



Conference Abstract

Non-syntrophic Methanogenic Hydrocarbon Degradation by an Archaeal Species

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Abstract

Methanogenic hydrocarbon biodegradation alters the composition of many subsurface oil reservoirs (Jones et al. 2007). This process reduced the crude oil quality by removing alkanes and thus increasing the oil viscosity. The process has been described for syntrophic associations of hydrocarbon-degrading bacteria and methanogenic archaea (Zengler et al. 1999, Dolfing et al. 2007). However, recent culture-independent studies suggest that the archaeon '*Candidatus Methanoliparum*' may combine alkane degradation and methanogenesis (Laso-Pérez et al. 2019, Borrel et al. 2019). Here we cultured *Ca. Methanoliparum* from a subsurface oil reservoir. To study this culture, situ hybridization, metagenomics and metatranscriptomics were combined with stable isotope probing and metabolite analyses for describing its functioning and assessing its potential role in reservoir chemistry.

Incubated an anoxic oily sludge of the Shengli oilfield with sulfate-free medium, we established a methanogenic culture. This culture consumed various different long-chain

alkanes, but also alkyl-benzenes and alkyl-cycloalkanes, and produced methane and CO₂ as products (Fig. 1a-b). Our analyses revealed that our culture is dominated by a single archaeon, *Ca. Methanoliparia* (green).

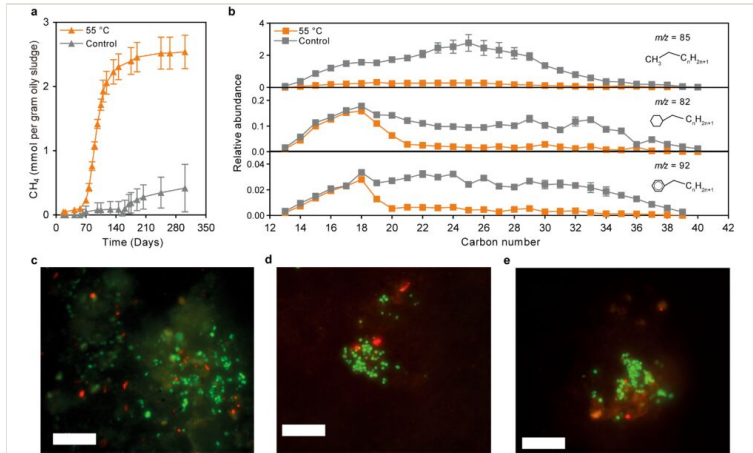


Figure 1. [doi](#)

Methanogenesis in the oily sludge and visualization of microorganisms.

To study the specific turnover of *n*-alkanes, the cultures were supplemented with 1,2-¹³C-labelled or unlabelled *n*-hexadecane (Fig. 2). Within 100 days of incubation, both compounds were quantitatively converted into methane and carbon dioxide. In the ¹³C-labelling experiment, around 0.46 mmol of ¹³CH₄ and around 0.15 mmol of ¹³CO₂ were produced, which was equal to 85% to 92% of the stoichiometric conversion of the supplemented labelled hexadecane according to $4C_{16}H_{34} + 30H_2O \rightarrow 49CH_4 + 15CO_2$ (Fig. 2a-d).

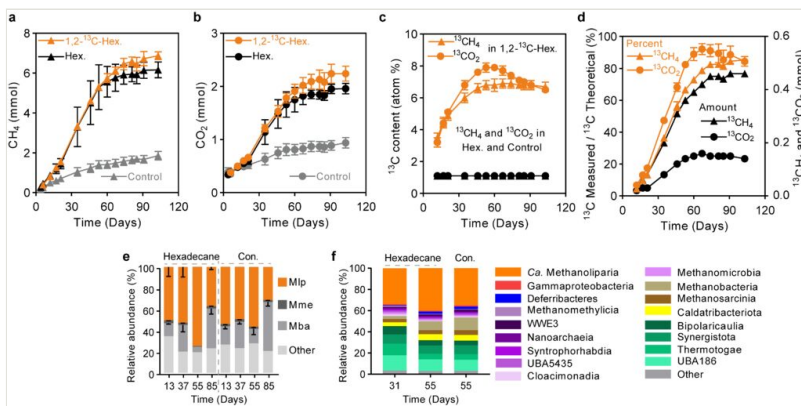


Figure 2. [doi](#)

Methanogenic hexadecane degradation by *Ca. Methanoliparum*.

We examined the functioning of *Ca. Methanoliparum* in the hexadecane-degrading culture using amplicon sequencing, metagenomics and metatranscriptomics. In the archaeal domain, the relative abundance of *Ca. Methanoliparum* in the hexadecane-degrading cultures comprised up to 75% of the total abundance according to analysis of archaeal 16S rRNA genes. Furthermore, *Ca. Methanoliparum* accounted for approximately 34–40% of the total microbial community as determined by metagenomic read recruitment estimation (Fig. 2e-f).

We analysed the gene expression patterns of *Ca. Methanoliparum* during methanogenic hexadecane degradation (Fig. 3). The genes encoding the methanogenic hexadecane degradation pathway ranked among the top 10% to 25% of all *Ca. M. thermophilum* transcribed genes. Moreover, genes of *Ca. M. thermophilum* encoding ACR and MCR ranked among the top 2% of all transcribed genes within the whole community (Fig. 3b). The MAGs of *Ca. M. thermophilum* also showed the highest transcription among all described MAGs (Fig. 3c). These analyses indicate that *Ca. M. thermophilum* performs both the degradation of hexadecane and the formation of methane.

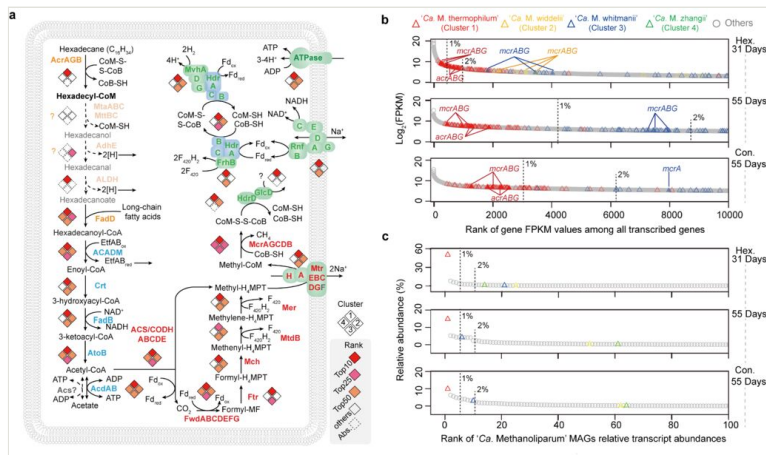


Figure 3. doi

Hexadecane degradation pathway of *Ca. Methanoliparum*.

We searched the cell extracts of the hexadecane-degrading cultures for hexadecyl-CoM formation using Q-Exactive Plus Orbitrap mass spectrometry. The unlabelled hexadecane culture contained a prominent mass peak of $m/z = 365.21868$ that matches the mass produced by synthesized authentic standard of hexadecyl-CoM. Fragmentation of both peaks yielded hexadecyl-thiol ($m/z = 257.23080$, $C_{16}H_{33}S^-$), ethenesulfonate ($m/z = 106.98074$, $C_2H_3SO_3^-$) and bisulfite ($m/z = 80.96510$, HSO_3^-). Moreover, cultures supplied with 1,2- ^{13}C -hexadecane produced a peak at $m/z = 367.22524$ for 1,2- ^{13}C -hexadecyl-CoM and the fragment 259.23721 for 1,2- ^{13}C -hexadecyl-thiol, with a mass shift of 2 units compared with the unlabelled group. These analyses confirmed the activation of n-hexadecane as hexadecyl-CoM (Fig. 4).

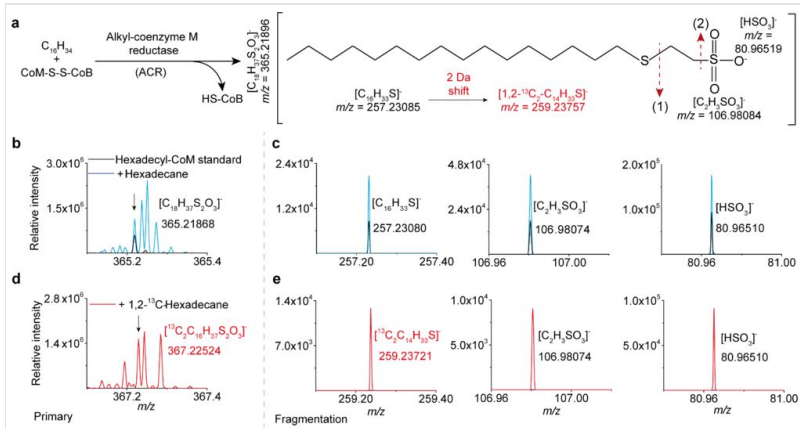


Figure 4. [doi](#)

Identification of the intermediate hexadecyl-CoM.

Here we demonstrate the activation of different hydrocarbon classes by ACRs of *Ca. Methanoliparum*, expanding the substrate range of this enzyme to an unforeseen number of compounds. *Ca. Methanoliparum* couples the degradation of long-chain alkanes and alkyl-substituted hydrocarbons to methane formation, proposed as alkylotrophy. Its metabolic pathways represent an additional mode of methanogenesis, adding to CO₂ reduction, methylotrophy, methyl reduction, acetate fermentation and the recently reported methoxydotrophy. *Ca. Methanoliparum* grows in a wide temperature range, at least between 35 and 55 °C, covering the temperature range of most biodegraded oil reservoirs. Indeed, sequences of *Ca. Methanoliparum* are present in various anoxic hydrocarbon-rich environments worldwide. Thus, the demonstration of the unique features of *Ca. Methanoliparum* in hydrocarbon conversion may fundamentally change our view of crude oil transformation and biogeochemical processes in subsurface oil reservoirs. Future studies with *Ca. Methanoliparum* cultures will resolve the biochemical mechanisms of methanogenic hydrocarbon degradation in archaea, and will be helpful for the application of microbial-enhanced energy recovery from depleted oil reservoirs.

Keywords

Alkane, Biodegradation, Methane

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Author contributions

L.C. and M.L. initiated the study. L.C., M.L., G.W. and P.-f.L. designed research. J.-z.L., W.-d.W. and Z.Z. collected the oily sludge samples. Z.Z., J.L., M.Y. and L.C. conducted cultivation experiments. Z.Z. and L.Y. performed oil analysis. C.-j.Z., P.-f.L., Z.Z., R.L.-P. and M.L. performed all bioinformatics analyses. R.L.-P. and L.C. designed CARD-FISH probes, and R.L.-P. performed CARD-FISH and cell visualization. L.F., L.C. and L.-p.B. performed metabolite analyses. P.-f.L., R.L.-P., G.W., M.L. and L.C. analysed data and wrote the manuscript with contributions from all of the co-authors.

Conflicts of interest

The authors have declared that no competing interests exist.

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