学位論文審査の要旨

博士の専攻分野の名称　博士（農学）　氏　名　Yanxia Nie

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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)

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 N_2O 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 N_2O emitters from the boreal acidic soils.

1. Isolation of N_2O emitting-bacteria from *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 μg $vial^{-1}$ 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 L^{-1} (*E)*-caffeic acid showed clear acceleration of its N_2O emission. 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*.

2. Comparison of N_2O-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_2O emission. Some gammaproteobacteria 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 μg $vial^{-1}$ 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.

3. Influence of plant polyphenols on N_2O emitters for their N_2O 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_2O emission upon exposure to 100 mg L^{-1} (*E*)-caffeic acid. Relatively low concentration of (*E*)-caffeic acid (≤ 0.1 g L^{-1}) also accelerated N_2O emission by an *Enterobacteriaceae* bacterium SC-L1 and *Serratia* sp. SC-K1. Among them, *Serratia* sp. SC-K1 was 13-fold higher of N_2O production with 0.1 g L^{-1} than that without (*E*)-caffeic acid. On *Pseudomonas* sp. SC-H2, N_2O emission significantly decreased when concentration of (*E*)-caffeic acid was supplemented more than 0.01 g L^{-1}.

4. Comparison of phylogenetic patterns of denitrification-associated genes and 16S rRNA genes among *Pseudomonas* N_2O-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_2O emission from degraded palsa bogs and *Sphagnum* moss-associating eubacteria, particularly those from *Sphagnum fuscum* and Sphagnum *capilifilium*. Characterization of these key players in N_2O emission may offer a clue into future prospects for regulation of N_2O 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.