Firmicutes and Their Roles in Uranium Immobilization

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Bioremediation of uranium in subsurface is a great challenge. To evaluate the natural occurring uranium-removing bacterial populations, we have anaerobically enriched uranium contaminated soil sediments (FW107, FW102-2, and FW102-3) collected from ORNL iFRC site (S3 area). Electron acceptors, NO^{3-}, Fe^{3+}, and U^{6+}, were supplemented in the enrichments as selection factors. We previously reported the shifts of bacterial community profiles in enrichments. After two serial transferring and enriching, bacterial 16S rRNA gene survey revealed that Fe^{3+} and U^{6+} supplemented enrichments shared similar community structures. Genus *Pelosinus* was found dominant in Fe^{3+} and U^{6+} enriched FW107 microcosms. This group of bacteria was also present in FW102-2 and FW102-3 enrichments but substantially less abundant. Unclassified *Clostridiales* and *Geobacter* groups were most abundant in Fe^{3+} and U^{6+} supplemented FW102-2 microcosms, respectively. Genus *Geobacter* was dominating the FW102-3 community after it was enriched with Fe^{3+} and U^{6+}. Hence, we suspect that Firmicutes might play a significant role in subsurface uranium removal.

With our goal of identifying unknown uranium immobilizers in mind, we have isolated 54 bacterial isolates from Fe^{3+} and U^{6+} supplemented enrichments. The growth rates of these isolates at 25ºC vary from 6 hours to 22 hours per generation. All isolates belong to phylum Firmicutes and can be categorized into four groups, *Clostridium* XI, *Clostridium* XIVa, *Clostridium sensu stricto*, and *Pelosinus*, based on their 16S rRNA gene sequences. Majority of the sequences in *Clostridium* XIVa group were identical to the 16S rRNA gene sequence of *Desulfotomaculum guttoideum*, which is a rarely studied sulfate-reducing bacterium. At 95% to 97% similarity, sequences in *Clostridium sensu stricto* group were closely related to *Clostridium acetobutylicum*, C*lostridium butyricum,* and *Clostridium puniceum*. While *Clostridium acetobutylicum* was recently reported as a uranium reducing bacterium, the other two were mostly studied for their chemical producing ability, such as butanol and 1,3-propandiol. The *Pelosinus* isolates we have identified were closely related to *Pelosinus sp*. strain UFO1, which was recently reported for its ability in multi-mode uranium immobilization. Out of 54 isolates, only one was identified as *Clostridium venationis* in group *Clostridium* XI. However, no studies have been reported on this species. In light of these taxonomy information and literature reports, we are in the process of evaluating the uranium immobilization rate by these isolates. Preliminary studies have shown that these isolates are resistant to 250μM of U^{6+}. We also performed rep-PCR to select the representative isolates to determine their U^{6+} removal rates. Currently, we are studying the U^{6+} immobilization by these representatives as well as the co-cultures of these isolates to evaluate the importance of mixed Firmicute populations in U^{6+} bioremediation.