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# "!...Comment...?"

***Microbiology* Comment** provides a platform for readers of ***Microbiology*** to communicate their personal observations and opinions in a more informal way than through the submission of papers.

Most of us feel, from time to time, that other authors have not acknowledged the work of our own or other groups or have omitted to interpret important aspects of their own data. Perhaps we have observations that, although not sufficient to merit a full paper, add a further dimension to one published by others. In other instances we may have a useful piece of methodology that we would like to share.

The Editors hope that readers will take full advantage of this section and use it to raise matters that hitherto have been confined to a limited audience.

*Jon Saunders*
**Editor-in-Chief**

## Microbial stress and culturability: conceptual and operational domains

The Comment article by Bloomfield and colleagues (2) proposes 'a unifying mechanism for cell destruction' by 'cell suicide' caused by 'oxidation damage generated from within the cell'. They propose that these sorts of phenomena might then serve to explain the so-called viable but non-culturable (VBNC or VNC) state allegedly adopted by certain prokaryotic micro-organisms. Yet, far from providing a unifying concept to account for the VNC phenomenon (*sic*), the Comment adds still further to the conceptual confusions that have clouded the important issues relating to the culturability of micro-organisms. It does so by providing ample illustrations of the experimental and terminological problems that we have attempted to address in a recent review (14).

As is all too common in this field, and notwithstanding the recognition (2) that the term 'VNC' is an oxymoron (1), the authors state that 'there is every reason to accept the practical existence of a VNC state'. Here the authors fail to make the distinction between the conceptual and operational (mis)uses of the term 'VNC'. In an operational context, for micro-organisms that reproduce vegetatively and asexually, only the [well-established (21, 22)] use of 'viable' to mean 'culturable' makes any logical and terminologically self-consistent sense, and the viability status of individual organisms must therefore be scored operationally and retrospectively (14, 23). Acceptance of this would allow authors to avoid curiosities such as 'the viability remains but culturability is lost through an inappropriate recovery protocol' (2). The paper also falls into the trap of assuming (or at least implying) that VNC is a single phenomenon, rather than recognizing that there are likely to be several underlying causes of the failure to culture different micro-organisms, or the same micro-organism under different culture conditions.

Moreover, the paper of Bloomfield and coworkers (2) contains factual errors that undermine even the arguments presented. For example:

(a) The cholera infection study (4), claimed as an example in which 'previously non-culturable vibrios can regain the capacity to multiply', makes no serious attempt to exclude the influence of culturable cells on their results. These authors recovered culturable *Vibrio cholerae* from two of nine volunteers who had been administered 10 billion cholera cells, having shown that culture of samples containing one billion cells failed to give colonies. Such statistics evidently do not stand up to scrutiny, and the only way one can hope to establish if animal passaging might indeed effect the resuscitation of cells that could not otherwise be cultured (14) is to treat each animal as a test tube in a most probable number (MPN) assay, and apply the appropriate statistical treatment (17, 22).

(b) The article confidently states that 'Recently the VNC concept has been extended to cover the vast majority of environmental microbiology'. We do not consider this to be the case and no reference is given to support this statement. Rather this statement appears to be a good example of the injudicious extrapolation that dogs this field and which we have reviewed at length (14). There is no reason (let alone evidence) to expect that any VNC-like phenomena in culturable organisms and/or 'as yet uncultured' organisms result from the same fundamental processes. Indeed, there are more than sufficient examples of organisms that have defied our efforts to culture them until the critical component had been added to the medium, well-known examples including *Legionella* spp. (16). In fact, an extension of this experimentally observed phenomenon to explain the majority of so-far uncultured organisms [many of which are indeed phylogenetically close to cultured relatives (15)] makes much more sense than the speculations provided (2). Indeed, the question of whether the abundant 'ultramicrobacteria' found in the marine environment are small forms of normal bacteria or normal forms of small bacteria (10) seems largely to have been answered in favour of the second of these (3, 24). Isolation of these facultative or obligate oligotrophs depends on the use of nutrient-poor media under effectively MPN conditions in the absence of competitors, while the recovery of starved cells of normally copiotrophic bacteria may similarly depend rather finely on the inclusion of low, but not high (i.e. 'normal'), nutrient concentrations in the recovery medium (19).

(c) To bracket together the phenomena referred to in *Escherichia*, *Micrococcus* and *Vibrio* makes no logical sense whatsoever. Quite apart from anything else, the dormant (or Not Immediately Culturable) state demonstrable in *Micrococcus luteus* (7, 8, 9, 10, 11, 12, 25) can now be explained at the

### ▶ *GUIDELINES*

Communications should be in the form of letters and should be brief and to the point. A single small Table or Figure may be included, as may a limited number of references (cited in the text by numbers, and listed in alphabetical order at the end of the letter). A short title (fewer than 50 characters) should be provided.

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molecular level. It clearly involves growth stimulation (9, 20), rather than prevention of suicide. Moreover, no VNC state has ever been proposed for *M. luteus*. As is again all-too-common in this field, the authors confuse and conflate the terms 'dormancy' and 'VNC'. Dormancy is a state of low metabolic activity from which cells can emerge and become culturable (10), i.e. by definition, dormancy [and related cryptobiotic states (13)] is reversible. 'VNC' in its usual usage is a state of measurable or even high metabolic activity in which the cells will not divide nor demonstrate that they are culturable. These states clearly could not be more different, and may in fact be the exact opposite. The confusion is exactly illustrated by the statement (2) 'Of course (*sic*) the possibility of cell suicide associated with attempts to culture starved microcosms does not preclude the possibility of transition to a "dormant" phenotype'. It does, since suicide is irreversible, dormancy is not.

None of these points excludes the authors' proposal as an interesting contribution to the list of mechanisms that might be used to account for the failure to cultivate normally culturable cells, and it is not our aim here specifically to criticize this hypothesis. It is of course widely recognized that many cells of a given species can enter a physiological state, e.g. stationary phase (6), in which they are significantly more resistant to environmental insults such as hydrogen peroxide addition. The usual feeling is that this is due to phenomena of the type in which heat-shock proteins bind to one or more sensitive targets and protect them from damage (5). The implicit assumption is that the target is itself no more resistant but that it is protected. Bloomfield *et al.* (2) stress, rather by inverting the argument, that a more subtle possibility exists: cells in exponential phase (or some appropriate non-growing state) are indeed more sensitive *but that they bring about their own destruction* (e.g. by making enzymes which are more likely to lead to free-radical production). A subtle distinction, but one arguably worth making. However, although it is well known both that reactive oxygen intermediates are antimicrobial and that the level of a cell's resistance depends on its composition, no unitary hypothesis can explain the cytotoxicity of dioxygen and its reduced forms (18).

It is therefore to be hoped that if and when the importance of free radical generation in affecting the culturability of normally culturable micro-organisms is tested (i) they do not use radical-trapping or other reagents that can themselves cause the production of free radicals and cytotoxic reduced oxygen intermediates, and (ii) they adopt terminologies for physiological states that are both logically self-consistent, and explicitly and operationally defined.

**Michael R. Barer,[1]* Arseny S. Kaprelyants,[2,3] Dieter H. Weichart,[2] Colin R. Harwood[1] and Douglas B. Kell[2]**

[1]Department of Microbiology, The Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH, UK.

[2]Cledwyn Building, Institute of Biological Sciences, University of Wales, Aberystwyth SY23 3DD, UK.

[3]Bach Institute of Biochemistry, Russian Academy of Sciences, Leninskii prospekt 33, 117071 Moscow, Russia.

*For correspondence. Tel: +44 191 222 8264. Fax: +44 191 222 7736. e-mail: m.r.barer@newcastle.ac.uk

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