



An evaluation of nine probiotics available in South Africa, August 2003

E Elliott, K Teversham

Background and objective. Although probiotics are not new, 5 new commercially available products have been launched onto the South African market in the last 2 years. Evaluations of products in the USA and Europe have shown poor correlation between label claims and actual contents. We undertook an evaluation of 9 products currently available on the shelves in South Africa.

Methods and analysis. An independent laboratory was used. A culture method involving serial dilutions on selective media was used to obtain a colony count per gram for the indicated organisms. A non-culture method, denaturing gradient gel electrophoresis (DGGE), was used to determine the organisms present in the products.

Results. Disturbingly, we found a relatively poor correlation between the advertised and determined bacterial content.

Only 3 of the 9 products tested contained the bacteria indicated on the label and 5 products contained sufficient bacteria for a probiotic effect. The *Enterococcus faecium*, a potential pathogen and *Saccharomyces cerevisiae* found in 2 of the products are of concern.

Conclusion. This evaluation confirms that the contents of several probiotics available in South Africa do not correspond to the label claims. This is of concern as clinical efficacy is dependent on strain specificity and organism numbers. Current regulatory requirements do not address this discrepancy. As such, we recommend that commercially available probiotic products be screened annually, and the results of such quality control measures be made available to the Medicines Control Council (MCC).

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Although the concept of probiotics is not new, the advent of commercial products has refocused attention on their potential uses and applications.¹ Probiotics are defined as 'live microbial supplements, which when given in sufficient amounts, offer health benefits beyond basic nutrition'. The organisms are lactic acid bacteria (LAB), usually *Lactobacilli* and *Bifidobacteria* species present as normal flora in healthy gastrointestinal tracts. Because of their fastidious growth requirements, a limited number of commercial products are available to the public and health care professionals in South Africa. However, in the last 2 years 5 new probiotic products have been introduced onto the market, with the inevitable competition for the consumers' disposable income. Additionally, the products have been introduced to health care professionals with a variety of therapeutic claims for health and benefit, often with extrapolated clinical evidence of efficacy.

Until recently, registration of these products was the domain of the Department of Health and they were registered as food supplements and designated as 'generally regarded as safe' (GRAS). Because therapeutic benefit is now substantiated with published clinical trials, this regulatory function has shifted to the Complementary Medicines Committee (CMC), set up by

the Medicines Control Council (MCC) as the appropriate body to regulate these products.

Unfortunately, assessment of these products is limited by the lack of independent technical expertise available in South Africa and the expense of setting up the infrastructure to do such testing. Therefore, products are currently not subjected to stringent scrutiny; the manufacturers' claims are difficult to validate and the regulatory body has no mechanism to do post-marketing surveillance. Standardising such evaluation with a validated method would provide a means to assess and compare products, confirm their contents and monitor the effect of storage on their shelf life.

In a recent European survey of commercially available probiotic products, the information on the labels did not correlate with the results of the assessment.² The same was true in a survey of probiotic veterinary and human products available in the USA.³ In a local assessment of commercial yoghurts in South Africa, the results correlated poorly with the label claims.⁴ Prompted by the European study by Temmerman *et al.*² and the USA study by Weese,³ we undertook an evaluation of 9 probiotic products commercially available in South Africa. Our aim was to determine if the label information was representative of the actual contents of the product.

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Ampath Laboratories, Johannesburg
E Elliott, MB ChB, DTH, PCPath (Micro) SA, MMedPath (Micro)

CareCross Health Pty Ltd., Cape Town
K Teversham, MB ChB, BPharm

Purchase and shipping procedure

An independent retail pharmacist ordered a selection of probiotic products via his usual wholesale suppliers. As there



was no way to predict the batch, this was a random selection. The products were kept refrigerated at 4°C until collected by the courier. They were sent under cold chain conditions to the Department of Microbiology at the University of Ghent in Ghent, Belgium. Receipt of products in good condition was acknowledged by e-mail. The Department of Microbiology at Ghent University was chosen as it has done similar analysis before and has no vested interest in any product.

Methods of analysis

Enumeration of the bacteria was performed on a culture-dependent basis. Three selective culture media were used, namely MRS (de Man Regosa and Sharpe) agar, M17 agar and Modified Columbia agar to isolate lactobacilli, streptococci and bifidobacteria respectively. One hundred microlitres of a ten-fold dilution series of each product were plated in triplicate onto the media. Plates were incubated aerobically for 24 hours and under anaerobic and microaerophilic conditions for 72 hours. After incubation, the visible colonies were counted and expressed as colony-forming units per gram (CFU/g), representing the number of viable bacteria present in each product.⁵

Identification of the bacteria present in the products was performed on a culture-independent basis, using the denaturing gradient gel electrophoresis (DGGE) technique.⁵ As this validated method does not require culture it can be used

for a variety of product types and can differentiate between the organisms when more than 1 is present in a product. The technique involves the extraction of bacterial DNA directly from the product, amplification of certain DNA fragments and separation by electrophoresis of these fragments through a polyacrylamide gel. After digital capturing of the DNA-band patterns, bacterial identification is achieved by comparing each DNA band pattern against a DNA pattern database of reference strains of organisms kept at the University of Ghent.

Results

As mentioned, previous studies have found a poor correlation between label and product.^{2,4} Our evaluation of the products available on the South African market shows a similar trend, with only 3 of the 9 products containing the bacteria indicated on the labels and only 5 products with sufficient bacteria for a probiotic effect. The results are shown in Tables I and II.

In Table I, 3 products contained the bacteria indicated on the label. These were the 2 BioPro Reuteri products, the drinking straws and tablets containing *Lactobacillus reuteri*, Infantiforte which contains *Bifidobacterium infantis*.

According to the label, Combiforte is a mixture of three bacteria, *Lactobacillus acidophilus*, *Bifidobacterium bifidus* and *B. longum*. Our evaluation showed that only 2 bacterial species are present and only the *L. acidophilus* corresponds to the label.

Table I. Results of the culture-independent analyses of 9 South African probiotic products in August 2003

Product	Expiry date	Organism on label	Detected using DGGE	Conclusion
BioPro Reuteri straws	Aug 04	<i>Lactobacillus reuteri</i>	<i>Lactobacillus reuteri</i>	Product = label
BioPro Reuteri tablets	Sep 04	<i>Lactobacillus reuteri</i>	<i>Lactobacillus reuteri</i>	Product = label
Combiforte capsules	Mar 05	<i>Lactobacillus acidophilus</i> <i>Bifidobacterium bifidus</i> <i>Bifidobacterium longum</i>	<i>Lactobacillus acidophilus</i> <i>Bifidobacterium infantis</i>	Partial correlation of label and product
Culturelle sachets	Jun 04	<i>Bifidobacterium longum</i> <i>Lactobacillus acidophilus</i> <i>Streptococcus thermophilus</i>	<i>Bifidobacterium lactis</i> <i>Lactobacillus paracasei</i> <i>Enterococcus faecium</i>	Mislabelling Poor identification
Culturelle tablets	Jun 06	<i>Bifidobacterium longum</i> <i>Lactobacillus rhamnosus</i>	<i>Bifidobacterium lactis</i> <i>Lactobacillus paracasei</i>	Poor correlation of product and label
Infantiforte capsules	Jun 04	<i>Bifidobacterium infantis</i>	<i>Bifidobacterium infantis</i>	Product = label
Lacteol Forte capsules	Jan 06	<i>Lactobacillus acidophilus</i>	DNA from heat-killed bacteria not detectable	Not measurable
Lacteol Forte sachets	Feb 05	<i>Lactobacillus acidophilus</i>	DNA from heat-killed bacteria not detectable	Not measurable
Lactovita capsules	Aug 04	Lactic acid bacillus	<i>Saccharomyces cerevisiae</i>	No bacteria present

DGGE = denaturing gradient gel electrophoresis.



Table II. Colony counts of viable bacteria in 9 South African probiotic products in August 2003 expressed as colony-forming units (CFU) per gram

Product	Expiry date	Detected on DGGE	Lactobacilli and other organism count (CFU/g)	Bifidobacteria count (CFU/g)	Conclusion
BioPro Reuteri straws	Aug-04	<i>Lactobacillus reuteri</i>	8.32×10^7 CFU/straw	0	Sufficient live bacteria
BioPro Reuteri chew tabs	Sep-04	<i>Lactobacillus reuteri</i>	1.51×10^8	0	Sufficient live bacteria
Combiforte capsules	Mar-05	<i>Lactobacillus acidophilus</i>	3.62×10^7	5.12×10^7	Sufficient live bacteria
Culturelle sachets	June-04	<i>Bifidobacterium infantis</i> <i>Bifidobacterium lactis</i> <i>Lactobacillus paracasei</i> <i>Enterococcus faecium</i>	1.05×10^5	1.16×10^7	Sufficient live bacteria
Culturelle tablets	June-05	<i>Bifidobacterium lactis</i> <i>Lactobacillus paracasei</i>	0	0	No living bacteria present
Infantiforte capsules	Jun-04	<i>Bifidobacterium infantis</i>	0	4.34×10^7	Sufficient live bacteria
Lacteol Forte capsules	Jan-06	N/A	0	0	No living bacteria present
Lacteol Forte sachets	Feb-05	N/A	0	0	No living bacteria present
Lactovita capsules	Aug-04	<i>Saccharomyces cerevisiae</i>	0	0	No living bacteria present

DGGE = denaturing gradient gel electrophoresis.

The *Bifidobacterium* species present was identified as *B. infantis* and those indicated on the label were not detected. There was therefore partial correlation of the label and the detected contents of the product.

The two Culturelle products showed no correlation between the label claim and the actual *Lactobacillus* and *Bifidobacterium* species identified. In addition, the *Streptococcus thermophilus* indicated on the label of the sachets was not detected. Instead, we detected *Enterococcus faecium*, a potential pathogen associated with invasive disease. In the report of the Joint Food and Agricultural Organisation/World Health Organisation (FAO/WHO) expert group issued in 2001,⁶ it was recommended that enterococci should not be used as probiotics as they can display or acquire resistance to vancomycin⁷ and are commonly associated with nosocomial infections in hospitals.

The label for Lactovita indicates that a lactic acid bacillus is present, but it does not provide any detail of the species. The product was found to contain only the yeast *Saccharomyces cerevisiae* and no bacteria. Rare cases of vulvovaginitis have been associated⁸ with this organism but it has low pathogenic potential. Furthermore, the probiotic property of *S. cerevisiae* is

not established. The discrepancy between label and contents is of concern.

The package inserts of the Lacteol Forte products indicate the contents as killed bacteria. The destruction of the viable bacteria involves exposing the organisms to a high temperature sterilising process. During this process, proteins and DNA are also denatured. This product therefore does not meet the definition of a probiotic, as it contains no viable bacteria and the sterilising process probably negates any potential effects of secreted peptides such as bacteriocins with antibacterial properties. Therefore, this product should not be registered as a probiotic.

Table II indicates the results of the viable bacterial colony counts. The expiry dates as indicated on the labels are included in the table. As none were due to expire in 2003, viable organisms should have been present. Of the 9 products tested, only 5 contained sufficient numbers of organisms to have probiotic potential.

In summary, according to our evaluation, only 3 products meet the criteria of a probiotic as they contain: (i) the organisms indicated on the label (Table I); and (ii) a sufficient number of organisms for a clinical effect (Table II).



Discussion

The clinical uses of probiotics and their potential benefits have recently been comprehensively reviewed by Mare and du Toit.¹ These include stimulation of the immune system, anticarcinogenic properties, cholesterol reduction, management of lactose intolerance, alleviation of constipation, management of infectious diarrhoea, management of peptic ulcer disease, management of inflammatory bowel disease and the treatment and prevention of urogenital infections.

The benefits show not only species-specific but also strain-specific effects and therefore cannot be generalised to all the LAB organisms. Due caution is therefore advised when assessing the claims of benefit. Where possible, claims of benefit of a particular product should be substantiated by the results of well-designed clinical trials. Unfortunately, many of the organisms in the products available in South Africa have not been substantiated in peer-reviewed publications. Rather, their claimed efficacies have been extrapolated from studies on other similar organisms. The notable exception involves *Lactobacillus reuteri* ATCC 55730 where the product available corresponds to the organism for which the clinical data are available. Although there are clinical data for *Bifidobacteria infantis* registered with Nestlé in a milk product,⁹ extrapolation of these data can be made to the Infantiforte *B. infantis*.

Although probiotics have GRAS registration, a few serious infections (but to date no deaths) have been reported as being directly attributable to probiotics. Therefore, finding *E. faecium*, a known potential pathogen isolated from immune-compromised patients is of concern as its presence is contrary to international guidelines. The potential for antimicrobial resistance is a further contraindication to its use in probiotic products. In the interests of safety, products containing this organism should be withdrawn.

The results of our evaluation clearly show the limitations of the current system for the evaluation of probiotics for registration and the lack of post-marketing surveillance. For probiotics to be effective, they must meet the minimum requirements of containing clinically validated species present in sufficient and viable quantities. It would be interesting to subject other new milk-based products such as yogurts and infant milk formulas containing probiotics to the same quality testing methods.

Conclusion

It is therefore in the interests of all that an evaluation system be implemented to protect the consumer. The evaluation should include a means of verifying label claims and assessment of the effects of storage on the products. We believe that our evaluation partly addresses the problem and that the DGGE method provides a suitable standard for organism identification together with standardised quantification.

Furthermore, it is suggested that an independent laboratory be used to do an annual random 'off the shelf' quality control of the commercially available products. It is also suggested that validated clinical data be the basis for registration of a probiotic at the Complementary Medicines Committee (CMC) of the Medicines Control Council (MCC).

Finally, the practice of substitution at pharmacy level should be viewed with scepticism. Certain health benefits of probiotics are strain specific. Strain definition of products should be linked to efficacy, eliminating the current extrapolation of data by some manufacturers. This together with questionable quality threatens credibility of all probiotic products because they cannot deliver the expected results.

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K Teversham is the medical advisor to Thebe Pharmaceuticals who market BioPro Reuteri and who initiated this study.

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