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The potential of stable isotope probing and metagenomics to unravel some of the mysteries of atmospheric methane consumption in forest soils

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It is estimated that 7-100 Tg y^{-1} of atmospheric methane is consumed by organisms in upland soils. The organisms believed to be largely responsible for this process have so far eluded cultivation. PCR surveys of *pmoA*, which encodes a subunit of the particulate methane monooxygenase (pMMO), have provided evidence suggesting that novel lineages of methane oxidizing bacteria are abundant in forest soils and may mediate the oxidation of atmospheric methane. Stable isotope probing (SIP) allows the isolation of nucleic acids from targeted metabolically active organisms in environmental samples and may provide a way of directly linking these *pmoA* sequences to the organisms responsible for atmospheric methane oxidation. For RNA-SIP, forest soil is incubated with $^{13}CH_4$ and after consumption of the substrate the ^{13}C-rRNA is purified by centrifugation. We have attempted to increase the sensitivity of the method such that the ^{13}C-rRNA can be sufficiently resolved from ^{12}C-rRNA of the total bacterial community to identify atmospheric methane oxidizers. We performed DNA-SIP experiments with forest soils and recovered genomic ^{13}C-DNA from a methane oxidizing population. This DNA was used to construct a metagenomic library of 2300 clones. The library was probed for key methylotrophy genes and a 15 kb clone containing *pmoCAB*, encoding a pMMO operon, was identified and sequenced. Analysis of the *pmoA* suggested that it was most similar to that of a *Methylocystis* sp. previously detected in this forest soil. Additional open reading frames were identified on the clone, including the gene encoding βRFAP synthase, which is involved in the biosynthesis of the “archaeal” C_1-carrier, tetrahydromethanopterin, also found in methylotrophs. The potential of this approach to identify and elucidate the metabolic capabilities of uncultivated atmospheric methane oxidizers is discussed.