Desert Actinobacterial Strains Increase Salt Stress Resilience in Crops†

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Abstract: The adaptation of crops to saline stress conditions generated by changes in rainfall and the current production systems is essential for maintaining many of them and minimizing possible damage or reductions in their productivity. The use of microorganisms to improve the conditions of plants from extreme environments, increasing their resilience, appears to be a possible alternative. In this work, we isolated strains from samples obtained in extreme environments, such as the Atacama Desert and Sahara Desert, and evaluated their capacity to promote the growth of plants directly and under stress conditions. We studied their ability to grow under salinity, and we selected some of these strains for their capacity to improve plant resilience.

Keywords: desert; soil; plant–bacteria interaction; climate change; microbiology; actinobacteria; Micromonospora; taxonomy; biodiversity; resilience

1. Introduction

One major problem facing the world is climate change. The decrease in rainfall in some areas and some of the agricultural practices developed for years are generating changes in the land of many countries, increasing the areas affected by high salinity [1].

Soils, in general, and desert soils, in particular, harbor a wide bacterial diversity with an immense potential for agriculture and biotechnology [2]. Soil microbes play essential roles in maintaining soil fertility through recycling nutrients, improving soil structure, degrading pollutants, and supporting healthy plant growth. Recent metagenomic and subsequent culturing studies on one of the most extreme biomes on Earth, the Atacama Desert, have revealed an abundant diversity of microbiological life harboring a remarkable potential to improve plant growth [3,4]. In general, microorganisms from deserts have evolved to develop a wide variety of strategies to survive under limiting conditions, including high salt tolerance [5]. On the other hand, plants’ evolution and adaptation capacity are slower, and it is possible that they rely on their microbiome to survive [6]. Therefore, the application of microorganisms with different origins and capacities to determine their effects on plants, both in generalized improvement of development [7] and in the increased tolerance to limiting conditions [8], are nowadays of strong interest.

During this work, several soil and plant tissues’ isolates from desertic areas were obtained, determining their capacity to grow under saline environments and their effect on the growth of *Medicago sativa* plants in the presence and absence of salt stress.

2. Materials and Methods

2.1. Strains of the Work

Strains from Atacama Desert were obtained from the collection of the team obtained in a previous work [9]. Other strains were recovered from nodules and roots of legumes...
recovered at the Sahara Desert. Those tissues were surface sterilized, as previously described [10], and strains were isolated on YMA media and subcultured on GYM media (M65 DSMZ medium).

2.2. Evaluation of In Vitro Salt Tolerance

Isolates were tested for their tolerance to grow over several NaCl (w/v) percentages (1, 3, 5, 7, 9, 12, 15, 18, and 21%) using GYM as basal media. For inoculation, strains were grown for 7 days at 28 °C and inocula were prepared in saline solutions with concentrations of around 1 × 10^8 UFC/mL. Ten microliters were inoculated in triplicate for each strain and kept at 28 °C for 1 month; we evaluated the capacity for growth weekly.

2.3. Plant Growth under Greenhouse Conditions

*Medicago sativa* seeds were germinated axenically, being sequentially immersed in 70% (v/v) ethanol for 30 s and 2.5% HgCl_2 (w/v) for 2 min, followed by several rinses with sterile distilled water. The seeds were then transferred to tap-water agar plates in the dark. After germination, the seedlings were placed on pots containing Rigaud and Puppo nutrient agar [11]. Three conditions of saline stress were tested (0, 1, and 3% NaCl (w/v)). The seedlings were kept in a plant growth chamber with fluorescent lighting with a photoperiod of 16 h light/8 h dark, a constant temperature of 21 to 22 °C, and 70% relative humidity. Plants were inoculated with the appropriate bacterial suspensions (1 × 10^8 CFU/mL), prepared as described before, and sterile distilled water as control. Plants were grown for 40 days with weekly evaluations of their development (stabilization, number of leaves, size) as well as a final evaluation, including wet and dry weights.

2.4. DNA Extraction and Identification

Extraction of genomic DNA for 16S ribosomal RNA (rRNA) gene sequence identification was carried out, as previously described [4]. A multiple sequence alignment of 16S rRNA genes was performed using DNASTAR with default options, and the strains were compared with type strains deposited in public databases using EzBioCloud. A phylogenetic tree was built using MEGA10. The 16S rRNA gene alignment was used to compute the genetic distances between all pairs of isolates.

3. Results

3.1. Isolation and Identification of Bacterial Strains

Fifteen strains presenting an actinobacterial morphology were isolated from nodules of autochthonous legumes growth in the Sahara Desert, while 17 strains were isolated from roots of the same plants. Nine strains previously recovered from Atacama Desert soils were also evaluated and analyzed. From them, a phylogenetic analysis of the 16S rRNA gene sequences showed that all the isolates from the Atacama Desert belonged to the *Micromonospora* genus, while, for the Sahara Desert, the strains belonged to several genera including *Micromonospora, Dermacoccus, Micrococcus*, and *Microbacterium* (Table 1).

| Table 1. Classification of the isolates obtained in the study, grouped by genera depending on the origin of the samples. |
|-------------------------------------------------------------|
| **Atacama Desert Soil** | **Sahara Desert Nodules** | **Sahara Desert Roots** |
| Dermacoccus sp. | - | 3 | - |
| Micromonospora sp. | 9 | 9 | - |
| Micrococcus sp. | - | 1 | 13 |
| Microbacterium sp. | - | 1 | 2 |

3.2. Tolerance to Saline Stress

All the strains were able to tolerate a minimum of 1% NaCl (w/v). In general, *Micromonospora* strains showed a lower tolerance to salt, with a maximum of 5%, while some strains of the genus *Micrococcus* reached 18%. Based on the results obtained here, six strains...
were selected for plant inoculation: three strains belonging to *Micromonospora* (S30, A3, and A9) that tolerated until 3, 5, and 5%, respectively, and three strains belonging to the *Micrococcus* genus (S27, S14, and SR36) that tolerated to 12, 15, and 18%, respectively.

3.3. Plant Growth Promotion Evaluation

Based on the data obtained, we were able to verify that some of the strains evaluated exerted a promoting effect on plant growth regardless of the presence of salt, improving and accelerating the development of plants (Figure 1), a result obtained mainly for the strains from the Atacama Desert (A). In addition, an improvement in the development of plants was observed in the presence of a moderate concentration of salinity of 1% in several of the strains analyzed, highlighting, in this case, the results obtained for the Sahara strain S14. On the other hand, it was observed that the negative effects of the high salt concentration could not be mitigated by the presence of the inoculated bacteria or a very slight mitigation was obtained.

![Figure 1.](image.png)

**Figure 1.** Plant growth promotion effects under salt stress of strains isolated from desert samples. (a) Comparison of the development of plants at 0, 1, and 3% of NaCl (w/v) in control vs. S14-inoculated plants; (b) differences observed in dry weight for all the samples tested under several saline concentrations.

4. Discussion

Our results showed the capacity of desertic strains to survive high concentrations of salinity in the media, with percentages as high as 18% for a root endophytic actinobacteria. In addition, we confirmed the strong relationship of actinobacteria with plants in desertic environments and their implication in increasing plant tolerance to salinity to similar levels as was described for the other type of bacteria: *Halomonas* and *Bacillus* [12]. *Micromonospora, Micrococcus,* and *Microbacterium* were previously described as endophytes [13]; however, this is the first time *Dermacoccus* strains were obtained from internal plant tissues. The study of microorganisms from extreme environments, such as deserts, in which the knowledge of their diversity is still very limited [3], is of special relevance, especially the determination of those that have a direct relationship with the development of plants under limiting conditions. The use of microorganisms to improve the development of plants and their adaptation to new stressful situations appears as a possibility to fight climate change consequences.

5. Conclusions

Our results show how several actinobacterial strains isolated from deserts present a high capacity to tolerate salinity as well as improving the plant’s tolerance to this stress. Based on the data presented here, we can say that, even if the *Micromonospora* isolates do not have the highest tolerant to salinity, they present a high capacity to improve plant growth and are also able to increase plant resilience to saline stress. The capacity of *Micromonospora* and *Micrococcus* as plant growth promotors has been exposed in several works, showing their capacity to produce IAA, chitinases, siderophores, or phosphatases as well as their
ability to inhibit the growth of several pathogens; however, this is the first time that their
capacity to increase plant tolerance to saline stress is described.

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**References**

1. Aragüés, R.; Medina, E.T.; Zribi, W.; Clavería, I.; Álvaro-Fuentes, J.; Faci, J. Soil salinization as a threat to the sustainability of
deficit irrigation under present and expected climate change scenarios. *Irrig. Sci.* 2015, 33, 67–79. [CrossRef]

2. Molina-Menor, E.; Gimeno-Valero, H.; Pascual, J.; Peretó, J.; Porcar, M. High Culturable Bacterial Diversity From a European
Desert: The Tabernas Desert. *Front. Microbiol.* 2021, 11, 583120. [CrossRef] [PubMed]

3. Idris, H.; Goodfellow, M.; Sanderson, R.; Asenjo, J.A.; Bull, A.T. Actinobacterial Rare Biospheres and Dark Matter Revealed in
Habitats of the Chilean Atacama Desert. *Sci. Rep.* 2017, 7, 8373. [CrossRef] [PubMed]

4. Carro, L.; Castro, J.F.; Razmilic, V.; Nouioui, I.; Pan, C.; Igual, J.M.; Jaspars, M.; Goodfellow, M.; Bull, A.T.; Asenjo, J.A.; et al.
Uncovering the potential of novel micromonosporae isolated from an extreme hyper-arid Atacama Desert soil. *Sci. Rep.* 2019,
9, 4678. [CrossRef] [PubMed]

5. Kothari, V.V.; Kothari, R.K.; Kothari, C.R.; Bhatt, V.D.; Nathani, N.M.; Koringa, P.G.; Joshi, C.G.; Vyas, B.R.M. Genome Sequence of
Salt-Tolerant Bacillus safensis Strain VK, Isolated from Saline Desert Area of Gujarat, India. *Genome Announc.* 2013, 1, e00671-13.
[CrossRef] [PubMed]

6. Carro, L.; Nouioui, I. Taxonomy and systematics of plant probiotic bacteria in the genomic era. *AIMS Microbiol.* 2017, 3, 383–412.
[CrossRef] [PubMed]

7. Ferreira, C.M.H.; Soares, H.M.V.M.; Soares, E.V. Promising bacterial genera for agricultural practices: An insight on plant
growth-promoting properties and microbial safety aspects. *Sci. Total Environ.* 2019, 682, 779–799. [CrossRef] [PubMed]

8. del Carmen Orozco-Mosqueda, M.; Glick, B.R.; Santoyo, G. ACC deaminase in plant growth-promoting bacteria (PGPB):
An efficient mechanism to counter salt stress in crops. *Microbiol. Res.* 2020, 235, 126439. [CrossRef] [PubMed]

9. Carro, L.; Razmilic, V.; Nouioui, I.; Richardson, L.; Pan, C.; Golinska, P.; Asenjo, J.A.; Bull, A.T.; Klenk, H.-P.; Goodfellow, M.
Hunting for cultivable Micromonospora strains in soils of the Atacama Desert. *Antonie Van Leeuwenhoek* 2018, 111, 1375–1387.
[CrossRef] [PubMed]

10. Benito, P.; Carro, L.; Bacigalupe, R.; Trujillo, M.E. From roots to leaves: The colonization capacity of an endophytic filamentous
actinobacteria. *Phytopathomes J.* 2022, 6, 35–44. [CrossRef]

11. Rigaud, J.; Pupo, A. Indole-3-acetic Acid Catabolism by Soybean Bacteroids. *J. Gen. Microbiol.* 1975, 88, 223–228. [CrossRef]

12. Kearl, J.; McNary, C.; Lowman, J.S.; Mei, C.; Aanderud, Z.T.; Smith, S.T.; West, J.; Colton, E.; Hamson, M.; Nielsen, B.L. Salt-tolerant
halophyte rhizosphere bacteria stimulate growth of alfalfa in salty soil. *Front. Microbiol.* 2019, 10, 1849. [CrossRef] [PubMed]

13. Parte, A.C.; Sardà Carbasse, J.; Meier-Kolthoff, J.P.; Reimer, L.C.; Göker, M. List of Prokaryotic names with Standing in Nomenclature
(LPSN) moves to the DSMZ. *Int. J. Syst. Evol. Microbiol.* 2020, 70, 5607–5612. [CrossRef] [PubMed]