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 N\textsubscript{2}O-emitting \textit{Proteobacteria} isolated from latent hot spots for N\textsubscript{2}O 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 (N\textsubscript{2}O) emission, major microbial contributors to the N\textsubscript{2}O efflux and mechanisms behind the frequent emergence of active N\textsubscript{2}O emitters from the acidic and fertilized soils still remain unclear. Screening of several active N\textsubscript{2}O emitters from soil or phytoepiphytic bacteria and analyses of their metabolic traits for the inorganic nitrogen and functional genes associated with N\textsubscript{2}O production were attempted to give an answer to this fundamental query.

1. \textbf{Isolation of N\textsubscript{2}O-emitting \textit{Pseudomonas} denitrifiers having lost their N\textsubscript{2}O-reductase activity from dent corn Andisol farmland in Hokkaido}

Ten bacterial isolates found as relatively active N\textsubscript{2}O emitters from Andisol corn farmland were identified as genus \textit{Pseudomonas} by 16S rRNA gene sequencing. As all of them accepted NO\textsubscript{3}\textsuperscript{-} as the substrate for N\textsubscript{2}O production but not NH\textsubscript{4}\textsuperscript{+}, they were characterized as heterotrophic denitrifiers. Remarkable acceleration of N\textsubscript{2}O emission by the \textit{Pseudomonas} bacteria in the presence of 1.5–15 mM sucrose supported this speculation. Acetylene inhibition assay showed negative responses on six active N\textsubscript{2}O emitters among the ten isolates, suggesting that these N\textsubscript{2}O emitters were likely atypical, incomplete denitrifiers that have lost their activity of N\textsubscript{2}O-reductase. Their negative results in PCR assay for detection of \textit{nos}Z also suggested that all of the six isolates are \textit{nos}Z gene-missing denitrifiers to omit their ability to reduce N\textsubscript{2}O into N\textsubscript{2}O.
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.