



HOKKAIDO UNIVERSITY

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学位論文内容の要旨

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Physiological and genetic traits of the N₂O-emitting *Proteobacteria* isolated from latent hot spots for N₂O emission, and their response to environmental factors including plant polyphenols

（潜在的N₂O放出ホットスポットから分離したN₂O放出能をもつグラム陰性細菌の細菌生理学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 (N₂O) emission, major microbial contributors to the N₂O efflux and mechanisms behind the frequent emergence of active N₂O emitters from the acidic and fertilized soils still remain unclear. Screening of several active N₂O emitters from soil or phytoepiphytic bacteria and analyses of their metabolic traits for the inorganic nitrogen and functional genes associated with N₂O production were attempted to give an answer to this fundamental query.

1. Isolation of N₂O-emitting *Pseudomonas* denitrifiers having lost their N₂O-reductase activity from dent corn Andisol farmland in Hokkaido

Ten bacterial isolates found as relatively active N₂O emitters from Andisol corn farmland were identified as genus *Pseudomonas* by 16S rRNA gene sequencing. As all of them accepted NO₃⁻ as the substrate for N₂O production but not NH₄⁺, they were characterized as heterotrophic denitrifiers. Remarkable acceleration of N₂O 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 N₂O emitters among the ten isolates, suggesting that these N₂O emitters were likely atypical, incomplete denitrifiers that have lost their activity of N₂O-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 N₂O into N₂.

2. N₂O emission potentials of *Burkholderia* species isolated from the leaves of a boreal peat moss *Sphagnum fuscum*

Using a culture-based N₂O emission assay, three active N₂O emitters were isolated from *Sphagnum fuscum* leaves and all identified as members of *Burkholderia*. These isolates showed N₂O emission in the medium supplemented with NO₃⁻ but not with NH₄⁺, and *Burkholderia* sp. SF-E2 showed the most efficient N₂O emission (0.20 µg·vial⁻¹·day⁻¹) at 1.0 mM KNO₃. In *Burkholderia* sp. SF-E2, the optimum pH for N₂O 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 N₂O emission was ignorable, but *Burkholderia* sp. SF-E2 upon exposure to 100 mg·L⁻¹ *E*-caffeic acid showed uniquely 67-fold higher N₂O emission than control. All of three N₂O emitters were negative in both acetylene inhibition assay and PCR assay for *nosZ*-detection, suggesting that N₂O reductase or the gene itself is missing in the N₂O-emitting *Burkholderia*.

3. Isolation of hyper-active N₂O 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 N₂O emission. Some gammaproteobacterium isolated from the *S. capillifolium* showed hyper active N₂O emitting capability in the culturing systems, and the most active N₂O emitter from the culturable community was identifiable as *Pseudomonas* sp. by 16S rRNA gene-targeted homology search. The N₂O emitting *Pseudomonas* sp. SC-H2 showed over 20 µg vial⁻¹ day⁻¹ of N₂O 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 N₂O in the neutral to alkaline regions to produce high level of N₂O.