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Bacterial Diversity in Al-Asfar Lake, Al Ahsa Oasis, Saudi Arabia

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Abstract

This study aimed to investigate bacterial diversity in Al-Asfar Lake, Al Ahsa Oasis, Saudi Arabia, based on a polyphasic approach. Water samples were collected and divided into two parts. For the culture-dependent approach, different media such as Tryptic Soy Agar (TSA), and Blood Agar (BA) were used. The obtained 29 isolates were diverse with respect to phenotypic characteristics revealed by VITEK. 18 isolates were selected for 16S rRNA sequencing based on the initial screening by VITEK. Since some types of bacteria do not grow on media, the DNA in the second part was isolated directly and subjected to metagenomic analysis. VITEK disclosed a total of 19 species belonging to 3 phyla: Pseudomonadota, Actinomycetota, and Bacillota, while the 16S rRNA sequencing revealed 12 species that could be amplified and sequenced. Metagenomic analysis exhibited variation in the relative abundance of 13 phyla, 18 classes, 20 families, 16 genera, and 24 species. Four dominant phyla are represented, including Firmicutes, Cyanobacteria, Bacteroidota, and Proteobacteria, respectively. Furthermore, functional gene screening revealed 33 functional categories including the metabolism of cofactors, vitamins, and xenobiotic biodegradation. This study affords insights into the bacterial diversity and fundamental biogeochemical processes in the lake and paves the way for the potential exploitation of microbial wealth in biotechnological applications.

Keywords: Al-Asfar Lake, Bacterial Diversity, Al-Ahsa, VITEK, 16S rRNA Sequencing, Metagenomic Analysis

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INTRODUCTION

Aquatic ecosystems are crucial elements of the earth's dynamic processes and are considered a massive contributor to biodiversity and ecological productivity; they also play fundamental roles in human economics and recreational activities.[1] Al-Asfar Lake is a significant shallow lake located about 70 km to the west of the Arabian Gulf in Al-Ahsa Oasis. It is considered the world's largest and most important oasis, covering 320 km^2 at about 150 m above sea level and containing numerous date palm and crop farms that play a vital role in economic development.[2]

Al-Asfar Lake, a vast man-made lake, was extensively eutrophied by Al Ahsa clay farm and animal waste and treated and untreated sewage water from a 1971 water network system.[3] The lake is characterized by a massive expanse of open water and extensive growth of flora much appears around the lake.[4,5] The lake has a shallow depth that inhibits thermal stratification where the maximum depth of the lake reaches 1.5 meters, and the water temperature of the lake follows the temperature of the surrounding air.[6] The lake water had a narrow alkaline nature pH range ranging from 7 to 8.3.[5]

l-Asfar Lake is marked by heavy metal pollution such as copper, zinc, and cadmium which is a significant problem with wide-ranging environmental impacts including the decline in water quality and accumulation of metals in aquatic organisms' tissues.[6] This bioaccumulation can lead to high concentrations of toxic metals in the food chain, potentially affecting human health if contaminated fish are consumed. Heavy metal pollution in lakes is a complex environmental problem that requires interdisciplinary efforts to find solutions and protect both the environment and public health.

The presence of halotolerant and moderately halophilic bacteria has been assured in Al-Asfar Lake water using the 16S rDNA sequencing method, including species of *Staphylococcus*, *Halobacillus,* and *Halomonas*, that can grow in the presence of NaCl.[4] Antimicrobial-resistant bacteria such as *E. coli*, *Salmonella typhimurium*, *and Staphylococcus* spp. included *S. aureus*, *S. intermedius*, *S. xylosus*, *S. capitis*, *S. saccharolyticus*, and *S. saprophyticus* were found in Al-Asfar Lake, where Asian, African, and European wild birds fly that may spread Al-Asfar Lake's antimicrobial-resistant bacteria.[3]

Microbial communities of Lake Elmenteita in Kenya revealed that the most abundant group in terms of numbers was Cyanobacteria. In contrast, the Firmicutes group was the second in terms of numbers but marked as the utmost diversity in terms of genera represented.[7] Chaohu shallow eutrophic lake in China disclosed high richness and diversity that showed that the dominant phyla of water were Cyanobacteria, Proteobacteria, Actinobacteria, and Bacteroidetes.[8] Water microbial communities of Lonar Lake showed that most were correlated to the phylum Firmicutes, with various genera*: Vagococcus, Alkalibacillus, Bacillus, Planococcus, Exiguobacterium, Paenibacillus,* and *Enterococcus*.[9]

Culturable and metagenomic approaches are two distinct methods used to characterize microbial communities in lakes. Molecular approaches are a transition point for microbial ecology studies that permit the simultaneous screening of hundreds of microbial communities.[10] Furthermore, it relates microorganisms to each other by creating phylogenetic tree maps based on gene sequences which disclosed that the essential extent of Earth's biodiversity is microbial.[11] However, culture-dependent methods remain very beneficial by permitting the isolation of individual species, making it possible to study their physiology and behavior in detail, providing a way to estimate the abundance of specific culturable microbes, and enabling the performance of functional assays, metabolic tests, and antibiotic resistance assessments, and more.[12] There is considerable argumentation regarding the most adequate approach, while it has been agreed on the sufficiency of the culture-independent molecular methods it fails to consider the distinction in cellular activity level as plentiful bacteria can live in dormant forms therefore it is very substantial to assess the activity of bacteria that participate to ecosystem functions and not inactive cells.[13] In summary, the choice between culturable and metagenomic approaches depends on the specific research goals and the level of detail required. In many cases, a combination

of both approaches, known as multi-omics, can provide a more holistic understanding of microbial communities in lakes.

Al-Asfar Lake holds significance as a prominent shallow wetland in the area and is considered a historical landmark in Al Ahsa. The few previously conducted studies in this lake mainly focused on isolating individual bacterial strains. However, the information about bacterial diversity and their community structure is unknown since none of the studies explored it. Therefore, this research aimed to conduct the first comprehensive study in this area to investigate the bacterial diversity and community structure using culture-dependent and culture-independent methods to better understand the lake ecosystems, provide basic information about the bacterial community composition, and will be an important implication for the optimization of integrated ecosystem assessment of the lake that provides interesting information for the subsequent ecosystem.

MATERIALS AND METHODS

Collection of water samples

Water samples (1500 ml) were collected from the upper layer of the water column, usually at a depth of 0.5 to 1 meter below the water surface in sterilized containers using a stainless-steel telescopic sampling rod container holder. The samples were collected on August 4, 2022, from three distinct locations of the lake (Figure 1) where the temperature reached 36.5° for water and 37° for the air with the following coordinates: First Location (WAT 1= Drainage site): 49°48'56.4"E longitude and 25°32'19.7"N latitude, Second Location (WAT 2=lake): 49°48'00.1"E longitude and 25°32'10.5"N latitude, and Third Location (WAT 3=lake): 49°48'33.3"E longitude and 25°32'09.0"N latitude." All samples were cooled and preserved immediately in an icebox at 4°C to be transferred into the laboratory under refrigerated conditions for further analysis. No pre-treatment or filtration for water samples has been performed before culture or DNA extraction.

Isolation and characterization based on culture-dependent methods

Isolation of bacteria and phenotypic characterization

A serial dilution in sterile saline (0.85% NaCl) was used for isolation. 150 µl of each dilution was inoculated onto Tryptic Soy Agar (TSA),

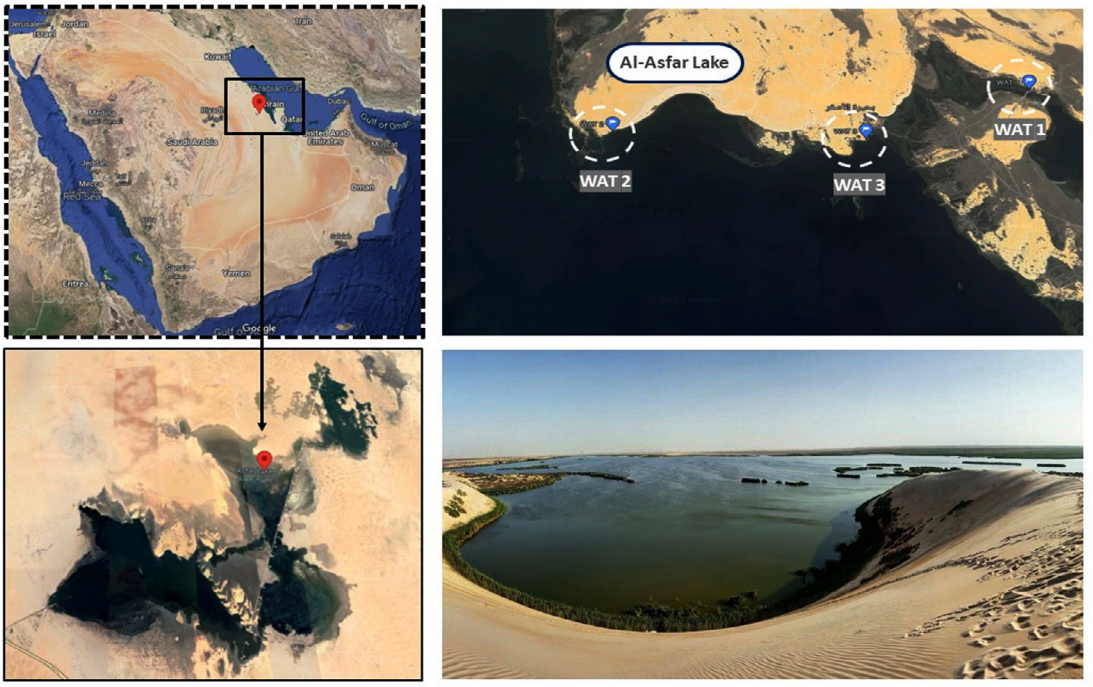

Figure 1. Map of Al-Asfar Lake study area. (Retrieved from https://earth.google.com/web)

Blood Agar (BA), King's B Agar (KB), Reasoner's 2A Agar (R2A), Nutrient Agar (NA), CHROMagar™ Orientation, Mueller Hinton agar *(*MHA*)*, Glucose Yeast Peptone Agar (GYP), Pikovskaya Agar (PVK), Casein Starch Agar (CSA), Tryptone Soya Yeast Extract Agar (TSYEA). All dilutions have been spread on agar with a sterilized L-shaped rod. After that, the plates were incubated at 28°C for 24-72 h. After incubation, colony-forming units per ml have been calculated.[14] Bacterial colonies were picked and streaked onto fresh media to get pure colonies. Isolates were preserved in tryptic soy broth containing 20% glycerol at -20°C freezer for long-term preservation.

The isolates were characterized based on colony color, margin, elevation, shape, and Gram stain. Following the manufacturer protocol, the bacterial isolates have been investigated using the VITEK 2® COMPACT (BioMérieux).

Hemolytic activity for the isolates was assessed in blood agar media after incubating at 38°C for 24 h. A transparent zone will be counted as a positive result in case of complete hemolysis (β-hemolysis) as well as a slight haze zone in case of incomplete hemolysis (α-hemolysis).[15]

Antibiotic susceptibility was assessed on Mueller Hinton agar plates. The Oxoid antibiotic discs of Erythromycin (E) 15 µg, Bacitracin (B) 10 µg, Vancomycin (VA) 30 µg, Novobiocin (NV) 5 µg, and Cephalothin (KF) 30 µg, were applied on the agar plate and then incubated at 38°C for 24 h.

Table 1. CFU of bacteria present in Al-Asfar Lake

Sample code	CFU/ml
WAT 1	2.1×10^4
WAT 2	1.2×10^5
WAT 3	3.0×10^4

Table 2. List of the identified bacteria by VITEK/ selected isolates (highlighted in yellow) for further testing

No.	Strain	VITEK identification %	Sample code
1.	*Brevundimonas diminuta / vesicularis*	Excellent identification (99%)	WAT 21
2.	*Aeromonas salmonicida*	Low discrimination (97%)	WAT 14
3.	*Acinetobacter lwoffii*	Low discrimination (95%)	WAT 25
4.	*Acinetobacter lwoffii*	Low discrimination (95%)	WAT 24
5.	*kocuria rosea*	Low discrimination (91%)	WAT 28
6.	*kocuria rosea*	Low discrimination (97%)	WAT 17
7.	*kocuria rosea*	Good identification (89%)	WAT 19
8.	*kocuria rosea*	Low discrimination (91%)	WAT 22
9.	*Alloiococcus otitis*	Low discrimination (94%)	WAT 1
10.	*Alloiococcus otitis*	Low discrimination (94%)	WAT 15
11.	*Sphingomonas paucimobilis*	Very good identification (93%)	WAT 29
12.	*Sphingomonas paucimobilis*	Excellent identification (97%)	WAT 3
13.	*Enterobacter cloacae complex*	Excellent identification (99%)	WAT 30
14.	*Enterobacter cloacae complex*	Very good identification (95%)	WAT 5
15.	*leuconostoc mesenteroides ssp cremoris*	Low discrimination (94%)	WAT 7
16.	*leuconostoc mesenteroides ssp cremoris*	Good identification (91%)	WAT 23
17.	*Pasteurella canis*	Very good identification (95%)	WAT 11
18.	*Providencia alcalifaciens*	Very good identification (95%)	WAT 8
19.	*Providencia alcalifaciens*	Very good identification (95%)	WAT 9
20.	*Pseudomonas stutzeri*	Low discrimination (96%)	WAT 13
21.	*Pseudomonas stutzeri*	Excellent identification (98%)	WAT 26
22.	*Rhizobium radiobacter*	Excellent identification (99%)	WAT 6
23.	*Gardnerella vaginalis*	Low discrimination (90%)	WAT 20
24.	*Pseudomonas alcaligenes*	Low discrimination (95%)	WAT 27
25.	*Moraxella group*	Low discrimination (91%)	WAT 10
26.	*Vibrio cholerae*	Excellent identification (98%)	WAT 2
27.	*Klebsiella pneumoniae*	Good identification (92%)	WAT 12
28.	*Streptococcus thoraltensis*	Very good identification (95%)	WAT 16
29.	*Staphylococcus lentus*	Good identification (90%)	WAT 4

Zones of inhibition were measured three times in millimeters (mm)

Molecular identification of isolates using 16S rRNA gene

Genomic DNA was extracted using boiling protocol.[16] PCR was carried out using Applied Biosystems GeneAmp™ PCR System 9700 thermal cycler following the previously reported conditions.[17] The 16S rRNA gene sequencing has been performed using Applied BioSystems model 3730XL automated DNA sequencing system. For phylogenetic analysis, the obtained sequences were subjected to computer software

Table 3. Calculate the Average value in mm for the diameter of each inhibition zone

Samples	Erythromycin	Bacitracin	Vancomycin	Novobiocin	Cephalothin
WAT 2	22	20	26	Resistant	40
WAT 4	2	Resistant	Resistant	Resistant	Resistant
WAT 5	Resistant				
WAT 6	22	Resistant	20	8	28
WAT 7	22	Resistant	18	8	28
WAT 8	Resistant	Resistant	6	Resistant	Resistant
WAT 10	10	8	4	2	Resistant
WAT 11	20	24	12	20	40
WAT 12	6	Resistant	Resistant	2	10
WAT 13	Resistant	Resistant	Resistant	8	Resistant
WAT 15	8	20	12	20	38
WAT 16	8	4	10	8	6
WAT 20	20	14	12	10	20
WAT 21	Unmeasured1	1	Unmeasured	18	Unmeasured
WAT 23	10	20	8	18	30
WAT 25	2	22	12	20	36
WAT 27	Resistant				
WAT 29	10	8	10	10	12

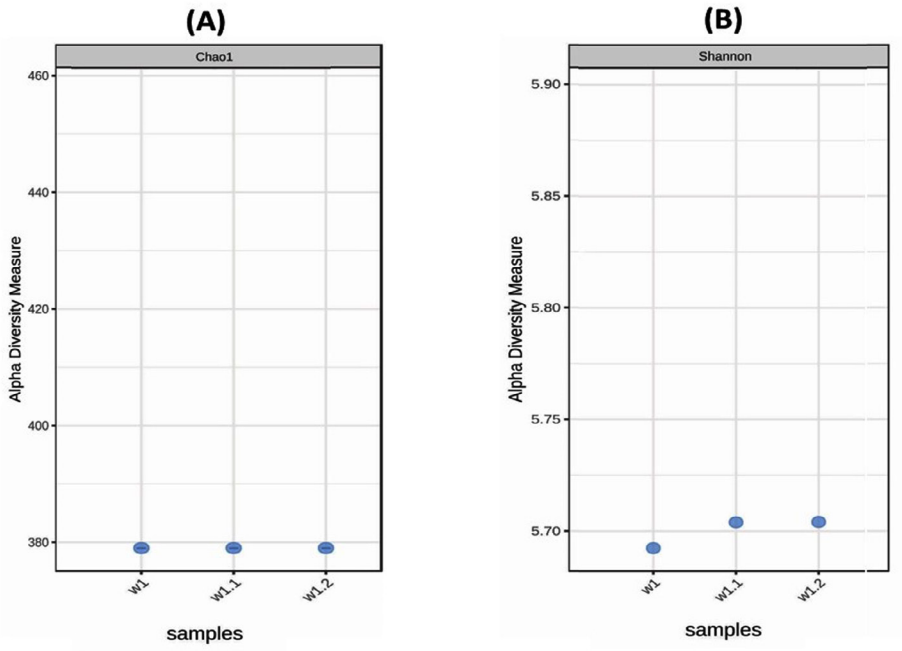

Figure 2. Bacterial diversity analysis using alpha-diversity: (A) Chao1 index, (B) Shannon index

analyses to be compared with other sequences using the GenBank Database by using the BLAST algorithm (National Center for Biotechnology Information, Maryland, USA). A phylogenetic tree was constructed in MEGA11.[18] The evolutionary history was inferred using the neighbor-joining method based on the Saitou and Nei model.[19] The evolutionary distances were computed using the Maximum Composite Likelihood method based on Tamura K, Nei M, Kumar S. model.[20]

Identification based on culture-independent methods

DNA was extracted using a QIAGEN DNeasy Power Water kit following the manufacturer's protocol. The extracted DNA was electrophoresed for assessment in 1% agarose gel and quantified using a NanoDrop 2000C Spectrophotometer (Thermo Fisher Scientific). The obtained extracts were kept at -20°C prior to pooling and sequencing. Equal amounts of High-quality and quantity DNA from each of the 3 DNA extracts were pooled and sent for sequencing.[21]

DNA extracts were subjected to 16S sequencing and shotgun analysis. Macrogen (Seoul, South Korea) sequenced the metagenome following the library technique using a TruSeq DNA PCR-Free Kit from Invitrogen (Madison, WI, USA).

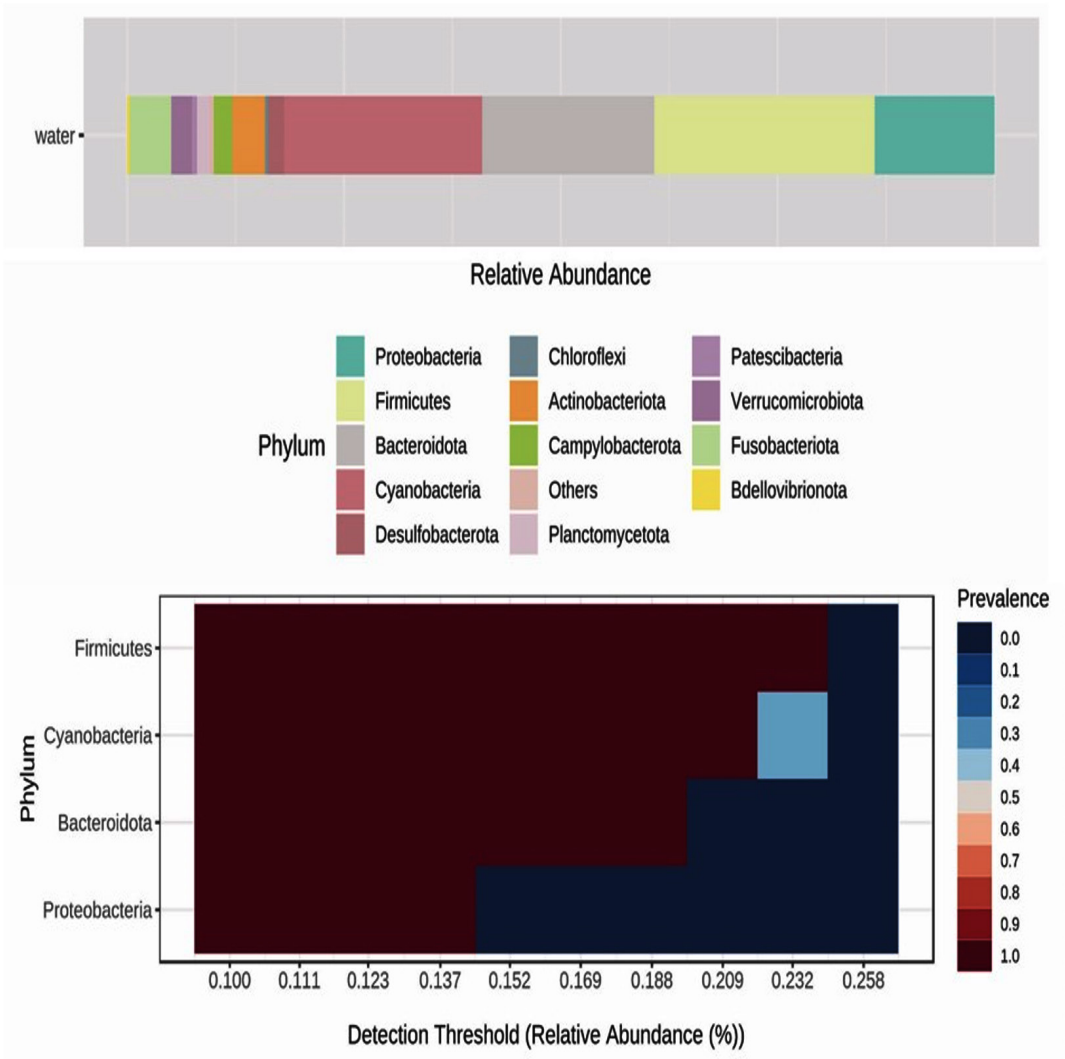

Figure 3. Bacterial phylum composition in Al-Asfar Lake

A 2 101 bp paired-end procedure on the Illumina HiSeq 2000 was used to evaluate each barcoded sample.

MiSeq FASTQ sequence files were processed and read clustered into Amplicon Sequence Variants (ASVs) using the dada2 pipeline (5) as explained in this tutorial https://benjjneb.github.io/dada2/tutorial.html. An ASV table is a higher-resolution analog of the traditional Operational Taxonomic Units table, which records the number of times each exact ASV was observed in each sample. ASV, taxonomy tables, and student metadata were then loaded into Microbiome Analyst (6) for further microbiome analyses. Microbiome Analyst is an open-access web-based tool (https://www.microbiomeanalyst.ca/faces/home.xhtml) for comprehensive statistical, visual, and meta-analysis of microbiome data. The Microbiome Analyst website also includes several detailed tutorials and datasets for learning microbiome analyses.[22]

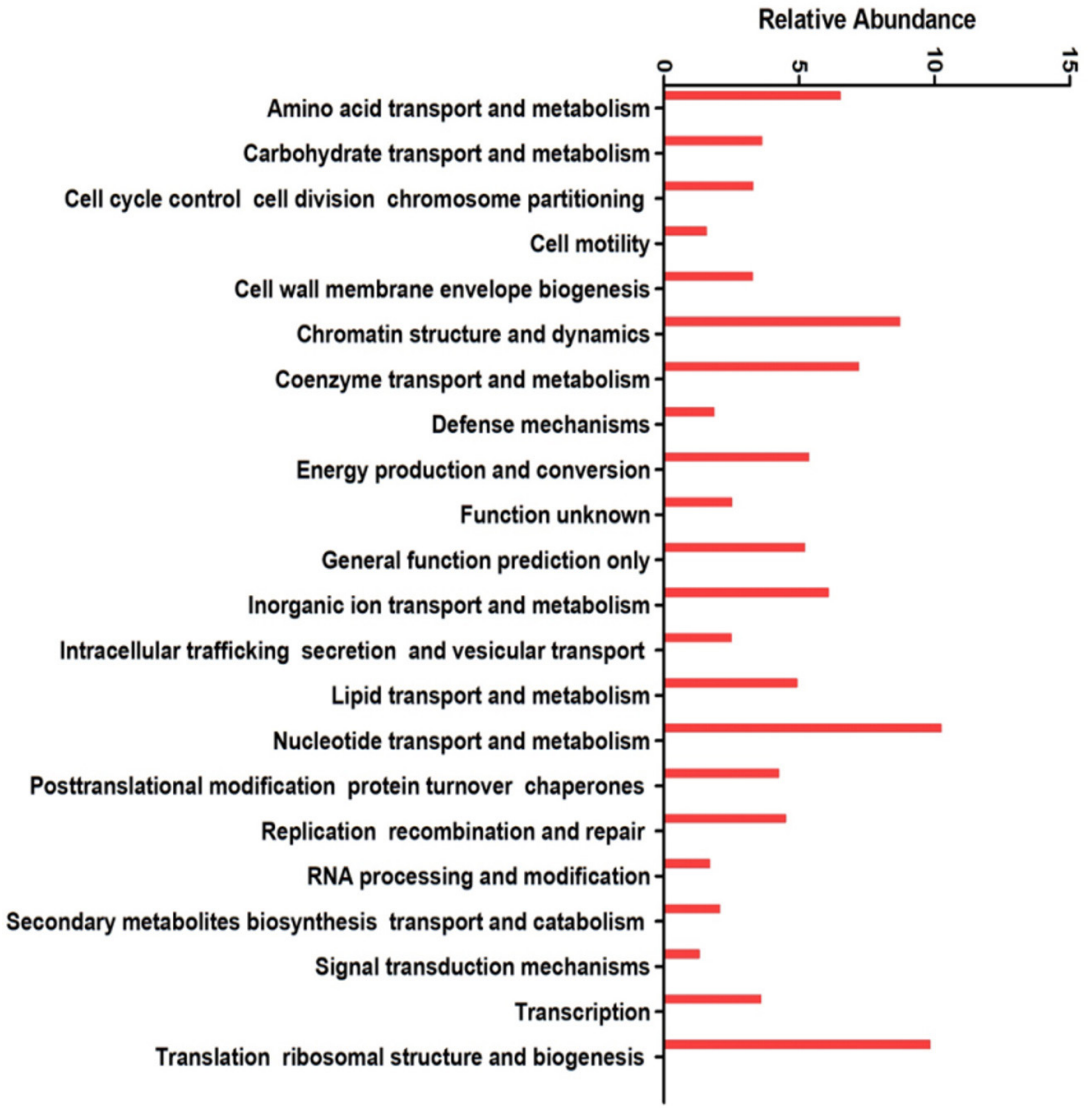

Figure 4. The abundance of observed functional categories among metagenomes

Table 4. Identity percentage of the isolates to the closely related recognized species

Sample	Closest species	% identity	Accession number
WAT 2	*Kosakonia oryzendophytica*	98.7%	JF795011
WAT 4	*Vibrio metoecus*	97.59%	KJ647312
WAT 5	*Salmonella enterica*	94.94%	EU014688
WAT 6	*Bacillus paralicheniformis*	95.10%	KY694465
WAT 7	*Metabacillus fastidiosus*	93.02%	AB681412
WAT 8	*Providencia alcalifaciens*	98.67%	ABXW01000071
WAT 12	*Pseudomonas guariconensis*	97.31%	FMYX01000029
WAT 13	*Pseudomonas gallaeciensis*	97.55%	FN995250
WAT 21	*Exiguobacterium profundum*	95.27%	AY818050
WAT 23	*Exiguobacterium profundum*	98.74%	AY818050
WAT 25	*Staphylococcus coagulans*	97.21%	AB233334
WAT 27	*Klebsiella granulomatis*	98.63%	AF010251

Nb: Sample WAT10, WAT11, WAT15, WAT16, WAT20, and WAT29 not amplified

RESULTS

Culture-dependent methods

Isolation of bacteria and phenotypic characterization

The total CFU on Tryptic Soy Agar media was found to be between 2.1×10^4 to 1.2×10^5 CFU/ml (Table 1). indicating a high abundance of bacterial communities harboring the lake.

Based on the morphological features of the colonies distinct bacterial isolates were selected (Figure 2). Diverse morphological characteristics were reported (Table 2).

19 diverse species belonging to three different phyla have been indicated in 29 samples, 62% belonged to the Pseudomonadota, 17.24% to Actinomycetota, and 20.68% to Bacillota. From the 29 identified isolates, 18 were chosen based on similarities in morphology, Gram stain, and VITEK results to be subjected to further phenotypic tests and 16S rRNA gene identification (Table 2).

The VITEK biochemical test identification for the selected 18 strains indicated positive results for 55 different substrates that varied among the isolates (Table 2).

The hemolysis activity detected in WAT 16 formed a clear zone due to the complete lysis of red blood cells (Beta hemolysis) and in WAT 15 that showed partial hemolysis characterized by greenish-brown discoloration (Alpha hemolysis).

Antibiotic susceptibility test was determined against different concentrations of 5 antibiotics using the disk diffusion method on Muller Hinton agar media. Triplicate measurements of the diameter inhibition zone were taken, and the average values have been calculated (Table 3). The susceptibility degree varied among the tested isolates. Many isolates had higher sensitivity towards Cephalothin (Kf) which recorded the most oversized diameter reaching 40 mm in two samples. While the susceptibility against VA, B, E, and NV was nearly convergent. Mean±Deviation values of the antibiotic inhibition zone's diameter are demonstrated.

Antibiotic resistance has been indicated in 10 samples. WAT 2 was resistant to NV, and WAT 4 was resistant to B, VA, NV, and KF. WAT 6 and WAT 7 were resistant to B, WAT 8 showed resistance towards all antibiotics except VA, WAT 10 was resistant to Kf, WAT 12 was resistant to B and VA, and WAT 13 was resistant to all except NV. While two of the samples WAT 5 and WAT 27 showed complete resistance to all used antibiotics.

Sequencing of 16S rRNA gene

Comparative sequences of analysis of the 16S rRNA gene revealed that the isolates belonged to different genera as shown in Table 4. WAT 12 and WAT 13 belonged to *Pseudomonas guariconensis* (accession number FMYX01000029) and *Pseudomonas gallaeciensis* (accession number FN995250) to which they exhibited 97.31% and 97.55% sequence homology, respectively. WAT 21 and WAT 23 belonged to *Exiguobacterium*

profundum (accession number AY818050) that had an identity of 95.27% and 98.74% respectively. The 16S rRNA gene sequences for isolates WAT 2, WAT 4, WAT 5, WAT 6, WAT 7, WAT 8, WAT 25, and WAT 27 shared 98.7%, 97.59%, 94.94%, 95.10%, 93.02%, 98.67%, 97.21%, and 98.63% identity with those of *Kosakonia oryzendophytica* (accession number JF795011), *Vibrio metoecus* (accession number KJ647312), *Salmonella enterica* (accession number EU014688), *Bacillus paralicheniformis* (accession number KY694465), *Metabacillus fastidiosus* (accession number AB681412), *Providencia alcalifaciens* (accession number ABXW01000071), *Staphylococcus coagulans* (accession number AB233334), and *Klebsiella granulomatis* (accession number AF010251), respectively. Six samples were not amplified.

The evolutionary history was inferred using the neighbor-joining method. The phylogenetic tree constructed using the 16S rRNA gene sequences of closely related recognized species shows the taxonomic affiliation of our isolates conducted by MEGA11 software. The evolutionary distances were computed using the Maximum Composite likelihood method and are in the units of the number of base substitutions per site.

Samples were clearly clustered together such as WAT 13 and WAT 12 which were clustered with members of *Pseudomonas* sp. As well as sample WAT 21 and WAT 23 that clustered with members of *Exiguobacterium* sp. Whereas other strains formed monophyletic subgroups such as WAT 4, WAT 2, WAT 27 with *Vibrio metoecus*, *Kosakonia oryzendophytica*, and *Klebsiella granulomatis*, respectively.

Culture-independent methods

The bacterial community structure of Al-Asfar Lake revealed a magnificent spectrum of diversity. After sequencing, the alpha diversity was measured using the Shannon index to assess the richness and evenness of bacterial taxa, which was around the value of 5.70. And the Chao1 index to estimate the total richness, which was 380 (Figure 2).

Taxonomic analysis at the phylum level showed that thirteen phyla were classified, and four predominant phyla are represented in (Figure 3). The Firmicutes, Cyanobacteria, Bacteroidota, and Proteobacteria were the most abundant phyla, respectively.

At the class level, eighteen classes were detected. Cyanobacteria, Clostridia, and Bacteroidia were the three most abundant classes, respectively (Figure 3). At the family level, twenty families have been disclosed. *Cyanobiaceae* and *Fusibacteraceae* were the most abundant families (Figure 3). At the genus level, sixteen genera were detected, and three genera were dominant, the first could not be assigned, while the two others were *Cyanobium_PCC_6307* and *Fusibacter*, respectively (Figure 3). At the species level, 24 bacterial species were observed. Two were dominant; one could not be assigned, while the second was *Fusibacter fontis* (Figure 3).

Functional gene expression of bacterial communities revealed many vital functions, such as secondary metabolite biogenesis and xenobiotic biodegradation. The most abundant functions included nucleotide and coenzyme transport and metabolism, translation, and ribosomal biogenesis. In addition, the metabolism of amino acids, cofactors, and vitamins (Figure 4).

DISCUSSION

This research examined the bacterial composition in Al-Asfar Lake using various methods, aiming to enhance our overall comprehension of the lake's ecosystems and to identify previously undiscovered species with potential applications. The goal was to study how the bacterial community responds to its environment, with the aim of promoting environmental sustainability and optimizing the assessment of the lake's integrated ecosystem, thereby providing valuable insights for future ecosystem management.

The CFU analysis revealed that the lake serves as a crucial habitat for a diverse range of bacteria. Our findings were consistent with prior research, although our study showed a higher CFU count.[23] Slight variations in CFU counts were observed across the three locations, which can be attributed to a combination of factors. In the drainage area of the lake (WAT 1), CFU counts were lower, possibly due to reduced nutrient availability compared to the lake basin. Additionally, the drainage site might receive contaminants that inhibit microbial growth and lead to lower CFU

counts. Whereas disparities in nutrient levels or water turbidity may explain the CFU count differences between locations WAT 2 and WAT 3 within the lake. These factors have been reported in previous study as a crucial factor that can significantly impact the microbial composition and diversity of certain ecological niches, that may exert their impact alone or in combination.[24]

Based on the colony morphology analysis, distinct single colonies were picked for purification for further identification. Many bacterial isolates have been obtained by using several culture media that aid in retrieving the bacterial diversity in the lake. The variation in the used media has proven its high efficiency in capturing bacteria in our study as has been proven in previous diversity studies.[25] The obtained twenty-nine isolates offered us an insight into the remarkable level of diversity with respect to morphological, biochemical, and molecular characterization. Similar observations have been previously reported in Lonar Lake in India,[9] in the Mongolian Baer Soda Lake,[26] and in El-Djerid Salt Lake (Tunisia).[27]

VITEK analysis has successfully identified 19 distinct species spanning three different phyla among the 29 tested isolates, based on their biochemical reactions. The diversity of the bacterial population was further substantiated by comparing our VITEK results with those from studies conducted in Alqueva water,[28] and Sapanca Lake (Turkey).[29] Notably, our VITEK findings revealed a higher level of diversity compared to the results reported in Alqueva water.[28] Additionally, we detected distinct *Staphylococcus* species not previously observed in Al-Asfar Lake in a prior study.[3] Furthermore, the presence of *Sphingomonas paucimobilis* in our findings has been associated with polluted environments in China.[30] Our VITEK biochemical findings have revealed significant phenotypic responses that hold the potential to enhance environmental sustainability. In aquatic environments, the presence of enzyme-mediated nitrogen cycling is essential. Several isolates, including WAT 4, WAT 6, WAT 20, and WAT 23, exhibit arylamidase enzymes. Enzymes like arylamidase, which is involved in nitrogen cycling, play a vital role in environmental processes.[31] Additionally, WAT 10 and WAT 21 possess lipase enzymes that could find applications in various industrial processes. Furthermore, WAT 6 and WAT 12 are equipped with beta-glucosidase enzymes, which are involved in biofuel production. We also observed resistance to Optochin, Bacitracin, Novobiocin, and Polymyxin in WAT 4, WAT 16, WAT 20, and WAT 23, with comparable resistance reported in previous research.[32] Similarly, our investigation into sugar substrate absorption and fermentation yielded similar results as previous studies.[32]

Interestingly, our study detected hemolysin production in *Streptococcus thoraltensis* "WAT 16," contrary to previous research that had categorized this species as non-hemolytic. Similar results were also observed for WAT 15.[33] It is important to note that further investigation is required to confirm the pathogenicity of these strains.

55.55% of isolates exhibited antibiotic resistance, with our results aligning with certain previous studies,[33] although conflicting findings were reported in other studies.[34] Notably, our research showed that the highest sensitivity was observed towards cephalothin, consistent with a previous study.[34]

Utilizing comparative 16S rRNA gene sequencing offers additional evidence for the presence of bacterial diversity within the lake. Our isolates' taxonomic association is shown by a neighbor-joining phylogenetic tree of closely related species' 16S rRNA gene sequences, our identified strains were reported previously that had identical characterization to Al-Asfar isolates.[35]

Investigation of bacterial communities using metagenomic DNA analysis revealed a magnificent spectrum of diversity. The lake bacterial communities are diverse according to the Shannon index which was near to what has been reported,[36] while the Chao1 index indicated higher diversity than stated in a former study.[37] Previous diversity analysis studies identified our phylum groupings.[9] Several sequences could not be assigned and were grouped as unclassified bacteria that might be novel, similar incidents were reported.[38] Firmicutes phyla were the most abundant identical to what has been reported in Lonar Lake,[9] and contradicts Kenya's study.[7] Similarities at the class level have been observed in Gammaproteobacteria and Desulfobacteria.[11] Propinquity was indicated at the family level.[39] lower abundance at the genus level was seen in a

previous study in comparison with Al-Asfar.[40] This approach uncovered a greater level of diversity in various species compared to earlier research.[41]

Functional diversity of Al-Asfar Lake bacterial communities revealed a high number of metabolic pathways that have been characterized in a preceding study in addition to the promising biodegradation capability.[42] Through the unveiling of the abundance of such bacterial communities with metabolic pathways associated with significant functions, this research has the potential to offer valuable insights into the structures of microbial communities and the ecological processes within the Al-Asfar Lake ecosystem.

In our study, discrepancies between the results of identification using 16S rRNA gene sequencing and the VITEK system have been indicated. This contradiction might be due to the differences between the 16S rRNA gene database used for sequencing and the database utilized by the VITEK system leading to different species assignments. VITEK database limitations have been documented in earlier research.[43] In addition, The VITEK system primarily relies on phenotypic characteristics and biochemical tests for bacterial identification, while 16S rRNA gene sequencing is a genotypic method. Differences in genotype and phenotype can lead to discordant results. Inconsistency between phenotypic and genotypic identification methods have been indicated in previous study.[44] Besides, 16S rRNA gene sequencing is generally considered a more robust and accurate method for bacterial identification, whereas automated systems like VITEK can have limitations, especially for less common or atypical strains.[45] In our belief, the VITEK identification test was a stress on the VITEK system since its database was challenged by various bacterial groups that were scarcely encountered in the clinical laboratory. From our perspective, the VITEK database is not suggested for any further future bacterial diversity studies.

Furthermore, differences were indicated among the distinct used approaches whereas the metagenomic sequencing looks more reliable by yielding higher diversity than the culture-dependent approach which aligns with findings from a similar study.[46] This disparity may refer to various reasons as reported in prior studies. For instance, certain species are challenging to cultivate in a laboratory setting due to their natural growth characteristics, such as dormancy, which makes them resistant to adaptation in controlled laboratory conditions. Moreover, many of the prevalent bacteria found in natural environments may not thrive in standard laboratory culture media and demand specific conditions for growth.[40]

In summary, large differences were indicated among the used approaches. Culturable approaches are valuable for studying individual species and their characteristics, while metagenomics provides a more comprehensive view of microbial diversity and functional potential but lacks the ability to isolate and study specific species. both approaches offer valuable insights into microbial communities. Therefore, combining these approaches often yields a more holistic understanding of microbial ecology, diversity, and function in various environments.

CONCLUSION

Our work was the first to investigate bacterial diversity in the Al-Asfar Lake using polyphasic approaches. Bacteria from 13 phyla have been found. The Firmicutes phylum dominated Al-Asfar Lake's bacterial taxonomic structure. Our investigation showed that medium variation captures bacteria well. Enzyme synthesis may be used to improve environmental sustainability. Some isolates may be bioremediation candidates. Hemolysis in certain strains suggests virulence factors. 55.55% of the tested isolates showed antibiotic resistance, indicating the prevalence of antibiotic-resistant bacteria in Al-Asfar Lake. Furthermore, our study supports the current view that multiple approaches are required to assess bacterial diversity in natural habitats.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

AK conceptualized the study and performed project administration. MA and AK supervised the study. ZAE performed literature review. AAM performed methodology and data curation. MAMG performed data analysis. AAM and AK wrote the manuscript.AAM, MAMG, ZAE and MA revised the manuscript. MAMG, ZAE and MA edited the manuscript. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

Not applicable.

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