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Actinobacteria from Extreme Niches in Morocco and Their Plant Growth-Promoting Potentials

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Received: 22 June 2019; Accepted: 14 August 2019; Published: 20 August 2019

Abstract: The objectives of this study were to assess actinobacterial diversity in five Moroccan extreme habitats and to evaluate their plant growth-promoting (PGP) activities. The soil samples were collected from different locations, including soils contaminated with heavy metals, from a high altitude site, from the desert, and from a marine environment. In total, 23 actinobacteria were isolated, 8 from Merzouga sand soil; 5 from *Cannabis sativa* rhizospheric soil; 5 from Toubkal mountain; 4 from a Draa sfar mining site; and 1 from marine soil. Based on their genotypic classification using 16S rRNA gene sequences, 19 of all belonged to the genus *Streptomyces* (82%) while the rest are the members of the genera *Nocardioides* (4.5%), *Saccharomonospora* (4.5%), *Actinomadura* (4.5%), and *Prauserella* (4.5%). Isolates *Streptomyces* sp. TNC-1 and *Streptomyces* sp. MNC-1 showed the highest level of phosphorus solubilization activity with 12.39 and 8.56 mg/mL, respectively. All 23 isolates were able to solubilize potassium, and 91% of them could grow under nitrogen-free conditions. The ability of the isolated actinobacteria to form indole-3-acetic acid (IAA) ranged from 6.70 to 75.54 µg/mL with *Streptomyces* sp. MNC-1 being the best IAA producer. In addition, all of the actinobacteria could produce siderophores, with *Saccharomonospora* sp. LNS-1 synthesizing the greatest amount (138.92 µg/mL). Principal coordinate analysis revealed that *Streptomyces* spp. MNC-1, MNT-1, MNB-2, and KNC-5; *Saccharomonospora* sp. LNS-1; and *Nocardioides* sp. KNC-3 each showed a variety of high-level plant growth-promoting activities. The extreme environments in Morocco are rich with bioactive actinobacteria that possess a variety of plant growth-promoting potentials that can further benefit green and sustainable agriculture.

Keywords: actinobacteria; extremophile; plant growth-promoting bacteria

1. Introduction

Microorganisms can colonize various ecological niches, including extremophilic habitats, due to their adaptive features, including structural as well as functional adaptations [1]. Several novel species have been isolated from these extreme habitats, including *Streptomyces thinghirensis* [2] and *Streptomyces youssoufiensis* [3]. Morocco possesses diverse ecosystems, many of which are considered extreme,

such as high mountain peaks, arid deserts, and polymetallic mines, but the actinobacterial diversity of these extreme habitats is lacking. These Moroccan untapped habitats represent hostile ecosystems enriched with immense biodiverse values worthy of being explored. Actinobacteria which occur in both terrestrial and aquatic habitats are among the most common groups of Gram-positive filamentous bacteria with high guanine-cytosine (G-C) content in their genomes and are able to form spores [4]. They have also been found to colonize various ecosystems, including extreme environments, and to produce extensive secondary metabolites with major importance for biotechnology and agriculture [5]. These bacteria have very high physiological and ecological plasticity, which makes them well adapted to survive in environments where the conditions may be extreme [6]. Actinobacteria which have incredible abilities to persist under extreme conditions have long been the focus of many studies and have been harnessed as valuable sources of novel bioactive metabolites [7]. The exploration of extreme habitats could even find new taxa. However, their ecological functions, which may be of benefit to agriculture, are not well known.

One of the primary sources of income in Morocco is agriculture, which requires a high input of chemical fertilizers resulting in a concomitant high cost of crop production. On the other hand, microorganisms play a significant role in soil fertility through the management of nitrogen (N), phosphorus (P) and potassium (K) cycles in the soil. For instance, some of the diverse bacteria called plant growth-promoting (PGP) rhizobacteria, which live within the rhizospheres of plants, can support plant growth by fixing atmospheric nitrogen and solubilizing inorganic P and K [8]. As with other PGP bacteria, actinobacteria also employ both direct and indirect mechanisms to influence plant growth and protection. The direct mechanisms involve the production of vigorous factors for crop growth, such as phytohormones and advantageous actions such as N fixation, P solubilization, and iron acquisition [9]. Additionally, PGP actinobacteria indirectly influence plant growth by controlling and minimizing the deleterious effects of external stresses from either biotic or abiotic sources [10]. Moreover, the use of actinobacteria as biofertilizers or biocontrol agents for improving and protecting crop yield has been highlighted in several research studies [11,12]. *Streptomyces* is the most abundant genus of actinobacteria found in the plant rhizosphere, and it often promotes plant growth by producing regulators such as indole acetic acid (IAA) that aid root growth, producing siderophores that can improve nutrient uptake, or through biocontrol activity to protect host plants from phytopathogens [13].

Furthermore, extremophilic actinobacteria have been thoroughly investigated for their unique mechanisms of adaptation to extreme environments, and also because they produce several unusual metabolites especially for the inhibition of human pathogens [14,15]. Numerous studies have investigated the ecology of actinobacteria, typically within common habitats [16]. However, very little data is available on the diversity and ecological functions of actinobacteria inhabiting soils from Moroccan extreme sites. The aims of this study were to: (1) investigate the generic diversity of culturable actinobacteria in different Moroccan extreme ecosystems (i.e., Toubkal mountain, Merzouga desert, Rif rhizospheric soil, Marchica marine environment and Draa Sfar mining site); and (2) evaluate the PGP potentials (i.e., N fixation, solubilization of P and K, and production of IAA and siderophores) of these bacteria.

2. Materials and Methods

2.1. Study Sites, Sample Collection, and Isolation of Actinobacteria

Five soil samples were collected at a depth of about 6 to 8 inches from different Moroccan ecosystems (Table 1). Immediately after their collection, the samples were stored at 4 °C before the isolation of actinobacteria.

Table 1. Origins and characteristics of the five Moroccan sites explored.

Sample	Origin	Site Characteristic	Geographic Coordinate	Physicochemical Property		
				pH	EC (mS/ms)	TOC (%)
Mountain soil	Toubkal	Highest mountain peak in Morocco and the Arab world	4167 m; 31.05917″ N −7.91583″ W	7.9	2.14	1.02
Desert soil	Merzouga	Highest dunes in Morocco	150 m; 31.147643″ N −3.974280″ O	8.04	0.95	0.96
Rhizospheric soil	Rif	Rhizosphere of *Cannabis sativa* grown in Rif plains	34.920059″ N −4.561078″ O	8.62	4.99	1.58
Marine soil	Marchica	Largest lagoon in Morocco	35.156468″ N −2.904342″ W	8.31	20.41	0.34
Mining soil	Draa Sfar	Significant potential for polymetallic ore	31.704270″ N −8.135748″ W	5.4	6.5	1.13

EC: electrical conductivity; TOC: total organic carbon.

Each soil sample (10 g) was suspended in 90 mL of a sterile saline solution (0.9% (*w*/*v*) NaCl in distilled water). The homogenous soil suspension was 10-fold serially diluted with the same sterile saline solution, and 0.1 mL of each dilution was spread onto saline nutrient agar for the marine soil [17] and onto Bennett agar [18] or chitin-vitamin agar [19] for the rest soil samples. The media were supplemented with nalidixic acid (100 µg/mL) and cycloheximide (50 µg/mL) to prevent the growth of Gram-negative bacteria and fungi, respectively. The seeded agar plates were incubated for 3 weeks at 28 °C and the colonies that developed were examined by light microscopy. After isolation, the purified isolates were stored in 25% (*w*/*v*) glycerol at −20 °C as the primary stocks.

2.2. Molecular Identification of Actinobacterial Isolates

Each actinobacterial isolate was grown in 100 mL of Bennett broth at 28 °C for 5 days. Its genomic DNA was extracted using a Bacterial DNA kit (MPure™, Ottawa, ON, Canada) according to the manufacturer's instructions. The 16S rRNA gene was amplified by PCR using universal primers: FD1 (5′AGAGTTTGATCCTGGCTCAG3′) [20] and S17 (5′CGGTCACGTTCGTTGC3′) [21]. The PCR reaction with a final volume of 50 µL contained GoTaq Reaction Buffer (Promega®), 1.5 mM of $MgCl_2$, 200 µM of each desoxyribonucleotides (dNTP), 1 µM of each primer, 0.5U of *Taq* DNA polymerase, and 1 µL (500 ng) of the purified bacterial DNA template. The amplification was carried out in a thermocycler (Thermo Scientific™, Munich, Germany) with the following profile: an initial denaturation at 95 °C for 5 min, 30 cycles of denaturation at 95 °C for 30 s, annealing at 53 °C for 30 s, extension at 72 °C for 1.5 min, and a final extension at 72 °C for 5 min. PCR products were separated using 1% (*w*/*v*) agarose gel electrophoresis and visualized by ultraviolet (UV) fluorescence (UV-transilluminators, CA, USA).

The same primers were then used separately in two sequencing reactions from the two ends of the amplified fragment (Eurofins Scientific, Berlin, Germany). The 16S rDNA sequences were compared with the publicly available nucleotide sequences in the EzBioCloud database [22]. The sequences were aligned using CLUSTAL W [23] in the Molecular Evolution Genetics Analysis (MEGA) software version 5.0 [24]. Phylogenetic trees were inferred using neighbor-joining analyses [25] based on the Tamura 3-parameter model.

2.3. Evaluation of Plant Growth-Promoting Potentials

2.3.1. P Solubilization

Each actinobacterial isolate was grown in 100 mL of National Botanical Research Institute's phosphate growth medium devoid of yeast extract (NBRIY) broth, supplemented with tricalcium phosphate ($Ca_3(PO_3)_2$) as a source of inorganic phosphate [26]. The inoculum was incubated at 28 °C on a rotatory shaker at 180 rpm. The pH of the culture broth was measured after 48 h up to 196 h with a pH meter. Sterile NBRIY broth without inoculation served as a baseline control. Bacterial cells and other insoluble materials were removed by centrifugation at 4830× *g* for 20 min, and the resulting supernatant was filtered through a 0.45 µm filter. The concentration of soluble P in the supernatant was measured using the molybdenum blue method described by Nagul et al. [27].

2.3.2. K Solubilization

The ability of each actinobacterial isolate to solubilize K was tested using Aleksandrov agar medium (composition per l:5 g glucose, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.1 g $CaCO_3$, 0.006 g $FeCl_3$, 2 g Ca_3PO_4, 20 g agar powder, and 5 g of Mica powder as a source of inorganic potassium) [28]. A single colony of each actinobacterium was streaked onto the agar medium and incubated at 28 °C for 7 days. The development of a clear zone surrounding a bacterial colony indicated a positive test for K solubilization. The level of K-solubilizing activity was determined by the size of the clear zones formed.

2.3.3. N Fixation

Each actinobacterial culture was previously grown in Bennett broth at 28 °C with shaking at 125 rpm for 5 days. The liquid culture was centrifuged at 6574× *g* for 10 min. The cell pellets were resuspended in sterile 0.9% (*w*/*v*) saline solution and streaked onto a solid N-free medium (NFM), then incubated for 7 days [29]. N-fixing isolates are those able to grow on this medium.

2.3.4. IAA Production

Each actinobacterial isolate was grown in 100 mL of Luria Bertani (LB) broth [30] supplemented with 1.02 g/L of L-tryptophan as a precursor of IAA. The culture broth was incubated at 28 °C with shaking at 125 rpm for 4 days. Then, the bacterial cells were removed by centrifugation at 8217× *g* for 10 min. The Salkowski's reagent (10 mM $FeCl_3$, 35% perchloric acid) and 2 drops of phosphoric acid were added to 1 mL of the bacterial supernatant. The test solution was subsequently incubated for 30 min in the dark before its absorbance was measured at 530 nm [31]. Sterile LB medium served as a blank. The amount of IAA produced was calculated using a standard curve of known IAA concentrations.

2.3.5. Siderophore Estimation

The supernatant (0.5 mL) of each actinobacterial isolate growing in LB broth at 28 °C for 5 days [32] was mixed with 0.5 mL of Chrome Azurol S (CAS) reagent. After 20 min of incubation at ambient temperature, the optical density of the test solution was measured at 630 nm [33]. The amount of siderophore produced was determined from a standard curve prepared with desferrioxamine mesylate as described by Baakza et al. [34].

2.4. Statistical Analysis

The results presented are means ± standard deviations (SDs) of four biological replicates per treatment for the in vitro tests. Statistical comparison of means was performed using a one way analysis of variance (ANOVA) with least significant difference (LSD) available in the COSTAT software. The results were compared using a Student, Newmann, Keuls (SNK) test. Significant differences at

$p < 0.05$ are indicated. P solubilization, siderophore, IAA production and their correlation with isolated actinobacteria were subjected to principal component analysis (PCA) using XLStat software.

3. Results and Discussion

3.1. Actinobacterial Diversity in Different Moroccan Extreme Environments

We isolated 23 actinobacteria from five soil samples collected from various Moroccan ecosystems (Table 2). All actinobacterial isolates belonged to one of five genera, namely: *Streptomyces*, *Nocardioides*, *Saccharomonospora*, *Actinomadura*, and *Prauserella* (Table 2 and Figure 1). The genus *Streptomyces* was found to be the most abundant actinobacteria, representing 82% of the total isolates. We also isolated some rare actinobacteria belonging to *Nocardioides* (4.5%), *Saccharomonospora* (4.5%), *Actinomadura* (4.5%) and *Prauserella* (4.5%) genera. *Actinomadura* sp. NDS-2 was isolated from Draa sfar, a heavy metal polluted area containing lead, copper, zinc, and zinc oxide [35]. Three other actinobacteria belonging to *Streptomyces* and *Prauserella* genera were also isolated from the same site. This result is consistent with previous research that reported metal-resistant strains belonging to several actinobacterial genera, such as *Streptomyces*, *Amycolatopsis* [36], *Nocardia*, *Micromonospora* [37], and *Frankia* [38].

Table 2. Molecular and plant growth-promoting (PGP) characteristics of the isolated actinobacteria.

		Molecular Characteristics			PGP Characteristics	
Site of Isolation	**Isolate Code**	**16 rRNA Gene Accession Number**	**Similarity %**	**Closest Type Strain**	**N_2 Fixation**	**K Solubilization**
Rhizospheric soil	KNC-1	MN161857	98.03	*Streptomyces koyangensis* DSM 41864^T	++	+
	KNC-2	MN161858	96.06	*Streptomyces actinomycinicus* RCU-197^T	+++	+++
	KNC-3	MN161861	98.23	*Nocardioides albus* KCTC9186^T	++	++
	KNC-4	MN161862	99.38	*Streptomyces chartreusis* NBRC 12753^T	+++	++
	KNC-5	MN161863	99.79	*Streptomyces setonii* DSM 40395^T	++	++
Desert soil	MNB-1	MN164446	96.20	*Streptomyces graminifolii* DSM 102004^T	+++	+
	MNB-2	MN164450	99.31	*Streptomyces luteus* NRRL B-59117^T	+++	+++
	MNB-3	MN164447	94.12	*Streptomyces pratensis* CH24^T	+	++
	MNC-1	MN161860	97.76	*Streptomyces asenjonii* KMN35-1^T	++	++
	MNC-2	MN161865	99.17	*Streptomyces plicatus* DSM 40319^T	+++	++
	MNC-3	MN161850	98.65	*Streptomyces violascens* ISP5183^T	+++	+++
	MNC-4	MN161866	98.45	*Streptomyces albidoflavus* DSM 40455^T	+++	+++
	MNT-1	MN161867	99.30	*Streptomyces hydrogenans* DSM 40586^T	++	++
Marine soil	LNS-1	MN161851	95.39	*Saccharomonospora azurea* NA-128^T	+	+
Mining soil	NDS-1	MN161854	97.80	*Streptomyces asenjonii* KMN35-1^T	+	+
	NDS-2	MN161853	98.03	*Actinomadura napierensis* B60^T	+++	++
	NDS-3	MN161864	99.68	*Streptomyces albidoflavus* DSM 40455^T	-	-
	NDS-4	MN161855	98.42	*Prauserella soli* 12-833^T	-	+
Mountain soil	TNB-1	MN164448	96.05	*Streptomyces jietaisiensis* DSM 41866^T	+++	+
	TNB-2	MN164449	98.85	*Streptomyces albidoflavus* DSM 40455^T	+++	+
	TNB-3	MN161859	97.11	*Streptomyces rutgersensis* ATCC 3350^T	++	+
	TNC-1	MN161852	98.56	*Streptomyces violascens* ISP5183^T	+++	+++
	TNC-2	MN161856	98.38	*Streptomyces hydrogenans* DSM 40586^T	+++	+++

-: Absence of activity; +: low activity; ++: moderate activity and +++: high activity. T: Type strain

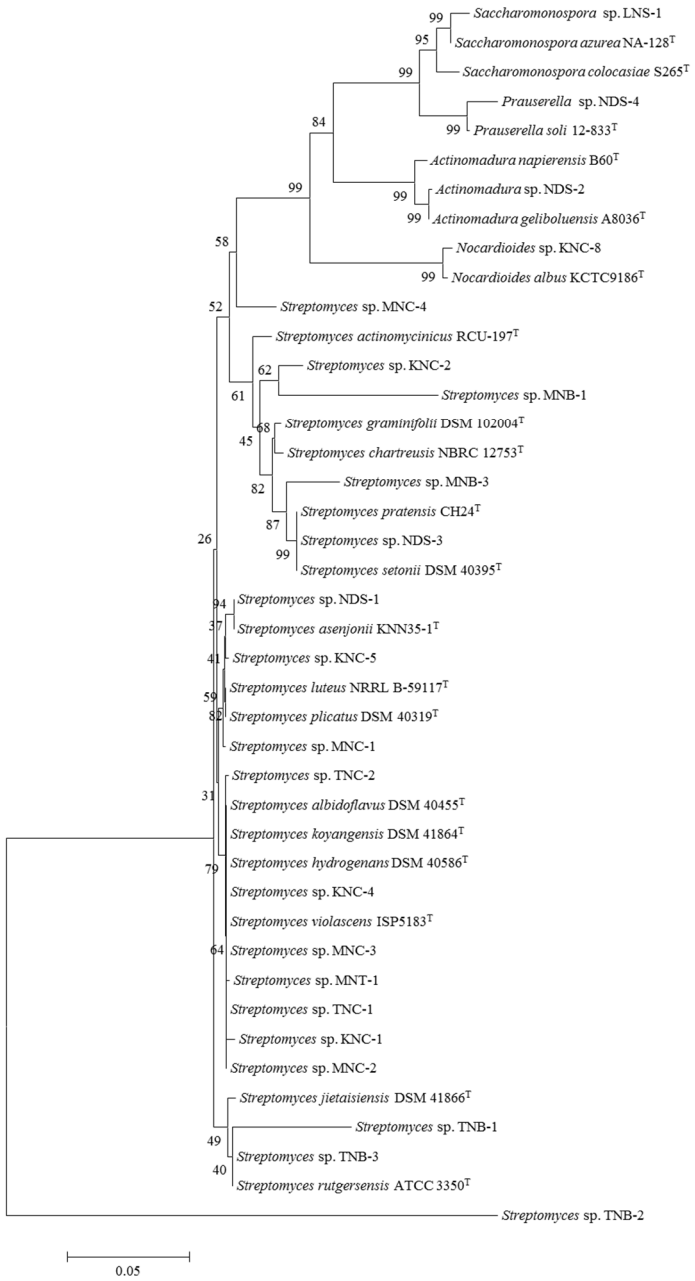

Figure 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences (>1300 nt) showing the relationships between 23 actinobacteria isolated from several Moroccan habitats and their closely related species. The numbers at the nodes indicate the levels of bootstrap support based on neighbor-joining analyses of 1500 resampled data sets. Bar, 0.05 substitution per nucleotide position.

Samples from the arid region "Merzouga" (desert soil), known for high temperatures, nanomolar concentrations of nutrients, low water activity, and intense radiation [39], permitted the isolation of eight isolates belonging to the *Streptomyces* genus. The same result was found for the Toubkal mountain sample with five *Streptomyces* isolates. These results are in agreement with the studies of Harwani, [40] and Santhanam et al. [41], who reported that some *Streptomyces* species (e.g., *Streptomyces deserti* and *Streptomyces bullii*) were isolated from the hyper-arid Atacama Desert. Kurapova et al. [42] have previously reported isolating a moderately thermophilic xerotolerant *Streptomyces* sp. 315 from Mongolian desert soil. These authors also isolated other species of *Streptomyces* with *Actinoplanes* and *Geodermatophilus* from the Negev and Mojave desert soils [42]. In addition, the Algerian Saharan soil

reveled the existence of several actinobacterial strains, with some of them belonging to rare genera such as *Actinomadura*, *Nonomuraea*, *Nocardiopsis*, *Saccharothrix*, *Spirillospora*, and *Streptosporangium* [43–45].

Unlike the mountain and the desert soils, the rhizospheric soil of *Cannabis sativa* exhibited the presence of five actinobacterial isolates. Among them we could identify *Nocardia*, which is a rare actinobacterium, in agreement with the study of Khamna et al. [46] who demonstrated that the rhizospheric soil of 16 medicinal plants provided a rich source of actinobacterial diversity. Overall, 89% of all actinobacteria that were found in this study belonged to the genus *Streptomyces*, supporting the suggestion that *Streptomyces* is the predominant actinobacterial genus in the soil [47,48].

The composition of the actinobacterial community may be affected by several environmental parameters, such as salinity [49], temperature [50], and heavy metals [51]. Furthermore, the apparent diversity may be affected by the nutrient composition of the culture media used during the isolation process [52]. In the marine soil sample, we successfully isolated a rare actinobacterium belonging to *Saccharopolyspora*. This isolate was able to grow in seawater, consistent with several studies that have discussed the isolation and diversity of actinobacteria in marine areas [53,54]. New marine actinobacterial taxa have been recovered, including *Salinispora*, the first marine obligate actinomycete isolated from ocean sediments [55], as well as from a sponge [56]. Many authors have reported *Streptomyces* as the major actinobacterial population in both terrestrial [57] and marine ecosystems [58,59]. Nevertheless, marine actinobacteria may have different characteristics from terrestrial ones and therefore might produce novel bioactive compounds, including new antibiotics [60].

3.2. PGP Potentials of Extremophilic Actinobacteria

The use of PGP actinobacteria as an alternative to chemical fertilizers is potentially a key strategy to enhance sustainable plant production and protection. The solubilization of inorganic P by the newly isolated actinobacteria is shown in Figure 2. After 8 days of incubation, the available P released ranged from 2.11 to 12.39 mg/mL with variations among different isolates. *Streptomyces* sp. TNC-1 and *Streptomyces* sp. MNC-1 were the most efficient isolates in releasing soluble P into the culture broth (12.39 and 8.56 mg/mL, respectively). The P solubilization by these isolates was often accompanied by a significant drop in the pH from an initial pH of 7.5 to as low as 4.83, after 192 h of incubation. With *Streptomyces* sp. TNC-1, the highest amount of soluble P was associated with a decrease in the pH to 4.93. These data indicate that the solubilization process of P is consistent with the bacterial excretion of substances that acidified the culture broth [61]. Several reports indicate that organic acid (OA) and siderophores production are among the mechanisms underlying inorganic P solubilization [62,63].

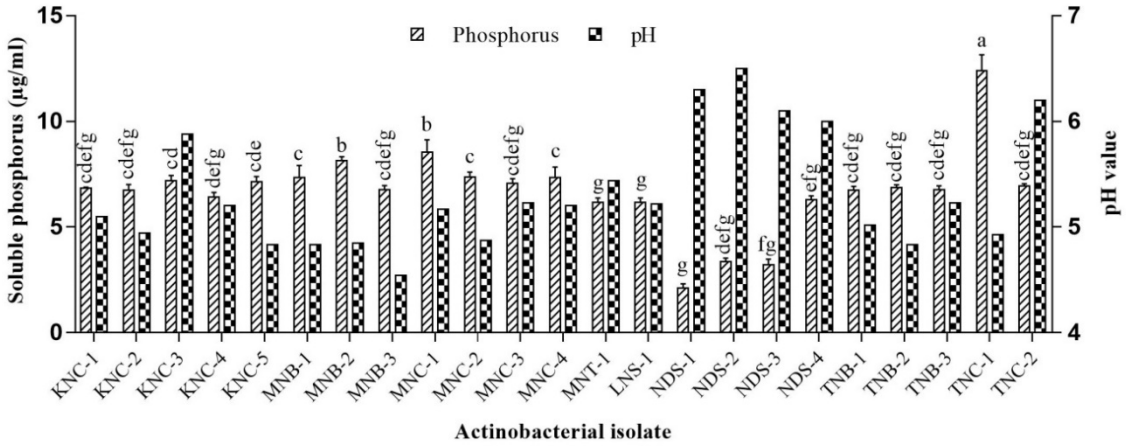

Figure 2. Determination of P solubilization and the evolution of pH in the culture supernatant of actinobacterial isolates. Means (±standard deviations) with in the same graphic followed by different letters are significantly different at $p < 0.05$.

Isolates belonging to *Streptomyces*, *Arthrobacter*, *Rhodococcus* and *Micromonospora* genera have been reported to solubilize inorganic P [9,64,65], which is consistent with our results. El-Tarabily et al. [66] demonstrated that *Micromonospora endolithica* has the capacity to solubilize P and improve the growth of bean plants. However, there has been very limited research on the P-solubilizing efficiency of actinobacteria isolated from either marine or arid environments [67,68].

Next, the isolates were screened for K solubilization ability (Table 2). Among all isolated actinobacteria, only one isolate was unable to form a significant zone of clearance on modified Aleksandrov agar plates, while six isolates (*Streptomyces* sp. KNC-2, *Streptomyces* sp. MNB-2, *Streptomyces* sp. MNC-3, *Streptomyces* sp. MNC-4, *Streptomyces* sp. TNC-1 and *Streptomyces* sp. TNC-2) showed relatively large zones of clearance. Currently, little information is available on K solubilization by rhizospheric actinobacteria. They may solubilize K and make it available to growing plants using various mechanisms, including the production of inorganic and organic acids and polysaccharides [69]. A wide range of rhizospheric bacteria have been reported as K solubilizers, including *Burkholderia*, *Acidithiobacillus ferrooxidans* [70], *Arthrobacter* sp. [71], *Enterobacter hormaechei* KSB-8 [72], *Paenibacillus mucilaginosus* and *Paenibacillus glucanolyticus* [73,74], and *Aminobacter* and *Sphingomonas* [75].

N fixation capacity was observed in 91% of the actinobacterial isolates (Table 2). Currently, there is no known N-fixing *Streptomyces*, other than the thermophilic, autotrophic *Streptomyces thermoautotrophicus* [76]. To our knowledge this is the first report of nitrogen fixation by the rare actinobacteria including *Nocardioides*, *Actinomadura* and *Saccharomonospora*. In addition, *Frankia*, a versatile N-fixing actinobacterium, fixes N under both free-living and symbiotic conditions [10]. It infects the root cells of actinorhizal plants through either intracellular root-hair infection or intercellular root invasion [77]. Moreover, various other endophytic actinobacteria have the ability to fix N, including *Arthrobacter*, *Mycobacterium* and *Propionibacteria* [78]. Additionally, other bacterial genera were reported as N-fixing actinobacteria, such as *Agromyces*, *Microbacterium*, *Corynebacterium* and *Micromonospora* isolated from root nodules of leguminous and actinorhizal plants [79].

Quantitative estimation of IAA production by actinobacterial isolates in the presence of L-tryptophan is shown in Figure 3. The IAA values ranged from 6.70 to 75.54 µg/mL after eight days of incubation. Isolate *Streptomyces* sp. MNC-1 isolated from the Merzouga desert produced the highest amount of IAA (75.54 µg/mL), followed by isolates: *Streptomyces* sp. MNB-2 (50.32 µg/mL), *Saccharomonospora* sp. LNS-1 (49.46 µg/mL), *Streptomyces* sp. MNT-1 (46.41 µg/mL) and *Streptomyces* sp. KNC-5 (41.92 µg/mL). *Streptomyces* MNC-1 has a similar level of IAA to the previously-reported work of Khamna et al. [46]. The lowest IAA production was observed for isolates *Streptomyces* sp. TNC-2 and *Streptomyces* sp. MNC-3 with 8.30 µg/mL and 6.70 µg/mL, respectively. These results agree with those reported by Anwar et al. [76] and Rodrigues et al. [31]. Several *Streptomyces* species, such as *Streptomyces olivaceoviridis*, *Streptomyces rimosus*, *Streptomyces rochei*, *Streptomyces griseoviridis*, and *Streptomyces lydi* also have the ability to produce IAA and promote plant growth [80–82]. In fact, the majority of actinobacteria produce IAA, which is responsible for an increased number of adventitious roots and root exudates [83]. In addition, IAA can also act as a signal for the production of secondary metabolites and sporulation of actinobacteria [84,85].

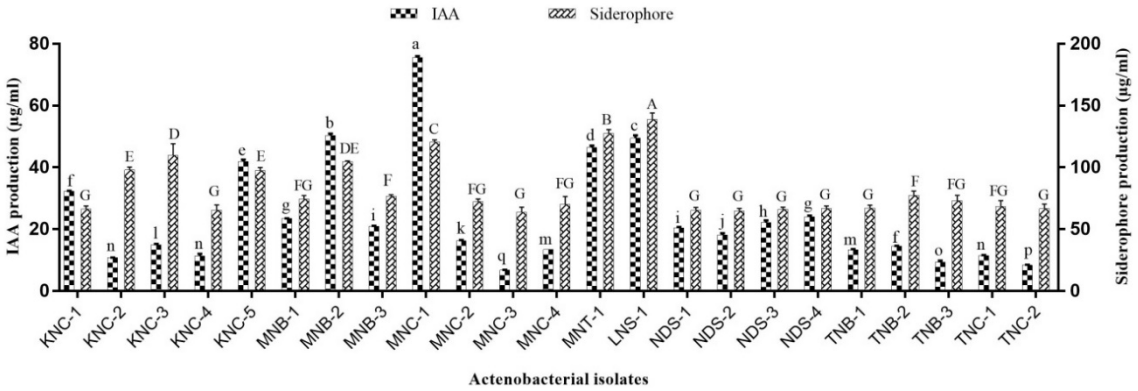

Figure 3. Indole acetic acid (IAA) and siderophore production by the isolated bacteria. Means (± standard deviations) within the same graphic followed by different small case letters from IAA production and capital letters for siderophore production are significantly different at $p < 0.05$.

The results of siderophore production in liquid medium by the isolated actinobacteria are shown in Figure 3. Isolate *Saccharomonospora* sp. LNS-1 was the highest siderophores producer (138.92 µg/mL), followed by isolates *Streptomyces* sp. MNT-1 (127.37 µg/mL) and *Streptomyces* sp. MNC-1 (120.35 µg/mL), while isolate *Streptomyces* sp. MNC-3 was the lowest siderophore producer (63.80 µg/mL). Siderophores help to satisfy an organism's iron needs by chelating it from inorganic sources and making it available to both bacteria and plants [86,87]. Siderophore production is implicated in growth promotion and in biocontrol of phytopathogens [33]. Numerous strains of actinobacteria have been reported as siderophore producers [13]. Oliveira et al. [88] found that *Saccharopolyspora erythraea* produces a hydroxamate type siderophore designated as erythrobactin. Similarly, the marine actinobacterium *Citricoccus* sp. KMM3890 produces a cyclic siderophore, nocardamine [89], while *Actinomadura* sp. DSMZ13491 produces the cyclic heptapeptide GE23077 with antibacterial activity [90]. Moreover, the novel heterobactin analog siderophore JBIR-16 is produced by *Nocardia tenerifensis* [91]. Kodani et al. [92] found that *Streptomyces* sp. TM-74 produces the siderophore tsukubachelin B.

Principal coordinate analysis (PCA) was used to select which isolates better expressed PGP activity (Figure 4). The two dimensions of the PCA (F1 and F2) were responsible for 91.19% of the total variation, with first axis accounting for 58.98% and the second axis for 32.21% of the variance. The PCA showed that isolates with higher activity in term of P solubilization, IAA and siderophores production were on the right of the first axis (F1), that is, they corresponded to isolates *Streptomyces* sp. MNC-1, *Saccharomonospora* sp. LNS-1, *Streptomyces* sp. MNT-1, *Streptomyces* sp. MNB-2, *Streptomyces* sp. KNC-5 and *Streptomyces* sp. KNC-3. Meanwhile, isolates with lower PGP activity are on the left in Figure 4. In comparison to the second axis, isolates *Saccharomonospora* sp. LNS-1, *Streptomyces* sp. MNT-1 and *Streptomyces* sp. KNC-5 displayed better production of IAA and siderophores and a medium level of P solubilization. In addition, isolates *Streptomyces* sp. MNC-1, *Streptomyces* sp. MNB-2 and *Streptomyces* sp. KNC-3 revealed better P solubilization than the other isolates. It's important to note that P solubilization ability is negatively correlated with IAA production. According to the results, we suggest that the most promising isolates were isolated from the desert soil, marine environment and rhizospheric soil of *C. sativa*. The interesting PGP activities of the promising isolates might be explained by their exhibition of a diversified metabolism and a strong enzymatic activity.

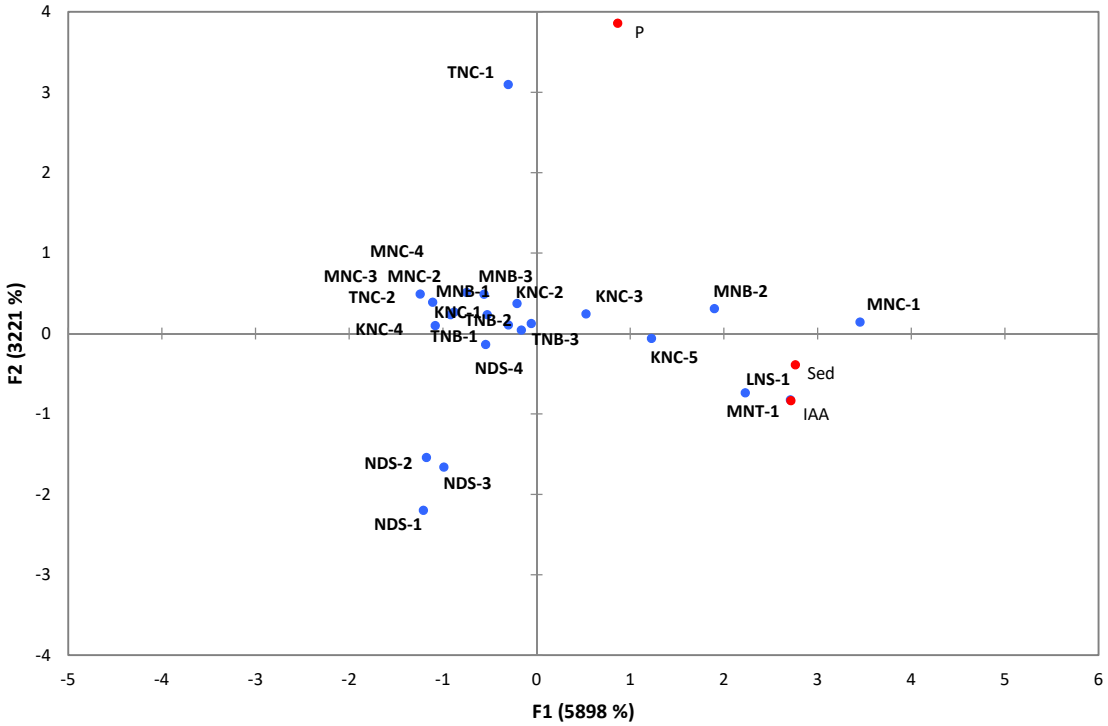

Figure 4. Principal coordinates analysis in term of phosphate solubilization (P), indole acetic acid (IAA) and siderophore (sid) production. The isolated actinobacteria are represented in blue; the PGP activities are presented in red.

4. Conclusions

Our findings revealed that the extreme ecosystems in Morocco are a rich source of valuable actinobacteria, especially for rare genera. This study is the first to report the discovery of *Nocardioides*, *Saccharomonospora*, *Actinomadura*, and *Prauserella* from a variety of extreme terrestrial and marine habitats in Morocco. Every actinobacterial isolate obtained exhibited distinctive PGP activities (i.e., IAA and siderophore production, P and K solubilization, and N-fixation), which can potentially be a promising microbial resource for optimizing green and sustainable agriculture in Morocco.

Author Contributions: Conceptualization, A.N.; methodology, A.N., A.R.; software, A.R., A.E.A.; formal analysis, A.N., A.R.; resources, F.E.K.; writing—original draft preparation, A.N., A.R., N.B.; writing—review and editing, B.R.G., Y.O., L.K. and M.H.; supervision, L.H.

Funding: This research received no external funding.

Acknowledgments: The principal author is grateful to all those who have participated in this work.

Conflicts of Interest: The authors have declared no conflict of interest.

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