Microbial footprints of tomato domestication

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Chapter 1

General Introduction and Thesis Outline

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Abstract

Plant domestication and breeding not only resulted in multiple phenotypic changes but also impacted the agricultural ecosystems in which our current crops are cultivated. Most crops to date rely on the extensive use of fertilizers and pesticides to support crop growth and health. To minimize the environmental impact of these management practices, the plant microbiome has gained renewed attention as a large yet untapped resource of microorganisms with beneficial effects on plant growth and health. In the past decade, it has become evident that the microbiome of plants plays a key role in nutrient acquisition, plant development, and tolerance to diverse abiotic and biotic stresses. Here, we review past and present knowledge of the microbiome of tomato as a model for unraveling the functional potential of plant microbiomes, the impact of domestication, and the underlying genetics of microbiome assembly and activity. We also provide perspectives on how this knowledge can be adopted to enhance crop productivity and strengthen the sustainability of agricultural management practices.

***Keywords*:** domestication, microbe-assisted breeding, microbial functionality, microbiome composition, production, tomato

General Introduction

Tomato is among the most valuable vegetables worldwide, with an estimated production of approximately 190 million tons, covering over five million hectares (FAO, 2020, 2021; Klee & Resende, 2020). Asia is the continent with the highest tomato production, accounting for 62.6% of the total tomato production, followed by the Americas (13.1%), Europe (12.2%), Africa (11.9%), and Oceania (0.2%) (FAO, 2020). Tomato is a source of carotenoids, vitamins, glycoalkaloids, and other metabolites with antioxidant, anti-inflammatory and anti-mutagenic activities (Chaudhary et al., 2018). Tomatoes are also a popular ingredient in several culinary traditions, which has triggered extensive breeding programs to develop an impressive variety of tomato cultivars with a multitude of flavors, colors, and shapes (Domínguez et al., 2020).

Domestication and subsequent breeding not only resulted in multiple phenotypic changes but also impacted the natural and agricultural ecosystems in which tomatoes are cultivated. These include the extensive use of fertilizers and pesticides to support tomato growth and health. For example, to control pests, diseases, and weeds in open-field tomatoes, chemicals have been extensively used due to their low cost and high control efficiency (Desneux et al., 2022). In addition, distinct pesticide classes are used in different countries, such as organophosphates, organochlorines, carbamates, triazines, pyrethroids, dithiocarbamates, benzimidazole, chloronitriles, liquid copper fungicides, and herbicides (Köhler & Triebskorn, 2013). Hence, alternative strategies are needed for sustainable pest and disease control. In addition, strategies for improving crop resilience to stresses posed by climate change, such as extreme temperatures, drought, and salinity, as well as soil degradation, will be essential. This has led to a renewed attention to the plant microbiome as a large yet untapped resource of microorganisms with beneficial effects on plant growth and health. For example, inoculating the tomato rhizosphere with microbial consortia can offer various beneficial functions to the plant, such as enhanced nutrient uptake efficiency and protection against pathogens (Gu et al., 2022; Hu et al., 2016; Schmidt et al., 2019). This scenario highlights the functional potential of the tomato microbiome as an integral component of a novel sustainable strategy of crop production. In this context, research programs have been initiated to investigate the impact of domestication on the tomato microbiome composition and functions (Alsharif et al., 2020; Barajas et al., 2020; Carrillo et al., 2019; Cordovez et al., 2021; Lee Díaz et al., 2022; Martínez-Romero et al., 2020; Oyserman et al., 2022). Central questions in these research programs include the following: (i) What is the impact of domestication on the taxonomic and functional diversity of the seed, root, and shoot microbiomes? (ii) What is the genetic basis of plant microbiome assembly and functioning? (iii) Which plant and microbiome functions were lost during domestication and can be reinstated to control (a)biotic stresses such as drought, salinity, nutrient

deficiency, diseases, and pests? Enhancing beneficial plant–microbe interactions can provide a substantial contribution to food security in the face of global climate change, land-use intensification, and increased (a)biotic stresses in agricultural production systems (Cordovez et al., 2019; Finkel et al., 2017; Porter et al., 2020; Porter & Sachs, 2020).

Tomato is an ideal plant species for studying the impact of domestication on microbiome assembly and functioning. Several compelling factors make tomato a suitable choice for such investigations. First, tomato plants have a relatively short life cycle, facilitating timely research and observation. Additionally, tomato possesses a diverse range of genetic resources, including multiple varieties and wild relatives, which offer ample opportunities for comparative analysis. Moreover, the availability of tools for genetic modification, such as CRISPR-Cas genome editing, further enhances the experimental capabilities for studying tomato–microbiome interactions. The knowledge of tomato chemistry, as well as access to native soils in the centers of origin, provides a valuable context for investigating the impact of domestication on microbiome assembly and functions. Lastly, the economic importance of tomato in both food production and research makes it a highly relevant model plant.

Here, we provide a comprehensive review of the tomato plant, including its taxonomy and origin, as well as the current knowledge of the taxonomic and functional diversity of its microbiome. We focus on bacterial communities, but whenever possible, we also highlight research on fungal communities associated with tomato. We specifically explore how the microbiome is modulated by the tomato genotype and environmental factors and how domestication affected microbiome assembly. Our aim is to provide an integrated perspective of the diversity of the tomato microbiome and its potential contribution to sustainable tomato production.

Taxonomy and geographic distribution of tomato

Botanical description

Most tomatoes are considered annual herbs, as they cannot withstand prolonged dry or cold seasons (Peralta & Spooner, 2000; Waheed et al., 2019). However, biennial and perennial members can be found in areas with favorable environmental conditions, allowing for a woody secondary growth with new shoots and adventitious roots with a lignified base (Peralta & Spooner, 2000). Tomato has a woody, hairy, and coarse stem, which first grows vertically and later becomes decumbent because of the weight of the branches. Generally, wild tomato plants show an indeterminate growth of several meters, whereas domesticated tomatoes have a determinate growth (Peralta & Spooner,

2000). The leaves have an imparipinnate arrangement comprising two to six opposite leaflet pairs and a terminal leaflet, all covered with glandular trichomes (Peralta & Spooner, 2000). Tomato flowers are arranged in a scorpioid cyme inflorescence with 6 to 12 flowers (Peralta et al., 2008). Fruits are fleshy, globose or ovoid, and generally bilocular in wild species or multilocular berries in cultivated varieties. Fruits typically contain 50 to 200 seeds per locule, which are covered with a jelly substance to avoid immediate germination (Peralta et al., 2008). The typical fruit color is red due to the accumulation of the pigment lycopene during fruit ripening (Howe & Smallwood, 1982; Zhong et al., 2013), whereas some varieties and wild species are yellow, orange, green, or purple due to the presence of carotenoids or anthocyanin (Peralta & Spooner, 2000; Waheed et al., 2019).

Taxonomy

Tomatoes belong to the section Lycopersicon of the genus *Solanum* of the family Solanaceae, which includes 13 species, all native to western South America (Knapp & Peralta, 2016; Waheed et al., 2019). Although other tomato relatives are found in the sections Lycopersicoides and Juglandifolia (Peralta et al., 2008), *Solanum pimpinellifolium* is considered the closest wild relative of the domesticated tomato *S. lycopersicum* (The Tomato Genome Consortium, 2012) (Figure 1). In some botanical books, tomatoes are referred to as nightshades. The term "nightshade" is derived from the fact that some members of the Solanaceae family thrive in shady environments, whereas others bloom at night. Additionally, the presence of psychoactive alkaloids in Solanaceae plants may have contributed to the name "nightshade" (Lee, 2006).

Figure 1. Taxonomy of tomato and its wild relatives (based on Peralta et al., 2008).

Native habitat

The *Solanum* species in sections Lycopersicon and Lycopersicoides are all wild species and native to western South America, spread from Ecuador (including Galápagos) to northern Chile (Knapp & Peralta, 2016). They are found in a wide range of habitats and conditions but prefer warm and dry climates in the region of the western Andes, including coastal areas, mountain slopes, and valleys (Kimura & Sinha, 2008; Peralta et al., 2008; Waheed et al., 2019). Some wild tomato populations, such as *S. chilense*, *S. habrochaites*, *S. pennellii*, *S. peruvianum*, and *S. arcanum*, occur in semi-highlands (5-30°S latitude) called "lomas" (i.e., hills) situated along the Pacific coast of Peru and Northern Chile. These lomas are areas of "fog-watered" vegetation in the Andes. The presence of this seasonal fog from September to December creates a microclimate with enough moisture to allow for flowering. During El Niño, this region experiences high rainfall, resulting in better growth, mass flowering, and high seed dispersal due to water runoff (Peralta et al., 2008). Nevertheless, *S. pimpinellifolium* populates coastal habitats or river valleys in the Low Andes region and has a wider distribution compared with the other wild species found in the lomas region (Peralta & Spooner, 2000, 2000; Ramón, 2008). Two species exclusive to the Galápagos Islands are *S. cheesmaniae*, found at various elevations, and *S. galapagense*, found in lower elevation habitats, typically on lava flows and affected by the Pacific Ocean spray (Peralta et al., 2008). This robustness to harsh environmental conditions, especially low relative humidity, is typical for the wild tomato species of the Lycopersicon group. Domesticated tomato populations can also survive in semidry conditions, but their ability to persist in the wild for many generations is limited (Peralta et al., 2008).

Center of domestication and diversification

Early domestication

Speciation between *S. pimpinellifolium* and *S. lycopersicum* var. *cerasiforme* occurred in South America 78,000 years ago without human intervention (Klee & Resende, 2020; Razifard et al., 2020). The domestication of tomato is thought to have started in Ecuador from *S. lycopersicum* var. *cerasiforme* by the agricultural culture Mayo-Chinchipe around 3,000 to 2,000 BCE (Blanca & Cañizares, 2021). Interestingly, the same culture was responsible for the domestication of cacao in the Andean region called the Low Andes (Zarrillo et al., 2018). This region in the south of Ecuador is considered a biodiversity hotspot, as it is characterized by a depression in the height of the Andes, with lower mountain ranges connecting the Pacific Coast to the Amazon rainforest, as well as the Northern Andes to the Central Andes (Ramón, 2008). The unique topography of this region is believed to have facilitated interactions between several cultures in both East-West and North-South directions and at various altitudes (Valdez, 2008),

which eventually marked the tomato's "journey" from South America to Mesoamerica (Klee & Resende, 2020; Figure 2).

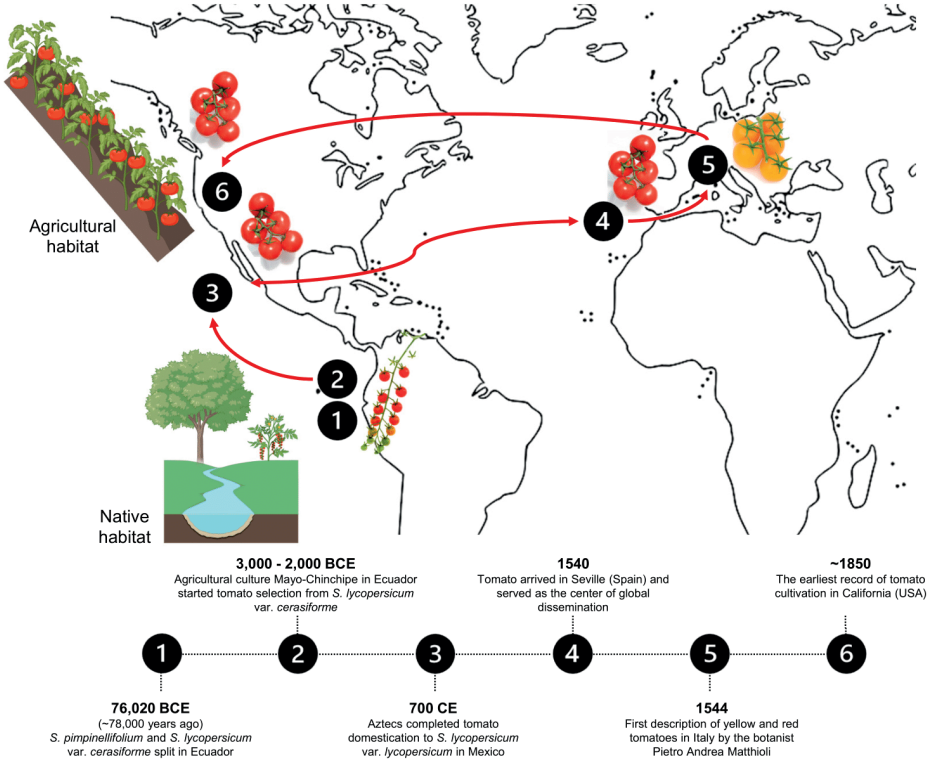

Figure 2. Historical view of the worldwide journey of tomato. Habitat figures were designed with BioRender.com.

S. lycopersicum var. *cerasiforme* underwent selection by humans for fruit traits (Blanca & Cañizares, 2021; Klee & Resende, 2020) and was grown on the high rainfall slopes of the Eastern Andes (Rick, 1983). When the ancestors of wild and early domesticated tomato arrived in Central America, they probably lost or reduced some of their original traits, including fruit size, sugar content, and floral display. This transformation likely occurred in Mexico with the fully domesticated tomato *S. lycopersicum* var. *lycopersicum* dating back to approximately 700 CE (Bai & Lindhout, 2007; Blanca et al., 2015; Jenkins, 1948; Keoke & Porterfield, 2009; Klee & Resende, 2020; Razifard et al., 2020). Interestingly, it was only in Mexico that this new crop received the name "tomato." This term was derived from the Nahuatl (Aztec language) word *xītomatl* (*xī*: peeled/skinned; *toma*: round fat thing; and *atl*: water, aqueous) to differentiate the fruit of *S. lycopersicum* from the husk tomatoes *Physalis philadelphica* and *P. ixocarpa* ("tomatl"

in Nahuatl language), which were also cultivated by Aztec families in small orchards known as "milpas" (Bowles, 2018; Díez & Nuez, 2008; Gran Diccionario Náhuatl, 2012; Jenkins, 1948; Long, 2022; Mediavilla, 2020; Wood, 2000).

Tomato distribution worldwide

Following contact with Indigenous communities in Mesoamerica, the Spanish conquerors were the first to bring the tomato to Europe in the 16th century (Díez & Nuez, 2008). These tomatoes first arrived in Seville (Spain), from where tomato quickly distributed throughout the country. The Spanish and Italians were the first to accept the use of tomato for consumption in a similar manner as Aztec culture, for instance, fried with oil, salt, and pepper (Caramante et al., 2021; Long, 2022; Peralta et al., 2008), unlike the French and North Europeans, who used tomatoes as ornamental plants, as they believed tomatoes to be toxic due to their similarity to poisonous European members of the Solanaceae family (Díez & Nuez, 2008; OECD, 2017).

In 1544, the botanist Pietro Andrea Matthioli first described the tomatoes that arrived in Italy as a flattened and segmented "mele rose" (variety of apple) that were green at first and ripened to a golden color. Later in 1554, Matthioli mentioned the red coloration in tomato and called tomatoes "pomi d'oro" ("golden apples"), which later became "pomodoro", the modern Italian word for tomato (McCue, 1952; Peralta et al., 2008). This revealed that the first tomatoes that arrived on the European continent had undergone a complete domestication process (big size) and that yellow varieties were initially more popular than the red ones (Peralta et al., 2008). Then, tomatoes were subjected to further domestication and bred into different varieties for human consumption (larger fruits, better flavor, higher yields) and compatible with new agricultural systems. Thus, Spain and Italy are acknowledged as secondary centers for tomato diversification and are resources of tomato landraces (Caramante et al., 2021; Cebolla-Cornejo et al., 2013; Lázaro, 2018).

Eventually, tomato was spread globally via commercial trading routes (Caramante et al., 2021; Díez & Nuez, 2008). For instance, in the 18th century, tomato plants were brought back to the Americas at commercial harbors on the eastern North American coast by European colonists (Díez & Nuez, 2008), where tomato was also registered first as an ornamental plant and only later, by the end of the 19th and beginning of the 20th century, was accepted as edible (Rick, 1978). The earliest record of tomato cultivation in the United States is in 1850 in San Diego, but only in 1867 did the United States start the production of commercial varieties (Díez & Nuez, 2008; Stevens & Rick, 1986).

Genetic resources

DNA-based analyses have demonstrated that domesticated tomatoes underwent a significant genetic bottleneck during their journey from South America to Central America and Europe. Compared with their wild relatives, domesticated tomatoes show less than 5% of genetic variation among them (Bai & Lindhout, 2007; Razifard et al., 2020). The tomato fruit enlargement and other improved agronomic traits were the result of genomic sweeps in two rounds, during the transition from the wild species *S. pimpinellifolium* to *S. lycopersicum* var. *cerasiforme* and then further improvement to the modern tomato *S. lycopersicum* (Lin et al., 2014). To preserve the genetic diversity of tomatoes, germplasm resources are essential. Pioneering work by Charles M. Rick led to a collection of thousands of wild tomato accessions from their natural habitats in the Andes and the Galapagos Islands. The Tomato Genetics Resource Center in Davis, California, maintains this tomato collection, as well as monogenic mutants. In the Netherlands, the Botanical and Experimental Garden maintains the most extensive ex situ non-tuberous Solanaceae species collection in the world (Bai & Lindhout, 2007; Barendse & van der Weerden, 1997). Additionally, the Solanaceae Genome Network (https://solgenomics.sgn.cornell.edu/) provides genomic information for the Solanaceae species, including tomato (*S. lycopersicum*), potato (*S. tuberosum*), eggplant (*S. melongena*), and pepper (*Capsicum annuum*) (Mueller et al., 2005). Remarkably, wild accessions with inferior phenotypes have been found to be a source of alleles that, when incorporated into cultivated backgrounds, lead to favorable phenotypes such as disease resistance and higher soluble solid content (Lin et al., 2014). Therefore, native germplasm and seed banks harbor large genetic potential for cultivated germplasm (Barone et al., 2008). Lastly, the significant contribution of the Andean and Mesoamerican regions in the early stages of tomato domestication should be recognized, as rural areas continue to harbor populations of wild tomatoes, landraces, and heirloom domesticated tomato varieties. These resources represent a valuable gene pool, in addition to existing seed banks, for new discoveries and modern breeding strategies (Bai & Lindhout, 2007; OECD, 2017; Rick, 1983). Hence, collaboration of governmental, nongovernmental, academic, and private-sector interest groups is essential to enhancing the generation of information and ensuring the proper management and conservation of tomato genetic resources (Bretting, 2018; Tanksley & McCouch, 1997).

Diversity of the tomato microbiome

Factors influencing microbiome assembly

The microbiome of plants plays an important role in development, growth, and health (Cordovez et al., 2019; Oyserman et al., 2018; Trivedi et al., 2020). The assembly of the microbiome and expression of specific microbial functional traits is affected by multiple abiotic and biotic factors (Dastogeer et al., 2020; Philippot et al., 2013; Trivedi et al., 2020; Turner et al., 2013). Here, we review current knowledge of host and environmental factors that influence tomato microbiome assembly and functions (Table 1).

Table 1. Microbial composition in tomato according to plant compartments.

Compartment	Microbial composition	Reference
Rhizosphere	Bacillaceae, Streptomycetaceae, Comamonadaceae, Oxalobacteriaceae, Mycobacteriaceae, Alphaproteobacteria, Burkholderiales, Pseudomonadaceae	Allard et al., 2016
Rhizosphere	Xanthomonadales, Nitrosomonadales, Myxococcales, Rhizobiales, Burkholderiales, Sphingobacteriales, Cytophagales, Acidobacteria Subgroup 4, Acidobacteria Subgroup 6	Cheng et al., 2020
Rhizosphere	Bacteria: *Paenibacillus, Bacillus, Patulibacter* and members of Gemmatimonadetes, Acidobacteria, Deltaproteobacteria Fungi: *Emmonsia, Alternaria, Cladosporium, Acremonium,* no identified Basidiomycota, *Conocybe, Hohenbuehelia* and *Rhodotorula*	Cordero-Ramírez et al., 2012
Rhizosphere	*Acinetobacter, Pseudomonas, Ensifer, Rhizobium*	Dong et al., 2019
Rhizosphere	Bacteria: *Nitrospira, Reyranella, Lactobacillus, Fibriiomonas, Rhodopila, Methylovorus*, *Dongia* Fungi: *Oidiodendron*, *Talaromyces*	Fuentes et al., 2020
Rhizosphere	Sphingomonadaceae, Micrococcaceae, Microbacteriaceae, Streptomycetaceae, Acidobacteria Subgroup 4	Lee et al., 2016
Rhizosphere	Bacteria: *Sphingobium, Sphingomonas, Microbacterium, Arthrobacter, Afipia, Leifsonia, Luteimonas* Fungi: *Hyphodiscus, Aspergillus, Trichoderma, Chrysosporium*, *Oidiodendron*	Lee et al., 2019
Rhizosphere	*Fusarium, Gibellulopsis, Penicillium, Phoma, Pyrenochaetopsis, Sarocladium*, *Trichoderma*	Poli et al., 2016
Rhizosphere	*Aspergillus, Penicillium, Trichoderma*, *Fusarium*	Tyagi and Tyagi, 2016
Rhizosphere	Soil Crenarchaeota Group (Thaumarchaeota), *Methanosarcina* (Euryarchaeota), *Methanoculleus* (Euryarchaeota)	Taffner et al., 2020
Phyllosphere	Flower: Xanthomonadaceae, Pseudomonadaceae, Microbacteriaceae, Enterobacteriaceae Fruit: Rhizobiaceae, Sphingomonadaceae, Microbacteriaceae, Pseudomonadaceae, Xanthomonadaceae	Allard et al., 2016
Phyllosphere	Leaf and stem: *Acinetobacter, Enterobacter, Pseudomonas*	Dong et al., 2019

Compartment	Microbial composition	Reference
Phyllosphere	Leaf: Bacteria: *Pantoea, Pseudomonas, Spingomonas, Xanthomonas,* Enterobacteriaceae, *Exiguobacterium, Bacillus* Fungi: Basidiomycota: *Rhodosporidiobolus, Filobasidium, Sporobolomyces, Tremellales;* Ascomycota: *Cladosporium*	Morella et al., 2020
Phyllosphere	Leaf: *Pseudomonas, Erwinia*, *Sphingomonas*	Ottesen et al., 2016
Phyllosphere	Leaf: Bacteria: *Pseudomonas, Sphingomonas, Methylobacterium, Variovorax, Rathayibacter, Cultibacterium, Microbacterium, Bacillus* Fungi: Basidiomycota: *Erythrobasidium, Symmetrospora, Filobasidium, Vishniacozyma, Cryptococcus*; Ascomycota: *Davidiella tassiana, Aureobasidium pullulans, Phoma medicaginis, Ophiosphaerella, Cladosporium, Alternaria*	Runge et al., 2022
Phyllosphere	Leaf: Bacteria: S*phingomonas, Methylobacterium, Pseudomonas, Deinococcus* Fungi: *Cladosporium, Dioszegia, Moesziomyces, Hannaella*	Toju et al., 2019
Phyllosphere	Leaf trichomes: *Bacillus, Deinococcus, Acinetobacter, Paracoccus, Sphingomonas, Massilia, Caulobacter, Capnocytophaga, Pseudomonas, Pedobacter, Luteimonas*	Kusstatscher et al., 2020
Endosphere	Root and leaf: *Acinetobacter*, *Enterobacter*, *Pseudomonas* Stem: *Acinetobacter*, *Enterobacter*, *Pseudomonas, Pantoea* Fruit: *Enterobacter, Tatumella, Acinetobacter, Weissella* Seed: *Enterobacter, Lachnospiraceae, Bacteroides*	Dong et al., 2019
Endosphere	Root: Deinococcacea, Enterobacteriaceae, Bacillaceae, Chromobacteriaceae, Halomonadaceae	French et al., 2019
Endosphere	Root: Bacteria: *Enterobacter, Acidovorax, Variovorax, Pseudomonas, Rhizobium, Streptomyces* Fungi: *Alternaria, Colletotrichum*	Lee et al., 2019
Endosphere	Leaf: *Methylobacterium radiotolerans*, *Shinella* sp., *Achromobacter xylosoxidans* Root: *Burkholderia cepacia, Pseudomonas* sp., *Sphingobium herbicidovorans*, *Rhizobium radiobacter*	Longoria-Espinoza et al., 2020
Endosphere	Leaf: *Pseudomonas, Propionibacterium, Streptococcus, Shinell*a. *Clavibacter* Root: *Curtobacterium, Clavibacter, Staphylococcus, Streptococcus, Acinetobacter*, *Pseudomonas*	López et al., 2020
Endosphere	Seed: *Bacillus aryabhattai, Bacillus nakamurai, Ralstonia pickettii, Stenotrophomonas maltophilia, Stenotrophomonas pavanii*	Bergna et al., 2018

Abiotic factors

Among the abiotic factors, soil physicochemical properties are the strongest determinant of the diversity of microbial communities (Bandyopadhyay et al., 2017; Jeanbille et al., 2016). Soil serves as the primary source of microorganisms, and its properties impact soil microbial diversity (Kandasamy et al., 2021; Lee et al., 2019; Philippot et al., 2013). Hence, agricultural practices such as the conversion of native habitats to crop production systems may have led to the loss of soil microbial diversity due to the external inputs of synthetic fertilizers and pesticides (Cordovez et al., 2019; Matson et al., 1997; Rodrigues et al., 2013; Schmidt et al., 2019). In the past, anthrosol (anthropogenic soil) was formed by farmers through organic soil amendments and management practices to improve soil fertility and plant productivity. These prac-

tices presumably also selected and enhanced populations of beneficial soil microbiomes (Martínez-Romero et al., 2020). The earliest recorded method of tomato cultivation in Mesoamerica was a farming system called "milpa," a polyculture association that included maize (*Zea mays*), beans (*Phaseolus* spp.), squash (*Cucurbita* spp.), and other species such as chili pepper, husk tomato, and tomato (Casas, 2001; Díez & Nuez, 2008; Long, 2022). Tomato underwent the same intensive selection process exerted by a habitat change as other useful weeds; that is, plants were collected from natural populations and protected within cultivation systems (Casas, 2001).

With the latest advances in agriculture technology, plants can now be grown under varying environmental and cultural conditions. These range from traditional open-field farms and small-scale gardens to large-scale production by the agroindustry. Innovative techniques include acclimatized greenhouses, plastic covers (Moreno & Moreno, 2008), hydroponics, and fertirrigation, as well as agroecology in alternative production systems (OECD, 2017).

Conventional management practices of tomato use mechanical structures, devices, and synthetic chemicals to condition the growing space, optimize the yield, and maintain the fruit's organoleptic traits along the food chain (Aghdam et al., 2012; Distefano et al., 2022; Garza Arizpe & Molina Velázquez, 2008; Islam et al., 2017; Lima et al., 2022; López Marín, 2017; Nicola et al., 2009; Rodrigues & Furlong, 2022).

Considering these large-scale changes in soil and crop management practices, the rhizosphere microbiome assembly of tomato is greatly affected. For example, field soils harbor more diverse bacterial communities than hydroponic systems do (Cheng et al., 2020; Chialva et al., 2018; Thomas et al., 2023; Vargas et al., 2021). More specifically, soil largely contributed to microbiome assembly in the tomato rhizosphere than tomato genotypes because they did not show statistically significant differences in bacterial diversity when grown in the same soil. Tomato rhizobacterial communities differed according to the soil source and organic matter; phosphorous, potassium, and manganese were the main soil properties that impacted rhizobacterial assembly (Cheng et al., 2020). The effects of the management practices on soil are indirect, as they impact soil properties as well as plant physiological traits that concomitantly alter the taxonomic and functional composition of the microbiome (Cai et al., 2017; Saleem et al., 2018; Schmidt et al., 2019). Hence, research focused on improving soil physicochemical properties on a large scale will be pivotal in promoting beneficial microbe–tomato interactions.

Biotic factors

Several studies have shown that the host genotype plays a relatively minor role (<10%) in microbiome assembly (French et al., 2020; Gutierrez & Grillo, 2022; Tabrett & Horton, 2020) and can decrease over time (Morella et al., 2020). In fact, human interventions have diminished the genetic diversity of domesticated crops and drastically changed plant morphology, physiology, and immune response (Chen et al., 2015; Doebley et al., 2006). Hence, domesticated tomatoes, as well as other plant species, have become more reliant on nutrients, water, and protective measures against biotic stresses (Raaijmakers & Kiers, 2022). Different tomato traits have changed drastically during domestication, including the shift from wild allogamous species to strictly autogamous commercial tomato cultivars (Razali et al., 2018; Rick, 1978; Taylor, 1986). This artificial selection has led to an increased fruit yield in domesticated varieties and enhanced disease, pest, and abiotic stress resistance (Stevens & Rick, 1986) but also reduced outcrossing and narrowed the genetic variation within tomatoes (Peralta et al., 2008). Both the native tomato *S. pimpinellifolium* and the landrace *S. lycopersicum* var. *cerasiforme* show higher genetic diversity compared with populations of the domesticated tomato *S. lycopersicum* var. *lycopersicum.* The latter displays higher frequencies of six loci linked to fruit traits, highlighting intense selection pressure for fruit weight and shape in modern tomato varieties (Blanca et al., 2015). The observed selective pressure may explain why different genotypes can exhibit varying degrees of microbiome assembly. For example, transgenic tomatoes deficient in salicylic acid (SA) or ethylene (ET) signaling showed significantly less alpha diversity in the root endosphere microbiome than the parental tomato lines, indicating that the tomato immune system affects microbiome assembly (French et al., 2019). In another study, Cordovez et al. (2021) highlighted the impact domestication and breeding can have on tomato microbiome assembly. They found that the bacterial alpha diversity decreased and beta diversity increased over time in wild and domesticated tomato in successive growth cycles, indicating that the host plant increased its selective pressure on microbiome composition, resulting in a more dissimilar microbiome between tomato genotypes. Sillo et al. (2022) found that two different domesticated *S. lycopersicum* cultivars showed changes in their microbiome composition under medium water stress in field conditions, with one of these cultivars displaying a decrease in Bacteroidetes and an increase in Firmicutes phyla in the rhizosphere.

Regarding genetics involved in microbiome assembly, Oyserman et al. (2022) found specific genomic regions in tomato associated with specific taxa and genes in rhizosphere bacteria. They observed associations between plant growth, stress, amino acid metabolism, iron and water acquisition, hormonal responses, and terpene biosynthesis and microbial traits such as metabolism of plant cell wall polysaccharides, vitamins, sulfur, and iron. Particularly, *Cellvibrio* and *Streptomyces* were shown to be differential

root colonizers of wild and modern tomato, respectively. Furthermore, these bacterial taxa harbored functions related to iron acquisition and carbohydrate metabolism that may allow them to profit from tomato rhizodeposition (Oyserman et al., 2022).

To date, domestication-associated changes in physiological and morphological traits such as root exudation and root architecture have been largely understudied. Specifically, more emphasis is given to the impact of root exudates (Gutierrez & Grillo, 2022) than root volatile emissions (Lee Díaz et al., 2022). Root exudation is affected by both plant host factors, such as genetics and developmental stages, and environmental conditions, such as water and nutrient availability (Hale et al., 1971). For instance, the composition of tomato root exudates varies during plant development, with amino acids, organic acids, and sugars being more prevalent in tomato roots compared with the fruiting stage (Vančura & Hovadík, 1965). This exudation profile in tomato roots likely attracts bacteria specialized in carbohydrate metabolism and conversion of sugars or toxic carbonyl compounds, enabling them to thrive in the root environment over time (Levy et al., 2018). For example, *Pseudomonas*, whose *mdh* operon encodes malate dehydrogenase, depends on organic acids from tomato root exudates for efficient root colonization (Lugtenberg et al., 2001).

In addition, stress by herbivorous insects as well as pathogens induces changes in the root exudation profile (Yi et al., 2011). In this context, the "cry for help" hypothesis has been coined and is defined as the mechanism by which plants change their root exudate chemistry to recruit or activate beneficial members of the microbiome, enabling plants to adapt to biotic stress (Berendsen et al., 2012; Rizaludin et al., 2021; Rolfe et al., 2019). The "cry for help" was first addressed by Rudrappa et al. (2008), who evidenced that *Pseudomonas syringae* pv. *tomato* elicited the secretion of L-malic acid from *Arabidopsis* roots to promote the recruitment of *Bacillus subtilis* and suppress subsequent pathogen attack. Moreover, recent studies showed significant differences in root volatile emissions between wild and domesticated tomatoes under leaf herbivory stress (Lee Díaz et al., 2022). More specifically, the wild tomato *S. pimpinellifolium* showed the largest change in root volatilome between *Spodoptera exigua*-stressed plants and the control. This and other studies highlight the importance of adopting a holistic view on microbiome assembly. Furthermore, metabolomics studies of water-soluble and volatile root compounds of wild and domesticated tomatoes, as well as transcriptomics of recruited microbiomes under stress conditions, are needed to understand the mechanisms involved in selective colonization and improved plant health.

Rhizosphere microbiome

The rhizosphere is the narrow zone of soil that is influenced by the presence and activity of living roots (Berendsen et al., 2012). The tomato rhizosphere microbiome composi-

tion is determined largely by the soil type. For example, Cheng et al. (2020) found no differences in the rhizosphere microbiome composition among 11 tomato genotypes grown in lawn soil collected from Fujian Normal University in Fuzhou, China. In this study, the tomato rhizosphere microbiome was defined by Proteobacteria (34%), Bacteroidetes (16%), and Acidobacteria (15%) as the most abundant phyla. However, when sowing a single tomato genotype (*S. lycopersicum* var. Meiguodahong 168) in seven different soils and substrates, they found that the rhizosphere microbiome differed greatly among soil sources (Cheng et al., 2020). Dong et al. (2019) showed that Proteobacteria even constituted more than 80% of the phyla detected in the tomato rhizosphere. Studies on the tomato rhizosphere microbiome in Mexico, the center of domestication, revealed that the rhizosphere microbiome of tomato grown in soil from this area differs from that of other studies. Members of the Firmicutes (45%), Proteobacteria (15%), Gemmatimonadetes (13%), Actinobacteria (11%), and Acidobacteria (10%) phyla were the most abundant bacterial taxa in a domesticated tomato grown under field conditions in Mexico (Cordero-Ramírez et al., 2012). When considering that over 30% of the world's tomato production occurs in greenhouses, Lee et al. (2016) found that the most abundant phyla in the rhizosphere microbiome of greenhouse tomatoes were Proteobacteria (78%, dominated by Alphaproteobacteria), Actinobacteria (9%), Bacteroidetes (4%), Acidobacteria (3%), and Planctomycetes (1%). Kim et al. (2006) found that Proteobacteria (53%) and Cytophaga–Flavobacterium–Bacteroides (35%) were the dominant phyla in the tomato rhizosphere under greenhouse cultivation. On the other hand, Resendiz-Nava et al. (2023) found bacterial differences in the tomato rhizosphere under a soilless culture system. They showed that Flavobacteriaceae and Rhodobacteraceae were more abundant in an organic fertilization regime, whereas Streptomycetaceae, Caulobacteraceae, and Chitinophagaceae were more abundant in the conventional fertilization regime.

In contrast to bacterial communities, less research has been conducted on fungal communities in the tomato rhizosphere. Nonetheless, soil was shown to be the main factor in shaping the mycobiome composition of the tomato rhizosphere (Poli et al., 2016). Following a culture-based approach, Cordero-Ramírez et al. (2012) and Poli et al. (2016) showed that most of the genera isolated belonged to the Ascomycota. There is, to our knowledge, no conclusive evidence supporting the occurrence of mycorrhizal fungi in significantly high numbers in the tomato rhizosphere, and their presence seems to be influenced by the plant genotype. Fuentes et al. (2020) showed a differential recruitment of members of the Glomeromycota by two native plants species from the Atacama Desert, *S. chilense* and *Billardiera scandens.* Although the tomato plants were not able to recruit Glomeromycota under natural conditions, they can respond better to symbiotic interaction when mycorrhizal fungi are inoculated. There is also a limited number of studies on the archaeal members of the tomato microbiome. In a study

by Taffner et al. (2020), members of the Thaumarchaeota (60.7%) and Euryarchaeota phyla (*Methanosarcina*: 12.6%, *Methanoculleus*: 3.4%) composed the tomato rhizosphere microbiome.

Phyllosphere microbiome

The phyllosphere, the aboveground plant compartment, including leaves, stems, blossoms, and fruits surfaces (Allard et al., 2016), is a microbial habitat strongly influenced by environmental conditions (Dong et al., 2019; Mechan Llontop et al., 2021; Morella et al., 2020; Müller & Ruppel, 2014; Ottesen et al., 2016). Proteobacteria dominate the tomato phyllosphere, such as leaves, stem, flowers, and ripe fruits, under both greenhouse and field conditions (Allard et al., 2016; Dong et al., 2019; Morella et al., 2020; Ottesen et al., 2016). A recent study on the leaves of wild tomatoes collected in their native habitat (Lima, Peru) revealed more bacterial diversity in *S. peruvianum* and *S. pimpinellifolium* compared with *S. habrochaites* and *S. corneliomulleri* (Runge et al., 2022). In addition, the genotype effect on microbiome composition was observed in *S. habrochaites* and *S. corneliomulleri* but not in *S. peruvianum* and *S. pimpinellifolium*. Also in this study, Proteobacteria were the most abundant in *S. habrochaites* and *S. corneliomulleri*, whereas Actinobacteria and Firmicutes were more abundant in the phyllosphere of *S. peruvianum* and *S. pimpinellifolium* (Runge et al., 2022). The fungal composition in the wild tomato phyllosphere was dominated by Basidiomycota (yeasts) and Ascomycota. Intriguingly, the native habitats assembled a more diversified community in the phyllosphere than in crops grown under greenhouse or field conditions (Morella et al., 2020; Runge et al., 2022). Moreover, Kusstatscher et al. (2020) found differences in the bacterial composition in tomato leaf trichomes between *S. habrochaites* LA1777 and *S. lycopersicum* LA4024. *S. habrochaites* harbored richer bacterial communities than did *S. lycopersicum*. Trichomes of *S. habrochaites* were significantly enriched in bacterial classes Alphaproteobacteria and Bacilli, whereas *S. lycopersicum* trichomes were dominated by Gammaproteobacteria and Bacteroidia.

Endosphere microbiome

The endosphere refers to the compartment inside plant tissue that is characterized by a distinct microbial assemblage and lower diversity than in the external compartments, such as the rhizosphere (French et al., 2020; Lee et al., 2019). López et al. (2020) studied the endosphere microbiome composition of healthy and diseased roots and leaves of tomato plants under greenhouse conditions. They discovered that the most prevalent classes in healthy roots were Actinobacteria (23.30%), Bacilli (15.02%), and Gammaproteobacteria (12.58%), whereas in diseased roots, Actinobacteria was reduced to 7.43% but Bacilli (17.58%) and Gammaproteobacteria (28.20%) increased. On the other hand, the endosphere of healthy leaves primarily harbored Proteobacteria (32.86%) and Actinobacteria (15.73%), in contrast with symptomatic leaves

that showed an increase of Actinobacteria (58.28%) and a decrease of Proteobacteria (25.72%). French et al. (2020) found that the tomato root endosphere was dominated by Proteobacteria (Gammaproteobacteria: 46.6%, Alphaproteobacteria: 22.5%, and Deltaproteobacteria: 3.4%), Actinobacteria (15.0%), and Firmicutes (6.8%) in eight tomato genotypes. Nevertheless, the root endosphere did not show a clustering pattern between wild *S. pimpinellifolium* and domesticated tomato *S. lycopersicum*, and Bacillaceae and Rhizobiaceae where the high-frequency endosphere colonizers (French et al., 2020). Likewise, Dong et al. (2019) and Longoria-Espinoza et al. (2020) showed that the endophytic community of root, shoot, leaf, fruit, and seed in tomato was characterized by the phylum Proteobacteria. For the endophytic fungal community, particularly in roots, Ascomycota was the major phylum of tomato roots (93.9%), followed by Basidiomycota (3.5%) and Zygomycota (0.2%) (Lee et al., 2019).

Seed microbiome

Microorganisms can be vertically transmitted from seeds over plant generations (Berg & Raaijmakers, 2018). A meta-analysis of the seed microbiome of 50 plant species revealed dominance of Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes and fungal classes Dothideomycetes, Sordariomycetes, and Tremellomycetes (Simonin et al., 2022). The core taxa included *Pantoea agglomerans*, *Pseudomonas viridiflava*, *Pseudomonas fluorescens*, *Rhizobium* spp., *Cladosporium perangustum*, and *Alternaria* spp. (Simonin et al., 2022). In addition, Bergna et al. (2018) found that seeds of *S. lycopersicum* (cultivars Moneymaker and Hildares F1) were selective for a few dominant bacterial taxa, such as Burkholderiaceae (19%), Pseudomonadaceae (7%), and Comamonadaceae (6%), which showed a continuous turnover after one generation. Similarly, Taffner et al. (2020) found that the seed endosphere of *S. lycopersicum* (Moneymaker and Hildares F1) was dominated by Thaumarchaeota and Euryarchaeota. Nevertheless, the archaeal diversity was low, and it exhibited a decline in the subsequent generation.

Functions of the tomato microbiome

The microbiome provides numerous life-support functions for plant growth and health. In tomato, a range of functions have been reported as well, including nutrient acquisition and tolerance to abiotic and biotic stresses.

Nutrition acquisition

Nitrogen (N), phosphorus (P), potassium (K), and iron (Fe) are essential elements for tomato development. Plant-associated microorganisms can improve plant nutrient uptake and, consequently, the overall health and productivity of plant systems through

different mechanisms. Below, we review the main microbial functions that facilitate nutritional absorption in tomato.

Nitrogen fixation

Nitrogen can be abundant in soil, but plants require it in a usable form, such as ammonium (NH_4^+) or nitrate (NO_3^-). The process of converting N_2 from the surrounding atmosphere to ammonium is known as nitrogen fixation (Singh et al., 2019). This process is carried out by specialized bacteria, either symbiotic bacteria such as *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, and *Frankia* or nonsymbiotic bacteria such as *Anabaena*, *Azoarcus*, *Azotobacter*, *Acetobacter*, *Azospirillum*, *Burkholderia*, *Diazotrophicus*, *Enterobacter*, *Gluconacetobacter*, *Nostoc*, and *Pseudomonas* (Prasad et al., 2019). Mohandas (1988) reported that *Azospirillum* present in the rhizoplane and endosphere of tomato was capable of fixing nitrogen under field conditions. Caballero-Mellado et al. (2007) found N_2-fixing *Burkholderia* species (*B. unamae*, *B. xenovorans*, *B. tropica*, and *B. kururiensis*) in the tomato rhizoplane in field crops in Mexico. Furthermore, Masood et al. (2020) determined that the addition of nitrogen into the soil activated the nitrogen fixation by *Bacillus pumilus* and increased nutrient uptake and tomato growth. Similarly, inoculating tomato plants with rhizobia (*Rhizobium etli* CE-3, *R. leguminosarum* SCR, and *R. leguminosarum* Semia-4088) promoted plant growth, and *R. etli* significantly increased tomato growth, NPK foliar content, and yield under field conditions compared with uninoculated plants, although the conclusive role of nitrogen fixation by the introduced rhizobia in growth promotion was not experimentally validated (Toledo Cabrera, 2021).

Phosphate solubilization

Phosphate-solubilizing bacteria release low molecular weight organic acids, such as gluconic and ketogluconic acid, that make inorganic phosphate available to the plant in the form of $H_2PO_4^-$ and HPO_4^{-2} ions (Goldstein, 1995). These bacteria can be found across various genera, including *Arthrobacter, Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Mesorhizobium*, *Microbacterium*, *Pseudomonas*, *Rhizobium*, *Rhodococcus*, and *Serratia* (Prasad et al., 2019). Under greenhouse conditions, Zhang et al. (2017) demonstrated that phosphate-solubilizing *Acinetobacter* (Gammaproteobacteria) and *Ochrobactrum* (Alphaproteobacteria), isolated from mushroom residues, promoted tomato growth. Similarly, Tchakounté et al. (2020) inoculated *Arthrobacter* and *Bacillus* on tomato plants, which increased phosphorous uptake in both high and low salt concentrations and promoted plant growth. Nassal et al. (2018) found significantly increased microbial phosphatase activity in the rhizosphere of tomato plants and plant growth promotion by *Pseudomonas* sp. strain RU47.

Iron acquisition

Iron is an element typically abundant in soil in an insoluble form and therefore not accessible to the plant. Thus, rhizobacteria play a role in solubilizing or chelating iron from complex organic or inorganic forms via the production of siderophores. The main types of siderophores produced by microbes are pseudobactins, pyoverdins, and pyochelins (Kannojia et al., 2019). Radzki et al. (2013) found that the siderophore produced by *Chryseobacterium* strain C138 increased plant growth, chlorophyl, and iron content in iron-starved tomato plants as compared with the uninoculated control plants. Similarly, Abbamondi et al. (2016) reported that *Pseudomonas*, *Rhizobium*, *Rhodococcus*, and *Agrobacterium* isolates from the endospheres of different tomato cultivars produced siderophores and promoted root hair formation in *Arabidopsis thaliana.* In a related study, Karuppiah et al. (2022) identified the rhizobacterium *Pseudomonas stutzeri* KRP8 as the highest siderophore producer, which enhanced the bioavailability of iron, improved plant tolerance to heavy metals, and promoted plant growth.

Enhanced tolerance to biotic and abiotic stresses

Regarding the plant health status, eubiosis refers to a balanced microbiome that provides beneficial effects to the host, whereas dysbiosis refers to a disturbed microbiome that causes deleterious effects on plant growth and health (Lee et al., 2021). For instance, it has been found that dysbiotic leaves (growth defects, leaf chlorosis and necrosis) of wild tomato exhibited lower bacterial richness (Runge et al., 2022). Many studies have indicated that specific single bacterial or fungal strains can provide protection to tomato plants against bacterial, fungal, and oomycete pathogens, as well insect pests, both below- and aboveground. These include *Pseudomonas* (García-Villaraco et al., 2021; Mohammed et al., 2020), *Bacillus* (Janahiraman et al., 2016; Zhou et al., 2021), and *Streptomyces* (Le et al., 2021; Ling et al., 2020), bacterial genera well known for the production of diverse antimicrobial compounds such as 2,4-diacetylphloroglucinol, amphisin, hydrogen cyanide, phenazine, oomycin A, tropolone, pyoluteorin, tensin, pyrrolnitrin, cyclic lipopeptides, kanosamine, oligomycin A, xanthobaccin, and zwittermycin. Additionally, *Pseudomonas, Streptomyces*, and *Bacillus* can counteract phytopathogens through production of fungal cell wall–degrading enzymes, such as chitinase or β-1,3-glucanase (Singh et al., 2019). Moreover, these and other bacterial genera were shown to induce systemic resistance in tomato against leaf pathogens such as *Botrytis cinerea* (Audenaert et al., 2002) and *Phytophthora infestans* (Tran et al., 2007). Taking a microbial community approach, Hu et al. (2016) showed that the suppression of *Ralstonia solanacearum* in tomato was dependent on the diversity of a *Pseudomonas* consortium. Subsequently, the study by Lee et al. (2021) revealed that *Ralstonia solanacearum* infections in tomato led to a decline in Firmicutes and Actinobacteria in the rhizosphere. Furthermore, they showed that treatment of the rhizosphere with the antibiotic vancomycin mimicked this dysbiotic tomato phenotype. However, when a

synthetic bacterial community (SynCom) was introduced, consisting of four isolates of Actinobacteria (*Brevibacterium frigoritolerans* HRS1) and Firmicutes (*Bacillus niacini* HRS2, *Solibacillus silvestris* HRS3, *Bacillus luciferensis* HRS4), disease protection of tomato against *R. solanacearum* was restored. The SynCom did not exhibit direct antagonism against the pathogen; therefore, they proposed that induced systemic resistance was the most likely mechanism of protection. This was supported by additional results showing that the SynCom upregulated the expression of jasmonic acid and SA signaling marker genes, such as *Pin2*, *AOS*, *LoxD*, *PR-P6*, *NPR1*, and *PR1a*, but did not activate the expression of ET and abscisic acid (ABA) signaling genes (Lee et al., 2021). Inoculation of *Aspergillus niger*, *A. flavus*, *Mucor circinelloides*, and *Penicillium oxalicum* on tomato (cv. Castlerock II PVP) reduced the disease severity of *Fusarium* wilt (Attia et al., 2022). Among these fungal species, *A. niger* showed the highest protection rate, whereas *M. circinelloides* showed the strongest increase in chlorophyll content, as well as in total soluble proteins and carbohydrates. In addition, growth promotion was observed on inoculation of tomato plants with *P. oxalicum*.

Salinization is one of the major stressors in agriculture systems of arid and semiarid regions, mainly due to the saline water used for irrigation and inadequate drainage. Salt stress causes metabolism imbalances induced by ion toxicity and water deficit due to salt accumulation in plant cells. Salinity leads to the formation of reactive oxygen species (ROS), such as superoxide (O^{2-}), singlet oxygen (O_2), hydroxyl (OH^-), and hydrogen peroxide (H_2O_2), resulting in redox imbalance and oxidation of cell membranes. Hence, an antioxidant enzyme system is activated during stress conditions, including H_2O_2 scavengers such as catalase, peroxidase, ascorbate peroxidase, glutathione peroxidase, glutathione S-transferases, glutathione reductase, superoxide dismutase, peroxiredoxin, scorbate, glutathione, α-tocopherol, and flavonoids (Bharti & Barnawal, 2019). Despite these adverse effects, salinity has also been reported to have a positive impact on the fruit quality of tomato landraces in comparison with commercial cultivars such as Moneymaker (Massaretto et al., 2018). Rhizobacteria such as *Pseudomonas*, *Azospirillum*, *Burkholderia*, *Arthrobacter*, *Bacillus*, *Enterobacter*, and *Azotobacter* can enhance the ability of crops to tolerate high levels of salt. These bacteria achieve this by producing antioxidants, including catalase and other ROS-scavenging enzymes (Bharti & Barnawal, 2019). For example, a synthetic community of five bacterial strains isolated from the root of *Indigofera argentea*, composed of *Massilia* sp. SA087, *Enterobacter* sp. SA187, *Ensifer* sp. SA403, *Bacillus* sp. SA436, and *Streptomyces* sp. SA444, successfully protected tomato plants against high salt stress (Schmitz et al., 2022). In other studies, *Enterobacter* sp. EJ01, *Achromobacter piechaudii* (Mayak et al., 2004), and *Pseudomonas mendocina* (Sadrnia et al., 2011) were found to promote growth and increase salt stress resistance in tomato plants through multiple mechanisms, such as the rapid upregu-

lation of salt stress–responsive genes and enhancement of ROS-scavenging activities (Kim et al., 2014; Mayak et al., 2004; Sadrnia et al., 2011).

When plants are subjected to salt stress, it triggers the overproduction of ET, which inhibits plant growth and development. However, certain rhizobacteria can produce ACC deaminase, an enzyme that cleaves ACC to α-ketobutyrate and ammonia, thereby decreasing the levels of ET in the host plant (Bharti & Barnawal, 2019). Among these rhizobacteria are *Pseudomonas fluorescens*, *P. aeruginosa*, and *P. stutzeri*, which were isolated from the tomato rhizosphere and enhanced salinity tolerance and promoted growth in tomato plants through ACC deaminase activity and phytohormones production (Tank & Saraf, 2010). Similarly, *P. putida* UW4 was observed to upregulate the expression of Toc GTPases genes, which are involved in facilitating the import of stress-responsive proteins into chloroplasts, resulting in increased salt stress tolerance in tomato plants (Yan et al., 2014).

With water scarcity posing a threat to global food security, one promising option is the use of rhizobacteria to promote the formation of lateral roots and increasing the length of primary roots, thus improving water and nutrient search and uptake. For example, Shintu and Jayaram (2015) showed that tomato inoculated with *Bacillus polymyxa* increased plant growth and yield under drought conditions compared with uninoculated plants. In another study, although the efficiency of the co-inoculation of *Rhizophagus irregularis* and *Variovorax paradoxus* 5C-2 in tomato recombinant inbred lines (RILs) under drought stress was variable, Calvo-Polanco et al. (2016) found that these inoculants positively impacted drought tolerance in RIL66, which was highly responsive to the inoculation and increased dry biomass, CO_2-assimilation rate, root hydraulic conductivity, and decreased proline accumulation under drought conditions. Additionally, different rhizobacteria, including *Alcaligenes*, *Azospirillum*, *Azotobacter*, *Bradyrhizobium*, *Burkholderia*, *Bacillus*, *Enterobacter*, *Mycobacterium*, *Pseudomonas*, *Rhizobium*, *Serratia*, and *Xanthomonas*, produce the phytohormone indole-3-acetic acid, which promotes root growth and enhances water and nutrient uptake (Barnawal et al., 2019). Also, the production of the phytohormone ABA may induce stomatal closure, thereby reducing water loss upon drought stress (Porcel et al., 2014). For example, Brilli et al. (2019) showed that in tomato roots inoculated with *Pseudomonas chlororaphis* subsp. *aureofaciens* strain M71, the leaf hormonal content of ABA and indole-3-acetic acid increased, resulting in drought tolerance through improved water use efficiency (Brilli et al., 2019). The study by Flemer et al. (2022) revealed that salinity stress in combination with *Verticillum dahliae* inoculation reduced the alpha diversity and significantly decreased the abundance of Deltaproteobacteria, Firmicutes, Planctomycetes, Chlamydiae, and Verrucomicrobia, whereas salinity stress alone increased bacterial phyla Proteobacteria and Bacteriodetes. Furthermore, isolated root endophytes, such

as *Microbacterium* from *V. dahliae*-infected plants and *Paenibacillus* from *Fusarium oxysporum* f. sp. *lycopersici*-infected plants, displayed salt and drought tolerance in vitro. These findings suggest that the tomato root endosphere was enriched by beneficial microorganisms that can mitigate the (a)biotic stress (Flemer et al., 2022).

Inoculation of tomato plants with arbuscular mycorrhizal fungi was shown to alleviate abiotic stress (Chandrasekaran et al., 2021). For instance, Leventis et al. (2021) found that tomato (cultivar EVIA F1) inoculated with the arbuscular mycorrhizal fungi *Funneliformis mosseae* and *Rhizophagus irregularis* displayed efficient drought stress mitigation and increased nutrient uptake when compared with the fully watered nonmycorrhizal controls. Ronga et al. (2019) also found that tomato plants inoculated with *Funneliformis mosseae* had higher leaf chlorophyll content, nitrogen balance index, and water use efficiency under drought conditions than *Rhizophagus intraradices* on the three tomato genotypes tested (Pearson, Everton, and H3402). Furthermore, Morsy et al. (2020) found fungal endophytes, such as *Ampelomyces* spp. and *Penicillium chrysogenum*, to mitigate drought and salinity stress, respectively. Halo et al. (2020) showed that the endophytic fungus *Talaromyces omanensis* conferred drought tolerance in tomato variety Platinum, as indicated by phloem and cortex thickness, shoot dry weight, root length, number of flowers, and fruit weight, as well as higher concentrations of gibberellic acid than control plants. These studies exemplify the enormous potential of fungal communities to enhance the stress resilience of tomato plants.

Breeding for beneficial microbiome–plant interactions

Restoring beneficial microbe–plant interactions in modern crops requires an interdisciplinary effort of breeders and scientists, with a focus on disentangling the complexity of these interactions (Oyserman et al., 2022; Wissuwa et al., 2008). Furthermore, when implementing microbial inoculants in agricultural systems, it is important to consider the coevolutionary trajectory of plant microbiome assemblage during the genetic improvement process to ensure persistence of beneficial microbes throughout the cropping season (Cordovez et al., 2019). Because rapid environmental changes can disrupt mutualistic relationships, finding the optimal match between the microorganism and plant genotype is crucial for rapid adaptation to new environmental conditions (O'Brien et al., 2021; Oyserman et al., 2021) Therefore, modification of the plant-associated microbiome should be viewed as a complementary strategy for developing a new and sustainable agriculture (Martínez-Romero et al., 2020).

Microbial inoculation

The integration of microbial inoculants into modern agricultural systems has revitalized interest in and applicability to microbiome engineering approaches. Thus, the addition of biofertilizers and inoculants to enhance plant fitness is a promising strategy, particularly in degraded agroecosystems impacted by the overuse of synthetic fertilizers and pesticides (Bandyopadhyay et al., 2017). The functional characterization of beneficial microorganisms and microbial communities, by both 'omics technologies and classical in vitro validation assays, is harnessed to develop SynComs, which are groups of three or more keystone microbial isolates that complement each other to enhance plant productivity (Bandyopadhyay et al., 2017; Mendes et al., 2011; van der Heijden & Hartmann, 2016). This strategy has been proposed to "artificially" engineer the plant microbiome (Marín et al., 2021; Vorholt et al., 2017). In this context, a meta-analysis of the application of microbial inoculants showed that crop yield was enhanced due to nutrient availability provided by the application of five bacterial genera: *Pseudomonas*, *Bacillus*, *Enterobacter*, *Burkholderia*, and *Rhizobium* (Li et al., 2022). The impact of fungal inoculants on tomato growth and health has also received attention, albeit less than bacterial inoculants. For example, the inoculation of tomato seedlings with the arbuscular mycorrhizal fungus *Funeliformis mosseae* led to modifications in the root architecture, such as shorter root length and greater root branching, as well as increased leaf area when compared with noninoculated plants (Cesaro et al., 2020). Moreover, *Rhizophagus irregularis* significantly improved, under drought stress, water use efficiency and enhanced the leaf area and nutritional status of tomato (Hart et al., 2015; Leventis et al., 2021; Volpe et al., 2018). *Trichoderma* species have also been widely applied in agriculture, and their products have demonstrated efficiency as biofertilizers and biocontrol agents against various pathogens in tomato (Natsiopoulos et al., 2022; Ye et al., 2020).

If plants and their associated microorganisms have coevolved for over hundreds of millions of years, then wild plants in their native ecosystems may still harbor ancient mutualistic plant–microbe associations, such as potential phytostimulants and biocontrol agents (Pérez-Jaramillo et al., 2016). Additionally, plants that grow in marginal soils and extreme conditions (drought, salt, heat, cold) similar to their natural habitats have established robust associations with microorganisms that enable them to survive these harsh environmental conditions (Barajas et al., 2020). Therefore, retrieving, identifying, and characterizing these microorganisms that associate with wild plants is essential to enhancing their applications in modern agriculture (Gutierrez & Grillo, 2022; Pérez-Jaramillo et al., 2018). Also, natural disease-suppressive soils remain a valuable resource for identifying individual species or consortia of microorganisms that counteract pathogen establishment or prevent disease in a determined crop species (Gómez Expósito et al., 2017). In this regard, a suppressive soil against *Fusarium oxysporum* f. sp. *lycopersici*,

from the Rosta region in Italy, elicited an alert status in tomato plant by showing up-regulation of phenylpropanoid metabolism genes and other defense responses in the presence of the fungus, as well as the expression of marker genes for plant–microbe interactions, when compared with disinfected soil and artificial substrate (Chialva et al., 2018). The selection of specific rhizobacteria from tomatoes, mainly belonging to the Firmicutes phylum, inhibited the growth of "helper" bacteria that stimulate the growth of the pathogen *Ralstonia solanacearum* and effectively reduced the density and incidence of the pathogen (Li et al., 2022).

Tomato breeding strategies

The process of domesticating plants has resulted in genetic erosion due to the loss of certain plant traits found in wild relatives (Priyadarshan, 2019). From the 1950s to the early 1980s, the tomato industry demanded the development of specific plant traits to meet market demands, which resulted in phenotypic selection (Foolad & Panthee, 2012). Classical breeding techniques were used to achieve phenotypic traits such as fruit morphology, flowers with inserted stigmas (strict autogamy), and high yield, whereas modern plant breeding was required for resistance to diseases and pests (Caramante et al., 2021; Ercolano et al., 2020; Fentik, 2017; Rick, 1950). To advance tomato genetics, both classical and modern breeding methods have been utilized. Classical breeding techniques include mass selection and pedigree methods, whereas modern methods include hybridization, backcrossing, and transgenesis (Fentik, 2017). Mass selection involves repeatedly planting several phenotypically superior lines over several years until desired traits are achieved. In the pedigree method, a controlled cross is performed, followed by several successive generations of a single selected plant. This strategy, which spans several generations (from F2 to F6), aims to develop a new variety from a single cross. Each generation must be grown in the same environment for genetic differences to be expressed, and selected traits are examined among individual plants in the early generations (Fentik, 2017).

Phenotypic selection has limitations, such as availability of screening environments, reduced response to low heritable traits, and the need for large populations and land areas for experimentation. In response, hybridization has been used to boost the heterosis potential of tomato (Foolad & Panthee, 2012). Hybridization improves traits such as earliness, total yield, resistance attributes, adaptability, and external appearance in the first F1 cultivar compared with the parental cultivars. Moreover, superior performance of hybrids has been attributed to the nullification of undesirable gene effects, such as low pollen fertility, poor fruit set, or necrosis due to heat stress, because these genes are in the heterozygous state in F1 hybrids (Cheema & Dhaliwal, 2004). In the same way, introgressive hybridization, also known as introgression, has enabled interspecific gene transfer. For example, disease resistance, a desirable trait in tomato cultivation, can

be readily inherited from wild species through introgression into tomato inbred lines with good horticultural traits (Díez & Nuez, 2008; Scott et al., 2013). These hybrids contain chromosome segments from a wild tomato crossed into the background of a modern domesticated tomato, making them useful for quantitative trait locus (QTL) mapping and gene identification studies using molecular markers (Martínez-Romero et al., 2020; OECD, 2017). However, developing some tomato phenotypes, such as insect resistance, is challenging because it requires the introgression of multiple "wild" QTLs, the combination of genes, and the development of numerous molecular markers associated with pest resistance (Kortbeek et al., 2021; Zeist et al., 2018). Nevertheless, efforts are underway to stack several desirable traits in one specific genotype. For example, developing tomato varieties with heat tolerance is a long-term research goal, as higher heat-stress sensitivity can reduce pollen viability. Although breeding for improved tomato flavor by increasing sugar and acid contents in fruits is possible, breeding for relating flavor, aroma, and volatiles to specific genes and molecular markers remains a challenge (Fentik, 2017; Tieman et al., 2017).

Marker-assisted selection has greatly advanced the study of tomato genetics by using molecular markers to select for desirable traits in crops. This technology helps in the identification of QTLs in tomato for improvement purposes, such as hybridity tests prior to hybrid seed sales, yield, fruit quality, and resistance to biotic and abiotic stresses (Azzi et al., 2015; Fentik, 2017; Foolad & Panthee, 2012; Scott et al., 2013). A range of molecular markers have been developed and used in tomato breeding, including restriction fragment length polymorphisms, simple sequence repeats, cleaved amplified polymorphic sequences, amplified fragment length polymorphisms, and single nucleotide polymorphisms. Recently, advanced molecular breeding strategies such as advanced backcross QTL mapping have been developed. Advanced backcross QTL mapping identifies the transference of favorable QTL alleles from wild or non-adapted to cultivated germplasm, and once the QTL alleles have been detected in segregating populations (BC_2 or BC_3), near isogenic lines or introgression lines can be developed and phenotyped to confirm the QTL effect and subsequently be used in the develop of an improved tomato variety (Barone et al., 2008; Grandillo et al., 2013). Whereas it is relatively easy to identify traits controlled by individual genes, identifying QTL regions is more complex and requires the development of sophisticated populations and genetic marker analysis over many generations to validate the QTLs for plant breeding (Ganal, 2013).

QTL analysis is referred to here as associated genetic studies that involve the identification of QTLs using several molecular markers in unrelated individuals with defined phenotypes. According to the marker variation observed, it is inferred which markers show significant associations with a trait of interest. On the other hand, single-nucle-

otide polymorphisms are not inherited as individual segregating units but as a small proportion within a genomic region called a haplotype and can be considered alleles at a given locus. Haplotypes can extend over smaller or larger genomic regions. The extent of the conserved haplotypes, also known as linkage disequilibrium, varies depending on the analyzed population, and its frequency of genetic recombination in the respective region can be estimated. Genome-wide association studies (GWASs) involve the identification of single-nucleotide polymorphisms, haplotype ranges, and linkage disequilibrium in many individuals influencing complex traits, such as a disease factor. Thereby, GWASs can reveal gene function, chromosomal location, and other available information (Ganal, 2013). Furthermore, GWASs can help to estimate the heritability of microbiome traits and predict the response of plant microbiomes to natural and artificial selection with high reliability (Gutierrez & Grillo, 2022).

Mutational approaches have also been developed to investigate the genetic and molecular bases of agronomic traits in tomato, such as TILLING (Targeting Induced Local Lesions In Genomes) (Azzi et al., 2015). Other methods include functional analyses of candidate genes through transgenesis on tomato plants using *Agrobacterium tumefaciens* (Sharma et al., 2009) gene repression through virus-induced gene silencing, and genetic edition via clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 mutagenesis system (Brooks et al., 2014).

To improve the microbiome of plants, it is essential to consider traits that are involved in recruiting and activating beneficial microbial communities (Oyserman et al., 2018, 2021; Pérez-Jaramillo et al., 2016). Quantitative profiling of microbiome-associated plant phenotypes, such as nutrient acquisition and pathogen protection, can be used to determine the association of a particular plant phenotype with a subset of the microbiome and to validate the compatibility between microbial consortia and the host (Oyserman et al., 2018). This approach can be complemented by assisted genotype or microenvironment modification for desired plant phenotypes (Cordovez et al., 2019; Oyserman et al., 2021; Vorholt et al., 2017). Root architecture and exudates are key factors in shaping belowground plant–microbiome interactions. Therefore, future breeding programs should aim to improve the microbiome based on these traits (Gutierrez & Grillo, 2022; Saleem et al., 2018). However, the use of microorganisms from native habitats may be challenging in domesticated crops due to modifications in root architecture, exudation, and plant defense mechanisms (Martínez-Romero et al., 2020). To develop more symbiotically robust cultivars, breeding strategies such as targeted introgression, hybridization, and gene editing could enhance symbiosis function in crops (Porter & Sachs, 2020). For example, studies have demonstrated microbiome selection by genomic regions in tomato. Smith et al. (1999) found significant phenotypic variation for *Pythium torulosum* disease suppression by *B. cereus* UW85

in a tomato RIL population (*S. lycopersicum* [cultivar UC204 C] × *S. cheesmanii* [LA 483]). Three QTLs were identified as contributors to microbial biocontrol by UW85, two from modern varieties and one from the wild parent, suggesting that the disease suppression was supported by biocontrol agent's ability to colonize the host and that the wild parent contributed with an allele at one of the three loci. Similarly, Oyserman et al. (2022) found that the 6.31 Mbp region in the tomato genome, which encodes the iron regulator FIT and the water channel aquaporin SlTIP2.3, was associated with differential recruitment of *Streptomyces* in the rhizosphere. Also, the genetic variation of bacterial genes in *Streptomyces* and *Cellvibrio*, involved in the metabolism of plant polysaccharides, iron, sulfur, trehalose, and vitamins, was associated with specific tomato QTLs.

Concluding remarks

The study of the tomato microbiome represents a vital avenue of research with significant implications for enhancing both agricultural productivity and sustainability. Researchers have dedicated their efforts to unravelling the intricate plant–microbiome relationship. The unique tomato plant attributes make it an excellent model for studying various aspects of microbiome research and developing new strategies for optimizing crop management, promoting plant health, and ensuring a resilient food supply in the face of a changing climate. Microbiome studies have traditionally focused on bacterial communities, but there is growing recognition of the importance of fungal communities (mycobiome) for improving the growth and health of crops, including tomatoes. Furthermore, the interplay between bacterial and fungal communities can have a profound impact on plant phenotypes, making it important for further microbiome research to consider both microbial kingdoms. Methods of studying the effects of domestication on the tomato microbiome include trait selection and innovative crop management practices. By exploring these aspects, we can gain valuable insights into establishing persistent mutualistic interactions between the plant and its associated microbiome. This knowledge will enable the optimization of agricultural practices to maximize tomato yield and quality. Furthermore, by using modern breeding tools such as GWASs, marker-assisted selection, CRISPR, and interspecific crosses, it is possible identify the genetics of wild and modern tomatoes to optimize the microbiome–plant mutualistic interactions into modern agroecosystems.

The novel approach of profiling tomato wild microbiomes in their centers of origin presents an exciting opportunity for revitalizing the fitness of cultivated tomato plants. By harnessing beneficial traits inherent in wild microbiomes and transferring them to modern cultivated varieties, we can develop effective strategies to counteract pests,

diseases, and environmental stresses that currently limit tomato productivity. Furthermore, investigating chemical signals in wild and domesticated tomatoes, such as root exudates and volatiles, offers a means of manipulating and controlling the microbiome composition. This knowledge can be leveraged to effectively engineer plants associated with microbial communities that promote plant growth, suppress pathogens, and enhance stress tolerance.

On the microbial front, the emerging field of microbial genomics provides a deeper understanding of the genetic mechanisms underlying the adaptation of microorganisms to their tomato hosts. By unraveling the genomic landscape of these interactions, targeted interventions and microbiome-based strategies can be developed to enhance tomato productivity and resilience. By addressing these areas of focus, we can advance our understanding of the tomato microbiome and harness its potential to improve plant health, increase productivity, and promote sustainable agricultural practices. This multidisciplinary approach presents a promising future for tomato cultivation and expands the field of microbiome research.

Thesis aim and outline

The overall aim of my PhD thesis was to decipher the impact of tomato domestication on rhizosphere microbiome assembly. More specifically, the objectives were to:

- explore the composition and functional potential of the native rhizosphere microbiome of wild tomato in its center of origin.
- investigate the impact of habitat and tomato genotype domestication on rhizosphere microbiome assembly.
- understand the functional role of the native soil microbiome in tolerance of wild and domesticated tomato to insect herbivory.
- disentangle microbial and plant genetic traits associated with tomato rhizosphere microbiome assembly.

To this end, the taxonomic and functional diversity of the tomato rhizosphere microbiome was investigated both in the tomato's center of origin in the Ecuadorian Andes and in controlled greenhouse assays with agricultural and native soils. Furthermore, the genetic basis for tomato rhizosphere microbiome assembly was investigated by using a recombinant inbred line (RIL) population of a cross between a wild and domesticated tomato species. Overall, the findings obtained in this thesis significantly increased our knowledge about the diversity and functions of the rhizosphere microbiome of wild

crop relatives in their native habitats and pinpointed specific microbiome members that may enhance resilience of the domesticated tomato cultivars to (a)biotic stressors.

Chapter 2 of this thesis explores the taxonomic and functional diversity of the rhizosphere microbiome of wild tomato in its center of origin in Ecuador. Here, the taxonomic composition of the bacterial and fungal rhizosphere microbiome of wild tomatoes grown in the Ecuadorian Andes was characterized by amplicon sequencing and the functional potential of this community was investigated by shotgun metagenome sequencing. We showed that in three different wild tomato populations growing in different soils with different microbiomes in the Ecuadorean Andes, wild tomatoes were able to recruit similar bacterial communities dominated by Enterobacteriaceae. These bacterial taxa showed features related to chemotaxis and motility, as well as phytohormone and antibiotic production, which likely conferred high competitiveness in the rhizosphere of wild tomato. **Chapter 3** describes the differences in the taxonomic composition and functional potential of the bacterial rhizosphere microbiome of different wild and domesticated tomato genotypes grown under controlled conditions in Ecuadorian native and agricultural soils as well as Dutch greenhouse soil. We found microbial composition significantly different along the tomato domestication trajectory, with habitat domestication having a major contribution on microbiome assembly. **Chapter 4** revealed that the soil microbiome plays a critical role in tolerance of wild tomatoes for leaf damage caused by the endemic insect *Prodiplosis longifila.* We found significant associations between specific members and functional traits of the tomato microbiome and tolerance to insect damage. We further showed that depletion of *Actinoplanes* from the rhizosphere microbiome by soil sterilization correlated with increased leaf damage in wild tomato by *P. longifila.* Functions of *Actinoplanes* associated with this phenotype encompassed motility, chorismate and secondary metabolite production. **Chapter 5** addresses the genetic basis in rhizosphere microbiome assembly of tomato. To disentangle the microbial and plant genetic traits associated with microbiome assembly, we studied the taxonomic and metagenomic diversity of the rhizosphere microbiome of a tomato RIL population (N = 100) from a cross between domesticated tomato *Solanum lycopersicum* and wild tomato *Solanum pimpinellifolium*. These analyses revealed reciprocal features associated between specific QTLs of wild and domesticated tomato with key taxa *Cellvibrio* and *Streptomyces* functionality, such as genes involved in metabolism of plant polysaccharides, iron, sulfur, trehalose, and vitamins. **Chapter 6** summarizes the most important findings of this thesis, connecting the results of the different experimental chapters on the impact of tomato domestication on rhizosphere microbiome assembly. I also provide an outlook on potential future research directions, highlighting how incorporating microbial features into management practices could enhance sustainability in modern agriculture.