Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 2725 - 2740
Received 25 April 2021; Accepted 08 May 2021.

So it is above, So it is below: Microbial Pathways Associated with Date Palm Trees

Faten Dhawi[1]* and Sumayah I. Alsanie[2]

[1]Biotechnology Department, College of Agricultural and Food Sciences, King Faisal University, Saudi Arabia
[2]Biology Department, College of Science, Imam Abdulrahman Bin Faisal University, Saudi Arabia

[*]*Corresponding author: E-mail:dr.faten.dhawi@gmail.com; falmuhanna@kfu.edu.sa*

Abstract. Among the many factors that influence beneficial plant–microbeinteraction, plant species affects the molecular interactions betweenplant roots and microbes. Plants'association with microorganisms was known to bespecies-specific in some plants, such as date palm trees (*Phoenix dactylifera*).In the current research, we explore microorganisms associated with date palmcultivars. Soil samples from the rhizosphere of Khalas date palm trees at the Al-Ahsaoasis in Saudi Arabia were subjected to DNA extraction, followed by metagenomic reassembly, quantification,and binning. The results revealed that most (86%) of the sequences were identified as bacteria, with 16% ofmicrobial communities identified specifically (5% Bacteroidetes, 3% of Verrucomicrobia, 3% of Proteobacteria, 3% ofPanactomycetes, and 2% of Acidobacteria);additionally,10% were identified asAscomycota fungi. These results were followed by functionalenzyme analysis, which identified microbial metabolism in diverse environmentsrelated to carbon fixation, carbohydrates, amino acids, and sulfur metabolism.

Keywords: *DNA, metagenomic, interaction, Bacteria, Khalas, Phoenix dactylifera*

Introduction

Changes in agricultural environments and habitats as a resultof climate change necessitate a deep understanding of microorganisms' position in ecosystems. Plant types, plant population diversity, agricultural practices used, and soil type are all factors that affect the growth-promoting bacteria that interact with plant roots [1],plant community diversity, agricultural practices applied, and soil type [2–3]. According to research conducted on natural habitats, various types of wild plants attract different types of growth-promoting bacteria, based on their needs and geoclimatic conditions [2 – 3]. For instance, asspeargrass roots in the Tunisian desert aren't selective, they attract whatever growth-promoting bacteria they can find in the soil[4];the date palm rootsinthe Tunisian Sahara Desert consistently attract two types of growth-promoting bacteria to their roots, regardless of their location[3].

Food security is amajor concern for all nations facingchallenges in cultivable area due to soil disintegration, saline soils, and the excessive use of pesticides, herbicides, and inorganic fertilizers [5]. The misuse of chemical fertilizers has led to accumulation of nitrate in plant tissues and groundwater,as well assurface runoff of phosphorus and nitrogen and the reshaping of microbial communities. Abuse of chemical fertilizers and pesticides have impacts on soil life forms that are comparable to human abuse of anti-microbes [6].

Soil microbial diversity is essential to a metabolic environment related to soil and plant health. In this manner, a fundamental understanding of differences insoil biota is required for thesustainability of soil characteristics and biological systems. The study of microbial differences in a specific living space is especiallycritical, as the organisms make a difference

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 2725 - 2740
Received 25 April 2021; Accepted 08 May 2021.

in the upkeep or adjustment of the environment. It is thusimportantto explore the relationship between the soil microbiota and plants,along withhow this relationship benefits both organismsdespite environmental changes [5].

The microbial diversity in soil is amazingly abundant and much greaterthan any other groupof eukaryotic organisms [7]. This diversity has been accomplished throughheterogeneity, multiphase nature, and the chemical and organic properties of the soils [8]. Microorganisms play a central role inthe nutrient cycle and inthe wellbeing of plants and soil [9]. Conventional methods forculturing microorganisms are very limited:approximately< 1% can be grown inlaboratory conditions[10]. The metagenomeobtained by NGS approaches gives more genetic data than any other culturable strategies [11].

Metagenomics combines genomics, bioinformatics, and frameworks science. Current information onculturable microbial strategies is unableto depictthe wholepicture of the differences and capacities of the microorganisms living in a single environment.Soil metagenomics is used to shed light on the capacitiesof microbial communities that drive the planet's energy and nutrient cycles, maintain the health of its inhabitants, and shape the evolution of life.

The date palm is known to have extraordinary financial value in Saudi Arabia, andfarmers utilize strategies to extend the financial value of a few zones of Saudi Arabia more than others. In this research, we utilized a soil metagenomics approach to investigate microorganisms related to date palm roots from the Khalas cultivar in Al-Ahsa oasis, where it originated.

Material and methods

A total of seven samples were collected from seven sites in the Al-Ahsa province of Saudi Arabia. Soil samples were from several locations of rhizosphere soil,1–2 inches of roots around date palm trees (*Phoenix dactylifera L*) of the Khalas cultivar.

DNA extraction, metagenomics library preparation, and sequencing

Soil samples were subjected to microbial DNA extraction using Soil Kit Qiagen DNeasy PowerMax, following the manufacturer's instructions. Extracted DNA was digested and adaptors were attached in a single step, using the tagmentase enzyme included in the Nextera DNA Flex library preparation kit (Illumina). The sequencing library was prepared using the Nextera DNA Flex library preparation kit (Illumina),closelyfollowing the manufacturer's instructions. Furthermore, the library was dual-indexed to allow for post-sequencing demultiplexing. The pool was sequenced in a fraction of an Illumina NovaSeq6000 PE150 lane, with the aim of obtaining 10 gigabases of raw data.

Quality control and pre-processing of sequencing data

The MetaWRAP pipeline [12]was used for the bioinformatic analysis, which includedquality control, taxonomy assignment, abundance estimation, and functional annotation. Raw metagenomic shotgun sequencing files consisted of forward (R1) and reverse (R2) reads, sorted by library and by their quality scores. The indices and sequencing primers weretrimmed during the demultiplexing step.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 2725 - 2740
Received 25 April 2021; Accepted 08 May 2021.

Taxonomic profile exploration and assignment

An analysis was conductedto explore the taxonomic profile of the whole dataset; we applied the novel assembly-free approach of CCMetagen [13], based on mapping the reads directly to a reference database. The assembly was inputted so as to map against the NCBI-Nucleotide database (nr/nt) using the KMA tool [14], and alignment was performed without similarity reduction. This tool includes an extra mapping step, wherein each mapped sequence was scored with the ConClave sorting scheme algorithm in order to reach the best score. Then, CCMetagen processed the results from the alignment to generate a Krona interactive pie chart [15].

Metagenomic assembly and binning

DNA sequence reads with high quality were filtered and assembled using the MetaWRAP pipeline by joining them into larger contigs and assigning them to different bins associated with draft genomes. The metagenomes were assembled with the software MEGAHIT [16],implemented in the metaWRAP-Asssembly module using default parameters. During the assembly, the shorter contigs (<1000 bp) were discarded. The software QUAST [17]was used to evaluate the assembled contigs for samples.

Metagenomic Reassembly, Quantification, and Binning

The resulting assemblies were binned with the metaWRAP-Binning module using three different softwares: metaBAT2, MaxBin2, and CONCOCT [18-19-20]. To consolidate and produce the highest-quality bin, the metaWRAP-Bin_refinement module was used to choose the best version of each bin, based on thecompletion (>70 %) and contamination (<10 %) values [21]. The metaWRAP-Bin_refinement module used a hybrid approach that tookthe three bin sets obtained during the initial binning to produce an improved binning set. The software CheckM [21]was run withthe improved bin set to generate completion and contamination rank plots,whichwere used to evaluate the success of the binning refinement process in both samples (see Figures 3 and 4). Additionally, the metaWRAP-Blobology module was used to visualize the results of the binning refinement in each sample. This module plots the GC content vs. the abundance of all the contigs across the metagenomic sample, including phylogenetic information [22]. The taxonomy of each contig wasestimated using MegaBLAST [23].

Furthermore, we ran the metaWRAP-Quant bins module to estimate the abundance of each bin in each metagenomic sample. This module used the software Salmon [24]to index the metagenomic assembly and then align the reads from each sample back to the assembly. The abundance table wasgenerated using the length-weighted average of the bin's contig abundances. We used the metaWRAP-Reassemble_bins module and reassembled the reads with metaSPAdes [25]. For each bin, three sets of reads were used: the reads mapping perfectly (strict mapping), the reads mapping with fewerthan three mismatches (permissive mapping), and the reads from the original bin. After reassembly, we used the software CheckM to evaluate itssuccess.

Results

Quality control and pre-processing of sequencing data

Using the metaWRAP-Read qc module to trim the raw sequence reads resulted in 100% of the reads passing quality reports for each sample (Table 1). Data was then subjected to

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 2725 - 2740
Received 25 April 2021; Accepted 08 May 2021.

assembly statisticsbased on contigs of size ≥ 1000 bp, unless otherwise noted (e.g., "# contigs (≥ 0 bp)" and "Total length (≥ 0 bp)" include all contigs) (Table 2).

Table 1: Quality control and pre-processing of sequencing data showed total of 100% reads from paired-end sequencing (i.e., forward (R1) and reverse (R2) reads).

% reads that passed filters	Number of reads after filtering*	Number of reads before filtering*
100%	37,751,837	37,758,313

Table 2: Assembly statisticsbased on contigs of size ≥ 1000 bp, unless otherwise noted (e.g., "# contigs (≥ 0 bp)" and "Total length (≥ 0 bp)" include all contigs).

Assembly	Sample
# contigs (≥ 0 bp)	84811
# contigs (≥ 1000 bp)	84810
# contigs (≥ 5000 bp)	3668
# contigs (≥ 10000 bp)	809
# contigs (≥ 25000 bp)	79
# contigs (≥ 50000 bp)	12
Total length (≥ 0 bp)	1.66E+08
Total length (≥ 1000 bp)	1.66E+08
Total length (≥ 5000 bp)	32330261
Total length (≥ 10000 bp)	12952359
Total length (≥ 25000 bp)	2973332
Total length (≥ 50000 bp)	777412
# contigs	84811
Largest contig	93021
Total length	1.66E+08
GC (%)	63.86
N50	1953
N75	1314
L50	21654
L75	48128
# N's per 100 kbp	0

Taxonomic profile exploration and assignment

The analysis conductedto explore the taxonomic profile of the whole dataset showed that 86% of the sequences were assigned to bacteria (Figures 1 and 2). Therefore, among the bioinformatic tools,we used metaWRAP-Classify_bins module to assign taxonomy to the reassembled bins. We estimated the taxonomy of each bin for each sample using MegaBLAST to align against the NCBI_nt as the reference database (Table 3).We used the metaWRAP-annotate_bins module to conductthe functional annotation of the reassembled bins. We used themodule PROKKA [26]to conductthe functional annotation and translation

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 2725 - 2740
Received 25 April 2021; Accepted 08 May 2021.

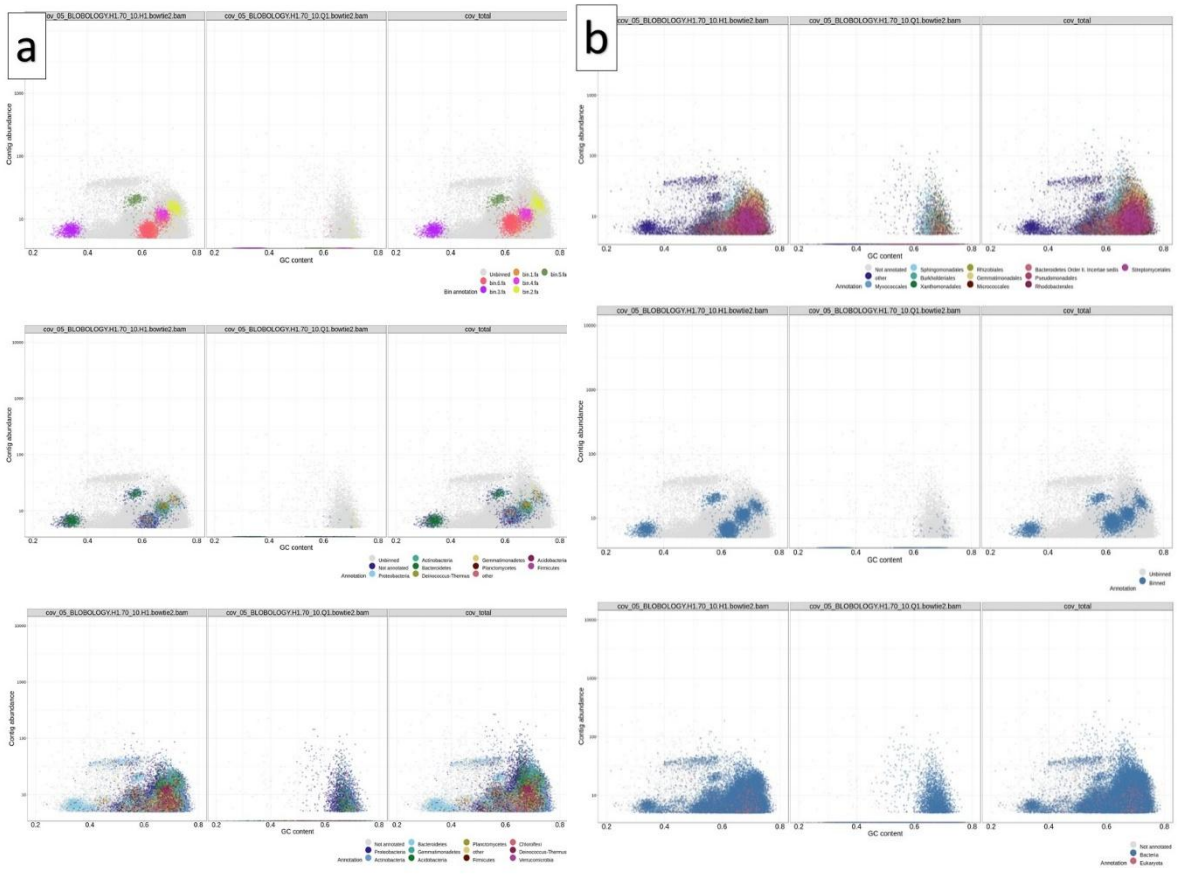
of genes in each bin. Swiss-Prot database from UniProt wasused for taxonomic division: bacteria identification [27-28].

Metagenomic assembly and binning

The abundance table wascomputed based on marker genes and the fraction of the dataset that was mapped against the marker genes of the reference database. Microbial reads belonging to taxa with no reference data available are reported in the table as unclassified. Table 3 representseach taxonomic unit in each sample. Additionally, the metaWRAP-Blobology module was used to visualize the results of the binning refinement in each sample. This module plotted the GC content vs. the abundance of all the contigs across the metagenomic sample, including phylogenetic information [22]. The taxonomy of each contig wasestimated using MegaBLAST [23]and the NCBI_nt database (Figure 3).

Table 3: Bin taxonomy assigned using Mega-BLAST for the identified soil microorganisms.

Genomic bins	Taxonomic identification
bin.5.orig.fa	Bacteria
bin.4.orig.fa	Bacteria
bin.2.orig.fa	Bacteria
bin.1.orig.fa	Bacteria
bin.6.orig.fa	Bacteria
bin.3.orig.fa	Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae



Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 2725 - 2740
Received 25 April 2021; Accepted 08 May 2021.

Figure1: Bins(a, b) membership of the contigs,visualized using the metaWRAP-Blobology module. This module plots the GC content vs. the abundance of all the contigs across the metagenomic sample including phylogenetic information.

Taxonomic profile exploration

The taxonomy of each contig wasestimated using MegaBLAST [23]. Figure 2 shows that the taxonomic analysis foundthat 86% of the sample sequences were assigned to bacteria (in which 70% were unknown, 5% Bacteroidetes, 3% Verrucomicrobia, 3% Proteobacteria,3% Panactomycetes, and 2% Acidobacteria). The eukaryotes were 14% of the taxonomy,with fungi represented by 10% Ascomycota, and 4% were unknown.

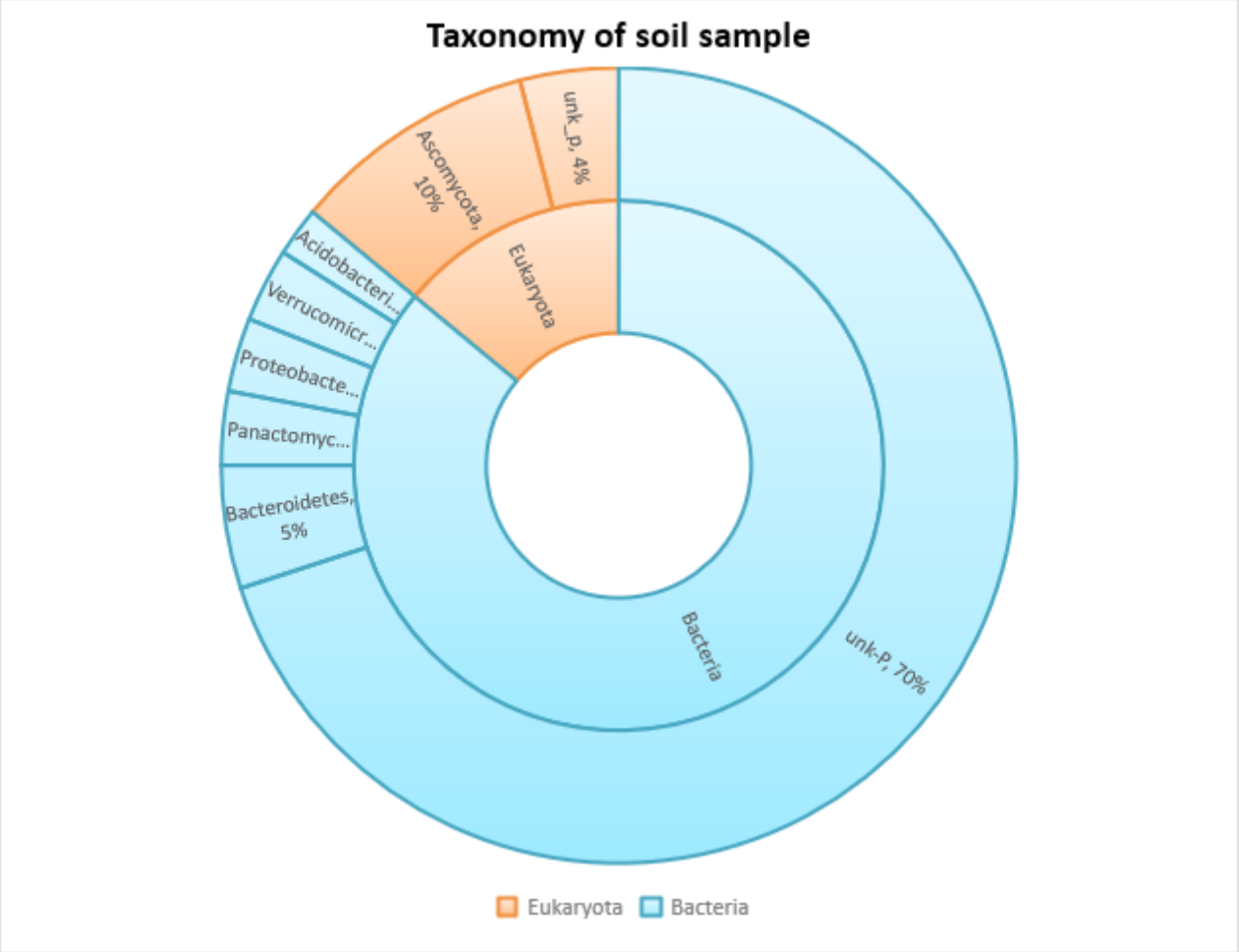


Figure2: A pie chart representing the taxonomy of each contig, estimated using MegaBLAST [23]and the NCBI_nt database.The taxonomic representation shows86% bacteria: 70% unknown, 5% Bacteroidetes, 3% Verrucomicrobia, 3% Proteobacteria,3% Panactomycetes, and 2% Acidobacteria. 14% of the taxonomy wereeukaryotes,with fungi represented by 10% Ascomycota.

Enzyme Commission enzyme codes (EC), Pathways, and Functional annotation

The analysis resulted in 44 enzyme codesassociated with 95 metabolites. The Sma3sv2 program was used to produce functional annotations with the most probable gene name, the most probable description, and the putative Enzyme Commission enzyme codes (EC). The

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 2725 - 2740
Received 25 April 2021; Accepted 08 May 2021.

iPath3.0 webapplication [29]was used for visualization and analysis of metabolic pathways from the EC numbers, to show functional annotations with putative ECsassociated withmicrobial metabolism in diverse environment pathways(Figure3). Further details of the list and the correlation between ECs, PubMed, GenBank, NCBI Protein, and KEGG GENESare presented in the appendix (Table1.s).

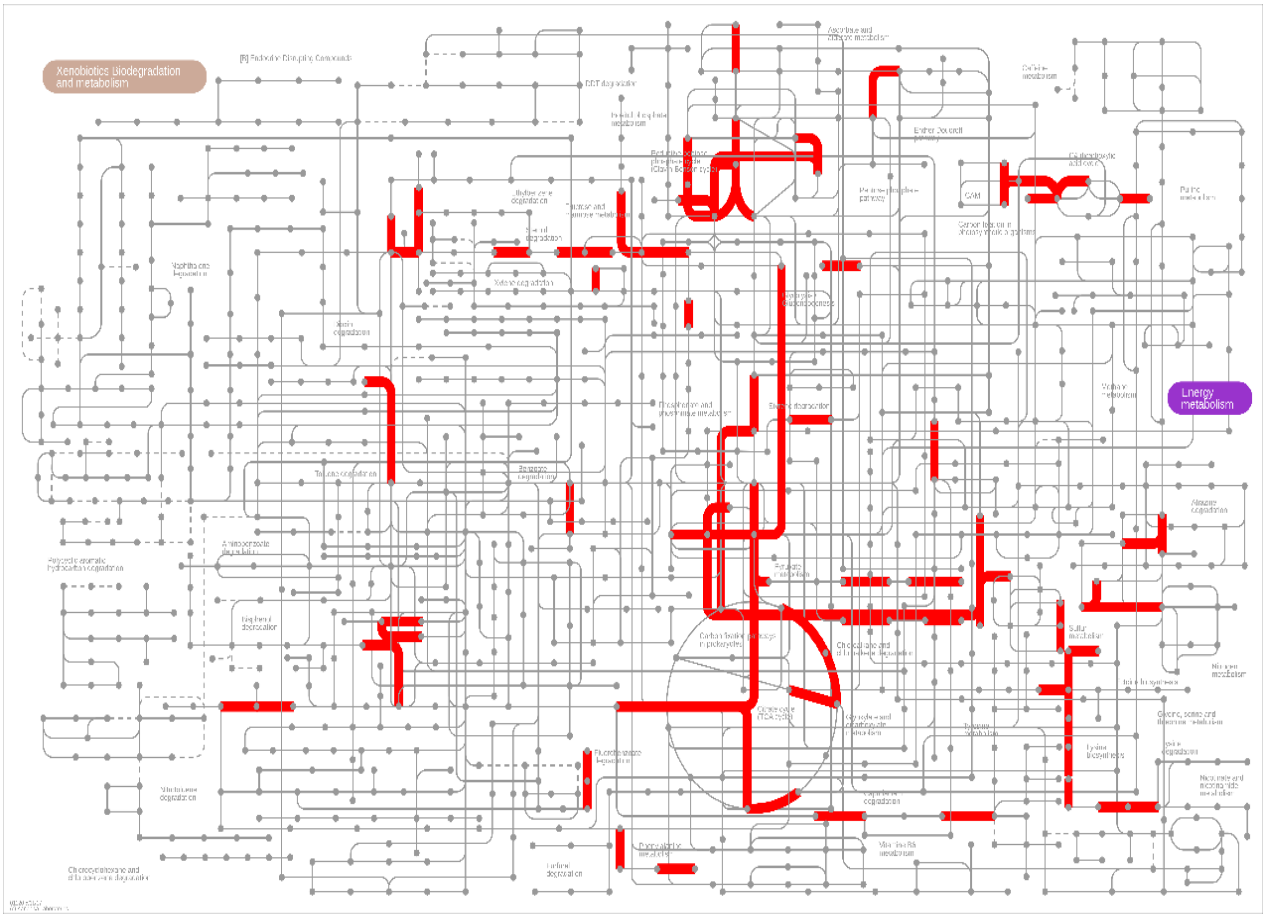


Figure 3: Microbial metabolism in diverse environment pathways for Enzyme Commission enzyme codes (EC),shown in red,using the iPath3.0 webapplication [29]; the lines allowus to navigate and find reactions involved in these metabolic processes on the pathway map.

Marker genes approach

In the following steps, we aimed to explore the taxonomic profile of the rest of the organisms that were potentially present in the soil samples (i.e., non-bacterial microorganisms). To this end, we used a different approach that is based on the analysis of taxonomically-informative marker genes present in the metagenomic dataset. The bioinformatic tool MetaPhlAn 3.0 [30] was used to generate the microbial community profile of samples. The MetaPhlAn takes metagenomic raw reads as input and maps them against a reference database composed of clade-specific marker sequences of bacteria, archaea, eukaryotes, and viruses.Consequently, only a small fraction of the dataset is mapped against the marker genes reference database. The results of this analysis include an estimation of the fraction of the metagenome, which is composed of unknown microbes.

We applied different bioinformatic pipelines to characterize the metagenomic information of the organisms present in the soil samples. Most of the metagenomic reads were identified as

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 2725 - 2740
Received 25 April 2021; Accepted 08 May 2021.

bacterial organisms;however, we argue that low and uneven coverage along the metagenomes, together with a lack of information in the available databases,prevented us from obtaining refined bins from the dataset and, consequently, assignments at lower taxonomic levels. Therefore, additional approaches (assembly-free and marker genes-based approaches) were included to identify the organisms present in the metagenomic sequencing data, which did not improve the taxonomic assignment.

Functional annotation

Functional annotations with the most probable gene name, the most probable description, and the putative ECwere used to explore possible pathways,followed by the EC classification scheme for enzymes based on the chemical reactions they catalyze. The iPath3.0 webapplication [29] was used to visualize and analyzethe environmental metabolic pathways from the EC numbers in order to find reactions involved in soil metabolic processes (Table 4).

Table 4: Soil EC Functional annotation associated pathways using iPath3.0 webapplication, associated pathways [extracted from the EC data listed in Table1s in the appendix].

Microbial metabolism in diverse environments
Glycolysis / Gluconeogenesis
Pyruvate metabolism
Carbon fixation pathways
Lysine degradation
Glyoxylate and dicarboxylate metabolism
Sulfur metabolism
Phenylalanine metabolism
Nitrogen metabolism
Pentose phosphate pathway
Methane metabolism
Ethylbenzene degradation
Atrazine degradation
Nitrogen metabolism
Aminobenzoate degradation
Purine metabolism
Benzoate degradation
Xylene degradation

Discussion

Despite the rapid and productive rural advancement that has occurred within the Kingdom of Saudi Arabia (KSA), a few areas remain challenged by low-quality soil along witha lack of adequate supplements, natural matter, and moisture. Therefore, around 3.22% (1.14 million hectares) of developed regions are exploited in Saudi Arabia. To address this, attempts have been made by the government to extend the agricultural region. Among the agricultural regions of the KSA, Al-Ahsa is considered one of the most productive areas. Soils of this region are used extensively for agricultural farming, to produce more food for the growing population. The spatial distribution of soil salinity along the Al-Ahsa Oasis, based on the

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 2725 - 2740
Received 25 April 2021; Accepted 08 May 2021.

FAO soil salinity classification,ranged from nosaline (0–2dS/m) to very strong saline (>16 dS/m),but the strong saline (8–16 dS/m) is the dominant class along the oasis. This oasis is primarily a date palm (*Phoenix dactylifera L*) region and is regarded as one of the leading date palms producers in Saudi Arabia, providing an important source of income for the government and for farmers [31].

The electrical conductivity is higher in date palm regions, with an average value of 18.97 dS/m,than in cropland and bare land,which show mean values of 10.25 and 5.20 dS/m, respectively. Soil salinity is likely to be higher in date palm and cultivated croplands,due to the accumulation of salts as a result of saline water flow from the water table and also due to using saline irrigation water [32].

Metagenomic taxonomic assignment

The metagenomic taxonomic assignment of each reconstructed bin (metagenome-assembled draft genomes) using the metaWRAP pipeline showed a domination of environmental bacteria in the rhizosphere of the Khalas date palm. The taxonomic representation showed 86% was bacteria: 70% were unknown bacteria, 5% were Bacteroidetes, 3% were Verrucomicrobia, 3% were Proteobacteria, 3% were Panactomycetes, and 2% were Acidobacteria. At the same time,known eukaryotes—with 10% being Ascomycota fungi—were identified in the sample.

The rhizosphere of the Khalas date palm showed that the5% of Bacteroidetes and the 3% of Proteobacteria were represented, respectively, by theFlavobacteriaceae class from the phylum of Bacteroidetes and by Proteobacteria, which is amajor phylum of Gram-negative bacteria. Members of the Flavobacteriaceae family are found in a wide variety of marine, freshwater, and soil habitats, and some are also associated with plants [33]. In the study of[3], the metagenomic analysis of the rhizosphere of the Deglet Nour date palm cultivar identified *Gammaproteobacteria*-and *Alphaproteobacteria*-dominated bacterial communities,and bulk soil consisted of Verrucomicrobia, Bacteroidetes,and Actinobacteria[3], which were identified in the rhizosphere of the Khalas date palm trees in our study. Proteobacteria are very common in soil environments and are related to a wide range of functions involved in carbon, nitrogen, and sulphur cycling [34],nitrogen fixation [35], and utilization of macromolecules like polysaccharides and proteins [33].

Verrucomicrobia appears to be dominant in many soil bacterial communities across the globe [36]; their structure and abundance are extremely sensitive to changes in chemical factors linked to soil fertility. The relative proportion of Verrucomicrobia declines in response to increased soil fertility. The rhizosphere soil of the Khalas date palm trees at Al-Ahsa showed 3% Verrucomicrobia, which is the normal range of Verrucomicrobia in good soil fertility. A previous analogous study by [37] reported that Verrucomicrobialcommunity abundance of the total bacterial signal in the sugarcane rhizosphere showed 2% under optimal soil conditions and 5% in deficient soil. Another bacterial community that showssignificant association with soil history and chemistry is *Planctomycetes,*in that the increase of *Planctomycetes* in soil is negatively correlated with cultivation. The cultivated soil (less than 50 years) has less Planctomycetes abundance and diversity [38], which was represented in our study by 3% Panactomycetes in the rhizosphere soil of Khalas date palm trees.

In the current study, Acidobacteria represented 2% of the soil sample taxonomy in the rhizosphere of the Khalas date palm trees.The known ecological roles of Acidobacteria in plant-soil ecosystem include facilitating modulation of critical biogeochemical cycles of

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 2725 - 2740
Received 25 April 2021; Accepted 08 May 2021.

carbon, nitrogen, and sulfur,as well as producingpolysaccharides that contribute to soil matrix formation and plant growth promotion [39].The presence of fungi in the current study'smetagenomic analysis, represented byby 10% Ascomycota in the rhizosphere soil of Khalas date palm trees, showed a healthy soil indicator,since Ascomycota is known to be associated with increased availability of phosphate,nitrogen, and carbohydrates[40].

Exploring in-depth genes and proteins and associated microbes

As reported in previous studies, bacteria regulates phytobeneficial traits through reciprocal protein stimulation via microbe plant interactions both during and after colonization, which isessential for plants' health and sustainability and provides a protective effect against stresses,such as drought [41-42-43]. In addition, fungi such as Ascomycota contribute significantly to soil and plant health by facilitating major soil elements. Functional annotations with the most probable gene namesand the putative EC were used to visualize the environmental metabolic pathways involved in soil metabolic processes. The ECused to check associated genes and proteins showed several microbial pathwaysthat are associated with soil environment where Khalas date palm trees grew. These pathways are essential to plant health and soil sustainabilityand are associated with the 16% of the microbialcommunity that was identified—bacteria at levels of 5% Bacteroidetes, 3% Verrucomicrobia, 3% Proteobacteria, 3% Panactomycetes and 2% Acidobacteria, as well as Ascomycotafungi at 10% of the taxonomy of the microbial community.

The primary pathways found in the current study were carbon fixation pathways,pyruvate metabolism, pentose phosphate pathway, glyoxylate phenylalanine,purine metabolism, and nitrogen metabolism. The results showed that the most significant pathways were carbohydrates and energy metabolisms,since these pathways are essential to microbial community and soil health. These pathways consisted of: carbon fixation pathways, glycolysis pathway (which converts glucose into pyruvate and generates small amounts of ATP energy),which is incorporated intoPyruvate metabolism. In parallel to glycolysis, the other source of energy is the Pentose phosphate pathway,which is used by bacteria to generate NADPH and sugar. In addition, environmental pathways of glyoxylate and dicarboxylate metabolism are also involved in the biosynthesis of carbohydrates from fatty acid.

The other essential environmental pathways are protein metabolisms,which consist of phenylalanine metabolismand purine metabolism. In addition, nitrogen metabolism is a set of pathways for forming nitrogenous end products from thedegradation of protein, atrazine, aminobenzoate, and lysine.Methane and sulfur metabolisms andbenzoate and xylene degradation metabolisms were the secondary pathways found in this study. Anaerobic bacteria degrade carbon in methanogens,which produce methane as a metabolic by-products[44],whereas sulfur is reduced or oxidized by bacteria from proteins, sulfate esters of polysaccharides, steroids, phenols, and sulfur-containing coenzymes to be metabolized and become available to plants.In addition, two bacterial pathways were involved in benzoate and xylene degradation, which are known to provide other nutrients such as carbon and acetyl CoA to bacteria.The primary pathways found in the current study were carbon fixation pathways,pyruvate metabolism, pentose phosphate pathway, glyoxylate phenylalanine,purine metabolism, and nitrogen metabolism, known to be associated with Ascomycota [40], which contributes significantly to soil phosphate transporter, nitrogen immobilization, and degradation of complex sugars and polysaccharide synthesis[40] and which was 10% of soil sample taxonomy in the current study.

Conclusion

The metagenomic analysis of the soil sample taxonomy from therhizosphere of Khalas date palm (*Phoenix dactylifera*)trees from Al-Ahsa Oasis identified a wide-ranging microbial community associated with the Khalas cultivar.Of this microbial community,26% was identified, with 16% of this being bacteria (5% Bacteroidetes, 3% Verrucomicrobia, 3% Proteobacteria, 3% Panactomycetes, and 2% of Acidobacteria)and 10% being fungi (Ascomycota). The study indicated that the plant cultivar species is significantly influenced by the bacterial community assembled in its root systems and environmental soil pathways. These results can be used to further identify soil–plant interaction related to specific species and to enhance the soil health with microbial communities related to the environmental essential pathways.

Acknowledgments

We would like to thank AllGenetics & Biology SL (www.allgenetics.eu)" for supporting us with Metagenomic analyses service.

[1] Dhawi, F., & Hess, A. (2017). Plant Growth-Prompting Bacteria Influenced Metabolites of Zea mays var. amylacea and Pennisetum americanum p. in a Species-Specific Manner. Advances in Biological Chemistry, 7(5), 161-169.

[2] Ferjani, R., Marasco, R., Rolli, E., Cherif, H., Cherif, A., Gtari, M., ... & Ouzari, H. I. (2015). The date palm tree rhizosphere is a niche for plant growth promoting bacteria in the oasis ecosystem. BioMed research international, 2015.

[3] Mosqueira, M. J., Marasco, R., Fusi, M., Michoud, G., Merlino, G., Cherif, A., & Daffonchio, D. (2019). Consistent bacterial selection by date palm root system across heterogeneous desert oasis agroecosystems. Scientific reports, 9(1), 1-12.

[4] Marasco, R., Mosqueira, M. J., Fusi, M., Ramond, J. B., Merlino, G., Booth, J. M., ... & Daffonchio, D. (2018). Rhizosheath microbial community assembly of sympatric desert speargrasses is independent of the plant host. Microbiome, 6(1), 215.

[5] Sabale, S. N., Suryawanshi, P. P., & Krishnaraj, P. U. (2019). Soil Metagenomics: Concepts and Applications. In Metagenomics-Basics, Methods and Applications. IntechOpen.

[6] Aktar W, Sengupta D, Chowdhury A. Impact of pesticides use in agriculture: Their benefits and hazards. Interdisciplinary Toxicology. 2009;2(1):1-2. DOI: 10.2478/ v10102-009-0001-7.

[7] Torsvik V, Øvreås L. Microbial diversity and function in soil: from genes to ecosystems. Current opinion in microbiology. 2002 Jun 1;5(3):240-5. https://doi.org/10.1016/S1369-5274(02)00324-7.

[8] Rolf D. The metagenomics of soil. Nat. Rev. Microbiol. 2005;3(6):p470-478. https://doi.org/10.1038/nrmicro1160.

[9] Chowdhury N, Marschner P, Burns RG. Soil microbial activity and community composition: impact of changes in matric and osmotic potential. Soil Biology and Biochemistry. 2011 Jun 1;43(6):1229-36. https://doi.org/10.1016/j.soilbio.2011.02.012.

[10] Mocali, S. and Benedetti, A., 2010. Exploring research frontiers in microbiology: the challenge of metagenomics in soil microbiology. Research in Microbiology, 161(6), pp.497-505. https:// doi.org/10.1016/j.resmic.2010.04.010

[11] Rondon, M.R., August, P.R., Bettermann, A.D., Brady, S.F., Grossman, T.H., Liles, M.R., Loiacono, K.A., Lynch, B.A., MacNeil, I.A., Minor, C. and Tiong, C.L., 2000. Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. Applied and environmental microbiology, 66(6), pp.2541-2547. https://doi.org/10.1128/aem.66.6.2541-2547.2000.

[12] Uritskiy, G.V., DiRuggiero, J., Taylor, J., 2018. MetaWRAP—a flexible pipeline for genome-resolved metagenomic data analysis. Microbiome 6, 1–13. https://doi.org/10.1186/s40168-018-0541-1

[13] Marcelino, V.R., Clausen, P.T., Buchmann, J.P., Wille, M., Iredell, J.R., Meyer, W., Lund, O., Sorrell, T.C., Holmes, E.C., 2020. CCMetagen: Comprehensive and accurate identification of eukaryotes and prokaryotes in metagenomic data. Genome Biology 21, 1–15. https://doi.org/10.1186/s13059-020-02014-2

[14] Clausen, P.T., Aarestrup, F.M., Lund, O., 2018. Rapid and precise alignment of raw reads against redundant databases with KMA. BMC Bioinformatics 19, 1–8. https://doi.org/10.1186/s12859-018-2336-6

[15] Ondov, B.D., Bergman, N.H., Phillippy, A.M., 2011. Interactive metagenomic visualization in a Web browser. BMC Bioinformatics 12, 385. https://doi.org/10.1186/1471-2105-12-385

[16] Li, D., Liu, C.-M., Luo, R., Sadakane, K., Lam, T.-W., 2015. MEGAHIT: An ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics 31, 1674–1676. https://doi.org/10.1093/bioinformatics/btv033.

[17] Gurevich, A., Saveliev, V., Vyahhi, N., Tesler, G., 2013. QUAST: Quality assessment tool for genome assemblies. Bioinformatics 29, 1072–1075. https://doi.org/10.1093/bioinformatics/btt086

[18] Alneberg, J., Bjarnason, B.S., De Bruijn, I., Schirmer, M., Quick, J., Ijaz, U.Z., Lahti, L., Loman, N.J., Andersson, A.F., Quince, C., 2014. Binning metagenomic contigs by coverage and composition. Nature Methods 11, 1144–1146. https://doi.org/10.1038/nmeth.3103

[19] Kang, D.D., Li, F., Kirton, E., Thomas, A., Egan, R., An, H., Wang, Z., 2019. MetaBAT 2: An adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies. PeerJ 7, e7359. https://doi.org/10.7717/peerj.7359

[20] Wu, Y.-W., Simmons, B.A., Singer, S.W., 2016. MaxBin 2.0: An automated binning algorithm to recover genomes from multiple metagenomic datasets. Bioinformatics 32, 605–607. https://doi.org/10.1093/bioinformatics/btv638

[21] Parks, D.H., Imelfort, M., Skennerton, C.T., Hugenholtz, P., Tyson, G.W., 2015. CheckM: Assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Research 25, 1043–1055. https://doi.org/10.1101/gr.186072.114

[22] Kumar, S., Jones, M., Koutsovoulos, G., Clarke, M., Blaxter, M., 2013. Blobology: Exploring raw genome data for contaminants, symbionts and parasites using taxon-annotated GC-coverage plots. Frontiers in Genetics 4, 237. https://doi.org/10.3389/fgene.2013.00237

[23] Morgulis, A., Coulouris, G., Raytselis, Y., Madden, T.L., Agarwala, R., Schäffer, A.A., 2008. Database indexing for production MegaBLAST searches. Bioinformatics 24, 1757–1764. https://doi.org/10.1093/bioinformatics/btn322

[24]Patro, R., Duggal, G., Love, M.I., Irizarry, R.A., Kingsford, C., 2017. Salmon provides fast and bias-aware quantification of transcript expression. Nature methods 14, 417–419.

[25] Nurk, S., Meleshko, D., Korobeynikov, A., Pevzner, P.A., 2017. metaSPAdes: A new versatile metagenomic assembler. Genome Research 27, 824–834. https://doi.org/10.1101/gr.213959.116

[26] Seemann, T., 2014. Prokka: Rapid prokaryotic genome annotation. Bioinformatics 30, 2068–2069. https://doi.org/10.1093/bioinformatics/btu153

[27] Boutet, E., Lieberherr, D., Tognolli, M., Schneider, M., Bairoch, A., 2007. Uniprotkb/swiss-prot, in: Plant Bioinformatics. Springer, pp. 89–112.

[28] Casimiro-Soriguer, C.S., Muñoz-Mérida, A., Pérez-Pulido, A.J., 2017. Sma3s: A universal tool for easy functional annotation of proteomes and transcriptomes. Proteomics 17, 1700071.

[29]Darzi, Y., Letunic, I., Bork, P., Yamada, T., 2018. IPath3.0: Interactive pathways explorer v3. Nucleic Acids Research 46, W510–W513. https://doi.org/10.1093/nar/gky299

[30] Segata, N., Waldron, L., Ballarini, A., Narasimhan, V., Jousson, O., Huttenhower, C., 2012. Metagenomic microbial community profiling using unique clade-specific marker genes. Nature methods 9, 811–814.

[31] Al-Abdoulhadi IA, Dinar HA, Ebert G, Büttner C. Influence of salinity levels on nutrient content in leaf, stem and root of major date palm (Phoenix Dactylifera L) cultivars. Int. Res. J. Agric. Sci. Soil Sci. 2012;2(8):341-6.

[32] Turk KG, Aljughaiman AS. Land use/land cover assessment as related to soil and irrigation water salinity over an oasis in arid environment. Open Geosciences. 2020 Jul 6;12(1):220-31.

[33] McBride M.J. (2014) The Family Flavobacteriaceae. In: Rosenberg E., DeLong E.F., Lory S., Stackebrandt E., Thompson F. (eds) The Prokaryotes. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-38954-2_130

[34] Spain, A. M., Krumholz, L. R., & Elshahed, M. S. (2009). Abundance, composition, diversity and novelty of soil Proteobacteria. *The ISME journal*, *3*(8), 992-1000.

[35] Madigan, M. T., & Martinko, J. (2005). Brock Biology of Microorganisms, 11th edn.

[36] Bergmann, G. T., Bates, S. T., Eilers, K. G., Lauber, C. L., Caporaso, J. G., Walters, W. A., ... & Fierer, N. (2011). The under-recognized dominance of Verrucomicrobia in soil bacterial communities. *Soil Biology and Biochemistry*, *43*(7), 1450-1455.

[37] Navarrete, A. A., Soares, T., Rossetto, R., van Veen, J. A., Tsai, S. M., & Kuramae, E. E. (2015). Verrucomicrobial community structure and abundance as indicators for changes in chemical factors linked to soil fertility. Antonie Van Leeuwenhoek, 108(3), 741-752.

[38] Buckley, D. H., Huangyutitham, V., Nelson, T. A., Rumberger, A., & Thies, J. E. (2006). Diversity of Planctomycetes in soil in relation to soil history and environmental heterogeneity. *Applied and Environmental Microbiology*, *72*(7), 4522-4531.

[39] Kalam, S., Basu, A., Ahmad, I., Sayyed, R. Z., El Enshasy, H. A., Dailin, D. J., & Suriani, N. (2020). Recent understanding of soil Acidobacteria and their ecological significance: A critical review. Frontiers in Microbiology, 11, 2712.

[40] Egidi, E., Delgado-Baquerizo, M., Plett, J. M., Wang, J., Eldridge, D. J., Bardgett, R. D., ... & Singh, B. K. (2019). A few Ascomycota taxa dominate soil fungal communities worldwide. Nature communications, 10(1), 1-9.

[41] Dhawi, F., Datta, R., & Ramakrishna, W. (2015). Mycorrhiza and PGPB modulate maize biomass, nutrient uptake and metabolic pathways in maize grown in mining-impacted soil. Plant Physiology and Biochemistry, 97, 390-399.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 2725 - 2740
Received 25 April 2021; Accepted 08 May 2021.

[42] Dhawi, F., Datta, R., & Ramakrishna, W. (2016). Mycorrhiza and heavy metal resistant bacteria enhance growth, nutrient uptake and alter metabolic profile of sorghum grown in marginal soil. Chemosphere, 157, 33-41.

[43] Dhawi, F. (2020). Plant Growth Promoting Rhizobacteria (PGPR) Regulated Phyto and Microbial Beneficial Protein Interactions. *Open Life Sciences*, *15*(1), 68-78.

[44] Lyu, Z., Shao, N., Akinyemi, T., & Whitman, W. B. (2018). Methanogenesis. Current Biology, 28(13), R727-R732.

Appendix:

Table1s: The correlation between Enzyme Commission codes (EC), PubMed, GenBank, NCBI Protein and KEGG GENES

KEGG GENES	NCBI Protein	GenBank	PubMed		EC
[ag:CAA43228]	CAA43228	X60835	1732207	1	
			9683650	2	
			10651637	3	
			17902707	4	
			19717587	5	E4.1.1.77
			5768862	1	
			12999785	3	
			20285042	4	
			4978445	5	
			13405917	6	E2.4.2.1
			9471964	1	E3.5.99.5
			9573204	2	
[ag:BAB03536]	BAB03536	AB020521	11081795	3	
			12620844	4	
[ag:BAA14135]	BAA14135	D90108	3162211	1	
[ag:BAA14135]	BAA14135	D90108	9882104	2	
[ag:BAA14135]	BAA14135	D90108	27393587	3	E3.4.21.111
			5418105	1	E3.2.1.22
			976079	2	
			10766746	1	
			12517450	2	
[mtu:Rv2429]			12761216	3	
			15178486	4	
			16368957	5	
			20463026	6	E1.11.1.28
			4356399	3	3.2.1.70
			6469959	1	
			6384215	2	E3.1.1.61
			6895223	2	E3.5.3.12
[ag:AAA83534]	AAA83534	M12799	3597405	2	
			10427017	3	
[ag:AAA83534]	AAA83534	M12799	9618487	5	E1.1.1.346

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 2725 - 2740
Received 25 April 2021; Accepted 08 May 2021.

[ag:BAB03531]	BAB03531	AB020521	9169437	1	
[ag:AAT35226]	AAT35226	AY605054	15580337	2	
[ag:AAT35226]	AAT35226	AY605054	23275161	3	E1.13.11.74
			810982	1	E1.1.1.47
			2530	3	
			12981017	4	
			4392298	5	
			21162	6	
			6791693	1	E3.4.21.50
			6168293	2	
			6359954	3	
			3090046	4	
[ag:AAA78946]	AAA78946	J05128	2684982	5	
[ag:AAA78946]	AAA78946	J05128	2492988	6	
			14167617	1	E1.14.19.1
			4382040	2	
			5543646	3	
			4373719	4	
[mja:MJ_1392]			9864346	1	E2.3.1.182
			15292141	2	
[ag:BAA04551]	BAA04551	D17669	1369078	1	E3.4.17.19
[ag:BAA04551]	BAA04551	D17669	7765282	2	
[ag:AAC45138]	AAC45138	U66917	9055410	1	E3.5.4.43
[ag:AAC45138]	AAC45138	U66917	17660279	2	
			16589307	1	E3.5.1.16
			13278318	2	
			10938279	2	E3.5.99.7
			21244019	3	
[hsa:112849]			22528483	2	E4.2.1.77
			4928623	1	E3.5.1.44
[mja:MJ_1140]			11589710	1	E3.1.3.71
			5641872	1	E3.5.4.20
			5354021	1	E1.7.2.1
			13295215	2	
			13782716	3	
			19396990	4	
[ag:CAA88564]	CAA88564	Z48635	1862344	6	
			7583671	7	
[tbd:Tbd_0077]			8639023	8	
[ag:CAA40150]	CAA40150	X53676	9409151	9	
			9667932	10	
			9308169	11	
			1094	1	E3.2.1.98
[tko:TK1765]	BAA88380	AB024740	11468293	1	E3.2.1.200
			15717865	2	
[ath:AT4G19810]			21390509	3	
[smw:SMWW4_v1c35890]	AGO05940	KC698971	23824666	4	

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 2725 - 2740
Received 25 April 2021; Accepted 08 May 2021.

			4171422	1	E1.6.5.2
			13705804	2	
			13765127	3	
			13846011	5	
			12226388	6	
			9485311	7	
			10683249	8	
[rno:24314]			7568029	9	
[mja:MJ_0430]			12538648	1	E3.5.4.30
			5487887	2	E1.11.1.5
			4318313	3	
			14189888	1	E5.1.1.8
			2722865	1	E3.5.3.18
[awo:Awo_c15700]			23315745	1	E2.8.3.23
[ag:BAI70378]	BAI70378	AB516431	20086163	1	E2.6.99.3
			23529730	2	