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The divergence between fungal and bacterial communities in seasonal and spatial variations of wastewater treatment plants

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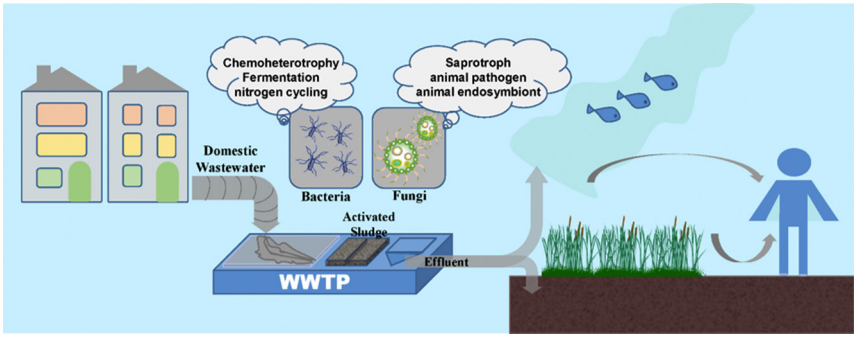
HIGHLIGHTS

- In activated sludge samples, fungal to bacteria ratio was less than 1%.
- Seasonal and spatial variations were observed in bacterial and fungal communities.
- Fungi exhibited lower divergence in taxonomy, alpha and beta diversities than bacteria.
- Bacteria and fungi owned different functions but worked jointly in activated sludge.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, quantitative PCR (qPCR) and high-throughput sequencing were used to simultaneously examine both bacteria and fungi across temporal and spatial scales in activated sludge from wastewater treatment plants (WWTPs). The ratio of fungi to bacteria was 0.43% on average after accounting for the multicopies in 16S rRNA gene (54.63%), indicating the number of fungi was far lower than bacteria in active sludge. The Miseq sequencing results revealed obvious seasonal and spatial variations in bacterial and fungal distribution patterns in WWTPs. Compared to bacteria, fungi showed a lower divergence in alpha and beta diversity, and exhibited less taxonomic diversity in both abundant and rare subcommunities at the class level, suggesting that the fungal community was less variable in this artificial ecosystem. Such variation of microbial communities was significantly correlated with geographical distance, DO, temperature, HRT, SRT, COD, TN and TP. In activated sludge, the main function of bacteria was chemoheterotrophy, fermentation, and nitrogen cycling processes, while the dominant functional guilds of fungi were saprotroph, animal pathogen, and animal endosymbiont. Moreover, both bacteria and fungi could play important roles in the degradation of toxicants, like hydrocarbon and aromatic compounds.

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1. Introduction

Integrated bio-engineered technologies, such as membrane bioreactor (MBR) (Park et al., 2017), anaerobic-anoxic-oxic (A2/O) (Ashfaq et al., 2017), combined orbal oxidation ditch (C-orbal OD) (He et al., 2013), and sequencing batch reactor (SBR) (Chen et al., 2017a), are widely applied in numerous wastewater treatment processes. Activated sludge plays crucial roles in these biotechnologies, as it is a highly complex microbial ecosystem composed of diverse bacteria, fungi, archaea, protozoa, and viruses (Zhang et al., 2012), capable of degrading various types of contaminants (Ashfaq et al., 2017; Louvet et al., 2017; Mal et al., 2017; Tiwari et al., 2017). There is plenty of evidence showing that bacteria play significant roles in activated sludge (Ju and Zhang, 2015; Li and Pagilla, 2017; Rodríguez et al., 2015; Shchegolkova et al., 2016), but the equally important fungi, which exhibit great diversity (More et al., 2010), have amounts of biomass comparable to bacteria (Anastasi et al., 2012), and are present in higher proportion than archaea, other microeukaryotes, or viruses (Liu et al., 2017), are far less studied.

Bacteria are a very important group of microorganisms in activated sludge, as they aid to form the heterogeneous structure of flocs (Ju and Zhang, 2015) and execute biodegradation functions (Shchegolkova et al., 2016). Meanwhile, the potential roles of fungi in activated sludge, such as fostering nitrification and denitrification (Guest and Smith, 2002), removal of toxic compounds, enhancing bioflocculation (More et al., 2010) and lipid accumulation (Liu et al., 2017), and improving bioconversion (Weber et al., 2007) and dewaterability (Zhou et al., 2015), have been much more slowly elucidated. Fungi are also believed to be potentially better than bacteria in terms of rate reaction, stoichiometry, and resistance to inhibitory chemicals like nitrification inhibitors and heavy metals (Guest and Smith, 2002). Therefore, application of a defined microbial consortium containing both bacteria and fungi in biological treatment might be a more effective strategy to improve this bioprocess in the future.

In the past, the diversity and function of fungi in activated sludge were mainly studied by culture-dependent methods (Guest and Smith, 2002; Liu et al., 2017; More et al., 2010; Zhang and Cui, 2015; Zhou et al., 2015). Recently, high-throughput sequencing has been applied to fungal communities in activated sludge from lab-scale bioreactors (Xiao et al., 2017), MBR (He et al., 2017) and biological aerated filters (BAFs) (Li et al., 2015). Unlike fungi, bacterial communities in activated sludge ecosystems have been widely surveyed using high-throughput methods (Joshi et al., 2017; Ma et al., 2016; Niu et al., 2016; Wang et al., 2016). It is known that the distribution patterns of bacteria communities vary over spatial scales (Ma et al., 2015; Wang et al., 2016), between seasons (Ju et al., 2014; Turki et al., 2017), as well as with environmental factors like pH (Joshi et al., 2017) and dissolved oxygen (Ma et al., 2016; Niu et al., 2016). However, whether fungal communities have seasonal and spatial variations similar to bacterial communities, remains unclear. In particular, high-throughput sequencing techniques also make the identification of abundant and rare microbes possible, which could help better reveal the individual and combined roles of these groups in shaping the diversity and function of ecosystems (Jiao et al., 2017; Logares et al., 2013). Previous research has shown there may be fundamentally distinct spatial distribution in marine microbial eukaryotes (Logares et al., 2014), or similar biogeographical patterns of bacteria in saline lake between abundant and rare groups (Logares et al., 2013). Until now, it has remained unclear whether abundant and rare taxa of bacteria and fungi have similar distribution patterns in wastewater treatment plants (WWTPs).

The marker genes, 16S rRNA gene and internal transcribed spacer (ITS) region, were chosen to represent the bacteria and fungi, respectively, in WWTPs. It should be noted that there are multiple copies of 16S rRNA gene present within the genomes of many bacterial species (Conville and Witebsky, 2007), and that a similar situation exists with the ITS of fungal species (Taylor et al., 2016). However, as yet, no available methods can correct for multiple copies in fungi (Taylor et al., 2016). In contrast, the overestimation of bacterial abundance could be avoided by use of the PICRUSt algorithm (Kembel et al., 2012; Langille et al., 2013), which could correct for the multiple copies in each OTU of 16S rRNA gene (Kembel et al., 2012). In this study, activated sludge from five domestic WWTPs across three Chinese cities (Beijing, Qingdao and Wuxi) was collected in two seasons, summer and winter. Quantitative PCR (qPCR) and high-throughput sequencing were employed to explore the abundance, diversity, function and variations of both bacteria and fungi in these samples. Specially, we mainly address following three questions. (i) Did the abundance and diversity of fungi alter similarly to bacteria with spatial distance and seasonal switching? (ii) How did the abundant and rare species of bacteria and fungi distribute among different WWTPs? (iii) What were the potential functional roles of bacteria and fungi in activated sludge of WWTPs?

2. Methods

2.1. Sample collection

A total of 30 activated sludge samples were collected from five urban sewage WWTPs at the capital and two eastern cities in China, including one from Beijing (BJ), two from Qingdao (QDN, QDS) and two from Wuxi (WXN, WXS), a region of 1070 km across (Fig. S1). These WWTPs mainly receive and treat domestic sewage, and the detailed operational parameters are summarized in Table S1. We collected the samples in triplicate from the aerobic tank in secondary treatment of each WWTP in December 2014 (winter samples, from north to south, defined as BJ12, QDN12, QDS12, WXN12, WXS12), and June 2015 (summer samples, defined as BJ6, QDN6, QDS6, WXN6, WXS6). In accordance with the Standard Method of Water and Wastewater Monitoring (Bureau, 2002), a suite of environmental parameters including dissolved oxygen (DO), pH, temperature, hydraulic retention time (HRT), sludge retention time (SRT), inflow and effluent of chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP) were determined to reflect the corresponding environmental conditions.

2.2. DNA extraction and quantification of 16S rRNA gene and ITS by qPCR

1 mL of each sample was centrifuged at 12,000 ×g for 10 min at 4 °C. DNA was extracted from 0.25 g of the resulting sediment pellet (wet weight) in duplicate by a FastDNA SPIN kit for soil (Qbiogene, Solon OH). In order to quantify the proportion of bacteria and fungi in activated sludge, the copy numbers of 16S rRNA gene (Zhang et al., 2017) and ITS (Ihrmark et al., 2012) were quantified via qPCR by CFX96 Touch Real-Time PCR Detection System (BioRad) in 30 DNA samples. The qPCR reactions, total volume of 20 μL, contained 2 μL of DNA template, and 0.3 μM of each primer. Reaction conditions for qPCR were as follows: denaturing for 15 min at 95 °C, following 40 cycles of 10 s at 95 °C, 30 s at annealing temperatures (50 °C for 16S rRNA gene or 60 °C for ITS), and a final melt curve stage with temperature ramping from 50 °C to 95 °C (0.5 per read, 5 s hold). Standard curves were generated by a ten-fold dilution series of the standard plasmids containing the targeted amplicons, with R^2 higher than 0.99 and amplification efficiencies in the range of 90% and 110% (Table S2). Each sample contained technical triplicates with parallel negative controls. The copy numbers of 16S rRNA gene and ITS generated according to the standard curves were defined as absolute abundance. The relative abundance of ITS was the ratio of ITS to 16S rRNA gene.

2.3. 16S rRNA, ITS sequencing and data processing

For MiSeq sequencing, the V4 region of 16S rRNA gene (Caporaso et al., 2012; Yarza et al., 2014) for both bacteria and archaea, and ITS2 region of ITS (Ihrmark et al., 2012) for fungi were amplified with barcode sequences at both ends. The PCR amplification was carried out on