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High diversity of methanotrophic bacteria in geothermal soils affected by high methane fluxes

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Volcanic and geothermal systems emit endogenous gases by widespread degassing from soils, including CH_4, a greenhouse gas 25 times as potent as CO_2. Recently, it has been demonstrated that volcanic/geothermal soils act as source, but also as biological filter for methane release to the atmosphere. For long time, volcanic/geothermal soils has been considered inhospitable for methanotrophic microorganisms, but new extremophile methanotrophs belonging to Verrucomicrobia were identified in three different areas (Pozzuoli, Italy; Hell's Gate, New Zealand; Kamchatka, Russia), explaining anomalous behaviours in methane leakages of several geothermal/volcanic sites. Our aim was to increase the knowledge of the relationship between methane emissions from volcanic/geothermal areas and biological methane oxidation, by investigating a geothermal site of Pantelleria island (Italy). Pantelleria Island hosts a high enthalpy geothermal system characterized by high temperature, high CH_4 and very low H_2S fluxes. Such characteristics are reflected in potentially great supply of methane for methanotrophs and scarce presence of inhibitors of their activity (H_2S and NH_3) in the Pantelleria soils. Potential methanotrophic activity within these soils was already evidenced by the CH_4/CO_2 ratio of the flux measurements which was lower than that of the respective fumarolic manifestations indicating a loss of CH_4 during the gas travel towards the earth's surface. In this study laboratory incubation experiments using soils sampled at Favara Grande, the main hydrothermal area of Pantelleria, showed very high methane consumption rates (up to 9500 ng CH_4 h^{-1} g^{-1}). Furthermore, microbiological and culture-independent molecular analyses allowed to detect the presence of methanotrophs affiliated to Gamma- and Alpha-Proteobacteria and to the newly discovered acidothermophilic methanotrophs Verrucomicrobia. Culturable methanotrophic Alpha-proteobacteria of the genus Methylocystis were isolated by enrichment cultures. The isolates showed a wide range of tolerance to pH (3.5 – 8) and temperatures (18 – 45°C), and an average methane oxidation rate of 450 ppm/h. A larger diversity of proteobacterial and verrucomicrobial methanotrophs was detected by the amplification of the methane mono-oxygenase gene *pmoA*.
This study demonstrates the coexistence of both the methanotrophic phyla Verrucomicrobia and Proteobacteria in the same geothermal site. The presence of proteobacterial methanotrophs was quite unexpected because they are generally considered not adapted to live in such harsh environments. Their presence at Favara Grande could be explained by not so low soil pH values (> 5) of this specific geothermal site and by the high methane availability. Such species could have found their niches in the shallowest part of the soils, were the temperatures are not so high, thriving on the abundant upraising methane. Understanding the ecology of methanotrophy in geothermal sites will increase our knowledge of their role in methane emissions to the atmosphere.