Phylogenetic and Functional Diversity of Denitrifying Bacteria Isolated from Rice Paddy Soil.

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Anaerobic layer of rice paddy soil, just a few millimeters below the top surface, has been shown to have a strong denitrifying activity. However, the microbial populations responsible for the denitrification in rice paddy have not been well characterized. Here, we report the diversity of denitrifying bacteria in paddy soil and their ability to reduce nitrous oxide (N₂O).

Microcosms were established using three different paddy soils of Japan by addition of nitrate and succinate as an electron acceptor and a donor, respectively. Denitrifying bacteria were isolated from the soil by our unique method using cell division inhibitors and a micromanipulator (functional single-cell isolation, FSC). The isolates were incubated under anaerobic condition in presence/absence of C₂H₂ (10%). The isolates were scored positive for N₂O-reducing activity if N₂O was accumulated only in the presence of C₂H₂.

A total of 212 denitrifiers isolated by FSC method were divided into 17 genera including Niastella, Massilia, and Dyella, which had not been reported previously as denitrifier. FSC method was shown as a powerful tool to isolate denitrifying bacteria actively growing in the soil under denitrifying condition. The dominant genera of the isolates were Bradyrhizobium and Chromobacterium. The dominant end-products of denitrification were different by isolate: Dyella and Niastella showed strong N₂O-reducing activity, whereas Cupriavidas, Vogesella, Aquabacterium and Bosea did not. Thus Dyella and Niastella could serve as N₂O reducer in rice paddy soil.