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Enrichment of hydrogen-consuming microorganisms and their interaction with metal ions in groundwater at KAERI Underground Research Tunnel site, Daejeon, South Korea

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In a radioactive waste disposal environment, hydrogen can be generated by the corrosion of copper [1] and steel [2] in an anaerobic environment. This accumulation of hydrogen leads pressure buildup, which can results in structural instability and fractures. As hydrogen-consuming bacteria present in groundwater are known to help mitigate these risks [3], interest in their metabolism and interactions with metal ions in radioactive waste disposal environments has been increasing. The objective of this study was to enrich hydrogen-consuming bacteria from the groundwater of the Korea Atomic Energy Research Institute (KAERI) Underground Research Tunnel (KURT) and to investigate the microbial diversity and characteristics of their interactions with metal ions.

Groundwater for microbial enrichment was collected from 340 m below the surface using a multi-packer system at the KURT facility in Daejeon, Korea. Enrichment of hydrogen-consuming microorganisms was performed at 25°C by adding 1 mL of groundwater to 100 mL of growth medium containing 5 mM ferric-citrate and a 5% H_2-95% N_2 gas. For comparison, the microorganisms were enriched in the same manner in a medium containing sodium acetate instead of hydrogen gas. Microbial growth was monitored by observing changes in the color and turbidity of the growth medium. The microbial diversity of the enriched cultures was assessed using next-generation sequencing (NGS) metagenomic analysis.

To date, the KURT groundwater sample was primarily composed of *Thiobacillus* (22.1%), *Herminiimonas* (18.6%), and other genera. However, the enriched culture in the H_2 gas-purged medium was predominantly *Paludirhabdus* (99.4%) and identified as the species *Paludirhabdus telluriireducens*. In contrast, the enriched culture with sodium acetate as an electron donor was predominantly *Neobacillus* (90%) and identified as the species *Neobacillus niacini*.

We are currently investigating how the enriched hydrogen-consuming microorganisms interact with metal ions such as iron and copper, and contribute to precipitate formation. These results offer insight into the role of hydrogen-consuming microorganisms in the geological environment of radioactive waste disposal and are expected to help improve the long-term stability of waste repositories.

[1] Hultquist et al. (2009); [2] Libert et al. (2011); [3] Collet et al. (2005)