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Application of Tea Polyphenols and Their Effects on Ultrafiltration Effluent Disinfection and Microbial Risk

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Abstract: To reduce the risk of by-products from traditional disinfection technology and to ensure safe water quality, tea polyphenols (TP) were used as disinfectants after the ultrafiltration (UF) process. The disinfectant effect of TP was tested on the total number of bacteria and changes in bacterial community structure, and pathogen virulence factors were detected by Illumina's high-throughput sequencing technology. The results showed that the recommended dosage of TP for water treatment after UF was 5 mg/L, which can effectively inhibit bacterial growth and maintain the disinfectant effect for up to 48 h later. In the disinfection process, the degradation of the tea polyphenols concentration was fitted to the equation of the second order reaction kinetics. The lower the initial concentration and the higher the reaction temperature, the faster the TP decay. The metagenomic analysis of the microorganisms indicated that disinfection with tea polyphenols reduced the diversity of microorganisms in the water and altered the structure of the bacterial community. The existence of tea polyphenols also significantly inhibited the growth of potential common Gram-negative pathogens, especially Mycobacterium. TP disinfectant can also reduce the diversity and abundance of pathogenic bacterial virulence factors and improve biological safety in drinking water.

Keywords: tea polyphenols; disinfection; pathogenic bacteria; risks; virulence factors

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1. Introduction

As a necessity of human life, the quality of drinking water affects human health. Therefore, disinfection of drinking water has attracted great attention at home and abroad. Currently, chlorine, chlorine dioxide, ultraviolet and ozone are commonly used as drinking water disinfectants. Its applications have the following characteristics: disinfection with chlorine contains an unpleasant taste and smell [1] and produces toxic by-products [2]. Disinfection with chlorine dioxide has the advantages of small-dosage, fast-action and high-sterilization rate, but its cost is high, the operation is complex in the process of preparation and use, leakage and explosion are easy to occur [3], and secondary carcinogens will be produced in the disinfection process [4]. Although ultraviolet disinfection has the advantages of a fast sterilization effect, no chemical addition, no formation of disinfection by-products, and cost savings [5], it does not have continuous sterilization capability, resulting in secondary pollution of drinking water in the process of transport pipes [6]. Ozone disinfection is highly efficient and can quickly kill bacteria and viruses in water without causing secondary pollution [7]. However, ozone lacks sustainable sterilization capability and has high cost, which is limited by location. Facing the main needs of the country in strengthening the guarantee of safe drinking water and improving water quality, it is urgent to study green and efficient treatment of drinking water. As a renewable green

resource, plant polyphenols have caught attention. Plant polyphenols are the secondary metabolites of plants. They are rich in reserves and derive from green sources. A large number of scientific studies have shown that plant polyphenols have antioxidant and other biological activities and physiological functions. The broad-spectrum antibacterial activity of plant polyphenols (including inhibition of filamentous fungi, bacteria and viruses) is the most important embodiment of its biological activity. Through the study of different plant polyphenol extracts, it is found that a variety of plant polyphenols can inhibit microbial growth. At present, the Bacteriostasis of plant polyphenols is mostly used in food, medicine, and other industries, and the research in the field of drinking water is still in the stage of laboratory exploration. The total number of bacteria in raw water is the key factor affecting the dosage of plant polyphenol disinfection, the time to reach the standard, and the disinfection duration. The higher the total number of bacteria in raw water, the greater the dosage of plant polyphenol required and the longer the time to reach the standard. For emergency water using natural water as a direct source, the contact time of plant polyphenols as disinfectant is long. When the dosage is 0.3 g/L, contact with water for more than 8 h can make the total number of bacteria reach the drinking standard. For the general pipe network, the water disinfected with plant polyphenols can maintain the total number of bacteria within 3 days without exceeding the standard, and there is no need to supplement chlorine [8]. Based on the extensive antibacterial activity of plant polyphenols, Cuimin Feng [8] and other researchers respectively added three types of plant polyphenols to the filter effluent of waterworks. The test showed that tea polyphenols (TP) had the best disinfection effect and the best disinfection sustainability.

As a type of plant polyphenols, tea polyphenols derive from green environmental protection, have mild performance, are non-toxic and harmless to the human body, and have a health care effect. It is a green and healthy natural bacteriostatic agent extracted from tea. It can have a good disinfection effect and disinfection durability when applied to drinking water disinfection treatment [9]. Therefore, it can be used for emergency and conventional disinfection of drinking water, showing durability and promising results [9–12]. Cuimin Feng et al. [9] and Wanxia Fu et al. [10] found that for emergency drinking water in rivers, the tea polyphenols dosage was 300 mg/L for 1 h; for emergency drinking water in reservoirs, the dosage was reduced to 50 mg/L for 30 min, which can meet the requirements [13]. The application of tea polyphenols in conventional water treatment plants as auxiliary disinfectants, replacing the use of chlorine together with decontamination by UV or ozone, may reduce the by-products generated from these treatments and improve the chemical safety of drinking water [13–15].

Currently, ultrafiltration membrane technology has been gradually applied in the renovation and expansion projects of water treatment plants [16–18]. This process achieves turbidity removal rates of greater than 95%, with the effluent turbidity being stable and basically unaffected by the turbidity of the influent water [16–19]. However, due to the individual growth and extrusion deformation of microorganisms in the ultrafiltration process, biological leakage or inevitable post membrane pollution will occur [20–22], and it is necessary to combine disinfection technology to ensure the biological safety of drinking water. Taking advantage of the ultrafiltration process, tea polyphenols can be considered instead of chlorine or chloride disinfection to further enhance the biosafety of the effluent and reduce the risk of disinfection by-products.

One way to assess the effect of disinfection is to estimate the total number of colonies. However, this assessment does not fully reflect the number of all microorganisms in the water. According to the research, the number of microorganisms that can generate colonies in natural water is only about 1%, which means that if the total number of colonies per ml of water is 100 UFC, there can be around 10^4 microorganisms present [16]. Metagenomics establishes a macrogenomic library by extracting all the genetic material of microorganisms from water samples to perform multidimensional assessments of species, abundance, and metabolism of microorganisms. This technique has advantages such as good coverage and detection of microorganisms, including at low concentrations [23],

which is of great importance for the detection of pathogens [24]. Based on the metagenomic technique, we can also obtain information about the pathogen, its composition, function, pathogenic mechanism and information about the genome of virulence factors, comparing it with the database in order to evaluate the control effect of disinfectants on hazardous microorganisms in water.

The conventional treatment process of waterworks effluent after filter was treated with an ultrafiltration membrane 0.01 μm (100 kDa), used as raw water for disinfection tests, and the effect of the disinfection of the ultrafiltration effluent was studied to control the length of microbial regeneration. Combined with the microbial community structure and pathogen risk analysis, the biosafety of disinfection with tea polyphenols in the effluent from the ultrafiltration process of hydroelectric plants was explored in order to establish a theoretical basis for improving the quality and safety of drinking water.

2. Experimental Materials and Methods

2.1. Experimental Materials

Tea polyphenols were purchased from Anhui Red Star Pharmaceutical Co., Ltd. as green tea extract with a purity of more than 98.0% (AR98.0%).

The test raw water was taken from a conventional treatment process water plant after the filtration tank and filtered through a 0.01 μm (100 kDa) ultrafiltration membrane. Membrane filtration uses water passing through the membrane to remove microbial particles such as bacteria and viruses, which are retained on the surface or inside the membrane. Viruses are the smallest of the disease-causing microorganisms in water, ranging in size from 0.02 to 0.45 μm . Pore size of 0.01 μm drinking water ultrafiltration technology can be used to effectively remove pathogenic microorganisms, including viruses. The raw water quality test is shown in Table 1.

Table 1. Raw water quality.

Parameter	Values	Instruments	Methods
Chroma (degree)	1 ± 1	PFXi-995 High Precision Automatic Colorimeter	
Turbidity (NTU)	0.1	HACH-2100AN Turbidity Meter	
SS (mg/L)	0.16		
UV ₂₅₄ (1/cm)	0.013 ± 0.002	DR6000 (HACH Instrument Corporation)	Direct Reading
TOC (mg/L)	0.90 ± 0.07	Shimadzu TOC-L Organic Carbon Analyzer	High Temperature Catalytic Combustion
NO ₃ -N (mg/L)	0.7 ± 0.2	DR6000 UV Spectrophotometers	Ultraviolet Spectrophotometry
NH ₄ ⁺ -N (mg/L)	0.06 ± 0.01	DR6000 UV Spectrophotometers	Salicylic acid method
Total phosphorus (mg/L)	0.69 ± 0.04	DR6000 UV Spectrophotometers	Digestive-Ascorbic Acid Method
Total number of bacteria (CFU/mL)	2 ± 2		Plate Counting
Total Coliforms (CFU/100 mL)	0		Multi-tubular fermentation
Escherichia coli (CFU/100 mL)	0		
pH	7.7 ± 0.2	FiveGo-Single Channel Portable pH Meter	
Dissolved oxygen (mg/L)	8.54 – 9.38		Iodometric method

The flat ultrafiltration membrane filtration test device used in the test is shown in Figure 1. The device consists of two parts: nitrogen cylinder and ultrafiltration cup (Merck group, UFSC05001, 50 mL). The transmembrane pressure required by the ultrafiltration device is provided by the nitrogen cylinder, and the constant transmembrane pressure difference is 0.10 MPa.

Figure 1. The device UF membrane filtration.

The ultrafiltration membrane used in the test was purchased from Merck & Co Inc. the material was regenerated cellulose (RC), the operation mode was immersion, the cutting molecular weight was 100 kDa, the filtration area was 41.8 cm² and the diameter was 76 mm.

2.2. Experimental Design

- (1) Disinfection test. Tea polyphenols were added to raw water at different gradient concentrations from 0 to 10.0 mg/L in a unit of 2.5 mg/L. The water temperature was adjusted to 10 °C. Samples were detected every 6 h for different times (0 to 48 h of treatment) to investigate the ideal dosage of tea polyphenols;
- (2) Factors that attenuate the tea polyphenols dose efficiency. Initial dosage amounts of tea polyphenols were 5.0, 7.5, and 10.0 mg/L. Water temperatures were adjusted to 4, 10, and 20 °C. The concentration of tea polyphenols was measured at 0, 12, 24, 36, and 48 h after contact of tea polyphenols with raw water, and the effects of relevant factors in the attenuation of tea polyphenols were investigated;
- (3) Microbial community structure and risk microorganism analysis. The best dosage of tea polyphenols was used, and changes in the microbial community structure in the water before and after disinfection were compared. Changes in species and abundance of the pathogenic bacteria virulence factor were analyzed to assess the biosafety of disinfection using tea polyphenols. The raw water sample number was Raw (Table 1), and the disinfected water sample was 5TP with the best dosage of tea polyphenols.

2.3. Data Detection and Processing

Total number of colonies: Plate counting method. A volume of 1 mL tea polyphenols disinfection water samples was evenly distributed on the agar of the prepared petri dish with cover and cultured at 30–37 °C for 48 h. Then, the total number of bacteria in each mL of original water sample was counted by the number of bacterial colonies on the plate.

Mass concentration of tea polyphenols: Ferrous tartrate method [14]. The principle is that ferrous tartrate will react with tea polyphenols to form blue purple complex, and the content of tea polyphenols is directly proportional to the color depth. The content of tea polyphenols can be determined by spectrophotometry.

Microbial community structure and bioinformatics analysis: Macro-genome sequencing commissioned by Bioengineering Co. (Shanghai, China).

Pretreatment of sequencing water samples: 100 L of water samples disinfected with the ideal dosage of tea polyphenols and 100 L of raw water were left to stand for 48 h under the same conditions. Subsequently, the microorganisms in the water samples were retained by a suction filtration device with a 0.01 μm pore filter membrane. Genetic material extracted from water enriched microorganisms, before and after with tea polyphenols disinfection, was analyzed by Illumina high-throughput sequencing technology.

Database comparison: Protein sequences from the gene pool were compared to the NR (non-redundant protein sequences, NCBI) database for blastp homology using DIAMOND obtaining information from homologous species. Screening conditions: E value $< 1 \times 10^{-5}$, score > 60 ; the protein sequences of the gene pool were compared with the setA part in the VFDB (pathogenic bacteria virulence factors) database for BLAST search to obtain information on pathogenic species, composition, function and abundance of virulence factors. Screening criteria: Blast Identity > 20 , Blast eValue $< 1 \times 10^{-5.2}$.

3. Results and Discussion

3.1. Disinfection Effect of Tea Polyphenols

The total count of raw water colonies was 0 CFU/mL, and the microbial growth condition with time under different dosages of tea polyphenols is shown in Figure 2.

Figure 2. Inhibitory effect of tea polyphenols on bacterial growth at 10 °C.

As seen in Figure 2, tea polyphenols have effective antibacterial properties. The initial dosage of tea polyphenols was 2.5 mg/L; after 12 h, this concentration decreased to 2.11 mg/L, which could control the number of colonies to 3 ± 2 CFU/mL. Thus, it significantly extended the period of bacterial growth retardation to 18 h, such that after that time, the bacteria grew rapidly. At that time, the concentration of polyphenols in tea was 1.56 mg/L. At 36 h, the total number of colonies was 103 ± 6 CFU/mL, not meeting the requirements of the drinking water quality standards. When the tea polyphenol dosage was 5.0, 7.5, and 10.0 mg/L, the total number of colonies did not grow. After 24 h, the total number of colonies showed a continuous downward trend, indicating that 5.0 mg/L can be used as the recommended dosage for disinfection with tea polyphenols, with the total number of colonies in the water being less than 10 CFU/mL in 48 h.

The justification for this behavior may be due to the fact that many compounds present in tea, such as hydroxyl-containing phenolic groups, can combine hydrogen with amino or carboxyl groups in protein molecules, and their hydrophobic benzene ring structure can also combine hydrophobically with protein. This multipoint bond between polyphenol and tea protein is one of the important reasons for its bacteriostasis. The antibacterial effect mainly results from the combination of tea polyphenols with proteins from the environment, which affects the use of proteins by microorganisms; In addition, tea polyphenols can also combine with intracellular proteins. Tea polyphenols act with cellular enzyme proteins to reduce enzyme activity. Tea polyphenols combine with membrane proteins to affect the absorption of microbial nutrients, reduce bacterial phosphorus metabolism, and affect the metabolism of bacterial life. There are several effects that inhibit bacterial growth and reproduction [25]. The treatment of ultrafiltration effluent with tea polyphenols as a disinfectant has high efficiency and sustainability.

3.2. Decline of Tea Polyphenols and Their Influencing Factors

(1) The effect of initial dosage on the decomposition of tea polyphenols.

When the water temperature reaches 4, 10, and 20 °C, the initial dosage of tea polyphenols is respectively 5.0, 7.5, and 10.0 mg/L, after processing zero, primary or pseudo-primary level, there is the analysis of fit of secondary and tertiary reaction kinetic equation. The decline of tea polyphenols in the disinfection process at a dosage of 5–10 mg/L agrees with the kinetic equation of the second order reaction. The R^2 values of regression equation of tea polyphenol concentration were greater than 0.93. The higher the initial dosage, the slower the attenuation of the tea polyphenols. The same results were observed by Yang Tongtong [13] and Guo Ziyu [14], with the order of the reaction increasing by one order compared to the dose of 10–300 mg/L [13,14,26]. It shows that when the dose of tea polyphenols is 5.0–10.0 mg/L, the effect of the initial concentration on its attenuation is more significant than at the concentration of 10–300 mg/L, as shown in Table 2.

Table 2. Reaction rate equation of tea polyphenol solution in disinfection process.

TP Dose/(mg/L)	Water Temperature/(°C)	Regression Equation	R^2	$k/(L \cdot (mg \cdot h)^{-1})$	$t_{1/2}/(h)$	Concentration of TP after 48 h (mg/L)
5.0	4	$y = 0.00240x$	0.9934	2.400×10^{-3}	76.53	3.22
	10	$y = 0.00460x$	0.9425	4.600×10^{-3}	39.93	2.11
	20	$y = 0.00720x$	0.9387	7.200×10^{-3}	25.51	1.56
7.5	4	$y = 0.00064x$	0.9688	0.643×10^{-3}	202.72	6.00
	10	$y = 0.00128x$	0.9755	1.280×10^{-3}	101.90	4.89
	20	$y = 0.00265x$	0.9895	2.650×10^{-3}	49.22	3.78
10.0	4	$y = 0.00033x$	0.9712	0.327×10^{-3}	292.92	8.78
	10	$y = 0.00062x$	0.9828	0.615×10^{-3}	155.63	7.67
	20	$y = 0.00119x$	0.9658	1.190×10^{-3}	80.46	6.00

(2) The effect of temperature on the decomposition of tea polyphenols.

Because the chemical structure of tea polyphenols is rich in phenolic hydroxyl groups, in the enzymatic oxidation polymerization reaction, these compounds produce o-quinone derivatives under the catalysis of tea polyphenols oxidase, and further polymerize to form dimers and polymers; under nonenzymatic conditions, these compounds can still be oxidized and polymerized under the catalysis of trace metal ions to form dimers and polymers and reactive oxygen radicals [27]. Temperature can affect the oxidation polymerization reaction in the disinfection process through tea polyphenols, which causes them to produce different degrees of attenuation [28]. Studies have shown that the higher the temperature, the faster the tea polyphenols decompose. According to Table 2, the decay rate of tea polyphenols solution at 20 °C is higher than that at 10 °C, and the lowest at 4 °C. At a dosage of 5.0 mg/L, the reaction rate constant k of 10 °C tea polyphenols is

4.6×10^{-3} L/(mg·h) with a half-life of 39.93 h. A concentration of 2.11 mg/L after 48 h, which is still higher than 2.0 mg/L, can inhibit bacterial growth and guarantee the total number of colonies at a lower level. While at 20 °C, the reaction rate constant k increases to 7.2×10^{-3} L/(mg·h), and the half-life is reduced to 25.51 h. The concentration of polyphenols in tea is less than 2.0 mg/L after 48 h, which may pose a threat to the biosafety of water. Therefore, it is advisable to increase the dosage of tea polyphenols in summer and maintain the dosage of tea polyphenols in water to ensure the inhibiting effect on microbial growth.

3.3. Analysis of the Structure of the Microbial Community in Water after Disinfection with Tea Polyphenols

Metagenome sequencing yielded a total of 97,166,304 reads. A total of 377,730 bp of gene number was obtained after data processing by Trimmomatic, splicing assembly by IDBA_UD software and ORF prediction by Prodigal. The Shannon and Simpson indices of the water samples are shown in Table 3. The Shannon and Simpson index of tea polyphenols from the water sample disinfected at 5TP (after 48 h of reaction between 5 mg/L of tea polyphenols tea and raw water) were lower than raw water. Thus, it demonstrates that the disinfection process with tea polyphenols will reduce the diversity of microorganisms in the water. This is consistent with the trend toward the effect of chlorine-based disinfectants on the microbial diversity of drinking water [29]. For example, chlorine and chloramines in the drinking water disinfection process reduce the diversity of microorganisms in the system [30–32].

Table 3. Community diversity statistics.

Sample	Number of Genes	Total Length of All Genes/(bp)	Average Gene Length/(bp)	Shannon Index	Simpson Index
Raw	309,360	199,399,269	644.55	11.13	1×10^{-4}
5TP	68,370	52,644,429	769.99	9.87	4×10^{-5}

Bacteria, archaea, viruses and fungi could be detected in Raw and 5TP, as shown in Table 4. Most sequences are related to the bacterial community, accounting for more than 98% of the total annotated protein sequences. Therefore, the structure of the bacterial community is the focus of the analysis.

Table 4. Proportion of microbial species at the kingdom level.

Pathogenic Microbes	Raw/(%)	5TP/(%)
Bacteria	98.7478	99.4904
Archaea	0.0004	0.0017
Viruses	0.0020	0.0017
Fungi	0.0070	0.0014
Else	1.2428	0.5048

At the phylum level, the flora in raw water includes *Proteobacteria*, *Bacteroidetes* and *Actinobacteria*, with percentages of 86.03%, 4.73% and 3.99% respectively, as shown in Figure 3a. This result is consistent with the research by Zhang [33], who studied the main bacterial groups in effluent water after membrane filtration, albeit with slightly different distribution ratios. *Proteobacteria* is the absolute dominant group in water after tap water treatment. After disinfection with tea polyphenols, the relative abundance of *Proteobacteria* increases from 86.03% to 96.63%, and that of *Bacteroidetes* and *Actinobacteria* decreases to less than 0.2%, as shown in Figure 3a.

Figure 3. Histogram of relative abundance at the level of phylum (a) and class classification (b).

At the class level, as shown in Figure 3b, the groups of bacteria in raw water are *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Flavobacteria* and *Actinobacteria*, representing 32.35%, 28.32%, 25.10%, 4.54% and 3.99%, respectively. The dominant bacterial groups consist of *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria*. After disinfection with tea polyphenols, the proportion of *Alphaproteobacteria* and *Betaproteobacteria* increased to 49.74% and 37.91%, respectively, while the proportion of *Gammaproteobacteria* decreased to 8.76%. The relative abundance of *Flavobacteria* and *Actinobacteria* decreased to 0.03% and 0.15%, respectively.

At the genus classification level, the heatmap of 39 strains with high relative abundance is shown in Figure 4. The dominant communities in raw water are *Sphingobium*, *Acinetobacter*, *Pseudomonas*, *Janthinobacterium*, *Methylophilus*, *Flavobacterium* and *Mycobacterium*. After disinfection with tea polyphenols, the dominant population decreased to *Sphingobium*, *Janthinobacterium* and *Pseudomonas*.

Figure 4. Heatmap of relative abundance at the genus level.

After disinfection with tea polyphenols, the relative abundance of *Sphingobium* increased from 19.60% to 38.84%, which belongs to an important bacterial group that increases the relative abundance of *Alphaproteobacteria*. *Sphingobium* can grow in a nutrient-poor environment due to its extensive and efficient metabolic capacity and multifunctional physiological characteristics [34,35]; thus, it is dominant after disinfection with tea polyphenols.

After disinfection with tea polyphenols, the relative abundance of *Janthinobacterium* increases from 5.69% to 18.52%. Certain *Janthinobacterium* bacteria can produce a secondary metabolite, such as *Violacein* [36], which has been widely shown to have antibacterial and antiviral activity [37,38]. This suggests that tea polyphenols promote the growth of colonies that can produce violacein, which exerts antibacterial and bactericidal effects along with secondary metabolites having antibacterial and antiviral activities. *Janthinobacterium* belong to the phylum *Betaproteobacteria* and are representative of the groups that increase the relative abundance of the phylum *Betaproteobacteria*. Conversely, the genus *Methylophilus*, which also belongs to *Betaproteobacteria*, is sensitive to disinfection with tea polyphenols, and its relative abundance decreased from 5.07% to 2.14%. After disinfection with tea polyphenols, the relative abundance of *Acinetobacter* and *Pseudomonas* belonging to *Gammaaproteobacteria*, as well as *Flavobacterium* to *Flavobacteriia* and *Mycobacterium* to *Actinobacteria*, decreased significantly. Among them, *Pseudomonas* represents a relatively high percentage. Most of these bacteria were Gram-negative cocci or bacilli, common in water, and all were conditioned pathogenic bacteria [15,39]. *Acinetobacter* and *Mycobacterium* are bacterial pathogens listed in the WHO Guidelines for *Drinking Water Quality*, and *Mycobacterium* has strong resistance to disinfectants due to its cell wall containing a large amount of lipids and forming capsules [15]. The significant decrease in the relative abundance of these groups of bacteria indicates that disinfection with tea polyphenols inhibited the growth of common Gram-negative pathogenic bacteria and had an obvious controlling effect on *Mycobacterium* with strong resistance to disinfection.

3.4. Analysis of Risk Microorganisms and Their Virulence Factors through Disinfection with Tea Polyphenols

The risk of diseases in drinking water is diverse, among which pathogenic bacteria are easily the cause of infectious diseases, which is the most common health risk in drinking water [40]. Its gene products that can cause colonization, reproduction and disease in the host environment are called virulence factors (VFS), which is the key to the virulence of pathogenic bacteria, in addition to their survival in the host and host disease [41].

The study uses the BLAST similarity comparison search method to build a BLAST analysis of the MvirDB protein database. This analysis on the MvirDB protein database showed that 247 virulence factors were detected in raw water and 191 were detected in 5TP. The diversity of virulence factors detected was reduced by 56, as shown in Table 5. After disinfection with tea polyphenols, the absolute abundance of various virulence factors decreased, and the total absolute abundance decreased by 51%. As can be seen in Table 5, the main virulence factors carried by pathogenic bacteria changed little after tea polyphenols disinfection, but the abundance of virulence factor genes (readings) decreased significantly, ranging from 32% to 67%. Among them, the absolute abundance of virulence factors closely related to the iron uptake system and the adhesion of pathogenic bacteria decreased the most, mainly, the virulence factor Pyoverdine and Type IV pili (*Pseudomonas aeruginosa* PAO1 and *Klebsiella pneumoniae* subsp. *Pneumoniae* NTUH-K2044). They play an important regulatory role in the release of virulence factors, forming biofilms and showing good adhesion [42]. The absolute abundance of the virulence factor CdpA [43] (*Burkholderia pseudomallei* K96243), which is closely related to the signal transducer system, was also significantly reduced. It regulates intracellular levels of C-DI-GMP (cyclic dimeric guanosine monophosphate) and affects a variety of phenotypes, such as flagella synthesis, bacterial movement, exopolysaccharide production, interbacterial aggregation, biofilm formation, cytotoxicity and host cell invasion [44].

Table 5. Gene abundance and relative abundance of virulence factors of major pathogens.

Main Pathogenic Bacteria	Virulence Factors	Absolute Abundance of Virulence Factor Genes/ (Reads)	
		Raw	5TP
<i>Pseudomonas aeruginosa</i> PAO1	Alginate, Alkaline protease, Deoxyhexose linking sugar, Flagella, HSI-I, LasB, LPS, Phenazines biosynthesis, Pyochelin, Pyocyanin, pyoverdine, Rhamnolipid, TTSS, Type IV pili, xcp secretion system	85,597	40,839
<i>Burkholderia pseudomallei</i> K96243	BimA, BoaA, BoaB, Bsa T3SS, Capsule I, CdpA, Flagella, Quorum-sensing, T6SS-1, Type IV pili	35,117	21,247
<i>Mycobacterium tuberculosis</i> H37Rv	Antigen 85, Erp, ESX-1, ESX-3, ESX-5, FadD33, HbhA, IdeR, Isocitrate lyase, LipF, MgtC, Mycobactin, PhoP/R, RegX3, RelA	26,086	14,371
<i>Neisseria meningitidis</i> MC58	Capsule, FarAB, FbpABC, HmbR, IgA1 protease, KatA, Lbp, MntABC, MsrAB, MtrCDE, Porin, RecN, Type IV pili	32,460	16,823
<i>Legionella pneumophila</i> subsp. <i>pneumophila</i> str. Philadelphia 1	T2SS, CcmC, Cytochrome c maturation (ccm) locus, Dot/Icm, enh loci, FeoAB, Flagella, Hsp60, IraAB, Iron acquisition/assimilation locus, KatAB, Legiobactin, LigA, lsp, Mip, Pht, Polar flagella, RtxA, SodB, SodC, type IV pili	22,205	7668
<i>Haemophilus influenzae</i> Rd KW20	Hgp, HitABC, HxuABC, IgA1 protease, LOS, P5 protein	18,942	6715
<i>Escherichia coli</i> CFT073	Aerobactin, Chu, Enterobactin, F1C fimbriae, Hemolysin, IroN, P fimbriae, Salmochelin, TcpC, Type 1 fimbriae	14,318	5076
<i>Aeromonas hydrophila</i> subsp. <i>hydrophila</i> ATCC 7966	Amonabactin, exe, Flp type IV pili, Hemolysin III, Hemolysin, HlyA, Mannose-sensitive hemagglutinin (Msh) pilus, type IV pili, Polar flagella, T6SS, Tap type IV pili, RTX, TH, Type I fimbriae, VgrG1	18,166	10,228
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium str. LT2	Agf, Bcf, AGF, Lpf, MgtBC, MisL, Rck, ShdA, SodCI, Spv, TTSS (SPI-1 encode), TTSS (SPI-2 encode), Type 1 fimbriae	9166	3014
<i>Bordetella pertussis</i> Tohama I	Brk, Cya, FHA, Fimbriae, LPS, Pertactin, Ptx, TcfA, TTSS	10,806	5145
<i>Streptococcus agalactiae</i> 2603V/R	Beta-hemolysin/cytolysin, Capsule, Lmb	11,946	6748
<i>Acinetobacter baumannii</i> ACICU	Bap, BfmRS, Csu fimbriae, Phospholipase C, PNAG, Quorum sensing	10,147	5165
<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> NTUH-K2044	Salmochelin, type 3 fimbriae (biofilm formation), yersiniabactin	14,992	10,062
<i>Campylobacter jejuni</i> subsp. <i>jejuni</i> NCTC 11168	CadF, Capsule, Capsule biosynthesis and transport, Flagella, LOS, PEB1, Pse5Ac7Ac, Pse5Ac7Am, Pse8OAc, Pse5Am7AcGlnAc	9462	3953
else	-	55,039	26,638

Virulence factors are important pathogenic genes encoded and transported by the isle of pathogenicity (PAI), which can adapt to a specific growth environment and meet the needs of life through horizontal microbial transfer along with PAI [45]. Huang [46] found that the diversity and abundance of bacterial virulence factors increased after disinfection with chlorine. However, the disinfection with tea polyphenols is different. After disinfection with tea polyphenols, the abundance of genes for major virulence factors significantly decreases, which is also the reason why tea polyphenols disinfection is selective, i.e., tea polyphenols can inhibit the growth of harmful bacteria common to the human body and promote the growth of beneficial bacteria [25].

4. Conclusions

This paper mainly explores the disinfection effect of tea polyphenols as a disinfectant in the treatment of ultrafiltration effluent, and the characteristics and changes of microbial community structure and community function in the water after disinfection. The disinfection effect of tea polyphenols in ultrafiltration effluent was discussed from the control of microbial regrowth and disinfectant attenuation. At the same time, through the study of macrogenomics of microorganisms in water after tea polyphenol disinfection, the microbial community structure, community functional characteristics, and changes after disinfection were discussed, and the principle of tea polyphenol disinfection was revealed. The conclusions are as follows:

- (1) The main function of the disinfection with tea polyphenols in the treatment of ultrafiltration effluent is to inhibit bacterial growth. The recommended dosage is 5.0 mg/L, which can meet the total number of colonies requirements in the drinking water quality standards within 48 h, and meet the microbial control needs of urban pipe networks of different sizes;
- (2) When the dosage of tea polyphenols is 5–10 mg/L, the attenuation of tea polyphenols is in accordance with the kinetic equation of the second order reaction. The lower the initial concentration and the higher the reaction temperature, the faster the attenuation of tea polyphenols. For water supply systems using surface water as a source, it is suggested to appropriately increase the amount of polyphenols in tea in summer;
- (3) Disinfection with tea polyphenols will reduce microbial diversity in the water and change the structure of the flora. Disinfection with tea polyphenols inhibited the growth of common Gram-negative conditional pathogens, especially *Mycobacterium* with strong resistance to disinfection. The increase in the relative abundance of *Janthinobacterium* strengthened the bactericidal capacity of tea polyphenols;
- (4) Disinfection with tea polyphenols reduced the diversity and abundance of virulence factor genes of pathogenic bacteria. Among which, the abundance of virulence factors, closely related to the iron uptake and adhesion system, decreased, followed by the virulence factors that control the signal transduction system. This makes disinfection with tea polyphenols selective, meaning tea polyphenols inhibit the growth of harmful bacteria and promote the growth of beneficial bacteria.

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