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Original

Recovery of viable bacteria from ten commercially available probiotic products in three cities in the State of São Paulo

Recuperação de Bactérias Viáveis de Dez Produtos Probióticos Disponíveis Comercialmente em Três Cidades do Estado de São Paulo

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ABSTRACT

Probiotic cultures are live microorganisms and, when administered in adequate quantities, provide health benefits to the host, however, the quality of products sold is questionable as some may not accurately meet label claims. **Objective:** This work aimed to evaluate ten commercially available probiotic products, purchased in three cities in the state of São Paulo, Brazil. **Methods:** To verify the viability of the probiotic strains, ten products in the form of sachets or capsules were subjected to serial decimal dilution, inoculated in MRS Agar culture medium (Man, Rogosa & Sharp - Difco®) supplemented with 0.05% cysteine-HCl (MRSc) and incubated at 37°C for 48 hours in an anaerobic atmosphere, to determine the total number of Colony Forming Units (CFU). **Results:** Additionally, the possible presence of contaminating microorganisms was verified. Approximately 83% showed bacterial viability according to the label. There were similarities for each probiotic product in relation to the cities where they were purchased. Based on the colonial morphology of the selective culture media used, no contaminating microbial species were detected in any of the ten products. **Conclusion:** We concluded that the viable bacteria in probiotics sold in Brazil, more precisely in the state of São Paulo, are generally in accordance with the product label.

Keywords: Probiotics, Bacteria viability, Culture medium, Conditioning, Colony forming unit.

RESUMO

As culturas probióticas são microrganismos vivos e, quando administradas em quantidades adequadas, proporcionam benefícios à saúde do hospedeiro; no entanto, a qualidade dos produtos vendidos é questionável, pois alguns podem não atender com precisão às reivindicações do rótulo. Objetivo: Este trabalho teve como objetivo avaliar dez produtos probióticos disponíveis comercialmente, adquiridos em três cidades do estado de São Paulo, Brasil. Métodos: Para verificar a viabilidade das cepas probióticas, dez produtos na forma

de sachês ou cápsulas foram submetidos à diluição decimal seriada, inoculados em meio de cultura MRS Agar (Man, Rogosa & Sharp - Difco®) suplementado com 0,05% de cisteína-HCl. (MRSc) e incubados a 37°C por 48 horas em atmosfera anaeróbia, para determinação do número total de Unidades Formadoras de Colônias (UFC). Resultados: Adicionalmente, foi verificada a possível presença de microrganismos contaminantes. Aproximadamente 83% apresentaram viabilidade bacteriana de acordo com o rótulo. Houve semelhanças para cada produto probiótico em relação às cidades onde foram adquiridos. Com base na morfologia colonial dos meios de cultura selectivos utilizados, não foram detectadas espécies microbianas contaminantes em nenhum dos dez produtos. Conclusão: Concluímos que as bactérias viáveis nos probióticos comercializados no Brasil, mais precisamente no estado de São Paulo, estão geralmente de acordo com o rótulo do produto.

Palavras-chave: Probióticos, Viabilidade de bactérias, meio de cultura, condicionamento, unidade formadoras de colônias.

INTRODUCTION

Probiotics are "live microorganisms that, when administered in adequate quantities, confer a health benefit on the host"[1-3]. Probiotics are "live microorganisms that, when administered in adequate quantities, confer a health benefit on the host" and the bacterial strains most commonly used as probiotics belong to the genera Lactobacillus spp. and Bifidobacterium spp. Some studies have highlighted the ability of probiotics to improve the function of the intestinal barrier, modulate the intestinal microbiota, improve the host's immune response, exert antimicrobial activities and possibly reduce the risk, duration or severity of diseases[4,5]. The level of knowledge about the benefits promoted by probiotic microorganisms has increased significantly in recent years, as has the global market for commercialized probiotic products[6-11]. As a consequence, the supply of probiotic products on the market has become increasingly diverse, with a wide variety of fermented food products and supplements, formulations, species and strains[12].

It is known that the regulatory status of probiotics is not currently established in all countries. In Europe, probiotic foods and dietary supplements are covered by the Food Products Directive and Regulation (regulation 178/2002/EC; directive 2000/13/EU). According to Report FAO/WHO[13], "it is necessary to accurately list the probiotic bacteria in food products to include them on the label, and the label must indicate the viable concentration of each probiotic present at the end of the shelf life". However, some studies have already detected, through the viability evaluation of probiotic products around the world, a general lack of correlation between the label and the actual content of probiotic products[14,15].

For this reason, our study aimed to evaluate ten commercially available probiotic products, purchased in three cities in the state of São Paulo, Brazil, describe the results obtained and compare them to the information on the labels. Additionally, the presence of contaminating microorganisms was tested.

METHODS

Microbiological analyzes were carried out in January, February and March for ten probiotic products commercially available in pharmacies and drugstores in three municipalities in the State of São Paulo (Ribeirão Preto, São Paulo and Sorocaba) identified in Table 1.

The probiotic samples, after homogenization in a tube shaker (Phoenix AP 65, Araraquara, SP, Brazil), were subjected to serial decimal dilution in sterile buffered saline solution (PBS) pH 7.4. Aliquots of 100 µL of each of the dilutions were deposited in duplicate in Petri dishes containing MRS Agar supplemented with 0.05% (w/v) cysteine-HCl (MRSc) and seeded using a sterilized angled glass rod. After seeding, the plates were incubated for 48 hours at 37° C in anaerobiosis (GASPAKTM EZ Anaerobe Container System, Sparks, MD, USA). Next, the number of colony forming units (CFU) was counted using a stereoscopic microscope (Leica S6E, Wetzlar, Germany)[16].

Additionally, in order to detect the possible presence of contaminating microorganisms (enterobacteria, coagulase-positive staphylococci,

enterococci and fungi), aliquots of 100 µL of the 10° dilution of probiotic samples were sown in duplicate, respectively, in the culture media. Mac Conkey Agar (DifcoTM), Mannitol-Sal Agar (BD DifcoTM), Agar M - Enterococcus (DifcoTM), Sabouraud Dextrose Agar (DifcoTM) and incubated aerobically at 37°C for 48 hours.

RESULTS

Table 1 shows tested products, the probiotic microorganisms (species), the form of preparation, minimum number of CFU declared on the label by the manufacturer, cities of purchase and number of CFU recovered through viability tests. Among the ten brands of commercially available probiotic products analyzed in this study, the recovery of viable bacteria ranged from 6.0 x 10^4 CFU to 3.0 x 10^{17} CFU. There were similarities for each probiotic product in relation to the cities where they were purchased.

The recovery of viable bacteria from probiotic products is mostly in accordance with the value reported by the manufacturer. Most products (83.3%) have viable probiotic bacteria counts equal to or greater than 1 billion (≥ 10^9 CFU).

The capsule or sachet format is not associated with a standard difference between announced quantity and confirmed quantity.

The Bridrilac® product purchased in the cities of Ribeirão Preto and São Paulo stood out numerically from the other probiotics evaluated, showing greater recovery (≥ 10^{17} CFU) of viable probiotic bacteria and higher than the value reported by the manufacturer.

Based on macroscopic morphology in the selective culture media used, no contaminating microbial species were detected in any of the ten probiotic products evaluated.

Table 1. Comparison of label information with bacterial count.

Product	Species	Form	Minimum Number of CFU According to the Manufacturer	Place of Purchase	Total number of CFU Recovered
Probiatop	*L. acidophilus* SD 5221 *L. rhamnosus* SD 5675 *L. paracasei* SD 5275 *B. lactis* SD 5674	Sachet	10^9 of each species	Ribeirão Preto - SP São Paulo - SP Sorocaba - SP	2.7 x 10^{10} 3.7 x 10^{10} 1.4 x 10^{10}
Bridrilac	*L. acidophilus* LA - 5 *B. lactis* BB - 12	Sachet	10^9	Ribeirão Preto - SP São Paulo - SP Sorocaba - SP	>3.0 x 10^{17} >3.0 x 10^{17} 1.2 x 10^{11}
Flora 5	*L. acidophilus* *L. casei* *Lactococcus lactis* *B. lactis* *B. bifidum*	Sachet	10^9 of each species	Ribeirão Preto - SP São Paulo - SP Sorocaba - SP	8.9 x 10^8 4.4 x 10^{10} 2.8 x 10^{10}
Lactoflor	*B. lactis*	Sachet	10^9	Ribeirão Preto - SP São Paulo - SP Sorocaba - SP	3.2 x 10^{10} 2.0 x 10^{10} 6.0 x 10^4
Bifilac	*L. acidophilus* NCFM *B. lactis* HN019	Capsule	10^9 of each species	Ribeirão Preto - SP São Paulo - SP Sorocaba - SP	1.8 x 10^{10} 7.0 x 10^9 2.8 x 10^{10}
20 Bí	*L.acidophilus* NCFM *L. paracasei* Lpc37 *B. lactis* Bi-04 *B. lactis* Bi-07 *B. bifidum* Bb-02	Capsule	2.0 x 10^{10}	Ribeirão Preto - SP São Paulo - SP Sorocaba - SP	1.5 x 10^{11} 2.5 x 10^{11} 3.5 x 10^{11}

Tamarine Probium	L. acidophilus B. lactis	Capsule	2.0×10^9	Ribeirão Preto - SP São Paulo - SP Sorocaba - SP	5.0×10^8 1.0×10^9 8.0×10^8
Florabac	*L. acidophilus* *B. lactis*	Capsule	2.0×10^9	Ribeirão Preto - SP São Paulo - SP Sorocaba - SP	1.2×10^8 2.2×10^{10} 2.0×10^9
Simbioflora	*L. acidophilus*NCFM *L. rhamnosus* HN001 *L. paracasei* LPC-37 *B. lactis* HN019	Sachet	10^9 of each species	Ribeirão Preto - SP São Paulo - SP Sorocaba - SP	4.5×10^9 5.6×10^9 9.3×10^9
Simfort	*L. acidophilus* *L. casei* *Lactococcus lactis* *B. lactis* *B. bifidum*	Sachet	10^9 of each species	Ribeirão Preto - SP São Paulo - SP Sorocaba - SP	1.7×10^9 1.2×10^{10} 2.5×10^{10}

DISCUSSION

The commercialization and consumption of probiotics, both as foods and dietary supplements, has increased. However, many studies have demonstrated that the action of probiotics is strain-dependent, in addition to the fact that the quantity of viable cells ingested influences their effectiveness. Therefore, each product should be properly labeled with guaranteed quality[2,13]. There is still much to be investigated regarding the use of probiotics for some health-related issues. For this reason, the literature questions whether the quantity of commercial probiotic products accurately meets the label claim, that is, concentrations equal to or greater than 10^9 CFU[17].

The present study was conducted to determine whether products presented in their freeze-dried form available for purchase in the main pharmacies and drugstores in three selected cities in the state of São Paulo were correctly labeled in terms of viable amounts of bacteria. However, it is known that the preservation of viable microorganisms in lyophilized form varies and depends on the preparation standard, transport conditions, storage at points of sale and even handling by the consumer himself. Therefore, the availability of viable strains could not reach the CFU dosages expected for a given product[18,19]. This fact may highlight issues related to the quality control of this type of product both at the time of production and post-marketing surveillance[17,20,21].

A Bulgarian study developed by Marinova et al.[22] tested the viability of 26 products, 16 of which were commercially available and 10 compounded in local pharmacies, and demonstrated that almost none of the probiotic products tested were of satisfactory quality. There were deviations in the information provided on the labels about the strain composition of the products, as well as one of the most common problems was the lower number of total viable counts in the products compared to those provided on the label. Furthermore, the presence of undesirable microorganisms was detected in some of the products. Therefore, the study concluded that available probiotic products must be subjected to routine and thorough inspections by the competent authorities.

Another Polish study conducted by Korona-Glowniak et al.[2] qualitatively evaluated 10 commercially available probiotic products and resulted in these tested products showing low quality, that is, only some products contained defined and viable quantities of probiotic microorganisms and concluded that there is a need for regulations regarding the true labeling of probiotic products, as well as their monitoring of microbiological quality.

In the present study, the probiotic Bidrilac, which reached concentrations of viable recovered bacteria in the order of 10^{17} CFU, was the only product to be numerically distinct from the other products. Surprisingly, in comparison with the literature, these results revealed counts that exponentially exceeded the UFC dosage described on the label.

Regarding the qualitative evaluation of the possible presence of contaminating microorganisms[17,20,23,24], we verified that enterobacteria, coagulase-positive staphylococci, enterococci and fungi were not detected in any of the 10 products tested in the present study.

One of the limitations of the study was the fact that we did not carry out the species identification assessment using the PCR method. Another limitation would be the dynamics of the trade of pharmaceutical products in the country, which naturally undergoes changes, improvements or seasonal variations, making this data valid for a given moment and location. Due to the scarcity of studies in this area, the data demonstrated here provide important information for a type of product that is increasingly used.

CONCLUSION

We concluded that the viable bacteria in probiotics sold in Brazil, more precisely in the state of São Paulo, are generally in accordance with the product label. Furthermore, no contamination by other bacteria was identified in the 10 brands of probiotics evaluated in this study. Therefore, we understand that all steps to maintain the conservation (temperature and humidity of the environment) of the product, from transport to establishments to its commercialization must be strictly followed, so that when they reach the consumer's hands, the probiotics correspond to the information contained on the label of the respective manufacturers. In view of our findings, we understand that there is a need to maintain rigor in monitoring microbiological quality control and effectiveness, as well as the implementation of regulatory standards in terms of assessing the viability and identification of species contained in commercialized products.

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