Discovery of Gram-Negative Sulfonamide Degraders from Municipal Activated Sludge

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Background/Objectives. The spread of antimicrobial resistance in the environment, especially its transfer into human pathogens, has emerged as a global concern. A culprit for the rise of antimicrobial resistance issues is misuse and/or unrestricted disposal of antibiotics, conducive to the prevalence of these persistent compounds that render the dissemination of antimicrobial resistance genes (ARGs). This is worse at municipal wastewater treatment plants (WWTPs) where antibiotics are frequently detected with relatively high concentrations. In this present study, we focus on characterizing sludge microorganisms that are capable of inactivating antibiotics and investigating their molecular foundations. Sulfamethoxazole (SMX) was selected as a representative for sulfonamides (SAs), a class of synthetic antibiotics that have been extensively used and frequently detected in surface and wastewater.

Approach/Activities. Active sludge samples were collected from two municipal WWTPs located in northern New Jersey. Prior to the enrichment, 25 mL of activated sludge was diluted equally with the wastewater obtained from the same aeration tank. Then, 50 µL of a filter-sterilized SMX stock (50 g/L in methanol) was transferred into an autoclaved amber 250-mL glass bottle. After methanol was evaporated, 50 mL of diluted sludge was added and incubated on a rotary shaker at 130 rpm and room temperature (25 ºC). Degradation of SMX was monitored during the enrichment. After two months of incubation, SMX-degrading consortia were streaked on ammonium mineral salts (AMS) agar plates containing 200 mg/L of SMX. After incubation at 30 ºC, morphologically distinct colonies were obtained. Individual colonies were inoculated to 10 mL AMS amended with 0.05 % yeast extract or 200 mg/L of sodium acetate and 50 mg/L of SMX to verify the biodegradation of SMX. The taxonomy of selected isolates was identified by 16S rRNA gene sequencing analysis. Complete genomes of these two isolates were sequenced using long-read Nanopore Minion Technology, which will be compared with those of 6 gram-positive actinomycetes available on NCBI.

Results/Lessons Learned. We successfully isolated two SMX degraders, *Lysobacter* sp. RD1 and *Xanthobacter* sp. LD2, from the enrichment of activated sludge samples from two different WWTPs located in northern New Jersey. In both strains, along with the degradation of SMX, accumulation of 3-amino-5-methylisoxazole (3A5MI) was observed by HPLC, indicating both strains can cleave the S-N bond and subsequently dismiss the antimicrobial effort of SMX. We also designed a set of degenerate primers targeting *sadA/sulX* genes that encode the class D FMNH2-dependent monooxygenases, known for their ability of catalyzing the breakdown of S-N linkage in SAs. Surprisingly, *sadA/sulX* genes have been positively detected in both RD1 and LD2. This is the first report revealing the occurrence of *sadA/sulX* genes in gram-negative bacteria prevailing in the diverse environments. We will primarily investigate the phyletic patterns of these antibiotic inactivation genes and surrounding mobile elements to elucidate molecular evidence for horizontal gene transfer (HGT) processes and predict donors, recipients, and co-acquired genes. This study will ultimately advance our understanding of HGT of antibiotic resistant genes, or, in other words, the evolution of antibiotic resistant bacteria. The identification of novel antibiotic inactivation genes and the associated molecular processes will enable us to design efficient methods for eliminate antibiotics and thus decelerate the dissemination of antibiotic resistance.