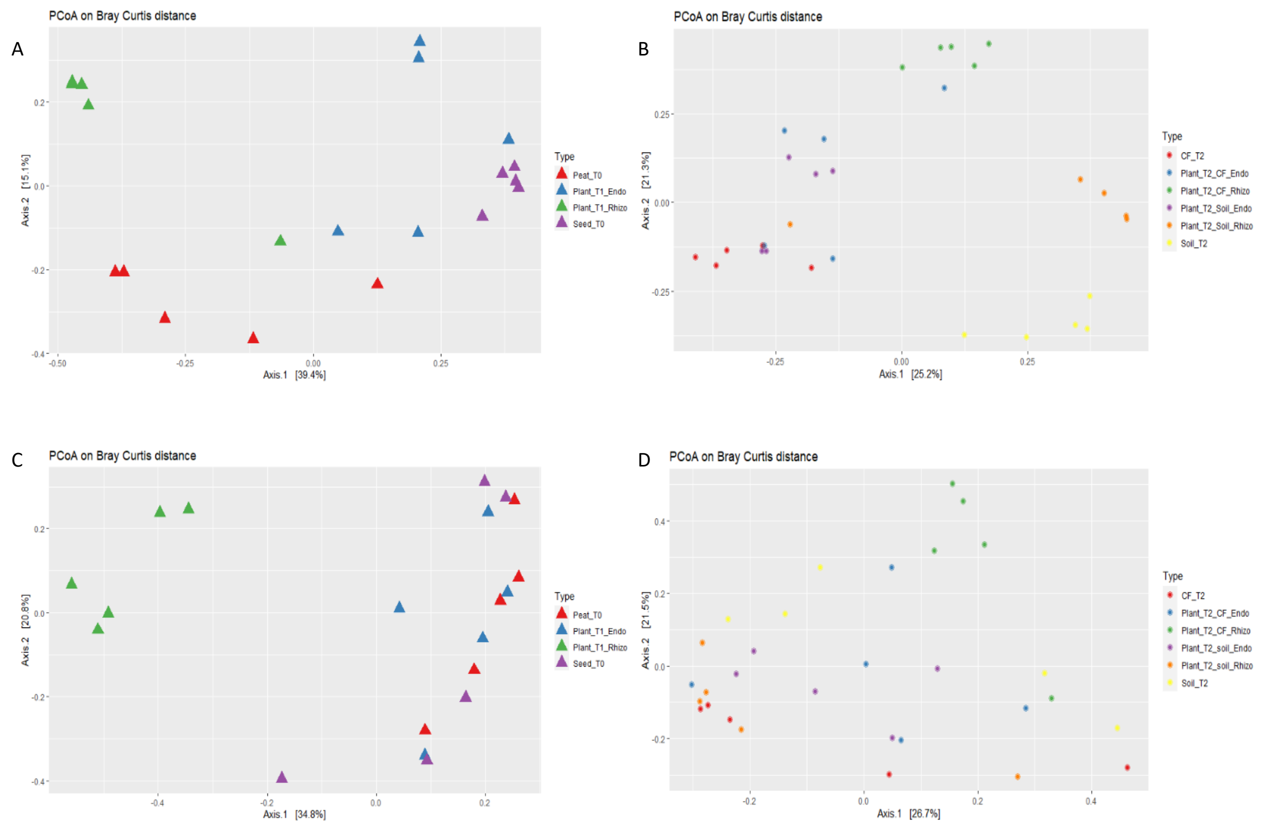

Figure S4: Mosaic plot of the traits showing the highest differentiation among endosphere samples, namely: siderophore production **(A)**, phosphate solubilitation **(B),** antagonistic activity against *Xep* **(C)**, and *Pco* **(D)**. Mosaic plots show the relative frequency of the presence (blue) or absence (red) of a trait (y axis) given the endosphere samples (x axis); the width of the columns is proportional to the numerosity of the accessions isolated on each environments.

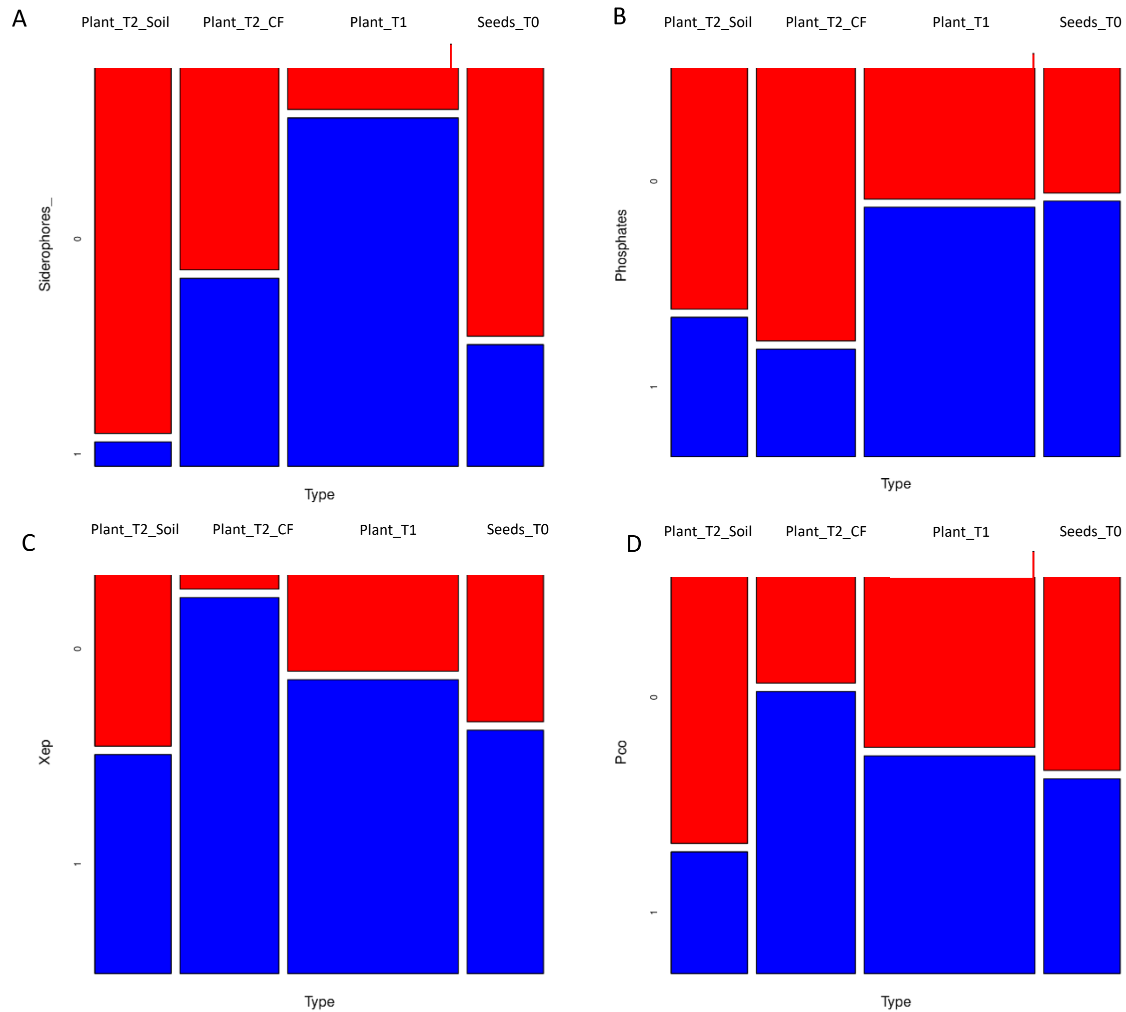

Table S1: Summary alpha diversity indices. The table illustrates the diversity indices in bacterial communities calculated for each sample.

#SampleID	Observed	Chao1	se.chao1	Shannon	Simpson
Seeds_T0.16S	81	81	0.124226	3.349929	0.917971
Seeds_T0.16S	69	69	0	3.525624	0.939527
Seeds_T0.16S	66	66	0	3.64745	0.954091
Seeds_T0.16S	67	67	0	3.440315	0.929239
Seeds_T0.16S	61	61	0	3.512637	0.944236
Peat_T0.16S	397	397.125	0.443815	5.019174	0.984473
Peat_T0.16S	150	150	0	4.49369	0.982366
Peat_T0.16S	394	394.2	0.621493	5.040208	0.985774
Peat_T0.16S	45	45	0	3.488675	0.961798
Peat_T0.16S	263	263	0	4.80541	0.983383
Soil_T2.16S	391	391.125	0.443814	5.00169	0.973697
Soil_T2.16S	396	396	0	4.795659	0.962626
Soil_T2.16S	484	484	0	4.943396	0.965979
Soil_T2.16S	178	178	0.099719	4.088661	0.934592
Soil_T2.16S	258	258.2	0.621431	4.424504	0.949122
CF_T2.16S	31	31	0	2.57644	0.841833
CF_T2.16S	126	126	0	2.106054	0.624686
CF_T2.16S	139	139	0	3.237509	0.871096
CF_T2.16S	67	67	0	2.102281	0.701799
CF_T2.16S	35	35	0	2.291113	0.795992
Plant_T1_Endo.16S	45	45	0	2.299812	0.824299
Plant_T1_Endo.16S	95	95	0	2.53245	0.775253
Plant_T1_Endo.16S	33	33	0	2.808593	0.908707
Plant_T1_Endo.16S	96	96	0	4.101503	0.976125
Plant_T1_Endo.16S	171	171	0	4.580438	0.983706
Plant_T1_Rhizo.16S	316	316	0.124802	4.570772	0.972613
Plant_T1_Rhizo.16S	383	383	0.099869	4.704082	0.979177
Plant_T1_Rhizo.16S	342	342	0.166423	4.545456	0.972271
Plant_T1_Rhizo.16S	165	165	0	4.471398	0.981749
Plant_T1_Rhizo.16S	398	398	0.083229	4.793127	0.978559
Plant_T2_Soil_Endo.16S	129	129	0.166019	3.54873	0.924881
Plant_T2_Soil_Endo.16S	9	9	0	1.906574	0.830158
Plant_T2_Soil_Endo.16S	88	88	0	3.613967	0.947675
Plant_T2_Soil_Endo.16S	49	49	0.494872	2.499089	0.789836
Plant_T2_Soil_Endo.16S	128	128	0	3.200205	0.872978
Plant_T2_Soil_Rhizo.16S	259	259	0.124758	3.895051	0.933667
Plant_T2_Soil_Rhizo.16S	319	320	2.342541	3.956	0.911675
Plant_T2_Soil_Rhizo.16S	399	399.1111	0.409692	4.332021	0.950843
Plant_T2_Soil_Rhizo.16S	62	62	0	3.023892	0.897515
Plant_T2_Soil_Rhizo.16S	445	445.125	0.443821	4.398594	0.946263
Plant_T2_CF_Endo.16S	82	82	0	3.260785	0.908135
Plant_T2_CF_Endo.16S	74	74	0	3.415206	0.924115
Plant_T2_CF_Endo.16S	116	116	0	3.975181	0.961656
Plant_T2_CF_Endo.16S	193	193	0	3.720545	0.922248
Plant_T2_CF_Endo.16S	170	170	0	4.359321	0.978587
Plant_T2_CF_Rhizo.16S	245	245	0	4.109481	0.939939
Plant_T2_CF_Rhizo.16S	214	214	0.099766	4.154926	0.955511
Plant_T2_CF_Rhizo.16S	207	207	0.083132	4.304182	0.970119
Plant_T2_CF_Rhizo.16S	205	205.2	0.621385	3.913612	0.938408
Plant_T2_CF_Rhizo.16S	167	167	0.166167	4.15861	0.972073

Table S2: Summary alpha diversity indices. The table illustrates the diversity indices in fungal communities calculated for each sample.

#SampleID	Observed	Chao1	se.chao1	Shannon	Simpson
Seeds_T0.ITS	288	291.3333	4.127892	4.279161	0.962702
Seeds_T0.ITS	248	248.1667	0.543482	4.273921	0.967122
Seeds_T0.ITS	173	173	0	3.951548	0.956465
Seeds_T0.ITS	167	167.25	0.736468	3.883076	0.952581
Seeds_T0.ITS	111	111	0	3.812441	0.959132
Peat_T0.ITS	258	258	0	4.311798	0.970672
Peat_T0.ITS	285	285	0	4.50421	0.974648
Peat_T0.ITS	299	299.5	1.027222	4.400636	0.970481
Peat_T0.ITS	388	388.5	1.298481	4.421487	0.970252
Peat_T0.ITS	309	309	0	4.272369	0.967034
Soil_T2.ITS	178	178	0	4.141206	0.973509
Soil_T2.ITS	349	349	0.099857	4.324085	0.968039
Soil_T2.ITS	347	347	0.166426	4.36313	0.973002
Soil_T2.ITS	330	330.5	1.298383	4.375939	0.97422
Soil_T2.ITS	90	90	0	3.831023	0.966648
CF_T2.ITS	248	248	0	4.121562	0.960618
CF_T2.ITS	300	300	0.124791	4.277109	0.963946
CF_T2.ITS	85	85	0	3.622246	0.953332
CF_T2.ITS	178	178.3333	0.925698	3.94311	0.95795
CF_T2.ITS	283	283	0	4.287516	0.962505
Plant_T1_Endo.ITS	218	218	0	4.364497	0.971363
Plant_T1_Endo.ITS	158	161	4.650185	4.028814	0.961784
Plant_T1_Endo.ITS	230	230	0	4.419227	0.970184
Plant_T1_Endo.ITS	331	331	0	4.186155	0.958936
Plant_T1_Endo.ITS	246	246	0	4.156024	0.961807
Plant_T1_Rhizo.ITS	266	267	1.817403	3.660056	0.903592
Plant_T1_Rhizo.ITS	426	426	0	3.293416	0.837121
Plant_T1_Rhizo.ITS	358	358	0.09986	3.564624	0.888577
Plant_T1_Rhizo.ITS	153	153	0.055374	2.79171	0.7653
Plant_T1_Rhizo.ITS	230	230	0.071273	2.794473	0.809301
Plant_T2_Soil_Endo.ITS	250	250	0	4.351238	0.977064
Plant_T2_Soil_Endo.ITS	181	181	0	4.224944	0.966717
Plant_T2_Soil_Endo.ITS	320	320.6	1.186191	4.289496	0.964663
Plant_T2_Soil_Endo.ITS	239	239	0.249476	4.447181	0.977746
Plant_T2_Soil_Endo.ITS	203	203	0	4.307315	0.975707
Plant_T2_Soil_Rhizo.ITS	249	249	0	3.00866	0.827303
Plant_T2_Soil_Rhizo.ITS	242	242	0	1.48242	0.422759
Plant_T2_Soil_Rhizo.ITS	196	196.3333	0.925753	2.761762	0.788128
Plant_T2_Soil_Rhizo.ITS	247	247	0	3.150441	0.838005
Plant_T2_Soil_Rhizo.ITS	203	203	0	3.94177	0.935464
Plant_T2_CF_Endo.ITS	231	231	0	4.389163	0.976715
Plant_T2_CF_Endo.ITS	231	236	6.041764	4.304742	0.967287
Plant_T2_CF_Endo.ITS	264	264	0.124763	4.435219	0.971006
Plant_T2_CF_Endo.ITS	267	267.2	0.621437	4.440334	0.972327
Plant_T2_CF_Endo.ITS	168	168	0	4.140865	0.967231
Plant_T2_CF_Rhizo.ITS	369	369	0.499322	4.422453	0.970997
Plant_T2_CF_Rhizo.ITS	423	423.5	1.298527	4.468542	0.970142
Plant_T2_CF_Rhizo.ITS	208	208	0.498797	4.202827	0.960464
Plant_T2_CF_Rhizo.ITS	289	289.2	0.621451	4.155921	0.961509
Plant_T2_CF_Rhizo.ITS	260	260	0.499038	3.667415	0.928188

Table S3: Molecular identification of the endophytic bacteria based on the 16S rRNA gene sequence.

	Best hit (ref_seq)							This study GenBank accession No.
Strain ID	**Order**	**Family**	**Genus**	**Species**	**Ident. %**	**Query length**	**NCBI Acc. No.**	
PSE74	Bacillales	Bacillaceae	*Bacillus*	*Bacillus velezensis*	99,86	698	CP054714.1	MZ066824
PSE35	Bacillales	Bacillaceae	*Bacillus*	*Bacillus velezensis*	99,71	699	CP054714.1	MZ066825
PSE32	Bacillales	Bacillaceae	*Bacillus*	*Bacillus subtilis*	100	698	MT571500.1	MZ066835
PSE31B	Bacillales	Bacillaceae	*Bacillus*	*Bacillus amyloliquefaciens*	97,29	707	MH260978.1	MZ066848
PFE52	Bacillales	Bacillaceae	*Bacillus*	*Bacillus velezensis*	100	698	CP054714.1	MZ066826
PFE47	Bacillales	Bacillaceae	*Bacillus*	*Bacillus subtilis*	100	698	MT571500.1	MZ066836
PFE45	Bacillales	Bacillaceae	*Bacillus*	*Bacillus velezensis*	100	698	CP054714.1	MZ066827
PFE43	Bacillales	Bacillaceae	*Bacillus*	*Bacillus velezensis*	100	697	CP054714.1	MZ066828
PFE42	Bacillales	Bacillaceae	*Bacillus*	*Bacillus velezensis*	100	698	CP054714.1	MZ066829
PFE41	Bacillales	Bacillaceae	*Bacillus*	*Bacillus velezensis*	100	698	CP054714.1	MZ066830
PFE40	Bacillales	Bacillaceae	*Bacillus*	*Bacillus subtilis*	100	698	MT571500.1	MZ066837
PFE39	Bacillales	Bacillaceae	*Bacillus*	*Bacillus subtilis*	100	698	MT571500.1	MZ066838
PFE38	Bacillales	Bacillaceae	*Bacillus*	*Bacillus nealsonii*	100	695	MN540832.1	MZ066850
PFE16	Bacillales	Paenibacillaceae	*Paenibacillus*	*Paenibacillus gansuensis*	99,41	698	JF496391.1	MZ066854
PFE11	Bacillales	Bacillaceae	*Bacillus*	*Bacillus subtilis*	100	698	MT571500.1	MZ066839
PFE9	Bacillales	Bacillaceae	*Bacillus*	*Bacillus spizizenii*	100	698	MT110989.1	MZ066851
PFE8	Bacillales	Bacillaceae	*Bacillus*	*Bacillus velezensis*	100	698	CP054714.1	MZ066831
PFE7	Bacillales	Bacillaceae	*Bacillus*	*Bacillus subtilis*	100	699	AM765842.1	MZ066840
PFE6	Bacillales	Bacillaceae	*Bacillus*	*Bacillus velezensis*	100	698	CP054714.1	MZ066832
S51	Bacillales	Bacillaceae	*Bacillus*	*Bacillus subtilis*	100	698	MT571500.1	MZ066841
PFE3	Bacillales	Bacillaceae	*Bacillus*	*Bacillus velezensis*	99,56	699	CP054714.1	MZ066833
PFE2	Bacillales	Paenibacillaceae	*Paenibacillus*	*Paenibacillus sp*	98,39	704	KC589227.1	MZ066856
POE68	Bacillales	Bacillaceae	*Bacillus*	*Bacillus velezensis*	100	698	CP054714.1	MZ066834
POE57	Bacillales	Bacillaceae	*Bacillus*	*Bacillus cereus*	99,86	701	MT611946.1	MZ066849
POE53	Bacillales	Bacillaceae	*Bacillus*	*Bacillus subtilis*	100	698	MT571500.1	MZ066842

POE52	Bacillales	Bacillaceae	*Priestia*	*Priestia aryabhattai*	100	699	KX443710.1	MZ066853
POE51	Bacillales	Bacillaceae	*Bacillus*	*Bacillus subtilis*	100	697	MT571500.1	MZ066843
POE48	Bacillales	Paenibacillaceae	*Paenibacillus*	*Paenibacillus gansuensis*	99,41	699	JF496391.1	MZ066855
S66	Bacillales	Staphylococcaceae	*Staphylococcus*	*Staphylococcus warneri*	100	698	MT642942.1	MZ066857
S65	Bacillales	Staphylococcaceae	*Staphylococcus*	*Staphylococcus warneri*	99,86	699	MN421083.1	MZ066858
S64	Bacillales	Staphylococcaceae	*Staphylococcus*	*Staphylococcus warneri*	100	698	MT642942.1	MZ066859
S63	Bacillales	Bacillaceae	*Priestia*	*Priestia aryabhattai*	100	699	MT525305.1	MZ066852
S61	Bacillales	Bacillaceae	*Bacillus*	*Bacillus subtilis*	100	698	MT571500.1	MZ066844
S60	Bacillales	Bacillaceae	*Bacillus*	*Bacillus subtilis*	100	698	MT571500.1	MZ066845
S59	Bacillales	Bacillaceae	*Bacillus*	*Bacillus subtilis*	100	698	MT571500.1	MZ066846
S57	Bacillales	Bacillaceae	*Bacillus*	*Bacillus subtilis*	100	698	MT571500.1	MZ066847
POE82	Burkholderiales	Comamonadaceae	*Delftia*	*Delftia sp*	100	683	EU252493.1	MZ066872
PSE34	Enterobacterales	Yersiniaceae	*Ewingella*	*Ewingella americana*	100	690	MT101745.1	MZ066912
POE84	Enterobacterales	Yersiniaceae	*Serratia*	*Serratia marcescens*	99,85	689	MT990452.1	MZ066911
POE76	Enterobacterales	Yersiniaceae	*Serratia*	*Serratia nematodiphila*	99,86	691	MN691296.1	MZ066906
POE74	Enterobacterales	Yersiniaceae	*Serratia*	*Serratia marcescens*	100	690	MT436395.1	MZ066907
POE70R	Enterobacterales	Yersiniaceae	*Serratia*	*Serratia marcescens*	99,86	690	MT436395.1	MZ066908
POE66	Enterobacterales	Yersiniaceae	*Serratia*	*Serratia marcescens*	100	690	MT436395.1	MZ066909
POE50	Enterobacterales	Yersiniaceae	*Serratia*	*Serratia marcescens*	100	690	MT436395.1	MZ066910
S67	Enterobacterales	Enterobacteriaceae	*Enterobacter*	*Enterobacter sp.*	99,13	691	LC484689.1	MZ066914
S62	Enterobacterales	Enterobacteriaceae	*Enterobacter*	*Enterobacter sp.*	98,98	689	AP019634.1	MZ066915
S58	Enterobacterales	Enterobacteriaceae	*Enterobacter*	*Enterobacter sp.*	99,13	690	LC484689.1	MZ066916
S55	Enterobacterales	Enterobacteriaceae	*Enterobacter*	*Enterobacter sp.*	99,13	690	LC484689.1	MZ066913
S52	Enterobacterales	Enterobacteriaceae	*Enterobacter*	*Enterobacter sp.*	99,13	689	LC484689.1	MZ066917
PSE36	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*	*Flavobacterium sp.*	97,75	678	MK311350.1	MZ066865
PSE31A	Flavobacteriales	Flavobacteriaceae		*Flavobacteriaceae bacterium*	99,53	678	MG980436.1	MZ066866
PFE44	Micrococcales	Micrococcaceae	*Glutamicibacter*	*Glutamicibacter halophytocola*	100	666	CP042260.1	MZ066869
PFE5	Micrococcales	Microbacteriaceae	*Microbacterium*	*Microbacterium foliorum*	100	669	MH669319.1	MZ066871

S68	Micrococcales	Microbacteriaceae	*Curtobacterium*	*Curtobacterium pusillum*	100	667	MK417938.1	MZ066870
S56	Micrococcales	Micrococcaceae	*Paenarthrobacter*	*Paenarthrobacter sp.*	99,11	671	MH698781.1	MZ066867
S54	Micrococcales	Micrococcaceae	*Arthrobacter*	*Arthrobacter sp*	99,7	675	MG569788.1	MZ066868
PSE78	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas gessardii*	99,36	695	MN069032.1	MZ066873
PSE77	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas azotoformans*	99,71	686	MW228158.1	MZ066901
PSE76	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas gessardii*	99,27	687	MN069032.1	MZ066874
PSE75	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas aeruginosa*	99,71	686	MN565979.1	MZ066902
PSE30	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas gessardii*	99,27	688	MN069032.1	MZ066875
PSE28	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas sp.*	99,56	686	MT140118.1	MZ066903
PFE51	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas fluorescens*	99,85	686	KP663368.1	MZ066889
PFE50	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas fluorescens*	100	684	KP663368.1	MZ066890
PFE49	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas fluorescens*	100	684	KP663368.1	MZ066891
POE78A	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas extremorientalis*	99,85	685	MT348509.1	MZ066895
POE81	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas simiae*	99,71	686	KR085825.1	MZ066900
POE69.2	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas gessardii*	100	684	MN069032.1	MZ066876
POE79	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas extremorientalis*	99,85	685	MT348509.1	MZ066896
POE78	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas sp.*	99,85	686	MH236005.1	MZ066904
POE75	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas veronii*	100	683	MN215443.1	MZ066897
POE73	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas veronii*	100	684	MN215443.1	MZ066899
POE72	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas gessardii*	99,85	684	MN069032.1	MZ066877
POE71	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas gessardii*	100	683	MN069032.1	MZ066878
POE70	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas gessardii*	100	684	MN069032.1	MZ066879
POE69	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas gessardii*	100	684	MN069032.1	MZ066880
POE65	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas gessardii*	100	684	MN069032.1	MZ066881
POE64	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas gessardii*	99,85	685	MN069032.1	MZ066882
POE63	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas gessardii*	99,85	685	MN069032.1	MZ066883

POE62	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas veronii*	100	684	MN215443.1	MZ066898
POE59	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas gessardii*	99,56	685	MN069032.1	MZ066884
POE58	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas gessardii*	99,85	685	MN069032.1	MZ066885
POE55	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas gessardii*	99,85	685	MN069032.1	MZ066886
POE54	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas fluorescens*	99,85	685	KP663368.1	MZ066894
POE49	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas fluorescens*	100	684	KP663368.1	MZ066892
POE47	Pseudomonadales	Moraxellaceae	*Acinetobacter*	*Acinetobacter lwoffii*	99,42	685	MG547698.1	MZ066905
POE46	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas gessardii*	100	683	MN069032.1	MZ066887
POE45	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas gessardii*	100	684	MN069032.1	MZ066888
POE44	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas fluorescens*	100	684	KP663368.1	MZ066893
PSE71	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	*Stenotrophomonas rhizophila*	99,71	694	CP050062.1	MZ066860
PSE29	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	*[Pseudomonas] hibiscicola*	100	690	MH669255.1	MZ066861
PSE27	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	*Stenotrophomonas sp.*	100	692	MT362666.1	MZ066862
PSE26	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	*[Pseudomonas] hibiscicola*	100	690	MH669255.1	MZ066863
POE60	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	*Stenotrophomonas maltophilia*	98,11	691	MH669295.1	MZ066864

Table S4: Identification and phenotypic qualitative evaluation of the presence (1) or absence (0) of beneficial traits of bacteria isolated

				PGP activity					Antagonistic activity					
ID samples	**Order**	**Family**	**Genus**	**Siderophore**	**ACC deaminase**	**HCN**	**NaCl 8%**	**Phosphates**	***Cmm***	***Pco***	***Pto***	***Xep***	***Fol***	***Bot***
S51	Bacillales	Bacillaceae	*Bacillus*	0	0	0	1	1	1	1	0	1	0	1
S52	Enterobacterales	Enterobacteriaceae	*Enterobacter*	0	0	0	1	0	1	0	1	1	1	1
S54	Micrococcales	Micrococcaceae	*Anthrobacter*	0	0	0	1	1	1	1	1	1	0	1
S55	Enterobacterales	Enterobacteriaceae	*Enterobacter*	0	1	0	1	1	1	1	1	1	0	0
S56	Micrococcales	Micrococcaceae	*Paenarthrobacter*	0	0	0	1	1	1	0	0	0	0	1
S57	Bacillales	Bacillaceae	*Bacillus*	1	0	0	1	1	0	0	0	0	1	1
S58	Enterobacterales	Enterobacteriaceae	*Enterobacter*	0	1	0	1	0	1	0	1	0	1	0
S59	Bacillales	Bacillaceae	*Bacillus*	0	1	0	1	1	1	1	1	1	1	1
S60	Bacillales	Bacillaceae	*Bacillus*	0	1	0	1	1	1	1	1	1	1	1
S61	Bacillales	Bacillaceae	*Bacillus*	0	1	0	1	0	1	1	1	1	1	1
S62	Enterobacterales	Enterobacteriaceae	*Enterobacter*	1	0	0	1	0	1	1	1	1	1	0
S63	Bacillales	Bacillaceae	*Priestia*	1	0	0	1	0	1	0	1	0	1	0
S64	Bacillales	Staphylococcaceae	*Straphylococcus*	0	0	0	1	1	1	0	1	0	1	0
S65	Bacillales	Staphylococcaceae	*Straphylococcus*	0	1	0	1	1	1	0	1	1	0	1
S66	Bacillales	Staphylococcaceae	*Straphylococcus*	0	0	0	1	1	1	1	1	0	1	1
S67	Enterobacterales	Enterobacteriaceae	*Enterobacter*	1	0	0	1	0	0	0	1	0	1	1
S68	Micrococcales	Microbacteriaceae	*Curtobacterium*	1	0	0	0	1	1	0	0	1	1	0
POE44	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	0	0	1	1	1	1	1	1	1	1
POE45	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	1	0	1	1	1	1	1	1	1	1
POE46	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	0	0	1	1	0	0	1	1	0	1
POE47	Pseudomonadales	Moraxellaceae	*Acinetobacter*	1	0	0	1	1	1	1	1	1	1	1
POE48	Bacillales	Paenibacillaceae	*Paenibacillus*	1	0	0	1	1	1	1	1	1	1	0
POE49	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	0	0	0	1	0	1	0	1	0	1	1
POE50	Enterobacterales	Yersiniaceae	*Serratia*	1	0	0	1	0	0	0	1	0	1	1
POE51	Bacillales	Bacillaceae	*Bacillus*	0	1	0	1	0	1	1	1	1	1	1
POE52	Bacillales	Bacillaceae	*Priestia*	1	1	0	1	1	1	1	1	1	1	0
POE53	Bacillales	Bacillaceae	*Bacillus*	0	1	0	1	0	1	1	1	1	1	1
POE54	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	1	0	0	1	1	0	1	1	1	1
POE55	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	0	0	1	0	0	1	1	0	0	1
POE57	Bacillales	Bacillaceae	*Bacillus*	0	0	0	1	0	1	0	1	0	1	1
POE58	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	1	0	1	1	1	0	1	0	1	1
POE59	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	0	0	0	1	0	1	1	1	0	1	1
POE60	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	1	0	0	1	1	1	0	0	1	1	1

				PGP activity					Antagonistic activity					
ID samples	**Order**	**Family**	**Genus**	**Siderophore**	**ACC deaminase**	**HCN**	**NaCl 8%**	**Phosphates**	***Cmm***	***Pco***	***Pto***	***Xep***	***Fol***	***Bot***
POE62	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	0	0	1	1	1	1	1	1	1	1
POE63	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	0	0	1	1	1	1	1	1	1	1
POE64	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	0	0	1	1	1	0	1	1	1	1
POE65	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	1	1	0	0	1	0	1	0	0	1
POE66R	Enterobacterales	Yersiniaceae	*Serratia*	1	0	0	1	1	0	0	1	1	1	1
POE67	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	0	0	1	0	1	1	1	0	0	0
POE68	Bacillales	Bacillaceae	*Bacillus*	1	1	0	0	1	1	0	1	1	1	1
POE69	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	0	0	1	0	1	1	1	1	1	1
POE70	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	0	1	1	1	1	1	1	1	1	1
POE70R	Enterobacterales	Yersiniaceae	*Serratia*	1	0	0	1	1	1	1	1	1	1	1
POE71	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	1	0	0	1	1	0	1	1	0	1
POE72	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	0	0	1	1	1	1	1	1	1	1
POE73	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	1	0	1	1	1	0	1	1	1	1
POE74	Enterobacterales	Yersiniaceae	*Serratia*	1	0	0	1	0	0	1	1	0	0	1
POE75	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	0	0	1	1	1	0	0	1	1	1
POE76	Enterobacterales	Yersiniaceae	*Serratia*	1	0	0	1	0	1	1	1	1	1	1
POE78	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	1	0	0	1	1	0	1	1	1	1
POE78A	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	0	0	1	1	1	1	1	1	1	1
POE79	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	0	0	0	1	1	0	1	1	0	0
POE81	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	1	0	1	1	1	0	0	1	1	1
POE82	Burkholderiales	Comamonadaceae	*Delftia*	0	0	0	1	0	0	0	1	0	0	0
POE83	Enterobacterales	Yersiniaceae	*Serratia*	1	0	0	1	0	1	1	1	0	1	1
PFE2	Bacillales	Paenibacillaceae	*Paenibacillus*	1	0	0	1	0	1	0	1	1	0	0
PFE3	Bacillales	Bacillaceae	*Bacillus*	0	0	0	1	0	1	0	1	0	0	1
PFE5	Micrococcales	Microbacteriaceae	*Microbacterium*	1	0	0	1	0	1	1	1	1	1	0
PFE6	Bacillales	Bacillaceae	*Bacillus*	1	0	0	1	0	1	0	1	1	1	1
PFE7	Bacillales	Bacillaceae	*Bacillus*	1	0	0	1	0	1	0	1	1	1	1
PFE8	Bacillales	Bacillaceae	*Bacillus*	1	0	0	1	1	1	1	1	1	1	1
PFE9	Bacillales	Bacillaceae	*Bacillus*	1	0	0	1	0	1	0	1	1	1	1
PFE11	Bacillales	Bacillaceae	*Bacillus*	1	0	0	1	1	1	1	1	1	1	1
PFE16	Bacillales	Paenibacillaceae	*Paenibacillus*	0	0	0	0	0	1	1	0	1	1	1
PFE38	Bacillales	Bacillaceae	*Bacillus*	0	0	0	0	0	1	0	0	0	0	0
PFE39	Bacillales	Bacillaceae	*Bacillus*	0	0	0	1	0	1	1	1	1	1	1
PFE40	Bacillales	Bacillaceae	*Bacillus*	0	0	0	1	0	1	1	1	1	1	1
PFE41	Bacillales	Bacillaceae	*Bacillus*	0	0	0	1	0	1	1	1	1	1	1
PFE42	Bacillales	Bacillaceae	*Bacillus*	0	0	0	1	0	1	1	1	1	1	1

				PGP activity					Antagonistic activity					
ID samples	**Order**	**Family**	**Genus**	**Siderophore**	**ACC deaminase**	**HCN**	**NaCl 8%**	**Phosphates**	***Cmm***	***Pco***	***Pto***	***Xep***	***Fol***	***Bot***
PFE43	Bacillales	Bacillaceae	*Bacillus*	1	0	0	1	1	1	0	1	1	1	1
PFE44	Micrococcales	Micrococcaceae	*Glutamicibacter*	0	0	0	1	0	1	1	1	1	1	1
PFE45	Bacillales	Bacillaceae	*Bacillus*	0	0	0	1	0	1	1	1	1	1	0
PFE47	Bacillales	Bacillaceae	*Bacillus*	0	0	0	1	0	1	1	1	1	1	1
PFE49	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	0	0	1	1	1	1	1	1	1	1
PFE50	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	0	0	0	1	1	1	1	1	1	1	1
PFE51	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1	0	0	1	1	1	1	1	1	1	0
PFE52	Bacillales	Bacillaceae	*Bacillus*	0	1	0	1	0	1	1	1	1	1	0
PSE26	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	0	0	0	1	0	0	0	1	0	1	0
PSE27	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	0	0	0	1	0	0	0	1	0	1	1
PSE28	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	0	0	0	1	0	0	0	1	1	1	1
PSE29	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	0	0	0	0	0	0	0	1	1	1	0
PSE30	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	0	0	0	1	1	1	1	1	1	1	1
PSE31A	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*	0	0	0	1	0	1	0	1	1	1	1
PSE31B	Bacillales	Bacillaceae	*Bacillus*	0	0	0	1	0	1	1	1	1	1	1
PSE32	Bacillales	Bacillaceae	*Bacillus*	0	0	0	1	0	1	1	1	1	1	1
PSE34	Enterobacterales	Yersiniaceae	*Ewingella*	1	0	0	1	1	1	1	1	1	1	0
PSE35	Bacillales	Bacillaceae	*Bacillus*	0	0	0	1	0	1	1	1	1	1	1
PSE36	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*	0	0	0	1	0	1	0	1	1	0	0
PSE71	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	0	0	0	0	1	1	0	0	0	0	0
PSE74	Bacillales	Bacillaceae	*Bacillus*	0	1	0	0	1	1	0	0	0	0	1
PSE75	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	0	0	0	1	1	1	0	1	0	0	0
PSE76	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	0	0	0	1	1	1	0	1	0	1	1
PSE77	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	0	0	0	1	0	1	0	1	0	1	1
PSE78	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	0	0	0	1	0	1	0	1	0	0	1

Table S5: Antagonistic activity of bacterial endophytes

Pathogens	N.o bacterial antagonistic	ID of bacterial antagonistic
Bot Cmm Fol Pco Pto Xep	30	S59,S60, S61, POE44, POE45, POE47, POE51, POE53, POE62, POE63, POE69, POE70, POE70R, POE72, POE76, POE78A, PSE30, PSE31B, PSE32, PSE35, PFE8, PFE11, PFE39, PFE40, PFE41, PFE42, PFE44, PFE47, PFE49, PFE50
Cmm Fol Pco Pto Xep	8	S62,POE48,POE52, PSE34, PFE5, PFE45, PFE51,PFE52
Bot Cmm Pco Pto Xep	1	S54
Bot Cmm Fol Pco Pto	3	S66, POE59, POE83
Bot Cmm Fol Pco Xep	1	PFE16
Bot Cmm Fol Pto Xep	11	S52, POE54, POE64, POE68, POE73, POE78, PSE31A, PFE6, PFE7, PFE9, PFE43
Cmm Pco Pto Xep	1	S55
Bot Cmm Pco Xep	1	S51
Bot Cmm Pto Xep	2	S65,POE71
Bot Cmm Fol Pto	3	POE49, POE57, POE58
Bot Cmm Fol Xep	3	POE60, POE75, ,POE81
Bot Fol Pto Xep	2	POE66R,PSE28
Cmm Pco Pto	1	POE67
Cmm Pto Xep	3	POE79, PSE36, PFE2
Cmm Fol Pto	3	S58,S63, S64
Bot Cmm Pto	2	POE65, PFE3
Cmm Fol Xep	1	S68
Bot Pco Pto	2	POE55,POE74
Fol Pto Xep	1	PSE29
Bot Pto Xep	1	POE46
Bot Fol Pto	3	S67, POE50, PSE27
Cmm Pto	4	PSE75, PSE76, PSE77, PSE78
Bot Cmm	1	S56
Fol Pto	1	PSE26
Bot Fol	1	S57
Cmm	3	PSE71, PSE74, PFE38
Pto	1	POE82

Table S6: Evaluation of antagonistic activity of bacterial endophytes against phytopatogenic bacteria (inhibition halo - cm) and the fungi (Percentage of growth reduction – PGI).

		Antagonistic activity					
ID samples	**Source**	***Cmm***	***Pcor***	***Pto***	***Xep***	***Fol***	***Bot***
S51	Seeds_T0	4.0	1.0	0	1.8	75.00	63.33
S52	Seeds_T0	3.5	0	1.8	1	87.50	65.00
S54	Seeds_T0	4.0	1.0	2.5	1.9	81.25	26.67
S55	Seeds_T0	4.5	1.3	3	1.8	81.25	0
S56	Seeds_T0	4.0	0	0	0	75.00	26.67
S57	Seeds_T0	0	0	0	0	62.50	46.67
S58	Seeds_T0	3.5	0	0.6	0	0	0
S59	Seeds_T0	4.5	0.5	3.0	1.8	75.00	56.67
S60	Seeds_T0	4.0	1.0	2.3	2.0	75.00	53.33
S61	Seeds_T0	5.0	1.0	2.0	1.7	75.00	50.00
S62	Seeds_T0	4.0	1.4	2.6	1.8	81.25	0
S63	Seeds_T0	2.5	0	2.5	0	0	0
S64	Seeds_T0	3.8	0	1.5	0	43.75	0
S65	Seeds_T0	3.5	0	2.0	1.8	0	6.67
S66	Seeds_T0	0.6	1.0	0.6	0	37.50	0
S67	Seeds_T0	0	0	0.5	0	0	16.67
S68	Seeds_T0	0.6	0	0	0.7	0	0.00
POE44	Plant_T1	1.2	1.0	1.9	2.0	25.00	36.67
POE45	Plant_T1	1.3	1.0	1.8	2.2	18.75	43.33
POE46	Plant_T1	0	0	1.8	1.2	0	33.33
POE47	Plant_T1	1.5	1.4	1.8	2.0	18.75	0
POE48	Plant_T1	2.5	1.3	2.2	1.8	6.25	0
POE49	Plant_T1	2.5	0	2.3	0	37.5	33.33
POE50	Plant_T1	0	0	1.9	0	81.25	20.00
POE51	Plant_T1	2.7	1.1	2	2.0	43.75	73.33
POE52	Plant_T1	2.6	1.3	1.9	2.2	18.75	0
POE53	Plant_T1	2.6	1.5	2.7	2.4	81.25	0
POE54	Plant_T1	3.0	0	3.1	1.9	25.00	30.00
POE55	Plant_T1	0	1.5	0.7	0	0	26.67
POE57	Plant_T1	2.5	0	2.0	0	31.25	33.33
POE58	Plant_T1	3	0	1.4	0	25.00	33.33
POE59	Plant_T1	2.5	0.5	2.0	0	25.00	63.33
POE60	Plant_T1	2.4	0	0	1.5	31.25	0
POE62	Plant_T1	2.0	0.7	1.2	1.3	25.00	23.33
POE63	Plant_T1	2.0	1.7	1.1	2.4	12.50	40.00
POE64	Plant_T1	2.8	0	2.0	2.3	37.50	36.67
POE65	Plant_T1	2.0	0	1.5	0	0	50.00
POE66R	Plant_T1	1.8	0	1.2	2.7	0	30.00
POE67	Plant_T1	2.0	0.5	1.0	0	0	0.00
POE68	Plant_T1	2.3	0	0.6	2.3	25.00	60.00
POE69	Plant_T1	2.3	0.8	1.9	2.5	31.25	46.67
POE70	Plant_T1	3.0	1.0	2.4	1.8	25.00	30.00
POE70R	Plant_T1	3	1.2	1.9	3.5	32	26.67
POE71	Plant_T1	3.0	0	2.4	1.9	0	36.67
POE72	Plant_T1	3.0	0.5	2.9	1.4	25.00	33.33
POE73	Plant_T1	3.0	0	1.6	1.6	31.25	3.33
POE74	Plant_T1	0	0.9	1.2	0	0	23.33
POE75	Plant_T1	2.5	0	0	1.2	18.75	20.00

		Antagonistic activity					
ID samples	**Source**	***Cmm***	***Pcor***	***Pto***	***Xep***	***Fol***	***Bot***
POE76	Plant_T1	3.0	0.5	1.3	2.7	25.00	23.33
POE78	Plant_T1	3.0	0	2.0	2.0	43.75	0
POE78A	Plant_T1	3	1.3	2.8	1.9	28	13.33
POE79	Plant_T1	2.5	0	1.8	2.0	0	0.00
POE81	Plant_T1	2.0	0	0	1.5	25.00	13.33
POE82	Plant_T1	0	0	1.2	0	0	0
POE83	Plant_T1	3	0.2	1.1	0	28	20.00
PFE2	Plant_T2_CF	3.5	0	2.0	2.3	0	0.00
PFE3	Plant_T2_CF	3.0	0	2.0	0	0	46.67
PFE5	Plant_T2_CF	4.0	0.5	3	2.0	81.25	0
PFE6	Plant_T2_CF	4.0	0	2.7	1.4	68.75	50.00
PFE7	Plant_T2_CF	5.0	0	3.0	1.8	68.75	26.67
PFE8	Plant_T2_CF	3.8	0.6	3.0	1.6	75.00	50.00
PFE9	Plant_T2_CF	4.0	0	2.5	1.9	93.75	53.33
PFE11	Plant_T2_CF	5.0	1.3	2.8	1.7	87.50	43.33
PFE16	Plant_T2_CF	2.8	0.3	0	0.5	25	0
PFE38	Plant_T2_CF	3	0	0	0	28	0
PFE39	Plant_T2_CF	3	1.2	1.7	3.3	68	66.67
PFE40	Plant_T2_CF	3	1.3	1.5	3.1	58	56.67
PFE41	Plant_T2_CF	3	1.3	1.6	2.8	58	53.33
PFE42	Plant_T2_CF	3	1.3	1.2	2.6	78	66.67
PFE43	Plant_T2_CF	0.3	0	1.3	1.4	57	50
PFE44	Plant_T2_CF	3	1.3	1.7	2.6	75	73.33
PFE45	Plant_T2_CF	3	1.6	2.3	3.3	29	0
PFE47	Plant_T2_CF	2.8	0.6	1.8	2.8	54	60.00
PFE49	Plant_T2_CF	3	1.3	2.4	1.6	28	36.67
PFE50	Plant_T2_CF	3	1.3	2.8	1.4	33	10.00
PFE51	Plant_T2_CF	3	1.4	2.8	1.5	23	0
PFE52	Plant_T2_CF	3	0.6	1.6	2.2	28	0
PSE26	Plant_T2_soil	0	0	0.3	0	21	0
PSE27	Plant_T2_soil	0	0	0.4	0	32	23.33
PSE28	Plant_T2_soil	0	0	0.5	0.5	18	16.67
PSE29	Plant_T2_soil	0	0	0.3	0.5	0	0
PSE30	Plant_T2_soil	2.8	1.3	2.8	1.9	62	43.33
PSE31A	Plant_T2_soil	3	0	1	2	65	66.67
PSE31B	Plant_T2_soil	3	1.4	1.5	2.8	70	63.33
PSE32	Plant_T2_soil	2.8	0.8	1.6	2.6	64	50.00
PSE34	Plant_T2_soil	3	0.9	0.7	2.3	21	0.00
PSE35	Plant_T2_soil	3	1	2	2.8	58	46.67
PSE36	Plant_T2_soil	3	0	0.6	0	27	0
PSE71	Plant_T2_soil	3.0	1.0	2.6	1.9	0	0
PSE74	Plant_T2_soil	2.0	0	0	0	37.50	40.00
PSE75	Plant_T2_soil	3.0	1.4	3.5	1.3	0	0
PSE76	Plant_T2_soil	1.9	0	0	2.9	0	13.33
PSE77	Plant_T2_soil	1.6	0	0	0	0	33.33
PSE78	Plant_T2_soil	1.7	0	1.3	0	0	26.67

7.3. Supplementary Material in chapter 4

Draft Genome Sequence of two Biocontrol Bacteria, P. citronellolis strain f1 and Pseudomonas sp. strain 172 Isolated from Tomato endorhizosphere

Supplementary Figures

Figure S1: Annotation of *Pseudomonas* sp. strains 172 **(A)** and f1 **(B)** genes against KEGG database. X-axis, Kegg pathway type; Y-axis, number of annotated genes.

Figure S2: NR annotation analysis of *Pseudomonas* sp. strains 172 **(A)** and f1 **(B)**. X-axis, species ID; Y-axis, number of annotated genes.

Figure S3: Phylogenetic trees of type strains of species belonging to the *Pseudomonas aeruginosa* and *P. putida* lineages and of *Pseudonomas* sp. f1 and 172 based on the partial sequences of 16S rRNA **(A)**, *gyr*B **(B)**, *rpo*B **(C)**, *rpo*D **(D)** genes.

Figure S4: Phylogenetic tree of partial sequences of genes 16S rRNA of the 13 strains clustered tight to strain 172 isolated from endorhizofere (Anzalone e al., 2021) comparated with eight type strains of species of *P. putida* group.

Supplementary Table

Table S1: Clusters of Orthologous Groups (GO) for *Pseudomonas* sp. strain 172, and *Pseudomonas citronellolis* strain f1.

Table S2: Summary of antiSMASH 5.0 results for *Pseudomonas* sp. strain 172, and *Pseudomonas citronellolis* strain f1, RIT 623

Figure S1: Annotation of *Pseudomonas* sp. strains 172 **(A)** and f1 **(B)** genes against KEGG database. X-axis, Kegg pathway type; Y-axis, number of annotated genes.

Figure S2: NR annotation analysis of *Pseudomonas* sp. strains 172 **(A)** and f1 **(B)**. X-axis, species ID; Y-axis, number of annotated genes.

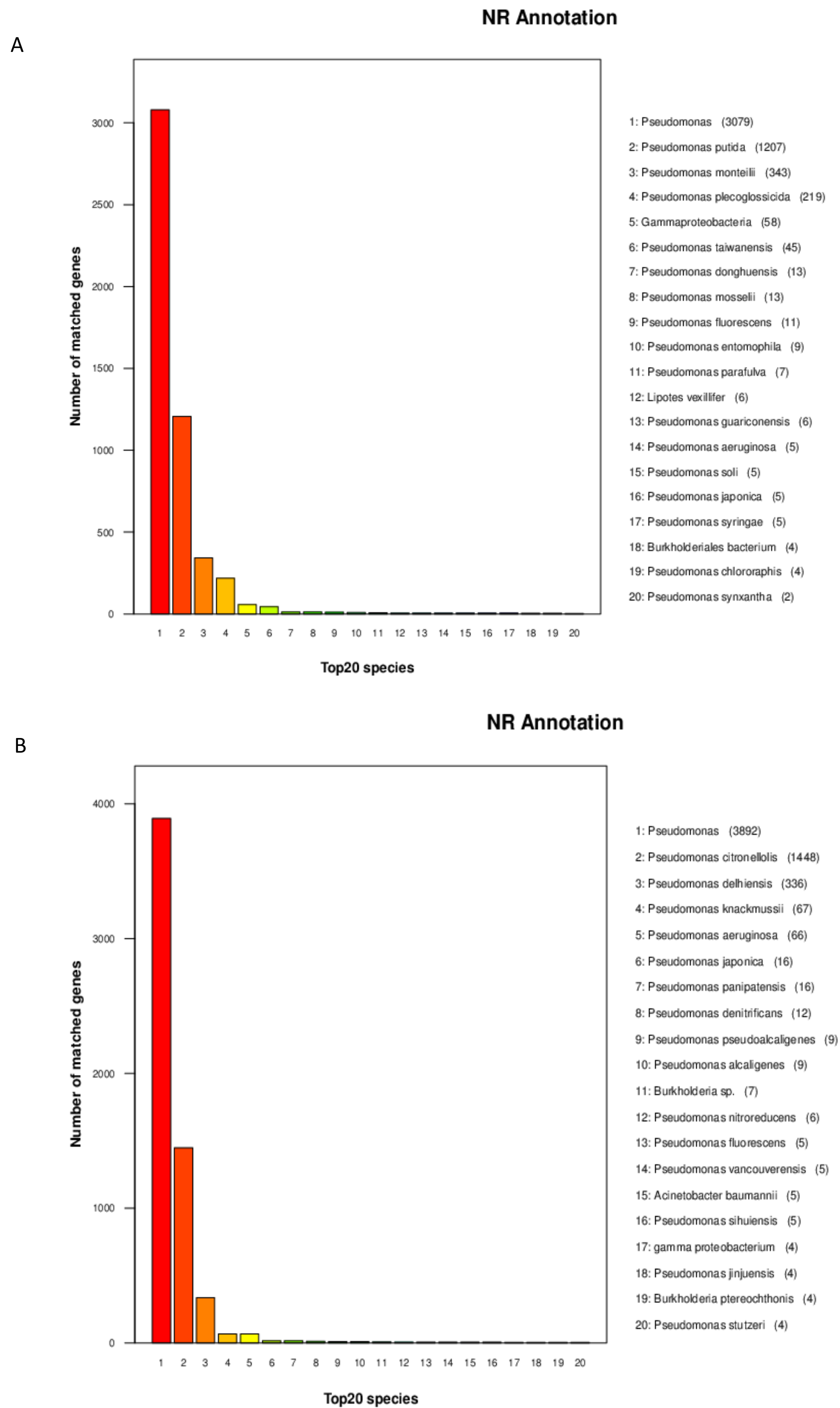

Figure S3: Phylogenetic trees of type strains of species belonging to the *Pseudomonas aeruginosa* and *P. putida* lineages and of *Pseudonomas* sp. f1 and 172 based on the partial sequences of 16S rRNA **(A)**, *gyr*B **(B)**, *rpo*B **(C)**, *rpo*D **(D)** genes.

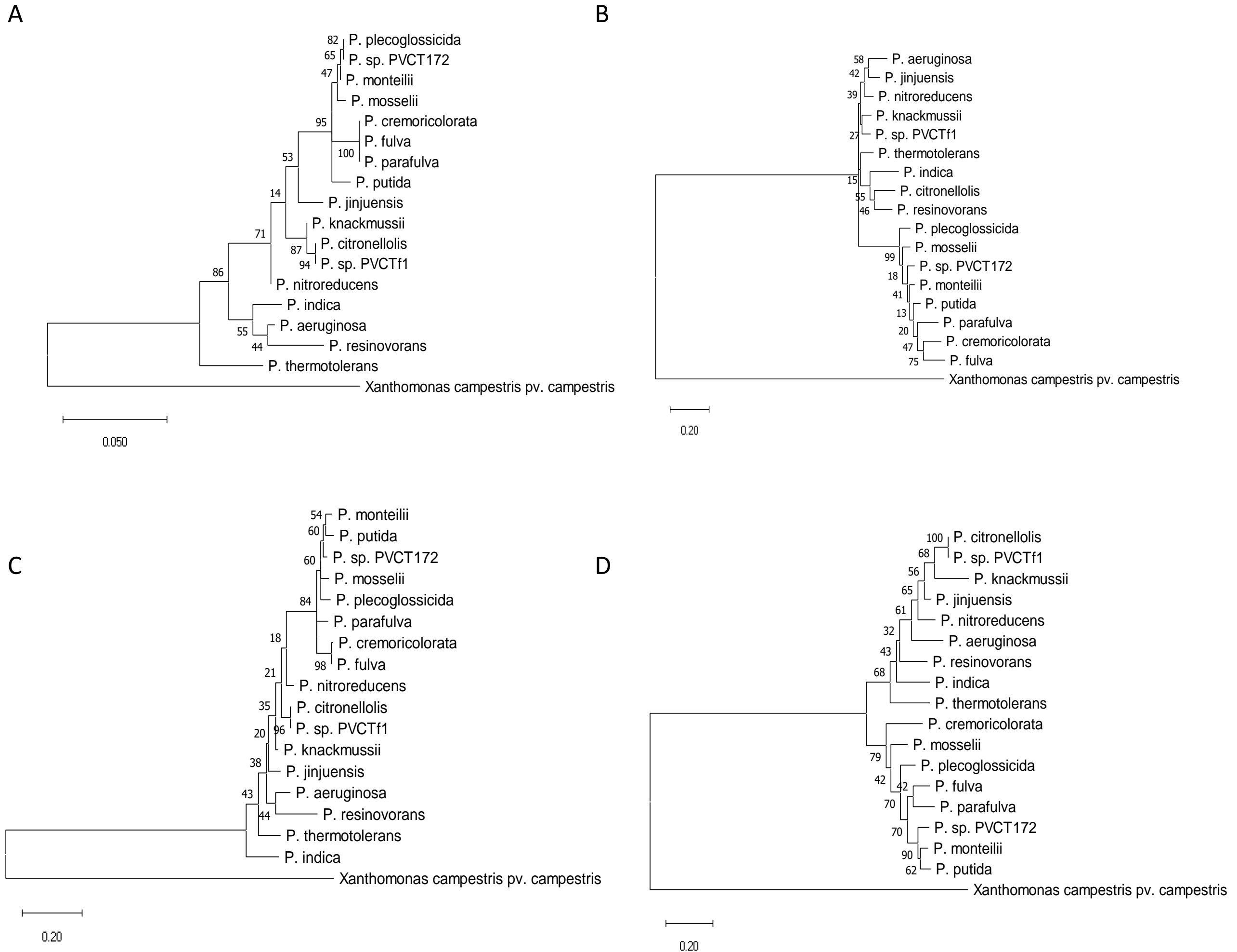

Figure S4: Phylogenetic tree of partial sequences of genes 16S rRNA of the 13 strains clustered tight to strain 172 isolated from endorhizofere (Anzalone e al., 2021) comparated with eight type strains of species of *P. putida* group.

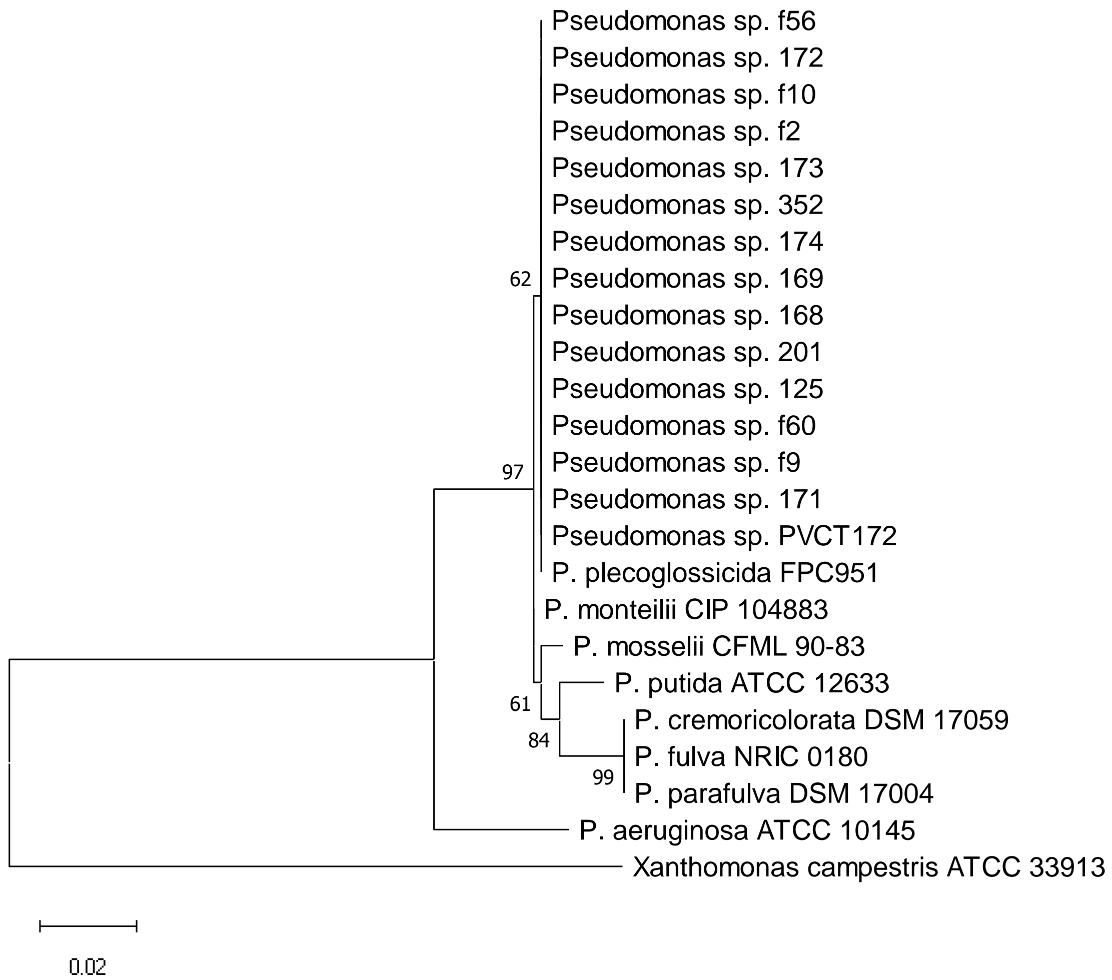

Table S1: Clusters of Orthologous Groups (GO) for *Pseudomonas* sp. strain 172, and *Pseudomonas citronellolis* strain f1.

Ontology	**Class**	**No of_Pseudo172**	**No of_PseudoF1**
biological_process	Biological Adhesion	19	24
	Biological Regulation	737	862
	Cell Killing	3	0
	Cellular Component Organization Or Biogenesis	157	169
	Cellular Process	2013	2356
	Death	1	2
	Developmental Process	21	34
	Establishment Of Localization	821	978
	Immune System Process	2	4
	Localization	839	1010
	Locomotion	47	63
	Metabolic Process	2070	2470
	Multi-Organism Process	35	47
	Multicellular Organismal Process	13	27
	Negative Regulation Of Biological Process	21	32
	Nitrogen Utilization	2	2
	Positive Regulation Of Biological Process	13	11
	Regulation Of Biological Process	718	850
	Reproduction	33	46
	Reproductive Process	22	31
	Response To Stimulus	385	426
	Signaling	254	295
	Viral Reproduction	22	27
cellular_component	Cell	1334	1563
	Cell Part	1334	1563
	Extracellular Region	23	30
	Extracellular Region Part	22	30
	Macromolecular Complex	201	217
	Membrane-Enclosed Lumen	19	22
	Organelle	199	205
	Organelle Part	97	90
	Virion	24	31
	Virion Part	24	31
molecular_function	Antioxidant Activity	14	15
	Binding	1630	2045
	Catalytic Activity	1868	2327
	Channel Regulator Activity	0	1
	Enzyme Regulator Activity	9	9
	Molecular Transducer Activity	274	332
	Nucleic Acid Binding Transcription Factor Activity	328	406
	Protein Binding Transcription Factor Activity	78	109
	Structural Molecule Activity	58	59
	Transporter Activity	317	365

Table S2: Summary of antiSMASH 5.0 results for *Pseudomonas* sp. strain 172, and *Pseudomonas citronellolis* strain f1, RIT 623

Pseudomonas sp. strain 172							
Scaffold	**Region**	**Type**	**From**	**To**	**Most similar known cluster**	**Known cluster**	**Similarity**
1	1.1	NRPS	1,68	49,92	pyoverdin	NRP	2%
	1.2	NRPS	275,614	328,567	pyoverdin	NRP	9%
4	4.1	RiPP-like	210,904	221,689			
5	5.1	RRE-containing	237,75	258,052			
6	6.1	NAGGN	72,222	87,052			
7	7.1	Ranthipeptide	58,938	80,368	pyoverdin	NRP	7%
11	11.1	redox-cofactor	117,325	139,466	lankacidin	CNRP + Polyketide	13%
38	38.1	NRPS	1	22,324	pyoverdin	NRP	1%
41	41.1	NRPS	1	20,261	pyoverdin	NRP	1%
48	48.1	NRPS	1	1,961	rhizomide A / rhizomide B / rhizomide C	NRP	100%
Pseudomonas citronellolis strain f1							
Scaffold	**Region**	**Type**	**From**	**To**	**Most similar**	**Known cluster**	**Similarity**
1	1.1	NAGGN	743,289	757,967			
4	4.1	Thiopeptide	219,383	245,128			
	4.2	Redox-cofactor	265,407	287,548	Lankacidin C	NRP + Polyketide	13%
7	7.1	RiPP-like	217,861	228,706			
10	10.1	Ranthipeptide	157,934	179,379	Pyoverdin	NRP	4%
12	12.1	Butyrolactone	71,5	82,519			
13	13.1	NRPS	23,269	77,287	Thiazostatin / watasemycin A / watasemycin B / 2-hydroxyphenylthiazoline enantiopyochelin / isopyochelin	NRP	26%
16	16.1	RiPP-like	33,33	44,163			
17	17.1	RiPP-like	89,574	100,383			
20	20.1	NRPS	1	80,493	Pyoverdin	NRP	19%

NRPS: non-ribosomal peptide synthetase
RiPP-like: Other unspecified ribosomally synthesised and post-translationally modified peptide product (RiPP) cluster.
RRE-containing: The RRE binds specifically to a precursor peptide and directs the post translational modification enzymes to their substrates
NAGGN: N-γ-acetylglutaminyl glutamine 1-amide

Publications

Index publications

- **Anzalone, A.,** Guardo, M. Di, Bella, P., Ghadamgahi, F., Dimaria, G., Zago, R., Catara, V. (2021). Bioprospecting of Beneficial Bacteria Traits Associated With Tomato Root in Greenhouse Environment Reveals That Sampling Sites Impact More Than the Root Compartment. *Front. Plant Sci.*12, 1–17. doi:10.3389/fpls.2021.637582.

- Licciardello, G., Caruso, A., Bella, P., Gheleri, R., Strano, C. P., **Anzalone, A.**, Trantas, A. T., Sarris, P. F., Almeida, N. F., Catara, V. (2018). The LuxR regulators PcoR and RfiA co-regulate antimicrobial peptide and alginate production in *Pseudomonas corrugata*. *Front. Microbiol.* 9, 521. doi.org/10.3389/fmicb.2018.00521.

Contributions at National and International conferences

- **Anzalone, A.,** Dimaria, G., Mosca, A., Musumeci, S., Privitera, G., Pulvirenti, A., Cirvilleri, G., Catara, V. (2021). Biological control of tomato bacterial diseases by Bacillus sp. and Pseudomonas sp. isolated from tomato endorhizosphere. 4th Annual Conference on Integrating science on Xanthomonadaceae for integrated plant disease management in Europe. 28-30 June 2021
- Panebianco, S, Lombardo, M., **Anzalone, A.,** Musumarra, A., Pellegriti, M.G., Catara, V., Cirvilleri G. (2021). Tomato carposphere-associated bacteria and their antagonistic activity against phytopathogens. Congress of XIII Giornate scientifiche SOI, I traguardi di agenda 2030 per l'ortoflorofrutticoltura Italiana, Catania, Italy 22-25 June 2021.
- Lombardo, M., Panebianco, S., **Anzalone, A.,** Catara, V., Cirvilleri, G. (2021). Effectiveness of sustainable alternatives to copper compounds for the biocontrol of citrus fungal diseases. Congress of XIII Giornate scientifiche SOI, I traguardi di agenda 2030 per l'ortoflorofrutticoltura Italiana, Catania, Italy 22-25 June 2021.
- **Anzalone, A.**, Bergna, A., Dimaria, G., Di Guardo, M., Mosca, A., Leonardi, C., Berg, G., Catara, V. (2021). Comunità batteriche e fungine del pomodoro: dal vivaio alla serra, in suolo e fuori suolo. Congress of XIII Giornate scientifiche SOI, I traguardi di agenda 2030 per l'ortoflorofrutticoltura Italiana, Catania, Italy 22-25 June 2021.
- Bella, P., Musumeci, S., Treccarichi, S., **Anzalone, A.**, Catara, V., Branca, F. (2021). Exploitation of *BRASSICA oleracea* complex species (n=9) for increasing the resistance to *Xanthomonas campestris* pv. *campestris* in the related crops. Congress of Breeding and seed sector innovations for organic food systems (EUCARPIA), 8-10 March 2021.
- **Anzalone, A.**, Bergna, A., Di Guardo, M., Dimaria, G., Leonardi, C., Berg, G., Catara, V. (2020). Beneficial bacteria communities from the tomato seed and root endosphere. Congress of Young Scientists for Plant Health , WEB Workshop (SIGA-SEI-SIBV-SIPAV), 16 December 2020, Poster Communication Abstract ISBN 978-88-944843-1-1.
- **Anzalone, A.**, Bella, P., Bergna, A., Cirvilleri, G., Catara, V. (2019). Microbial diversity of root-associated bacteria in tomato plants grown in the greenhouse environment.

Congress of 5th International Conference on Microbial Diversity, Catania, ITALY 25-27 September 2019. Book of abstract 326-327.

- **Anzalone, A.**, Bella, P., Bergna, A., Cirvilleri, G., Catara, V. (2019). Identification and characterization of tomato root bacterial endophytes for integrated disease control program. Congress of the XXV Congress of the Italian Phytopathological Society (SIPaV), Milan, Italy, September 16-18, 2019. *J. Plant Pathol.* 101, 81. doi.org/10.1007/s42161-019-00394-4.
- Aiello, D., Vitale, A., Contarino, G., **Anzalone, A.**, Catara, V., Cirvilleri, G., (2019). Evaluation of antibacterial products for the control of *Xanthomonas* pv. *perforans* in organic tomato farming. Congress of the 4th International Symposium on Biological Control of Bacterial Plant Diseases, Viterbo, Italy 9-11 July. *J. Plant Pathol.* 101, 873.2019. doi.org/10.1007/s42161-019-00395-3.
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- **Anzalone**, **A.**, Licciardello, G., Strano, C.P., Bella, P., Bertani, I., Venturi, V., Catara, V. (2018). *Pseudomonas corrugata* genome mining reveals a Luxr 'Solo' with a role in virulence in tomato. (Selection a oral presentation and Poster). Congress of the XXIV Congress of the Italian Phytopathological Society (SIPaV), Ancona, Italy, 5-7 September 2018. *J. Plant Pathol.* 100, 615. doi.org/10.1007/s42161-018-0130-y.
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