



Comprehensive insights into the key components of bacterial assemblages in pharmaceutical wastewater treatment plants

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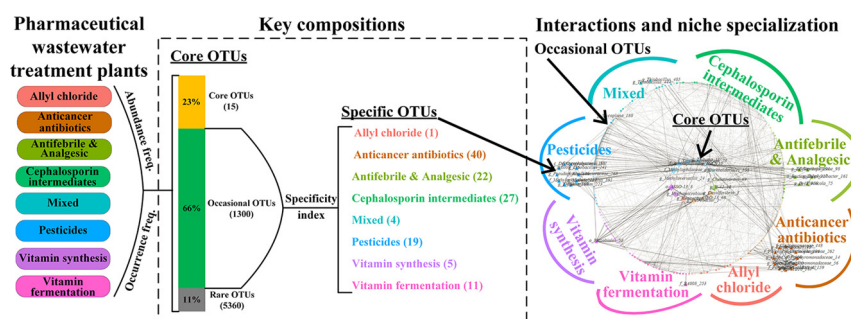
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HIGHLIGHTS

- Meta-analysis was conducted to explore key components of bacterial assemblages in pWWTPs.
- pWWTPs showed relatively lower bacterial diversity and unique community composition.
- Type of wastewater mainly drove the divergence of bacterial community in pWWTPs.
- pWWTPs contain core and specific species as key components of bacterial community.
- Bacterial assemblages within specific community tend to co-occur in pWWTPs.

GRAPHICAL ABSTRACT



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ABSTRACT

Due to complexity and variety of pharmaceutical wastewater composition, little is known as for functionally important microflora of pharmaceutical wastewater treatment plants (pWWTPs). We compared bacterial composition and diversity of pWWTPs (27 sludge samples collected from 12 full-scale pWWTPs) with those of other industrial (iWWTPs) (27 samples) and municipal wastewater treatment plants (mWWTPs) (27 samples) through meta-analysis based on 16S rRNA gene amplicon sequencing, and identified putatively important organisms and their ecological correlations. Non-metric multidimensional scaling indicated that the pWWTPs, iWWTPs and mWWTPs showed distinctive differences in bacterial community composition ($P < 1e-04$), and the pWWTPs had significantly lower bacterial diversity than the mWWTPs ($P < 1e-06$). *Thermotogae* and *Synergistetes* phyla only strictly dominated in the pWWTPs, and 26, 30 and 6 specific genera were identified in the pWWTPs, mWWTPs and iWWTPs, respectively. Totally, 15 and 1300 OTUs were identified as core and occasional groups, representing 23.2% and 66.2% of the total read abundance of the pWWTPs, respectively. Permutational multivariate analysis of variance revealed that the bacterial components were clearly clustered corresponding to the types of pharmaceutical wastewater, and a total of 129 local specific OTUs were identified in the pWWTPs, among which anticancer antibiotics pWWTPs had the highest number of specific OTUs (40 ones). Co-occurrence network revealed that the species dominating in the same type of pWWTPs tended to co-occur much more frequently than theoretical random expectation. The results may extend our knowledge regarding the ecological status and correlation of the key microflora in pWWTPs.

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1. Introduction

Pharmaceutical wastewater is considered as one type of the most complicate industrial wastewater usually containing high concentrations of chemical oxygen demand (COD), ammonia nitrogen (NH_4^+ -N), saline and heavy metals (Gadipelly et al., 2014; Rana et al., 2014). Biological treatment processes (i.e. bioreactors), such as up-flow anaerobic sludge blanket (Chelliapan et al., 2011), anaerobic-aerobic (Shi et al., 2014), sequencing batch reactors (Lefebvre et al., 2014), and membrane bioreactor (MBR) (Ng et al., 2016), are widely used in the pharmaceutical wastewater treatment plants (pWWTPs) due to their low operational cost. Pharmaceutical wastewater often contains a variety of antimicrobial compounds that can inhibit the growth of microbes, resulting in instable operation and low efficiency of the bioreactors (Chelliapan et al., 2011; Rana et al., 2014).

Understanding microbial ecology of the bioreactors can practically help to find effective and feasible measures or strategies to solve frequent running problems (Daims et al., 2006), such as failure in nitrogen or phosphorus removal (Lu et al., 2014; Ju and Zhang, 2015; Mielczarek et al., 2013), and sludge bulking and foaming (Wang et al., 2016; Jiang et al., 2016). However, few studies have been conducted to explore the key components of bacterial assemblages in pWWTPs treating diverse types of pharmaceutical wastewater, although municipal wastewater treatment plants (mWWTPs) have received great concerns (Ju et al., 2014; Ju and Zhang, 2015; Saunders et al., 2016).

Growing studies have focused on the partition of microbial community into three ecological groups, i.e. core, occasional, and rare species or operational taxonomic units (OTUs) (Chaillou et al., 2015; Lundberg et al., 2012; Reveillaud et al., 2014; Rui et al., 2015; Saunders et al., 2016; Toju et al., 2018; van der Gast et al., 2011). Core community is usually defined as being frequently present and abundant in most samples, with an extensive resource utilization range to metabolize complex and fluctuant chemicals and a stronger anti-perturbation ability to resist a variety of factors inhibiting microbial growth (Peers et al., 2012; Rui et al., 2015). Meanwhile, occasional community is deemed as being occasionally abundant in a few of samples (Saunders et al., 2016), with a narrow resource utilization range only to degrade partial chemicals and a more sensitive response to fluctuating environmental stresses (Peers et al., 2012; Rui et al., 2015; Saunders et al., 2016). Conversely, rare community with low abundance in all samples has the potential of being abundant when the external environments are changed (Saunders et al., 2016). Occasional community may be transferred to the specific species for certain types of pWWTPs, due to the presence of different inhibitors in different types of pharmaceutical wastewater (Gadipelly et al., 2014; Rana et al., 2014). Specific species have a specific resource utilization range (Mariadassou et al., 2015; Peers et al., 2012), but little information is currently available for pWWTPs. Network interactions of microbial community in ecosystem can reveal the potential mutualism associations among the bacterial taxa (Ju et al., 2014; Ju and Zhang, 2015; Rui et al., 2015) and strong niche specialization (Faust et al., 2012) in the environment.

In order to comprehensively explore the key components of bacterial assemblages in pWWTPs, this study determined the bacterial community composition of 12 full-scale pWWTPs treating eight types of pharmaceutical wastewater by using 16S rRNA gene amplicon sequencing. The types of pharmaceutical wastewater were differentiated based on the classification of the pharmaceutical products manufactured, including allyl chloride, anticancer antibiotics, antifebrile and analgesic, cephalosporin intermediates, mixed pharmaceutical wastewater, pesticide, vitamin A and E (chemical synthesis), and vitamin C (biological fermentation). The microbial communities of the pWWTPs were also compared with those of other industrial wastewater treatment plants (iWWTPs) (including whey filtering, petroleum refinery, printing and dyeing, acrylic polymer manufacturing and food fermentation, 27 samples) and mWWTPs (27 samples) through a meta-analysis of publicly available sequence data. An integrated strategy combining core

community concept and specificity ecological models was applied to identify putatively important organisms, whose ecological interconnections were defined by occurrence network analysis. This study may help to better understand the bacterial assemblages and their interrelations in pharmaceutical wastewater treatment bioreactors.

2. Materials and methods

2.1. Samples collection

During January to March 2014, we collected 27 sludge samples from anaerobic and aerobic bioreactors of 12 full-scale pWWTPs of China treating 8 types of pharmaceutical wastewater, including allyl chloride, anticancer antibiotics, antifebrile and analgesic, cephalosporin intermediates, mixed pharmaceutical wastewater (collected from wastewater treatment plants of a pharmaceutical industrial park), pesticide, vitamin A and E (chemical synthesis), and vitamin C (biological fermentation). The process flow of 12 full-scale pWWTPs and the corresponding sampling sites are shown in Fig. S1. The differences in the composition of the eight types of pharmaceutical wastewater were introduced in detail in Text S1. Table S1 shows the detailed information of the different types of pharmaceutical wastewater and their water quality. All the samples were fixed on site using 100% ethanol (1:1, v/v) before immediately delivered to the laboratory and stored at $-20\text{ }^\circ\text{C}$ for DNA extraction (Ibarbalz et al., 2013; Ju et al., 2014).

2.2. DNA extraction and sequencing

The methods of extraction and quality check of metagenomic DNA from the 27 sludge samples were described in Text S3. V1-V2 regions of the 16S rRNA gene was amplified by PCR, and the purified PCR products were sent for sequencing on a Miseq platform (Illumina, San Diego, CA, USA) at the Jiangsu Zhongyijinda Analytical & Testing Co., Ltd., Yixing, China to produce 2×250 bp paired-end reads (details shown in Text S4). The obtained raw sequence data have been deposited into the NCBI SRA under the accession number of SRR5683095-SRR5683121. To compare the microbial communities of pWWTPs with those of iWWTPs and mWWTPs, we also downloaded the 16S rRNA gene amplicons sequencing datasets of iWWTPs (27 samples) and mWWTPs (27 samples) from the publicly available NCBI database under the accession numbers of SRR987663 (Ju et al., 2014), SRR987664 (Ju et al., 2014), SRA061963 (Ibarbalz et al., 2013), SRR1060401 (Lu et al., 2015) and SRP066236 (Tang et al., 2016), and GenBank under the accession numbers of GU481685 and GU549391 (Kwon et al., 2010). The iWWTPs included the wastewater treatment plants of whey filtering, petroleum refinery, printing and dyeing, acrylic polymer manufacturing and food fermentation. Table S2 shows the detailed information of the obtained datasets for all the 81 samples.

2.3. Sequence processing

For the raw sequences generated by Miseq platform, the quality trimming, denoising, and chimera and non-bacterial sequences removal were conducted by using Mothur (Schloss et al., 2009) (details shown in Text S5) to generate a total of 914,784 clean reads. The normalized reads from all the samples were subjected to processing with QIIME (Caporaso et al., 2010) using the uclust algorithm for open reference OTU clustering at an identity threshold of 0.94 (Edgar, 2010), approximately corresponding to the taxonomic level of genus (Saunders et al., 2016). To fairly compare all samples at the same sequencing depth, the library size of each sample was normalized (each having 14,460 sequences for alpha and beta diversity analysis) by removing singleton OUT. For taxonomic assignment, the representative sequences were aligned by RDP Classifier (Wang et al., 2007) against the Greengenes taxonomy (Desantis et al., 2006) released in May 2013 at the confidence threshold of 50% (McDonald et al., 2012). To compare the three types of

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