Analysis of formulations of probiotics accessible to the population of Tabasco, México

Análisis de formulaciones de probióticos accesibles a la población de Tabasco, México

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DOI: 10.19136/hs.a20n2.4140

Received date: November 22, 2020 Accepted date: January 26, 2021

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Abstract

Objective: To analyze the composition of formulations of probiotics sold in establishments in the State of Tabasco or that can be purchased online.

Materials and methods: A descriptive study in which formulations of probiotics sold in 21 establishments in the city of Villahermosa were identified and compared with 30 probiotic supplements sold online. Product information was organized in a database and analyzed according to the dosage form, probiotic genera, species/subspecies contained and their classification as probiotics or synbiotics and as drugs or supplements.

Results: Thirty-one local products and 30 online products formulated with probiotics in 6 different dosage forms were analyzed. Only five local products and no online products are certified by COFEPRIS as drugs. Forty-eight percent of the formulations are monostrain and the rest are multistrain. Seventy-two percent of the formulations are probiotics and the rest are synbiotics. Among the 61 products analyzed, 46 species belonging to 13 different genera were identified, and 39% were common to local and online products. Many of the rest are synbiotics. Among the 61 products analyzed, 46 species belonging to 13 different genera were identified, and 39% were common to local and online products. Many of the rest are synbiotics.

Conclusions: The population of the State of Tabasco can find at least 31 formulations of probiotics in local establishments. The variety increases if we consider the dietary supplements available for sale online. Multistrain supplements are particularly abundant in online retailers. The products certified by COFEPRIS ensure that the benefits of the formulation are supported by clinical trials in humans and are manufactured following good manufacturing practices.

Keywords: Probiotics; synbiotics; dietary supplements; Lactobacillus; Bifidobacterium.

Resumen

Objetivo: Analizar la composición de formulaciones de probióticos que se venden en establecimientos del Estado de Tabasco o que se pueden comprar en línea.

Materiales y métodos: Estudio descriptivo en el que se identificaron formulaciones de probióticos comercializados en 21 establecimientos de la ciudad de Villahermosa y se compararon con 30 suplementos probióticos comercializados en línea. La información del producto se organizó en una base de datos y se analizó de acuerdo con la forma de dosificación, los géneros y especies / subspecies de probióticos presentes y su clasificación como probióticos o simbióticos y como medicamentos o suplementos.

Resultados: Se analizaron 31 productos locales y 30 productos de venta en línea formulados con probióticos en 6 formas de dosificación diferentes. Solo cinco productos locales y ningún producto en línea están certificados por COFEPRIS como medicamentos. El 48% de las formulaciones son monocepa y el resto son multicépicas. El 72% de las formulaciones son probióticos y el resto son simbióticos. Entre los 61 productos analizados, se identificaron 46 especies pertenecientes a 13 géneros diferentes y el 39% fueron comunes a productos locales y de venta en línea. Muchos de los productos contienen especies de los géneros Lactobacillus y Bifidobacterium. El género Bacillus fue el único que nunca se combinó con otros géneros en las formulaciones analizadas en este estudio.

Conclusiones: La población del estado de Tabasco puede encontrar al menos 31 formulaciones de probióticos en establecimientos locales. La variedad aumenta si tenemos en cuenta los suplementos dietéticos disponibles de venta en línea. Los suplementos multicépicas son particularmente abundantes en los productos en línea. Los productos certificados por COFEPRIS aseguran que los beneficios de la formulación están respaldados por ensayos clínicos en humanos y se fabrican siguiendo buenas prácticas de fabricación.

Palabras clave: Probióticos; simbióticos; suplementos dietéticos; Lactobacillus; Bifidobacterium.
Introduction

According to the Scientific Association of Probiotics and Prebiotics (ISAPP) consensus, probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit”. Microorganisms must be well characterized, be safe, and provide a general benefit to humans, supported by scientific evidence, to be considered probiotics. Lactobacillus, Bifidobacterium, and Saccharomyces species are the most commonly used probiotics. Other less frequently used probiotics include species of Bacillus, Propionibacterium, Streptococcus, and Escherichia. Evidence supports that probiotics can improve the immune system response, gastrointestinal health, and vaginal lactobacilli concentration. However, they do not promote persistent changes in gut microbiota.

Formulations containing probiotics must ensure an effective microorganism dose viable until the expiration date to be considered supplements and be allowed to declare a specific beneficial property. In addition, a risk and benefit assessment and the compliance with regulatory standards in each country are required to register the formulations as drugs. In addition to probiotics, many formulations include substrates used selectively by host microorganisms that also confer a health benefit (prebiotics). This combination of probiotics and prebiotics is called a synbiotic.

In Mexico, we can find formulations containing probiotics that have been approved by the Federal Commission for the Protection against Sanitary Risks (COFEPRIS, for its acronym in Spanish; https://www.gob.mx/cofepris) as drugs. Additionally, we can find formulations described as food or dietary supplements that, in some cases, declare the benefit they confer. The population should know what probiotic dietary supplements that, in some cases, declare the benefit they confer. In this way, 31 products formulated with probiotics were identified, hereinafter referred to as local products. Likewise, 30 products formulated with probiotics that can be purchased online were identified and compared with those sold locally.

Materials and methods

Identification of formulations containing probiotics sold in local establishments.

Nineteen pharmaceutical establishments and two health food stores located in Villahermosa, Tabasco were visited between October and November 2019, and the products formulated with probiotics that are offered to the population were identified. A total of 33 different products were obtained; two were discarded since no reliable information was found in the scientific literature or in the National Center for Biotechnology Information (NCBI) taxonomy database to support the strain declared on the label. Therefore, this study analyzed the information of 31 products, which we refer to as local products. Some of these products are of national manufacture, while others are imported.

Identification of formulations containing probiotics sold online.

An internet search was carried out using the term probiotic supplement, and information on 30 products that can be purchased online was collected. All these products are imported.

Information recording and data analysis.

The information on local and online products was recorded in a database and analyzed according to several criteria, such as dosage form, genera, species/subspecies of probiotics contained, and classification as probiotics or synbiotics and as drugs or supplements.

Results

It was determined which products formulated with probiotics are sold in 19 pharmacies and two health food stores in Villahermosa by asking the salesmen to identify the probiotic products sold by the establishment. When it was detected that the concept of probiotics was not familiar to the seller, we asked him or her about supplements or medications that help restore or preserve the intestinal flora (the colloquial term used to name the gut microbiota). On some occasions, the best-known probiotic genera (Lactobacillus, Bifidobacterium, and Saccharomyces) were named to guide the seller. In this way, 31 products formulated with probiotics were identified, hereinafter referred to as local products. Likewise, 30 products formulated with probiotics that can be purchased online (products for sale online) were identified and compared with those sold locally.

The establishments visited belong to large pharmaceutical chains that operate regionally (south-southeast of the country) and nationally and have branches in the 17 municipalities of Tabasco. In addition, several of them also offer online sales. Thus, we can assume that the products analyzed as local can be purchased in the 17 municipalities of the State of Tabasco.

Classification of the formulations as drugs or supplements

The 31 local products, along with the 30 products sold online, were classified as supplements or drugs using the label information and verifying their registration by the
Federal Commission for Prevention against Sanitary Risks (COFEPRIS) as drugs. Regarding products sold online, 100% (30) were described on the label as a food supplement and contained warnings that they are not regulated by the FDA. In general, these products stated that they contribute to digestive health. On the other hand, 83.87% (26) of local products were food supplements, and only 16.13% (5) were identified as drugs figure 1, some of which specify on the label their functions as regulators of intestinal flora, assistants in the treatment of diarrhea, or both.

Dosage forms of the formulations analyzed.

Figure 2 shows the dosage forms of the local and online formulations analyzed. The dosage form of 83% (25) of the products sold online was the capsule, and the rest were presented as tablets, chewable tablets, or gummies. By contrast, the variety of dosage forms is better represented in local products: 32% (10) are administered in capsules, 30% (9) in powder, 19% (6) in tablets, and 19% (6) in suspension. The dosage forms common to both types of products were the capsule and the tablet, representing 57% and 13% of the total products, respectively.

Figure 1. Percentage of supplements and drugs formulated with probiotics that are sold locally (31 in total)

Figure 2. Dosage forms of the formulations analyzed (61 in total).
Genera and species present in the formulations analyzed.

Species belonging to 8 bacterial genera and five yeast genera were identified in the 61 formulations analyzed. As shown in figure 3, local products contain species from only six genera, while online products contain species from 12 genera. In both cases, the most represented genera were *Lactobacillus* and *Bifidobacterium*. Some local products [64% (20)] and online products [36% (11)] contain species of a single genus, either *Bifidobacterium*, *Lactobacillus*, *Saccharomyces*, or *Bacillus* (Figure 3A and 3B). Unlike local products [22% (7)], 47% (14) of online products contained species of 2 genera, which, in most cases, were a mixture of *Lactobacillus* and *Bifidobacterium* (Figure 3A). In addition, the species *Streptococcus thermophilus* was present in 8 products. It is worth noting that *Bacillus* species were not mixed with species of other genera in any products. Finally, among the online products, there were 2 cases containing species from 10 different genera.

Analyzing the species/subspecies present in the products revealed that 61% (19) of the local products contained a single bacterial species, and 39% (12) contained 2 to 10 different species (Figure 3C). By contrast, 30% (9) of the products sold online contained a single species, while 60% (18) contained 2 to 10 species, and the remaining 10% (3) contained 18 to 31 species from 10 different genera.

Figure 4 shows the 46 species and subspecies identified in the formulations analyzed. Thirty-nine percent (18) were found both in local and in online products, whereas 7% (3) were only present in local products and the remaining 54% (25) were only found in products sold online. *Lactobacillus* was the genus with the highest abundance of species (18) in the formulations analyzed. *Lactobacillus acidophilus*, followed by *L. rhamnosus*, *L. plantarum*, and *L. casei*, were the most represented. In all the products, specific strains of each species were mentioned. Although *Lactobacillus* species diversity in online products was vast, 44% of the species were only present in 3% (2) or less of products.

**Figure 3.** The Venn-Euler diagram represents the number of formulations containing species from the different genera in local (A) and online products (B), where the set called various genera * represents the genera Brettanomyces, Debaryomyces, Kluyveromyces, Torulaspora, and Leuconostoc. The number of products that contain a variable number of species/subspecies in their formulation (C).

Fuente: Elaboración propia
The second most represented genus was *Bifidobacterium*, with seven species. It is worth pointing out that *Bifidobacterium infantis* and *B. lactis* are subspecies of *Bifidobacterium longum* and *B. animalis*, respectively. However, they were described with the old classification due to the importance of subspecies in studying and using probiotics. *B. infantis* is the most abundant subspecies in the infant gut microbiota, and *B. lactis* was discovered in dairy products and has been widely used in their production. The *Bifidobacteria* species most represented in local products were different from those most represented in the products sold online. In addition, the number of products sold online containing the most represented species [*B. lactis* (53% (16)), *B. bifidum* (40% (12)), and *B. longum* (33% (10))] was elevated compared to the *Bifidobacteria* species most represented in local products [*B. infantis* (13% (4)) and *B. lactis* (10% (3))]. Few products mentioned specific strains of *Bifidobacteria*.

The third-most abundant species were yeasts of the genus *Saccharomyces* (5), and only *Saccharomyces boulardii* was common to local and online products. Most of the remaining genera (7) were represented by a single species. *S. thermophilus* stood out for being the ninth most represented species in all of the products, preceded only by *Bifidobacteria* and *Lactobacillus* species.

It should be noted that *Bacillus coagulans* was formerly named *Lactobacillus sporogenes*, and this name is preserved on product labels.

### Classification of formulations as probiotics or synbiotics

The 61 formulations analyzed were classified as probiotics or synbiotics using the nutritional information, the list of ingredients declared on the label, or both. For this, a synbiotic was considered a mixture containing live microorganisms and substrate(s) used selectively by the host microorganisms that confer a health benefit, according to the consensus reported by Swanson in 2020. The substrates mentioned in the labels that allowed classifying the products as synbiotics were inulin, fructooligosaccharides, and oligofructose. It was observed that 32% and 23% of local products and online products, respectively, were synbiotic, even though some were declared as probiotics on the label.
Discussion

The results show that the Tabasco population has access to various supplements and drugs made with probiotics sold by local establishments. Additionally, more complex formulations than those sold locally can be purchased online. It should be noted that only 5 of the 31 local products are registered as drugs by COFEPRIS figure 1. Three of those products contain only Bacillus clausii and are administered in suspension (2) and capsule form (1). The other two products are administered in powder form, with one composed of Saccharomyces boulardii and the other composed of a mix of Lactobacillus fermentum and L. delbrueckii. Interestingly, none of the species contained in these drugs was one of the most represented in the 61 formulations analyzed (Figure 4). Multiple studies have demonstrated the effectiveness of B. clausii in the treatment of acute diarrhea in children and S. boulardii in reducing the risk of acquiring antibiotic-associated diarrhea in children and adults.

Regulatory approaches in some countries, such as Canada and Italy, agree that products must have a minimum amount of \(1 \times 10^9\) CFU of well-studied microbial species per serving to be considered probiotic foods or supplements. Health Canada lists the following as probiotic species: Bifidobacterium adolescentis, B. animalis subsp. animalis and lactis, B. bifidum, B. breve, B. longum subsp. longum and infantis, L. acidophilus, L. casei, L. fermentum, L. gasseri, L. johnsonii, L. paracasei, L. plantarum, L. rhamnosus, and L. salivarius. Except for L. johnsonii, all these strains were present in at least one formulation that we analyzed (Figure 4). The number of microorganisms declared in the five drugs identified in 64% (17) of local supplements (25) and all products sold online (30) is above \(1 \times 10^9\) CFU, although some products clarify that this is the amount when manufactured and that the effective number of bacteria until the expiration date is \(1 \times 10^7\) CFU.

The most commonly used dosage form was the capsule, followed by the tablet figure 2. However, 4 of the five drugs were in powder or suspension form. The strain and the dosage form are critical to ensure that the probiotics remain viable since, after being ingested, the probiotic faces various fluids (bile salts, pancreatic enzymes, gastric juice, among others) during its passage through the gastrointestinal tract. In addition, during encapsulation, factors such as pH, pressure, temperature, moisture content, amount of oxygen, and water activity affect the viability of microorganisms. Improper storage or distribution of these products, or both, can also affect the viability of the microorganisms. Notably, several studies show that probiotics that have been killed, destroyed by heat, or inactivated by ultraviolet light, and even cellular

Figure 5. Classification of the analyzed products (61 in total) as probiotics or synbiotics

Fuente: Elaboración propia
fractions, can confer health benefits and be as effective as living organisms and, in some instances, safer for the host in the treatment of some conditions.\textsuperscript{10,11}

Regarding the complexity of the formulations analyzed, 46\% (28) contained only one probiotic strain of the \textit{Lactobacillus}, \textit{Bifidobacterium}, \textit{Saccharomyces}, or \textit{Bacillus} genera, so they were considered monostains. In contrast, the rest of the formulations were multistrain and contained two to 32 species from 14 different genera figure 3. The abundance of species of the genera \textit{Lactobacillus} and \textit{Bifidobacterium} is evident. These species have been widely studied with favorable results in multiple gastrointestinal tract disorders, such as irritable bowel syndrome, ulcerative colitis, Crohn’s disease, and infectious and antibiotic-associated traveler’s diarrhea.\textsuperscript{2} The strain included is essential when choosing probiotic formulations for the treatment of gastrointestinal diseases. \textit{L. acidophilus} LB is effective in the treatment of traveler’s diarrhea, while other \textit{L. acidophilus} strains are not.\textsuperscript{3} For this reason, the strain must be specified in the formulations.

Although monostrain products are the most studied, the efficacy of multistrain formulations in treating different conditions is being increasingly studied. For example, a probiotic composed of 4 strains of \textit{Lactobacillus} (3) and \textit{Enterococcus} (1) species was well tolerated and associated with a decrease in intestinal inflammation in patients with ulcerative colitis.\textsuperscript{14} Likewise, a symbiotic formulation composed of fructooligosaccharides and six strains of \textit{Lactobacillus} (4), \textit{Bifidobacterium} (1), and \textit{Bacillus} (1) genera significantly improved glycemic control in subjects with type 2 diabetes mellitus.\textsuperscript{15} Another formulation with eight strains of \textit{Lactobacillus} (4), \textit{Bifidobacterium} (2), and \textit{Lactococcus} (2) genera given to patients with type 2 diabetes mellitus for six months without prior medication significantly decreased the HOMA-IR index, reduced inflammation, and improved the cardiometabolic profile of patients.\textsuperscript{16} These findings show that these types of formulations may be a promising adjuvant in anti-diabetic therapy. Treating diseases is not the only benefit that can be obtained from ingesting multistrain formulations. The administration of a probiotic composed of 4 strains of \textit{Lactobacillus} (3) and \textit{Saccharomyces} (1) species reduced the toxicity of a mixture of phthalates and bisphenol A in Wistar rats, and very recently, a probiotic formulation of 8 strains of \textit{Lactobacillus} (4), \textit{Bifidobacterium} (3) and \textit{Streptococcus} (1) genera cultivated in consortium from \textit{in vitro} studies showed that it could absorb toxic elements such as Cd, Hg, and Pb. Additionally, the concentration of Cd in the feces of 15-day-old infants whose mothers consumed this formulation was significantly reduced.\textsuperscript{18} These findings open the possibility of using probiotic consortia as a method for bioprotection and detoxification of different pollutants. On the other hand, the addition of different probiotic strains could also be considered a technological strategy. \textit{Lactococcus lactis} and \textit{S. thermophilus} were present in some of the products analyzed, always combined with \textit{Lactobacillus} and \textit{Bifidobacteria}. \textit{L. lactis} and \textit{S. thermophilus} have shown to assist the survival of other lactic acid bacteria in food products.\textsuperscript{19} In addition, \textit{S. thermophilus} has high oxygen consumption, so it is added to anaerobic microorganisms to reduce the oxygen considered toxic during encapsulation, avoiding affecting the viability of the microorganisms.\textsuperscript{2} It has been shown that \textit{L. rhamnosus} GG, the second most represented species in the products analyzed, promotes interactions between crucial elements of the microbiota, such as \textit{Bifidobacteria} and the host epithelium, and significantly modifies the microbiota of healthy individuals.\textsuperscript{20} These properties make it attractive for the formulation of both single and multistrain products.

Among the formulations analyzed, 2 contained 32 species of 10 different genera. The yeasts \textit{Brettanomyces anomalus}, \textit{Debaryomyces hansenii}, \textit{Kluyveromyces marxianus}, and \textit{Torulaspora delbrueckii}, and the four species of \textit{Leuconostoc} were exclusive to these products. These yeasts have been identified and used as starters for various dairy products, whereas various species of \textit{Leuconostoc} have been identified in various fermented products and have shown to inhibit pathogen growth.\textsuperscript{22} In addition to the probiotic strains, these formulations contain some vitamins, minerals, and enzymes that promote digestion. It is important to note that more is not always better. Therefore, it is important to have trials that show that a complex formulation promotes a more significant benefit to consumer health.

Finally, 28\% of the products analyzed figure 5 were classified as symbiotic since their components include some prebiotic carbohydrates such as inulin, fructooligosaccharides, and oligofructose. These products are complementary synbiotics since the selective use of prebiotics by autochthonous microbiota has already been established. There are no studies that show that the addition of prebiotics to probiotic formulations leads to a more significant benefit to be considered synergistic synbiotics.\textsuperscript{4}

Evidence supporting the benefits conferred by living, dead, or remnants of probiotics, as well as discrepancies in the effectiveness of monostrain and multistrain probiotics in the treatment of several conditions, reinforces the need to have well-characterized formulations supported by \textit{in vitro}, preclinical and clinical trials to ensure that a formulation is effective to promote health or to assist in the treatment of specific conditions.\textsuperscript{8} In addition, scientific evidence should support that more than one strain, additional ingredients, or both in the same formulation provides more significant benefits. In this sense, probiotics registered as drugs by regulatory agencies such as COFEPRIS in Mexico are safer
since this agency certifies that the formulations are supported by clinical trials in humans that prove their benefits and certifies that they have been prepared following good manufacturing practices. The comparison between local and online products shows that although online products widen the variety of formulations that we can purchase, they are marketed in Mexico and abroad following regulations different from those that govern drugs; therefore, they are not required to support the expected benefits with scientific evidence, and consequently, there is no obligation to mention the benefits on the label.

Conclusions

The population of the state of Tabasco can find at least 31 formulations containing probiotics. The variety of products increases if the supplements that can be purchased for sale online are considered, particularly with regard to multistrain supplements. Moreover, online product offerings increase and change quickly. Our findings show that probiotic products that can be purchased in stores and online are not governed by the same health regulations as those found locally. Considering that they are designed with living organisms and that many of their characteristics depend on their viability, health regulations must be reviewed and include aspects that guarantee their quality, especially those declared as supplements. On the other hand, we must not forget that the distribution of any product for sale online does not reach all places, and it is still necessary to have the products available in local establishments. A more in-depth exploration of the local market over time will allow an understanding of if the formulations offered increase and diversify. In addition, exploring the use of probiotics in local clinical practice would be useful to determine if health professionals demand a greater diversity of these products and if they have pertinent information about them to prescribe and recommend them.

Acknowledgments

We thank CONACYT SEP-Ciencia Basica no. 288403 project and 30394 for financial support.

Conflicts of interest

The authors declare that there are no conflicts of interest.

References


### Appendix 1. Local and online products included in this study.

<table>
<thead>
<tr>
<th>Name (Manufacturer or distributor)</th>
<th>Dosage form</th>
<th>Name (Manufacturer or distributor)</th>
<th>Dosage form</th>
</tr>
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<tbody>
<tr>
<td>Enterogermina (Sanofi)*</td>
<td>Suspension</td>
<td>Align Probiotic (Align)</td>
<td>Capsule</td>
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<tr>
<td>Sinuberase (Laboratoire Unither)*</td>
<td>Suspension</td>
<td>Align Digestive Care Probiotic (Align)</td>
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<tr>
<td>Sinuberase (Laboratoire Unither)*</td>
<td>Capsule</td>
<td>Align Fuerza Extra (Align)</td>
<td>Capsule</td>
</tr>
<tr>
<td>Lacteol fort (Carnot)*</td>
<td>Powder</td>
<td>Kids daily probiotic (Culturelle)</td>
<td>Ch e w a b l e tablet</td>
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<tr>
<td>Floratil (Biocodex 1)*</td>
<td>Powder</td>
<td>Digestive Health Extra Strength (Culturelle)</td>
<td>Ch e w a b l e tablet</td>
</tr>
<tr>
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<td>Probiotix complex (GNC)</td>
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<tr>
<td>Lactipan baby (Italmex pharma)</td>
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<td>Lactipan Kids (Italmex pharma)</td>
<td>Suspension</td>
<td>Probiotix (GNC)</td>
<td>Capsule</td>
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<td>Simi bacilos forte (Manufacturas Naturex)</td>
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<td>RAW probiotics women (Garden of life)</td>
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<td>Simi probacils (Manufacturas Naturex)</td>
<td>Tablet</td>
<td>RAW probiotics colon care (Garden of life)</td>
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<td>Ultimate cultures complex (Mayfair nutrition)</td>
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<td>Alkabiotics 7 probiotics (Alka balance)</td>
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<td>PRO-Moms (Hyperbiotics)</td>
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<td>Capsule</td>
<td>Probiotic colon support (Walgreens)</td>
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</tr>
<tr>
<td>Proteflor (Siegfried Rhein)</td>
<td>Capsule</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Products registered by COFEPRIS.