# Isolation of the first thermophilic and actively nitrogen-fixing bacteria in the deep branching phylum *Aquificae*

*Arisa Nishihara[1], Katsumi Matsuura[1], Marcus Tank[1], Shawn E McGlynn[2,3,4], Vera Thiel[1], Shin Haruta[1]

1. Tokyo Metropolitan University, 2. Earth-Life Science Institute, Tokyo Institute of Technology, 3. Biofunctional Catalyst Research Team, RIKEN Center for Sustainable Resource Science, 4. Blue Marble Space Institute of Science

Thermophilic nitrogen-fixing bacteria have been suggested to occur in hydrothermal vents and terrestrial hot springs from molecular and functional-based analyses of prokaryotic communities. Nif genes, biomarkers for nitrogen-fixing bacteria, already have been found in some chemosynthetic thermophilic isolates, too. Although nitrogen-fixing ability was observed in methanogenic archaea at 92ºC, active nitrogen fixation in thermophilic bacteria more than 70ºC has not been demonstrated yet.
In this study, we isolated two novel *Aquificae*, a deeply branching bacterial phylum, into axenic culture under nitrogen-fixing conditions from chemosynthetic microbial communities at 70-77ºC in sulfidic alkaline hot springs (Nakabusa, Nagano, Japan). Phylogenetic analysis based on 16S rRNA gene classified both strains within the genus *Hydrogenobacter*; strain 1-6 showed 98.7% nt identity to *Hydrogenobacter subterraneus* HGP1 and strain 2-18 had 97.6% identity to *H. hydrogenophilus* DSM 2913. Both isolated strains contained *nifH* gene sequences, encoding a key enzyme component of nitrogen fixation, with 96.5% and 97.4% amino acid identity to *Hydrogenobacter thermophilus* TK-6. Nitrogenase activities were confirmed in both strains incubated at 70ºC using the acetylene reduction test. Both strains showed nitrogen-gas-dependent growth under lowered aerobic conditions with approximately up to 10% oxygen using $CO_2$ as a sole carbon source and $N_2$ as sole nitrogen source with $H_2$ or thiosulfate as electron donors at 70ºC.
This is the first demonstration of active nitrogen-fixation in thermophilic bacteria (more than 70ºC) and in the phylum *Aquificae*. The potential impact of nitrogen fixation in thermophilic chemosynthetic bacteria will be discussed with respect to life on earth before the appearance of photosynthesis.

Keywords: Aquificales, nitrogen fixation, geothermal spring, thermophile, chemosynthetic bacteria

 