生理学的及び遺伝的性質を有するN₂O排出微生物の探る研究：暖かい場所でのN₂O排出，及ぼす環境因子への応答

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theses (doctoral - abstract and summary of review)

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Nie_Yanxia_review.pdf (審査の要旨)
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

The thesis of 109 pages consists of 6 sections in the body of text with 136 literatures cited, 41 figures, 5 tables, and 4 schemes. One original article is attached as a reference paper. This study first performed screening, isolation, and characterization of N₂O emitting bacteria from Sphagnum mosses in palsa bogs in Finland. Physiological traits of the active isolates were further investigated, and environmental factors including plant polyphenols and pH provided acceptable speculations for fundamental mechanisms behind the frequent emergence of active N₂O emitters from the boreal acidic soils.

1. Isolation of N₂O emitting-bacteria from 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 clear acceleration of its N₂O emission. 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.
2. **Comparison of N\textsubscript{2}O-emitting bacteria in epiphytic and/or endophytic bacterial community of *Sphagnum fuscum* and *Sphagnum capillifolium***

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\textsubscript{2}O emission. Some gammaproteobacteria isolated from the *S. capillifolium* showed hyper active N\textsubscript{2}O emitting capability in the culturing systems, and the most active N\textsubscript{2}O emitter from the culturable community was identifiable as *Pseudomonas* sp. by 16S rRNA gene-targeted homology search. The N\textsubscript{2}O emitting *Pseudomonas* sp. SC-H2 showed over 20 μg vial\textsuperscript{-1} day\textsuperscript{-1} of N\textsubscript{2}O 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\textsubscript{2}O in the neutral to alkaline regions to produce high level of N\textsubscript{2}O.

3. **Influence of plant polyphenols on N\textsubscript{2}O emitters for their N\textsubscript{2}O emission***

All three *Burkholderia* isolates from leaves of *S. fuscum* were responsive to gallic acid and (E)-caffeic acid, and the most activated *Burkholderia* sp. SF-E2 showed 67-fold higher N\textsubscript{2}O emission upon exposure to 100 mg L\textsuperscript{-1} (E)-caffeic acid. Relatively low concentration of (E)-caffeic acid (≤ 0.1 g L\textsuperscript{-1}) also accelerated N\textsubscript{2}O emission by an *Enterobacteriaceae* bacterium SC-L1 and *Serratia* sp. SC-K1. Among them, *Serratia* sp. SC-K1 was 13-fold higher of N\textsubscript{2}O production with 0.1 g L\textsuperscript{-1} than that without (E)-caffeic acid. On *Pseudomonas* sp. SC-H2, N\textsubscript{2}O emission significantly decreased when concentration of (E)-caffeic acid was supplemented more than 0.01 g L\textsuperscript{-1}.

4. **Comparison of phylogenetic patterns of denitrification-associated genes and 16S rRNA genes among *Pseudomonas* N\textsubscript{2}O-emitters isolated from Andisol***

For *Pseudomonas* denitrifiers isolated from Andisol corn farmland in Hokkaido, phylogenetic trees of *nosZ*, *nirS*, and *narG* genes were compared with that of their 16S rRNA genes. The *nirS*, and *narG* genes had a close relationship with phylogeny of 16S rRNA genes. In contrast, there was no similarity between *nosZ* and 16S rRNA gene phylogenetic trees, suggesting that *nosZ* gene is more dynamic and often missing in the bacterial genome.

Thus, the thesis study revealed the presence of a clear linkage between N\textsubscript{2}O emission from degraded palsa bogs and *Sphagnum* moss-associating eubacteria, particularly those from *Sphagnum fuscum* and Sphagnum capillifilium. Characterization of these key players in N\textsubscript{2}O emission may offer a clue into future prospects for regulation of N\textsubscript{2}O production in disturbed boreal peatlands.

Therefore, we acknowledge that the author is qualified to be granted the Degree of Doctor of Philosophy in Agriculture from Hokkaido University.