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Abundance and distribution of Macrolide-Lincosamide-Streptogramin resistance genes in an anaerobic-aerobic system treating spiramycin production wastewater

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ABSTRACT

The behaviors of the Macrolide-Lincosamide-Streptogramin (MLS) resistance genes were investigated in an anaerobic-aerobic pilot-scale system treating spiramycin (SPM) production wastewater. After screening fifteen typical MLS resistance genes with different mechanisms using conventional PCR, eight detected genes were determined by quantitative PCR, together with three mobile elements. Aerobic sludge in the pilot system exhibited a total relative abundance of MLS resistance genes (per 16S rRNA gene) 2.5 logs higher than those in control samples collected from sewage and inosine wastewater treatment systems ($P < 0.05$), implying the presence of SPM could induce the production of MLS resistance genes. However, the total relative gene abundance in anaerobic sludge (4.3×10^{-1}) was lower than that in aerobic sludge (3.7×10^{0}) despite of the higher SPM level in anaerobic reactor, showing the advantage of anaerobic treatment in reducing the production of MLS resistance genes. The rRNA methylase genes (*erm*(B), *erm*(F), *erm*(X)) were the most abundant in the aerobic sludge (5.3×10^{-1}–1.7×10^{0}), followed by esterase gene *ere*(A) (1.3×10^{-1}) and phosphorylase gene *mph*(B) (5.7×10^{-2}). In anaerobic sludge, *erm*(B), *erm*(F), *ere*(A), and *msr*(D) were the major ones (1.2×10^{-2}–3.2×10^{-1}). These MLS resistance genes (except for *msr*(D)) were positively correlated with Class 1 integron ($r^2 = 0.74$–0.93, $P < 0.05$), implying the significance of horizontal transfer in their proliferation.

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1. Introduction

Antibiotic production generates waste streams with high concentrations of chemical oxygen demand (COD_{Cr}), residual antibiotics and ammonium, which are, in general, treated by biological processes (Larsson et al., 2007; Ma et al., 2009). However, high antibiotic concentrations and bacterial densities in biological wastewater systems may favor the proliferation of antibiotic resistance genes (ARGs), which could pose health risks to humans via various pathways (Li et al., 2010; Munir et al., 2011; Zhang et al., 2013). Our previous study has revealed that large amounts of tetracycline resistance genes were generated during aerobic treatment of oxytetracycline (OTC) production waste stream (Liu et al., 2012). On the other hand, the anaerobic process has been widely used for the treatment of industrial wastewater containing high concentration of COD_{Cr} (Lettinga, 1995). At the same time, anaerobic digestion of excess sludge has been found to effectively reduce ARGs (Diehl and Lapara, 2010). However, the behaviors of ARGs during anaerobic wastewater treatment processes under high antibiotic levels have been seldom reported.

Macrolides, which could inhibit a wide range of bacteria, are a group of antibiotics frequently used in clinics and feedlots (Iacoviello and Zinner, 2002). Macrolides share an overlapping binding site on bacterial ribosome with structurally distinct lincosamides and streptogramins, leading to clusters of genes encoding resistance to these antibiotics, called Macrolide-Lincosamide-Streptogramin (MLS) resistance genes (Sutcliffe and Leclercq, 2002). MLS resistance could be divided into three groups according to their resistance mechanisms: rRNA methylase genes, efflux genes, and enzymatic modification genes (Roberts et al., 1999). To date, 79 MLS resistance genes have been identified in a variety of gram-positive and gram-negative bacteria from human and animal origins (http://faculty.washington.edu/marilynr/). Recently, the occurrence of MLS resistance genes has gained increasing concerns. Chen et al. (2007) and Koike et al. (2010) surveyed rRNA methylase genes in swine feedlot environment and found that *erm*(B) and *erm*(F) had the highest relative abundance. Negreanu et al. (2012) reported that the relative abundances of *erm*(B) and *erm*(F) were at the levels of 10^{-4}–10^{-3} in the raw influent and aerobic sludge of sewage treatment plants. However, the behaviors of MLS resistance genes during the treatment of macrolide production wastewater with high antibiotic levels have not yet been clarified.

In this work, we surveyed the occurrence, abundance, and distribution of MLS resistance genes in a pilot-scale biological system consisting of anaerobic, anoxic, and aerobic units in succession to treat spiramycin production waste stream (SPM wastewater) and its mixture with two other antibiotic production waste streams (paromomycin and ribostamycin) over a period of 9 months. Fifteen MLS resistance genes, which conduct different resistance mechanisms and have been reported in the environment previously, were screened by conventional PCR. Eight detected genes (*erm*(B), *erm*(F), *erm*(T), *erm*(X), *msr*(D), *mef*(A), *ere*(A), *mph*(B)) were determined using quantitative PCR (qPCR), together with three mobile elements (*intI1*, ISCR1, and Tn916/1545). At the same time, clone libraries were constructed to analyze bacterial compositions and diversities of MLS resistance genes. This study will help understand the fate of ARGs during anaerobic and aerobic wastewater treatment of high antibiotic levels.

2. Materials and methods

2.1. Pilot-scale wastewater treatment systems and sampling

SPM is a 16-member macrolide widely used in veterinary clinics. Samples were taken from a pilot-scale SPM wastewater treatment system consisting of sequential up-flow anaerobic sludge bed (UASB), anoxic reactor, and oxic reactor (Fig. 1). The effective reactor volumes of the three reactors were 600 L, 200 L, and 400 L, respectively. The flow rate of raw wastewater was 200 L/d. The hydraulic retention time (HRT) was 48 h for UASB and oxic reactors, and 24 h for the anoxic reactor. The UASB effluent was recirculated to the inlet at a dilution ratio of 2:1 (flow rate ratio of UASB effluent to raw wastewater). The mixed liquor from the oxic reactor was recirculated to the anoxic reactor at a ratio of 2:1 (flow rate ratio of oxic effluent to UASB effluent). Sludge from the sedimentation tank was returned to the anoxic reactor at a ratio of 1:1. Sludge from a citrate production wastewater treatment plant and a sewage treatment plant was inoculated into the UASB reactor at a volume ratio of 1:1. Inoculum for the anoxic and oxic reactors (A/O reactors) was collected from a full-scale SPM wastewater treatment plant in Jiangsu Province. The operation of the system was divided into three stages: receiving (i) SPM wastewater, (ii) mixture of SPM wastewater and paromomycin wastewater, and (iii) mixture of SPM

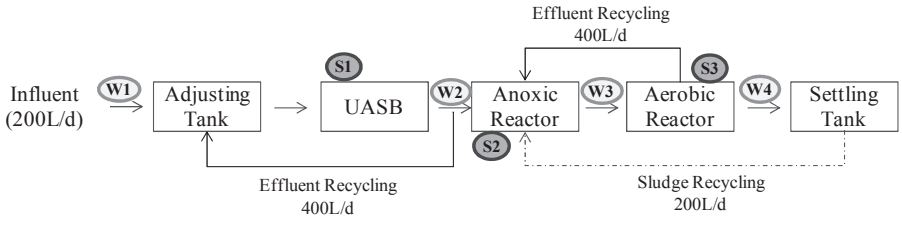

Fig. 1 – Scheme of the SPM wastewater treatment systems and sampling sites. UASB: Up-flow Anaerobic Sludge Bed Reactor. W1, W2, W3, W4: Raw influent and effluent from UASB, Anoxic, and Aerobic reactors. S1, S2, S3: Sludge from the UASB, Anoxic, and Aerobic reactors, respectively. $S1_1$ – $S3_1$; $S1_2$ – $S3_2$; $S1_3$ – $S3_3$: sludge samples from the three stages treating spiramycin, spiramycin and paramomycin, spiramycin and ribostamycin, respectively.