

Plant-microbe Synergy: Employing Coastal Plant Bacteria for Wheat Prosperity under Combined Saline and Heat Stress

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Abstract

Environmental stresses due to climate changes, such as high temperatures and land degradation, significantly impact crop yield, making innovative strategies necessary to increase plant stress tolerance. This study investigates the potential of plant growth-promoting rhizobacteria (PGPR) to enhance wheat resilience under multiple environmental stresses, such as high salinity and temperature. For this, 15 bacterial strains were isolated from the rhizosphere and roots of *Panocratium maritimum*, for their ability to withstand high salinity (50–600 mM NaCl) and elevated temperatures (up to 42°C). The isolates were identified by 16S rRNA sequencing and tested for their PGP traits under combined abiotic stresses. Most of the strains exhibited PGP features, such as biofilm formation, phosphate solubilization and phytohormone production. To enhance the growth of wheat plants, used as a model crop of commercial interest, three different *consortia* were designed and tested *in vitro*. The *consortium* (CONSIII), composed of *Serratia marcescens* ERA6, *Enterobacter cloacae* ERA9, and *Bacillus proteolyticus* ESOB2, provided synergistic effects that led to an enhancement in plant growth and stress resilience *in vitro*. This positive effect was confirmed in pot trials under double abiotic stress (37°C, 132 mM NaCl), where CONSIII was able to boost the root and shoot growth, increase chlorophyll and carotenoid content, and enhance antioxidant activity, mitigating reactive oxygen species accumulation. These findings underscore the potential of PGPR consortia as bioinoculants for sustainable agriculture, demonstrating their effectiveness in the simultaneous presence of salinity and heat stresses—a challenging and under-investigated environmental scenario.

Introduction

Over the last decades, the cumulative impact of human activity on the planet has led to numerous extreme environmental conditions being introduced into ecosystems and agricultural lands (Grimm et al., 2008, Lehmann et al., 2014, Sala et al., 2000, Teuling, 2018). These conditions encompass climate change-driven extreme and fluctuating weather events, such as heat waves, cold snaps, flooding, and prolonged droughts, in combination with adverse soil conditions (saline, alkaline, and/or acidic soils) (Rosenzweig et al., 2000), anthropogenic contaminants (heavy metals, microplastics, pesticides, antibiotics, and persistent organic pollutants) (Kallenborn et al., 2012), radiation (UV) (McKenzie et al., 2011), limited nutrient availability (Brouder et al., 2008), and elevated levels of airborne molecules and gases (ozone, combustion particles, CO₂) (Chakraborty et al., 2000). Plants are continuously exposed to these multi-stress factors, which adversely affect their reproduction, survival, and resistance to phytopathogens, ultimately contributing to ecosystem deterioration and reduction in crop yields (Cohen et al., 2020; Desaint et al., 2021; Hamann et al., 2021; Lee et al., 2023). Since the frequency and intensity of these abiotic stress combinations are expected to rise in the coming years (Alizadeh et al., 2020; Mazdiasni et al., 2015; Rillig et al., 2019; Zandalinas et al., 2022), there is a strong need to understand their combined effects on plants. While previous studies have traditionally focused on the impact of individual stressors, the scientific community is increasingly shifting toward studying the complexity of multiple stressors affecting plants in natural environments and on the search for eco-friendly strategies

to address the problem (Coolen et al., 2016; Defo et al., 2019). In this context, there is an increasing interest in endophytic microbial communities as microbial-based strategies for enhancing crop yield in multi-stress conditions. A specific group of microorganisms known as plant growth-promoting rhizobacteria (PGPR) have garnered attention for their beneficial effects on plant growth. PGPR positively influences plant growth and offers promising and sustainable solutions to increase plant biomass production under multi-stress environments (Lindemann et al., 2016; Umesha et al., 2018). These microorganisms directly contribute to plant growth by synthesizing phytohormones such as indole-3-acetic acid (IAA), gibberellins, and cytokinins and performing functions such as phosphate solubilization and nitrogen fixation. In addition, these beneficial bacteria release various secondary metabolites and volatile compounds that inhibit phytopathogen growth and alleviate abiotic stresses such as drought and salinity (Lugtenberg et al., 2002; Singh et al., 2022). In line with this premise, the current study aimed to isolate and characterize plant growth-promoting microorganisms associated with the sea daffodil *Pancratium maritimum* L., a perennial species from the Amaryllidaceae family that grows in the Mediterranean coast's nutrient-deficient, saline, sandy soils. This species is adapted to withstand extreme environmental stresses like high temperatures, severe sunlight, and extremely low availability of freshwater (Defo et al., 2019). The fact that plants can cope with such harsh conditions suggests that it is, at least in part, mediated through interactions with halotolerant PGPRs, making it a prime candidate for isolating stress-tolerant bacterial strains. To this aim, 15 PGPR strains were isolated from root-associated microbiome, selecting those capable of withstanding high temperatures and salinity. Their plant growth-promoting (PGP) activities were assessed, and five top-performing strains were further evaluated *in vitro* for their ability to enhance plant growth under temperature and salinity stress using wheat (*Triticum* spp.) as a model crop.

Material and methods

Sampling and Isolation of Bacteria from Pancratium maritimum roots

Given the adaptation of *Pancratium maritimum* to a particular ecosystem, the plant's roots were sampled from the nearby beach in Diamante (Cosenza, Italy). Three roots' samples were taken, collected in sterile containers and kept in sterile Phosphate buffer (1XPBS) at 4°C until processed. No specific permissions were required for sampling in that place because the plant is not endangered, according to IUCN (International Union for Conservation of Nature), and sampling was not destructive. To isolate exophytic bacteria from the root surface, 1XPBS of storage solution containing root material was used. Endophytic bacteria were isolated by washing the plant roots with ethanol 70%v/v for 5 min, followed by several washes with sterile distilled water. To check the efficiency of root disinfection, 0.1 mL of the final washing water was spread on LB agar. Surface disinfected roots (1.0 g) were then homogenized in 10 mL sterile 1XPBS. Serial dilutions (up to 10^{-6}) of the homogenate roots and the roots storage solutions were plated onto LB agar (8 g L⁻¹ NaCl, 10 g L⁻¹ tryptone, 5 g L⁻¹ yeast extract, 15 g L⁻¹ agar) supplemented with three different NaCl concentrations (50 mM, 132 mM, and 330 mM). The plates were incubated at 25°C, 37°C, and 42°C for 24–48 h. Colonies exhibiting distinct morphological characteristics were selected and streaked onto fresh LB agar to obtain pure isolates. Each bacterial

isolate was characterized by visual inspection for colony colour and morphology, such as colony shape, size, margin, and appearance. Every bacterial isolate was cultivated also on DSM agar plates (8 g L⁻¹ Nutrient Broth, 1 g L⁻¹ KCl, 1 mM MgSO₄, 1 mM Ca(NO₃)₂, 10 µM MnCl₂, 1 µM FeSO₄, Sigma–Aldrich, Germany) (Vittoria et al., 2023a) at the three temperatures to understand if the isolates were spore-forming bacteria (Nicholson et al., 1990). Glycerol stocks of the isolates were prepared and stored at -80°C.

16S rRNA Sequencing and Phylogenetic Analysis

Exponentially growing cells were used to extract chromosomal DNA using the DNeasy PowerSoil kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. 16S rRNA gene was PCR amplified by using chromosomal DNA as a template and oligonucleotides forward 8F (50-AGTTTGATCCTGGCTCAG-30 annealing at position + 8/+ 28) and reverse 1517R (50-ACGGCTACCTTGTTCGACT-30 annealing at position + 1497/+ 1517). These two oligonucleotides were designed to amplify a ~ 1500 bp DNA fragment and the reaction was carried out according to Haiyambo et al. (2015) in an Esco SwiftTM MaxPro Thermal Cycler. The 1500 bp DNA amplified fragment was purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The purified products were sequenced at the BMR Genomics sequencing facility. The quality of the sequences was analysed using SeqTrace, the minimum acceptable base call quality (Phred score) has been set to 30 and the minimum consecutive bases at 20; after this, SeqTrace was also used to auto-align and generate consensus sequences (Stucky, 2012). The trimmed and paired sequences were analysed using Basic Local Alignment Search Tool (BLAST). Phylogenetic analysis based on 16S rRNA gene sequences of bacterial isolates. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 9,418 is shown in Fig. S1 (Supplementary Materials). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1.000 replicates) are shown next to the branches. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. The rate variation among sites was modelled with a gamma distribution (shape parameter = 1,00). The analytical procedure encompassed 48 coding nucleotide sequences using 1st, 2nd, 3rd, and non-coding positions. The pairwise deletion option was applied to all ambiguous positions for each sequence pair resulting in a final data set comprising 1.600 positions. Evolutionary analyses were conducted in MEGA12 utilizing up to 7 parallel computing threads. The 16S rRNA sequence of *Aquifex aeolicus* (AJ309733.1) was used to assign an outgroup species. Evolutionary analyses were conducted in MEGA12 (Kumar et al., 2024) utilizing up to 7 parallel computing threads. All 16S rRNA sequences were deposited in the NCBI Sequence Read Archive and identified with the accession number as shown in Table S2. All the isolated and identified strains are deposited in the bacterial collection of Microalab of the Department of Biology at the University of Naples "Federico II" under the supervision of prof. Rachele Isticato; all of them are stored in cryopreserved cultures at -80°C in the presence of Glycerol 20% v/v and are publicly accessible under request to Rachele Isticato (isticato@unina.it).

Evaluation of the physiological properties

Bacterial isolates were analyzed for their swarming motility using LB plates prepared at the 0.7%w/v of agar and spot inoculating 5 μL of fresh bacterial culture. Plates were incubated overnight at the three temperatures, and the swarming motility was evaluated by measuring the radius of the grown colony. The isolates were also tested for their ability to produce biofilm using the Congo Red Agar method as reported in Lee et al. (2016). Congo Red Agar (CRA) contains: 37 g L^{-1} Brain Heart Infusion (BHI; Thermo Scientific™), 0.36 g L^{-1} sucrose, 0.008 g L^{-1} Congo red dye (Sigma–Aldrich, St. Louis, MO, USA), 18 g L^{-1} Agar; CRA was supplemented with the three concentrations of NaCl (50 mM, 132 mM and 330 mM). Bacteria were spot inoculated on CRA by adding 5 μL of fresh bacterial culture and incubated at the respective temperature. The morphology and colour of the resulting colonies were then assessed. A positive result for biofilm formation was indicated by black colonies with a dry crystalline consistency. Conversely, smooth orange to red colonies and colonies that remained pink indicated non-biofilm producers.

Phosphate Solubilization

Pikovskaya medium with some modification (Schoebitz et al., 2013) was used to observe the phosphate-solubilizing ability of the bacterial isolates by the dissolution of calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$). The ability to solubilize inorganic phosphate was tested by spotting 5 μL of exponential bacterial cultures on Pikovskaya agar complemented with three concentrations of NaCl (50 mM, 132 mM and 330 mM) for 10 days at 25°C, 37°C, 42°C. The development of a transparent halo zone around the inoculated bacterial strain confirmed phosphate solubilization activity.

Indole Acetic Acid (IAA) Production

Indole acetic acid production by bacterial isolates was determined using a modified quantification method developed by Gordon et al. (1951). To detect the IAA production, the bacteria were grown in LB broth, with and without the presence of 0.5 mg mL^{-1} tryptophan (SigmaAldrich, Germany) complemented with three concentrations of NaCl (50 mM, 132 mM and 330 mM) for 48 h at 25°C, 37°C, 42°C with shaking at 150 rpm. After the incubation, bacterial cultures were centrifuged (7000 rpm at 4°C for 10 min) then 67 μL of bacteria supernatant was transferred to a 96 multiwell and mixed with 133 μL of Salkowski reagent ($\text{H}_2\text{O}:\text{H}_2\text{SO}_4:\text{FeCl}_3$ 0.5 M in a ratio 50:30:1 respectively) and was finally incubated for 30 min at room temperature. The absorbance at 530 nm was measured using a Synergy™ HTX Multi-Mode Microplate Reader (BioTek, United States). The un-inoculated medium mixed with the Salkowski reagent was used as a negative control. The development of pink colour in the well indicated the production of IAA, and the amount of IAA produced was estimated against a standard curve prepared with different concentrations of IAA (from 500 to 3.9 $\mu\text{g } \mu\text{L}^{-1}$, eight stocks were obtained with serial

dilutions and all the concentrations were tested in triplicate) (Sigma-Aldrich, Germany) (Gordon et al., 1951, Tsavkelova et al., 2006).

Detection of ammonia

Bacteria were grown in Peptone 1%w/v broth supplemented with different NaCl concentrations (50 mM, 132 mM and 330 mM) for 72 h at 25°C, 37°C, 42°C with shaking at 150 rpm. After the incubation, bacterial cultures were centrifuged (7000 *g* at 4°C for 10 min) then 20 µL of bacteria supernatant was transferred to a 96 multiwell and mixed with 176 µL of H₂O and 4 µL of Nessler's reagent. The development of brown to yellow colour in the well indicated the production of ammonia that was evaluated by measuring the optical density at 450 nm using a Synergy™ HTX Multi-Mode Microplate Reader (BioTek, United States). The concentration of ammonia was estimated based on a standard curve of ammonium sulphate prepared with 9 concentrations ranging from 3.9 to 1000 µg L⁻¹, obtained with serial dilutions of the initial ammonium sulphate stock (Demutskaya et al., 2010).

DPPH Assay

The α,α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging method was used to evaluate the potential production of biomolecules with antioxidant activity of the isolated strains as described by (Xiang et al., 2018). Briefly, 0.2 mL of fresh bacterial culture, grown at the different conditions of salinity and temperature (50 mM, 132 mM and 330 mM, and 25°C, 37°C, 42°C) were incubated in a final volume of 1 mL of methanol containing 0.1 mM of freshly prepared DPPH (dissolved in methanol). The reaction was allowed to proceed for 30 min in the dark at room temperature. The DPPH free radical scavenging activity was then monitored by determining the absorbance at 517 nm and calculated according to the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \bullet 100$$

where Abs_{sample} is the absorbance of the reacted mixture of DPPH with the extract sample, and Abs_{control} is the absorbance of the DPPH solution (Vittoria et al., 2023b).

Screening for Hydrolytic Enzymatic Activity

The hydrolytic enzyme assays (amylase, protease and cellulase) were performed on solid media. All enzymatic activities were performed by growing the bacterial isolates in LB broth for 24 h at 37°C. After incubation 5 µL of each fresh bacterial culture were spot inoculated on the different assay plates complemented with different salt concentrations (50 mM, 132 mM and 330 mM) and incubated at the three temperatures (25°C, 37°C, 42°C). To detect the amylase activity were used Starch Agar plates as previously reported (Nimisha et al., 2019). After 72 h of incubation, the plates were flooded with Gram's iodine solution and the hydrolysis of starch was observed as a colourless zone around grown colonies. For the proteolytic activity, isolated bacteria were spot inoculated on Skimmed Milk Agar (SMA) plates.

The formation of clear halos around the colony was confirmed as proteolytic activity (Morris et al., 2012). For the detection of cellulase and xylanase activities, Xylanase Production Medium (XPM) (Meddeb-Mouelhi et al., 2014) agar plates were used with 0.5%w/v xylan from beechwood (Megazyme) and a minimal medium with 0.5%w/v carboxymethylcellulose (CMC) (Hankin et al., 1977) as a sole carbon sources, both media were complemented with the three concentrations of salt (50 mM, 132 mM, 330 mM). The plates were incubated at the three temperatures (25°C, 37°C, 42°C) for 3 days after which hydrolysis zones were visualized by flooding the plates with 0.1%w/v Congo Red aqueous solution for 30 min and then destained by washing twice with 1 M NaCl. Plates, where CMC and xylan were omitted, were used as non-substrate controls. Transparent-yellowish hydrolytic zones around the colonies were considered positive.

Dual-culture method for the evaluation of antifungal activity

The isolated strains were examined *in vitro* for antifungal activity against pathogenic fungus *Parastagonospora nodorum* (Ragucci et al., 2023) and *Pyrenophora tritici-repentis* (Carmona et al., 2006). The two fungal strains used in this study were kindly supplied by prof. Marcelo Anibal Carmona (Facultad de Agronomía, Cátedra de Fitopatología, Universidad de Buenos Aires, Buenos Aires, Argentina) (Hafez et al., 2023). Pure cultures were grown for 5 days at 25 ± 1°C on PDA (potato dextrose agar) medium consisting of 200 g L⁻¹ potato, 20 g L⁻¹ dextrose, 18 g L⁻¹ agar and deposited in the fungal culture collection of the Biology Department of the University of Naples, Federico II, Italy.

The *in vitro* antifungal bioassays were carried out based on the dual-culture method as previously described by Khamna et al. (2009) with some modifications. Fungal plugs of 5 mm diameter were placed at the centre of PDA plates and 5 µL of bacteria strains grown overnight in LB broth were placed on the opposite side of the plates at 1.5 cm away from the fungal disc. Plates containing the fungal plugs without bacterial inoculation were used as control plates. All plates were incubated at 28°C for five days. The percentage of inhibition of the fungal growth was calculated using the following formula:

$$\text{Inhibition (\%)} = \left(\frac{R_c - R_i}{R_c} \right) \bullet 100$$

where R_c is the radial growth of the test pathogen in the control plates (mm), and R_i is the radial growth of the test pathogen in the test plates (mm). The experiment was repeated three times. Bacterial strains that showed an inhibition of the growth of pathogenic fungus were observed by stereoscopic microscope with 10X magnification (Castaldi et al., 2023).

Compatibility assay

To determine strains combinations that have potential for additive and/or synergistic effects on plants, compatibility between any two isolates was determined by conducting a modified agar diffusion test. Single colonies of each strain were inoculated in 5 mL of LB broth and incubated at 37°C, 150 rpm overnight. The bacterial suspension was then quantified and diluted in soft LB agar (0.7%w/v) to a final

concentration of $\sim 10^8$ CFU mL⁻¹. Once solidified, 10 μ L of an overnight culture of the other 14 strains, grown as reported for the target strains, were spotted on the soft agar. Plates were incubated at 37°C and observed at 24 h intervals over a period of 7 days. Two microorganisms were considered compatible when the colonies overlapped; on the contrary, they were identified as incompatible when a clear zone of inhibition was observed around the colony. For each bacteria-bacteria combination, experiments were performed in three independent replicates using the same bacteria as control for compatibility.

Plant material and in vitro growth conditions

All experiments were carried out with wheat seeds (*Triticum durum* cv. creso) that were kindly provided by prof. Sheridan Lois Woo (Department of Pharmacy, University of Naples Federico II) (Silletti et al., 2021). Seeds sterilization was performed as described in Barbulova et al. (2005). Unsynchronized seedlings were discarded five days after sowing on H₂O agar Petri dishes in sterile conditions. Synchronized germinated seedlings were incubated with bacterial suspension if necessary and transferred in Petri dishes containing H₂O agar (1%w/v) supplemented with different NaCl concentrations. The plates were incubated in the dark at 25°C or 37°C with different salt concentrations for 7 days.

Effects of PGPR on growth of wheat

Bacteria were grown overnight in LB broth at 37°C at 150 rpm, the pellets were washed three times with sterile 1XPBS and then resuspended in a final volume of 10 mL of sterile 1XPBS to be quantified with a Bürker chamber (Sigma, USA; BR719505) under an optical microscope (Olympus BH-2 with 100× lens), and diluted to 10^8 cells mL⁻¹ in 30 mL of 1XPBS for seed-bioprimering. The synchronized seedlings were incubated with bacterial suspensions overnight at room temperature under continuous shaking and the transferred in Petri dishes with H₂O agar (1%w/v) supplemented with different concentrations of NaCl (0 mM or 132mM) and incubated at 25°C or 37°C, in the darkness.

Pot experiments

Seeds were sterilized as described above and inoculated with bacteria (10^8 cells mL⁻¹ in 30 mL of 1X PBS) when needed while controls were incubated in sterile 1X PBS; a schematic representation of the experiment is described in Table S6. After the incubations, ten seeds were sown in each pot at a depth of 1 cm in autoclaved soil and irrigated regularly in order to overcome the losses for evapotranspiration and to avoid water stress. Plants were cultivated in a controlled growth chamber with a light intensity of 200 μ mol m⁻²sec⁻¹ at 25°C or 37°C with a 16 h: 8 h, light: night cycle.

Evaluation of biochemical parameters in plants

Total chlorophyll and carotenoid content in leaves

Five leaves per treatment were collected to determine total chlorophyll ($a + b$) and total carotenoid ($x + c$) concentrations following the procedure reported by Petrillo et al. (2022). Fresh samples (10 mg) were powdered in liquid nitrogen and treated with ice-cold 100% acetone and centrifuged (Labofuge GL, Heraeus Sepatech, Hanau, Germany) at 5000 rpm for 5 min. The absorbance of supernatants was read using a spectrophotometer (Cary 100 UV-VIS, Agilent Technologies, Santa Clara, CA, USA) at 470, 645, and 662 nm. The pigment concentration was determined through the Lichtenthaler equations and expressed in mg g^{-1} of fresh weight (mg g^{-1} FW) (Lichtenthaler, 1987).

Antioxidant activity in leaves and roots

The antioxidant activity was determined in leaves and roots on six replicates per treatment performing the DPPH (α, α -diphenyl- β -picrylhydrazyl) free radical scavenging activity assay, according to Castaldi et al. (2024). Briefly, fresh samples (50 mg) were powdered in liquid nitrogen and extracted in methanol overnight. Then, the extracts were centrifuged at 14.000 rpm for 15 min at 4°C, mixed with a 6×10^{-5} M DPPH methanolic solution and incubated at 37°C for 20 min. The absorbance was measured at 515 nm with a spectrophotometer (BioTek Synergy HTX Multimode Reader, Agilent Technologies, Palo Alto, CA, US) and converted in percentage of DPPH radical inhibition through the formula:

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \bullet 100$$

where A_{control} is blank absorbance on the DPPH methanolic solution and A_{sample} is the sample absorbance.

Statistical analysis

All statistical analyses were performed using GraphPad Prism (GraphPad Prism 8.0.1 Software, San Diego, CA), and the data were expressed as the mean $\pm$ SEM. As indicated in Figure legends, differences among groups were compared with One-way ANOVA test by Tukey's test ($\alpha = 0.05$) or with unpaired t-test (two-tailed $\alpha = 0.05$). Differences were considered statistically significant at $p < 0.05$.

Results

Root samples of *Pancratium maritimum* were collected to isolate bacteria capable of withstanding multiple stress conditions. Exophytic bacteria were isolated by washing the roots in 1XPBS, while endophytic bacteria were obtained following surface sterilization and subsequent root grinding. The isolated bacteria were subjected to a gradual enrichment process involving incubation under increasing temperatures and salinity levels. Specifically, the temperatures tested included 25°C as a control and 37°C and 42°C, which represent extreme summer temperatures commonly observed in the Mediterranean region (Tejedor et al., 2024). Salinity concentrations tested were 50, 132, 330 and 600 mM of NaCl, corresponding to low-salinity conditions, typical slightly saline environments, moderately saline environments, and high salinity environments, respectively (van Zelm et al., 2020). Through this

approach, 12 endophytic (ERA strains) and three exophytic (ESO strains) isolates were selected based on their unique cultural characteristics, including colony size, shape, elevation, surface texture, consistency, pigmentation, and growth response to varying salt concentrations and temperatures (Table S1). As shown in Table S1, 10 of the 15 strains of bacteria were tolerant to up to 600 mM NaCl, while 9 strains grew at temperatures of up to 42°C. Notably, strains ERA3, ESOB2, ERAS1, ERAS3, and ERAS4 were identified as spore-forming bacteria. Amplification and sequencing of the 16S rRNA gene were performed to achieve taxonomic identification of all isolated bacteria. BLAST analysis of the sequences against the NCBI database (Table S2) found strains belonging to three genera: *Bacillus*, *Pseudomonas*, and *Enterobacter* (Table S2). Analysis of the phylogenetic tree, constructed using the neighbor-joining (NJ) algorithm, also clustered the strains into three, representing *Bacillus*, *Pseudomonas*, and *Enterobacter*, as shown in Fig.S1.

Screening for hydrolytic enzymes activity

PGPR possess the ability to enhance plants' rhizosphere environment by modulating soil enzyme activity and improving its fertility. The enzymatic activities of PGPR in the rhizosphere thus represent a valuable indicator for assessing soil stress levels (del Carmen Rivera-Cruz et al., 2008). Several studies have shown that many rhizobacteria can synthesize extracellular hydrolytic enzymes that are involved in breaking down complex macromolecules, leading to higher nutrient availability for plants (Pang et al., 2004, Shaikh et al., 2014). However, despite some progress in understanding the effects of soil salinity on enzyme activity (Pan et al., 2020), few studies have focused on the enzymatic responses of PGPR under combined temperature and salinity stress conditions (Pal et al., 2006, Ren et al., 2021). Figure 1 shows the positive results obtained from five bacterial strains (*Bacillus proteolyticus* ESOB2, *Bacillus stercoris* ERAS3, *Bacillus cabrialesii* ERAS1 *Bacillus paramycoides* ERA3, and *Serratia marcescens* ERA6) out of the fifteen tested, on three representative enzymatic activities (protease, amylase, and cellulase) associated with PGPR. As illustrated in Fig. 1a, *B. cabrialesii* ERAS1 and *B. paramycoides* ERA3 exhibited the strongest amylase activity in all tested multi-stress conditions, underscoring their robust enzymatic potential. Meanwhile, *B. proteolyticus* ESOB2 and *B. stercoris* ERAS3 showed moderate to high activity levels. Cellulase activity, assessed through the degradation of Carboxymethyl cellulose (CMC), revealed similarly promising trends. As shown in Fig. 1b, *B. proteolyticus* ESOB2, *B. stercoris* ERAS3, and *B. paramycoides* ERA3 demonstrated medium-to-high cellulase activity under all conditions. Notably, *B. cabrialesii* ERAS1 stood out as the top-performing strain, exhibiting the highest cellulase activity under the combined temperature and salinity stress scenarios. Finally, the protease activity analysis, reported in Fig. 1c, revealed distinct trends. *S. marcescens* ERA6 and *B. paramycoides* ERA3 exhibited strong protease activity at 25°C in the presence of 50 mM NaCl, although their activity declined under more extreme temperature and salinity conditions. Remarkably, *B. cabrialesii* ERAS1 was the only strain capable of maintaining a moderate activity level even under the harshest tested conditions (42°C).

Antagonistic activity of isolates against fungal plant pathogen

Hydrolytic enzymes not only contribute to nutrient acquisition but also hold potential antifungal properties by targeting the cell walls of phytopathogenic fungi. Given the promising hydrolytic activities of the strains *B. albus* ESOB2, *B. stercoris* ERAS3, *B. cabrialesii* ERAS1, *S. marcescens* ERA6 and *B. paramycooides* ERA3 (Fig. 1), their biocontrol activity was also evaluated. A dual culture assay was performed to evaluate their effectiveness against two wheat-pathogenic fungi, *Parastagonospora nodorum* (Carmona et al., 2006) and *Pyrenophora tritici-repentis* (Khamna et al., 2009) (Fig. 2). Both the phytopathogenic fungi utilized in our experiment infect wheat, the model plant used in this study, severely impairing photosynthesis and inducing necrosis in tissues, decreasing plant growth and yield. Furthermore, the high evolutionary potential of both fungi has allowed them to develop genetic resistance to environmental stresses and commonly used pesticides, making them significant threats in wheat cultivation worldwide (Downie et al., 2021, Guo et al., 2020).

The dual-culture assays revealed variability in the inhibitory effects of the selected strains against the fungal pathogens, with some exhibiting strong antimicrobial activity while others showing limited or no effect. As illustrated in Fig. 2, strains *B. proteolyticus* ESOB2 and *B. stercoris* ERAS3 demonstrated slight inhibition against *P. nodorum* and showed more pronounced activity against *P. tritici-repentis*. In contrast, *B. paramycooides* ERA3 and *S. marcescens* ERA6 exhibited minimal activity against both phytopathogens. Interestingly, *B. cabrialesii* ERAS1, which showed the strongest hydrolytic activities under multi-stress conditions (Fig. 1), also exhibited notable antifungal activity, as evidenced by the inhibition zones observed between the bacterium and the fungal colonies (Fig. 2). To further determine the inhibitory action of *B. cabrialesii* ERAS1, a microscopic analysis of the co-cultured plates was performed to assess the morphology of the fungal hyphae (Fig. 3).

The analysis revealed a visible impact of the bacteria on the morphological characteristics the hyphae. The panels in Fig. 3b and 3d (*P. tritici-repentis* and *P. nodorum* co-cultured with *B. cabrialesii* ERAS1, respectively), show that the bacteria caused dramatic alterations in comparison to the control (Fig. 3a and 3c, *P. tritici-repentis* and *P. nodorum* without *B. cabrialesii* ERAS1, respectively). The hyphae of the treated sample exhibited a less uniform structure, with the presence of obvious swellings in the cells that point toward autophagosome formation, as reported by Li et al. (2014).

In vitro characterization of potential PGPR

The 15 bacterial isolates were then assessed for their plant growth-promoting traits under the selected multi-stress conditions, including their abilities for swarming motility, biofilm production, phosphate solubilization, and polymer hydrolysis as previously described by Vasseur-Coronado et al. (2021). Among these, only the strains *E. cloacae* ESOA and *B. albus* ESOB2 demonstrated swarming motility, suggesting their potential for root colonization (Table S1). Seven isolates (*E. hormaechei* ERA1, *B. paramycooides* ERA3, *K. cowanii* ERA4, *S. marcescens* ERA6, *E. cloacae* ERA9, *E. cloacae* ESOA, *E. cloacae* ESOB1) were capable of biofilm production, a key feature for adhesion to the root surface. Phosphate solubilization, an important trait for improving nutrient availability (Table S3) was observed in five isolates, with four strains (*E. hormaechei* ERA1, *E. cloacae* ERA9, *E. cloacae* ESOA, and *B. proteolyticus* ESOB2) maintaining

activity even under high temperature and salinity conditions. Eight bacterial strains produce Indole-3-Acetic Acid (IAA), but only *E. cloacae* ESOA, *B. stercoris* ERAS3, and *E. hormaechei* ERA1 remained efficient under stress, with IAA production ranging from $1.05 \mu\text{g mL}^{-1}$ to $218.42 \mu\text{g mL}^{-1}$ (e.g. *B. stercoris* ERAS3 under 50 mM NaCl and 37°C). Furthermore, nitrogen fixation and subsequent ammonia reduction were observed in five strains (*R. aquimaris* ERAS4, *B. stercoris* ERAS3, *B. cabrialesii* ERAS1, *S. marcescens* ERA6, and *B. paramycoides* ERA3) as shown in Fig. 4.

The characterization identified five top-performing strains based on their combined PGP traits:

- ERA1: biofilm formation, phosphate solubilization, and IAA synthesis
- ERA6: biofilm formation, ammonia production, and protease secretion
- ERA9: biofilm formation, phosphate solubilization, and antioxidant activity
- ESOA: swarming, ammonia production, and IAA synthesis
- ESOB2: swarming, ammonia production, and antioxidant activity

No single strain displayed all tested PGP activities under increasing stress, prompting the formulation of bacterial *consortia* to combine complementary functionalities. Three *consortia* were formulated:

- CONSI: ERA1 + ESOA + ESOB2
- CONSII: ERA1 + ESOA + ERA6
- CONSIII: ERA6 + ERA9 + ESOB2

Compatibility tests confirmed that the following consortia could grow together without mutual inhibition.

Effect of salt-tolerant plant growth-promoting bacteria on wheat growth under salt and temperature stress

In vitro experiments

The five selected bacterial strains and the three consortia were tested on wheat (*Triticum durum* cv. creso), a staple crop sensitive to environmental stresses, to assess their ability to promote plant growth under physiological (25°C without salt) and stress conditions (37°C with 132 mM NaCl) (Canton, 2021, Upadhyay et al., 2012). Wheat seeds inoculated with bacterial strains (10^8 cells mL^{-1}) were grown on H₂O-agar plates, and growth was evaluated after seven days by measuring the combined root and shoot lengths (Fig. 5).

Under physiological conditions, all bacterial strains enhanced wheat growth compared to the control, with *E. cloacae* ERA9 showing the most pronounced effect in plant height (Fig. 5a). Under stress, seed germination was completely inhibited without bacterial inoculation (Fig. 5b). However, the inoculation of bacterial strains restored the growth of wheat plants, with *E. cloacae* ERA9 once again standing out for producing the most beneficial effect on the plant. Interestingly, it primarily enhances root growth under physiological conditions while it significantly boosts shoot growth under multi-stress conditions (Table

S4). Consortia testing revealed that CONSIII (composed of *S. marcescens* ERA6, *E. cloacae* ERA9, and *B. proteolyticus* ESOB2) significantly improved wheat growth in both physiological and stress conditions (Fig. 5c,d, Table S5). CONSIII increased plant length by 60.3% under physiological conditions and 81.0% under stress. This synergistic effect likely stems from the complementary PGP traits of the constituent strains, including phosphate solubilization, ammonia production, and antioxidant activity. In contrast, CONSI and CONSII showed limited or moderate growth promotion and were less effective than CONSIII, for this reason only CONSIII was used in subsequent experiments.

Pot experiments

Pot trials further validated the *in vitro* findings under more realistic conditions. Wheat plants were grown in pots under both physiological conditions and double abiotic stress conditions (37°C with 132 mM NaCl). Growth and some biochemical markers were measured after 21 days from germination, and the results are reported in Fig. 6 and Fig. 7, respectively.

Wheat plants treated with CONSIII exhibited significant growth improvement under both non-stress and stress conditions, particularly in plant height. As shown in Fig. 6a, wheat plants treated with CONSIII at 25°C exhibited an 82% increase in height under normal conditions and a 95% increase under salt stress. Similarly, at 37°C (Fig. 7d), CONSIII enhanced plant height by 49% in the absence of salt stress and by 122% under osmotic stress, increasing from 8.48 cm in the control to 18.86 cm. Even if no statistically significant differences were observed in terms of germination rate and plant fresh weight (Fig. 6b,e,c,f), a positive trend was observed, confirming the increase in plant length treated with CONSIII, and reinforcing the synergistic effect of the three PGPR strains in promoting wheat growth and improving stress resilience in a more natural setting. Biochemical analyses indicate that temperature influences chlorophyll and carotenoid production, as shown in Fig. 7a.

As shown in Fig. 7a, carotenoids and chlorophyll levels increase in all the conditions tested compared to the control, especially in the physiological conditions (25°C without salt stress). Conversely, in Fig. 7b, the antioxidant activity of roots and leaves is strongly affected by temperature and salt concentration, while there are no significant differences at 25°C among the samples. These findings lead to further interpretation of how bacterial inoculation modulates plant physiological and biochemical responses under stress, as discussed below.

Discussion

This work highlights the potential of halotolerant PGPR strains isolated from *Pancratium maritimum* roots to improve wheat growth under combined stress, high temperature and salinity. Through selective enrichment under double stress, 15 bacterial strains have been isolated and identified, with the majority of them belonging to the genera of *Bacillus*, *Pseudomonas*, and *Enterobacter*, commonly associated with stress-resilient PGPR in saline and arid soils (Ruppel et al., 2013; Ngumbi and Kloepper, 2016). Among the isolated strains, *Bacillus cabrialesii* ERAS1 and *Enterobacter cloacae* ERA9 emerged for their ability to produce hydrolytic enzymes and key PGP characteristics such as IAA release, ammonia production,

phosphate solubilization and biofilm formation, especially in multi-stress environments. The production of hydrolytic enzymes not only contributes to nutrient acquisition but also holds potential antifungal properties by targeting the cell walls of phytopathogenic fungi. Given the promising hydrolytic activities of the strains *B. albus* ESOB2, *B. stercoris* ERAS3, *B. cabrialesii* ERAS1, *S. marcescens* ERA6 and *B. paramycoides* ERA3, their biocontrol activity was also evaluated through dual culture assay against two major wheat pathogens, *P. nodorum* and *P. tritici-repentis*. In particular, the strain *Bacillus cabrialesii* ERAS1 exhibited a strong antifungal activity against both wheat pathogens, and microscopic analyses further revealed morphological alterations in fungal hyphae, suggesting a disruption of cellular integrity and possible autophagy induction (Li et al., 2014; Dutta et al., 2020). This result suggests a synergistic antifungal activity given by the enzymatic degradation of fungal cell wall and the production of diffusible antifungal compounds, as previously reported by Pal et al. (2006) and Ren et al. (2021).

After the characterization of the strains, three different consortia were assembled based on complementary PGP traits of the single bacteria. Trials with plants, both *in vitro* and *in pot*, confirmed that the strongest synergistic effect of the strains was obtained with CONSIII, composed of *S. marcescens* ERA6, *E. cloacae* ERA9, and *B. proteolyticus* ESOB2, highlighting the value of combining functionally complementary strains to enhance plant resilience in multi-stress environments. In particular, CONSIII was able to promote the germination of the wheat seeds and the growth of the seedlings *in vitro* under stress conditions, achieving an 81% increase in plant height compared to the control under salt and temperature stress. The results from the pot experiments further validated these findings, with CONSIII-treated plants showing a 122% increase in plant height under stress conditions.

Although the pot test did not reveal statistically significant differences between the groups in terms of germination rate and plant fresh weight, a positive trend was observed in the groups treated with the beneficial bacteria, confirming that the inoculant with CONSIII tends to improve the plant fitness in every condition tested.

The beneficial effects of CONSIII were also extended to biochemical markers such as pigments concentration and antioxidant capacity. Interestingly, the CONSIII demonstrates a more effective response than CTRL in inducing a significant increase in carotenoids at 37°C, suggesting that the presence of bacteria reduces plant stress, thereby supporting light harvesting, photosynthesis, and plant growth even under unfavourable temperature conditions. The enhanced antioxidant activity at higher temperatures, mainly in roots, could be due to the observed increase of carotenoids, which are known to play an active role in scavenging processes related to oxidative stress and reactive oxygen species (ROS) removal (Zhang et al., 2012). Conversely, in roots, the absence of pigments may contribute to explaining the different response of antioxidant activity compared to leaves. Indeed, roots being more exposed to salt stress in the cultivation mean, likely need to recruit higher antioxidant defences (Bandoğlu et al., 2004). These findings reinforce the idea that carefully selected microbial consortia can be a sustainable strategy to enhance crop productivity and stress tolerance in the context of global climate change.

Conclusions

The cumulative impact of human life on our planet over the past few decades has led to the emergence of many extreme environmental conditions affecting ecosystems and agricultural land. Global warming, climate change and pollution expose plants to unique combinations of multiple abiotic and biotic stresses simultaneously. This study highlights the potential of PGPR isolated from *P. maritimum* in promoting the growth of plants and their resilience under abiotic stress conditions, like salinity and high temperature. Using an enrichment approach, a core group of halotolerant and thermotolerant PGPR capable of maintaining multiple PGP functions under abiotic stress conditions was isolated. Five top-performing strains were combined in three consortia and tested for their ability to promote wheat growth under physiological and double abiotic stress conditions. This study clearly demonstrates the importance of PGPR in mitigating the harmful consequences of abiotic stress on plants. The results observed with CONSIII-treated plants suggest that bacterial inoculation can sustain plant growth and photosynthesis under adverse conditions, counteracting the accumulation of ROS and supporting overall plant health. These benefits are probably due to complementary PGP traits of CONSIII, lying in the fact that phosphate solubilization, ammonia production, and antioxidant activity act together to promote plant growth and stress tolerance. In conclusion, CONSIII represents a promising microbial *consortium* for developing sustainable agricultural solutions to improve crop productivity under challenging environmental conditions.

Declarations

Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Author Contribution

Ivana Staiano and Stefany Castaldi: Investigations; formal analysis; methodology; visualization; writing—original draft; writing—review, data curation and editing. Ermenelgilda Vitale: methodology and formal analysis. Carmen Arena: Data curation. Rachele Isticato: Conceptualization; formal analysis; methodology; project administration; supervision; validation; visualization; writing—original draft; writing—review and editing.

Data Availability

Sequence data that support the findings of this study have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive. The accession numbers of 16S RNA sequences are provided in the supplementary information files (Table S2).

References

1. Alizadeh MR, Adamowski J, Nikoo MR, AghaKouchak A, Dennison P, Sadegh M (2020) A century of observations reveals increasing likelihood of continental-scale compound dry-hot extremes. *Sci Adv* 6:eaaz4571. <https://doi.org/10.1126/sciadv.aaz4571>
2. Ashraf M, Harris PJC (2013) Photosynthesis under stressful environments: An overview. *Photosynthetica* 51:163–190. <https://doi.org/10.1007/s11099-013-0021-6>
3. Bandoğlu E, Eyidoğan F, Yücel M, Öktem A, H (2004) Antioxidant responses of shoots and roots of lentil to NaCl-salinity stress. *Plant Growth Regul* 42:69–77. <https://doi.org/10.1023/B:GROW.0000014891.35427.7b>
4. Barbulova A, D'Apuzzo E, Rogato A, Chiurazzi M (2005) Improved procedures for *in vitro* regeneration and for phenotypic analysis in the model legume *Lotus japonicus*. *Funct Plant Biol* 32:529–536. <https://doi.org/10.1071/FP05015>
5. Brouder SM, Volenec JJ (2008) Impact of climate change on crop nutrient and water use efficiencies. *Physiol Plant* 133:705–724. <https://doi.org/https://doi.org/10.1111/j.1399-3054.2008.01136.x>
6. Canton H (2021) Food and Agriculture Organization of the United Nations — FAO, twenty-thi. ed. Routledge
7. Carmona MA, Ferrazini M, Barreto DE (2006) Tan spot of wheat caused by *Drechslera tritici-repentis*: Detection, transmission, and control in wheat seed. *Cereal Res Commun* 34:1043–1049. <https://doi.org/10.1556/CRC.34.2006.2-3.236>
8. Castaldi S, Lorenz C, Vitale E, Santorufo L, Isticato R, Arena C (2024) Potentialities of Technosol-isolated PGPB *consortium* in promoting plant growth in lettuce seedlings. *Plant Soil*. <https://doi.org/10.1007/s11104-024-06746-z>

9. Castaldi S, Zorrilla JG, Petrillo C, Russo MT, Ambrosino P, Masi M, Cimmino A, Isticato R (2023) *Alternaria alternata* Isolated from Infected Pears (*Pyrus communis*) in Italy Produces Non-Host Toxins and Hydrolytic Enzymes as Infection Mechanisms and Exhibits Competitive Exclusion against *Botrytis cinerea* in Co-Infected Host Fruits. J Fungi. <https://doi.org/10.3390/jof9030326>
10. Chakraborty S, Tiedemann AV, Teng PS (2000) Climate change: potential impact on plant diseases. Environ Pollut 108:317–326. [https://doi.org/https://doi.org/10.1016/S0269-7491\(99\)00210-9](https://doi.org/10.1016/S0269-7491(99)00210-9)
11. Cohen SP, Leach JE (2020) High temperature-induced plant disease susceptibility: more than the sum of its parts. Curr Opin Plant Biol 56:235–241
12. Coolen S, Proietti S, Hickman R, Davila Olivas NH, Huang P, Van Verk MC, Van Pelt JA, Wittenberg AHJ, De Vos M, Prins M (2016) Transcriptome dynamics of *Arabidopsis* during sequential biotic and abiotic stresses. Plant J 86:249–267
13. Defo MA, Gendron AD, Head J, Pilote M, Turcotte P, Marcogliese DJ, Houde M (2019) Cumulative effects of cadmium and natural stressors (temperature and parasite infection) on molecular and biochemical responses of juvenile rainbow trout. Aquat Toxicol 217:105347
14. del Rivera-Cruz C, Narcía M, Ballona AT, Kohler GC, Caravaca J, Roldan F, A (2008) Poultry manure and banana waste are effective biofertilizer carriers for promoting plant growth and soil sustainability in banana crops. Soil Biol Biochem 40:3092–3095
15. Demutskaya L, Kalinichenko I (2010) Photometric determination of ammonium nitrogen with the nessler reagent in drinking water after its chlorination. J Water Chem Technol 32:90–94. <https://doi.org/10.3103/S1063455X10020049>
16. Desaint H, Aoun N, Deslandes L, Vailleau F, Roux F, Berthomé R (2021) Fight hard or die trying: when plants face pathogens under heat stress. New Phytol 229:712–734
17. Downie RC, Lin M, Corsi B, Ficke A, Lillemo M, Oliver RP, Phan HTT, Tan K-C, Cockram J (2021) *Septoria Nodorum* Blotch of Wheat: Disease Management and Resistance Breeding in the Face of Shifting Disease Dynamics and a Changing Environment. Phytopathology 111:906–920. <https://doi.org/10.1094/PHYTO-07-20-0280-RVW>
18. Gordon SA, Weber RP (1951) Colorimetric estimation of indoleacetic acid. Plant Physiol 26:192
19. Grimm NB, Foster D, Groffman P, Grove JM, Hopkinson CS, Nadelhoffer KJ, Pataki DE, Peters DPC (2008) The changing landscape: ecosystem responses to urbanization and pollution across climatic and societal gradients. Front Ecol Environ 6:264–272
20. Guo J, Shi G, Kalil A, Friskop A, Elias E, Xu SS, Faris JD, Liu Z (2020) *Pyrenophora tritici-repentis* Race 4 Isolates Cause Disease on Tetraploid Wheat. Phytopathology® 110:1781–1790. <https://doi.org/10.1094/PHYTO-05-20-0179-R>
21. Hafez M, Gourlie R, McDonald M, Telfer M, Carmona MA, Sautua FJ, Moffat CS, Moolhuijzen PM, See PT, Aboukhaddour R (2023) Evolution of the ToxB Gene in *Pyrenophora tritici-repentis* and Related Species. Mol. Plant-Microbe Interact. 37, 327–337. <https://doi.org/10.1094/MPMI-08-23-0114-FI>
22. Haiyambo DH, Chimwamurombe PM, Reinhold-Hurek B (2015) Isolation and Screening of Rhizosphere Bacteria from Grasses in East Kavango Region of Namibia for Plant Growth Promoting

- Characteristics. *Curr Microbiol* 71:566–571. <https://doi.org/10.1007/s00284-015-0886-7>
23. Hamann E, Blevins C, Franks SJ, Jameel MI, Anderson JT (2021) Climate change alters plant–herbivore interactions. *New Phytol* 229:1894–1910
 24. Hankin L, Anagnostakis SL (1977) Solid Media Containing Carboxymethylcellulose to Detect Cx Cellulase Activity of Micro-organisms. *Microbiology* 98:109–115.
<https://doi.org/https://doi.org/10.1099/00221287-98-1-109>
 25. Kallenborn R, Halsall C, Dellong M, Carlsson P (2012) The influence of climate change on the global distribution and fate processes of anthropogenic persistent organic pollutants. *J Environ Monit* 14:2854–2869. <https://doi.org/10.1039/C2EM30519D>
 26. Khamna S, Yokota A, Lumyong S (2009) Actinomycetes isolated from medicinal plant rhizosphere soils: diversity and screening of antifungal compounds, indole-3-acetic acid and siderophore production. *World J Microbiol Biotechnol* 25:649–655. <https://doi.org/10.1007/s11274-008-9933-x>
 27. Kumar S, Stecher G, Suleski M, Sanderford M, Sharma S, Tamura K (2024) *Mol Biol Evol* 41:msae263. <https://doi.org/10.1093/molbev/msae263>. MEGA12: Molecular Evolutionary Genetic Analysis Version 12 for Adaptive and Green Computing
 28. Lee H, Calvin K, Dasgupta D, Krinner G, Mukherji A, Thorne P, Trisos C, Romero J, Aldunce P, Barret K (2023) 2023 IPCC: Climate Change 2023: Synthesis Report, Summary for Policymakers. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, Lee H, Romero J (eds.)]. IPC
 29. Lee J-S, Bae Y-M, Han A, Lee S-Y (2016) Development of Congo red broth method for the detection of biofilm-forming or slime-producing *Staphylococcus sp.* *LWT* 73:707–714.
<https://doi.org/https://doi.org/10.1016/j.lwt.2016.03.023>
 30. Lehmann J, Rillig M (2014) Distinguishing variability from uncertainty. *Nat Clim Chang* 4:153
 31. Li H, Zhang S, Lu J, Liu L, Uluko H, Pang X, Sun Y, Xue H, Zhao L, Kong F, Lv J (2014) Antifungal activities and effect of *Lactobacillus casei* AST18 on the mycelia morphology and ultrastructure of *Penicillium chrysogenum*. *Food Control* 43:57–64. <https://doi.org/10.1016/j.foodcont.2014.02.045>
 32. Lichtenthaler H (1987) [34] Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes, in: *Plant Cell Membranes*. Academic Press, pp. 350–382.
[https://doi.org/https://doi.org/10.1016/0076-6879\(87\)48036-1](https://doi.org/https://doi.org/10.1016/0076-6879(87)48036-1)
 33. Lindemann SR, Bernstein HC, Song H-S, Fredrickson JK, Fields MW, Shou W, Johnson DR, Beliaev AS (2016) Engineering microbial *consortia* for controllable outputs. *ISME J* 10:2077–2084.
<https://doi.org/10.1038/ismej.2016.26>
 34. Lugtenberg BJJ, Chin-A-Woeng TFC, Bloemberg GV (2002) Microbe–plant interactions: principles and mechanisms. *Antonie Van Leeuwenhoek* 81:373–383.
<https://doi.org/10.1023/A:1020596903142>
 35. Mazdiasni O, AghaKouchak A (2015) Substantial increase in concurrent droughts and heatwaves in the United States. *Proc. Natl. Acad. Sci.* 112, 11484–11489

36. McKenzie RL, Aucamp PJ, Bais AF, Björn LO, Ilyas M, Madronich S (2011) Ozone depletion and climate change: impacts on UV radiation. *Photochem Photobiol Sci* 10:182–198.
<https://doi.org/10.1039/C0PP90034F>
37. Meddeb-Mouelhi F, Moisan JK, Beauregard M (2014) A comparison of plate assay methods for detecting extracellular cellulase and xylanase activity. *Enzyme Microb Technol* 66:16–19.
<https://doi.org/https://doi.org/10.1016/j.enzymictec.2014.07.004>
38. Morris LS, Evans J, Marchesi JR (2012) A robust plate assay for detection of extracellular microbial protease activity in metagenomic screens and pure cultures. *J Microbiol Methods* 91:144–146.
<https://doi.org/https://doi.org/10.1016/j.mimet.2012.08.006>
39. Nicholson WL, Setlow P (1990) Sporulation, germination and outgrowth, in: Wiley, Chichester (Eds.), *Molecular Biological Methods for Bacillus*. pp. 391–450
40. Nimisha P, Moksha S, Gangawane AK (2019) Amylase Activity of Starch Degrading Bacteria Isolated from Soil. *Int J Curr Microbiol Appl Sci* 8:659–671. <https://doi.org/10.20546/ijcmas.2019.804.071>
41. Pal KK, Scholar V, Gardener BM (2006) Biological Control of Plant Pathogens 1–25.
<https://doi.org/10.1094/PHI-A-2006-1117-02.Biological>
42. Pan J, Huang C, Peng F, Zhang W, Luo J, Ma S, Xue X (2020) Effect of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting bacteria (PGPR) inoculations on *Elaeagnus angustifolia* L. in saline soil. *Appl Sci* 10:945
43. Pang Z, Otaka K, Suzuki Y (2004) Purification and characterization of an endo-1, 3- β -glucanase from *Arthrobacter* sp. *J Biol Macromol* 4:57–66
44. Petrillo C, Vitale E, Ambrosino P, Arena C, Istatico R (2022) Plant Growth-Promoting Bacterial *Consortia* as a Strategy to Alleviate Drought Stress in *Spinacia oleracea*. *Microorganisms* 10.
<https://doi.org/10.3390/microorganisms10091798>
45. Ragucci S, Castaldi S, Landi N, Istatico R, Di Maro A (2023) Antifungal Activity of Ageritin, a Ribotoxin-like Protein from *Cyclocybe aegerita* Edible Mushroom, against Phytopathogenic Fungi.
<https://doi.org/10.3390/toxins15090578>. *Toxins* (Basel)
46. Ren H, Lv C, Fernández-García V, Huang B, Yao J, Ding W (2021) Biochar and PGPR amendments influence soil enzyme activities and nutrient concentrations in a eucalyptus seedling plantation. *Biomass Convers Biorefinery* 11:1865–1874
47. Rillig MC, Ryo M, Lehmann A, Aguilar-Trigueros CA, Buchert S, Wulf A, Iwasaki A, Roy J, Yang G (2019) The role of multiple global change factors in driving soil functions and microbial biodiversity. *Science* 366:886–890 (80-.)
48. Rosenzweig C, Hillel D (2000) Soils and global climate change: challenges and opportunities. *Soil Sci* 165. <https://doi.org/10.1097/00010694-200001000-00007>
49. Sala OE, Stuart Chapin F, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A (2000) Global biodiversity scenarios for the year 2100. 287:1770–1774 *Science* (80-.)

50. Schoebitz M, Ceballos C, Ciamp L (2013) Effect of immobilized phosphate solubilizing bacteria on wheat growth and phosphate uptake. J soil Sci plant Nutr
51. Shaikh SS, Sayyed RZ (2014) Role of plant growth-promoting rhizobacteria and their formulation in biocontrol of plant diseases. Plant Microbes Symbiosis: Applied Facets. Springer, pp 337–351
52. Silletti S, Di Stasio E, Van Oosten MJ, Ventorino V, Pepe O, Napolitano M, Marra R, Woo SL, Cirillo V, Maggio A (2021) Biostimulant Activity of *Azotobacter chroococcum* and *Trichoderma harzianum* in Durum Wheat under Water and Nitrogen Deficiency. Agronomy. <https://doi.org/10.3390/agronomy11020380>
53. Singh R, Pandey KD, Singh M, Singh SK, Hashem A, Al-Arjani A-BF, Abd_Allah EF, Singh PK, Kumar A (2022) Isolation and Characterization of Endophytes Bacterial Strains of *Momordica charantia* L. and Their Possible Approach in Stress Management. <https://doi.org/10.3390/microorganisms10020290>. Microorganisms
54. Stucky BJ (2012) SeqTrace: a graphical tool for rapidly processing DNA sequencing chromatograms. J Biomol Tech 23:90–93. <https://doi.org/10.7171/jbt.12-2303-004>
55. Tejedor E, Benito G, Serrano-Notivoli R, González-Rouco F, Esper J, Büntgen U (2024) Recent heatwaves as a prelude to climate extremes in the western Mediterranean region. npj Clim Atmos Sci 7:218. <https://doi.org/10.1038/s41612-024-00771-6>
56. Teuling AJ (2018) A hot future for European droughts. Nat Clim Chang 8:364–365
57. Tsavkelova EA, Klimova SI, Cherdyntseva TA, Netrusov AI (2006) Microbial producers of plant growth stimulators and their practical use: A review. Prikl Biokhim Mikrobiol 42:133–143
58. Umesha S, Singh K, Singh PP (2018) R., Chapter 6 - Microbial Biotechnology and Sustainable Agriculture, in: Singh, R.L., Mondal, S.B.T.-B. for S.A. (Eds.),. Woodhead Publishing, pp. 185–205. <https://doi.org/https://doi.org/10.1016/B978-0-12-812160-3.00006-4>
59. Upadhyay SK, Singh JS, Saxena AK, Singh DP (2012) Impact of PGPR inoculation on growth and antioxidant status of wheat under saline conditions. Plant Biol 14:605–611. <https://doi.org/https://doi.org/10.1111/j.1438-8677.2011.00533.x>
60. van Zelm E, Zhang Y, Testerink C (2020) Salt Tolerance Mechanisms of Plants. Annu Rev Plant Biol 71:403–433. <https://doi.org/https://doi.org/10.1146/annurev-arplant-050718-100005>
61. Vasseur-Coronado M, du Boulois HD, Pertot I, Puopolo G (2021) Selection of plant growth promoting rhizobacteria sharing suitable features to be commercially developed as biostimulant products. Microbiol Res 245:126672. <https://doi.org/https://doi.org/10.1016/j.micres.2020.126672>
62. Vittoria M, Saggese A, Di Gregorio Barletta G, Castaldi S, Isticato R, Baccigalupi L, Ricca E (2023a) Sporulation efficiency and spore quality in a human intestinal isolate of *Bacillus cereus*. Res Microbiol 174:104030. <https://doi.org/https://doi.org/10.1016/j.resmic.2023.104030>
63. Vittoria M, Saggese A, Isticato R, Baccigalupi L, Ricca E (2023b) Probiotics as an Alternative to Antibiotics: Genomic and Physiological Characterization of Aerobic Spore Formers from the Human Intestine. <https://doi.org/10.3390/microorganisms11081978>. Microorganisms 11

64. Xiang H, Sun-waterhouse D, Cui C, Wang W, Dong K (2018) Modification of soy protein isolate by glutaminase for nanocomplexation with curcumin. *Food Chem* 268:504–512.
<https://doi.org/https://doi.org/10.1016/j.foodchem.2018.06.059>
65. Zandalinas SI, Balfagón D, Gómez-Cadenas A, Mittler R (2022) Plant responses to climate change: metabolic changes under combined abiotic stresses. *J Exp Bot* 73:3339–3354
66. Zhang Q, Wang L, Kong F, Deng Y, Li B, Meng Q (2012) Constitutive accumulation of zeaxanthin in tomato alleviates salt stress-induced photoinhibition and photooxidation. *Physiol Plant* 146:363–373

Figures

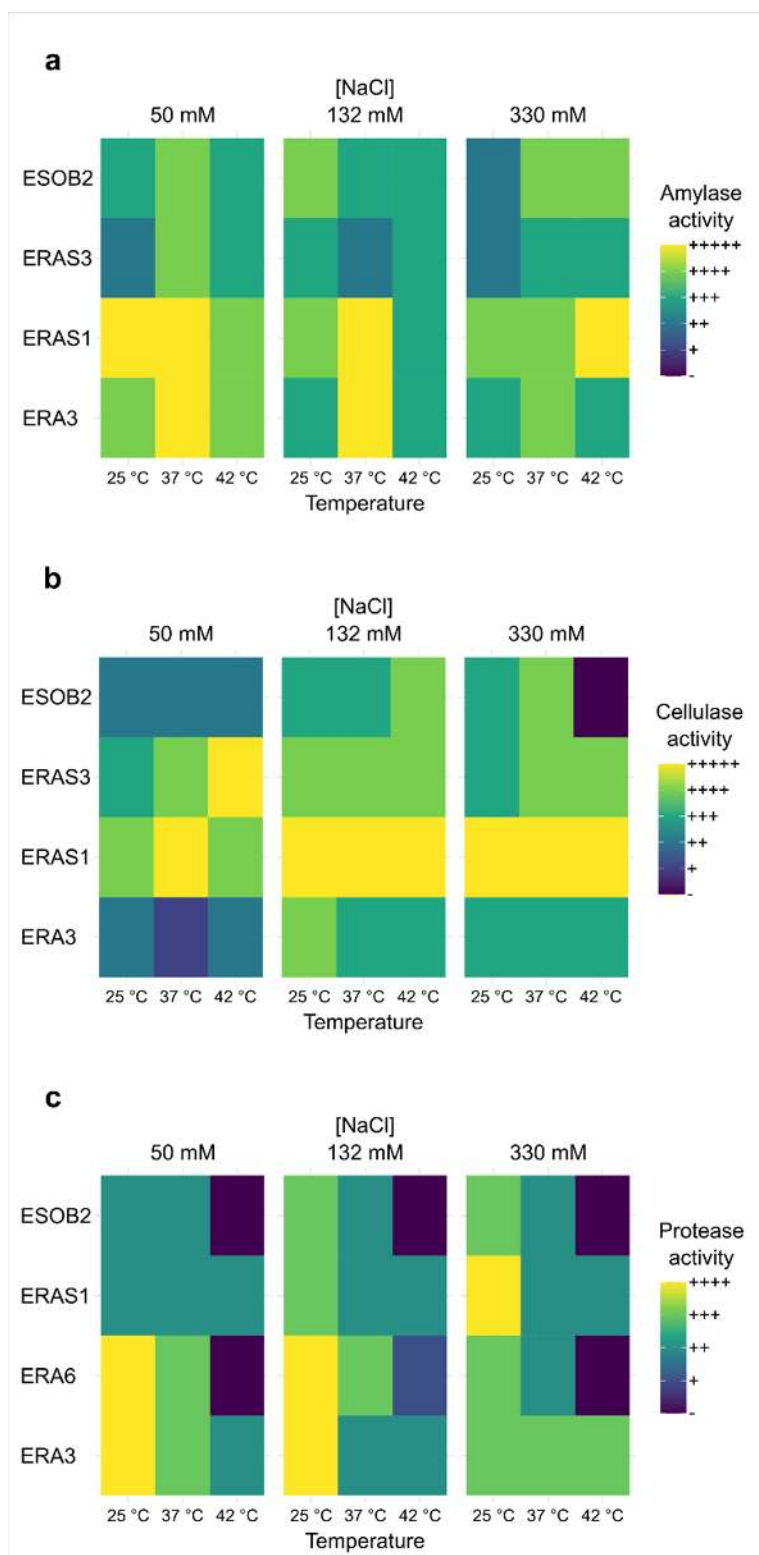


Figure 1

Higher hydrolytic activity observed among 15 isolated strains (only the 4 most efficient strains are reported) under temperature and salt stress. Results of amylase production (a), cellulase production (b) and protease production (c) are reported as means of at least three replicates ($p < 0.05$) and are expressed as no activity (-), hydrolysis zone diameter (hzd) < 5 mm (+), hzd $\geq$ 5 mm (++), hzd $\geq$ 10 mm (+++), hzd $\geq$ 15 mm (++++), hzd $\geq$ 20 mm (+++++)

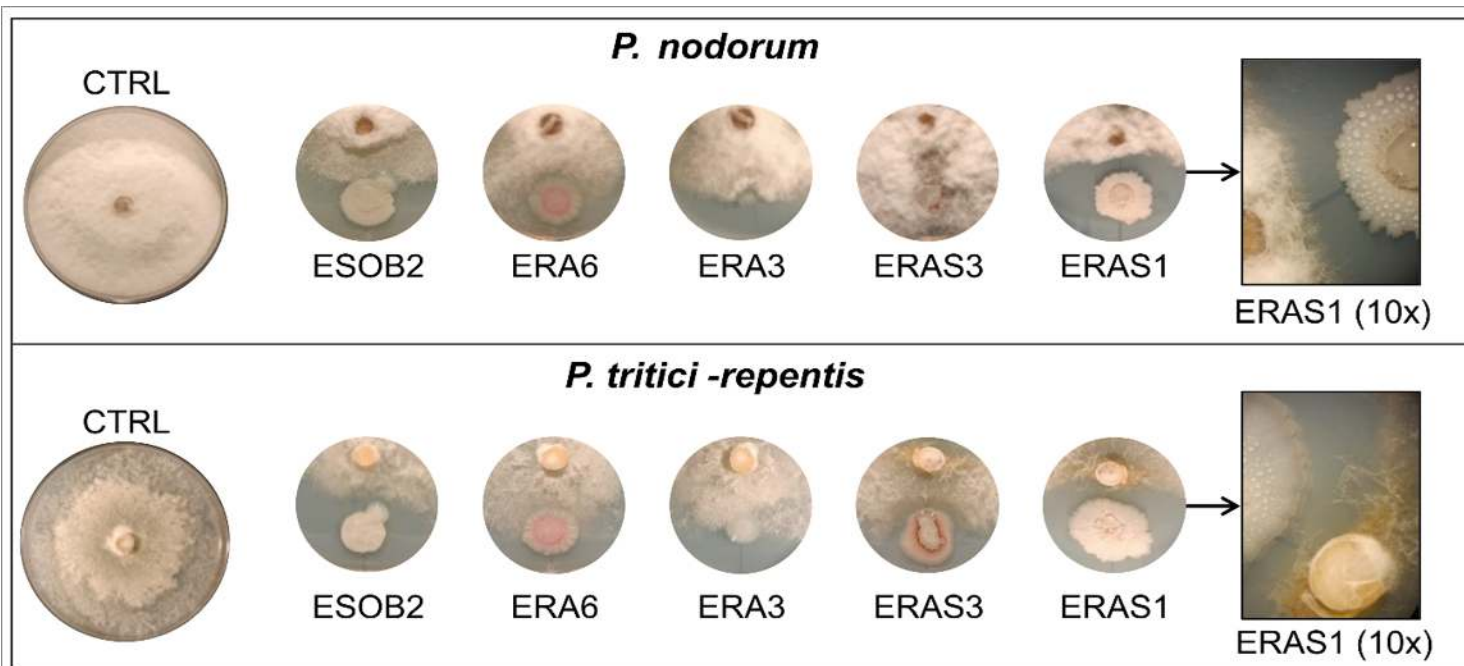


Figure 2

Antagonism assays on PDA medium. Photographs of dual-culture assay for in vitro inhibition of mycelial growth of *P. nodorum* and *P. tritici-repentis* by selected bacterial strains. Rectangles on the right represent the interaction zone of strain ERAS1 and the two fungi acquired with a stereoscopic microscope (10× magnification). All experiments were performed in triplicate with three independent trials

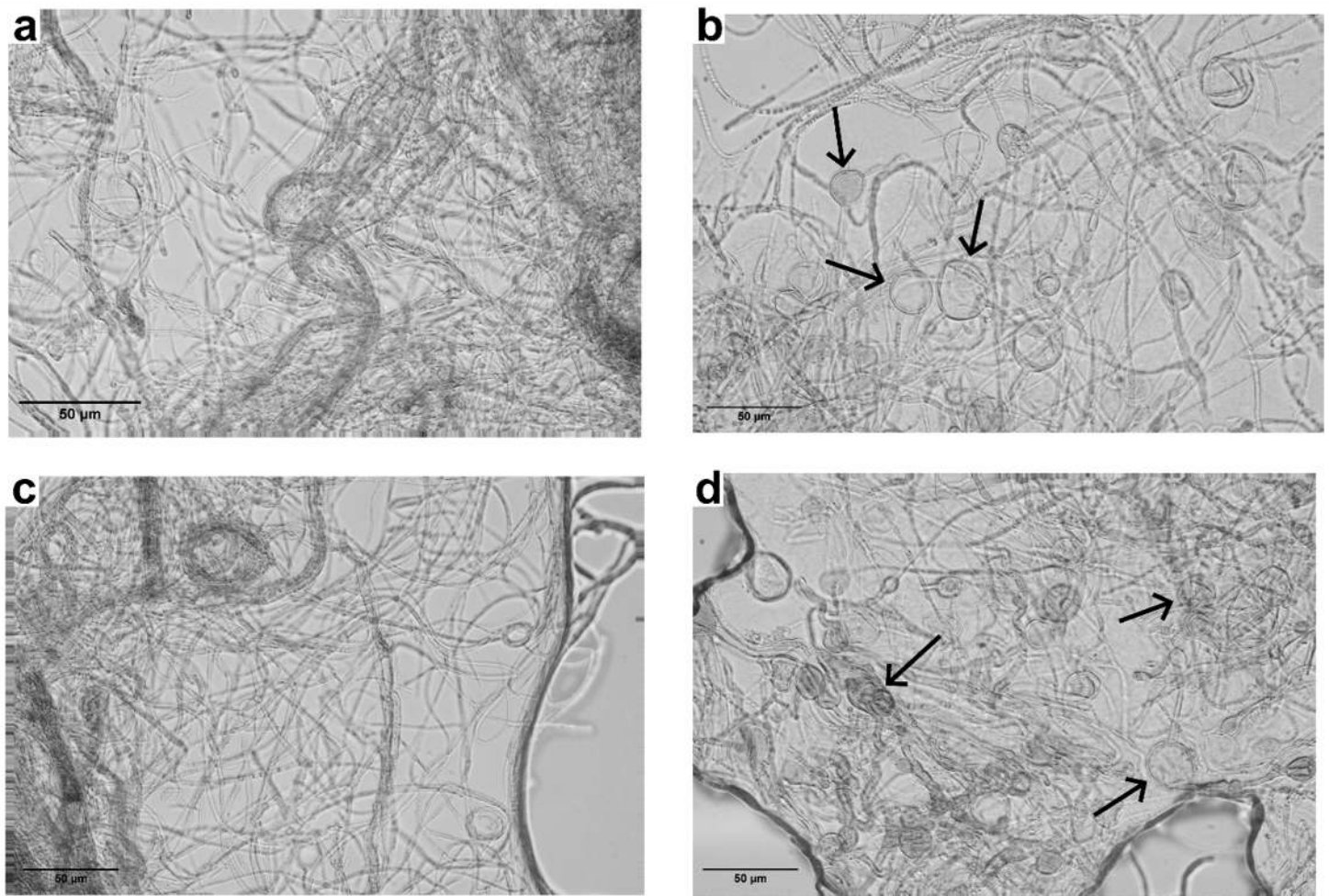


Figure 3

Observation of the effect of *B. cabrialesii* ERAS1 on the hyphae of *P. tritici-repentis* (a): control, fungus grown without bacteria; (b): hyphae infected with the bacteria. Effect on *P. nodorum*; (c): control, fungus grown without bacteria; (d): hyphae infected with the bacteria. All the pictures are obtained with a phase contrast light microscope with 40X magnification. Arrows indicate the potential autophagosomes formed in the hyphae grown in presence of the strain ERAS1

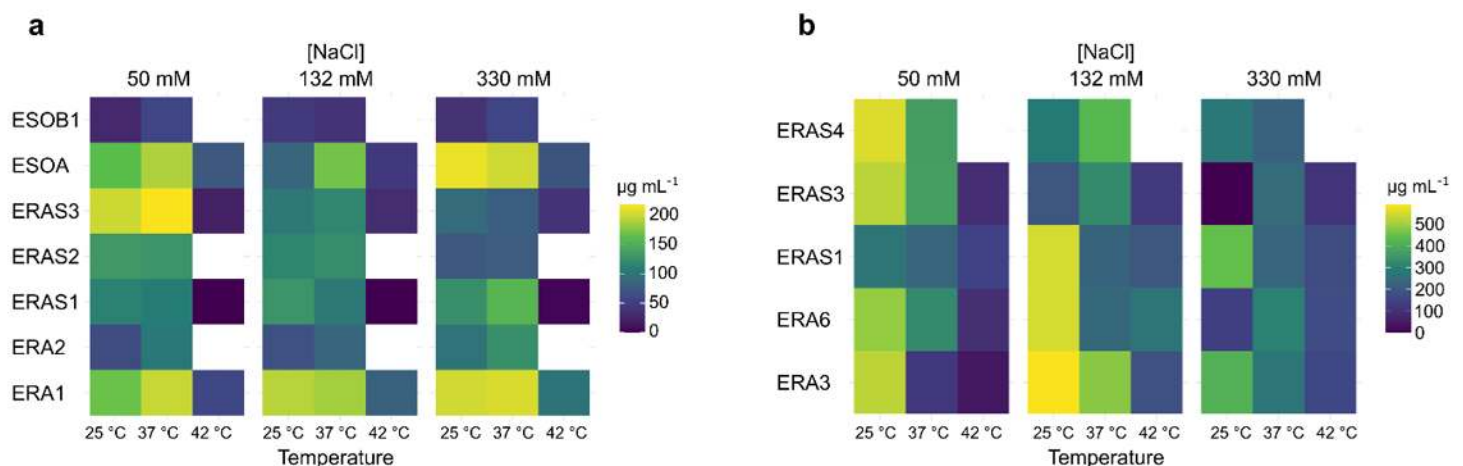


Figure 4

PGP traits evaluation under temperature and salt stress of top-performing strains. Results of indole-3-acetic acid (IAA) production (a) and ammonia production (b) are reported as means of at least three replicates ($p < 0.05$). The presented data of IAA production are obtained by growing the bacteria in presence of tryptophan (0.5 mg mL^{-1}) as reported above. Blank spaces in the Figure represent the strains that were not able to grow in the defined media at 42°C .

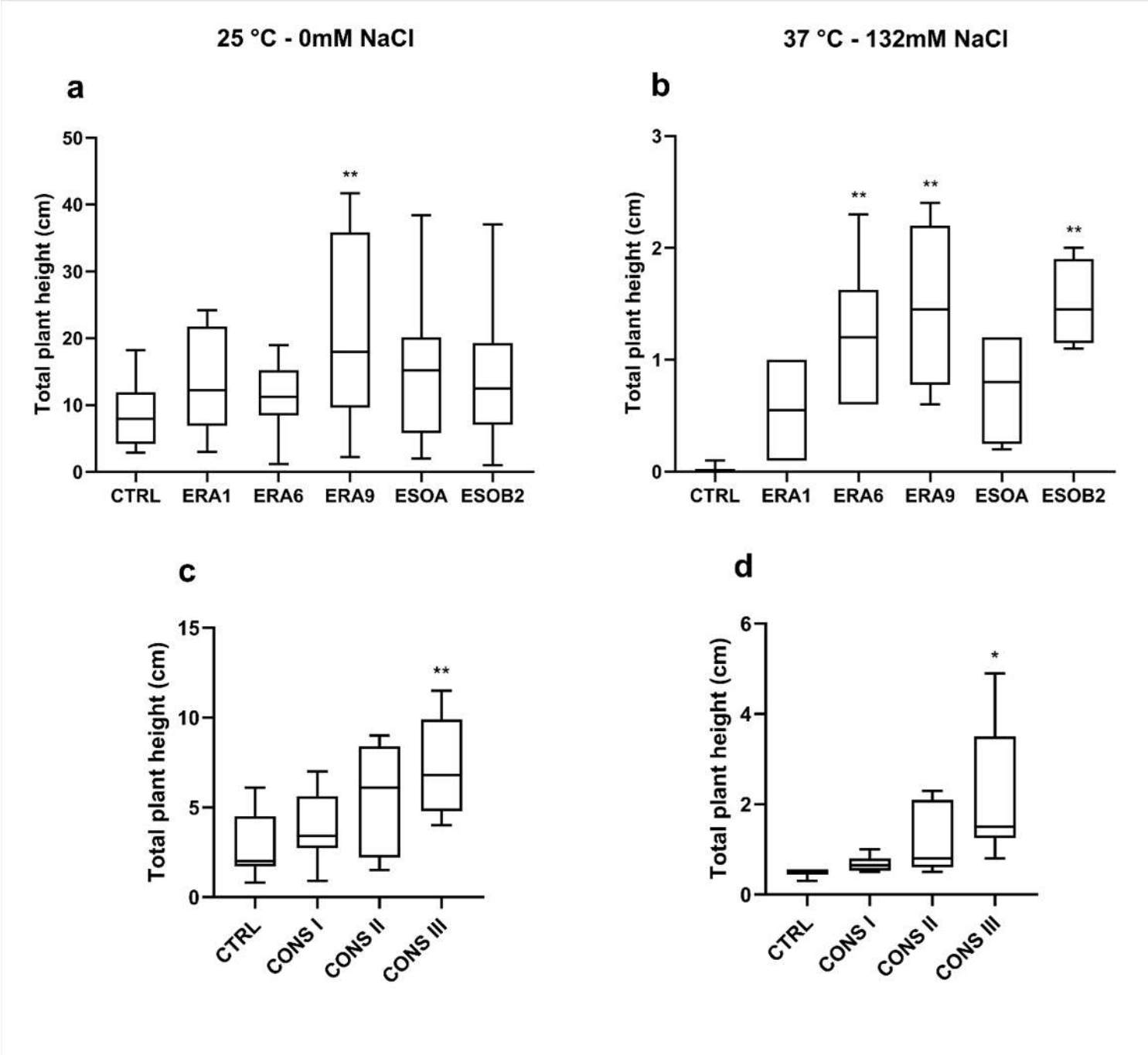


Figure 5

In vitro germination of wheat seedlings in presence of PGPR. Wheat seedlings were incubated overnight with each strain or consortium and deposited on water-agar plates at 25°C without salt (a,c) and at 37°C with 132 mM NaCl (b,d).

°C with 132 mM of NaCl (b,d). The total plant length is represented as the sum of shoot and root length. Results are reported as mean ($n = 24$) $\pm$ S.E. of three independent experiments. One-way ANOVA test was performed to compare the groups of data, and p-values are shown in the figure. Asterisks indicate significant differences with the control (CTRL) according to Tukey's test, *: $p < 0.05$; **: $p < 0.005$

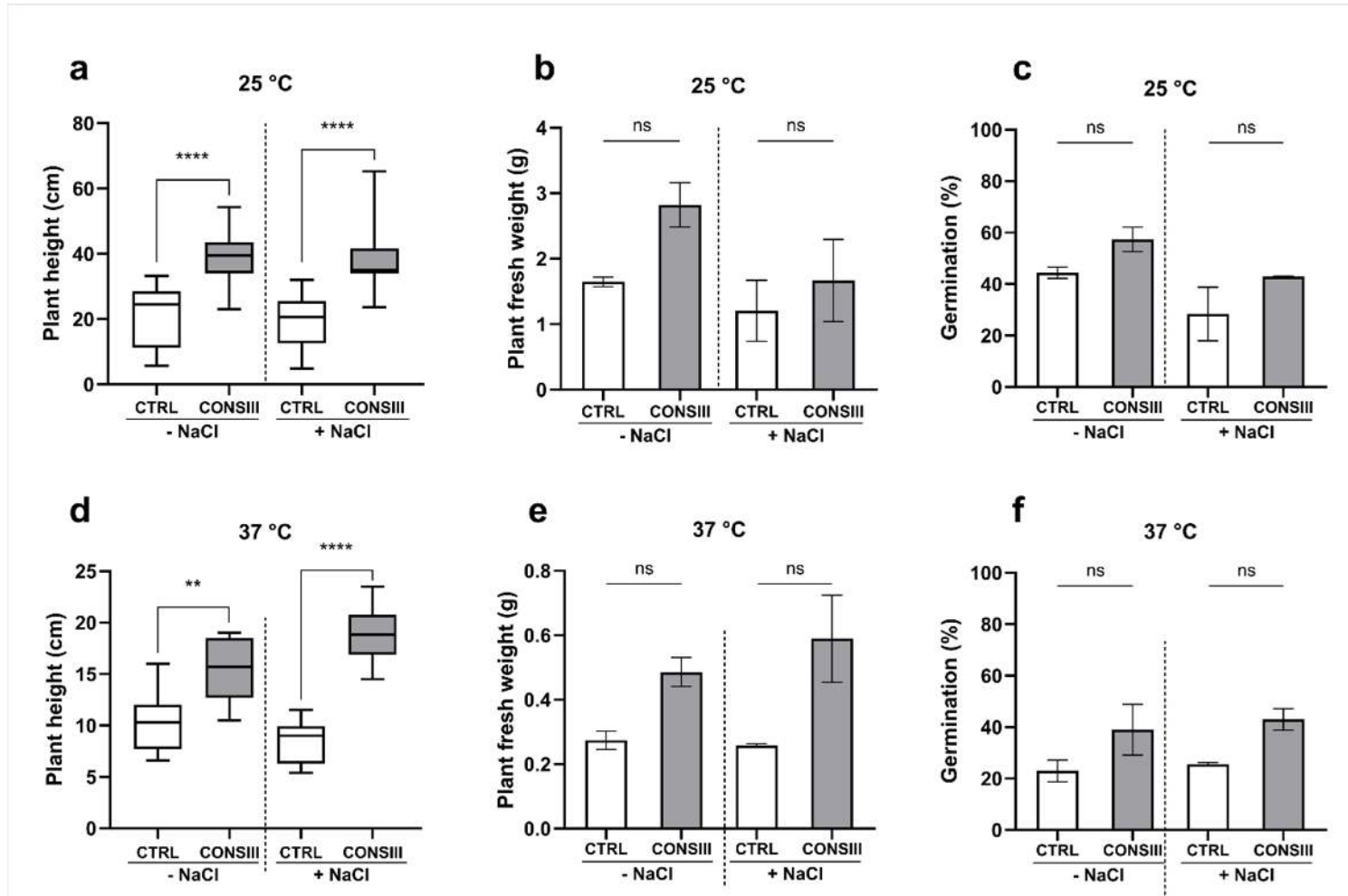


Figure 6

Effect of CONSIII on morphological characteristics of wheat plants under different salt concentrations and temperatures (a,b,c: 25 °C; d,e,f: 37 °C). Wheat seeds were incubated overnight with CONSIII and sown in sterile soil-filled pots, then incubated at the respective temperatures with a 16 h: 8 h, light: night cycle. The total plant length (B and E) is represented as the sum of shoot and root length. Results are reported as mean ($n = 28$) $\pm$ S.E. of two independent experiments. Statistical analysis was performed using unpaired t-test, comparing each treatment with CONSIII with the respective control without bacteria (CTRL). Statistical significance is reported with asterisks on the graphs, ns: not significant; **: $p < 0.01$; ****: $p < 0.0001$

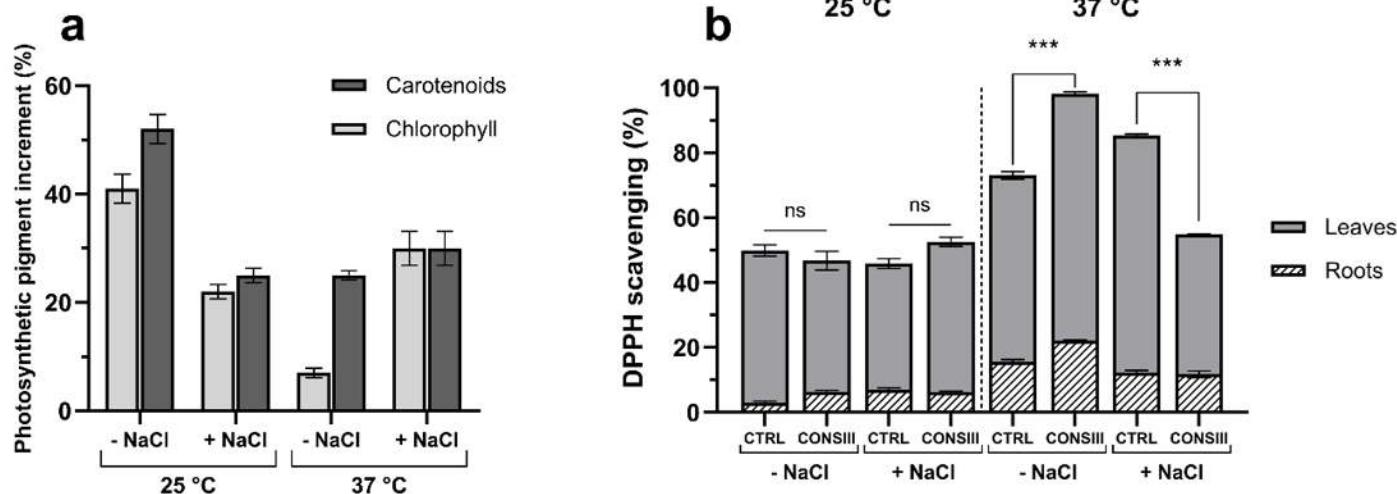


Figure 7

Effect of CONSIII on biochemical markers of wheat plants under different salt concentrations and temperatures. Chlorophylls and carotenoids (a) contents are reported as mg of pigment per gram of fresh weight (FW). The antioxidant activity is reported as a percentage of 2,2- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity observed in leaves and roots (b). Results are reported as mean $\pm$ S.E. (n = 5). Statistical analysis was performed using unpaired t-test, comparing each treatment with CONSIII with the respective control without bacteria (CTRL). Statistical significance is reported with asterisks on the graphs, ns: not significant; **: $p < 0.01$; ****: $p < 0.0001$

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterials.docx](#)