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PNAS LETTER

No evidence for methanotrophic growth of diverse marine methanogens

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"Growth, in the culture of microorganisms, is more than cellular growth since it implies the growth of a population—that is, the growth of a group of cells from division to division…" Wayne W. Umbreit (1).

This definition of microbial growth is foundational to microbiology research. While rarely stated explicitly, it is implied that microbial growth only occurs if an individual cell can grow from division-to-division ad infinitum so long as resources are not limited. When assigning microorganisms to metabolic guilds, microbiologists are not making statements about transient chemical phenomena that may occur in their presence. Rather, these assignments represent statements about the nutritional conditions that sustain—often exponential—increases in populations that can be maintained indefinitely through serial passages (2).

In their article entitled "Respiration-driven methanotrophic growth of diverse marine methanogens" (3), Yan et al. make the bold claim that two axenic microbial cultures should be assigned to one of the few remaining metabolic guilds that lack cultured representatives: anaerobic methanotrophs.

To support this claim, heavy inocula of the methanogens *Methanosarcina acetivorans* and *Methanococcoides orientis* were added to media supplemented with methane and various electron acceptors. Under these conditions, the authors report methane oxidation, a reversal of these organisms' central energy metabolism. In the case of *M. acetivorans* incubated with ferrihydrite, this chemical phenomenon is accompanied by an increase of total protein from 75 μg/mL to 135 μg/mL (Fig. 1C) and an increase of cell count from 1.18×10^7/mL to 1.90×10^7/mL (Fig. S2), typical values for either organism with either electron acceptor.

These data do not rise to the level of demonstrating microbial growth. Two key points are inescapable: 1) The organism completes at most a single doubling based on cell counts and protein increase, and 2) this "growth" ceases while significant quantities of electron donor and acceptor remain. Critically, no attempt was made to passage the organisms, a simple and mandatory prerequisite for the claim of microbial growth under novel conditions.

In light of this, phrases like "methanotrophic growth of… methanogens" (title), "…respiratory energy conservation that drives methanotrophic growth…" (introduction), "Methanotrophic growth dependent on reduction of Fe(III) or humic acids" (results), and "The present findings end a decades-long absence of any axenic wild-type anaerobic methanotroph…" (discussion) are significant overinterpretations of the data that promote an inaccurate *state of the art* to nonexperts and quench any further scientific inquiry into this important topic.

It is quite possible that Yan et al. are on the right track. Perhaps canonical methanogens will indeed be shown to grow as anaerobic methanotrophs. However, the present data should inspire the authors to investigate why these organisms are apparently incapable of methanotrophic growth, not to claim that they "…definitively show that canonical methanogens are capable of growth as methanotrophs…" (conclusions). We note that this is not the first time such claims have been made within the field; thus, our concerns are not merely academic quibbling over semantics. It is our hope that addressing this issue in a public forum will lead to a more careful and valid assessment of the science, both in retrospect and going forward.

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The authors declare no competing interest.

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