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 CO_2 as products (Fig. 1a-b). Our analyses revealed that our culture is dominated by a single archaeon, *Ca*. Methanoliparia (green).

To study the specific turnover of *n*-alkanes, the cultures were supplemented with 1,2-^{13}C-labelled or unlabelled *n*-hexadecane (Fig. 2). Within 100 days of incubation, both compounds were quantitatively converted into methane and carbon dioxide. In the ^{13}C-labelling experiment, around 0.46 mmol of $^{13}CH_4$ and around 0.15 mmol of $^{13}CO_2$ were produced, which was equal to 85% to 92% of the stoichiometric conversion of the

supplemented labelled hexadecane according to $4C_{16}H_{34} + 30H_2O$ -- $49CH_4 + 15CO_2$ (Fig. 2a-d).

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.

We searched the cell extracts of the hexadecane-degrading cultures for hexadecyl-CoM formation using Q-Exactive Plus Orbitrap masss pectrometry. 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).

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 CO_2 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*. Methanoliparia cultures will resolve the biochemical mechanisms of methanogenic hydrocarbon degradation in archaea, and will be helpful for the application of microbial-enhanced energyrecovery 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 manuscriptwith contributions from all of the co-authors.

Conflicts of interest

The authors have declared that no competing interests exist.

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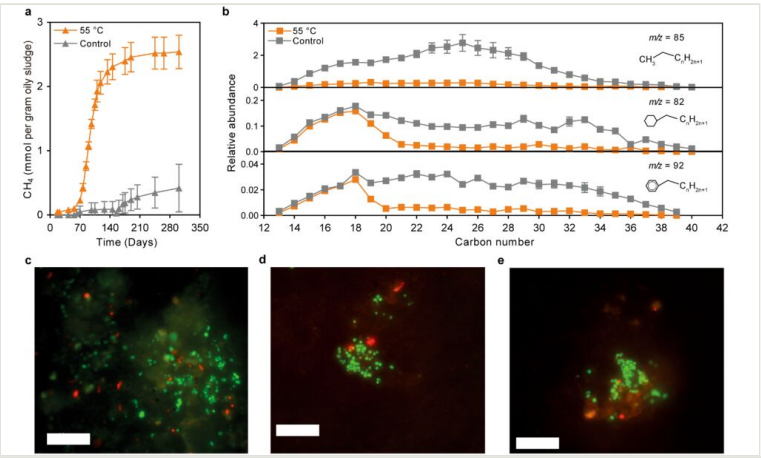

Figure 1.

Methanogenesis in the oily sludge and visualization of microorganisms.

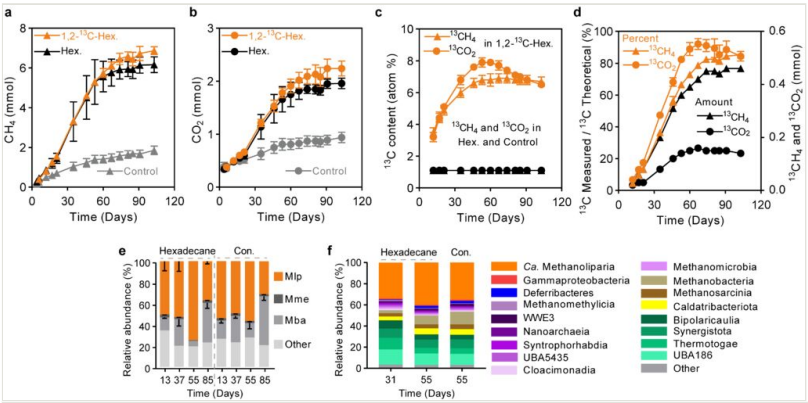

Figure 2.

Methanogenic hexadecane degradation by *Ca.* Methanoliparum.

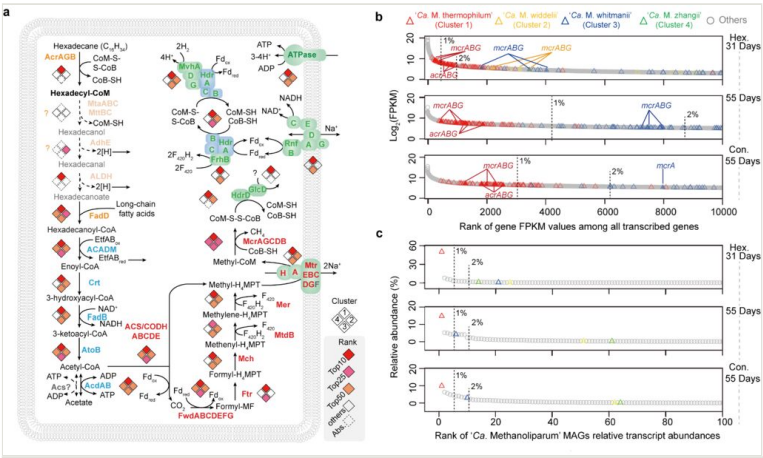

Figure 3.

Hexadecane degradation pathway of *Ca*. Methanoliparum.

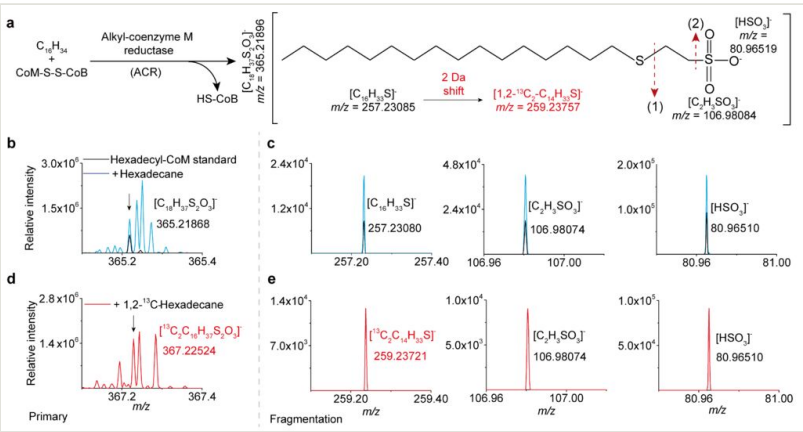

Figure 4.

Identification of the intermediate hexadecyl-CoM.