Identification and application of microorganisms responsible for nitrogen fixation and methane metabolism in paddy soil

Yoko Masuda (Graduate School of Agricultural and Life Sciences, The University of Tokyo)
yokomasuda@g.ecc.u-tokyo.ac.jp, ygigico@gmail.com

In paddy soil, microorganisms actively drive reductive nitrogen transformation (denitrification, dissimilatory nitrate reduction to ammonium (DNRA), and nitrogen fixation) and carbon metabolism (straw decomposition, methane generation/elimination, etc.). These microbial reactions are important for sustainable soil nitrogen fertility and greenhouse gases (N_2O and methane) emission. Previously, numerous studies attempted to identify the microbial communities involved in these reactions by culture-dependent methods or PCR-based methods. However, these methods underestimated microbial functions and diversity due to the presence of unculturable microorganisms and PCR bias. In this study, metatranscriptomic analysis was performed to elucidate the whole picture of microbial communities that actively drive reductive nitrogen transformation and methane metabolism in paddy soil.

Methane is emitted from paddy soil as a result of various microbial reactions, hydrogen production, acetogenesis, methanogenesis in lower reduced soil layer and methane oxidation in surface oxidized layer. Paddy soil metatranscriptomics revealed the microbial drivers of each reaction: *Anaeromyxobacter*, unclassified *Chloroflexi*, *Candidatus* Solibacter generate hydrogen, and *Bradyrhizobium, Azoarcus, Burkholderia,* and *Anaeromyxobacter* generate acetate. By utilizing hydrogen and acetate, *Methanocella*, *Methanoregula,* and *Methanosaeta* archaea generate methane. Part of the generated methane was oxidized by methanotrophs belonging to *Methylocystis* and *Methylogaea*. This study was the first to simultaneously elucidate the microbial consortia responsible for methane emission from paddy soil [1)].

Metatranscriptomics unveiled previously overlooked microbial consortia involved in reductive nitrogen transformation [2)]. Most surprisingly, *Anaeromyxobacter* and *Geobacter*, known as iron-reducing bacteria predominant in paddy soils, were suggested to be primary drivers of nitrogen fixation and contribute to soil nitrogen fertility. We therefore isolated a number of *Anaeromyxobacter* and *Geobacter* strains from paddy soils and verified their nitrogen-fixing ability in culture medium and paddy soil conditions [3)].

Iron-reducing bacteria utilize ferric iron as an electron acceptor for respiration. We supposed that adding ferric iron oxide could enhance nitrogen-fixing activity of *Anaeromyxobacter* and *Geobacter* in paddy soil. Actually, soil nitrogen-fixing activity significantly increased after adding ferrihydrite or Fe_2O_3 to laboratory soil microcosm and iron powder to paddy field soil, due to the enhancement of nitrogen fixation by the iron-reducing bacteria. This study would lead to novel paddy soil management strategies to increase soil nitrogen fertility and ensure rice yields with reduced nitrogen fertilizer input and lower environmental nitrogen burdens.

References

1) Masuda Y., Itoh H., Shiratori Y., and Senoo K.: Soil Sci Plant Nutr 64:455-464 (2018).
2) Masuda Y., Itoh H., Shiratori Y., Isobe K., Otsuka S., and Senoo K.: Microbes Environ 32:180-183 (2017).
3) Masuda Y., Yamanaka H., Xu Z., et al.: Appl Environ Microbiol 86: e00956-20 (2020).