学位論文内容の要旨

博士の専攻分野名称：博士（農学）　　氏　名：Yanxia Nie

学位論文題名

Physiological and genetic traits of the N_2O-emitting *Proteobacteria* isolated from latent hot spots for N_2O emission, and their response to environmental factors including plant polyphenols

（潜在的N_2O放出ホットスポットから分離したN_2O放出能をもつグラム陰性細菌の細菌生理学的および分子遺伝学的性状と，植物ポリフェノールを含めた環境諸因子に対するそれら分離細菌株の応答）

Although acidic lands all over the world are known to have potentials to be an active spot of nitrous oxide (N_2O) emission, major microbial contributors to the N_2O efflux and mechanisms behind the frequent emergence of active N_2O emitters from the acidic and fertilized soils still remain unclear. Screening of several active N_2O emitters from soil or phytoepiphytic bacteria and analyses of their metabolic traits for the inorganic nitrogen and functional genes associated with N_2O production were attempted to give an answer to this fundamental query.

1. Isolation of N_2O-emitting *Pseudomonas* denitrifiers having lost their N_2O-reductase activity from dent corn Andisol farmland in Hokkaido

Ten bacterial isolates found as relatively active N_2O emitters from Andisol corn farmland were identified as genus *Pseudomonas* by 16S rRNA gene sequencing. As all of them accepted NO_3^- as the substrate for N_2O production but not NH_4^+, they were characterized as heterotrophic denitrifiers. Remarkable acceleration of N_2O emission by the *Pseudomonas* bacteria in the presence of 1.5–15 mM sucrose supported this speculation. Acetylene inhibition assay showed negative responses on six active N_2O emitters among the ten isolates, suggesting that these N_2O emitters were likely atypical, incomplete denitrifiers that have lost their activity of N_2O-reductase. Their negative results in PCR assay for detection of *nosZ* also suggested that all of the six isolates are *nosZ* gene-missing denitrifiers to omit their ability to reduce N_2O into N_2O.

2. N_2O emission potentials of *Burkholderia* species isolated from the leaves of a boreal peat moss *Sphagnum fuscum*

Using a culture-based N_2O emission assay, three active N_2O emitters were isolated from *Sphagnum fuscum* leaves and all identified as members of *Burkholderia*. These isolates showed N_2O emission in the medium supplemented with NO_3^- but not with NH_4^+, and *Burkholderia* sp. SF-E2 showed the most efficient N_2O emission (0.20 $\mu g \cdot vial^{-1} \cdot day^{-1}$) at 1.0 mM KNO_3. In *Burkholderia* sp. SF-E2, the optimum pH for N_2O production was 5.0, close to that of the phyllosphere of *Sphagnum* mosses, while the optimum temperature was uniquely over 30 °C. The stimulating effect of additional 1.5 mM sucrose on N_2O emission was ignorable, but *Burkholderia* sp. SF-E2 upon exposure to 100 $mg \cdot L^{-1}$ *E*-caffeic acid showed uniquely 67-fold higher N_2O emission than control. All of three N_2O emitters were negative in both acetylene inhibition assay and PCR assay for *nosZ*-detection, suggesting that N_2O reductase or the gene itself is missing in the N_2O-emitting *Burkholderia*.

3. Isolation of hyper-active N_2O emitting *Pseudomonas* sp. SC-H2 from *Sphagnum capillifolium* in palsa bog

As *Sphagnum* moss-dominant palsa bogs mainly composed of *Sphagnum capillifolium* and *S. fuscum,* and degrading palsa bog often becomes a hot spot for N_2O emission. Some gammaproteobacterium isolated from the *S. capillifolium* showed hyper active N_2O emitting capability in the culturing systems, and the most active N_2O emitter from the culturable community was identifiable as *Pseudomonas* sp. by 16S rRNA gene-targeted homology search. The N_2O emitting *Pseudomonas* sp. SC-H2 showed over 20 $\mu g\ vial^{-1}\ day^{-1}$ of N_2O production in 10 mL culture medium containing 0.05% sucrose only at neutral pH (6.8-7.3) but not at acidic regions. As its unique characteristic, *nosZ* gene-harboring *Pseudomonas* sp. SC-H2 skipped reduction process for N_2O in the neutral to alkaline regions to produce high level of N_2O.