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Interaction between common antibiotics and a *Shewanella* strain isolated from an enhanced biological phosphorus removal activated sludge system

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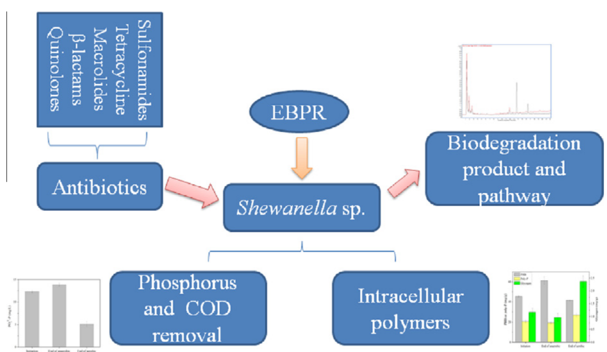
HIGHLIGHTS

- A *Shewanella* strain was isolated from an EBPR system.
- This strain could remove phosphorus and chemical oxygen demand.
- Antibiotics affected this strain through metabolism of intracellular polymers.
- These effects varied with the structure and concentration of the antibiotic.
- The *Shewanella* strain could degrade the cefalexin to 2-hydroxy-3-phenyl pyrazine.

GRAPHICAL ABSTRACT



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ABSTRACT

With increasing production and consumption, more antibiotics are discharged into wastewater treatment plants and generally cannot be sufficiently removed. Because of the complexities of biological treatment processes, the fates of antibiotics and their effects on microorganisms, particularly those involved in the phosphorus removal system, are still unclear. Here, a *Shewanella* strain was isolated from an enhanced biological phosphorus removal (EBPR) system and was found to have the ability to remove phosphorus (P) and chemical oxygen demand (CODcr). Antibiotics affected the *Shewanella* strain through metabolism of the three main intracellular polymers, altering the ability of the strain to remove P and CODcr. These effects varied with the structure and concentration of the antibiotics. The *Shewanella* strain removed cefalexin and amoxicillin by degradation or adsorption, producing 2-hydroxy-3-phenyl pyrazine from cefalexin. This study enabled the recognition of the effect and removal of antibiotics during wastewater treatment.

1. Introduction

Pharmaceuticals and personal care products (PPCPs), including antibiotics, have attracted attention worldwide owing to their beneficial effects. However, even when used at low concentrations, antibiotics can also have adverse effects on organisms and the environment. In recent years, increased use of antibiotics has been

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shown to promote the emergence of antibiotic-resistant bacteria and antibiotic-resistance genes; this is regarded as a major threat to the ecological environment and human health by the World Health Organization (Pruden et al., 2013).

In general, antibiotics cannot be fully assimilated by humans and livestock (Marx et al., 2015b); thus, excreted antibiotics and their metabolites originating from industrial, hospital, and household sources can enter into wastewater treatment plants (WWTPs) (Giger et al., 2003). Because most municipal WWTPs are designed to remove conventional chemical oxygen demand (CODcr), pathogens, and nutrient substances, antibiotics cannot be removed completely (Zhang and Li, 2011). The concentrations of antibiotics in influents and effluents from WWTPs can range from ng/L to μg/L; these concentrations have obvious spatial and temporal variations due to differences in production, consumption, and usage (Kasprzyk-Hordern et al., 2009). β-Lactams, sulfonamides, quinolones, tetracyclines, and macrolides are often detected in WWTPs (Luo et al., 2014; Marx et al., 2015a; Zhou et al., 2013). However, the fates of antibiotics during different biological wastewater treatment processes and their mechanisms of degradation remain unclear.

Because of their bacteriostasis functions, antibiotics have negative effects on biological wastewater treatment processes. Additionally, the remaining antibiotics in wastewater can promote the emergence of antibiotic-resistant bacteria and antibiotic-resistance genes, which have been detected in many WWTPs around the world (Marti et al., 2014; Xu et al., 2015; Zhang et al., 2015). Considering the insufficient removal of antibiotics and their toxicities to microorganisms involved in biological processes, studies are needed to elucidate the fates of antibiotics during wastewater treatment processes and determine their effects on microbes.

Several studies have examined the influences of antibiotics on biological wastewater treatment processes. For example, the impact of chronic exposure to 50 mg/L tetracycline on biomass with enriched nitrifying communities was studied, and the ammonia oxidizing bacterial community was found to be the most sensitive to this antibiotic (Katipoglu-Yazan et al., 2015). Mitchell et al. (2013) evaluated the effects of four common animal husbandry antibiotics on anaerobic digestion treatment and their potential to degrade during digestion. Additionally, Zhang et al. (2013) studied the influence of trace tetracycline on the microbial community of sequencing batch reactors (SBRs) and the development of tetracycline-resistance genes. Analysis of the inhibitory effects of acetaminophen and doxycycline on the activity of short-cut nitrifying, denitrifying, and anammox biomass and phosphate-accumulating organisms (PAOs) has shown that all biomass types are affected by these two antibiotics (Alvarino et al., 2014). Continuous sulfamethoxazole dosing at 50 mg/L suppresses acetate utilization in fast-growing microbial cultures and leads to decreased diversity of bacteria, with accumulation of acetate-degrading species (Kor-Bicakci et al., 2014). Previous studies have mainly focused on the effects of antibiotics on growth and nutrient substance utilization of activated sludge. In contrast, few studies have examined the influence of antibiotics on phosphorus removal systems, and these existing studies have been based on a single type of antibiotic; moreover, the fates and effects of different categories of antibiotics on intracellular metabolites have not been elucidated.

Shewanella strains have the ability to use many types of inorganic and organic compounds for respiration and can therefore be applied in bioremediation of pollutants (Hau and Gralnick, 2007). Thacher et al. (2015) used columns charged with *Shewanella onediensis* to conduct bioactive sand reactor studies and found that *Shewanella MR-1* was effective for reducing chromium in simulated aquifers. Yang et al. (2013) discovered that *Shewanella* was involved in polybrominated diphenyl ethers (PBDE)-degrading microbial communities. Additionally, *Shewanella* was reported to be capable of reducing ferrihydrite (Dippon et al., 2015), absorbing cadmium (Yu and Fein, 2015), and taking up mercury and arsenic (Szczuka et al., 2015). Zhao et al. (2015) isolated *Shewanella xiamenensis* from an estuarine water sample and showed that the strain had multidrug resistance.

In this study, the characteristics of the *Shewanella* strain isolated from EBPR activated sludge and its ability to remove phosphorus were analyzed. Batch experiments were conducted to investigate the effects of typical antibiotics on the characteristics of the *Shewanella* strain, including its ability to remove phosphorus and CODcr as well as its intracellular metabolic pathways. The mechanism through which cefalexin was removed by the *Shewanella* strain was studied. Furthermore, the microbial degradation product was identified.

2. Materials and methods

2.1. Bacterial strain and chemicals

The bacterial strain was isolated from an EBPR SBR system with a working volume of 8 L (Chen et al., 2004; Li et al., 2008; Wang et al., 2015b). The SBR was operated with three cycles each day, and every cycle consisted of 2-h anaerobic, 4-h aerobic, 0.5-h settling, and idle phases. The components of synthetic wastewater were as follows: 583 mg/L CH_3COONa, 76.4 mg/L NH_4Cl, 43.9 mg/L KH_2PO_4, 11 mg/L $CaCl_2{\cdot}2H_2O$, 35 mg/L $MgCl_2$, 0.045 mg/L $FeCl_3{\cdot}6H_2O$, 0.045 mg/L H_3BO_3, 0.009 mg/L $CuSO_4{\cdot}5H_2O$, 0.054 mg/L KI, 0.036 mg/L $MnCl_2{\cdot}4H_2O$, 0.018 mg/L $Na_2MoO_4{\cdot}2H_2O$, 0.036 mg/L $ZnSO_4{\cdot}7H_2O$, 0.045 mg/L $CoCl_2{\cdot}6H_2O$, and 3 mg/L EDTA. Seed sludge was collected from an aerobic tank at a local sewage treatment plant. After a 40-day cultivation period, the phosphorus and TOC removal efficiencies were both above 90% during the stable phase.

The wastewater sample containing activated sludge from the EBPR SBR system was mixed with LB medium plus 10 mg/L PO_4^{3-}-P at a ratio of 1:20 and was cultivated in a shaking incubator at 30 °C and 140 rpm. The cultures were then serially diluted with LB with addition of 10 mg/L PO_4^{3-}-P. The diluted cultures were then spread plated onto LB plate medium. After 3–4 times successive streaks, the purified strains were used to investigate their abilities to remove phosphorus and COD_{cr}.

Common antibiotics (three sulfonamides: sulfamethoxazole [SMX], sulfadiazine [SDZ], and sulfamethazine [SMZ]; two macrolides: erythromycin [ERY] and roxithromycin [ROX]; two β-lactams: cefalexin [CLX] and amoxicillin [AML]; two tetracyclines: tetracycline [TET] and oxytetracycline [OTC]; three quinolones: ofloxacin [OFL], ciprofloxacin [CIP], and norfloxacin [NOR]) were chosen from antibiotic categories that were detected frequently in influents and effluents of WWTPs.

The properties of the antibiotics are shown in Table S1. The antibiotics were dissolved in synthetic wastewater, and stock solutions (100 mg/L) were stored in brown bottles at 4 °C. Poly (3-hydroxybutyric acid-co-3-hydroxyvalerio acid) was purchased from Sigma-Aldrich (St. Louis, MO, USA). High-performance liquid chromatography (HPLC)-grade methanol, acetonitrile, and formic acid were obtained from Fisher (USA). The glucose oxidase method kit was purchased from Baoding Great Wall Clinical Reagents Co., Ltd. (China).

2.2. Culture medium

The *Shewanella* strain was activated and cultivated on Luria-Bertani (LB) agar plates and with LB medium containing an additional 10 mg/L PO_4^{3-}-P. The LB medium at pH 7.0 contained 10 g/L tryptone, 5 g/L NaCl, 5 g/L yeast extract, and 0.0439 g/L KH_2PO_4,