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COMMENT

The '1% culturability paradigm' needs to be carefully defined

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In their commentary in ISME Journal, Steen and coworkers expressed concerns over my recent analysis about the culturability of microorganisms [1, 2]. My analysis challenged the long-standing and oft-repeated '1% culturability paradigm' stating that most microbes cannot be cultured. First and foremost, I argue that the paradigm is poorly defined but can be classified into at least three hypotheses: (H1) *1% of cells* in a community can be cultivated, (H2) *1% of taxa* in a community can be cultivated or (H3) *1% of cells* in a community grow when plated on *a standard agar medium*. A second complication centers on how one defines whether two organisms are the same vs. different, and studies abound with discussions of this point [3]. A narrow definition of similarity will invariably lead to a low representation of microbial communities in culture collections due to the sheer abundance and sequence variants of microorganisms. Depending on how one defines the problem, when something is the same or different, and the specific environments in which it is tested, you get different answers. However, I show that if you apply the most common interpretation of the paradigm (H1) and the usual definition of taxon similarity (~97% 16S rRNA similarity), you reach the conclusion that many and especially abundant lineages have already been cultured across diverse environments.

Steen and coworkers commentary challenge this conclusion but do not identify which version(s) of the paradigm they challenge. I do not doubt the quality of their analysis, and the differences in their conclusion would seem to support my original point that framing the problem matters. I interpret their analysis as a test of H2 as they dereplicate their sequence libraries and thereby remove taxon-abundance effects. In my original analysis, the interpretation of H2 is sensitive to the inclusion of rare sequence variants (Figure S5 in the original paper). Some of these rare variants are real organisms, whereas others may represent sequencing errors. Many recent culturing efforts have focused on abundant lineages. Thus, the increased representation of either rare members or sequencing errors in your analysis should lead to a low observed culturability. Steen and coworkers also advocate for a narrow definition of taxon similarity. They state 'that it is impossible to know whether a microbe is culturable until it has been cultured' and that 'a great deal of genomic and phenotypic diversity can exist among strains of the same species.' Thus, it is not surprising that they come to a conclusion that most microbial taxa have not been cultured.

The different interpretations can be well illustrated with the abundant marine cyanobacterium *Prochlorococcus*. There are ~10^{27} *Prochlorococcus* cells in the ocean [4], and we have ~50 diverse isolates in culture representing most subclades [5]. Culture experiments have revealed much about the shared physiology (and variation) of *Prochlorococcus*, but sequencing has also revealed that key traits and specific genotypes are missing among the isolates [6–8]. Is *Prochlorococcus* culturable and/or cultured? The answer differs depending on your criteria.

Personally, I have for many years taken the paradigm of 1% culturability as a discouragement for trying to isolate microorganisms—and I suspect many others have been discouraged, too. We are developing increasingly sophisticated techniques for molecular in situ analyses of microbial communities, whereas very few cultivation-based model systems are proposed. However, some (but too few) studies have revealed that traditional techniques like dilution-to-extinction or using substrate-poor agar plates can lead to the isolation of many abundant microbial lineages [9–11]. I hope to illustrate with this work that, since the 1% culturability paradigm was proposed, we have made great strides in culturing representatives of abundant lineages from multiple environments, and we should continue down this path.

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