

Comparison of the viability of probiotics from various cultured-milk drinks in a simulated pH study of the human gastrointestinal tract

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Abstract: Different probiotic strains are incorporated into cultured-milk drinks by respective manufacturers with the common aim of providing health benefits to the consumers. Four common cultured-milk products (brands N, S, V and Y) were evaluated for their quality of probiotic strains used, based on the susceptibility of the probiotics to various pH levels simulated to mimic the gastrointestinal system. Results showed that brands Y and V have higher initial probiotic inoculum compared to brands N and S although probiotics from brands N and S were more tolerant to pH 3. Generally, all probiotic strains preferred higher pH with highest viability of *Lactobacillus* spp., *Bifidobacterium* spp., and *Streptococcus thermophilus* observed at pH 8.1. Our study also discovered that among the four brands tested, brand V contained probiotic strains which are most likely to remain viable after passage through the gastrointestinal system.

Keywords: cultured milk, *Bifidobacterium* spp., *Lactobacillus* spp., *Streptococcus thermophilus*, pH tolerance

Introduction

Probiotics are live microorganisms that when administered or consumed in adequate amounts, act in the gastrointestinal tract to confer health benefits to the host (Rolfe, 2000; Montville and Matthews, 2005). They are used mainly in food fermentation such as for yoghurt production (Heller, 2001; Mortazavian *et al.*, 2006); incorporated into pasteurized milk (Rolfe, 2000); or consumed as live probiotic cells in many other pharmaceutical products (Ray, 2004; Lavermicocca *et al.*, 2005). Consumption of probiotics is known to reduce symptoms in lactose-intolerant individuals (Ray, 2004) and the cholesterol levels in individuals at risk (Ray, 2004; Parvez *et al.*, 2006). In the recent years, probiotics are also established to have "anti-cancer" properties, by detoxifying ingested carcinogens and altering metabolic activities of cancer-carrier bacteria (Ray, 2004; Parvez *et al.*, 2006).

The health benefits from probiotics were conferred mostly by *Lactobacillus* spp., *Bifidobacterium* spp. and *Streptococcus thermophilus* (Saarela *et al.*, 2000; Shah, 2000; Ray, 2004; Aslim and Beyatli, 2004). These three probiotic strains are highly desirable as they can be ingested, thus are often incorporated

into food for consumption. They also have resistance to antibiotics (Saarela *et al.*, 2000; Ishibashi and Yamazaki, 2001; Aslim and Beyatli, 2004), good adhesion to cell lines (Saarela *et al.*, 2000; Ishibashi and Yamazaki, 2001), and have antagonistic potential against pathogen (Verschuere, 2000; Ishibashi and Yamazaki, 2001; Maragkoulakou *et al.*, 2006). They can also neutralize harmful products from foods in the gastrointestinal tract, thus providing further protection to human health (Heller, 2001; Ray, 2004).

For probiotics to render its numerous benefits, they must be able to survive their passage through the human gastrointestinal system. Their tolerance to pH is therefore a critical factor influencing their probiotic functionality (Matto *et al.*, 2006). The low pH of the stomach (pH 1.5 to 2.5) and the antimicrobial action of pepsin are known to provide an effective barrier against entry of most bacteria into the intestinal system (Bourlioux *et al.*, 2003; Huang and Adams, 2004). Thus, it is important that a study is conducted to evaluate the impact of pH on the viability of the probiotics upon ingestion.

Consumption of cultured milk drinks is one of the most accessible and convenient source of introducing probiotics into the daily diet routine. It is most common among the public, thus it is important to

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understand the benefits that can be truly obtained by consuming these probiotics from cultured milk. In our study, we evaluated the tolerance of probiotics from four cultured-milk drinks from brands N, S, V and Y, representing the four popular brands in Malaysia, to pH conditions mimicking the gastrointestinal system. The probiotics selected for the assessment were *Lactobacillus* spp., *Bifidobacterium* spp. and *Streptococcus thermophilus*. We aim to determine the susceptibility of the different probiotics strains to various pH conditions, and their relatedness to the brands of cultured milk drinks.

Materials and methods

Samples

Four different brands of cultured milk with a variety of probiotics were chosen for this study. They were acquired from a hypermarket in Wangsa Maju, Selangor. Due to ethical concerns and legislative compliance, the brand of each cultured milk drink is not revealed, instead identified as brand S, N, V and Y.

Effect of pH conditions

Five ml of cultured milk was dispensed into a sterile 15 ml glass tube. The pH conditions of pH 3.0, pH 7.4 and pH 8.1 were achieved by incorporating 0.1 M of sodium hydroxide (System[®]) or 0.1 M hydrochloric acid (PC Lab Research) into the cultured milk, adjusted using a pH meter (DELTA 320) to obtain basic and acidic conditions, respectively. The pH of the stomach was simulated at pH 3.0 as pH

1.5 to pH 2.5 was too acidic and unsafe for handling. pH 7.4 and pH 8.1 represents the pH condition of the saliva and the intestine, respectively. The cultured milks were then incubated in a water bath (Memmert) at $36.9 \pm 2^\circ\text{C}$ for 3 hr. The parameters to mimic the human digestion such as the pH and the time of the human digestion (incubation time) was modified from Marteau et al. (1997) and Sheerwood (2004). Cultured milk without pH adjustment served as control with a mean pH value of 3.7.

After incubation, a serial dilution was performed using sterilized distilled water. From each dilution factor, 0.1 ml aliquot was transferred and spread onto three replicates of agar plates containing De Man Rogosa (MRS) media, De Man Rogosa-Lithium Propionate (MRS-LP) media and *Streptococcus thermophilus* (ST) media, to isolate *Lactobacillus* spp., *Bifidobacterium* spp. and *Streptococcus thermophilus*, respectively (Roy, 2001; Castele et al., 2005). MRS agar and MRS-LP agar plates were incubated for 5 days, while ST agar plates were incubated for two days, in an incubator (37°C) (Memmert) (modified from Dave and Shah, 1996; Tharmaraj and Shah, 2003). Plates containing 30 to 300 colonies were then enumerated and analyzed statistically. This method of enumeration was performed for all tested brands.

Statistical analysis

The data recorded for all the parameters assessed, were analyzed with Analysis of Variance (ANOVA) and the means compared with Tukey's Studentized Range Test ($\text{HSD}_{(0.05)}$) using the Stastical Analysis System (SAS) version 6.12.

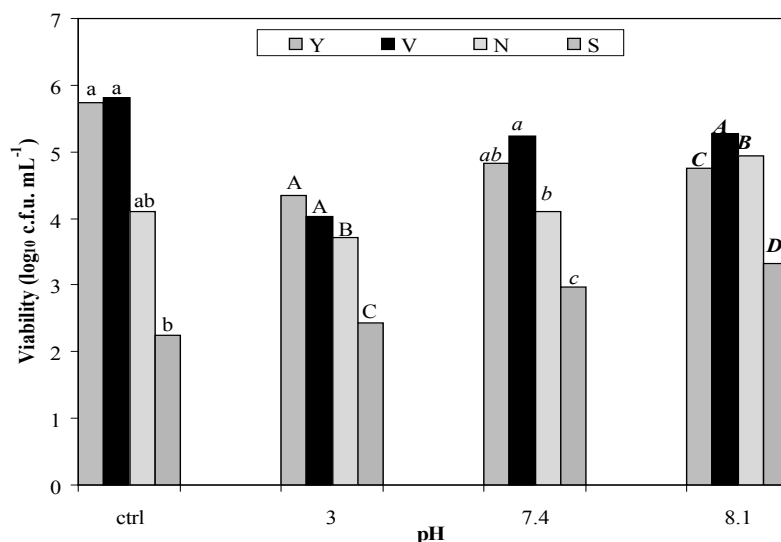


Figure 1. Viability (\log_{10} c.f.u. mL^{-1}) of probiotic bacteria in cultured milk from brands Y, V, N and S incubated at pH 7.4, pH 3.0 and pH 8.1. Means with the same letters and font type are not significantly different ($\text{HSD}_{(0.05)}$)

Results and discussion

Results from this study revealed that the initial inoculum concentration of probiotics differs according to the brands of the cultured milk drink. Brands Y and V both have approximately $5.8 \log_{10}$ cfu ml^{-1} viable cells recovered from samples in control, while brand S had the least initial inoculum with only $2.2 \log_{10}$ cfu ml^{-1} (Figure 1). The differences in the number of viable cells derived from various brands were insignificant between brands Y and V for all pH levels tested. This suggested that probiotics used by brands Y and V were most likely inoculated with similar concentrations. Viable cells from brands N and S were significantly lower compared to brands Y and V for all levels of pH tested. The lower initial concentrations in the cultured milk drinks therefore, resulted in the overall lesser number of viable cells recovered for brands N and S compared to brands V and Y throughout the study.

However, regardless of the brands used, probiotics generally showed an increase in viable cell recovery from pH 3 to pH 8.1. Exposure to pH 3, generally reduced the viable cell count compared to control (pH 3.7), with mean of $3.63 \log_{10}$ cfu ml^{-1} compared to $4.53 \log_{10}$ cfu ml^{-1} , respectively (Figure 1). At pH 8.1, mean viability of probiotics improved to $4.58 \log_{10}$ cfu ml^{-1} . This suggested that most of the strains used in all four brands preferred pH 8.1. Therefore, it is summarized that the viability is affected when the probiotics enter the stomach which has very low acidity (pH 1.5 to 2.5). The few surviving probiotics cells then continued to proliferate and multiply when entered into the

intestinal system with a more alkaline pH condition (pH 8.1). Thus, strains from brands Y and V were suggestively more adaptable to pH changes as these strains were able to give the highest number of viable cells upon transit within the simulated pH conditions of the gastrointestinal system.

All three bacterial strains have a preference for higher pH levels (pH 8.1) compared to pH 3 (Figure 2). *Lactobacillus* spp. is the least susceptible to the changing pH conditions, while *Bifidobacterium* spp. and *Streptococcus thermophilus* were more susceptible to pH 3 (Figure 2). This influenced the total probiotics enumerated after exposure to pH conditions. The recovery rate of viable *Lactobacillus* spp. cells was significantly highest in pH 3, 7.4 and 8.1, consistently more than $4.6 \log_{10}$ cfu ml^{-1} for all pH conditions, while significant reduction in viable cell count was seen for *Bifidobacterium* spp. in pH 3 (Figure 2). Survivability of strains to acidity in the stomach varies greatly as some strains are proven resistant to acid environment (Ray, 2004). In our study, the viable cell count for *Lactobacillus* spp. is relatively higher than *Bifidobacterium* spp. and *Streptococcus thermophilus* due to their more acid-resistant nature (Marteau *et al.*, 1997). All three probiotic strains showed positive response to the increasing pH levels, with higher probiotics count in pH 8.1 compared to pH 3 (Figure 2).

Of the four brands evaluated, the mean viable cell count for the probiotics in cultured milk from brand V was the highest, followed by brands Y, N and S with approximately $5.8 \log_{10}$ cfu ml^{-1} , $5.76 \log_{10}$ cfu ml^{-1} , $4.1 \log_{10}$ cfu ml^{-1} and $2.23 \log_{10}$ cfu

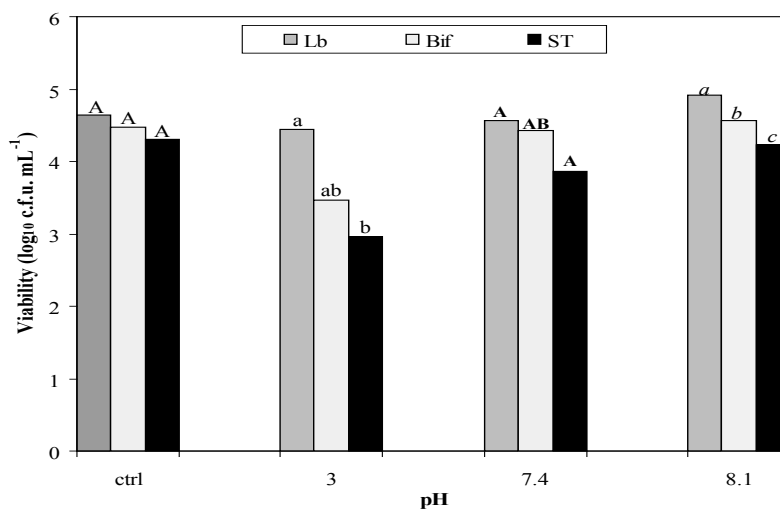


Figure 2. Viability (\log_{10} c.f.u. mL^{-1}) of *Lactobacillus* spp. (Lb), *Bifidobacterium* spp. (Bif) and *Streptococcus thermophilus* (ST) incubated at pH 3.0, pH 7.4 and pH 8.1. Means with the same letters and font type are not significantly different ($\text{HSD}_{(0.05)}$)

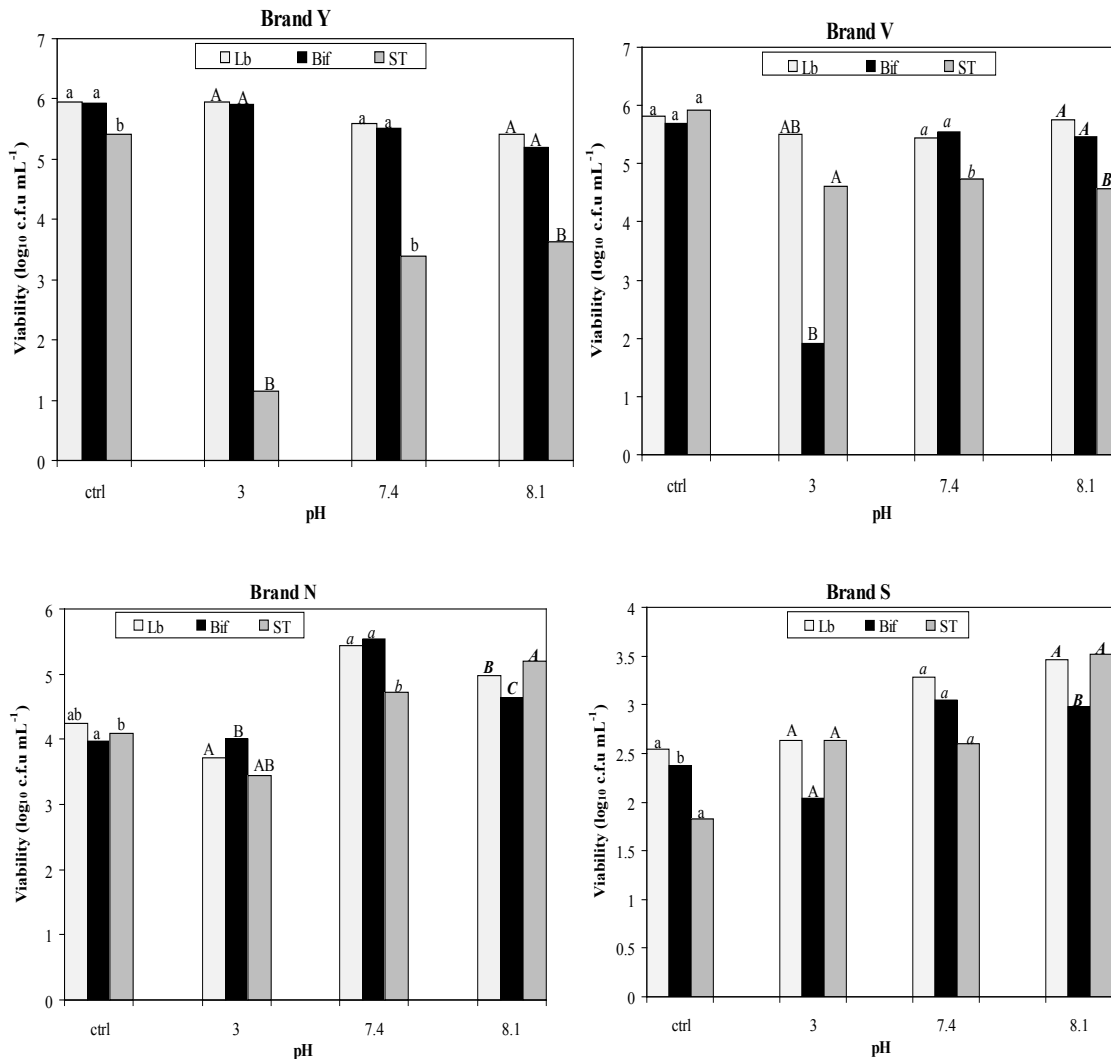


Figure 3. Viability (\log_{10} c.f.u. mL⁻¹) of *Lactobacillus* spp. (Lb), *Bifidobacterium* spp. (Bif) and *Streptococcus thermophilus* (ST) in cultured milk drinks from brands Y, V, N and S incubated at pH 7.4, pH 3.0 and pH 8.1. Means with the same letters and font types are not significantly different (HSD(0.05))

ml⁻¹, respectively (Figure 3). Total number of cells for each strain type was not significantly different for brand V, although brand Y and S had lower number of cells for *Streptococcus thermophilus* (Figure 3). At pH 8.1, mean probiotics recovered were in a similar manner, whereby highest viable cell count was derived from cultured milk from brand V, followed by brand Y, N and S. However, the viable cell counts for brands V and Y were 0.6 \log_{10} cfu ml⁻¹ and 1.03 \log_{10} cfu ml⁻¹ lesser than the control, respectively, indicating that the final number of viable cells recovered after the simulated gastrointestinal pH test was lesser than what was in the original content in the cultured milk. In contrast, viable number of cells from brands S and N were 1.07 \log_{10} cfu ml⁻¹ and 0.6 \log_{10} cfu ml⁻¹ higher than the control, respectively.

To conclude, our study suggested that different brands of cultured milk may have used different strains of *Lactobacillus* spp., *Bifidobacterium* spp. and *Streptococcus thermophilus*, inoculated with different number of cells. Probiotics from brands Y and V are better sources of probiotics compared to brands N and S, with strains able to withstand the pH conditions in the gastrointestinal system.

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