Isolation and Characterization of Nitroguanidine-Degrading Bacteria

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Background/Objectives. Nitroguanidine (NQ) is one of the insensitive munitions that are resistant to exposure due to unanticipated stimuli such as impact, friction, heat, or sparks. NQ is also used to synthesize various organic compounds and herbicides. Exposure to NQ can cause acute and chronic toxicity to mice, aquatic organisms, or plants. Despite the wide application of NQ and its associated toxicity, cost-effective treatment of NQ is currently not available. Mostly, little is known about biodegradation of NQ and only one bacterium, *Variovorax* strain VC1, capable of using NQ as a sole nitrogen source has been reported. Thus, the goal of this study is to enhance our knowledge of NQ degradation particularly with respect to isolation and characterization of microorganisms capable of utilizing NQ for growth.

Approach/Activities. Enrichment cultures were first established using one for the following inocula: soils, sediments, and biomass from a membrane bioreactor treating various types of insensitive munitions including NQ. The established enrichment cultures were used for agar plate streaking. Colonies showing ability to grow on glucose with NQ as a sole nitrogen source were further tested for their ability to grow in liquid medium containing glucose and NQ. The confirmed isolates were further screened for their ability to use different nitrogen sources including analogs of NQ, NH_4^+, NO_3^-, and NO_2^-. Phylogenetic analysis was conducted to identify taxa of the isolates. Further tests were conducted to evaluate whether inorganic nitrogen addition could inhibit isolate growth during NQ degrading conditions.

Results/Lessons Learned. Three isolates (designated as NQ4, NQ7, NQ5) capable of using NQ as the sole nitrogen source, not as a carbon source, were obtained. The isolates degrade NQ with varying rates. NQ5 grew 5~6 times faster than NQ4 or NQ7. Strains NQ4 and NQ7 were closely associated with *Pseudarthrobacter* spp. while NQ5 was closely related to *Pseudomonas* spp. Strains NQ4 and NQ7 can utilize NH_4^+, NO_3^-, or NO_2^- as the sole nitrogen source while NQ5 is unable to utilize NO_2^-. All isolates were able to utilize urea and guanidine while NQ5 solely utilized guanylurea and ethyl allophanate as sole nitrogen sources. All strains did not grow on biuret. The addition of other nitrogen sources such as NH_4^+ or NO_3^- to NQ-grown NQ5 appeared to inhibit the use of NQ by the strain. On-going research efforts are to identify catabolic genes responsible for NQ degradation, and whole genome sequencing and gene annotation.