One size doesn't fit all

In his Comment article in the May issue of *Microbiology* (Ferenci, 2006), Dr Ferenci provides a very timely 'pull on the reigns' and reminds us that what we all have been taught classically about continuous cultures is a gross oversimplification. Pragmatically, or by necessity if you like, the theory of the continuous culture is based upon a simple mathematical model. If we look at the constants in that model, $\mu_{\max}$ and K_s , we really should stop to think. These are not invariable within a population of cells and, as such, mutations will sweep through a population due to selective pressures (as indicated by Dr Ferenci). These mutant 'take-overs' have been described in several studies, and have been shown to be strain-dependent. Indeed, our laboratories work mainly with *Streptomyces coelicolor*, where visually obvious mutants (asporulent and pigmented antibiotic variants) occur frequently upon prolonged continuous culture. These strain variabilities and mutational effects are approached in our laboratories by restricting prolonged studies (reduction in generation times) when the mutational aspect is not the reason for study, thus material required for analysis is harvested on or at the establishment of (quasi-) steady-state, especially for global studies. With respect to our previous article (Hoskisson & Hobbs, 2005), we failed to indicate that the establishment of (quasi-) steady-state under empirically established conditions may be problematic in the initiation of studies, and a careful experimental design strategy is required.

Mutations aside, one should also carefully consider K_s , the substrate affinity constant. Substrate uptake mechanisms are often composed of multiple transporters for nutrients. These nutrients can often have carrier molecules facilitating their 'capture' and binding (e.g. siderophores involved in iron binding). These transporters and siderophores are not mutually exclusive and considering the term K_s as a 'single' constant is probably the greatest fault in the

mathematical model. Monod's approach of producing a simple mathematical model remains valid today. However, it should be tempered by an appreciation of the fact that what we are dealing with are highly complex biological systems.

It is not doubted that continuous culture systems are of huge benefit to a multitude of studies, and the study of bacterial evolution and selection processes are a major beneficiary of the versatility of continuous culture. However, I feel that, providing the pitfalls outlined by Dr Ferenci are appreciated, the initiation of global studies of transcription, proteomics and metabolomics in continuous culture systems will provide useful insights into the biology of bacterial physiology.

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On the enhancement of anti-neurotoxin antibody production by subcomponents HA1 and HA3b of *Clostridium botulinum* type B 16S toxin-haemagglutinin

In a recent article in this journal, Lee *et al.* (2005) reported that immunization of mice with formaldehyde-treated botulinum neurotoxin B (BoNT/B) in the presence of haemagglutinin subcomponents HA1 and HA3b, normally present in the 16S toxin, produced higher levels of anti-BoNT/B antibodies (Abs) than immunization with BoNT/B alone. Because of the paper's potential clinical therapeutic implications, this comment discusses three major issues in the paper: quality of the antigen, dose and frequency of immunization. I show that these were quite different from those used in therapeutic applications of active BoNT/B (or any BoNT) complex.

BoNTs (mostly types A and B) are used to treat a variety of clinical conditions associated with involuntary muscle spasm and contractions as well as in cosmetic and other therapeutic applications (Jankovic, 2002, 2004; Atassi & Oshima, 1999; Brashear *et al.*, 2004; Dressler & Bigalke, 2005). The therapeutic benefits are of limited duration and toxin injections need to be repeated every 3–6 months. In a very small percentage of patients (less with toxin A than with toxin B) the treatment elicits blocking Ab responses against the correlate toxin, which reduce or completely terminate the patient's responsiveness to further treatment (Göschel *et al.*, 1997; Atassi & Oshima, 1999; Atassi, 2002, 2004;

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Charles J. Dorman, Editor-in-Chief