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Novel syntrophy driven by methylotrophic methanogens

(メチル基利用メタン生成アーキアによる新規栄養共生)

Methanogenic degradation of organic matter plays a vital role in the global carbon cycle. In methanogenic ecosystems, organotrophic bacteria and methanogenic archaea cooperatively drive chemical transformations via interspecies H₂/formate transfer or direct interspecies electron transfer (DIET) to combat energetic and thermodynamic limitations, an interaction termed syntrophy. It was thought the syntrophic interaction was only associated with hydrogenotrophic methanogens or acetoclastic methanogens. However, it can not explain the prevalence of methylotrophic methanogens across the Earth, especially in the methylated compounds lacking environments.

In this study, a hitherto overlooked metabolic symbiosis was discovered between a formate-utilizing anaerobe *Zhaonella formicivorans* K32 and obligate methylotrophic methanogens *Methermicoccus shengliensis* ZC-1 or AmaM, marking a fourth mode of syntrophy facilitated by methanol. Subsequently, the metabolic pathway of this novel syntrophy was studied by genomic and transcriptomic analysis. Furthermore, its survival strategy and the ecological role were discussed. The novel methanol-mediated syntrophy greatly expands the possibilities for anaerobic carbon flow by bridging two processes assumed to be independent until now - syntrophic organics degradation and methylotrophic methanogenesis.

1. Establishing formate-fed syntrophic communities driven by methylotrophic methanogens

Formate is one of the main metabolic products of anaerobic organic matter degradation, and it might be the precursor of methylated compounds. The formate utilizer *Z. formicivorans* K32 and methylotrophic methanogen *M. shengliensis* ZC-1 or AmaM were both thermophiles derived from oil reservoirs where methyl compounds were lacking. Incubation of concentrated K32 resting cells with formate resulted in a slight accumulation of methanol, indicating its methanol metabolism ability. Further

cocultivation of *Z. formicivorans* with *M. shengliensis* showed a clear formate consumption and methane formation, which were consistent with the reaction $4\text{HCOO}^- + \text{H}_2\text{O} + \text{H}^+ = 3\text{CH}_4 + 3\text{HCO}_3^-$. Additional H_2 (i.e., the inhibitory by-product of typical H_2 -mediated syntrophy) did not inhibit formate utilization and methane production of K32/ZC1 coculture, indicating that H_2 was not the primary electron carrier. K32 and ZC-1 could continue formate degradation when physically separated by a dialysis membrane, suggesting the primary route was not DIET either. It was further convinced by the non-effect of conductive material amending. In tracer experiments using ^{13}C -formate and ^{12}C -bicarbonate, the proportion of ^{13}C - CH_4 was constant despite the increasing ^{13}C - CO_2 , indicating that the major methanogenesis route for *M. shengliensis* was not CO_2 -reducing. The results indicated the only methane production pathway that could support such behavior was methylotrophic methanogenesis, namely the novel syntrophy was mediated by methylated compounds.

2. Construction of metabolic pathway of the formate-driven syntrophy

The mechanism of formate-driven syntrophy remains unknown. *Z. formicivorans* genome encodes enzymes of the Wood-Ljungdahl pathway, the glycine/serine pathway, and the formate-methanol-reducing pathway for formate utilization. The gene expression profiles of *M. shengliensis* ZC-1 clearly showed a methylotrophic methanogenesis, which agreed with the hypothesis that methanol was the primary compound mediating symbiosis. Combination of gene expression profiles and electron balance revealed that strain K32 conducted methanol generation by aldehyde ferredoxin oxidoreductase and alcohol dehydrogenase with the oxidation of formate to CO_2 by glycine/serine-involving reversed Wood-Ljungdahl pathway. Strain K32 possesses ion gradient-driven phosphorylation performed by electron bifurcation enzyme Nfn and Hdr-flox complex for its energy conservation during the syntrophic lifestyle.

3. Prediction of the survival strategy and the ecological niche by thermodynamics

Strain K32 employs two thermodynamic/energetic options for formate catabolism, making it flexible for optimizing energy acquisition to dynamic/heterogeneous environmental conditions. The formate disproportionation pathway used by K32 encounters no thermodynamic limit of their partners and is unencumbered by thermodynamic competition, making it has thermodynamic/energetic advantages over typical H_2 -mediated syntrophic formate oxidation.