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Soil Bacteria from the Namib Desert: Insights into Plant Growth Promotion and Osmotolerance in a Hyper-Arid Environment

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Abstract: The Namib Desert is characterized by a number of abiotic stresses, including high temperature, high salinity, osmotic pressure, alkaline pH, and limited water availability. In such environments, dry soils typically exhibit a low water potential, scarce nutrients, and high concentrations of dissolved ions, collectively creating a challenging habitat for microbial life. In this study, 89 bacterial isolates belonging to 20 genera were identified. Bacteria demonstrated significant osmotolerance, with some strains thriving at polyethylene glycol (PEG) concentrations exceeding 20%. Furthermore, these bacteria demonstrated halotolerance, high pH tolerance, and capacity to produce plant growth-promoting (PGP) traits under conditions of osmotic stress. Osmotolerant bacteria exhibited higher proficiency in siderophore production, potassium solubilization, and phosphorus solubilization, all of which are critical for supporting plant growth in nutrient-scarce and stressful environments, such as deserts. However, alginate production was higher in isolates that were less osmotolerant, indicating the potential for a compensatory mechanism in strains that were more sensitive. These findings highlight the complex strategies employed by desert bacteria to survive and support host plants in extreme environments. The present study not only enhances our understanding of microbial adaptations in arid ecosystems, but also provides important information for the development of potential applications for these bacteria in the reclamation of arid land and agricultural practices aimed at improving crop resilience to abiotic stress.

Keywords: drought; desertification; plant growth promoting rhizobacteria; plant growth promotion traits; abiotic stress tolerance

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1. Introduction

Arid and semi-arid regions, which make up approximately one-third of the Earth's land surface, are distinguished by extreme environmental conditions that present considerable challenges to both plant and microbial life [1,2]. Among these harsh environments, deserts represent some of the most extreme ecosystems, with factors such as limited water availability, high temperatures, and nutrient scarcity creating a unique and challenging habitat [1,3]. The Namib Desert, situated along the southwest zone of Africa, represents one such extreme environment that is distinguished by its hyper-arid conditions, sporadic rainfall, and unique soil composition, which collectively makes it an intriguing subject for studying soil microbiota, with potential for different applications [3–5].

The soil microbiome plays a pivotal role in ecosystem functioning, contributing to nutrient cycling, soil structure, and plant health [6,7]. In arid environments, the capacity of microbial communities to adapt to osmotic stress, nutrient deficiency, and other extreme conditions is essential for plant survival and growth [8,9]. Among these microorganisms, bacteria have received considerable attention owing to their versatility and range of functions, including the promotion of plant growth and tolerance to different constraints [10–12].

Plant growth-promoting rhizobacteria (PGPR) have been extensively studied in various environments because of their ability to enhance plant growth [1]. These beneficial bacteria possess the ability to promote plant growth through a variety of mechanisms such as the production of phytohormones, facilitation of nutrient acquisition, and inhibition of plant pathogens [13–16]. One of the key mechanisms by which PGPR enhance plant growth is the production of indole-3-acetic acid, a crucial phytohormone that regulates various aspects of plant development, including root growth, shoot elongation, and nutrient uptake [17]. Additionally, PGPR can produce siderophores, which aid in the solubilization and acquisition of iron, an essential micronutrient for plants [18]. In desert soils, where nutrient availability is often limited, PGPR can solubilize and mobilize essential macronutrients such as phosphate and potassium, making them more available to plants [19–21].

One of the principal adaptive strategies utilized by these bacteria is osmotolerance, which refers to the capacity to survive and sustain cellular function under conditions of low osmotic potential, a phenomenon frequently associated with drought and salinity [5,22]. Osmotolerance in bacteria can be conferred through various mechanisms, including the synthesis of osmoprotectants such as proline and alginate, and the regulation of cell processes and structures [23,24].

An understanding of the diversity, functionality, and potential applications of soil bacteria from the Namib Desert is not only a valuable contribution to the existing body of knowledge, but also of significant practical importance. As global climate change continues to exacerbate water scarcity and expand desertification, the development of sustainable agricultural practices that can thrive under these conditions is becoming increasingly urgent [25]. By studying microbial communities in one of the Earth's most extreme environments, insights can be gained into the mechanisms of osmotolerance and plant growth promotion and tolerance under extremely challenging conditions [1,26]. This may result in the creation of strategies that can enhance crop resilience in arid and semiarid regions globally.

The aim of this study was to examine the soil culturable bacterial communities of the Namib Desert, with particular emphasis on their abiotic tolerance and plant growth-promoting attributes. To achieve this objective, bacteria were isolated from the roots of plants from the Namib Desert and identified. Subsequently, the isolates were characterized based on their tolerance to different abiotic stresses (osmotic, salt, acidity, and alkalinity) and their PGP capabilities were evaluated (IAA synthesis, alginate production, siderophore production, phosphate, and potassium solubilization). Using this approach, we intend to contribute to a more comprehensive understanding of the ecological roles of desert soil rhizobacteria and their potential applications in sustainable agriculture, particularly in areas threatened by aridity.

2. Material and Methods

2.1. Bacteria Isolation

Bacteria were isolated from the roots of *Tetraena simplex*, *Tetraena stapffii,* and *Stipagrostis* sp. harvested in the Namib Desert (15°08′06.2″ S–12°12′51.7″ E). Plants were collected in February 2022 from a site where the soil is characterized by its aridity and undergrowth (see Supplementary Figure S1). A spade was used to collect the hole system from a depth of 10–20 cm. Soil was carefully removed from the root material to avoid dislodging secondary roots, and the collected plant material was transported to the laboratory in an icebox. In the laboratory, samples were used immediately or stored in a refrigerator at 4 °C for further bacterial isolation according to the procedures described by Somasegaran and Hoben [27].

Roots were laid on the surface of yeast mannitol agar (YMA) plates [27] and allowed to grow at 26 °C. Colony growth was monitored twice per day, and the isolated colonies were transferred to YMA plates to obtain a pure culture. A total of 150 isolates were obtained and stored for further identification and plant growth promotion (PGP) traits assessment.

2.2. BOX-PCR, 16S rRNA Gene Amplification and Phylogenetic Analysis

To identify unique genetic profiles, isolates underwent BOX-PCR typing prior to 16S rRNA gene amplification. The 150 isolates were cultured on YMA plates and a loopful of an individual colony was used to create a bacterial suspension in 100 µL of sterilized deionized water. The PCR reaction mixture consisted of 1 µL bacterial suspension, 1 µL BOXA1R primer (5′-CTACGG CAAGGCGACGCTGAC-3′; [28]) diluted to 10 µmol/µL in sterile Milli-Q water, 6.25 µL NZYTech 2× Taq Green Master Mix (NZYTech, Lisbon, Portugal), and sterile Milli-Q water to reach a total volume of 25 µL. The amplification protocol consisted of an initial cycle at 95 °C (7 min), followed by 30 cycles of 94 °C (1 min), 53 °C (1 min), and 65 °C (8 min), concluding with a final cycle at 65 °C (16 min) using a Bio-Rad iCycler Thermal Cycler (Hercules, CA, USA). Cluster analysis was conducted using GelCompar II (Applied Maths, Sint-Martens-Latem, Belgium) to calculate the Pearson correlation coefficient and apply the unweighted pair group method with arithmetic mean (UPGMA). The generated dendrograms were examined to identify clusters of isolates sharing at least 85% similarity. This threshold was established to ensure that patterns known to be identical (molecular weight markers) were classified within the same group. A representative isolate was randomly chosen from each fingerprint group and 89 strains were obtained.

The 16S rRNA gene from representative isolates was amplified using primers 27f (5′-AGAGTTTGATCCTGGCTCAG-3′; [29]) and 1492r (5′-GGTTACCTTGTTACGACTT-3′; [29]), together with NZYTaq 2× Green Master Mix (NZYTech, Lisbon, Portugal). The master mix contained dNTPs, NZYTaq II DNA polymerase (0.2 U/µL), MgCl2 (2.5 mM), and two dyes (blue and yellow) that allowed monitoring the progress of the electrophoresis. Amplification was performed in 25 µL tubes with an initial cycle of 94 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1.5 min, and a final extension at 72 °C for 10 min. The resulting PCR products were sent to GATC Biotech (Constance, Germany) for sequencing, with each reaction tube containing 5 µL PCR product, 2.5 µL 27f primer, and 2.5 µL autoclaved Milli-Q water. Sequences were identified using software FinchTV V1.4.0 (Geospiza, Inc., Seatle, WA, USA). A BLAST search against Genbank (core_nt, August 2024; E-value threshold $< 1 \times 10^5$) and Ezbiocloud [30] databases were performed to identify bacteria at the genus level. Partial 16S rRNA gene sequences from the representative isolates were submitted to GenBank (PQ201021:PQ201075) (Supplementary Table S1).

2.3. Bacteria Tolerance to Abiotic Stresses

2.3.1. Osmotolerance

The osmotolerance of the isolated bacteria was assessed using the methodology described by Sá et al. [23]. Bacteria were grown in yeast mannitol broth (YMB) supplemented with different polyethylene glycol 6000 (PEG) concentrations (0, 5, 10, 15, 20%, and 25%), with five replicates for each strain and PEG concentration. Bacteria were grown for 48 h at 26 °C in an orbital shaker (180 rpm) and the optical density (OD) was recorded at 600 nm for bacterial growth. The results were used to calculate IC50, which corresponds to the PEG concentration that inhibits 50% bacterial growth. For the IC50 calculation, Excel software (V. 16.89.1) was used to calculate the PEG concentration that inhibits 50% the bacterial growth. The exponential curve was the one that best fitted the data (curve fitting was always > 90%), and the equation was $y = a \times e^{bx}$, in which y is the bacterial OD at 600 nm, a is the y-intercept, b is the base, and x is the PEG %. Bacterial osmotolerance was classified according to the IC50: sensitive (S)—IC50 < 10% PEG; moderately tolerant (MT)—10% PEG $\leq$ IC50 < 15% PEG; tolerant (T)—15% PEG $\leq$ IC50 < 20% PEG; and highly tolerant (HT)—IC50 $\geq$ 20% PEG.

2.3.2. Halotolerance

Salt tolerance of the isolated bacteria was screened in YMB supplemented with different NaCl concentrations (0, 2, 4, 6, 8, 10, and 12%). Three replicates were performed for

each isolate and for each salt concentration. The inoculated tubes were incubated at 26 °C on an orbital shaker (180 rpm) for 48 h [31]. For the growth measurements, the optical density (600 nm) was determined [32]. The growth variation (GV), following the formula $GV = \frac{(x-control)}{control} \times 100$ at 6% relative to the control (0% NaCl) was chosen to represent the salinity tolerance of the isolates, since it was the NaCl concentration at which the average growth inhibition of all isolates was 50%. Five levels of tolerance were considered: very sensitive, GV $\leq -75\%$; sensitive, -75% < GV $\leq -50\%$; moderately tolerant, -50% < GV $\leq -25\%$; tolerant, -25% < GV $\leq 25\%$; and highly tolerant, >25%.

2.3.3. Tolerance to pH

Tolerance to acidic and alkaline conditions was screened in YMB at pH 4.0 and 10.0, respectively, and compared to growth in YMB (pH 7.0). The pH was adjusted using 1 M HCl or 1 M KOH to lower or increase the pH of the medium. For each isolate and pH three replicates were performed. The inoculated tubes were incubated at 26 °C on an orbital shaker (180 rpm) for 48 h. For the growth measurements, the optical density (600 nm) was determined. Growth variation at pH 4 and 10 relative to the control (pH 7) was used to determine the tolerance of the isolates to acidic and alkaline conditions. Five levels of tolerance were considered: very sensitive, GV $\leq -75\%$; sensitive, 75% < GV $\leq -50\%$; moderately tolerant, 50% < GV $\leq -25\%$; tolerant, -25% < GV $\leq 25\%$, and highly tolerant, > 25%.

2.4. Plant Growth Promoting (PGP) Traits

The ability of the bacteria for plant growth promotion (PGP) was evaluated. Siderophore production was determined according to the method described by Alexander and Zuberer [33]. The ability to solubilize phosphate was measured according to the procedure described by Pikovskaya [34], whereas potassium solubilization was determined according to the method described by Meena [35]. The results for siderophore production and phosphate and potassium solubilization are expressed as the ratio of halo diameter to colony diameter (SI = halo diameter/colony diameter). Each strain was tested five times.

The indole-3-acetic acid (IAA) production was quantified according to the method of Solon and Robert [36] and the alginate production was determined according to the method of Johnson [37] with modifications by Sá et al. [23]. IAA and alginate concentrations were measured under osmotic stress conditions (10% PEG) and expressed as micrograms of IAA or alginate per optical density at 600 nm (μg/mL/OD). Each strain was tested at least three times.

2.5. Data Analysis

Hypothesis testing for different osmotolerance groups regarding their capacity to produce PGP traits was performed using a one-factor nonparametric permutational analysis of variance with encapsulated bacteria. The analysis was performed using PRIMER v.7 with PERMANOVA+ add-on [38]. The dataset was square root transformed and a similarity matrix was built using a Euclidean distance metric, followed by a Monte Carlo test (9999 permutations). Pairwise comparisons assessed significance, and differences were considered significant only at $p < 0.05$ and are marked with different lowercase letters in the figures. The null hypothesis tested was that there would be no difference between the different osmotolerance groups.

3. Results

3.1. Diversity

Molecular typing of the bacterial isolates using BOX-PCR revealed 89 distinct profiles. The most common genera were *Pseudomonas* (17%) and *Enterobacter* (9%) which were isolated from different plant hosts. The remaining strains belonged to different genera, with five strains belonging to *Siccibacter*, four strains belonging to *Acinetobacter*, and four strains belonging to *Stenotrophomonas*. The genera *Microbacterium*, *Pantoea*, *Cronobacter*, and

Rhizobium were each represented by two strains. Most genera (*Aeromonas*, *Bradyrhizobium*, *Caulobacter*, *Chryseobacterium*, *Flavobacterium*, *Massilia*, *Paenibacillus*, *Priestia*, *Pseudarthrobacter*, *Rhodococcus*, and *Xanthomonas*) were represented by single strains (Figure 1).

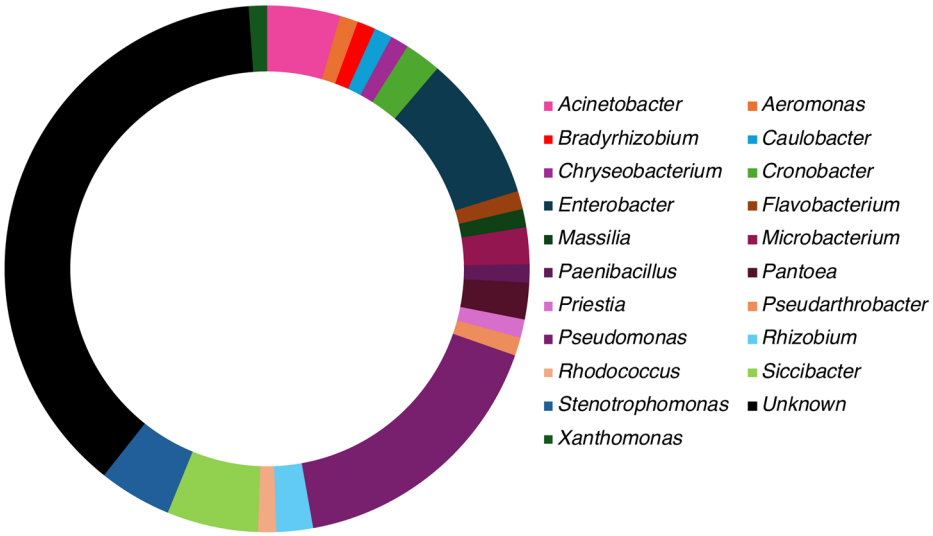

Figure 1. Bacterial diversity. Circular chart showing the different bacterial genera identified after BOX-PCR and 16S rDNA gene isolated from three different host plants (*Tetraena simplex*, *Tetraena stapffii*, and *Stipagrostis* sp.) growing in the Namib Desert.

3.2. Bacterial Tolerance to Abiotic Factors

3.2.1. Osmotolerance

Most bacteria (89%) showed osmotolerance between 10% and 20% (Figure 2). These bacterial isolates were separated into two levels, moderately tolerant (10% $\leq$ IC50 < 15%) and tolerant (15% $\leq$ IC50 < 20%). Some isolates (7%) showed low osmotolerance (IC50 < 10%), and only four (4%) displayed IC50 values higher than 20%.

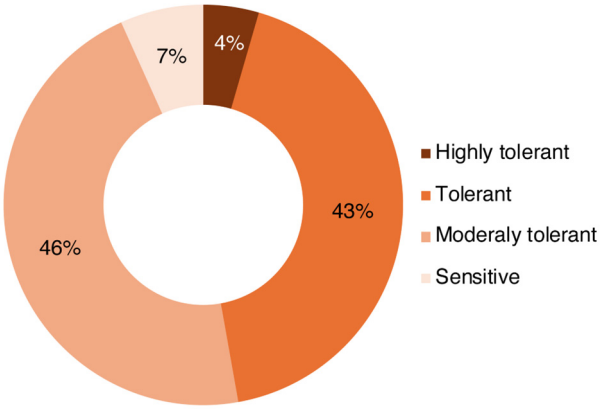

Figure 2. Bacterial osmotolerance (calculated by PEG concentration inhibiting 50% growth, IC50). Different levels of osmotolerance were identified: sensitive (IC50 < 10% PEG); moderately tolerant (10 % PEG $\leq$ IC50 < 15% PEG); tolerant (15% PEG $\leq$ IC50); and highly tolerant (IC50 $\geq$ 20% PEG).

3.2.2. Halotolerance

All bacteria were able to grow in 6% NaCl, albeit with different responses. Although no significant differences in NaCl tolerance were observed among osmotolerance levels, the growth of HT isolates was less inhibited (17% to 40%) than that of isolates from S, MT, and T (Figure 3A). Differences in the dispersion of halotolerance values were noted within each osmotolerance level (Figure 3B), with isolates S and MT showing similar responses, and T

presenting some isolates with increased growth compared to 0% NaCl and HT, indicating less dispersion of values (Figure 3B).

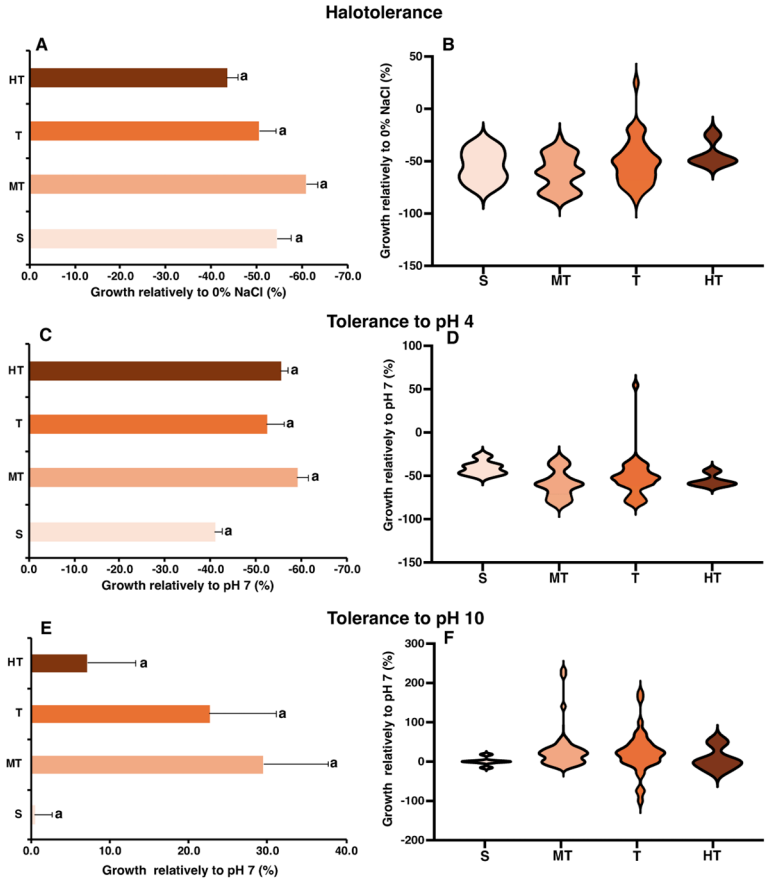

Figure 3. Bacterial tolerance to abiotic stress grouped by osmotolerance levels (S—sensitive; MT—moderately tolerant; T—tolerant; HT—highly tolerant). For each parameter, the general responses (bar charts) and distributions (violin plots) are presented. (**A**,**B**): Halotolerance at 6% NaCl relative to the control (0% NaCl). (**C**,**D**): Tolerance at pH 4 (acidity) relative to the control (pH 7). (**E**,**F**): Tolerance to pH 10 (alkalinity) relative to the control (pH 7). Values represent the mean of at least three replicates + standard error. Different lowercase letters indicate significant differences ($p < 0.05$) among the different osmotolerance levels.

3.2.3. Tolerance to Acidity

Acidity caused an overall decrease in growth, with no significant difference among osmotolerance levels. However, the S isolates showed a smaller reduction in growth (around 40%) compared to the remaining levels, which showed a decrease of more than 50% (Figure 3C). The responses of MT and T isolates were more heterogeneous, especially T, with the growth of some isolates stimulated by acidity (Figure 3D).

3.2.4. Tolerance to Alkalinity

In contrast to that at pH 4, growth was not inhibited at pH 10. Although there were no significant differences among osmotolerance levels, the MT and T isolates showed higher growth induction (Figure 3E). The S isolates were poorly influenced by alkaline conditions and showed little variation in their response. At the remaining levels, the response was more heterogeneous, including growth decreases and increases compared with the control (pH 7), especially at the MT and T levels, with some isolates doubling their growth (Figure 3F).

3.3. Bacterial PGP Traits

3.3.1. Indole Acetic Acid Production

Overall, IAA production was higher in the less osmotolerant isolates (S and MT), although the differences were not statistically significant (Figure 4A). All isolates showed the capacity to produce IAA (Figure 4B), with differences in the dispersion of values; the HT isolates showed lower and higher values, and the S isolates showed little variation (Figure 4B).

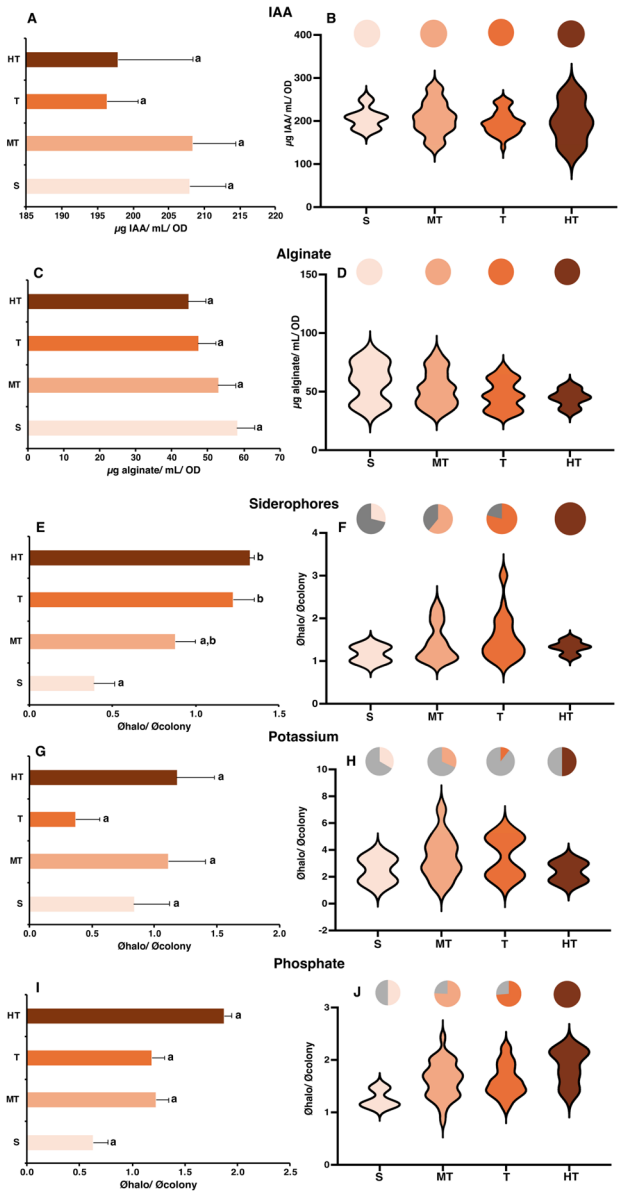

Figure 4. Plant Growth Promotion traits grouped by osmotolerance levels (S—sensitive; MT—moderately tolerant; T—tolerant; HT—highly tolerant). For each parameter, the general response (bar charts), ratio of isolates with (color) and without (grey) ability (pie chart), and distribution (violin plots) are presented. (**A**,**B**): IAA production under osmotic stress (10% polyethylene glycol). (**C**,**D**): Alginate production under osmotic stress (10% polyethylene glycol). (**E**,**F**): Siderophore production. (**G**,**H**): Potassium solubilization. (**I**,**J**): Phosphate solubilization. Values are means of at least three replicates + standard error. Different lowercase letters indicate significant differences ($p < 0.05$) among the different osmotolerance levels.

3.3.2. Alginate Production

Alginate production increased with a decrease in osmotolerance. Although no significant differences were observed, the S isolates produced 23% more alginate than the HT isolates (Figure 4C). All strains were able to produce alginate (Figure 4D), with differences in the dispersion of values, which decreased with an increase in the osmotolerance level (Figure 4D).

3.3.3. Siderophore Production

Isolates with higher osmotolerance (T and HT) produced significantly more siderophores than those with lower osmotolerance (S isolates) (Figure 4E). The number of isolates without the capacity to produce siderophores decreased with the level of osmotolerance: 67%, 39%, 21%, and 0% in S, MT, T, and HT, respectively (Figure 4F), with differences in the dispersion of values, higher in MT and T, and lower in HT (Figure 4F).

3.3.4. K Solubilization

The ability to solubilize K was lower in the T isolates (Figure 4G). Most isolates were unable to solubilize K, especially T isolates (89%), but half of the HT isolates were able to solubilize K (Figure 4H). The isolates with a higher capacity to solubilize K belonged to the MT group (Figure 4H).

3.3.5. P Solubilization

HT isolates showed 35% to 64% higher capacity to solubilize P than isolates from other osmotolerance levels, although the difference was not significant (Figure 4I). The number of isolates capable of solubilizing P increased with osmotolerance: 50%, 76%, 74%, and 100% in S, MT, T, and HT, respectively (Figure 4J), with differences in the dispersion of values, smaller in S, and higher in MT, T, and HT (Figure 4J).

4. Discussion

The present study aimed to examine the diversity of bacteria isolated from the roots of plants growing in an arid environment in the Namib Desert. The prevailing conditions in these environments, such as high osmotic stress, high salinity, and neutral to alkaline pH, directly affect the survival of organisms, and only well-adapted organisms thrive. Plant–microbe interactions help plants cope with the harsh conditions of deserts, improve nutrient availability, enhance water absorption, and induce stress tolerance [39,40]. Bacteria are known to provide all these benefits to plants; thus, the isolation of bacteria from these environments is a way to obtain strains with the capacity to tolerate osmotic stress and maintain plant growth capabilities under such conditions [41,42]. To persist in harsh environments, bacteria have evolved mechanisms to overcome constant adverse conditions. Studies have described specific bacterial communities associated with plants in desert environments but focus largely only on bacterial diversity and their PGP abilities [43–47] and also patterns and diversity of microbiomes under extreme environments [48,49]. Our study focuses on the bacterial diversity of rhizosphere bacteria isolated from dry soil and evaluates their PGP traits and their tolerance to different abiotic stresses, correlating the PGP traits with the different tolerance capacities. The 20 genera found in our study associated to the rhizosphere of plants from the Namib desert were already described in other studies (*Pseudomonas* [50], *Acinetobacter* [51], *Stenotrophomonas* [51], *Paenibacillus* [52], *Flavobacterium* [51], *Rhodococcus* [53], *Massilia* [53], *Microbacterium* [54], *Bradvrhizobium* [54], *Pantoea* [54], *Rhizobium* [54], *Chryseobacterium* [55], *Priestia* [56], *Pseudarthrobacter* [57], *Siccibacter* [58], *Cronobacter* [58], *Aeromonas* [59], *Caulobacter* [60], *Enterobacter* [61], *Xanthomonas* [62]), highlighting the distribution of these genera over different arid environments and confirming the existence of a specific bacterial community associated with plants in desert environments [63].

Most of these studies have focused on the capacity of bacteria to promote plant growth and have neglected other aspects, such as the capacity to tolerate abiotic stresses. Our study aimed to fill this gap and determined the ability of 89 isolates from 20 bacterial genera to tolerate high levels of osmolarity, salinity, acidity, and alkalinity as well as their capacity to produce PGP traits under osmotic stress.

4.1. Tolerance to Abiotic Stresses

The osmotolerance of microorganisms generally reflects their habitat conditions. In our study, bacteria from the rhizosphere of plants growing in the Namib Desert evidence high osmotolerance since the isolates considered sensitive were able to grow at 8% PEG. The majority of the bacterial isolates demonstrated moderate (10% to 15% PEG) to high osmotolerance (15% and 20% PEG), and four strains were able to grow at PEG concentrations higher than 20%, which corresponds to conditions with low water potentials (−0.7 to –1.67 MPa) [64]. Sharma et al. [65] isolated bacteria from the Thar Desert (India) and found isolates able to grow at 26% glycerol (−0.3 MPa). Desert soils generally have water potentials ranging from −1 to −10 MPa [66]. Even the most osmotolerant bacteria in our study hardly survived in environments with −10 MPa. However, bacteria isolated from the rhizosphere of plants retain soil moisture near the roots, conferring protection to bacteria during periods of higher water scarcity [67]; therefore, the level of osmotic stress to which they are exposed is lower than in bare soil and closer to rhizospheric bacteria in environments with higher water availability. Indeed, Sá et al. [23] and Cardoso et al. [68] determined the osmotolerance of isolates from sites in Portugal under different rainfall regimes and found that most strains were moderately tolerant to osmotic stress (IC50 between 8 and 15% PEG), with some strains being able to grow at PEG > 20%.

The bacterial osmotolerance level appears to be related to other stress responses. For instance, highly osmotolerant (HT) isolates showed higher halotolerance. The same was already reported in other studies, where drought-tolerant bacteria demonstrated the capability to grow under high-salinity conditions [69]. In nature, these two stresses are frequently associated, as low moisture increases the concentration of dissolved ions and, therefore, salinity [70,71]. Cardoso et al. [31] found a high halotolerance (IC50 > 5.4% NaCl) in bacteria isolated from dry soils in southern Portugal. In our study, bacteria displayed even higher tolerance, with some increasing growth at 6% NaCl, and most surviving at 12% NaCl. Identical results were obtained by Belov et al. (2018) in the Gibson (Australia) and Sahara (Egypt) deserts, with bacteria being able to grow at 15% NaCl. The mechanisms conferring osmotolerance also enhance halotolerance, such as the increase in osmolytes that adapt cells to low osmotic potentials, an effect arising from both osmotic and salt stresses [72,73].

Our study showed that bacteria were much more tolerant to high pH than to low pH, reflecting the prevailing conditions of the environment from which they were isolated. The soil pH in the Namib Desert varies but generally tends to be alkaline, often ranging from 8.0 to 9.0 [74]. In our study, most bacteria showed increased growth at pH 10 and severely reduced growth at pH 4 compared to that at pH 7. Bacteria isolated from the deserts of Gibson and Sahara also showed high inhibition under acidic conditions (pH 3 and 4), but these bacteria were also inhibited under alkaline conditions (pH 8, 9, and 10) [75]. The dissimilarity in results to alkaline conditions between our study and Belov's [75] highlights the differences in tolerance to abiotic constraints that may exist in bacterial communities among desert environments, reflecting differences in the bedrock mineral composition and rainfall regime [76]. The microenvironment generated by exuded from plants through roots can also influence the root microbiome, as the diversity of bacteria from each plant species in our study evidence. There are a number of bacterial genera which are common to the different plants in this study, but there are also genera which are specific to each plant. In particular, *Rhizobium* was detected exclusively in the roots of *Tetraena stapffii*, *Pantoea* was specific to the roots of *Tetraena simplex*, and *Pseudarthrobacter* was uniquely associated with

Stipagrostis sp. These results suggest that plants have the ability to modulate their root microbiomes in response to their specific physiological or ecological requirements.

4.2. Osmotolerance and PGP Traits

In desert environments where conditions are harsh and resources are scarce, the interaction between plants and rhizobacteria becomes even more critical. Rhizobacteria induce root growth, allowing plants to access deeper and moist soil layers by producing hormones such as indole acetic acid (IAA). Several studies have described the importance of IAA in plant tolerance to water stress by inducing root elongation [77,78] and regulating stomatal closure [79,80]. Our study evidenced that all isolates were able to produce IAA, some at high levels. Although IAA production is relatively common among rhizobacteria, not all possess this ability, and in most cases IAA production is low [68,81,82]. Thus, the high ability that most isolates from our study show must be an important feature in plant–bacteria interactions in desert environments.

Alginate, a polysaccharide produced by some bacteria, plays a significant role in enhancing the survival of both bacteria and their associated plants under water stress conditions through water retention and hydration, biofilm formation, desiccation resistance, plant–bacteria interaction improvement, and stress-responsive mechanisms induction [83,84]. These responses help plants cope better with stress experienced in desert environments. Sá et al. [23] described the relation between the ability to synthesize alginate and bacteria osmotolerance. However, in our study less osmotolerant isolates evidenced a higher ability to produce alginate than more osmotolerant ones. Therefore, it appears that in harsh environments alginate production is a mechanism on which more sensitive bacteria rely to tolerate osmotic stress.

Desert soils often have low levels of bioavailable iron due to their high calcium carbonate content and high pH, which reduces iron solubility and forms precipitates, reducing the amount available for plants and microorganisms to uptake [85]. Iron is one of the most important elements for the growth of bacteria and plants. It is involved in important metabolic processes (reduction of ribonucleotides and molecular nitrogen) and in energy-producing electron transfer reactions in mitochondria and photosystems [86]. The oxidative stress generated by alkaline conditions and water stress [87,88] can also be scavenged by iron containing antioxidant enzymes, such as catalase [89] and superoxide dismutase [90]. A large proportion of soil bacteria (71–79%) have the ability to produce siderophores under iron deficiency [33], and also, root exudates (organic acids, sugars, and amino acids) regulate the expression of siderophores in bacteria [91,92], demonstrating the synergy in the interaction between plants and bacteria to overcome iron deficiency. Organisms with adequate iron levels can maintain better energy production, and in the case of plants higher photosynthetic rates [93,94], which are crucial for survival in harsh desert environments. Several studies have reported the ability of bacteria to produce siderophores [68,95–97] even in harsh environments [81,82,95]. Our study showed a relationship between the ability to produce siderophores and the level of osmotolerance, with more osmotolerant isolates producing significantly more siderophores than sensitive ones. Iron is a cofactor of antioxidant enzymes such as catalase and superoxide dismutase, which protect organisms from oxidative damage. In desert conditions, where bacteria and plants often experience increased oxidative stress due to high osmotic stress, salinity, temperature, and intense sunlight, siderophores help to ensure that organisms have sufficient iron to support these protective mechanisms.

Most of the potassium in desert soils is unavailable, such as feldspar, mica, and other silicate minerals [98]. Potassium-solubilizing bacteria (KSB) produce organic acids, exoenzymes, and other metabolites that can break down these minerals and release potassium, which can be absorbed by bacteria and nearby plants. Our study showed that only 25% of the isolates were able to solubilize K, and this percentage was much lower in the less osmotolerant isolates (11% to 33%) than in the highly tolerant isolates (50%). The intracellular accumulation of potassium enables organisms, such as bacteria and plants, to maintain

turgor pressure, allowing them to adjust to low osmotic pressures [99–102]. By facilitating access to potassium, KSB can increase the tolerance of organisms to osmotically stressed environments, which is supported by our data, since the ability to solubilize K is more frequent in highly osmotolerant isolates.

In desert soils, phosphorus is often found in insoluble mineral forms such as calcium phosphate, iron phosphate, and aluminum phosphate. Phosphate solubilizing bacteria (PSB) convert these insoluble phosphates into soluble forms, such as orthophosphate, which can be readily absorbed [103–105]. Our results showed a higher proportion of isolates with the capacity to solubilize P, as well as a higher solubilization efficiency in isolates with higher osmotolerance. Cruz et al. [82] also found that most bacteria isolated from an arid and saline environment also evidenced the ability to solubilize P [106,107]. Phosphorus is a key component of ATP (energy related molecule) and NAP(P)H (reduction power), involved in the energy and redox status of cells and in metabolism regulation through phosphorylation. By ensuring adequate phosphorus availability, PSB can help organisms to tolerate harsh desert conditions.

5. Conclusions

This study evidences the remarkable resilience of bacterial communities isolated from the rhizosphere of plants in the Namib Desert to high osmotic stress, salinity, and pH variations. The capacity of these bacteria to produce plant growth-promoting traits in the presence of considerable stress highlights their potential to facilitate plant survival and growth in arid ecosystems. The positive correlation between osmotolerance and other stress-related traits, including halotolerance, siderophore production, and potassium and phosphorus solubilization, indicates that these bacteria possess multifaceted mechanisms to cope with the challenges of desert environments. The knowledge gained from this study not only contributes to our understanding of microbial life in extreme environments, but also opens new avenues for developing bio-inoculants that could enhance plant growth and productivity in arid and semi-arid regions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/land13101678/s1, Figure S1: Photograph of the plant collection site in the Namib Desert (15°08′06.2″ S–12°12′51.7″ E; Table S1: Bacterial genera, accession number and plant origin of isolates collected from root plants (*Tetraena simplex*, *Tetraena stapffii*, and *Stipagrostis* sp.) in the Namib desert.

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