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Research Article

Evaluation of Commercial Probiotic Products

Mansa Fredua-Agyeman*[1,2], Shruti Parab[1], Simon Gaisford[1]

[1]UCL School of Pharmacy, University College London, 29-39 Brunswick Square, London, WC1N 1AX, United Kingdom

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*Corresponding author.
Tel.: +233(0)544836318
E-mail: mansa.fredua-agyeman.11@ucl.ac.uk

[2] Present address: School of Pharmacy, University of Ghana, Legon Accra LG43

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ABSTRACT

Although there is a vast number of probiotic products commercially available due to their acceptability and increasing usage, their quality control has continuously been a major concern. This study aimed to assess some commercially available probiotics on the UK market for content in relation to their label claim. Seven products were used for the study. The bacteria content were isolated, identified and enumerated on selective media. The results revealed that all products evaluated contained viable probiotic bacteria but only three out of the seven products (43%) contained the claimed culture concentration or more. None of the multispecies product contained all the labelled probiotic bacteria. Misidentification of some species occurred. The results concurred with previous studies and showed that quality issues with commercial probiotics remain. Since probiotic activity is linked with probiotic concentration and is strain specific, the need exist for a global comprehensive legislation to control the quality of probiotics whose market is gaining huge momentum.

INTRODUCTION

Probiotics are described as single or multispecies live microorganisms that when administered, beneficially affect the host health beyond inherent basic nutrition (Guarner and Schaafsma, 1998; Dunne et al., 1999). Interest in using probiotics is gaining momentum and this is for several reasons, including availability in several forms, better understanding of their mechanism of action and scientific evidence of health benefits (Ng et al., 2009; Oelschlaeger, 2010; Masood et al., 2011; Iqbal et al., 2014). With their increased usage and commercialization, probiotics are still not properly regulated with respect to their quality control and effectiveness because they are considered food and dietary supplements not drugs (FAO/WHO, 2001, 2002). Consequently, the quality of commercial probiotic products is poor and most commercial probiotic products do not accurately meet their label claim (Temmerman et al., 2001; Weese, 2002; Drago et al., 2004; Elliot and Teversham, 2004; Aureli et al., 2010; Drago et al., 2010; Weese and Martin, 2011). For instance, a study by Weese (2002) on both human and veterinary probiotics reported that only 15% of products accurately described and contained their claim content (Weese, 2002). Similarly, another study by Weese and Martin (2011), which assessed 25 commercial probiotics used in animal health reported that only 4 out of 15 products that stated the concentration of viable content on the label, met their label claim. Also, Drago et al. (2010) evaluating commercial probiotic products available on the USA market in 2009 reported that only 4 out of the 13 products fulfilled their label claim. These authors recommended the need for adequate control of

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probiotic production, the periodical screening of probiotic products and monitoring for effect of storage on product quality (Drago et al., 2010).

The aim of this study was to evaluate whether some probiotic food and dietary supplements available on the UK market contained their claimed probiotic bacteria and were present in quantities stated on their label.

MATERIALS AND METHODS

Seven commercial products were used in the study (Table 1). They were mainly purchased from local supermarkets or pharmacies at Brunswick Centre (London, UK) or from the manufacturer. After purchase, the products were stored appropriately in cool, dry places, away from light or in the fridge at 4°C as per the information on their labels. They were all used before the expiry dates printed on the labels.

The products were either lyophilised powders packed in capsules or sachet or liquid products. For isolation and identification of viable probiotic species in the products, the solid (lyophilised) commercial products (Biobalance Support, Biobalance Travel, Digestive Health and OptiBac) were hydrated in 3 mL de Man Rogosa and Sharpe broth (Oxoid, Basingstoke, UK) supplemented with 0.05% (w/v) L-cysteine hydrochloride (MRSc broth). A loopful of the hydrated solid products or the liquid products was streaked onto MRSc agar plates and incubated at 37°C anaerobically using an Oxoid anaerobic jar with an AnaeroGen GasPak System (Oxoid, Basingstoke, UK). After 48 h of incubation, the colonies obtained, which could be differentiated by their morphology (size, shape and appearance) were sub-cultured to obtain pure cultures of the selected colonies. Gram staining was performed on the pure cultures isolated. Biochemical tests for identification of the bacteria isolated were carried out using the commercial kits API 50 CHL for *Lactobacillus* and related genera and API Rapid ID 32A for anaerobes. The tests were conducted according to the manufacturers' instructions.

For enumeration of probiotic bacteria in the commercial products, the content of a capsule or sachet of the solid products was dispersed in 10 ml sterile phosphate buffered saline (PBS) (pH 7.4) and vortexed thoroughly. Serial dilutions of the mixtures were made and spread-plated on MRSc agar. The liquid products were serially diluted and spread-plated on MRSc agar. The inoculated plates were incubated at 37°C anaerobically for 48 h. Colonies were counted at the end of incubation. The number of viable bacteria present in each product was expressed as colony forming units per capsule/sachet or volume.

RESULTS AND DISCUSSION

The last decade has seen a rise in usage of probiotic food and supplement worldwide. Although no agreement has been made globally in terms of how much viable probiotic bacteria should be consumed per serving or daily for health benefit, some national guidelines advocate that one could consume a total of 10^9 probiotic bacteria per serving or daily to effect a health benefit (Health Canada, 2009; Italian Ministry of Health, 2013). It is also globally recognized that the activity of probiotics is strain-specific and that adequate number of viable probiotic bacteria should be maintained in a product throughout its shelf life although the number is not globally defined (FAO/WHO 2001, 2002).

The results of the study indicated that all the products evaluated contained viable probiotic bacteria. Almost all the products contained at least one probiotic bacteria indicated on their label but none of the multispecies product contained all the labelled bacteria. Only three out of the seven products (43%) contained the claimed culture concentration or more.

According to the label, Biobalance Support, Biobalance Travel and Digestive Health contained three probiotic bacteria mixture: *Lactobacillus acidophilus, Bifidobacterium bifidum* and *Bifidobacterium lactis.* In this study only two types of colonies were isolated from these products. A Gram stain of the colonies revealed Gram-positive, straight, rounded end rods in chains or presented singularly. The API 50 CHL test established that both colonies were lactobacilli.

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Table 1. *Comparison of labelled culture with recovered culture.*

Product	Form	Probiotic species/strains claimed on label	No. of viable probiotic species isolated and identified	Claimed culture concentration	Recovered culture concentration
Actimel ®	Liquid (milk-based)	*Lactobacillus casei* DN 114 001 (main strain), *Lactobacillus bulgaricus* and *Streptococcus thermophilus*	1	1 x 10^{10} cfu per 100 mL	5.80 x 10^{10} cfu per 100 mL
Biobalance support	Solid (capsule)	*Bifidobacterium bifidum*, *L. acidophilus*, and *Bifidobacterium lactis*	2	1.25 x 10^{10} cfu per capsule	3.16 x 10^{8} cfu per capsule
Biobalance Travel	Solid (capsule)	*B. bifidum*, *L. acidophilus*, and *B. lactis*	2	1 x 10^{10} cfu per capsule	3.42 x 10^{7} cfu per capsule
Digestive Health	Solid (capsule)	*L. acidophilus, B. bifidum, B. lactis*	2	1.25 x 10^{10} cfu per capsule	2.39 x 10^{6} cfu per capsule
OptiBac	Solid (powdered sachet)	*L. acidophilus* Rosell-52, *L. casaei* Rosell-215, *Lactococcus lactis* Rosell-1058, and *B. bifidum* Rosell-71	2	5 x 10^{9} cfu per sachet	7.84 x 10^{8} cfu per sachet
Symprove™	Liquid (non-milk)	*Lactobacillus rhamnosus, Lactobacillus plantarum, L. acidophilus, and Enterococcus faecium*	2	1 x 10^{10} cfu per 50 mL	1.04 x 10^{10} cfu per 50 mL
Yakult®	Liquid (milk-based)	*L. casei* Shirota	1	6.5 x 10^{9} cfu per 65 mL	1.30 x 10^{10} cfu per 65 mL

The API rapid ID 32A test however suggested that they could be bifidobacteria except one of the isolates of Digestive Health, which was identified as *L. acidophilus*. All three products contained fewer viable bacteria than claimed.

OptiBac, which was labelled to contain *L. acidophilus*, *Lactobacillus casaei*, *Lactococcus lactis* and *B. bifidum,* also gave two isolates. The API rapid ID 32A test suggested that the isolates were *L. acidophilus* and *Bifidobacterium* spp. The API 50 CHL however suggested that the isolates were *Lactobacillus paracasei* and *L. acidophilus.* The product also showed a lower amount of viable bacteria than declared (7.8 x 10^{8} cfu per sachet versus 5 x 10^{9} cfu per sachet claimed).

Symprove™, was labelled to contain four species: *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Enterococcus faecium* but only two of the species indicated on the label were isolated. The API 50 CHL identified the species as *L. plantarum* and *L. rhamnosus*. The recovered viable culture concentration corresponded to the label claim (1 x10^{10} per 50 mL).

Actimel® also indicated it contained *Lactobacillus casei* DN 114 001 as the main strain. Only one type of colony was isolated from it. The colony was identified as *L. paracasei* with the API 50 CHL test kit. The other yoghurt cultures, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were not isolated. The product contained higher viable culture concentration than the label claim (5.8 x 10^{10} cfu per 100 mL versus 1 x 10^{10} cfu per 100 mL).

A single isolate was obtained from Yakult®, which was also labelled to contain *L. casei* Shirota. The species was identified as *L. paracasei* with the API 50 CHL test kit. It contained in excess of the claimed culture concentration (1.3 x 10^{10} cfu per 65 mL versus 6.5 x 10^{9} cfu per 65 mL claimed).

The Food and Agriculture Organization (FAO) and World Health Organization (WHO) recommend that microbial species be stated on the label and the number of viable probiotic bacteria present at the end of shelf life be stated as well. Nonetheless,

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many studies, which have evaluated the quality of probiotics, have shown widespread deficiencies in identification and enumeration and generally poor correlation between labelled and claimed (Temmerman et al., 2001; Weese, 2002; Elliot and Teversham, 2004; Drago et al., 2004; Lin et al., 2006; Aureli et al., 2010; Drago et al., 2010; Weese and Martin, 2011).

Similar to the previous studies, the number of viable probiotic bacteria in the products, especially the solid products were low. This could be attributed to the stressful processes the cells are subjected to during manufacture, which may result in injury of the cells (Champagne et al., 2011). Moreover, inadequate packaging, storage and transport conditions after production may decrease the survival of the bacterial cells. This therefore highlights the importance of carefully selecting suitable species/strains that can withstand the manufacturing processes or selecting suitable manufacturing processes for selected strain. It also highlights the need for control of rehydration of the solid products as rehydration could be a critical step in viable cell recovery (De Valdez et al., 1985) but unfortunately is usually left to the discretion of the consumer. Furthermore, the packaging, storage and transport of the products must be evaluated as factors such as temperature, oxygen, moisture and light may affect the viability of the bacteria in the products (Morgan et al., 2006). For example, studies by Abadias et al. (2001), Costa et al. (2002) and Savini et al. (2010) have all shown the advantage of storing lyophilised probiotics or bacteria at 4^{o}C than at room temperature, however some of the solid products insisted the products did not require refrigeration and should be stored preferably in cool dry places away from light. This could account for the low viable concentration in these products compared to the liquid products, which were all stored at 4^{o}C.

Probiotics properties are usually strain specific (Sanders and Huis in't Veld, 1999) and therefore to control a cohort of diseases, which they are often targeted against, it was suggested that probiotic products should consists of a combination of strains (Sanders, 1993; Dunne et al., 1999; Famularo et al., 1999; Sanders and Huis in't Veld, 1999). Multistrain (or multispecies) probiotic products are therefore commonly available preparations although practical superior benefit has been controversial (Chapman et al., 2011; Chapman et al., 2012; Tejero-Sarinena et al., 2013; Chapman et al., 2013). Our evaluation showed that none of the multispecies products contained all the labelled species. Only two isolates per product were produced and identified even though up to four species were declared in two products. The reason for this could be a result of inhibition amongst the species (Be'er et al., 2009; Chapman et al., 2012). It is also possible that some of the species could not grow well or were outgrown by other species on the selective medium used or that the species were not viable or were not included in the products. Whilst one could argue that the selective medium used may not cater for all probiotic species, it is one of the widely used medium in probiotic bacteria propagation and has received recognition by International Organisation for Standardization (ISO) and International Dairy Federation (IDF) for enumeration of lactic acid bacteria and bifidobacteria with antibiotic supplementation (ISO and IDF 2006; 2010). It is thus more likely the non-isolated species were inhibited, non-viable or not in the products.

Bacterial identification was based on morphological characterization, Gram staining and biochemical profiling without any genotypic method which could be a limitation of this study. However it must be noted that although genotypic methods of identification are now fairly popular and more accurate, most of these sequence-based identification methods have a potential bias for detection and identification of non-viable cells. Also, assay cost could be high with these methods. Equally, the culture methods are not without limitations (evident in this study) but they offer the simplest way to detect and quantify viable microbes. One main challenge is the differentiation of colonies of various lactobacillus species in mixed culture. To circumvent this, the products were also assessed on MRSc agar supplemented with 0.002% w/v of bromophenol blue (which can differentiate between species based on characteristic pH change during growth; Lee and Lee, 2008) to confirm number of isolated species.

Misidentification was encountered with the two biochemical tests; lactobacilli were identified as

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bidifobacteria with the API Rapid ID 32A for anaerobes and bifidobacteria could not be identified at the species level. The Gram stain results however corroborated well with the API 50 CHL data which test was more detailed and rigorous, consisting of fermentation assays with longer growth incubation duration compared with the API rapid ID 32A, which depended on preformed enzymes by mostly previously lyophilised species. This misidentification has also been previously reported (Moll et al., 1996) and shows that the biochemical tests may not in particular accurately discern phenotypic variability within members of the different genus.

Labelled *L. casei* was also identified as *L. paracasei* by the biochemical test. It must be noted that *L. casei* and *L. paracasei* form a closely related taxonomic group within the heterofermentative lactobacilli (Ward and Timmins, 1999). Hence these two species though well distinguishable from other lactobacilli species (except *L. rhamnosus*), have proven to be difficult to differentiate using traditional fermentation profiles, which often identify *L. casei*, as *L. paracasei* (Ward and Timmins, 1999; Yeung et al., 2002).

One of the products was labelled to contain *E. faecium*. Whilst this species was not isolated from the product, it is not a generally regarded as safe (GRAS) bacterium as it is a potential pathogen in immunocompromised patients with high level of antimicrobial resistance and as such should not be included in probiotic products (Lund and Edlund, 2001; FAO/WHO, 2001).

CONCLUSIONS

Although some limitations exist in this study, it has revealed some information about the quality of some commercial probiotics on the UK market and indication from the study is that the quality of probiotics is not improving. Probiotic activity is linked to the health, attribute and amount of a specific strain in a product. There is therefore the need for a worldwide legislation for the proper and standardized control of probiotics for quality and efficacy in the absence of which the quality and effectiveness of probiotics would continue to be poor.

CONFLICT OF INTEREST

Part of this work has been previously included in an abridged form in an article entitled "Comparative survival of commercial probiotic formulations: Tests in biorelevant gastric fluids and real-time measurements using microcalorimetry" published in Beneficial microbes, 2015, 6 (1), 141-151.

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