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Synergistic Impacts of Plant-Growth-Promoting Bacteria and Selenium Nanoparticles on Improving the Nutritional Value and Biological Activities of Three Cultivars of *Brassica* Sprouts

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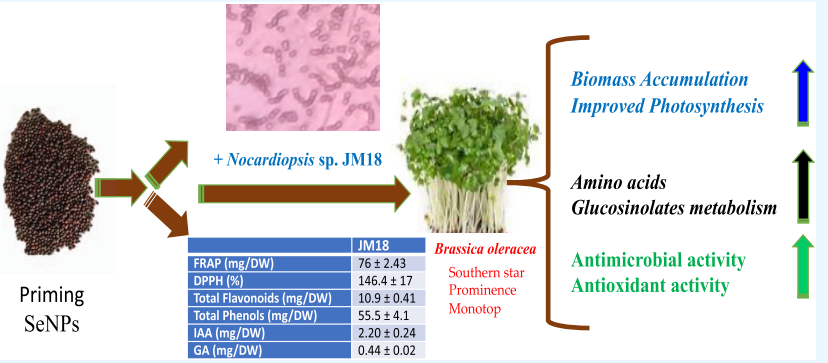

ABSTRACT: Due to the growing world population and increasing environmental stress, improving the production, nutritional quality, and pharmaceutical applications of plants have become an urgent need. Therefore, current research was designed to investigate the impact of seed priming using plant-growth-promoting bacteria (PGPB) along with selenium nanoparticles (SeNPs) treatment on chemical and biological properties of three *Brassica oleracea* cultivars [Southern star (VA1), Prominence (VA2), Monotop (VA3)]. With this aim, one out of five morphologically different strains of bacteria, namely, JM18, which was further identified via 16S rRNA gene sequencing as a *Nocardiopsis* species with strong plant-growth-promoting traits, isolated from soil, was used. To explore the growth-promoting potential of *Nocardiopsis* species, seeds of three varieties of *B. oleracea* were primed with JM18 individually or in combination with SeNP treatment. Seed treatments increased sprout growth (fresh and dry weights) and glucosinolate accumulation. The activity of myrosinase was significantly increased through brassica sprouts and consequently enhanced the amino-acid-derived glucosinolate induction. Notably, a reduction in effective sulforaphane nitrile was detected, being positively correlated with a decrease in epithiospecifier protein (EP). Consequently, the antioxidant activities of VA2 and VA3, determined by the ferric reducing antioxidant power (FRAP) assay, were increased by 74 and 79%, respectively. Additionally, the antibacterial activities of JM18-treated cultivars were improved. However, a decrease was observed in SeNP- and JM18 + SeNP-treated VA2 and VA3 against *Serratia marcescens* and *Candida glabrata* and VA1 against *S. marcescens*. In conclusion, seed priming with the JM18 extract is a promising method to enhance the health-promoting activities of *B. oleracea* sprouts.

1. INTRODUCTION

In recent years, there has been a lot of interest in plants as a source of healthy food, pharmaceuticals, and energy, as they are good providers of carotenoids, phenolic compounds, minerals, vitamins, and dietary fiber. One of the top 10 economic crops and the most utilized in the world is Brassica (i.e., Chinese cabbage, broccoli, kale, and cauliflower). Brassica is a well-known component of a healthy diet since it is a significant source of minerals, vitamins, carbohydrates, amino acids, and phytochemicals that improve health.[1] The synergistic effects of plant phytochemicals, which enhance the immune system functions and meanwhile help prevent cancer, heart diseases, and chronic diseases, are very beneficial to human health.[2] Biofertilizers, such as plant-growth-promoting bacteria (PGPB), are promising alternatives to avoid soil and environmental degradation introduced because

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of over usage of chemical supply of macronutrients such as nitrogen, phosphate, and potassium, necessary for plant growth, in particular under intensive cropping.[3] PGPB inoculation of seeds can stimulate the growth of plants by improving nutrient availability, protecting plants from diseases, triggering plant hormones, decreasing of ethylene levels in plants, and making plants more resistant to environmental stressors.[4,5] Besides their role in rhizosphere soil fertility through organic matter recycling, some PGPBs can create symbiotic interactions with their host plants.[6] PGPBs have a range of strategies to promote plant growth and health, including symbiotic fixation of atmospheric nitrogen; the release of siderophores and phytohormones including gibberellins, auxins, and cytokinin; solubilizing phosphorus (P) and other minerals; and synthesis of enzymes that relieve stress.[7,8]

One of the efficient methods for enhancing the development, growth, and yield of plants is seed treatment before germination, an inexpensive and easy way to induce a priming effect that can improve strength and surviving capacities in challenging environments; as a consequence, this technique improves the percentage of appearance, biomass production, crop production, and stability of ionic homeostasis.[9] Sprouts are premature seedlings generated by the sprouting and germination processes; they are a nutritious food containing a variety of nutrients with a favorable impact on human health, including some with antioxidant capacity and cancer and cardiovascular disease protection.[10] In detail, broccoli sprouts are rich in dietary fiber, sulfur-containing compounds, and glucosinolate metabolites that are rare in other vegetables.[11] These phytochemicals show beneficial impacts on human health, and their pharmacological potential is linked to their degrading products, indoles, and isothiocyanates.[12] Besides their role in human nutrition, the high concentrations of glucosinolates, amino acids, and antioxidants present in broccoli sprouts are well known for their exceptional biological activities and beneficial effects on plant metabolism.[11] Glucosinolates play an important role in plant protection systems. However, environmental factors can alter their production both in terms of quantity and quality.[10] It was reported that broccoli sprouts have 10−100 times glucosinolates when compared to adult tissues. Sulforaphane is the most prevalent and effective glucosinolate in broccoli. Sulforaphane and sulforaphane nitrile are byproducts of glucosinolate hydrolysis by cytosolic endogenous myrosinase.[13] The myrosinase enzyme activity, the presence of other proteins like epithiospecifier protein (ESP), and conditions like temperature and pH play a role in this hydrolysis process and compounds that are generated.[14]

Today, mineral deficiency, especially of those minerals that are essential for the nourishment of both human beings and animals through various agricultural products, is a determining factor in crop production.[15] Nanotechnology provides a good alternative to chemical fertilizers and pesticides for the development of environmentally safe and useful substances. Nanoparticles (NPs) have been shown to have unique and innovative physicochemical properties that are superior to those of their bulk counterpart due to their enormous surface-to-volume proportion and the size range of 5−100 nm.[16]

Selenium is a common trace component that is essential for maintaining plant physiology.[15] This element is essential for the full functioning of a living plant. Previous research has demonstrated the benefits of selenium nanoparticles in plant growth, nutritional quality, crop production, and antioxidant capacity.[17]

Improving plant growth and its nutritional status are among the most important concerns of agricultural researchers. This study aimed at investigating the combined effects of useful bacteria and nanoparticles on Brassica species. Several bacterial strains were isolated from the rhizospheric soil and molecularly identified. The isolated strains were preliminarily surveyed for plant-growth-promoting traits like IAA, phosphate, and ammonia production, as well as potassium solubilization, chitinase and glucanase production, and their ability to promote plant growth. The selected strain along with selenium nanoparticles was evaluated either separately or combined for enhancing growth, glucosinolate metabolism, and antioxidant and antimicrobial activities of three cultivars of *Brassica*.

2. MATERIALS AND METHODS

2.1. Isolation of Plant-Growth-Promoting Bacteria (PGPB). Soil samples were randomly collected from local grass fields (Giza, Egypt) at 3−4 cm below the soil surface. Sterilized zip-lock polythene bags were used to collect and transfer the soil samples to the laboratory. For further processing, each sample was kept in a refrigerator at 4 °C. The standard serial dilution plate method was used to isolate bacteria from soil samples, resuspending 10 g of the soil sample in sterile distilled water. Afterward, the samples were 10-fold serially diluted. From each soil dilution, a 100 μL aliquot was inoculated onto sterile nutrient, plated on yeast extract mannitol agar, which was incubated for a week at 30 °C.[18] The number of colonies obtained on each plate was determined, and the characteristics of each colony were recorded. For further identification, separate colonies were kept in nutrient agar slopes at 4 °C for morphological and biochemical characterization.

2.2. Biological Activities of Bacterial Isolates. *2.2.1. Antioxidant Activities.* The antioxidant activities of the bacterial isolates (JM18, JM19, JM20, JM21 and JM22) were determined by the ferric reducing antioxidant power method (FRAP) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Briefly, the isolates were extracted by placing 0.1 g of sprouts in 80% ethanol, and the mixture was centrifuged for 20 min at 14,000 rpm. C. In order to assess the antioxidant activity, 0.1 mL of the supernatant was mixed with DPPH and FRAP reagents. For FRAP, 170 μL of a freshly prepared FRAP reagent (acetate buffer (0.3 M, pH 3.6), TPTZ (0.01 mM), $FeCl_3 \cdot H_2O$ (0.002 mM)), was mixed with 30 μL of diluted ethanol extracts at 25 °C incubation for 30 min. The antioxidant capacity was calculated using a Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) standard. For the DPPH assay, each plant extract was mixed with 0.4 mL of a DPPH solution (0.24 mM in 96% ethanol). After shaking at room temperature for 30 min, 2 mL of ddH_2O was added, and the inhibition % was calculated. After incubation for 35 min at 29 °C, the absorbance was measured at 517 nm and 590 nm for DPPH and FRAP, respectively,[19,20] using a microplate reader (Synergy Mx, Biotek Instruments Inc., Vermont, VT).

2.2.2. Phytochemical Analysis. Previously described methods[21] were used for the phytochemical analysis of the bacterial isolates using total phenolic content and total flavonoid content. About 0.25 mL of the methanolic extract was added to 2.25 mL of distilled water and 0.25 mL of Folin−Ciocâlteu's reagent for 90 min. The solution was incubated at room temperature, and 2.50 mL of Na_2CO_3 (7%) was used to neutralize the reaction in complete darkness. Using a standard

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curve of gallic acid, total phenolic content was calculated in the form of gallic acid equivalency (mg g^{-1} dry weight). Regarding flavonoid contents, a 15% $NaNO_2$ solution was added after mixing 250 μL of the extract with 1000 μL of distilled water. $AlCl_3$ (10%) in the quantity of 75 μL was added; after 6 min of incubation at room temperature, 1000 μL of NaOH (4%) was added, followed by distilled water up to 2500 μL. After mixing, the mixture was left to stand for 15 min, and the absorbance was measured at 510 nm against a blank. The amount of catechin equivalent was used to express the total flavonoid contents in 1 g of the dry extract (mg CE g^{-1} extract).

2.2.3. Antibacterial Activity. The antibacterial activity of different bacterial extracts was determined using the agar well diffusion method.[22] Each extract was concentrated (20 mg mL^{-1}) by dissolving 200 mg of each extract in 10 ml of 99.9% dimethyl sulfoxide (DMSO). A negative control consisted of pure DMSO, while a positive control was of cefotaxime (2 mg mL^{-1}). *Escherichia coli* and *Streptococcus* sp. bacterial strains were used in triplicate tests for each microorganism, and results were presented as arithmetic averages.

2.3. Molecular Identification of the Bacterial Isolate. The bacterial isolate JM18 was identified using 16S rDNA sequencing.[23] The eubacterial universal primers, i.e., 5′-AGAGTTTGATCCTGGCTCAG-3′ primers (27F) and 5′-GGTTACCTTGTTACGACTT-3′ primers (1492R) were used to amplify the 16S rRNA gene. The polymerase chain reaction (PCR) conditions were as follows: 30 cycles, 95 °C predenaturation for 5 min, 94 °C denaturation for 1 min, 60 °C annealing for 1 min, 72 °C extension for 1 min and 30 s, 72 °C final extension for 10 min, 4 °C hold. Then, 50 ng μL^{-1} each PCR product was used to prepare the samples, which were sent to MacroGen Company (Seoul, Republic of Korea) for molecular identification. The 16S rDNA sequence of the isolate was analyzed by the GenBank database and the BLAST search program to search for nucleotide sequence homology. Highly similar sequences were aligned, and neighbor-joining trees were generated using the MEGA version. Bootstrap replication was used to statistically support the nodes in the phylogenetic trees (1000 replications).

2.4. Growth Experiment. Broccoli cultivar seeds were collected from the market [(*Brassica oleracea*, variety Sourthern star (VA1); *B. oleracea*, variety Prominence (VA2); *B. oleracea*, variety Monotop (VA3))]. For the study, healthy seeds with similar size and form were selected, and the seeds were put in sterile Petri plates and bacterized aseptically in a laminar airflow cabinet by soaking in 5 g l^{-1} sodium hypochlorite for 1 h and then washed. Sterilized seeds were also treated with selenium nanoparticles (SeNPs) by dipping them in a 25 ppm solution of SeNPs for 6 h (control soaked in distilled water) before sowing. The seeds were spread on trays (four trays per treatment) filled with vermiculite, which contained a liquid suspension of inoculants (2.5×10^{-7} cfu mL^{-1}). Afterward, the seeds were transferred to a controlled growth chamber and kept at 25 °C air temperature, photosynthetically active radiation (400 mol m^2 s^{-1}), and a 16/8 h day/night photoperiod and 63% humidity. After 10 days, sprouts were harvested, and fresh and dry weights were measured. To measure the dry weights, some sprouts were determined after oven-drying at 70 °C until constant weight. Other sprouts were frozen in liquid nitrogen and kept in a −80 °C freezer for future biochemical analyses.

2.5. Determination of the Photosynthetic Rate. An EGM-4 infrared gas analyzer (PP Systems, Hitchin, U.K.) was used for the determination of the photosynthetic rate from 180 s measurements of net CO_2 exchange (NE).

2.6. Determination of Amino Acid Content. For the determination of amino acid content in treated and untreated *Brassica* cultivars, 200 mg of the seedlings was dissolved in 1 mL of 80% ethanol before being centrifuged (14,000 rpm for 20 min). The pellets were resuspended in 1 mL of chloroform, and the centrifugation was repeated. Thereafter, the extract was centrifuged (14,000 rpm for 20 min), and the aqueous phase was microfiltered (0.2 μm) using Novarline as an internal control. The amino acids were separated using ethylene bridged hybrid (BEH) amide 2.1 × 50 columns (UPLC-tqd system) at 30 °C.[24]

2.7. Determination of Glucosinolates. Glucosinolate levels in sprouts from all three *Brassica* varieties were determined as fully described by Dawoud et al.[24] Myrosinase activities were inhibited before glucosinolate extraction as sprout samples were heated for 2 min. The sprout samples were then extracted in a solution of 70% methanol and trifluoroacetic acid. After a while, the extracts were then placed in stoppered flasks with continuous stirring at 70 °C for 30 min. The extracts were left to cool, and then centrifugation (14,000 rpm for 20 min) was done. The supernatants were collected, filtered, and then evaporated. The residues were then resuspended in HEPES−KOH. To determine enzyme activity, thioglucosidase 0.12 U in HEPES−KOH 0.2 mM, pH 7.0, was treated with the total glucosinolate extract for 24 h at 37 °C. To stop the process, perchloric acid was added to the mixture. Instead of sample extracts, a buffer was used to represent the control samples. The glucosinolate content was estimated by calculating the amount of glucose produced by hydrolyzing glucosinolates with thioglucosidase, considering one mol of glucosinolates equivalent to one mol of the produced glucose. Total glucose was determined using a glucose oxidase/peroxidase assay.[24]

2.8. Determination of Biological Activities. *2.8.1. Antioxidant Activity.* The antioxidant activity of the sprouts was measured using the FRAP assay.[19] Extraction was done with 80% ethanol, followed by centrifugation for 20 min at 14,000 g. The sprout extracts were then mixed with FRAP reagents in a concentration of 0.1 mL; after a 25 °C incubation for 30 min, the absorbance was measured at 590 nm (for more details, see Section 2.2.1).

2.8.2. Antibacterial Activity. The antibacterial activity of the sprouts was analyzed against different bacterial strains: *Staphylococcus saprophyticus*, *Streptococcus salivarius*, *E. coli*, *Salmonella Typhimurium*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Serratia marcescens*, *Candida glabrata*, and *Aspergillus flavus*. Before use, the bacterial species were cultivated in brain heart infusion agar (BHIA) and incubated at 37 °C for 24 h. Then, the bacterial suspension was dispersed on Muller Hinton agar, as well as the extracts were loaded on filtered discs (6 mg per disc), which were placed on agar plates for 24 h at 37 °C. Finally, the inhibition zones were measured using a vernier caliper.[21]

2.9. Statistical Analysis. The results of each experiment were presented as means ± standard deviations for each triplicate experiment. One-way analysis of variance (ANOVA) was used to compare the results of the extract activities to those of the positive controls in order to determine significant differences. SPSS 22 (SPSS Science, Woking, U.K.) was used, and significant differences between means of treatments were identified using Duncan's test ($P < 0.05$).

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Table 1. Screening of the Five Different Bacterial Isolates Isolated from Local Grass Fields for their Biological Activity as Indicated by Flavonoids and Phenolics Levels and Total Antioxidant Capacity Measured by FRAP Assays[a]

	JM18	JM19	JM20	JM21	JM22
antioxidant activity (FRAP) (mg/DW)	76 ± 2.43a	51.32 ± 7.75b	55.69 ± 6.78b	33.75 ± 0.91c	59.55 ± 2.35b
antioxidant activity DPPH (%)	146.44 ± 17.30a	46.36 ± 2.59c	58.15 ± 4.41b	27.51 ± 0.22d	52.96 ± 5.98b
total flavonoids (mg/DW)	10.95 ± 0.41a	5.50 ± 1.06b	5.45 ± 0.04b	5.28 ± 0.81b	6.14 ± 0.83b
total phenols (mg/DW)	55.55 ± 4.10a	31.05 ± 2.72c	37.92 ± 4.50b	26.9 ± 1.57d	36.34 ± 0.99b
IAA (mg/DW)	2.20 ± 0.24a	1.20 ± 0.15b	2.3 ± 0.18b	1.15 ± 0.09c	1.55 ± 0.30b
GA (mg/DW)	0.44 ± 0.02a	0.31 ± 0.01b	0.15 ± 0.00c	0.39 ± 0.02a	0.16 ± 0.05c

[a]Values are represented by means ± standard deviations of three independent replicates. Different letters indicate significantly different between treatments at a P value < 0.05.

3. RESULTS

3.1. Isolation and Identification of PGPB. The bacteria were isolated from the soil using a serial dilution method and preliminarily identified by their phenotypic characteristics. Potential PGPB colonies were characterized for colonial size, color, margins, spore chain, and pigment synthesis, as well as cellular morphology, using light microscopy. Five isolated bacteria (JM18-JM22) were then submitted for further investigations (Table 1). Interestingly, the bacterial isolates JM18 and JM19 showed the maximum number of colonies. To determine the bioactivity of genera of isolated bacteria, some biochemical tests were performed.

3.2. Biological Activities of the Bacterial Isolate. *3.2.1. Antioxidant activity.* All isolates (JM18, JM19, JM20, JM21 and JM22) were screened for their overall antioxidant activities through DPPH and FRAP assays (Table 1). Regarding DPPH and FRAP analyses, the scavenging activities of JM18 were the highest, followed by JM22 and JM19, respectively.

3.2.2. Phytochemical Analysis. The total phenolic content of different bacterial extracts was calculated using the Folin−Ciocalteu technique. The results were given in micrograms of GAE per milligram of extracts (Table 1). Notably, the JM18, JM19, JM20, JM21, and JM22 showed a total phenolic content with values ranging between 26.9 and 55.5 μg GAE mg^{-1}; the JM18 extract had a significantly higher total phenolic content than the other extracts. Further, the total flavonoid contents of JM18, JM19, JM20, JM21, and JM22, determined in methanolic extracts and expressed as quercetin equivalent (QE), showed significantly higher values in the JM18 extract (10.9 ± 1.2 mg QE g^{-1} extract) in comparison to the others (Table 1). The selected five isolates were tested for phytohormone production, indole acetic acid (IAA) and gibberellic acid (GA). According to our results, the five isolates revealed considerable activities, with JM18 displaying the highest levels (Table 1).

3.3. Morphological Characteristics of Strain JM18. The existence of aerial hypha, either unbranched or moderately and irregularly branched, was revealed in the morphological examination of the actinobacterial isolate JM18 (Table 2). They were lengthy, zig−zag, and fragmented into irregularly sized spores.

3.4. Molecular Characterization. The 16S rRNA gene sequence of the isolate JM18 comprised 568 nt. According to the analysis using NCBI blast, isolate JM18 was most closely related to *Nocardiopsis* sp. JS-C45 (100%) (Figure 1 and Supporting Table 1 and Figure 1). *Nocardiopsis* sp. strain FXJ6.077, *Nocardiopsis dassonvillei* subsp. *albirubida* strain OAct926, *Nocardiopsis synnemataformans* strain SIL2, and *N. dassonvillei* subsp. *dassonvillei* strain MDF2 as well as the represented strains were 99.06% (Supporting Table 1). The percent of similarity between the isolate JM18 and the actinomycetes *Nocardiopsis* spp (Figure 1 and Supporting Table) indicated that there is no difference in the 16S sequence, i.e., the sequences are identical. The 16S rRNA gene data from the *Nocardiopsis* JM18 strain have been deposited in the NCBI and GenBank nucleotide sequence databases under accession number ON936864.

Table 2. Screening of the Five Different Bacterial Isolates for their Morphological and Biochemical Characterization[a,b]

property				antimicrobial activities against			
NaCl	Gr	AM	SM	B.s	E.c	P.a	F.o
0%	+	creamy to faint yellow	pale yellow	+	+	+	+
2.5%	+	creamy to faint yellow	yellow	+	+	+	+
5%	+	creamy to faint yellow	yellow	+	+	+	+
7.5%	+	faint yellow	yellow	+	+	+	+
pH							
6	+	creamy to faint yellow	yellow	+	+	+	+
7	+	creamy to faint yellow	deep yellow	+	+	+	+
8	+	creamy to faint yellow	yellow	+	+/−	+/−	+
9	W	faint yellow	yellow	−	−	−	−
temp							
20 °C	+	creamy to faint yellow	deep yellow	+	+	+	+
30 °C	+	creamy to faint yellow	deep yellow	+	+	+	+
40 °C	+	faint yellow	yellow	+	+	+	+
50 °C	−		yellow				

[a]Signs + and − indicate presence or absence, respectively. [b]Gr: Growth; AM: aerial mycelium; SM: substrate mycelium; W: weak; B.s: *Bacillus subtilis*; E.c: *E. coli*; P.a: *P. aeruginosa*; and F.o: *Fusarium oxysporum*.

3.5. Physiological and Antimicrobial Properties of Nocardiopsis Strain JM18. The *Nocardiopsis* sp. strain (JM18) was studied for abiotic stress tolerance activities such as different pH and temperature ranges and salt concentrations (Table 2). *Nocardiopsis* sp. strain JM18 tolerated salinity at various NaCl concentrations with no color change, but at 7.5%, the isolate (JM18) color turned to a faint yellow aerial mycelium. Furthermore, the isolated strain JM18 showed high antimicrobial activity against *B. subtilis*, *E. coli*, *Pseudomonas, and F. oxysporum*. For the pH tolerance test, the isolated strain JM18 grew well on pH 6 and pH 7, with the aerial mycelium

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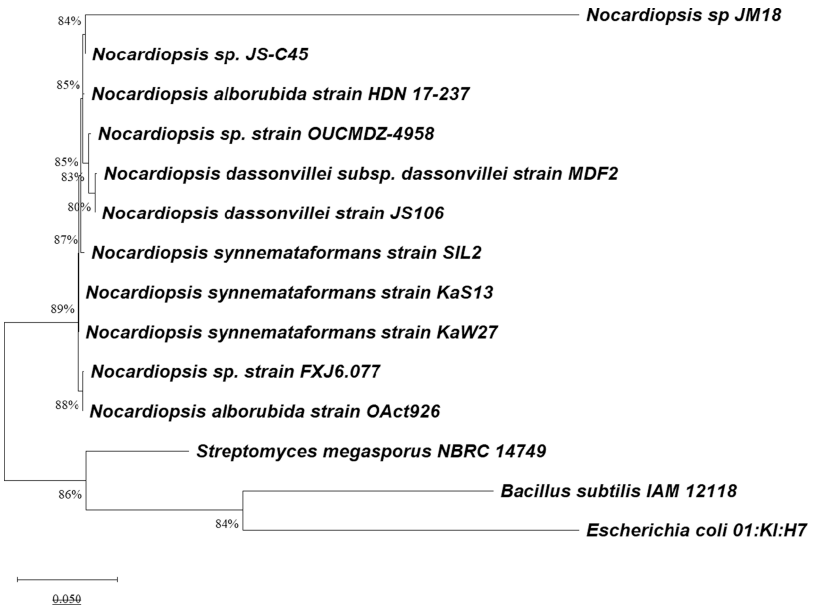

Figure 1. Neighbor-joining phylogenetic tree for the isolate JM18 was identified as *Nocardiopsis* sp. with the closest NCBI (BLASTn) strains based on the 16S rRNA gene sequences.

that was creamy to light yellow in color and the substrate mycelium that was a deep yellow color. However, JM18 was unable to grow at higher pH levels. For the temperature tolerance test, the optimal growth of isolate JM18 was observed at 20−30 °C, and the medium had high antistrain activity. On the other hand, JM18 was unable to grow and revealed no activity at 50 °C. There were no diffusible pigments in the substrate mycelium, which was pale yellow to deep yellow. At the antimicrobial level, the antibacterial activity of isolates was determined against *E. coli* and *Streptococcus* sp. The inhibitory zones were measured, and the results are reported in Table 2. JM18 revealed antibacterial potential against *E. coli* and *Streptococcus* sp.

3.6. Effect of JM18 and SeNPs on Growth and Photosynthesis of Brassica Cultivars. Seed priming of *Brassica* cultivars (*B. oleracea*, variety Sourthern star (VA1); *B. oleracea*, variety Prominence (VA2); *B. oleracea*, variety Monotop (VA3)) was carried out by JM18, SeNP, and JM18 + SeNP treatments. The results revealed that JM18, SeNP, and JM18 + SeNP treatments enhanced the growth of all *Brassica* cultivars under the study, compared with untreated controls (Figure 2). In VA1, the total biomass values of JM18, SeNP, and JM18 + SeNP treatments were 3.06 ± 0.41, 3.12 ± 0.55, and 3.04 ± 1.01 g FW, respectively. As a result, the SeNP-treated group showed a significantly higher growth rate than other groups. Among the several seedlings, JM18 induced the maximum increase in biomass in the case of VA2 seeds i.e., 3.93 ± 0.37 g FW, as compared with the control. In VA2, SeNP treatment increased the biomass by 0.9-fold, whereas JM18 + SeNPs increased the biomass by 0.7-fold. In contrast, VA3 showed the highest growth (0.7-fold) when treated with JM18 + SeNPs. The treatment with SeNPs resulted in a 0.6-fold increase in biomass, whereas the treatment with JM18 induced a 0.5-fold biomass increase. The photosynthesis rate was significantly increased when different *Brassica* cultivars were individually treated with JM18 or SeNPs compared to control plants. In addition, the combined treatments with JM18 and SeNPs significantly increased photosynthesis relative to the control plants (Figure 2).

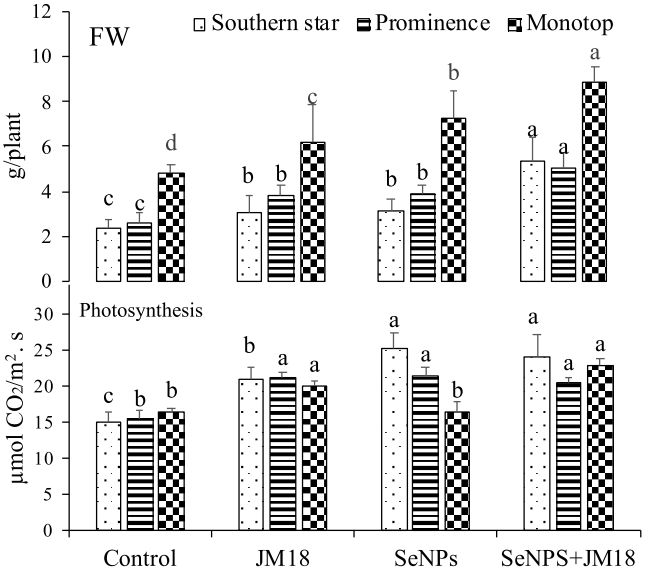

Figure 2. Effect of JM18 and SeNPs on growth and photosynthesis of *Brassica* cultivars (Southern star, Prominence and Monotop). Values are represented by means ± standard deviations of three independent replicates. Different letters indicate significantly different between treatments at a P value < 0.05.

3.7. Effect of JM18 and SeNPs on Amino Acids of Brassica Cultivars. We also determined the content of several amino acids in JM18, SeNP, and JM18 + SeNP-treated *Brassica* sprouts. The concentrations of various amino acids increased in treated *Brassica* cultivars after seed priming with different extracts (JM18, SeNPs, or JM18 + SeNPs) (Table 3). There were significant increases in the levels of glutamic acid, asparagine, leucine, glutamine, valine, methionine, serine, phenylalanine, and tyrosine in all varieties treated with JM18, SeNPs, and JM18 + SeNPs when compared with their respective controls, while a nonsignificant increase was observed in alanine and glycine (by about 60 and 40%, respectively). Meanwhile, alanine was significantly changed in

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Table 3. Effect of JM18 and SeNPs on Amino Acids of Brassica Cultivars (Southern Star, Prominence and Monotop)[a]

	control	JM18	SeNPs	SeNPs + JM18
		Southern Star		
leucine	10.44 ± 0.14b	18.85 ± 0.46a	12.30 ± 0.16b	19.67 ± 0.48a
tyrosine	7.06 ± 0.18d	10.07 ± 0.24c	49.70 ± 0.66b	70.04 ± 1.11a
glutamic acid	142.33 ± 1.95b	136.52 ± 4.22b	412.51 ± 42.01a	592.44 ± 68.21b
glutamine	66.93 ± 5.10c	60.06 ± 5.00c	114.21 ± 29.10b	141.45 ± 7.61a
asparagine	83.21 ± 1.41a	84.13 ± 4.32a	34.87 ± 6.42b	44.80 ± 8.51b
valine	11.93 ± 0.16d	17.32 ± 0.48c	59.11 ± 0.79b	76.77 ± 1.84a
serine	17.50 ± 0.34a	12.05 ± 0.16b	8.99 ± 1.11c	9.67 ± 2.16c
alanine	1.88 ± 0.05b	1.72 ± 0.05b	4.81 ± 3.82a	4.95 ± 2.59a
methionine	10.54 ± 0.28b	17.74 ± 0.42a	6.05 ± 2.01c	18.16 ± 1.61a
phenylalanine	4.77 ± 0.09c	9.24 ± 0.24a	5.86 ± 2.06c	7.89 ± 2.70b
glycine	1.62 ± 0.02c	1.97 ± 0.02c	5.63 ± 2.13b	7.48 ± 2.83a
		Prominence		
leucine	8.11 ± 0.11 c	14.86 ± 0.21 b	9.55 ± 0.13 c	17.51 ± 0.23a
tyrosine	7.33 ± 0.10 c	17.25 ± 1.71 c	51.58 ± 0.69 b	75.57 ± 2.56a
glutamic acid	77.54 ± 1.06 d	179.99 ± 30.11 c	687.42 ± 22.9 b	702.5 ± 13.07a
glutamine	106.56 ± 1.51 c	143.58 ± 32.01 b	254.12 ± 75.04 a	147.40 ± 51.09b
asparagine	100.93 ± 1.72 a	171.73 ± 6.31 a	46.37 ± 11.11 c	76.18 ± 17.11b
valine	10.45 ± 0.41 d	24.12 ± 1.52 c	49.47 ± 0.93 b	64.63 ± 3.71a
serine	23.35 ± 0.95 b	28.20 ± 0.66 a	18.58 ± 0.41 bc	16.90 ± 0.34c
alanine	3.71 ± 0.02 c	3.38 ± 0.08 c	11.63 ± 0.63 b	14.05 ± 1.14a
methionine	6.77 ± 0.18 b	17.39 ± 0.30 a	4.64 ± 1.58 b	18.91 ± 2.76a
phenylalanine	13.22 ± 0.57 a	3.56 ± 0.06 c	4.83 ± 1.52 c	8.77 ± 2.78b
glycine	2.40 ± 0.01 c	2.49 ± 0.01 c	4.37 ± 1.60 b	8.10 ± 3.04a
		Monotop		
leucine	7.15 ± 0.11b	17.18 ± 0.24a	8.83 ± 0.12b	20.23 ± 0.27a
tyrosine	6.63 ± 0.09d	14.8 ± 0.48c	46.67 ± 0.62b	62.97 ± 1.56a
glutamic acid	113.40 ± 3.31d	194.45 ± 3.54c	444.16 ± 33.12b	590.37 ± 45.13a
glutamine	101.84 ± 5.14d	114.92 ± 22.32c	244.99 ± 71.21b	300.97 ± 87.87a
asparagine	155.76 ± 2.14b	180.18 ± 4.12a	96.12 ± 7.81c	90.95 ± 21.32c
valine	12.81 ± 1.15c	23.38 ± 5.10b	63.47 ± 0.85a	63.96 ± 11.43a
serine	21.75 ± 0.30b	36.33 ± 3.34a	8.23 ± 1.11d	15.95 ± 1.40c
alanine	7.97 ± 0.03c	8.81 ± 0.02c	13.89 ± 1.5b	17.96 ± 0.83a
methionine	6.41 ± 0.09c	28.06 ± 0.41a	4.30 ± 1.46c	10.38 ± 3.17b
phenylalanine	7.09 ± 0.13d	24.96 ± 0.66a	4.37 ± 1.40c	30.11 ± 3.22b
glycine	2.62 ± 0.02c	2.47 ± 0.04c	4.06 ± 1.52b	9.29 ± 3.50a

[a]Values are represented by means ± standard deviations of three independent replicates. Different letters indicate significantly different between treatments at a P value < 0.05.

the three varieties of *Brassica* treated with JM18 + SeNPs. Furthermore, only JM18 + SeNP treatment increased glycine by about 50% in the three varieties of *Brassica*.

3.8. Effect of JM18 and SeNPs on Glucosinolates of Brassica Cultivars. The present study showed that certain treatments significantly increased the levels of total glucosinolates in sprouts of all treated *Brassica* varieties (Figure 3). According to the results, the highest level of glucosinolates was increased by 0.6-fold in VA1, as observed in JM18 and SeNP treatments. Furthermore, SeNP treatment increased glucosinolate values in the VA2 *Brassica* variety by 0.7-fold, whereas 0.6-fold in JM18 treatment and 0.4-fold in JM18 + SeNP treatment. Moreover, SeNP treatment increased glucosinolates in the VA3 *Brassica* variety by 88%. Additionally, dominant glucosinolates, i.e., glucoraphanin, showed a significant increase in all treated *Brassica* varieties. For instance, glucoraphanin in VA1 showed elevations of 0.7-fold, 0.6-fold, and 0.3-fold when treated with SeNPs, JM18, and JM18 + SeNPs, respectively (Figure 3). Also, JM18, SeNP, and JM18 + SeNP treatments increased glucoraphanin in VA2 by 40, 30, and 28%, respectively. Among the various seedlings, SeNP treatment induced the highest increase (71%) of glucoraphanin in VA3. Concerning myrosinase activity, significant increases were recorded in the three *Brassica* cultivars. In VA1 and VA3 treated with SeNPs, myrosinase activity was increased by 79% and by 72% in VA2 (Figure 3). Furthermore, the action of ESP was significantly decreased in treated sprouts of VA1, VA2, and VA3 cultivars (Figure 3). Consequently, the concentration of sulforaphane nitrile was greatly decreased when compared with untreated plants. These treatments also significantly enhanced the concentrations of isothiocyanate products, i.e., sulforaphane and sulforaphane nitrile in VA1, VA2, and VA3. The outcome showed that sulforaphane was increased in VA1 by 42%, VA2 by 30%, and VA3 by 52% after SeNP treatment (Figure 1). Regarding sulforaphane nitrile, VA3 displayed the highest increase (0.8-fold) through SeNP treatment, while a 0.7-fold elevation was observed in both VA1 and VA2 (Figure 3).

3.9. Effect of PGPB and Nanoparticles on Biological Activities of Brassica Cultivars. *3.9.1. Antioxidant Activity.* The antioxidant activity of untreated control and JM18, SeNP, and JM18 + SeNP-treated *Brassica* sprouts was evaluated using

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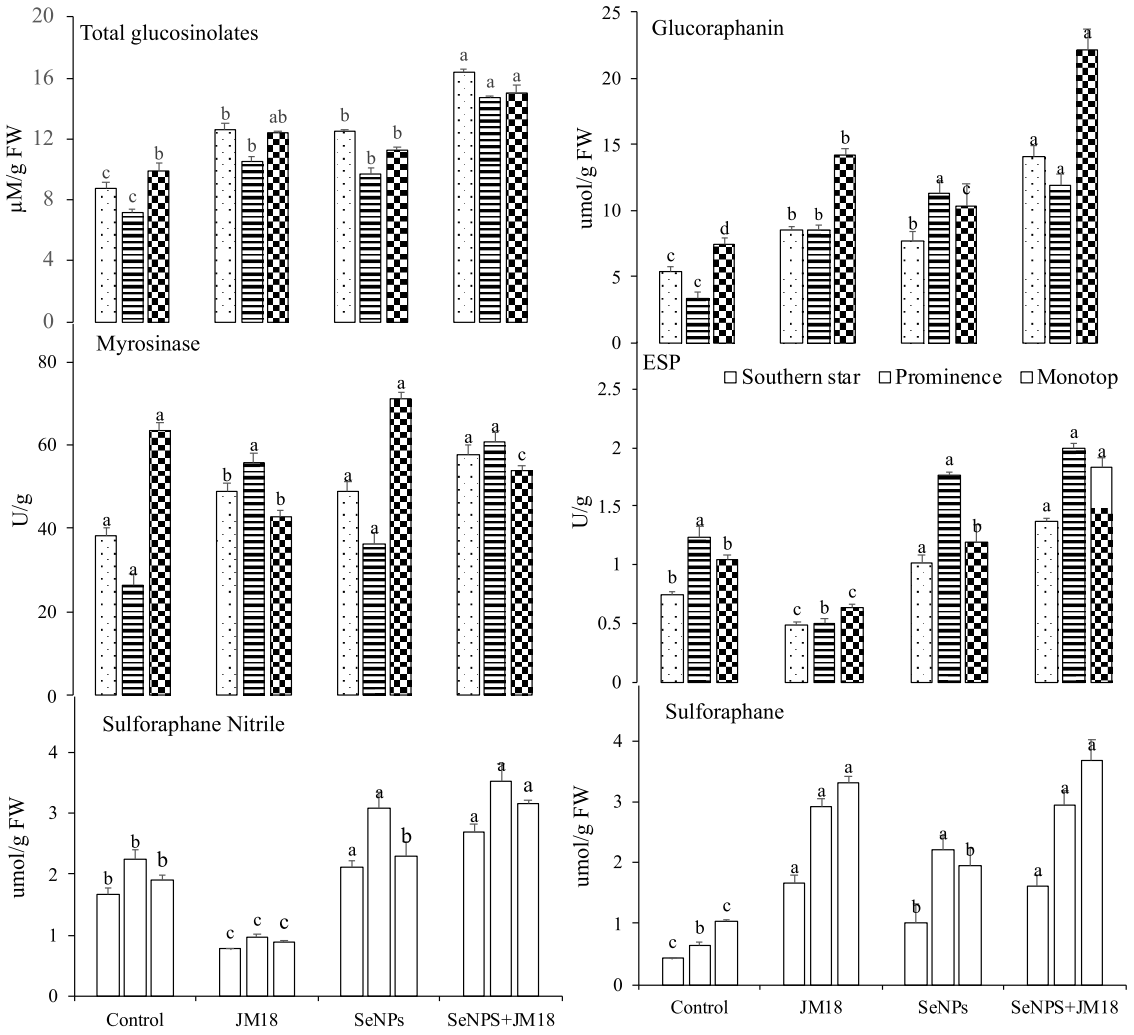

Figure 3. Effect of JM18 and SeNPs on the levels of (a) total glucosinolates, (b) glucoraphanin, (c) myrosinase activity, (d) epithiospecifier protein, (e) sulphoraphane nitrile, and (f) sulforaphane in different cultivars (Southern star, Prominence and Monotop) of *Brassica* sprouts. Values are represented by means ± standard deviations of three independent replicates. Different letters indicate significant differences between the treatments at a P value < 0.05.

the FRAP test. The three varieties displayed considerable antioxidant potentials, which were further improved after seed priming with JM18, SeNPs, and JM18 + SeNPs (Figure 4). When compared to controls, the findings showed that JM18 + SeNP treatment caused a significant increase in the antioxidant activity of VA2 and VA3. Furthermore, the JM18 treatment presented similar effects on the antioxidant activities of VA1 and VA2, while there was a minor difference in VA3. The antioxidant activity of VA1 was increased by 0.7-fold in the JM18 treatment and 0.6-fold in SeNP and JM18 + SeNP treatment. In the case of VA2, the antioxidant activity was increased by 0.7-fold, 0.6-fold, and 0.4-fold by JM18, SeNP, and JM18 + SeNP treatments, respectively. The antioxidant activity of VA3 was significantly increased by 79% in the JM18 treatment and nonsignificantly by SeNP (68%) and JM18 + SeNP (51%) treatments.

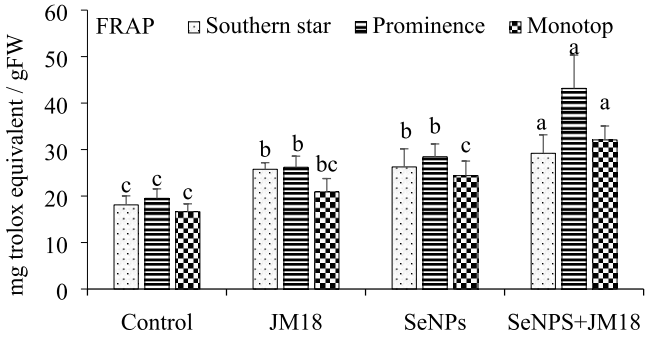

Figure 4. Effect of JM18 and SeNPs on the antioxidant activities in different cultivars of *Brassica Brassica* (Southern star, Prominence and Monotop). Values are represented by means ± standard deviations of three independent replicates. Different letters indicate significantly different between treatments at a P value < 0.05.

3.9.2. Antimicrobial Activity. The antimicrobial activity of the untreated control and JM18, SeNP, and JM18 + SeNP-treated *Brassica* sprout extracts was evaluated via disc diffusion assay (Table 4). The antibacterial activity was evaluated against *E. coli*, *S. saprophyticus*, *S. salivarius*, *A. flavus*, *S. Typhimurium*, *E. aerogenes*, *S. marcescens*, *C. glabrata*, and *P. aeruginosa*. Interestingly, the JM18, SeNP, and JM18 + SeNP treatments resulted in the enhancement of the antibacterial effect of *Brassica* sprout extracts against a few strains, while the reduction was noticeable in others. In VA1, seedlings treated

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Table 4. Antimicrobial Activity of the Untreated Control and JM18, SeNPs,and JM18 + SeNP-Treated Brassica Sprout Extracts[a]

	control	JM18	SeNPs	SeNPs + JM18
		Southern Star		
S. saprophyticus	13.88 ± 0.18b	26.41 ± 0.42a	16.56 ± 0.21b	23.65 ± 0.56a
S. salivarius	6.58 ± 0.72b	6.49 ± 0.23b	5.25 ± 0.12c	7.99 ± 0.62a
E. coli	4.53 ± 0.06c	9.12 ± 0.85a	5.34 ± 0.08c	7.63 ± 1.91b
S. typhimurium	11.67 ± 1.24a	10.83 ± 0.76b	7.91 ± 0.11c	12.75 ± 0.73a
P. aeruginosa	22.11 ± 2.4a	19.90 ± 2.14a	19.85 ± 0.20a	22.94 ± 1.11a
E. aerogenes	5.63 ± 0.07d	12.94 ± 0.30a	6.71 ± 0.08c	9.73 ± 1.53b
S. marcescens	2.49 ± 0.02a	3.25 ± 0.17a	2.81 ± 0.25a	2.98 ± 0.26a
S. typhimurium	16.24 ± 0.22a	10.71 ± 0.16b	7.85 ± 0.24c	15.27 ± 1.25a
C. glabrata	2.37 ± 0.03c	5.58 ± 0.27a	2.79 ± 0.05c	4.07 ± 0.19ab
A. flavus	19.39 ± 0.39c	30.21 ± 2.30a	23.23 ± 2.07b	23.43 ± 0.16b
		Prominence		
S. saprophyticus	15.36 ± 0.04b	24.39 ± 0.90a	15.64 ± 0.41b	23.65 ± 0.19a
S. salivarius	6.39 ± 0.08c	7.56 ± 0.08b	8.97 ± 0.03b	14.68 ± 0.07a
E. coli	4.99 ± 0.01b	7.87 ± 0.29a	5.89 ± 0.04b	8.08 ± 0.22a
S. typhimurium	11.02 ± 0.12a	12.89 ± 0.14a	7.21 ± 0.02b	11.84 ± 0.06a
P. aeruginosa	22.58 ± 0.22a	22.97 ± 0.33a	17.58 ± 0.02b	20.21 ± 0.09a
E. aerogenes	6.23 ± 0.02c	10.57 ± 0.44b	12.49 ± 0.08ab	14.41 ± 0.12a
S. marcescens	2.07 ± 0.03c	3.37 ± 0.06b	4.09 ± 0.04b	6.34 ± 0.05a
S. typhimurium	13.97 ± 0.05a	13.86 ± 0.49a	6.37 ± 0.01c	11.91 ± 0.13b
C. glabrata	2.61 ± 0.01c	4.78 ± 0.22b	6.78 ± 0.02a	7.26 ± 0.06a
A. flavus	21.48 ± 0.27a	27.43 ± 0.61a	16.77 ± 0.24b	18.11 ± 0.02b
		Monotop		
S. saprophyticus	13.78 ± 0.11d	27.26 ± 0.29a	16.31 ± 0.12c	21.69 ± 1.31b
S. salivarius	7.49 ± 0.04c	10.37 ± 0.11b	10.57 ± 0.28b	15.50 ± 2.21a
E. coli	5.45 ± 0.04b	9.08 ± 0.15a	6.61 ± 0.043b	8.43 ± 0.63a
S. typhimurium	10.27 ± 0.04b	12.58 ± 0.16a	6.97 ± 0.040c	10.28 ± 0.68b
P. aeruginosa	21.81 ± 0.07a	22.22 ± 0.23a	16.05 ± 0.16c	18.62 ± 0.18b
E. aerogenes	8.59 ± 0.04c	13.89 ± 0.18b	15.61 ± 0.41a	15.6 ± 1.31a
S. marcescens	3.27 ± 0.04d	4.53 ± 0.05c	5.66 ± 0.10b	7.74 ± 1.12a
S. typhimurium	12.16 ± 0.16a	13.2 ± 0.17a	5.73 ± 0.09c	9.15 ± 0.52b
C. glabrata	4.13 ± 0.02c	6.27 ± 0.07b	8.46 ± 0.21a	8.43 ± 0.83a
A. flavus	20.39 ± 0.26b	27.33 ± 0.3a	13.73 ± 0.40d	16.61 ± 1.06c

[a]Values are represented by means ± standard deviations of three independent replicates. Different letters indicate significantly different between treatments at a P value < 0.05.

with JM18 and JM18 + SeNPs showed significant increases in antibacterial activity against *S. saprophyticus* ($p < 0.05$), whereas SeNP treatment had no significant increase ($p > 0.05$). JM18 + SeNP treatment significantly boosted the zone of inhibition against *S. salivarius*, while JM18 and SeNP treatments, separately, revealed no significant increase ($p > 0.05$). *E. coli* resistance to JM18-treated seedlings was significantly increased. However, JM18 + SeNP treatment of *Brassica* sprouts significantly inhibited *E. coli*, while SeNP treatment did not make a significant change. Maximum zones of inhibition against *S. Typhimurium* and *P. aeruginosa* were shown by JM18 + SeNP treatment. JM18 treatment presented the best antibacterial activity against *E. aerogenes*, *C. glabrata*, and *A. flavus*. An insignificant increase in antibacterial activity against *S. marcescens* was observed in JM18, SeNP, and JM18 + SeNP treatments. In VA2, after seed priming with JM18, there were significant increases in antimicrobial potential against all bacterial strains, whereas slight increases were noticeable in the case of SeNP and JM18 + SeNP treatments. The zones of inhibition against *S. saprophyticus*, *E. coli*, *E. aerogenes*, *C. glabrata*, *and A. flavus* were significantly enhanced in VA3 treated with JM18 as compared to control VA3, although it was reduced greatly in SeNP- and JM18 + SeNP-treated seedlings.

4. DISCUSSION

In this study, for the first time, we investigated the combined effects of PGPB and SeNP priming on different *B. oleracea* varieties. Seed priming can improve crop productivity by maintaining ionic homeostasis,[25] enhancing water relations and the photosynthetic rate.[26] Moreover, we hypothesized the use of a bioactive PGPB as a biofertilizer[27] by producing a variety of regulatory biochemicals.[28]

Therefore, we isolated some soil bacteria and, based on the in vitro plant-growth-promoting activities of isolates, found JM18 to be the most efficient PGPB that could produce IAA, HCN, and ammonia, among many other characteristics. As a tool for preliminary bacterial identification and a widely used technique by microbiologists worldwide, morphological and biochemical characterizations of bacterial isolates were carried out.[29] Similarly, it has been reported that various techniques are developed depending on the nutrients, metabolism, bioactive molecules, or biochemical reactions of the bacteria to help group and identify them according to the genus and species level.[30,31]

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Similar to our results, researchers have isolated a number of potential microorganisms from plants, including *Azotobacter* species, *Klebsiella* species, *Streptomyces* species, etc.[16] Here, we selected the bioactive JM18 strain (*Nocardiopsis* sp.) according to its high antioxidant capacity (DPPH radical, 31) and high phenolic and flavonoid production. Interestingly, the JM18 strain can tolerate different abiotic stresses introducing it as a bioresource for improving agricultural production under environmental challenges, including salinity.[28]

The seeds of three varieties of *B. oleracea* cultivars (Southern star, Prominence, and Monotop) were primed with the JM18 bacterial isolate and SeNPs either individually or combined. Different parameters were measured to evaluate the impacts of JM18 seed priming on *B. oleracea* seeds. Results revealed significant impacts of the JM18 extract on the nutritional quality and biological activities of the three cultivars of *B. oleracea* sprouts (VA1, VA2, and VA3). In a previous study, higher plant growth was observed in *Camelina sativa* (L.) Crantz when its seeds were primed with Sorghum water.[9] Similarly, Ahmad and colleagues also reported that sorghum extract treatment induced a higher shoot length and shoot and root dry and fresh weights.[32] In the current study, seeds primed with JM18, SeNPs, and JM18 + SeNPs have been used for a pot trial. After 10 days of inoculation, seeds coated with the JM18 bacterial inoculant, SeNPs, or JM18 + SeNPs showed induced biomass accumulation. JM18, followed by SeNP treatment, produced an increase in seed germination and shoot and root length measurements. In contrast to seeds treated with JM18 and SeNPs separately, the combination of JM18 + SeNPs did not produce any appreciable results. Plant growth and NPs may be related in a dose−response manner.[15] Furthermore, NPs are likely able to penetrate the seed coat and enhance germination. According to Nawaz and Bano,[33] AgNPs were ineffective when combined with PGPB since it decreased the effectiveness of PGPB. Additionally, previous studies demonstrated that early in the ontogenesis process, SeNPs have a significant influence on seedling germination and development.

Furthermore, it is widely recognized that a crop's amino acid composition correlates with its increased nutritional and health-promoting quality.[5,10] The free amino acids in *B. oleracea* are precursors of metabolites like glucosinolates, which have strong effects on human health. Thus, increasing the amino acid levels in *B. oleracea* sprouts by using natural sources could improve the nutritional advantages and increase the likelihood that they would be used as beneficial food additives. The present study examines the amino acid profile in JM18, SeNP, and JM18 + SeNP-treated and untreated *B. oleracea* cultivars. Noticeably, the aforementioned treatments exhibited significant effects on the amino acid contents of all cultivars. Results showed that JM18, SeNP, and JM18 + SeNP treatments significantly augmented the levels of glutamic acid, asparagine, leucine, glutamine, valine, methionine, tyrosine, serine, and phenylalanine in the three cultivars, as compared with their corresponding untreated controls, while insignificant increases were observed in alanine and glycine. It is well known that amino acids contribute to the production of glucosinolates, whereas aliphatic glucosinolates are produced from methionine, leucine, valine, alanine, and isoleucine. Meanwhile, phenylalanine, tryptophan, or/and tyrosine are used to make indole glucosinolates and aromatic glucosinolates, respectively.[34] Previous research showed that sprouts of *B. oleracea* contain significantly higher glucoraphanin contents than their mature counterparts.[12] The dynamic variations in glucosinolate levels depend on glucosinolate production, degradation, and mobilization and their control.[35] In the present study, the JM18, SeNP, and JM18 + SeNP treatments increased the amino acid concentration and boosted the formation of glucosinolates, as observed in all treated cultivars. Similar to our results, seed priming increased the amount of total glucosinolates in the sprouts of *B. oleracea*, increasing it from 34 to 100%.[11] In the current study, JM18, SeNP, and JM18 + SeNP treatments increased glucoraphanin levels in the three cultivars. Sulforaphane, hydrolyzed from glucoraphanin, is a kind of isothiocyanate and is a highly desirable agent due to its anticarcinogenic potential.[12] *B. oleracea* sprouts are considered a high source of sulforaphane.[36] Myrosinase is an enzyme that regulates the hydrolysis of glucosinolates into isothiocyanates, or efficient glucosinolates (sulforaphane), and ineffective glucosinolates (sulforaphane nitrile).[14] Contrarily, ESP causes the synthesis of sulforaphane nitrile rather than sulforaphane; as a result, the lower ESP activity promotes a higher quantity of sulforaphane. In this regard, VA1, VA2, and VA3 were found to have significantly lower ESP activities. As a result, when compared to control plants, the content of sulforaphane nitrile was much lower. In addition, the myrosinase activity increased, causing the breakdown of sulforaphane into its active form. Overall, the interaction between priming and sprouting may lead to improved glucosinolate synthesis. We also evaluated how treatments with JM18, SeNPs, and JM18 + SeNPs affected the bioactivities of *Brassica* cultivars. By using FRAP assays, the antioxidant capacity of the JM18, SeNP, and JM18 + SeNP-treated cultivars was assessed and compared to untreated controls. Results showed that JM18 + SeNP treatment stimulated the antioxidant property of *Brassica* sp. The results were significant for VA2 and VA3 in the FRAP assay. Former studies showed that priming *Vigna mungo* seeds with plant extracts increased the function of antioxidant enzymes, which, in turn, increased the antioxidant activity of treated seeds.[37] Moreover, this study presented that the antibacterial properties of the JM18, SeNP, and JM18 + SeNP-treated cultivars were elevated, which may be connected to an increase in glucosinolate synthesis and stimulation of its hydrolytic pathway in treated cultivars. Similar to our findings, earlier research found that isothiocyanates exhibit antibacterial capabilities against both Gram-negative and Gram-positive bacteria, including *Listeria monocytogenes*, *P. aeruginosa*, *E. coli*, and *Staphylococcus aureus*.[38] The ability of glucosinolates to change the membranes' properties and reduce its surface charge, which causes potassium to seep out and propidium iodide to be taken up, may provide an explanation for these antibacterial effects. Overall, treated sprouts of various cultivars showed hierarchical variations that could be attributable to species diversity and ontogeny.

5. CONCLUSIONS

To improve the production, nutritional quality, and pharmaceutical applications of sprouts of three *B. oleracea* cultivars, the impact of seed priming using *Nocardiopsis* sp. JM18 along with SeNPs was evaluated, assessing sprout growth, glucosinolate metabolism, and biological activities (antioxidants, antibacterial) of *Brassica* cultivars. Glucosinolate metabolism was enhanced because of high precursor (amino acids) availability. The variation among *Brassica* cultivars in growth and the contents of the bioactive compound was

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cultivar specific. These results increase the potentiality of using *Brassica* sprouts grown and treated with *Nocardiopsis* sp. JM18 and SeNPs as promising nutritional and health-promoting functional food or food additives.

■ ASSOCIATED CONTENT

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c02957.

Similarities between sequences from strain JM18 and other close bacterial strains and image of the actinobacterial isolate Nocardiopsis sp. JM18 (PDF)

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Notes

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