Journal of Experimental Biology and Agricultural Sciences, February - 2024; Volume – 12(1) page 60 – 75

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Exploration and Profiling of Potential Thermo-alkaliphilic *Bacillus licheniformis* and *Burkholderia* sp. from varied Soil of Delhi region, India and their Plant Growth-Promoting Traits

Charu Singh[1], Abhishek Chauhan[2*], Jayati Arora[1], Anuj Ranjan[3], Hardeep Singh Tuli[4], Moyad Shahwan[5,6], Vishnu D. Rajput[3], Tatiana Minkina[3], Sambasivan Venkat Eswaran[7], Tanu Jindal[2,*]

[1]Amity Institute of Environmental Sciences, Amity University, Noida, Uttar Pradesh, India
[2]Amity Institute of Environmental Toxicology, Safety, and Management, Amity University, Noida, Uttar Pradesh, India
[3]Academy of Biology and Biotechnology, Southern Federal University, Rostov-on-Don, Russia
[4]Department of Bio-Sciences and Technology, Maharishi Markandeshwar Engineering College, Maharishi Markandeshwar (Deemed to Be University), Mullana, Ambala, 133207, India
[5]Department of Clinical Sciences, College of Pharmacy and Health Sciences, Ajman University, Ajman 346, United Arab Emirates
[6]Centre of Medical and Bio-Allied Health Sciences Research, Ajman University, Ajman 346, United Arab Emirates
[7]Ex-Head (Chemistry Department) and Dean (Academics), St Stephen's College, Delhi, 110007; Amity University, Noida, Uttar Pradesh-201313, India

Received – January 15, 2024; Revision – February 21, 2024; Accepted – March 05, 2024
Available Online – March 15, 2024

DOI: http://dx.doi.org/10.18006/2024.12(1).60.75

KEYWORDS

PGPR

Sustainable Agriculture

Crop Health

Oryza sativa

ABSTRACT

Soilless cultivation has emerged as a fundamental alternative for large-scale vegetable production because it generates high-quality yields and uses resources efficiently. While plant growth-promoting bacteria (PGPB) are known to enhance growth and physiological aspects in crops grown in soil, their application in soilless cultivation has been relatively less explored. This study aimed to isolate potential PGPBs from soil samples collected from five locations in and around the Delhi-National Capital Region (NCR), India, which were further screened for significant PGPB attributes. Among these, 51 isolated were selected for assessing the impact on *Oryza sativa* (rice) growth and yield grown on a hydroponic set. The results indicated that isolates AFSI16 and ACSI02 significantly improved the physiological parameters of the plants. For instance, treatment with AFSI16 showed a 23.27% increase in maximum fresh shoot mass, while ACSI02 resulted in a 46.8% increase in root fresh mass. Additionally, ACSI02 exhibited the highest shoot length (34.07%), whereas AFSI16 exhibited the longest root length (46.08%) in *O.sativa*. Treatment with AFSI16 also led to significant increases in total protein content (4.94%) and chlorophyll content (23.44%), while ACSI02 treatment showed a 13.48% increase in maximum carotenoid

* Corresponding author
E-mail: akchauhan@amity.edu (Abhishek Chauhan); tjindal@amity.edu (Tanu Jindal)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

content in the leaves. The potential PGPBs were identified through 16S rRNA sequencing, as the two most effective strains, AFSI16 and ACSI02, belonged to thermo-alkaliphilic *Bacillus licheniformis* and *Burkholderia* sp., respectively. This study demonstrated the potential of these identified PGPB strains in enhancing crop performance, specifically in soilless cultivation systems.

1 Introduction

In the twenty-first century, the world's agricultural system confronts more challenges, including declining productivity and degradation of agroecosystem sustainability. According to United Nations projections, the global human population is anticipated to reach 9 billion by 2050, leading to a persistent rise in food demand coupled with a continuous shortage in supply and adverse changes in suitable climate for agriculture (Wood 2001; Alexandratos and Bruinsma 2012; Meena et al. 2017, Zeifman et al. 2022). Agricultural practices have undergone many important transformations worldwide, especially after the Green Revolution. To enhance plant productivity and crop yield, the use of new high-yielding seed varieties, synthetic fertilizers, pesticides, and other agrochemicals are being used for better agricultural practices, which have been posing serious risks to humans, ecosystems, and the environment at large (Kaushik et al. 2009; Pingali 2012; Basu et al. 2021). Scientists, farmers, and agricultural representatives are gradually focusing on new sustainable solutions for growing agriculture issues with minimal environmental footprint. Sustainable agriculture practices require products that can enhance crop health by improving nutrient uptake efficiency, mitigating biotic challenges, and reducing the support on fertilizers, including other benefits such as improving soil fertility by remediation of organic pollutants and heavy metals (Hirel et al. 2011; Singh and Ryan 2015).

Sustainable agriculture encourages reducing synthetic agrochemicals and emphasizing the utilization of natural materials biowaste obtained from biowaste and the inherent capabilities of microorganisms and plants to promote the health and productivity of crops (Fascella et al. 2018). In this context, chicken droppings manure (CDM) has the potential to enhance soil fertility through alterations in soil microbial dynamics, which helps nutrient cycling. Additionally, they offer a sustainable and cost-effective method for enhancing soil health (Liu et al. 2016; Minkina et al. 2023).

The plant rhizosphere is considered a significant region in the soil of the root zone point for microbial activity where the root system, microorganisms, and soil form an association to create a micro-ecosystem to support the plant's health at indirect levels (Li et al. 2019; De La Fuente Cantó et al. 2020). The rhizosphere microbial community varies among plant species, growth stages, and environmental habitat and often includes PGPB (Hayat et al. 2010; Hussain et al. 2011). Some of the PGPB strains improve the resilience of plants to abiotic stresses such as drought and salinity (Baha and Bekki 2015; Delshadi et al. 2017). It is also crucial in heavy metal remediation and degrading organic pollutants under their enzymatic activities and metabolic pathways (El-Meihy et al. 2019). They assist in immobilizing, transforming, and detoxifying heavy metals in the soil, contributing significantly to environmental cleaning efforts (Arora 2020). In mitigating biotic stresses, PGPBs act as natural defenders for plants against pathogens, pests, and other stress-inducing agents. They stimulate the plant's immune responses, produce antimicrobial compounds, and enhance its tolerance to diseases and pests (Yu et al. 2022; Ranjan et al. 2023). Hence, these strains of PGPB are evident to serve as biological stimulants for increasing plant growth and development to support the United Nations Sustainable Development Goals (Bhardwaj et al. 2023a).

PGPBs can improve biological nitrogen fixation, increase phosphate solubility, produce phytohormones and other compounds, encourage beneficial mycorrhizal-plant interactions, and protect plants from pathogenic bacteria (Di Benedetto et al. 2017). PGPB, which include well-studied genera like *Azospirillum, Azotobacter,* and *Nitrobacter* (although only a few are highly capable at root colonization), along with other genera such as *Bacillus, Pseudomonas, Bradyrhizobium, Acinetobacter, Klebsiella, Mesorhizobium,* and *Rhizobium,* have demonstrated capabilities in colonizing the root surface, surviving, and competing with other microbiota (Ahemad and Kibret 2014; Walker et al. 2003). Implementing rhizobacteria is one of the best approaches to enhance phytoremediation efficiency (Rajput et al. 2022).

Plants and microorganisms form different relationships that accelerate beneficial (both symbiotic and non-symbiotic) and pathogenic interactions. As plants grow, microorganisms inhabit the rhizosphere and connect with roots, producing substances controlling plant growth. Conversely, plants differentiate compounds derived from microbes and adjust their defence and growth mechanisms in response to the specific type of microorganism (Ortíz-Castro et al. 2009; Walker et al. 2003).

In agricultural practices, hydroponics involves cultivating plants in soilless systems, carrying essential nutrients through a water-based solution. Hydroponic systems yield substantially more than soil-based systems and encourage quicker growth (Touliatos et al.

2016). This might be due to the more accessible nutrient supply and the deficiency of inhibitions to root growth from soil-related systematic factors (Lee and Lee 2015; Sharma et al. 2018). Using PGPB presents an innovative solution for some of the major challenges in hydroponic cultivation. They prevent pathogen outbreaks, increase plants' strength to environmental stress, and strengthen crop yield per square meter. These combined benefits lead to a reduced benefits period for the initial capital investment (Stegelmeier et al. 2022).

In this study, bacterial strains were isolated from diverse soil types and evaluated for their ability to promote plant growth under hydroponic conditions. The research also highlights the application of the identified PGPB on hydroponically grown *Oryza sativa* growth. The study emphasizes sustainable agriculture and explains isolated bacterial strains' plant growth-promoting (PGP) potential, offering a promising way for eco-friendly and resilient agricultural practices.

2 Materials and Methods

All the chemicals used in the extraction and characterization stages were high purity (analytical research grade). The seeds of *Oryza sativa* were procured from the Indian Agriculture Research Institute (IARI), New Delhi, India.

2.1 Sample collection

The soil samples were collected from 0-20 cm depths from five different locations Delhi-NCR region, India. The collected soil samples varied in nature and included forest soil, soil from agricultural fields, riverbank soil, soil from landfill sites, and clayey soil. The soil from the forest area near Gwal Pahadi, Gurugram, Haryana, India (Aravalli Hills), has remained untouched by human activities, leading to its classification as an 'undisturbed' soil and was located 17 km from Gurugram. The soil from the agricultural field was located 20 km from Gautam Buddha Nagar, Uttar Pradesh, India. The soil from the riverbank was collected from the River Yamuna bank at Kalindi Kunj, Delhi, India. Soil from the landfill region was collected near the Ghazipur landfill site, Ghaziabad, Uttar Pradesh, India. The clayey soil was near the soil of a pond in the Bulandshahr District, Uttar Pradesh, India. These collected samples were designated as AFSI (Amity Forest Soil Isolates), AASI (Amity Agriculture Soil Isolates), AYSI (Amity Yamuna Soil Isolates), ALSI (Amity Landfill Soil Isolates), and ACSI (Amity Clayey Soil Isolates) respectively. Table 1 contains the comprehensive sampling particulars, while Figure 1 illustrates the sampling locations.

Five specific sampling sites were selected due to their significant variations in paedogenetic factors, including parent material, landform, land use, and management practices. Additionally, molecular-level diversity in soil samples was suspiciously considered during the selection process.

Several sub-samples were collected from the same locations, air-dried, and sieved through a 2 mm mesh for chemical analysis. All experiments were carried out at 25±1°C and atmospheric pressure. The glassware used was cleaned properly with neutral washing reagents and distilled water.

2.2 Physicochemical properties of the soil samples

Soil samples were assessed for estimation of organic carbon (OC), nitrogen (N), available phosphorus (P), potassium (K), pH, and temperature of the soil by standard methodology. The Walkley and Black approach assessed OC (Walkley and Black 1934; Xiao et al. 2021). The available N, P and K were estimated using the Kjeldahl method, Bray's P-1 method and ammonium acetate ($C_2H_7NO_2$) extraction method, respectively (Richer and Holben 1950; Bray and Kurtz 1945; Ashworth and Mrazek 1995). The pH of the compost samples was analyzed with a digital pH measuring device (Labman, LMPH-10) by making soil-to-water suspension in 1:2 (w/v)(Behera and Shukla 2015).

2.3 Quantification and isolation of bacterial population from soil samples

To determine the bacterial population per gram of soil, a 1 mL sample was taken from each prepared dilution and streaked onto nutrient agar (NA) plates for multiple streak isolations. The count of colonies, distinguished by their morphological features, was conducted using a digital colony counter. To calculate the number of colonies, the equation of Chauhan and Jindal (2020) was

Table 1 Sampling locations for the soil sample collection sites

S. No.	Sample Codes	Sampling Regions	Latitude	Longitude
1	AFSI	Forest soil	28.457523	77.026344
2	AASI	Agricultural field soil	28.411331	77.848434
3	AYSI	River Yamuna bank soil	28.545267	77.306092
4	ALSI	Soil near landfill	28.625242	77.327989
5	ACSI	Clayey soil	28.514580	77.377594

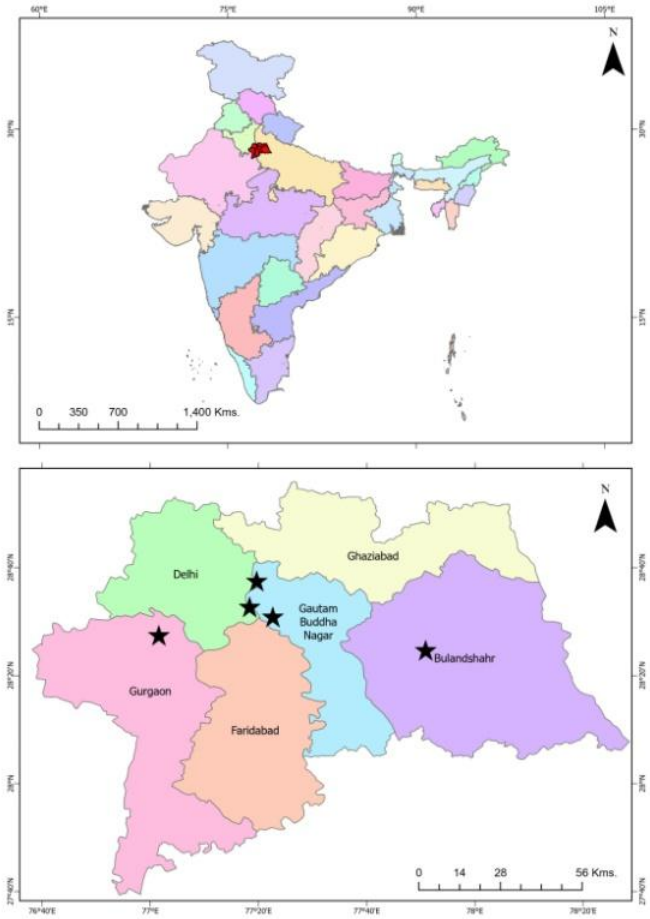

Figure 1 Sampling locations map of Delhi-NCR region

employed to express the count in Colony Forming Units per gram (CFU g^{-1}) of soil:

$$N = \frac{\Sigma C}{(n1+0.1n2)d} \times \frac{10}{\text{weight of sample taken}}$$

In this context, N represents the total colony-forming units within 1 mL of the sample, where 'd' signifies the dilution factor used for the initial counts, 'n1' and 'n2' denote the count of plates considered for the first and second dilutions, and ΣC sums up all the colonies observed across the plates. Upon microscopic examination, identical colonies were isolated and transferred onto NA plates to cultivate pure cultures. These cultures were subsequently assessed for their plant growth-promoting traits, as Chauhan and Jindal (2020) described.

The bacterial isolates were obtained through the pour-plate technique, using NA as the growth medium. The procedure involved serial 10-fold dilutions. Initially, 10 g of the sample was mixed with 90 mL of 0.9% saline solution. To evaluate the PGP potential of these isolates, distinct agar media were employed. The spread-plate cultures were sealed using parafilm and incubated in a dark chamber at a controlled temperature of 25±1°C until the bacterial colonies became visible. This process was carried out in triplicate for each dilution. Subsequently, the colonies were differentiated based on morphological characteristics, isolated, and purified using the streak plate method. Based on their source of isolation, each isolate was assigned a unique code, and further subculturing was undertaken to ensure the isolation of pure colonies, which were then cultured on NA slants and stored at a temperature of 4°C for short-term usage (Chauhan and Jindal 2020).

2.4 Inoculum preparation

The selected bacterial isolates were aseptically inoculated into the nutrient broth and placed in an incubator at 30°C for 48 hours while shaking at 150 rpm. Centrifugation was carried out at 5000 rpm for 12 minutes to separate the cells. The resulting cell pellets were then resuspended in sterilized normal saline to attain an optical density of 1.0, corresponding to approximately 7×10^8 CFU mL^{-1}. These prepared cultures served as a 1.0% (v/v) inoculum for investigating PGP attributes (Bhardwaj et al. 2023b).

2.5 Screening of bacterial isolates for PGP attributes

Using standard methods, the selected bacterial isolates were screened for various PGP attributes such as IAA production, siderophore production, PO_4^{2-} solubilization, NH_3, and HCN production.

2.5.1 Indole acetic acid production

IAA production by bacterial isolates was assessed using the Salkowski reagent method. Bacterial cultures were grown in tryptophan-containing nutrient broth and subjected to centrifugation after 48 hours of incubation at 30 ± 0.5°C and 180 rpm in the dark to obtain the supernatants for testing IAA production (Gang et al. 2019). Two groups of 2 mL supernatants from nutrient broth cultures (one with tryptophan and one without) were incubated with 1 mL of Salkowski reagent for 30 minutes in the dark. The emergence of a pink colour confirmed IAA production, and its presence was further confirmed by measuring absorbance at 530 nm (Ehmann 1977).

2.5.2 Siderophore production

Siderophore production was assessed using the Chrome Azurol S (CAS) agar plate method (Schwyn and Neilands 1987). In this procedure, 48-hour-aged bacterial cultures were individually placed on CAS agar plates and then incubated for 48 hours at 28 ± 1°C. These bacteria were cultured in nutrient broth for 72 hours at 28°C with continuous shaking at 150 rpm. The absence of the blue colour on the CAS agar plates indicated the presence of siderophores.

2.5.3 Phosphate solubilization

The assessment of PO_4^{2-}-solubilizing activity relied on observing clear halo zones surrounding bacterial colonies with the ability to solubilize calcium phosphate. Following the established protocol by Mehta and Nautiyal (2001), the qualitative examination of PO_4^{2}-solubilization was conducted using Pikovskaya media. Each bacterial isolate was subjected to three replicates on these media plates, which were subsequently incubated at 30°C for 7 days. The formation of halo zones around the bacterial colonies was then examined as an indicator of PO_4^{2}-solubilization.

2.5.4 Ammonia production

To assess NH_3 production by the isolates, they were initially cultivated in Ashby's N-free liquid medium for 24 hours at 37 °C. Subsequently, these cultures were streaked and incubated on Ashby's N-free agar plates at the same temperature for 24 hours. NH_3 production was observed using the standard method (Kumar et al. 2012). The liquid medium-grown cultures underwent centrifugation for 10 minutes at 3000 rpm, and 0.2 mL of the supernatant was mixed with 1 mL of Nessler's reagent, with the final volume adjusted to 8.5 mL using NH_3-free distilled water. A colour change from brown to yellow indicated NH_3 production, while test tubes with no colour change were considered negative.

2.5.5 Hydrogen cyanide production

HCN production by the bacteria was examined using the method outlined by Lahlali et al. (2020). The bacteria were cultured on solid Luria Bertani (LB) medium supplemented with 4.4 gL^{-1} glycine. Each box's lid was lined with Whatman paper soaked in alkaline picrate and incubated for four days at 30°C. The presence of a red-orange colour indicated the production of HCN.

2.6 Evaluation of the potential effects of bacterial isolates on the growth of *O. sativa*

2.6.1 Seed procurement

The seeds of *O. sativa* were procured from National Seeds Corporation, IARI, Pusa, New Delhi, India, and were tested for germination potential. The seeds were sanitized for one minute with 70% ethanol (v/v), then for 20 min with 2.5% sodium hypochlorite with three washes with sterilized distilled water (Bhardwaj et al. 2023b).

2.6.2 Effects of Isolates on the seed germination rate (GR)

Sterilized square filter paper was placed in petri dishes and loaded with 45 seeds. Subsequently, 5 mL of sterilized distilled water and 0.1 mL of each bacterial isolate were added. The petri plates were sealed and incubated at 20 ± 5°C for 20 days. Control petri dishes contained uninoculated seeds (without bacterial isolates). Each day, the total number of germinated seeds was tallied, and the GR was calculated after 20 days using the provided equation (Islam et al. 2016).

$$\text{Germination rate } \% = \frac{\text{Germinated seeds}}{\text{Total seeds}} \times 100$$

2.6.3 Plant materials and growth conditions

Rice seeds (*Oryza sativa* L.) were procured from IARI, Delhi, India. Before use, seeds of consistent size underwent surface sterilization using a 10% (v/v) sodium hypochlorite solution for 10 minutes. Following this, they were thoroughly rinsed with distilled water and soaked for 4 hours. Subsequently, the healthy and uniformly sized seeds were placed in 150 mm petri plates, lined with Whatman no. 1 filter paper, and moistened with half-strength Hoagland's solution (pH 6.5) as described by Arditti and Dunn (1969). The bacterial inoculum was added in 10^8 CFU mL^{-1}. The seeds were then germinated in darkness at a temperature of 28 ± 2°C for 4 days. The ensuing seedlings were cultivated under a photon flux density (PFD) of 150 µmol photons m^2s^{-1} and

maintained at a relative humidity range of 50–60%. This environment followed a 12-hour day/night cycle at a consistent temperature of 28 ± 2°C and humidity of 50% for 8 days within a growth chamber. Following this period, seedlings of uniform size were selected and transferred into half-strength Hoagland's solution for a 7-day acclimatization period. Subsequently, the leaves were carefully stored at -86 °C until further analyses. After the 7-day treatment period, root and shoot samples were collected from both control and treated seedlings, and various parameters were analyzed (Arditti and Dunn 1969).

2.6.4 Morphological and Biochemical Characterization

The bacterial isolates exhibiting potential plant growth-promoting activity were subjected to morphological identification and biochemical tests. Colony morphology for colour, size, shape, margin, and elevation was observed under a compound microscope. Biochemical tests like oxidase, citrate, catalase, nitrate, starch, Indole, and Voges-Proskauer were performed (Chauhan and Jindal 2020).

2.6.5 Inoculation effects on growth parameters

The study assessed plant growth by measuring fresh and dry root and shoot weights. For each sample, ten randomly chosen seedlings underwent measurements. Dry weight was obtained by wrapping root and shoot sections in butter paper and oven-drying at 65-75 °C for 48 hours. Chlorophyll and carotenoid levels were determined by extracting 25 mg of fresh leaves with 80% acetone, using methods by Arnon (1949) and Ikan (1991).

The growth response was observed, focusing on chlorophyll content, shoot and root dry weight, and fresh weight. Measurements involved using a vernier calliper, manual counting, and spectrophotometric methods. Weight variations were determined using a standard balance during harvesting. Photosynthetic pigment analysis included harvesting the top leaf of matured plants, mixing a 0.2 g leaf sample with 80% acetone, and measuring absorbance at 652 nm using a UV-visible spectrophotometer(Lau et al. 2020).

2.7 DNA Isolation, PCR amplification, and phylogenetic assessment

DNA extraction process employed the use of GenElute^{TM} Bacterial Genomic DNA kit. A 1.5 kbp 16S-rDNA segment was PCR-amplified with high-fidelity polymerase and the primers 16S forward (GGATGAGCCCGCGGCCTA) and 16S reverse (CGGTGTGTACAAGGCCCGG). The PCR product was purified, visualized on agarose gel, and then subjected to bi-directional sequencing. NCBI's Blast software was used for the identification of the bacterium and to list its close relatives. Based on maximum identity scores, the top ten sequences were subjected to multiple alignment software, Clustal W, where homologous nucleotide sequences were aligned. A phylogenetic tree was constructed using the neighbour-joining algorithm in MEGA 10 software, with tree-clade stability assessed through a 1000-replication bootstrap analysis (Kumar et al. 2018).

2.8 Statistical analysis

The gathered data experienced statistical analysis through IBM SPSS Statistics 23. An independent t-test was utilized to identify significant differences among the physiochemical properties of the 12 isolates. For assessing multiple comparisons between isolates and their impacts on plants, ANOVA Tukey's tests (T-test) were utilized. Germination results were compared against a control group, and all findings were visually presented using graphs created with MS Excel 2021.

3 Results

3.1 Soil physicochemical analysis

The soil physicochemical analysis unveiled notable variations in key parameters across the distinct soil samples. Organic carbon content exhibited a gradient, with AASI showcasing the highest at 4.2%, followed by AFSI at 3.2%, AYSI at 0.7%, ALSI at 0.2%, and ACSI at 2.6%. Nitrogen levels were highest in AFSI at 42 $mgkg^{-1}$, with AASI and ACSI recording intermediate values of 39 and 38 $mgkg^{-1}$, respectively. AYSI demonstrated a moderate nitrogen content of 26 $mgkg^{-1}$, while ALSI had the lowest at 21 $mgkg^{-1}$. Available phosphorus content followed a similar trend, with AFSI leading at 623 $mgkg^{-1}$, closely trailed by AASI at 611 $mgkg^{-1}$. AYSI exhibited a moderate level at 563 mg kg^{-1}, whereas ALSI and ACSI displayed lower values at 495 and 587 $mgkg^{-1}$, respectively. Further, the pH values varied distinctly, with ALSI characterized by a highly alkaline pH of 8.5 ± 0.4, while AYSI and AFSI had pH values of 7.9 ± 0.2 and 7.8 ± 0.2, respectively. ACSI samples were almost with a neutral pH of 7.2 ± 0.1. Temperature differences were observed, with AFSI experiencing the highest at 48°C, while the lowest at 36°C with ACSI and AASI, AYSI, and ALSI exhibiting intermediate temperatures of 39, 38, and 40°C, respectively. These findings underscore the diverse soil characteristics (Table 2).

3.2 Quantification and isolation of bacteria

The forest area exhibited a total bacterial population of 1.9×10^{9} $CFUg^{-1}$, while the agricultural area, Yamuna region, and disposal site displayed bacterial populations of $3.1\times10^{6}CFUg^{-1}$, 1.6×10^{6} $CFUg^{-1}$, and 1.1×10^{6} $CFUg^{-1}$, respectively. Clayey soil recorded a bacterial population of 2.8×10^{7} $CFUg^{-1}$. Subsequently, based on observed colony morphology on NA during the enumeration process, 51 isolates with identical colonies were specifically chosen for further screening to identify potential PGP strains (Figure 2).

Table 2 Physicochemical analysis of soil samples

S. No.	Parameters	AFSI	AASI	AYSI	ALSI	ACSI
1	Organic carbon	3.2%	4.2%	0.7%	0.2%	2.6%
2	Nitrogen ($mgkg^{-1}$)	42	39	26	21	38
3	Available phosphorus ($mgkg^{-1}$)	623	611	563	495	587
5	pH	7.8 ± 0.2	7.6 ± 0.1	7.9 ± 0.2	8.5 ± 0.4	7.2 ± 0.1
6	Temperature (°C)	48	39	38	40	36

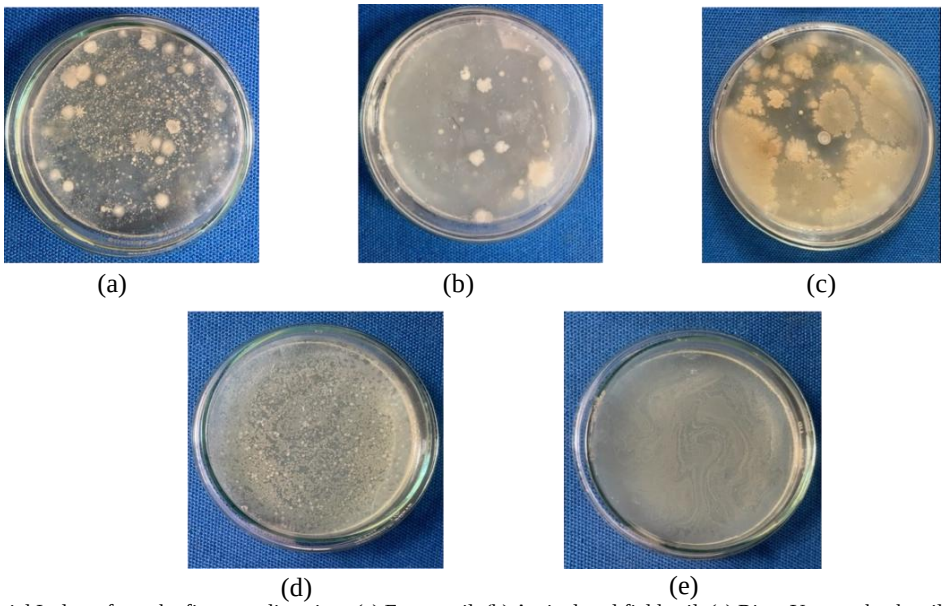

Figure 2 Bacterial Isolates from the five sampling sites: (a) Forest soil, (b) Agricultural field soil, (c) River Yamuna bank soil, (d) Soil near the landfill, and (e) Clayey soil

3.3 Screening of bacterial isolates for PGP attributes

In this study, 51 bacterial strains were isolated from various soil samples and their PGP attributes were evaluated. Twelve isolates were confirmed to be positive for IAA and siderophore production and the formation of HCN and NH_3, along with phosphate solubilization. IAA production was determined by a colour change from pale yellow to pink in Salkowski treatments; among the isolated bacterial strains, AFSI16 was identified as the highest IAA producer among the 51 isolates. Siderophore production was evidenced by a colour change from blue to orange in CAS medium for all 12 isolates. Clear zones surrounding bacterial colonies on Pikovskaya's agar confirmed the ability to solubilize phosphate. Among the isolates, only 12 demonstrated efficient emission of a high ammonia concentration, as indicated by a deep yellow colour in nesslerized spent broth. Additionally, HCN production was observed in the 12 isolates, evidenced by a colour change from pale yellow to red-orange in the LB medium. Thus, the twelve strains, namely AFSI06, AFSI07, AFSI10, AFSI16, AFSI19, AASI03, AASI07, AASI13, ACSI02, ACSI06, ACSI08, ACSI09, and control were identified as exhibiting all PGP attributes and were selected for further experiments in a hydroponic setup.

3.4 The effect of PGPB isolates on the growth of *O. sativa* seedlings

In the current study, 12 bacterial strains were selected after a preliminary study, and it demonstrated that the inoculation of isolates had an impact on SFW, RFW, root length, shoot length, chlorophyll content, protein content, and carotenoid content of *O. sativa.* Significantly enhanced SFM was observed in AFSI16 (0.795 ± 0.012) and ACSI024 (0.795 ± 0.020), and it was found to be 23.27% higher compared to the control T0 (0.645 ± 0.020) ($p<0.05$). Concurrently, RFM showed substantial elevation in AFSI16 (0.321 ± 0.005) and AASI14 (0.322 ± 0.005), rising by 45.45% and 46.8%, respectively, compared to the control (0.221 ± 0.007) ($p<0.05$). However, except for AFSI16 and ACSI02, the other treatments did not exhibit significant changes ($p<0.05$) following the inoculation of PGP isolates on rice seedlings (Figure 3).

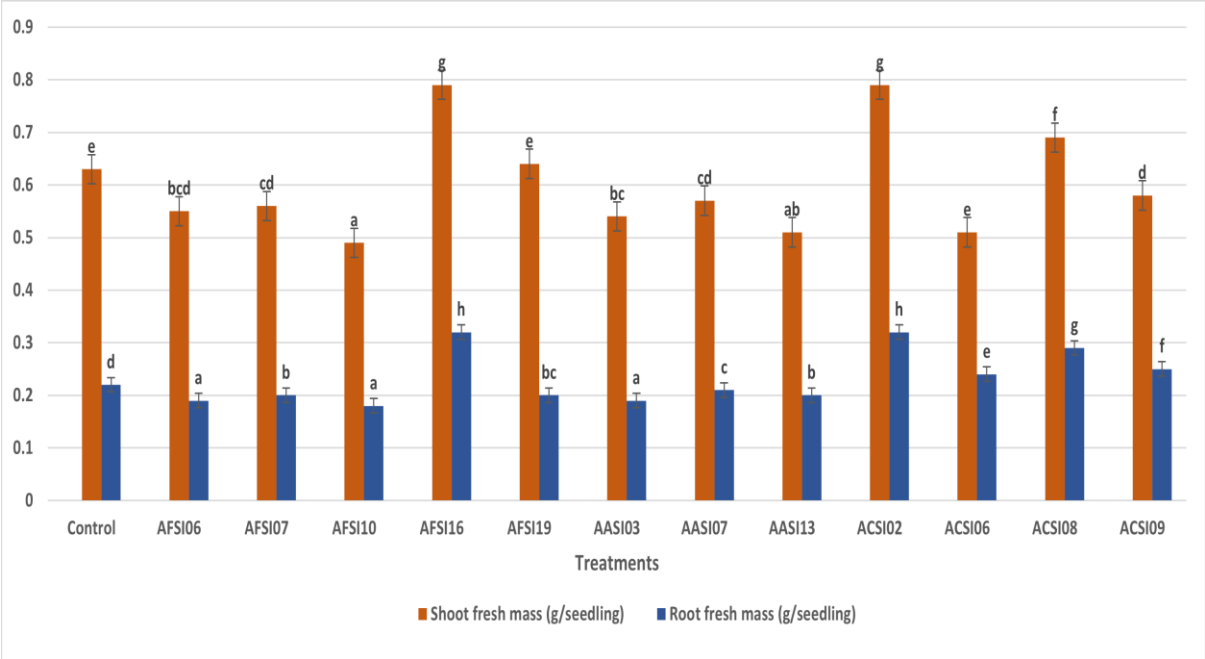

Figure 3 Effects of 12 isolates inoculated in the *O. sativa* crop in the Hydroponics system on shoot fresh mass and root fresh mass of plants. Error bars represent the mean ± S.D of three replicates. Different letters above columns indicate statistically significant differences at $p \leq 0.05$

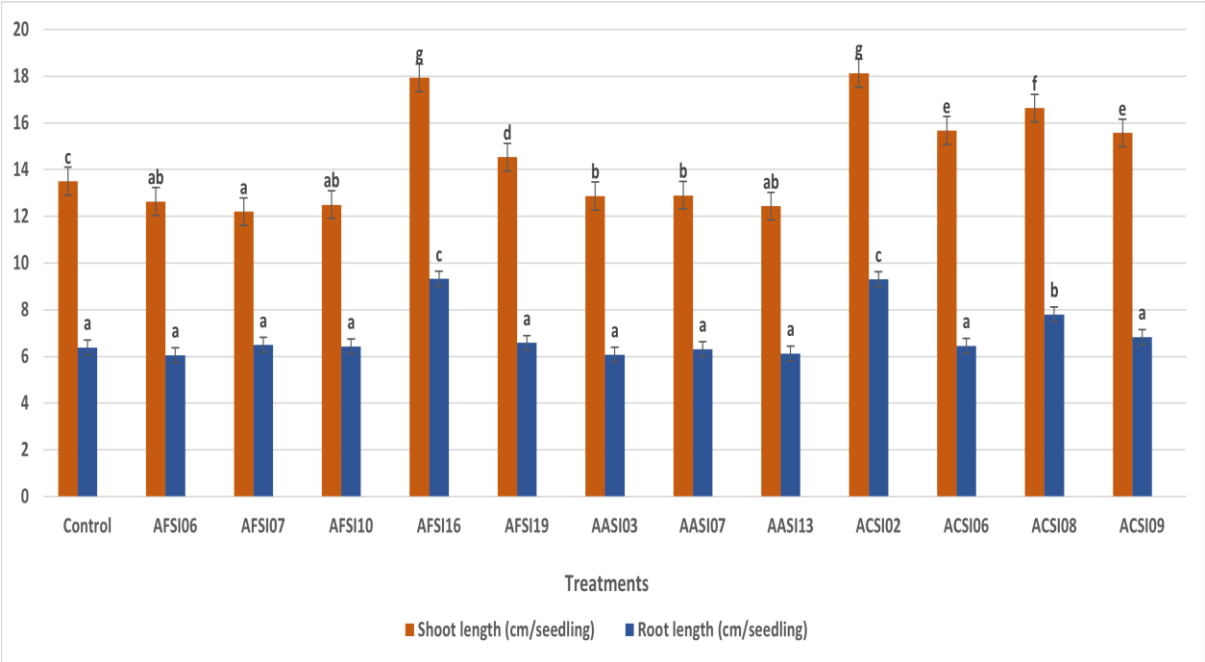

Figure 4 Effects of 12 isolates inoculated in the *O. sativa* crop in the Hydroponics system on plants' shoot and root length. Error bars represent the mean ± S.D of three replicates. Different letters above columns indicate statistically significant differences at $p \leq 0.05$

Like shoot and root mass, significant improvements were observed in SL for AFSI16 (17.93 ± 0.145) and ACSI02 (18.13 ± 0.12) and it is demonstrating increases of 32.5% and 34.07%, respectively, compared to the control (13.5 ± 0.23) ($p<0.05$). Meanwhile, RL exhibited significant enhancements in AFSI16 (9.32 ± 0.023) and ACSI02 (9.3 ± 0.03), rising by 46.08% and 45.76%, respectively, in comparison to the control (6.38 ± 0.31) ($p<0.05$). However, except for AFSI16 and ACSI02, the remaining treatments did not exhibit significant changes ($p<0.05$) after the inoculation of PGP isolates on rice seedlings (Figure 4).

Significant improvements were observed in TP; among the tested 12 strains, bacterial strain AFSI16 (17.44 ± 0.76) displayed a 4.94% increase compared to the control (16.58 ± 0.46) ($p<0.05$). Similarly, TC exhibited significant increases in AFSI16 (1.79 ± 0.18) and ACSI02 (1.73 ± 0.15), rising by 23.44% and 19.31%, respectively, compared to control (1.45 ± 0.05) ($p<0.05$). Additionally, carotenoid content significantly increased in AFSI16 (481.48 ± 15.74) and ACSI02 (493.66 ± 26.57), showing increments of 23.44 and 19.31%, respectively, compared to the control (435 ± 12.68) ($p<0.05$). However, apart from AFSI16 and ACSI02, the other treatments did not exhibit significant changes ($p<0.05$) after the inoculation of PGP isolates on rice seedlings (Figure 5).

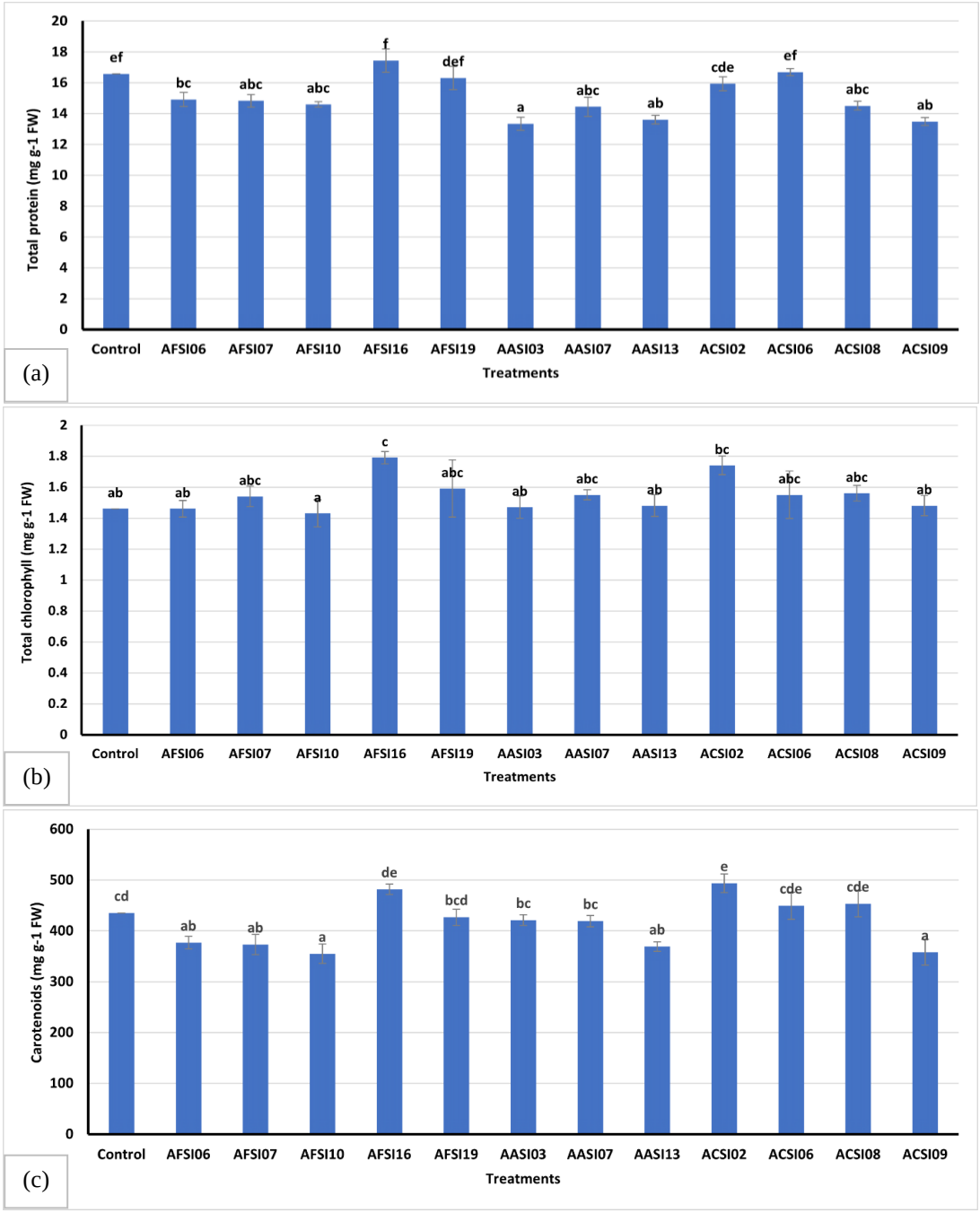

Figure 5 Effects of 12 isolates inoculated in the *O. sativa* crop in the Hydroponics system on (a) total protein, (b) total chlorophyll, and (c) carotenoid content of plants. Error bars represent the mean ± S.D of three replicates. Different letters above columns indicate statistically significant differences at $p \leq 0.05$

Table 3 Percentage growth promotion of PGP isolates on rice seedlings as compared to control

Treatments	Shoot fresh mass %	Root fresh mass %	Shoot length %	Root length %	Total protein %	Total Chlorophyll %	Carotenoid %
AFSI16	23.27	45.45	32.5	46.08	4.94	23.44	10.68
ACSI02	23.25	46.8	34.07	45.76	4.1	19.31	13.48

3.5 Efficacy of PGP isolates on the growth promotion of rice seedlings

Among the various treatments applied to rice seedlings, distinctive outcomes were observed. Treatment AFSI16 and ACSI02 exhibited the highest increase in shoot fresh mass by 23.27% and 23.25%. In a similar pattern, treatment ACSI02 resulted in the greatest surge in root fresh mass at 46.8%, followed by 45.5% of treatment AFSI16. Moreover, treatment ACSI02 has remarkably enhanced shoot length by 34.07% and treatment AFSI16 by 32.5%. The highest root length was observed in AFSI16 treatment (46.08%), followed by treatment ACSI02 (45.76%). Treatment AFSI16 exhibited the highest total protein content of 4.94%, while it was reported 4.1% in treatment ACSI02. Regarding chlorophyll content, 23.44% was the highest total chlorophyll content value observed in treatment AFSI16, while this % was reported 19.31% in treatment ACSI02. Carotenoid percentage of 13.48% was the maximum percentage reported in treatment ACSI02, followed by 10.68% in treatment AFSI16 (Table 3).

In general, two of the twelve bacterial isolates used in this study improved the growth parameter of rice seedlings as compared to the control; however, the AFSI16 isolate showed better enhancement (Table 3).

3.6 Morphological and Biochemical Characterization

Among the isolated bacterial strains, AFSI16 is characterized by irregular colonies with an undefined margin and demonstrates adaptability with a shiny, moist surface and pale colouration. Gram staining revealed a positive result, which indicated the presence of a robust peptidoglycan layer. The rod-shaped, endospore-forming bacterium is actively motile, enhancing its exploration capabilities. Biochemically, it thrives aerobically and facultative anaerobically, showing positive catalase and citrate production. Negative results in Indole, oxidase, and urease tests contrast with positive nitrate reduction. These combined traits depict AFSI16 as a versatile and resilient bacterium adapted for diverse environments with potential clinical significance.

On the other hand, ACSI02 was characterized by circular colonies with entire margins (2-5 mm), presenting a convex, opaque surface that is white and glistering. Gram staining is negative, revealing curved rod-shaped vegetative cells without endospore formation. Despite the absence of endospores, ACSI02 is actively motile. Biochemically, it prefers aerobic growth and displays negative indole production. Positive catalase and citrate production highlights its ability to break down hydrogen peroxide and utilize citrate as a carbon source. ACSI02 is oxidase-positive, lacking urease activity and nitrate reduction. Importantly, it shows no haemolysis on blood agar, signifying non-destructive behaviour towards red blood cells. This collective profile defines ACSI02 as a motile, non-endospore-forming bacterium with specific colony morphology and metabolic traits (Table 4 and 5).

3.7 16SrRNA sequencing

A total of 168 ng and 172 ng of DNA were successfully extracted from the AFSI16 and ACSI02 strains, respectively. Subsequent 16S rRNA sequencing revealed that AFSI16 exhibited an impressive

Table 4 Morphological tests of *Bacillus licheniformis* (AFSI16) and *Burkholderia* sp. (ACSI02)

Morphological characteristics	AFSI16	ACSI02
Colony shape	Irregular	Circular
Margin	Irregular	Entire
Size of colony	-	2-5 mm
Elevation	Flat	Convex
Surface	Shiny and moist	Opaque
Colour	Pale	White and glistering
Odour	Yes	-
Gram staining	Positive	Negative
Shape of vegetative cells	Rod shaped	Curved rods
Endospore formation	Yes	No
Motility	Actively motile	Actively motile

Table 5 Biochemical tests of *Bacillus licheniformis* (AFSI16) *Burkholderia* sp. (ACSI02)

Biochemical tests	AFSI16	ACSI02
Growth	Aerobic and facultative anaerobic growth	Aerobic growth
Indole production	Negative	Negative
Catalase production	Positive	Positive
Citrate production	Positive	Positive
Oxidase production	Negative	Positive
Urease test	Negative	Negative
Nitrate reduction test	Positive	Negative
Haemolysis on blood agar plate	β-haemolytic	Negative

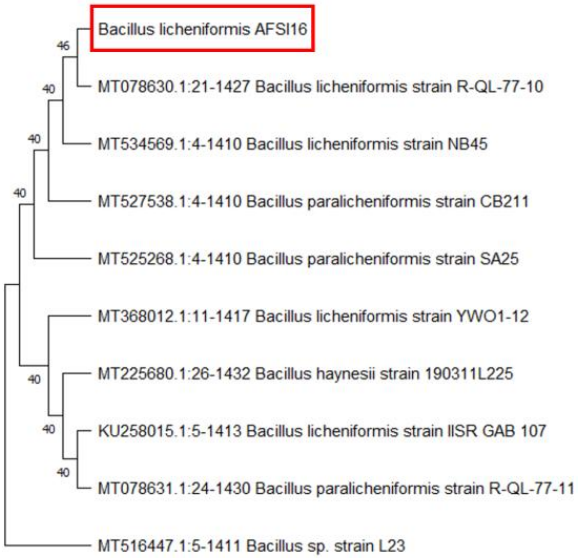

Figure 6 Phylogenetic tree of the selected isolates AFSI16 with its nearest neighbours

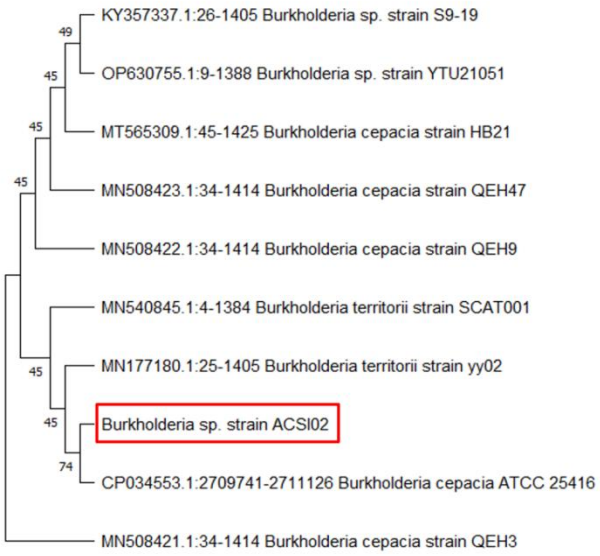

Figure 7 Phylogenetic tree of the selected isolate ASCI02 with its nearest neighbours

99.86% genetic similarity with *Bacillus licheniformis* strain IISR GAB 107, while ACSI02 demonstrated a 97.40% similarity to *Burkholderia* sp. strain S9-19. The 16S rRNA phylogenetic tree analysis further substantiated these findings, confirming that AFSI16 aligns with *B.licheniformis* (Figure 6) and ACSI02 corresponds to *Burkholderia* sp. (Figure 7).

4 Discussion

Holistic understanding is essential for informed agricultural strategies, promoting environmentally intentional approaches for sustainable crop health and enhanced productivity in diverse ecosystems. In this study, diverse soil samples from Delhi-NCR exhibited significant variations in organic carbon (OC), nitrogen (N), phosphorus (P), pH, and temperature. Among the collected soil samples, AASI had the highest organic carbon (4.2%), ALSI had the lowest (0.2%), and the nitrogen and phosphorus levels followed similar trends. pH values varied from highly alkaline in ALSI to slightly acidic in AYSI and AFSI. Temperature differences were observed, with AFSI experiencing the highest (48°C). Bacterial quantification showed varying populations, with the forest area having the highest at 1.9×10^{9} $CFUg^{-1}$ and the disposal site the lowest at 1.1×10^{6} $CFUg^{-1}$. Further screening of 51 isolates for PGP attributes added depth to microbial exploration. These findings align with research emphasizing the influence of soil physicochemical properties on microbial activities and community composition. For instance, the study by Chen et al. (2003) on *Burkholderia pseudomallei* highlighted the importance of soil pH and temperature in microbial growth, reflecting parallels with the Delhi-NCR soil variations. Sharma et al. (2019) demonstrated the correlation between physicochemical properties and soil microbial diversity, reinforcing that soil conditions drive microbial community shifts. Similarly, Mhete et al. (2020) investigated bacterial abundance and diversity under different land-use regimes, connecting these variations to soil properties, which resonates with the observed variations in Delhi-NCR soils. The study by Sapkota et al. (2020) on antimicrobial-producing *Actinomycetes* further emphasizes the impact of diverse soil conditions on microbial functional capabilities.

In the current study, the results obtained from the hydroponics experiments, wherein isolates AFSI16 and ACSI02 exhibited a substantial enhancement in the growth parameters of *O. sativa*, align closely with other studies highlighting the favourable influence of *Bacillus* sp. and various other plant growth-promoting bacteria on a wide range of crops. Especially the results align with studies investigating the growth promotion effects of *Bacillus* sp. on rice seedlings, tomato plants, and paddy plants (Awlachew and Mengistie 2022; De O. Nunes et al. 2023; Kumari et al. 2022). The observed improvements in shoot and root parameters, as well as the promotion of root exudation of essential metabolites, parallel the positive effects reported in the hydroponics experiments (Rekha et al. 2020). The study by Arimurti et al. (2022), focusing on bacteria isolated from hydroponic rock wool, aligns with our hydroponics results, showcasing positive influences on total protein, chlorophyll, and carotenoid content in *O. Sativa*. The concept of using specific bacterial isolates to enhance nutrient availability and alleviate stress demonstrated in this study is similar to the Wang et al. (2023) and Khan et al. (2021) studies. The similarity between our study and these diverse investigations provides additional context and highlights the key role of specific bacterial isolates in promoting plant growth, nutrient uptake, and stress tolerance across various crops.

Our study revealed that AFSI16, characterized as *Bacillus licheniformis*, and ACSI02, identified as *Burkholderia* sp., exhibit growth-promoting attributes in hydroponic *O. sativa* cultivation. In comparison, Mohammad et al. (2017) emphasize thermophilic *Bacillus licheniformis* enzyme production, while Pande et al. (2017) and O'Hair et al. (2020) explore *Burkholderia cepacia-related* strains for phosphate solubilization and biochemical production. The findings of this study contribute unique insights into the specific growth-related features of *B. licheniformis* and *Burkholderia* sp. in hydroponic rice systems.

Thermo-alkalophilic bacteria represent a fascinating group of microorganisms renowned for their ability to thrive in extreme conditions of both high temperature and alkalinity. These resilient bacteria have adapted to environments characterized by elevated temperatures, often exceeding 50° C, and alkaline pH levels ranging from 8-10 or higher; therefore, they could be categorized as thermos-alkalophilic bacteria as reported in previous studies (Brock 1978; Perrry and Staley 1997; Souza and Martins 2001; Olsson et al. 2003). The genus *Bacillus* is adaptable to high-temperature settings and resilient in harsh environmental conditions (Connor et al. 2010; Kawasaki et al. 2011; Aanniz et al. 2015). Furthermore, Manachini et al. (1998) identified three distinct groups within 182 isolated *B. licheniformis* strains. Similarly, this strain has been found in various hot springs across the globe (Manachini et al. 1998).

Earlier studies in Jordan recognized the isolation of thermophilic bacteria categorized under the genus *Bacillus* (Mohammad et al. 2017). Thermo-alkalophilic bacteria play pivotal roles in bioremediation efforts and the production of enzymes and bioactive compounds. Their exceptional capacity to flourish and function well in demanding settings makes them different subjects for scientific investigation and raises the intriguing potential for various sectors (Tizazu et al. 2022). The unique contributions of *B.licheniformis* and *Burkholderia* sp. in hydroponic rice systems illuminate a path for eco-friendly practices, resonating with the broader movement towards environmentally conscious agricultural approaches for heightened productivity and improved crop health.

Conclusion

This study investigated the potential of two different species as plant growth-promoting bacteria (PGPB) in soilless cultivation: thermo-alkaliphilic *B. licheniformis* (AFSI16) and *Burkholderia* sp. (ACSI02). Following a rigorous screening procedure, 12 isolates with various PGP characteristics were identified and isolated, which suggested their potential to boost plant development. Among these twelve strains, AFSI16 and ACSI02 have remarkably impacted *O. sativa* (rice) growth in the hydroponics cultural system, especially by greatly enhancing root and shoot development. Two PGPB strains, AFSI16 and ACSI02, helped encourage crucial components essential for plant health. They particularly amplified the root length, possibly due to their capacity to stimulate root proliferation and elongation. Additionally, the presence of these strains led to heightened levels of proteins, carotenoid, and chlorophyll content within the plants, indicating their role in enhancing physiological processes vital for growth, possibly through nutrient uptake facilitation or enhanced metabolic activities. The conclusive identification of these PGPB strains through 16S rRNA sequencing further solidifies their significance in soilless cultivation. This discovery presents an exciting opportunity for sustainable and resource-efficient vegetable production in systems without traditional soil and offers a pathway to maximize growth and nutrition.

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