

Article

# Seasonal Variability in the Microbial Community and Pathogens in Wastewater Final Effluents

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**Abstract:** Numerous bacteria, especially pathogens, exist in wastewater final effluents, which can lead to possible human health and ecological security risks when effluents are reused or discharged. However, the diversity, composition, and spatiotemporal dynamics of bacteria in wastewater final effluents remain poorly understood. In this study, a comprehensive analysis of the microbial community and pathogens in wastewater final effluents was performed using high-throughput sequencing. The results revealed that wastewater final effluents in autumn exhibited the highest bacterial community richness and diversity, while those in winter exhibited the lowest. Bacteria in wastewater final effluents predominantly belonged to five phyla, in the order of Proteobacteria, Actinobacteria, Planctomycetes, Bacteroidetes, and Firmicutes. At the species level, there were 8~15 dominant species in the wastewater final effluent in each season, and *Dokdonella immobilis*, *Rhizobium gallicum*, *Candidatus Flaviluna lacus*, and *Planctomyces limnophilus* were the most dominant species in spring, summer, autumn, and winter, respectively. The seasonal variability in bacteria suggested that the microbial diversity and community in wastewater final effluents were mainly influenced by temperature, salinity, disinfection methods, and flocculants. Notably, pathogenic bacteria in wastewater effluents had both the highest relative abundance and species abundance in summer. *Arcobacter* spp., *Legionella* spp., and *Mycobacterium* spp. were the dominant pathogenic bacteria, and all pathogenic bacteria were mainly associated with dermatosis, enteropathies, septicemia, and pneumonia.

**Keywords:** wastewater final effluent; bacterial community; pathogens; seasonal variability

## 1. Introduction

Municipal wastewater is a matrix consisting of microorganisms, biodegradable organic materials and compounds, metals, and other inorganic materials [1]. The treatment of wastewater is important to prevent the pollution of the environment and water bodies. More importantly, wastewater treatment protects public health by preventing the spread of pathogenic diseases [2–4]. While routine wastewater treatment diminishes the load of microorganisms and organic nutrients, the limitation of microbial standards has caused wastewater final effluents to be regarded as reservoirs of pathogens [5].

The presence or abundance of *fecal coliforms* universally serves as the index of the presence of human pathogens in wastewater final effluents [6]. However, *fecal coliforms* are far more sensitive to disinfection than many other bacteria and should be absent immediately after disinfection, which enables the use of their presence as an indicator of the efficacy of wastewater final effluent treatment [7].

For example, pathogenic strains of *Vibrio* have been isolated from wastewater final effluents in the Eastern Cape of South Africa and in other places [4,8], and Odjadjare EEO found that municipal wastewater effluents were a source of *Listeria* pathogens in the aquatic milieu of the Eastern Cape Province of South Africa [9]. In addition, the discharge of treated effluent often results in an increased number of microbial pathogens in the receiving water, which may lead to a range of waterborne-related diseases, such as giardiasis and gastroenteritis [8]. Therefore, it is very important to gain insights into the microorganisms in effluents to prevent public health issues.

Researchers have performed substantial work on the microbiological safety of wastewater final effluents. Anthropogenic microorganisms in wastewater are the main microbial pathogens in wastewater final effluents [10]. The wastewater treatment process plays an important role in the retention of microbial pathogens in wastewater, and the retention efficiency is related to dissolved oxygen and temperature [11]. Disinfection by chlorination, ultraviolet light exposure, or ozonation can rapidly reduce the abundance of microbial pathogens, although vastly different sterilization effects on different pathogens have been detected for certain disinfection technologies [12–14]. Muhammetoglu A found that there was a weak correlation between *fecal coliforms* and common microbial pathogens, while *Clostridium perfringens* has a significant correlation with common microbial pathogens [6].

However, the objects of most studies above were *E. coli* and *fecal coliforms*. Only a few studies have evaluated *fecal Streptococci* and *Enterococci*, *Salmonella* spp., *Shigella* spp., and *Vibrio* spp., or other pathogens and potential pathogens. Moreover, there are few studies on the diversity and community structure of microbial pathogens in wastewater final effluents, and thus, the identities of the species of pathogens and potential pathogens present in wastewater final effluents and their seasonal variability remain unknown. Therefore, the investigation of the microbial diversity and community structure of wastewater final effluents is very important, both for understanding effluent microbes and for public health. To the best of our knowledge, the study described here represents the most comprehensive temporal analysis of microbial communities in wastewater final effluents to date.

## 2. Materials and Methods

### 2.1. Sampling Procedure

The wastewater final effluent samples were collected from Li Cun He municipal wastewater treatment plant in Qingdao, Shandong Province, during each season (23 April 2017; 17 August 2017; 24 October 2016; 25 January 2017). The treatment process included an A<sup>2</sup>O reactor followed by a microfiltration (MF) system, while filtration fabric and chlorine disinfection were used as the disinfection method. This sewage treatment plant treats  $1.7 \times 10^5$  m<sup>3</sup> wastewater every day, on average 70% of which is industrial sewage, and the rest is domestic sewage each year. Samples were taken from the wastewater final effluent after fabric filtration and chlorine disinfection. The wastewater final effluent is discharged into Li Cun He through a ditch with a dimension of 1 meter wide, 0.5 meters deep. Two L wastewater samples were collected from the top 10 cm of the profile (to minimize the impact of disturbances from the biofilm on the ditch wall) and thoroughly mixed immediately. The samples were taken by using sterile sampling bags, were kept at 4 °C, and then sent back to the laboratory immediately for subsequent analyses. The environmental factors were detected during sampling. Biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solid (SS), total nitrogen (TN), and total phosphorus (TP) were determined using the titration method described in Standard Methods (APHA, 1995). The values are listed and shown in Table 1.

**Table 1.** Environmental factors of sampling stage in different seasons.

Season	Date	T (°C)	BOD <sub>5</sub> (mg/L)	COD <sub>cr</sub> (mg/L)	SS (mg/L)	TN (mg/L)	TP (mg/L)	pH Values
Spring	2017.04.23	15.3	9	47	8	15	0.6	6.8
Summer	2017.08.17	25.1	8	42	6	12	0.5	6.8
Autumn	2016.10.24	20.0	11	56	10	17	0.7	6.9
Winter	2017.01.25	1.0	10	52	7	16	0.6	6.9

## 2.2. DNA Extraction and Purification

DNA was extracted using the Fast DNA SPIN Kit for Soil (OMEGA, China) according to the manufacturer's instructions. The crude DNA was purified by using the Wizard plus sv Minipreps DNA purification system (Promega Corporation, Madison, WI, USA).

## 2.3. 454 Pyrosequencing

The 16S rRNA universal bacterial primers 28F (5'-GAGTTTGATCNTGGCTC AG-3') and 519R (5'-GTNTTACNGCGGCKGCTG-3') were used to amplify approximately 500 bp of the variable regions V1 to V3. The PCR products were purified by the MiniBest DNA Fragment Purification Kit Ver.4.0 (TaKaRa, Japan). Purified PCR products were mixed and sent to Shanghai Personal Biotechnology Co., Ltd. for pyrosequencing. Pyrosequencing of the PCR products was performed on a GS FLX sequencing instrument using the manufacturer's suggested methods and reagents. Initial image collection was performed on the GS FLX instrument, in addition to subsequent signal processing, quality filtering, and generation of nucleotide sequences. All filtered sequences were compared with the Silva database [15] by the local BLASTN tool, and the output sequences were then assigned to NCBI taxonomies with MEGAN [16] to profile the bacterial communities. Sequences identified as archaea and eukaryon were filtered out. The 16S rRNA gene database [17] was downloaded from a homepage of the University of Hong Kong (<http://web.hku.hk/~{zhangt/ZhangT.htm>).

## 3. Results

### 3.1. Community Richness and Diversity of Wastewater Final Effluents in Four Seasons

Between 24 October 2016 and 17 August 2017, we collected four samples of wastewater final effluents from Li Cun He municipal wastewater treatment plant in Qingdao, Shandong Province. These four samples represent the wastewater final effluents in different seasons. We obtained a total of 57,116 DNA sequences, with an average of 14,279 sequences per sample. Of those sequences, 43,139 sequences were high-quality sequences, and those high-quality sequences were selected using PANGEA and subsequently pooled. Operational taxonomic units (OTUs) were determined at the genus (95%) level. Analysis by weighted Fast UniFrac was used to compare the bacterial communities across different effluent samples (Hamady et al., 2010). In this study, there were  $1800 \pm 200$  OTUs per sample.

To compare the genotypic diversity, the community richness (Chao and Ace indexes) and community diversity (Shannon index) were calculated (Table 2). A greater Chao/ACE index indicates higher community richness, while higher Shannon index indicates higher community diversity. Table 2 shows that the wastewater final effluents sampled in summer and autumn had significantly higher bacterial diversity than the wastewater final effluents sampled in spring and winter, and the samples in autumn displayed the highest diversity, while the lowest bacterial diversity was found in samples collected in winter.

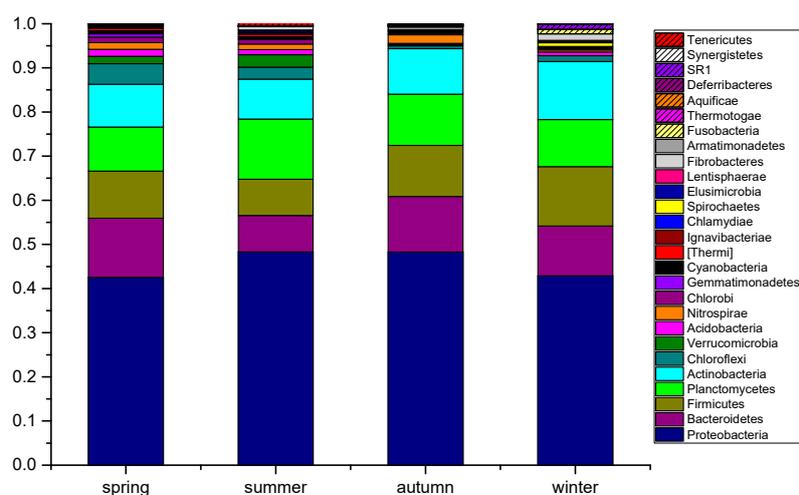
**Table 2.** Community richness (Chao and Ace indexes) and diversity (Shannon index) of the wastewater final effluents in four seasons.

Sample	Chao	Ace	Chao/ACE	Shannon
Sample in spring	1732	1940	0.89	8.56
Sample in summer	5381	5397	0.99	8.54
Sample in autumn	6075	6121	0.99	9.38
Sample in winter	3588	5079	0.71	6.37

### 3.2. Identification of the Bacterial Community in the Wastewater Final Effluents

The analyses of the phylogenetic compositions revealed 27 phyla in the wastewater final effluents in the four seasons. The bacteria in the wastewater final effluent from different seasons mainly belonged to 5 phyla. Phylum Proteobacteria was the most dominant phylum in the wastewater final effluent in all seasons; however, the community composition of bacteria varied greatly.

As shown in Figure 1, there were 22 bacterial phyla in the wastewater final effluent in spring, and the most dominant phylum was Proteobacteria, with a relative abundance of 42.5%. The phyla Chloroflexi, Bacteroidetes, Actinobacteria, and Firmicutes all increased in relative abundance in the wastewater final effluent in spring. The 5 phyla above accounted for 86% of the bacterial relative abundance in the wastewater final effluent in spring. In contrast, there were 5 phyla that accounted for no more than 0.5% of the bacterial relative abundance in the wastewater final effluent in spring: phylum Chlamydiae, phylum Spirochaetes, phylum Fibrobacteres, phylum Lentisphaerae, and phylum Fusobacteria.

**Figure 1.** Seasonal variability of bacterial community in wastewater final effluents.

In summer, there were 22 bacterial phyla in the wastewater final effluent, and the relative abundance of Proteobacteria was 48.3%. The phyla Planctomycetes, Actinobacteria, Bacteroidetes, and Firmicutes were all dominant. The 5 phyla above accounted for 87% of the bacterial relative abundance in the wastewater final effluent in summer. However, the phyla Cyanobacteria, Spirochaetes, and Gemmatimonadetes were minor phyla, accounting for no more than 1% of the bacterial relative abundance.

The wastewater final effluent in autumn was the most diverse, with 23 phyla. The relative abundance of Proteobacteria was 48.3%. The phyla Bacteroidetes, Firmicutes, Planctomycetes, and Actinobacteria were also dominant in the wastewater final effluent in autumn, and all 5 phyla above accounted for 94% of the bacterial relative abundance. The other 18 phyla, excluding Nitrospirae, accounted for less than 1% of the bacterial relative abundance.

Similar to other seasons, Proteobacteria was the most dominant phylum in the wastewater final effluent in winter, with a relative abundance of 42.9%. The phyla Firmicutes, Actinobacteria,

Bacteroidetes, and Planctomycetes had high relative abundances in the wastewater final effluent in winter, while the phyla Acidobacteria, Spirochaetes, Verrucomicrobia, Nitrospirae, Chlorobi, Lentisphaerae, Cyanobacteria, and Gemmatimonadetes were minor phyla, accounting for no more than 1% of the relative abundance.

### 3.3. Seasonal Variability in the Dominant Species in Wastewater Final Effluents

A total of 305 OTUs shared more than 0.2% of the relative abundance, the representative sequence of which was identical in 203 species. Thirty-five species were dominant species with relative abundances greater than 1% in the wastewater final effluents. The dominant species are listed in Table 3. The table shows that only *Planctomyces limnophilus* was detected in the four seasons, while the distribution of all other dominant species varied greatly in different seasons.

**Table 3.** The dominant species in wastewater final effluents.

Serial Number	Similar Species of Geneticrelationship	Accession Number in NCBI	Spring (%)	Summer (%)	Autumn (%)	Winter (%)
1	<i>Acidisoma tundrae</i>	NR_042705	—	—	2.27	—
2	<i>Anaerolinea thermophila</i>	NR_074383	2.40	—	—	—
3	<i>Arcobacter cryaerophilus</i>	NR_025905	—	1.76	0.67	3.59
4	<i>Blastopirellula cremea</i>	NR_118153	—	0.99	1.19	1.89
5	<i>Candidatus Flaviluna lacus</i>	NR_125496	—	2.86	5.50	0.54
6	<i>Candidatus Nitrospira defluvi</i>	NR_074700	1.16	—	0.69	0.23
7	<i>Comamonas terrigena</i>	NR_114856	—	—	—	2.20
8	<i>Corynebacterium deserti</i>	NR_118005	—	—	1.98	0.62
9	<i>Demequina flava</i>	NR_113566	—	—	1.85	0.26
10	<i>Dokdonella immobilis</i>	NR_108377	4.97	—	—	—
11	<i>Ferruginibacter alkalilentus</i>	NR_044588	0.56	—	—	2.08
12	<i>Flavobacter iumanseonense</i>	NR_132690	2.51	—	—	—
13	<i>Geobacter anodireducens</i>	NR_126282	—	2.50	—	—
14	<i>Gracilimonas rosea</i>	NR_109751	—	1.31	—	—
15	<i>Helicobacter cetorum</i>	NR_074476	—	1.01	5.15	1.59
16	<i>Lactococcus piscium</i>	NR_043739	1.65	—	—	—
17	<i>Levilinea saccharolytica</i>	NR_040972	2.40	0.37	—	—
18	<i>Limisphaera ngatamarikiensis</i>	NR_134756	—	—	—	1.17
19	<i>Limnobacter thiooxidans</i>	NR_025421	—	1.59	0.51	—
20	<i>Massilia aflava</i>	NR_117901	0.35	2.03	—	1.15
21	<i>Mycoplasma cynos</i>	NR_102477	—	—	—	1.15
22	<i>Nautilia abyssi</i>	NR_042690	—	—	2.15	0.60
23	<i>Neoskia ramosa</i>	NR_025269	—	—	0.59	1.47
24	<i>Noviherbaspirillum aurantiacum</i>	NR_118040	—	—	6.06	—
25	<i>Noviherbaspirillum soli</i>	NR_118041	1.22	—	—	—
26	<i>Pedobacter oryzae</i>	NR_116174	1.45	2.00	—	—
27	<i>Pirellula staleyi</i>	NR_074521	—	1.76	—	—
28	<i>Planctomyces limnophilus</i>	NR_074670	0.27	0.22	1.88	3.99
29	<i>Rhizobium gallicum</i>	NR_036785	—	4.67	—	—
30	<i>Salimicrobium flavidum</i>	NR_104548	—	1.86	—	1.40
31	<i>Schlesneria paludicola</i>	NR_042466	—	1.65	1.03	1.76
32	<i>Sphingomonas starnbergensis</i>	NR_109485	—	1.02	—	—
33	<i>Sulfuricurvum kujiense</i>	NR_074398	—	—	0.27	1.11
34	<i>Thermogutta hypogea</i>	NR_134825	—	—	—	1.34
35	<i>Thermogutta terrifontis</i>	NR_134826	—	—	2.22	1.94

Note: “—” representatives this species is not detected under the experimental conditions.

In spring, eight species were dominant in the wastewater final effluent: *Dokdonella immobilis*, *Flavobacterium anseonense*, *Anaerolinea thermophila*, *Levilinea saccharolytica*, *Lactococcus piscium*, *Pedobacter oryzae*, *Noviherbaspirillum soli*, and *Candidatus Nitrospira defluvi*, in that order. All eight species accounted for 17.76% of the relative abundance, and the most dominant species, *Dokdonella immobilis*, accounted for 4.97% of the relative abundance. However, *Dokdonella immobilis* was not detected in the wastewater final effluent in other seasons. In addition to *Dokdonella immobilis*, other

dominant species, such as *Anaerolinea thermophila*, *Flavobacterium anseonense*, *Lactococcus piscium*, and *Noviherbaspirillum soli*, were also detected only in the wastewater final effluent in spring, and *Candidatus Nitrospira defluvi* and *Levilinea saccharolytica* exhibited low relative abundances in all seasons excluding spring. This indicated that dominant species in the wastewater final effluent in spring had obvious differences from those in the other seasons.

In summer, there were 13 dominant species with relative abundances greater than 1% in the wastewater final effluents, and those 13 species altogether accounted for 26.02% of the relative abundance. The five most dominant species were *Rhizobium gallicum*, *Candidatus Flaviluna lacus*, *Geobacter anodireducens*, *Massilia flava*, and *Pedobacter oryzae*, with relative abundances of 4.67%, 2.86%, 2.50%, 2.03%, and 2.00%, respectively. Similar to the most dominant species in spring, the most dominant species, *Rhizobium gallicum*, in the wastewater final effluent in summer was not detected in the effluent samples from the other seasons. However, eight species (*Arcobacter cryaerophilus*, *Candidatus Flaviluna lacus*, *Helicobacter cetorum*, *Limnobacter thiooxidans*, *Massilia flava*, *Pedobacter oryzae*, *Salimicrobium flavidum*, and *Sphingomonas starnbergensis*) of the 13 dominant species were detected in the other seasons, which was different from the dominant species detected in spring.

In autumn, there were 11 dominant species (greater than 1% relative abundance) in the wastewater final effluents. Those 11 species accounted for 31.28% of the relative abundance, which was higher than the dominant species in other seasons. Except for *Noviherbaspirillum aurantiacum*, the 10 other dominant species in the wastewater final effluent in autumn were detected in the three other seasons. The six most dominant species were *Noviherbaspirillum aurantiacum*, *Candidatus Flaviluna lacus*, *Helicobacter cetorum*, *Acidisoma tundrae*, *Thermogutta terrifontis*, and *Nautilia abyssi*, with relative abundances of 6.06%, 5.50%, 5.05%, 2.27%, 2.22%, and 2.15%, respectively. Three species in the wastewater final effluent had relative abundances greater than 5% and were detected in the sample in autumn, while the most dominant species in the other three seasons had relative abundances no more than 5%. The reason for this finding may be that, in autumn, the temperatures drop rapidly, which leads to a decrease in the relative abundance of temperature-sensitive species and an increase in the relative abundance of temperature-insensitive species.

There were 15 dominant species in the wastewater final effluents in winter, which was more than the number of dominant species in the other three seasons. The reason for this finding may be that *Dokdonella immobilis*, the most dominant species in spring, grew at 15–32 °C, with the optimum 25 °C [18], *Rhizobium gallicum*, the most dominant species in summer, grew at 17–37 °C, with the optimum 28 °C [19], *Herbaspirillum aurantiacum*, the most dominant species in autumn, grew at 22 °C as the optimum temperature [20], and it was poorly suited for the growth and reproduction of those species in winter. As a result, those most dominant species exhibited reduced relative abundances, which lead to an increase in the relative abundance of other species. However, no species assumed absolute dominance in the wastewater final effluents in winter, and the relative abundance of each species among those 15 dominant species was not high. Except for *Planctomyces limnophilus* and *Arcobacter cryaerophilus*, which had relative abundances of 3.99% and 3.59%, respectively, all other dominant species had relative abundances of no more than 2.2%.

#### 3.4. Functional Profiling of Bacteria in the Wastewater Final Effluents

According to the relative abundances of the OTUs, functional profiling of microbial communities were predicted using 16S rRNA marker gene sequences [21], and the results are shown in Figure 2. These functional genes presented stable distributions with slight seasonal variations. They were mainly related to cellular processes, environmental information processing, genetic information processing, human diseases, metabolism, and organismal systems. Of these, the genes related to metabolism were the most dominant, with relative abundances of more or less than 50% in each season. In addition, genes related to human diseases had relative abundances of approximately 1% in each season, and the main diseases related to the annotated genes were infectious diseases, neurodegenerative diseases, cancers, metabolic diseases, immune system diseases, and cardiovascular diseases.

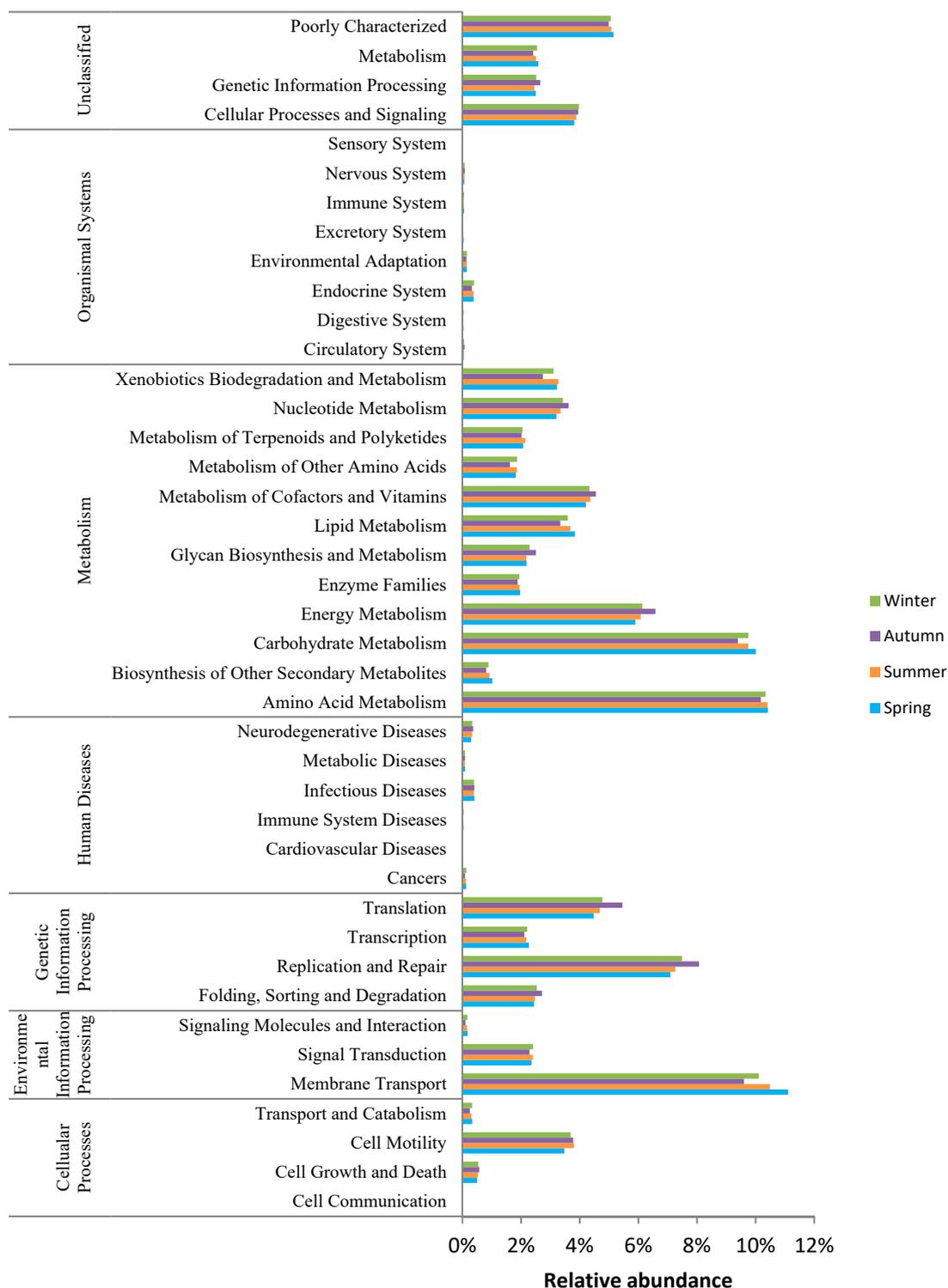


Figure 2. Functional profiling of bacterial in the wastewater final effluents.

#### 4. Discussion

There are numerous bacteria, especially pathogens, in wastewater final effluents. Although many researchers have performed substantial work on the microbiological safety of wastewater final effluents, the diversity and community structure of bacteria, pathogens, and potential pathogens in wastewater final effluents remain unknown. Therefore, this study focused on the community composition and seasonal variability in bacteria in wastewater final effluents.

This study reveals that the bacteria in the wastewater final effluent in the four seasons were mainly dominated by Proteobacteria. Bacteria in phylum Proteobacteria have a strong tolerance to salt, ultraviolet radiation, low and high temperatures, and organic pollutants. Therefore, bacteria in the phylum Proteobacteria are dominant both in the wild and in structured environments, such as soil, atmosphere, rivers, lakes, groundwater, phyllospheres, rhizospheres, and wastewater treatment systems, thus resulting in the highest relative abundance in wastewater final effluents. In addition to the phylum Proteobacteria, the phyla Actinobacteria, Planctomycetes, Bacteroidetes, and Firmicutes also had elevated relative abundances in the wastewater final effluent. Those 5 phyla were consistently dominant in the wastewater final effluent, and the bacterial community was relatively stable at the phylum level; however, the composition of dominant species varied greatly in different seasons.

In spring, the most dominant species was *Dokdonella immobilis*, followed by *Flavobacterium anseonense*. *Dokdonella immobilis* is frequently detected in industrial wastewater treatment systems, and it has been shown to play an important role in the degradation of organic wastes that are noxious or difficult to decompose [22]. Moreover, bacteria in wastewater final effluents mainly originate from the wastewater treatment system, which may lead to *Dokdonella immobilis* having the highest relative abundance in wastewater final effluents. Notably, *Dokdonella immobilis* is resistant to many antibiotics, such as novobiocin, penicillin, ampicillin, and clarithromycin, and possesses highly proficient DNA double-strand break (DSB) repair mechanisms [18], which enable it to accurately reassemble its genome from hundreds of DNA fragments produced by ultraviolet irradiation and  $\gamma$ -rays. These findings imply that ultraviolet disinfection alone for treated wastewater is not sufficient in spring. The second most dominant species was *Flavobacterium anseonense*, and it is a potential pathogen to fish. Therefore, *Flavobacterium anseonense* in treated wastewater should be removed before discharge to safeguard fish in the receiving water.

In summer, there were three dominant species: *Rhizobium gallicum*, *Candidatus Flaviluna lacus*, and *Geobacter anodireducens*. *Rhizobium gallicum* has a variable ability to tolerate salt stress [23], and some strains can grow at NaCl concentrations up to 500 mM. Salinity is one of the major yield-limiting factors that hinders plant growth by positively or negatively affecting a number of physiological processes. Many researchers have investigated the induction of salt tolerance in plants by halotolerant bacteria, and *Rhizobium gallicum* was one of these bacteria [24]. Most of the *Rhizobium* strains were salt tolerant and performed well for the growth promotion of *Vigna radiata* under salt-stress conditions. The halotolerance mechanism of *Rhizobium gallicum* may be related to a reduction in ethylene levels [25], the direct stimulation of rhizobial growth/survival in the soil, and the enlargement of the root system by hormone production for enhanced nutrient uptake. Considering the salt tolerance and high relative abundance of *Rhizobium gallicum*, wastewater final effluents were suggested to be used for irrigation to increase the salt tolerance in crops. *Candidatus Flaviluna lacus* is a new species [26], and it has also been isolated from eutrophic freshwater lakes or ponds [27]. The reason for the increased relative abundance of *Geobacter anodireducens* is also speculated. *Geobacter anodireducens* is a Fe (III)-reducing bacterium. Moreover, the assistants in wastewater treatment plants use flocculants to improve sludge settling properties, which often contain iron or aluminum. It would be wise to use flocculants with iron considering their cost. The iron in the wastewater treatment system provides the electronic acceptor for *Geobacter anodireducens*. This may be one reason for its increased relative abundance in wastewater final effluents.

In autumn, *Candidatus Flaviluna lacus*, *Noviherbaspirillum aurantiacum*, and *Helicobacter cetorum* were the three most dominant species in the wastewater final effluent. *Noviherbaspirillum aurantiacum* is a novel species, and it is detected in glacier forefields [28], volcanic mountain soils [20], biopharmaceuticals [29], and contaminated oil [30]. This indicates that the adaptability of *Noviherbaspirillum aurantiacum* is relatively strong; therefore, it still has a large number of clones in the wastewater final effluent in autumn when the temperature decreases rapidly. *Helicobacter cetorum* and other *Helicobacter* species have been previously detected in the stomach, stool, dental plaque, and gastric fluid of humans and many marine mammals, including dolphins (*Tursiops* spp., *Lagenorhynchus*

*acutus*, *Lagenorhynchus obliquidens*, *Delphinus delphis*), beluga whales (*Delphinapterus leucas*), and seals (*Phoca groenlandica*, *Arctocephalus* spp.) [31]. *Helicobacter* spp. are considered to be responsible for the most common gastric infections in humans and animals worldwide. This indicates that the wastewater treatment plant should pay increasing attention to the abundance of *Helicobacter cetorum* in the wastewater final effluents to protect human and animal health when it is reused or discharged.

In winter, the two most abundant species in the wastewater final effluent were *Planctomyces limnophilus* and *Arcobacter cryaerophilus*. *Planctomyces limnophilus* is a member of the order Planctomycetales, a group of budding microorganisms lacking peptidoglycan and representing a phylogenetically distinct lineage within the domain Bacteria. Interestingly, *Planctomyces limnophilus* is a major player in the global nitrogen and carbon cycles and performs reactions such as the anaerobic oxidation of ammonium with the aid of subcellular organelles known as anammoxosomes [32]. It can be speculated that *Planctomyces limnophilus* plays an important role in the wastewater treatment system. *Arcobacter cryaerophilus* has been associated with gastrointestinal and extragastrointestinal disease and is considered an emerging enteropathogen and potential zoonotic agent. *Arcobacter cryaerophilus* has been found in humans, animals, food, such as poultry, cattle, milk, retail meat, shellfish, and ready-to-eat meals, and water, with frequent detection in products of animal origin. Due to the frequent isolation of *Arcobacter cryaerophilus* from food and water, it has been suggested that these are the most likely transmission routes to humans and animals. Additionally, *Arcobacter cryaerophilus* has been shown to be resistant to common antimicrobials and survive several physical and chemical treatments [33]. Therefore, it is recommended that *Arcobacter cryaerophilus* be set as a part of the microbial standards for wastewater final effluent.

This study also analyzed pathogenic bacteria or opportunistic pathogenic bacteria in the wastewater final effluent. Among the 203 species that shared more than 0.2% relative abundance, 23 species showed identity to pathogenic bacteria or opportunistic pathogenic bacteria in the wastewater final effluent. Detailed information on these 23 pathogenic bacteria or opportunistic pathogenic bacteria is listed in Table 4. Table 4 shows that the relative abundance of pathogenic or opportunistic pathogenic bacteria was the highest in summer (6.58%) and lowest in autumn (2.82%). Interestingly, the relative abundance of pathogenic bacteria or opportunistic pathogenic bacteria in winter reached 5.62%, which was far higher than that in spring and autumn.

These 23 pathogenic bacteria or opportunistic pathogenic bacteria in the wastewater final effluent belong to 14 genera, of which *Arcobacter* spp., *Legionella* spp., and *Mycobacterium* spp. were dominant. *Arcobacter* spp. have attracted increasing attention because they are considered emergent enteropathogens and potential zoonotic agents [34]. In recent years, *Arcobacter* spp. have been proven to be related to diarrhea, bacteremia, endocarditis, and peritonitis in humans [35] and implicated in abortions, mastitis, and gastrointestinal disorders in animals [36]. Thirty-five years after the discovery of *Legionella pneumophila*, *Legionella* spp. have grown in number considerably, as the genus now contains 59 species. Some *Legionella* spp., e.g., *Legionella pneumophila*, cause legionellosis [37]. *Legionella pneumophila* was recognized as a human pathogen during an outbreak of severe pneumonia in 1976 in Philadelphia, PA [38]. Since then, outbreaks occur annually, particularly in the industrialized world, and legionellosis remains a current health problem. *Legionella pneumophila* is the primary cause of Legionnaires' disease worldwide, as it causes more than 90% of the diagnosed cases. *Legionella longbeachae* is the second cause of legionellosis, responsible for 3.9% of cases [39]. Three species of *Legionella* were detected in this study, of which *Legionella fallonii* and *Legionella longbeachae* can cause infection in humans. *Mycobacterium* spp. is composed of a large number of clinically important pathogens, both obligate and opportunistic [40]. The impact of mycobacteria on human morbidity and mortality is hard to overstate. The incidence of disease due to nontuberculous mycobacteria has been steadily increasing worldwide [41] and has likely far surpassed the incidence of disease due to tuberculosis (TB) [42]. In addition, skin and tissue infections caused by *Mycobacterium* spp. constitute a significant animal health problem for veterinarians involved in the care of livestock and companion animals [43]. In addition to *Arcobacter* spp., *Legionella* spp., and *Mycobacterium* spp., all other

pathogenic or opportunistic pathogenic bacteria in the wastewater final effluent were mainly associated with dermatosis, enteropathies (such as diarrhea, enterogastritis, and gastrohelcoma), septicemia, and pneumonia.

**Table 4.** The pathogenic bacteria or opportunistic pathogenic bacteria existed in wastewater final effluents.

Serial Number	Similar Species of Geneticrelationship	Spring (%)	Summer (%)	Autumn (%)	Winter (%)	Total	Disease Category
1	<i>Aeromonas hydrophila</i>	—	0.61	—	—	0.61	dermatosis/diarrhea [44]
2	<i>Arcobacter cryaerophilus</i>	—	1.76	0.67	3.59	6.02	diarrhea [45]
3	<i>Arcobacter defluvii</i>	—	0.26	—	—	0.26	enteropatia [46]
4	<i>Arcobacter skirrowii</i>	0.15	0.24	—	—	0.39	enterogastritis [47]
5	<i>Arcobacter trophiarum</i>	—	—	—	0.35	0.35	septicemia [48]
6	<i>Arcobacter venerupis</i>	0.32	—	0.87	—	1.19	enteropatia [49]
7	<i>Campylobacter curvus</i>	—	—	—	0.53	0.53	enterogastritis [50]
8	<i>Chlamydophila pneumoniae</i>	0.21	—	—	—	0.21	pneumonia
9	<i>Comamonas kerstersii</i>	—	0.73	—	—	0.73	diarrhea [51]
10	<i>Desulfovibrio legallii</i>	—	0.41	—	—	0.41	Dermatosis [52]
11	<i>Helicobacter brantae</i>	—	—	0.39	—	0.39	Gastrohelcoma [53]
12	<i>Helicobacter mastomyrinus</i>	0.24	—	—	—	0.24	Gastrohelcoma [53]
13	<i>Legionella fallonii</i>	0.98	0.55	—	—	1.53	Legionellosis [54]
14	<i>Legionella longbeachae</i>	0.63	0.44	—	—	1.07	Dermatosis [55]
15	<i>Legionella oakridgensis</i>	—	0.7	—	—	0.7	fever and dyspnea [56]
16	<i>Mycobacterium mageritense</i>	0.29	0.2	0.89	—	1.38	Dermatosis [57]
17	<i>Mycoplasma cynos</i>	—	—	—	1.15	1.15	pneumonia [58]
18	<i>Mycoplasma edwardii</i>	—	0.22	—	—	0.22	pneumonia/septicemia [59]
19	<i>Pseudomonas putida</i>	0.42	—	—	—	0.42	urinary infections/dermatosis
20	<i>Prevotella copri</i>	0.25	—	—	—	0.25	pneumonia
21	<i>Rickettsia sibirica</i>	—	0.24	—	—	0.24	Dermatosis [60]
22	<i>Staphylococcus Equorum</i>	0.55	—	—	—	0.55	Dermatosis/septicemia
23	<i>Tatlockiamic dadei</i>	—	0.22	—	—	0.22	pittsburgh pneumonia [61]
	total	4.04	6.58	2.82	5.62		

Note: “—” representatives this species is not detected under the experimental conditions.

Overall, there are more pathogenic microorganisms in wastewater final effluents than expected, and this study provides valuable references for the prevention of infection diseases from wastewater final effluents and reused water.

## 5. Conclusions

This study investigated the diversity, composition, and spatiotemporal dynamics of the microbial community and pathogens in wastewater final effluents. Proteobacteria was the most dominant phylum, with a relative abundance of more than 40% in each season, followed by Actinobacteria, Planctomycetes, Bacteroidetes, and Firmicutes. However, the bacterial diversity and community composition of the bacteria in the wastewater final effluent had significant seasonal variations. The main factors influencing microbial communities in wastewater effluent may be temperature, salinity, disinfection methods, and flocculants. The analysis of the pathogenic microorganisms or opportunistic pathogenic microorganisms in the wastewater final effluents revealed that the relative abundance of pathogenic microorganisms or opportunistic pathogenic microorganisms in summer was the highest. The main pathogenic microorganisms or opportunistic pathogenic microorganisms were *Arcobacter* spp., *Legionella* spp., and *Mycobacterium* spp. These pathogenic species are mainly associated with dermatosis, enteropathies (such as diarrhea, enterogastritis, and gastrohelcoma), septicemia, and pneumonia. Therefore, city disease control departments may need to pay additional attention to these pathogenic microorganisms or opportunistic pathogenic microorganisms. Notably, some pathogenic species are tolerant and resistant to ultraviolet, which indicates that wastewater treatment plants should pay increasing attention to the disinfection rate and disinfection method of wastewater final effluents.

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