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

*西原 亜理沙[1]、松浦 克美[1]、Tank Marcus [1]、McGlynn Shawn[2,3,4]、Thiel Vera[1]、春田 伸[1]
*Arisa Nishihara[1], Katsumi Matsuura[1], Marcus Tank[1], Shawn E McGlynn[2,3,4], Vera Thiel[1], Shin Haruta[1]

1. 首都大学東京、2. 東京工業大学 地球生命研究所、3. 理化学研究所 環境資源科学研究センター、4. Blue Marble Space Institute of Science
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.

キーワード：Aquificales、nitrogen fixation、geothermal spring、thermophile、chemosynthetic bacteria
Keywords: Aquificales, nitrogen fixation, geothermal spring, thermophile, chemosynthetic bacteria

