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 [an abstract of dissertation and a summary of dissertation review]

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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

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 N₂O.
2. N$_2$O emission potentials of *Burkholderia* species isolated from the leaves of a boreal peat moss *Sphagnum fuscum*

Using a culture-based N$_2$O emission assay, three active N$_2$O emitters were isolated from *Sphagnum fuscum* leaves and all identified as members of *Burkholderia*. These isolates showed N$_2$O emission in the medium supplemented with NO$_3^-$ but not with NH$_4^+$, and *Burkholderia* sp. SF-E2 showed the most efficient N$_2$O emission (0.20 μg·vial$^{-1}$·day$^{-1}$) at 1.0 mM KNO$_3$. In *Burkholderia* sp. SF-E2, the optimum pH for N$_2$O 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$_2$O emission was ignorable, but *Burkholderia* sp. SF-E2 upon exposure to 100 mg·L$^{-1}$ E-caffeic acid showed uniquely 67-fold higher N$_2$O emission than control. All of three N$_2$O emitters were negative in both acetylene inhibition assay and PCR assay for nosZ-detection, suggesting that N$_2$O reductase or the gene itself is missing in the N$_2$O-emitting *Burkholderia*.

3. Isolation of hyper-active N$_2$O 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$_2$O emission. Some gammaproteobacterium isolated from the *S. capillifolium* showed hyper active N$_2$O emitting capability in the culturing systems, and the most active N$_2$O emitter from the culturable community was identifiable as *Pseudomonas* sp. by 16S rRNA gene-targeted homology search. The N$_2$O emitting *Pseudomonas* sp. SC-H2 showed over 20 μg·vial$^{-1}$·day$^{-1}$ of N$_2$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$_2$O in the neutral to alkaline regions to produce high level of N$_2$O.