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Research article

Biodegradation of typical pharmaceutical compounds by a novel strain *Acinetobacter* sp.

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ABSTRACT

A novel sulfamethoxazole (SMX)-degrading strain, *Acinetobacter* sp., was used to degrade other pharmaceutical compounds, including sulfadiazine (SD), sulfamethazine (SMT), trimethoprim (THM), triclosan (TCS), diclofenac (DFC) and carbamazepine (CBZ). The experimental results showed that *Acinetobacter* sp. can completely degrade SMX, SD and SMT, but with different mineralization efficiency. *Acinetobacter* sp. can mineralize 98.8% of SMX, while only 17.5% and 20.5% for SD and SMT, respectively. The intermediate products of SMX, SD and SMT degradation were tentatively identified. Based on the intermediates, it is inferred that the initial step for degrading sulfonamides by *Acinetobacter* sp. was the amidation of the amino groups in the benzene ring. The presence of methyl in the heterocyclic ring could induce the formation of methylase. By comparing the intermediates of SMX, SD and SMT degradation, it is concluded that *Acinetobacter* sp. preferred attacking the oxazole ring. However, *Acinetobacter* sp. cannot degrade THM, TCS, DFC and CBZ, while *Acinetobacter* sp. can still degrade SMX in the respective presence of THM, DFC and CBZ, although the degradation rate decreased. Moreover, the presence of TCS could completely inhibit the degradation of SMX by *Acinetobacter* sp.

1. Introduction

Pharmaceuticals and personal care products (PPCPs) are becoming ubiquitous pollutants (Wang and Chu, 2016), they are detected in the waterbody with concentration ranging from ng/L to μg/L (Miège et al., 2009; Verlicchi et al., 2012; Zhao et al., 2016), and exerted adverse effect on the aquatic organisms and human health (Baran et al., 2011; Boxall et al., 2012; Overturf et al., 2015; Yu and Wu, 2015). Sulfamethoxazole (SMX) is one of the sulfonamides antibiotics and has been widely used in the world. Various technologies have been developed for the removal of refractory organic pollutants, including physical, chemical and biological processes (Wang and Xu, 2012; Liu et al., 2017; Song et al., 2017; Wang and Bai, 2017; Wang and Wang, 2018), because the conventional wastewater treatment plants (WWTPs) was ineffective for the removal of SMX evidenced by the significant fluctuation of the removal efficiency of SMX (Benotti and Brownawell, 2009; Larcher and Yargeau, 2011; Gao et al., 2016; Wang and Wang, 2016), which could be attributed to the different environmental conditions, especially the composition of microbial communities in the activated sludge. The presence or absence of SMX degrading-strains in the activated sludge is critical for the removal of SMX. Study has reported that mixed culture in the activated sludge can degrade SMX by co-metabolism (Kassotaki et al., 2016). In comparison with the mixed culture, several pure cultures, including *Microbacterium* sp. SMXB24 (Herzog et al., 2013), *Achrombacter* sp. BR3 (Bouju et al., 2012), *Pseudomonas psychrophila* HA-4 (Jiang et al., 2014), have been identified to be capable of degrading SMX. In addition, in our previous study, *Acinetobacter* sp. was found to be capable of degrading SMX with the concentration up to 240 mg/L within 12 h (Wang and Wang, 2017). Considering that the sulfonamides have the similar physiochemical properties, it is meaningful to investigate the performance of *Acinetobacter* sp. W1 in removing other sulfonamides. In addition, THM, CBZ, TCS and DFC usually coexisted with SMX in the wastewater (Papageorgiou et al., 2016). It is thus necessary to investigate the capacity of *Acinetobacter* sp. to degrade THM, CBZ, TCS and DFC, and the effect of THM, CBZ, TCS and DFC on the degradation of SMX.

The objectives of this study were to investigate: (1) the performance of *Acinetobacter* sp. in degrading typical pharmaceutic compounds, including SMX, SD, SMT, THM, CBZ, TCS and DFC; (2)

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the degradation kinetics and pathway; (3) the degradation capacity of *Acinetobacter* sp. to THM, CBZ, TCS and DFC; (4) and the effect of THM, CBZ, TCS and DFC on the degradation of SMX by *Acinetobacter* sp.

2. Materials and methods

2.1. Chemicals

THM (>99% purity), SMX (>98% purity), CBZ (>98 purity) and TCS (97% purity) was obtained from Aladdin Industrial Corporation (China). SD and SMT were purchased from Alfa Aesar Company (Tianjin, China) with the purity of 99%. DFC was obtained from Tokyo Chemical Industry (Japan) with the purity of >98%. The relevant information of the selected pharmaceutic compounds have been provided in Table 1.

All the stock solutions of the selected compounds were prepared by directly adding into 1 L of mineral medium and trace elements (MMTE). The concentration of stock solution was determined based on the solubility of each selected compound published by the National Center for Biotechnology Information (https://pubchem.ncbi.nlm.nih.gov/compound/), and was 100 mg/L, 200 mg/L, 10 mg/L, 10 mg/L, 40 mg/L and 100 mg/L for THM, SMX, CBZ, TCS, SD and SMT, respectively. All other chemicals and solvents used in this study were reagent grade. Table 2 listed the composition of mineral medium and trace elements used in the cultivation of *Acinetobacter* sp.

2.2. Enrichment of Acinetobacter sp. and preparation of cell suspensions

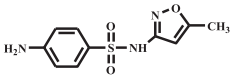
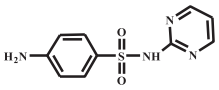
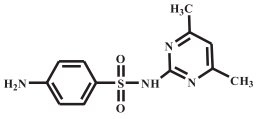
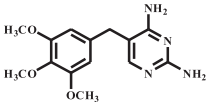
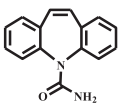
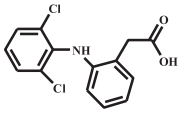
The strain was isolated from the activated sludge which was acclimated to SMX for 3 months. The strain was identified as *Acinetobacter* sp. based on the analysis of 16S rRNA gene sequence. The detailed isolation procedure has been reported in the previous study (Ye and Zhang, 2013). The GenBank accession number of this strain was of KX622562, and it has been deposited in China General Microbiological Culture Collection with the number of 13236. Three glass bottles with the volume of 250 mL (Reeko Company, China) were used to enrich the cultures. Each glass bottle has 200 mL sterilized MMTE (121 °C for 30 min) with the SMX initial concentration of 240 mg/L. *Acinetobacter* sp. was inoculated in MMTE. During the enrichment process, SMX was served as the sole carbon and energy source. All cultures were incubated at 25 °C at pH 7, which was the optimal condition for *Acinetobacter* sp. to degrade SMX (Wang and Wang, 2017). To prevent the degradation of SMX caused by photo-degradation, all cultures were incubated at the darkness with the shaking speed of 150 rpm.

For the biodegradation experiments, exponentially growing cells were harvested by centrifugation (10000 g for 5 min) and washed three times with 0.1 M phosphate buffer at pH 7.0 before use.

Table 2
The composition of mineral medium and trace elements.

Chemicals	Concentration	Chemicals	Concentration
Mineral medium			
Na_2CO_3	1 mM	$MgSO_4 \cdot 7H_2O$	0.24 mM
$(NH_4)_2SO_4$	1.8 mM	KH_2PO_4	2.5 mM
$Na_2HPO_4 \cdot 12H_2O$	2.5 mM	$CaCl_2$	35 μM
$FeSO_4 \cdot 7H_2O$	1 μM		
Trace elements			
$FeCl_2 \cdot 4H_2O$	7.5 mM	$CoCl_2 \cdot 6H_2O$	0.8 mM
$MnSO_4 \cdot 7H_2O$	0.35 mM	$ZnCl_2$	0.51 mM
$NiCl_2 \cdot 6H_2O$	0.1 mM	$Na_2MoO_4 \cdot 2H_2O$	10 μM
$MnCl_2 \cdot 4H_2O$	30 μM	$CuCl_2 \cdot 2H_2O$	12 μM

Table 1
Relevant information of the selected pharmaceuticals.

Compounds	Classification	Molecular weight	Log*Kow*	Structure
SMX	Antibiotics	253.276	0.89	
SD		250.276	−0.09	
SMT		278.33	0.89	
THM		290.323	0.91	
CBZ	Anticonvulsants	236.34	2.45	
DFC	Nonsteroidal anti-inflammatory drugs	296.147	4.51	
TCS	Disinfectants	289.536	4.76