Egypt. J. Bot. **Vol. 62**, No. 2, pp. 493-505 (2022)

Egyptian Journal of Botany
http://ejbo.journals.ekb.eg/

Influence of Irrigation Water on the Diversity and Distribution of the Endophytic Bacterial Microbiome Associated with *Mentha longifolia*; Metagenomics Profiling

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MENTHA longifolia is one of the most vital medicinal and economic plants in Saudi Arabia. This study aimed to describe the bacterial endophytic diversity associated with the leaves and root of *M. longifolia* from two different locations in Al-Madinah region, Saudi Arabia, using metagenomics approach, then, correlate their deviation with the environmental stress conditions. Chemical analysis of the essential oils from *M. longifolia* leaves revealed the predominance of carvenone in the first location (Abyar-Ali) and pulegone in second one (Abyar-Almashi). When the irrigation water in the two locations was chemically analyzed, the results showed high content of total dissolved solids in Abyar-Ali area compared to Abyar-Almashi. On the other hand, Abyar-Almashi irrigation water had higher levels of the total organic nitrogen. Illumina MiSeq analysis of the endophytic microbiome using V3-V4 amplicons of the rRNA gene detected 114 distinct OTUs. These 114 OTUs were grouped under eight different bacterial phyla most of which belonged to Proteobacteria followed by Cyanobacteria. Within the eight phyla, all taxa were clustered into 14 class, 25 order, 41 family and 57 genera. Among the detected endophytic microbial taxa, nitrogen fixing bacteria that play a crucial role in pulegone biosynthesis, *Pseudomonas*, as well as *Enterobacter*, both excellent degrader of organic compounds were found with high abundance in Abyar-Almashi. While genus *Bacillus* long with other genera, either dominated or were unique to Abyar-Ali in response to the high salt stress in that location. Collectively, the previous results designate that, the dynamics in the number, type and distribution of the bacterial endophytic population between the two areas varies in response to the type of stress to which the plant is exposed.

Keywords: Endophytic bacteria, *Mentha longifolia*, Metagenomics, 16S rDNA.

Introduction

Endophytes are group of microorganisms that inhabit various host plant tissues in a commensal or beneficial association without showing any symptoms. Endophytes commonly comprise different types of microbes including bacteria, fungi and actinomycetes (Kaul et al., 2016). Endophytes greatly affect plant's health and yield (Tian et al., 2015; Abd El-Megeed & Mohiy, 2022). The complex relations between the microbe and its host is valued source of new metabolites and biotechnologically important products (Kaul et al., 2016). Those metabolites are the same or similar to their associated plant enzymes (Heinig et al., 2013). The host plants benefit from their endophytes as they help with nutrient solubilization and absorption, improving plant growth in addition to increasing their tolerance to both biotic and abiotic stress as well as soil contaminants (Rodriguez et al., 2008; Alvin et al., 2014).

Mentha (mint) is a pharmaceutically important plant that is cultivated worldwide, it is classified as genus in family Lamiaceae and contains about 42 species. *Mentha* naturally grows in Europe, Africa, Asia, Australia, and North America (Kręgiel et al., 2018). Because it has many medical benefits as well as refreshing odor and taste, mint is one of the most common herbs and it is widely used in food preparation, as well as cosmetics manufacturing (Saeed et al., 2006). Different *Mentha* species

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Received 27/10/ 2021; Accepted 07/03/ 2022
DOI: 10.21608/ejbo.2022.102867.1821
Edited by: Prof. Dr.: Wael S. El-Sayed, Faculty of Science, Ain Shams University, Cairo, Egypt.

are used as treatment for ulcerative colitis, throat infection, bronchitis, nausea and liver problem (Işcan et al., 2002). Furthermore, *Mentha* essential oils are reported to have very effective antioxidant, antifungal, and antibacterial activity against bacterial growth (de Sousa Barros et al., 2015).

Mentha species are well studded considering their medical and pharmaceutical properties, or otherwise for their essential oils composition and effects. However, no published data discussed *Mentha* microbiome. Moreover, very few studies covers *M. longifolia* in Saudi Arabia. The metagenomics is an important and helpful technique that allow the detection and analysis of the entire microbial population including both culturable and unculturable endophytic communities (Dinsdale et al., 2008). It escapes the necessity and disadvantages of isolation and cultivation methods (Kaul et al., 2016). Previously, metagenomics analysis have enhanced the understanding of the microbial population diversity in several environments including plants (Akinsanya et al., 2015). Such studies are important to elucidate how internal microbes affect stress tolerance and promote its host plant growth (Kaul et al., 2016). Implying metagenomics in analyzing the multiplicity of mint microbiome will deliver improved understanding of *M. longifolia* growth requirements and will emphasize the great prospective of the endophytic microbiome in enchanting plant growth, production and improvement of plant stress tolerance. This study is aiming to analyze the endophytic microbiome inhabiting *M. longifolia* leaves and roots; in addition to investigating the effect of irrigation water on their diversity and distribution in Medina region, KSA using metagenomics approach. We understand that, this is the first article to investigate both cultivable and uncultivable bacterial population inhabiting this plant.

Materials and Methods

Plant sample preparation

The *M. longifolia* plants were collected from two different sites in Al- Medina region, Saudi Arabia, Abyar-Ali (24°25'33.6"N, 39°33'52.8"E) and Abyar-Almashi (24°06'51.8"N, 39°38'09.2"E). Good looking healthy plant materials were randomly selected directly from the field. Each sample was thoroughly washed until all soil particles were removed then left to dry. The plants were detached to leaves and roots, then surface sterilized according to Anjum & Chandra (2015), with some modifications. Briefly, plant parts were submerged in 70% ethanol for 2min, then treated with sodium hypochlorite (2.5%) solution (1:30min for leaves and 3min for roots). Samples were then washed three times in sterile distilled water and dried out. One hundred fifty microliters of the last washing water were cultivated onto nutrient agar plates, to ensure the efficiency of the surface sterilization technique. Any bacterial or fungal colonies in the inoculated plates within 24-72h of incubation (30°C ± 2°C) indicates ineffective surface sterilization and samples discarded.

Isolation of microbial community metagenome

One gram from the different sterile plant parts were separately mashed with glass beads in phosphate-buffered saline (pH 7.0) to crush tissue. The resulted slurry was transferred to a sterile 50mL falcon tube and placed in vortex for one hour in the refrigerator to release the microbes out of the tissues. The resulting homogenized solution was filtered through sterile gauze to remove the host plant large material. The pooled solution was then centrifuged at 14,000rpm for 5min to obtain endophytic microbial sediment. Later, the total genomic DNA of each sample was extracted from the endophytic microbial community using ZymoBIOMICS™ DNA Miniprep Kit following the manuals' instructions. The purity and concentration of the collected genomic DNA was checked at Å260/280nm using (NanoDrop 2000c spectrophotometer, Thermo Scientific, U.S.A.). DNA samples integrity was detected using 0.8% agarose gel. Samples stored at −80°C for further use in the metagenomics sequencing analysis.

Metagenomics analysis

The bacterial community was identified based on ribosomal DNA (16S rDNA) sequencing, using the next generation MiSeq Illumina Sequencing Platform available at Beijing Genomics Institute (BGI), China.

Illumina library preparation

Four qualified metagenomics DNA samples were used to construct a library, leaves (MA1) and roots (MA3) from Abyar-Ali; Leaves (MM1) and roots (MM3) from Abyar-Almashi. The hyper variable V3-V4 regions (400bp length) of the bacterial 16S rRNA gene were amplified. Two fusion primers that included, specific index sequence for each sample; the Illumina adapter; as well as the universal primers 341F (GCCTACGGGNGGCWGCAG) and 806R (ACTACHVGGGTATCTAATCC),

were used to generate the amplicon library. Each PCR reaction (50µL) has 30ng of metagenomics DNA, and the PCR protocol was as follows, initial denaturation at 98°C for 3min then 30 cycles of 45sec at 98ºC, 45sec at 55ºC, and 72ºC for 45sec, finally an extension step at 72°C for 7min. To remove the unspecific amplicons from the PCR library, the products were purified with Ampure XP beads (AGENCOURT). Library quality and size distribution was determined using the Agilent 2100 bioanalyzer. Each library was qualified by real-time quantitative PCR (EvaGreen™). Only the qualified libraries were loaded and sequenced pair end onto Illumina MiSeq platforms for a 2×300 paired end sequencing with dual index reads.

Processing of sequencing data and data analysis

The raw sequence data (forward & reverse read) were trimmed and filtered to eliminate reads with adapter contaminated sequences, barcodes, primer pollution, ambiguous and low-quality bases to obtain clean reads. Then the high-quality paired-end reads with overlap were combined to tags. Sequences with high quality were analyzed for chimeric reads, and cleaned out by UCHIME v4.2.40 (Edgar et al., 2011). The resulting tags were grouped into operational taxonomic units (OTUs) with 97% clustering threshold using UPARSE pipeline (Edgar, 2013) then the OTU abundance was calculated using USEARCH. Then, representative sequence of each OTU was taxonomically classified using UNITE database (Abarenkov et al., 2010), using 0.6 confidence values as cutoff. Venn diagram; the OUT relative abundancoye histograms and OTU rank curve illustration as well as the principal component analysis (PCA) and species heatmap analysis calculations were generated using software R (R Core Team, 2018). OTUs at the genus level were used to reconstruct phylogenetic tree using the QIIME v1.80 (Caporaso et al., 2010) and imaged by software R v3.1.1.

The complexity of taxa diversity was calculated in both alpha and beta directions. The rarefaction curve based on Shannon analysis calculations was used to evaluate the alpha diversity in each sample, the curve was drawn by software R v3.1.1. Bray-Curtis analysis was calculated by QIIME v1.80 software (Caporaso et al., 2010) to measure beta diversity.

Chemical analysis: The chemical analysis was performed at the national research center, Egypt.

Irrigation water chemical analysis

Water samples from each sampling site were collected and chemical analysis was carried out to measure the nutrients and salt concentrations in each sample. Chloride concentration was measured according to the argentometric method (Yoder Lester, 1919), while the total phosphorous was measured calorimetrically according to Vanado-Molybdate method (APHA, 2005). The standard macro-Kijeldhal colorimetric method was used to measure the total Kjeldahl Nitrogen (TKN). Ammonium (NH4–N) concentration was determined by the salicylate method (Kempers & Kok, 1989) and measured at 650 nm using spectrophotometer. Nitrate was analyzed using sodium salicylate method. Sodium (Na), Calcium (Ca), Magnesium (Mg), Sulfates (SO4), and Potassium (K) were measured according to Ion Chromatography method (Dione X ICS-5000T DS-Thermo).

Essential oil components determination in the plant

Aerial parts of mint of both samples were hydro-distillated for 3 h, using Clevenger-type apparatus. The extracted essential oils were analyzed using gas chromatography–mass spectrometry GC-MS (Laboratory Equipment Company [LECo], USA), and separated on trace GC ultra-chromatography (Thermo Scientific, USA), equipped with ISQ-Mass (Thermo Scientific, USA) and TG-5MS capillary column (Thermo Scientific, USA). The separation program was as follow; 50°C for 3min and temperatures increase at rate 4°C/min to 140°C then isothermal hold for 5min, followed by 6ºC/min to 260°C with 5min isothermal hold. The carrier gas was helium, and the ionization energy was set at 70 eV. The national institute of standards and technology (NIST, USA) library was used for compounds identification.

Results

The present study aimed to determine the endophytic bacterial communities associated with *M. longifolia* cultivated in two locations (Abyar-Ali and Abyar-Almashi) in Medina region, KSA that are irrigated with two different types of water. Morphological variation between leaves and roots samples collected from different sites was observed. Abyar-Ali leaves were bigger and have light color (Fig. 1a) with strong odor and the plants' roots were more branched than Abyar-Almashi samples (Fig. 1b).

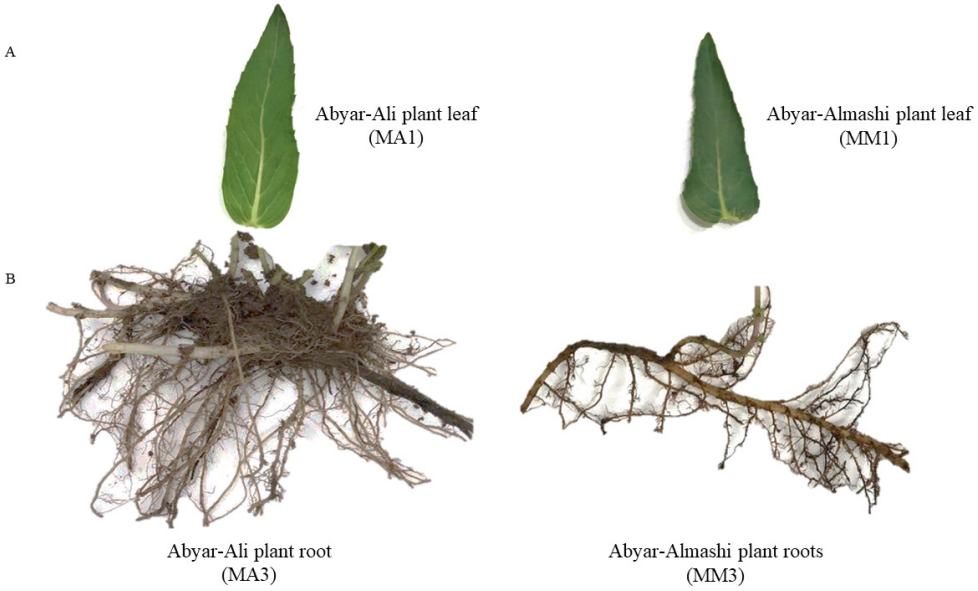

Fig. 1. Variations in the morphology of *M. longifolia* plant grown in two separate locations, (A) Large size and light color of the leaf detached from the plant grown in Abyar-Ali compared to that grown in Abyar-Almashi; (B) root branching of *M. longifolia* plant grown in Abyar-Ali compared to that grown in Abyar-Almashi

Chemical analysis

The analysis of irrigation water clarified that, the water from Abyar-Ali had high total dissolved solids (TDS= 3000 mg/L) content compared to that of Abyar-Almashi location (800 mg/L). The analysis also showed that Abyar-Ali has higher levels of salts such as Sulfate (200.9 mg/L); Calcium (514.55 mg/L) and Sodium (765.86 mg/L) compared to Abyar-Almashi that reported (65, 97.7 and 146.89 mg/L) from each salt respectively. Abyar-Almashi irrigation water on the other hand had higher levels of the total nitrogen (9.8 mg/L) compared to (5.7 mg/L) in Abyar-Ali.

Chemical analysis of the volatile oils in the plant leaves from each site showed that Abyar-Ali samples had higher relative abundance levels of carvenone (16.8 Peak Area %) compared to (1.49 Peak Area %) in Abyar-Almashi samples. Alternatively, Abyar-Almashi had higher relative abundance levels of the pulegone (13.42 Peak Area %) compared to (0.83 Peak Area %) in Abyar-Ali. Both samples had similar levels of 2-pinene-4-one (~ 14 Area %) with 18 other compounds that represented very small percentage.

Metagenomics data

The main targets of this study were to define the diversity of *M. longifolia* bacterial endophytes and to assess the effect of irrigation water on their population complexity. The metagenome profiling targeting the V3-V4 region of the 16S ribosomal RNA gene revealed high level of bacterial diversity. The raw sequence reads were (362703*2). After quality processing of initial sequence, a total of (308709*2) high-quality clean reads were obtained. Based on overlaps, the clean sequence was combined into 307576 tags in total, with average 76894 tags per sample and the average length was 446 bp. The tags were assigned to 114 different OTUs across all four samples after clustering at a 97% similarity level. Obviously, the root samples had higher richness than the leaves represented in the number of OTUs, detailed information about the sequencing data is found in Table 1.

According to Venn diagram analysis, a reliable overlap patterns of OTU clusters among different samples were obtained (Fig. 2). From the total 114 OTUs obtained, 29 OTUs representing about 25.4%, were common to both regions, nine were found to be unique to the samples collected from Abyar-Ali (MA) while 28 unique OTUs observed in Abyar-Almashi samples (MM) none of which were unique to the leaves while 39 OTUs were distinctively found in the root tissue. The root samples from the two areas shared high percentage of OTUs (30.5%), in contrast, the leaves shared a lower number of OTUs (5.2%).

TABLE 1. Sequencing data and OTUs of endophytic bacteria in *M. longifolia* tissues at 97% similarity. OTU numbers per sample primarily represents the degree of sample diversity

Sample name	Raw data (Mbp)	Clean data (Mbp)	Raw reads	Clean reads	Connect tag number	Average length/ SD[π]	Tag number	OTU[£] number	Shannon index[*]
MA1	52.64	43.97	88478*2	78537*2	78424	445/1	169	1	0
MA3	53.23	42.44	89620*2	75886*2	75528	446/3	712	38	2.807556
MM1	57.66	45.16	97241*2	80771*2	80537	446/3	1177	18	1.809075
MM3	51.72	41.01	87364*2	73515*2	73087	448/6	5637	57	2.321069

[*] Alpha diversity statistics using Shannon index

[π] Stander deviation

[£] Operational taxonomic units

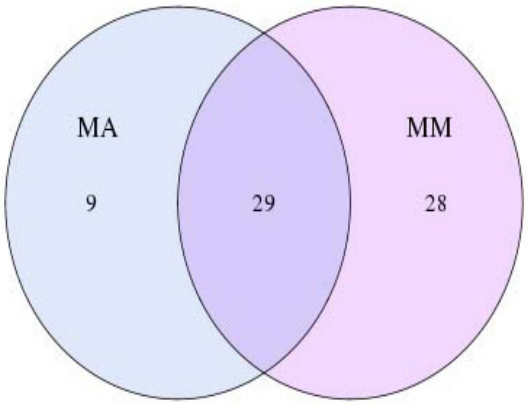

Fig. 2. Venn diagrams analysis showing the number of shared/unique OTUs across the two sampling locations Abyar-Ali and Abyar-Almashi

The OUTs composition between the two sample groups showed a discrete separation based on the sampling area as illustrated by the principal component analysis (PCA). It also indicated that the diversity between the samples from Abyar-Ali is higher than that between Abyar-Almashi samples (Fig. 3).

Rank abundance analysis showed that MA3 as well as MM3 has the best species richness followed by MM1. The analysis also showed that all the samples displayed some level of abundance with one or more of the detected OTUs as indicated by the variable levels of steepness in their rank curve gradient with the sample MM1 showing the lowest level of evenness (Fig. 4).

The considered diversity indices that calculated the Alpha-diversity, indicated that, the highest level of bacterial complexity (expressed as species diversity) was observed in MA3 followed by MM3, however no complexity was observed in MA1, Shannon estimator analysis values are shown in Table 1. On the other hand, beta diversity distance analysis calculated based on the OTU data pointed out that the bacterial population in MM is discretely separated from MA samples as indicated in the Bray-Curtis distance matrix based heat-map (Fig. 5), but it shows relatively similar OTU composition between the leaves and the root from Abyar-Almashi area which confirms the PCA analysis.

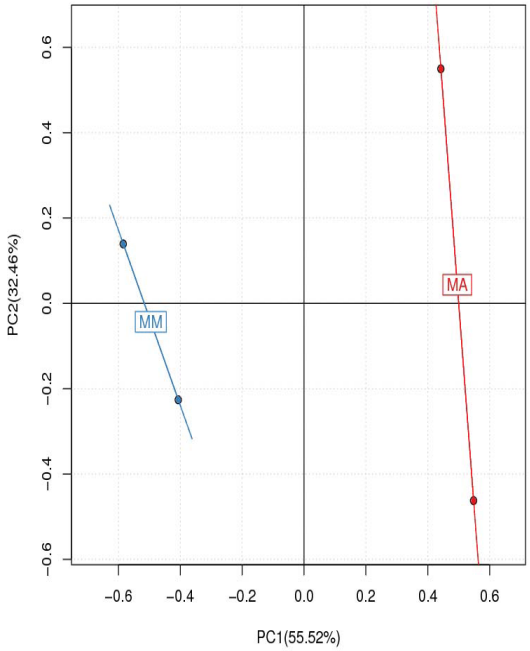

Fig. 3. PCA analysis based on OTU composition showing the diversity among samples [X-axis represents the 1st principal component while the Y-axis is the 2nd principal component. The assorted colors signify diverse groups (red is Abyar-Ali; blue is Abyar-Almashi), and a dot denotes the different samples]

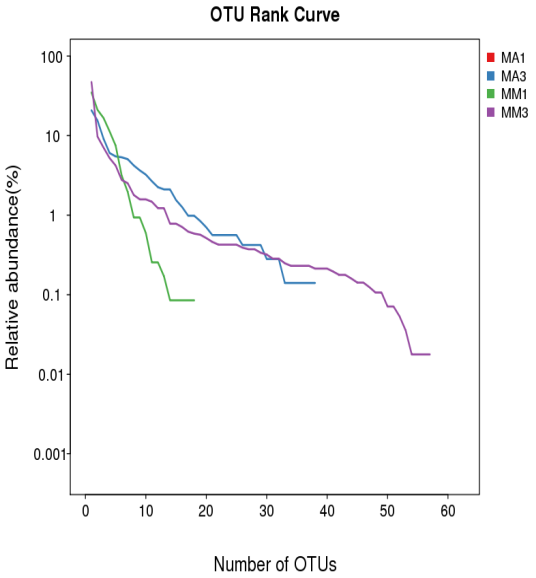

Fig. 4. OTUs rank curve showing species richness that can be viewed as the number of distinct species on the X-axis, while the numbers on the Y-axis represents the log of the OTU relative abundances

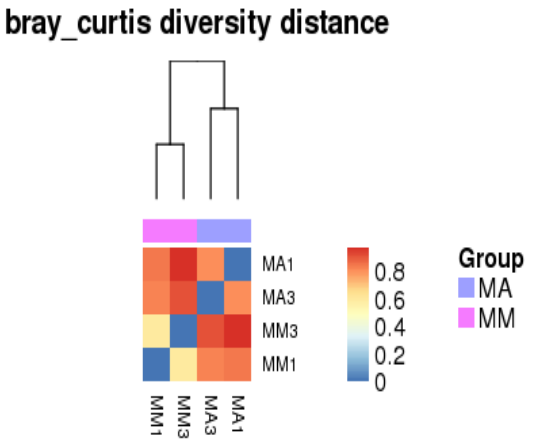

Fig. 5. Bray Curtis analysis represented as heat map showing beta-diversity distance distribution among samples, it reflect the differences between any two communities [Bigger index indecates greater difference]

Taxonomic analysis that included species composition and abundance indicated that the 114 OTUs detected in the metagenomes isolated from the four different tissues of *M. longifolia* were taxonomically grouped into eight different bacterial phyla, mostly (78.8%) belong to Proteobacteria that were found to be represented in three samples (MA3, MM1 and MM3) at different rates but it was not detected in the last sample (MA1). The remaining (21.2%) were classified as; Cyanobacteria (8.7%); Firmicutes (4.45%); Actinobacteria (4.2%); Bacteroidetes (2.3%); TM7 (1.15%); Verrucomicrobia (0.3%) and finally with very small percentage Acidobacteria (0.026%). This was also confirmed by the phylogenetic analysis as shown in Fig. 6 that represents the evolutionary relationship between the identified genera.

At the class level 14 different classes were categorized, with noticeable percentage (35.0%) of unclassified OTUs. Alphaproteobacteria was dominant in three of the samples with the highest relative distribution (36.9%). The abundance analysis showed that the absolute number of classified orders is 25, however the OTUs with less than 0.5% abundance in all samples were grouped into 'others'. More than third (38.9%) of the OTUs were not classified into a defined family, however the remaining (61.1%) were successfully categorized into 34 different families. Families *Enterobacteriaceae* (29%) and *Moraxellaceae* (22%) were dominant in MM samples, while family *Bacillaceae* (12.4%) dominated MA samples. Genus *Enterobacter* showed relatively high prevalence among *Enterobacteriaceae* of MM. On the other hand, genus *Bacillus* of the family *Bacillaceae* was found in MA samples in high level. At the genus level, 57 genera were determined, 40.6% of them were not classified into a defined genus. Samples from MM had relatively high abundance of genera *Enterobacter* and *Pseudomonas* with the genus *Comamonas* unique to that sampling location. The absolute abundance of each genus across samples are presented in Fig. 7.

Most of the OTUs (70.5%) were not classified under any previously identified species which indicate that *M. longifolia* is rich with new species, with the plants from Abyer-Ali harbor higher percentage (90%) of unclassified species than Abyar-Almashi plants that had 47.4% of its endophytic bacterial population as unknown species. The root and leaves samples of Abyar-Almashi were highly populated with the species *Enterobacter hormaechei* (47.1%) and *Acinetobacter johnsonii* (34.8%). While the species *Pseudoxanthomonas mexicana* was uniquely found in the root microbiome. On the other hand, *Lysinibacillus massiliensis* and *Sphingobacterium multivorum* were unique to the root sample of Abyar-Ali. *Methylotenera mobilis* and *Mycobacterium vaccae* are common species between the root samples.

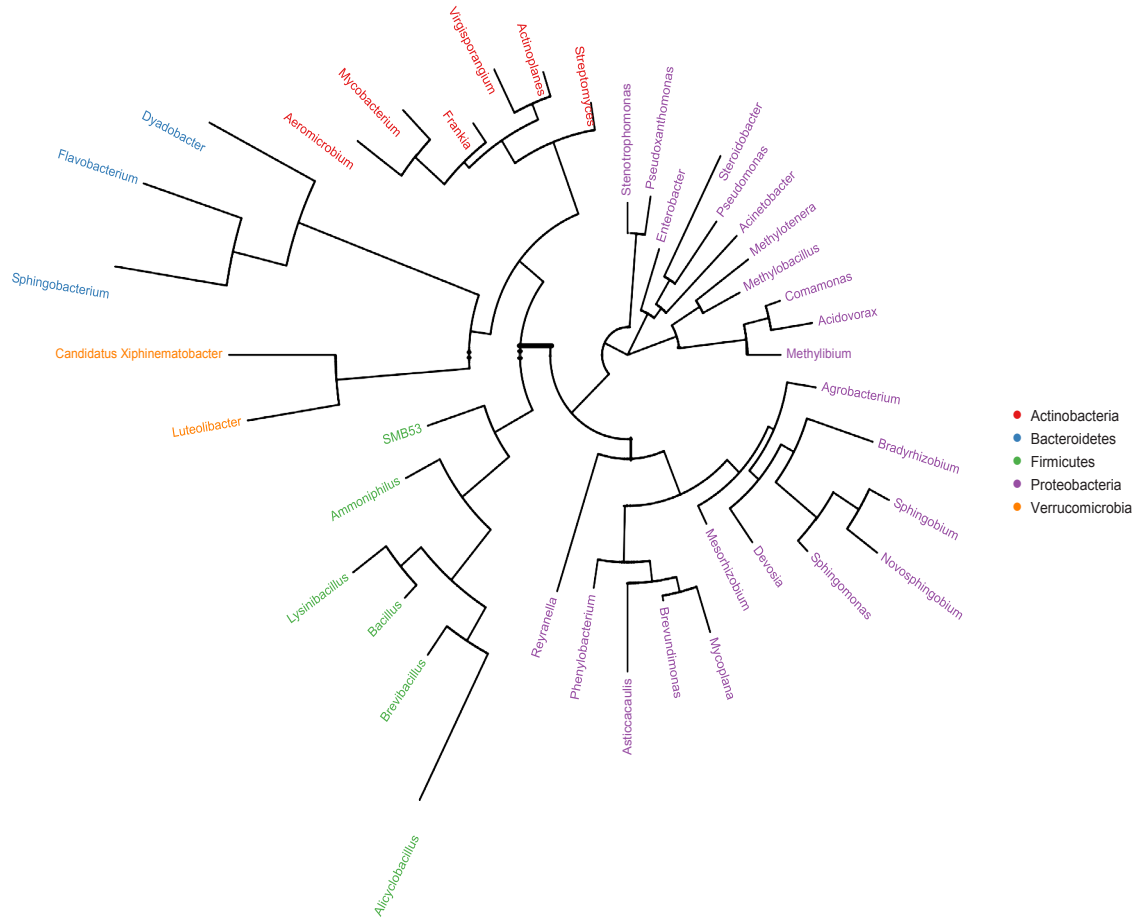

Fig. 6. Phylogenetic tree at the genus level across all samples using QIIME (v1.80) [The genera of the same phylum are shown in the same color]

Fig. 7. Taxonomic composition distribution histograms, the bacterial population of each sample were shown at the genus level [For the unclassified genera, the closest classified level is stated between brackets]

Discussion

The underestimation of non-cultivable microorganisms beneficially living inside the plant tissue and the limitations of direct isolation techniques has become more and more relevant with the advent of metagenomics techniques. These strains can be obtained through enrichment procedures (Thompson et al., 2005), aiming to isolate autochthonous microorganisms that usually produce large amount of metabolites which can be used for plant benefit as well as in different biotechnological applications. Knowing the diversity of the bacterial community associated with a medically important plant, like mint, and the effect of irrigation water in the distribution of that community will greatly affect our understanding of that plant needs and development methods. It also would provide a microbial population that can be used for the benefit of other crops. In this work, we assessed whether the type of irrigation water had significant effects on the microbial community arrangements.

The results obtained showed a discrete separation in the bacterial population based on the sampling location indicating the effect of irrigation water, as groundwater well was the source of irrigation in Abyar-Ali. Groundwater is known to contain various types of dissolved inorganic chemicals that originate from chemical and biochemical interactions between water and the geological materials as well as from dissolution or weathering of the rocks and soil (Şener et al., 2021) which explains the high amount of total dissolved solids and salts concentrations detected in MA water. The high salt concentration may also the cause for the accumulation of aminocyclopropane-1-carboxylic acid (ACC) deaminase, indole acetic acid (IAA), and proline to help plants tolerate salinity stress. The production of the previous compounds reduces the ethylene level in plants, leading to better root growth and leaf biomass (Qureshi et al., 2013; Raghuwanshi & Prasad, 2018; Saikia et al., 2018), which explains the morphological variation between plants across sampling locations. On the other hand, the high level of nitrogen in MM water is probably due to irrigation with treated wastewater in this location. During wastewater management with activated sludge, its high level of nitrogen result in many complications, since nitrification process is often inhibited by high ammonia and nitrite content and macronutrient deficiency hinders denitrification, all of which has negative influence on the treatment processes (Zhang et al., 2021).

The result also indicated that, Abyar- Almashi samples revealed high OTU abundance (75), while samples from Abyar-Ali showed lower richness (39 OTUs). The root sample from Abyar-Almashi (MM3) had the highest number of OTUs, whereas the least number of OTUs was observed in the leaves sample of Abyar-Ali region (MA1). The low number in this sample is not an indication of sequencing problem nor to low coverage since the raw and clean data and reads of this sample are very similar and even higher compared to other samples as shown in Table 1. However, it may indicate low bacterial diversity in the leaves as it is completely occupied by single bacterial genus. This could possibly be explained by the high salinity level in Abyar-Ali irrigation water leading to the accumulation of phenol and hydrogen peroxide (H_2O_2) in the leaves of *M. longifolia*. Both are primary stressors produced in plants in response to salinity stress and are probably responsible for the significant reduction in the endophytic population of the leaves (Raghuwanshi & Prasad, 2018; Fan et al., 2020). The low diversity in MA1 affected its curve at the rank abundance analysis while the curves of the other samples indicated that MA3 as well as MM3 have the best species richness followed by MM1. Similar results were reported by Huo and his colleagues in 2020, in which root samples had higher OTU richness than other tissues. This may be explained by the fact that, most endophytic bacteria originate from soil (Huo et al., 2020). Previous researches also observed different levels of diversity among the endophytic community isolated from various plant parts (Liu et al., 2017; Yang et al., 2017).

The taxonomic analysis was in agreement with other reports. Generally, phylum Proteobacteria, with all its classes α, β and γ-Proteobacteria, is described as the most dominant in diversity analysis of endophytes. However, classes of the Firmicutes and Actinobacteria phyla are also among the most consistently found as endophytes (Romero et al., 2014; Alibrandi et al., 2020). At class level, the result does not come as a surprise since Alpha proteobacteria is a diverse class that comprises several genera which show a great ability to adapt to a broad range of environments (Ettema & Andersson, 2009). Also, class Alpha

proteobacteria contains several nitrogen-fixing as well as plant-growth promoting (PGP) bacteria, that have been reported to abundantly occur within plant tissue as endophytes (Kim et al., 2019; Mahmud et al., 2020; Shi et al., 2021). To the best of our knowledge, there are no previous studies that involved next generation metagenomics analysis of *M. longifolia* endophytic bacterial community, however similar results were reported for the culturable bacterial endophytes isolated from the same plant (Golinska et al., 2015; Ameen et al., 2019). Only recently, *Enterobacteriaceae* have been established as indigenous components of the plant microbiome (Erlacher et al., 2015; Cernava et al., 2019). The result in this study comes in good agreement with previous findings regarding the predominance of enterics in lettuce microbiome due to the type of irrigation water (Williams et al., 2013). Also, members of *Enterobacteriaceae* family are considered as excellent degraders of organic compounds along with the presence of recycled irrigation water containing high nitrogen content like the one used in Abyar-Almashi which will promote their proliferation (Alegbeleye et al., 2018; NandaKafle et al., 2018). On the other hand, recent studies hypothesized that plant species can recruit specific endophytic bacterial communities especially *Bacillus* spp. in response to abiotic stress factors such as: climate change, temperature, drought, and salinity (Mackenzie, et al. 2013; Zhao et al., 2017; Cheng et al., 2018). In this context, Abyar-Ali could be considered as extreme environment due to the high levels of salts found in its irrigation water which explains the domination of family *Bacillaceae* (Ibekwe et al., 2021).

A few *Pseudomonas* (a dominant genus in Abyar-Almashi samples) strains were reported as endophytes in the literatures (Miliute et al., 2015), some of which acted as an endophytic nitrogen-fixing bacteria (Rediers et al., 2003), others were reported to have beneficial interaction with plants such as secretion of phytohormones, enhanced plant growth releasing antimicrobial substances or producing siderophores that induce plant systemic resistance to pathogens (Lally et al., 2017; Wicaksono et al., 2018). Recently, it was also demonstrated that ketosteroid isomerase (KSI) an enzyme produced by *Pseudomonas putida* can act as isopulegone isomerase (IPGI) that produces pulegone from cis-isopulegone (Leavell et al., 2016; Currin et al., 2018). This could explain the high concentration levels of pulegone detected in Abyar-Almashi leaves samples. Pulegone is a naturally occurring organic compound that has been previously reported as a major essential oil in *M. longifolia* plant grown in Saudi Arabia (Alam et al., 2016). Pulegone is also a powerful naturally occurring insecticides in many mint species and is used as flavoring agents and in perfumery (Božović et al., 2015). These results are in agreement with previous studies showing that the components of the oil from the same plant could differ quantitatively due to environmental conditions (Hassan et al., 2020; Burneo et al., 2021).

Despite that Abyar-Ali area samples were not rich with the genera of *Brevibacillus*, *Lysinibacillus* and *Sphingobacterium* still they have high relative abundance percentage as unique genera to this location. Endophytic bacteria belonging to *Brevibacillus* and *Lysinibacillus* have been isolated and identified in other crops (Deng et al., 2011; de Oliveira Costa et al., 2012). *Both* species have been recognized as potential bio-control agents (van Zijll de Jong et al., 2016; Chen et al., 2017; Santana-Martinez et al., 2019). *Sphingobacterium* on the other hand contains *salt tolerant bacterial* species *that have been reported to improve plant tolerance to salinity through inducing antioxidant systems and energy metabolism of plant roots, leading to systemic tolerance and providing the whole plant with protection during salt stress (*Vaishnav et al., 2020). *Collectively,* these results indicated that the type of irrigation water played a crucial role in shaping up the microbial community associated with the *M. longifolia* growing in Abyar-Ali in respect to types and numbers causing it to be more diverse from those collected from Abyar-Almashi as supported by the Alpha and beta analysis. Alpha and beta analysis also suggests that the improved growth in Abyar-Ali plant parts may be related to the diversity in their bacterial endophytic associates.

Acknowledgment: The author greatly appreciate Lubna Badr's efforts and contribution in funding this work that made it possible for the manuscript to be completed.

Ethical approval: Not applicable

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تأثير مياة الري على تنوع وتوزيع المجتمع الميكروبى الداخلى لنبات النعناع: النمط الميتاجينومي

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يعتبر نبات النعناع من أهم النباتات الطبية والاقتصادية في المملكة العربية السعودية. هدفت هذه الدراسة إلى توصيف التنوع الداخلي البكتيري المرتبط بأوراق وجذر *Mentha longifolia* من موقعين مختلفين (أبيار على وأبيار الماشي) في منطقة المدينة المنورة، بالمملكة العربية السعودية، باستخدام طرق دراسة الميتاجينوم. ثم ربط الانحراف الكمى لهذه الميكروبات وتنوعها بظروف الإجهاد البيئي. كشف التحليل الكيميائي للزيوت الأساسية لأوراق *M. longifolia*عن ارتفاع تركيز مادة carvenone في "أبيار علي" ومادة pulegone في "أبيار الماشي". كما أظهر التحليل الكيميائي لمياه الري في منطقة "أبيار علي" نسبة عالية من المواد الصلبة الذائبة الكلية مقارنة بمنطقة "أبيار الماشي". بالإضافة إلى ذلك أوضح تحليل Illumina MiSeq للحمض النووي الجيني للميكروبيوم الداخلي باستخدام rDNA عن 114 وحدة OTU مختلفة تم تجميعها في ثماني شعب بكتيرية ينتمي معظمها إلى شعبة Proteobacteria تليها شعبة Cyanobacteria. تم تصنيف الوحدات الناتجة في 14 فئة و25 رتبة و41 عائلة و57 جنسًا. من بين هذه الميكروبات، البكتيريا المثبتة للنيتروجين التي تلعب دورًا رئيسيًا في التخليق الحيوي لمادة Pulegone، *Pseudomonas*، وكذلك البكتيريا المعوية *Enterobacter*؛ والقادرة على تحليل المركبات العضوية بكثرة في "أبيار الماشي". بينما كان جنس *Bacillus* مهيمناً أو فريدًا "لأبيار علي" استجابة لارتفاع ضغط الملح في ذلك الموقع. تشير هذه النتائج إلى أن التباين في المحتوى البكتيري الداخلي يدعم احتياجات النبات وفقًا لنوع الإجهاد الذي يتعرض له. قد يكون الإجهاد الملحي المكتشف عاملاً رئيسيًا يسبب ديناميكيات في عدد ونوع وتوزيع البكتيريا بين المنطقتين تحت الدراسة.