Viability and Gastrointestinal Tolerance of Commercial Probiotic Products

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***Abstract*—The basic principle for probiotic microorganisms to be beneficial to the host is that they must show enough viability when arriving at the intestine as the site of action. Thus, they must be able to stay alive in the lower pH environment in the gastrointestinal tract. The extremely low pH in the human stomach and pepsin enzyme as antimicrobial agents provide an effective barrier toward foreign microorganisms in the human gastrointestinal system. Therefore, the primary purpose of this work is to evaluate the initial viability of the commercial probiotic product and the transit tolerance of the probiotic samples toward low pH in the human gastrointestinal system. In the present study, ten commercial probiotic products available in Malaysia with different types of dosage form were chosen for viability tests and in vitro tolerance toward the human gastric acid environment. The acid tolerance test was conducted at pH 2 for 3 h incubation at 37 °C. The viability was evaluated using a flow cytometer method to determine the cell count. Six out of ten products showed similar or higher viable organism count than the stated label. However, all of the products still met the minimum initial viability requirement for commercial probiotic products, which is 10^6 CFU per g or mL sample. Generally, all the probiotic product strains cannot tolerate the lower gastric pH environment except for those obtaining enteric coating protection or possessing acidic tolerance.**

***Index Terms*—Enteric coating, gastric tolerance, probiotic, viability**

I. Introduction

Probiotics have been postulated to have a positive impact on the consumer by maintaining a microbial balance and healthy intestinal microflora. The probiotic microorganisms are mostly bacteria, namely *Bifidobacterium* spp. and *Lactobacillus* spp., and some of the yeasts like *Saccharomyces* spp. They play an essential role in the defense system to protect the host from harmful microorganisms and also improve the host immune system [1]. However, the beneficial effect of probiotics is different toward human health based on different probiotic strains and the amount of live culture consumed.

According to the International Scientific Association of Probiotics and Prebiotic (ISAPP), probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [2]. This definition is inclusive of a broad range of microbes and applications while capturing the essence of probiotics (microbial, viable, and beneficial to health). Nowadays, commercial probiotic products are commonly found in functional food and supplements in liquid, powder, tablet, and capsule dosage forms.

The basic principle for the probiotic microorganisms to be beneficial to the host is that they must show enough viability when arriving at the intestine as the site of action. Thus, they must be able to stay alive in the lower pH environment in the gastrointestinal tract [3]. pH tolerance is one of the critical factors that have an impact on probiotics viability at the target site. The extremely low pH in the human stomach and pepsin enzyme as an antimicrobial agent provide an effective barrier toward foreign microorganisms in the human gastrointestinal system. Several studies [4]-[9] have examined that some products in the market that contain probiotic cells lack the viability of live cells after being consumed. Therefore, it shows the significance of assessing the influence of extremely acidic environment toward the viability of probiotic products. One of the reasons that reduce the survivability of the probiotic cells during transition through the stomach is the condition of the gastrointestinal tract with low pH condition [10]. Thus, ten commercial probiotic products with different types of dosage form were selected from a local pharmacy store in Kuantan, Pahang, Malaysia. An in vitro technique was applied in this investigation to evaluate the resistance and tolerance of probiotic samples toward low pH in the human gastrointestinal system.

II. Materials and Methods

A. Sources of Probiotic Products

Ten commercial probiotic products were obtained from a local pharmacy store at Kuantan, Pahang. These products were labeled A to J for different brands, and several types of dosage forms were chosen, such as liquid, capsule, powder, and tablet. The brands of each product are not revealed due to ethical concerns and legislative compliance. All samples were stored at the optimal storage environment, as stated in the product label description (room temperature or 4 °C) until utilization.

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B. Evaluating the Viability of Probiotic Products

Enumeration of the viable organisms was modified from Agyeman *et al*. [4]. For tablet and capsule products, one tablet or capsule was added to 100 mL of simulated intestinal fluid (SIF) and incubated at 37 °C with 200 rpm until dissolved. For powder products, one sachet of the product was dispersed in 100 mL of SIF and incubated at 37 °C and 200 rpm until fully dissolved. For liquid products, the amount of one serving size recommended by the manufacturer was added to 100 mL of SIF and incubated at 37 °C and 200 rpm. After the products were dissolved, 1 mL of each sample was collected, and the viability was evaluated using the flow cytometer method.

C. Preparation of Simulated Gastric Fluid (SGF)

The SGF was prepared according to a modified USP 35 method [11]. Two grams of sodium chloride and 3.2 g of purified pepsin were dissolved in sterile purified water. Next, 1 mL of concentrated hydrochloric acid was added to adjust pH, and sterile water was added to make 1 L. The pH of the test solution was approximately 2.

D. Preparation of Simulated Intestinal Fluid (SIF)

The SIF was prepared according to USP 35 method [11]. First, 6.8 g of monobasic potassium phosphate was dissolved in 250 mL of purified water. Next, 77 mL of sodium hydroxide (0.2 N), 10 g of pancreatin, and 500 mL of purified water were added to the solution. The solution was added with 0.2 N of sodium hydroxide or 0.2 N of hydrochloric acid to pH 6.8 ± 0.1. Lastly, purified water was added to make 1 L.

E. Treatment of Probiotic in SGF

This procedure was modified from Jamilah *et al*. [12]. All samples were incubated for 3 h in 100 mL of SGF at 37 °C to mimic the human stomach condition with stirring at 200 rpm using an incubator shaker to simulate bowel movement. After 3 h, the undissolved samples were then transferred to 100 mL of SIF. If the samples dissolved in the SGF, 1 mL of SGF was collected and transferred to SIF. One millilitre of each suspension was removed and evaluated for the survivability of the probiotic cells.

F. Treatment of Probiotic in SIF

This procedure was modified from Jamilah *et al*. [12]. After the gastric treatment using SGF, the undissolved samples or 1 mL of SGF samples (for dissolved products) were transferred into 100 mL of SIF and incubated at 37 °C and 200 rpm for 2 h. Then, 1 mL of each suspension was removed and evaluated for the survivability of the probiotic cells.

G. Preparation of Simulated Gastric Fluid (SGF)

The assessment of probiotic microbial cell survival was based on Chiron *et al*. [13]. For each sample, 500 µL of cell suspension was collected. Next, 5 µL of thiazole orange (TO) solution and propidium iodide (PI) dye solution (BD cell viability kit) was added to the samples. The samples were then vortexed and incubated for at least 5 min at room temperature. The samples were passed through a flow cytometer (Accuri C6 brand), and the data were collected by using the FL1 versus FL3 dot plot. All experiments were conducted in triplicate.

H. Statistical Analysis

For each probiotic product, total microbial viability was evaluated from three independent samples. Values are given as mean ± standard deviation. Data were analyzed using *t*-test with Microsoft Excel. A *p*-value of less than 0.05 was regarded as statistically significant.

III. Results and Discussions

A. The Initial Count of Probiotics

All probiotic products met the minimum viability requirement for commercial products. The number of probiotic viability is recorded in Table I. Brand E had the highest viability count, while brand G had the lowest viability count but still meets the minimum requirement for the live culture for a commercial product, which is 10^6 CFU per g or mL sample. Several researchers suggested that enough live probiotic microorganisms must be maintained at least 10^6 to 10^7 CFU/g or mL of a product to make it beneficial [14]. Others said it must be maintained at 10^8 [15] and 10^9 to 10^{12} CFU/g or mL [16].

To achieve high viability after product consumption, that microbial strain must withstand the harsh condition and thermal tolerance during processing and, most importantly, during transportation and storage [15]. Processing methods such as spray- or freeze-drying are also essential in maintaining viability. Furthermore, the types of substances or protectant mixed with the live culture during processing affect the storage viability and result in a different number of initial count during consumption [16].

B. Evaluating the Viability of Probiotic Products

The viability and microbial content accuracy of the product label is the most crucial aspects of probiotic product preparation. Stable viability and accurate label content are the key features that determine the quality of probiotic products. The results of the enumeration test for all commercial products are shown in Fig. 1. The viability of the microorganisms was analyzed and compared with the claimed viability based on the serving size displayed on the label. All quantifications of microbial content were performed using the flow cytometer method.

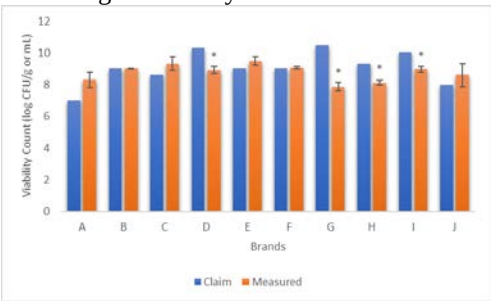

Figure 1. Viability content evaluation of commercial products compared to the product label claim. Measured values are mean of three samples ± SD. Cell count values with an asterisk (*) indicate significantly lower ($p < 0.05$) than the claimed value.

TABLE I. COMMERCIAL PROBIOTIC PRODUCT DOSAGE FORM, LIST OF MICROBIAL STRAINS CONTAINED AND INITIAL MICROBIAL VIABILITY

Brand	Dosage form	Species	CFU/g or mL
A	Coating tablet	*Lactobacillus gasseri, Bifidobacterium bifidum, and Bifidobacterium longum*	$1.99 \pm 1.38 \times 10^8$
B	Coating capsule	*Lactobacillus acidophilus, Lactobacillus salivarius, Bifidobacterium bifidum, and Streptococcus thermophilus*	$2.63 \pm 0.04 \times 10^9$
C	Capsule	*Saccharomyces cerevisiae*	$8.48 \pm 2.22 \times 10^8$
D	Capsule	*Lactobacillus rhamnosus*	$3.25 \pm 0.95 \times 10^9$
E	Capsule	*Saccharomyces boulardii*	$8.51 \pm 1.61 \times 10^9$
F	Powder	*Lactobacillus fermentum*	$5.70 \pm 0.23 \times 10^8$
G	Powder	*Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus lactis, Bifidobacterium bifidum, Bifidobacterium infantis and Bifidobacterium longum*	$3.06 \pm 0.80 \times 10^7$
H	Powder	*Lactobacillus paracasei*	$8.80 \pm 0.52 \times 10^8$
I	Powder	*Saccharomyces boulardii, Lactobacillus acidophilus, Bifidobacterium lactis, and Streptococcus thermophilus.*	$3.79 \pm 1.60 \times 10^8$
J	Liquid	*Lactobacillus reuteri*	$4.14 \pm 2.82 \times 10^9$

Value are mean of three samples ± SD

Among the products tested for viable microbial count, products A, B, C, E, F, and J showed similar or higher than that shown on the label. The other four products had lower microbial count compared with the value shown on the label. Only the liquid and tablet products showed similar viable microbial count with the stated claim on the label, while the powder and capsule products showed lower microbial count than the one on the label.

Many factors influence the viability of the microorganisms in the product such as preservation or drying method, protective agent, rehydration and storage condition, and packaging [17]. In this test, all the powder dosage forms showed lower microbial count compared to the stated label claim. This kind of product undergoes a drying method such as freeze- or spray- drying to change the product into a solid form. The viability after the drying process varies according to several factors, such as the protective agent used, type of microbial strain, and also the method of rehydration process [18]. In this case, the low microbial count recorded from capsule and powder products might be because of the different types of rehydration medium used compared to the commercial product method, affecting the total viability of the microorganisms. Besides, in recent years, inaccurate label content has become an issue, and several findings have emphasized the inaccuracy in commercial probiotic products labeled content and the viability of the probiotic strains in European [19] and USA [20] markets.

C. Evaluating Tolerance to Gastric Fluid

Probiotic microorganisms should be viable and reach their target of action alive which is the small intestine. The pH in the gastric juice ranges around 2-3 after food consumption and can decrease to pH 1 during fasting. Therefore, pH 2 was chosen for this analysis of simulated gastric resistance, and 3 h was selected because the total time for the food to move through the human stomach ranges around 2.5-4 h. Table II summarizes the results of probiotic microbial survivability after in vitro gastric treatment. It clearly shows the different acid tolerance of each product. Most of the probiotic species in the products cannot survive at lower pH except for brands A, B, H, and J.

TABLE II. MICROBIAL SURVIVABILITY AFTER IN VITRO GASTRIC TREATMENT

Brand	Initial count (log CFU)	Post-SGF count (log CFU)	Survivability (%)
A	8.42 ± 0.09	8.11 ± 0.02	49.69 ± 0.05
B	8.06 ± 0.01	8.06 ± 0.01	98.60 ± 0.01
C	8.26 ± 0.16	-	-
D	8.49 ± 0.13	-	-
E	8.67 ± 0.08	-	-
F	8.28 ± 0.18	-	-
G	8.48 ± 0.04	-	-
H	8.21 ± 0.06	7.67 ± 0.06	28.85 ± 0.03
I	9.10 ± 0.04	-	-
J	7.09 ± 0.14	6.55 ± 0.14	28.84 ± 0.05

Value are mean of three samples ± SD

Naturally, most of the probiotic microbial strains are sensitive toward lower pH except for particular strains that possess acid resistance or have some protection toward the acidic environment. The gastric environment in the human stomach is highly acidic because of the presence of hydrochloric acid. Hydrochloric acid is the first defense mechanism that inhibits any infectious agent from entering the intestinal tract [21]. Therefore, most of the probiotic products are sensitive to the lower pH in the stomach since only a few microorganisms can tolerate the extremely low pH of the stomach. According to Tennant [22], microorganisms exposed to human gastric acid more than 15 minutes will be dead because of the hydrochloric acid and pepsin enzymes in the gastric juice. These results are consistent with the literature findings [4], [7], [8], where the viability of the probiotic microorganism decreases when introduced to pH lower than 2 for 3 h incubation time. It emphasizes the importance of gastric protection for probiotic products.

From the results, brands A and B survived the lower pH of gastric juice around 50% survivability and above. This finding is expected since brands A and B are the coated tablet and coated capsule types, respectively. An enteric coating is an outer layer used on the oral pharmaceutical dosage that protects the drugs against gastric environmental conditions, especially for lower pH [23]. The enteric coating tolerates certain pH in the extreme environment of the stomach, and then starts to disintegrate and dissolve once it gets to the intestines. Therefore, it avoids the release of sensitive probiotic microorganisms in the stomach and allows the delivery of this probiotic strain to the target site, which is the intestine. This observation demonstrates consistency with previous studies [24]-[26], where protection such as

enteric coating is needed to improve the viability of the probiotic cell once it reaches the intestine as a target site. Furthermore, besides the enteric coating application, other methods have been proposed to improve the viability of the probiotic cells, such as encapsulation and microencapsulation techniques [27], [28].

Apart from brands A and B that can withstand the low pH because of the enteric coating application, brands H and J also survived in extremely low pH for 3 h without any coating applied. Even though the survivability percentage is not more than 30%, the log CFU count was still above six, which means that it still meets the minimum requirement for probiotic product viability because brands H and J contain microbial strains that have acidic resistance. Results illustrated in Table I. show that both brand H and J comprise probiotic microorganisms from *Lactobacillus paracasei* and *Lactobacillus reuteri*, respectively. As reported by Kou [29], *L. paracasei* is the most acid-resistant probiotic strain compared to other *Lactobacillus* strains. This result is consistent with the recent study demonstrated by Xu [30] that reported *L. paracasei* has a survival rate of 98.73% after 3 h incubation in a medium at pH 2. A similar finding also reported that *L. reuteri* also has a high survival rate when exposed to low acidic pH [31].

Brands C, D, E, F, G, and I showed zero CFU count after incubation in SGF for 3 h, indicating that most of the cells were killed in this harsh pH environment. Low pH environment is thought to inhibit the metabolism and growth of the microbial strains, thereby reducing the viability of the probiotic. However, the results acquired in this in vitro study may not truly indicate their performance in vivo because many other physiological conditions might also affect the survival of the strains.

IV. Conclusion

This study is limited and does not claim to provide a comprehensive analysis of the ability of commercially available probiotics products to resist gastric conditions. However, the outcome of this analysis leads to the conclusion that most of the probiotic brands met the minimum initial viability requirement for commercial probiotic products, which is 10^6 CFU per g or mL sample. However, not all commercial probiotic supplier provides an accurate product label regarding the viability of the probiotic strain. Additionally, most probiotic products will lose their viability during the transition toward the target site, which is the small intestine, unless some protection is provided to the strain such as enteric coating or that particular probiotic strains have resistance to gastric acid.

In consequence, the effectiveness of the probiotic products without any protection can be questionable since they do not provide the patient with the benefit related to the consumption of the probiotic supplement.

Finally, recommendations can be made to the manufacturer to provide some additional information on product labels or websites that can be beneficial to assess the quality of probiotic products in a more comprehensive way, such as the quality control information, mainly regarding the purity and viability of the probiotic microorganism.

Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

Mohd Akmal Azhar, Mimi Sakinah Abdul Munaim, Muzamir Hasan and Zularisam Ab Wahid developed the project and designed the research; Mohd Akmal Azhar and Mimi Sakinah Abdul Munaim performed the experiments and wrote the paper.

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