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A cultural divide on the use of chemostats

The attractions of chemostat studies are indeed too often overlooked as suggested by Hoskisson & Hobbs (2005). My laboratory routinely uses continuous cultures and I fully agree that there is a great deal of research that would benefit from using cells grown in this way. Still, before labs gear up for chemostat studies to provide reproducible conditions for global regulation and proteomics, the over-simplifications of Hoskisson &

Hobbs (2005) need to be balanced by pointing out some of the pitfalls as well.

The first obvious point is that chemostats induce a particular nutrient-limited state. Nutrient limitation results in bacterial responses not only specific for the type of limiting nutrient, but also responses that are far from uniform for a particular nutrient. The choice of different growth or dilution rates will critically influence the expression patterns obtained (Ferenci, 1999a; Harder & Dijkhuizen, 1983). For example, a glucose-limited *Escherichia coli* cell grown at a dilution rate of 0.1 h⁻¹ is near starvation and has highly induced stress responses, whereas bacteria grown at 0.6 h⁻¹ have elevated hunger responses and increased nutrient scavenging activities (Ferenci, 2001). Dilution rates in between have a varying mix of both responses. This aspect is poorly appreciated and not well integrated into many of the studies cited in Hoskisson & Hobbs, where mostly an arbitrary dilution rate is chosen.

A second point that needs elaborating is that nutrient limitation is an extremely strong selection condition for mutational changes that rapidly sweep populations. Indeed, the historical introduction misses the aim of Novick & Szilard (1950) who were more interested in the evolutionary applications of continuous culture than in the production of reproducible, steady-state bacteria (which was Monod's aim). The conditions to establish a steady state suggested by earlier workers (multiple chemostat culture volumes to achieve a physiological 'steady state') are indeed sufficient for *E. coli* populations to be swept by *rpoS* mutations (Notley-McRobb *et al.*, 2002). An added complication is that the type of takeover is very strain-dependent, even amongst laboratory strains of *E. coli* (King *et al.*, 2004). So before functional genomic assays are assessed, the time-course of changes needs to be carefully controlled. The dichotomy between the desire for 'steady state' in functional genomics studies and the rapid selection for change needs to be appreciated.

The third point missed by Hoskisson & Hobbs (2005) is that there is actually no true steady-state in chemostats for the simple reason that the residual concentration of limiting nutrient

continues to drop for hundreds of hours in a culture (Kovarova-Kovar & Egli, 1998). So a lot of the kinetic assumptions and the estimations of supposed constants, like K_s , are subject to complex environmental effects and changes in populations (Ferenci, 1999b).

As with all methodology, adoption requires an appreciation of the drawbacks and limitations as well as the advantages cited by Hoskisson & Hobbs (2005). In my personal opinion, chemostats are indeed useful for looking at growth rate effects on expression and metabolism as long as the complexities above are taken on board. However, continuous cultures are even more suited to studies of evolution and the processes of mutation and selection in response to controlled environments.

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