Accessing the metabolism of viable but non-culturable (VBNC) microbes

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Abstract

While many microbes could be cultivated on common nutrient medium from environmental samples, there is perhaps a larger consortium of microbes that could not be brought under cultivation. Known as viable but non-culturable (VBNC) microbes, many facets of cell biology, biochemistry and physiology remain hidden from view given the inability to culture them in the laboratory. Without the ability to culture VBNC, many modern genetic tools could not be used to interrogate intrinsic metabolic capabilities and regulatory mechanisms of the cells. A more important question is perhaps what defines the VBNC state. Specifically, what is the level of metabolic activity in such cells and which branch of metabolism remains active in helping cells maintain cellular sensory system essential to understanding extracellular nutrition and environmental conditions crucial for activating vegetative growth under the right conditions? To answer the questions, we first need to develop methods for identifying cells in the VBNC state. One possibility involves screening environmental microbes for their ability to grow in rich medium under standard laboratory incubation conditions using 96 well plate assay where single cells are inoculated into each well. Cells that fail to grow would subsequently be selected for single cell RNA sequencing to understand the transcriptome that could be correlated to the VBNC state. In parallel, single cell whole genome sequencing could also be conducted to obtain the reference genome on which expression of different genes in the transcriptome could be assessed. Specifically, automated gene annotation pipelines could be used for gene detection; thereby, yielding an ensemble of genes useful for understanding the transcriptome. But, detection of mRNA transcripts does not mean the successful translation of mRNA into proteins. More importantly, while single cell proteomics might be achievable on a routine basis in future, conventional methods lack the sensitivity for profiling cellular proteome at the global level in single cell given the inability to massively amplify proteins unlike the case for DNA or RNA. Similarly, single cell metabolomics, which is essential to obtaining a complete picture of cellular metabolism in VBNC state faces challenges associated with sensitivity and detection of a broad range of intermediates and compounds. Thus, at present, efforts to access the metabolic state associated with VBNC would most likely stop at probing the global transcriptome at the single cell level. But, future developments in single cell proteomics and metabolomics would hopefully provide new tools for biologists to revisit the important question on what is the metabolic status of cells in VBNC, and more importantly, which metabolic branch remain active in maintaining sensory awareness of the cell's immediate environment.

Keywords: metabolism, transcriptome, single cell RNA sequencing, whole genome sequencing, single cell proteomics, metabolomics, cellular physiology, viable but not-culturable (VBNC), metabolic state, cell sensory system,

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