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Changes of microbiome in response to supplements with silver nanoparticles in cotton rhizosphere

Abhijeet Joshi ⬤ | **Radhika Joshi** | **Prushti Koradiya** | **Hetvi Vank**

Department of Microbiology, Atmiya
University, Rajkot, Gujarat, India

Correspondence
Abhijeet Joshi, Department of
Microbiology, Atmiya University,
Kalawad Rd, 3rd Floor, A Wing, Rajkot,
360005 Gujarat, India.
Email: joshiabhijeets@gmail.com

Abstract

The current study focuses on analyzing the effects of supplements containing silver nanoparticles (AgNPs) on plant growth and rhizospheric bacterial communities. Specifically, the impact of AgNP supplements was assessed on both plant growth promoting traits and bacterial communities in the soil. To do this, a screening process was conducted to select bacteria capable of synthesizing AgNPs through extracellular biosynthesis. UV-Visible spectrophotometer, Fourier transform infrared, X-ray diffraction, scanning electron microscope, and field emission scanning electron microscopy all confirmed, produced AgNPs is in agglomerates form. The resulting AgNPs were introduced into soil along with various supplements and their effects were evaluated after 10 days using next generation sequencing (Illumina—16S rDNA V3–V4 region dependent) to analyze changes in bacterial communities. Seed germination, root-shoot biomass and chlorophyll content were used to assess the growth of the cotton plant, whereas the bacterial ability to promote growth was evaluated by measuring its culturable diversity including traits like phosphate solubilization and indole acetic acid production. The variance in Bray–Curtis β diversity among six selected combinations including control depends largely on the type of added supplements contributing to 95%–97% of it. Moreover, seed germination improves greatly between 63% and 100% at a concentration range of 1.4 to 2.8 mg/L with different types of supplements. Based on the results obtained through this study, it is evident that using AgNPs along with fructose could be an effective tool for promoting *Gossypium hirsutum* growth and enhancing plant growth traits like profiling rhizospheric bacteria. The results that have been obtained endorse the idea of boosting the growth of rhizospheric bacteria in a natural way when AgNPs are present. Using these supplements in fields that have been contaminated will lead to a better understanding of how ecological succession occurs among rhizospheric bacteria, and what effect it has on the growth of plants.

KEYWORDS

AgNPs with supplements, *Gossypium hirsutum*, microbial community profiling, plant growth promoting traits

1 | INTRODUCTION

The field of nanotechnology has seen an increase in its utilization due to its availability, significant impact, and potential applications. The ability to manipulate materials at the nanoscale has opened up new opportunities for various industries, from electronics to medicine. It is noteworthy that the use of AgNPs, or silver nanoparticles, has tripled within a decade as more research shows their potential in antimicrobial properties and improving wound healing. The advancements in nanotechnology continue to pave the way for new discoveries and innovations in various fields [1–3]. Although numerous studies have demonstrated the positive impacts of released AgNPs, it is crucial to recognize that these studies also reveal their toxicological impacts on the environment. These impacts can include detrimental effects on both plants and animals, as the nanoparticles can accumulate in soil and water. As such, it is imperative that we continue to study the effects of AgNPs on the environment to ensure that their use does not cause unintended harm [4, 5]. In the complex world of plant growth, the presence of nanoparticles can present a variety of outcomes. These tiny particles, known as AgNPs, can have both favorable and unfavorable effects on different types of plants. Some plants seem to have a natural inclination for eliminating the stress effects generated by AgNPs, while others struggle to do so. Understanding the unique response of each plant species to these nanoparticles is important for developing strategies that can maximize plant growth and protect our natural ecosystems [6, 7]. As cotton plant is concern Gujarat, India is the leading cotton-producing state in the country, with 29% of the country's cotton production. This is achieved through the use of 94% hybrid cotton species [8]. To improve yield, farmers in Gujarat are using nanobased composites, such as nanofertilizers and nanopesticides. According to a study conducted by Yang et al. [9], it was found that silver nanoparticles (AgNPs) accumulated less in Bt cotton plants compared with non-Bt ones. This suggests a potential difference in the interaction between AgNPs and different cotton varieties which allows its use in cotton. The task of separating AgNPs from contaminated sites is a challenging one due to various physicochemical changes that occur such as reduction, oxidation, aggregation, dissolution, complexation and secondary reactions. These changes can alter the properties of the nanoparticles, making them difficult to detect and separate. Despite the challenges, it is important to find ways to effectively remove AgNPs from contaminated sites to prevent potential harm to the environment and human health [10–13]. Research

has shown that the presence of AgNPs (silver nanoparticles) can have a significant impact on microbial communities within soil. In addition to changing the soil's carbon and nitrogen cycles, AgNPs also have a negative impact on the nitrification profile of the soil. Additionally, transcriptomic studies have revealed that AgNPs can have an impact on DNA replication and repair mechanisms, further emphasizing the potential long-term effects of this substance on the environment [14–16]. According to a study by Tripathi et al. [17], many bacteria living in the rhizosphere have evolved resistance to AgNPs, but this process has taken a long time and involved changes to the bacteria's cellular makeup, metabolic processes, and interactions with other organisms. The resilience of these bacteria, despite the challenges they faced, highlights the adaptability of microorganisms and the importance of understanding their behavior in response to different stimuli. Recent research conducted by Mahmud et al. [18] and Chaudhary et al. [19] has highlighted the crucial role of the rhizospheric microbiome in plant growth and yield. Alterations in this microbiome can lead to a range of negative effects, such as poor seed germination, stunted plant growth, low yield or even no growth at all. These findings emphasize the importance of maintaining a healthy rhizospheric microbiome through practices such as crop rotation, organic farming, and the use of microbial inoculant. After taking into account the aforementioned information, research was conducted on the rhizospheric microbiome of cotton plants by exposing them to various sources of carbon, nitrogen, and vitamins. The study also validated the effects of these supplements on the expression of two traits that promote plant growth, as well as on changes in both the microorganisms present in the rhizosphere and overall plant development. This marks an initial effort to augment crucial nutrients to facilitate natural expansion of beneficial bacteria within the rhizosphere.

2 | MATERIALS AND METHODS

2.1 | Soil sampling and physicochemical analysis

Samples of soil from the rhizosphere of cotton plants at the seedling stage were collected in sterile polythene bags from the Cotton Research Center located at Nanded, Maharashtra, at coordinates 19.1361° N; 77.3464° E. The samples were carefully transported with ice bags while keeping them between 2°C and 4°C [20] until analysis was carried out as per APHA standards for physical and chemical properties [21].

2.2 | Isolation of AgNPs synthesizing *Pseudomonas* species

The isolates were subjected to screen for their capacity to synthesize AgNPs through the supernatant addition method, as described by Deepak and Kalishwaralal [22]. Isolates supernatants that exhibited a color change in a 1 mM solution of AgNO$_3$ were treated as AgNPs producer and such isolates were further characterized through cultural, morphological, and 16S rRNA gene sequencing [23, 24]. The confirmation of AgNP production was primarily conducted through UV Visible spectroscopy, while scanning electron microscope (SEM), dynamic light scattering (DLS), field emission scanning electron microscopy (FESEM), and X-ray diffraction (XRD) analyses were used to characterize them. Additionally, functional groups were identified through Fourier transform infrared (FTIR) analysis (Supporting Information Method 1.1).

2.3 | Antifungal activity of AgNPs and *Pseudomonas* species

The efficacy of AgNPs that were synthesized was tested against two plant pathogens, namely *Aspergillus niger* and *Fusarium oxysporum*, using the Poisson method. Meanwhile, the antifungal activity of *Pseudomonas* was determined using the dual culture method (Supporting Information Method 1.2).

2.4 | Impact of AgNPs on community growth profiling and plant growth promoting traits

The joint effect of AgNPs and particular carbon, nitrogen and vitamin (CNV) was assessed to analyze the community composition of cultivable bacteria using various combinations. These combinations included only soil, soil with AgNs, soil with AgNPs and carbon sources, soil with AgNPs and organic nitrogen sources, soil with AgNPs and vitamins, soil with AgNPs plus carbon, nitrogen, and vitamins, as well as other combinations involving the addition of carbon alone or in combination with either nitrogen or vitamins to the original soils (Supporting Information: Table S1 for list of used carbon, vitamin and amino acids). A medium was prepared and 100 μL of soil suspensions were added to it. The tubes containing the mixture were then kept in an incubator at a temperature of 30°C. After every interval of 24 h, the optical density of the solution was measured at a wavelength of 580 nm [25–28]. Two plant growth promoting traits, namely IAA production and phosphate solubilization were also investigated for the aforementioned combinations (Supporting Information Method 1.3).

2.5 | Plant growth promotion activity and metagenome analysis

A pot assay was conducted to study the combined effects of AgNPs and selected CNV sources. Ten different pot combinations were created, each with varying levels of soil, AgNPs, carbon sources, organic nitrogen sources, and vitamins. Before planting, the seeds were sterilized with a mixture of ethanol and HgCl$_2$. After planting three seeds per pot and watering with sterile distilled water, the pots were kept in a dark area at a temperature of 38°C until germination occurred. After 10 days, the seedlings were measured for root-shoot length, dry weight, and photosynthetic pigments. This experiment was performed three times to ensure accuracy. The plant growth promotion and plant growth promoting traits rhizobacteria exhibited high levels of success in the combinations, leading to their selection for metagenome analysis. A soil sample of 1 g of seedling staged rhizospheric was collected and DNA was extracted using a modified method suggested by Shah et al. [29]. The lysed soil samples were washed twice with chloroform and then precipitated with 5% polyethylene glycol (PEG-10,000). The purified DNA was then analyzed with a UV spectrophotometer and a 1% agarose gel. DNA was amplified using V3F_CCTACGGGNBGCASCAG, V4R_GACTACNVGGGTATCTAATCC primers before being constructed into a library as per the instructions of NEBNext UltraTM DNA Library Prep Kit for Illumina and then sequenced at Illumina Hiseq. 2500. Finally, QIIME 2 was used to determine the taxonomical, structural diversity and functional annotation study of the raw sequences (fastq).

3 | RESULTS

3.1 | Physicochemical analysis of soil samples

Soil samples were physico-chemically analyzed, and no significant impact of AgNPs and augmented CNV on soil physicochemical parameters was identified (Table 1).

3.2 | Isolation and characterization of AgNPs producing *Pseudomonas* species

The isolates were screened for extracellular biosynthetic ability of AgNPs production and identified

TABLE 1 Physicochemical analysis of soil samples.

| Sr. no.	Sample Parameter / soil samples	pH	Electric conductivity (MS/cm)	Organic carbon (%)	Phosphorus (kg/h)	Potassium (kg/h)	Ferrous (ppm)	Manganese (ppm)	Zinc (ppm)	Copper (ppm)
1	Only soil	7.26	0.57	0.59	52.01	443.59	4.76	2.40	0.83	0.38
2	Soil + AgNPs	7.28	0.54	0.53	52.03	443.46	4.72	2.38	0.84	0.36
3	Soil+AgNPs + carbon	7.27	0.55	0.55	52.02	442.86	4.73	2.39	0.84	0.36
4	Soil + AgNPs + nitrogen	7.28	0.53	0.55	52.02	443.46	4.73	2.39	0.84	0.36
5	Soil+AgNPs + vitamin	7.26	0.56	0.54	52.04	442.96	4.74	2.37	0.84	0.36
6	Soil + AgNPs + vitamin + carbon + nitrogen	7.27	0.56	0.56	52.01	442.98	4.75	2.39	0.84	0.36
7	Soil + carbon	7.26	0.55	0.56	52.02	443.56	4.75	2.38	0.84	0.36
8	Soil + nitrogen	7.27	0.56	0.57	52.03	442.98	4.74	2.39	0.84	0.36
9	Soil + vitamin	7.27	0.54	0.58	52.04	442.97	4.74	2.39	0.84	0.36
10	Soil + vitamin + carbon + nitrogen	7.28	0.57	0.58	52.03	442.99	4.76	2.39	0.84	0.36
11	SD	0.01	0.01	0.02	0.01	0.29	0.01	0.01	0.01	0.01
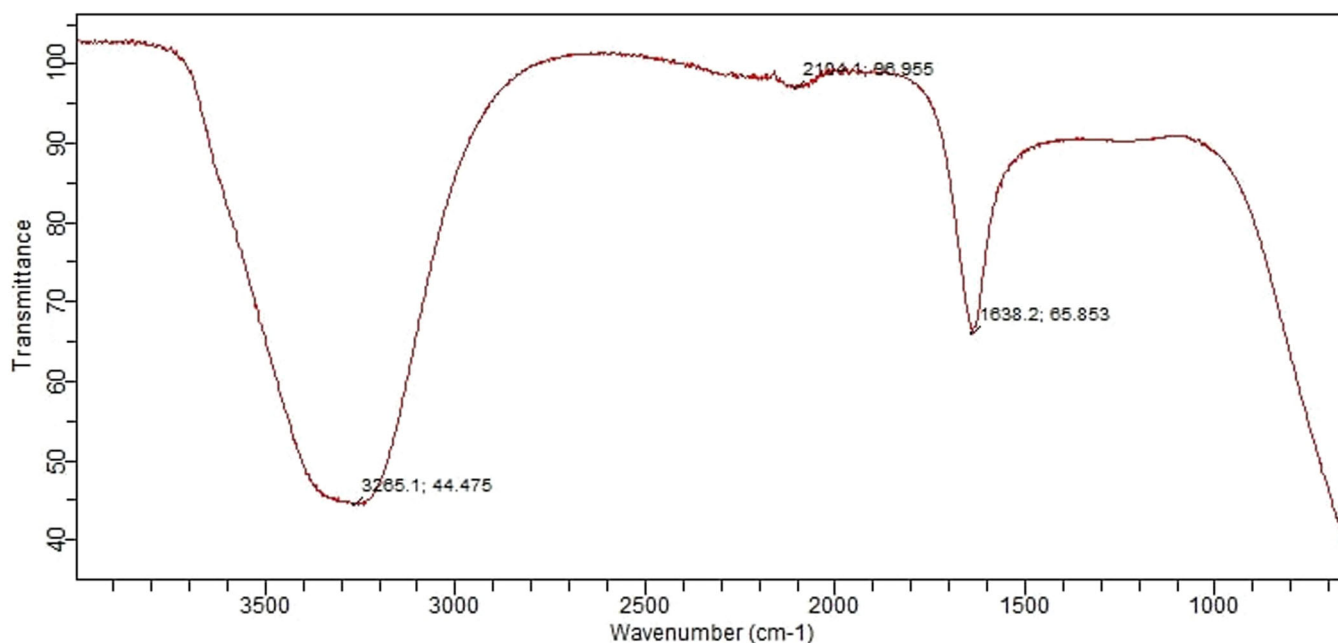
FIGURE 1 Fourier transform infrared spectrum of silver nanoparticles (control).

as *Pseudomonas fluorescens* based on its morphological, biochemical, and molecular characteristics (NCBI accession numbers MG234532 and MG230469) (Supporting Information: Tables S1 and S2) [30].

3.3 | Characterization of AgNPs

3.3.1 | UV visible spectrophotometer

Scanning of the broth from 200 to 650 nm revealed its absorption maxima close to 420 nm. The contaminants may alter the absorption peak. The maximum absorption at the 420 nm wavelength validates the presence of silver nanoparticles in the broth solution.

3.3.2 | FTIR analysis

The extracellular synthesis of AgNPs by *Pseudomonas fluorescens* is validated by FTIR analysis. Except for the peaks 1058.6 and 1015.6, it resembles the control. Bond stretches at 3267 confirm alcoholic O–H stretch, whereas a faint peak at 2098.6 indicates C–C stretch and C=C at 1638.2. The primary alcohol's C=O stretch at 1058.6 and ring vibration at 1015.6 in the sample indicate the existence of extra functional groups associated with AgNPs, which could have accelerated the conversion of Ag^{2+} ions into Ag0 (Figures 1 and 2).

3.3.3 | DLS

To determine the stability, particle size, and presence of charge over them, the zeta potential of a sample was measured. The results showed that 70% of AgNPs had dimensions of 101.2 nm (d) × 22.4 nm (w), while 30% had larger dimensions measuring at 399 (d) × 124.3(w). All AgNPs carried a negative charge with a conductivity of 278 μS/cm at analysis conducted at temperatures of 26°C and pH levels at seven. The high conductivity and size for the latter group suggests possible agglomeration among them. Dispersion or aggregation in AgNPs was confirmed through FESEM and SEM analysis as well [31].

3.3.4 | SEM and FESEM

The pictures displayed particles that were irregular in shape and clustered together to form agglomerates (Figures 3 and 4).

3.3.5 | XRD

Figure 5 shows experimental diffraction patterns of AgNP samples. The XRD pattern of AgNPs, with diffraction peaks at 26.622°, 33.097°, 36.520°, 40.263°, and 45.763°, can be attributed to a face centered cubic (FCC) structure.

We determined the miller indices (hkl) values for all of the peaks and found that hkl values related to the peak
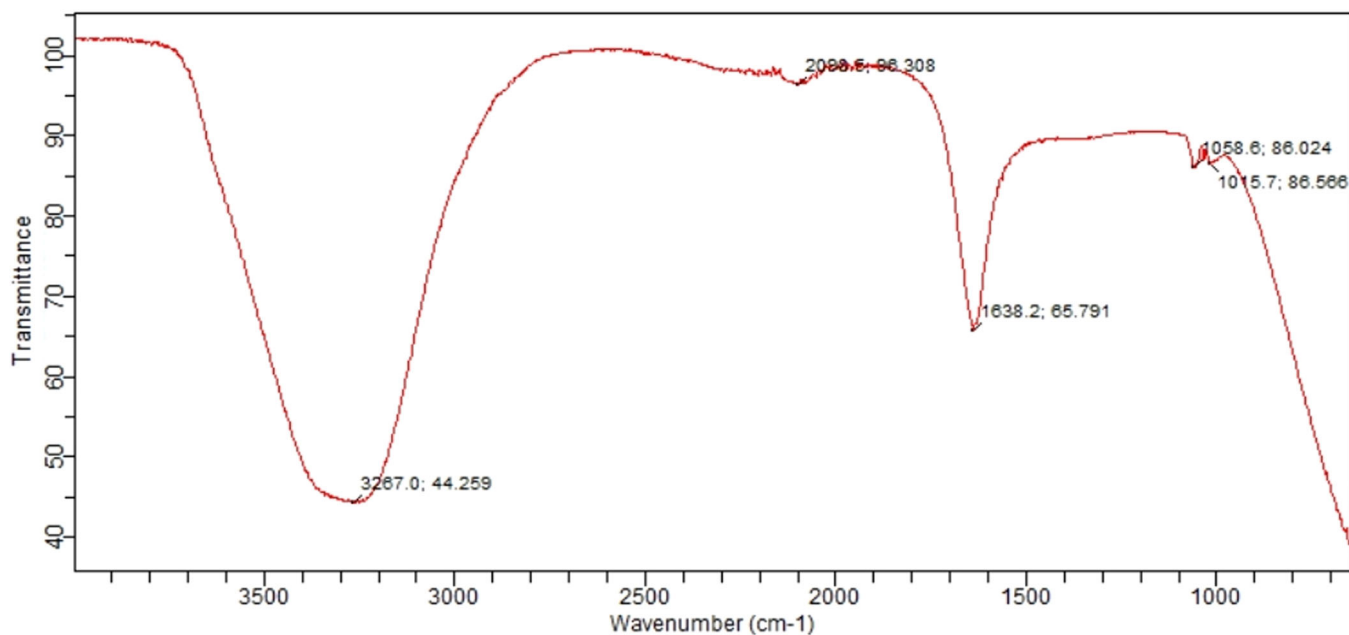
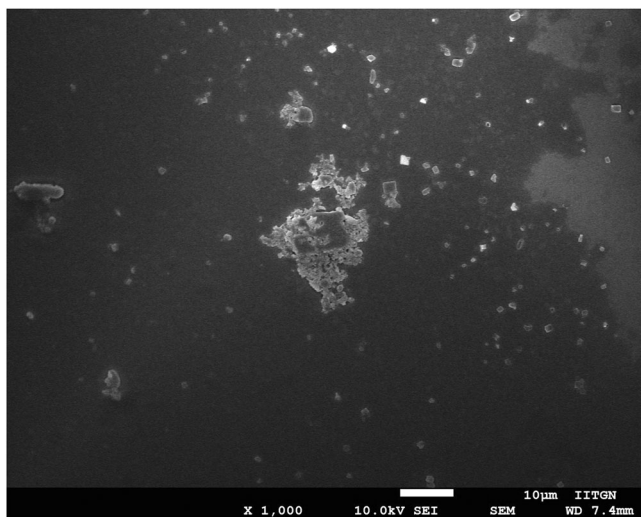**FIGURE 2** Fourier transform infrared spectrum of silver nanoparticles (synthesized by isolate).

FIGURE 3 Field emission scanning electron microscopy image of silver nanoparticles.

FIGURE 4 Scanning electron microscope image of silver nanoparticles.

are either odd or even, indicating that it belongs to the FCC. The other peaks in graph highlight the presence of organic (protein) impurities associated with AgNPs.

3.4 | Antifungal activity of AgNPs

Table 2 illustrates the evaluation of AgNPs and *Pseudomonas fluorescens* against *F. oxysporum* and *A. niger*, indicating a high level of activity (Avg. 71.66% for AgNPs and 92.1% for isolates) against *F. oxysporium* but a
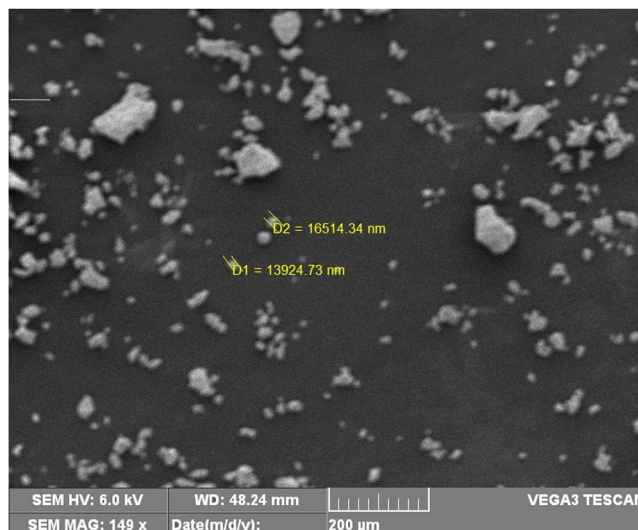
low level of activity (Avg. 61.39% for AgNPs and 69.65% for isolates) against *A. niger* was observed in both cases.

3.5 | Impact of AgNPs on community growth profiling and plant growth promoting traits

The effects of AgNPs on community growth profiling of rhizobacteria have been made evident by our study. To
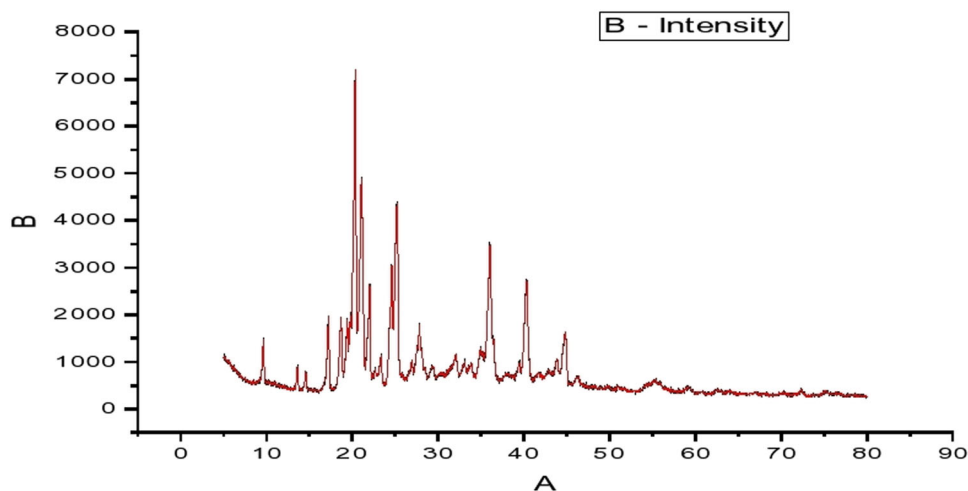
FIGURE 5 X-ray diffraction pattern of silver nanoparticles.

TABLE 2 Antifungal activity of silver nanoparticles and *Pseudomonas fluorescens*.

Sr. no.	Incubation	*Fusarium oxysporum* control = 4.5 cm % of inhibition	*Aspergillus niger* control = 8.5 cm % of inhibition
1	AgNPs after 24 h	100 ± 0	68.2 ± 1.2
2	AgNPs after 48 h	83.3 ± 0.7	60.9 ± 1.6
3	AgNPs after 72 h	31.6 ± 1.1	54.9 ± 1.9
4	*Pseudomonas fluorescens*	92.4 ± 0.8	69.1 ± 0.9

combat these effects, we utilized 16 varying carbon sources, five types of vitamin sources and eight amino acids in the experiment. Through our findings, it was determined that rhizobacteria displayed notable turbidity levels when subjected to certain carbon sources such as glucose, fructose, and adonitol. Additionally, sialic acid and ascorbic acid were highlighted as effective vitamin sources along with the use of asparagine as a nitrogen source (Supporting Information: Table S1). The impact of different combinations on the growth-promoting traits of rhizobacteria was re-examined, revealing that fructose, asparagine and sialic acid were the main sources contributing to the expression of two studied plant growth promoting traits: IAA and phosphate solubilizers in combination with AgNPs. The study emphasizes that combining fructose, sialic acid, and asparagine supplements allows for optimal expression of IAA and phosphate solubilization; when used alone, they allow for maximal propagation of IAA producers (Figure 6).

3.6 | Plant growth promotion activity

The use of AgNPs has an effect on both the germination process of seeds and growth of plants. In particular, a decrease in seed germination and chlorophyll levels were noted when exposed to combined actions of AgNPs-sialic acid and AgNPs-aspargine. On the other hand, plant growth was notably enhanced with the use of AgNPs-fructose based on Table 3 data.

3.7 | Metagenome analysis

The metagenome analysis of the rhizospheric soil sample was conducted using the 16S (V3–V4 region dependent) technique, as depicted in Supporting Information Data-2. The resulting bacterial community structure in the cotton rhizosphere is represented in Figure 6 (Supporting Information: Figures S1 and S2). Among all obtained reads, bacteria accounted for 95.50%. Actinobacteria emerged as the most common phylum with a proportion of 44.70%, followed by Firmicutes at 15.9% and unclassified bacteria at 14.4%. Additionally, Actinobacteria were found to be abundant when classified by class while unclassified bacteria and *Actinomycetales* dominated communities on an order level basis, as observed during analysis. The study revealed the presence of unclassified bacteria at both family and genus level, with
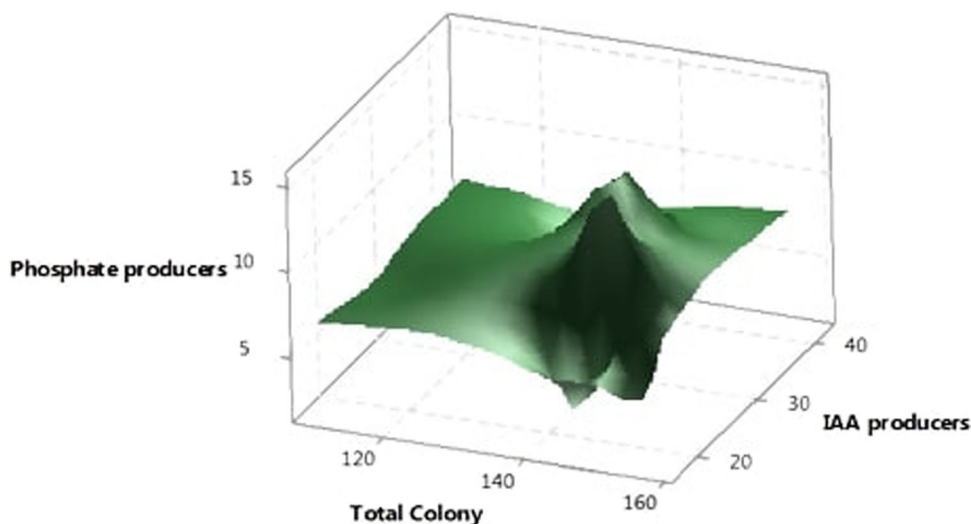
FIGURE 6 Impact of silver nanoparticles associated carbon, nitrogen, vitamin treatment on plant growth promoting traits of rhizobacteria.

actinobacteria families showing a significant proportion. Upon exposure to AgNPs, changes were observed in the bacterial community. The phylum Proteobacteria was found to be most dominant (19.91%), followed by Firmicutes (8.84%) and Actinobacteria (3.94%). Gamma-proteobacteria proved to be the most prominent class, while *Pseudoalteromonadales* and *Pseudomonadales* emerged as the dominating communities at the order level. Additionally, *Pseudoalteromonadaceae* bacteria exhibited dominance at both family and genus level; however, there were also significant proportions of families belonging to *Pseudomonadaceae*, as illustrated in Figure 7 (Supporting Information: Figures S2–S6).

To address the problem of fluctuating bacterial communities, additional substances such as fructose, sialic acid, and asparagine were introduced both individually and in combination with AgNPs. After conducting experiments, it was determined that fructose had the most significant impact on altering microbial populations. When combined with AgNPs, fructose successfully increased population levels of Actinobacteria, Chloroflexi, Cyanobacteria, Planctomycota and Proteobacteria. On the other hand, using a mixture of fructose along with sialic acid and asparagine proved particularly effective in promoting growth among firmicutes (as shown in Figure 8). The increase in bacterial community had an impact on the growth of plants and an augmentation of chlorophyll content. The phylogenetic analysis provided clear evidence of this change. Alpha-proteobacteria were mainly promoted by supplementing AgNPs with fructose and asparagine, while sialic acid with AgNPs resulted in the promotion of Acidobacteria and Actinomycetes (Figure 9). The distribution of

actinomycetes was highlighted across all treatments through a heat map analysis (Figure 9) and phylogeny relationship was expressed in Figure 10.

4 | DISCUSSION

The focus of our recent investigation revolved around the impact of biosynthesized AgNPs and the supplementation they provided to *Gossypium hirsutum*. We found that AgNPs had a notable influence on the germination of Bt cotton seeds. While there were only slight variations in shoot length (measured in cm), leaf count per plant, dry weight produced by the shoots (measured in g), seed germination percentage, and photosynthetic properties compared with the control group, the addition of AgNPs yielded positive outcomes. This aligns with previous studies conducted by Farghaly and Nafady [32] as well as Latif et al. [33], which demonstrated that changes in nitrogen metabolism brought about alterations in photosynthetic properties. Further research by Qian et al. [34] revealed that when AgNP concentration exceeds 3 mg/Liter, it can initiate the apoptosis process in plants, as observed in their study on *Arabidopsis thaliana*. Meanwhile, Tripathi et al. [17] review concluded that smaller AgNPs (10–50 nm) have more severe toxic effects compared with larger ones. In our study, we utilized AgNPs larger than 100 nm with a concentration below 3 mg/L, resulting in minimal direct stimulation of plant growth and inhibition of plant pathogens.

The examination of AgNPs and isolate revealed that AgNPs surpasses isolate in terms of effectiveness. However, it should be noted that the synthesized AgNPs,

TABLE 3 Effect of silver nanoparticles (AgNPs) on *Gossypium hirsutum* seed germination and growth.

| AgNPs (mg/L)	Shoot length (cm)	Leaves (no./plant)	Shoot dry wt./plant (g)	% Seed germination	Chl a	Chl b	Carotenoids	Total pigments
Only soil	10	3	0.76	80	1.089	0.442	0.28	1.811
Soil + AgNPs	9.6 ± 0.8	2.5	0.72	63.33	0.876	0.234	0.189	1.432
Soil + AgNPs + carbon	16 ± 1.20	4 ± 0.79	0.95 ± 0.13	100 ± 0	1.39 ± 0.1	0.66 ± 0.04	0.296 ± 0.02	2.311 ± 0.1
Soil + AgNPs + nitrogen	14.2 ± 1.1	3 ± 0.54	0.96 ± 0.12	100 ± 0	1.13 ± 0.23	0.52 ± 0.03	0.278 ± 0.01	2.44 ± 0.17
Soil + AgNPs + vitamin	14.1 ± 1.5	3 ± 0.63	0.95 ± 0.13	100 ± 0	1.14 ± 0.21	0.56 ± 0.04	0.289 ± 0.023	2.6 ± 0.15
Soil + AgNPs + vitamin + carbon + nitrogen	13.9 ± 1.23	4 ± 0.54	0.98 ± 0.12	100 ± 0	1.17 ± 0.24	0.60 ± 0.04	0.294 ± 0.026	2.8 ± 0.20
Soil + carbon	11.2 ± 0.44	4 ± 0.66	0.88 ± 0.40	83.33	0.92 ± 0.2	0.36 ± 0.03	0.196 ± 0.018	1.8 ± 0.1
Soil + nitrogen	11.4 ± 0.42	4 ± 0.36	0.90 ± 0.18	83.33	0.96 ± 0.2	0.38 ± 0.03	0.212 ± 0.023	2.1 ± 0.11
Soil + vitamin	11.4 ± 0.42	4 ± 0.36	0.89 ± 0.17	83.33	0.98 ± 0.2	0.42 ± 0.03	0.230 ± 0.023	2.1 ± 0.11
Soil + vitamin + carbon + nitrogen	12.2 ± 1.2	4 ± 0.30	0.94 ± 0.12	90%	1.04 ± 0.1	0.44 ± 0.04	0.240 ± 0.02	2.2 ± 0.11
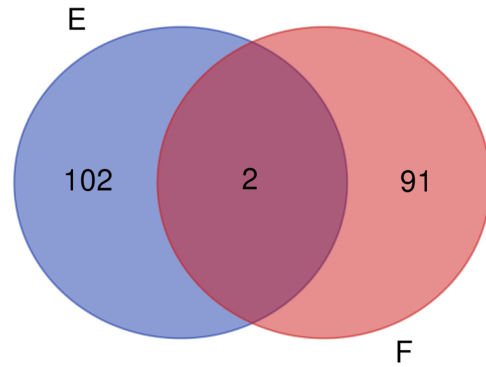
FIGURE 7 Impact of silver nanoparticles (AgNPs) on bacterial communities. E, only soil; F, soil + AgNPs.
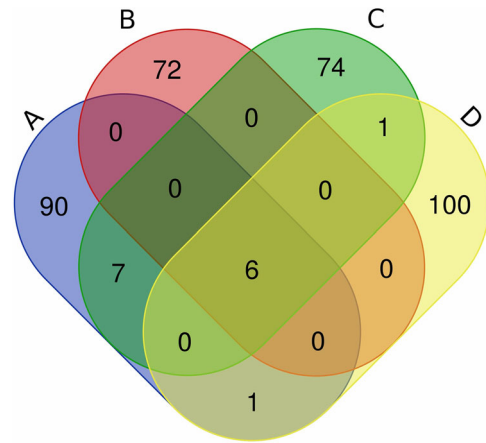

FIGURE 8 Impact of silver nanoparticles (AgNPs) with supplemented carbon, nitrogen and vitamin sources over bacterial communities. A, AgNPs + carbon (fructose); B, AgNPs + vitamin (sialic acid); C, AgNPs + amino acid (asparagine); D, AgNPs + carbon (fructose) + vitamin (sialic acid) + amino acid (asparagine).

which exists in agglomerate form and exceeds 60 nm, does not exhibit complete inhibition of *Fusarium oxysporum* and *Aspergillus niger*. These findings align with the conclusions drawn by Jian et al [35].

An investigation into the influence of AgNPs and provided supplements on rhizospheric bacterial communities was carried out using metagenome analysis. Given the intricacy of the metagenomes, it is impossible for any software pipeline to encompass complete functional and taxonomic annotation, as pointed out by Sharpton [36]. Consequently, the data collected was analyzed using MG RAST and QIIME software. The findings from both software applications were found to be similar.

Based on the QIIME 2 analysis of the control study, it was discovered that Actinobacteria was the most prevalent phylum in the soil, accounting for 44.70%. Following closely behind were Firmicutes (15.9%) and unclassified
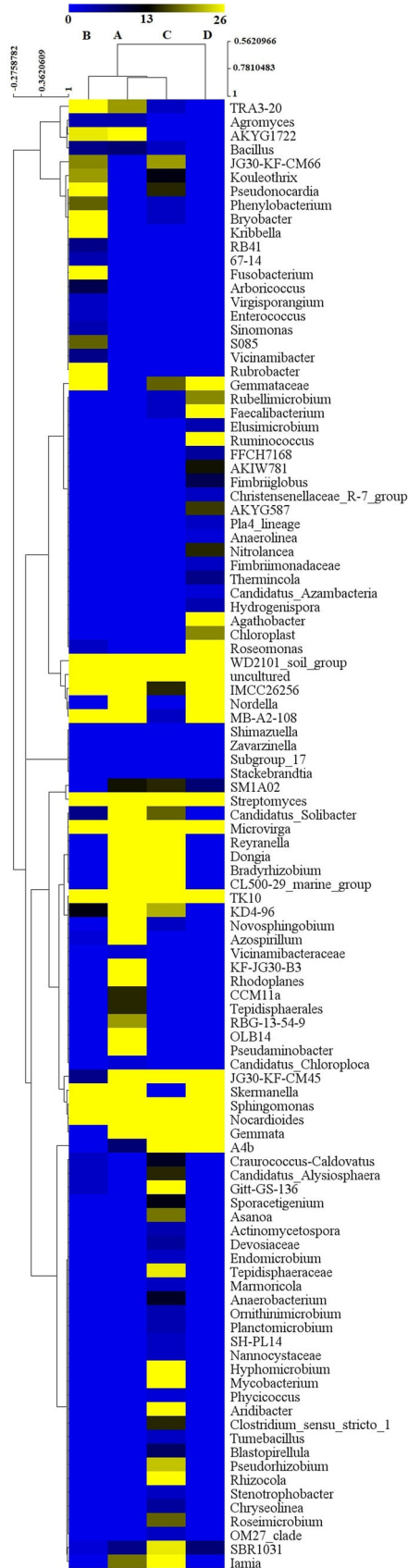
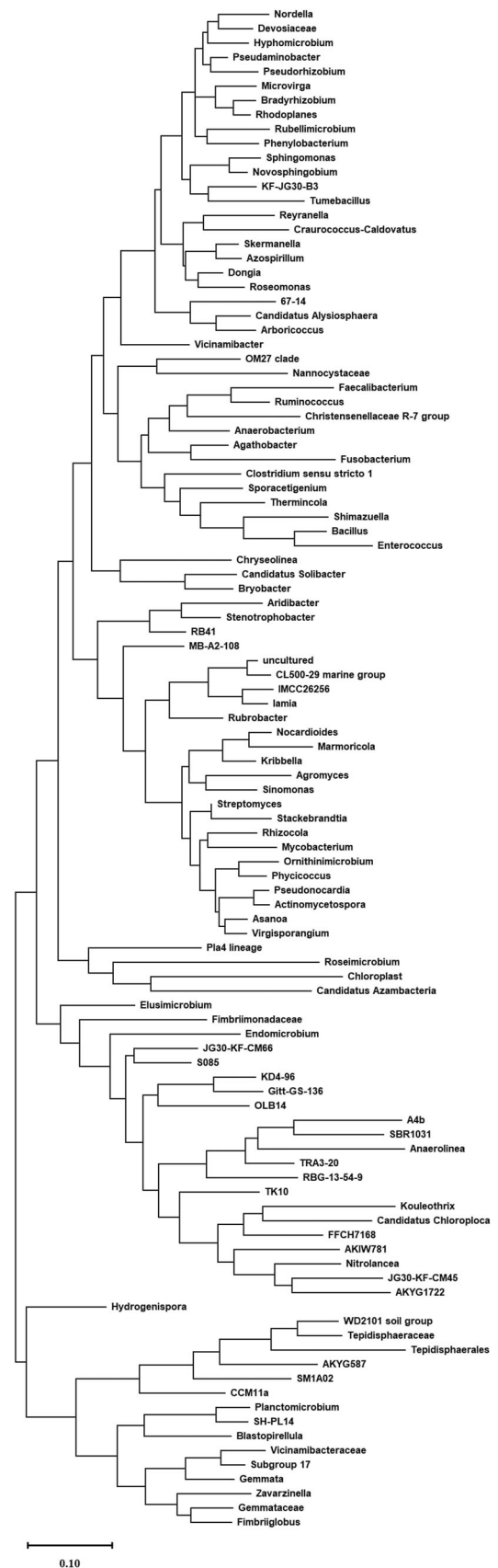**FIGURE 9** Genus heat map of isolates.

FIGURE 10 Phylogeny tree of isolates.

bacteria (14.4%). However, when compared with Ullah Abid [37] findings on Bt cotton rhizosphere, it was observed that Proteobacteria emerged as the dominant species at 31.7%, followed by Actinobacteria (29.6%), Gemmatimonadetes (9.8%), Chloroflexi (9%), Cyanobacteria (5.6%), and Acidobacteria. These results suggest a potential alteration in microbial composition due to abiotic factors.

When AgNPs was introduced into the rhizospheric samples, a shift in taxonomic units occurred. Proteobacteria took the lead with a dominance of 19.9%, followed by Firmicutes at 8.84%, and Actinobacteria trailing behind at a mere 3.95%. Actinobacteria is often studied using fructose as a marker because it promotes the growth of specific genera within this phylum [38] Notably, fructose has been found to induce changes in the gut microbiota of rats and stimulate the growth of actinobacteria [39]. Our research sheds light on similar effects in soil and demonstrates how the combination of fructose and AgNPs can impact plant growth. We also observed that fructose promotes the growth of other plant-growth-promoting phyla such as Chloroflexi, Cyanobacteria, Planctomycota, and Proteobacteria [40]. In previous studies conducted by Sharma et al. [41] the support is required to improve phosphate solubilization. However, our own research did not uncover any evidence suggesting that fructose alone had a similar effect [42, 43]. Exploring the role of sialic acid in relation to the gut microbiome, studies by North et al. [44] and Bell et al. [45] have highlighted its significance in the transportation mechanism of phosphate among various bacteria [46]. Interestingly, our findings indicate that there is no direct correlation between growth promotion within a phylum and its phylogenetic connection. This research represents a pivotal initial step toward manipulating natural rhizospheric microbiomes while incorporating AgNPs, showcasing a linear relationship between ecological succession and the promotion of plant growth, all while taking into consideration environmental safety precautions [47]. Further investigations may contribute to a deeper understanding of how microbiomes can be shaped to ensure sustained plant viability.

Through biosynthesis, we discovered that AgNPs possess exceptional effectiveness in combating fungal infections caused by *F. oxysporum*. Surprisingly, under the experimental conditions, no significant effects on shoot length (measured in centimeters) were observed in either of the tested plants. Similarly, there was minimal observable change in the number of leaves per plant or the dry weight of the shoots (measured in grams per plant). Although there were slight variations in seed germination percentages, these differences were not statistically significant and our results are in-line with

the results of Mazumdar [48]. Furthermore, there were no particularly noticeable alterations in the photosynthetic properties of either plant, suggesting that further investigation is necessary to fully comprehend the complete impact of the experimental treatment.

The results of this study indicate that combining AgNPs with fructose has the potential to enhance the growth of *G. hirsutum*, a commonly used cotton-producing plant, and also foster metabolic profiling of rhizospheric bacteria. This discovery holds significant implications for agricultural practices, especially in regions where the soil may lack nutrients or be contaminated.

ORCID

Abhijeet Joshi http://orcid.org/0000-0003-1053-9678

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.