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Rapid enumeration of *Bifidobacterium lactis* in milk powders using impedance

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Abstract

An impedance method was evaluated to enumerate *Bifidobacterium lactis*, a probiotic, added to milk powder. Impedance changes were measured at 40 °C and recorded using the BacTrac™ 4100 microorganism growth analyser. Five different media were compared for the optimum impedance response. A raffinose-based medium (*B. lactis* medium) produced the fastest and most reproducible results. Good correlations were obtained between cell numbers from pure cultures of *B. lactis* (DR 10™) on reinforced clostridial agar plates and the impedance changes in the *B. lactis* medium. Enumeration of these bacteria in milk powder using the BacTrac™ 4100 impedance system showed no significant difference when compared with agar plate count results. The impedance counts estimated the cell counts of 10^6 cells g^{-1} within 15 h and was faster than the 3 days required to obtain a result using the agar plate count method.

Keywords: *Bifidobacterium lactis*; Impedance; Bacterial enumeration; Probiotics

1. Introduction

The availability of dairy products containing probiotic bacteria is increasing. The natural habitat for probiotic bacteria is the intestinal tract where they are thought to have beneficial effects on human health (Robinson, 1989). The selection of strains used in dairy products is important as different strains exhibit diverse responses to the adverse conditions found in dairy products. Factors important in selecting probiotic strains, from a commercial perspective, include the capability to grow at high cell density in inexpensive media, ease of culturing, the ability to retain their probiotic properties throughout the industrial process, and efficacy in the gastrointestinal tract. Efficacy includes the ability to adhere to epithelial cells and coaggregation ability (Marth & Steele, 2001; Tuomola, Crittenden, Playne, Isolauri, & Salminen, 2001).

The addition of these bacteria into dairy products requires accurate, rapid, and reliable methods for quality assessment. Various methods have been developed for counting and detecting these microbes such as enzymatic-colourimetric assays (Bibiloni, Pérez, & de Antoni, 2000), plate count methods (Hartemink, Kok, Weenk, & Rombouts, 1996; Roy, Mainville, & Mondou, 1997; Roy, 2001; Sozzi, Brigidi, Mignot, & Matteuzzi, 1990), and DNA-based methods (Kok et al., 1996). Plate count methods using either reinforced clostridial agar (RCA) or De Man Rogosa Sharpe (MRS) medium supplemented with cysteine, are the media of choice for industrial quality control laboratories (Roy, 2001).

The accuracy of the detection and counting of active probiotic bacteria in dairy products may be affected by the ability of the cells stressed during the dairy product manufacture, to grow; and by clumps of bacteria formed during product manufacture. The aim

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in the manufacture of products containing probiotics is to minimise stress on the bacteria as the efficacy of stressed cells as probiotics is unknown. However, the cells are still in a dried and stressed state and a drop in viability is not uncommon (Kirsop & Snell, 1984). Stressed cells often grow better in liquid media than on agar media. It could be argued that the revival of stressed cells can over-estimate the probiotic value of the product. On the other hand, direct microscopic counts carried out, in our laboratory, show that bifidobacteria have the tendency to form clumps during dairy manufacturing processes. Growth of colonies on agar plates from clumps of bacteria rather than from individual cells may give false counts of viable cells. Consequently, agar plate counts may underestimate the enumeration of bifidobacteria and alternative liquid media tests may be more accurate.

An alternative method for microbial counting using impedance is widely used to enumerate bacteria in foods (Brooks, 1986). Nieuwenhof and Hoolwerf (1987) have used this method for the total enumeration of bacteria in raw milk. However, there are no reports on using this system to enumerate bifidobacteria.

Advantages of the impedance system include the use of a broth medium which will revive stressed bacteria, the ability to enumerate cells in clumps, the ease to automate the analysis of samples, and rapid results. The BacTrac™ 4100 microorganism growth analyser (Sy-Lab, Purkersdorf-Vienna, Austria) measures electrical changes resulting from microbial metabolic processes in both the growth medium and the electrode surfaces (impedance splitting). These changes can be correlated to the numbers of bacteria in the inoculum added to the growth medium.

In this study, cultures of *Bifidobacterium lactis* (DR10™) were enumerated using impedance and the response compared with agar plate counts. Different media were compared to select the optimum conditions for detection of impedance changes in the medium (*M*-value) and on the electrode surface (*E*-value). A calibration curve was prepared using the medium giving the fastest response using serial ten-fold dilutions of isolates and this curve was used for the estimation of viable cells in experimental and industrial milk powders containing *B. lactis* (DR10™).

2. Methods

2.1. Cultures

B. lactis (DR10™) from the Fonterra Research Centre culture collection was used for the impedance calibration. The samples containing *B. lactis* (DR10™), used for testing the calibration, were both experimental and industrial batches of Cygnet Fernleaf™ milk powder, Batch Codes: experimental-K, L, M, N; industrial-11504, 11506, 11507 and 22505.

2.2. Culture media

Four culture media whose compositions were published, and an experimental formulation, were screened to determine if they were suitable for the detection of *B. lactis* using impedance. These were *Bifidobacterium* selective medium (BSM; Beerens, 1990), Wilkins–Chalgren anaerobe broth (WCAB; Oxoid Ltd, Basingstoke, Hampshire, England); reinforced clostridial medium (RCM; Merck KgaA, Darmstadt, Germany); raffinose broth medium (RBM; Hartemink et al., 1996) and *B. lactis* medium (BLM). This last medium was an experimental formulation and was comprised of the following components ($g\,L^{-1}$): sodium thioglycolate (Sigma-Aldrich Corporation, St Louis, Missouri, USA), 0.5; cysteine HCL (Merck KgaA, Darmstadt, Germany), 0.5; sodium caseinate (Sigma-Aldrich Corporation), 5.0; yeast extract (Difco Laboratories, Becton Dickinson Microbiology Systems, Sparkes, Maryland, USA), 1.5 raffinose (Sigma-Aldrich) (which was selected as the best from three different sugars tested), 15.0. This medium was prepared by adding the above ingredients to one-third of the final volume of water, then 150 μL of 6 M NaOH was added and mixed quickly. This reduced the precipitation of proteins in the medium. The remainder of the water was added and the pH was adjusted to 6.8 ± 0.1 before autoclaving at $121 \pm 1\,^{\circ}C$ for 15 min.

All media were prepared and autoclaved in 10 mL amounts in the tubes for the BacTrac™ and cooled to room temperature immediately before use. The tubes of media were inoculated with 0.1 mL of sample and then overlayed with 1 mL of sterile paraffin oil.

Each medium was screened three times to determine the most suitable one for the development of a calibration curve for a more detailed study. Suitability of the media was determined on the basis of the time taken to detect an inoculum containing approximately 10^8 cells mL^{-1} in a 10% solution of milk powder, using triplicate tests, in the impedance monitor. The 10% solution was prepared from whole milk powder, containing <10 cfu g^{-1} aerobic plate count, by adding a 10 g sample to 90 mL 0.1% peptone prewarmed to 37 °C. The milk powder was reconstituted by mixing in a peristaltic blender for 2 min. The reconstituted milk powder, without *B. lactis*, was incubated at 37 °C for 30 min as a control step to replicate the conditions used to revive stressed cells for routine plate assays. A 1 mL sample of *B. lactis* (DR10™), grown overnight in the RCM medium at 37 °C to give approximately 10^9 cells mL^{-1}, was added to the milk powder solution, then mixed again in the peristaltic blender for 1 min