Summary of Doctoral Dissertation (Doctoral Program (Science))

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Title: Growth capability on ammonium as the electron source found in sulfate-reducing bacteria and anoxygenic photosynthetic bacteria

(Japanese): 硫酸還元細菌および酸素非発生型光合成細菌に見出したアンモニアを電子源とする増殖能（英文）

Summary

Ammonium is a thermodynamically valuable electron source, but organisms that oxidize ammonium for growth under anaerobic conditions have yet to be isolated. Anaerobic ammonium oxidizing pathway is a missing link in the global nitrogen cycle. In this study, I explored sulfate-reducing ammonium oxidizing bacteria (SR-AOB) and photosynthetic ammonium oxidizing bacteria (PS-AOB).

For SR-AOB, sediments collected from Onikobe Hot Springs (Miyagi) were anaerobically cultivated in an autotrophic medium containing NH_4^+ and SO_4^{2-} at 55°C. After the repetitive subcultivations, a sulfate-reducing bacterium, strain WS belonging to the genus *Thermodesulfomicrobium*, was successfully isolated from the enrichment culture. When NH_4^+ in the medium was replaced with another nitrogen source, NO_3^-, no growth was observed, indicating NH_4^+ supported the growth of this strain as the electron source and nitrogen source. During the growth, NH_4^+ consumption was confirmed, but N_2, N_2O, NO_2^-, and NO_3^- were not detected. I assumed that NO was the possible oxidized product and that this free radical suppressed the growth. Thus, I examined the effects of a strong NO-scavenging reagent on growth. The addition of the NO-scavenger stimulated the growth on NH_4^+. These results indicated that the isolated strain WS oxidized NH_4^+ to NO by sulfate respiration.

For PS-AOB, I examined the NH_4^+-dependent growth capability in thermophilic anoxygenic photosynthetic bacteria in the genus *Chloroflexus*. *Chloroflexus* has been known to be ancient photosynthetic bacteria in the deeply branching lineage and metabolically versatile, *e.g.*, anaerobic photoautotrophy and aerobic chemoautotrophy. Among strains tested, stable growth on NH_4^+ as the sole electron source was observed in *Chloroflexus aggregans* NA9-6, isolated from Nakabusa Hot Springs (Nagano). NH_4^+ consumption during the growth was confirmed, and the consumed amount was larger than the expected amount of NH_4^+, which was required as the nitrogen source. Productions of NO_2^-, NO_3^-, and gaseous compounds, including N_2 and N_2O, were not detected. A NO-scavenging reagent did not affect the initial growth on NH_4^+ but increased the final growth yield. These results suggested that NO was the oxidized product of NH_4^+ by this photosynthetic bacterium.

To the best of my knowledge, these were the first findings showing that sulfate-reducing bacteria and anoxygenic photosynthetic bacteria can utilize NH_4^+ as the electron source for the growth. Enzymes that anaerobically oxidize NH_4^+ to NO have never been known. These phylogenetically and physiologically different two bacteria possibly possess novel enzymes for NH_4^+ oxidation.