Pak. J. Bot., 56(6): 2441-2445, 2024. DOI: http://dx.doi.org/10.30848/PJB2024-6(21)

SOIL MICROBIOTA AND IDENTIFICATION OF MICROORGANISMS USING 16S rRNA GENE SEQUENCING BY ILLUMINA MISEQ IN THE HAIL REGION OF SAUDI ARABIA

NAWAF ALSHAMMARI[1*], MARYAM OMAR MUSA ALHDAIRES[1], HUSAM QANASH[2], YAZEED ALBULAIHED[1] AND KHAN MOHD. SARIM[4]

[1]Department of Biology, College of Science University of Hail, Hail, Saudi Arabia
[2]Department of Medical Laboratory Science, College of Applied Medical Sciences, University of Ha'il, Ha'il, Saudi Arabia
[4]Institut Ruđer Bošković Bijenička cesta 54, 10000, Zagreb, Croatia
[*]Corresponding author's email: naib.alshammari@uoh.edu.sa

Abstract

The microbiota, which refers to the microbial populations, wields significant influence over human and animal immune systems, geochemical nutrient cycles, and the health and productivity of plants. The interplay of these microbes with soil and their natural plant hosts within their ecosystems plays a pivotal role in understanding the development and pathogenic mechanisms of various diseases. Metagenomics has introduced a revolutionary technique for extracting DNA from environmental samples like soil, bypassing the need for culturing these microorganisms. This research primarily concentrated on identifying various bacterial species present in soil samples collected from the Hail region in north-central Saudi Arabia. Our approach involved leveraging 16S rRNA gene-based metagenomics analysis through the Illumina Miseq platform, coupled with the use of the NCBI nucleotide collection. Highly similar sequences underwent rigorous scrutiny via the Megablast program, and the obtained sequences were meticulously compared with the query sequences. The examination of over a thousand sequences unveiled that over 95% of these sequences exhibited significant alignment, with a total of 600 sequences corresponding to 100 distinct taxa. These taxa included pathogenic (comprising 40 species), non-pathogenic (comprising 15 species), parasitic (comprising 16 species), symbiotic (comprising 16) and additionally, 13 unidentified bacterial species. These findings underscore the diversity within the bacterial strains present in the soil, shedding light on the dynamic nature of soil microbiomes. This information not only serves as an indicator of soil health but also holds relevance for understanding plant-related pathogens and identifying beneficial microbes conducive to plant growth. Consequently, the primary objective of this study was to employ innovative molecular techniques in identifying bacterial species that pose a threat to plants. However, further research is imperative to delve deeper into the actual relationship between these isolated bacterial species and their ecological preferences, as well as to ascertain their potential medicinal properties.

Key words: Microbiota, Microbes, 16S rRNA, Illumina Miseq., Hail region Northern Saudi Arabia.

Introduction

The ecosystem is composed of two major components: the biotic and abiotic, which interact with each other (Fadiji *et al*., 2022). Within this intricate web of life, numerous species occupy various trophic levels, forming the foundation of ecological pyramids. Among these species, certain insects within the microbiota play a pivotal role in the ecosystem, sometimes leading to the emergence of pathogenic pest problems (Fahad *et al*., 2022).

Microorganisms, including protists, bacteria, archaea, and fungi, exert a significant influence on the agroecosystem of plant cultivation (Zhao *et al*., 2023). They are instrumental in steering the development of plants and the production of crops through the regulation of essential processes like the nitrogen and carbon cycles. However, our understanding of these microorganisms, their interactions, diversity, and ecological roles in our ecosystem remains limited (McDonald & Stukenbrock, 2016).

In a single soil sample, there exists a vast and diverse microbial population, which plays a crucial role in the development of pathogenicity in plant species. To gain insights into this intricate microbial world, a novel soil metagenomic technology (Senf, 2022) has been employed. This approach involves screening various clone libraries of microorganisms, followed by the isolation and analysis of DNA from soil samples. Through metagenomics, multiple genomes can be analyzed from environmental samples of interest (Ahmad *et al*., 2023).

Within this microbial tapestry, the Actinobacteria genus, such as Blastococcus, has been extensively studied and described. *Blastococcus atacamensis*, for instance, hails from the Atacama Desert and was first isolated from an exceedingly arid soil sample in November 2011. In the Geodermatophilaceae 16S rRNA gene tree, Blastococci forms a well-supported clade (Lee, 2006). Distinguishing features set them apart from other genera within this family, and these features are a result of their unique phenotypic characteristics.

Streptomyces species, found ubiquitously in fertile soil, are notable as major producers of natural products (Narayanan *et al*., 2023). Their contribution amounts to nearly 8,000 natural products, representing roughly 45% of all such products from microbial sources(Thomas & Singh, 2019). Streptomycetes are recognized by their resilient, filamentous colonies and play a critical ecological role in breaking down organic matter in the soil, utilizing complex organic materials as sources of carbon and energy [(Sholkamy *et al*., 2020)]. The composition of microbial communities, including streptomycetes, is influenced by a combination of biotic and abiotic factors, including vegetation, soil type, and climate (Shahbaz *et al*., 2023).

The diversity of actinomycetes, particularly that of streptomycetes, has been extensively documented for various purposes, including the continued isolation of environmental strains for pharmaceutical, biodegradative, and biotechnological research (Chang *et al*., 2022). These microorganisms remain integral to the ongoing exploration of their remarkable capabilities.

The primary objective of present research work is to assess the occurrence of pathogenic or non-pathogenic microorganisms in soil samples from a specific area. This evaluation will be achieved through high-throughput sequencing of the 16S rRNA gene using the Illumina Miseq platform. The study aims to shed light on the potential influence of soil microbiota on the pathogenicity of plants in the designated area, offering valuable insights into the role of specific microorganisms.

Material and Methods

Soil sampling: A survey of the sample collection site was conducted in January 2022. All healthy and diseased plants used for soil sample collection were photographed, and data on environmental conditions were recorded. Soil samples were gathered from the Hail region, located at coordinates, 27.984950 41.762861in the northern part of Saudi Arabia (Fig. 1). Rhizospheric soil samples were obtained from various depths using sterile hatchets and placed in sterile polybags. To prevent further oxidation, all samples were stored at 4ºC and subsequently utilized for physico-chemical analysis.

DNA Extraction, PCR and Electrophoresis: Total DNA was extracted from freshly collected soil samples from each specified different area, using an E.Z.N.A. HP Plant DNA Kit (Omega), according to the manufacturer's instructions. The purity of extracted DNA from each sample was calculated by $A_{260/280}$ and $A_{230/260}$ absorption ratio in spectrophotometer/nanodrop. Genomic DNA is amplified using 1541R 5' AAGGGGTGATCCAGCCAGCCCA-3' and 8F 5' AGAGTTTGATCCTGGCTCAG-3' primers (Al Othaim *et al*., 2020). PCR reaction was prepared in 25-µl mixture containing 1 µl of each universal primer (8F and 1541R); 3 µl of DNA template; 7.5 µl of Nuclease-Free Water, and 12.5 µl of GoTaq® GreenMasterMix (Promega™ Corporation, WI). The 16S ribosomal RNA gene is amplified in a thermal cycler (BioRad) and the reaction condition were as follows: initial denaturation at 95ºC for 3 min, followed by 35 cycles at 98ºC for 30 s, primer annealing at 55ºC for 30 sec, 72ºC for 1 min, and at the end final extension for 5 min at 72ºC). Finally, by using 1 µl of SYBR Safe DNA Gel Stain (Thermo Fisher Scientific) fluorescent dye, agarose gel electrophoresis was carried out to confirm the amplification. The amplified products were subjected for library preparation and 250bp paired end sequencing using Illumina MiSeq plaform (Illumina, San Diego, USA).

16S rRNA sequencing analysis: bioinformatics: The raw reads in the form of FASTQ file from sequencer were preprocess through quality check using Fast QC, and cutadapt (v1.14) for primer removal, as described by (Parada *et al*., 2016). All the short read less than 25bp were eliminated and clean high-quality reads were used for further processing of similarity sequence. QIIIME2 (v2020.8) pipeline was used to analyse the data, clean data was imported to QIIME2 through sample data function, and subsequently subjected to dada2 pipeline in order to remove noise and chimeric sequence by denoising and filtering. Subsequently SILVA curated database used for taxon identification and 97% threshold value set for genus and species sequence similarityKruskal Wallis test was used to confirm he statistical significance whereas to assess the beta diversity OTUs were identified through Jaccard coefficient. The sample depth in both cases were set 100. In addition to this permutation-based ANOVA (PerMANOVA) was also used to evaluate statistical significance among different groups.

The 16S rRNA sequencing data available at the NCBI Sequence Read Archive (SRA) (https://www.ncbi.nlm.nih.gov/search/all/?term=16SRNA) (accessed on 14 September 2022), used and examined each sequence individually by entering it into the NCBI nucleotide collection in which highly similar sequences through megablast program were retrieved and compared. The obtained results were checked for cultured or uncultured sequence. Any sequence scored more than 95% matching was taken into consideration for further analysis. In case of highly similar matching, 95% and above, if it was a cultured sequence, the sequence from gene bank was extracted and documented. It was noteworthy that most of the collected samples partially sequences were belonged to uncultured bacteria. Cultured sequences were further examined to determine if each individual type of bacteria to classify as "Symbiotic", "Pathogenic", or "Parasitic".

Results and Discussion

Pre-processing and sequencing: To explore the microbiome within the rhizosphere and bulk soil, A total of three samples were collected and for each sample, soil from five spots was collected and pooled to make a composite sample. The quantified DNA from all samples underwent 16S rRNA sequencing on the Illumina MiSeq platform. This yielded a total of [number] reads. Subsequently, the raw reads obtained from the Illumina platform were meticulously processed to remove chimeric sequences and improve quality scores. These cleaned reads were then aligned to the 16S rRNA database available on NCBI.

16S rRNA based taxonomic profiling: The obtained sequencing reads from the hypervariable region V3-V4 were analysed and categorized into family, genus, and species levels. Another crucial objective of this study was to identify and classify the output sequences into bacterial groups, distinguishing between pathogenic and non-pathogenic, symbiotic, and parasitic categories.

We employed partial sequences from the "Soil Microbial Community" file to establish associations between these sequences and well-documented bacterial types in the NCBI database. For this purpose, we conducted a standard nucleotide blast search using a nucleotide query, accessible at https://www.ncbi.nlm.nih.gov/nucleotide/ (accessed on 14 September 2022). In the "Enter Query Sequence" field, we initiated the identification process by individually submitting sequences from the specified file and executing the Blast sequence query search. Sequences exhibiting a minimum of 95% significant alignment in the results were documented and reported. The scientific name associated with each sequence indicated whether it was cultured, uncultured, or unidentified.

In our study, we examined more than 1000 sequences; those with a minimum of 95% significant alignment were considered for further investigation. However, any sequence with a significant alignment of less than 95% was discarded. Out of the 1000 path sequence, 600 sequences were significant (>=95%). They were grouped as uncultured (496 sequences), cultured (100 sequences), and unidentified (4 sequences). The obtained bacterium types were individually reviewed to check if they were pathogen, symbiotic, or parasitic. Their families were identified and reported in the form ID% and SGN. We identified 100 taxa of cultured bacteria and among which pathogenic, parasitic, non-pathogenic, and symbiotic-associated bacterial species were found. Moreover, Table 1 exhibited the unidentified four bacteria species.

The current study insights into the microbial communities inhabiting agricultural soil, shedding light on the consequences of pathogenic bacteria for agricultural productivity, following the categorization of bacterial species into pathogenic, parasitic, or symbiotic groups (Fig. 2). The soil samples analyzed exhibited a phylogenetically diverse range of microbial communities with a consistently stable structure (Alshammari *et al*., 2022). Alotaibi *et al*., (2020) have previously documented a multitude of fungal and bacterial species in extreme environments, such as Sabkha and hot deserts, some of which hold medicinal significance.

Several microbial species were isolated from the Al-Aushazia soil, including bacterial species like *Lactobacillus murinus* and *Bacillus subtilis*. Additionally, unique fungal species specific to the region, such as *Fusarium proliferatum*, *Myzostoma* spp., *Gymnoascus reesii*, *Fusarium proliferatum*, and *Actinomyces elegans*, were identified (Alotaibi *et al*., 2020). Soil microbiotas play a pivotal role, as they can mitigate soil-borne diseases and enhance the inherent suppressiveness of the soil. Disturbances within the microbiome can lead to specific disruptions, reshaping the interconnected web of soil microbial communities, ultimately bolstering resistance against phytopathogens and diseases (Lee, 2006; Oliveira *et al*., 2022).

Recent findings emphasize the close-knit relationship between plants and their microbiota, highlighting the fact that plants host unique and diverse microbial communities crucial for their survival (Lee, 2006).

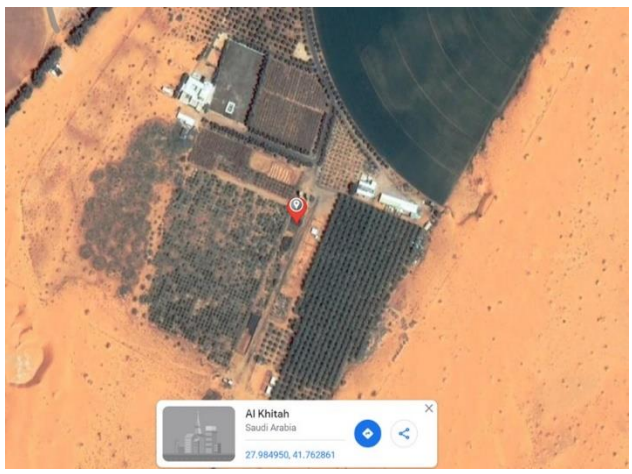

Fig. 1. Sample site.

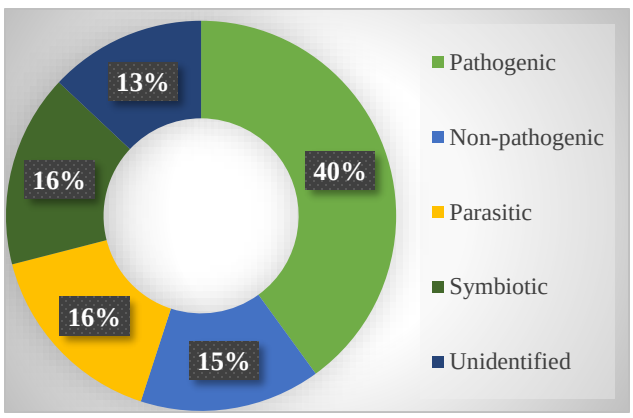

Fig. 2. The figures show the proportion of the Pathogenic, Parasitic, Symbiotic, and non-Pathogenic of taxon.

Table 1. The detail of taxon as pathogen and non-pathogen, parasitic, symbiotic and unidentified sp with their SGN.

No.	Taxon	Pathogen	Non-pathogen	Parasitic	Symbiotic	SGN
1.	*Geodermatophilaceae (Blastococcus)*		√			MH479063.1 98%
2.	*Xanthomonadaceae (Pseudoxanthomonas mexicana)*	√				MT549102.1 99%
3.	*Xanthomonadaceae (Lysobacter)*		√			KY194260.1 99%
4.	*Streptomycetaceae (Streptomyces)*	√				LC387249.1 98%
5.	Unidentified sp. 1					MH671375.1 99%
6.	*Devosiaceae (Devosia riboflavin)*				√	MT023386.1 100%
7.	Xanthomonadaceae (Lysobacter)	√				KY194260.1 99%
8.	Rhizocolahellebori		√			NR_126184.1 99%
9.	Xanthomonadaceae (*Pseudoxanthomonas mexicana*)	√				MT549102.1 99%
10.	Planococcaceae (*Planomicrobium glaciei*)		√			MN826480.1 99%
11.	Sphingomonadaceae (Sphingomonas)	√	Not highly pathogenic			LN833305.1 100%
12.	Flavobacteriaceae (Flavobacterium)	√				MT316501.1 99%
13.	Pseudomonadaceae (*Pseudomonas indoloxydans*)	√				MH725279.1 100%
14.	Rhizobiaceae (*Rhizobium alvei*)				√	MT373608.1 100%
15.	Thermomonosporaceae (Actinocoralliaherbida)	√				LC066303.1 95%
16.	Thermomonosporaceae (Actinocoralliaherbida)	√				LC066303.1 99%
17.	Unidentified sp. 2					MT815721.1 97%
18.	Actinobacteria, (Nocardioides)		√			KU560416.1 95%
19.	Bacteroidaceae (Bacteroidetes)	√				KP412837.1 100%
20.	Halobacteriaceae (Halococcus)			√		MG758766.1 99%
21.	Halobacteriaceae (Halococcus)			√		MG758766.1 98%
22.	Chitinophagaceae (Chitinophaga)			√		MT542328.1 99%
23.	Xanthomonadaceae (Lysobacter)		√			KY194260.1 100%
24.	Xanthomonadaceae (Lysobacter)		√			KY194260.1 100%
25.	Geodermatophilaceae (Blastococcus)	√				AB540016.1 95%

Table 1. (Cont'd.).

No.	Taxon	Pathogen	Non-pathogen	Parasitic	Symbiotic	SGN
26.	Pseudomonadaceae (*Pseudomonas indoloxydans*)			√		MT435025.1 97%
27.	Streptomycetaceae (Streptomyces)	√				OL587648.1 100%
28.	Unidentified sp. 3					MN966872.1 99%
29.	Chitinophagaceae (Chitinophaga)			√		KU379669.1 100%
30.	Sphingomonadaceae (*Sphingomonas crocodyli*)	√				LT840126.1 95%
31.	Blastocatellaceae (*Stenotrophobacter terrae*) Symbiotic				√	NR_146023.1 99%
32.	Unidentified sp. 4					MN492040.1 100%
33.	Chitinophagaceae (Chitinophaga)			√		KU379669.1 99%
34.	Unidentified sp. 5					CP019389.1 95%
35.	Pseudomonadota (*Qipengyuania pelagi*)	√				MN492070.1 99%
36.	Streptomycetaceae (Streptomyces)	√				CP054920.1 99%
37.	Devosiaceae (Devosia)	√				MH094645.1 99%
38.	Rhizobiaceae (Rhizorhabduswittichii)				√	MK696354.1 99%
39.	Unidentified sp. 6					KF193202.1 98%
40.	Flavobacteriaceae (Flavobacterium)	√				MN310902.1 99%
41.	Flavobacteriaceae (Flavobacterium)	√				MT316501.1 99%
42.	Cytophagaceae (Dyadobacter)			√		KY056227.1 99%
43.	Flavobacteriaceae (Flavobacterium)	√				MN310902.1 99%
44.	Cytophagaceae (Dyadobacterfermentans)			√		MT072107.1 99%
45.	Verrucomicrobiaceae (Luteolibacter luteus)	√				CP051774.1 99%
46.	Sphingomonadaceae (Sphingomonadales)	√				JQ402869.1 99%
47.	Unidentified sp. 7					LN876541.1 98%
48.	Unidentified sp. 8					MK519137.1 99%
49.	Phyllobacteriaceae (Mesorhizobium)				√	MT386299.1 99%
50.	Unidentified sp. 9					LC490854.1 97%
51.	Thermomonosporaceae (Actinocoralliaherbida)	√				OK384304.1 99%
52.	Halomonadaceae (Halomonas)	√				CP053032.1 95%
53.	Aeromicrobiumkwangyangensis			√		MT197356.1 98%
54.	Sphingomonadaceae (Sphingomonas)	√				LN876444.1 95%
55.	Unidentified sp.10					KY445633.1 99%
56.	Flavobacteriaceae (Empedobacter)	√				KC525956.1 97%
57.	Bradyrhizobium				√	AM086010.1 99%
58.	Xanthomonadaceae (Pseudoxanthomonas Mexicana)	√				MT540251.1 98%
59.	Rhizobiaceae (Rhizobium pseudoryzae)				√	CP049244.1 98%
60.	Priestiaaryabhattai			√		LC667800.1 99%
61.	Alicyclobacillaceae (Tumebacillus avium)		√			CP021434.1 99%
62.	Flavobacteriaceae (Flavobacterium)	√				CP042831.1 99%
63.	Actinomycetota (Actinomycetia bacterium)				√	MT613865.1 99%
64.	Acidobacteriaceae (Acidobacteria bacterium)				√	Z95735.1 95%
65.	Erythrobacteraceae (Aurantiacibacteraquimixticola)		√			MT628737.1 98%
66.	Flavobacteriaceae (Flavobacterium)	√				MT316501.1 99%
67.	Flavobacteriaceae (Flavobacterium)	√				MT316501.1 99%
68.	Pseudomonadaceae (Pseudomonas indoloxydans)			√		MH725279.1 99%
69.	Xanthomonadaceae (Pseudoxanthomonas Mexicana)	√				MT560351.1 99%
70.	Pseudomonadaceae (Pseudomonas indoloxydans)			√		MH725279.1 99%
71.	Pseudomonadaceae (Pseudomonas indoloxydans)			√		MH725279.1 95%
72.	Alteromonadaceae (Marinobacternanhaiticus)				√	KU320883.1 99%
73.	Alcaligenaceae (Achromobacter)	√				MT585879.1 99%
74.	Rhodobacteraceae (Rhodobacter)				√	KC174860.1 99%
75.	Unidentified sp. 11					AJ252709.1 98%
76.	Unidentified sp. 12					AJ252709.1 98%
77.	Erythrobacteraceae (Erythrobacter)	√				KX989393.1 95%
78.	Streptosporangiaceae (Nonomuraea)		√			MG770687.1 99%
79.	Rhizobiaceae (Rhizorhabduswittichii)				√	MK696354.1 99%
80.	Xanthomonadaceae (Stenotrophomonas pavanii)	√				MT534145.1 99%
81.	Micromonosporaceae (Polymorphospora rubra)		√			JQ899229.1 99%
82.	Erythrobacteraceae (Erythrobacter)	√				MH704967.1 98%
83.	Xanthomonadaceae (Lysobacter)		√			EU374884.1 99%
84.	Micrococcaceae (Arthrobacter)	√				OM060450.1 99%
85.	Micrococcaceae (Arthrobacter)	√				LN812280.1 95%
86.	Erythrobacteraceae (Erythrobacter)	√				MT829536.1 99%
87.	Micromonosporaceae (Micromonospora)		√			MT374969.1 98%
88.	Hyphomicrobiaceae			√		KC921198.1 99%
89.	Flavobacteriaceae (Flavobacterium)	√				MG232343.1 99%
90.	Flavobacteriaceae (Chryseobacterium)	√				MN326730.1 99%
91.	Flammeovirgaceae (Tunicatimonas)				√	MZ292242.1 98%
92.	Rhizobiaceae (Rhizobium alvei)				√	MT373608.1 99%
93.	Isosphaeraceae (Ellin6059)		√			AY234711.1 96%
94.	Chitinophagaceae (Chitinophaga)			√		MT542328.1 99%
95.	Unidentified sp. 13					AJ252709.1 95%
96.	Xanthomonadaceae (Lysobacter)		√			LC025471.1 99%
97.	Cytophagaceae (Niastellapopuli)				√	AB682649.1 99%
98.	Bradyrhizobium				√	MZ478068.1 96%
99.	Xanthomonadaceae (Xanthomonas)	√				KC439359.1 99%
100.	Saccharimonadaceae (*Candidatus saccharibacteria bacterium*)			√		CP065013.1 95%

Conclusion

Research work accessed the microbial diversity of above-mentioed farming region of Hail, Saudi Arabia using 16S rRNA approach through Illumina alongwith available database in NCBI, and categorized obtained microbes based on pathogenic, parasitic, or symbiotic. Such a high level of soil microbial biodiversity in present study suggests that microbiota play a prominent role in the generation or progression of disease associated with the plant. The authors have no knowledge of any published studies regarding soil microbes and there are no ongoing initiatives to compile a catalog of the taxa present in the hail region. However, the microbiological taxa of the Hail region in northern Saudi Arabia and its neighboring areas have been extensively studied using molecular techniques (Table 1). Genetic sequences of certain microbial taxa did not correspond to any entries in the GenBank database. This suggests that they may be relatively rare (i.e., mentioned but not yet analyzed) or potentially even new to the field of science. The authors anticipate that more comprehensive investigations will yield a significant number of additional records. Furthermore, intense research is needed to back up this claim. Insight from this result can be utilized further in designing bioremediation, plant growth promotion and disease suppression research strategies.

Acknowledgemnt

This research has been funded by Scientific Research Deanship at University of Ha'il – Saudi Arabia through project numbe GR-22 090.

References

Ahmad, Z., S.M. Khan, S.E. Page, H. Balzter, A. Ullah, S. Ali, S. Jehangir, U. Ejaz, R. Afza, A. Razzaq and A.S. Mukhamezhanova. 2023. Environmental sustainability and resilience in a polluted ecosystem via phytoremediation of heavy metals and plant physiological adaptations. *J. Clean. Prod.*, 385, 135733. https://doi.org/10.1016/j.jclepro.2022.135733.

Alotaibi, M.O., H.S. Sonbol, S.S. Alwakeel, R.S. Suliman, R.A. Fodah, A.S. Abu Jaffal, N.I. Al-Othman and A.E. Mohammed. 2020. Microbial Diversity of Some Sabkha and Desert Sites in Saudi Arabia. *Saudi J. Biol. Sci.*, 27(10): 2778-2789. https://doi.org/10.1016/j.sjbs.2020.06.038

Al-Othaim, A., D. Marasini and F. Carbonero. 2020. Impact of increasing concentration of tart and sweet cherries juices concentrates on healthy mice gut microbiota. *Food Frontiers*, 1(3): 224-33. https://doi.org/10.1002/fft2.46

Alshammari, N., M. Alazmi, N.A. Alanazi, A.M.E. Sulieman, V.N. Veettil and M. Ponce-Alonso. 2022. A comparative study on the microbial communities of *Rhynchophorus ferrugineus* (Red Palm Weevil)-infected and healthy palm trees. *Arab. J. Sci. Engin.*, 47(6): 6735-6746. https://doi.org/10.1007/s13369-021-05979-9

Chang, P.C., S.C. Liu, M.S. Ho, T.W. Huang and C.H. Huang. 2022. A soil-isolated *Streptomyces spororaveus* species produces a high-molecular-weight antibiotic AF1 against Fungi and Gram-Positive Bacteria. *Antibiotics*, 11(5). https://doi.org/10.3390/antibiotics11050679

Fadiji, A.E., O.O. Babalola, G. Santoyo and M. Perazzolli. 2022. The potential role of microbial biostimulants in the amelioration of climate change-associated abiotic stresses on crops. *Front. Microbiol.*, 12. https://doi.org/10.3389/fmicb.2021.829099

Fahad, S., M. Adnan, S. Saud and L. Nie. 2022. *Climate change and ecosystems: challenges to sustainable development*. CRC Press.

Lee, S.D. 2006. Blastococcus jejuensis sp. nov., an actinomycete from beach sediment, and emended description of the genus Blastococcus Ahrens and Moll 1970. *Int. J. Sys. Evol. Microb.*, *56*(10): 2391-2396. https://doi.org/10.1099/ijs.0.64268-0

McDonald, B.A. and E.H. Stukenbrock. 2016. Rapid emergence of pathogens in agro-ecosystems: Global threats to agricultural sustainability and food security. *Philosophical Transactions of the Royal Society B: Biol. Sci.,* 371(1709): 20160026. https://doi.org/10.1098/rstb.2016.0026

Narayanan, M., S.S. Ali and M. El-Sheekh. 2023. A comprehensive review on the potential of microbial enzymes in multipollutant bioremediation: Mechanisms, challenges, and future prospects. *J. Environ. Manag.*, 334: 117532. https://doi.org/10.1016/j.jenvman.2023.117532

Oliveira, C., E. Shakiba, D. North, M. McGraw, E. Ballard, M. Barrett-D'amico, G. Glazko and Y. Rahmatallah. 2022. 16S rRNA Gene-Based metagenomic analysis of rhizosphere soil bacteria in arkansas rice crop fields. *Agronomy*, 12(1): https://doi.org/10.3390/agronomy12010222

Parada, A.E., D.M. Needham and J.A. Fuhrman. 2016. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ Microbiol*., 18(5): 1403-14. https://doi.org/10.1111/1462-2920.13023

Senf, C. 2022. Seeing the System from Above: The use and potential of remote sensing for studying ecosystem dynamics. *Ecosystems*, 25(8): 1719-1737. https://doi.org/10.1007/s10021-022-00777-2

Shahbaz, A., N. Hussain, T. Mahmood M. Ashraf and N. Khaliq. 2023. Environment-friendly microbial bioremediation. In: *Microbial Bioreactors for Industrial Molecules* (pp. 397-419). Wiley. https://doi.org/10.1002/9781119874096.ch19

Sholkamy, E. N., P. Muthukrishnan, N. Abdel-Raouf, X. Nandhini, I.B.M. Ibraheem and A.A. Mostafa. 2020. Antimicrobial and antinematicidal metabolites from *Streptomyces cuspidosporus* strain SA4 against selected pathogenic bacteria, fungi and nematode. *Saudi J. Biol. Sci.*, 27(12): 3208-3220.

Thomas, L. and I. Singh. 2019. Microbial biofertilizers: types and applications. *Biofert. Sustain. Agri. Environ.*, 1-19.

Zhao, Y., Y. Lu, H. Zhuang and S. Shan. 2023. In-situ retention of nitrogen, phosphorus in agricultural drainage and soil nutrients by biochar at different temperatures and the effects on soil microbial response. *Sci. Total Environ.*, 904: 166292.

(Received for publication 12 May 2023)