

Research Article

Molecular diagnosis using metagenomic technology on wastewater from the Danville Valley in the city of Mosul, Iraq

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ARTICLE INFO

Article history:

Received 5 Jul 2024

Accepted 12 Aug 2024

Published 30 Dec 2024

DOI: [10.35192/jjoas-n.v18i1.1897](https://doi.org/10.35192/jjoas-n.v18i1.1897)

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Keywords:

Water pollution
Danfeli Valley
Tigris River
Metagenomics
Molecular diagnosis

ABSTRACT

Background: The wastewater of the Danville Valley is one of the most polluted valleys in the city, as it consists of sewage and industrial wastewater, in addition to excreta from fodder residues and animal waste, along with rainwater and agricultural waste.

Objective: This study aims to perform molecular diagnosis of Danville Valley waste to determine the types of bacteria present in sewage wastewater in the city of Mosul.

Methods: The PCR results were sent to Zymo Research Europe in Freiburg, Germany, in order to analyze the entire genetic material in the sample using metagenomic technology. Special primers were used to amplify the (V3-V4) region of the complete 16S rRNA gene.

Results: The analysis of the wastewater sample from the study area revealed varying percentages of bacterial levels. The phylum (Proteobacteria) was the dominant bacterial phylum in the wastewater at a rate of 82.9%. The class (Epsilonproteobacteria) and the order (Campylobacteriales) both recorded rates of 42.7%, while the family (Campylobacteraceae) recorded a rate of 41.1%. The dominant bacterial genus was (Arcobacter) at 39.9%.

Conclusion: The phylum (Proteobacteria) is the dominant bacterial phylum in the wastewater of the study area at a rate of 82.9%. The class (Epsilonproteobacteria) recorded a rate of 42.7%, the order (Campylobacteriales) at 42.7%, and the family (Campylobacteraceae) at 41.1%. The dominant bacterial genus was (Arcobacter) at 39.9%.

Introduction

Water pollution is considered one of the most important problems facing humanity currently. Concern has increased in recent years about water in the world. Population growth in large cities and industrial development are among the most important reasons for disturbing the environmental balance. Water pollution is one of the most dangerous types of pollution for water works on vast areas of the Earth, and that the final fate of soil and air pollutants is water bodies, whether directly or indirectly (1). The World Health Organization has proven that about 80% of bacterial, viral and parasitic diseases are transmitted through contaminated water, in addition to that 5% of child deaths around the world are due to poor water quality, and diarrhea, which is one of the most common and dangerous diseases, is transmitted through intestinal viruses. found in drinking water (2; 3). Untreated wastewater is also one of the most dangerous pollutants that cause diseases. The arrival of these pollutants into drinking water sources due to the wrong activities of the population led to the release of various types of pollutants, especially biological ones (4).

The method of detecting biological contamination by partial methods is one of the modern methods, which relies on isolating organisms from various contaminated media and growing them in nutrient media in order to obtain the largest possible number of cells. After the process of extracting deoxyribonucleic acid from these cells, it is amplified using primers. It depends on the type of organism, as the 16S rRNA primer is used to detect bacteria (5), while the ITS primer is used to detect fungi (6), and to detect algae, the 18S rDNA primer is used according to (7). Then, an analysis of the nitrogenous bases is performed to determine its DNA sequence, then it is matched with the National Center for Biotechnology Information (NCBI) to determine the type and gender of the organism and record what is new.

Metagenomic technology is considered one of the most prominent techniques discovered in biology due to its importance in study-

ing microorganisms through their detection and investigation in all fields, such as environmental and medical research, clinical biology, and the discovery of many pathogens, such as viruses and bacteria (8; 9). This technology has a distinct role in forensic and criminal medicine (10). Through metagenomic studies, a large number of microorganisms have been discovered in different environments that cannot be diagnosed and their types and sequences cannot be known due to the inability to cultivate them (11).

In its work, metagenomics relies on two main methods: the Shotgun Metagenomic method, which when used gives accurate results for the samples studied, as DNA is used randomly without the need for a copy process, which ensures the detection of many previously undiscovered microorganisms (12). And the Metagenomic Targeted method, which works to target a specific area containing multiple organisms. This method relies in its work on the use of primers such as 16S rRNA for bacteria. This method is considered one of the methods with high accuracy in showing results, because it targets most of the microorganisms present in the examination samples (13).

Materials And Methods

Description of the study area

The study area included the Danville Valley in the city of Mosul/Iraq, as shown in Figure 1. The valley's water represents sewage and industrial wastewater, as well as waste from fodder and animal waste, in addition to rainwater and agricultural waste.

Molecular Test

Genomic DNA was extracted directly from the water (liquid) sample, using the Presto Mini gDNA Bacteria Kit provided by GeneAid, following the steps recommended by the manufacturer, and genomic DNA was stored at 20°C (14). As for the polymerase chain reaction, it was prepared using the PCR Master Mix[®]AccuPower kit and according to the steps recommended by the producing company

(Promega). The PCR result was then sent to a company to Zymo Research Europe, Freiburg, Germany to study the entire genetic ma-

terial in the sample, and special primers were used to duplicate the (V.-V.) portion of the complete 16S rRNA gene.



Figure 1. Paths of the mouth of the Danville Valley

Results and Discussion

Table 1. Results of the current study

level	Bacteria	Percentage (%)
Phylum	Proteobacteria	82.9
	Bacteroidetes	7.8
	Fusobacteria	6.6
	Firmicutes	1.2
	Cyanobacteria	0.1
	Gracilibacteria	0.4
	Other	1
Class	Epsilonproteobacteria	42.7
	Gammaproteobacteria	27.3
	Betaproteobacteria	12.1
	Fusobacteriia	6.6
	Bacteroidia	4.4
	Flavobacteriia	3.3
	Other	3.6
Order	Campylobacteriales	42.7
	Aeromonadales	16.6
	Pseudomonadales	9.6
	Burkholderiales	9.1
	Fusobacteriales	6.6
	Bacteroidales	4.3
	Other	11.1
Family	Campylobacteraceae	41.1
	Aeromonadaceae	15.9
	Comamonadaceae	9.0
	Moraxellaceae	7.8
	Leptotrichiaceae	6.5
	Enterobacteriaceae	0.3
	Other	19.4

Genus	Arcobacter	39.9
	Tolomonas	8.8
	Acinetobacter	7.2
	Aeromonas	7.1
	Comamonas	2.9
	Flavobacterium	2.4
	Hydrogenophaga	2.1
	Acidovorax	2.1
	Pseudomonas	1.7
	Sulfurospirillum	1.2
	Bacteroides	1
	Other	23.6

The results of the metagenomic analysis of the Danville Valley wastewater sample showed varying percentages of bacteria levels, as the phylum (Proteobacteria) recorded the highest percentages of phyla, reaching (82.9%). It is one of the main and dominant phyla in the world of bacteria. It is negative for the Gram stain, includes six classes, and is found in a number of environments such as brackish water, estuaries, and in the digestive system of warm-blooded animals (15), some of its types are used to remove sulfate ions in industrial wastewater (16). As for the phylum (Bacteroidetes), it was the second highest phylum. Its percentage reached (7.8%) and includes three classes of bacteria, widely spread in the environment, and often found in the intestines and in patients suffering from irritable bowel syndrome (17). Regarding the classes of bacteria in the study area, the class recorded as (Epsilonproteobacteria) had the highest percentage of classes (42.7%) and belongs to the phylum (Proteobacteria). These bacteria are found in the digestive system of animals and act as symbionts or pathogens (18; 19). The second highest class was (Gammaproteobacteria) (27.3%). It also belongs to the Proteobacteria phylum and contains approximately 250 genera (20). It is used as biological filters for methane and in wastewater treatment (21), and is considered distinctive in the analysis of crude oil after Oil spills (22).

The highest rank recorded in the current study is the order (Campylobacteriales) with a rate of (42.7%) and it belongs to the class (Epsilonproteobacteria) and the phylum (Proteobacteria), while the order (Aeromonadales) recorded the second highest rank in the study with a rate of (16.6%) and it belongs to the class (Gammaproteobacteria) and the phylum (Proteobacteria). The Campylobacteraceae family, which recorded the highest percentage

(41.1%) at the family level, belongs to the order (Campylobacteriales), class (Epsilonproteobacteria), and phylum (Proteobacteria). The second highest family was (Aeromonadaceae) with a percentage of (15.9%). This family belongs to the order (Aeromonadales), class (Gammaproteobacteria), and phylum (Proteobacteria), As shown in Table 1 and Figure 2.

As for the genera level, a number of bacterial genera were recorded during the metagenomic analysis of the sample of the current study. The genus (Arcobacter) recorded a percentage of (39.9%) of the total genera present in the sample, and (Arcobacter) belongs to the phylum (Proteobacteria) and is widespread in different environments, and pathogens in humans and animals (23), some evidence indicates that livestock are the main reservoir for this Bacteria, and its presence in surface and groundwater has been widely reported in recent years (24). As for the genus (Tolomonas), which constituted (8.8%), it is from the family (Aeromonadaceae), which has the ability to produce toluene, which is a trace of human pollution (25).

The genus (Acinetobacter) recorded a percentage of (7.2%). It is a genus of Gram-negative bacteria that belongs to the class (Gammaproteobacteria) (26). Its species are widely distributed in nature. They are usually found in soil and water, and their species are considered a source of major cause of infection among patients in hospitals due to resistance to various disinfectants (27). Regarding the genus (Aeromonas), which constituted a percentage of (7.1%), it is a Gram-negative, facultative anaerobic bacteria, and most of its species are considered pathogens for humans. They are found in rivers and freshwater lakes and have been frequently observed in drinking water (28).

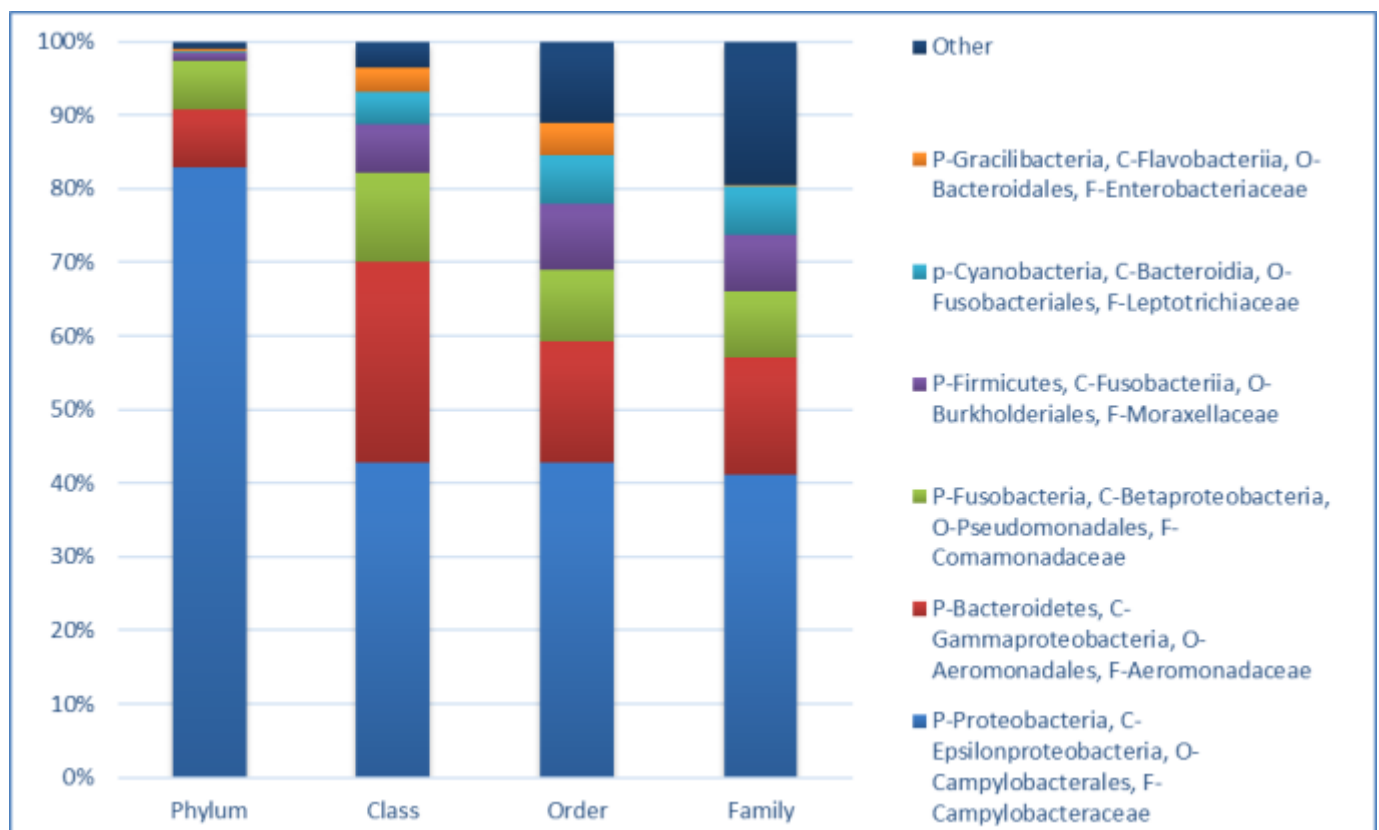


Figure 2. Percentage (%) of bacteria levels in the current study area

The genus (*Comamonas*) recorded (2.9%) and belongs to the phylum (Proteobacteria), and is considered a pathogen in humans (29). While the genus (*Flavobacterium*) recorded a percentage of (2.4%), which is a gram-negative Bacteria and consists of 130 species (30), bacteria of this genus are found in soil and fresh water, and many of its species are considered pathogens for freshwater fish. (31). As for the genera (*Hydrogenophaga* and *Acidovorax*), which recorded a percentage of (2.1%), they belong to the family (*Comamonadaceae*), and the genus (*Acidovorax*) is considered a pathogen of plants (32; 33).

The genus (*Pseudomonas*) showed a percentage of (1.7%). It is a gram-negative Bacteria and belongs to the class (Gammaproteobacteria). It consists of 313 species and is considered a pathogen in humans, animals, and plants (34). While the genus (*Sulfurospirillum*) showed a percentage of (1.2%) belonging to the family (*Campylobacteraceae*), and this Bacteria is found in soil, groundwater, and polluted environments (35). Finally, the genus (*Bacteroides*) recorded a percentage of (1%) over the rest of the genera. This Bacteria is found in the human colon and can be toxic (36). A new bacterial gene was registered as (*Arcobacter* sp. RaMaSh) with code (PP793006) as shown in Table 1 and Figure 3.

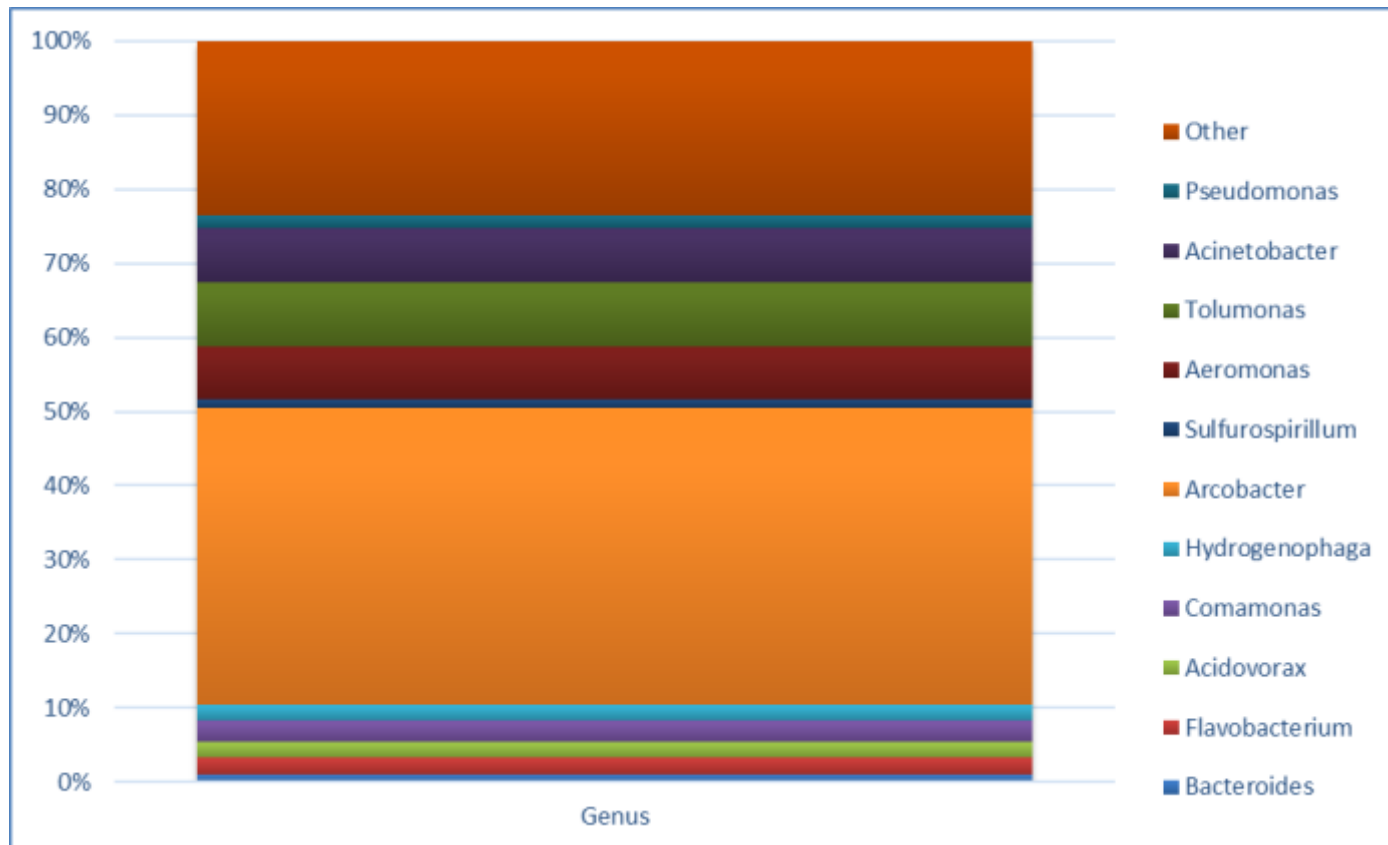


Figure 3. Percentage (%) of bacterial genera in the current study area

Conclusions

The phylum (Proteobacteria) is the dominant bacterial phylum in the wastewater of the study area at a rate of 82.9%, while the class (Epsilonproteobacteria) recorded a rate of 42.7%, the order (*Campylobacteraceae*) at a rate of 42.7%, and the family (*Campylobacteriales*) at a rate of 41.1%. As for the dominant bacterial genus, it was (*Arcobacter*) by 39.9%.

Authors contribution

Sample collection: Al-Hadedi, R. Writing: Al-Hadedi, R. and Abdullah, Sh. Discussion Al-Hadedi, R. and Mohammed, M.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Data Availability Statement

No new data were generated or analyzed in support of this research.

Funding

This work was extracted from a master thesis of the first author and supported.

Acknowledgment

The authors acknowledge the support provided by the Jordanian Journal of Applied Sciences in publishing this research.

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