

## Metagenomics of Bacterial Communities Associated with the Halophytic Plant

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### Abstract

Soil associated with plants host various microorganisms. These microbial communities interact with plants to adopt biotic and abiotic stresses promoting plants growth and development. These microorganisms have a potential promising future with the applications of commercial, medical and environmental biotechnology. Halophytes are salt-tolerating plants which can grow in soil or water with presence of high salt concentration and can also tolerate high drought and high temperature. In order to better understand the mechanism of salt and drought tolerance in halophytes, it is important to elucidate how the bacterial community interacts with halophytes. Recent advancement in the metagenomics techniques facilitates the analysis of genome of large number of uncultured and cultured bacteria from an environment in order to understand their composition and function. This review is to provide the latest development in the field of metagenomics associated with halophytic plants. We will present in-depth insights about the role of bacteria in abiotic stress management in halophytes and its future prospective in biotech applications.

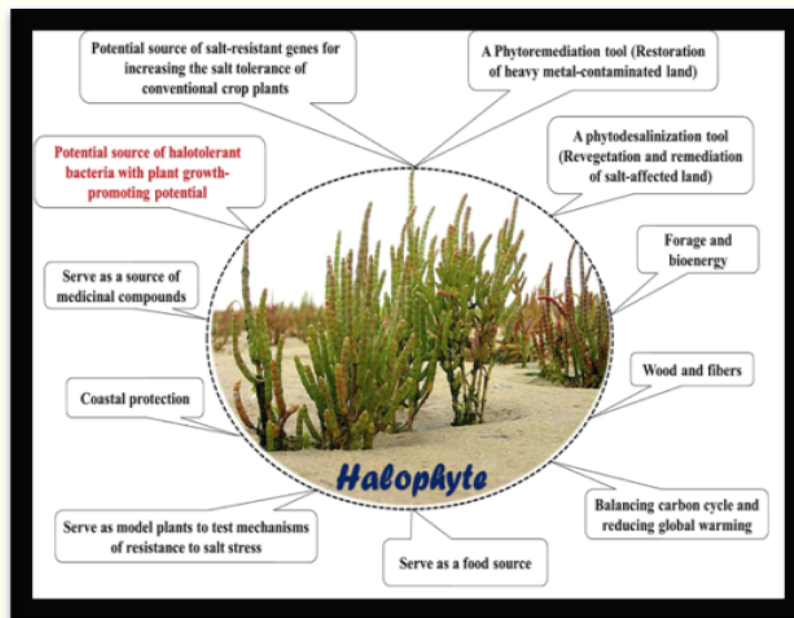
**Keywords:** Halophyte; Bacillus; Salinity; Biotic; Abiotic; Metagenomics

### Introduction

Soil is one of the most complex natural environments that have large numbers of diverse microorganisms. Soil provides a favorable ecosystem for the growth and development of many types of microorganisms [5]. On the other hand, these microorganisms are involved in the decomposition of biomass that contribute in the plant nutrition, growth and the maintenance of soil fertility [6]. Diversity and complexity of the microbial communities can vary according to the chemical and physical properties of the soil and its geographical location [7]. However, various soil ecosystems have not been investigated, and therefore a majority of microorganisms has not yet discovered [8]. Molecular investigations of ecosystems proved that most soil microorganisms are not known, because they could not be cultured at the standard laboratory conditions [9]. Thus, the limitation of non-cultured microbes poses a great challenge to study the diversity and composition of microorganisms in the soil. The composition of microorganisms can vary drastically among soil samples taken from different locations. It has been estimated that one gram of cultivated soil may contain up to  $2 \times 10^9$  microorganisms [10], while a gram of forest soil would contain  $4 \times 10^7$  microorganisms [11]. With the advent of metagenomics filed, it become possible to investigate the vast majority of non-cultured microbes across diverse environments on earth including soil, fresh water and ocean [12]. Therefore, using the means of metagenomics to study and analyze the total genomic DNA extracted from environmental samples is useful in discovering and exploring the world of microorganisms [13].

## Halophytes

Soil salinity is a major issue influencing crop production throughout the world [14]. It is estimated that one billion hectares (20% of the world's cultivated land and 33% of irrigated lands) are affected by high concentrations of toxic salts leading to crop decline [15]. However, there are plant species which resist or tolerate all these conditions and effectively adapt and grow in high salinity [16]. Halophytes are salt-tolerating plants which are able to grow in soil or water with presence of high salt concentration and could also tolerate the dry season [17]. Though, they constitute about 2% of terrestrial plant species, while the majority of plant species are glycophytes which cannot withstand against high salt concentration [18]. Halophytes are found in arid and semi-arid areas as well as wetlands with high salinity [19]. Halophytes are plants which may withstand abnormal amounts of salts from 1 to 33% [1,17]. Halophytes have been divided into two groups; Hydro-halophytes which are described by the plants that can develop in hydrous conditions or on wet soils while Xero-halophytes are described by the plants which may be seen in dry and saline soil [19]. Moreover, these plants have various benefits of maintaining the ecosystem including agrarian improvement, and ecological restoration in regions that influenced by salinity and sand dunes stabilization that located on the waterfront (Figure 1) [20,21]. They also provide food and sanctuary to a very large number of species including amphibian and reptiles [22].



**Figure 1:** Various benefits for halophytes [1].

Halophytes are armed with salt-responsive genes and proteins to counter the harmful effects of salinity [23,24]. Though, the mechanisms of salt tolerance in halophytes are not completely understood. However, halophytes evolve more advanced mechanism of osmotic adjustment using  $\text{Na}^+$  and  $\text{Cl}^-$  transporters, where  $\text{NaCl}$  and other inorganic salts are accumulated in the cell vacuoles, the organic solutes are accumulated in the cell cytoplasm [25]. Moreover, halophytes adjust themselves with soil salinity by various mechanisms, which are engaged with complex molecular, biochemical, physiological, and morphological changes (Figure 2) [1]. For example, A. Controlling the ion from reduction particularly ( $\text{K}^+$ ) ion [26]. B. Controlling the gene expression that is engaged in plant saltiness resistance, where halophytes can react to salt stress by up-regulating countless genes, and transcription factors [19,27,28]. C. Modifying photosynthetic pathways [29,30].

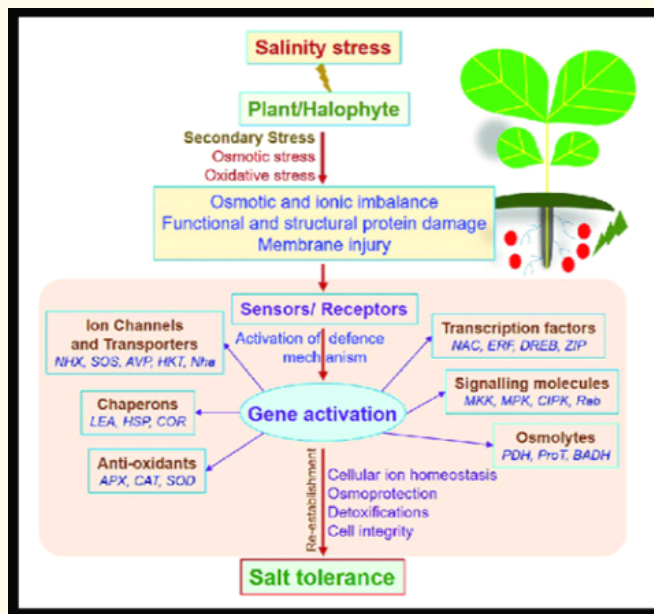


Figure 2: Adaptive strategies for salt tolerance in plants [2].

Soil bacterial communities associated with plants’ roots help them to adopt the survival in biotic and abiotic stress condition. Metagenomic approaches revolutionize the understanding of bacterial community and their function in diverse environment including soil, cold and hot deserts, forests, grasslands, and tundra [31]. The diverse bacterial communities associated with halophytes and their roles in salt tolerance have not been well explored so far. Therefore, metagenomic methods could be used to understand the diversity of bacterial community and their function associated with halophytes [32]. The detailed study will give an opportunity to characterize the bacteria and identifying the important genes helping salt tolerance in halophytes [33]. Understanding the mechanism of tolerating high salinity in halophytes would have potential to engineer the crop plants to grow in saline soil and seawater with high yield biomass. Therefore, studies on halophytes may provide potentials to grow more crops to feed demanding population of human and animals as well as using large body of seawater and saline soil which are not fit for agriculture (Figure 3) [1].

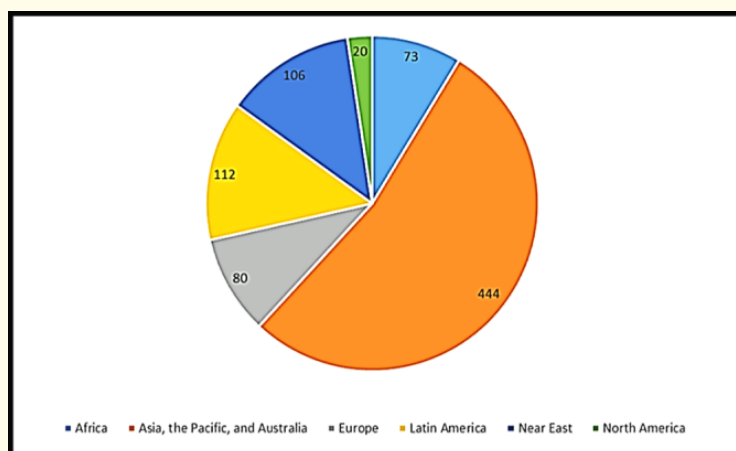


Figure 3: Regional distribution of salt-affected soils, in million hectares [3].

**The relationship between the halophytes and plant growth-promoting rhizobacteria**

Plant development might be affected by soil salinity through a roundabout way which can disserve the activities of beneficial bacteria that present in the rhizosphere [34]. The bacteria that tolerates the soil salinity are capable of extending the growth of many crops that present in salt-affected soils [35] and this is noticed at halophytes as they possess different types of bacteria at the aerial parts of the plant as so at the rhizosphere area that have many functions [36]. One of the most advanced researches to control and reduce the toxicity of soil affected by salinity is the use of Plant Growth-Promoting Rhizobacteria (PGPR) that present in the plant root. PGPR has effective role in reducing the stress conditions affecting plants by fixing atmospheric nitrogen into rhizosphere area [37], diseases resistance [38,39] and also the major ability to promote direct plant growth approach by nutrient absorption. The environmental conditions affect the capacity of PGPR to convert nutrients and increase plant tolerance to abiotic stress [40]. Moreover, the plant-bacterial cooperation lead the plant to format the microbiome in the rhizosphere and endosphere [41]. Under stress conditions, plants need the presence of associated bacteria to tolerate stress, so bacteria have been developed and become part of the plant biological system [42]. And so on, plants rely on bacteria to tolerate the stress conditions, especially the symbiotic bacteria, which may be an important factor in plant stress tolerance [1]. *Bacillus* species represents a good example of PGRP as they are considered as the most bacterial species that were isolated from soils because they can adapt to different environments at any conditions [43]. Moreover, many studies have confirmed that *Bacillus* species were found and isolated from desert soil under harsh conditions [44]. *Halopeplis perfoliata* (Forssk) is considered as an important halophytic desert plant, and as one of the dominant halophytes that grow on the Red Sea coastline with limited resources and abilities to resist and tolerate the saline conditions (Figure 4). The phenomenon of changing fruits and other parts of the plant to red is remarkable in *H. perfoliata*, and the expression of these segments may be related to the salinity tolerance (Figure 5) [45-47].



**Figure 4:** The area that the *Halopeplis perfoliata* grows, and it is clear that the area affected by salinity.



Figure 5: Halopeplis perfoliata resists the saline conditions.

Bibi and colleagues studied the bacteria that produce the hydrolytic enzymes from seven different halophytes including: *Salsola imbricata*, *Avicennia germinans*, *Avicennia marina*, *Halopeplis perfoliata*, *Halocnemum strobilaceum*, *Zygophyllum qatarense* and *Cyperus conglomeratus* [48].

These halophytes were collected at the coastal area of Thuwal, Saudi Arabia. They used the carboxymethyl cellulose (CMC) agar medium to isolate the bacteria, and later on the 16S rRNA sequence was used to identify the bacterial strains (Table 1).

PGRP genera	Specific Strains	Mechanisms	Reference
<i>Acinetobacter</i>	<i>A. calcoaceticus</i>	Gibberellin production, phosphate solubilization	[50]
<i>Azetobacter sp.</i>	<i>A. paspali</i>	Nitrogen fixation, auxin production	[51]
<i>Azospirillum sp.</i>	-	Nitrogen fixation, increase in root development	[52]
	<i>A. brasilense</i>	Auxin production	[53]
<i>Bacillus sp.</i>	<i>B. amyloliquefaciens</i>	Release of volatiles (antifungal)	[54]
	<i>B. cereus</i>	Antibiotic production	[52]
	<i>B. subtilis</i>	Release of volatiles, antibiotic production, activation ISR	[54, 55]
	<i>B. thuringiensis</i>	Biocontrol against insects	
<i>Bradyrhizobium sp.</i>	-	Nitrogen fixation/root nodulation	[52]
<i>Enterobacter sp.</i>	<i>E. cloacae</i>	Release of volatiles	[56]
<i>Paenibacillus</i>	<i>P. polymyxa</i>	Activation ISR	[57]
<i>Pseudomonas sp.</i>	<i>P. fluorescens</i>	Auxin production, activation ISR, plant iron nutrition	[52, 56]
	<i>P. putida</i>	Biocontrol	[58]
<i>Rhizobium sp.</i>	-	Nitrogen fixation/root nodulation, production of ACC deaminase	[52]
<i>Sphingomonas sp.</i>	=	Chitinase production	[59, 60]
<i>Streptomyces sp.</i>	-	Antibiotic production, siderophore production, auxin production (in planta tests still necessary)	[61]
	<i>S. platensis</i>	Volatile production (antifungal)	[62]

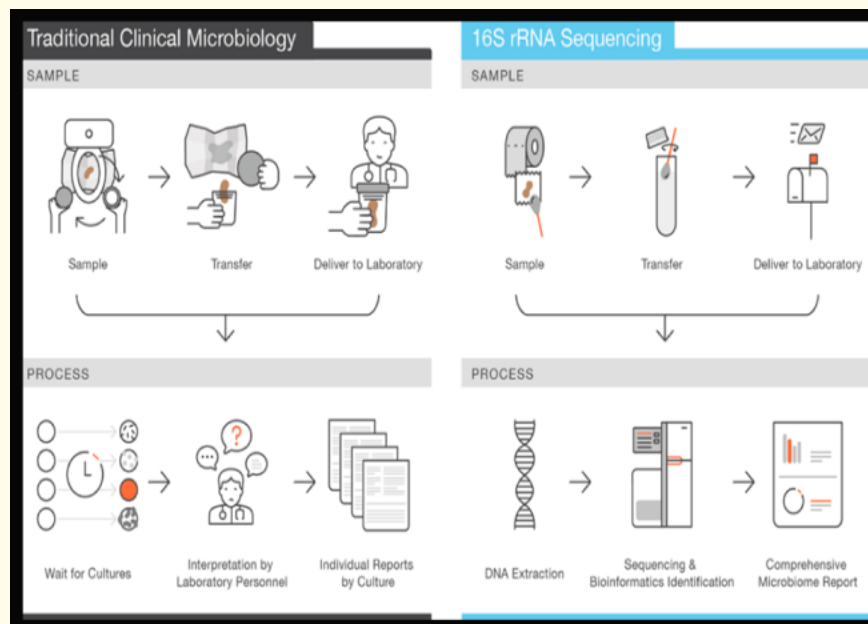
Table 1: The most common (PGPR) and their mechanism.

### Significance of metagenomics

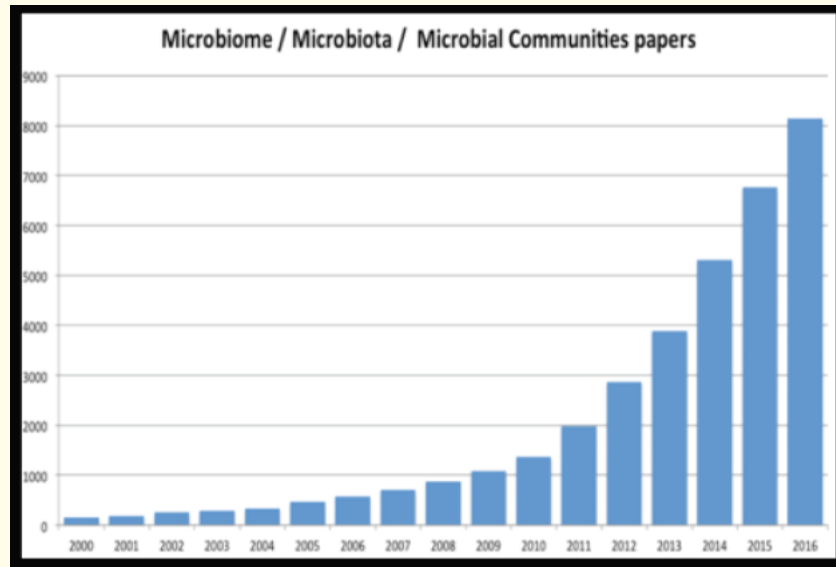
Metagenomics field became an essential method to identify and classify the uncultured microbiota [12]. Before metagenomics was known, several traditional methods were used to discover new strains of bacteria [63]. These traditional methods includes gram stains, physiological and biochemical methods which they rely on the phenotypic properties of bacteria [64]. However, these methods can be used only for isolates that can be cultured in the laboratory, but not the unculturable ones [65,66]. Metagenomic approaches could facilitate the discovery of new strains, novel genes, antibiotics, metabolites and proteins with novel function that would be useful in biotechnological industries [67].

### Metagenomics and the discoveries of uncultured bacteria

Over 70% of bacteria that are present in an environment cannot be cultured readily [9]. The majority of these bacteria are significant players in cycles of vitality, and they can live as free-living, symbiotic or as parasitic organisms. Researchers have started to expose this immense bacterial world by metagenomic techniques which are effective more than using traditional molecular biology techniques (Figure 6) [4,68]. Moreover, the investigation of uncultured bacteria has given an important bit of knowledge into disregarded catalysts that could not be discovered by traditional techniques (Figure 7) [69]. The field of metagenomics has demonstrated productive methods pinpointing the uncultured bacteria [12]. The advancements in the metagenomic analysis have given an extensive picture of the structure and function of both cultured and uncultured bacteria, which provides a significance to consider their environment and development. Moreover, the improvements of the PCR, progress in DNA sequencing innovation, and utilization of the 16S rRNA quality as a taxonomic marker, have authorized to discover genes of bacteria that were in various environmental samples [70]. Exploring the genetic identity of uncultured bacteria can be done through total genomic DNA isolation from collected samples, amplifying, and sequencing the fragments of the 16S rRNA gene that elected by primers [71]. Metagenomics approach have the credit for the development of molecular biology techniques, especially in the uncultured bacterial field, and the evolution in metagenomics field will ensure further discoveries in near future [12].



**Figure 6:** The traditional method versus 16S rRNA gene sequencing [4].



**Figure 7:** Evolution of the number of microbiome. Microbial communities papers as registered in PubMed (microbiomedigest.com, 9 January 2017).

### Future Potential and Perspective Applications

Several metagenomics studies were carried out to understand the soil microbiome of different ecosystems, however, very few studies were focused on the soil microbial communities associated with halophytes. Furthermore, it is also imperative to understand how the soil microbiome interacts and influences halophytes to sustain in the abiotic stress, and how the halophytes response to the microbiome. Therefore, in addition to metagenomics, transcriptome analysis of halophytes is also required to understand the interaction between microorganism and halophytes at molecular level. In the other hand, the future of industrial, medical, environmental biotechnology will depend on the field of metagenomics and environmental genomics hence for the fields of agriculture, animal feed, aquacultures and bio-energy.

### Concluding Remarks

As the Kingdom of Saudi Arabia is characterized by unique environments starting from the harsh desert of Arabia, passing by the saline areas and salt marshes and ending by the Red Sea which is known by its unique diversity, therefore, these rich environments must be investigated to explore the different worlds of microbiota and their abilities to improve the future of humanity and its resources. Microbiomes are the treasures hidden in nature, and now is the time to unveil them and interduce them to the interested scientific communities who have the knowledge for corresponding methodologies.

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