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► **To cite this version:**

Guillaume Borrel. A microbe that uses crude oil to make methane. *Nature*, 2022, 601 (7892), pp.196-197. 10.1038/d41586-021-03729-3 . pasteur-04075884

**HAL Id: pasteur-04075884**

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Submitted on 21 Apr 2023

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## Biogeochemistry

### A microbe that uses crude oil to make methane

A microorganism that dwells in an underground oil reservoir has been found to degrade various petroleum compounds and use them to produce methane through a previously unreported biochemical pathway. See p.XXX

Microbial communities tend to use the most energy-rich and easily metabolized compounds that they have at their disposition. This leads to a progressive enrichment of compounds that are difficult to break down and provide little energy, particularly in the absence of oxygen or other inorganic electron acceptors. Under these conditions, the use of hydrocarbons — molecules made of carbon and hydrogen, such as alkanes — has been thought to rely entirely on a collaboration (known as syntrophy) between bacteria that break down these compounds into acetate and  $H_2$ , and microorganisms called methanogenic archaea that use these molecules to produce methane ( $CH_4$ ), the simplest hydrocarbon<sup>1,2</sup>. Writing in *Nature*, Zhou *et al.*<sup>3</sup> overturn this long-standing account of a division of labour for the methanogenic degradation of hydrocarbons by reporting that a single type of microorganism can degrade various large hydrocarbons into methane (Fig. 1).

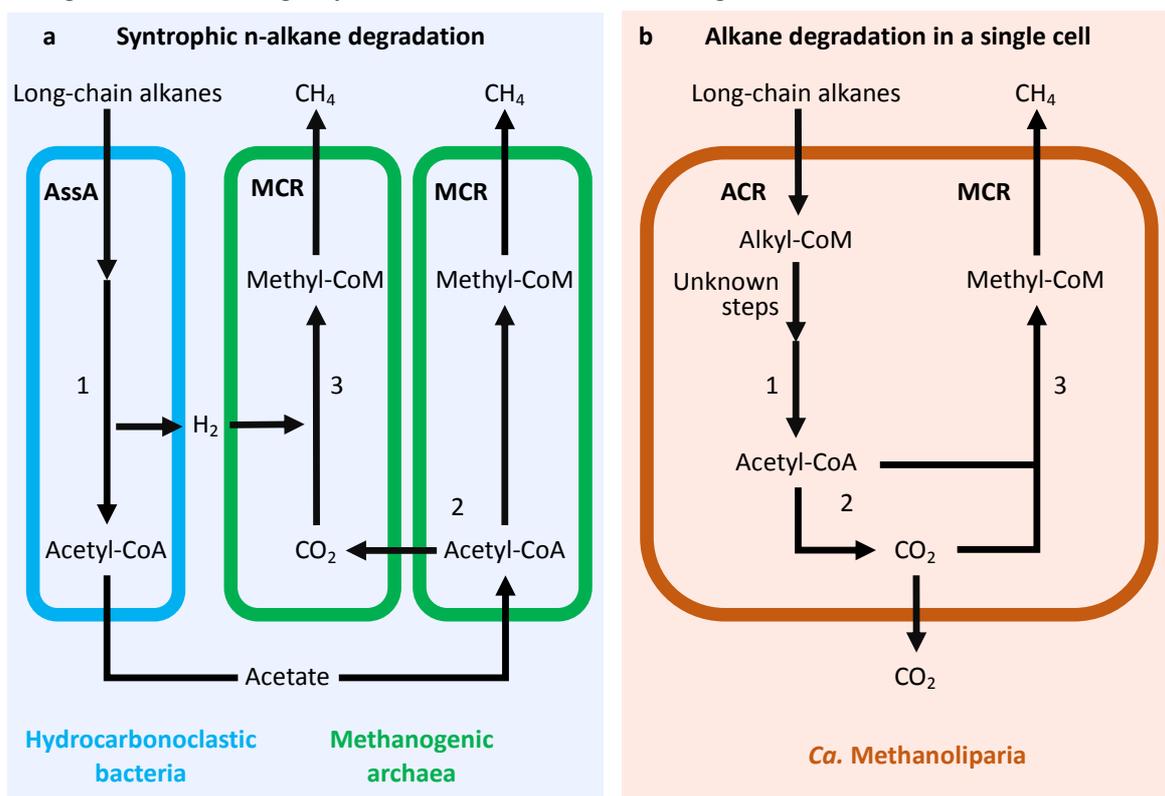


Figure 1 | Two microbial processes of methane production from hydrocarbon molecules. a, Hydrocarbons, entirely made of hydrogen and carbon, are broken down and used to generate methane ( $CH_4$ ) through a collaboration (syntrophy) between bacteria and archaea. In this process, long-chain alkanes are degraded into acetate and hydrogen ( $H_2$ ) by hydrocarbonoclastic bacteria. One group of methanogenic archaea transforms acetate into carbon dioxide ( $CO_2$ ) and  $CH_4$  and another group of methanogenic archaea makes  $CH_4$  using  $H_2$  and  $CO_2$ . b, Zhou *et al.*<sup>3</sup> identified that an archaeon belonging to *Candidatus Methanoliparia* can couple hydrocarbon degradation and methane generation. Several enzymes (MCR and those of steps 1 [Beta-oxidation], 2 [CODH] and 3 [methyl-branch of the Wood-Ljungdahl pathway + MTR]) and intermediates (acetyl-coenzyme A (acetyl-CoA) and methyl-coenzyme M (methyl-CoM)) are similar between *Ca. Methanoliparia* and the syntrophic microbes. Conversely, the initial step of alkane breakdown is mediated by different enzymes, ACR in *Ca. Methanoliparia* and AssA in hydrocarbonoclastic bacteria.

Whereas many microorganisms can use a large range of substrates to obtain energy, methanogenic microorganisms (methanogens) are very specialized. Most of them can only obtain energy by reducing carbon dioxide (CO<sub>2</sub>) into CH<sub>4</sub> using molecular hydrogen (H<sub>2</sub>) as an electron donor, and a few others can use acetate and methylated compounds (such as methanol and methylamines). Over the past five years, genomics studies have described several lineages of previously unknown and uncultured methanogenic microbes from various oxygen-free environments, including marine sediments, hot springs and subsurface oil reservoirs<sup>4-7</sup>.

Although most of these newly discovered methanogens were inferred to also rely on simple compounds, primarily methylated compounds reduced with H<sub>2</sub><sup>4,8</sup>, a great surprise was the prediction of a previously undescribed pathway for generating methane from multi-carbon alkanes and possibly long-chain fatty acids<sup>4,9</sup>. This metabolic pathway was initially predicted from genomes belonging to a class of uncultured archaea that was named *Candidatus Methanoliparia*<sup>4</sup> because its members mostly occur in oil reservoirs or environments contaminated with petroleum.

Cheng *et al.* collected crude oil from Shengli oilfield (northeast China) and found that *Ca. Methanoliparia* represented about half of the archaeal community in these samples. When these samples were incubated between 35°C and 65°C, without oxygen or other inorganic electron acceptors, long-chain alkanes (linear chains of 13 to 39 carbons) were completely depleted, and a large amount of methane was produced. The authors similarly observed reductions in the levels of hydrocarbon molecules composed of a carbon ring bond to a long-chain alkyl, such as n-alkylcyclohexanes and n-alkylbenzenes.

Because *Ca. Methanoliparia* are far from being the only microorganisms in these cultures, one might ask at this stage whether the production of methane from petroleum compounds might have resulted from the activity of conventional bacteria–archaea associations. Indeed, bacteria known to break down hydrocarbons were present, even if they represented only 4% of the total microbial community in the original culture. However, after several transfers of the culture into fresh medium, interspersed by weeks of incubation, the proportion of these bacteria dropped to less than 0.1%. By contrast, the abundance of *Ca. Methanoliparia* (around 40% of the microbial community) and the rate of methanogenic degradation of long-chain alkane was maintained over these transfers.

Further supporting the absence of syntrophic associations involving *Ca. Methanoliparia*, microscopy revealed that these cells generally occurred alone and not in aggregates of multiple species<sup>3,9</sup>. Moreover, *Ca. Methanoliparia* genomes lack genes encoding enzymes and nanowire structures that are involved in electron transfer between syntrophic partners<sup>4,9</sup>, corroborating the idea that *Ca. Methanoliparia* work alone.

The predicted metabolic route for methanogenic degradation of long-chain hydrocarbons by *Ca. Methanoliparia* involves a combination of enzymes that are otherwise partitioned between syntrophic archaea and bacteria, as well as enzymes specific to *Ca. Methanoliparia* (Fig. 1). Among the enzymes that are used by both *Ca. Methanoliparia* and the syntrophic archaea and bacteria association is methyl-coenzyme M reductase (MCR) complex, which performs the final step of methane production in all methanogens.

*Ca. Methanoliparia* species also carry the genes encoding an enzymic complex called alkyl-coenzyme M reductase (ACR) complex, which was recently described to mediate the first step of the degradation of short-chain alkanes — forming alkyl-coenzyme M (alkyl-CoM) — in non-methanogenic archaea<sup>10-12</sup>. *Ca. Methanoliparia* members are the first microorganisms reported to have both an MCR and an ACR complex, an observation that initially led to propose that they may be capable of methanogenesis based on multi-carbon alkanes<sup>4,9</sup>.

Following the same principle as for short-chain alkanes, the activity of ACR complex on long-chain alkanes should also lead to the formation of alkyl-CoM. In cultures enriched with *Ca. Methanoliparia* and fed hexadecane (a long-chain alkane containing 16 carbons), Cheng et al.<sup>3</sup> detected the presence of hexadecyl-CoM, confirming that the ACR complex is involved in the first step of the degradation of long-chain alkanes. The authors made similar observations for long-chain monoaromatic hydrocarbons: addition of such molecules to the culture led to the formation of monoaromatic alkyl-CoM. The genes encoding MCR and ACR complexes in *Ca. Methanoliparia* were among the most highly expressed in the culture, supporting the proposal that these microorganisms couple hydrocarbon degradation with methane production. Altogether, these findings largely expand the range of substrates that can be used by the ACR complex, and methanogens in general.

Could it be that short-chain alkanes are also targets of *Ca. Methanoliparia* ACR, as previously described in the non-methanogenic archaea? Apparently not, as no alkyl-CoM and no methane were produced in response to the addition of two to eight carbon alkanes to the culture. This suggests that some ACR complexes might use short-chain hydrocarbons, whereas others use long-chain hydrocarbons. Now that cultures of *Ca. Methanoliparia* are available, it will be exciting to resolve the 3D structure of their ACR complex and compare it to that of evolutionary related MCR and ACR complexes<sup>12,13</sup>. Such investigation will likely reveal the molecular mechanisms enabling the activity of these enzymes, including how they discriminate hydrocarbons depending on their length.

As for other archaea that use ACR to initiate the breakdown of hydrocarbons, such as *Ca. Syntrophoarchaeum*<sup>10</sup>, several steps of the process of hydrocarbon degradation in *Ca. Methanoliparia* are still unknown (Fig. 1). The authors suggest that one of these steps might involve a type of enzyme called long-chain fatty-acid CoA ligases. Intriguingly, although this study focused on the most enriched *Ca. Methanoliparia* species (*Ca. Methanoliparum thermophilum*), which contains both ACR and MCR, another *Ca. Methanoliparia* species whose genome was sequenced by the authors lacks the genes encoding the ACR complex, but encode long-chain fatty-acid CoA ligases. Thus, the diversity of methanogenic metabolic pathways in this class of archaea could be even greater than that proposed by the authors, as some species might perform methanogenic degradation of long-chain fatty acids directly, instead of starting from hydrocarbons.

Future studies on *Ca. Methanoliparia* members will probably uncover intriguing novel molecular mechanisms of methanogenesis and reveal even more of their already impressive range of substrates. *Ca. Methanoliparia* also represent a useful model to better understand which evolutionary and environmental factors favour the division of labour between two species or the ‘do it yourself’ approach of this class. Investigating these microbes further will also provide a better understanding of the role of microorganisms in the fate of petroleum in oil reservoirs deep underground.

Finally, the findings shed light on a previously unappreciated source of methane production. It will be crucial to consider the contribution of this natural process to methane emissions and, in turn, to global warming.

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The author declares no competing interests.

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*[Thank you for suggesting a figure. I have discussed it with our graphics editor, and we feel it needs to be simplified quite a bit in order to be visually clear, especially to non-specialists. And, the figure also needs to be simplified for the figure legend to keep relatively short, since all technical terms need to be defined in the legend and the legend must keep within 150 words. Therefore, we suggest removing the red font parts (which didn't seem necessary), the different colours/style of arrows, and reducing the number of arrows in total.*

*I have included two options of what this could look like, below - with all labels in black and in the same size font, in line with our figure style.*

*We did have one question about part b, which is why I've included two options. We weren't sure whether in part b, acetyl-CoA and CO<sub>2</sub> could be shown together to feed into pathway, rather than separately (since technically, it seems that both acetyl-CoA and CO<sub>2</sub> are generated and are both involved in the 2 pathway). Please could you let me know your thoughts on this?*

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Figure 1 | Two microbial processes of methane production from hydrocarbon molecules. a, Hydrocarbons, entirely made of hydrogen and carbon, are broken down and used to generate methane (CH<sub>4</sub>) through a collaboration (syntrophy) between bacteria and archaea. In this process, long-chain alkanes are degraded into acetate and hydrogen (H<sub>2</sub>) by hydrocarbonoclastic bacteria. One group of methanogenic archaea transforms acetate into carbon dioxide (CO<sub>2</sub>) and CH<sub>4</sub> and another group of methanogenic archaea makes CH<sub>4</sub> using H<sub>2</sub> and CO<sub>2</sub>. b, Zhou et al.<sup>3</sup> identified that an archaeon belonging to *Candidatus* Methanoliparia can couple hydrocarbon degradation and methane generation. Several enzymes (MCR and those of steps 1, 2 and 3) and intermediates (acetyl-coenzyme A (acetyl-CoA) and methyl-coenzyme M (methyl-CoM)) are similar between *Ca.* Methanoliparia and the syntrophic microbes. Conversely, the initial step of alkane breakdown is mediated by different enzymes, ACR in *Ca.* Methanoliparia and AssA in hydrocarbonoclastic bacteria.

