



Biodiversity in Bacterial Phyla Composite in Arid Soils of the Community of Desert Medicinal Plant *Rhazya stricta*

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NAB, MNB, SON and JPH designed and conceived research. Authors MNB, RMH, DAAZ and AMAH harvested plants, isolated DNA. Authors SON, MNB, AAA, RSJ and MAM assisted in data analyses and figure production, and wrote the manuscript. All authors read and approved the final version of the manuscript.

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ABSTRACT

Rhazya stricta is an important medicinal plant used as native herbal drug to cure various diseases across Asia. Plant-microbe interactions represented by different environment abiotic stress factors that shows effects also on the host plants or on the associated microbial communities. Soil microorganism can be adapted to specific stress conditions and evolve tolerance to this stress. The

adjustment of the plant microbiome can productively improve the yield production of essential crops. In the present study, metagenomics analysis of bacterial communities associated with five desert plants (*Rhazya stricta*, *Enneapogon desvauxii*, *Citrullus colocynthis*, *Senna italica*, and *Zygophyllum simplex*) was used to highlight the bacterial diversity in the communities of these desert plants, as they also have an importance as folkloric medicinal plants, in addition to their important roles in the ecological and agricultural applications. The results of the bacterial community's taxonomy showed eleven phyla in each sample, and the most abundant were highlighted for further analysis as it was unassigned phyla. Results indicated that the most commonly known phyla were; *Actinobacteria*, *Proteobacteria*, *Chloroflexi*, *Firmicutes*, *Acidobacteria*, *Gemmatimonadetes*, and *Bacteroidetes*. Further bioinformatics and functional analysis of bacteria are needed to investigate their effectiveness in promoting plant growth and understanding symbiotic relationships under drought-stress conditions, meanwhile, introducing the pinpointed ones in this study will help in achieving this task.

Keywords: Drought stress; *Rhazya stricta*; *Enneapogon desvauxii*; *Citrullus colocynthis*; *Senna italica*; *Zygophyllum simplex*; metagenomics; plant growth-promoting bacteria (PGPB); environmental stress; desert plants.

1. INTRODUCTION

Arid and semiarid ecosystems exhibit about 40% of the worldwide surface area, The main concern in these ecosystems is water availability [1]. Plants that grow in arid and semi-arid soils suffer from a lack of water and nutrients, but these plants have developed various mechanisms by genes and proteins responsive to salinity and other abiotic stress factors to counter the harmful effects and to adapt to these stressful conditions [2,3,4]. Plant development may indirectly be affected by soil salinity, which can frustrate the activities of beneficial bacteria in the rhizosphere. However, the salinity-tolerant bacteria can extend the development of many crops that present in soils that affected by salinity, as observed at the halophytes [5,4,6]. Many research demonstrates that symbiotic bacteria have beneficial impacts on plant fitness and adaptation under diverse stress conditions [7,8,9,10,11,12], and the adjustment of the plant microbiome can productively improve the yield production of essential crops [12]. Soil microorganisms can adapt to specific abiotic stress conditions such as salinity, drought, heat, and other conditions and evolve tolerance to these stress factors [13,14], as the microbial communities that exist in the desert soil enter into a recession with the limited activities of the surrounding species, however, when the rain season starts, the bacterial species begin to reactivate relatively quickly and uniformly, especially in the soil crust [15]. Soils are the initial depository wherefrom roots recruit their microbiomes; the microbiome of drought-exhibited root with difficulty dependent on the response of soil microorganism diversity to moisture the soil-water limitation [16].

Soil chemistry also affect the various responses of bacterial communities through several factors such as pH, total nitrogen, and organic carbon content [17]. On the other hand, as a consequence of plant-microbe interactions that leads to different environment abiotic stress resistance which reflects positively on the host plants or in the associated microbial communities [18].

Molecular studies of the ecosystems have pinpointed that many microbial communities in the soil, which are unknown due to the inability to be cultured under the standard laboratory conditions [19]. Thus, the metagenomic approach highlights the understanding of bacterial society and its functions across different environments [20]. Advances in metagenomic analysis have given a broad illustration of the structure and function of both cultured and non-cultured bacteria, giving significance to the thought of their condition and development. The main focus of this study was to highlight the diversity of the isolated bacterial communities from the associated rhizosphere with the host plant, and how microbiome metagenomics influence the plant health and resistance to drought stress by using the metagenomics analysis.

2. MATERIALS AND METHODS

2.1 Sample Collection

We used the meta genomics tools to identify the bacteria that exist in the rhizosphere from the soils of five different desert plants; (1) *Rhazya stricta* plants member of the *Apocynaceae* family (2) *Enneapogon desvauxii* plants member of the *Poaceae* family (3) *Citrullus colocynthis*

plants member of the *Cucurbitaceae* family(4) *Senna italic* plants member of *Fabaceae* family (5) *Zygophyllum simplex* plant's member of *Zygophyllaceae* family, has been described by Prof. Nabih Baeshen based on the Plant collection of King Abulaziz University herbarium. The soil samples were collected from Hadda, Makkah-Jeddah old road, Saudi Arabia (Lat: 21.444271, Lon: 39.5316938) in June 2014 during the morning, where the temperature was 40°C. The soil samples were coded by A1, A2, A3, A4, and A5 respectively to the five plant species(Fig. 1).

2.2 Extraction of Genomic DNA and Metagenomics

Total genomic DNA were extracted from each soil sample by "MO BIO's Power Soil DNA Isolation Kit" and shipped to Beijing Genome Institute (BGI) in China for library preparation and next generation sequencing. Based on PCR results, original high-quality, library preparation was done by Illumina kits. Sequencing was performed on Illumina 16S Metagenomic Sequencing by HiSeq 2000 platform. Sequenced reads were checked for quality using FASTQC.

3. RESULTS

Our results determined the phyla total sequence reads were 3807, 1882, 2455, 2653, and 4213, respectively to sample codes (A1, A2, A3, A4, and A5). The taxonomic results of the bacterial communities gave eleven phyla in each sample. However, the most abundant were highlighted among the samples for further analysis, as they indicate that the unassigned bacteria were the most significant phyla as they appeared among the samples with an average of 36.28%, followed by Actinobacteria with an average of 34.39%, then followed by; Proteobacteria with an average of 15.44%, and finally Chloroflexi with an average of 2.78%. In contrast, the Firmicutes with an average of 3.61% did not exist in the rhizosphere of *Enneapogon desvauxii* and *Zygophyllum simplex* plants. The Bacteroidetes, with an average of 2.84% has not been found in the *E. desvauxii* and *Citrullus colocynthis* rhizospheres. In comparison, the Gemmatimonadetes with an average 2.29% did not appear in the *Rhazya stricta*, *C. colocynthis*, and *Senna Italica* rhizospheres. Acidobacteria, with an average of 1.89%, has not been found in the *R. stricta* and *Z. simplex* rhizospheres(Fig. 2).



Fig. 1. The distribution of the soil samples collocations, bacterial groups of the study were found in the rhizosphere of five different plants: (A) *Rhazya stricta*, (B) *Enneapogon desvauxii*, (C) *Citrullus colocynthis*, (D) *Senna italica*, (E) *Zygophyllum simplex*

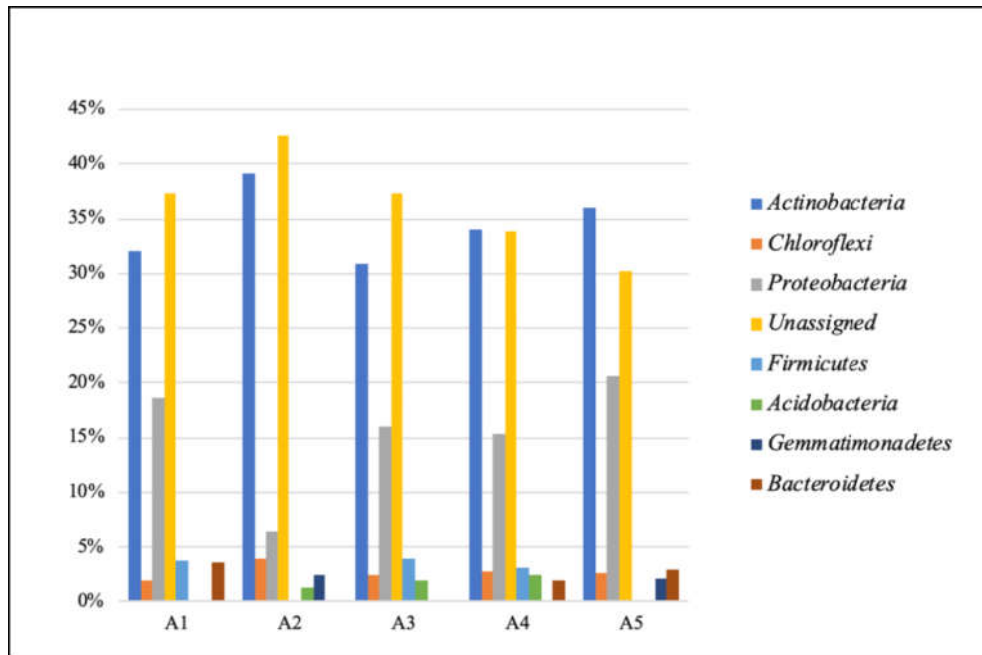


Fig. 2. The most abundant of the phyla among the samples

The dominant phylum in the *R. stricta* rhizosphere was unassigned bacteria (average; 37.36%) followed by; Actinobacteria (average; 31.97%), Proteobacteria (Average; 18.67%), Bacteroidetes (average; 3.58%), Firmicutes (average 3.75%), and finally Chloroflexi(average 1.92%) (Fig. 3-A1). While the most significant phylum in the *E.desvauxii* sample was unassigned bacteria (average; 42.60%), followed by; Actinobacteria (average; 39.20%), Proteobacteria (average; 6.41%), Chloroflexi (average; 3.99%), Gemmatimonadetes (average; 2.50%), and finally Acidobacteria(average; 1.26%) (Fig. 3-A2). The unassigned phylum was found with the most significant amount with an average of 37.30% in the *C. colocynthis* rhizosphere, followed by; Actinobacteria (average; 30.80%), Proteobacteria (average; 16.09%), Firmicutes (average; 3.95%), then Chloroflexi (average; 2.48%), and finally Acidobacteria(average; 1.91%) (Fig. 3-A3). The *S. Italica* rhizosphere had unassigned bacterial phylum as a dominant with an average of 33.92%, followed by; Actinobacteria (average; 34%), then Proteobacteria (average; 15.42%), Firmicutes (average; 3.13%), Chloroflexi (average; 2.86%), Acidobacteria(average; 2.49%), and finally Bacteroidetes (average; 1.96%) (Fig. 3-A4). The dominant phylum in the fifth sample of *Z. simplex* rhizosphere, was Actinobacteria with an average of 36%, followed by; unassigned bacteria (average; 30.24%), then Proteobacteria (average; 20.60%), Bacteroidetes

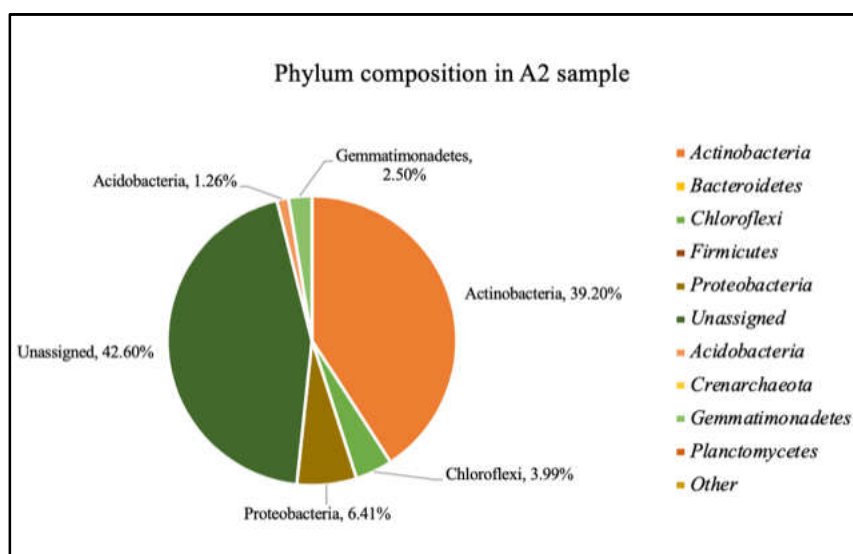
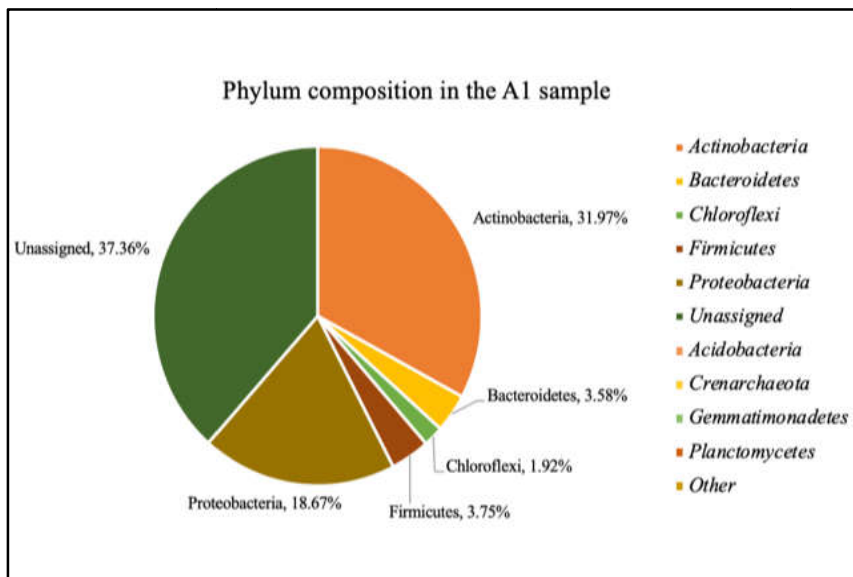
(average; 2.99%), Chloroflexi(average; 2.63%), and finally Gemmatimonadetes (average; 2.07%) (Fig. 3-A5).

4. DISCUSSION

The microbial communities associated with the different plant species in this study are shaped by the environmental conditions and the association with the host plants, including geographic site, plant phenotype and genotype, soil structure, and other environmental seasonal factors. This study demonstrates that bacterial communities associated with five different desert plants were essentially influenced by the plant type and the surrounded soils. This group of plants widespread in the desert region of the Kingdom of Saudi Arabia and considered as medicinal shrubs were used in the folk medicines to treat various diseases such as the use of the *Senna italica* plant to treat the infants diarrhea and to treat hair-related diseases [21], *Zygophyllum simplex* plant was used as an anti-inflammatory agent and for the treatment of gout and asthma [22]. Furthermore, *Citrullus colocynthis* plant used to react with different disorders and purposes such as a laxative, diuretic, insect bites, and skin diseases [23,24]. *Rhazya stricta* plant is used to treat various disorders such as diabetes, sore throat, syphilis, helminths, infectious diseases and rheumatism. Moreover, this plant has many properties such as anti-microbial activities, anesthesia and anti-

cancer effects [25,26,27]. However, the examination of organelle genomes of *R. stricta* may give more accurate information for the use of this plant or other medicinal plants in the fields of pharmacy and biotechnology [28]. *R. stricta* plant has been studied extensively as these studies have indicated important roles of the plant in C₃ photosynthesis under that harsh environmental conditions, which may be a major factor in adaptation to its environment [29,30], and so on for the other plants and the interacted microbiota which lives within the community *R. stricta*. Eight common phyla among all the collected plant species were indicated: *Actinobacteria* and *Proteobacteria*, *Chloroflexi*, *Firmicutes*, *Acidobacteria*, *Gemmatimonadetes*, and *Bacteroidetes* in addition to the unassigned phyla were obtained in all samples. These

various bacterial communities have more likely an important connection for the abundance of bacterial diversity in desert soils [31], and this will have an effect on many factors such as soil structure, nutrient uptake, plant variety, and the total production of the desert environments [32,33]. Under the drought stress conditions, plants depend on the interactions with the symbiotic bacteria to tolerate drought stress, considering the bacteria as significant tools used by the plant's tolerance against the stressful condition [34,3]. Therefore, these bacteria are considered as Plant Growth-Promoting Rhizobacteria (PGPR). PGPR plays a significant role in regulating the stress conditions and promoting directly the growth in plants by introducing the absorbing nutrients to the plant [35].



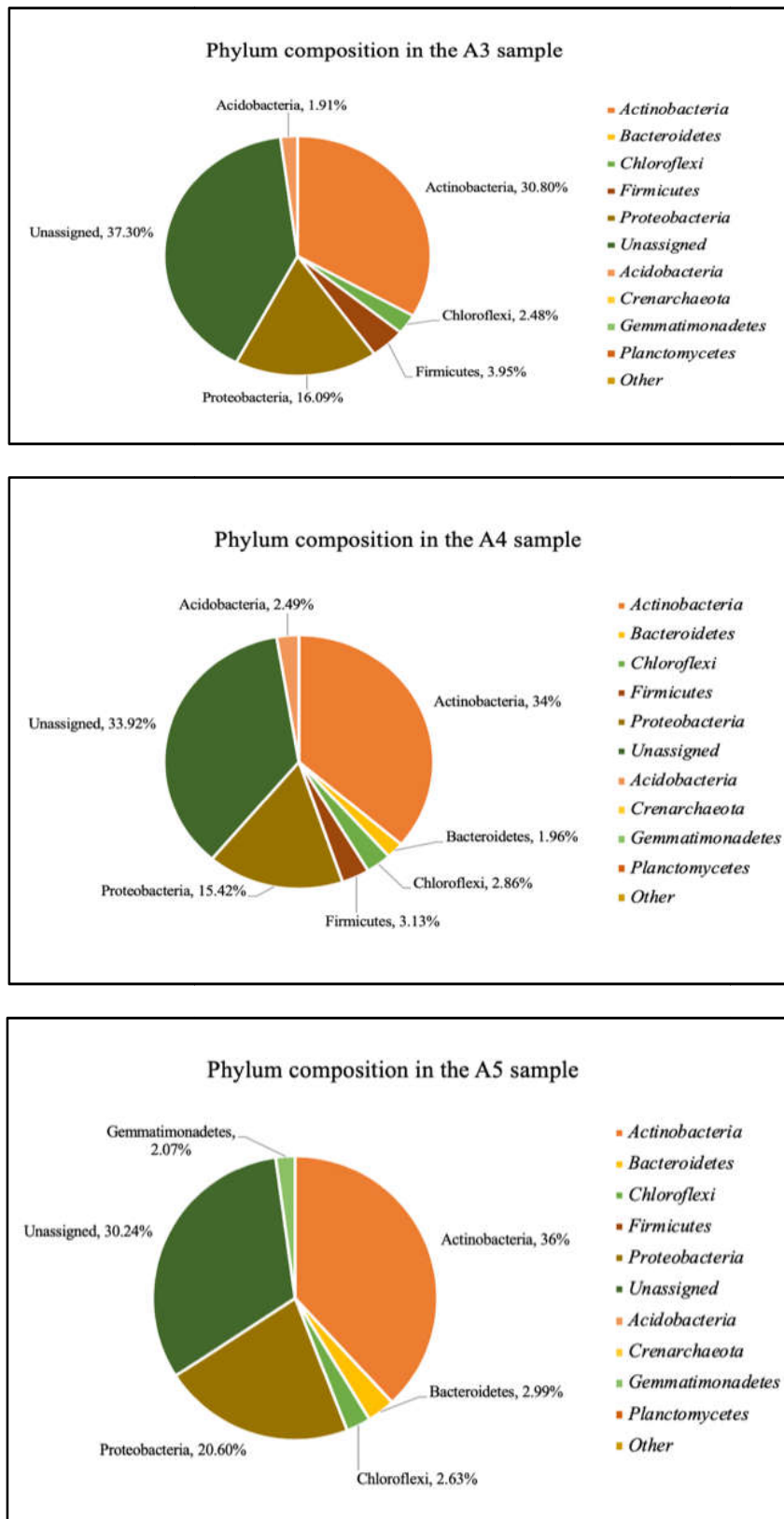


Fig. 3. The microbial communities in the five different soil samples: Phylum compositions of the rhizosphere of: (A1) *Rhzyastricta*, (A2) *Enneapogon desvauxii*, (A3) *Citrullus colocynthis*, (A4) *Senna italica*, (A5) *Zygophyllum simplex*

Often, the composition of the bacterial community is extremely affected by dehydration, and it was observed that the percentage of Gram-positive bacteria is much higher than Gram-negative bacteria [36,37]. Our results have validated this study by the metagenomics analysis. The results have indicated that *Actinobacteria* have the enormous bulk among the samples, while *Proteobacteria* and *Bacteroidetes* have appeared but with an insignificant amount among the samples (Fig. 2). Several studies indicate that this is because the Gram-positive bacteria can utilize the inorganic nitrogen to create extracellular enzyme that separates the complex organic enzymes compounds, which are moderately accessible in dry soil. Whereas, the Gram-negative have reproductive properties, since they incline toward contaminated carbon mixes and organic nitrogen, particularly as plant root secretions [38,39,40,41,42]. *Chloroflexi* have appeared in all the samples. *Chloroflexi* is an important and beneficial bacteria among the bacteria that present in drought soil, which they play a role in treating the plants by removing nitrogen (N) and phosphorus (P). Moreover, they break down the complex polymeric organic compounds into low molecular weight substrates to support their and other bacteria growth [43]. *Gemmatimonadetes* have appeared with more abundance in A2 and A5 samples. It has been reported that *Gemmatimonadetes* can adapt to the low soil moisture due to their capability to survive under aerobic and anaerobic conditions as they are also well-known as hypersaline microorganisms associated with biogeochemical transformations [44,45]. The existence of *Acidobacteria* with significant soil abundance indicates a decrease in its fertility, as they have appeared in the soils of A2, A3, and A4 samples. However, little is known about the physiology and metabolic functions of *Acidobacteria* [46]. *Firmicutes* have found with more abundance in A1, A3, and A4 samples. *Firmicutes* presented by *Bacillus* species can produce salt-stress compounds to tolerate salt, which causes osmotic pressures. It is worth noting that many studies have confirmed that species of *Bacillus* can grow in the desert soil broadly in relative proportions after stress [47,48].

Unassigned bacteria have appeared with high abundance among the samples. The emergence of the unclassified bacteria at the phylum-level may be due to the lack of a reference sequence in the database, and these bacteria might include a potential candidate that is still not identified.

These results indicate that the explored soil microbiome could enhance the plant resistance against environmental stress conditions, and this privileged feature is found among all the presented phyla members of the study. Although, a combination of diverse bacteria in each region seems to maintain the plant growth fitness and health under these harsh conditions.

5. CONCLUSION

Finally, further bioinformatic analysis and functional analysis need to be done to investigate the effect of this microbiome on the plant growth-promoting mechanisms and to understand the symbiotic relationships with the host plants under drought stress conditions at the molecular level.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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