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EDITORIAL

Viable but non-culturable and dormant bacteria: time to resolve an oxymoron and a misnomer?

For many years microbiologists have accepted the view that the capacity of a cell to replicate to detectable levels (a colony or broth turbidity) reflects its viability [1]. The proposal that several pathogens which are readily culturable, including *Vibrio cholerae, Campylobacter jejuni* and *Helicobacter pylori*, may enter a state in which they retain viability but fail to grow in conventional culture [2–5] strikes at the core of the equation, viability = culturability, and has given rise to the apparently self-contradictory expression 'viable but non-culturable' (VNC or VBNC). The observations that led to this proposal fall into two main categories.

Firstly, the application of so-called rapid viability assays to stressed populations of bacteria in which the total number of intact cells demonstrable by microscopy exceeds the number capable of immediate growth by several orders of magnitude [6]. These assays have regularly revealed 'signs of life' or 'absence of signs of death' in large numbers of cells that could not be cultured [e.g., 3, 7–9]. In a limited number of instances it has been claimed that special culture methods or animal passage [e.g., 7, 10–12] can induce a return to the culturable state.

Secondly, the distribution of certain infectious diseases is at variance with our ability to culture the causal organisms from implicated sources or reservoirs. The possibility that these apparent discrepancies could be attributed to failure of culture-based systems to detect 'VNC forms' of the organisms concerned presents an alarming prospect and a potentially serious challenge to public health microbiology. These concerns are heightened by the studies in which evidence for the presence of non-culturable pathogens has been obtained [e.g., 13, 14].

However, in spite of >15 years research in this area, there is no consensus on the physiological basis for, or the medical significance of so-called VNC bacteria. Moreover, much of the debate has been fuelled by a failure of many authors to define what they mean by 'VNC' in the first place and this has tended to obscure key scientific questions. Critically, the whole area raises concerns about how reliable culture-based methods are as means of determining the distribution of pathogens and their responses to antimicrobial agents. These concerns will remain until we are able to achieve a clearer understanding of the basis for the phenomena that led to the proposal of a VNC state. Thus, even though the direct evidence for involvement of VNC forms in human infection is far from conclusive, the practical need for confidence in our methods for bacterial detection and assessment of cidal activity require that we investigate the problem further.

The central scientific issue raised by authors who refer to bacteria in the VNC state is the view that non-culturable cells may result from a genetically determined differentiation process, perhaps comparable to sporulation. Against this view is the lack of specific information on the nature of the stimuli required to initiate the process, the phenotypes of the cells that result from it (the connection with coccoid transformation is by no means clear) and the stimuli required to return cells to the culturable state. In particular, it has not been possible to integrate observations on the biochemical changes associated with VNC phenomena with the mass of information emerging from studies on the molecular and genetic basis of adaptive responses in bacteria. Until this can be achieved, the notion of a unitary 'VNC state' as an aspect of bacterial physiology will remain controversial.

An alternative and perhaps more conventional view of VNC phenomena sees some cells which are classified as non-culturable as injured [15]. Classically, such cells may be recognised by their failure to grow under selective isolation conditions (e.g., on media containing bile salt) and recovery or repair leads to restoration of this particular growth property. In this context, the recovery medium through which cells apparently return to culturability becomes critical. Perhaps man is a particularly good recovery medium [16], better in practice than any available set of laboratory media.

Irrespective of the true basis for the phenomena that have been discussed in the VNC domain, the medical issues remain. Moreover, they are heightened by examples of studies in which it is quite clear that conventional short-term culture has failed either to predict the infectivity or the ultimate culturability of bacteria [e.g., 11, 17–19].

A critical aspect of further progress in studying these issues is the terminology. Although the acronyms VNC and VBNC have provided stimulating headlines, the notion of viable but non-culturable bacteria belonging to species that can normally be cultured is not logically sustainable in an operational sense. This conclusion is based on the view that ultimate demonstration of culturability remains the only practically acceptable definition of viability for these organisms. Any cell which has the capacity to be cultured in the future but does not grow under standard conditions for that organism is better termed 'not immediately culturable', as it remains cultur*able* in an ultimate sense. The suggestion that measures of cellular activity or integrity can supplant culturability as assays of viability can be supported only in very special circumstances and the equation, cellular viability = cellular activity, is demonstrably false in a universal sense [6, 20]. Consequently it is important to recognise metabolic activity and culturability as independent measurable properties of bacteria. Thus, metabolically active cells may be culturable (viable) or non-culturable (non-viable).

Practical microbial science must proceed with terms that are applicable without operational ambiguity. Microbiologists regularly use the term viability in both operational and conceptual senses and much confusion has arisen from failure to differentiate between these two contexts. In fact, use of 'viability' is generally unnecessary and can often be replaced by more practically explicit terms such as colony or most probable number counts.

A further issue of medical significance relates to use of the term 'dormancy'. Microbial physiologists apply this to describe a reversible state of metabolic shut-down [20] and spores provide the prime example. However, medical use has inadvertantly connected the well-recognised dormant or latent phase between primary and post-primary stages of clinical tuberculosis with a so-called dormant state in *Mycobacterium tuberculosis*. Whether clinical latency or subclinical tuberculosis are attributable to host- or organism-related factors is not known. Moreover, in the Wayne model of mycobacterial dormancy [21], although the cells are not growing, they remain metabolically active; thus they would not meet the physiologist's criteria for dormancy as the converse of metabolic activity (at least by some measures). This confusion is further compounded by a tendency to describe putatively VNC cells as dormant.

The time has come to end this terminological anarchy. Medically oriented work on phenomena which have been related to the putative VNC state and bacterial 'dormancy' has flagged up very substantial gaps between the fundamental science of bacterial physiology and the practical concerns of medical microbiology. At the very least, re-integration of these two fields, first by establishing a unified terminology (preferably rooted in physiological definitions) and second by recognising the potential significance to medicine of new insights into bacterial physiology [e.g., 22, 23], should be beneficial to both disciplines.

M. R. BARER
Department of Microbiology,
The Medical School
Framlington Place
Newcastle upon Tyne
NE2 4HH.

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