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Probiotic and paraprobiotic potential of *Bacillus coagulans*: Impact of processing and storage on viability and resistance in the gastrointestinal tract

Potencial probiótico e paraprobiótico de *Bacillus coagulans*: Impacto do processamento e do armazenamento na viabilidade e na resistência ao trato gastrointestinal

Potencial probiótico y paraprobiótico de *Bacillus coagulans*: Impacto del procesamiento y almacenamiento en la viabilidad y resistencia en el tracto gastrointestinal

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Abstract
Probiotic microorganisms of the genus *Bacillus* spp. are attractive due to their inherent stability of spore-forming bacteria. Among them, *Bacillus coagulans* has demonstrated great prospects as a probiotic for food production. This review explores the impacts of the processing and storage on the viability of *B. coagulans* and how this influences its applicability on different food matrices. In addition, the resistance to the gastrointestinal tract, which is clearly a crucial factor to probiotics, is approached. In parallel, the paraprobiotic potential of *B. coagulans* is also discussed. Studies evidenced a great survival range of this microorganism during different processing/storage conditions: *B. coagulans* maintains its availability during thermal and acidic conditions and also can survive in foods with intermediate water activity. Vegetative and sporulated cells of *B. coagulans* showed great resistance to in vitro simulations of gastrointestinal tract, surviving adverse conditions of pH, osmotic concentration, and temperature. These results suggest that *B. coagulans* has the potential to be used in the production of different probiotic food matrices.
Keywords: Functional food; Microbial resistance; Probiotic; Spores; Thermophilic.

Resumo
Microrganismos probióticos do gênero *Bacillus* spp. são atraentes devido à sua estabilidade inerente de bactérias formadoras de esporos. Dentre eles, o *Bacillus coagulans* tem demonstrado grandes perspectivas como probiótico na

produção de alimentos. Esta revisão explora os impactos do processamento e armazenamento na viabilidade de *B. coagulans* e como isso influencia sua aplicabilidade em diferentes matrizes alimentares. Além disso, aborda-se a resistência ao trato gastrointestinal, que é claramente um fator crucial para os probióticos. Em paralelo, o potencial paraprobiótico de *B. coagulans* também é discutido. Estudos evidenciaram uma grande faixa de sobrevivência desse microrganismo em diferentes condições de processamento/armazenamento*:* *B. coagulans* mantém sua disponibilidade em condições térmicas e ácidas e pode sobreviver em alimentos com atividade de água intermediária. As células vegetativas e esporuladas de *B. coagulans* mostraram grande resistência às simulações in vitro do trato gastrointestinal, sobrevivendo a condições adversas de pH, concentração osmótica e temperatura. Esses resultados sugerem que o *B. coagulans* tem potencial para ser utilizado na produção de diferentes matrizes alimentarem probióticas.
Palavras-chave: Alimento funcional; Resistência microbiana; Probiótico; Esporos; Termofílico.

Resumen
Microorganismos probióticos del género *Bacillus* spp. son atractivos debido a su estabilidad inherente a las bacterias formadoras de esporas. Entre ellos, *Bacillus coagulans* ha mostrado grandes perspectivas como probiótico para la producción de alimentos. Esta revisión explora los impactos del mayor procesamiento y almacenamiento en la viabilidad de *B. coagulans* y cómo esto influye en su aplicabilidad en diferentes matrices alimentarias. Además, aborda la resistencia del tracto gastrointestinal, que es claramente un factor crucial para los probióticos. Paralelamente, también se discute el potencial paraprobiótico de *B. coagulans*. Los estudios han demostrado una amplia gama de supervivencia de este microorganismo en diferentes condiciones de procesamiento/almacenamiento: *B. coagulans* mantiene su disponibilidad en condiciones térmicas y ácidas y también puede sobrevivir en alimentos con actividad de agua intermedia. Las células vegetativas y esporulantes de *B. coagulans* mostraron gran resistencia a las simulaciones in vitro del tracto gastrointestinal, sobreviviendo a condiciones adversas de pH, concentración osmótica y temperatura. Estos resultados sugieren que *B. coagulans* tiene potencial para ser utilizado en la producción de diferentes matrices alimentarias probióticas.
Palabras clave: Alimento funcional; Resistencia microbiana; Probiótico; Esporas; Termófilo.

1. Introduction

The concern with the body, well-being and health has favored special attention to healthy eating. The search for foods with functional properties became a reality in the diet of many people. Functional foods represent a series of food products with benefits for several physiological aspects and can be described as those foods that have properties beneficial to the human body in addition to basic nutrition (Konar et al., 2018; Grochowicz et al., 2021; Ahmed et al., 2022). Foods, or even functional ingredients, are characterized by offering health benefits and can play a potentially valuable role in reducing the risk of disease.

Allied to the concern of consumers with nutrition, the consumption of functional products has formed different niche markets. In this context, the probiotic-based diet has gained prominence in recent years due to its well-documented health benefits and for being added to a wide variety of products (De Mauri et al., 2020; Tang et al., 2021).

According to the United Nations Food and Agriculture Organization and the World Health Organization (FAO/WHO), probiotics are "living microorganisms that, when administered in adequate quantities, confer a health benefit to the host". The addition of microorganisms in food or supplements can be an indication of health only if their benefits are proven in clinical and documented studies (FAO/WHO, 2002). For a probiotic product to present the health benefit claim on the label, the National Health Surveillance Agency (ANVISA) establishes that the minimum viable quantity of the culture must be between 10^8 to 10^9 CFU per daily portion of the product (Brasil, 2008). According to ANVISA, proofing the safety of probiotics and their health benefits requires the characterization and identification of the lineage of the microorganism. The health benefit associated with the use of the probiotic must be clearly identified and its proof requires demonstration of survival to the conditions of the human digestive tract and evidence of the effects obtained through studies (Brasil, 2018).

Among the most common probiotic foods, dairy products like yoghurts and fermented milks, dominate the market. These live microorganisms, when ingested in a specific amount, have a beneficial effect on health and well-being, since they are able to prevent intestinal infections, improve the immune system, assist in the metabolism of lactose and neutralize the effects of pathogenic microorganisms (Panghal et al., 2018). Due to these benefits attributed to probiotics, the interest in the consumption and commercialization of functional foods containing these microorganisms has grown considerably (Champagne et al., 2018).

According to a market analysis report released recently, the global probiotic market in 2018 was $ 48.38 billion, and these results were driven by consumers' growing concern for health and well-being (Grand View Research, 2019).

Probiotic cultures have been successfully applied to different types of food matrices. Different food products, including dairy products, meats, beverages, cereals, vegetables and fruits and bakery products, have been used as matrices to support probiotic microorganisms (Shori, 2015). The intrinsic characteristics of the distinct foods represent great challenges for the survival and viability of these microorganisms. Factors such as low water activity (Aw), acidic pH, restricted availability of nutrients and oxygen can affect microbial viability during food processing and storage (Reale et al., 2019). In addition, foods that will be exposed to thermal processes with temperatures above 60 °C may result in decreased probiotic viability (Pimentel et al., 2019).

The most used probiotics are *Lactobacillus* and *Bifidobacterium*. However, most of these strains are not resistant to high temperatures, as well as to the acid present in the stomach, digestive enzymes, and bile salts, requiring the characterization of other types of probiotics due to technological difficulties with their release mechanisms (Zhou et al., 2020). Therefore, many strains of *Bacillus* spp. have been used as probiotic dietary supplements, including *Bacillus coagulans*.

B. coagulans is a gram-positive, facultative anaerobic, non-pathogenic, spore-forming, and lactic acid-producing bacterium (Konuray & Erginkaya, 2018). The growth temperature for *B. coagulans* is 35 to 50 °C and the ideal growth pH is 5.5 to 6.5 (Zhou et al., 2020). *B. coagulans* has been reported to be safe by the Food and Drug Administration (FDA) and the European Union Food Safety Authority (EFSA) and is on the list of Generally Recognized As Safe (GRAS) and Qualified Presumption of Safety (QPS) (Figure 1).

The terminal spores of *B. coagulans* have strong resistance, rebirth, and stability, which can be activated in the acidic environment of the stomach and begin to germinate and proliferate in the intestinal tract. This microorganism may accommodate in the intestinal environment with low oxygen content and reach the gastrointestinal tract to play the role of lactic acid bacteria (BAL) in the intestinal tract (Shinde et al., 2019). Once germinated, *B. coagulans* is able to produce a bacteriocin called coagulin, which has antibacterial activity against a wide spectrum of enteric microorganisms (Hyronimus et al., 1998). Due to this technological robustness *B. coagulans* is important from an industrial point of view (Konuray & Erginkaya, 2018).

An *in vitro* and *in vivo* toxicology studies conducted by Endres et al. (2009) evaluated the safety of *B. coagulans* GBI-30 (GanedenBC 30™; 6086). The results evidenced that a dose of 9.52×10^{11} CFU over 90 days has proved to be well tolerated and safe. Moreover, the authors verified that the toxicological safety assessment indicated that GanedenBC 30™ does not show mutagenic, clastogenic or genotoxic effects. Therefore, based on scientific procedures and supported by its use history, GanedenBC 30 ™ is considered safe for chronic human consumption (Endres et al., 2009).

Heat-treated food products are generally not used to add probiotic microorganisms in the form of free cells, due to factors that affect their viability and stability. To avoid this difficulty, *B. coagulans*, *Bacillus racemilacticus* and *Bacillus laevolacticus*, as well as the genus *Sporolactobacillus*, could be used as probiotics due to their heat resistant spore forms (Cao et al., 2020). These spores protect the cell's genetic material from the heat and pressure processes (Song et al., 2012). Spores can survive in industrial conditions and therefore provide long-term survival of the bacteria (Sanders et al., 2001).

The conditions applied in the of food processing, such as heat treatment, can limit the growth of the probiotic strain. Thus, it is essential to ensure an adequate survival rate of probiotics during production, as well as during the product shelf life (Sharifi et al., 2021). Thus, some alternatives such as the use of thermoresistant probiotic microorganisms become essential for the food industry (Table 1).

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Table 1. Application of *Bacillus coagulans* in different food matrices.

Food matrix	Process conditions	Viability after process	Viability after storage	Effect on quality	References
Semolina pasta	The dough was dried at 50ºC.	8.3 log CFU/g (after drying)	6.0-7.8 log CFU/g.	The use of the probiotic microorganism did not affect the sensory, textural and color characteristics of the samples during storage.	Konuaray & Erginkaya (2020)
Coffee	The coffee was prepared with water at 80ºC.	9.34 log CFU/200 ml		Resistance to high temperatures was observed, without changing the sensory characteristics of the product.	Majeed et al. (2019)
Tea	The tea was prepared with water at 80ºC.	9.1 log CFU/200 ml	8.62 log CFU/200 ml	Resistance to high temperatures was observed, without changing the sensory characteristics of the product.	Majeed et al. (2019)
Cream cheese	Pasteurization (65ºC / 30 min); Coagulation (70ºC), fusion (90ºC)	*B. coagulans* spores maintained their viability after heat treatments.	7-8 log CFU/g	No impact on the profile of proteolysis and fatty acids.	Soares et al. (2019)

Source: Author's elaboration.

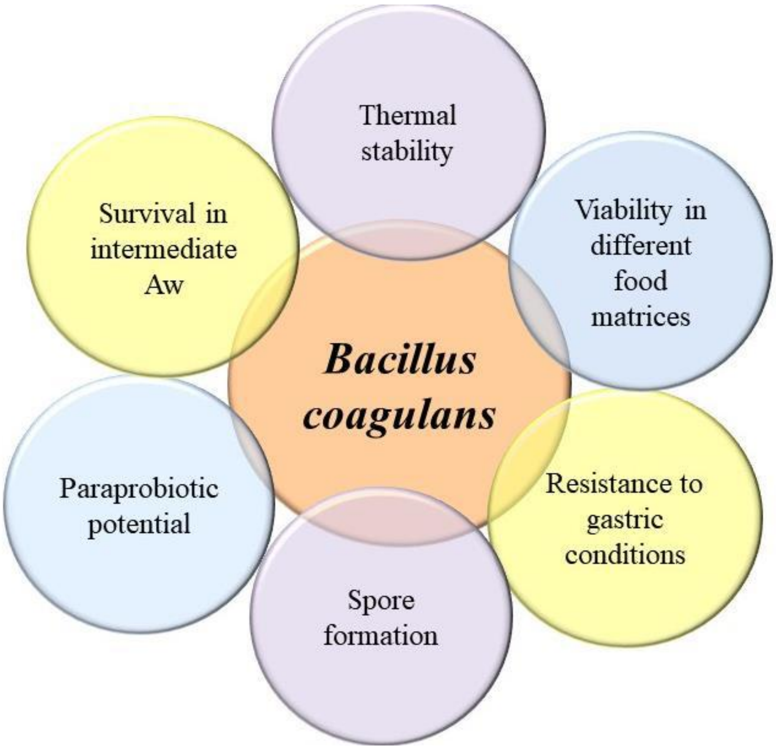
Due to the intensification of studies exploring the beneficial effects of probiotic microorganisms, the food industries have followed the technological trend and launched many products, both dairy and probiotic food products based on fruits, cereals, and vegetables, or even a combination of these. The periodic updating of the strains of microorganisms used in these raw materials should be done, to accompany the research and published data on probiotics, contributing with improved information. Thus, there is a need to investigate the potential of other microorganisms and their association with different food matrices.

However, although there are several studies regarding the probiotic potential of *B. coagulans*, there are no reviews in the literature that addresses its use. Thus, this work presents a narrative review on *B. coagulans* and how this microorganism is affected by food processing. In addition, main applications, impacts of processing on viability and resistance to the TGI, as well as the paraprobiotic potential are also addressed and discussed.

2. Impact of Main Processing and Storage Conditions on the Viability of *Bacillus coagulans*

Spore-forming probiotic microorganisms have attracted researchers' attention as an alternative to increase the diversity of probiotic foods (Konuray & Erginkaya, 2020). The ability to form spores provides to these microorganisms a potential tolerance to adverse environmental conditions (Majeed et al., 2016). Among the microorganisms, *B. coagulans* has demonstrated a great potential in functionalizing food products (Figure 1). In the next items, the detailed analysis of the main conditions on the food supply chain that affect the resistance and viability of *B. coagulans* are highlighted.

Figure 1. Characteristics of the microorganism, possibilities of use and its advantages.



Source: Author's elaboration.

2.1 Heating/cooling

Studies have demonstrated the use of *B. coagulans* in different food matrices, submitted to different processing and storage temperatures. One of the main steps of food processing is heat treatment, which aims to guarantee the microbiological safety of the products. *B. coagulans* MTCC 5856 and *B. coagulans* GBI-30 6086 demonstrated excellent thermal stability when inoculated into milk and subjected to pasteurization at 65 °C for 30 min to produce creamy probiotic curd. *B. coagulans* GBI-30 6086 was the most resistant strain, and the spore count remained practically unchanged (Soares et al., 2019).

In addition, *B. coagulans* GBI-30 6086 did not impact the proteolysis of the curd and the fatty acid profile during storage at 6 °C for 45 days (Soares et al., 2019). This is an important observation regarding undesirable reactions, such as lipid oxidation and increased rancidity, which negatively affect the food quality. Therefore, the result indicates that the use of *B. coagulans* GBI-30 6086 can be a promising option in the production of creamy curd maintaining viability and with minimal changes in the sensory characteristics of the product.

The thermal resistance and viability of *B. coagulans* is a technological characteristic that was observed in other matrices. In the processing of Nabat candy, *Bacillus coagulans* Unique IS-2 spores were added to the candy coating. Under thermal stress during dissolution in hot liquid at 80 °C and drying of the coating at 40 °C, the survivability of the spores used in the coated sweets did not significantly decrease. The evaluation of the viability of probiotic in the product, after six months of storage at room temperature, revealed 90.39% viability (Adibpour et al., 2019). The spore-forming ability of *B. coagulans* helps it survive in products that are subjected to high temperatures, being a major factor in food processing, since one of the main barriers to the commercialization of probiotic foods is to maintain the viability of the microorganism when the product undergoes heat treatment.

It is important to note that the use of thermal shock, depending on its intensity, can cause a slight negative influence on the spore count of *B. coagulans* in food. For example, as with *B. coagulans* ATCC 31284 during the production of probiotic sausage. However, although the number of spores was significantly lower after the thermal shock at 80 °C for 10 minutes, the spore counts were still above the recommended minimum daily therapeutic dose (>10^6 CFU/g), which was maintained during storage for 45 days under refrigeration at 4 °C (Jafari et al., 2017).

The ability of spore-forming bacteria such as *B. coagulans* to withstand heat treatment and refrigerated storage propose their ability to be used in cooked foods, expanding the options of food matrices for formulating new products with probiotic properties.

Considering low temperatures, the survival of *B. coagulans* MTCC 5856 was demonstrated in apple juice, stored for 6 months at temperatures of 4 to 6 °C (Majeed et al., 2016). Under freezing conditions at temperatures of -20 °C, the viability of the microorganism was 92%, and 86% after 12 months of storage in muffins and waffles, respectively (Majeed et al., 2016). When added in chocolate syrup topping, hot syrup topping, peanut butter, strawberry jam and vegetable oil, the bacterium showed an improved viability (95%) for up to 12 months, after storage at room temperature (Majeed et al., 2016).

Therefore, the sporulation is a condition that gives greater resistance to the microorganism. This allows its use in different matrices and processing conditions, such as heat treatment, which is one of the main steps used in food preservation and represents one of the barriers for the development of probiotic products.

2.2 Acidification

In general, conventional probiotic microorganisms have ideal pH values ranging from 5.0 to 7.0. In *Bifidobacterium* species there are values between 6.0 and 7.0. On the other hand, species of the *Lactobacillus* genus are aciduric with an optimum pH between 5.5 and 6.2. This range is close to the optimal growth pH of *Bacillus coagulans* species, that present values between 5.5 and 6.5 (Konuray & Erginkaya, 2018; Macedo et al., 2008).

The growth of most spore-forming bacteria is inhibited at low pH, but there are some exceptions, such as *B. coagulans*, which can survive in acidic and thermally processed foods (Silva & Gibbs, 2004). For this reason, this bacterium is considered thermoacidophile. Therefore, in addition to its ability of surviving thermal conditions, its capability of growth under low-pH environment made *B. coagulans* a probiotic microorganism of interest to several researchers and the industry (Konuray & Erginkaya, 2018).

In juçara and passion fruit jelly candies, the ability of *B. coagulans* to survive under acid pH conditions (3.81) was verified, with counts greater than 6.4 log CFU/g over 90 days (Miranda et al., 2020). Furthermore, the presence of *B. coagulans* did not alter the acidity and the overall quality of the product. These results indicate the possibility of using *B. coagulans* in plant matrices (Miranda et al., 2020).

Numerous factors contribute to the resistance and viability of a microorganism during processing, such as pH, pressure, and temperature. A study evaluated the influence of these parameters on the germination and inactivation of *B. coagulans* spores in tomato sauce and concluded that under acidic conditions, high pressure treatment can induce germination of spores of this microorganism, indicating the acidophilic character of these bacteria. However, high pressure treatments conducted at a moderately high temperature (ca. 60 °C), can inactivate these spores (Vercammen et al., 2012).

Based on these studies, it is highlighted that the probiotic microorganisms used in food in the form of spores can present benefits when compared to non-sporulates, as they are less susceptible to acidic conditions during the processing and storage stages. However, it is necessary to evaluate all manufacturing conditions since the sum of its effects can reduce the resistance and viability of these microorganisms.

2.3 Drying

Another important factor that affects the probiotic viability during food processing/storage is the water activity (Aw) (Vesterlund et al., 2012). Dry foods are usually stored at room temperature and probiotic foods with low Aw should be stable at this temperature. Aw values between 0.5 and 0.6 still represent a challenge for the survival of probiotics. In this context, spores of the probiotic *Bacillus* spp. may be more suitable to survive in such food matrices (Marcial-Coba et al., 2019).

Research has shown that *B. coagulans* is little affected in products with intermediate Aw. Different formulations of dry date paste with *B. coagulans* BC4 were evaluated with Aw of 0.48 - 0.59. The count of viable cells and spores was not significantly affected by Aw after 45 days of storage at 25 °C. The spore and viable cells count between 7.85 - 8.22 and 7.90 - 8.28 log CFU/g, respectively (Marcial-Coba et al., 2019).

In Nabat candy with Aw of 0.62, spores of *B. coagulans* Unique IS-2 maintained viability of 90.39% after six months of storage at room temperature (Adibpour et al., 2019). Spores of *B. coagulans* MTCC 5856 added in glucose syrup concentrate with Aw around 0.7 showed 99% viability when stored for 24 months at 4 °C (Majeed et al., 2016). Capsules containing 10^9 spores of *B. coagulans* GBI-30 6086 were added in gummy candies. After 90 days of storage at 25 ° C, the Aw of the candies was around 0.71, and the *B. coagulans* GBI-30 6086 count was greater than 6.4 log CFU/g (Miranda et al., 2020).

Thus, it is observed that *B. coagulans*, mainly in the form of spores, can survive under conditions of intermediate Aw. Spores have advantages over vegetative cells, due to their ability to resist adverse conditions, maintaining their probiotic potential. Therefore, this microorganism can be added in several food matrices, with intermediate water activity, increasing the availability and diversity of probiotic carrier products.

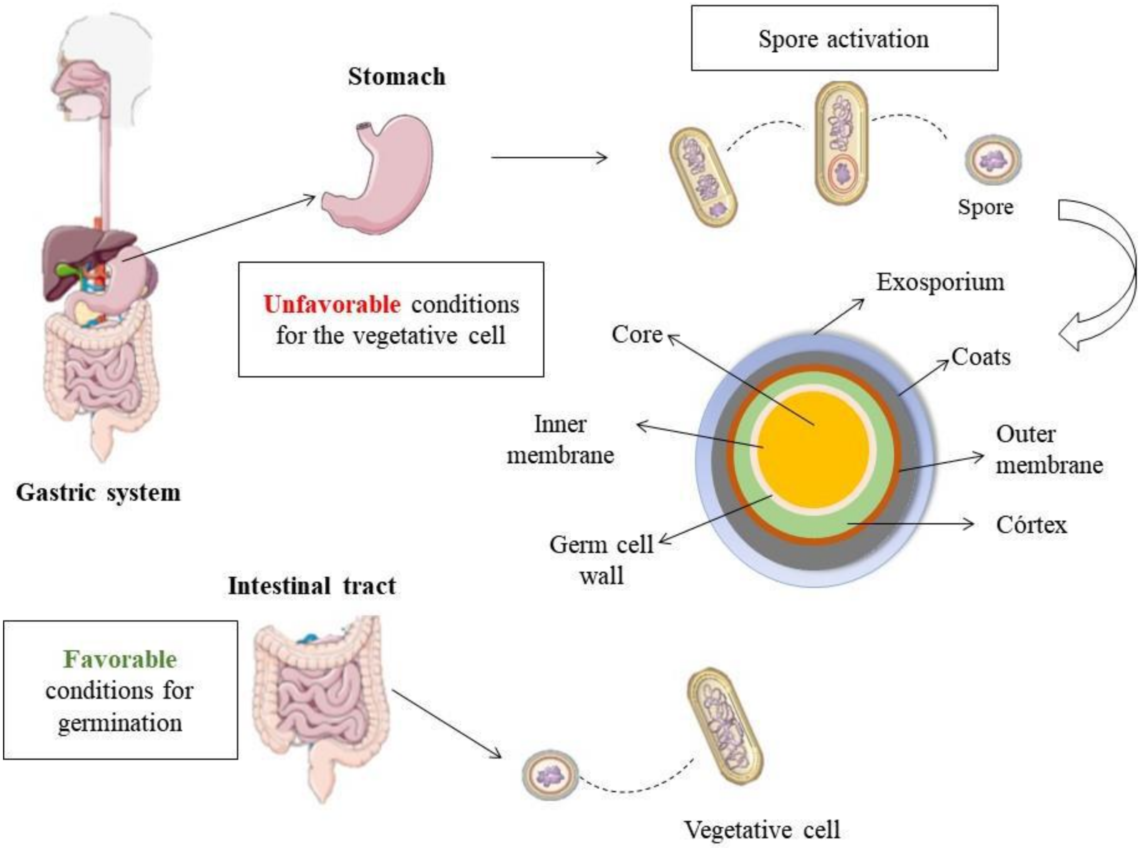
3. Resistance of *Bacillus coagulans* to Gastrointestinal Tract Conditions

The study of the resistance of probiotic microorganisms during passage through the gastrointestinal tract (TGI) is essential to ensure that viable cells reach the end of the passage in sufficient quantity to promote benefits to the host.

Several studies evaluating *B. coagulans* resistance to TGI have been carried out. Among them, the tolerance of *B. coagulans* T242 to TGI simulating exposure to gastric juice and bile salt was evaluated at 37 ° C for 3 hours. The results of exposure to gastric juice and bile salt indicated a reduction of 13% and 52% in viability after a period of 3 hours, respectively (Sui *et al.*, 2020). Based on these results (count reduction less than one log cycle), *B. coagulans* T242 can be considered suitable for use as a probiotic, since resists adverse conditions like acidic pH and bile salt.

Most conventional probiotics, such as non-sporulating bacteria and yeasts, are more sensitive to gastric acidity and bile. This is due to the existence of an external layer in the spore structure, the exosporium, which consists of proteins, lipids and carbohydrates that provide hydrophobic characteristics to the spore and make it insoluble. The low permeability of the inner membrane prevents hydrophobic and hydrophilic molecules from entering the nucleus. The intrinsic and unique composition of the bacterial spores provides high survival in viable amounts, necessary to promote health benefits to the host (Bernardeau *et al.*, 2017). The survival strategy of *Bacillus coagulans*, including its spores' structure, against the TGI adversities can be observed at Figure 2. Spores formed by lactic acid bacteria have the advantage over vegetative probiotic strains due to their ability to withstand various industrial processes and more severe gastrointestinal conditions (Soccol et al., 2010; Gu et al., 2015).

Figure 2. Adversities of intrinsic factors such as pH and the survival strategy of *Bacillus coagulans*.



Source: Author's elaboration.

Analyzing the behavior of *B. coagulans* spores inoculated in fruit jelly candies during the *in vitro* passage through the TGI, it was found that there was no reduction in microbial viability (> 6.57 Log CFU/g) after the gastric and enteric phases (Miranda et al., 2020). In other studies, there was an increase in the count of *B. coagulans* throughout the digestive phases (Ahire, Neelamraju, & Madempudi, 2020; Miranda et al., 2020). Spore germination in the first part of the small intestine is advantageous for reproduction due to low microbial competition and high nutrient concentration (Bernardeau et al., 2017). It is estimated that about 8% of the germinating spores are sufficient to influence the colon microbiota and provide the probiotic effects to the host (Hatanaka et al., 2012).

The high viability of *B. coagulans* at the end of TGI indicates its promising use in food matrices as a probiotic microorganism. Adverse conditions of pH, osmotic concentration and temperature did not prevent the survival of this microorganism, which allows its use in foods with different restrictions.

4. *Bacillus coagulans* as a Paraprobiotic

The usual concept for probiotics highlights the need to maintain viable cells to ensure the desired beneficial effects on host health. However, recent reports show that bacterial viability is not fundamental to confer these probiotic benefits, given the identified evidence that bacterial fragments have equivalent functionalities, even though cell metabolism is inactivated. Based on this, the term "paraprobiotic" emerged, conceptualized as "non-viable bacterial cells or fragments of microorganisms, probiotics or not, that have biological activity in the host (Taverniti & Guglielmetti, 2011; de Almada et al., 2016).

Paraprobiotics are derived from inactivated microorganisms that have completely lost their viability after exposure to factors that alter microbial cellular structures, such as breaking DNA filaments, cell membrane rupture, or inactivation of metabolic activity. In addition, this includes loss of viability due to changes in microbial physiological functions, such as inactivation of key enzymes or deactivation of membrane selectivity (De Almada et al., 2016).

Jensen et al. (2010) investigated the differential effects of the isolated components of *Bacillus coagulans* GBC30 fragments compared to bacterial metabolites. The authors found that the contents of the cell wall provide anti-inflammatory effects, while supporting key aspects of immune defense mechanisms. This demonstrates that bacterial viability is not a requirement to achieve all probiotic effects, since some clinical benefits are related to non-viable bacteria (Cuevas-González et al., 2020). Thus, *Bacillus coagulans* have been investigated for its potential use as paraprobiotic and has presented properties that stimulate its use in several food matrices.

5. Conclusion

Studies in the literature have helped to understand the efficiency of *Bacillus coagulans* as a probiotic microorganism. From the review carried out, the resistance of these microorganisms to various conditions of food processing is highlighted, as well as its high resistance to the gastrointestinal tract. Thus, this microorganism has been the focus of recent studies due to its great potential for use in different probiotic food matrices. Finally, to confirm the functionality of this microorganism as a paraprobiotic, further research is needed to expand the range of information.

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