Methanotrophic Bacterial Diversity in Soils

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Abstract

Soil samples under long-term (50 years) no-tillage, plow-tillage, grasslands and forests were collected and analyzed for methanotrophic bacterial diversity using *pmoA* and *16s rRNA* gene sequences as phylogenetic markers. DGGE of the amplicons were carried out, followed by sequence analysis of the excised bands. Phylogenetic analysis revealed 84-97% sequence similarity to *M. album* and *M. capsulatus and over 98% sequence similarity to M.* sp. SC2, *M. parvus*, *M. albus* and *M.* sp. LW14. Soils from no-till plots showed greater methanotrophic species diversity as compared to that from plow-till. Between grass and forest soils, the latter exhibited higher species diversity.

Fig. 1. Agricultural fields showing plow (left) and no-tillage (right) practices.

Introduction

Methanotrophs, or methane-oxidizing bacteria (MOB) present in aerobic soil, serve as the only known biological sink for atmospheric CH_4. Agriculture, amongst other land-use practices, impacts the rate of CH_4 oxidation with a number of studies indicating that varying tillage practices have different rates of CH_4 oxidation[1].

Methanotrophs in soil are grouped into Type I and Type II categories based on the oxidation pathway followed and use methane monooxygenases to catalyze the oxidation of CH_4. The enzymes catalyze the following process:

$$CH_4 + O_2 + NADH + H^+ \rightarrow CH_3OH + H_2O + NAD^+$$

Sequences of the methane monooxygenase gene and the16s rRNA gene can be used as phylogenetic markers to characterize the nature and abundance of methanotrophic communities in different soils. In this study, denaturing gradient gel electrophoresis was used to separate gene specific amplicons. Individual bands were excised and sequenced. The diversity of methanotrophic bacteria in different soil types under long-term (48-50 years) no-tillage, plow tillage, grasslands and forests was determined using phylogenetic data analyses of the sequences.

References

1. Hütsch, B.W. 2001, "Methane oxidation in non-flooded soils as affected by crop production", *European Journal of Agronomy*, vol. 14, pp. 237-260.
2. Chen, Y., Dumont, M.G., Cebron, A. & Murrell, J.C. 2007, "Identification of active methanotrophs in a landfill cover soil through detection of expression of 16S rRNA and functional genes", *Environmental Microbiology*, vol. 9, pp. 2855-2869.
3. Jacinthe P-A, Dick W.A., Lal R, Shrestha R.K., & Bilen S. 2013, Effects of no-till duration on the methane oxidation capacity of Alfisols. *Biology and Fertility of Soils* (in press).

Study Design

- Soil cores, in triplicate, were collected from:
  - No-Till Continuous Corn (NT-CC)
  - No-Till Corn Soybean (NT-CS)
  - Plow-Till Continuous Corn (PT-CC)
  - Plow-Till Corn Soybean (PT-CS)
  - Grassland (G)
  - Forest (F)
- DNA was isolated from the soil samples.
- PCR amplifications of *pmoA* and 16S rRNA genes were carried out. The primer sets included[2]:
  - For target *pmoA* gene: A189f-A682r and A189f-mb661r.
  - For target *16srRNA* gene: Type IIF and Type IIR.
- Denaturing Gradient Gel Electrophoresis (DGGE) was conducted to determine microbial diversity. Clear distinct bands were cut and sequenced. Phylogenetic relationships between the sequences were determined by the neighbor-joining method using MEGA 6.0 software.
- Future scope of work includes designing Fluorescent *In-Situ* Hybridization (FISH) probes to detect and quantify methanotrophic bacteria in soil from a mixed population, using a flow cytometer, according to the amount and type of probes present.

Results

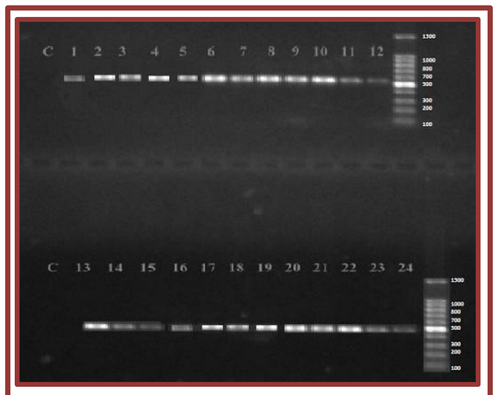

Figure 2. PCR amplification of the *pmoA* gene using primer sets A189f-A682r (top) and A189f-mb661r (bottom).

The primer sets were successful in amplifying a ~480 bp region of the *pmoA* gene sequence. The amplicons were reamplified using primer sets specific for DGGE analysis. These primer sets had a GC-clamp attached to the forward primers.

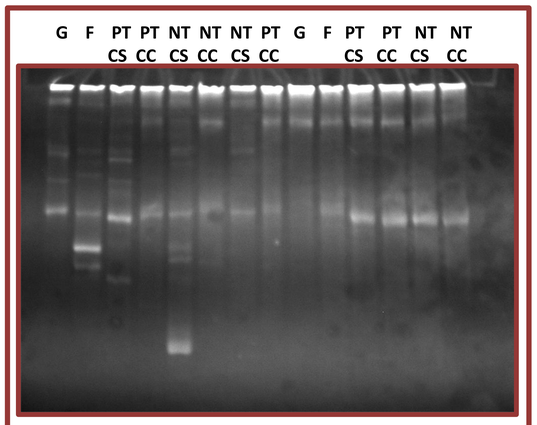

Figure 3. DGGE analysis of amplified *16sDNA* gene sequences using primer set Type IIF and Type IIR. Visually, a comparison can be drawn from the number of bands observed in the reps. A total of4 bands from G, 7 bands from F, 5 bands from PT-CS, 2 bands from PT-CC, 3 bands from NT-CC and 10 bands from NT-CS were excised. The bands were purified followed by re-amplification with the primer set Type IIF and Type IIR. The amplicons were sequenced to determine species diversity.

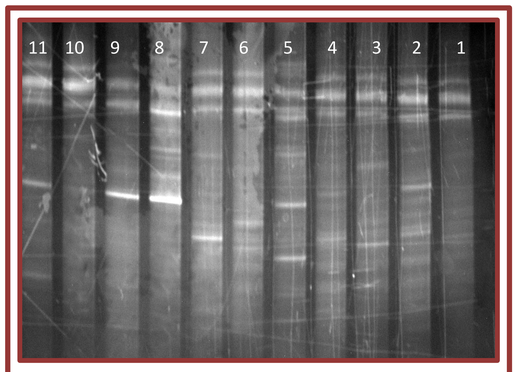

Figure 4. DGGE analysis of amplified *pmoA* sequences using primer set (GC-A189f)-A682r of samples for no-till (lanes 1-6) and plow-till (lanes 7-11) soils.
Visually, a comparison can be drawn from the number of bands observed in each lane. Lanes 1-6 have 3, 7, 5, 5, 10 and 6 unique bands, respectively, while lanes 7-11 have 5, 7, 3, 3 and 5 unique bands, respectively. The bands were excised and purified followed by re-amplification with the primer set A189f-A682r. The amplicons were sequenced to determine species diversity.

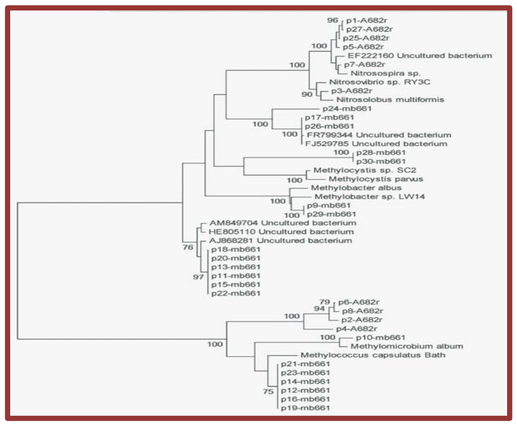

Figure 5. Neighbor-joining phylogenetic tree of pmoA gene sequences amplified by the primer sets A189f-A682r and A189f-mb661r. Bootstrap values greater than 70 are shown. The scale bar represents 0.1 substitutions per nucleotide position.

The *pmoA/amoA* phylogeny (A189f-A682r) – Ten sequences were obtained and six showed close similarity to the *amoA gene of Nitrospira* sp., *Nitrosovibrio* sp. RY3C and *Nitrosolobus multiformis* (96-98%).The remaining four sequences showed 84% similarity to the *pmoA* gene of *Methylomicrobium album* and *Methylococcus capsulatus.*

The *pmoA* phylogeny (A189f-mb661r) – Twenty sequences were obtained and two showed 99% identity to the *pmoA* gene of *Methylocystis* sp. SC2 and *Methylocystis parvus*. Two sequences showed 99% identity to the *pmoA* gene of *Methylobacter albus* and *Methylobacter* sp. LW14. Seven sequences were highly similar (97-98%) to the *pmoA* gene of *Methylomicrobium album* and *Methylococcus capsulatus* Bath. Nine sequences showed 85-92% similarity to the *pmoA* gene of clusters of uncultured methanotrophs recovered from Genbank.

Discussion

Data from the long term (50 years) tillage plots have shown that NT plots have greater methane oxidation than PT plots[3]. This may be due to the greater diversity of methanotrophs in no-till versus plow-till soils. Further comparisons between agricultural (no-till and plow-till), grasslands and forest soils will hold implication in climate change mitigation strategies in terms of changing agricultural and land-use practices contributing to increased biological fixation of methane gas.

Acknowledgment

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