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The molecular identification of airborne bacteria in Basrah, IRAQ

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Abstract

The objective of this study is to elucidate the environmental significance and potential health implications of airborne microorganisms through their molecular characterization, thereby enhancing our comprehension of atmospheric health and its broader impact on human well-being. Amplification of the 16sRNA gene was carried out based on the multiplex polymerase reaction and used Sanger method determining the nucleotide sequence of DNA to determine the evolutionary relationships, as it is a clear and reliable diagnostic method instead of the traditional inaccurate methods. The study included five stations from Basra Governorate, Iraq, represented by the areas of Qurna, Shuaiba, Tanumah, Khor Al-Zubair and Old Basra, as it revealed The study covers five genera belonging to 16 Gram-negative and Gram-positive bacteria species: *Mammliicoccus lentus, Staphylococcus saprophyticus, Erwinia, Staphylococcus hominis, Lysinibacillus, Exiguobacterium aurantiacum, Staphylococcus epidermidis, Staphylococcus equorum, Staphylococcus succinus, Bacillus pumilus, Bacillus safensis, Staphylococcus xylosus, Pseudomonas aeruginosa, Atlantibacter hermannii, Bacillus subtilis, and Pantoea agglomerans)*. The isolated bacteria were submitted to the NCBI GenBank database for registration. Using the MEGA 11 software, a phylogenetic tree was constructed to analyze the evolutionary relationships among the study isolates and compare them to internationally available samples.

Keywords: 16S Sanger sequencing, VITEK 2, 16S rRNA gene, phylogenetic analysis

التشخيص الجزيئي للبكتريا المحمولة جوا في محافظة البصرة، العراق

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الخلاصة

يهدف هذا البحث إلى تسليط الضوء على الأهمية البيئية والآثار الصحية المحتملة للبكتيريا المحمولة جواً من خلال توصيفها بالتقنيات الجزيئية مما يساهم في نهاية الامر الى فهم واضح لصحة الغلاف الجوي والرفاهية العامة. تم اجراء تضخيم الجين 16 sRNA بالاعتماد على تفاعل البلمرة المتعدد لطريقة سانجر (Sanger method) لتحديد تسلسل النوكليوتيدات الخاص بالـ DNA لتحديد العلاقات التطورية،،اذ تعد طريقة تشخيصية واضحة ومعتمدة بدلا من الطرق التقليدية الغير دقيقة .شملت الدراسة خمس محطات من محافظة البصرة ،العراق متمثلة بمنطقة القرنة ،الشعيبة ،التومة ،خور الزبير والبصرة القديمة،اذ كشفت الدراسة عن خمس اجناس تنتمي ل16 نوعا بكتيريا سالبة وموجبة لصبغة كرام وهي *Mammliicoccus*

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lentus, Staphylococcus saprophyticus, Erwinia, Staphylococcus hominis, Lysinibacillus, Exiguobacterium aurantiacum, Staphylococcus epidermidis, Staphylococcus equorum, Staphylococcus succinus, Bacillus pumilus, Bacillus safensis, Staphylococcus xylosus, Pseudomonas aeruginosa, Atlantibacter hermannii, Bacillus subtilis, and Pantoea agglomerans تم تقديم البكتيريا المعزولة إلى قاعدة بيانات جين بانك الخاصة بـ NCBI للتسجيل. باستخدام برنامج MEGA 11 ، تم بناء شجرة تطورية لتحليل العلاقات التطورية بين عزلات الدراسة ومقارنتها بالعينات المتاحة دوليًا.

1. Introduction

Airborne particles carry bacteria or their organic molecules, spreading them through the air in the form of bioaerosols. Biological activity accounts for a significant portion of the makeup of Earth's atmosphere [1]. Exposure to bioaerosols can lead to severe and common health problems [2]. Many infectious diseases affecting the respiratory system, whether acute or chronic, are a result of this exposure, including influenza and bronchitis. One of the potential causative agents of these diseases is pathogenic bacteria that can be transmitted through airborne particles from infected individuals via oral and respiratory fluids, leading to the spread of various illnesses [3]. The identification of bioaerosols can be achieved through the sequencing of nucleic acids. Many studies utilize the traditional Sanger sequencing method, as it provides sufficiently long sequences to determine genera or individual species through comparison with online databases such as the National Centre for Biotechnology Information (NCBI) [4]. To distinguish markers those decide the degree of the connection between bacterial presence and irresistible microorganisms in people, creatures, and plants, as well as the effect of climatic circumstances on bacterial presence and diversity. Numerous factors contribute to the proliferation of hazardous aerosols and microorganisms in urban, rural, and surrounding areas, leading to various medical issues associated with microbial bioaerosols [5]. The presence of airborne germs is widespread in the environment and has the potential to induce a range of severe to mild negative health consequences in human beings. Hence, the monitoring and regulation of airborne microbes are of utmost importance to ensure the protection of public health and occupational safety [6]. The aim of the present study is to detect bacterial diversity and distribution in the selected study sites.

2. Materials and methods

2.1 Sample Collection Sites

From March 1, 2023 to November 1, 2023, samples were collected from five locations in the Basrah region of Iraq: North, South, Central (Basrah City), West, and East. The collection was conducted in two phases: weekly and monthly collections, as shown in **Figure** 1.

2.2 Airborne microbial sampling methods

Microbial samples were collected from the outdoor environment of the Basrah Province using the airborne sampling sedimentation technique. Sampling took place from 9:00 AM to 1:00 PM. Over an 8-month period that included both dry and humid seasons, collection was conducted using 9-cm plates. Nutrient agar, mannitol salt agar, blood agar, and MacConkey agar were poured into the plates for collection purposes. Subsequently, the plates were exposed to the air for 3 to 5 minutes, after which they were sealed and transported to the laboratory. The plates were then incubated in an incubator at a temperature of 37 °C [7]. The visible colonies on the agar plates were counted using the equation $CFU/m^3 = 5a*10^4 (bt)^{-1}$, where 'a' represents the number of bacterial colonies on the plates, 'b' represents the surface area of the dish in cm2, and 't' represents the exposure time in minutes [8]. The plates were

then sub-cultured by streaking, returned to the incubator for 24 hours at 37 °C, and subsequently subjected to microbiological methods for bacterial species identification [9]. The isolated pure cultures were maintained in brain heart infusion broth supplemented with 20% glycerol and stored at -20°C for future molecular identification.

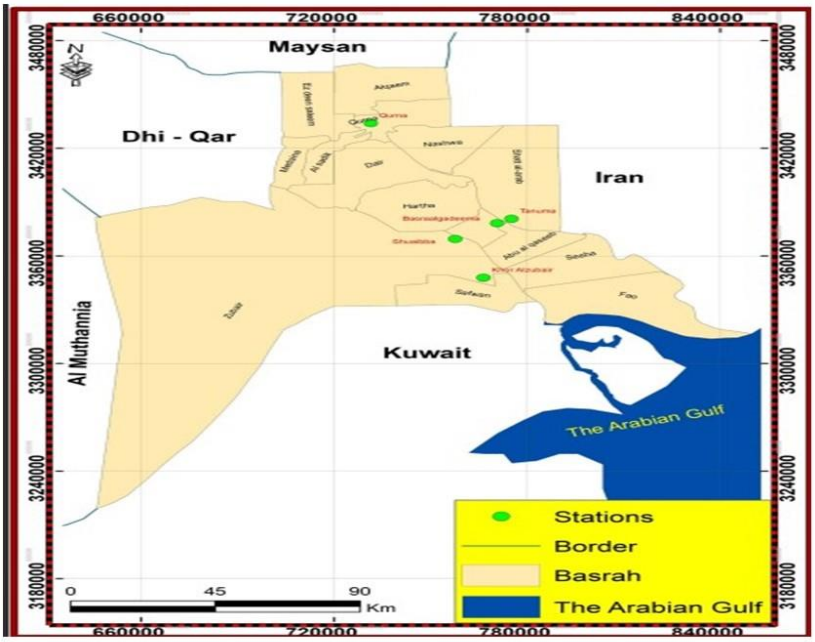

Figure 1:- Districts of Basra Province showing the study areas

2.3 Bulk genomic DNA extraction from bacteria

The genomic DNA extraction process was conducted using the Wizard Genomic DNA Purification Kit, which was supplied from Promega in the United States. The concentration of the extracted DNA was measured using a nanodrop spectrophotometer after DNA extraction from the samples. The purity of the extracted DNA samples was evaluated using a Nanodrop spectrophotometer.

2.4 Amplification and sequencing of bacteria 16sRNA

The polymerase chain reaction (PCR) was run on the study samples to evaluate the gene that was present [10]. The 16S rRNA gene was amplified using the universal 16S RNA forward and reverse primers. The Wizard Genomic DNA Purification Kit, manufactured by Promega in the USA, was employed to extract DNA from the isolated bacteria for subsequent gene amplification. A final volume of 50 µL was achieved for PCR amplification by utilizing the following components: 5 microliters of DNA template, F primer (2.5 µL), R primer (2.5 µL), and nuclease-free water (17 µL). To the Master Mix tube, an extra 17 µL was added. The reaction mixture underwent a denaturation step by being heated to 95 °C for 5 minutes. Next, the processes of denaturation, annealing and extension were repeatedly cycled 30 times. Each cycle involved denaturation at 94 °C for 30 seconds, annealing at 50 °C for 30 seconds, and extension at 72 °C for 30 seconds. The complete cycling process took a total of 1.30 minutes to perform. The total volume of the reaction was 50 µL, an extension was carried out for 5 minutes at 72 °C. The 5 µl of 16S rDNA that was recovered from the polymerase chain reaction (PCR) was electrophoresed on a ladder for 1.5 hours at a voltage of 50V. In a casting tray, 1.5% agarose powder was dissolved in 100 ml of TBE buffer, which contained 0.5 µL of ethidium bromide in 100 ml of agarose solution, to conduct the

electrophoresis. The items were observed using a UV laser system, and the presence of a 1500-bp band signified the presence of 16SRNA. The extracted 16S rRNA gene was amplified using universal 16S RNA reverse and forward primers. The primers employed for amplifying the gene from the isolated microorganisms were as follows:
27F5'-AGAGTTTGATCCTGGCTCAG-3
1492R 5'GGTTACCTTGTTACGACTT-3 (Eden et al., 1991)

The various components described above consist of both primary and secondary constituents, which were thoroughly mixed using a vortex machine. After preparing the PCR reaction mixture in the tubes, the tubes were then placed into a thermal cycler instrument, also known as a thermocycler for PCR machine. DNA sequencing was performed using standard shotgun sequencing reagents according to the manufacturer's instructions [11].

2.5 Gel electrophoresis

1.5% of the agarose granules were dissolved in 100 ml of TBE buffer. The solution was then heated to a gentle simmer in an autoclave. Following the sedimentation of the mixture, the ethidium bromide dye was introduced. Subsequently, the resultant mixture was introduced into the gel casting tank, wherein the combs were strategically placed to generate cavities within the gel formulation. The gel that was poured was allowed to undergo solidification at room temperature for a duration of thirty to forty minutes. After the comb was removed, the gel was treated with TBE buffer. After the ladder and samples were placed in their designated wells, an electrophoresis procedure began using a voltage of 70 watts for one hour. The gel was then transferred to a UV transilluminator operating at a wavelength range of 260–280 nanometers. High-resolution images were subsequently captured using a digital camera, as shown in Figure 2.

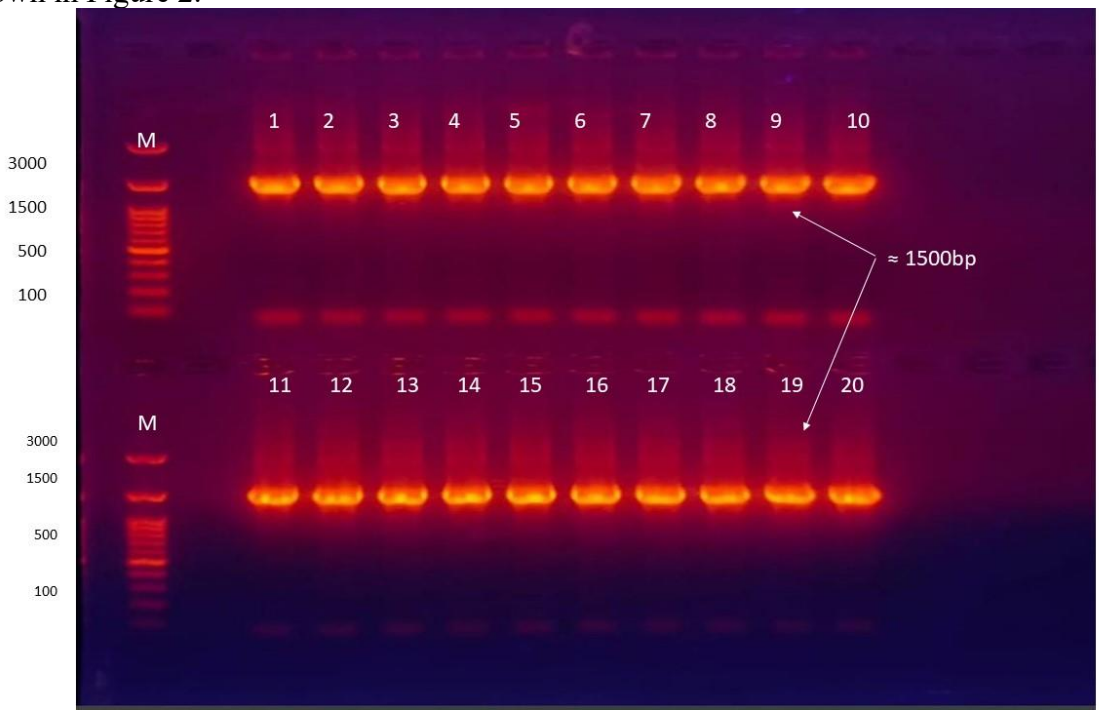

Figure 2:- Agarose gel electrophoresis of PCR –amplified for16 sRNA from bacterial isolate at 75 volt for 25 minutes

2.6 Bioinformatics analysis

Processing of 16SRNA gene sequences was conducted through MEGA11 software, and sequences were aligned using GenBank/Basic Local Alignment Search Tool (BLASTn), as shown in Table 1.

Table 1:- Accession number of 16S rRNA gene sequence

List	accession numbers	Name of isolates in Gen Bank	size of the sequences gene 16sRNA
1	PP419973	***Staphylococcus hominis* strain NWMA11**	962
2	PP419974	***Mammaliicoccus lentus* strain NWMA12**	682
3	PP419975	***Staphylococcus saprophyticus* strain NWMA13**	809
4	PP419976	***Staphylococcus succinus* strain NWMA14**	775
5	PP419977	***Staphylococcus succinus* strain NWMA15**	865
6	PP419978	***Staphylococcus succinus* strain NWMA16**	773
7	PP419979	***Staphylococcus xylosus* strain NWMA17**	511
8	PP419980	***Staphylococcus epidermidis* strain NWMA18**	645
9	PP419981	***Staphylococcus epidermidis* strain NWMA19**	757
10	PP419982	***Staphylococcus equorum* strain NWMA20**	680
11	PP419972	***Staphylococcus hominis* strain NWMA10**	742
12	PP417749	***Bacillus subtilis* strain NWMA2 16S**	762
13	PP417750	***Bacillus pumilus* strain NWMA3**	750
14	PP417748	***Bacillus safensis* strain NWMA1**	620
15	PP417751	***Lysinibacillus sp*. strain NWMA4**	826
16	PP417928	***Erwinia sp*. strain NWMA6**	623
17	PP417927	***Atlantibacter hermannii* strain NWMA5**	812
18	PP417930	***Pantoea agglomerans* strain NWMA8**	857
19	PP417931	***Pseudomonas aeruginosa* strain NWMA9**	556
20	PP417929	***Exiguobacterium aurantiacum* strain NWMA7**	839

2.7 Aligning the sequences

The sequences were registered in GenBank, a database maintained by the National Centre for Biotechnology Information NCBI, and subjected to similarity analysis using the BLAST algorithm [12,13]. The objective was to discern sequences exhibiting homology or analogous traits when benchmarked against known sequences archived in the GenBank registry. The sequences underwent multiple alignments using the Multiple Alignment feature in MEGA 11.013 [14].

3. Results and discussion

Throughout this study, 20 distinct airborne bacterial species were identified, representing 5 genera. These included various Bacillus species (B. pumilus, B. safensis, B. subtilis) and Lysinibacillus. Staphylococcus (*Hominis, Lentus, Saprophyticus, Succinus, Xylosus, Epidermidis equorum), Erwinia sp., Atlantibacter hermannii, Pantoea agglomerans, Pseudomonas aeruginosa, and Exiguobacterium aurantiacum*). Extraction and amplification of DNA were performed on the majority of Gram-positive bacteria. The concentration of DNA ranged from 25 to 50 ng, while the quality of the DNA was assessed to be within the range of 1.6 to 1.8. The DNA was subsequently transferred onto an agarose gel that included the 16sRNA gene and subjected to analysis using ethidium bromide dye. The results obtained using ultraviolet light indicated the presence of DNA bundles with a length of 1500 base

pairs. The sequencing technique was used for the diagnosed isolates after the completion of the PCR examination, and the results of the examination were sent to Macrogen Company in South Korea to sequence the nitrogenous bases using the AB DNA sequencing device. The search results indicate that molecular methods have proven to be highly effective for the identification of bacterial pollution sources. Several studies have demonstrated the advantages of using molecular techniques compared to traditional phenotypic approaches [15,16]. Therefore, we utilized 16s RNA sequencing and blast analysis in our study. Some of the isolated bacteria were reported as pathogenic bacteria in the literature, such as *Pseudomonas aeruginosa*. In addition, the identified bacteria showed variable responses to antibiotic susceptibility testing. To our surprise, several were resistant to antibiotics, as noted in Nalidixic Acid. This indicates a serious threat to public health as these antibiotic markers could circulate and spread among other species of bacteria that have a greater tendency to cause diseases. However, the variation in the outdoor bacterial load could be attributed to many factors, such as population density, number and types of animals, pressure, temperature, and dust Figure 3, showed the findings of the study indicate that there were notable variations in the presence of aerosol bacteria in different places over the duration of the research [17,18]. The findings of the study indicate that there were notable variations in the presence of aerosol bacteria in different places over the duration of the research. The Algadeema in Tanuma, Basrah, exhibited a notably higher magnitude compared to other districts characterized by highly inhabited commercial zones, potentially attributable to a diverse range of businesses. The viability of airborne microorganisms varies among different genera. The major bacterial elements in the atmosphere were bacilli. As spore-forming organisms, bacilli exhibit the capacity to endure the high temperatures characteristic of both the summer and winter seasons. A phylogenetic tree made from the sequenced 16sRNA regions of 20 pathogenic bacterial isolates and identified, the evolutionary analyses were conducted in the MEGA X program. The phylogenetic grouping indicated that strains with similar sequences were typed in the same group and probably were considered close relatives. Phylogenetic analysis revealed that the majority of isolates were closely related to Staphylococcus and Bacillus strains [19], as illustrated in Figures 4, 5, and 6.

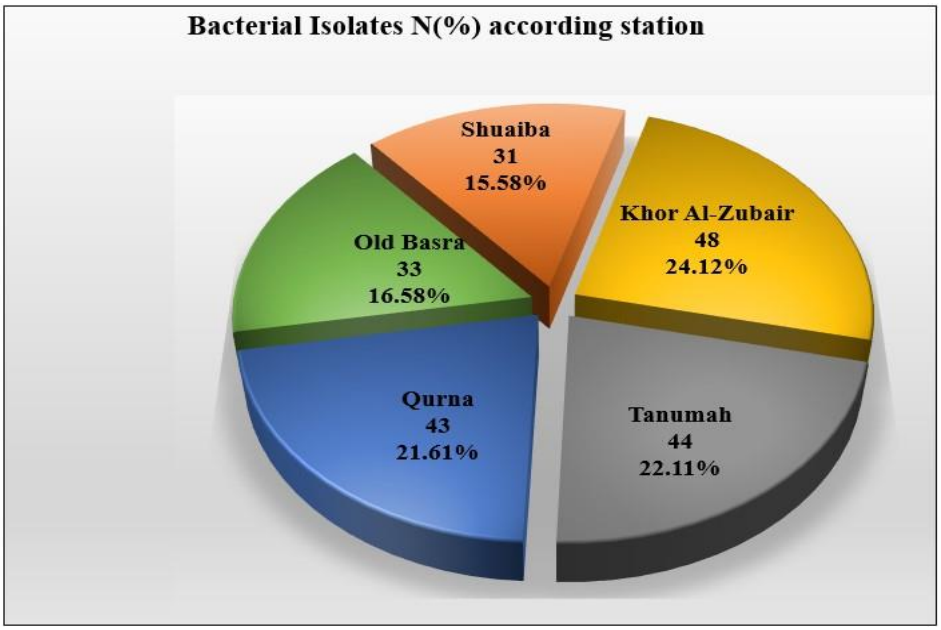

Figure 3:- Mean total viable counts of air borne bacteria during March 1, 2023, to November 1, 2023

Conclusion

Airborne microorganisms affect both physical processes in the atmosphere, such as cloud formation and precipitation, as well as the atmosphere itself. Microorganisms can be incorporated into cloud droplets through nucleation processes: by providing surface condensation to water vapor and acting as CCN (Cloud Condensation Nuclei) or by inducing ice formation and acting as IN (Ice Nuclei). The study examined airborne bacteria levels in Basrah, Iraq, during a period of high heat and humidity. These bacteria are known to have significant health implications. Airborne bacteria have potential as biotechnological agents due to their distinctive enzymatic and receptor-mediated metabolic activities, and ability to tolerate stresses such as desiccation and exposure to UV radiation. There is little question that the influence of airborne bacteria will stimulate a wide range of microbiological studies on the atmosphere. Bacterial species previously unknown in such conditions were discovered, including *E. aurantiacum* and *A. hermannii*, as well as the use of the 16sRNA gene to gain a better understanding of airborne bacterial species.

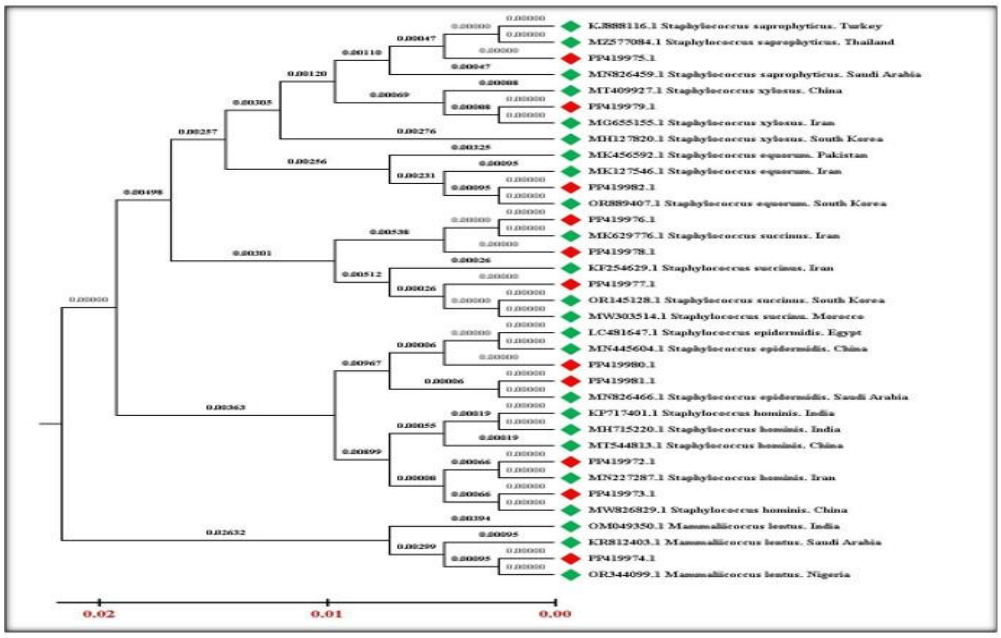

Figure 4:- Phylogenetic tree of *staphylococcus sp* group

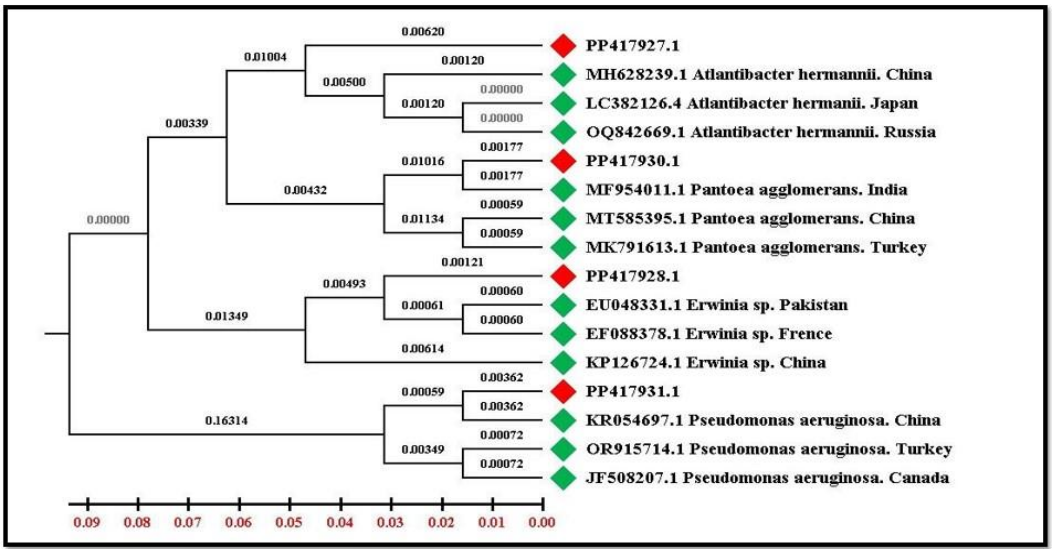

Figure 5:- Phylogenetic tree of *Enterococcus* group

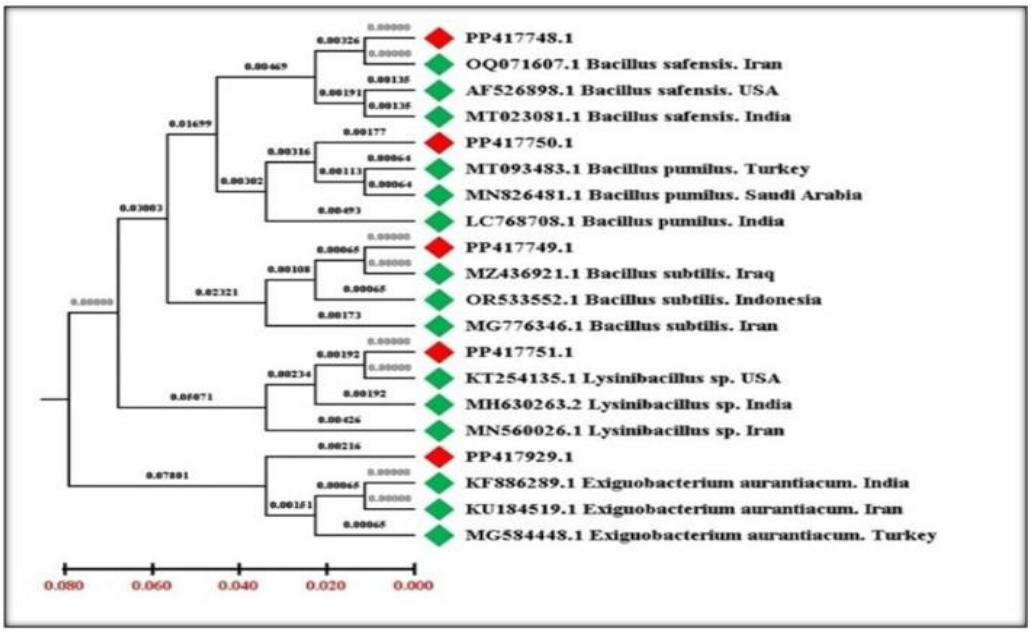

Figure 5: Phylogenetic tree of *Bacillus* group.

Registered links in NCBI:

https://www.ncbi.nlm.nih.gov/search/all/?term=PP419973
https://www.ncbi.nlm.nih.gov/search/all/?term=PP419974
https://www.ncbi.nlm.nih.gov/search/all/?term=PP419975
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-Ethics approval and consent to participate

Ethical approval was received from the ethical and research committee of the university of Basrah, College of Education for Pure Science, Department of Biology, Basrah, Iraq

-Availability of data and material

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

-Competing interests

The authors declare that they have no competing interests

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Reference

[1] P. Cariñanos, I. Foyo-Moreno, I. Alados, J. L. Guerrero-Rascado, S. Ruiz-Peñuela, and G. Titos, "Bioaerosols in urban environments: Trends and interactions with pollutants and meteorological variables based on quasi-climatological series", *Journal of Environmental Management*, vol. 282, p. 111963, 2021.

[2] A. Sadigh, E. Fataei, M. Arzanloo, and A. A. Imani, "Bacteria bioaerosol in the indoor air of educational microenvironments: Measuring exposures and assessing health effects", *Journal of Environmental Health Science and Engineering,* vol. 19, pp. 1635-1642, 2021

[3] S. O Yagoub and A. El Agbash, " Isolation of potential pathogenic bacteria from the air of hospital-delivery and nursing rooms", *Journal of Applied Sciences,* vol. 10, pp. 1011-1014, 2010.

[4] J. Fröhlich-Nowoisky, C. Ruzene Nespoli, D. A. Pickersgill, P. Galand, I. Müller-Germann, andT. Nunes, "Diversity and seasonal dynamics of airborne archaea", *Biogeosciences,* vol. 11, pp. 6067-6079, 2014.

[5] F. Zhou, M. Niu, Y. Zheng, Y. Sun, Y. Wu,and T. Zhu, "Impact of outdoor air on indoor airborne microbiome under hazy air pollution: A case study in winter Beijing", *Journal of Aerosol Science,* vol. 156, p. 105798, 2021.

[6] S. Menteşe, "Airborne bacteria and sick building syndrome (SBS) ", *in Viruses, bacteria and fungi in the built environment*, ed: Elsevier, 2022, pp. 147-178.

[7] Z. A. Nasir and I. Colbeck, "Assessment of bacterial and fungal aerosol in different residential settings ", *Water, Air, & Soil Pollution,* vol. 211, pp. 367-377, 2010.

[8] J. Vandepitte, "*Basic laboratory procedures in clinical bacteriology*",World Health Organization, 2003.

[9] Z. Gizaw, M. Gebrehiwot, and C. Yenew, "High bacterial load of indoor air in hospital wards: the case of University of Gondar teaching hospital, Northwest Ethiopia" , *Multidisciplinary respiratory medicine,* vol. 11, pp. 1-7, 2016.

[10] E. A. Mustafa, S. M. Hamdoon, and E. Y. Shehab, "Molecular detection and identification of Enterococcus faecium isolated from dental root canals", *Iraqi Journal of Science*, pp. 1447-1451, 2021

[11] X. Zhao, Y. Ni, H. Zhao, X. Liu, B. He, B. Shi, "Plant growth-promoting ability and control efficacy of Penicillium aurantiogriseum 44M-3 against sesame Fusarium wilt disease", Biocontrol *Science and Technology,* vol. 31, pp. 1314-1329, 2021.

[12] N. Sharma, S. Patiyal, A. Dhall, A. Pande, C. Arora, and G. P. Raghava, "AlgPred 2.0: an improved method for predicting allergenic proteins and mapping of IgE epitopes", *Briefings in Bioinformatics*, vol. 22, p. bbaa294, 2021.

[13] Q. Mohamed and H. R. R. Al-Taai, "Phylogenetic Analysis of Klebsiella pneumoniae Isolated from Nosocomial and Community Infection in Diyala, Iraq", *Iraqi Journal of Science*, pp. 2726-2740, 2023.

[14] M. A. Thamer and A. A. Shareef, "Molecular detection of some Vancomycin Pathogenic bacteria in Basrah Governorate", *J. Basrah Res.(Sci.),* vol. 48, pp. 27-34, 2022.

[15] K. Locher, C. R. Belanger, E. Eckbo, M. Caza, B. Velapatino, and M. K. Charles, "Automated 16S sequencing using an R-based analysis module for bacterial identification", *Microbiology Spectrum,* vol. 10, pp. e00408-22, 2022.

[16] N. K. Younus, " Phenotypic and Genotypic Characterization of Multidrug-resistant Escherichia coli and Klebsiella pneumoniae Isolated from Women with Urinary Tract Infections in Mosul City", *Iraqi Journal of Science*, pp. 24-35, 2024

[17] P. A. Eden, T. M. Schmidt, R. P. Blakemore, and N. R. Pace, "Phylogenetic analysis of Aquaspirillum magnetotacticum using polymerase chain reaction-amplified 16S rRNA-specific DNA", *International journal of systematic and Evolutionary microbiology,* vol. 41, pp. 324-325, 1991

[18] M. R. Green and J. Sambrook, "Analysis of DNA by agarose gel electrophoresis", *Cold Spring Harbor Protocols,* vol. 2019, p. pdb. top100388, 2019.

[19] J. Chun, K. Y. Kim, J.-H. Lee, and Y. Choi, "The analysis of oral microbial communities of wild-type and toll-like receptor 2-deficient mice using a 454 GS FLX Titanium pyrosequencer", *BMC microbiology,* vol. 10, pp. 1-8, 2010.