



Probiotics — let's get the strains right before we even try to count them!

To the Editor: In response to rebuttals published in the March¹ and April^{2,3} 2004 issues of the *SAMJ*, the study 'An evaluation of nine probiotics on the South African market'⁴ published in the February issue is less about the recognised benefits and more about the quality of the probiotics tested. The starting point is the claim relating to the organism's strain stated on the label of, and the quantity of micro-organisms contained in, some products on the market in South Africa.

1. The study was done independently by a team of microbiologists at the University of Ghent in Belgium, using internationally recognised methods and experienced in analysing products of this nature. The results were reviewed and written up by an independent medical microbiologist, Dr Elliott, reviewed by two senior peers prior to submission, and reviewed by the *SAMJ* peer review committee before publication.

2. All the samples were sent to Belgium under the same consignment conditions (cold-chain conditions) under which Pharmadynamics and Thebe Pharmaceuticals import their products from Scandinavia.

3. Denaturing gradient gel electrophoresis (DGGE) is not a method used to quantify bacteria but, as clearly stated in the article, a culture-independent technique of direct identification of bacterial strains in probiotic products. DGGE is an internationally recognised method, validated by Temmerman *et al.*,^{5,6} of evaluating bacterial strains in a sample especially where multiple bacteria exist. *Bergey's Manual* provides renowned and approved methods of phenotypic culture-dependent analysis. In the chapter on lactobacilli, one example states that the identification of *Lactobacillus acidophilus* cannot be based on simple phenotypic tests, to reliably distinguish it from *L. gasseri*, *L. crispatus* and *L. amylovorus*. DGGE offers a new validated method of more accurate strain identification of bacteria than the traditional culture-dependent methods. Where a product contains heat-killed bacteria, the DGGE method and probably also PFGE and RAPD are not reliable owing to denatured DNA. Only one product in the study contained a heat-killed lactobacillus. That was Lactéol Forte, not claimed as a probiotic but included in the study because it is has been promoted as a probiotic (personal communication with pharmacists).

4. Experience through preparing and testing many samples from different dosage forms has allowed the Ghent microbiologists to develop a standardised and validated protocol that most consistently provides the highest bacterial counts. Furthermore, where growth was low or zero, a further sample was submitted to the same triplicate procedure

(internationally recognised) as well as broth culture to give the bacteria the best chance (personal communication with Dr Temmerman). This standardisation is also the reason why the overall counts achieved in this analysis are lower than the generally accepted counts for probiotic efficacy of between 10^7 and 10^{10} according to clinically proven doses of different probiotic strains depending on their robustness and ability to survive the human digestive tract. This standardisation further meant that, for the purposes of interpretation of this study, the cut-off count of 10^6 was used as a threshold value for physiological probiotic effects and not higher as suggested in the literature.⁷ Information on methodology was referenced in the original article and exclusion of methodological detail from the article does not detract from the conclusions. Overall, we maintain that the article is a fair and accurate interpretation of the report received from the laboratory in Ghent, and therefore of the quality of the probiotics randomly selected and analysed at that point in time.

5. The Joint FAO/WHO working group guidelines for the evaluation of probiotics in food advocate that the nomenclature of the bacteria must conform to the current, scientifically recognised names to prevent consumers and regulatory agencies making assumptions about the identity of the real bacterium being sold.⁸ While probiotic effects are strain-specific and not species-specific, DGGE is capable of distinguishing between *Bifidobacterium longum* and *B. infantis*. It is noted that *B. suis*, *B. infantis* and *B. longum* are three different biovars and should be unified as *B. longum*.⁹ Using the species names *infantis* and *longum* interchangeably on labels creates confusion which will probably be resolved when their reclassification is published in the *International Journal of Systemic and Evolutionary Microbiology*.

6. The importance of strain identification is further illustrated in a recent letter to pharmacists in South Africa dated 20 February 2004 (J Smith, 'Blatant misinformation on probiotics') which indicates that 'they have proven that their product yields millions of bacteria as claimed'. A second letter to the pharmacists, dated 1 March 2004 (J Smith, 'Levels of viable cells per capsule'), reports counts of 'viable cells' in various batches ranging from 30 to 150 million per capsule with no identification. The label claims that the product contains 'Lactic Acid Bacillus' which could mean any one of many lactobacilli or bifidobacteria. At present it is not known what organism is in this product. If we don't know what it is, how do we know it's safe?

7. Regular analysis of off-the-shelf probiotics is essential to ensure and maintain quality, and as such the Complementary Medicines Council of the Medicines Control Council is now aware of the lack of regulation of this emerging market and has established an investigation following a mandate issued by the registrar of medicines.



8. There are 55 probiotic products with registered NAPPI codes available to pharmacies and health shops. Various independent investigators, among whom is Dr Temmerman from the University of Ghent, have analysed many different probiotic products, of which the trade names are cited in the references.^{5,10-12} In our opinion, using trade names is upfront, honest, and enables clear communication with the health care profession and the consumer needing to be educated to make a decision about products that contain a number of difficult-to-pronounce bacterial names (unlike products containing a generic active like 'paracetamol').

We contend that it is the manufacturer's and distributor's minimum responsibility to ensure that their product complies with its label claim, and that it is the right of the health care worker as well as the consumer to know that the probiotic product recommended or purchased contains what it claims to contain. Accurate strain identification and viable count are paramount. Proof of label claims would provide an appropriate starting point for the industry to assist regulatory bodies in drawing up guidelines for trustworthy probiotic products. What needs to follow is a comparative review of the various claimed health benefits and published literature of the different probiotic strains.

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Probiotics — consensus of analysis

To the Editor: I have some important issues to raise with regard to the paper by Elliott and Teversham¹ and the rebuttals and opinions emanating from it.

While traditional starter cultures used in the dairy industry are selected for their ability to rapidly produce desirable organoleptic qualities of cultured dairy products, probiotic bacteria are selected for the potential to provide specific health or nutritional benefits following consumption. It must be realised that such a selection addresses several criteria including safety, technological and functional aspects.² The latter aspect has been extensively studied and reviewed, and the editorial 'Probiotics — how functional are they?' by H J Koornhof in the April *SAMJ*³ clearly demonstrates the paramount fact about probiotics: their functionality in terms of health benefits to man and animal.

Two frequently overlooked and ignored aspects of safety and technological difficulties remain. The safety of probiotic strains is of major importance and guidelines for the safety assessment have been addressed in several articles.^{4,6} The prerequisite of microbiological safety is the *identification of the strain*. The current state of evidence includes that probiotic effects are strain-specific, meaning that correct identification of the strain level is important to link a strain to a specific health effect as well as to enable accurate surveillance and epidemiological studies.⁷ Furthermore, the origin of the strain and its potential GRAS (Generally Regarded as Safe) status,⁸ antibiotic resistance profile and possible adverse side-effects are a few of the many issues to be addressed in the process of pre-marketing a probiotic.

To succeed in promoting the consumption of probiotic products, the food industry has to meet the demands of the consumer: in other words all probiotic foods should be safe, functional and attractive to the senses. Before probiotic strains can be delivered to consumers, they must first be capable of being included into industrial fermentations. Next, they need to survive and retain their functionality during storage as frozen or freeze-dried cultures as well as in the commercial food products to which they are added. Finally, the packaging material and storage conditions are important factors influencing the quality of the probiotic product during its shelf life. In most cases, the probiotic properties are affected by the way in which the strain or culture has been produced,⁹ meaning that each strain and its production process should be characterised extensively in order to enable an effective probiotic product to enter the consumer market.

Regulatory and product labelling issues in the functional foods area (probiotics are not considered separately in this field) primarily involve two concerns: safety and assuring that product labelling and promotion, while communicating the