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NEXT GENERATION MICROBIAL BIOTECHNOLOGY UNLOCKING ADVANCED-LEVEL PLANT BREEDING POTENTIAL FOR SUSTAINABLE AGRICULTURE: A REVIEW

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ABSTRACT

The integration of microorganisms into advanced-level plant breeding has revolutionized sustainable agriculture by providing precise, and acceptable solutions to the posed several challenges by climate changeability, soil degradation, and food security. Beneficial plant-soil-microbes interaction like plant growth-promoting rhizobacteria (PGPR), endophytes, mycorrhizae etc. play a key role in stimulating plant traits through different mechanisms involving hormone modulation, stress tolerance, and nutrient exchange. Beyond conventional biofertilizer importances, recent advances expose the involvement of microorganisms in completed breeding strategies for example genome editing technique CRISPR-CAS, microbe-assisted genomic selection, epigenetic modulation, and marker-free transgenic strategies. Moreover, microbes work as accelerator for boosting QTL mapping, genome-wide association studies (GWAS), and precision phenotyping by shaping plant-microbe interactions and effecting gene expression. Horizontal gene transfer (HGT) processed by microbial contributions to natural genetic innovation, facilitating gene transfer and functional diversity in crop genomes. The application of synthetic microbial consortia and microbial-composed biomolecules further ongoing new frontiers in crop improvement. As research detailed study of multi-omics approaches like microbial genomics, metagenomics, and synthetic biology, the integrated application of microbial biotechnology and plant breeding holds immense capable to develop resilient, high-yielding, and environmentally acclimatize crop cultivars. This paradigm shift refines breeding structures, moving toward a microbiome-centric approach in next-generation microbial-biotechnology engine of advanced-level plant breeding for crop improvement in sustainable agriculture.

Key words: Microbial breeding, Plant-microbe interaction, CRISPR, PGPR, Endophytes, Epigenetics, QTL mapping, GWAS, Horizontal gene transfer, Synthetic microbiome, Precision agriculture, Natural genetic engineering

Introduction

The integration of next generation microbial biotechnology into advanced- level plant breeding represents a transformative shift toward in sustainable

agriculture for crop improvement (Das *et al.*, 2023; Khan *et al.*, 2024; Riaz *et al.*, 2025). Historically microorganisms are regarded as passive soil inhabitants or biocontrol agents and now recognized as dynamic participants in plant

development, genome diversity and plasticity (Ciancio *et al.*, 2019; Muhammad *et al.*, 2025). With the convergence of omics approaches, synthetic biology, and precision agriculture, the microbiome is being recognized as a critical “second genome” of plants (Huo and Wang, 2024; Singh *et al.*, 2025), directly effecting phenotypic expression, epigenetic modulation, and trait heritability (Busby *et al.*, 2017; Compant *et al.*, 2025).

In the current scenario day by day climate change, soil quality deterioration, and the urgent requirement for resilient crop varieties, microbial approaches provide a bio-based alternative to conventional breeding bottlenecks (Mikiciuk *et al.*, 2024; Nwankwo *et al.*, 2025; Pehlivan *et al.*, 2025). Unlike traditional approaches (Wankar, 2025; Verma *et al.*, 2025) that act as solely on Mendelian inheritance, microbe-assisted breeding exhibits the natural and synthetic capabilities (Marco *et al.*, 2022; Srivastava *et al.*, 2025) of microbes to enhance genetic diversity (Thoenen *et al.*, 2023), regulate hormonal crosstalk, alter stress responses, and increase nutrient-use efficiency in crops (Glick, 2023; Niu *et al.*, 2017). Most important microorganism such as endophytes (Anand *et al.*, 2022) rhizobacteria, mycorrhizal fungi, and actinomycetes play essential roles in shaping host plant physiology (Ansari *et al.*, 2020) by effecting gene expression, secondary metabolite synthesis pathway, and even horizontal gene flow (Sena *et al.*, 2024).

Recent breakthroughs in CRISPR- Cas based editing applying microbial enzymes e.g., Cas9, Cas12a (Dudeja *et al.*, 2025) and metagenome-wide association studies (mGWAS) (Gai *et al.*, 2025) have further explored the potential of microbiota in precise genome editions (Chaudhari *et al.*, 2024) and trait predictions. Moreover, plant-microbiome interaction studies have demonstrated (Chambial *et al.*, 2025) that host genotype microbial assembly and that microbial heritability can be harnessed in next-generation breeding pipelines (Mueller and Linksvayer, 2022).

Thus, microorganisms are not only longer viewed as ancillary agents but also as central elements of advance-level plant breeding programs. The future of crop improvement will likely be based on a synergistic integration approach of plant genomics, microbial ecology, and artificial intelligence-driven microbiome engineering to make climate-smart, high-yielding cultivars.

Microbe-Assisted Breeding for Stress Tolerance

The enhancing frequency of abiotic stresses (Marco *et al.*, 2022) like drought, cold, salinity, heat, extreme temperatures, and minerals and nutrient deficiencies (Srivastava *et al.*, 2025) poses an important role threat

to world’s food security (Irfan *et al.*, 2023). Conventional plant breeding for stress resilience is generally developed by limited genetic diversity, long breeding cycles, and unpredictable genotype-environment interactions (Hafeez *et al.*, 2023). In this context microbe-assisted breeding (MAB) (Ahanger *et al.*, 2023) explores as a promising complementary strategy, using beneficial microorganisms to enhance plant stress tolerance and inform the selection of resilient genotypes (Basu *et al.*, 2022).

Mechanisms of Microbial Stress Alleviation

Plant-associated microbes (Dai *et al.*, 2024, Shrivastava and Kumar, 2015) particularly plant growth-promoting rhizobacteria (PGPR) (Sharma *et al.*, 2020) and endophytes (Bhagya *et al.*, 2019; Emitaro *et al.*, 2024), reservoir stress resilience through multiple direct and indirect mechanisms, including: Phytohormone (Kloepper *et al.*, 2004; Santosh *et al.*, 2024; Vishwakarma *et al.*, 2024), microbes modulate plant hormones like abscisic acid (ABA), indole-3-acetic acid (IAA), salicylic acid (SA) (Brar *et al.*, 2025) and ethylene to plant responses under stress. For example, ACC deaminase enzymes producing bacteria lower ethylene levels under stress, promoting root growth and cellular homeostasis (Glick, 2023). Osmolyte bioactive accumulation and antioxidant defence (Hardoim *et al.*, 2015). Microbial bio-inoculants enhance the bio-synthesis pathway of proline, glycine betaine, and secretion of antioxidant enzymes like SOD, CAT, APX (Nadeem *et al.*, 2014; Sachdev *et al.*, 2021) which protect cellular environment from oxidative damage caused by reactive oxygen species (ROS) or free radical under stress condition such as drought and salinity etc., (Muhammad *et al.*, 2024). Stress-responsive gene expression recognition by colonization certain PGPR triggers effective systemic tolerance through upregulation of genes related to osmotic adjustment, cellular membrane stability and heat shock proteins (HSPs), (Fig. 1). For

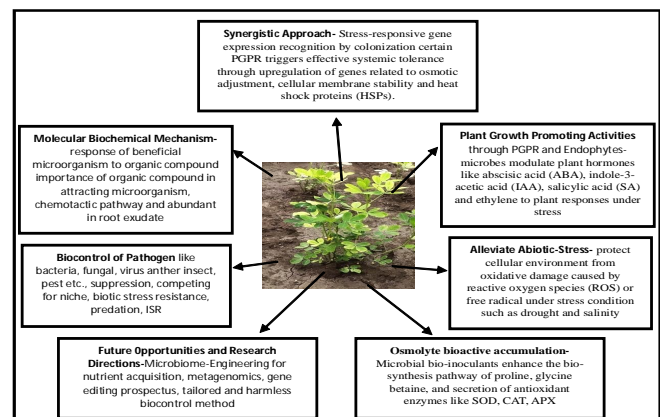


Fig. 1: Mechanisms of Microbial Stress Alleviation.

instance, using bio-inoculation of wheat with *Bacillus velezensis* introduced the expression of DREB2A and NHX1, increasing tolerance against fluctuation salt and drought (Giannelli *et al.*, 2023).

Application in Breeding Programs

Microbe-assisted breeding (MBC) integrates microbiome functionality into the phenotyping and selection processes of plant breeding (Ogidi and Richard-Nwachukwu, 2024). Trait-based screening breeding lines can be evaluated based on their ability to recruit and respond to beneficial microbes under stress conditions (Barnes *et al.*, 2025). This includes assessing root exudate composition, rhizosphere microbial profiles, and plant physiological responses (Compant *et al.*, 2025). Core microbiome selection recent advances in microbiome-wide association studies (mGWAS) and metagenomics approaches (Gai *et al.*, 2025; Chaudhari *et al.*, 2024) permits breeders to identify plant genotypes that harbour a durable and functional core microbiome associated traits with stand stress resilience (Marco *et al.*, 2022; Srivastava *et al.*, 2025) synthetic microbial consortia or genome engineered microbial (Chaudhari *et al.*, 2024) communities enhanced to target plant genotypes are being developed to standardize beneficial microbial effects in wider agroecosystem and reproducible trait enhancement in breeding trials (Portal-Gonzalez *et al.*, 2015).

Recent Advances

According to Misu *et al.*, (2025) studied rice and drought tolerance bacteria in which inoculation of rice with *Azospirillum lipoferum* and *Burkholderia phytofirmans* enhanced water-use efficiency and yield

under water deficit condition by modulating ABA biosynthesis pathway and stomatal behaviour expression. Singh *et al.*, (2022) strongly suggested that maize and heat stress endophytic *Enterobacter cloacae* was observed to enhance thermotolerance in maize by increasing chlorophyll membrane rigidity and reactive oxygen species (ROS) capturing enzymes, leading to long duration survival and productivity. In case of wheat Huang *et al.*, (2022) studied on salinity stress, application of halotolerant *Pseudomonas stutzeri* in saline soils effectively increased tiller number, grain filling, and sodium exclusion, suggesting its application in saline breeding programs.

In conclusion, microbe-assisted breeding serves a powerful approach to increase crop stress tolerance in an eco-safely, cost-effective, and programmable manner. By maintain microbial symbiosis association, breeders can choose plant genotypes with enhanced resilience traits and stability under fluctuating climatic conditions. Integration of multi-omics, AI-based microbiome modelling, and core microbiome engineering will further accelerate this synergy in future breeding frameworks.

Microbial-Induced Epigenetic Modulation

According to Kaya *et al.*, (2023) recent advances in plant-microbe interactions have studied those beneficial microorganisms can alter plant epigenomes for improvement in plant breeding and stress adaptation. Epigenetic regulation with several strategies such as DNA methylation, histone modifications, and non-coding RNAs, plays a key role in managing gene expression without altering the DNA sequence. Microbial colonization can enhance heritable epigenetic changes in plants, boosting growth, immunity, and environmental adaptability making it a promising technique for epigenetic-breeding strategies (Saijo and Loo, 2020; López-Arredondo *et al.*, 2023).

Mechanisms of Microbial-Induced Epigenetic Changes

Microorganisms several types like endophytes, rhizobacteria, and mycorrhizal fungi modulate epigenetic frameworks via the following effective mechanisms (Bhimani *et al.*, 2024), (Fig. 2).

- (a) **DNA Methylation Changes:** Kapazoglou *et al.*, (2025) studied Microbial colonization affect cytosine methylation (5mC) patterns in plants, especially in promoters of stress-putative genes, leading to transcriptional-level reprogramming. Yang *et al.*, (2022) conducted experimental study on *Pseudomonas simiae* WCS417 colonization in *Arabidopsis thaliana* caused extensive changes in DNA methylation at defence-related

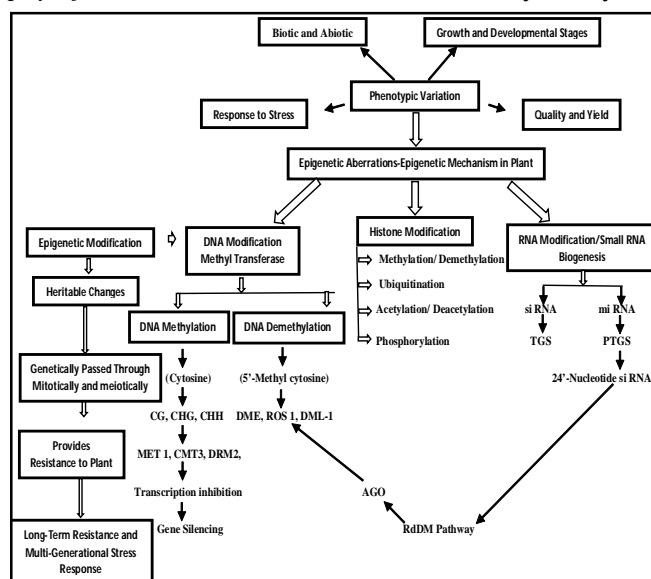


Fig. 2: Mechanisms of Microbial-Induced Epigenetic Changes.

loci, leading to long-lasting priming against pathogens.

- (b) **Histone Modification:** In epigenetic approach, microbial interactions affect histone acetylation (H3K9ac) and methylation (H3K4me3, H3K27me3), altering chromatin accessibility and transcriptional activation of stress-related genes. For example, Roy *et al.*, (2021) suggested that colonization by *Piriformospora indica* induced H3K4me3 enrichment in defence genes, promoting resistance to *Fusarium oxysporum* in tomato plants.
- (c) **Small RNAs and non-coding RNAs:** According to Salunke *et al.*, (2025) beneficial microbes can regulate plant microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), which in turn control downstream gene array included in growth, development, and immunity. Jia *et al.*, (2022) conducted experimental studied on *Oryza sativa*, inoculation with *Azospirillum brasilense* altered the expression of miRNAs targeting auxin signalling and defence regulators, contributing to drought tolerance.

Heritability and Application in Epibreeding

Bashir and setyawati, (2025), one of the most promising implications of microbial-induced epigenetic changes is their potential heritability across generations: studies have shown transgenerational inheritance of stress-induced DNA methylation changes following microbial priming, providing new way for epigenetic breeding (Epibreeding). Epigenetic memory conferred by microbial associations could be enhanced in breeding programs to develop “primed” cultivars or varieties that exhibit quick responses to stress with mild genetic modification (Williams, 2024).

Biotechnological Applications and Tools

According to Kruthi *et al.*, (2024), Epigenome-wide association studies (EWAS) and single-cell epigenomics are being employed to plant epigenetic responses to microbial bio-inoculants. CRISPR- Cas based tools like dCas9-TET1 or dCas9-HDACs guided by microbial signals are under exploration for desired epigenome editing in plants (Zhou *et al.*, 2023).

In, summary microbial-induced epigenetic alteration opens a novel frontier in plant breeding, providing an additional information of heritable phenotypic plasticity or durability without genetic alteration. As our understanding of microbiome–epigenome interactions deepen, targeted manipulation of plant epigenetics through

efficient microbes could revolutionize breeding strategies for climate resilience, yield improvement, durability and stability, and improved stress tolerance.

Horizontal Gene Transfer and Natural Genetic Engineering

According to Jonkheer, (2024), Horizontal gene transfer (HGT), the non-Mendelian transmission of genetic material across different species or kingdoms, is increasingly recognized as a significant driver of plant genome evolution and adaptation. Unlike vertical inheritance, HGT enables organisms including plants to acquire novel traits rapidly from microbial partners, bypassing the slow processes of mutation and selection. This phenomenon is not only central to microbial evolution but also has profound implications in plant breeding and genetic engineering.

Microbes as Agents of Horizontal Gene Transfer

Husnik and Cutcheon, (2018) strongly suggested that microorganisms, particularly bacteria and fungi, act as both donors and mediators of genetic exchange. Several mechanisms facilitate HGT. According to Gelvin, (2023) *Agrobacterium*-mediated transformation, the soil bacterium *Agrobacterium tumefaciens* is a natural genetic engineer that transfers T-DNA from its Ti plasmid into the plant genome through a type IV secretion system. This process has been extensively harnessed in plant genetic engineering for the introduction of agronomically important genes, including those for disease resistance, herbicide tolerance, and nutritional improvement. Endophytic gene transfer emerging evidence Chen *et al.*, (2019) suggests that certain endophytic microbes can facilitate inter-kingdom gene transfer via mobile genetic elements such as transposons, integrative conjugative elements (ICEs), and plasmids, introducing adaptive traits directly into the plant host genome fungal symbionts Arbuscular mycorrhizal fungi (AMF) and certain pathogenic fungi have been found to carry and potentially transfer effector genes or biosynthetic gene clusters to plant hosts, influencing plant immunity and metabolism (Sperschneider *et al.*, 2015), (Fig. 3).

Evidence of HGT in Plants

Mitochondrial HGT; several plant mitochondrial genomes contain genes of bacterial origin, particularly from proteobacteria and cyanobacteria, suggesting historical HGT events that contributed to metabolic adaptations (Keeling *et al.*, 2024). Nuclear genome insertions; genes related to stress tolerance and metabolite biosynthesis in some angiosperms have shown strong homology with microbial genes, indicating ancient or recent HGT from symbiotic or epiphytic microbes. CRISPR

elements in plants; while rare, some plant genomes exhibit sequences homologous to microbial CRISPR loci or Cas-like proteins, possibly acquired through environmental microbial associations or endosymbiotic events (Singh *et al.*, 2021).

Biotechnological Applications of Natural Genetic Engineering

Understanding and mimicking HGT pathways and synthetic biology applications in breeding; *Agrobacterium*-based transformation strategies remain the most adaptable and widely used method for stable plant genome modification. New protocols have enhanced its efficacy in recalcitrant crops like wheat, maize, and legumes (Gelvin, 2017). De novo domestication using microbial vectors with synthetic T-DNA has enabled the recombinant-engineering of wild species into cultivars or varieties with desired traits (Hefferon and Herring, 2017). Bioengineering of endophytes to carry efficient traits and deliver genetic circuits into plant cells is under exploration as a novel in planta gene delivery system, potentially replacing the need for tissue culture in genetic transformation (Thakur *et al.*, 2023).

Challenges and Bioethical Considerations

James, (2024) strongly suggested that HGT provides powerful tools for crop improvement, it raises concerns about gene containment, harmful ecological effects, and biosafety issues. Regulatory body must evolve to distinguish between naturally occurring genetic exchange and synthetic HGT (via GMOs), and especially as precision breeding tools like CRISPR-Cas are employed microbial vectors.

Conclusion, horizontal gene transfer and natural genetic engineering denotes the outer line of plant genome plasticity and diversity. By managing microbial systems for gene delivery and transformation, plant breeders and

biotechnologists can successfully rapid trait introgression, introducing the potential for developing resilient, high-yielding, and climate-smart crops. As omics approaches and bioinformatics tools continue to illuminate the complexity of inter-kingdom gene transfer, the next era of breeding will increasingly rely on microbial based genetic innovation.

Microbial Metabolites as Natural Mutagens and Growth Regulators

According to Marks *et al.*, (2025) microbial metabolites have enhanced as essential biochemicals in plant biotechnology and breeding due to their ability to fluctuate plant morphogenesis and genetic makeup. These bioactive promoters like auxins, cytokinins, gibberellins, and volatile organic compounds (VOCs) play dual roles (Fincheira and Quiroz, 2018); firstly, they act as natural plant growth regulators (PGRs) and another have mutagenic agents that creates genetic and phenotypic diversity. These properties are especially exploited in plant tissue culture, where microbial metabolites can be introduced through somaclonal variation, callus induction or differentiation, and somatic embryogenesis, thereby providing clonal propagation and the development of novel plant genotypes (Yapýcý *et al.*, 2021).

Somaclonal Variation Induced by Microbial Metabolites

According to Cusson *et al.*, (2017) somaclonal variation refers to the genetic and epigenetic changes observed in plants regenerated from in vitro cultures. Certain microbial metabolites can act as mild natural mutagens, enhance chromosomal rearrangements or activate transposable compounds, thus introducing variability without application of harmful chemicals. Volatile organic compounds (VOCs) secreted by *Bacillus subtilis* and *Pseudomonas fluorescens* like 2,3-butanediol and acetoin have been found to induce systemic signalling pathways and epigenetic level modifications in plant tissue cultures. These VOCs can affect function of histone acetylation and DNA methylation patterns, contributing to heritable epigenetic variation in regenerants (Kumar *et al.*, 2023).

Microbial Auxins in Callus Induction and Somatic Embryogenesis

Jha and Saraf, (2025) studied Auxins, particularly indole-3-acetic acid (IAA), are central to cell division, root growth and differentiation in plant tissue culture. Microorganisms like *Azospirillum*, *Rhizobium*, and *Pseudomonas* spp. Bio-synthesize of IAA through tryptophan- pathways. *Azospirillum brasilense* is a well-known PGPR secreting IAA, which promotes root

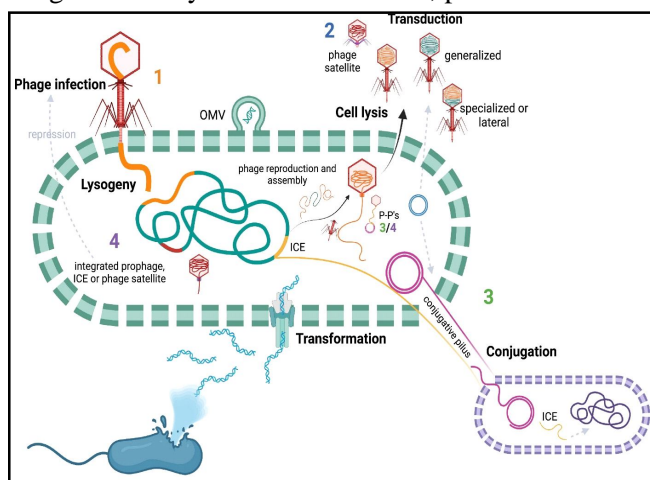


Fig. 3: Mechanism of Horizontal Gene Transfer Adapted de *et al.*, (2023).

elongation and inductions somatic embryogenesis in different plant species cultured *in vitro* (Eid *et al.*, 2021). Rodriguez-Molina *et al.*, (2023) studies have shown that microbial auxin phytohormone can stimulate dedifferentiation of explants, induction callus formation, and promote pro-embryo development in legumes and cereals crops.

Cytokinin-Producing Microbes and Shoot Regeneration

Ahmad *et al.*, (2023) strongly suggested that Cytokinins promote cell division and shoot initiation in tissue cultures. Endophytic and rhizospheric microbes like *Methylobacterium spp.*, *Agrobacterium tumefaciens*, and *Bacillus megaterium* are natural secreters of cytokinins. These microbial cytokinins enhance shoot meristem initiation, callus greening, and bud formation, essential for direct organogenesis *in vitro* (Wang *et al.*, 2025). The synergistic action of microbial auxin and cytokinins is reported to increase regeneration ability in recalcitrant species like *Capsicum annuum* and *Oryza sativa* (Singh *et al.*, 2021).

Gibberellins and Enhancement of Somatic Embryogenesis

According to Zdravkoviæ-Koraæ *et al.*, (2023) Gibberellins released by fungi (*Gibberella fujikuroi*) and bacteria (*Bacillus subtilis*, *Acinetobacter spp.*) are known to promote embryo growth, maturation and elongation in plant tissue cultures. Microbial GAs enhances pro-embryo induction to plantlets and enhances elongation of shoots and hypocotyls during regeneration processes (Keswani *et al.*, 2022). Application of *Bacillus*-derived GA to *Solanum lycopersicum* cultures has been reported to enhance the frequency and size of somatic embryos (Tiwari *et al.*, 2023).

VOCs and Embryogenic Competence

According to Fincheira and Quiroz, (2018) Microbial VOCs act as signalling molecules that effect plant growth and development at a distance. These gaseous metabolites can able to promote cellular totipotency, induce callus regeneration, and upregulate expression of embryogenesis-related genes (Cusson *et al.*, 2017). VOCs like acetoin and 2-pentylfuran were observed to upregulate embryogenic markers such as *WUSCHEL* and *LEC1* in *Arabidopsis* and rice tissue cultures, enhancing embryogenic capacity (Ikeda *et al.*, 2020; Xu *et al.*, 2024; Singh *et al.*, 2024).

Applications in Breeding and Biotechnology

In advance plant breeding, (Altaf *et al.*, 2024) natural mutagenic activity of microbial metabolites can be

increased for developing novel plant genotypes with improved gene expression. Somatic hybridization; increased callus induction, embryogenesis process and hybrid production via somatic cell fusion or protoplasm fusion. Clonal propagation; improved regeneration ability through microbial metabolites accelerates the micropropagation of elite genotypes. Stress-tolerant compounds; VOC, induced somaclonal variation (mutation) may produce cultivars with improved abiotic or biotic stress tolerance.

In summary, microbial metabolites offer a natural and sustainable alternative to synthetic phytohormones and mutagens in plant tissue culture. By modulating genetic expression, promoting morphogenetic pathways, and inducing somaclonal variation, these compounds play a pivotal role in plant regeneration, breeding, and clonal propagation. With recent advancements in microbial biotechnology strategies and omics-based metabolite sequencing, the use of microbial metabolites in precision plant breeding and *in vitro* micropropagation systems is poised to expand further.

Endophytes as Gene Reservoirs for Novel Trait Introduction

Dubey *et al.*, (2020); Tiwari and Bae, (2020) strongly suggested that Endophytic microorganisms, comprising both bacteria and fungi, are emerging as essential genetic reservoirs for the discovery and incorporation of novel traits in host genome due to their long-term association results co-evolution, and symbiotic relationship with plants, endophytes have developed dynamic genomic adaptations that enable them to survive under diverse and stressful extreme environmental or climatic condition and include gene clusters that are responsible for abiotic stress tolerance such as drought, salinity, temperature extremes, and biotic stress resistance like against pathogens and herbivores, nutrient acquisition, phytohormone biosynthesis, and secondary metabolite production.

Genetic Potential of Endophytes

Dinesh *et al.*, (2027) studied the genomes of endophytes contain biosynthetic gene clusters (BGCs) that are not present in their free-living or without plant environment. These BGCs code for a wide range of bioactive compounds, like antimicrobial peptides, siderophores, polyketides, and non-ribosomal peptides, many of which are important in agriculture and pharmaceuticals (Brader *et al.*, 2017). Furthermore, endophytes produce signalling molecules such as quorum sensing compounds and hormones (e.g., indole-3-acetic acid, gibberellins) that act as defence mechanism, modulate plant physiology system and stress responses

biotic as well as abiotic (Compant *et al.*, 2010).

Omics Approaches for Gene Mining

Recent advancements in next generation multi-omics approaches such as metagenomics, comparative genomics, transcriptomics, and functional genomics have facilitated the identification of molecular level beneficial genes within endophyte genomes (Wani *et al.*, 2022; Bastas and Kumar, 2023). Metagenomic strategies permits for the exploration of the unculturable majority of endophytic communities, whereas functional genomics can link gene presence with phenotypic expressions in plants. These approaches are valuable in identifying candidate genes for use in genome engineering and synthetic biology platforms aimed for crop improvement (Kaur *et al.*, 2024; Shishodia *et al.*, 2025).

In Case of *Piriformospora indica* (Gill *et al.*, 2016) a good example is the root-colonizing fungal endophyte. Genomic-level analyses of this fungus have identified multiple genes or loci linked to drought stress resistance, iron uptake, and responsible for plant growth promotion (Li *et al.*, 2023; For instance, Zuccaro *et al.*, (2011) identified gene families that are involved in iron mobilization, osmoregulation, free radical and reactive oxygen species (ROS) detoxification, which introduced to enhanced plant resilience. Such genes are being used for biofortification strategies, and trait transfer and introduced in breeding programs aimed at nutrient enrichment and stress adaptation or mitigation.

Synthetic Biology and Trait Transfer

With the advancement of CRISPR-Cas genome editing tools and synthetic biology systems, genes cluster from endophytes can be systematically transferred into

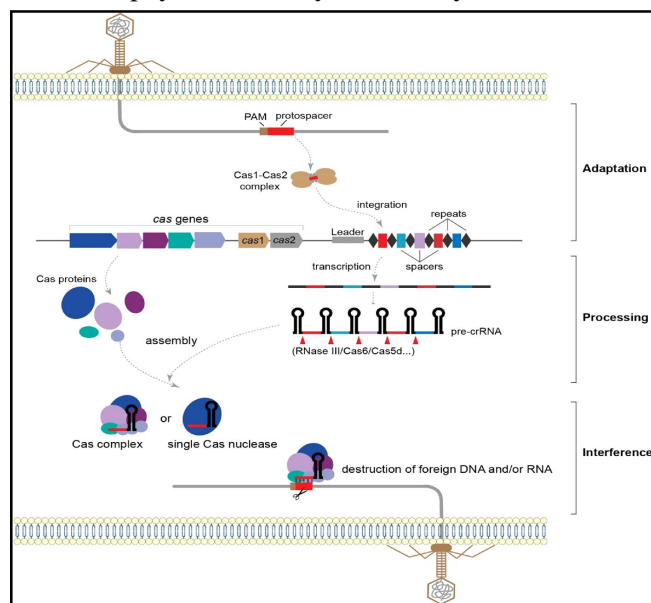


Fig. 4: CRISPR-Cas System Adapted by Wei *et al.*, (2023).

plants or microbial bio-inoculants (Sharma *et al.*, 2023; Thankappan *et al.*, 2024). This opens doors for designing efficient microbial consortia or genetically improved crops with specific resilience traits like phosphorus solubilization, biotic and abiotic stress resistance (drought tolerance). Endophyte-derived genes also hold potential in transgenic strategies for increasing host plant adaptability with respect to climate change (Argente-Martínez *et al.*, 2025).

CRISPR and Microbial Enzymes for Genome Editing

CRISPR terms refers as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and associated with Cas enzymes as alike endonuclease enzyme has revolutionized genome editing techniques across biological systems, with beneficial implications for sustainable agriculture. This gene editing, originally discovered as an adaptive immune system in prokaryotic bacteria namely *Streptococcus pyogenes*- using microbial enzymes like Cas type Cas9, Cas12a, and Cas13 to execute precise and programmable alterations of nucleic acid DNA or RNA sequences (Ghosh *et al.*, 2024), (Fig. 4).

Microbial Origin and Mechanism

Jinek *et al.*, (2012) discovered Cas9, a microbial endonuclease derived from *Streptococcus pyogenes*, functions by nicking double-stranded breaks (DSBs) at targeted genomic loci, guided by a synthetic single guide RNA (sgRNA) in laboratory. These double-stranded nicks are repaired by two mechanisms either non-homologous end joining (NHEJ) or homology-directed repair (HDR) mechanisms in the host genome, and based on gene knockouts, insertions, or precise base modifications (Ganger *et al.*, 2023), (Fig. 5).

Other microbial strains, like Cas12a (Cpf1) from *Francisella novicida* and Cas13 endonuclease systems that target RNA, have expanded the CRISPR toolkit, able for precise base editing, transcriptional regulation, and epigenome modulation without nicking DSBs (Zetsche *et al.*, 2015; Bandyopadhyay *et al.*, 2020; Kordys *et al.*, 2022).

Applications in Precision Plant Breeding

CRISPR/Cas genome editing tools are now widely usable in precision advanced- level breeding for crop improvement. Unlike transgenic approaches that introduce foreign DNA, CRISPR enables cisgenic or non-transgenic modification by editing desire genes. This is valuable in elite cultivars, where targeted editing can incorporate desire traits like disease resistance, abiotic stress tolerance, nutritional improvement, yield

enhancement and reduced the effect of allergenicity or antinutrients. For example, in case of disease-resistant crops Chen *et al.*, (2019) studied a notable application is the development of disease-resistant rice and tomato lines against bacterial and fungal disease. In rice, the CRISPR/Cas9 tools has been used to knock out *OsSWEET* genes, which are susceptibility loci caused by *Xanthomonas oryzae* in tomato, precise editing of the *Mlo* gene has conferred resistance against powdery mildew disease, demonstrating the efficiency of microbial enzymes in developing resistance crop varieties (Kumar *et al.*, 2020).

Furthermore, CRISPR has been incorporation with RNA-guided transcriptional regulators for gene expression control and CRISPR activation (CRISPRa)/CRISPR interference (CRISPRi) techniques for reversible and programmable modulation of crop traits (Ganger *et al.*, 2023).

Synthetic Biology and Multiplexing

According to Jiang *et al.*, (2025) the use of microbial CRISPR approaches in plants is also possible by synthetic biology systems, like artificially designing sophisticated sgRNA arrays to simultaneously edit multiple genes. This is necessary for polygenic or quantitative trait improvement and has been reported in crops such as wheat, maize, and soybean.

Microbial Bioinformatics and Predictive Breeding

The integration of microbial bioinformatics approaches into crop improvement has denoted a paradigm shift in how plant breeders understand and alteration the plant microbiome. Traditionally focused on plant genomics, advance-level breeding strategies are now enriched by data-driven approaches that use microbiome profiling, computational bioinformatics modelling, and predictive data analytics. These techniques help in the rational selection of microbial consortia that enhance crop productivity, stress tolerance, and overall agriculture and ecosystem sustainability.

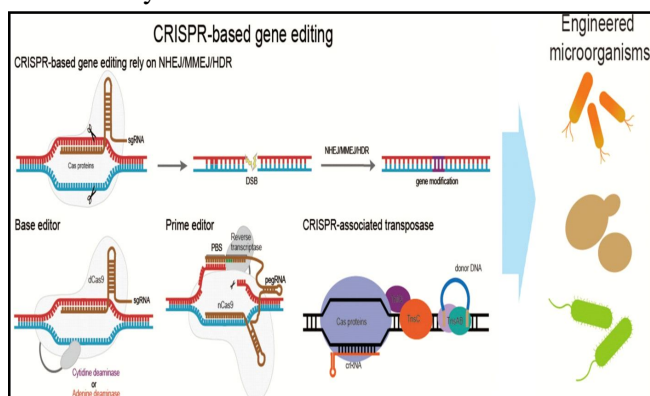


Fig. 5: CRISPR and Microbial Enzymes for Genome Editing Adapted by Wei *et al.*, (2023).

Microbiome Profiling and Genotype Interactions

According to Ullah *et al.*, (2023) plants host complex microbial communities especially in the rhizosphere, phyllo-sphere, and endo-sphere which are correlated by both host genotype and environmental conditions. Advances in next-generation sequencing (NGS) technologies and multi-omics like metagenomics have able provide for high-resolution microbial taxa profile associated with particular plant genotypes. (Minipara *et al.*, 2022). Studies have shown that different cultivars need distinct microbial assemblages due to variations in root exudate chemistry, immune signaling interaction, and developmental traits.

For instance, Bulgarelli *et al.*, (2012) demonstrated that different accessions of *Arabidopsis thaliana* selectively shape their root microbiome, providing key proof that host genetics interaction microbial community structure. These findings have opened up the possibility of predictive breeding, where microbial compatibility is considered parallel plant genetic traits.

Computational Modelling and Predictive Tools

Jain *et al.*, (2024) strongly suggested that microbial bioinformatics exhibits a range of computational tools, including; Machine learning algorithms tools help to prediction of recognition pattern and expression of crops and microbes and data analysis to study microbe-microbe and plant-microbe interactions. Multi-omics integration strategies like metagenomics, transcriptomics, proteomics for functional inference. Ecological modelling computational platform to enhance community dynamics under different environmental conditions. These models help breeders predict beneficial microbial integrations that can be leveraged for synthetic microbial consortia design or crop-specific bioformulations. For example, the use of Random Forests and Support Vector Machines (SVMs) can correlate microbiome strata with plant health metrics, disease suppression, or nutrient uptake efficiency (Chang *et al.*, 2017).

Integration with Genomic Selection

According to Xu *et al.* (2022) microbial bioinformatics complements genomic selection (GS) by providing additional information of biological system. While GS based on DNA markers to predict phenotypic performance, integrating microbiome data permits breeders to account for plant-microbe-soil-environment interactions. This integrated breeding structure can; improve trait heritability. Predict microbial inoculant success across genotypes and soils and guide climate-resilient cropping strategies. In Case of *Arabidopsis thaliana*, Bulgarelli *et al.*, (2012) conducted an elaborate

survey of the *A. thaliana* root microbiome and discovered that the host genotype significantly effects the microbial community composition. Specific bacterial taxa were discovered to be efficient in response to specific genotypes, demonstrating a genetically encoded ability to include beneficial microbes. This work manages the stage for host-mediated microbiome engineering in crops, where breeders can choose for genotypes that naturally foster beneficial microbial relationships.

Future Directions

With advancement of next generation technology and increasing access to AI-drive predictive modelling and large-scale microbiome databases e.g., MG-RAST, NCBI SRA, IMG/M, breeders can now make decision-support systems for microbial selection. Tailor microbial applications to specific soil types, climates, and crop varieties. Enhance field-position predictability of microbial bio-inoculant performance. This emergence of bioinformatics, microbiology, and genomics is paving the way for next-generation breeding programs that are not only gene-centric but also holobiont-aware, determining the plant and its microbiota as a single evolutionary.

Conclusion

Microorganisms are not only longer passive symbionts but also active agents in advanced-level plant breeding systems. Their importances span from genome engineering and epigenetic modulation techniques to stress tolerance and predictive breeding. Integrating next generation microbial biotechnology with multi-omics such as genomics, transcriptomics, metabolomics and genome editing CRISPR tools provides an unprecedented opportunity to breed resilient, high-yielding crop varieties or cultivars to support sustainable agriculture.

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