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Diversity of Culturable Bacteria from Iraqi Marine Water

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Iraqi Territorial water, Marine bacteria, Bacillus spp., Actinomycetes, Planomicrobium.

Abstract

The current study focused on the isolation and identification of bacteria from Iraqi Marine Waters. A total of 21 water samples were collected from seven stations within the Iraqi marine waters. The temperature ranged between 13-19°C. The pH values range from 7.5 to 8.2. which revealed that Iraqi maritime waters exhibit alkaline characteristics. The salinity and Total Dissolved Salts (TDS) concentrations were rather high. Their maximum concentrations were 44.1 ppt and 39.5 gm/L, respectively, while their minimum values were 27.4 ppt and 26.4 g/L. The dissolved oxygen (DO%) ranged from 2.01 to 3.37 g/L. A total of 37 bacterial isolates were collected from marine water. Their diagnosis was based on their morphological, biochemical, and molecular characteristics, utilizing the *16SrDNA* gene. The *Bacillus* spp. exhibited a significant prevalence, with a total of 30 isolates. Three strains of Actinomycetes (*Streptomyces spinoverrucosus*, *Pseudarthrobacter siccitolerans*, and *Kocuria flava*) were obtained. One species was identified as *Planomicrobium okeanokoites*, two species were identified as *P. koreense* and one species was identified as *Desemzia incerta.*

Introduction

The marine environment is a dynamic ecosystem that supports a diverse range of life. Critical to the existence of life on Earth, these ecosystems are of utmost significance as they serve as the origin of life. Their biodiversity is significant as a result of their exceptional capacity. This diversity has expanded the possibilities for discovering creatures that offer advantages to humanity, as its potential as an abundant and untapped resource can be harnessed through effective exploitation [1] [2]. Bacterial species living in marine waters face challenges due to extreme environmental conditions, including high salinity concentrations. These conditions increase the risk of dehydration and loss of cellular water for the bacteria, as there is a significant difference in osmotic pressure between their cells and the

surrounding environment. To mitigate the risk of dehydration, they can address it by augmenting the intracellular levels of sodium and potassium ions. Several factors, including high hydrostatic pressure from the water column, dissolved oxygen levels, and temperatures influence the marine environment. These factors play a crucial role in determining the presence of bacterial species. Additionally, marine waters contain significant amounts of toxic heavy metals and petroleum hydrocarbons which are introduced by ships and boats navigating through these waters and harbors. Oil, as well as the discharge of wastewater, industrial operations, and agricultural practices are also included as toxins. This requires bacterial species that are well-suited to these circumstances, resulting in different characteristics and metabolisms in marine and terrestrial waters. Marine ecosystems contain highly intricate species of bacteria, making them promising locations for finding bacterial species that have adapted to survive in extreme conditions. These bacteria are metabolically active and serve as a valuable source of enzymes and secondary metabolites with significant medical and industrial applications. They exhibit efficacy against germs, viruses, pathogenic parasites, cancer cells, and other advantages, hence offering the potential for numerous breakthroughs [3] [4].

The bacterial community serves as an indicator of the overall well-being of the marine ecosystem. Bacteria have a dominant role in the primary productivity and energy flow within this ecosystem, comprising 90% of the living organisms in the marine ecosystem [5] [6]. Hence, analyzing the bacterial community composition in the marine environment provides valuable insights into the environmental behavior of its members. Moreover, certain members of this microbial community can be utilized to generate materials with potential health benefits. The metabolic efficacy of this organism makes it suitable for both environmental and industrial applications. The moderate temperatures in the marine environment of Iraq, which typically increase during the summer, will enhance the metabolic activity of bacterial species and reduce the lifespan of the generation, resulting in a steady increase in their population with rising temperatures. Moreover, high temperatures may accelerate the rate of genetic mutations caused by ultraviolet radiation [7] [8].

Gram-positive bacteria, particularly Bacillus spp., are abundant in the marine environment. Additionally, Actinomycetes and other species coexist with them [9] [10]. The majority of the species in these two types of bacteria are characterized by their capacity to produce extracellular metabolic products. These secondary metabolites, which are physiologically active enzymes, are secreted by bacteria outside their cells. They serve as a defense mechanism to minimize competition from other microbes or to break down and detoxify hazardous chemicals in their environment [10]–[15].

The *16S rDNA* gene was used in molecular taxonomic research by Woese and Fox (1977) who constructed the life phylogenetic tree at the first time. This gene is unique to prokaryotic lineages,

including archaea and bacteria [16]. The utilization of it as a taxonomic guide encountered obstacles, which were subsequently resolved with the revelation of the polymerase chain reaction (PCR) technique in 1983 by the American biochemist Kary Mullis. The Sanger technique played a significant role in determining the DNA sequences of nitrogenous bases, both in general and specifically for genes of taxonomic significance. This technique has greatly advanced the study of genes and their application in classifying various bacterial species and constructing their evolutionary trees.

The *16S rDNA* gene emerged as a prominent tool in molecular classification and evolutionary research. It accurately classifies about 99% of bacterial and archaeal species; information about these species can be found in global databases and gene banks [17]. The length of this gene is approximately 1542 base pairs (bp). The gene encodes the synthesis of 16S rRNA, which interacts with around 19-21 distinct proteins to assemble the 30S ribosomal subunit [18]. This gene consists of conserved regions with a consistent and unchanging arrangement of nitrogenous bases across generations in all prokaryotic species. Besides, it contains nine variable regions with differing arrangements of nitrogenous bases. Researchers utilized the unique structure of this gene at the genus or species level to classify individual bacteria at the molecular level. They achieved this by exploiting conservative areas to create universal or specialized primers specific to a particular genus or species, scientists can exploit the variation in the arrangement of nitrogenous bases in heterogeneous regions to differentiate between similar species and genera. This technique is particularly useful in evolutionary studies and comparisons between species. However, there is a lack of recent research on the use of this gene in classification processes [19].

Al-Dhabi *et al.* (2018)[11] isolated *Streptomyces* sp. from water and sediments of the Arabian Sea, whereas Alotaibi *et al.* (2018)[20] isolated thirty-six bacterial isolates from the coast of the Red Sea in the city of Jeddah, Saudi Arabia. Parasuraman *et al.* (2020) [21] conducted a study on the marine bacterium *Bacillus subtilis*. Khalil *et al*. (2022 a,b) [13], [14] conducted two distinct research on the marine bacteria *Nocardiopsis dassonvillei* and *Streptomyces catenulae*, which were collected from the Red Sea coast in Jeddah. Jaafar *et al.* (2022) [6] isolated marine bacteria from Iraqi Marine Water, which included both *Bacillus* spp. and some species of actinomycetes. The present study aimed to investigate the bacterial population in Iraqi marine waters and employ molecular techniques by amplification of *16Sr DNA* gene to diagnose it.

Materials and Methods

Study area:

The study area included four stations, commencing from the Shatt Al-Arab estuary, northwest of the Arabian Gulf, at Ras al-Bisha in Al-Faw District, southern of Basra Governorate, extended towards Khor Al-Amaya Oil Port and Basra Oil Port, in addition to three stations within the Grand Al-Faw Port, at the western and eastern breakwaters (Table 1, Figure 1):

Table 1 The study area and the coordination of sampling stations

NO.	Station name	Longitude	Latitude
St. 1	Shatt Al-Arab estuary	48.64883	29.90209
St. 2	Iraqi marine water entrance	48.71714	29.85125
St. 3	Neer Khor Al-Amaya Oil Port	48.75630	29.82482
St. 4	Neer Basra Oil Port	48.76860	29.77656
St. 5	Grand Al-Faw Port- The Eastern Breakwater	48.30146	29.503855
St. 6	Grand Al-Faw Port-The Western Breakwater 1	48.28264	29.512192
St. 7	Grand Al-Faw Port-The Western Breakwater 2	48.27168	29.521888

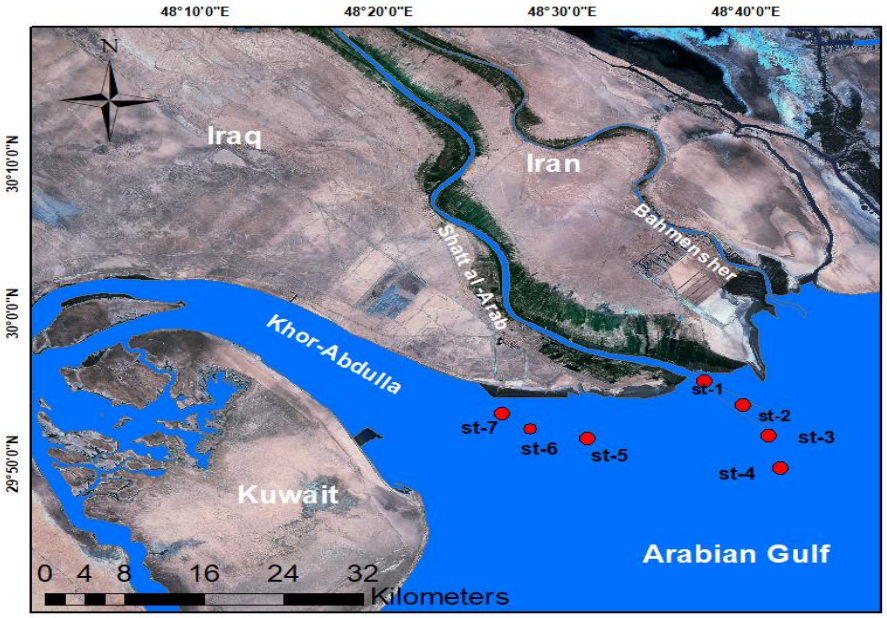

Figure 1 Marine water sampling stations in Iraqi Territorial Waters.

Collection of water samples:

Marine water samples were collected in January and March/ 2022 with a water sampler (Wildco, USA). The specimens were collected at a depth ranging from 10 to 15 cm and preserved in 500 ml sterile glass bottles. These bottles were stored in an ice box and then transferred to the Microbiology laboratory for postgraduate studies at the Department of Biology, College of Education for Pure Science, University of Basrah. Throughout the data collection process, many environmental parameters were measured,

including temperature, pH, conductivity (EC), salinity, total dissolved salts (TDS), and percentage of dissolved oxygen (DO%). These measurements were obtained using a multimeter (HORIPA, Japan) [22].

Isolation of bacteria:

A serial dilution from collected marine water was performed using sterile normal saline (85% NaCl). Then, each dilution was cultivated on Zobell marine agar 2216 plates (HiMedia, India), and the pH of the medium was ultimately set to 7.5± 0.2. The plates were incubated at 25±2 ºC for 5-7 days. After the incubation, the bacterial isolates were then cultivated on a nutrient agar to obtain pure colonies [23].

Identification of bacteria:

Morphological and biochemical identification:

The morphological characteristics of the bacterial colonies on agar plates were examined using a dissecting microscope to determine their shape, edge, texture, and coloration. The 3% potassium hydroxide (KOH), Gram stain (HiMedia, India), and cultivation on MacConkey agar were used to distinguish between Gram-positive and Gram-negative bacteria. The light microscope was utilized to examine the cellular morphology, encompassing diverse morphologies such as cocci, bacilli, and others. The isolate's ability to generate catalase was evaluated using 3% hydrogen peroxide (H_2O_2) [23].

Molecular identification:

The pure bacterial isolates underwent molecular identification to validate their diagnosis. The genomic DNA (gDNA) of isolates was extracted using the Presto TM Mini gDNA bacteria kit (Geneaid, Taiwan), and the success of the gDNA extraction process was confirmed by electrophoresis using a 1% agarose gel (Bioneer, Korea). The gel was submerged in a Tris-Borate-EDTA (TBE) solution. To visualize the DNA, ethidium bromide (Promega, USA) was utilized as a dye. The DNA solution was stored at a temperature of -20°C. A universal primer set was utilized to amplify the 16S rDNA gene using Polymerase Chain Reaction (PCR). The forward primer used was 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and the reverse primer was 1492R (5′-GGTTACCTTGTTACGACTT-3′)[24][25]. The PCR mixture included 25 µl master mix (Promega, USA), 2 µL forward and reverse primers, 5 µL gDNA, and 16 µL nuclease-free water (Bioneer, Korea). The 50 µL mixture was prepared in a 200 µL PCR tube. PCR tubes were placed in the Thermocycler (DLAB, USA). The amplification method begins with 5 minutes of denaturation at 94°C, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 55.5°C, and 90 seconds at 72°C. Finally, the final elongation is repeated once at 72°C for 5 minutes [26]. The PCR products were electrophoresed on a

2% agarose gel in TBE buffer. The amplified gene was stained with ethidium bromide. The PCR findings were sent to Macrogen in Seoul, South Korea, to determine the *16SrDNA* gene nucleotide sequence. BLAST was used to compare the *16SrDNA* gene sequence to the NCBI GeneBank reference sequence.

Results and Discussions

The environmental properties of sampling stations:

According to the measurements of environmental factors in the sampling stations where water samples were collected in this study, it is evident that these areas fall within the marine environment. This is indicated by the high levels of salinity, moderate acidity, and a tendency towards basicity due to the presence of high concentrations of dissolved salt ions (TDS). The temperatures during the period of sample collection were low in all stations (Table 2).

Table 2 The environmental factors measurement of sampling stations

Sampling station	Temp. (℃)	DO (mg/L.)	Salinity (ppt)	pH	TDS (g/L.)
St. 1	13.0	2.01	27.40	8.0	26.4
St. 2	13.6	2.58	37.90	8.0	34.7
St. 3	14.7	2.11	44.10	8.2	39.5
St. 4	14.9	3.37	43.96	8.3	35.5
St. 5	18.0	3.05	40.00	7.5	32.4
St. 6	18.5	2.80	40.30	7.6	32.7
St. 7	19.0	3.00	40.70	7.6	32.9

The environmental parameters were selected in this study due to their influencing both microbial diversity and density. Temperature is the main physical measure of water quality and its ability to support life in aquatic ecosystems. It impacts bacteria by altering the concentration of dissolved oxygen in water, it influences the percentage of dissolved oxygen in water [27]. The values observed in the present study fell within the annual mean for the specific geographic area, which spans from 10 to 21°C [28].

Wave propagation increases water oxygen concentration and dissolution. Photosynthesis by algae and aquatic plants produces oxygen in water. Aquatic biodiversity depends on oxygen availability. All living things, including aerobic bacteria, need oxygen as the final electron acceptor for energy synthesis. Sodium chloride and other dissolved salts determine water salinity, these concentrations are vital to the aquatic ecology and its inhabitants. Each bacterial species has a distinct range of salt tolerance within which it can survive, grow, and reproduce. The pH values indicate that Iraqi marine waters are generally neutral and slightly alkaline due to the presence of calcium and sodium salts. Microbial diversity in the marine environment has a direct impact on the solubility of many chemicals

in water, including ammonia, chloride, and mineral elements, which are influenced by alterations in pH values [29].

The rise in salinity and total dissolved salts, coupled with the decline in dissolved oxygen and temperatures during the sampling period, along with the moderation of acid function values and their inclination towards basicity, serve as evidence of the challenging and extreme conditions in the sampled areas. These conditions have a direct impact on the bacterial diversity in those areas. The environment necessitates the existence of halotolerant or halophilic bacteria that are specifically suited to thrive in conditions characterized by elevated levels of total dissolved salts and relatively cool temperatures. The environmental parameters measured in the present study closely align with those documented by Jaafar et al. (2022) [6].

Identification of bacteria:

A total of 21 seawater samples were collected from the study area, resulting in the isolation of 37 bacterial isolates. The isolates underwent initial identification using phenotypic and microscopic examination, as well as some biochemical tests, to determine their genus. Subsequently, the molecular diagnosis was conducted to validate the diagnosis of these bacterial isolates and achieve.

Morphological and biochemical identification:

The findings revealed that all the isolates were aerobic and Gram-positive bacteria, as confirmed by Gram staining and testing with 3% KOH solution, and none of the bacterial isolates exhibited growth on MacConkey agar. Additionally, the isolates demonstrated catalase activity. The prevalence of Gram-positive bacteria in the study areas can be attributed to their capacity to acclimate to the challenging conditions of the marine environment. They exhibit resilience against high hydrostatic pressure in marine waters, which can otherwise lead to the deterioration of functional enzymes and structural proteins. This adaptability is achieved through alterations in gene expression and augmentation. The cell membrane's stiffness is enhanced by the alignment of unsaturated fatty acids which firmly pack together. This, in addition to the cell wall, is composed of many layers of peptidoglycan and teichoic acid that enhances the rigidity of these cells [30]. The findings align with the results of Syakti *et al.* (2019) [9], who discovered 51 bacterial isolates from the Indonesian waters of the Pacific Ocean, all of which were Gram-positive bacteria.

A total of 30 isolates from the genus *Bacillus* spp. were obtained. The colonies exhibited diverse shapes and colors. Microscopic analysis of these isolates revealed that the cells displayed varying lengths of rod-shaped morphology, ranging from short to relatively elongated. In some cases, the cells appeared slightly curved. They were arranged in pairs or short chains, while the cells in other isolates were

solitary, these isolates produced single, central, or subcentral endospores. The growth of the bacteria on the nutrient agar was rapid, occurring within 24-48 hours only. Many studies support this finding [9], [10], [31]–[33]. The abovementioned considerations may explain this phenomenon, as most of its constituents have a wide temperature range that can resist pH changes. Members of this genus have sodium pumps and live in alkaline conditions, these pumps keep cells neutral, counteracting the basic marine environment. Cellular mechanisms that resist salinity and high osmotic pressure, and maintain cellular water allow it to endure various salinities. It can move potassium, sodium, and chloride ions into its cells by active transport, and can maintain high concentrations which raise the cell's osmotic pressure to match the environment. Thus, it controls cell water content and prevents depletion. Furthermore, it possesses the capability to synthesize extracellular enzymes such as proteinase, amylase, and cellulase. Meso-diaminopimelic acid is present in the peptidoglycan, enhancing the cell wall's stiffness. The fats in the plasma membrane consist of saturated fatty acids arranged in branched chains, these fatty acids enhance the durability of cell wall components, such as glutamic acid, diaminopimelic acid, muramic acid, and glucosamine. This arrangement makes the plasma membrane resistant to hydrostatic pressure and enables it to form endospores that exhibit high resistance to extreme conditions [34][9][10]. Syakti *et al.* (2019) [9] reported that certain species of *Bacillus* spp. possess the capacity to metabolize petroleum hydrocarbons as a carbon source. This finding sheds light on the potential abundance of these bacteria in Iraqi marine waters, which are exposed to petroleum hydrocarbons from both Iraqi and foreign oil ports, the riparian zone, as well as the navigation of maritime vessels such as diverse cargo ships and fishing boats. However, endospores not only as a stable form for *Bacillus*, but also as a site for the action of numerous biologically active enzymes involved in redox reactions, these enzymes help the spore withstand the harmful effects of various elements and heavy compounds that are continuously present in marine habitat [35].

Anju *et al.* (2015) [36] observed that *Bacillus* species found in marine environments frequently exhibit unique metabolic pathways and possess the capability to produce secondary metabolites that inhibit the growth of other bacterial species. This ability gives them a competitive advantage in acquiring space and nutrients in their habitat. Additionally, these metabolic compounds enable them to withstand the toxic effects of metals and other heavy metals present in the marine environment. They achieve this by employing extracellular enzymes to detoxify and reduce the toxicity of these compounds, transforming them into less harmful substances or utilizing them for their benefit and serving as a carbon source [37].

Three Actinomycetes isolates were isolated, characterized by their slow growth rate and requiring 5-7 days of incubation on a nutrient agar. One of these isolates had the trait of producing colonies that

resembled delicate, whitish cotton-like mycelia on the nutrient agar medium. Furthermore, these isolates exhibited the existence of aerial hyphae during their development. The colonies exhibit strong adherence to its surface and possess the capacity to secrete a pigment, resulting in a dark brown coloration of the medium. The cells exhibited an elongated shape and were arranged in elongated, branched chains. The isolates were classified as the genus *Streptomyces* sp. The second isolate displays colonies that demonstrate a yellow coloration while being cultivated on nutrient agar. When examined with a light microscope, the cells displayed a small size and a round form. The cells were organized singly, in pairs or short chains, but not in clusters, without the ability to create endospores, and were identified as the genus *Kocuria* sp. In the third isolate upon microscopic analysis, it was observed had elongated, rod-shaped cells as well as short, slightly spherical cells. These cells were arranged in the form of short, branching chains. The isolate was classified as a member of the genus *Arthrobacter* sp. Marine waters may include Actinomycetes bacteria which are capable of adapting to a broad temperature spectrum. These bacteria can thrive in cold seas with temperatures ranging from 0-10 ºC, as well as in tropical seas and oceans with high temperatures. The majority of bacteria in this group can produce distinct enzymes and metabolites that allow them to tolerate high levels of salinity and pH in marine water [38]. Besides, the majority of these bacterial species serve as a reservoir for distinctive secondary metabolites which aid in their ability to outcompete other organisms. On the one hand, microbes are obtained from sources other than themselves, while on the other hand, these metabolic products enabled them to withstand the harmful effects of heavy metals and compounds present in the marine environment. The fact that it is separated from the marine environment is supported by various studies [39] [40] [11] [13][14] [6]. These studies have found that it makes up the majority of the microbial community in marine waters and sediments. Grossart *et al.* (2004)[41] also indicate that actinomycetes make up approximately 10% of the marine microbial community. However, four isolates were obtained that exhibited rod-shaped cells but lacked spores, thereby rendering them non-existent, and they do not belong to the genus *Bacillus*.

Molecular Identification:

Molecular identification was conducted on bacterial species that were isolated from Iraqi marine water, specifically those belonging to the genus *Bacillus* spp., these bacteria share many identical traits, making traditional methods of diagnosis based on physical characteristics and biochemical tests inadequate [42]. Through the use of electrophoresis for gDNA which are extracted from 37 bacterial isolates, distinct gDNA bands were showed on a 1% agarose gel, this indicates the success of the gDNA extraction process (Figure 2). The use of universal primers 27F and 1492R yielded good outcomes in the amplification of the *16SrDNA* gene using PCR. This resulted in distinct bands at about 1550

nitrogen base pairs (bp) in size when compared with molecular weight indicator (Marker) using electrophoresis on a 2% agarose gel, this indicates the success of amplification process of *16SrDNA* gene of 37 bacterial isolates which are obtained from Iraqi marine water by using the universal primers (Figure 3). After the nitrogenous base sequence of this gene was established, the alignment procedure using the BLAST tool was carried out with the sequences maintained in NCBI GenBank. The molecular diagnosis of 37 bacterial isolates revealed the presence of ten species belonging to the genus *Bacillus*, three species from Actinomycetes, three species from the genus *Planomicrobium*, and only one species from the genus *Desemzia* (Table 3). Upon molecular identification, it was observed that there were discrepancies in certain nitrogenous bases among the reported bacterial species when compared to the reference strains due to the disparity in environmental conditions between the Iraqi marine environment and the ecosystems where the reference strains were obtained, as well as the variation in pollution and nutrient levels.

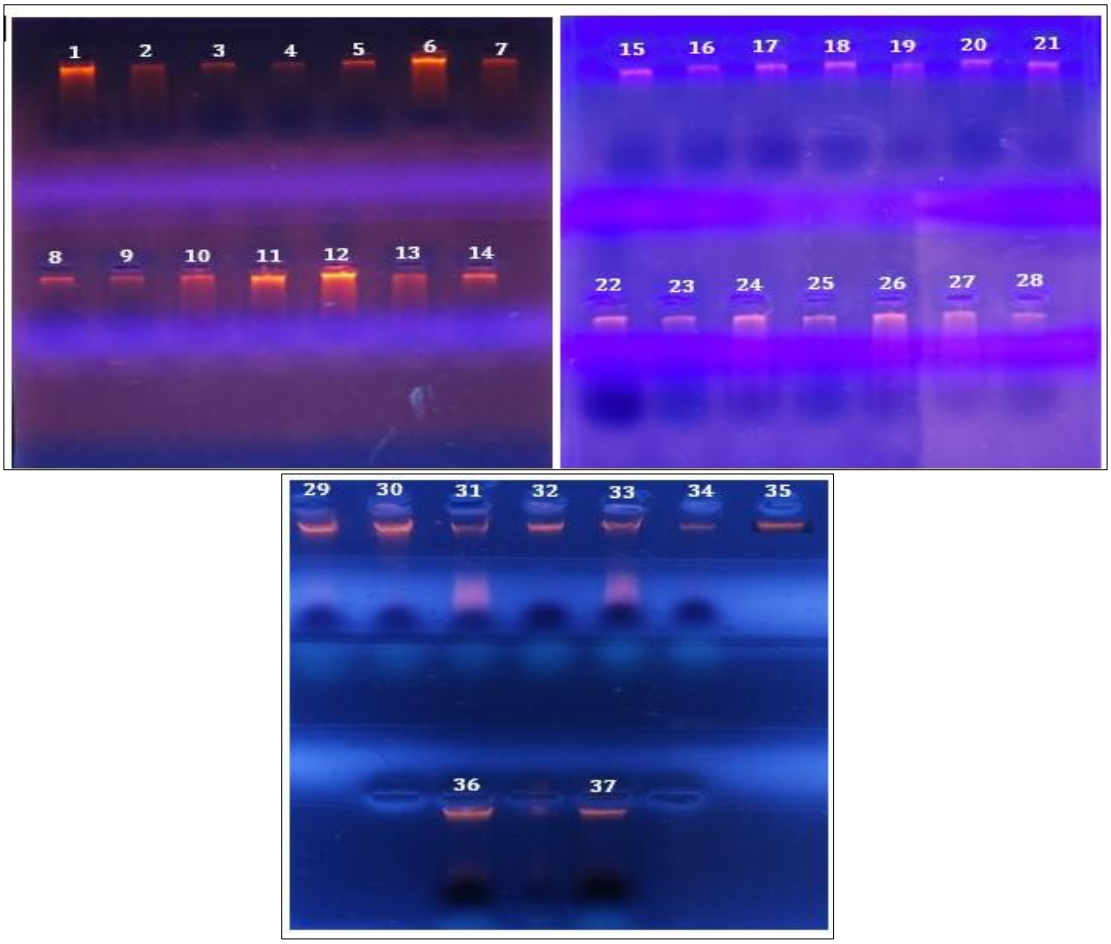

Figure 2: The results of gDNA electrophoresis in 1% agarose gel of 37 bacterial isolates obtained from Iraqi marine water

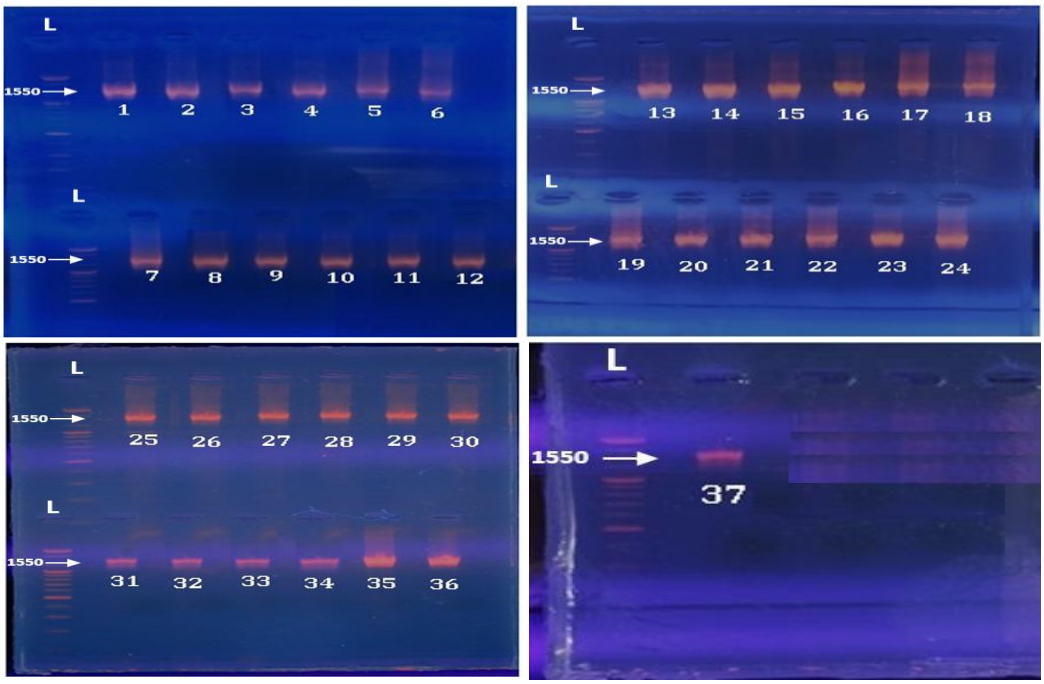

Figure 3: The results of 16S rDNA gene amplification by PCR in 2% agarose gel for 37 bacterial isolates

Table 3. The results of molecular diagnosis of bacterial species isolated from Iraqi marine water and identity% with reference strains

No.	Isolate symbol	Bacterial species	Reference	Identity %
1.	F1	*Bacillus aquimaris*	OL979380.1: *Bacillus aquimaris* strain DIP71	100%
2.	F3	*Bacillus aquimaris*	MT125713.1: *Bacillus aquimaris* strain SLA-183	100%
3.	F5	*Bacillus aquimaris*	MT125713.1: *Bacillus aquimaris* strain SLA-183	99.48%
4.	F7	*Bacillus aquimaris*	MT337422.1: *Bacillus aquimaris* strain LGMT10	99.56%
5.	F12	*Bacillus aquimaris*	MT337422.1: *Bacillus aquimaris* strain LGMT10	100%
6.	F14	*Bacillus aquimaris*	MT337422.1: *Bacillus aquimaris* strain LGMT10	99.92%
7.	F17	*Bacillus aquimaris*	MT125748.1: *Bacillus aquimaris* strain SLA-302	100%
8.	F18	*Bacillus aquimaris*	MK250512.1: *Bacillus aquimaris* strain SD20	100%
9.	F39	*Bacillus aquimaris*	MT125713.1: *Bacillus aquimaris* strain SLA-183	100%
10.	F40	*Bacillus aquimaris*	MK250512.1: *Bacillus aquimaris* strain SD20	100%
11.	F41	*Bacillus aquimaris*	MF077120.1: *Bacillus aquimaris* strain 133-CR9	99.93%
12.	F44	*Bacillus aquimaris*	MT125713.1: *Bacillus aquimaris* strain SLA-183	100%
13.	F61	*Bacillus aquimaris*	MT337422.1: *Bacillus aquimaris* strain LGMT10	99.86%
14.	F67	*Bacillus aquimaris*	LN995469.1: *Bacillus aquimaris* strain SR1-69A	99.39%

No.	Isolate symbol	Bacterial species	Reference	Identity %
15.	F34	*Mesobacillus foraminis*	CP033044.1: *Mesobacillus foraminis* strain Bac44	99.90%
16.	F35	*Mesobacillus foraminis*	CP033044.1: *Mesobacillus foraminis* strain Bac44	99.78%
17.	F36	*Mesobacillus foraminis*	CP033044.1: *Mesobacillus foraminis* strain Bac44	99.78%
18.	F38	*Mesobacillus foraminis*	MT176179.1: *Mesobacillus foraminis* strain DST26	97.95%
19.	F49	*Bacillus proteoluticus*	CP033044.1: *Bacillus proteolyticus* strain LPB0288	99.93%
20.	F56	*Bacillus proteoluticus*	CP033044.1: *Bacillus proteolyticus* strain LPB0288	99.93%
21.	F65	*Bacillus seohaeanensis*	HE586585.1: *Bacillus seohaeanensis* strain DV3	99.93%
22.	F8	*Bacillus boroniphilus*	KU601261.1: *Bacillus boroniphilus* strain Y3	100%
23.	F46	*Bacillus boroniphilus*	MN384451.1 *Bacillus boroniphilus* strain ABFSP07	100%
24.	F25	*Bacillus safensis*	OR098488.1: *Bacillus safensis* strain DG-40	99.70%
25.	F32	*Bacillus safensis*	OR083363.1: *Bacillus safensis* strain N6	100%
26.	F19	*Bacillus halosaccharovorans*	MK785124.1: *Bacillus halosaccharovorans* strain VS-10	100%
27.	F47	*Bacillus halosaccharovorans*	MK785124.1: *Bacillus halosaccharovorans*strain VS-10	99.88%
28.	F42	*Bacillus firmus*	MT605507.1: *Bacillus firmus*strain ZJTZ-3	99.79%
29.	F48	*Cytobacillus oceanisediminis*	MH283842.1: *Cytobacillus oceanisediminis* strain CSIO_43758	100%
30.	F66	*Peribacillus frigoritolerans*	MN098854.1: *Peribacillus frigoritolerans* strain S2.1-10	99.85%
31.	F30	*Streptomyces spinoverrucosus*	MK519101.1: *Streptomyces spinoverrucosus* strain Ng2-6	99.47%
32.	F31	*Pseudarthrobacter siccitolerans*	MF681903.1: *Pseudarthrobacter siccitolerans* strain D126	99.85%
33.	F57	*Kocuria flava*	ON843620.1: *Kocuria flava* strain AUMC B-459	99.86%
34.	F33	*Planomicrobium koreense*	KF219799.1: *Planomicrobium koreense* strain KBM-2-20	99.64%
35.	F62	*Planomicrobium koreense*	KC844819.1: *Planomicrobium koreense* strain AL-A18	99.73%
36.	F64	*Planomicrobium okeanokoites*	HQ848119.1: *Planomicrobium okeanokoites* strain QT-30	99.68%
37.	F43	*Desemzia incerta*	MT225670.2: *Desemzia incerta* strain 190311L243	99.93%

The species *Bacillus aquimaris* was initially isolated by Yoon *et al.* (2003) [43] from the Yellow Sea in South Korea. The members of this species thrive in the presence of salt. The ideal salinity for growth falls between the range of 20-50 ppt, and they possess the ability to withstand salinity levels as high as 160 ppt. Additionally, it exhibits a high tolerance for a broad temperature spectrum, spanning from 10-

45 °C, it thrives within a pH range of 6.0-8.0, which aligns with the conditions found in Iraqi marine waters. Cherian *et al.* (2019)[44] documented this species in Indian marine water, while Syakti *et al.* (2019)[9] isolated it from marine waters in Indonesia, and Chaida *et al.* (2021)[10] isolated it from marine waters in Algeria.

Ettoumi *et al.* (2009)[45] isolated various species of *Bacillus* spp. from marine waters in the North Atlantic Ocean, including *M. foraminis*. They found that the individuals of this species are exclusively found in marine environments and can withstand high levels of salt. Because of its enzymatic capacity to oxidize different metals in the marine environment, deposit them in marine sediments, and retain high concentrations of these metals within its cells, it is regarded as a significant contributor to the geochemical cycles of metals in the seas and oceans [46]. Al-Amoudi *et al.* (2016)[47] isolated many species of *Bacillus* spp., including this species from marine sediments of the Red Sea, near Jeddah, Saudi Arabia.

Liu *et al.* (2017)[48] reported that *B. proteolyticus* thrives in a temperature range of 10-39 °C, and the pH value is constrained to 5.0-10.0, which aligns with the conditions seen in Iraqi marine waters. Furthermore, members of this species possess the capacity to generate a set of extracellular enzymes which allows them to depend on many energy sources [49]. Registration *B. seohaeanensis*, was conducted by Lee *et al.* (2006) [50] after isolating it from the soil of salt lakes along the western coast of South Korea. They observed that this type thrives within a temperature range of 15-50 °C and a pH range of 5.0-8.0. Roohi *et al.* (2014) [51] isolated this species from the Karak salt mines in Pakistan. Ahmed *et al.* (2007) [52] isolated *B. boroniphilus* from soil contaminated with boron on the Turkish coast adjacent to the Aegean Sea. It thrives within a temperature range of 15-37 °C and a pH range of 6.5-9.0. Furthermore, this species is capable of growing in the presence of sodium chloride salt at concentrations as high as 70 ppt.

Individuals of *B. safensis* exhibit a broad thermal tolerance spanning from 10 to 50 °C and may thrive within a pH range of 4.0 to 9.0. This organism is halophilic, i.e., it can withstand high levels of sodium chloride salt, specifically up to 100 ppt [53]. This species can secrete a range of extracellular enzymes, including amylase, lipase, protease, cellulase, chitinase, keratinase, and others. This ability has allowed it to adjust to various environmental circumstances and withstand organic pollutants and toxins derived from complex molecules, eliminating their harmful properties by pulverizing them and utilizing certain materials as a source of energy and carbon [54]. Roohi *et al.* (2014)[51] isolated ten strains of *B. safensis* from salt mines in Pakistan. Galaviz-Silva *et al.* (2018) [55] also isolated this species from various sources such as algae, crustaceans, snails, and marine sediments along the coast of Mexico. Hanh *et al.* (2018) [56] found *B. safensis* in marine sponges in the waters surrounding the South Korean

island of Jeju. Additionally, Perumal (2020)[57] isolated *B. safensis* from marine sediments in the Bay of Bengal, India.

Mehrshad *et al.* (2013)[58] isolated *B. halosaccharovorans* for the first time from a high-salinity lake in Aran-Bidgol, Iran. They found that this species thrives in high salinity, and can grow in sodium chloride concentrations ranging from 50 to 150 ppt. The temperature varies between 20 and 45 °C, while the pH value goes from 6.0 to 9.0.

The species *B. firmus* isolated by Pane *et al.* (1996) [59] from marine waters, has been found to tolerate high levels of sodium chloride salt, it thrives best with a pH range of 7.0-8.0, and can survive in temperatures ranging from 8-26 °C. Additionally, this species is known for its production of various enzymes. However, it is important to note that this agent also carries hazardous properties. Sabdono (2008)[60] identified and extracted these bacteria from Coral colonies in the northern Java Sea, Indonesia. Keung *et al.* (2009)[61] also recovered this bacterium from coastal sediments in Hong Kong, while Xiong *et al.* (2015)[62] isolated this species from the South China Sea.

Zhang *et al.* (2010)[63] identified and isolated *Cytobacillus oceanisediminis* from marine water sediments in the South China Sea, this species is capable of growing within a broad temperature range of 4-45°C and can tolerate a pH level between 6.0-10.0, it exhibits high tolerance to salinity, with the ability to withstand concentrations up to 130 ppt. Roohi *et al.* (2014)[51] obtained an isolate of *C. oceanisediminis* from the Karak salt mines in Pakistan. Jung *et al.* (2016)[64] found genes that resist cadmium, nickel, copper, lead, and zinc in *C. oceanisediminis*, so, it can flourish in metal-contaminated habitats. Feng *et al.* (2020) [65] isolated this species from sediments in Lake Weiming, China. Nabil-Adam *et al.* (2023)[66] obtained one isolate of *C. oceanisediminis* from Abu Qir Gulf in Egypt.

The species *Peribacillus frigoritolerans* is capable of thriving within a temperature range of 4-40 °C and a pH range of 5.0-10.0. Furthermore, it exhibits tolerance towards salt concentrations of up to approximately 60 ppt [67]. Hence, the marine water environment in Iraq provides favorable conditions for the existence of this species. Montecillo and Bae (2022) [68] reassigned the species *B. frigoritolerans* to the genus *Peribacillus*. This reclassification was based on DNA hybridization, the similarity in nucleotides and amino acids, and the difference in the quinine-cytosine ratio, and the affiliation of this species was changed to the genus *Peribacillus*. Omuzbuken *et al.* (2022)[69] described Various bacterial species, such as P. frigoritolerans from in marine environments.

Diab and Al-Gounaim (1982) [70] isolated *Streptomyces spinoverrucosus* from the air in Kuwait. Hu et al. (2012) [71] isolated this species from marine sediments in Trinity Bay, located in the state of Texas in the United States. SantaCruz-Calvo et al. (2013) [72] documented the discovery of a new bacterial species called *Arthrobacter siccitolerans*, they reported that this species can thrive within a

temperature range of 15-35 °C and a pH range of 5.0-9.0. It is capable of growing in the presence of sodium chloride salt. This elucidates the potential for its proliferation in the maritime waters of Iraq which are distinguished by these environmental attributes. Furthermore, it possesses the capacity to excrete various enzymes that facilitate its use of diverse energy sources, as well as its ability to withstand harmful substances, elements, and contaminants that may come into contact with its surrounding environment. Busse (2016) [73] conducted a reference study to reclassify individuals of the genus *Arthrobacter* based on the peptidoglycan structure. Accordingly, the species A. siccitolerans was reclassified and added to the genus Pseudarthrobacter. Consequently, this species is now known as *Pseudarthrobacter siccitolerans*. Karaevskaya *et al.* (2021) [74] isolated some species of the genus *Pseudarthrobacter* from marine sediments of the Arctic Ocean in Norway. Sulieman *et al.* (2022) [75] referred to the species *P. siccitolerans* and other species within the same genus as having a significant ecological function in eliminating contaminants from the environment and thriving in conditions of environmental adversity.

Zhou *et al.* (2008) [76] obtained the species *Kocuria flava* from the air in Xinjiang, China, and designated it as a reference strain in the NCBI Gene Bank. They indicated that it thrives within a pH range of 7.0-9.0 and can withstand high levels of sodium chloride salt surpasses 100 ppt. This species possesses the capacity to secret numerous enzymes, which allows them to utilize multiple energy sources. These enzymes aid in their resistance against the harmful impact of organic pollutants and heavy metals, facilitating the detoxification process. Ba-akdah and Satheesh (2021) [77] isolated *K. flava* from marine algae leaves in the Red Sea near the coast of Saudi Arabia.

Zobell and Upham (1944) [78] were the initial researchers to separate the species *Flavobacterium okeanokoites* from marine water and categorize it as a member of the genus Flavobacterium. Subsequently, Nakagawa *et al.* (1996) [79] reclassified this species as belonging to the genus *Planococcus* after conducting a genetic study of the nitrogenous base sequences of the *16SrDNA* gene and Chemotaxonomy. In their study, individuals of this species inhabit a temperature range of 20-37 °C and can withstand salt concentrations ranging from 30 to 50 ppt. Yoon *et al.* (2001) [80] transfer *Planococcus okeanokoites* into genus *Planomicrobium* to become *Planomicrobium okeanokoites*. Boontanom and Chantarasiri (2020) [81] isolated *P. okeanokoites* from marine algae and marine sediments on the coast of the Thailand Gulf. Saini *et al.* (2023) [82] isolated this species from the leaves of marine algae in the Antarctic Ocean, in East Antarctica. The species *Planomicrobium koreense* was initially described by Yoon *et al.* (2001) [80] following its isolation from seafood dishes in South Korea. According to their statement, it thrives within a temperature range of 4-38 °C and a

pH range of 7.0-8.5. The members of this species thrive in environments with salt concentrations of up to 60 ppt.

The species *Desemzia incerta*, initially identified as *Bacterium incertum* by Steinhaus (1941) [83] after isolating it from insect ovaries, was later reclassified by Breed (1953)[84] and renamed *Brevibacterium incertium*. However, Stackebrandt *et al.* (1999)[85] reclassified this species based on chemotaxonomy and analysis of the *16SrDNA* gene's nitrogenous base sequences. As a result, they proposed that the species previously known as *B. incertum* should be reclassified as a new species called *Desemzia incerta*. Avendano-Herrera *et al.* (2016) [86] isolated this species from the internal organs of fish samples obtained from Fields Bay in the Antarctic Ocean. Singh *et al.* (2020) [87] isolated this species from large marine organisms, such as sponges and crustaceans, collected from the Indian marine coast.

Conclusion

The present study reveals the prevalence of Gram-positive bacteria in Iraqi marine waters, particularly those of the *Bacillus* species. Furthermore, it highlights the significant function of the *16SrDNA* gene in accurately identifying bacterial isolates obtained from this habitat.

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