TITLE: METHANE METABOLISM ASSOCIATED GENES AND THEIR MICROBIAL SPECIES IN ANAEROBIC INCUBATED AMAZONIAN FLOODPLAIN SOILS WITH FOUR DIFFERENT CARBON SOURCES

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ABSTRACT:

Methane (CH_4) is a greenhouse gas with approximately 34 times the potential of carbon dioxide (CO_2). The Amazonian floodplains are an important natural source of CH_4 in the tropics, and the CH_4 emissions will depend on different environmental and biological factors. The methanogenic microbial community can use different substrates for energy production and growth, and can convert acetate, formate, H_2/CO_2, or methanol and methylamines into CH_4. To better understand the dynamic of the methanogenic community of the soils from the Amazonia floodplains, we conducted a microcosm experiment with soil from a floodplain seasonally inundated with the waters of the Solimões River incubated with different sources of carbon. Soils from forest and agroforest sites were collected during the rainy season and incubated anaerobically with four different carbon sources: sodium acetate, sodium formate, glucose, and methanol. The control treatment was composed of the soil inoculum incubated with only water. Total DNA was isolated from 10 soil samples in triplicate (4 carbon sources + control × two biological replicates) using the DNeasy PowerSoil Total DNA Kit. The final library was sequenced on an Illumina Nextseq Sequencing System. All reads were assembled and annotated through the DiTing pipeline. Genes associated with methane metabolism were selected for further analysis and the annotated contigs belonging to the mcrABCDG operon had their taxonomy assigned through BLAST protein-protein (nr database). Finally, assembled contigs were binned using MetaBAT2 v1.7, and high-quality archeal bins were annotated and distilled with DRAM v0.1.2. The relative abundance of genes belonging to methane metabolism was higher in the reactors incubated with glucose, followed by the ones with methanol. The incubation with both acetate and formate did not significantly affect the abundance of genes, even the ones associated with the acetate and formate conversion to acetyl-CoA. The contigs belonging to the mcrABCDG operon were mostly associated with different *Methanosarcina* and *Methanobacterium* species, but we also detected lower abundances of *Methanomassiliicoccus* and *Methanocella*. In the control microcosms, we also detected the presence of *mcrBDG* contigs belonging to the archaeon *Candidatus Methanoperedens nitroreducens*, an archaea capable of methanotrophy through a reverse methanogenesis pathway coupled with the reduction of nitrate to nitrite. Finally, we also isolated a high-quality (>95% completeness, 0% contamination) metagenome-assembled genome (MAG) that was assigned as a member of the *Methanosarcina* genus and displayed the complete pathway for acetoclastic, hydrogenotrophic, and methylotrophic methanogenesis, and had a relatively high abundance in the reactors incubated with glucose (4.72% of all reads). This metagenomic approach highlighted the importance of the acetoclastic, hydrogenotrophic, and methylotrophic pathways of methanogenesis and how their interaction in the Amazonian floodplain soils is substrate-dependent. We also explored the genome of a highly abundant *Methanosarcina sp.,* that encoded all enzymes necessary for all three pathways of methanogenesis. This emphasizes the importance of understanding the niche of these dominant

methane producers and how the analysis of their metabolism can impact future action to mitigate climate change.

Keywords: Methanogenesis, shotgun metagenome sequencing, carbon cycle, microbial ecology.

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