



HOKKAIDO UNIVERSITY

Title	Studies on physiological characteristics of Pseudomonas denitrifiers isolated from post-harvest soil of dent corn Andisol farmland, and regulative approach of those nitrous oxide (N ₂ O) emitters relevant to active N ₂ O efflux from the soil [an abstract of dissertation and a summary of dissertation review]
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Studies on physiological characteristics of *Pseudomonas* denitrifiers isolated from post-harvest soil of dent corn Andisol farmland, and regulative approach of those nitrous oxide (N₂O) emitters relevant to active N₂O efflux from the soil

（収穫後デントコーン畑地黒ボク土壌から分離した*Pseudomonas*属脱窒細菌群の生理学的特徴と、その土壌の高い亜酸化窒素（N₂O）放出を担うN₂O生成細菌群の制御に関する研究）

The Andisol of Shizunai Experimental Livestock Farm of Hokkaido University is known to be a relatively active source of nitrous oxide (N₂O)-emittable eubacteria. Using a reproducible, culturing N₂O emission assay, culturable N₂O-emitting eubacteria were isolated from Andisol corn farmland in Hokkaido, and all the active N₂O emitters were identified as *Pseudomonas* spp., most of which are characterized as incomplete denitrifiers. Using these active pseudomonad N₂O emitters, effects of some chemicals on N₂O emission by soil microbial communities in farmed soils were investigated.

1. Isolation of N₂O-emittable *Pseudomonas* spp. and their characteristics

Andisol soil (5 g) obtained from a post-harvest dent corn farm at the Hokkaido University Shizunai Livestock Farm emitted N₂O at rate of 6 ng·d⁻¹. In contrast, the same soil supplemented with 1.5 mM sucrose and 0.1 mM substrates (regardless of NO₃⁻ or NH₄⁺, or both) produced 20- to 30-fold higher N₂O than the control. Denaturing gradient gel electrophoresis (DGGE), however, indicated that no significant differences in bacterial communities were observed among the soils, irrespective of the presence or absence of C- and/or N-source supplementation. Both *nosZ*-negative and *nosZ*-positive denitrifiers of *Pseudomonas* spp. were isolated in a screen for N₂O-emitting bacteria in post-harvest Andisol farm soil. The *nosZ*-negative bacterial isolates from post-harvest soil showed greater acceleration of the N₂O production than isolates obtained from unfertilized grasslands.

Corn farm soils collected in spring of the third year showed higher N₂O-emitting capabilities than those collected post-harvest. Therefore, it was speculated that the high N₂O emission potential of the corn farm soil is attributed to the high content of

saprophytic, incomplete pseudomonad denitrifiers. An active supply of organic substances from corn roots and sufficient fertilization of acidic soils could assist active N₂O emission via incomplete denitrification by saprophytic *Pseudomonas* spp. and their yearlong survival in soils. It was thus likely that seasonal change of the soil microbial communities in the Andisol farmland is highly related to development of crop root systems along with active N₂O emission from the soils.

2. Effects of herbicides and their related chemicals on N₂O emitters

Some commercial herbicides, a representative antimicrobial secondary metabolite of corn, 6-methoxy-2-benzoxazolone (MBOA), and *N*-heterocyclic compounds structurally related to MBOA were examined their effects on N₂O-emitting soil bacteria. In the N₂O emission assay, two N₂O-emitting eubacteria, the incomplete denitrifier *Pseudomonas* sp. 10CFM5-1B and *Pseudomonas* sp. 10CFM5-2D (both isolated from post-harvest Andisol corn farmland in Hokkaido), were used. It was found that methyl viologen dichloride (Paraquat®) at 2 µM significantly repressed N₂O emission by the active denitrifying bacteria. MBOA also repressed pseudomonad denitrifiers at a concentration of 10 µM. Other herbicides such as simazine (6-chloro-*N,N'*-diethyl-1,3,5-triazine-2,4-diamine) and amitrole (3-amino-1,2,4-triazole) accelerated N₂O emission by *Pseudomonas* sp. 10CFM5-1B at 2 or 10 µM. This study suggested that methyl viologen dichloride may have somewhat contributed to the repression of global warming by suppressing N₂O production from farm soils. Some herbicides, including amitrole and other triazole-type chemicals, may instead have potentials to activate N₂O emission from the fertilized soils.

3. Effect of C- and N-sources on Andisol microbial community structures and N₂O emission

In the preliminary soil incubation assay carried out in duplicate, supplementation of 1.5 mM sucrose to 5 g soil led to an N₂O emission at 46 ng N₂O·vial⁻¹·d⁻¹ (i.e., approximately 7-fold higher production than the control [6 ng N₂O·vial⁻¹·d⁻¹]), but supplementation of inorganic nitrogen showed almost no effects on N₂O emission. Conversely, a synergistic effect of the supplemented mineral nitrogen (NH₄⁺, NO₃⁻ or their equivalent mixture at 0.1 mM) and 1.5 mM sucrose was 21-31-fold higher than the control without sucrose (soil only). In this drastic change in N₂O emission, all the soils including the control showed almost the same DGGE profiles. This direct soil incubation assay in the gas-chromatographic vial suggested that both denitrifiers and unculturable ammonia-oxidizing bacteria in soil most contribute to active N₂O emission.