Research article

Multifaceted Plant Growth Promoting Traits and Abiotic Stress Resistance Abilities Exhibited by Chrysanthemum Rhizobacteria

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Abstract

Plant growth-promoting rhizobacteria (PGPR) boost plant growth and agricultural sustainability in an ecologically friendly way. The cultivation of chrysanthemum, a globally significant flower crop, has relied heavily on substantial agrochemical inputs that have detrimental impact on the environment. To assess the potential of chrysanthemum rhizobacteria to reduce this reliance, bacterial strains were retrieved from the plant rhizosphere and subjected to an assessment of various plant growth-promoting traits. Out of the 34 rhizobacterial isolates, 21 demonstrated the production of the plant growth hormone auxin, 21 had phosphate solubilization ability, 22 were capable of nitrogen fixation, and 21 could produce ammonia. Based on these findings, seven preeminent PGPR strains, characterized by multifaceted plant growth-promoting traits, were selected for subsequent studies and identified as species belonging to *Acinetobacter*, *Bacillus*, *Pantoea*, *Serratia* and *Staphylococcus*. The selected strains were systematically analyzed for their capacity to endure an array of abiotic stresses. A majority of these strains demonstrated adaptation under osmotic stress ranging from -0.15 to -0.49 MPa, temperatures of 20°C and 30°C, and salt stress within the range of 3 to 7% NaCl, which suggests their potential to promote plant growth across diverse environmental conditions. Additionally, the secretion of hydrolytic enzymes such as protease, pectinase and amylase was

Keywords

chrysanthemum rhizobacteria;
PGPR;
phosphate solubilizing bacteria;
auxin producing bacteria;
abiotic stress tolerance;
protease production;
sustainable agriculture

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examined, and only the *Staphylococcus hominis* PGPR-12 strain demonstrated the ability to produce all three extracellular hydrolases. These findings underscore the potential application of multiple isolates possessing promising plant-probiotic properties to enhance plant growth across various conditions, thereby necessitating further exploration through pot and field assays.

1. Introduction

Plant growth promoting rhizobacteria (PGPR) represent a beneficial group of root-colonizing microorganisms that offer a natural approach to enhancing plant growth and health [1]. PGPR employ an array of mechanisms to foster plant growth including enhanced nutrient uptake, production of plant hormones, solubilization of insoluble minerals, nitrogen fixation, disease suppression, root growth promotion, improvement of soil structure and so on. [2, 3]. Integrating PGPR into agroecosystems constitutes a pivotal facet of sustainable agriculture since it has the potential to decrease the reliance on synthetic fertilizers and pesticides, while promoting plant growth and soil health. Sustainable agriculture refers to a systematic approach of producing agricultural products ensuring the long-term health and productivity of the ecosystem [4]. However, achieving sustainable agriculture is a major challenge to the worldwide agricultural community as it requires a fundamental shift in the way agriculture is currently practiced. Sustainable agriculture involves the adoption of environmentally friendly agroecological practices such as crop rotation, intercropping, use of cover crops, organic farming, etc. The United Nations (UN) has also recognized the importance of sustainable agriculture and included it as one of the Sustainable Development Goals (SDGs). SDGs represent global appeal for a concerted action to eliminate poverty and protect the environment [5]. The integration of PGPR into agriculture can help achieve these goals. Specifically, SDG 2 is aimed at eradicating hunger, attaining food security, and enhancing nutrition whilst promoting sustainable agricultural practices.

Chrysanthemums are among the most important and most popular flowers around the world [6, 7]. They are primarily cultivated as a source for cut and loose flowers which find widespread application in garland making, general decoration, gift preparation, hair adornment, social functions, and other activities [8]. Besides their ornamental use, chrysanthemum plants are also cultivated for their essential oils, sesquiterpenoids, and medicinal properties including antimicrobial, anti-inflammatory, immuno-modulatory, and neuro-protective effects, among others [9-12]. Moreover, chrysanthemum flowers are considered a valuable source of natural quercitrin and myricetin which are essential components in the formulation of several pharmaceutical products [13-15]. Due to their ornamental and medicinal values, chrysanthemums are in high demand and are cultivated extensively worldwide. Therefore, a significant quantity of synthetic fertilizers is required for their cultivation. Although the synthetic fertilizers can increase crop yield, their use may lead to a lack of other vital nutrients and induce unfavorable changes in the chemical, physical, and biological properties of soil, ultimately resulting in long-term harm to soil quality and plant growth [16]. The excessive use of agrochemicals can also lead to soil hardening, reduced fertility, increased pesticide potency, and pollution of water and air [17]. Moreover, overuse of fertilizers also results in the acidification or basification of soil, depletion of beneficial microbial communities, and increased vulnerability to harmful insects, thereby posing a serious challenge to the achievement of balanced and sustainable growth [16, 17]. Long-term fertilization also decreases total organic carbon and basic cation contents [18]. These changes have been found to have a considerable impact on soil quality and productivity. Consequently, the ongoing use of synthetic fertilizers is of critical concern in the preservation of agricultural yield and sustainability. In this regard, the PGPR have emerged as a better alternative to agrochemicals in integrated nutrient management systems [19].

The current study was conducted to investigate the plant growth promoting characteristics of chrysanthemum rhizobia. The research was aimed at reducing reliance on agrochemicals. To this end, bacterial strains were recovered from the rhizospheric soil of chrysanthemum plants and subjected to a comprehensive evaluation of their plant growth promoting activities *in vitro*. Selected isolates were taxonomically characterized and assessed further for their ability to tolerate various stress conditions. The utilization of these bacteria as biofertilizers can assist farmers to reduce their reliance on chemical fertilizers and can contribute to the development of more efficient, eco-friendly, and sustainable agricultural practices in the future.

2. Materials and Methods

2.1 Isolation of rhizobia

To isolate rhizobacteria from chrysanthemum, twenty grams of root-associated soil were collected from five chrysanthemum plants, placed in sterile aluminum foil, and promptly transported to the laboratory. The chrysanthemum plants were obtained from a private garden near the North campus of the University of Chittagong. Bacteria were harvested from the soil adhering to the plant roots. Five grams of the soil sample were suspended in 100 mL of sterile distilled water and agitated at 200 rpm for 30 min at room temperature. The soil suspension was then serially diluted eight times at 1:5 ratio and 100 µL of each dilution was spread evenly onto Luria-Bertani (LB) and tryptone soya broth (TSB) media [20, 21] using 4.0 mm glass beads. The plates were then incubated at 30°C for 12-24 h. Subsequently, 36 colonies with distinct morphological characteristics were selected. To acquire pure cultures, each of the isolated rhizobacteria was repeatedly streaked on LB and TSB plates and preserved in 20% glycerol at -20°C or -80°C [22, 23].

2.2 Activation and maintenance of the isolates

The bacterial isolates were cultured under aerobic conditions in LB or TSA media at 30°C for 24 h with agitation in an incubator shaker at 180 rpm. Prior to each experiment, preculture was prepared by inoculating bacterial cells from the frozen glycerol stock and incubating them overnight at 30°C. For the actual experiment, 0.1 to 1% of the preculture was transferred to fresh media and incubated aerobically at 30°C for 24 to 48 h under agitation at 180 rpm.

2.3 Determination of auxin production

To determine auxin production, the bacterial isolates were inoculated in test tubes containing 3 mL of LB broth supplemented with 1 g/L of L-tryptophan, and incubated at 30°C for 48 h. Subsequently, 2 mL of the culture was subjected to centrifugation at 9500 × g for 2 min. Following centrifugation, 1 mL of the supernatant was combined with 2 mL Salkowski reagent (consisting of 1 mL of 0.5 M $FeCl_3$ and 49 mL of 35% $HClO_4$), and incubated for 30 min in the dark at room temperature. The presence of a reddish-pink color indicated the production of indole-3-acetic acid [24, 25].

2.4 Estimation of phosphate solubilization

The isolates were examined on Pikovskaya's agar plates for the detection of phosphate solubilization activity. The Pikovskya's medium contained 5.0 g $Ca_3(PO_4)_2$, 10.0 g glucose, 0.2 g NaCl, 0.2 g KCl, 0.5 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.002 g $MnSO_4 \cdot H_2O$, 0.002 g $FeSO_4 \cdot 7H_2O$, 0.5 g yeast extract, and 15.0 g agar in 1000 mL distilled water (pH 7.0). The isolates were streaked on the medium and

incubated at 28°C for 7 days. Positive activity was recorded for bacterial isolates that produced clear zones surrounding their colony in the medium [24, 26].

2.5 Detection of ammonia production

The bacterial cultures were introduced into individual tubes each containing 10 mL of peptone water, and subsequently incubated at 30°C for a period of 48 to 72 h. Following this, an aliquot of 0.5 mL of Nessler's reagent was introduced into each tube. The emergence of a color transition from brown to yellow was interpreted as an indication of ammonia production [24, 27].

2.6 Determination of nitrogen fixation

The isolated rhizobacteria were examined for their ability to fix nitrogen using modified nitrogen-free Jensen's medium which contained 20.0 g sucrose, 1.0 g dipotassium phosphate, 0.5 g sodium chloride, 0.005 g sodium molybdate, 0.5 g magnesium sulphate, 0.1 g ferrous sulphate, 2.0 g $CaCO_3$, 15.0 g agar and 2 mL filter sterilized bromothymol blue solution (5 g L^{-1} in 0.2 N KOH) in 1000 mL distilled water with a final pH of 7.0. The bacterial cultures were grown on the Jensen's medium with or without 0.2% NH_4Cl used as a nitrogen source. After 8 days of incubation, bacterial isolates that were able to grow on both plates were selected as nitrogen fixers [28, 29].

2.7 Amplification and sequencing of 16S rRNA gene

Amplification of the isolates' 16S rRNA genes by polymerase chain reaction and their sequencing were carried out according to previously described methods using the 27F (5′-AGAGTTTGATCC TGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) primers [30]. The sequences were archived in the GenBank repository and are accessible via accession numbers OQ845739 to OQ845745.

2.8 Analysis of 16S rRNA gene for taxonomic identification

The 16S rRNA gene sequences of the isolated strains was analyzed using the BLAST application of NCBI and 16S based ID of EzBioCloud. The default parameters were used in BLAST except that the "16S ribosomal RNA sequences (Bacteria and Archaea)" was selected as the database and the maximum target sequences were set to 1000. The number of hits against a particular taxon, as well as the % identity of the sequences that produced significant alignments, were recorded. The closest type strain was determined using EZBioCloud's 16S based ID. Taxonomy was decided considering the number of hits, % identity with top phylotypes, and that with the closest type strain. If a considerable number of hits was not obtained against a particular species, or if more than one species showed significant sequence similarity, the respective isolate was classified up to the genus level.

2.9 Enzyme assay

Assays for extracellular proteolytic, amylolytic and pectinolytic enzymes were performed following previously described method [23]. For the protease assay, the bacterial isolates were inoculated onto gelatin-containing agar plates (1% w/v gelatin) for 48 h and subsequently stained with a solution of mercuric chloride (150 g/L $HgCl_2$ in 20% v/v HCl). For the amylase assay, the isolates grown on starch-agar plates for 48 h were subjected to staining using an iodine-potassium iodide solution (1% iodine in 2% potassium iodide). Pectinase enzyme assay was performed on 48-hour cultures grown on pectin-agar media, and the resulting plates were also stained with the iodine-potassium iodide

solution. The appearance of distinct clear zones surrounding the bacterial colonies in each assay was interpreted as positive enzyme activity.

2.10 Determination of abiotic stress resistance

For the determination of salt tolerance, TSB was prepared with varying concentrations of NaCl (3%, 5%, and 7%) and inoculated with freshly grown cultures. Optical density at 600 nm was monitored at 0, 2, 4, 8, 24, and 48 h to estimate cell growth. For the drought tolerance assay, TSB with water potentials of -0.15, -0.49, -1.03 and -1.76 MPa were prepared by adding 10%, 20%, 30% and 40% PEG-6000, respectively. The broth was then inoculated separately with each of the isolates and cell growth was estimated spectrophotometrically at 600 nm. For thermal assay, growth of cells was monitored at 20 and 30°C for 48 h in TSB.

3. Results and Discussion

3.1 Plant growth promoting traits of the chrysanthemum rhizobacteria

Plant growth promoting rhizobacteria (PGPR) play a substantial role in fostering plant growth and health by providing plants with essential nutrients and helping them adapt to biotic and abiotic stresses. In total, 34 morphologically dissimilar strains were obtained from rhizospheric soil of chrysanthemum. These strains were screened for various plant probiotic traits including phosphate solubilization, nitrogen fixation, ammonia production, and secretion of the plant hormone auxin. Of all isolates, 21 were found to produce auxin, 17 had phosphate solubilization activity, 22 were capable of nitrogen fixation, and 21 could produce ammonia, indicating the presence of plant probiotic properties in a significant proportion of the rhizobacteria (Figure 1). While most of the isolates exhibited two or more plant probiotic properties, only six of them (PGPR-12, PGPR-13, PGPR-14, PGPR-15, PGPR-24, and PGPR-34) displayed all four plant growth promoting characteristics and were therefore selected for further investigations. In addition, strain PGPR-27 (identified as a *Serratia* strain; see following section) was included in the analysis due to its potential as an emerging plant probiotic strain according to recent studies [31, 32].

Auxin is a plant hormone known to regulate several aspects of plant growth and development including cell division, root and shoot elongation, and the formation of lateral roots [33]. The production of auxin by the PGPR isolates signifies their capacity to improve plant growth and yield which makes them promising candidates as biofertilizers. Studies previously reported production of auxin by several rhizobacterial species that are members of *Azospirillum*, *Pseudomonas*, *Rhizobium* and *Bacillus*, and are currently being used as PGPR [34, 35]. These studies also highlighted the strengthening potential of the identified bacteria to be utilized as effective plant growth promoting agents. Moreover, nitrogen is also an essential macronutrient for plant growth, and its availability is a key factor in determining crop yield. One strategy to increase nitrogen availability to the plants is to use nitrogen-fixing PGPR as biofertilizers. Presently, *Rhizobium* species are widely used in agriculture for their ability to fix nitrogen and form symbiotic relationships with leguminous plants [36]. *Azospirillum*, a gram-negative genus encompassing both rhizobacterial and endophytic strains, can also increase nitrogen uptake and plant growth [37, 38].

The present study additionally revealed that 21 of the isolated bacteria could produce ammonia, another essential nutrient for plant growth. Ammonia is a primary source of nitrogen for plants. It is essential for the biosynthesis of amino acids, nucleic acids, and chlorophyll. The ability to produce ammonia is considered as an essential characteristic of plant probiotics and has been

Isolates	Plant Growth Promoting Traits				Hydrolytic Enzyme Production		
	Auxin Production	Phosphate Solubilization	Nitrogen Fixation	Ammonia Production	Protease	Amylase	Pectinase
PGPR-1	-	+	-	+	+	-	-
PGPR-2	+	-	+	+	+	+	+
PGPR-3	+	-	+	-	+	-	+
PGPR-4	-	-	-	-	-	-	-
PGPR-5	-	+	+	+	+	-	+
PGPR-6	+	+	-	-	-	-	-
PGPR-7	+	-	-	-	-	-	-
PGPR-8	+	-	-	-	+	-	-
PGPR-9	-	-	+	-	-	-	-
PGPR-10	+	-	+	-	-	-	-
PGPR-11	+	+	-	+	+	+	-
PGPR-12	+	+	+	+	+	+	+
PGPR-13	+	+	+	+	-	-	-
PGPR-14	+	+	+	+	+	-	-
PGPR-15	+	+	+	+	-	-	-
PGPR-16	-	+	+	+	+	-	+
PGPR-17	+	-	-	+	-	-	-
PGPR-18	-	-	+	+	+	+	+
PGPR-19	+	+	-	-	-	-	-
PGPR-20	-	+	+	+	+	-	-
PGPR-21	-	+	+	+	+	-	-
PGPR-22	-	-	+	+	-	-	-
PGPR-23	-	+	-	+	-	-	-
PGPR-24	+	+	+	+	-	-	-
PGPR-25	+	-	+	+	-	-	+
PGPR-26	-	-	+	-	+	-	+
PGPR-27	+	+	-	+	+	-	-
PGPR-28	+	-	+	+	+	-	-
PGPR-29	-	-	+	+	-	-	-
PGPR-30	+	+	-	-	-	-	-
PGPR-31	+	-	-	-	+	-	-
PGPR-32	+	-	+	+	-	-	+
PGPR-33	-	-	+	-	+	-	-
PGPR-34	+	+	+	+	-	-	+

Figure 1. Plant growth promoting activities and extracellular hydrolytic enzymes detected in the chrysanthemum associated rhizobacteria; + and – indicate presence and absence of each of the activities, respectively.

observed in several strains of PGPR. For example, bacterial strains from the genus *Rhizobium*, *Bacillus*, *Pseudomonas*, and *Azotobacter* exhibit the ability to produce ammonia and have been shown to have beneficial impact on plant growth [39, 40]. Furthermore, 17 of the isolated bacteria displayed phosphate solubilization activity which is important for plant growth as it enables the uptake of phosphate, another key nutrient for plants. Phosphate solubilizing bacteria convert insoluble phosphorus present in the soil into soluble forms which can easily be taken up by plants. For instance, certain species of *Bacillus* are recognized for their capacity to enhance plant growth by solubilizing phosphates [41]. These bacteria can produce organic acids and enzymes that break

down complex phosphorus compounds in the soil, making them available to plants. This makes the phosphate solubilizing isolates promising candidates for sustainable agriculture as they can mitigate reliance on chemical fertilizers.

3.2 Taxonomy of the PGPR isolates

The taxonomic characterization of the selected isolates was based on the analysis of their 16S rRNA gene sequences. Taxon was assigned to each isolate based on its sequence identity with GenBank strains and the number of hits generated for its 16S rRNA gene against a particular phylotype, which was further confirmed by phylotype of the nearest type strain (Table 1). Consequently, the seven PGPR isolates were identified as *Staphylococcus* sp. PGPR-12, *Bacillus* sp. PGPR-13, *Acinetobacter* sp. PGPR-14, *Staphylococcus* sp. PGPR-15, *Pantoea* sp. PGPR-24, *Serratia* sp. PGPR-27, and *Pantoea* sp. PGPR-34. Their taxonomic distribution suggested that the isolates were affiliated with the two major taxonomic classes of bacteria, namely Gammaproteobacteria and Bacilli. Moreover, the identification of two distinct isolates of *Pantoea*, which could be identified to the species level as *P. dispersa* (PGPR-24) and *P. anthophila* (PGPR-34), and two isolates of *Staphylococcus*, *S. hominis* (PGPR-12) and *S. epidermidis* (PGPR-15) indicate the predominance of these genera in the rhizospheres of chrysanthemums.

Previous studies frequently reported *Pantoea* as a PGPR. Similar to the findings of the current study, *Pantoea* spp. isolated from the rhizospheres of other plants were found to demonstrate various plant probiotic properties such as phosphate solubilization, nitrogen fixation, and auxin production [42, 43]. *Bacillus cereus* and *S. nematodiphila*, two other isolates in the present study, were encountered in the rhizosphere of several plants. *Serratia nematodiphila* is a gram-negative species commonly found in soil and in association with plants. This bacterium has been shown to possess plant growth-promoting properties such as phosphate solubilization, nitrogen fixation, and production of phytohormones. *Serratia nematodiphila* NII-0928 that had been isolated from soil in Western Ghat forest, exhibited several plant growth-promoting features including phosphate solubilization, and the production of auxin, siderophores and HCN, according to a study by Dastager *et al*. [44]. Furthermore, the strain was found to promote the growth of black pepper cuttings. In another study, Kang *et al*. investigated the effects of *S. nematodiphila* on the growth of pepper plants under low-temperature stress [45]. The results demonstrated that the application of *S. nematodiphila* strain PEJ1011 enhanced the growth of pepper plants while concurrently ameliorating the adverse impact caused by low temperature [46]. *Bacillus cereus*, commonly found in soil, has also gained attention as a promising PGPR for its various plant growth enhancing activities. Similar to other PGPR, *B. cereus* employs key mechanisms to foster plant growth, primarily via the synthesis of plant growth-promoting compounds and exerting biocontrol activities against plant pathogens [47, 48]. However, it is important to acknowledge that some strains of *B. cereus* have been implicated in foodborne illness in humans. Therefore, it is important to ensure the safety of its use in agricultural practices. The other strains identified in the present study, such as the *Staphylococcus* isolates and *A. oleivorans* are relatively less explored as PGPR. Nevertheless, they were previously isolated from the rhizosphere of one or multiple plants and exhibited various plant probiotic traits [49-52]. The ubiquitous presence of these bacterial isolates in the soil and rhizosphere, and the associated plant growth promoting abilities that align with previous research findings, reinforces the notion that they represent promising candidates for practical applications as plant growth-promoters.

Table 1. Taxonomic affiliations of the selected isolates

Isolates	Top Strain(s) in GenBank from BLAST Result				Nearest Type Strain				Identification		Aceession No.
	Strain	Score	Query Coverage	% Identity	Strain	Pairwise Similarity (%)	Mismatch Total nt	Completeness (%)	Strain	Class	
PGPR-12	*Staphylococcus hominis* (DM 122)	2512	100%	100	*Staphylococcus hominis* DSM 20328	99.93	1/1358	100	*Staphylococcus* sp. PGPR-12	Bacilli	OQ845739
PGPR-13	*Bacillus cereus* (IAM 12605) *Bacillus cereus* (ATCC 14579) *Bacillus cereus* (JCM 2152) *Bacillus cereus* (CCM 2010) *Bacillus cereus* (NBRC 15305)	2566	100%	100	*Bacillus cereus* ATCC 14579	100	0/1389	100	*Bacillus* sp. PGPR-13	Bacilli	OQ845740
PGPR-14	*Acinetobacter oleivorans* (DR1)	2433	100%	100	*Acinetobacter oleivorans* DR1	100	0/1317	100	*Acinetobacter* sp. PGPR-14	Gammaproteobacteria	OQ845741
PGPR-15	*Staphylococcus epidermidis* (NBRC 100911)	2580	100%	99.93	*Staphylococcus epidermidis* NCTC 11047	99.93	1/1399	100	*Staphylococcus* sp. PGPR-15	Bacilli	OQ845742
PGPR-24	*Pantoea dispersa* (DSM 30073)	2555	100%	99.57	*Pantoea dispersa* LMG 2603	100	0/1343	92.01365	*Pantoea* sp. PGPR-24	Gammaproteobacteria	OQ845743
PGPR-27	*Serratia nematodiphila* (DZ0503SBS1)	2453	100%	99.92	*Serratia nematodiphila* DSM 21420 *Serratia marcescens* ATCC 13880	99.92 99.77	1/1329 3/1329	100	*Serratia* sp. PGPR-27	Gammaproteobacteria	OQ845744
PGPR-34	*Pantoea anthophila* (LMG 2558)	2471	98%	99.56	*Pantoea anthophila* LMG 2558	99.78	3/1350	95.89603	*Pantoea sp.* PGPR-34	Gammaproteobacteria	OQ845745

3.3 Production of extracellular protease, amylase and lipase

The PGPR strains were assessed for their capacity to produce various extracellular hydrolytic enzymes such as protease, amylase and pectinase which can decompose complex organic matters of soil to make nutrients available to the plants. Proteolytic activity was identified as the predominant hydrolytic activity present in 17 of the isolates (Figure 1). Pectinolytic enzyme was detected in ten isolates and amylolytic enzyme in four isolates. Only three strains could produce all the hydrolytic enzyme activities, including only one strain (PGPR-12) among the seven selected isolates. These enzymes participate in the degradation of complex organic substances within the soil, converting them into more accessible forms that are readily assimilated by plants. Among the selected strains, proteases were the most commonly detected enzyme. Proteases hydrolyze peptide bonds in proteins breaking them down into amino acids that can be used as a source of nitrogen for plants. This process is particularly important in nutrient-poor soils where the availability of organic matter is limited. Moreover, proteases can also help to protect plants from pathogenic microorganisms by degrading the proteins of the pathogen cell walls resulting in cell wall lysis and death of the pathogen [53].

3.4 Abiotic stress tolerance

Assessing the ability of plant growth-promoting bacteria to withstand abiotic stresses is important considering their potential exposure to a diverse range of environmental conditions in the soil. The ability of the strains to thrive in different abiotic stresses was examined in this study. The findings suggested that the selected strains of plant growth-promoting bacteria had moderate to excellent resistance towards osmotic, salt, and temperature stresses, which was an important factor for their application as biofertilizers in agricultural systems. Drought stress was evaluated under -0.15, -0.49, -1.03 and -1.76 MPa induced by 10%, 20%, 30% and 40% of PEG 6000, respectively. All isolates exhibited the ability to tolerate up to 20% PEG concentration (Figure 2). Two of the isolates, PGPR-14 and PGPR-34, exhibited relatively better tolerance and were able to thrive at a higher PEG concentration. The isolates also showed resistance to salinity stress at all NaCl concentrations from 0.5 to 7.5% (Figure 3), and temperatures of 20 and 30°C (Figure 4).

Several studies have also reported on the isolation of abiotic stress resistant rhizobial strains. For instance, Basharat *et al*. [24] reported the isolation of PGPR strains from rice rhizosphere that exhibited high tolerance to salinity and drought stress. In another study, Mohammed *et al*. [54] isolated PGPR strains from grass pea rhizosphere that were significantly tolerant to salinity, drought, and high temperatures. Similarly, Kumar *et al*. [55] isolated plant growth promoting strains from rhizosphere and nonrhizosphere soil that also demonstrated tolerance to salinity, drought, and high temperature stresses. In the present work, the ability of the isolated PGPR strains to survive in conditions of high temperature, salinity, and low water availability, suggests their ability to survive and thrive in different environments while still promoting plant growth.

Looking ahead to future research, the planned genome sequencing and comprehensive analysis of the most distinguished PGPR strains will unveil key genes associated with their plant growth-promoting functions. This genomic exploration aims to provide the genetic foundation and deeper insights into their plant probiotic activities. Our forthcoming investigations will involve *in vivo* studies using varied systems, commencing with growth analysis through pot assays and progressing to direct field assays employing selected PGPR strains. The aim of these subsequent projects is to further elucidate the impact of these strains on plant growth and development under practical conditions.

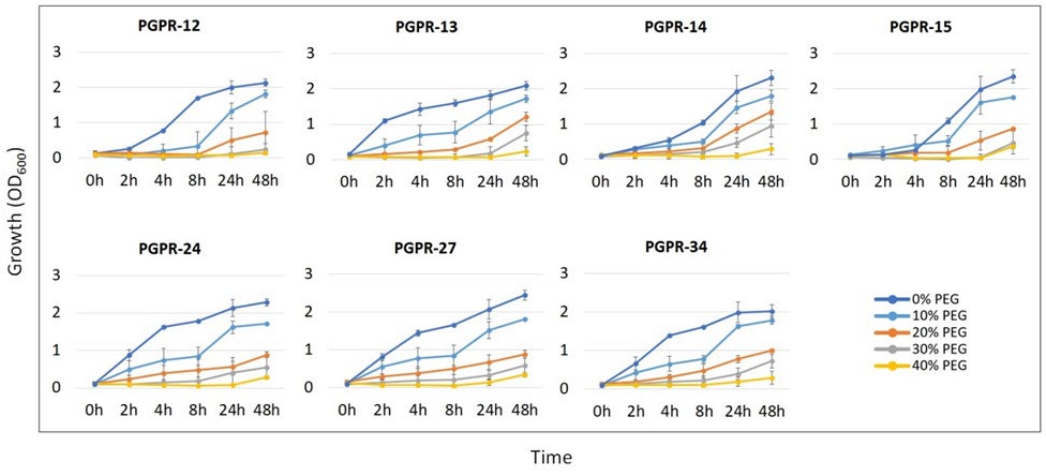

Figure 2. Drought tolerance of the bacterial isolates. The isolates were grown in TSB with the osmotic stress induced by various concentrations of PEG-6000. The density of the culture suspension was measured at specific time points at 600 nm.

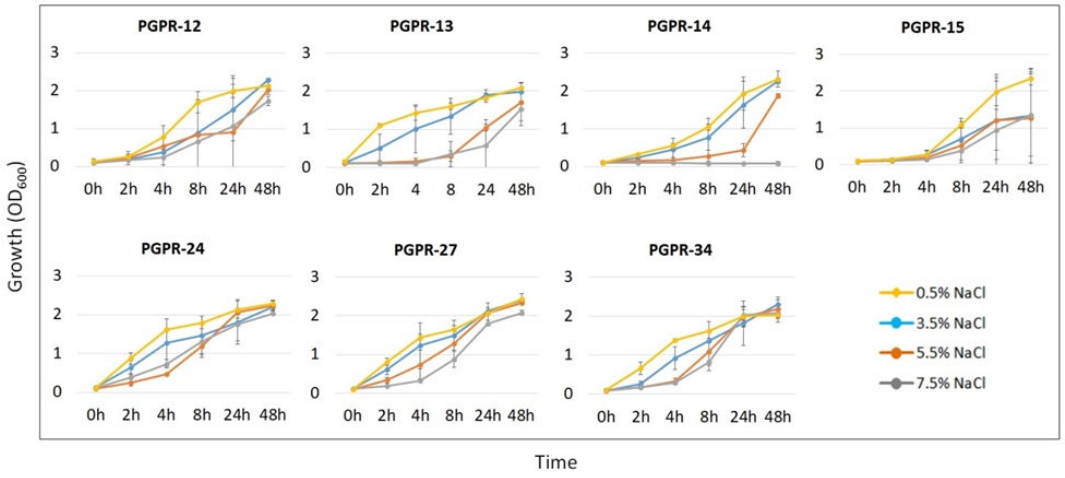

Figure 3. Salt tolerance of the isolates. The salt tolerance was determined by measuring their growth in TSB media containing varying concentrations of NaCl. Growth was measured at specific time points by monitoring absorbance at 600 nm.

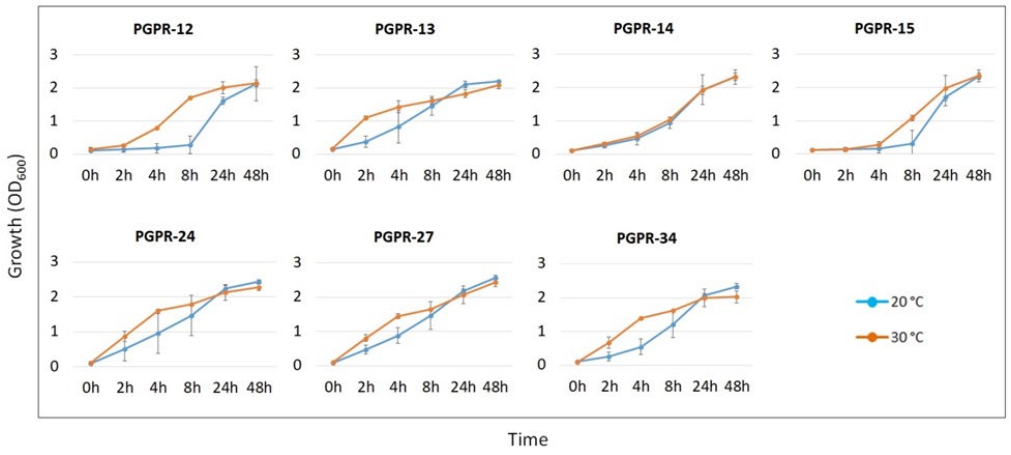

Figure 4. Growth profile of the isolates across different temperatures. The cell densities of the cultures were measured at 600 nm after growth at 20°C and 30°C.

4. Conclusions

Several of the chrysanthemum rhizobial strains examined in the present research exhibited substantial abilities to foster plant growth and confer resistance to abiotic stress under *in vitro* conditions. The production of auxin and ammonia, the solubilization of phosphate, the capacity to fix atmospheric nitrogen, and the secretion of protease, amylase and pectinase enzymes make these bacteria promising candidates for use as biofertilizers which can reduce the reliance on synthetic fertilizers. In order to practically employ these strains in crop-cultivation, they must be further evaluated through pot assay and field assay to determine their effectiveness in practical settings. Their use as bioinoculants may provide significant benefits for sustainable agriculture practices, including enhanced crop yield, improved soil health and fertility, reduced environmental impact, and reduced production costs for farmers.

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