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## Bacterial Diversity in Different Positions in the Iraqi Marine Area

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### Abstract:

Microorganisms establish both structural and functional construction in the marine environment, despite scientific advances, the identification of marine bacterial species is still considered as a common challenge in microbiology. Nevertheless, the present study aims to make an effort, although it seems modest, but it could establish a basis for studying the bacterial diversity in the Iraqi marine area, because of what this aspect entails of the poverty of studies related to this aspect in the studied area. The current results show the marine studied area are classified within warming area, where the average temperature ranged from 23.17 to 26.17 °C. The recorded number of bacteria was increased with temperature increasing (0.210, 0.250, 0.305, 0.517, 0.553 and 0.625) through the studied period (January, February, March, April, May and June) respectively. The results showed that the identified bacteria were related to three bacterial phyla (Firmicutes, Proteobacteria, and Actinobacteria), with the clear superiority to the phyla (Firmicutes). The most occurrence bacterial genera were (*Staphylococcus lentus*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Sphingomonas paucimobilis* and *Aeromonas hydrophila/caviae*), which have different percentage of frequency (50, 40, 60, 30 and 20%) respectively. The present discoveries provide initial evidence on the bacterial diversity in the Iraqi marine waters. Further studies are necessary to make a more comprehensive image of bacterial diversity within these marine regions.

Running Title: Marine Bacterial Diversity

**Keywords:** Bacteria, Diversity, Firmicutes, Proteobacteria, Actinobacteria, Iraqi Marine Area,

### Introduction:

When describing environments according to their bacterial diversity, it can be said that the marine environment is the most diverse ecosystem, that the situation in the marine environment is neither selective, nor discouraging to a specific group<sup>1</sup>. The bacterial diversity in the marine environment reflects their health status and wellbeing<sup>2</sup>. Bacteria are responsible for the largest percentage of marine primary productivity, and it is the key factor in the processes of energy flowing through biogeochemical process, and influencing the climate of our planet, as they constitute approximately 90% of the marine environment<sup>3</sup>. Determining the societal composition of bacteria as well as their distribution patterns has an effective role in forming an important vision about the ecological and biogeochemical performance of the marine ecosystem<sup>2</sup>.

There are many environmental factors to which bacteria in marine environment are exposed, such as temperature, oxygen concentration, and salinity<sup>4</sup>. Marine bacterial diversity is affected by different environmental factors, but the temperature is considered as the most important ones. The previous studies have shown that there is a decrease in bacterial diversity in the polar regions due to decrease in temperature. In high temperatures, the metabolic rate increases and the age of generation is shortened, also high temperature leads to accelerate environmental and physiological processes and finally increases the rate of mutation and speciation resulting in a large site diversity<sup>5, 6, 7</sup>. All microbes have complex proton pumps that are involved in bioenergetics, but it is not clear how microbes might respond to changes in environmental proton balance<sup>8</sup>. Studies indicated that although the bacteria have ability to adapt with the change in the

see water acidity and conserve their abundance, yet in response to such situation they lose their diversity<sup>9</sup>. Expensively, salinity is referred to as an important factor affecting microbial assemblies in both lakes, coastal waters and oceans<sup>10</sup>. Salinity has also been suggested to be the major determinant of microbial community composition, exceeding the influence of temperature and pH<sup>11</sup>. The effect of increase salinity on the microbial component is quite complex. The studies showed different responses in the moderate salinity, including a decrease in bacterial diversity<sup>12</sup>, no effect<sup>10</sup>, and occurrence of a peak in diversity at moderate salinity<sup>13</sup>. The concentration of oxygen in the marine environment has an important role in shaping the ecosystem, as it controls the temporal and spatial distribution of all living organisms, whether microbial organisms or higher organisms. The differential solubility of oxygen and consumption via microbial-mediated oxidative processes are also known to affect the oxygen concentration in marine water.<sup>14</sup> Studies have shown that there are factors restricting microbial diversity in marine waters, but the gradient in the concentration of oxygen remains the limiting factor for that diversity<sup>14</sup>.

The viral presence in the marine environment is one of the controllers in the biomass of bacteria and has a role in maintaining the biological balance as well as its role in highlighting biological diversity as it is an intermediate factor in the genetic exchange between living organisms<sup>15,16</sup>

The unlimited possibilities of services that can be gained for human society as a result of microbial diversity have not been exploited yet. And one of the most important obstacles to exploiting these potentials is the lack of a database on the bacterial community through which it is possible to assess and determine future changes. Given the importance of the availability of a database of the quantity and diversity of bacteria in Iraqi marine areas, the current study aims to determine the quantity and diversity of bacteria in the current study areas.

## Materials and Methods:

### Study areas

The study area included three stations located within the Iraqi marine borders. Table 1 shows the coordination and the deep of this station.

**Table 1. Coordination and deep of sampling stations**

Sampling Stations	Latitude	Longitude	Deep (M)
1-Khour Al-Zubair	30 06 47.3	47 55 14.7	13
2-Khour Abdullah	29 53.504	048 21.446	10
3-Al- Skhra	29 37.628	048 48.693	7.5

### Sample collection

Samples were collected randomly from a depth of half a meter using 500 ml of glass bottles already sterilized. "The samples were transferred under refrigeration to the laboratory and the samples continued to be collected monthly during the period from the January to the July /2018.

### Physical and chemical properties of water samples

Some physical and chemical properties of water samples like; Temperature, pH, Electrical conductivity, Dissolve oxygen, Total dissolved substance, and Salinity were measured during sample collection using Multimeter device (Sensodirect, Germany).

### Enumeration of Bacteria

For bacterial enumeration 0.1 ml of each water sample aseptically inoculated in 10ml tube containing nutrient broth (Hi media). The medium was prepared according to the manufacturer's instructions and sterilized in an autoclave. A duplicate tube was used for each sample with one as a control. Tubes were incubated with shaking incubator at 120 rpm for 24hs at 25°C<sup>17</sup>.

### Absorbance of bacterial growth

Different assays for viability can be used, however the optical method is the best one to be adapted into high-throughput setups as it can be done on standard plate readers<sup>18</sup>. The OD. method is done by sending light at a specified wavelength, in this case 600 nm (OD600). The obtained optical density is a number that depends on the amount of diffracted light. The number is directly related to the amount of bacteria<sup>19</sup>. In the current study the absorbance of bacteria was measured using spectrophotometer (Uv-1800, double beam, Shimadzu, Japan) under scanning wave length. The readings of absorbance at 600 nm wave length were recorded and tabulated by taking only nutrient broth media as blank standard; (Absorbance readings at other than 600nm wave length recorded not relevant, hence were not shown in the data)<sup>17</sup>.

### Isolation of marine Bacteria

For isolation of marine bacteria 1.0 ml from each water sample was mixed with 9 ml of distilled water, then serial dilution was performed up to  $10^{-5}$ . 0.1ml from the final dilution was spread on the surface of the nutrient agar, (Hi media). The inoculated plates were incubated at 30°C for 24-48 hs. Colonies developed after incubation were purified by repeated sub culturing on nutrient agar and finally, bacterial colonies with distinct characteristics such as pigmentation, size, opacity, elevation, margin and surface appearance were chosen for further characterization<sup>20</sup>

### Identification of bacteria

Morphological and some biochemical test (Grams stain) were carried out for diagnosis the bacterial colony from nutrient agar media. The automated instrument for bacterial identification (Vitek II) has been used for accurate identification.

### Results and Discussion:

#### Physical and chemical properties of sea water

As the current study focus on the diversity of bacteria, so it takes care of assessing the most effectiveness physical and chemical properties of water in the studied area. Table 2. shows the results of these studied properties. The temperature ranges from, 23.17- 26.17 °C during the studied period (table.2). Temperature is an elementary water quality and it governs the suitability of water for the different forms in aquatic environment. Depending

on the geographic location the mean annual temperature varies in the range of 10 to 21 °C<sup>21</sup>. Effect of temperature on the bacterial diversity related to its effect on the rate of oxygen solubility and photosynthesis by algae and other aquatic plants, metabolic and production rate<sup>22</sup>. The present study recorded that the pH value is ranges from, 8.34-8.76 (Table.2). Usually any extreme changes in the hydrogen potential play a crucial role in changing the concentrations of other substances in the water bodies to a more toxic form, for example, the ammonia toxicity, chlorine decontamination effectiveness, and metal solubility are all dependent to changes in pH value<sup>23</sup>. Salinity of sea water usually related to the sodium chloride, generally water salinity effecting on the aquatic life, and every type of organism has an ideal salinity range that it is suitable for their presence and proliferation. The present study recorded that salinity ranges from, 46.00-43.41(ppt), and general aquatic life, occupation well in a salinity range of 6.0 to 9.0(ppt).<sup>23</sup>. Dissolved oxygen is a result of the diffusion from the air during rapid movement and as byproduct of photosynthesis, the amount of dissolved oxygen is crucial for all living organisms and eventually effecting their diversity<sup>23</sup>. The range of dissolved oxygen recorded in the present study was 3.61-2.93 mg/l (Table.2). The total dissolved solid is a term defining the amount of inorganic salts and small amounts of organic matter present in the water, the amount of TSS in the present study was within the range 44.85-39.09 g/l (Table.2).

**Table 2. Range of some physical and chemicals properties of sea water recorded during the study period**

Stations	Temperature (°C)	pH	Salinity (ppt)	Electrical conductivity (mS/cm)	Dissolved oxygen (mg/l)	Total dissolves substance (g/l)
1	23.17	8.34	46.00	47.74	3.61	44.85
2	25.37	8.40	43.16	42.75	4.21	40.1
3	26.17	8.76	43.41	41.91	2.93	39.09

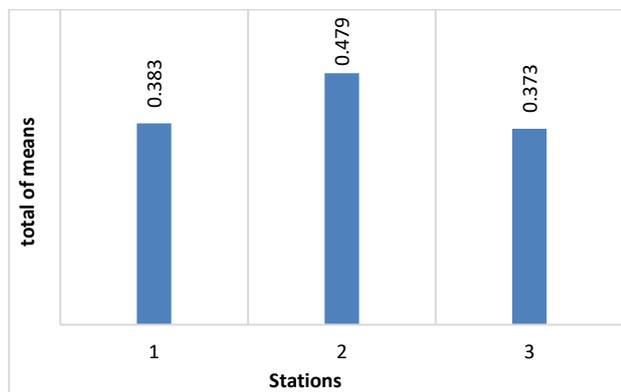
#### The absorbance of bacterial growth

In the present study the obtained results (Table.3), shows the number of bacteria through the studied period and at the three studied stations. The statistical analysis shows no significant difference ( $P>0.05$ ) among station, and significant difference ( $P< 0.05$ ) presented among the studied months. Results also indicated that the means number of bacteria in the studied stations follow this order station 2> station 1> station 3(Fig.1), The logical explanation for these results; is the high drainage and the influx into the Arabian Gulf in addition to the high influxes of nutrients provided by the Shatt Al-,Arab, additionally as a result of dilution in salinity by Shatt Al-Arab River or any near resource

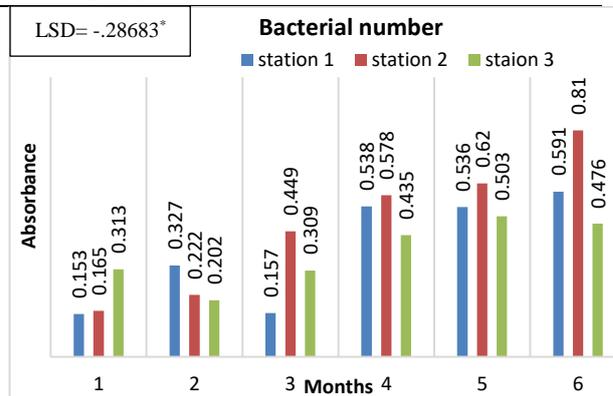
of fresh water<sup>24</sup> In relation to the number of bacteria during the study period, the order was June> May> Apr.> Mar.> Feb.> Jun. (Fig.2.). In temperate marine areas, the microbial community varies according to "changes in temperature and the length of the day"<sup>25,26</sup> indicated that communities were separated into two main groups, corresponding to two main periods: from November to April (colder months), and from May to October (warmer months). Previous studies have shown that in temperate regions, the change in the bacterial community ranges between a gradual change throughout the year or that the change is continuous and rapid between cold and warm months<sup>27</sup>.

**Table 3. The bacterial number through the studied period in the studied stations**

Stations	Mean of absorbance (600nM)						Mean of total
	Jun.	Feb	Mar	Apr.	May.	June	
1	0.153	0.327	0.157	0.538	0.536	0.591	0.383
2	0.165	0.222	0.449	0.578	0.620	0.810	0.479
3	0.313	0.202	0.309	0.435	0.503	0.476	0.373
Mean of total	0.210	0.250	0.305	0.517	0.553	0.625	0.367



**Figure 1. The mean number of bacteria in the three studied stations**



**Figure 2. The mean number of bacteria in the studied stations through different studied period with the LSD value**

### Types of bacteria identified

Totally, 100 bacterial isolates were isolated from the water of the three sampling stations by dilution-plating technique. After occurrent purification, 18 pure bacterial colonies, were subjected to morphological, and microscopic examination by Gram stain test, to make sure of the bacterial staining ability Finally, 18 pure isolates were identified using the VitekII instrument (Table 4). Among the 18 identifying bacteria five genera

(*Staphylococcus lentus*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Sphingomonus paucimobilis* and *Aeromonas hydrophila/caviae*), shows the respected occurrence in the studied stations, with different percentage of frequency (Table.4). Bacterial communities differ in different marine environments, while they are similar in marine environments that are similar but separated by long distances<sup>27,28</sup>

**Table 4. Identifying bacteria and their related phyla and some morphological and biochemical reactions, The most occurrence bacterial genera and the percentage of their frequency (%)**

Name of The Bacteria	Related phylum	Shape	color	Gram s reaction	Most occurrence	The percentage of the frequency%
<i>Staphylococcus pseudintermedius</i>	Firmicutes	cocci	grey-white	+	<i>Staphylococcus lentus</i>	50
<i>Staphylococcus lentus</i>	Firmicutes	cocci	yellow	+	<i>Vibrio parahaemolyticus</i>	40
<i>Staphylococcus schleiferi</i>	Firmicutes	cocci	pale	+	<i>Vibrio alginolyticus</i>	60
<i>Staphylococcus epidermidis</i>	Firmicutes	cocci	white	+	<i>Sphingomonus paucimobilis</i>	30
<i>Staphylococcus lugdunensis</i>	Firmicutes	cocci	tan	+	<i>Aeromonas hydrophila/caviae</i>	20
<i>Pantoea spp</i>	Proteobacteria	cocci	yellow	-		
<i>Kokoria kristinae</i>	Actinobacteria	cocci	Pale cream	+		
<i>Sphingomonus paucimobilis</i>	Proteobacteria	bacilli	white	-		
<i>Leconostoc mesenteroides</i>	Firmicutes	cocci	grayish	+		
<i>Lactococcus lactis</i>	Firmicutes	cocci	bright red	+		
<i>Streptococcus sp</i>	Firmicutes	spherical	white	+		
<i>Aerococcus viridans</i>	Firmicutes	cocci	bright white	+		
<i>Pasteurella testudines</i>	Proteobacteria	rod	gray	-		
<i>Aeromonas hydrophila/caviae*</i>	Proteobacteria	straight rods	white	-		

<i>Vibrio fluvialis</i>	Proteobacteria	Rod	yellow	-
<i>Vibrio parahaemolyticus</i>	Proteobacteria	rod	green	-
<i>Vibrio alginolyticus</i>	Proteobacteria	rod	Bluish Green	-

## Conclusions:

The results of the current study confirm that the Iraqi marine area is classified among the warm regions, as the temperature during the study period ranges between (23.17-26.17 °C). In relation to the bacterial diversity in the study area, the results show the superiority of three bacterial phyla( Firmicutes , Proteobacteria, and , Actinobacteria),with the clear superiority to the phyla( Firmicutes). The most occurrence bacterial genera is( *Staphylococcus lentus*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Sphingomonas paucimobilis* and *Aeromonas hydrophila/caviae*), which has different percentages of frequency ,(50,40,60,30 and 20%)

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## Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Basrah.

## Authors' contributions statement:

R.SH.J. contributed to developing the research idea and preparing for it, as well as collecting samples, conducting tests and analyzing the results. R.SH.J wrote the paper and sent it to the journal for publishing. A.T. contributed to developing the research idea and preparing for it, as well as collecting samples, conducting tests and analyzing the results

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## التنوع البكتيري في المياه البحرية العراقية

فاضل نعمة الكناني

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### الخلاصة:

تشكل الكائنات الحية الدقيقة كل من البناء التركيبي والوظيفي في البيئة البحرية، ولكن رغم التطور العلمي تبقى دراسة التنوع البكتيري في المناطق البحرية من المهمات الصعبة التي تواجه الباحثين في هذا المجال. وعليه هدفت الدراسة الحالية الى بذل جهد بهذا الصدد بالرغم من كونه متواضع الا انه قد يشكل الحجر الركيزة في دراسة التنوع البكتيري في المياه البحرية الإقليمية حيث ان الدراسات تكاد تكون معدومة في هذا الجانب المهم والحيوي. اعتماداً على نتائج الدراسة الحالية يمكن القول ان المياه البحرية العراقية تصنف من ضمن المناطق الدافئة حيث تراوح معدل درجات الحرارة خلال فترة الدراسة من 23.17 الى 26.17 م<sup>0</sup>. اظهرت نتائج العد البكتيري باستخدام طرق الامتصاص زيادة في عدد البكتيريا في المياه مع ارتفاع درجة الحرارة خلال اشهر الدراسة ( كانون الثاني , شباط , اذار , نيسان , أيار , حزيران) حيث بلغ العدد كالتالي ( 0.210,0.250,0.305,0.517,0.553 and 0.625). اظهرت النتائج ان السيادة كانت لثلاث عوائل بكتيرية (*Firmicutes*, *Proteobacteria*, *Actinobacteria*) مع تفوق واضح للعائلة *Proteobacteria* كانت الاجناس البكتيرية الأكثر ظهوراً كالتالي: *Staphylococcus lentus*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Sphingomonas paucimobilis* and *Aeromonas hydrophila/caviae* والتي اظهرت نسب تردد مختلفة بالظهور 50,40,60,30 and 20% وعلى التوالي. تقدم نتائج الدراسة الحالية ادلة أولية على التنوع البكتيري في المياه البحرية العراقية. ولذلك فإن القيام بمزيد من الدراسات ضرورية لعمل صورة أكثر شمولاً للتنوع الميكروبي داخل هذه المناطق البحرية.

الكلمات المفتاحية: البكتيريا، التنوع، Firmicutes، Proteobacteria، Actinobacteria، المنطقة البحرية العراقية.