METHANOTROPHIC COMMUNITIES IN SOILS

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Key words: methane monooxygenase, pmoA, saline alkaline soils

Introduction. Methane is an important greenhouse gas (GHG) emitted under strict anaerobic conditions as contribute substantially to the 'global warming'. The soil is not only a source of CH_4, but it can also be a sink. The metanothophs, a specialized group of bacteria and archaea, are mostly responsible for methane oxidation under aerobic and anaerobic conditions (Hanson and Hanson, 1996). The key enzyme for aerobic methanotrophy is methane monooxygenase (MMO) that converts methane to methanol. The pmoA gene encoding a subunit from particulate MMO has been used as molecular marker to characterize the aerobic methanotrophic communities in environmental samples.
The objective of this study was to characterize methane-oxidizing bacteria (MOB) in a saline alkaline soil and compare them with those found in agricultural soils.

Methods. Soils with high electrical conductivity (85 dS m^{-1}) and pH 10.3, were sampled from the former lakebed Texcoo - México. Arable soils, one from Chiapas (oxidize CH_4 rapidly) and one from the central highlands of Mexico (Alcholoya) served as control. More details about arable soils can be found in Ruiz-Valdiviezo *et al*. (2010) and Serrano-Silva *et al*. (2011). A sub-sample of 20 g soil was taken from each sample and stored at –80°C until extracted for DNA. Total DNA was extracted from three 0.5 g soil samples by using a modified method based on direct cell lysis technique of Valenzuela-Encinas *et al*. (2008). Total pooled DNA was used as template for PCR amplification of pmoA genes. The amplification was done via the semi-nested PCR technique as described by Horz *et al*. (2005). PCR products were used to construct pmoA clone libraries using pGEM T-Easy Vector Cloning System I kit (Promega, Madison, Wis.). Representatives of each OTU were analysed phylogenetically to determine the affiliation of the PmoA sequences. A maximum-likelihood tree was constructed using PmoA-deduced fragments and the sequences were affiliated with the respective target group.

Results. Overall, 132 clones were sequenced. The mean coverage of the clone libraries was 97%, 98% and 96% for Alcholoya, Chiapas, and Texcoco soils respectively. Seven OTUs were found in Alcholoya and in Chiapas soil and four in Texcoco soil. Most sequences of the arable soils, i.e. Chiapas and Alcholoya, were located within previously described PmoA clusters or near them: methanotrophs type I, such as USC-γ, JR-2 and JR-3, but also to the group USC-α from the methanotrophs type II. Most of the clones (21%) of the Alcholoya soil were closely related to *Methylocaldum tepidum* (U89304). In the Chiapas soil, however, most of the sequences could not be affiliated within previously described clusters and showed 88–99% similarity with uncultured bacterial clones from other soils (paddy field soils and alpine meadow soils). All the clones from the Chiapas soil belonged to the methanotrophic group type I. In the Texcoco soil, two sequences were identical to PmoA from *Methylomicrobium japanense* (BAE86885) and just one sequence matched with partial AmoA related to *Nitrosomonas europaea*. A high percent of clones (> 92%) in the librarie of the Texcoco soil, showed 94−97% similarity with the reported but unpublished AmoA sequence from ammonia oxidizing *Nitrosococcus halophilus* Nc4 (YP003526243) and with AmoA sequence from *Nitrosococcus oceani* ATCC 19707 (YP344485).

Conclusions. The pmoA-like sequences obtained from this study showed that a potential aerobic methanotrophic community exists in all the studied soils.
By using pmoA as a functional marker, it was found that the methanotrophic communities in soil of the former lake Texcoco differed from the Chiapas and Alcholoya soils. Most of the sequences from the Texcoco libraries (> 92%) were related to the *Nitrosococcus*-like clade thus, further experiments are needed to confirm that the metanotrophic community found in this study oxidizes methane under these extreme conditions.

Acknowledgements. N. S.-S. and C. V.-E. received grant-aided support from CONACYT.

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