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Nanotechnology and bacterial biofilms in sustainable agriculture: mechanisms, stress mitigation, and rhizosphere applications

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Abstract

Increasing demand for sustainable agriculture technologies has stimulated research on nanotechnology and beneficial microbial approaches as alternatives to conventional agricultural inputs. Among these approaches, bacterial biofilms and nanomaterials offer complementary functions that can increase plant growth, nutrient availability, stress tolerance, and disease control. This review examines how nanomaterials interact with bacterial biofilms, focusing on the mechanisms involved and their potential agricultural applications. It covers biofilm formation, extracellular polymeric substances (EPS), quorum sensing (QS), and root colonization, as well as the physicochemical properties and agricultural functions of various nanomaterials. Particular attention is given to how nanoparticles affect bacterial viability, EPS production, biofilm architecture, cellular metabolism, and QS pathways. The review also covers the differential effects of nanomaterials on beneficial and pathogenic biofilms, including their ability to promote microbial colonization or inhibit pathogen colonization. It explores how they can be used together for nutrient management, plant growth promotion, abiotic stress mitigation, disease suppression, and soil remediation. However, despite promising results in laboratory and controlled experiments, practical applications are limited by dose-dependent nanoparticle toxicity, compatibility with microbes, formulation stability, and uncertainty about long-term environmental effects. Determining how reliable these systems are under agricultural conditions will require mechanistic studies, advanced imaging, standardized safety assessments, and field trials at multiple locations. Combining nanotechnology with biofilm-based microbial approaches opens up new possibilities for designing effective, eco-friendly strategies for sustainable crop production.

Keywords: Abiotic stress; Biocontrol; Biofilms; Extracellular polymeric substances; Sustainable agriculture

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1. Introduction

Agricultural production is negatively affected by several abiotic stresses, including salinity, drought, and extreme temperatures, as well as biotic stresses such as plant pests and pathogens (Uniyal *et al.*, 2025). These stressors affect photosynthesis, metabolic processes, nutrient uptake, and plant growth, particularly when multiple stressors co-occur. Despite being major agricultural solutions, agrochemicals such as pesticides and fertilizers can pollute the environment, degrade soil quality and health, and harm

non-target organisms, including humans, when used excessively (Uniyal *et al*., 2024). Consequently, there has been considerable interest in sustainable alternatives that utilize beneficial microbes and new farming techniques (Backer *et al*., 2018; Fanai *et al*., 2024; Kah *et al*., 2018; Sodhi *et al*., 2025). Plant growth-promoting bacteria (PGPB) aid plant development by fixing nitrogen, solubilizing phosphate, producing siderophores, regulating phytohormones, expressing 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, and biologically suppressing plant pests and pathogens (Backer *et al*., 2018; Glick, 2012; Lugtenberg & Kamilova, 2009; Uniyal *et al*., 2025). However, their agricultural impact depends largely on successful root association, colonization, and persistence amid changing soil conditions (Wheatley & Poole, 2018). Biofilm formation supports these processes by producing an extracellular polymeric substance (EPS) matrix that is made up of polysaccharides, proteins, and extracellular DNA (eDNA). This matrix promotes microbial attachment, retains moisture, facilitates nutrient exchange, and protects against environmental stress, making biofilm-forming PGPB a promising approach for sustainable crop production (Ajijah *et al*., 2023; Flemming & Wingender, 2010; Y. Li *et al*., 2024; Venturi & Keel, 2016).

Nanotechnology offers additional opportunities to improve agricultural productivity through the application of metallic, metal-oxide, silica-based, carbon-based, and polymeric nanomaterials. Their physicochemical properties can influence nutrient delivery, plant physiological responses, antimicrobial activity, and the controlled release of agricultural inputs (Fraceto *et al*., 2016; Kah *et al*., 2018, 2019). Importantly, nanoparticles can interact with bacterial cells and EPS matrices, influencing microbial viability, biofilm architecture, motility, and quorum-sensing (QS)-associated processes. While selected nanomaterials may stimulate beneficial microbial functions or enhance microbial delivery, others can inhibit pathogenic biofilms. These responses are strongly dependent on nanoparticle composition, concentration, surface characteristics, bacterial strain, and environmental conditions (Fulaz *et al*., 2019; Handa & Kalia, 2024; Lowry *et al*., 2012; Panichikkal *et al*., 2022). Despite advances in agricultural nanotechnology and microbial biofilm research, their combined roles in plant–microbe interactions and stress management require further integration. This review examines the mechanisms of bacterial biofilm formation, EPS-mediated functions, QS, and rhizosphere colonization, followed by the interactions of nanomaterials with beneficial and pathogenic biofilms. Particular attention is given to their individual and combined contributions to abiotic and biotic stress mitigation, agricultural applications, and environmental biosafety. The review also identifies current limitations and research priorities for developing effective, stable, and environmentally compatible nano-enabled microbial formulations.

2. Biofilms in plant–microbe interactions

This section provides an overview of bacterial biofilm organization and development, highlighting the roles of EPS and QS in microbial communication and root colonization. It further discusses how biofilm-forming plant-associated bacteria contribute to nutrient mobilization, plant growth promotion, stress tolerance, pathogen suppression, and soil structure maintenance.

2.1. Biofilm structure and development

Microbial biofilms are structured, three-dimensional populations of microorganisms that remain permanently attached to biotic or abiotic surfaces, including plant roots, soil particles, pipelines, or living tissues—at solid–liquid interfaces—and are embedded in a self-produced extracellular matrix (Arampatzi *et al*., 2011; Di Martino, 2018; Karygianni *et al*., 2020). EPS makes up 50%–90% of the total organic matter in major biofilms and consists mostly of water, together with polysaccharides, proteins, lipids, and eDNA (Flemming & Wingender, 2010). Water maintains hydration and supports nutrient transport. Polysaccharides form an adhesive structural scaffold, anchoring cells to surfaces and to one another. Proteins and eDNA stabilize the matrix architecture through non-covalent interactions while mediating extracellular enzymatic breakdown (Flemming *et al*., 2025; Limoli *et al*., 2015; Rather *et al*., 2021; Whitchurch *et al*., 2002). The EPS matrix promotes cooperative micro-consortia and shields resident bacteria against phagocytes, bacteriophages, desiccation, and antibiotic substances by keeping cells close together (Arampatzi *et al*., 2011; Flemming & Wingender, 2010).

In agricultural environments, root-associated biofilm formation progresses through four dynamic, environmentally responsive stages: (i) initial attachment, (ii) irreversible adhesion and microcolony formation, (iii) maturation into structured three-dimensional architectures, and (iv) dispersal (Coenye *et al*., 2024; Luo *et al*., 2022):

(i) Initial attachment: Hydrodynamic forces, chemotaxis toward root exudate gradients, and flagella-driven motility transport planktonic bacteria to solid surfaces, where extracellular appendages including flagella, pili, fimbriae, and outer membrane proteins overcome long-range repulsive forces depending on substrate charge and hydrophobicity (Chapman, 2015;

Luo *et al*., 2022). Transcriptome profiling of the plant growth-promoting rhizobacterium (PGPR) *Bacillus amyloliquefaciens* SQR9 and FZB42 exposed to maize root exudates and soil extract revealed marked early upregulation of chemotaxis and flagellar genes (*cheA*, *cheB*, *cheW*, *mcpB/C*, *fli*, *flg*, *flh*, and *hag*), confirming that early attachment relies on active motility and chemical recognition of plant-derived signals (Fan *et al*., 2015; N. Zhang *et al*., 2015).

(ii) Irreversible adhesion and microcolony formation: Following surface contact, cells change from reversible Brownian motion to irreversible attachment and the formation of microcolonies by limiting flagellar movement and releasing adhesins and EPS (Chapman, 2015). Fourier-transform infrared spectroscopy (FTIR) of 21 strong biofilm-forming rhizobacterial isolates from drought-prone soils confirmed active accumulation of polysaccharides, proteins, lipids, and nucleic acids during early colonization (M. d. M. Haque *et al*., 2020). Specific plant metabolites directly regulate this transition; in *B. amyloliquefaciens* SQR9, maize root exudate organic acids, specifically fumaric and citric acids, upregulated the matrix biosynthesis genes *epsD* and *tapA* by more than 10-fold (N. Zhang *et al*., 2015).

(iii) Maturation: As microcolonies expand, they develop complex three-dimensional architectures such as towers or mushroom-like clusters permeated by nutrient channels featuring a regulatory basal zone, a dense central microbial layer, and an outer dispersal-ready region (Coenye *et al*., 2024; Karygianni *et al*., 2020; X. Wang *et al*., 2023). Confocal laser scanning microscopy of plant-associated *Pseudomonas donghuensis* P482 demonstrates that progression into dense, three-dimensional clusters depends on time and nutrient availability (X. Wang *et al*., 2023). Moreover, biofilms dynamically remodel EPS quantity and carbohydrate enrichment under hydrodynamic shear stress in soil pores to preserve mechanical integrity (Perez *et al*., 2021).

(iv) Dispersal: Certain cell subpopulations regain motility and exit the mature matrix to migrate and colonize new root zones or soil microhabitats (Liaqat *et al*., 2025). Comparative transcriptomic analysis of *B. amyloliquefaciens* SQR9 and FZB42 under rhizosphere-mimicking conditions reveals distinct changes in motility and chemotaxis gene expression across early and late growth phases, revealing a coordinated shift from attachment to maturation and dispersal (Fan *et al*., 2015; N. Zhang *et al*., 2015).

2.2. Quorum sensing and communication

Quorum sensing is a density-dependent communication system that coordinates gene expression across bacterial communities. It works through the production, release, and detection of signaling molecules, including acyl-homoserine lactones (AHLs) in Gram-negative bacteria, autoinducing peptides in Gram-positive bacteria, and autoinducer-2 (AI-2) across diverse bacterial populations (Miller & Bassler, 2001; Papenfort & Bassler, 2016; Waters & Bassler, 2005). Alongside nutrient availability, surface-associated signals, and environmental stress, QS regulates biofilm formation and maturation, secondary metabolite synthesis, phytohormone production, nitrogen fixation, and ACC deaminase activity in beneficial rhizobacteria (Danhorn & Fuqua, 2007; Jung *et al*., 2020; Von Bodman *et al*., 2003). Biofilm formation and QS are closely interconnected: QS controls genes governing bacterial adhesion, EPS production, and dispersal, while high cell density and spatial organization within biofilms concentrate QS signals to promote coordinated expression of plant-beneficial traits (Danhorn & Fuqua, 2007; Pandit *et al*., 2020).

Beyond regulating bacterial physiology, bacterial AHL QS molecules act as interkingdom signals that prime plant defense responses against pathogens (Lazarus & Easwaran, 2024). In particular, long-chain AHLs such as 3-oxo-C14-HSL activate plant mitogen-activated protein kinase (MAPK) cascades—specifically MPK3 and MPK6—triggering cell wall reinforcement through callose deposition, stomatal closure, and phenolic/phytoalexin accumulation (Schenk *et al*., 2014; Schikora *et al*., 2011). Furthermore, medium-chain AHLs such as 3-oxo-C8-HSL modulate salicylic acid (SA)-mediated defense pathways to enhance host resistance against foliar bacterial pathogens such as *Pseudomonas syringae* (F. Liu *et al*., 2020), underscoring the potential of QS-associated molecules as sustainable biostimulants (Lazarus & Easwaran, 2024).

2.3. Biofilms in soil and root function

In natural soil environments, most microorganisms do not exist as free-swimming planktonic cells. Instead, they inhabit surface-associated micro-aggregates and biofilm-like structures bound by EPS, which shape edaphic microbial interactions and biogeochemical cycling (Q. Chen *et al*., 2024; B. Zhang *et al*., 2024). Biofilm-forming PGPB, including *Pseudomonas fluorescens, Bacillus subtilis*, *Azospirillum brasilense, Rhizobium leguminosarum*, and *Burkholderia phytofirmans*, convert nitrogen (N), phosphorus (P), and potassium (K) into plant-available forms through fixation, solubilization, and retention (Q. Chen *et al*., 2024; Glick, 2012; Y. Li *et al*., 2024; Sahu *et al*., 2019). In wheat (*Triticum aestivum* L.), inoculation with the biofilm-forming PGPR strains BF10 and BF15 significantly improved plant growth, grain yield, and

nutrient accumulation. Under pot conditions, grain nitrogen and phosphorus contents increased by 58.8% and 63.0%, respectively, with consistent improvements also observed under field conditions (Rafique *et al*., 2024).

Beyond nutrient mobilization, biofilm-associated PGPB promote plant performance by modifying root system architecture, synthesizing phytohormones (such as indole-3-acetic acid and gibberellic acid), enhancing micronutrient uptake, and stimulating host antioxidant defenses (M. d. M. Haque *et al*., 2020), as demonstrated by biofilm-forming *B. amyloliquefaciens* 54 reducing oxidative damage and enhancing drought tolerance in tomato (D. C. Wang *et al*., 2019). Bacterial biofilms also establish tripartite associations with arbuscular mycorrhizal fungi and plant roots, where continuous bacterial cell layers interact closely with fungal hyphae embedded in self-secreted exopolysaccharides to improve nutrient transfer (Pandit *et al*., 2020; C. Zhang *et al*., 2024). At the soil scale, adhesive EPS binds mineral particles into water-stable aggregates, improving soil porosity, aeration, erosion resistance, and moisture retention in a positive feedback loop that supports root and microbial growth (Abdian *et al*., 2024; Y. Li *et al*., 2024).

The taxonomic diversity of the soil rhizosphere further shapes biofilm architecture. Plant-associated Actinobacteria, such as *Streptomyces* and *Micromonospora*, grow as filamentous hyphae and release bioactive secondary metabolites that help nutrient uptake and suppress pathogens. When selecting inoculants, however, their filamentous colony networks must be experimentally distinguished from the classical matrix-enclosed, unicellular biofilms formed by *Bacillus, Pseudomonas,* and symbiotic rhizobia (Viaene *et al*., 2016). Finally, root colonization spans three interconnected compartments: the rhizosphere (root-influenced soil), the rhizoplane (root surface), and internal plant tissues (endophytic compartment) (Bhattacharyya *et al*., 2023; Huang *et al*., 2024; Y. Liu *et al*., 2024). Because robust *in vitro* biofilm formation does not always translate into rhizosphere persistence against indigenous microbial competition, candidate inoculants require integrated validation via microscopy, viable-cell recovery, and soil-based plant trials.

3. Nanotechnology in sustainable agriculture

This section explains the agricultural nanomaterials, their physicochemical characteristics, and their applications in crop protection, nutrient delivery, and stress management. It also highlights green nanoparticle synthesis, the importance of physicochemical characterization, and the emerging use of nanocarriers for delivering PGPB while maintaining microbial viability and effective root colonization.

3.1. Nanomaterial types and agricultural roles

Nanomaterials possess unique physicochemical properties including high specific surface area, tailored particle size and morphology, surface charge (zeta potential), and catalytic reactivity that enable diverse applications in sustainable agriculture as nanopesticides, nanofertilizers, nano-based sensors, and protective seed coatings (Fraceto *et al*., 2016; Lee & Je, 2013; Mazaherinia *et al*., 2010; Padda *et al*., 2017; Prasad *et al*., 2012). Based on chemical composition and biological function, agricultural nanomaterials are broadly categorized into metallic, metal-oxide, silica-based, carbon-based, polymeric, and hybrid nanocomposites, distinguishing direct antimicrobial agents from protective microbial and chemical carriers (Khot *et al*., 2012; Duhan *et al*., 2017; Kah *et al*., 2018; Mgadi *et al*., 2024; Wahab *et al*., 2024; Sodhi *et al*., 2025). Experimental research shows that nanoparticles affect bacterial biofilms differently depending on their composition and concentration, as well as the characteristics of the bacteria. Representative findings and their potential agricultural implications are summarized in Table 1.

Metal and metal-oxide nanoparticles, including silver nanoparticles (AgNPs), zinc oxide nanoparticles (ZnO-NPs), and iron oxides, are important for pest management and nutrition. AgNPs show antimicrobial properties against bacteria, fungi, and viruses by disrupting membranes and releasing Ag^{+} ions. However, precise dosing is needed to avoid harming beneficial rhizobacteria (Durán *et al*., 2016; McShan *et al*., 2014; Shelar *et al*., 2023). ZnO-NPs deliver Zn micronutrients and inhibit pathogens, while iron-oxide nanoparticles regulate iron availability in the rhizosphere, siderophore competition, and chlorophyll-linked metabolism. (Kah *et al*., 2018; Sodhi *et al*., 2025; Zaman *et al*., 2025).

Non-metallic and carbon-based nanomaterials (multi-walled carbon nanotubes, fullerenols, graphene oxide) and silicon dioxide substantially enhance seed germination, water uptake, and plant vigor. Metal and carbon nanoparticles increase the surface area available for electron transport with biomolecules. They also penetrate seed coats and support enzymatic metabolism (Lahiani *et al*., 2013). For example, OH-functionalized fullerenes (fullerenol) aid cell division and hypocotyl elongation in *Arabidopsis*, whereas carbon nanotube seed treatments increase water uptake and bioactive metabolite accumulation (such as lycopene and cucurbitacin-B) in crops such as *Momordica charantia*, resulting in improved

fruit number, size, and total yield (Husen & Siddiqi, 2014; S. Li *et al*., 2021). Additionally, silica-based nanomaterials help structural integrity and reduce abiotic stress, while biodegradable polymers such as chitosan and alginate support the controlled release of agrochemicals (Elsayed *et al*., 2025; Prokisch *et al*., 2024). Nanomaterial-based seed coatings also induced plant defenses and systemic acquired resistance against pathogens (Selvakesavan *et al*., 2023; Shelar *et al*., 2023).

3.2. Nanoparticle synthesis and characterization

Biosynthesis uses plant extracts, bacteria, fungi, and algae to produce nanoparticles in an environmentally friendly way, with natural biomolecules serving as reducing and capping agents (Bahrulolum *et al*., 2021; Lithi *et al*., 2025; Zineb *et al*., 2025). For example, endophytic *Bacillus siamensis* C1 can successfully synthesize AgNPs with antibacterial and antibiofilm activity against rice pathogens (Ibrahim *et al*., 2019). However, biosynthesis does not ensure biosafety; rather, it is a manufacturing process. Ecotoxicological testing on non-target organisms and strict downstream purification and standardization are necessary to remove residual capping biomolecules and dissolved ionic substances.

To trace nanoparticle alterations in complex soil environments, such as dissolution, homo- and hetero-aggregation, and natural organic matter adsorption, thorough analytical characterization is necessary. Techniques including transmission and scanning electron microscopy, dynamic light scattering, zeta potential measurement, X-ray diffraction, and FTIR enable accurate monitoring of how physicochemical properties and environmental coronas alter nanoparticle bioavailability in rhizosphere soils compared with simplified aqueous suspensions (Cornelis *et al*., 2014; Lowry *et al*., 2012; Kah *et al*., 2018).

3.3. Nanocarriers for plant growth-promoting bacteria

Nanomaterials serve as advanced delivery systems and slow-release fertilizer matrices that enhance nutrient use efficiency, improve soil structure, stimulate microbial diversity, and increase photosynthetic capacity (Alharbi *et al*., 2022; Chaudhary *et al*., 2021; Daniel *et al*., 2022; Karnwal *et al*., 2023; Majumder *et al*., 2023; Qiu *et al*., 2022; Tymoszuk *et al*., 2022; Vinzant *et al*., 2023). However, designing nanocarriers for living PGPB differs fundamentally from traditional pesticide or fertilizer encapsulation: the carrier matrix must maintain high bacterial cell viability during prolonged storage and release metabolically active cells onto root surfaces without disrupting beneficial biofilm formation, root colonization, or rhizospheric community stability (Handa & Kalia, 2024; Sodhi *et al*., 2025; Zaman *et al*., 2025).

4. Nanoparticle–biofilm interactions

This section covers the interactions between nanoparticles and bacterial biofilms, focusing on nanoparticle attachment, EPS binding and penetration, membrane damage, and oxidative stress (Table 1). It further highlights how nanomaterials affect EPS architecture, QS, bacterial motility, and metabolic activity, emphasizing concentration-dependent effects that determine whether beneficial biofilms are stimulated or pathogenic biofilms are inhibited (Table 2).

4.1. Attachment, EPS sequestration, and penetration

Nanoparticles initially interact with bacterial cells through electrostatic forces, hydrophobic interactions, van der Waals forces, hydrogen bonding, and chemical associations with cell-envelope macromolecules including membrane phospholipids, surface proteins, lipoteichoic acids, and lipopolysaccharides (Arakha *et al*., 2015; Karnwal *et al*., 2023). Because bacterial adhesion and cell-to-cell aggregation represent the critical early stages of biofilm establishment, nanoparticle-induced alterations in cellular aggregation, surface attachment, and envelope responses directly govern early colonization dynamics (Karnwal *et al*., 2023). Consequently, physicochemical characteristics such as particle size, surface charge, and exposure concentration strongly influence how beneficial and pathogenic bacteria interact with surrounding surfaces (Arakha *et al*., 2015; Karnwal *et al*., 2023).

The EPS matrix of a biofilm functions as an active binding compartment after it is developed. Electrostatic attraction, van der Waals forces, and hydrogen bonding can either concentrate nanoparticles at the biofilm's periphery or sequester them from deeper bacterial cells (Bhatia *et al*., 2021; Flemming & Wingender, 2010; Lowry *et al*., 2012; Slavin *et al*., 2017). Nanoparticle transport into the biofilm interior relies on hydrodynamic diameter, surface charge, and EPS pore architecture (Fulaz *et al*., 2019; Joo & Aggarwal, 2018). Moreover, nanoparticles entering the biofilm microenvironment may dissolve, aggregate, or acquire biomolecular coronas. Spatial imaging is therefore important to differentiate superficial surface retention from deep penetration into the matrix (Fulaz *et al*., 2019; Joo & Aggarwal, 2018).

4.2. Membrane damage and oxidative stress

Antimicrobial nanoparticles such as AgNPs and copper oxide nanoparticles disrupt bacterial membrane lipid packing, alter transmembrane ion gradients, impair cellular

Table 1. Mechanisms of nanomaterial–biofilm interactions and associated microbial outcomes

Interaction mechanism	Nanomaterial type	Mode of action on cells & matrix	Observed biofilm & physiological outcome	Key references
Surface attachment & EPS sequestration	Size- and charge-dependent NPs	Electrostatic, hydrophobic, van der Waals, and H-bonding with phospholipids, proteins, lipoteichoic acids, LPS, and EPS	Alters cell aggregation and early adhesion; concentrates or sequesters NPs at biofilm periphery; induces dissolution, aggregation, or corona formation	Bhatia *et al.* (2021); Flemming & Wingender (2010); Fulaz *et al.* (2019); Joo & Aggarwal (2018); Karnwal *et al.* (2023); Lowry *et al.* (2012); Slavin *et al.* (2017)
Membrane disruption & permeability	AgNPs, CuO-NPs	Disrupts membrane lipid packing, alters ion gradients, impairs respiration, and causes intracellular leakage	Differential sensitivity between vulnerable outer biofilm layers and EPS-shielded inner layers	Durán *et al.* (2016); Hajipour *et al.* (2012); McShan *et al.* (2014)
Oxidative stress & ROS generation	Metal & metal-oxide NPs	Triggers superoxide, H_2O_2, and hydroxyl radicals that overwhelm catalase, SOD, and peroxidases	Oxidative damage to lipids, proteins, and nucleic acids; cellular inactivation and virulence loss	Joo & Aggarwal (2018); Kalu *et al.* (2026)
EPS matrix remodeling	ZnO-NPs vs. chitosan NPs (CNPs)	Destabilizes EPS (ZnO-NPs) or reorganizes 3D matrix topography (CNPs)	ZnO-NPs reduce biofilm formation; CNPs remodel *Paenibacillus* sp. CCB36 biofilm and boost *Rhizoctonia solani* antagonism	Fulaz *et al.* (2019); Panichikkal *et al.* (2022)
Quorum sensing & motility inhibition	Biogenic & endophyte-derived AgNPs	Interferes with QS signaling, extracellular polysaccharide synthesis, and flagellar swimming motility	7%–93% biofilm reduction in *Pseudomonas aeruginosa* (5–25 μg/mL); suppressed swimming motility in *Xanthomonas oryzae* pv. oryzae and *Acidovorax oryzae*	Akther *et al.* (2020); Ibrahim *et al.* (2019); Kalu *et al.* (2026); Trzcińska-Wencel *et al.* (2023)
Metabolic suppression vs. stimulation	Fungal AgNPs vs. vermiculite & saponite NPs	Inhibits hydrolytic enzymes (AgNPs) or stimulates bacterial proliferation and oxidoreductases (mineral NPs)	AgNPs reduce FDA hydrolytic activity in mature biofilms; vermiculite NPs boost *Azotobacter vinelandii* dehydrogenase and *Bacillus subtilis* peroxidase	Karnwal *et al.* (2023); Trzcińska-Wencel *et al.* (2023)

Abbreviations: AgNPs: Silver nanoparticles; CuO-NPs: Copper oxide nanoparticles; EPS: Extracellular polymeric substances; FDA: Fluorescein diacetate; LPS: Lipopolysaccharide; NPs: Nanoparticles; QS: Quorum sensing; ROS: Reactive oxygen species; SOD: Superoxide dismutase; ZnO-NPs: Zinc oxide nanoparticles.

respiration, and induce intracellular leakage (Durán *et al.*, 2016; Hajipour *et al.*, 2012; McShan *et al.*, 2014). Within structured biofilms, EPS shielding and physiological stratification cause outer, metabolically active layers to respond differently from protected inner subpopulations (Durán *et al.*, 2016; Hajipour *et al.*, 2012; McShan *et al.*, 2014).

Concurrently, nanoparticles trigger the generation of reactive oxygen species (ROS), including superoxide anions, hydrogen peroxide, and hydroxyl radicals, that oxidize membrane lipids, proteins, and nucleic acids once endogenous bacterial antioxidant defenses (such as catalase, superoxide dismutase, and peroxidases) are overwhelmed (Joo & Aggarwal, 2018). Together, physical membrane disruption, respiratory inhibition, and oxidative stress compromise bacterial viability and persistence within biofilm communities (Joo & Aggarwal, 2018; Kalu *et al.*, 2026).

4.3. EPS remodeling, quorum sensing, and motility

Nanomaterials can either destabilize EPS polymers or, at sublethal concentrations, stimulate stress-induced EPS secretion; normalizing EPS biochemical assays to viable cell biomass is therefore essential to distinguish structural

Table 2. Experimental evidence for nanoparticle–biofilm interactions in agriculture

Nanomaterial	Bacterial species/target	Major findings	Agricultural relevance	References
Zinc oxide nanoparticles (ZnO-NPs) and chitosan nanoparticles	*Paenibacillus* sp. CCB36	ZnO-NPs reduced biofilm formation, whereas chitosan nanoparticles modified biofilm architecture and enhanced antagonistic activity against *Rhizoctonia solani*.	Shows that nanoparticle type can differentially regulate beneficial bacterial biofilms and biocontrol activity.	Panichikkal *et al.* (2022)
Biogenic silver nanoparticles (AgNPs)	*Xanthomonas oryzae* pv. oryzae and *Acidovorax oryzae*	AgNPs suppressed bacterial growth, biofilm-associated traits, and swimming motility.	Potential application in managing bacterial rice diseases.	Ibrahim *et al.* (2019)
Fungal-derived silver nanoparticles	*Agrobacterium tumefaciens*, *Pseudomonas syringae*, *Xanthomonas campestris*, and *Pectobacterium carotovorum*	AgNPs inhibited biofilm formation and hydrolytic enzyme activity in a concentration-dependent manner.	Potential antibiofilm approach against phytopathogens.	Trzcińska-Wencel *et al.* (2023)
Vermiculite and saponite nanoparticles	*Azotobacter vinelandii* and *Bacillus subtilis*	Mineral nanoparticles enhanced bacterial proliferation and selected metabolic enzyme activities.	Suggests that selected mineral nanomaterials may support beneficial microbial functions.	Karnwal *et al.* (2023)
Rice-husk-derived nanosilica	Rhizosphere bacteria	Nanosilica application was associated with increased rhizobacterial populations and improved plant-growth-related responses.	Nanosilica positively affects beneficial bacterial populations and the soil's nutrient value.	Karunakaran *et al.* (2013)

matrix remodeling from general growth inhibition (Fulaz *et al.*, 2019). Demonstrating how different nanomaterials selectively modulate beneficial biofilms, Panichikkal *et al.* (2022) investigated ZnO-NPs and chitosan nanoparticles on the endophytic bacterium *Paenibacillus* sp. CCB36 (isolated from *Curcuma caesia* rhizomes). While ZnO-NPs reduced biofilm formation, supplementation with chitosan nanoparticles remodeled the three-dimensional biofilm architecture, as confirmed by atomic force microscopy, and enhanced the antagonistic biocontrol activity of *Paenibacillus* sp. CCB36 against the fungal pathogen *Rhizoctonia solani*.

In addition to altering matrix architecture, nanomaterials interfere with intercellular communication and flagellar motility required for pathogen establishment (Kalu *et al.*, 2026). Biologically synthesized AgNPs inhibited *Pseudomonas aeruginosa* biofilm formation by 7% to 93% at concentrations of 5–25 μg/mL by disrupting bacterial growth, extracellular polysaccharide production, and QS signaling (Akther *et al.*, 2020; Trzcińska-Wencel *et al.*, 2023). Similarly, endophyte-derived AgNPs significantly suppressed swimming motility in *Xanthomonas oryzae* pv. oryzae and *Acidovorax oryzae* (Ibrahim *et al.*, 2019). Because flagellar motility drives bacterial movement, surface interactions, and plant tissue colonization, simultaneous suppression of motility, QS signaling, and biofilm development allows nanomaterials to target multiple virulence pathways in plant pathogens (Ibrahim *et al.*, 2019; Kalu *et al.*, 2026).

4.4. Metabolic responses and dose effects

Beyond preventing initial attachment, nanoparticles directly modulate the metabolic and enzymatic activities of bacteria embedded within established biofilms (Karnwal *et al.*, 2023; Trzcińska-Wencel *et al.*, 2023). Fungal-derived AgNPs suppressed both biofilm formation and the hydrolytic enzyme activity of preformed biofilms in a concentration-dependent manner, as demonstrated using fluorescein diacetate assays (Trzcińska-Wencel *et al.*, 2023). Conversely, selected mineral nanoparticles stimulate the metabolic and plant growth-promoting functions of beneficial bacteria: vermiculite and saponite

nanoparticles enhanced bacterial proliferation, with vermiculite nanoparticles increasing dehydrogenase activity in *Azotobacter vinelandii* and peroxidase activity in *B. subtilis* (Karnwal *et al*., 2023).

Across both pathogenic and beneficial strains, nanomaterial responses frequently follow concentration- and time-dependent patterns, shifting from sublethal metabolic stimulation or EPS induction at low doses to bactericidal disruption at high doses (Ahmed *et al*., 2020). To establish true nanoparticle-specific mechanisms rather than artifacts from dissolved metal ions (Ag^{+}, Zn^{2+}, Cu^{2+}) or residual plant and microbial synthesis extracts, experimental designs must incorporate untreated controls, nanoparticle-free synthesis controls, matched soluble-ion controls, particle-free leachates, vehicle/stabilizer controls, and assay-interference controls (Joo & Aggarwal, 2018; Lowry *et al*., 2012; McShan *et al*., 2014).

5. Biofilms under abiotic stress

Soil salinization is a major constraint on agricultural productivity worldwide, primarily due to the accumulation of soluble salts and associated sodium toxicity in the soil. (Y. Li *et al*., 2024). Within the rhizosphere, PGPB biofilms chelate free Na^{+} cations via negatively charged functional groups in their EPS matrix, restricting root Na^{+} uptake and maintaining favorable cytosolic K^{+}/Na^{+} ratios (Y. Li *et al*., 2024). For example, EPS secreted by *Pseudomonas* sp. AK-1 sequestered soil Na^{+} and sustained normal soybean growth under saline conditions of up to 200 mM NaCl (Kasotia *et al*., 2015). Salt-stressed roots also exude specific sugars, such as trehalose and rhamnose, which recruit halotolerant biofilm-forming bacteria to the root zone; these microbes further bolster plant resistance through compatible solute accumulation, phytohormone modulation, and ACC deaminase activity (Ansari & Ahmad, 2019; M. M. Haque *et al*., 2022; Morcillo & Manzanera, 2021).

Under drought and water-deficit conditions, rhizosphere biofilms bind soil particles into water-stable aggregates and form a hydrophilic microenvironment around roots that buffers matric water loss during drying–rewetting cycles (Ansari & Ahmad, 2019; Brokate *et al*., 2024). Enrichment of uronic acids, such as *D*-glucuronate, within bacterial EPS during drought increases matrix water-holding capacity and contributes antioxidant functional groups that protect root tissues from desiccation injury (Ajijah *et al*., 2023).

Hydrated EPS microenvironments buffer root-associated microbes against extreme thermal fluctuations and desiccation, though severe heat or cold alters membrane fluidity and matrix polymer synthesis, necessitating multi-stress screening rather than single-temperature assays (Ansari & Ahmad, 2019; Morcillo & Manzanera, 2021). Across these abiotic challenges, steep oxygen and nutrient gradients within biofilms create localized redox microenvironments. There, bacterial antioxidant enzymes such as catalase, superoxide dismutase, and peroxidases work alongside PGPB-induced plant antioxidant systems to limit cellular lipid peroxidation (M. M. Haque *et al*., 2022).

In soils contaminated by industrial activities, intensive pesticide use, and nitrogen–phosphorus–potassium (NPK) fertilizers, bacterial biofilms immobilize non-biodegradable heavy metals through EPS biosorption, redox transformation, precipitation, and complexation. This prevents plant roots from absorbing the metals and enhances phytoremediation performance (Roy *et al*., 2024; Xing *et al*., 2022). Biofilm consortia can also tolerate and biotransform certain organic pesticide residues, but analytical chemistry is needed to distinguish true biodegradation from physical sorption by the matrix (Brokate *et al*., 2024; Morcillo & Manzanera, 2021). Across all abiotic stressors, EPS supports hydration, selectively binds ions and contaminants, promotes soil aggregation, and maintains stable rhizoplane attachment. Its functional efficacy depends on polymer chemistry, not just total matrix mass (Flemming & Wingender, 2010; Morcillo & Manzanera, 2021).

6. Biofilms under biotic stress

Biofilm formation is a key virulence factor in phytopathogenic bacteria: it protects cells from host antimicrobials, UV radiation, desiccation, and nutrient deprivation, while harboring metabolically dormant persister cells that are nearly impossible to eradicate (Carezzano *et al*., 2023). For example, while the plant defense compound rosmarinic acid effectively suppresses planktonic bacteria, pathogens protected within biofilms resist its antimicrobial effects (Walker *et al*., 2004). Foliar and vascular pathogens rely heavily on biofilm matrices for infection: *P. syringae* pv. syringae depends on biofilms to cause brown spot disease on leaves (Monier *et al*., 2004); *Xanthomonas campestris* pv. campestris utilizes the exopolysaccharide xanthan gum and cell-wall-degrading enzymes to colonize cruciferous xylem and induce black rot (Dow *et al*., 2003); *Clavibacter michiganensis* subsp. sepedonicus forms exopolysaccharide biofilms to cause bacterial ring rot in potato (Marques *et al*., 2003); and *Ralstonia solanacearum* and *Pantoea stewartii* rely on QS-regulated EPS matrices and persister cells to colonize xylem vessels and evade host defenses (Mina *et al*., 2019; Von Bodman *et al*., 2003).

Conversely, beneficial rhizoplane bacteria including *Bacillus, Pseudomonas*, and *Azospirillum*

establish protective root-surface biofilms that suppress phytopathogens through competitive niche exclusion, antibiosis (secreting lipopeptides such as surfactins and iturins, as well as phenazines), siderophore-mediated iron competition, lytic enzymes, and elicitation of host immunity (Haas & Défago, 2005; Lugtenberg & Kamilova, 2009; Nair & Vijayaraghavan, 2025; Riseh *et al*., 2025; Rudrappa *et al*., 2008). In *B. subtilis*, conserved biofilm formation genes are strictly required for effective root colonization and plant protection (Y. Chen *et al*., 2013). Furthermore, beneficial biofilms trigger jasmonic acid/ethylene-dependent induced systemic resistance (ISR) through lipopolysaccharides, siderophores, and volatile organic compounds. Biofilm-forming PGPB show stronger expression of these elicitors than biofilm-deficient mutants (Pieterse *et al*., 2014; Salwan *et al*., 2023; Shrestha *et al*., 2020). Complementary quorum-quenching strategies, such as enzymatic signal degradation or receptor interference, further reduce pathogen virulence without imposing lethal selection pressure. However, target specificity must be verified to avoid disrupting beneficial AHL-mediated plant priming (Von Bodman *et al*., 2003; Zhu *et al*., 2023).

7. Synergy between nanoparticles and biofilms

Nanoparticle–biofilm interactions provide a complementary approach to sustainable agriculture, combining the physicochemical properties of nanomaterials with the protective, colonization, and biocontrol functions of beneficial microbial biofilms. Together, they may increase plant tolerance to abiotic stress, enhance host defense against pathogens, and help maintain microbial stability under multiple environmental challenges. However, their effectiveness depends on nanoparticle characteristics, microbial compatibility, and application dosage.

7.1. Abiotic stress mitigation

Under abiotic stress, nanomaterials complement biofilm-forming PGPB by directly scavenging ROS, increasing plant antioxidant enzyme activity, shielding photosynthetic machinery, and improving nutrient availability (Simonin *et al*., 2018; Thabet & Alqudah, 2024; Wu *et al*., 2017; Xiong *et al*., 2021). For example, foliar application of ZnO-NPs on cucumber increased dry weight and chlorophyll content while elevating leaf catalase and superoxide dismutase activities (S. Li *et al*., 2021; Sharma *et al*., 2023; Singh D. *et al*., 2021). Cerium oxide nanoparticles (CeO_2 NPs) cycle between Ce^{3+} and Ce^{4+} oxidation states to catalytically scavenge superoxide anions, hydrogen peroxide, and hydroxyl radicals into water and oxygen, protecting chloroplast function and boosting carbon-assimilation rates by up to 67% under abiotic stresses such as excess light, heat, and dark chilling (Wu *et al*., 2017). Similarly, nano-formulated iron applications (such as nano-encapsulated $FeSO_4$ and iron oxide nanoparticles) enhance chlorophyll biosynthesis, accelerate CO_2 assimilation rates, optimize photosystem II efficiency, improve tissue iron content, and limit foliar salt accumulation under saline conditions (Singh *et al*., 2021). Nanoparticles such as silicon nanoparticles also upregulate drought-responsive stress signaling and promote stomatal closure to curb transpirational water loss and improve plant water-use efficiency (Camara *et al*., 2019).

As a complementary approach, integrating nanomaterials with stress-adapted, biofilm-forming bacteria allows nanoparticles to influence soil microbial dynamics and nutrient cycling (Simonin *et al*., 2018), while the bacterial EPS matrix buffers ionic toxicity and stabilizes root-surface colonization (Brokate *et al*., 2024). Similarly, combined seed treatment of ZnO-NPs (0.2 mg/cm^3) with *B. subtilis* in red cabbage seedlings improved germination, seedling length, photosynthetic pigments, and antioxidant compounds compared with individual treatments, though higher doses (0.4 mg/cm^3) reduced efficacy (Krzepiłko *et al*., 2025). Achieving true agronomic synergy therefore requires factorial validation to confirm that nanoparticle doses preserve bacterial viability, EPS integrity, and root colonization under stress (Brokate *et al*., 2024).

7.2. Pathogen control and host defense

Under biotic stress, engineered and biogenic nanomaterials reinforce beneficial rhizosphere biocontrol by directly dismantling pathogen biofilms, inhibiting fungal growth, and lowering conventional agrochemical phytotoxicity (Ansari, 2023; Kalu *et al*., 2026; Pérez-de-Luque & Rubiales, 2009). Mycosynthesized spherical AgNPs (15.56 ± 9.22 nm, zeta potential −38.43 mV) produced using *Fusarium culmorum* JTW1 inhibited biofilm formation and hydrolytic enzyme activity in a concentration-dependent manner across major bacterial phytopathogens (Trzcińska-Wencel *et al*., 2023). Specifically, 0.125 μg/mL AgNPs reduced *Agrobacterium tumefaciens* biofilm formation to 63% of the untreated control and achieved an 83% reduction with complete hydrolase inhibition at 1 μg/mL, while 0.5 μg/mL AgNPs reduced biofilm formation in *P. syringae*, *X. campestris*, and *Pectobacterium carotovorum* by 80%, 73%, and 56%, respectively, and inhibited spore germination in *Botrytis cinerea, Phoma lingam*, and *Sclerotinia sclerotiorum* (Trzcińska-Wencel *et al*., 2023). Similarly, biogenic AgNPs synthesized using endophytic *B. siamensis* C1 or plant extracts (*Arctium lappa, Solanum melongena, Taraxacum mongolicum*) suppressed biofilm development and swimming motility in *X. oryzae* pv.

oryzae and *A. oryzae* while improving rice seedling growth and reducing bacterial leaf blight symptoms (Ibrahim *et al*., 2019; Mishra *et al*., 2020).

Metal-oxide, composite, and carbon-based nanomaterials provide additional targeted antimicrobial and host-priming actions alongside beneficial biofilms. Saffron (*Crocus sativus*)-synthesized nickel–silica nanocomposites (Ni-SiO_2-NPs; 200 μg/mL) reduced *X. oryzae* pv. *oryzae* growth by 89.07% and biofilm formation by 80.40% while inducing 99.61% bacterial apoptosis (Abdallah *et al*., 2023; Kalu *et al*., 2026). Papaya (*Carica papaya*)-derived CuO nanoparticles inhibited *R. solanacearum* biofilm formation, swarming motility, cellular ATP synthesis, and virulence gene expression, reducing bacterial wilt severity in tobacco (J. Chen *et al*., 2019), while green-synthesized CuO nanoparticles directly inhibited bacterial pathogen proliferation by disrupting cell membrane integrity (Sujitha *et al*., 2017). Additionally, CuO nanoparticles anchored on reduced graphene oxide nanosheets (1 mg/L) perforated fungal membranes and outperformed the commercial fungicide Kocide 2000 (2.5 g/L) against *Fusarium oxysporum* in tomato and pepper (El-Abeid *et al*., 2020), whereas molybdenum-based nanofungicides disrupted fungal mycelia and conidiophore development (Salama *et al*., 2022). Carbon nanotubes and graphene oxide also interact with microbial envelopes to disrupt membrane integrity and inhibit bacterial biofilm formation (Kalu *et al*., 2026).

Co-application studies further demonstrate disease suppression alongside soil health gains: co-applying biosynthesized selenium (Se) and titanium dioxide (TiO_2) nanoparticles with *Pseudomonas* sp. and *Enterobacter cloacae* in *F. culmorum*-infected wheat reduced disease severity, increased thousand-grain weight, and enhanced available soil phosphorus, respiration, and microbial biomass carbon, while *B. subtilis* Vru1 encapsulated in an alginate–bentonite–TiO_2 carrier suppressed *R. solani* in bean (Saberi-Rise & Moradi-Pour, 2020). Beyond direct antimicrobial action, nanomaterials reinforce host defense signaling and antioxidant systems during pathogen attack: ZnO-NPs reduced *Fusarium wilt* symptoms in tomato by 28.57% (providing 67.99% overall protection), while AgNPs lowered rice bacterial leaf blight severity by 49.2% (Kalu *et al*., 2026).

7.3. Combined stress and microbial compatibility

In agricultural fields, crops frequently experience simultaneous salinity (osmotic and ionic Na^+/Cl^- toxicity), drought (matric water deficit), chemical pesticide residues, and pathogen infection (Backer *et al*., 2018; Nadeem *et al*., 2014). Under combined stress, salt- and drought-adapted biofilm-forming PGPB buffer water potential via EPS and regulate root architecture, while compatible nanomaterials supply micronutrients, stabilize inoculant formulations, and operate within a selective dosing window that suppresses pathogen biofilms without impairing beneficial rhizoplane biofilms or host ISR signaling (Haas & Défago, 2005; Handa & Kalia, 2024; Kah *et al*., 2018; Lowry *et al*., 2012; Pieterse *et al*., 2014; Slavin *et al*., 2017).

However, experimental evidence demonstrates that nanomaterial compatibility is highly strain- and dose-dependent. Screening nitrogen-fixing, phosphate-solubilizing, and biofilm-forming bacteria from Indian agricultural soils showed that AgNPs are bactericidal at relatively low minimum inhibitory and bactericidal concentrations (2–22 μg/mL) and alter the relative abundance of *Proteobacteria, Actinobacteria*, and *Firmicutes*. In contrast, ZnO-NPs inhibit beneficial strains only at substantially higher concentrations (Chavan & Nadanathangam, 2019; Karnwal *et al*., 2023). Similarly, trials in wheat using *Bacillus* isolates coated with mesoporous silica, ZnO, or CuO nanoparticles confirmed that growth outcomes depend strongly on the specific bacterium–nanoparticle pairing and crop cultivar. Multi-strain consortia may also show uneven sensitivity among their members (Karunakaran *et al*., 2024; Timofeeva *et al*., 2023).

Consequently, evaluations of combined stress must use full factorial controls that compare untreated, pathogen-only, nanoparticle-only, bacterial-inoculant-only, and combined treatments. They must also quantify microbial endpoints (viable cell counts, EPS production, rhizoplane colonization, and pathogen abundance) alongside plant physiological metrics (biomass, root morphology, relative water content, membrane stability, chlorophyll, enzymatic antioxidants, lipid peroxidation, proline, and Na^+/K^+ ratios) across multi-season field trials (Bhatia *et al*., 2021; Cornelis *et al*., 2014; Kwaslema & Michael, 2024; Mina *et al*., 2019; Sodhi *et al*., 2025; Verma *et al*., 2024).

8. Agricultural applications of nano-microbial systems

Nano-biofertilizers and nano-biopesticides pair nitrogen-fixing, nutrient-solubilizing, or antagonistic biofilm-forming strains with biocompatible carriers such as mesoporous silica, chitosan, or alginate. These carriers extend shelf life, improve root adherence, and help suppress pathogens (Ajijah *et al*., 2023; Handa & Kalia, 2024; Karunakaran *et al*., 2024; Mgadi *et al*., 2024). For example, rice-husk-derived nanosilica doubled soil rhizobacterial populations, increased NPK availability, and enhanced maize germination. When co-applied with *P. fluorescens*,

it also strengthened biocontrol responses in maize (Karunakaran *et al*., 2013; Rangaraj *et al*., 2014). Applied as seed coatings or seedling root dips, these formulations deliver inoculants directly to early colonization sites in cereals, legumes (with rhizobial nodulation compatibility to be verified), horticultural transplants, and climate-resilient crops. Carrier matrices must preserve cell viability during storage without limiting bacterial attachment or EPS synthesis (Backer *et al*., 2018; Karunakaran *et al*., 2024).

Halotolerant, EPS-producing PGPB combined with organic amendments and precision irrigation support crop establishment in salt-affected and drought-prone soils, while adsorptive or catalytic nanomaterials paired with pesticide-degrading biofilms facilitate soil remediation, provided that complete mineralization and reduced residual toxicity are analytically verified (Ajijah *et al*., 2023; Morcillo and Manzanera, 2021; Vitale *et al*., 2022). Commercial translation requires standardized quality control (viable cell counts, particle dispersion stability, and shelf life), life-cycle assessment of green-synthesis routes, and replicated multi-location trials demonstrating net economic return over conventional agrochemicals (Kah *et al*., 2018; Sundararajan *et al*., 2024). The integration of nanomaterials with beneficial microbial systems offers opportunities for developing advanced agricultural formulations. However, their practical application requires validation of microbial compatibility, formulation stability, plant responses, and environmental safety (Table 3).

Table 3. Agricultural applications of nanoparticle–biofilm combinations

Agricultural application	Proposed nanoparticle–microbial combination	Expected function	Important evaluation parameters	References
Nano-biofertilizers	Beneficial nitrogen-fixing and phosphate-solubilizing bacteria with biocompatible nanocarriers	Improved nutrient availability, microbial survival, and root colonization.	Viable cell count, nitrogen fixation, phosphate solubilization, root colonization, and plant biomass.	Backer *et al*. (2018); Karunakaran *et al*. (2024)
Nano-enabled biocontrol	Antagonistic biofilm-forming bacteria combined with silica or chitosan nanoparticles	Improved pathogen suppression through microbial antagonism and nanoparticle-mediated antimicrobial activity.	Pathogen inhibition, biofilm formation, extracellular polymeric substances (EPS) production, disease incidence, and plant growth.	Panichikkal *et al*. (2022); Rangaraj *et al*. (2014)
Seed coating and targeted delivery	Beneficial bacterial inoculants incorporated into alginate-, chitosan-, or silica-based formulations	Improved inoculant delivery, seed adherence, and early root colonization.	Seed germination, bacterial viability, release kinetics, root colonization, and seedling growth.	Backer *et al*. (2018); Handa & Kalia (2024)
Stress-resilient microbial formulations	Halotolerant or drought-adapted EPS-producing bacteria combined with compatible mineral nanomaterials	Improved microbial persistence, soil-water retention, and plant stress tolerance.	EPS production, soil moisture, Na^+/K^+ ratio, antioxidant activity, and plant biomass.	Brokate *et al*. (2024); Morcillo & Manzanera (2021)
Soil remediation	Pollutant-transforming microbial biofilms combined with adsorptive or catalytic nanomaterials	Potential immobilization or transformation of soil contaminants.	Pollutant concentration, degradation products, residual toxicity, microbial activity, and soil enzyme activity.	Ajijah *et al*. (2023); Vitale *et al*. (2022)
Commercial nano-microbial products	Stabilized bacterial inoculants with biodegradable or mineral nanocarriers	Improved shelf life, formulation stability, and field application performance.	Shelf life, viable cell count, particle stability, storage conditions, production cost, and field efficacy.	Kah *et al*. (2018); Sundararajan *et al*. (2024)

9. Future research prospects

Future work should be directed at improving the consistency, safety, and practicality of nano-enabled microbial technologies in agriculture. There is a need to determine the nanoparticle concentrations required to support the viability of beneficial microbes, their EPS production, and their ability to colonize the root, without being toxic (Karunakaran *et al*., 2024). The integration of genomics, transcriptomics, proteomics, and metabolomics will be key to understanding the mechanisms by which nanomaterials affect microbial metabolism, biofilm formation, and plant–microbe interactions (Joo & Aggarwal, 2018; Mortimer *et al*., 2021). More sophisticated imaging techniques (confocal laser scanning microscopy, electron microscopy, etc.) could also improve our understanding of biofilm formation and nanoparticle distribution in soil (Flemming *et al*., 2016).

The development of functionalized nanocarriers and predictive modeling approaches, including machine learning, may help optimize nanoparticle formulations and improve their agricultural performance (Singh & Gurjar, 2022). Equally important is the assessment of environmental safety, particularly nanoparticle accumulation, dissolution, interactions with soil organic matter, effects on non-target microbial communities, and changes in soil enzyme activities following repeated applications (Cornelis *et al*., 2014; Kah *et al*., 2018; Lowry *et al*., 2012). Standardized nanoparticle characterization, toxicity testing, and viability-based biofilm assessment should therefore be incorporated into future investigations (Mgadi *et al*., 2024; Organization for Economic Co-operation and Development, 2020, 2021, 2022). Finally, multi-season and multi-location field trials are essential to establish formulation stability, shelf life, long-term effectiveness, and economic feasibility under diverse agricultural conditions.

10. Conclusion

Bacterial biofilms and engineered or biogenic nanomaterials are two very different (though complementary) biological and technological approaches to improving sustainable agriculture. Their interactions may be beneficial for nutrient acquisition, plant growth promotion, stress tolerance, root colonization, and suppression of plant pathogens. The outcome of such interactions, however, depends heavily on nanoparticle composition, concentration, surface properties, bacterial strain, biofilm characteristics, and the environmental conditions. At appropriate concentrations, nanomaterials can be shown to stimulate microbial activity and alter the biofilm architecture, whereas at higher or inappropriate concentrations they can have the opposite effect and inhibit the beneficial microbes and their functions. Individual studies have shown promise in this area, but the efficacy and robustness of combined nanoparticle–biofilm systems under field conditions are still not well established. Future work in this area will need to focus on mechanistic validation, strain compatibility, formulation optimization, environmental safety, and long-term field performance. This integrated research will be key to developing reliable and environmentally sound nano-enabled biofertilizers and biocontrol products.

Conflict of Interest

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