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REVIEW ARTICLE

Culturomics of the plant microbiota: the emerging in situ *similis* cultivation strategies to meet the complexity of nutritional requirements of microbiota associated with plants of multiple species, growth stages and compartments

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Abstract
Background The holobiont" refers to the plant and its associated microbiota that are pivotal to the plant's health, fitness, and survival. By in vitro culturing and functionally characterizing members of the plant microbiota, their specific roles in influencing plant responses to environmental changes can be determined and manipulated to foster sustainable agriculture and ecosystem management.
Aims The review presents a comprehensive survey and current updates on culturomics of plant microbiota within the overall context of: a) the importance of understanding the plant holobiont composition and functioning; b) the necessity to in vitro track down and explore environmental microbiomes, entailing the plant microbiome with its myriad composition and spatio-temporal dynamics and mobility in various plant species, compartments and growth stages and c) the recent developments of the emerging *in-situ similis* cultivation strategies grounded on plant-based culture media.
Conclusions The review highlights the urgent need to explore in vitro cultivation strategies built on compatible plant-based culture media, and the transformative role of omics technologies in refining these strategies. By bridging fundamental research and cultivation-based applications, such tools offer a gateway towards more sustainable and efficient in vitro cultivation systems, leading to a deeper understanding and potential manipulation of the plant holobiont.

Keywords Plant holobiont · Compartment-affiliated microbiota · Culturomics strategies · Plant-based culture media · In situ *similis* cultivation · Omics technologies and AI developments

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The plant holobiont

The plant holobiont: the concept

The concept of the plant holobiont has gained great prominence in recent years as our understanding of plant–microbe interactions deepens. The term "holobiont" refers to the assemblage of the plant and its

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associated microbiota, including bacteria, fungi, archaea, and viruses, that collectively form a complex and dynamic system (Berg et al. 2024). This integrated entity is pivotal to the plant's health, fitness, and survival, especially in the face of environmental challenges such as climate change, nutrient limitation, drought, salinity and pathogen pressure. Traditionally, plant microbiome studies have focused on individual organisms such as beneficial bacterial or fungal strains which were selected and applied; and the plant nutritional and health responses as well as causal mechanisms of interactions were studied. However, the holobiont approach emphasizes the interdependence of plants and their complex microbiomes as a unified entity. The microbiome's specific role in influencing how plants respond to these environmental changes has been an emerging area of research, offering insights into potential applications in sustainable agriculture and ecosystem management (Arnold et al. 2025; Lyu et al. 2021; Shah et al. 2021). Particularly, roots are a hotspot of microbial activity, where microbes occur in several micro-niches, such as the rhizosphere soil surrounding the roots, the root surface (rhizoplane), and even the endo-rhizosphere (Reinhold-Hurek et al. 2015). Some of these endophytes can spread systemically into shoots or are specific to endosphere colonization in aerial parts; however, the colonization density is lower than in roots.

The plant microbiome significantly influences plant health by enhancing nutrient availability, disease resistance, and tolerance to environmental stresses. Rhizospheric and endophytic microbial communities help the plant by providing essential services like biological nitrogen fixation, phosphate solubilization, and production of growth-promoting compounds. Atmospheric nitrogen-fixing bacteria, such as root nodule symbionts like *Rhizobium*, or associative N_2-fixing bacteria in close association with "friendly" fungi, provide plants with essential nutrients (Pang et al. 2023; Hurek et al. 2002). Similarly, endophytic fungi as well as mycorrhiza fungi extend the root network improving water and nutrient absorption and affecting the plant holobiont functioning (Wahab et al. 2023; Sharma et al. 2021). This mutually beneficial relationship strengthens the plant's ability to thrive in diverse environmental conditions.

The plant microbiome: spatio-temporal dynamism

One of the hallmarks of plant holobionts is the ability of their microbial communities to dynamically shift in response to spatial and temporal changes. Such spatio-temporal impacts modulate the plant microbiota and provide deep insight into the dynamism of plant –microbe interactions. Further, they are expected to guide efforts for identifying diversity, abundance and functions of multiple taxa. Concomitantly, providing recommendation towards cultivability in respect of sampling time, tested plant compartments and/or substrate composition that simulate an in situ milieu for ad hoc in vitro cultivation.

Environmentaly, the ongoing changes do impact shifts in microbial composition enabling plants to adapt rapidly to stress. Research indicates that environmental stresses, such as changes in temperature, pH, or nutrient availability, can cause a significant reconfiguration of the microbial community, selecting for taxa that can better support plant stress responses (Jansson et al. 2023). Under high salinity, halotolerant microbes, *e.g.* certain species of *Halomonas* and/or *Pseudomonas* became dominant in the rhizosphere, providing the plant with enhanced salt tolerance (Moner et al. 2025). Drought conditions are often associated with the recruitment of microbial taxa that produce drought-mitigating substances like trehalose, a compound that helps plants retain water (Poudel et al. 2021). Such microbial plasticity is essential for plant survival, and allowing the holobiont to respond to rapid and unpredictable environmental changes. In addition, human activities, such as intensive agriculture, deforestation, and the widespread use of chemical fertilizers and pesticides, are disrupting natural microbial communities (Dixit et al. 2024).

The plant microbiota: mobility and repositioning from below- to aboveground organs/compartments of plants

The microbiota in its latent or active forms occupies plant organs, from the roots to the aerial parts, ectophytes and endophytes. And, they are in direct or indirect contact with the surrounding environment, mainly the soil and atmosphere which are the main repositories of surrounding microorganisms, besides the host plant itself via the seeds. These microorganisms, bacteria, archaea and fungi, thrive in multiple plant compartments, *i.e.*, rhizosphere, rhizoplane, endosphere, phyllosphere, caulosphere, carposphere, anthrosphere and spermosphere, which are interconnected all over the plant, cultivated or wild, terrestrial or aquatic (Lombardo et al. 2024; Krstić Tomić et al. 2023; Wan et al. 2023; Sangiorgio et al. 2022; Swift et al. 2021; Lee et al. 2019; Wallace et al. 2018; Lemanceau et al. 2017; Coleman-Derr et al. 2016; Müller et al. 2016). To the extent that the plant host selection of its root and organ microbiota prevails after transfer to non-native soil (Singer et al. 2019). In return, plants do modulate their composition and function through intimate trophic interactions and molecular signaling (Lemanceau et al. 2017).

The soil itself harbors an extraordinarily rich diversity of bacteria and acts as a primary repository for the onset of effective invasion via a number of gateways to the roots and ultimately colonizes the root cortex to the vascular system (Fig. 1). Following the colonization of rhizosphere and rhizoplane, microorganisms migrate to colonize the root endosphere to continue spreading within the aerial parts (Bettenfeld et al. 2022; Wei et al.2021; Deyett and Rolshausen 2020; Compant et al. 2011). Within the host plant, such plant compartments furnish a network of ecological niches for the microorganisms inhabiting them, where detected habitat-specific gene enrichment indicates ongoing functional traits relevant for effective host colonization (Müller et al. 2016). Following such horizontal/vertical transmission of microbiota (Wei and Ashman 2018), it is reported that substantial percentages of the microbial communities, originating from soil/rhizosphere, are shared among the bulk soil, rhizosphere, and aerial compartments of the plant (Arnold et al. 2025; Lombardo et al. 2024; Krstić Tomić et al. 2023; Wan et al. 2023; Bettenfeld et al. 2022; Deyett and Rolshausen 2020; Singer et al. 2019; Zarraonaindia et al. 2015; Bai et al. 2015). However, the host plant exercises a selective pressure/filtration power, which together with the fierce inter-microbe competition, results in a limited number of endophytes, obligate, facultative and/or passive, thriving in specific compartments and driving a symbiotic lifestyle with the host plant (Wan et al. 2023; Singer et al. 2019; Reinhold-Hurek et al. 2015; Bulgarelli et al. 2012; Hardoim et al. 2008). The active selection power is exercised on the interacting communities of microorganisms through multiple factors, *e.g.* root exudates, rhizodeposition and rhizosheath (Zhang et al. 2022; Li et al. 2021; Othman et al. 2004; Pillay and Nowak 1997). For the aerial parts, microorganisms make their way through multiple entries, *e.g.* leaf stomata, surface wounds and/or via sucking insects (Bettenfeld et al. 2022; Mercado-Blanco 2015; Bodenhausen et al. 2013). It appeared that the host plant, whether annual or perennial, and its nutrients imprint and impact its microbiome recruitment (Arnold et al. 2025; Sangiorgio et al. 2022; Cai et al. 2017). An effect that extends to the differential nutritional makeups of the plant organs. This particular effect of compartment niches was reported to be coupled with host ecotypes (Wei et al. 2021; Coleman-Derr et al. 2016) and with additional neutral processes, *e.g.* dispersal limitations, ecological drift and speciation events (Moroenyane et al. 2021).

It is reported that differences in microbiota composition and function are pronounced among microbiota of belowground and aboveground compartments, with a decreasing and selective gradient in bacterial richness/diversity from the soil to the aerial parts (Lombardo et al. 2024; Krstić Tomić et al. 2023; Sangiorgio et al. 2022; Wei et al. 2021; Dong et al. 2019; Lee et al. 2019; Müller et al. 2016; Bai et al. 2015; Zarraonaindia et al. 2015; Martins et al. 2013). Several reports with various plants distinguished unique bacterial phylotypes associated specifically with relevant organs of field, vegetable and fruit crops which differ from those of the underground compartments (Zarraonaindia et al. 2015; Ottesen et al. 2013). A phenomenon that is even reported as well for

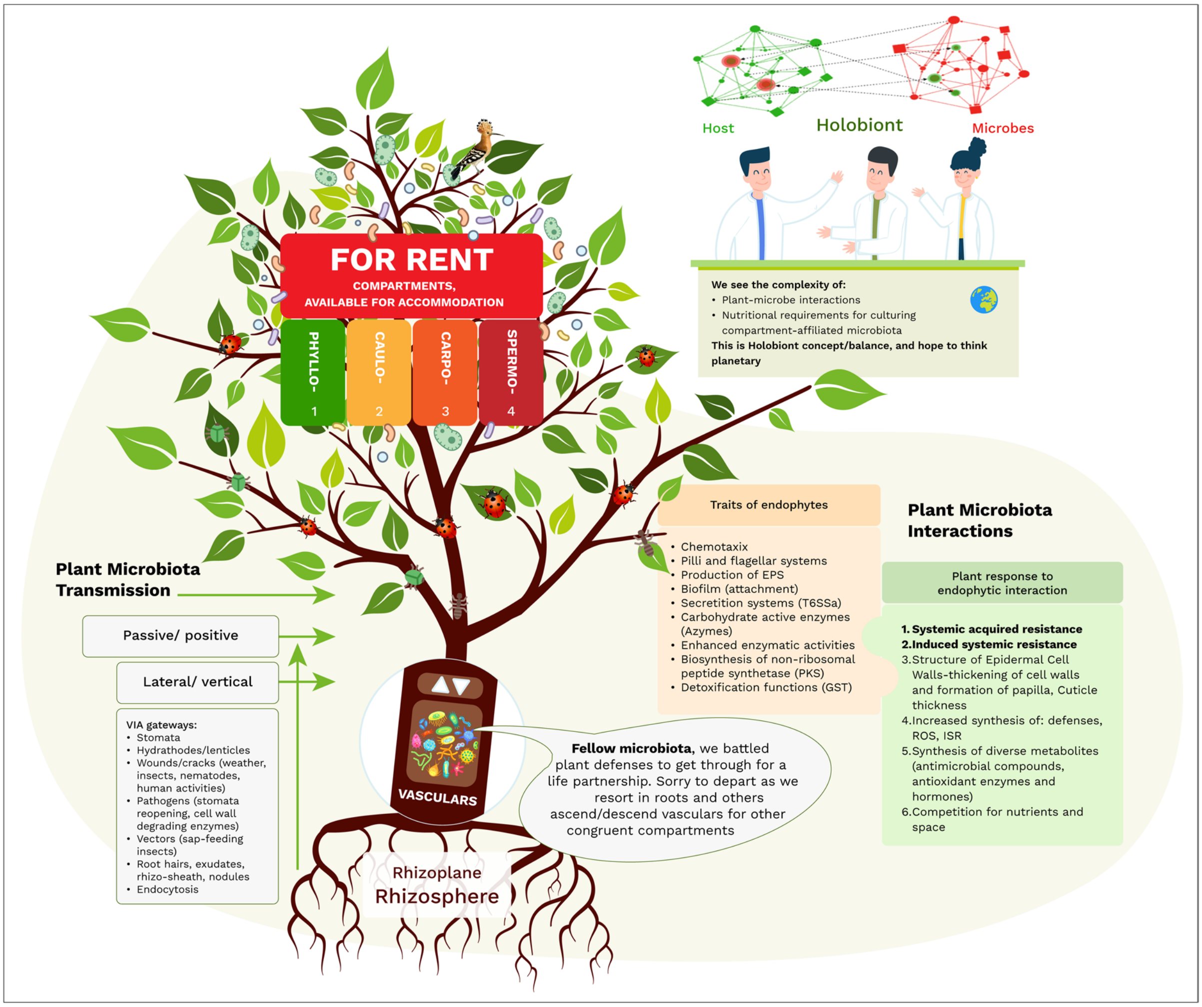


Fig. 1 The complexity of the plant holobiont: Transmission, interaction and mobility of microbiota within plants to occupy various organs/compartments and to act as a constitutive part of the plant holobiont system

life wood trees where the partitioning of prokaryotes, archaea and fungi is demonstrated by distinctive taxonomic signatures between heartwood and sapwood (Arnold et al. 2025). Such microbial communities residing in the plant endospheres are reported to possess more genes involved in membrane transport compared to bulk soil which indicates active exchange of diverse compounds among microorganisms and plant organs (Lee et al. 2019).

In response to the surrounding environmental conditions, microbial communities are believed to be compartment-dependent; however, with a relatively lesser magnitude on the aboveground compared to the underground compartments. Such compartmental effects extend to all aspects of the bacterial community, including taxa (OTUs) distribution, community composition, diversity, co-occurrence networks, and mutualism between potential functions of microbiomes and different plant compartments (Arnold et al. 2025; Krstić Tomić et al. 2023; Wan et al. 2023; Zhang et al. 2022; Moroenyane et al. 2021; Hamonts et al. 2018; Coleman-Derr et al. 2016; Robinson et al. 2016; Zarraonaindia et al. 2015). This does not contradict the reported substantial overlap between the leaf and root microbiota phylogenetically and functionally, with individual functional differences that may reflect specialization at the respective niche level (Bai et al. 2015). Here, the metabolites and/or signals differentially emitted by plant organs may explain organ preferential colonization by various groups of microbiota with multiple functions (Arnold et al. 2025; Sangiorgio et al. 2022), taking into consideration the intervention of the physico-chemical properties of the soil and geographical location on microbial communities not only in the root zone but also in the aboveground compartments.

The existence of a continuum of species from the underground parts to the aerial parts lead to the origin of the concept of core microbiota, bacteria and fungi (Arnold et al. 2025; Lombardo et al. 2024; Bettenfeld et al. 2022; Zhang et al. 2022; Neu et al.2021; Stopnisek and Shade 2021; Hamonts et al. 2018; Frank et al. 2017; Bai et al. 2015; Lundberg et al. 2012). The core microbiota of bacteria/fungi is a set of species found in all of the studied compartments of a given individual host or found in a specific compartment on different individuals, independent of their terrestrial/aquatic life, annual/perennial nature, wild/cultivated, genotype, age, agronomic practices, regions, seasons, climate and/or edaphic properties (Wan et al. 2023; Bettenfeld et al. 2022; Swift et al. 2021; Cregger et al. 2018; Frank et al. 2017; Coleman-Derr et al. 2016; Zarraonaindia et al. 2015; Zaura et al. 2009). Such core microbiota is especially efficient in plant colonization, functional performance, nutrient acquisition and stress tolerance thereby supporting crop health and nutrition (Arnold et al. 2025; Mapelli et al. 2023; Hamonts et al. 2018; Tian et al. 2017; Müller et al. 2016).

However, the assembly of the core microbiomes and their collective functions overlooked other important plant compartments, such as vascular tissues that is considered as a long-distance transport system connecting the plant root and shoot (Fricke 2017). They guarantee smooth transport of solutes and signals among plant organs (Lucas et al. 2013), in addition to the holes in the perforated plates between xylem elements that are large enough to allow passage of microbes (Compant et al. 2005; Bove and Garnier 2002). Reports are accumulating in regards to the spread and migration of microbiota to the above ground plant compartments via transpiration–driven xylem flow, that may additionally require the secretion of cell-wall-degrading enzymes such as cellulases and pectinases (Cregger et al. 2018; Wei and Ashman 2018; Compant et al. 2005). This particular nature of xylem vessels creates a highly dynamic endosphere which was demonstrated by culture-dependent/- independent analyses, and confirmed the presence of most representative bacterial genera in field, vegetable and fruit crops as well as wood trees (Table 1; Table S1). With maize (Zhang et al. 2022), it is reported that: a) the root (endosphere) and leaf-associated (endophyllosphere) bacterial communities varied significantly with environmental factors, b) the stem xylem recruited highly conserved taxa independent of geographic and climatic distances and c) the majority of bacteria in the root endosphere derived from the rhizosphere soil and a significant percentage of endophytes in the phyllosphere derived from xylem-inhabitant endophytes. A finding that was reported for other plants, not only herbaceous species (*e.g.* barley, maize and rice; Sangiorgio et al. 2022; Bulgarelli et al. 2015; Edwards et al. 2015; Peiffer et al. 2013) but also perennial grapevine (Deyett and Rolshausen 2019) and olive trees (Anguita-Maeso et al. 2020). Quite recently, Arnold et al. (2025) reported that the phenomenon extends to wood trees, as a huge microbial load of prokaryotic cells and fungi, $> 10^{9} - 10^{12}$ cells tree $^{-1}$, do occupy the woody tissues of living trees, with distinguished partitioning between heartwood and softwood tissues of more than 150 tested living trees of 15 species. Although living wood communities may overlap with those of better studied model plants, they also include microbial groups uncommon in other plant microbiomes that are subjected to dynamic composition/structure fluctuations in response to seasonal effects.

Currently, it is acknowledged that the potential and functional roles of plant microbiota are not restricted only to the rhizosphere but extend as well to all of the plant organs/compartments, and that the joint use of both culture – dependent and—independent approaches do consistently provide deeper understanding of compartment-affiliated plant microbiota (Table 1 and Table S1).

Ultimately, research on and engineering of the microbiota of the plant holobiont offers future opportunities and advancements for enhancing plant resilience to environmental stress. One potential application lies in microbiome engineering-tailoring, reintroducing microbial communities to improve plant productivity and stress tolerance

Table 1 Culture-dependent analysis of plant microbiota in various plant compartments, and culture media commonly employed

Culture- dependent methods				
Types of crops	Host plants	Compartments	Culture media	References
Fruit/vegetable crops	Olive	Xylem sap	General standard chemically-synthetic culture media: BCYE, PD2, R2A and NA	Anguita-Maeso et al. (2020)
	Strawberry	-Below ground: bulk soil, rhizosphere soil, roots -Above-ground tissues: leaves, stems, crown	General standard chemically-synthetic culture medium: LB agar	Sangiorgio et al. (2022)
	Grapevine	Endophytes of roots, flowers, berries without seeds, seeds and rhizosphere	General standard chemically-synthetic culture medium: R2A	Compant et al. (2011); Bettenfeld et al. (2022)
	Tomatoe	Bulk soil; rhizosphere; endorhizosphere; phyllosphere; endocaulosphere	General standard chemically-synthetic culture media: NA, CCM and NBRIP	Helal et al. (2022)
Root/tuber crops	Sugar beet	Rhizosphere; phyllosphere (endophytic and epiphytic)	-Plant-based culture media: leaf extract agar, leaf extract phytagel, root extract agar, root extract phytagel -Soil extract culture medium -General standard chemically synthetic culture media: TSA and NA	Krstić Tomić et al. (2023)
	Potato	Endo-rhizosphere, endo-caulosphere, carposphere, ecto/endo spermosphere	General standard chemically-synthetic culture medium: R2A	Elsayed et al. (2021)
Cereal crops	Maize	Xylem sap	General standard chemically-synthetic culture media: TSB, R2A, and Ashby's nitrogen-free	Zhang et al. (2022)
Herbaceous plants (model plants)	Arabidopsis	Roots and leaves	General standard culture media: TYG, YEM, M408, M715, Flour, TWYE, MYX, MM + MeOH, and R2A	Bai et al. (2015)

(Berruto and Demirer 2024), reduce reliance on chemical inputs, promote sustainable crop production and to increase food security. However, a prerequisite to handle such microbial reintroduction is efficient in vitro culturing and gain-assessing of plant promoting microorganisms. Still we are only able to cultivate less than 1% of the already detected bacteria using artificial chemically-synthetic culture media. Bringing these not yet-cultured organisms into culture is essential for advancing our understanding of the plant holobiont and its complex interactions with environmental factors. By focusing on the prerequisites of microbial ecophysiology, environmental mimicry, co-cultivation, and tailored growth conditions, researchers are unlocking new microbial diversity that can provide new insights into plant–microbe interactions. The successful isolation of these elusive microorganisms will have broad applications in agriculture, ecology and biotechnology; that potentially transform our ability to manipulate and enhance plant holobiont function for sustainable solutions. According to Armetta et al. (2025), combining multi-omics approaches with new culturomics techniques can accelerate the success in bringing the most important taxa of the plant microbiome into culture.

Microbiome dynamics and rearrangement along with plant development stages

As the large and diverse microbiota live intimately with their host plants and have simultaneously coevolved, they constitutively impact a range of aspects of plant performance and development (Martin et al. 2017). It is strongly postulated that the host plant by itself has strong selection effects on its microbiome via intrinsic host characteristics, e.g. genetic networks, immune system and nutritional profiles (Shakir et al. 2021; Walters et al. 2018). Further, recent studies have highlighted the significant contribution of plant developmental stages on plant microbiome assembly, coping with plant physiological requirements and composition, nutritional sinks and exudates that vary with the plant's growth phases (Zhao et al. 2021; Chen et al. 2019; Viviane et al. 2019; Zhang et al. 2018). It has been suggested that the host plant may actively and selectively modulate microbial interactions to meet its developing requirements throughout phases of plant growth, as microbial network hubs are supposed to play crucial roles in maintaining plant health and nutrition (Coyte et al. 2015). In fact, the effects of plant developmental stage reflect the dynamic changes in plant metabolism, exudation and immune-associated traits during plant growth (Viviane et al.2019; Sasse et al.2018). In this respect, it is reported that associated microbes develop powerful chemotaxis towards plant signal molecules such as organic acids and sugars (Hu et al. 2020; Chen et al. 2019; Kudjordjie et al. 2019; Chaparro et al. 2013).

Unfortunately, limited information is available regarding the plant microbiome assembly and function throughout plant developmental stages, especially in the field and particularly across the soil–plant continuum subjected to multiple environmental factors, *e.g.* climate, edaphic properties and human perturbations (Xiong et al. 2021). However, there are examples in literature that demonstrate the remarkable effects of plant developmental stages on the structure of associated microbiota (Table S2). This included several examples of a variety of host plants grown in a wide spectra of plant-soil environments. All such reports detected significant changes and variations of microbiota structure across multiple levels of phylogenies, including phyla, classes, orders, families and genera.

As a good example of non-legumes, Xiong et al. (2021) demonstrated that plant developmental stages had a much stronger influence on the maize microbiome in respect of diversity, composition and interkingdom networks in plant compartments than in soils, with the strongest effect in the phyllosphere. The detected plant microbiome assembly was: a) mainly influenced by compartment niche and developmental stage regardless of farming regions and fertilization regimes and b) more sensitive to plant developmental stage than soil microbiomes. Also, they reported that maize phylloplane microbiome was found to possess a higher functional diversity at the seedling stage, with more abundant genes associated with nutrient provision and glycosyltransferases at the seedling stage while N assimilation-and C-degradation-related genes were enriched at later stages. More abundant, bacteria may take an important ecological role in the plant microbiome and host performance at the early stage, while fungi do so at the late stage. Actinobacteria, known for their antagonistic/antibiotic effects, were more numerous at the seedling stage than at two later stages in plant compartments providing necessary protection against plant pathogens (Lee et al. 2021; Alvarez-Perez et al. 2017).

With legumes, microbiota composition of the soybean rhizosphere changed during growth with alterations in the relative contributions of various detected phyla (Xu et al. 2009). Among the bacterial communities of the soybean rhizosphere during growth in the field, Proteobacteria increased, while Actinobacteria and Firmicutes decreased in rhizosphere soil during growth (Sugiyama et al. 2014). This was accompanied by a higher abundance of OTUs of potential plant growth promoting rhizobacteria, including *Bacillus*, *Bradyrhizobium,* and *Rhizobium.*

A better understanding of the mechanisms and temporal dynamics of plant microbiome assembly, functions and co-occurrence networks provide: a) appreciation of the prevalence of their *insitu* communities (Xiong et al. 2021) and b) an improved ability to define and tailor the growth milieu and conditions required for in vitro culturability of associated microbiota in their real time and at a given plant development stage (Sarhan et al. 2019).

The development of culturomics: strategies applied to diverse environmental microbiomes

Environmental microbiomes are much more diverse than expected, and it is reported that the majority of the existing microbiota are difficult to culture. Here, the conventional/historical approach of culture-based methods has an exceedingly restricted view for studying complexes of microbial niches present. So far, the fundamental basis of conventional techniques of in vitro cultivation relies on the ability of microorganisms to grow under specific and artificial conditions, *i.e.* mainly using selective differential culture media. However, certain microorganisms do not thrive or grow on chemically-synthetic culture media that limits the outcome of culture-based approaches (Hegazi et al. 2017). In fact, the less than 1% of bacteria that can grow in today's culture media limits our ability to cultivate and study microorganisms *en masse*, despite their microscopic nature (Amann et al. 1995). The uncultured genera and phyla could comprise 81% and 25% of microbial cells, respectively, and that where phyla were over-represented in metatranscriptomes relative to metagenomes suggesting that they are viable (Lloyd et al. 2018).

During the last decade, innovative culturomics approaches were advanced that can be divided into culturomics-based traditional methods and culturomics -next generation culture-based methods (Lagier et al. 2012, 2015, 2016). Those authors experimented > 200 different culturing conditions and successfully recovered > 30,000 bacterial colonies from human feces that belonged to hundreds of varied bacterial species. In another study, more than 70 culture conditions were approved to expand the human gut repertoire leading to the identification of additional new bacterial species from human stool samples (Pfleiderer et al. 2013; Seng et al. 2010). Currently, culturomics as the newest member of OMICS family represents a high-throughput cultivation approach based on the extensive use of multiple combinations of culture media and growth conditions that has significantly expanded our knowledge regarding in vitro cultivation of hundreds of new taxa. The technology acts as a breakthrough in throughput characterization/identification of microbiota and their association with the health and sustainability of their hosts in various environments (Kambouris et al. 2018). However so far, such culturomics approaches applied in plant microbiome studies have been rudimentary (Sarhan et al. 2019).

Several reports advance a variety of culturomics approaches that have been developed to cultivate and isolate microbiota of a number of terrestrial and aquatic environments (Table S3). Additional information on the culturomics of microbiota of other environments is available as well (Table S4).Various combinations of enriched culture methods have successfully isolated previously unculturable anaerobic thermophiles from compost and autotrophic ammonia-oxidizing archaea from marine as well as from multiple other environments (Klein et al. 2022; Bae et al. 2005; Könneke et al. 2005). Modulating the composition of culture media and incubation conditions has allowed for the immobilization and cultivation of thermophilic and hyperthermophilic anaerobic microorganisms from marine ecosystems, rare soil bacteria, extremophilic yeast from the stratosphere, and various haloalkaliphilic and halotolerant bacteria from unique environments (Gómez-Acata et al. 2021; Molina-Menor et al. 2021; Bender et al. 2020; Kurm et al. 2019; Pulschen et al. 2018; Landreau et al. 2016). Co-culture techniques have successfully isolated thermophilic-syntrophic-anaerobic consortia, unculturable variants of marine *Prochlorococcus* and novel species like *Lucifera butyrica* from sediments; and heme-dependent bacteria from marine sediments using innovative methods like the sandwich agar plates (Zhang et al. 2024; Sánchez-Andrea et al. 2018; Morris et al. 2008; Plugge and Stams 2002). The soil substrate membrane system (SSMS) has been instrumental in recovering unculturable soil microbiota strains related to various genera (Ferrari et al. 2005, 2008). The combination of flow cytometry and gel microdroplets (GMD) has facilitated the cultivation of micro-colonies of previously uncultivated genera (Dichosa et al. 2014; Zengler et al. 2002; Álvarez-Barrientos et al. 2000). The combination of live-FISH and FACS has been effective in isolating bacterial species from marine microbiota (Batani et al. 2019). Automated techniques such as micromanipulators and laser manipulation systems have isolated hyperthermophilic archaea and new archaea species from deep-sea environments (Antunes et al. 2008; Huber et al. 2000). The 'isolation chip' or 'ichip' platform was introduced to: a) increase microbial recovery from 5 to 300-fold; b) provide access to inaccessible sets of microbes, and c) uncover species of significant phylogenetic novelty. The method is well suited for both fundamental and applied research, and simple protocols were designed for the cultivation of bacteria in soil as well as a variety of other environments (Berdy et al. 2017; Nichols et al. 2010). Reverse genomics has led to the isolation of human oral Saccharibacteria species using antibodies against specific cell surface proteins (Ibrahim et al. 2022; Cross et al. 2019). Lately, automation and machine learning approaches have facilitated the cultivation of diverse bacterial genera from human fecal samples (Huang et al. 2023).

Complexity of in vitro cultivation of compartment-affiliated microbiota in view of their dynamics, unique positioning and multiple nutritional identities

During the last decade, increased information has indicated that host-associated microbial communities do not form randomly, but follow defined phylogenetic paths within their host plants (Müller et al. 2016) and along their development stages (Xiong et al. 2021). Furthermore, there is increasing awareness to include all plant compartments in microbiome studies.

In spite that plant microbiota are reported to be cultivable on a myriad of standard laboratory culture media (Thompson et al. 1993; Table 1), the new developments regarding organ/compartment-dependent populations require more attention to meet their specific nutritional requirements for substantial improvement of in vitro cultivation. Therefore, improved methods and conditions of cultivation are advantageous to continue the isolation of strains from various plants of different growth stages and multiple specific organs. This will support the expanding of culture collections as well as improving the analogy of SynComs, synthetic communities, to natural plant microbiota.

In view of the complexity of microbiota colonizing the multiple plant compartments, it is imperative to develop innovative culturomic strategies to minimize the gap between gene-detected (culture-independent) and cultivable (culture-dependent) bacterial diversity. Such targeted isolation strategies are the main approach to address/satisfy the varying nutritional requirements of organ-specific microbiota and to retrieve their core taxa in pure cultures. Here, the need arises to tailor combinations of culture media to simulate nutritional profiles of various plant compartments. In this context, devising plant culture media based on various host plant materials/organs is a practical approach to satisfy the nutritional requirements of cultivated microbiota of different plant organs (Elsawey et al. 2023; Krstić Tomić et al. 2023; Sarhan et al. 2019). Such culturing strategies create "*in-situ-similis*" vegan nutritional matrices that favor in vitro cultivability of plant microbiota very much similar to conditions *in planta* (Nemr et al. 2020, 2021; Elsawey et al. 2020; Sarhan et al. 2020). This is in an effort to realize conformity of in vitro cultivation with in vivo conditions to culture the hidden members of plant microbiota.

In situ *similis* culturomics strategies based on plant-based culture media to emulate the complex nutritional demands of compartment-affiliated microbiota

In contrast to human gut microbiota, it was rationalized that plant microbiota are more appropriately cultured on vegetal- not bovine-based culture media (Elsawey et al. 2020; Sarhan et al. 2019; Hegazi et al. 2017). As plants live together and in coevolution with their hosted microbial communities, natural plant-based culture media do provide multiple nutrients compatible to the micro-residents of various plant compartments. They are prepared from plant materials in various forms, *e.g.* plant juices, slurries, broths and dehydrated powders, to reproduce the natural and particular nutritional composition and concentrations normally found in the natural plant's environment. The message was delivered in a number of publications reviewed by Sarhan et al. (2019) that was the dawn of the era of plant-based culture media for the cultivation of plant microbiota. Very clearly and convincingly, such plant materials without any additives are compatible vegan substrates, which very competitively and efficiently support in vitro cultivability. They greatly expanded the diversity of culturable plant microbiota and significantly enriched representatives of previously unculturable and fastidious microbiota (*e.g.* members of Acidobacteria, Chloroflexi, Cyanobacteria, Elusimicrobia, Gemmatimonadetes, Planctomycetes, and Tenericutes which includes "Candidatus Phytoplasma" as well as those of candidate divisions/phyla, *e.g.* BRC1, Omnitrophica (OP3), Atribacteria (OP9), Dependentiae (TM6), Latescibacteria (WS3) (Mourad et al. 2018; Sarhan et al. 2018, 2016; Hegazi et al. 2017; Youssef et al. 2016; Nour et al. 2012). The plant-based culture media were further adjusted for a number of additional achievements, *e.g.* formulations based on plant pellets were introduced to support the large-scale cultivation of value-added biomass of rhizobacteria that is having strong impacts on future agro-technologies (Daanaa et al. 2020).

The plant-based culturing strategies are well established by now and advanced enough to provide a breadth of natural plant nutrients. This represents the in situ-*similis* approach that was recently introduced in a number of strategies to simulate the plant milieu, in terms of composition and concentrations of nutrients, and to cope with the compatible nutritional requirements of plant compartment- affiliated microbiota (Abdel-Fattah et al. 2025; Moner et al. 2025; Elsawey et al. 2020, 2023; Nemr et al. 2020, 2021; Sarhan et al. 2020). They are briefly outlined in Table (2) and illustrated in Fig. (2). The following is a brief synopsis on the major strategies reported in literature and the important outcomes obtained. They mainly depend upon the use of: a) liquid inocula prepared from the tested host plant organs as a dual source of both nutrients and contained microbiota, b) the intact plant organs, *e.g.* leaves, roots and fruits, as a nutritional pad and/or support for growth of associated microbiota, c) an adjusted plant-broth-based seawater culture medium for the specific isolation/domestication of halophyte microbiota, and d) culture media that are based on plant broths of homologous, not heterologous, tested host plants for better nutritional compatibility.

A. The inoculum-dependent cultivation strategy (IDC)

The inoculum-dependent cultivation strategy (IDC) depends primarily on the direct inoculation of the prepared dilutions of plant organs onto nutrient-deficient water agar plates, allowing the growth of the existing bacterial endophytes on the expense of nutrients contained within the administered inoculum (Sarhan et al. 2020; Fig. 2IA). The extensively diluted nutrients in such culture conditions together with longer incubation allow the recovery of fastidious bacteria in the form of micro-colonies (µCFUs). A phenomenon that was brought into focus while asserting that multi-omics information awaits integration into the development of novel cultivation approaches for increasing the culturability of environmental microbiomes (Gutleben et al. 2018; Lagier et al. 2018; Overmann et al. 2017; Zengler et al. 2002). Providing real time and compatible nutrients of the tested homologous plant organ, the strategy brought into cultivation the endophytic Actinobacteria, including the genera of *Curtobacterium* spp., *Plantibacter* spp., *Agreia* spp., *Herbiconiux* spp., *Rhodococcus* spp., and *Nocardioides* spp.; in addition to novel species belonging to *Agreia* spp. and *Herbiconiux* spp. (Fig. 2IB). The method is a simple and promising culturomic tool to: a) unearth the hidden and novel members of the plant microbiota, and b) undertake screening programs that require handling of numerous samples for securing large numbers of isolates from diverse plants/organs as well as other environmental microbiomes (Sarhan et al. 2020).

B. The use of intact plant organs, e.g. leaves, roots and fruits, as nutritional pad and/or support for growth of associated microbiota

In situ similis culturing strategy based on plant leaf blades as innate nutritional pads

The strategy is based on the direct application of plant inoculant and/or indirectly on membrane filters, onto the host plant leaf surfaces to create an in situ *similis* environment, that allows the direct exchange of multiple plant nutrients in their natural/concentrations and gradients (Nemr et al. 2020; Fig. 2IIA). The strategy supported good recovery and increased the overall diversity of bacteria associated with the endo-phyllosphere compared to the endo-rhizosphere of sunflower plants (Fig. 2IIB). The 16S rRNA gene analysis of hundreds of representative isolates showed the predominance of 13 genera of > 30 potential species, belonging to Firmicutes, Proteobacteria, and Actinobacteria. Preferentially, the leaf cultivation strategy enriched genera of Alphaproteobacteria and Gammaproteobacteria that were only reported for sunflower by culture-independent analysis, namely *Rhizobium* sp., *Aureimonas* sp., *Sphingomonas* sp., *Paracoccus* sp., *Stenotrophomonas* sp., *Pantoea* sp., *Kosakonia* sp., and *Erwinia* sp. (*e.g.*, Oberholster et al. 2018; Alsanius et al. 2017). Further, a number

Table 2 Basic information on various approaches to in situ *similis* cultivation of plant microbiota available in literature, that are grounded on the sole use of plant-based culture media and their major outcomes

In situ *similis* strategies (Related references)	The concept	Tested host plants, and compartments	Methods of cultivation, culture media and microbiota analysis	Major outcomes and advantages
An inoculum-dependent culturing strategy (IDC) for the cultivation of environmental microbiomes and the isolation of novel endophytic Actinobacteria (Sarhan et al. 2020)	Direct inoculation of plant serial dilutions onto plain water agar plates, allowing bacterial growth onto natural nutrients contained in the administered inoculum	• Maize plants (*Zea mays* L.) • Rhizosphere and phyllosphere	• Surface-inoculated agar plates • Standard R2A, plant broth • 16S rRNA gene sequencing of pure isolates	• Recovered several isolates of endophytic bacteria, in particular Actinobacteria, *e.g. Curtobacterium* spp., *Plantibacter* spp., A*greia* spp., *Herbiconiux* spp., *Rhodococcus* spp., and *Nocardioides* spp. • Two isolates likely novel species belonging to *Agreia* spp. and *Herbiconiux* spp.
Plant broth- (not bovine-) based culture media provide the most compatible vegan nutrition for in vitro culturing and in situ probing of plant microbiota (Elsawey et al.2020)	Culture media based on the broth of cooked aqueous mixtures of host plants	• Berseem clover (*Trifolium alexandrinum* L.) and wheat (*Triticum aestivum* L.) • Endo-rhizosphere and endo-phyllosphere	• Surface-inoculated agar plates • Plant broth; plant powder teabags, nutrient agar; standard R2A, N-deficient combined carbon sources medium (CCM) • 16S rRNA gene sequencing and MALDI-TOF analysis of pure isolates	• Improved in vitro growth and extended in situ recovery of plant microbiota • With clover, the predominance of Firmicutes, Alphaproteobacteria and Gammaproteobacteria, and less frequently Bacteroidetes and Actinobacteria, whereas R2A exposed overwhelming diversity of Firmicutes, • Wider diversity of clover endophytes beyond rhizobia, *e.g.* multiple genera of *Chryseobacterium*, *Cronobacter*, *Kosakonia*, *Tsukamurella*, and a potentially/presumptive novel species • MADI-TOF analysis clustered isolates according to their plant niches, endo-phyllosphere/endo-rhizosphere

Table 2 (continued)

In situ *similis* strategies (Related references)	The concept	Tested host plants, and compartments	Methods of cultivation, culture media and microbiota analysis	Major outcomes and advantages
"In situ *similis*" culturing of plant microbiota: A novel simulated environmental method based on plant leaf blades as nutritional pads (Nemr et al. 2020)	Intact leaves used in different preparations as the sole supportive substrate, *i.e.*, culture pad, for culturing bacteria present in the various plant compartments	• Sunflower (*Helianthus annuus* L.) • Rhizosphere, endo-rhizosphere, ecto-phyllosphere, endo-phyllosphere	• Surface-inoculated agar plates; membrane filter technique • Plant powder teabags; standard nutrient agar, R2A • 16S rRNA gene sequencing and MADI-TOF analysis of pure isolates	• Cultivated a diverse set of microorganisms with the predominance of 13 genera of > 30 potential species, belonging to Firmicutes, Proteobacteria, and Actinobacteria; while the standard R2A medium mainly pertain to Firmicutes, especially *Bacillus* spp. • Recovered genera not commonly reported for sunflower, *e.g.*, *Rhizobium*, *Aureimonas*, *Sphingomonas*, *Paracoccus*, *Stenotrophomonas*, *Pantoea*, *Kosakonia*, and *Erwinia* • Extended diversity and richness in the endophyllosphere compared to the endorhizosphere • MALDI-TOF clustered isolates according to their niche and potential functions, as the majority of isolates of the endorhizosphere clustered away from those of the endophyllosphere

Table 2 (continued)

In situ *similis* strategies (Related references)	The concept	Tested host plants, and compartments	Methods of cultivation, culture media and microbiota analysis	Major outcomes and advantages
Culture media based on leaf strips/root segments create compatible host/organ setup for in vitro cultivation of plant microbiota (Nemr et al. 2021)	Most probable number (MPN) culturing strategy based on leaf strips and/or root segments of tested host plants, as sole sources of nutrients, immersed in semi-solid water agar tubes	● Sunflower (*Helianthus annuus* L.); ● Endo-rhizosphere and endo-phyllosphere	● Leaf strips and root segments immersed in semi-solid culture tubes ● Plant powder teabags culture medium; standard R2A ● PCR-DGGE analysis; 16S rRNA gene sequencing of pure isolates	● PCR-DGGE analyses indicated divergence in community composition of cultivable endophytes primarily attributed to culture media, and signalling a certain degree of plant organ affinity/compatibility ● Based on 16S rRNA gene sequencing of pure isolates, 20 genera comprising 32 potential species were enriched; belonged to Bacteroidetes, Firmicutes, and Alpha-/Gammaproteobacteria ● Cultivation extended to > 8 genera: *Bosea*, *Brevundimonas*, *Chitinophaga*, *Pseudoxanthomonas*, *Sphingobacterium* *Caulobacter*, *Scandinavium*, not previously reported for sunflower, and possible unknown species or even genera
In situ *similis* culturomics strategies based on vegetable (veggie)-discs extend diversity of in vitro-cultivated microbiota of vegetables (Abdel-Fatah et al. 2025; available in the present PLSO Special issue)	Veggie-discs of homologous (tomato) and heterologous vegetables (potato and taro) are used as nutritional pads	● Tomato (*Solanum lycopersicum*) ● Phyllosphere and endo-rhizosphere	● Veggie discs embedded in water agar; inocula overlaid in semi-solid thin agar layer ● Surface inoculated plates prepared from plant broth-based culture medium ● PCR-DGGE analysis and 16S rRNA gene sequencing of pure isolates	● Uncovered the highly divergent composition of tomato culturable community that extended to representatives of Actinomycetota, Bacillota, Bacteroidota and Pseudomonadota ● Brought into cultivation additional 18 genera not previously reported for tomato microbiota ● Novel cultivation of unique isolates that showed higher similarity to previously-uncultured clones representing Pseudomonadaceae, Oxalobacteraceae and Sphingomonadaceae

Table 2 (continued)

In situ *similis* strategies (Related references)	The concept	Tested host plants, and compartments	Methods of cultivation, culture media and microbiota analysis	Major outcomes and advantages
In vitro domestication of halophyte microbiota for future SynCom application (Moner et al. 2025; available in the present PLSO Special issue)	Plant-broth-sea water-based culture medium used for CFUs counting and liquid batch culturing of halophyte microbiota	• The common halophyte glasswort *Salicornia europaea* L.; • Endo-rhizosphere and endo-phyllosphere	• Plant broth-sea water based-culture medium (PBSW) • Standard N-deficient combined carbon sources culture medium (CCM) supplemented with NaCl • PCR-DGGE analysis; 16S rRNA gene sequencing of pure isolates	• PBSW supported higher CFUs counts; and related to 16S rRNA gene copy numbers (qPCR), increased (> 40 fold) culturability compared to NaCl-salted-standard CCM • Successive in vitro domestication/batch cultures in PBSW boosted bacterial growth, diminished differences among tested culture media and shortened doubling times (DT) • PCR-DGGE showed divergence in culturable community composition primarily attributed to culture media • 16S rRNA gene sequencing of pure isolates indicated greater diversity in endo-phyllosphere than endo-rhizosphere; abundant phyla were Pseudomonadota/Bacillota/Actinomycetota; the dominance of *Halomonas* among 15 genera identified; the genera of *Gracilibacillus*, *Metabacillus*, *Mixta*, *Salinicoccus*, *Zhihengliuella*, Marinobacter, *Marinimicrobium* and *Planomicrobium* were first reported/cultivated for *S. europaea* • In vitro domestication resulted in dominance of genera of Pseudomonadota/Bacillota for endophyllosphere and *Halomonas* sp. of Pseudomonadota for endo-rhizosphere

of isolates was unique and might represent putative novel species that displayed significant separation from all of the deposited members of the related genera/species (*Rhizobium* sp. and *Erwinia* sp.). The strategy extended cultivation to slow-growing taxa reluctant to cultivation, which often follow k-strategy, *e.g.* the three genera of Actinobacteria (C*urtobacterium* sp., *Microbacterium* sp. and *Kocuria* sp.).

In situ similis setup of leaf strips/root segments immersed in semi-solid water agar for in vitro cultivation of compartment-affiliated microbiota

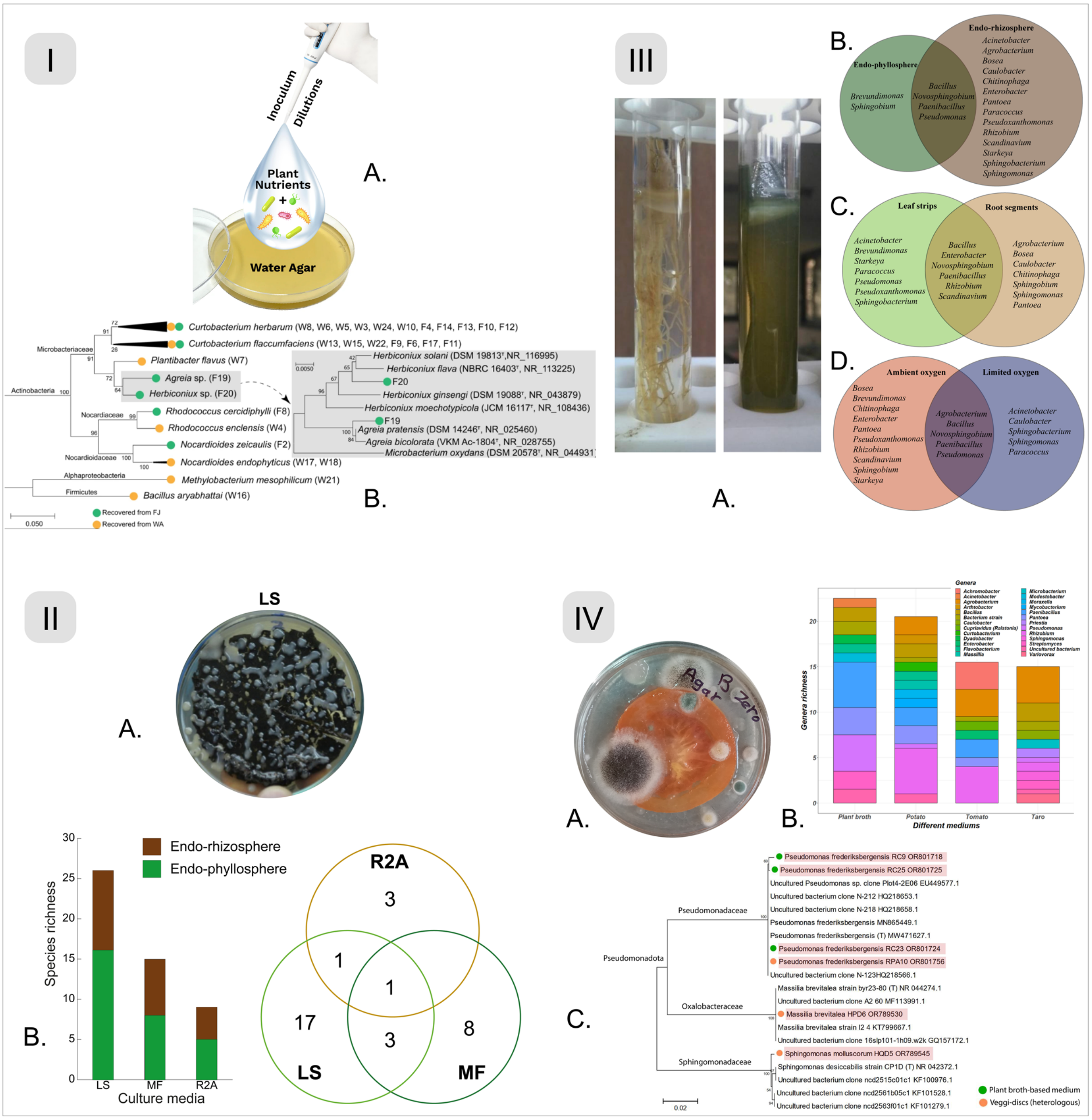
The in situ-*similis* leaf-based cultivation strategy was further developed by exploring compatible cultivation of plant microbiota when grown on corresponding plant organ, *i.e.* leaf/root-based semi-solid culture media (Nemr et al. 2021). Adding the advantages of the semi-solid MPN- enrichment methodology to such natural plant organ- based culture media, the introduced method efficiently simulates the nutritional milieu of the relevant plant organ/compartment, *i.e.* leaf strips and/or root segments immersed in plain semi-solid water agar. Very likely, the bacteria at the constructed semi-solid interface provide an in situ *similis* environment of varying concentrations of nutrients and oxygen gradients that favor active colonization and development of bacterial biofilms (Fig. 2IIIA). Appreciable MPN estimates ($>\log 4.0$ $-7.0\ \mathrm{g}^{-1}$) were estimated for plant compartments (endo-rhizosphere/endo-phyllosphere) of tested maize and sunflower plants. Compatible culturability, demonstrated as a certain degree of plant organ specificity/preference, was reported and based on homologous plant organ, *i.e.*, endo-phyllosphere bacteria preferred a culture medium based on leaf strips while endo-rhizosphere bacteria favored a culture medium based on root segments. Incubation under restricted oxygen concentrations favored the growth of endo-phyllosphere community compared to that of endo-rhizosphere. With 16S rRNA gene sequences of representative isolates, the tested culturomic combinations extended the diversity and richness of more than 32 potential species belonging to 20 genera of the phyla Alphaproteobacteria, Gammaproteobacteria, Firmicutes, and Bacteriodetes. Among the genera identified, differential prevalence is reported in response to plant compartments, organ-based culture media and/or oxygen availability (Nemr et al. 2021; Fig. 2IIIB, C, D). Both plant compartments shared the presence of 4 genera while 14 genera were confined to the endo-rhizosphere and only 2 genera to the endo-phyllosphere. This aligns with the established concept of the greater microbial diversity and richness in belowground compared to aboveground plant organs (Wagner et al. 2016; Zarraonaindia et al. 2015). The method exhibited compatible preferences of culturability, where the leaf strips-based culture medium enriched rare species of the endo-phyllosphere, *Bacillus* sp. and *Sphingobium* sp., while the root segments-based culture medium favored the cultivation of the multiple genera of the endo-rhizosphere, *Agrobacterium* sp., *Bosea* sp., *Caulobacter* sp., *Chitinophaga* sp., *Pantoea* sp. and *Scandivavium* sp.

Vegetable (veggie)-discs as appropriate in situ similis milieu for in vitro-cultivation of vegetable microbiota

As a practical strategy, veggie-discs of vegetable crops, *e.g.* tomatoes, potatoes and taro, were used as the nutritive platform for in vitro cultivation of vegetable microbiota. Detailed information on the protocol applied and the results obtained are included in the current PLSO Special Issue (Abdel-Fatah et al. 2025). The method supported well-developed CFUs, and both DGGE and 16S rRNA gene sequencing confirmed the cultivation of diverse taxa of bacteria, Actinobacteria and fungi (Fig. 2IVA). The diversity of culturable taxa was significantly extended to include representatives of Actinomycetota, Bacillota, Bacteroidota and Pseudomonadota (Fig. 2IV B). The strategy brought into cultivation an additional 18 genera not previously reported for tomato microbiota, and also recovered unique isolates that showed high similarity to previously uncultured clones representing Pseudomonadaceae, Oxalobacteraceae and Sphingomonadaceae (Fig. 2IVC).

C. In vitro simulation of saline plant-soil environments for efficient isolation/domestication of halophyte microbiota

The plant broth-based-seawater culture medium has been advanced for in vitro cultivation of the microbiota of endo-rhizosphere/endo-phyllosphere of halophytes (Moner et al. 2025; Saleh et al. 2017). With *Salicornia europaea*, this particular set up simulated in situ environments in terms of real time composition



and concentrations of host plant nutrients and prevailing natural salt stress. Detailed information on the protocol applied and the results obtained are included in the current PLSO Special Issue (Moner et al. 2025). Related to 16S rRNA gene copy numbers (qPCR), the introduced strategy significantly increased (*ca.* > 40 fold) culturability compared to NaCl-salted-standard culture medium (Fig. 3). 16S rRNA gene sequencing of representative isolates indicated: a) greater diversity in endo-phyllosphere than endo-rhizosphere (Fig. 3I), b) abundant phyla were Pseudomonadota, Bacillota and Actinomycetota (Fig. 3.IIA) with the dominance of *Halomonas* among 15 genera identified and c) *Gracilibacillus*, *Metabacillus*, *Mixta*, *Salinicoccus*, *Zhihengliuella*, *Marinobacter*, *Marinimicrobium* and *Planomicrobium* were first reported/cultivated for *S. europaea* (Fig. 3IIB). Throughout successive steps of in vitro domestication in liquid batch cultures, differences among all tested culture media including NaCl-salted standard

◂**Fig. 2** The various approaches of in situ *similis* culturing strategies based on plant-based culture media: I- The inoculum-dependent cultivation: A, The inoculum acts as a dual source of both nutrients and contained microbiota. B, Exceptionally, the method brought into cultivation slow growing genera of endophytes, including Actinobacteria.*(Adapted from* Sarhan et al. 2020*)*. II- Plant leaf blades as nutritional pads on water agar plates: A, Well developed CFUs on leaf surfaces; B, The method increased the overall diversity of endo-phyllosphere bacteria compared to those in the endo-rhizosphere; 16 s RNA sequences identified fewer common isolates and more unique isolates according to the culture media used *(Adapted from* Nemr et al. 2020*)*. III- Leaf strips/root segments in MPN tubes: A, Leaf strips/root segments immersed in semi-solid water agar supported copious bacterial growth; among 32 genera identified, differential prevalence is reported in response to plant compartments (B), organ-based culture media (C), oxygen availability (D) *(Adapted from* Nemr et al. 2021*)*. IV-Vegetable (veggie)-discs on water agar plates: A, Discrete colonies of bacteria, Actinobacteria and fungi developed on tomato veggie discs; B, Veggie-discs of homologous (tomato)/heterologous (potato, taro) and plant-broth based culture media extended cultivation to members of Actinomycetota, Bacillota, Bacteroidota and Pseudomonadota.; C, The neighbor-joining phylogenetic tree of unique bacterial isolates developed on plant-based culture media that showed higher similarity to previously uncultured clones *(Adapted from* Abdel-Fatah et al. 2025*; for more details refer to the original article published in the current PLSO Special Issue)*

culture media, in terms of populations (CFUs) and biomass production (Optical Density- OD), were significantly diminished and doubling times (DT) shortened (Fig. 3III). Such in vitro domestication resulted in dominance of genera of Pseudomonadota and Bacillota for endo-phyllosphere and *Halomonas* sp. of Pseudomonadota for endo-rhizosphere (Fig. 3IV). The strategy is applicable not only for recovering and identifying halophyte core microbiota but also for in vitro domestication and propagation of representative core microbiota, instead of the laborious work of constructing consortia of single pure isolates for SynCom applications.

D. Host plant compatibility and cross cultivation in culture media based on plant broth of homologous/heterologous tested plants

Despite the promiscuous nature of a given plant material to cultivate rhizobacteria associated with different tested host plants, preliminary results indicated a certain affinity and preference of cultivation on culture media based on the corresponding/homologous tested host plant, at least at the family level and/or from the same environments (Mourad et al. 2018). Additional reports on the microbiota of sunflower indicated a certain degree of divergence in community that signaled a kind of plant organ (leaves/roots) affinity/compatibility (Nemr et al. 2021). This inspired the investigation and comparison of diversity in response to in vitro cross cultivation on homologous/heterologous plant- broth culture media. This would reveal the inter-plant species chemo-diversity (He et al. 2015a), which is reflected in the multiplicity and complexity of primary and secondary metabolites that are synthesized and characterize various host plants (Fang et al. 2018; Maeda 2019). Such a manifold of metabolites strongly interacts with plant growth and development in continually changing environmental conditions subjected to abiotic and biotic stresses, and further extends to the plant–microbe specialized metabolic interactions that are expressed in reciprocal interactions (Van der Hooft et al. 2020). Therefore, the complex nutritional matrices that recruit the accompanying microbiota strongly compel the use of compatible culture media based on the tested homologous host plants with their real time and fingerprinted complex chemical composition. In this context, Elsawey et al. (2023) demonstrated the distinct differences in the chemical/nutritional composition of the vegetative parts of maize and sunflower plants that are used in the preparation of plant broth based-culture media. Furthermore, they analyzed the culturable community of the maize endo-phyllosphere developed on a plant broth culture medium based on homologous (maize) compared to another developed on a medium based on heterologous plants (sunflower). Amplicon sequence data and related UPGMA and PCA analyses confirmed the separation of the mother culture-independent communities apart from the culturable communities (Table 3; Fig. S1A, C). As to community composition, the culture-independent bacterial community showed the highest diversity and relative abundance of Proteobacteria, with the majority of Gammaproteobacteria (Pseudomonadales, Enterobacterales, and Xanthomonadales), followed by Alphaproteobacteria (Rhizobiales and Sphingomonadales) and Betaproteobacteria (Burkholderiales). Less abundant were Bacteroidetes (Flavobacteria), Firmicutes (Bacillales) and Actinobacteria. In vitro growth cultivation demonstrated less diversity with unequivocal differentiation that is based on

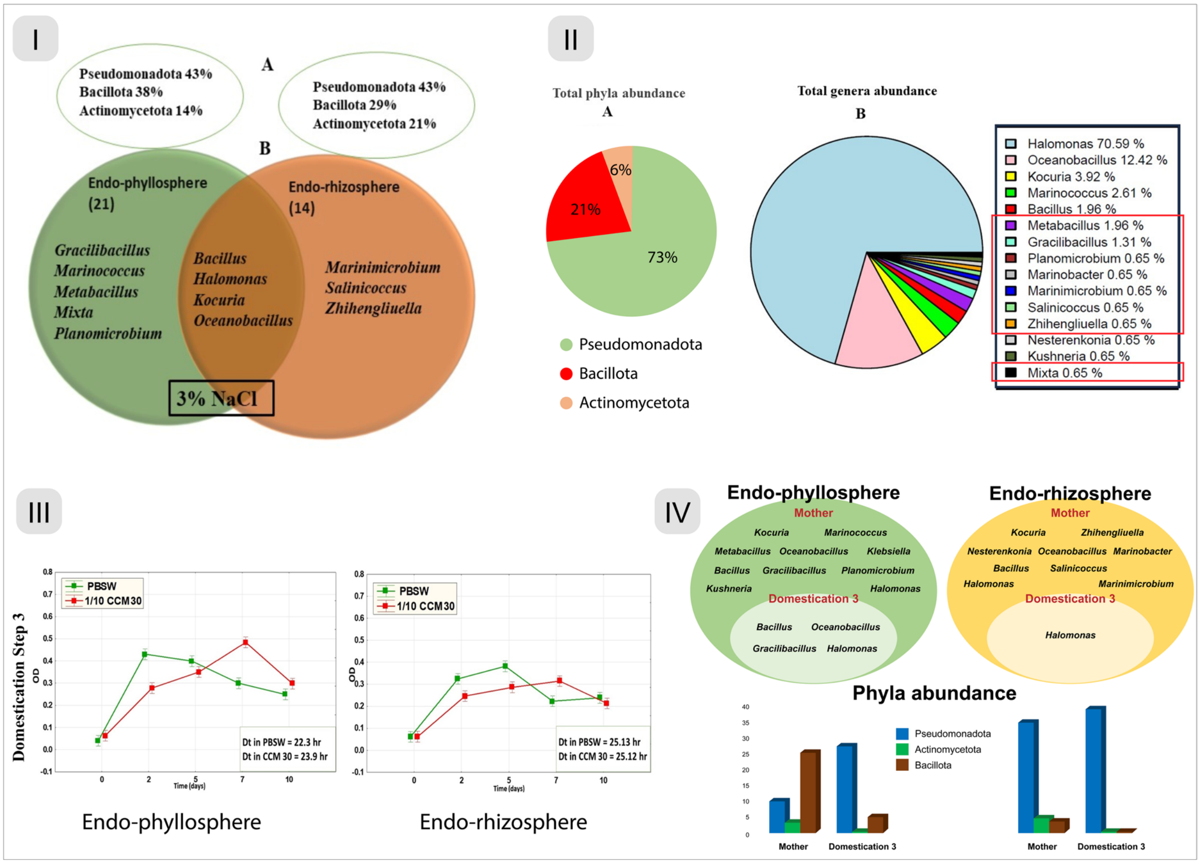


Fig. 3 Plant broth-based-seawater culture medium successfully in vitro cultivated and domesticated halophyte microbiota: I) significantly recovered microbiota (A, Phyla and B, genera) of endo-phyllosphere compared to endo-rhizosphere; II) exhibited dominance of *Halomonas* sp. and recovered 8 genera reported for the first time for *S.europaea* (contained in box); III) successive steps (third step) of in vitro domestication in liquid batch cultures significantly diminished differences in biomass production (OD) and shortened doubling time; IV) in vitro domestication resulted in the dominance of genera of Pseudomonadota/Bacillota for endo-phyllosphere and *Halomonas* sp. of Pseudomonadota for endo-rhizosphere.(*Adapted from* Moner et al. 2025*; for more details refer to the original article published in the current PLSO Special Issue)*

homologous versus heterologous plant broth culture medium, in terms of over/less abundance of bacterial taxa on multiple levels. The homologous cultivation on maize plant broth distinguishably enriched taxa of the phylum Proteobacteria, in particular those belonging to the Pseudomonadaceae and Moraxellaceae families of Gammaproteobacteria (Fig. S1A and B). On the other hand, heterologous cultivation on sunflower plant broth and standard chemically-synthetic R2A favored the enrichment of the members of Firmicutes (Fig. S1C and D). The LEfSe analysis (Linear discriminant analysis (LDA) effect size; Segata et al. 2011) was applied to predict biomarkers that characterize the existing bacterial community (Table 3; Fig. S1E, F). The culture-independent communities were distinctively characterized by the strong representation of both clades of Alphaproteobacteria, order Rhizobiales (Family Hyphomicrobiaceae), and Gammaproteobacteria, order Xanthomonadales (Family Xanthomonadaceae). With homologous cultivation, Actinomycetales were the distinguished phylogenetic unit while cultivation on heterologous plant broth of sunflower over-represented the families Alcaligenaceae and Rhizobiaceae. Extraordinarily, the chemically-synthetic standard R2A culture medium enriched the taxa of Firmicutes.

Today, microbiologists are becoming more and more aware that culture media are not set recipes for

Table 3 Community composition of microbiota of the maize endo-phyllosphere revealed by culture independent analysis, comparing cross cultivation on plant broth-based culture media prepared from homologous (maize) and heterologous (sunflower) host plants

Points of comparison	Culture-independent analysis	Culture-dependent analysis (in situ *similis* cultivation)	
		Homologous cultivation	Heterologous cultivation
Amplicon sequence data and related UPGMA and PCA analyses	**Highest abundance**: Proteobacteria: (Gammaproteobacteria; Pseudomonadales; -Enterobacterales, and Xanthomonadales), **Followed by**: -Alphaproteobacteria; (Rhizobiales and Sphingomonadales) -Betaproteobacteria (Burkholderiales) **Less abundant:** Bacteroidetes (Flavobacteria), -Firmicutes (Bacillales)—Actinobacteria	**Highest abundance**: Proteobacteria (Pseudomonadaceae, Moraxellaceae)	**Highest abundance**: Firmicutes members **(Similarly, standard R2A culture medium highly represented Firmicutes)**
The LEfSe analysis (Linear discriminant analysis (LDA) effect size (to predict biomarkers)	**Strong representation**: -Alphaproteobacteria (Rhizobiales; Hyphomicrobiaceae) -Gammaproteobacteria (Xanthomonadales, Xanthomonadaceae)	**Over-represented**:Actinomycetales (Microbacteriaceae, *Microbacterium* sp.)	**Over-represented**: -Betaproteobacteria (Alcaligenaceae, *Achromobacter* sp.) -Alphaproteobacteria (Rhizobiaceae, *Shinella* sp,) **(R2A standard culture medium enriched Firmicutes, Bacilli, Bacillales, and Paenibacillaceae**, *Brevibacillus* sp)

laboratories to follow, but rather to be tailored for compatibility with the tested environment/holobiont (Elsawey et al. 2023). As to the plant microbiota, the dynamic structure of the chemical composition of host plants at various development stages and with various compartments compels the need to approximate and simulate comparable real-time nutritional matrices in any tailored culture media. Mechanistically, the sole presence of host plant materials provides a mimicked environment that: a) supplies plant nutrients in their natural composition and complexity, b) supports culturing/co-culturing of compatible microbiota, and c) encourages inter and intraspecific interplay among culturable communities (Cleary et al. 2017; Nemr et al. 2021). Nutritionally, such plant materials provide more diverse plant macromolecules, major and minor elements, and growth factors in the form of amino acids and other compounds of unknown composition and concentration compared to chemically-synthetic culture media. This particular milieu facilitates the in vitro exposure of not-yet cultured genera and less abundant or hard-to-culture bacterial phyla (Sarhan et al. 2018, 2019). Further, the reproduction of in vitro chemical/nutritional diversity of plants is fairly accomplished through the use of representative plant materials of specific plant organs, *e.g.*, leaves/roots (Nemr et al. 2021) especially those of the tested homologous plant (Elsawey et al. 2023).

Culturomics on the tail of other OMICS technologies and AI

While the significance of culturomics and omics technologies is well-documented in medical system sciences, such as infectiomics and pharmacomicrobiomics (Kambouris et al. 2018), they also play a crucial role in understanding soil and plant microbiome-holobiont interactions (Sarhan et al. 2019). Both approaches are well-established tools for exploring microbiome-related resistomes in diverse environments (Nowrotek et al. 2019). For instance, the recently developed BGC Atlas, a web resource dedicated to exploring secondary metabolite diversity encoded in bacterial genomes, has unveiled numerous novel antibiotic gene cluster variants in soil microbes (Bağcı et al. 2024). Additionally, omics technologies have facilitated the identification and classification of microbial marker taxa that mitigate drought and salinity stresses in crops, critical for advancing desert farming systems and developing tailored bioinoculants (Lian et al. 2023). In the following sections, we will explore how major omics technologies contribute to the study of plant-associated microbes and how they can facilitate the cultivation of previously uncultured microbes by targeting individual compartments of the plant holobiont. This data-driven approach leverages insights from the known to predict and uncover the unknown, paving the way for novel strategies in plant cultivation and microbiome management.

Integration of OMICS technologies in current culturomics

Omics technologies provide comprehensive insights into the structure and function of plant-associated microbial communities. A number of omics layers contributes unique data streams that collectively elucidate the complex organismal and molecular interactions among different members and components of the holobiont (Table 4). They are represented in 16S RNA and ITS-based metataxonomics, shotgun metagenomics, metatranscriptomics, proteomics, metabolomics, metalipidomics and metavolatilomics.

As early as 1980s –1990s, 16S rRNA and ITS-based metataxonomics were introduced as phylogenetic markers that revolutionized culture-independent microbial identification. They are using selective amplification and sequencing of 16S rRNA gene or gene fragments that enables high-resolution taxonomic profiling of bacterial and archaeal communities. As well, fungal microbiota components can be analogously analyzed by ITS amplification and sequencing of the internal transcribed spacer (Abarenkov et al. 2022). Both techniques are widely used and highly targeted but also have major disadvantages (Bartoš et al. 2024; Douglas et al. 2020; Mongad et al. 2021; Wemheuer et al. 2020; Pauvert et al. 2019). This was followed by Shotgun metagenomics techniques that extend beyond taxonomic profiling by capturing the entire genetic repertoire of microbial communities. Combining metagenome assembly strategies and/or long read sequencing approaches with metabolic pathway reconstruction and transcription data integration are key elements for microbial genome-scale metabolic modeling (Leonidou et al 2023a; Gutleben et al. 2018). In that context, single-cell genomics approaches that result in single-amplified microbial genomes further increase the understanding of yet uncultured microbes and their potential metabolic complexity (Chijiiwa et al. 2020), and guide the development of tailored nutrient-enriched culture media and individual growth conditions (Liu et al. 2022; Table 4).

Beyond genetic potential (metagenomics), metatranscriptomics provides a functional perspective by identifying actively transcribed genes associated to the alive microbial fraction (Gutleben et al. 2018). The combination of metatranscriptomics and comparative genomics has led to an innovative enrichment culture system for uncultured strains that also revealed an efficiency of resuscitation of dormant species residing in the environment (Mu et al. 2018; Bomar et al. 2011). Proteomics complements transcriptomics by quantifying the functional protein output of organisms' communities under a given state. It delivers valuable insights into the metabolism of amino acids and proteins within and between organisms. Proteomics can elucidate not only plant responsiveness to biotic and abiotic stresses, but also communication with microbes (Jain et al. 2021; Liu et al. 2019). The MALDI-TOF MS is applied for high throughput classification of cultured and uncultured microbial strains based on their protein profiles (Huschek and Witzel 2019; Seuylemezian et al 2018). Understanding the plasticity of protein profiles of plants and microbes during interaction will give hints on alternative protein-based culture-media formulations to get the uncultured fraction into culture (Arulanantham et al. 2012).

Differently, metabolomics are offering insights beyond genomics or proteomics, as metabolites are immediate indicators of physiological changes. It focuses on the small-molecule metabolites produced by both plants and their associated microbiota. High-throughput targeted or untargeted metabolomics has the potential to identify metabolic profiles of known, novel or difficult-to-grow bacteria that can foster enhanced cultivation strategies (Fiorini et al. 2022). Already, metabolomics prediction from genome data is a good basis for identification of target metabolites. Enriching such derived data with transcriptomics, proteomics and/or metabolic quantitative

Table 4 Examples of integration of OMICS technologies in the development of current culturomics strategies

Omics Technology	Methodology	Key insights	Key limitations	Examples
16S rRNA & ITS-based metataxonomics	Amplicon sequencing of 16S rRNA gene or ITS region	Taxonomic profiling of bacterial, archaeal and fungal communities; alpha and beta diversity metrics	- Limited taxonomic resolution that may lead to errors in taxonomical and functional profiling; reference databases may introduce systematic biases if microbial communities are not well-represented (Roy et al. 2024)	- Multiomics profiling, including metataxonomics and metabolomics, reveals cross-kingdom signalling to recruit beneficial rhizosphere bacteria (Sun et al. 2024)
Shotgun metagenomics	Whole-genome sequencing	Functional gene discovery; pathway reconstruction; metabolic potential assessment	- Variations in sample collection methods, sequencing technologies, and bioinformatics pipelines can lead to conflicting results; as many microorganisms remain poorly characterized, it is challenging to analyse metagenomic data comprehensively (Roy et al. 2024)	- Metagenomics-data driven culturing of the uncultured microbes (Liu et al. 2022) *e.g.*, identifying medium additives (caffeine) to enhance taxa recovery (e.g., Lachnospiraceae, Oscillospiraceae, Ruminococcaceae (Armetta et al. 2025) - Based on correlation with meta-analysis data, an optimized culture medium was formulated for in vitro cultivation of the infant gut microbiota (Alessandri et al. 2022)
Metatranscriptomics	RNA-seq of microbial RNA	Identification of actively transcribed genes; functional responses to environmental conditions	- Expensive and requires significant computational power - Non-dominant microbial transcripts from fungi/archaea are challenging to detect due to limited reference databases -Many microbial proteins are hypothetical/poorly characterized, making functional interpretation difficult. - Correlation of metatranscriptome to microbial viability and activity is not always clear. -Separating prokaryotic mRNA from rRNA is challenging and can affect data quality (Butowski, et al. 2025)	- Enrichment culture system for uncultured strains (Mu et al. 2018) *e.g.*, replacing glucose by mucin leading to the recovery of *Rikenella*-like bacterium from leech gut (Bomar et al. 2011)

Table 4 (continued)

Omics Technology	Methodology	Key insights	Key limitations	Examples
Proteome analysis	Mass spectrometry (MS), MALDI-TOF of proteins	Protein-level functional characterization; enzymatic activity profiling	- Expensive instrumentation that limits accessibility for larger samples and broader research -Different protein extraction methods, lack of standardized workflows and quality control making comparisons across labs difficult - Host cells complicate protein extraction and analysis - Low-abundance proteins and theoretically present proteins challenge accurate representation (Zhang et al. 2025)	- Alternative protein-based culture media for bacterial growth (Arulanantham et al. 2012) - Metabolic labelling-based quantitative proteomic method used to compare natural and laboratory community metabolic profiles and confirm the presence and activity of low abundance members, by improving culturability and increasing growth rates in laboratory communities (Belnap et al. 2010)
Metabolomics	LC–MS/MS, NMR of metabolites	Identification of plant- and microbe-derived metabolites; insights into biochemical interactions	- Requires advanced and expensive technologies (GC–MS, LC–MS, and NMR) -Lack standardized methods for sample processing and data analysis. - Integrating metabolomics with other omics data is challenging due to complexity of interactions and lack of robust bioinformatics tools (Zheng et al. 2025)	- Viable soil defined media using quantitative metabolomics (Jenkins et al. 2017) - Provide insights into the unculturability, pathogenicity, and lifestyle of the uncharacterized TM7 phylotype as a possible parasitic epibiont of *Actinomyces odontolyticus* XH001 (He et al. 2015b)
(Meta-) Lipidomics	GC–MS of fatty acids	Identification of plant- and microbe-derived fatty acids and lipids	- Limited database information and standards. -Accurate quantification of single lipid molecules is difficult and depends on the quality/concentration of internal standards and control samples. - Targeted lipidomics approaches have limited coverage and cannot identify unknown lipids; untargeted lipidomics generates large amounts of data difficult to process and interpret (Appala et al. 2020; Jia et al. 2022)	- SCFAs as potential resuscitation factors of dormant bacteria (Sun et al. 2023)

measurements can lead to innovative media formulations. Such environment-based media formulations are highly desired to explore real ecological patterns (Jenkins et al. 2017; Wang and Carvalho 2023). Metalipidomics and metavolatilomics are another common practice for the analysis of the cellular fatty acid profiles, using *e.g.* GC–MS, to describe novel strains added to culture collections (*e.g.* DSMZ) (Vieira et al. 2021; Sasser 2001). But like the proteomics issue, lipid profiles might alter depending on the environmental pressure, caused by *e.g.* heat, chemicals or the plant immune response (Kuźniak and Gajewska 2024; Singh et al. 2013). Identification of novel microbial lipidome members might be an indicator for a yet-uncultivated strain (Ding et al. 2021; Keymer et al. 2017). When applied in a hybrid approach together with predicted proteomics, they guide different culture modes of microbial strains (Hasni et al. 2020; Sun et al. 2023). Distinctively, volatilomics focuses on the volatile organic compounds (VOCs) fraction that plays, for instance, a crucial role in plant communication with pollinators, enemies, and microorganisms (Meredith and Tfaily 2022; Bouwmeester et al. 2019; Schulz-Bohm et al. 2017).

A number of examples available in literature clearly demonstrated that metagenomics and reverse-genomics approaches have improved microbial isolation strategies by designing unique culture media for the recovery of uncultivable taxa. In this respect, Tyson et al. (2005) while studying *Leptospirillum* population groups in acid mine drainage biofilm detected a single *nif* gene operon in the genomic data from the *Leptospirillum* group III population, which was lacking in group II. So, the growth medium was modified to lack nitrogen and was used to successfully isolate the first cultivated representative of *Leptospirillum* group III. As well, metagenome-assembled genomes of *Acidobacteria* revealed the ability to utilize complex plant-derived polysaccharides, facilitating cultivation on low-nutrient media supplemented with hemicellulose, cellulose, and chitin (Ward et al. 2009; Kielak et al. 2016). The metagenomic profiling of members affiliated to Succinovibrionaceae supported the development of a defined medium, which contained starch and urea as the sole carbohydrate and nitrogen sources, respectively, as well as the antibiotic bacitracin, (Pope et al. 2011). The genomic analyses of *Tropheryma whipplei*, a Gram-positive bacterium that causes Whipple's disease, revealed auxotrophy for amino acids, nucleotides, and lipids, which directly lead to the development of media enriched with amino acids, nucleosides, and fatty acids, enabling its isolation (Renesto et al. 2003). Currently, it is well accepted that the dependencies and interplay of OMICs-derived data can lead to novel cultivation strategies and culture media formulations. While genomics-based data provide potential hypotheses for predictive microbe and microbe-host modeling approaches, transcriptomics- and chemical-based approaches can drive the proof and aid in optimization of metabolic models.

Predictive culture media optimization using artificial intelligence (AI)

The application of omics technologies spans both natural and controlled environments, allowing for a comparative approach for understanding plant holobiont dynamics. By examining microbes in their native habitats and under in vitro conditions, researchers can identify key metabolites, genes, and microbial functions that are critical for growth and/or plant-metabolic niche adaption.

Data derived from natural habitats serve as a reference point for holobiont composition and functionality under optimal conditions. Machine learning (ML) approaches have the power to manage the amount of data that must be monitored and analyzed simultaneously to transform omics data interpretation. One example is to learn from stress-mitigating microbes and their assemblage in association with different plant species (genotypes):

(i) Starting from a feature importance analysis, microbial stress-mitigating marker taxa, for *e.g.* drought or salinity, can be identified (Abdelfadil et al. 2024; Hagen et al. 2024).
(ii) Then the microbial metabolic potential has to be examined through metabolic reconstruction.
(iii) Further, analyzing the microbial stress-mitigating potential dependent on altering plant genotypes and their metabolic plasticity (*e.g.* of root exudates) will make it possible to identify relevant plant compounds, that provide specific metabolic niches.

(iv) Such compounds might be important to bring as yet uncultured microbial plant-associated strains into culture.

Related information has been already collected intensively, albeit rudimentarily, and can be applied with Deep Learning (DL) approaches in form of Convolutional Neuronal Networks or the development of Large Language Models (LLMs) that can bring light into the ecological darkness.

Practically, specific examples are reported in literature *e.g.* Liu et al. (2025). They analyzed nutrient compositions from the MediaDive database to construct a dataset of 2369 media types; and when combined with microbial 16S rRNA sequences, they developed 45 binary classification models using the XGBoost algorithm. As a result, they predicted growth conditions for various human gut microbes, confirming their practical utility and highlighting the potential of machine learning to optimize culture media selection. Zhang et al. (2023) combined machine learning (ML) with active learning to fine-tune the medium components for the selective culture media of differing bacteria, i.e., *Lactobacillus plantarum* and *Escherichia coli*, and demonstrated the efficiency and practicality of active learning in medium optimization for selective culture media.

It is anticipated that AI will have a great impact on future culturomic approaches. The related techniques, *e.g.* Machine Learning Algorithms and Deep Models, are trained by analyzing large datasets of experimental data, including nutritional components, culture conditions, and microbial responses to find complex patterns in microbial growth. This is to recognize the ideal ratios of nutrients and other components to predict and optimize microbial culture media. They can also accurately display the results of various growth conditions and parameters, *e.g.* growth rate, pH changes, and desired cell activities. In turn, they can lead to active learning by recommending the next experiments to perform. Ultimately, they guide the optimization of culture media compositions, reducing traditional experimental trial-and-error, and treat multiple goals simultaneously.

FAIR data

Integration of various omics datasets into culturomics requires simple but strict principles for data collection and handling in a FAIR manner to guarantee highest compatibility and comparability. Structured and controlled terms are needed for generation of the comparable data quantity required for AI and for computational models, as *e.g.* suggested through the Systems Biology Ontology (SBO) (Leonidou et al 2023b). The resulting semantics can then be integrated into LLMs supporting the selection of chemical compounds for fractional microbial cultivation. In fact, while omics technologies offer transformative potential, several challenges must be addressed to fully integrate their outputs into culture media optimization:

- **Validation Across Systems:** Omics methodologies must be validated for diverse holobionts, accounting for differences in plant species, plant development stages, plant compartments, plant-based metabolites, microbial assemblages and environmental conditions.
- **Standardization of Protocols:** The lack of standardized protocols hampers cross-study comparisons. Establishing uniform workflows for sample preparation, data acquisition and bioinformatics analyses is imperative.
- **Integrative Analysis:** The full potential of omics lies in the integration of data across multiple layers. Advanced computational tools for multi-omics integration will be a key to achieving holistic insights into holobiont functioning.
- **Curated Databases and Ontologies:** Linked organism and media databases, like Media*Dive* of the DSMZ, are needed to store information about conditional cultivation (Oberhardt et al. 2015). Further, host-microbe genetic and metabolic dependencies have to be reflected much stronger in the form of extended or novel ontologies or semantic models.
- Future research should prioritize the development of predictive frameworks that link omics-derived insights to practical outcomes, such as improved growth rates, stress tolerance, and metabolite production under optimized culture conditions.

General conclusions

Within the plant holobiont, alliances of microbiota with their hosts are masked by the inability of in vitro cultivation of their bulk. The pure cultures stockpiled in international centers originated from dissimilar environments and hosts, which makes them inadequate for future interventions and the application of microbiota-mediated strategies. To meet the complexity of the cultivation of microbiota endophytic to various plant compartments, the review presents the recent developments regarding plant-based in situ *similis* culturomic strategies. Irrespective of their shortcomings regarding standardization of the composition of the used plant-based culture media and unavailability of well-structured protocols, they do: a) simulate/fingerprint the nutritional composition of tested host plants, b) advance real-time in vitro cultivation and lab-keeping of compatible plant microbiota, c) expose the magnificent diversity of plant microbiota, d) bring into cultivation masked members of the plant microbiota resources, and e) expose preferential pairing of plant–microbe partners towards future synthetic community (SynComs) research and use in agriculture. The review also outlines members of the OMICS family which could contribute to the future progress and development of culturomics of the plant microbiota.

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Data availability Data sharing does not apply to this article, as no datasets were generated or analyzed for the current study.

Declarations

Consent to participate and publish All authors have expressed their consent to participate in the writing and publication of the article.

Conflict of interest The authors declare no conflict of interest.

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