ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

Alginate Beads Encapsulated Auxin-Producing PGPR as a Biofertilizer Promotes *Triticum aestivum* Growth

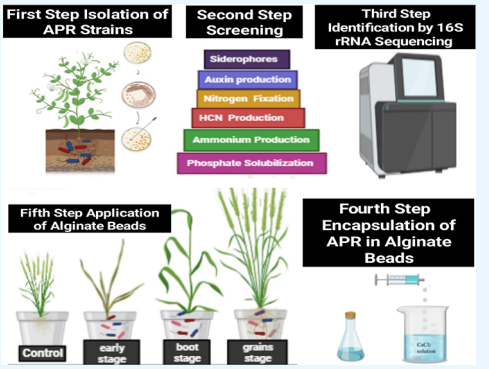
Nimra Mushtaq, Atia Iqbal,* Shumaila Batool, Sara Janiad, Mehboob Ahmed, Fahad Al-Asmari, Muhammad Abdul Rahim,* Mohamed Fawzy Ramadan, and Eliasse Zongo*

Cite This: *ACS Omega* 2025, 10, 4303−4314

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Plant growth-promoting rhizobacteria (PGPR) can be utilized to enhance plant growth and production. The use of efficient PGPR is one of the effective ways to improve *Triticum aestivum* (wheat) growth and nutrition. The exploration of native rhizobacterial strains as biofertilizers with suitable carriers in immobilized form is an alternative way to prevent soil ecosystem pollution. In order to evaluate the potential of biofertilizers and multiple plant growth-promoting attributes, numerous experiments were conducted on isolated strains. The impact of auxin-producing rhizobacterial strains (APRS) on various growth parameters of wheat was examined through laboratory and field experiments. This study aimed to investigate the potential of rhizobacteria (isolated from various crop types) to encourage wheat growth by immobilization in alginate beads. The PGPR, obtained from different rhizospheric soils, were identified based on their colony morphology and biochemical characteristics. In addition, they were evaluated for their ability to produce indole-3-acetic acid (IAA), hydrogen cyanide (HCN), ammonia (NH_3), and siderophores. The 16S rRNA-based identification revealed that auxin-producing rhizobacterial strains showed homology to various genera, including *Bacillus, Brevundimonas*, and *Exiguobacterium* spp. Selected strains showed plant growth-promoting (PGP) attributes and hydrolytic enzyme-producing abilities. Most of the strains had multifaceted plant growth-promoting attributes. Growth potential, assessed under laboratory and natural environments, confirmed the efficacy of bacterial strains as alginate beads biofertilizers. Encapsulation with alginate beads showed a 70−80% improvement in seed germination and a 60−70% enhancement in root and shoot length than control. The results revealed that the selected strain can be used as biofertilizers. Screening and the application of efficient PGPR encapsulation with alginate beads can be a better option to promote the production and yield of wheat.



1. INTRODUCTION

Triticum aestivum (wheat) is widely used as a staple food in Pakistan, and 35% of the world's population consumes it as a major calorific source of nutrition. Pakistan's agriculture sector is considered a major pillar of the economy, wherein the wheat crop shares 1.9% of the agriculture sector and 1.7% of the country's gross domestic product (GDP). Global environmental changes, i.e., biotic and abiotic stresses and the unexpectedly rapid increase in population, impose adverse impacts on the agriculture sector, reducing crop production rates by 50%.[1] Chemical fertilizers are used to increase crop yield and plant growth. The broad utilization of chemical fertilizers has adversely affected human well-being and the environment. Haphazard use of these chemical fertilizers causes deleterious effects on the soil matrix and its microbiota due to soil fertility loss.[2] Thus, there is an acute need to generate alternative strategies that promote crop production in an eco-friendly manner.[1,3]

Rhizobacteria are involved in plant growth through direct or indirect mechanisms.[4] Direct mechanisms include phytohormone production (auxin, phosphate, nitrogen, ammonium, and siderophores), while indirect mechanisms involve HCN production and lytic enzyme production.[4,5] Auxin is a key organic molecule synthesized by PGPR, found in extremely low amounts, which influences the physiological, morphological, and biochemical processes of plants.[6] The most active form of auxin is indole-3-acetic acid,[3] which helps in the elongation of root structure, i.e., root hairs and lateral roots that regulate uptake in nutrients, increase the permeability of water, induce protein synthesis, and enhance gene regulation and cellular processes such as cell differentiation, expansion, and division.[7] It influences apical dominance, early flowering, and fruiting and can potentially regulate cellulase production.[3]

Received: July 1, 2024
Revised: October 31, 2024
Accepted: December 27, 2024
Published: January 28, 2025

Therefore, soil rhizobacteria used as biofertilizers/bioinoculants enhance growth capabilities and innate microbiota, which overturn the harmful effects of pathogenic microbes and are safe for the ecosystem.[1,3]

Despite the effectiveness of biofertilizers, when applied directly into the soil, they may sometimes be leached down or face difficulties related to the survival of microorganisms. They lose their colonization with roots and proper functioning.[8] Therefore, it is necessary to find suitable carrier materials or techniques beneficial for microorganisms functioning in the soil, having long-lasting effects on plant growth promotion.

Encapsulation or immobilization of rhizobacteria with a suitable carrier material is a good technique/technology that provides a healthy environment for both microbes and plants and protects them from external or internal hazardous factors. This material helps microbe cells remain alive or active with their metabolic and catalytic activities.[4] Literature showed that different types of polymeric materials are used to encapsulate PGPR, e.g., Arabic gum, wax, chitosan, carrageenan, and alginate.[4,9,10] Alginate is a natural polymeric material widely used for encapsulation and enhances the viability and activity of microorganisms.[10] Alginate is an environmentally friendly, biodegradable, and cheap biopolymer that slowly releases microbes. It can also easily be converted into liquid and solid biofertilizers. Therefore, many researchers use this biopolymer for agricultural improvement.[4,9,10] In the scenario described above, the primary purpose of the present study is to explore the potential of Auxin-producing rhizobacterial strains (APRS) to boost wheat growth by immobilizing alginate beads.

2. MATERIALS AND METHODS

2.1. Sample Collection and Isolation. All research was conducted in the postgraduate laboratory of the MMG department, The Women University, Multan, Pakistan. Eighty-eight soil samples were collected from the rhizospheric regions of different plants from different districts of Punjab, Pakistan, e.g., Vehari, Multan, Sahiwal, and Burewala, then brought into the laboratory in sterile polythene bags for further process.[11] For isolation, 1 g of soil from each rhizospheric sample was mixed in respective 9 mL of sterilized water and placed in a shaker for 30−40 min. Afterward, serial dilutions were prepared in the range of 10^{-1}−10^{-6}, and 0.1 μL of the bacterial suspension was spread onto Luria−Bertani agar (LB), preceded by 24 h incubation at 28 °C.[12] Distinct colonies were selected, purified by streak plating/restreaking until pure cultures were obtained, and stored at 4 °C in a refrigerator for further analysis.[39] The physiochemical properties of the gathered rhizospheric soil, such as soil temperature, texture, and pH value, were also determined. Soil pH was checked with a pH meter, soil texture was noted based on physical appearance, and temperature was noted with the help of a thermometer.

2.2. Qualitative and Quantitative Analysis of Auxin-Producing Rhizobacterial Strain (APRS). Screening of efficient auxin-producing rhizobacterial strains was done using Salkowski's reagent test.[12,13] LB broth media were prepared with the amendment of L-tryptophan (1000 μg/mL), inoculated with selected rhizobacteria, and placed in a shaking incubator (DW-SI-100B, Chongqing, China) at 28 °C for 72 h at 120 rev/min. After that, the broth was centrifuged (Mikro200 Hettich, Tuttlingen, Germany) at 14,000g for 5 min, and the supernatant was collected.[14,42] For color development, 1 mL of Salkowski's reagent was mixed with 2 mL of the supernatant and placed in the dark for 30 min. Bacterial strains that showed a red or pink color were considered positive for auxin production. A spectrophotometer (Varian Cary 300 UV−vis Spectrophotometer, Spectralab Scientific Inc., Canada) was used to determine the absorbance (O.D) at 535 nm for 1 mL of the mixture, with the control consisting of 1 mL of Salkowski's reagent and 2 mL of culture medium.[41,49] Then, 50 mL of methanol was used consisting of 2.5 mg of synthetic indole acetic acid (IAA) (Oxoid). Afterward, 2 mL of the methanol mixture was used, and 1 mL of Salkowski's reagent was added, homogenized, and incubated in the dark for 30 min at room temperature so that the solution would turn pink, and the absorbance was measured using a spectrophotometer (Varian Cary 300 UV−vis Spectrophotometer, Spectralab Scientific Inc., Canada) at a wavelength of 535 nm.[40] A standard curve of synthetic IAA with different concentrations of 0, 10, 20, 50, 100, 150, and 200 μg/mL was plotted.[15] The results were expressed in μg/mL.

2.3. Morphological and Biochemical Identification of Selected APRS. The morphological characteristics of the selected strains were determined by growing them on LB agar. Gram staining was done to reveal the cell morphology of the bacterial strains.[16] Various biochemical tests, e.g., the catalase test, growth on MacConkey's and EMB, production of acid from sugars (lactose, mannitol, glucose, maltose, and sucrose), oxidative fermentation, Voges−Proskauer, and methyl red tests were performed, and the strains were identified by using Bergey's manual.

2.4. Molecular Identification of Selected APRS. Phylogenetic identification of the selected strains was carried out through 16S rRNA sequencing. Total genomic DNA was isolated for PCR analysis, and the sequence data were used for BLAST analysis (http://www.ncbi.nlm.nih.gov/blast), followed by sequences submission to GenBank for accession numbers. A phylogenetic tree was constructed with the neighbor-joining algorithm using the NCBI database and MEGA 11 software.[13]

2.5. Optimization at Different Physical Parameters of APRS. For optimization, APRS was grown in LB broth with the amendment of L-tryptophan at different ranges of temperature (25, 30, 37, 40, 45, and 50 °C)[17] and pH.[4−8,17] Shaking cultures (120 revs/min) were incubated for 72 h, and auxin production was measured by Salkowski's method.[17,18]

2.6. Estimation of Auxin Production by Thin-Layer Chromatography (TLC) Technique. For thin-layer chromatography (TLC), selected APRS strains were grown in LB broth with the amendment of L-tryptophan and incubated at 28 °C for 72 h. After that, the broth was centrifuged (Mikro200 Hettich, Tuttlingen, Germany) at 10,000 g for 15 min, and the resultant supernatant was separated from the pellet in single aliquots; then, this supernatant was mixed with ethyl acetate in an equal volume (1:1, v/v) and shaken vigorously for 10−15 min. Two layers were found; the organic and inorganic layers were separated. A rotary evaporator evaporated the organic layer. After that, the remaining dried mass was dissolved in 1 mL of methanol per litter. TLC plates were developed using a solvent system comprising formic acid:chloroform:ethyl acetate (10:50:40, v/v) and sprayed with Salkowski's reagent for color development. The standard IAA [retention factor (Rf) 0.60] was compared with the rhizobacterial culture.[19]

https://doi.org/10.1021/acsomega.4c06066
ACS Omega 2025, 10, 4303−4314

2.7. Evaluation of Plant Growth-Promoting Traits of Auxin-Producing Rhizobacteria (APR). The phosphate (P) solubilizing ability of APRS was tested on NBRIP media (National Botanical Research Institute's phosphate medium). Bacterial strains (24 h fresh microbial culture) were streaked on NBRIP media. After 7−14 days of incubation, clear zones around bacterial colonies showed positive P solubilization results. Colony and zone sizes were measured, and the phosphate solubilization index was calculated according to the following formula: SI = Colony (C) + Zone (Z) /Colony (C).[20]

Hydrocyanic acid (HCN) production and nitrogen fixation assays were used to determine the ability of hydrocyanic acid production.[49] APRS was streaked on LB agar with the amendment of 0.44% glycine. The filter paper was dipped in a mixture of 2% (2 g/100 mL) sodium carbonate solution and 0.5% (0.5 g/100 mL) picric acid solution and kept on the upper side of the Petri plates, and sealed with parafilm, followed by incubation for 24−48 h at 28 °C. Changes in the color of the filter paper from light brown (+) to dark brown (++) to orange (+++) indicate positive results. The efficiency of nitrogen fixation of APRS was observed in solid Jensen's media. Selected strains were streaked on Jensen's media and incubated at 28 °C for 7 days in a dark place. Bacterial growth indicates that the selected strains can fix nitrogen.[20]

With respect to ammonium production, ammonium production was checked by bacterial strains inoculated in 10% peptone water for 72 h at 28 °C. After incubation, 0.5 mL of Nessler's reagent was added to 1 mL of the supernatant. The appearance of a yellow-to-orange color indicates ammonium production.

Regarding the assay for siderophore production, CAS dye and nutrient agar were separately autoclaved and mixed before pouring. The stab test of APRS was performed and incubated for 2−7 days. The orange zone around the bacterial strains indicates positive results.[20]

The extracellular enzyme production assay was carried out by streaking APRS on LB agar containing 0.1% colloidal chitin and incubating for 24 h at 28 °C. Following incubation, clear zones around the strains indicated chitinase production. For protease enzyme production, LB agar was prepared with 1% skim milk. Clear zones around the bacterial strains gave positive results. For pectinase enzyme production, the media were prepared, streaked with fresh 24 h bacterial culture, and incubated for 48 h. After that, the hexadecyl trimethylammonium bromide solution (1.0 g/100 mL) was flooded, and the clear zone indicated pectinase production.[15]

2.8. Plant Microbial-Interaction Experiment. *2.8.1. Seed Germination and Rooting Assay in Petri plates.* Wheat seeds (Var. Faisalabad 2008) were taken from the Punjab Seed Corporation. The seeds were sterilized with a 0.1% sodium hypochlorite solution for 2−3 min and washed several times with autoclaved distilled water. For the seed germination assay, sterilized seeds were soaked in bacterial culture for 30 min. Uninoculated LB broth was used as a control. After that, five seeds were transferred to wet filter paper in Petri plates and placed in a dark place for 3 days. Then, the seed germination percentage was noted, and the Petri plates were placed in the room (light:dark = 16:8) for 5 days. For the rooting bioassay and root colonization, a bacterial cell suspension was prepared in phosphate buffer solution at a concentration of 10^8 colony-forming units (CFU)/mL by measuring the optical density (OD) at 600 nm; then, for 5 days, wheat seedlings were dipped into the bacterial suspension for 2 h at 28 °C. The root was taken and washed with distilled water, and a piece of the root was cut, dipped into a 0.1% acridine orange solution, and placed on a slide. Under a light and fluorescent microscope, different parameters were measured, namely, the length of root and shoot, the number of roots, root hairs, root colonization of bacteria, and seed germination. Noninoculated seeds were used as a control. The whole experiment was done in duplicate.

2.8.2. Encapsulation of Selected Auxin-Producing Rhizobacterial Strains (APRS) in Alginate Beads. Alginate beads were prepared according to Bashan's method,[21,5,43] with some modifications. A 24 h fresh bacterial liquid culture was obtained and centrifuged at 10,000 rpm for 10 min to get a pellet and washed with phosphate buffer solution. The bacterial pellets (OD600 nm) were mixed with phosphate-buffered saline and a presterilized 2% sodium alginate solution for the preparation of bacterial beads. A 5 mL sterilized syringe was used to add the homogenized solution drop by drop into a solution of 1% (w/v) $CaCl_2$ while the solution was being stirred magnetically for 30 min. Freshly alginate beads made of bacteria were collected by passing them through Whatman No. 1 paper (filter paper) (Figure 1).[44]

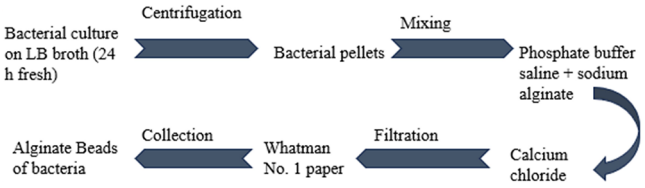


Figure 1. Preparation of the alginate beads.

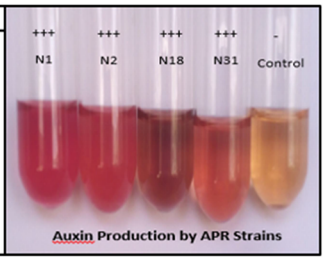
2.8.3. Laboratory and Field Experiment. Ten of the best strains with good PGPR characteristics were selected for laboratory and field trials. The following treatments were used in the experiment: P1(WumN1), P2(WumN2), P3(WumN3), P4(WumN15), P5 (WumN17), P6(WumN18), P7-(WumN19), P8(WumN20), P9(WumN31), P10(WumN32), and P11(control without inoculum). Before seed sowing, soil physical parameters were also determined. In the laboratory experiment, sterilized soil was filled into pots (200 g/pot), five seeds per pot were sown, and three alginate beads/seeds were applied in each pot. After 15 days, plant growth parameters, including seed germination rate, length, and weight of root and shoot, were calculated.[20] In the field experiment, 5 kg of soil per pot was filled, and 15 seeds with 45 beads per pot were applied. Readings of plant growth parameters, i.e., seed germination, shoot and root length, spike length, and seed weight, were measured every 2 weeks until crop harvest. The whole experiment was performed in duplicate. Besides physical parameters, biochemical contents, e.g., auxin, protein, alkaloids, terpenoids, and steroid contents were also measured.

2.9. Phytochemical Analysis. *2.9.1. Auxin Estimation.* A paste of plant seedlings was prepared and transferred into a test tube, and 2 mL of ethyl ether was thoroughly mixed and kept at 4 °C for 4 h. After that, 2 mL of 0.1 M sodium bicarbonate was mixed with the supernatant. This step was repeated twice, followed by the acidification of the sodium bicarbonate layer to pH 3 with 6 N HCl. The inorganic layer was discarded, while 2 mL of Salkowski's reagent was mixed into the organic phase. One mL of ethyl ether and 2 mL of Salkowski's were considered blank. Auxin was estimated with the help of a

https://doi.org/10.1021/acsomega.4c06066
ACS Omega 2025, 10, 4303−4314

(a) Qualitative Estimation of Auxin

Plant name	Isolated Strains	Auxin	Color Code	Location
Control	No strain	-	Yellow	Multan
Cucurbita spp.	WumN1	++++	Reddish pink	Multan
Cucurbita spp.	WumN2	++++	Reddish pink	Multan
Gossypium spp.	WumN3	++++	Reddish pink	Multan
Raphanus raphanitrum spp.	WumN15	++++	Reddish pink	Vehari
Helianthus auus	WumN17	+++	Reddish pink	Vehari
Cucurbita spp.	WumN18	+++	Reddish pink	Multan
Oryza sativus	WumN19	+++	Reddish pink	Multan
Oryza sativus	WumN20	+++	Reddish pink	Sahiwal
Gossypium spp.	WumN31	++	Pinkish	Sahiwal
Saccharum officiarum	WumN32	+++	Reddish pink	Burewala



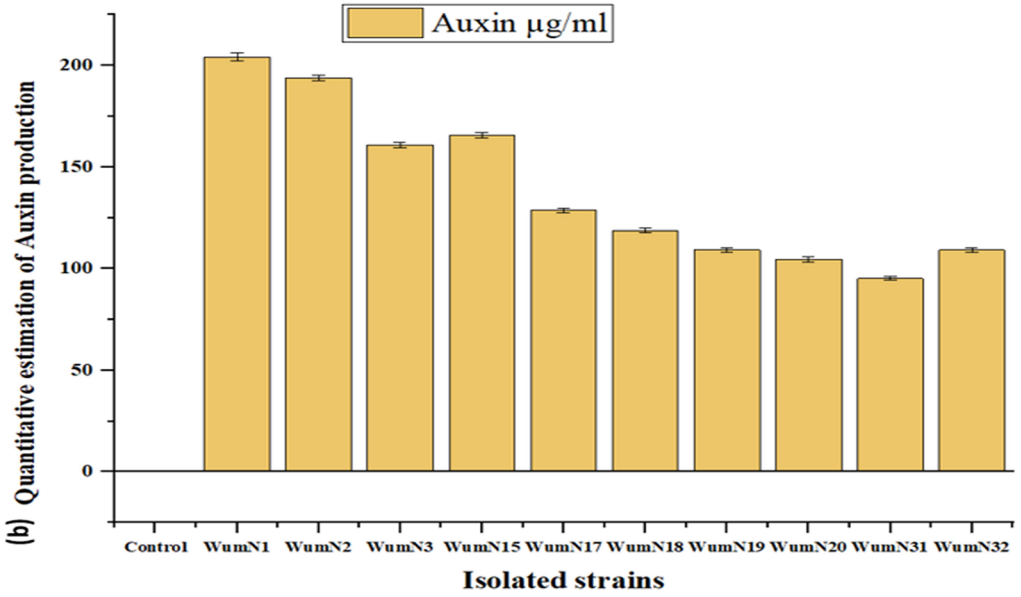


Figure 2. (a) Qualitative screening of APRS based on color and (b) quantitative estimation of APR strains in LB broth μg/mL.

spectrophotometer by measuring the optical density at a wavelength of 535 nm.

2.9.2. Total Soluble Protein Content. One gram of frozen seedling material was extracted by adding 4 mL of phosphate buffer. The residue was centrifuged at 5000 *g* for 10 min. 0.4 mL of the supernatant was mixed with 2 mL of Folin's reagent and placed at room temperature for 15 min; then, 0.2 mL of Folin's and Ciocalteus' phenol reagent was added and incubated for 45 min. The same process was repeated with the control. After the color was developed, the optical density was measured at 750 nm with a spectrophotometer.

2.9.3. Alkaloid Estimation. Fifteen milligrams of the extract of fresh leaves was mixed with 6 mL of HCl solution (1%, v/v) and placed in a water bath for 5 min. Then, a few drops of Wagner's reagent were added, and brown precipitates appeared, which were considered positive for alkaloids.[22]

2.9.4. Terpenoid Estimation. One hundred grams of fresh leaves were mixed with 2 mL of chloroform, and 2 mL of concentrated sulfuric acid was added. The appearance of a reddish-brown color at the interface was considered positive for terpenoids.[22]

2.9.5. Steroid Estimation. One hundred grams of the extract of leaves were mixed with chloroform, and a few drops of acetic anhydride were added. Then, the mixture was boiled in a water bath for 30 min and rapidly cooled in ice water. Afterward, 2 mL of concentrated sulfuric acid was added. The appearance of a brown ring at the junction of the two layers showed the presence of steroids, and the red color showed the presence of triterpenoids.[22]

2.10. Statistical Analysis. All statistical analyses were conducted using SPSS software (version 20.0, IBM Corp., Armonk, NY, USA). Posthoc analysis was conducted using Duncan's multiple range test to identify significant differences between group means. This method was chosen for its ability to control the type I error rate while allowing for multiple comparisons. Differences were considered statistically significant at a p-value of less than 0.05. Results are presented as means ± standard deviations (SD).

3. RESULTS

3.1. Physiochemical Analysis of Rhizospheric Soil Samples. Rhizospheric soil samples were gathered from different cultivated and noncultivated districts of Punjab, such as four different locations, viz. Multan, Burewala, Sahiwal, and Vehari. The soil samples showed different temperature ranges of 25−40 °C with different soil textures. Multan and Vehari showed a loamy texture, Burewala showed a loamy to sandy loam texture, while the soil collected from Sahiwal showed a clayey texture.[23] The soil samples showed a neutral pH, but rhizospheric soil samples showed different pH levels, varying from acidic to alkaline.[20]

3.2. Qualitative and Quantitative Estimation of Auxin-Producing Rhizobacteria. A total of 88 rhizobacterial strains were isolated from rhizospheric soil sampled from different districts of Punjab (Vehari, Multan, Burewala, and Sahiwal). The screening of these rhizobacterial strains focused on their ability to produce auxin. Out of the 88 strains, 10 of the best strains, which showed pink to dark red color, were selected, and their optical density was compared (WumN1,

https://doi.org/10.1021/acsomega.4c06066
ACS Omega 2025, 10, 4303−4314

WumN2, WumN3, WumN15, WumN17, WumN18, WumN19, WumN20, WumN31, and WumN32) (Figure 2a). Auxin production of the selected strains ranged from 95.1 to 204.2 μg/mL (Figure 2b). Maximum auxin production was examined in WumN1 (204.2 μg/mL), followed by the WumN31 strain with an auxin production potential of up to 95.1 μg/mL (Table 1).

Table 1. IAA Production in 10 Isolates

isolated strains	absorbance (nm)	IAA concentration (μg/mL)
WumN1	0.642	204.4
WumN2	0.62	193.9
WumN3	0.552	160.9
WumN15	0.568	165.6
WumN17	0.483	128.6
WumN18	0.466	118.6
WumN19	0.448	109.2
WumN20	0.435	104.5
WumN31	0.412	95.1
WumN32	0.446	109.2

3.3. Morphological and Biochemical Characterization. Auxin-producing strains varied in size from 0.9 to 2.5 mm and were Gram-positive and rod-shaped. The textures of most of the strains were smooth, except for WumN3, WumN17, and WumN19, which showed a rough texture. All selected strains exhibited irregular margins, but WumN1, WumN2, and WumN17 had regular margins. Catalase, methyl red, Voges−Proskauer, indole production, and oxidation fermentation tests were positive for all strains, while one strain, WumN3, tested negative for the nitrate reduction test; the remaining were all positive (Table 2).

3.4. Phylogenetic Identification of Auxin-Producing Rhizobacteria. For the identification of APRS, 16S rRNA sequencing is the most reliable method. The auxin-producing rhizobacterial strains WumN1, WumN2, WumN3, WumN15, WumN17, WumN19, and WumN20 showed maximum similarity with the genus *Bacillus*. WumN3, WumN15, and WumN17 were identified as *Bacillus subtilis*, WumN1 as *Bacillus cereus*, WumN2 as *Bacillus sonorensis*, WumN19 as *Bacillus vallismortis* , and WumN20 as *Bacillus paralicheniformis*. Likewise, WumN18 showed maximum homology with *Brevibacillus borstelensis*, WumN31 showed maximum similarity with *Brevundimonas vancanneytii* , and WumN32 showed maximum similarity with *Exiguobacterium indicum*. The neighbor-joining method constructed the phylogenetic tree with selected APRS, which showed a dot in the tree at the end of the node (Figure 3).

3.5. Optimization of Selected APRS Strains. APRS showed maximum auxin production at pH 6−7. Among the strains, WumN20, WumN15, WumN17, WumN31, and WumN32 showed growth at pH 4, whereas one strain, WumN20, grew at pH 5. At pH 8, all strains showed growth (Figure 4a). The current study indicated that the ability of auxin production is also affected by the pH of the culture media, and the maximum auxin produced at pH 7 indicated it as optimum pH (responsible for maximum auxin production).

Table 2. Morphological and Biochemical Characterization of Selected APR Strains[a]

morphological and biochemical characterization	selected rhizobacterial strain									
	WumN1	WumN2	WumN3	WumN15	WumN17	WumN18	WumN19	WumN20	WumN31	WumN32
size (mm)	2.5	1	1.3	1.5	0.9	2.1	2	2.4	1.6	1.9
cell morphology	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod
colony shape	R	R	IR	IR	R	IR	IR	IR	IR	IR
Gram staining	+	+	+	+	+	+	+	+	+	+
MacConkey's agar	−	−	−	−	−	−	−	−	−	−
growth on EMB	−	−	−	−	−	−	−	−	−	−
catalase	+	+	+	+	+	+	+	+	+	+
motility	−	+	+	−	−	−	−	−	+	+
indole production	+	+	+	+	+	+	+	+	+	+
citrate test	−	−	−	−	−	−	−	−	−	−
urease test	−	−	−	−	−	−	−	−	−	−
methyl red	+	+	+	+	+	+	+	+	+	+
Voges−Proskauer	+	+	+	+	+	+	+	+	+	+
oxidation	+	+	+	+	+	+	+	+	+	+
starch hydrolysis	−	−	−	+	+	+	+	−	−	+
arginine	−	−	+	−	−	−	−	−	−	−
hydrolysis	+	−		+	−	−	−	−	−	−
gelatin	−	−	+	+	−		−	−	+	−
hydrolysis	−	+	+	−	−	−	−	−	+	+
nitrate reduction test	+	+	-	+	+	+	+	+	+	+
H_2S production	−	−	−	−	−	−	−	−	−	−
acid from glucose	−	−	−	−	−	−	−	−	−	−
acid from maltose	−	+	−	+	+	−	−	−	−	+
acid from mannitol	−	−	+	−	−	−	−	+	−	−
acid from lactose	−		−	+	+	+	+	−	−	+
acid from sucrose	−	−	+	−	−	−	−	−	−	−

[a]Symbols description: + = positive for production of required chemical; − = negative for production of required chemical; R = regular; IR = irregular.

https://doi.org/10.1021/acsomega.4c06066
ACS Omega 2025, 10, 4303−4314

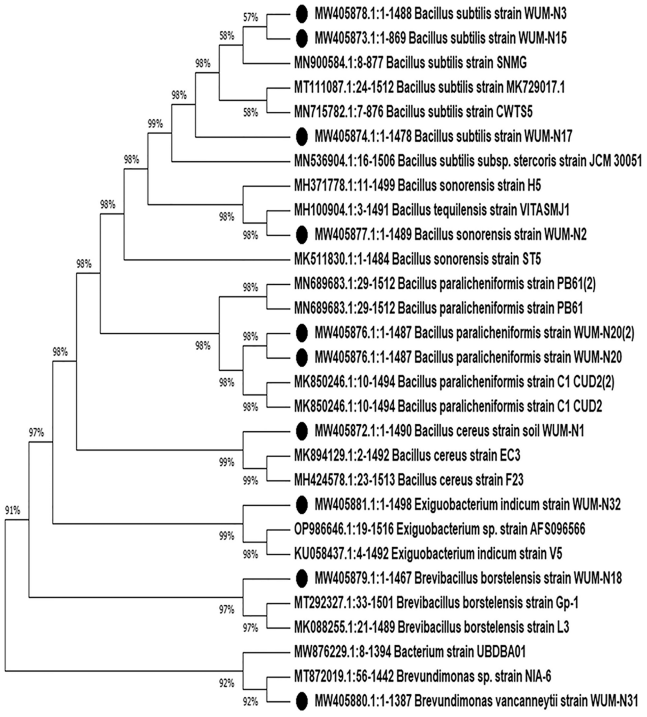


Figure 3. Evolutionary history was inferred by using the neighbor-joining method. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The evolutionary distances were computed using the maximum composite likelihood method. This analysis involved 20 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1522 positions in the final data set. Evolutionary analyses were conducted in MEGA11 software, and APR strains showed the presence of a dot at the end of the node.

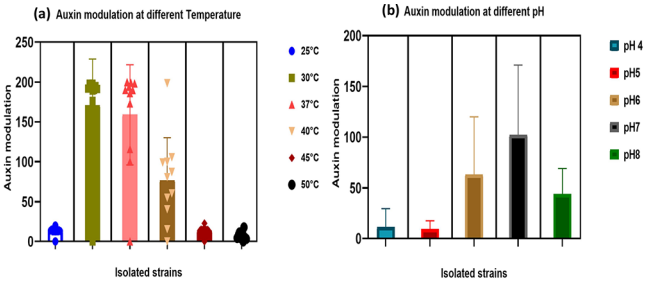


Figure 4. Optimization of APRS under different physical conditions. (a) Temperature: in the above graph, at temperatures of 25, 45, and 50 °C, the auxin production level decreased as compared to 30, 37, and 40 °C, at which all strains produce auxin; (b) pH: at pH 4, 5, and 8, the auxin production level decreased as compared to pH 6 and 7.

The temperature range, where APRS showed an increase in auxin production, was 30−37 °C, whereas at 40−50 °C, auxin production seemed to be decreased (Figure 4b). The optimum temperature was 37 °C, at which all rhizobacterial strains exhibited maximum auxin production potential.

3.6. Confirmation of IAA Production by Thin-Layer Chromatography. The selected APRS were confirmed as indole acetic acid producers by thin-layer chromatography, as they showed the same Rf value of 0.60, comparable with the Rf value 0.60 of the standard IAA, which indicated the ability of the selected strains to synthesize IAA (Figure 5).

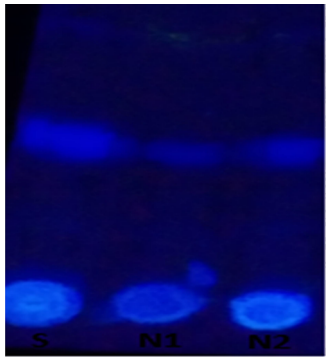


Figure 5. Thin-layer chromatography of the selected APR strains under a UV illuminator showed the same bands and Rf value of IAA compared to synthetic IAA (S = synthetic auxin; N1 = WUMN1; N2 = WUMN2).

3.7. Plant Growth-Promoting Characteristics. The plant growth-promoting abilities of APRS showed that these strains are potential candidates for biofertilizers. All APRS gave a positive result for nitrogen fixation, except for WumN1, while four strains—WumN2, WumN15, WumN20, and WumN32—produced ammonium. All auxin-producing strains produced HCN, except for WumN1. Except for WumN17, all other strains produced siderophores, and two APRS, WumN31 and WumN32, exhibited phosphate solubilization activity (Table 3).

Extracellular enzyme production is also a significant characteristic of rhizobacterial strains. Out of 10 strains, six strains (WumN3, WumN15, WumN17, WumN19, WumN20, and WumN32) produced the pectinase enzyme by producing clear zones ranging from 0.9 to 4.3 mm, while WumN1, WumN2, WumN18, and WumN31 were unable to produce the pectinase enzyme. One strain, WumN2, produced a clear zone on the chitinase medium, while the remaining strains could not produce the chitinase enzyme. For protease enzyme production, WumN1, WumN3, WumN17, WumN19, WumN20, and WumN32 showed clear zones of 3.1 to 4.9 mm, but WumN2, WumN15, WumN18, and WumN31 were unable to produce the protease enzyme (Table 3).

3.8. Estimation of APR Alginate Beads Biofertilizer Effect on Wheat Growth Promotion. *3.8.1. Petri Plate Bioassay.* Auxin-producing strains displayed significant ($P < 0.05$) effects on seed germination, root hairs, root numbers, and the lengths of shoots and roots of wheat plants. Germination of seed increased by 70−90% compared to that of the control group (Figure 6). WumN31 and WumN2 showed maximum increases (49−50%) in root length, followed by WumN18 (43−33%) and WumN17 (37−80%) related to the control. A significant rise was observed in shoot lengths of WumN32- and WumN18-inoculated seedlings (27−52%) compared to the control. Root hairs and root numbers were also increased. The maximum increase of plant growth was observed by strain WumN20 (39−75%), followed by biofertilized seedlings by WumN31 (34−21%) as compared to unfertilized seedlings (Table 4).

3.8.2. Light and Fluorescent Microscopy. Under a light microscope, root hair proliferation of *Triticum aestivum* with auxin-producing strains was observed. Inoculation of auxin-producing strains has a distinct effect on the growth of root hairs as compared to the control. Strains WumN2, WumN3, WumN18, WumN19, WumN20, WumN31, and WumN32

https://doi.org/10.1021/acsomega.4c06066
ACS Omega 2025, 10, 4303−4314

Table 3. Plant Growth-Promoting Attributes of Selected APRS[a]

	PGP activities					extracellular enzyme production		
strains	growth on N-free media	HCN production	NH_3 production	phosphate solubilization (SI = C + Z/C)	siderophore production	pectinase (mm)	protease (mm)	chitinase (mm)
WumN1	−	−	−	−	+++	−	3.9	−
WumN2	+	+	++	−	+	−	−	4.2
WumN3	+	++	−	−	++	2.4	4.4	−
WumN15	+	+	+++	−	+	1.9	−	−
WumN17	+	++	−	−	−	2.1	3.1	−
WumN18	+	+++	−	−	++	−	−	−
WumN19	+	+	−	−	+++	4.3	5	−
WumN20	+	++	++	−	+++	0.9	4.7	−
WumN31	+	+	−	2	+	−	−	−
WumN32	+	+	+	1.77	++	2.3	3.7	−

[a]Symbol description: − = negative; + = positive; ++ = medium positive; +++ = strong positive; S = sensitive; R = resistant.

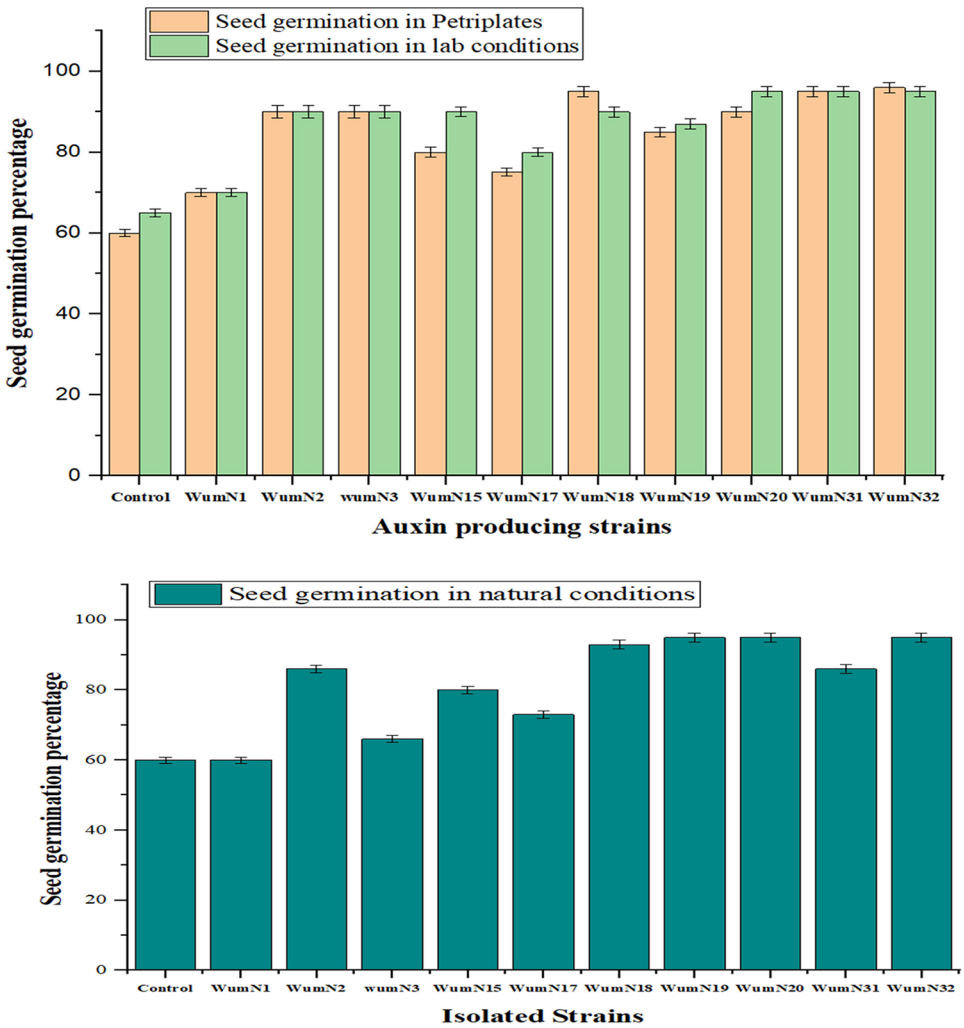


Figure 6. Effect of APRS on seed germination percentage of wheat under different conditions. Values are mean of $n = 5$.

exhibited a strong (+++) effect on the length of root hairs, while WumN1, WumN15, and WumN17 exhibited a medium (++) impact on root hair length in comparison to the control. Under a fluorescent microscope, a root colonization assay unveiled that roots were effectively colonized with bacteria. All selected strains were split into three kinds, viz. strong, medium, and weak root colonizers. Therefore, WumN2, WumN3, WumN18, WumN19, and WumN32 showed strong colonization with roots, while WumN15, WumN17, WumN20, and WumN31 were medium, and WumN1 were weak root colonizers (Table 4).

3.8.3. In Laboratory Condition Pot Experiment. Alginate bead biofertilizer showed a noteworthy ($P < 0.05$) rise in plant growth under both conditions (laboratory and natural). The laboratory showed a 70−80% rise in seed germination, while root and shoot lengths were increased by 60−70%. Fresh

https://doi.org/10.1021/acsomega.4c06066
ACS Omega 2025, 10, 4303−4314

Table 4. Effect of APR Strains on Wheat Growth in Petri Plates[a]

strain	shoot length	root length	root no.	leaves number	root hairs	root colonization
control	6.9 ± 1.5[a]	8.5 ± 1.7[a]	5 ± 1[a]	1.3 ± 0.5[a]	+	+
WumN1	8.6 ± 0.2[b]	9.8 ± 2.1[b]	7.6 ± 0.5[f]	2.3 ± 0.5[b]	+	+
WumN12	9.8 ± 0.4[f]	11.6 ± 0.2[f]	5.6 ± 0.5[c]	3 ± 1[d]	++	++
WumN3	11.5 ± 0.4[j]	14.8 ± 0.4[g]	6.33 ± 0.5[d]	2.66 ± 0.5[c]	+++	+++
WumN15	10.4 ± 0.2[h]	15.8 ± 0.8[i]	5.33 ± 0.5[b]	2.66 ± 0.5[c]	++	++
WumN17	10.2 ± 0.9[g]	15.06 ± 0.3[h]	6.3 ± 0.5[d]	2.66 ± 0.5[c]	++	++
WumN18	11.4 ± 0.2[i]	18.5 ± 0.9[k]	6.4 ± 0.5[d]	2.66 ± 0.5[c]	+++	+++
WumN19	9.5 ± 0.4[d]	10.4 ± 0.4[c]	6.3 ± 0.5[d]	2.66 ± 0.5[c]	+++	+++
WumN20	9.3 ± 0.3[c]	11.2 ± 0.3[e]	8.3 ± 0.6[g]	2.66 ± 0.5[c]	++	++
WumN31	9.7 ± 0.2[e]	10.6 ± 0.1[d]	6.4 ± 0.5[e]	2.66 ± 0.5c	++	++
WumN32	11.9 ± 0.1[k]	19.6 ± 0.2[l]	6.3 ± 0.5[d]	2.66 ± 0.5[c]	+++	+++

[a]Symbols: + = positive, ++ = medium positive, +++ = strong positive. Values are the mean of $n = 5$; mean values (mean ± standard error). The significant difference among different APRS and control plants was expressed as different letters by using Duncan's multiple range test; sharing the same letter does not differ significantly at the means of ($P < 0.05$).

Table 5. Effect of Alginate Bead Biofertilizer on Wheat Growth under Laboratory Conditions[a]

strain	shoot length	root length	roots no.	leaves number	root hairs	root colonization
control	9.78 ± 0.5[a]	10.6 ± 0.5[a]	6 ± 1[a]	2.3 ± 0.5[a]	+	+
WumN1	22.9 ± 0.7[j]	13.9 ± 0.5[b]	7.6 ± 0.5[f]	3.3 ± 0.5[b]	+++	++
WumN2	22.1 ± 0.4[h]	16.6 ± 1.0[d]	6.6 ± 0.5[c]	3.5 ± 1[d]	+++	+++
wumN3	22.4 ± 0.5[i]	22.8 ± 1.1[j]	6.33 ± 0.5[d]	3.46 ± 0.5[c]	+++	+++
WumN15	14.1 ± 0.4[d]	26.7 ± 0.9[l]	7.33 ± 0.5[b]	3.55 ± 0.5[c]	++	+++
WumN17	15.2 ± 0.7[e]	22.4 ± 0.5[i]	8.3 ± 0.5[d]	3.16 ± 0.5[c]	++	+++
WumN18	13.0 ± 0.8[c]	15.8 ± 0.4[c]	6.4 ± 0.5[d]	3.66 ± 0.5[c]	+++	+++
WumN19	12.6 ± 0.2[b]	18.4 ± 0.4[e]	6.3 ± 0.5[d]	3.66 ± 0.5[c]	+++	+++
WumN20	15.8 ± 0.4[f]	27.2 ± 0.8[m]	8.3 ± 0.6[g]	3.46 ± 0.5[c]	++	++
WumN31	23.0 ± 0.2[k]	25.6 ± 0.8[k]	6.4 ± 0.5[e]	3.36 ± 0.5c	+++	+++
WumN32	25.6 ± 0.8[m]	19.4 ± 0.5[f]	6.3 ± 0.5[d]	3.86 ± 0.5[c]	+++	+++

[a]Symbols: + = positive, ++ = medium positive, +++ = strong positive. Values are the mean of $n = 5$; mean values (mean ± standard error). The significant difference among different auxin-producing rhizobacterial strains and control plants was expressed as different letters by using Duncan's multiple range test; sharing the same letter does not differ significantly at the means of ($P < 0.05$).

Table 6. Effect of Alginate Bead Biofertilizer on Wheat under Natural Conditions[a]

	increase in shoot length with passage of time								
strains	7 days	14 days	21 days	28 days	42 days	70 days	98 days	spike length	weight of 100 seeds/g
control	7.13 ± 1.4[a]	9.56 ± 0.2[a]	10.8 ± 0.2[a]	12.1 ± 0.1[a]	14.7 ± 0.4[a]	18.3 ± 0.2[a]	22.7 ± 0.7[a]	7.05 ± 0.3[a]	1.35 ± 0.01[a]
WumN1	12.3 ± 0.6[c]	14.6 ± 0.6[c]	18.1 ± 0.3[e]	22 ± 0.4[g]	23.9 ± 1.3[e]	25.3 ± 1.2[c]	34.9 ± 1.8[i]	7.35 ± 0.5[b]	3.58 ± 0.3[c]
WumN2	13.3 ± 0.1[d]	15.5 ± 0.1[e]	19.6 ± 0.4[f]	23.2 ± 0.8[i]	25.1 ± 0.8[i]	27.7 ± 0.6[g]	31.9 ± 1.7[d]	7.4 ± 0.7[b]	4.05 ± 0.02[d]
WumN3	14 ± 0.3[g]	16.5 ± 0.7[h]	18.4 ± 0.7[e]	21.6 ± 1.0[f]	25.3 ± 1.2[j]	28.7 ± 0.9[i]	36.3 ± 1.2[k]	8.36 ± 0.6[b]	5.63 ± 0.07[f]
WumN15	13.6 ± 0.4[e]	16.3 ± 0.3[g]	20.6 ± 0.4[h]	23.6 ± 0.6[j,k]	28.6 ± 3.9[m]	33.8 ± 0.9[l]	39.7 ± 3.0[m]	8.15 ± 0.8[b]	3.46 ± 0.3[b]
WumN17	11.7 ± 0.4[b]	15.1 ± 0.5[d]	18.3 ± 0.04[e]	19.2 ± 0.7[d]	23.2 ± 1.3[e,f]	26.4 ± 1.8[d]	30.2 ± 2.2[b]	7.23 ± 0.7[b]	4.00 ± 0.05[d]
WumN18	15.4 ± 0.4[i]	15.9 ± 0.4[f]	19.8 ± 0.8[g]	22.7 ± 0.8[h]	23.8 ± 0.3[h]	27.2 ± 0.3[f]	34.7 ± 2.3[h]	7.67 ± 0.6[b]	5.83 ± 0.1[g]
WumN19	16.3 ± 0.7[k]	19.4 ± 0.1[k]	21 ± 0.1[i]	23.5 ± 0.4[j]	24.5 ± 0.7[b]	29.9 ± 0.8[k]	31.3 ± 0.6[c]	7.28 ± 0.9[b]	3.22 ± 0.2[b]
WumN20	12.1 ± 0.8[c]	13.4 ± 0.4[b]	16.1 ± 0.8[c]	18.8 ± 0.3[c]	26.6 ± 0.8[l]	29.4 ± 1.0[k]	33.3 ± 0.8[f]	7.22 ± 0.7[b]	3.83 ± 0.03[d]
WumN31	11.7 ± 0.9[b]	13.5 ± 0.9[b]	15.7 ± 0.6[b]	17 ± 0.08[b]	22.6 ± 0.5[d]	27.9 ± 0.4[h]	35.8 ± 0.9[j]	7.4 ± 1.0[b]	5.42 ± 0.2[f]
WumN32	15.6 ± 0.5[j]	18.5 ± 0.02[j]	20.9 ± 0.3[i]	23.9 ± 0.1[l]	24.4 ± 0.1[f,g]	26.9 ± 0.7[e]	32.3 ± 0.5[e]	7.34 ± 0.7[b]	4.81 ± 0.02[e]

[a]Symbols: + = positive, ++ = medium positive, +++ = strong positive. Values are the mean of $n = 5$; mean values (mean ± standard error). The significant difference among different auxin-producing rhizobacterial strains and control plants was expressed as different letters by using Duncan's multiple range test; sharing the same letter does not differ significantly at the means of ($P < 0.05$).

weight of the plant increased from 40 to 70%, while dry mass increased from 70 to 80% compared to the control (Table 5).

3.8.4. In Natural Condition Pot Experiment. Alginate bead biofertilizer increased seed germination by 40−80% and showed a 27.8−44.4% enhancement in shoot length compared to the control, whereas a 27−58.2% enhancement in seed weight was also seen. The APRS also affected spike length and increased from 24 to 32% compared to the control. The results revealed that auxin-producing rhizobacterial strains immobilized in alginate beads showed a significant ($P < 0.05$) increase in the impact on seed germination, length of root and shoot, and the biomass of *Triticum aestivum* L. WumN15, WumN20, WumN31, and WumN32 showed better results than other strains. However, compared to noninoculated plants, all strains positively affected plant growth (Table 6).

3.9. Phytochemical Analysis. The APR alginate biofertilizer also showed a significant effect on the phytochemical content of wheat. Strain WumN1 showed the highest optical

https://doi.org/10.1021/acsomega.4c06066
ACS Omega 2025, 10, 4303−4314

density (1.55), while other strains showed low optical density, between 0.78 and 1.23, but it was higher than that of the control. Regarding protein content, strains WumN1, WumN2, WumN17, WumN19, WumN20, and WumN32 showed the highest optical density of 1.1−1.4. Other compounds like alkaloids, terpenoids, and steroids were also present in trace concentrations, but their concentrations were higher than the control (Figure 7). These results revealed that alginate bead biofertilizer improves physical traits and enhances the wheat crop's phytochemical production.

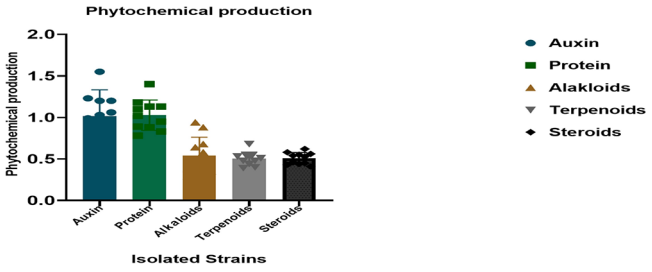


Figure 7. Effect of alginate bead-encapsulated APRS on phytochemicals (auxin, protein, alkaloids, terpenoids, and steroids) production in wheat under natural conditions.

4. DISCUSSION

In the present era, it is a big challenge to enhance crop production by utilizing eco-friendly fertilizers and reducing the use of chemical fertilizers. To solve this problem, plant growth-promoting rhizobacteria (PGPR) as biofertilizers play a vital role in the uptake of nutrients and provide an eco-friendly environment. Plant development-advancing rhizobacteria are fundamental microorganisms that colonize the root surface, called the rhizosphere, ordinarily utilized as biofertilizers, take an imperative part in take-up of supplements, and give eco-accommodating climate. The goal of this study was to evaluate novel approaches that may reduce the use of chemical fertilizers and improve the growth of wheat. After a lot of experiments/testing, it was discovered that the use of PGPR in the form of alginate beads could be a useful strategy for increasing the yield of wheat.

The data acquired from screening techniques can aid in determining the most favorable isolates with plant growth-enhancing characteristics and their subsequent application in agricultural research. They boost plant growth by involving direct and indirect mechanisms, i.e., auxin production, siderophore production, nitrogen fixation, P solubilization, and phytohormone production activities.[24,25] The selection of plant growth-promoting rhizobacteria (PGPR) is based on their ability to produce auxin, which is a direct feature that promotes plant development. Various studies have recorded that the genus *Bacillus* is involved in the production of auxin and plant growth promotion.[24,26,27] In the present research, out of 88 strains isolated from different areas of Punjab, only 10 PGPR strains were selected based on auxin production ability; eight strains belonged to the genus *Bacillus,* while only two belonged to another genus (*Brevundimonas* and *Indicum*). These findings were correlated with previous studies, which showed that most auxin-producing bacteria belong to the genus *Bacillus.*[1,10,28]

Auxin production is an essential ability of PGPR strains under harsh environmental conditions, which boosts the immunity of plants. Most bacterial isolates have the ability to synthesize auxins, such as indole-3-acetic acid (IAA), as a natural part of their metabolic processes. These auxins are crucial for promoting plant growth and development.[29] In our study, isolated bacterial strains were selected for their ability to promote plant development by producing indole-3-acetic acid (IAA). A colorimetric assay was used to determine the ability of these rhizobacterial strains to produce auxin in in vitro conditions in the presence of L-tryptophan,[30] and the results ranged from 95.1 to 204.2 μg/mL, which are comparable with different studies.[31−33] Increasing the concentration of L-tryptophan has shown a significant increase in the auxin production of PGPR in media.[31,33,34] It is reported that *Brevundimonas* and *Exiguobacterium* bacterial strains were capable of IAA production. Similarly, it is also reported that *Bacillus* spp. was capable of producing IAA.[1,10,28]

Rhizobacterial strains showed fluctuations in auxin production under different environmental conditions. The optimum temperature for all auxin-producing rhizobacterial strains was 37 °C and pH was 7, but selected strains can also survive at high temperatures of 40−50 °C and pH,[4−8] which is comparable with a study[18] that described that most PGPR grow at 37 °C and neutral pH but can also survive at high temperatures and pH values.

Along with indole-3-acetic acid production, the selected strains also produced other phytohormones that help plants to grow. Like indole-3-acetic acid, 90 percent of the strains were siderophore and HCN producers and had ACC deaminase/nitrogen fixation ability. Microorganisms possessing ACC deaminase enzymatic activity/nitrogen-fixing activity break down ethylene and utilize the resulting substances as a nitrogen source to support their growth.[45,47] In our data, nine isolated bacteria showed positive nitrogen fixation ability and identified *Bacillus* bacteria as capable of performing nitrogen fixation/ACC deaminase activity.[46,47]

Based on the phosphate solubilization index, our two isolated bacteria produced phosphate. Bacterial isolates were identified as *Brevundimonas* and *Exiguobacterium*. In our study, out of 10 isolates, four produced ammonium. Identified *Bacillus* bacteria were capable of producing ammonium.[1,36] Along with indole-3-acetic acid, the isolated bacteria also produced ammonium. In comparison, 20% could solubilize phosphate, and 40% of the strains were ammonium producers. These results correlated with the studies of Zahra et al. and Sarode et al.,[1,36] which showed that auxin-producing bacteria could also produce other phytohormones, but their concentrations varied according to environmental conditions. The production of lytic enzymes is indirectly involved in plant growth promotion. These enzymes provide mechanisms to control soil-borne phytopathogens,[37] and the selected strains also produce different lytic enzymes. Several studies indicate the benefits of rhizobacterial inoculation on plants, which protects plants from harsh environmental conditions and improves their plant growth characteristics (root and shoot length, root and shoot biomass), ultimately enhancing plant yield.[1,2,6]

The current study selected auxin-producing strains in encapsulation form as alginate bead bioformulations, which showed significant effects on wheat crops. Results revealed that auxin-producing rhizobacterial strains led to a significantly ($P < 0.05$) increased rate of seed germination, length of root and shoot, and biomass of *Triticum aestivum* L. Previous studies also revealed that auxin-producing rhizobacterial strains are

https://doi.org/10.1021/acsomega.4c06066
ACS Omega 2025, 10, 4303−4314

found to be involved in enhancing the productivity of crops.[18,38] After Petri plate bioassays, pot trials under laboratory conditions were performed. In pot trials, shoot and root lengths, as well as fresh and dry weight, were measured after seed germination. Encapsulation with alginate beads showed a 70−80% improvement in seed germination and a 60−70% enhancement in the length of the root and shoot in comparison to the control and also showed a significant increase in fresh and dry weight as compared to the control. Our results correlated with studies[18,35] that observed that auxin-producing rhizobacteria have pronounced effects on growth parameters of wheat. The encapsulation of alginate bead's effects corroborated with the study of Mohite et al.[38] Results revealed that the seed germination percentage increased under laboratory conditions because laboratory environmental conditions are controlled compared to natural conditions, (Figure 8). As we observed significant effects of

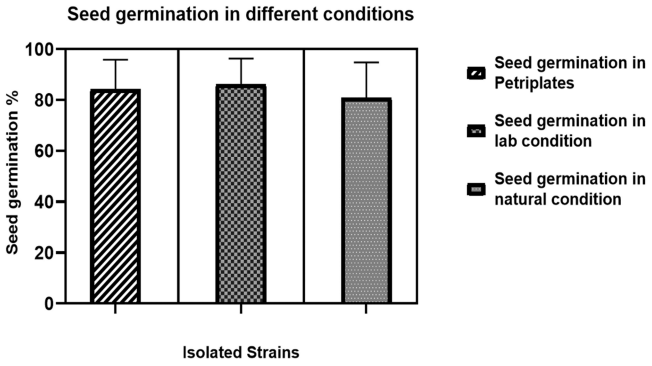


Figure 8. Comparison of seed germination in Petri plates, laboratory conditions, and natural conditions.

alginate beads in pot trials, we also applied these under natural conditions, and the results confirmed their positive effects on wheat crops. Compared to the plants that were noninoculated, the plants that were treated with PGPR and biochar exhibited enhanced growth and increased yield. The use of alginate beads as biofertilizers showed a significantly positive impact on physiological parameters, plant growth, and yield-related features as compared to the control treatment. The synthesis of phytohormones, such as indole-3-acetic acid, which could be closely associated with plant productivity and plant components, might have a role in enhancing plant growth and development, as stated by Ijaz et al.[48]

Alginate beads biofertilizers improve physical traits and enhance the phytochemical compounds of the wheat crop, such as alkaloids, terpenoids, and steroids. Overall, auxin-producing rhizobacterial strains showed pronounced effects on the productivity of *Triticum aestivum* L., which showed their potential for commercial utilization. Using active PGPR strains as biofertilizers in the form of alginate beads holistically seems to be a good environmentally friendly approach to boost growth and development in crop plants (wheat).

Our research findings indicated that the wheat yield was enhanced with the application of biofertilizers. Moreover, future investigations of genomic studies should provide the most promising PGPR, which helps in fully understanding the relationship between plant hosts and their mechanisms.

5. CONCLUSIONS

The current study has shown that the application of alginate beads with PGPR is a sustainable approach for improving wheat growth and yield as compared to the control; especially, strains belonging to the *Bacillus* category significantly effect seed germination, root length, shoot length, and yield. Alginate bead inoculation also increased the concentrations of plant hormones, proteins, steroids, and terpenoids. Moreover, future investigations of genomic studies should provide the most promising PGPR, which helps to fully understand the relationship between plant hosts and their mechanisms. Thus, it is concluded that the application of alginate beads enhanced the growth of wheat as compared to the control.

By incorporating promising bioinoculants as biofertilizers, it is possible to reduce the use of chemical fertilizers and protect the environment, resulting in improved crop productivity. Furthermore, their application has the potential to improve food security by increasing the yield of important crops like wheat. Further research is needed to investigate the impact of various sources of biofertilizers that have been inoculated with plant growth-promoting microorganisms (PGRP) in diverse cropping systems.

■ ASSOCIATED CONTENT

Data Availability Statement

Data are contained within the article.

■ AUTHOR INFORMATION

Corresponding Authors

Atia Iqbal − *Department of Microbiology and Molecular Genetics, Faculty of Life Science, The Women University, Multan 60000, Pakistan*; Email: atiaiqbal01@gmail.com

Muhammad Abdul Rahim − *Department of Food Science & Nutrition, Faculty of Medicine and Allied Health Sciences, Times Institute, Multan 60650, Pakistan*; Email: mabdulrahim@t.edu.pk

Eliasse Zongo − *Laboratory of Research and Teaching in Animal Health and Biotechnology, Universite NAZI BONI, Bobo-Dioulasso, Burkina Faso*; orcid.org/0000-0001-9885-8301; Email: zongoeliasse@yahoo.fr

Authors

Nimra Mushtaq − *Department of Microbiology and Molecular Genetics, Faculty of Life Science, The Women University, Multan 60000, Pakistan*

Shumaila Batool − *Department of Microbiology and Molecular Genetics, Faculty of Life Science, The Women University, Multan 60000, Pakistan*

Sara Janiad − *Department of Microbiology and Molecular Genetics, Faculty of Life Science, The Women University, Multan 60000, Pakistan*

Mehboob Ahmed − *Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore 54590, Pakistan*

Fahad Al-Asmari − *Department of Food and Nutrition Sciences, College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa 31982, Saudi Arabia*

Mohamed Fawzy Ramadan − *Department of Clinical Nutrition, Faculty of Applied Medical Sciences, Umm Al-Qura University, Makkah 21421, Saudi Arabia*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.4c06066

https://doi.org/10.1021/acsomega.4c06066
ACS Omega 2025, 10, 4303−4314

Author Contributions

N.M. and A.I. contributed to the conception and design of the study. N.M. and S.B. performed the experiment and statistical analysis. N.M., M.A., and S.J. wrote the first draft of the manuscript. S.J., F.A., M.A.R., M.F.R., E.Z., and M.A. wrote sections of the manuscript, critically revised the manuscript and funding acquisition. All authors contributed to the manuscript revision and read and approved the submitted version.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors received no financial support for the research, authorship, and/or publication of this article. The research was performed as part of the employment of the authors.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors acknowledge support from the Department of Microbiology and Molecular Genetics, Faculty of Life Science, The Women University, Multan, and the Department of Food Science & Nutrition, Times Institute, Multan, Pakistan, where all research activities were conducted. This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [Grant A289].

■ ABBREVIATIONS

APRS	auxin-producing rhizobacterial strains
CFU	colony-forming units
GDP	gross domestic product
HCN	hydrocyanic acid
HCN	hydrogen cyanide production
IAA	indole-3-acetic acid
NBRIP	National Botanical Research Institute's phosphate medium
OD	optical density
P	phosphate
PSR	phosphate-solubilizing rhizobacteria
PGPR	plant growth-promoting rhizobacteria
Rf	retention factor
TLC	thin-layer chromatography

■ REFERENCES

(1) Zahra, S. T.; Tariq, M.; Abdullah, M.; Azeem, F.; Ashraf, M. A. Dominance of Bacillus species in the wheat (*Triticum aestivum* L.) rhizosphere and their plant growth promoting potential under salt stress conditions. *PeerJ.* **2023**, *9* (1), No. e14621.

(2) Omara, A. E.; Hafez, E. M.; Osman, H. S.; Rashwan, E.; El-Said, M. A.; Alharbi, K.; Abd El-Moneim, D.; Gowayed, S. M. Collaborative impact of compost and beneficial rhizobacteria on soil properties, physiological attributes, and productivity of wheat subjected to deficit irrigation in salt affected soil. *Plants.* **2022**, *11* (7), 877−885.

(3) Mishra, B. S.; Sharma, M.; Laxmi, A. Role of sugar and auxin crosstalk in plant growth and development. *Physiol plant.* **2022**, *174* (1), 13546−13555.

(4) Gupta, G.; Parihar, S. S.; Ahirwar, N. K.; Snehi, S. K.; Singh, V. Plant growth promoting rhizobacteria (PGPR): Current and future prospects for development of sustainable agriculture. *J. Microb. Biochem. Technol.* **2015**, *7*, 96−102.

(5) Conde-Avila, V.; Ortega-Martinez, L. D.; Loera, O.; Perez-Armendariz, B.; Martinez Valenzuela, C. Encapsulation of *Azotobacter vinelandii* ATCC 12837 in Alginate-Na Beads as a Tomato Seedling Inoculant. *Curr. Microbiol.* **2022**, *79* (4), 112−125.

(6) Khan, A.; Singh, A. V.; Kumar, R.; Kukreti, B.; Bundela, V.; Upadhayay, V. K. Relative impact of PGPR inoculation on biofortification and yield of wheat under field conditions and their performance assessment through statistical tools. *Pharm. Innov.* **2022**, *11* (8), 490−495.

(7) Konstantinova, N.; Korbei, B.; Luschnig, C. Auxin and root gravitropism: Addressing basic cellular processes by exploiting a defined growth response. *Int. J. Mol. Sci.* **2021**, *22* (5), 2749−2767.

(8) Strobel, S. A.; Allen, K.; Roberts, C.; Jimenez, D.; Scher, H. B.; Jeoh, T. Industrially-scalable microencapsulation of plant beneficial bacteria in dry cross-linked alginate matrix. *Indus Biotechnol.* **2018**, *14* (3), 138−147.

(9) Saberi-Riseh, R.; Moradi-Pour, M.; Mohammadinejad, R.; Thakur, V. K. Biopolymers for biological control of plant pathogens: Advances in microencapsulation of beneficial microorganisms. *Polymers* **2021**, *13* (12), 1938.

(10) Moradi Pour, M.; Saberi Riseh, R.; Skorik, Y. A. Sodium alginate−gelatin nanoformulations for encapsulation of *Bacillus velezensis* and their use for biological control of pistachio gummosis. *Mater.* **2022**, *15* (6), 2114.

(11) Motamedi, M.; Zahedi, M.; Karimmojeni, H.; Baldwin, T. C.; Motamedi, H. Rhizosphere-Associated Bacteria as Biofertilizers in Herbicide-Treated Alfalfa (*Medicago sativa*). *Journal of Soil Science and J. Plant Nutr.* **2023**, *23* (2), 2585−2598.

(12) Ouali, K. O.; Houali, K.; Cruz, C.; Melo, J.; Benakli, Y.; Ousmer, L.; Madani, Z.; Nabti, E. H. Halophilic Plant Growth-Promoting Rhizobacteria as Producers of Antifungal Metabolites under Salt Stress. *Agronomy* **2024**, *14* (4), 845.

(13) Park, S.; Kim, A. L.; Hong, Y. K.; Shin, J. H.; Joo, S. H. A highly efficient auxin-producing bacterial strain and its effect on plant growth. *J. Genet. Eng. Biotechnol.* **2021**, *19*, 179.

(14) Gilbert, S.; Poulev, A.; Chrisler, W.; Acosta, K.; Orr, G.; Lebeis, S.; Lam, E. Auxin-producing bacteria from duckweeds have different colonization patterns and effects on plant morphology. *Plants* **2022**, *11* (6), 721.

(15) Oo, K. T.; Win, T. T.; Khai, A. A.; Fu, P. Isolation, screening and molecular characterization of multifunctional plant growth promoting rhizobacteria for a sustainable agriculture. *Amer J. Plant Sci.* **2020**, *11* (6), 773−792.

(16) Van Elsas, J. D.; Trevors, J. T.; Jansson, J. K.; Nannipieri, P. *Modern soil microbiology*; CRC Press, 2006; pp 7−10.

(17) Widawati, S. Isolation of Indole Acetic Acid (IAA) producing Bacillus siamensis from peat and optimization of the culture conditions for maximum IAA production. *IOP Conf. Ser.: Earth Environ. Sci.* **2020**, *572* (1), No. 012025.

(18) Aziz, K.; Nawaz, M.; Nazir, J.; Anjum, A. A.; Yaqub, T.; Ahmad, M. U.; Rehman, M. U.; Aziz, G.; Khan, M. Isolation, characterization and effect of auxin producing bacteria on growth of *Triticum aestivum*. *J. Anim. Plant Sci.* **2015**, *1* (25), 1003−1007.

(19) Tsavkelova, E. A.; Volynchikova, E. A.; Potekhina, N. V.; Lavrov, K. V.; Avtukh, A. N. Auxin production and plant growth promotion by *Microbacterium albopurpureum* sp. nov. from the rhizoplane of leafless *Chiloschista parishii* Seidenf. orchid. Frontiers. *Plant Sci.* **2024**, *15*, No. 1360828.

(20) Batool, S.; Iqbal, A. Phosphate solubilizing rhizobacteria as alternative of chemical fertilizer for growth and yield of *Triticum aestivum* (Var. Galaxy 2013). *Saudi J. biol sci.* **2019**, *26* (7), 1400−1410.

(21) Tirry, N.; Ferioun, M.; Laghmari, G.; Kouchou, A.; Bahafid, W.; El Omari, B.; El Ghachtouli, N. Effect of co-immobilized tri-bacteria into alginate beads on growth and root mycorrhizal colonization potential of Medicago sativa plants. *Biointerface Res. Appl. Chem.* **2022**, *13* (5), 451.

(22) Joshi, A.; Bhobe, M.; Sattarkar, A. Phytochemical investigation of the roots of *Grewia microcos* Linn. *J. Chem. Pharm. Res.* **2013**, *5* (7), 80−87.

https://doi.org/10.1021/acsomega.4c06066
ACS Omega 2025, 10, 4303−4314

(23) Jamil, M.; Akhtar, N.; Iqbal, M. M.; Khan, M. U. H.; Muslim, N.; Qazi, M. A. Indexing of physico-chemical variables and fertility status of district Sahiwal soils, Punjab, Pakistan. *Soil Environ.* **2021**, *40* (1), No. 202520.
(24) Bano, I.; Tanveer, S.; Ali, B. Plant Growth Promoting Potential of Rhizobacteria Isolated from *Cannabis sativa* L. *J. Med. Life Sci.* **2022**, *5* (2), 291−300.
(25) Riaz, U.; Murtaza, G.; Anum, W.; Samreen, T.; Sarfraz, M.; Nazir, M. Z.; Hakeem, K. R.; Dar, G. H.; Mehmood, M. A.; Bhat, R. A. Plant Growth-Promoting Rhizobacteria (PGPR) as biofertilizers and biopesticides. Microbiota and biofertilizers: a sustainable continuum for plant and soil health. *Horizones* **2022**, 181−196.
(26) Rayavarapu, V. B.; Padmavathi, T. Bacillus sp. as potential plant growth promoting rhizobacteria. *Int. J. Adv. Life Sci.* **2016**, *9* (1), 29−36.
(27) Tanveer, S.; Ali, B. Evaluation of Bacillus and Rhizobium Strains to Enhance the Growth of *Vigna radiata* (L.) under Drought Stress. *J. Med. Life Sci.* **2022**, *5* (1), 101−112.
(28) Batista, B. D.; Dourado, M. N.; Figueredo, E. F.; Hortencio, R. O.; Marques, J. P.; Piotto, F. A.; Bonatelli, M. L.; Settles, M. L.; Azevedo, J. L.; Quecine, M. C. The auxin-producing *Bacillus thuringiensis* RZ2MS9 promotes the growth and modifies the root architecture of tomato (*Solanum lycopersicum* cv. Micro-Tom). *Arch. Microbiol.* **2021**, *203* (7), 3869−3882.
(29) Moradi Pour, M.; Saberi Riseh, R.; Skorik, Y. A. Sodium alginate−gelatin nanoformulations for encapsulation of Bacillus velezensis and their use for biological control of pistachio gummosis. *Mater.* **2022**, *15* (6), 2114.
(30) Noor, A.; Ziaf, K.; Naveed, M.; Khan, K. S.; Ghani, M. A.; Ahmad, I.; Anwar, R.; Siddiqui, M. H.; Shakeel, A.; Khan, A. I. L-Tryptophan-Dependent Auxin-Producing Plant-Growth-Promoting Bacteria Improve Seed Yield and Quality of Carrot by Altering the Umbel Order. *Horticulture.* **2023**, *9* (9), 954.
(31) Verma, J. P.; Jaiswal, D. K.; Maurya, P. K. Screening of bacterial strains for developing effective pesticide-tolerant plant growth-promoting microbial consortia from rhizosphere soils of vegetable fields of eastern Uttar Pradesh, India Energy, Ecology and Environment. *Plant Sci.* **2016**, *1*, 408−418.
(32) Rahman, A.; Sitepu, I. R.; Tang, S. Y.; Hashidoko, Y. Salkowski's reagent test as a primary screening index for functionalities of rhizobacteria isolated from wild dipterocarp saplings growing naturally on medium-strongly acidic tropical peat soil. *Biosci, biotech biochem.* **2010**, *74* (11), 2202−2208.
(33) Park, S.; Kim, A. L.; Hong, Y. K.; Shin, J. H.; Joo, S. H. A highly efficient auxin-producing bacterial strain and its effect on plant growth. *J. Gen Eng. Biotechnol.* **2021**, *19*, 179.
(34) Ali, B.; Sabri, A. N.; Ljung, K.; Hasnain, S. Quantification of indole-3-acetic acid from plant associated Bacillus spp. and their phytostimulatory effect on *Vigna radiata* (L.). *W J. Microbiol Biotechnol.* **2009**, *25*, 519−526.
(35) Raheem, A.; Ali, B. The Microphenotron: a novel method for screening plant growth-promoting rhizobacteria. *PeerJ.* **2022**, *10* (9), No. e13438.
(36) Sarode, P. D.; Rane, M. P.; Chaudhari, B. L.; Chincholkar, S. B. Screening for siderophore producing PGPR from black cotton soils of North Maharashtra. *Curr. Trends Biotechnol. Pharm.* **2007**, *1* (1), 96−105.
(37) Verma, J. P.; Jaiswal, D. K.; Maurya, P. K. Screening of bacterial strains for developing effective pesticide-tolerant plant growth-promoting microbial consortia from rhizosphere soils of vegetable fields of eastern Uttar Pradesh. *India. Ener Ecol Environ.* **2016**, *1* (13), 408−418.
(38) Mohite, B. Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. *J. Soil Sci. Plant Nutr.* **2013**, *13* (3), 638−649.
(39) Ganesh, J.; Hewitt, K.; Devkota, A. R.; Wilson, T.; Kaundal, A. IAA-producing plant growth promoting rhizobacteria from Ceanothus velutinus enhance cutting propagation efficiency and Arabidopsis biomass. *Front. Plant Sci.* **2024**, *15*, No. 1374877.
(40) Gusmiaty; Restu, A. M.; Payangan, R. Y. Production of IAA (Indole Acetic Acid) of the rhizosphere fungus in the Suren community forest stand. *IOP Conf. Series: Earth Environ. Sci.* **2019**, *343*, No. 012058.
(41) Ganesh, J.; Hewitt, K.; Devkota, A. R.; Wilson, T.; Kaundal, A. IAA-producing plant growth promoting rhizobacteria from Ceanothus velutinus enhance cutting propagation efficiency and Arabidopsis biomass. *Front. Plant Sci.* **2024**, *15*, No. 1374877.
(42) Gilbert, S.; Poulev, A.; Chrisler, W.; Acosta, K.; Orr, G.; Lebeis, S.; Lam, E. Auxin-Producing Bacteria from Duckweeds Have Different Colonization Patterns and Effects on Plant Morphology. *Plants* **2022**, *11*, 721.
(43) Bashan, Y. Alginate beads as synthetic inoculant carriers for slow release of bacteria that affect plant growth. *Applied and environmental microbiology* **1986**, *51* (5), 1089−1098.
(44) Shoaib, A.; Shafique, H.; Anwar, A.; Javed, S.; Malik, B.; Mehnaz, S. Iron-Supplemented Alginate Beads of Pseudomonas chlororaphis Improves Charcoal Rot Disease Resistance and the Productivity in Tomato Plants. *J. Plant Growth Regul.* **2024**, *43*, 4038−4061.
(45) Glick, B. R. Plant growth-promoting bacteria: Mechanisms and applications. *Scientifica* **2012**, *2012*, 1−15.
(46) Khamwan, S.; Boonlue, S.; riddech, N.; Jogloy, S.; Mongkolthanaruk, W. Characterization of endophytic bacteria and their response to plant growth promotion in Helianthus tuberosus L. *Biocatal. Agric. Biotechnol.* **2018**, *13*, 153−159.
(47) Qarni, A.; Billah, M.; Hussain, K.; Shah, S. H.; Ahmed, W.; Alam, S.; Sheikh, A. A.; Jafri, L.; Munir, A.; Malik, K. M.; Khan, N. Isolation and characterization of phosphate solubilizing microbes from rock phosphate mines and their potential effect for sustainable agriculture. *Sustainability* **2021**, *13* (4), 2151.
(48) Ijaz, M.; Tahir, M.; Shahid, M.; Ul-Allah, S.; Sattar, A.; Sher, A.; Mahmood, K.; Hussain, M. Combined application of biochar and PGPR consortia for sustainable production of wheat under semiarid conditions with a reduced dose of synthetic fertilizer. *Braz. J. Microbiol.* **2019**, *50*, 449−458.
(49) Khan, N.; Bano, A.; Rahman, M. A.; Guo, J.; Kang, Z.; Babar, M. A. Comparative physiological and metabolic analysis reveals a complex mechanism involved in drought tolerance in chickpea (Cicer arietinum L.) induced by PGPR and PGRs. *Sci. Rep.* **2019**, *9* (1), 2097.

https://doi.org/10.1021/acsomega.4c06066
ACS Omega 2025, 10, 4303−4314