Al-Farabi Kazakh National University (KazNU)

Faculty of Biology and Biotechnology

Department of Molecular Biology and Genetics

Discipline: «Modern Problems of Plant Genetics»

Lecture 7

The Plant Microbiome

Holobiont co-evolution, rhizosphere-phyllosphere-endosphere niches, root exudate signaling, multi-omics profiling, PGPB mechanisms, and SynCom engineering

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Aim and Structure of the Lecture

Aim: understand how plant-associated microbial communities shape host nutrition, stress tolerance, immunity, and breeding

KEY FOCUS

Lecture Plan & Modules

Key sections:

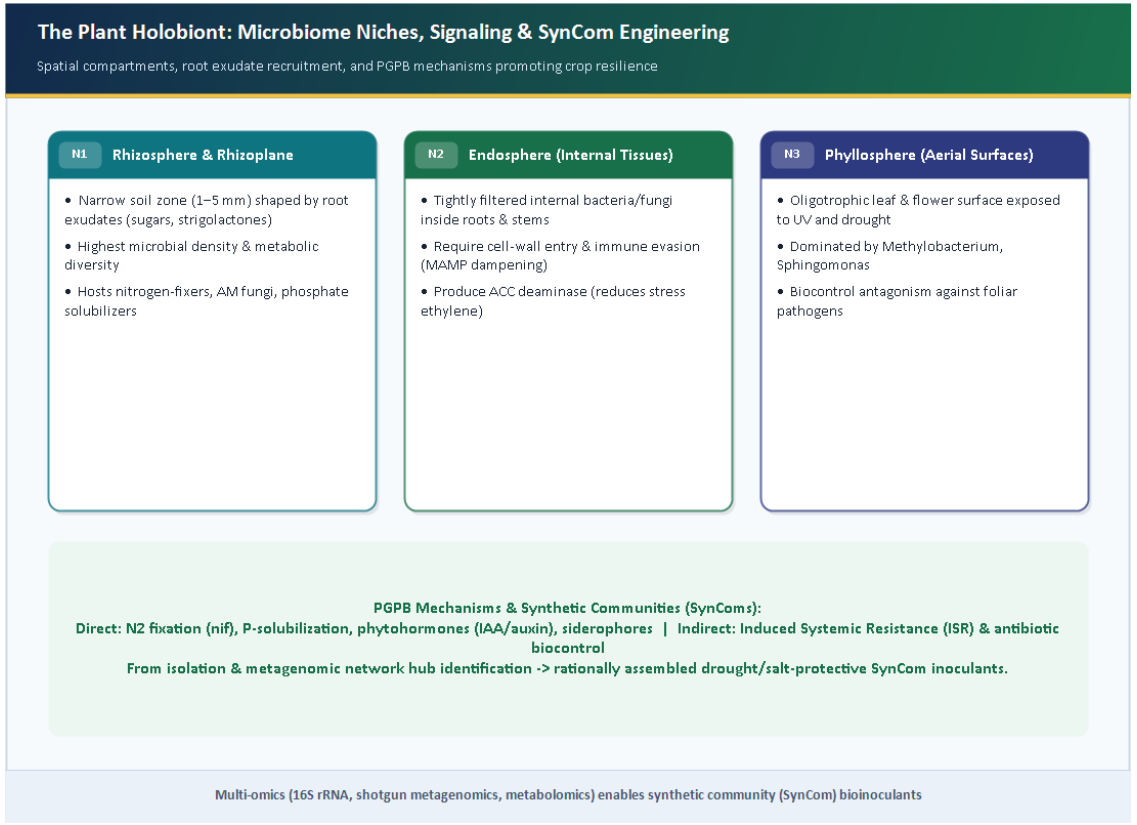
1. The Plant Holobiont Concept
2. Spatial Microbiome Compartments
3. Chemical Cross-Talk & Assembly
4. Multi-Omics Profiling Toolkit
5. Beneficial PGPB & Immunity
6. Pathobiome & SynCom Design

- **The Plant Holobiont Concept:** Co-evolution of host plant and associated bacteria, fungi, archaea, protists, and viruses as an ecological unit
- **Spatial Microbiome Compartments:** Distinct diversity gradients across bulk soil, rhizosphere, rhizoplane, endosphere, and phyllosphere
- **Chemical Cross-Talk & Assembly:** Root exudate recruitment, flavonoids, strigolactones, coumarins, and the «cry for help» stress response
- **Multi-Omics Profiling Toolkit:** 16S rRNA/ITS amplicons, shotgun metagenomics, metatranscriptomics, metaproteomics, and metabolomics
- **Beneficial PGPB & Immunity:** Nitrogen fixation, P-solubilization, IAA/ACC deaminase, and ISR (JA/ET) vs SAR (SA) defense pathways
- **Pathobiome & SynCom Design:** Pseudomonas syringae virulence (T3SS, ice nucleation), disease-suppressive soils, and mGWAS breeding

The Plant Holobiont: Host Genome Plus Extended Metagenome

Plants never grow axenically in nature—microbial partners extend metabolic capacity beyond plant chromosomes

- **Evolutionary & Genetic Perspective:** Holobiont = macroorganism (host plant) + all stably associated symbiotic, commensal, and transient microbiota
- Hologenome combines host nuclear/organellar DNA with orders-of-magnitude larger microbial gene repertoire
- **Microbial genes supply absent enzymatic functions:** atmospheric N_2 fixation (nitrogenase), insoluble P cleavage
- Co-selection favors mutualistic traits, though horizontal gene transfer and environmental drift remain dynamic
- **Ecological Assembly Filters:** Two-step recruitment model (Bulgarelli et al.): soil physicochemical biome provides initial microbial seed bank
- **Step 1:** Root rhizodeposits enrich copiotrophic Proteobacteria, Actinobacteria, and Bacteroidetes in rhizosphere



The plant holobiont: rhizosphere, endosphere and phyllosphere niches

Structural and Functional Niches of the Plant Microbiome

Diversity drops from bulk soil to internal tissues as host immune selection intensifies

Compartment	Spatial Boundary	Dominant Taxa	Primary Ecological Functions
Rhizosphere	1–5 mm soil zone directly influenced by root exudates	Proteobacteria, Actinobacteria, Bacteroidetes, Glomeromycota	Nutrient mineralization, pathogen competition, iron chelation, stress priming
Rhizoplane	Root epidermis & mucilage biofilm interface	Pseudomonas, Bacillus, Rhizobium, Azospirillum	Root colonization, quorum sensing, biofilm protection, phytohormone release
Endosphere	Internal root, stem, leaf & seed apoplast/symplast	Alphaproteobacteria, Burkholderia, dark septate endophytes	Biological N_2 fixation, systemic ISR activation, abiotic drought buffering
Phyllosphere	Aerial leaf/flower cuticle surface (~10^6–10^7 cells/cm^2)	Methylobacterium, Sphingomonas, Pseudomonas, yeasts	Methanol utilization, UV/desiccation adaptation, foliar pathogen antagonism
Spermosphere	Transient soil zone surrounding germinating seed	Inherited endophytes + fast-growing soil colonizers	Early seedling vigor, damping-off suppression, germination hormone crosstalk

Root Exudate Assembly and the «Cry for Help» Strategy

Plants actively invest 10–30% of fixed photosynthate carbon into shaping a protective rhizosphere consortium

1 Carbon Allocation

- Sucrose exported via SWEET phloem transporters
- Organic acids, amino acids & mucilage secreted

2 Stress Reprogramming

- Infection or nutrient deficit alters JA/SA signals
- Exudate metabolome shifts dynamically

3 Chemotaxis Signal

- Coumarins, flavonoids & strigolactones diffuse
- Attract motile beneficial flagellated taxa

Phase 4 Selective Enrichment

- Beneficial Bacillus/Pseudomonas proliferate
- Biofilms exclude opportunistic pathogens

Phase 5 Soil Legacy Effect

- Enriched beneficials persist in soil bank
- Protect subsequent crop generation in rotation

Example: Downy mildew foliar infection triggers Arabidopsis roots to exude malic acid, selectively recruiting Bacillus subtilis FB17.

Molecular Signals Governing Plant–Microbe Cross-Talk

Small secondary metabolites mediate partner recognition while preventing pathogen exploitation

Canonical Symbiotic Signal Cascades

- **Legume–Rhizobium:** root isoflavonoids (luteolin, genistein) activate bacterial NodD -> Nod factor lipo-chitooligosaccharides
- Host LysM receptors (NFR1/NFR5) trigger nuclear Ca^{2+} spiking and root nodule organogenesis
- **Plant–AMF Mycorrhiza:** strigolactones stimulate spore germination and hyphal branching toward roots
- Fungal Myc factors share the Common Symbiosis Signaling Pathway (SYMRK, CCaMK, CYCLOPS)

Nutrient Starvation & Defense Metabolites

- **Iron scarcity:** Arabidopsis CYP82C4/F6H1 coumarins (scopoletin) mobilize Fe^{3+} and selectively suppress competitors
- **Maize benzoxazinoids (DIMBOA):** reshape rhizosphere bacteria/fungi and prime jasmonate defenses in progeny
- **Quorum Sensing (AHL lactones):** regulates bacterial biofilm maturation and plant root architecture
- Strigolactone/ABA hormonal loops coordinate microbial recruitment with stomatal water conservation

Multi-Omics Technologies for Plant Microbiome Research

Moving beyond culture-dependent biases—over 50–90% of plant-associated microbes remain uncultured on standard agar

Omics Layer	Molecular Target	Analytical Resolution	Key Strengths & Constraints
Amplicon Sequencing	16S rRNA (V3–V4 bacteria) & ITS1/ITS2 (fungi)	Genus / ASV level	Low cost, massive sample throughput; PCR/primer bias & no direct function
Shotgun Metagenomics	Total microbial + host DNA	Strain / MAGs & gene pathways	Captures functional genes (nifH, acdS) & viruses; requires host DNA depletion
Metatranscriptomics	Microbial mRNA transcripts	Active metabolic pathways	Distinguishes live active genes from dormant DNA; rapid RNA turnover challenge
Metaproteomics	Expressed enzymes (LC-MS/MS)	Translated effector machinery	Validates protein abundance & host–microbe interactions; complex databases
Metabolomics	Exudates, VOCs & hormones	Small biochemical signals	Directly maps chemical communication; challenging isomer/origin assignment

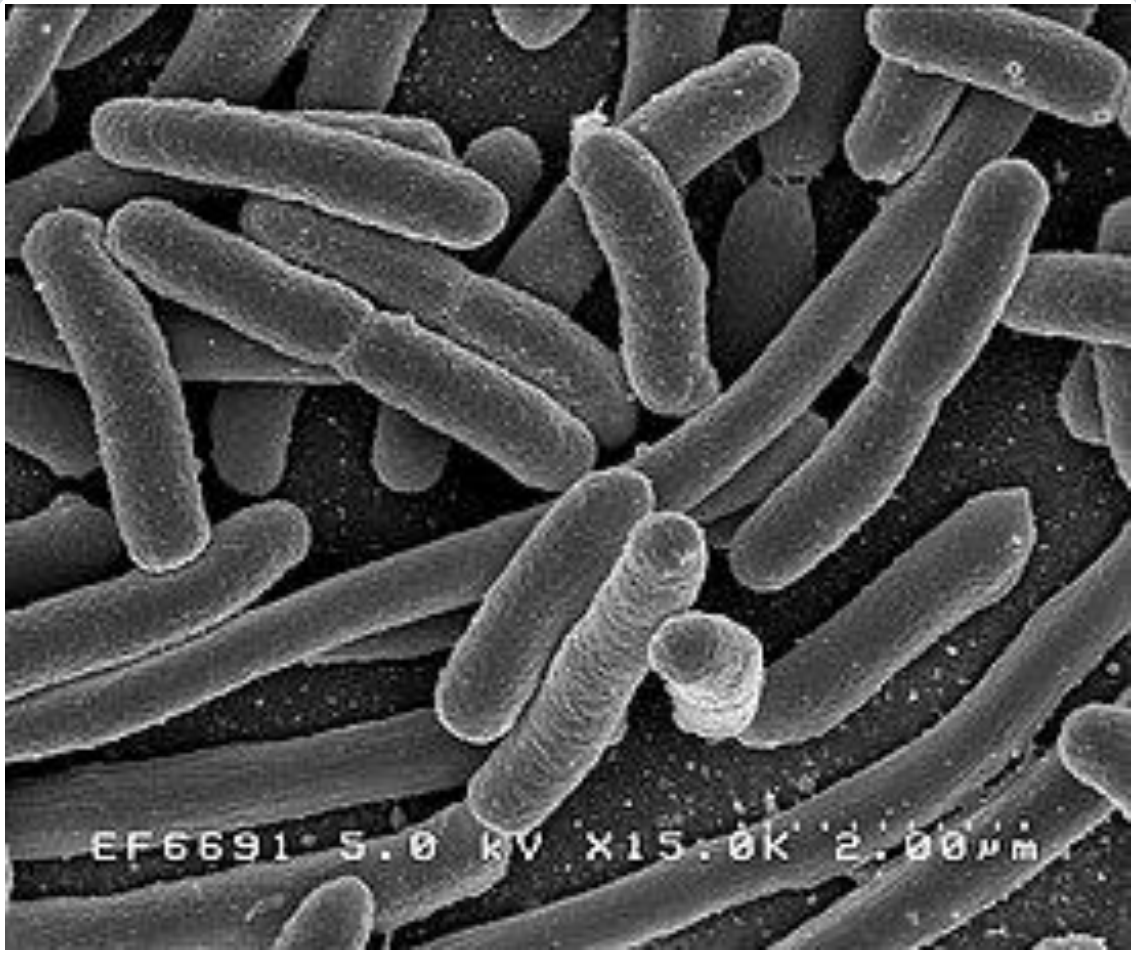
★ Key Takeaway

- Moving beyond culture-dependent biases—over 50–90% of plant-associated microbes remain uncultured on standard agar
- **Comparison scope (5):** Amplicon Sequencing; Shotgun Metagenomics; Metatranscriptomics; Metaproteomics; Metabolomics
- **Priority synthesis:** Metabolomics — Directly maps chemical communication; challenging isomer/origin assignment

Six Mechanisms of Plant Growth-Promoting Bacteria (PGPB)

Direct nutritional facilitation and hormonal tuning enhance crop resilience under low-input farming

- **01 — Biological N_2 Fixation:** Nitrogenase (nifH/D/K) reduces atmospheric $N_2 \rightarrow 2NH_3$; Symbiotic (Rhizobium, Frankia) & associative (Azospirillum)
- **02 — Phosphate & K Solubilization:** Gluconic/citric acid excretion releases Ca/Fe/Al-bound PO_4^{3-}; Phytases mineralize recalcitrant soil organic phosphorus
- **03 — Siderophore Fe^{3+} Capture:** High-affinity pyoverdines/enterobactin scavenge insoluble Fe^{3+}; Feeds host while starving soilborne fungal pathogens of iron
- **04 — Phytohormone Modulation:** Microbial IAA stimulates lateral root proliferation & hair growth; Gibberellins & cytokinins boost leaf expansion & grain fill
- **05 — ACC Deaminase (acdS):** Cleaves ethylene precursor ACC into α-ketobutyrate + NH_3; Prevents growth arrest under drought, salt, & flooding stress
- **06 — VOCs & Osmoprotectants:** 2,3-Butanediol & acetoin volatiles promote growth & immunity; Bacterial trehalose, proline & EPS biofilms retain root moisture



Microbial colonization and Plant Growth-Promoting Bacteria (PGPB)

Symbiotic Biofertilization: Rhizobia and Arbuscular Mycorrhiza

Harnessing evolutionary nutrient symbioses to lower synthetic N and P fertilizer dependency

Symbiotic & Endophytic Nitrogen Fixation

- Legume nodules deliver low-O_2 micro-environment via leghemoglobin protecting O_2-labile nitrogenase
- **Energetically demanding:** 16 ATP required per N_2 molecule reduced
- Free-living & endophytic diazotrophs (Azospirillum, Gluconacetobacter, engineered Klebsiella) colonize cereals
- **Frontiers (2024–2026):** CRISPR-tuning root exudation and synthetic nitrogenase modules for non-legumes

Arbuscular Mycorrhizal Fungi (AMF) Hyphal Networks

- Glomeromycota (Rhizophagus irregularis) form symbiotic arbuscules inside >80% of terrestrial plant families
- Extraradical hyphae extend meters beyond the root phosphate-depletion zone
- Host PT4 (phosphate transporter 4) specifically imports mycorrhiza-delivered inorganic phosphate (Pi)
- Common Mycorrhizal Networks redistribute water, nutrients, and early herbivory warning signals between plants

Microbial Priming of Plant Immunity: ISR vs SAR

Beneficial microbes train plant immunity for rapid response without chronic yield-limiting defense activation

01

Induced Systemic Resistance (ISR): Triggered by non-pathogenic root-colonizing PGPB (Pseudomonas simiae, Bacillus velezensis, Trichoderma); Signaling relies primarily on Jasmonic Acid (JA) and Ethylene (ET) pathways, independent of SA bursts; Key transcription factor MYC2 primes callose deposition, lignification, and phytoalexin synthesis; Low fitness cost: defenses ramp up rapidly only upon actual insect or necrotrophic pathogen attack

02

Systemic Acquired Resistance (SAR): Triggered locally by necrotizing foliar/root pathogens via effector recognition and cell death; Dependent on Salicylic Acid (SA) biosynthesis (ICS1) and master co-regulator NPR1 translocation; Induces constitutive systemic accumulation of Pathogenesis-Related (PR-1, PR-2, PR-5) proteins; Strong broad-spectrum protection against biotrophic fungi, oomycetes, and bacterial pathogens

Agronomic Potential and Market Scale of Plant Microbiomes

Synthetic bioinoculants increasingly complement chemical inputs in regenerative agriculture (2024–2026)

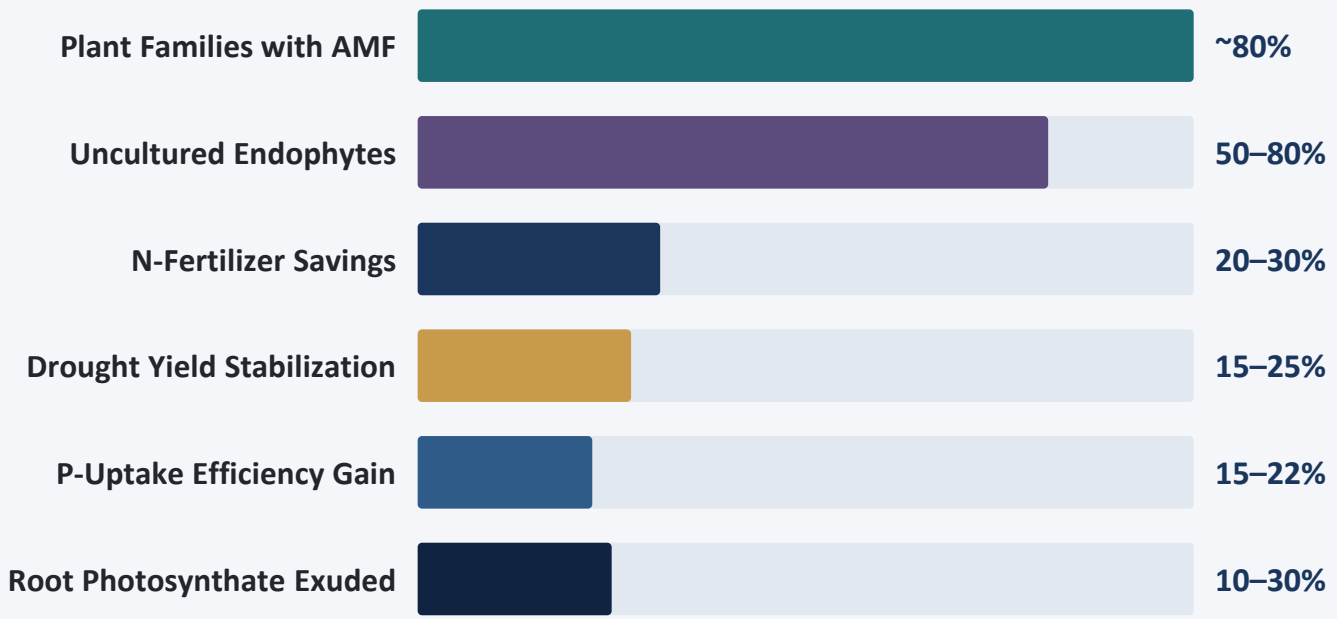
20–30%
Synthetic N Reduction

+10–25%
Yield Under Stress

$3.5B+
Global Biofertilizer Mkt

>50%
Taxa Yet Uncultured

Reported Field Gains & Microbial Resource Metrics (%)



Translation Bottlenecks

- Single-strain bioinoculants often fail due to soil competition & native microbiome priority effects
- Multi-strain SynComs paired with compatible host genotypes stabilize field colonization
- Biotic/abiotic carrier formulation & shelf-life dictate commercial farm adoption

Synthesized from Trivedi et al. (Nature Reviews Microbiology), Busby et al. (PLoS Biology), and FAO biofertilizer assessments.

Pathobiome Dynamics: Pseudomonas syringae & Dysbiosis

How bacterial pathogens breach host defenses and why healthy microbiomes suppress disease

Pseudomonas syringae Pathogenesis

- Type III Secretion System (T3SS) injects ~30–40 virulence effectors directly into plant cytoplasm
- Effectors (AvrPto, HopM1) dismantle FLS2/BAK1 PAMP-triggered immunity and hijack vesicle trafficking
- Phytotoxin coronatine structurally mimics JA-Ile, forcing stomata open for apoplastic bacterial entry
- Ice Nucleation Protein (InaZ) catalyzes ice crystal formation above -2°C, rupturing cells to liberate nutrients

Microbiome Dysbiosis vs Suppressive Soils

- Dysbiosis occurs when abiotic shock or monoculture collapses alpha-diversity and protective taxa
- Disease-suppressive soils naturally curb take-all (Gaeumannomyces) and Fusarium wilt via DAPG/phenazines
- Phyllosphere commensals (Sphingomonas, Methylobacterium) outcompete P. syringae for limiting leaf sugars
- Phage cocktails and antagonistic Bacillus biofungicides offer precision pathobiome control

Rational Engineering of Synthetic Microbial Communities (SynComs)

Bottom-up construction of defined, reproducible consortia with predictable plant phenotypes

1 Biobanking & WGS

- High-throughput culturomics (dilution-to-extinction)
- Whole-genome annotation of functional traits

2 Network Hubs

- Co-occurrence modeling identifies keystone hubs
- Select complementary metabolic guilds

3 Compatibility Test

- Exclude mutual antagonism & antibiotic kill
- Verify co-growth on root exudate mimics

Step 4 Drop-Out Assays

- Test in gnotobiotic microcosms (leave-one-out)
- Quantify causal host biomass & immunity gain

Step 5 Field Formulation

- Alginate/biochar seed-coat encapsulation
- Validate resilience across diverse soil types

Minimal SynComs (5–15 strains) offer ecological redundancy without overwhelming regulatory complexity.

Breeding the Holobiont: Host Genetics Shapes Microbiota

Integrating microbiome traits into crop improvement programs after decades of breeding under high chemical inputs

- **Domestication Bottleneck:** Green Revolution cultivars selected under high NPK often lost strong responsiveness to wild AM fungi & rhizobia; Wild crop ancestors harbor distinct exudate alleles recruiting stress-protective microbiota
- **Microbiome GWAS (mGWAS):** Maps host plant SNPs regulating abundance of keystone rhizosphere taxa as quantitative genetic traits; Identifies host immune, cell-wall, and transporter loci controlling microbial heritability
- **CRISPR Exudate Tuning:** Editing root exudate transporters (MATE, SWEET, ABC) boosts beneficial colonization without pathogen feeding; Fine-tunes flavonoid output in cereals to favor associative nitrogen fixers
- **Engineered Bioinoculants:** Commercial release of gene-edited associative diazotrophs (e.g., constitutively fixing nifA mutants in maize); Supplies 20–30 kg N/ha directly at the root surface while reducing nitrate leaching



Breeding the holobiont: GWAS and genomic modeling of host-microbiome traits

Control Questions and Key Takeaways

Integrating microbial ecology with modern plant molecular genetics

? Control & Review Questions

- 1. Define the plant holobiont and explain the two-step model of rhizosphere and endosphere microbiome assembly.
- 2. How do root exudates such as flavonoids, strigolactones, and coumarins coordinate the «cry for help» response?
- 3. Compare the informational resolution and biases of 16S/ITS amplicon sequencing vs shotgun metagenomics and metatranscriptomics.
- 4. Contrast the signaling molecules, master regulators, and fitness costs of ISR vs SAR immunity.
- 5. Outline the five stages of designing and validating a synthetic microbial community (SynCom) for field crops.

✓ Key Takeaways

- Plants actively curate distinct microbial consortia in rhizosphere, endosphere, and phyllosphere niches via exudates.
- Multi-omics links community composition (16S/ITS) directly to active nitrogen, phosphorus, and stress pathways.
- PGPB enhance nutrient uptake, cleave stress ethylene (ACC deaminase), and prime JA/ET-dependent ISR immunity.
- Next-generation breeding treats host–microbiome assembly as a heritable target via mGWAS and SynComs.

Recommended Literature and Microbiome Databases

Foundational reviews and bioinformatic platforms for plant microbiome analysis

1 Main Literature

- 1. Trivedi, P., Leach, J.E., Tringe, S.G., Sa, T., Singh, B.K. (2020). Plant–microbiome interactions: from community assembly to plant health. Nature Reviews Microbiology 18: 607–621.
- 2. Bulgarelli, D., Schlaeppi, K., Spaepen, S., van Themaat, E.V.L., Schulze-Lefert, P. (2013). Structure and functions of the bacterial microbiota of plants. Annual Review of Plant Biology 64: 807–838.
- 3. Compant, S., Samad, A., Faist, H., Sessitsch, A. (2019). A review on the plant microbiome: ecology, functions, and emerging trends. Journal of Advanced Research 19: 29–37.
- 4. Pieterse, C.M.J. et al. (2014). Induced systemic resistance by beneficial microbes. Annual Review of Phytopathology 52: 347–375.

2 Additional Literature & Databases

- 1. Song, C. et al. (2021). Designing synthetic microbial communities for crop protection and soil resilience. Trends in Microbiology.
- **2. Xin, X.F., Kvitko, B., He, S.Y. (2018). Pseudomonas syringae:** what it takes to be a pathogen. Nature Reviews Microbiology 16: 316–328.
- **3. Reference Taxonomic & Analysis Databases:** SILVA (arb-silva.de), UNITE Fungal ITS, QIIME 2, GTDB.
- 4. Escudero-Martinez, C., Bulgarelli, D. (2023). Tracing host genetic determinants of the plant microbiota (mGWAS). Plant Journal.

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