

COMMENT

Is the CTC dye technique an adequate approach for estimating active bacterial cells?

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In the paper 'Coherence of microbial respiration rate and cell-specific bacterial activity in a coastal planktonic community' by Smith (1998), *Aquat Microb Ecol* 16:27-35, the author, using the fluorogenic tetrazolium dye 5-cyano-2,3 ditolyl tetrazolium chloride (CTC)-as a measure of cell-specific metabolic activity, and comparing the numbers of CTC reducing cells with respiratory activity, reported that CTC-active (CTC+) cell abundance was highly correlated with respiration rates within the microplankton community, explaining 80% or more of the variations in these rates. This finding is a new and interesting observation which had not previously been reported.

However, the fraction of CTC+ cells made up on average only 14% of the total bacterial numbers although a eutrophic site, Chesapeake Bay, was investigated. The author concluded that this low fraction of CTC+ bacteria '...is likely responsible for the bulk of bacterial community metabolic activity...'. On the other hand, the portion of active bacteria has been reported to be 25 to 94% of total bacterial numbers using 2-(*p*-iodo-phenyl)-3-(*p*-nitrophenyl)-5 phenyltetrazolium chloride INT in the same environment (Tabor & Neihof 1984). To explain this discrepancy the less direct coupling of INT to respiratory activity compared to CTC was cited (Smith & McFeters 1996, 1997), necessarily leading '...to lower estimates of that fraction of the total population quantitatively engaged in respiratory metabolism...'. But does this mean that INT-positive bacteria can be neglected with respect to the metabolic activity of bacterial communities?

There are, indeed, a number of methods which yield distinctly higher levels of metabolically active bacterial cells compared to the CTC method. In a recent study of

marine bacterial assemblages (aged offshore water compared to rich harbour water), metabolically active bacteria were simultaneously determined using different methods (Karner & Fuhrman 1997). In this study, universal 16S rRNA probe counts averaged about half of the corresponding DAPI counts and were highly correlated to microautoradiographic counts (using a [³H]amino acid mixture), while the number of CTC+ cells were 2 orders of magnitude below DAPI counts, and thus well below the estimates obtained using the other methods. In another study, CTC+ counts were compared to esterase active cells (Yamaguchi & Nasu 1997). While CTC+ cells amounted to only 10 to 15% and 30% of total bacteria numbers in an unpolluted and a highly polluted river, respectively, esterase active cells made up 50 and 90%, respectively.

As mentioned by Smith (1998), low fractions of active cells were generally reported if the *in situ* activity of natural communities was determined using CTC (e.g. Schaule et al. 1993, Gasol et al. 1995, Choi et al. 1996, Lovejoy et al. 1996, Ullrich et al. 1996, Kalmbach et al. 1997, Karner & Fuhrman 1997, Yamaguchi & Nasu 1997). But the conclusion (derived from the correlation between the number of CTC-reducing cells and respiration) that CTC+ cells are '...responsible for the bulk of community processes such as production and respiration...' can be misleading in this generalised form. The low portions of active cells generally observed under natural conditions using the CTC method may be instead due to methodological uncertainties related to this approach. For example, it was observed that several bacterial strains tested were difficult to stain with CTC (Yamaguchi & Nasu 1997). Smith & McFeters (1997 and literature cited therein) reported that some active bacteria do not reduce CTC under certain conditions. Furthermore, it is known from a number of investigations that the portion of CTC-reducing

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bacteria increased with greater nutrient concentrations (e.g. Rodriguez et al. 1992, Schaule et al. 1993, Smith et al. 1994, Smith & McFeters 1996 and literature cited therein, Kalmbach et al. 1997). Only a few exceptions to this pattern have been reported so far (Coallier et al. 1994, Pyle et al. 1995). Therefore, the use of CTC might be problematic if the method is applied to study sites characterised by low nutrient concentrations. Karner & Fuhrman (1997) reported CTC+ counts below 1% of DAPI counts for a marine environment (Santa Monica Bay). In the central Arabian Sea (oligotrophic conditions), CTC+ counts amounted to about 2 to 3% of total bacteria numbers, clearly ranging below counts determined by microautoradiography (6 to 46%, n = 11). Even under coastal upwelling conditions off the Omani coast (enhanced nutrient conditions), the portion of CTC+ bacteria made up only 7% of total bacterial counts (Ullrich et al. unpubl. data).

Indeed, it is uncertain whether the CTC method is a suitable approach for the determination of the actual number of respiring bacteria. The relationship between CTC reduction and CO₂ production was recently investigated in mixed microbial communities under changing nutrient conditions (Cook & Garland 1997). A general lack of coincidence between the formazan-containing cell population and changes in respiration was found, contradicting the view that CTC reduction and respiration are generally closely linked. Inhibitory effects of CTC on bacterial respiration and metabolic activity were reported by Ullrich et al. (1996). There was evidence from this investigation that bacterial production (calculated from [³H]leucine uptake) and respiration ([¹⁴C]glucose) were greatly reduced to 1–14%, and 4–44%, respectively, by the addition of CTC compared to untreated controls, leading to an underestimation of actively respiring cells by CTC. Additionally, inhibitory effects of CTC were demonstrated by means of luminescence in a Microtox bioassay. CTC concentrations of 0.1 and 5.0 μM required only 15 min for decreases of approximately 50 and 100%, respectively. Furthermore, it was calculated that the low fraction of active cells determined by the CTC method would result in unrealistically high per cell production and implausibly short generation times.

Many of these critical results were not considered or discussed in the commented paper. The number of actively respiring cells might be underestimated due to the methodological insufficiencies of the CTC method mentioned above. Even if CTC+ cells represent the most active bacteria (as claimed by Smith), this pattern cannot be clearly defined and standardised. Not taking into account the results of former publications, Smith concluded that the CTC method '...provides an ecologically meaningful measure of active bacterial abundance...'. This general view is inconsistent with the

results of the comparative methodological studies cited here. One must distinguish between the number of CTC-reducing bacteria determined in special *in situ* conditions, and the actual number of active bacteria as determined by more sensitive methods.

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