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Microbial community functional structure in response to antibiotics in pharmaceutical wastewater treatment systems

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ABSTRACT

It is widely demonstrated that antibiotics in the environment affect microbial community structure. However, direct evidence regarding the impacts of antibiotics on microbial functional structures in wastewater treatment systems is limited. Herein, a high-throughput functional gene array (GeoChip 3.0) in combination with quantitative PCR and clone libraries were used to evaluate the microbial functional structures in two biological wastewater treatment systems, which treat antibiotic production wastewater mainly containing oxytetracycline. Despite the bacteriostatic effects of antibiotics, the GeoChip detected almost all key functional gene categories, including carbon cycling, nitrogen cycling, etc., suggesting that these microbial communities were functionally diverse. Totally 749 carbon-degrading genes belonging to 40 groups (24 from bacteria and 16 from fungi) were detected. The abundance of several fungal carbon-degrading genes (e.g., glyoxal oxidase (*glx*), lignin peroxidase or ligninase (*lip*), manganese peroxidase (*mnp*), endochitinase, exoglucanase_genes) was significantly correlated with antibiotic concentrations (Mantel test; $P < 0.05$), showing that the fungal functional genes have been enhanced by the presence of antibiotics. However, from the fact that the majority of carbon-degrading genes were derived from bacteria and diverse antibiotic resistance genes were detected in bacteria, it was assumed that many bacteria could survive in the environment by acquiring antibiotic resistance and may have maintained the position as a main player in nutrient removal. Variance partitioning analysis showed that antibiotics could explain 24.4% of variations in microbial functional structure of the treatment systems. This study provides insights into the impacts of antibiotics on microbial functional

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structure of a unique system receiving antibiotic production wastewater, and reveals the potential importance of the cooperation between fungi and bacteria with antibiotic resistance in maintaining the stability and performance of the systems.

1. Introduction

Since the introduction of penicillin, antibiotic production and application for the treatment of bacterial infections and diseases have continued to increase (Kümmerer, 2003). Along with the fermentative production of antibiotics, large volumes of antibiotic production wastewater containing antibiotics of up to several mg L^{-1} are routinely generated (Larsson et al., 2007; Li et al., 2008a), which are much higher than previously reported in other environments (Kümmerer, 2009).

Activated sludge processes, a biological approach utilizing bacterial metabolic functions for the removal of organic nutrients (Seviour and Nielsen, 2010; Wagner and Loy, 2002) have been widely applied for the treatment of antibiotic production wastewater. As antibiotics are designed to target bacteria, there is a concern that high concentrations of antibiotics in wastewater may seriously inhibit bacterial growth, leading to deterioration in bacterial functions for the removal of organic nutrients. A reduction in the number of bacteria and alterations in microbial populations were observed in model sewage purification systems when different antibiotics were added with concentrations equivalent to those in hospital wastewater (Al-Ahmad et al., 1999; Kümmerer and Al-Ahmad, 2000). Our previous studies using clone libraries and quantitative PCR demonstrated that under high antibiotic levels (mainly with streptomycin), aerobic wastewater treatment communities may maintain system stability through adjusting bacterial and eukaryal compositions (Deng et al., 2012). Fungi, which are able to utilize organic nutrients in wastewater (Adav et al., 2007), were abundant with *Ascomycota* being the dominant phyla.

At the same time, the high antibiotic concentrations and bacterial densities in biological antibiotic production wastewater treatment systems make it easy for bacteria to acquire resistance and for antibiotic resistance genes to proliferate (Kim et al., 2007; Tenover, 2006). Our previous studies have consistently shown that the high antibiotic levels in antibiotic production wastewater could lead to the occurrence of abundant antibiotic resistance in bacterial isolates from antibiotic production wastewater treatment systems (Li et al., 2009, 2010). So it is possible that bacteria possessing antibiotic resistance may play an important role for nutrient removal. However, little has been done to evaluate the impacts of residual antibiotics in antibiotic production wastewater on the functions of microbial communities in biological treatment systems from a holistic view.

The rapid development of high throughput metagenomic approaches like GeoChip (He et al., 2010a) has made it possible to easily examine microbial functional diversity, composition and structure in a rapid fashion. GeoChip 3.0, which contains more than 57,000 gene variants from 292 functional gene families, including antibiotic resistance genes (He et al., 2010a), has been extensively employed to analyze the functional gene structure of microbial communities in different environments (He et al., 2010b; Trivedi et al., 2012; Wang et al., 2009). It is, therefore, a suitable tool for examining the impact of antibiotic residues on microbial communities with regard to microbial functional gene structures as well as the occurrence of antibiotic resistance in biological antibiotic production wastewater treatment systems.

This study aimed to reveal how the microbial functional communities in biological antibiotic production wastewater treatment systems respond to the high concentrations of residual antibiotics and maintain their nutrient removal functions. To answer this question, the functional gene structures of microbial communities in two biological antibiotic production wastewater treatment plants mainly receiving oxytetracycline-containing wastewater were analyzed with GeoChip 3.0 in combination with bacterial 16S rRNA gene clone library and quantitative PCR (for bacterial 16S rRNA genes, fungal 18S rRNA genes, and tetracycline resistance genes (*tet* genes)). The results of this study will provide useful information for the establishment of a sound process and operational strategy for successful antibiotic production wastewater treatment.

2. Material and methods

2.1. Study facilities, sampling sites and characterization of samples

Worldwide annual output of the tetracycline class of antibiotics has reached more than 20,000 tons. Over 1000 and 6000 tons of oxytetracycline are produced every year in the two studied antibiotic manufacturing plants – North China Pharmaceutical Group Corporation (HY) and Huashu Pharmaceutical Company (HS), respectively, in Shijiazhuang city, north China. These are among the largest oxytetracycline production facilities in the world. Biological antibiotic production wastewater treatment plants in HY and HS have been in use since 2000 and 2001, respectively. The wastewater treatment system in HY receiving oxytetracycline crystal mother liquor (HY-ML) consists of a sequential batch reactor (SBR) (hydraulic retention time, 8 h) and a two-stage submerged biological contact reactor using fiber bundle as the bio-carrier (hydraulic retention time, 8 h for each) (Fig. 1a). The dilution water (HY-W1) composing of the equipment washing water, penicillin G (Penicillin) production wastewater and a small amount of sewage are used to dilute the HY-ML. The total production of excess sludge from HY is approximately 125 tons (dry weight) per year. The final effluent in HY (HY-W4) is discharged to a nearby river. The mixed liquor suspended solids in the SBR is maintained at 4000–5000 mg/L. One cycle of the SBR includes