Journal of Functional Foods 64 (2020) 103643

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Probiotic characteristics of *Bacillus coagulans* and associated implications for human health and diseases

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ARTICLE INFO

Keywords:
Bacillus coagulans
Digestion
Metabolism
Microbiota
Immune system
Human diseases

ABSTRACT

As a spore-forming probiotic bacterium, *Bacillus coagulans* has become a focus of research due to its high tolerance of extreme environments and probiotic characteristics. Several beneficial effects of *B. coagulans* have been reported. Firstly, *B. coagulans* can promote intestinal digestion. For example, *B. coagulans* strains can produce various enzymes that facilitate excretion and digestion. Secondly, *B. coagulans* can regulate host symbiotic microbiota and inhibit the growth of pathogenic bacteria. Lastly, due to its ability to normalize both the quantitative parameters of the immune system and immune cells' functional activity, *B. coagulans* can significantly benefit the host immune system. Due to the evidence supporting various probiotic effects of *B. coagulans*, many *B. coagulans strains* have been studied in the management and alleviation of several human diseases. Therefore, the administration of *B. coagulans* may be an attractive preventive and/or therapeutic approach for human diseases.

1. Introduction

Probiotics are defined by the World Health Organization as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2001). In recent years, the role that probiotics play in human health and diseases has attracted the attention of researchers. This may be due, in part, to the excellent performance of probiotics in preventing and alleviating diseases, as well as the public's increasing desire for natural therapies. At present, studies on probiotic microorganisms have mainly focused on the treatment of gastrointestinal (GI) conditions, with some traditional probiotics, such as *Lactobacillus* spp., *Bifidobacterium* spp., *Propionibacterium* spp., *Streptococcus* spp. as well as some *Saccharomyces* species (Duc, Hong, Barbosa, Henriques, & Cutting, 2004; Islam, 2016). However, these microorganisms mentioned above can hardly survive in an extremely harsh environment (Keller, Verbruggen, Cash, Farmer, & Venema, 2019). For example, traditional probiotics, such as *Lactobacillus* and *Bifidbacterium* species, show outstanding probiotic activities, but their survival is generally low, in the order of 1–15%, with some strains performing even more poorly (Keller et al., 2019; Ruiz et al., 2011). Therefore, spore-forming probiotic microorganisms have attracted the interest of researchers. Some non-pathogenic *Bacillus*

Abbreviations: B. coagulans, *Bacillus coagulans*; *B. subtilis*, *Bacillus subtilis*; *C. difficile*, *Clostridium difficile*; BLIS, Bacteriocin-like inhibitory substance; LAB, Lactic acid bacteria; GI, Gastrointestinal; GIT, Gastrointestinal tract; FDA, Food and Drug Administration; EFSA, European Union Food Safety Authority; SCFAs, Short-chain fatty acids; TLRs, Toll-like receptors; ROS, Reactive oxygen species; PMN, Polymorphonuclear; PBMCs, Peripheral blood mononuclear cells; NKT, Natural killer T; NK, Natural killer; Th1, T Helper 1; Th2, T Helper 2; IL, Interleukin; IFN-gamma, Interferon-gamma; TNF, Tumor necrosis factor; BV, Bacterial vaginosis; SIBO, Small intestinal bacterial overgrowth; IBD, Inflammatory bowel disease; IBS, Irritable bowel syndrome; AAD, Antibiotic-associated diarrhea; HIV, Human immunodeficiency virus; RA, Rheumatoid arthritis; MDD, Major depressive disorder; NEC, Necrotizing enterocolitis; FAP, Functional abdominal pain

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https://doi.org/10.1016/j.jff.2019.103643
Received 23 July 2019; Received in revised form 2 October 2019; Accepted 17 October 2019
Available online 29 October 2019

species, which are not as well-known as lactic acid bacteria (LAB) and yeasts, are being used as probiotics (Hyronimus, Marrec, Sassi, & Deschamps, 2000). The survival and stability of these bacteria are considerably better when compared to other probiotics as a result of their spore-forming abilities. Thus, it is an ideal choice to develop some spore-forming probiotics for functional food (Fares et al., 2015).

Among the probiotic *Bacillus* species, *B. coagulans* has been studied for a long time. It was firstly isolated from spoiled canned milk by Hammer in 1915 (Weerkamp, 1991). As a kind of lactic acid-producing bacteria, some *B. coagulans* strains were mislabeled as *Lactobacillus sporogenes* (Dutta et al., 2011; Shirodkar, Sankholkar, Ghosh, & Nulkar, 1980). Subsequent taxonomic study demonstrated that this specie should be classified within the genus *Bacillus* (Drago & Vecchi, 2009). *B. coagulans* is lactic acid-producing, spore-forming, catalase positive and facultative anaerobic bacterium (Özüsağlam & Aksaray, 2010). Its optimum growth temperature is 35–50 °C and its optimum pH is close to 6. During the growth process, *B. coagulans* can decompose glucose, sucrose, maltose, and mannitol to produce L-lactic acid. Biotin and thiamine are major growth factors that promote the growth of *B. coagul*ans (Sneath, Mair, Sharpe, & Holt, 1986; Weerkamp, 1991). Some studies have shown that *B. coagulans* differs from other *Bacillus* probiotics in several physiological characteristics, including microscopic observations, growth conditions, biochemical reactions and carbon sources utilizations (Clerck et al., 2004; Konuray & Erginkaya, 2018). For instance, the compostion of *B. coagulans* cell wall is unique since the teichoic acid from *B. coagulans* cell has a higher lipid content (12.6%) than many Gram-positive bacteria (1–2%). Moreover, compared with other *Bacillus* probiotics, the teichoic acid from *B. coagulans* lacks amino acid substituents (Forrester & Wicken, 1966). In the past, researchers have found *B. coagulans* can be applied in livestock, aquaculture and human health (Gu et al., 2015; Hung et al., 2012). Animal and preclinical studies of *B. coagulans* have mainly focused on the prevention and treatment of gastrointestinal tract (GIT) disorders, such as irritable bowel syndrome (IBS), antibiotic-associated diarrhea, inflammatory bowel disease and colorectal cancer (Dolin, 2009; Sudha, Jayanthi, Aasin, Dhanashri, & Anirudh, 2018). Notably, among these disorders, *B. coagulans* has frequently been reported as an effective treatment for IBS. In addition to its application in GIT diseases, *B. coagulans* has also been used to treat and/or prevent non-GI medical conditions including bacterial vaginosis, obesity, and major depression induced by IBS (Sudha, Ratna, Yelikar, & Sonali, 2012). Compared with traditional commercial probiotics, *B. coagulans* is more likely to exert its probiotic role in the intestinal tract than traditional live probiotics due to its ability to produce spores. *B. coagulans* can survive from the stomach in the form of spores and germinate in the intestine, thereby exerting its probiotic effect. However, several studies have demonstrated the poor ability of *B. coagulans* to colonize in the intestinal tract of mammals (Casula & Cutting, 2002; Maathuis, Keller, & Farmer, 2010). It seems that *B. coagulans* can only affect the intestinal microbiota through temporary proliferation in the human intestine (Abhari, Shekarforoush, Sajedianfard, Hosseinzadeh, & Nazifi, 2015).

B. coagulans has been granted Generally Recognised As Safe (GRAS) status by the US Food and Drug Administration (FDA). A study has assessed its safety *in vivo*, and a dose as high as 9.52×10^{11} CFUs was shown to be well tolerated and safe for a 70 kg human (Endres et al., 2009). In addition, the genomic analysis of *B. coagulans* suggested that the antibiotic resistance related genes in this species were not easily transferrable to other bacteria, and no other genes with potential safety risks were retrieved (Salvetti et al., 2016). Some researches have reported fermentation characteristics of *B. coagulans in vitro*, indicating *B. coagulans* strains, such as *B. coagulans* MTCC 5855, *B. coagulans* PTA-6086 and *B. coagulans* 15B, could be applied in food industry (Juturu & Wu, 2016; Konuray & Erginkaya, 2018). At present, there are many kinds of *B. coagulans* containing functional foods on the market, such as pasta, chocolate and ice cream. The main reason is that compared to other probiotic bacterial strains, the spore-forming nature of *B. coagulans* guarantees its stability and vitality in functional food (Majeed, Majeed, et al., 2016). For instance, the spores of *B. coagulans* provide a resistance to high temperature food processing such as baking and boiling. This makes *B. coagulans* an ideal choice for the development of functional cereal-based products (Fares et al., 2015; Konuray & Erginkaya, 2018). On the other hand, a previous study indicated that the addition of *B. coagulans* did not exhibit an adverse effect on the sensory and nutritional characteristics of the products (Kobus-Cisowska et al., 2019).

To the best of our knowledge, there were only a limited number of reviews described the probiotic effects of *B. coagulans* on human health. A study had summarized the probiotic characteristics of *B. coagulans in vitro*, including the resistance to acid and bile salt, adhesion properties, and antimicrobial abilities (Drago & Vecchi, 2009). Jäger, Purpura, Farmer, Cash, and Keller (2017) reported the ability of *B. coagulans* PTA-6086 to aid protein absorption and utilization in animals and humans (Jäger et al., 2017). Therefore, in this review, we focused on the beneficial functions of *B. coagulans* in human health and disease prevention.

2. The gastrointestinal life cycle of *B. coagulans* in humans

2.1. Endospores: A unique form of B. coagulans that can survive in the GIT

Previous studies have indicated that dead microorganisms and cell fragments can generate beneficial biological responses (Adams, 2010). However, the important role of live probiotics in the GIT is widely recognized. Therefore, increasing gastrointestinal survival of probiotics has long been an intense focus of research. For example, a novel *B. coagulans* spore-based oral carrier loading curcumin has been developed for colon cancer treatment (Yin et al., 2017). The ability to generate spores is a unique feature of *Bacillus* probiotics, and improves their survival rate in the harsh environment of the GIT (Bressuire-Isoard, Broussolle, & Carlin, 2018). *B. coagulans* has attracted considerable attention due to its ability to produce spores. Researchers have elucidated the mechanism of formation and structure of the spores of *B. coagulans* and demonstrated that it sporulates in harsh conditions (e.g. nutrient limitation) and due to quorum sensing signals through a complex developmental process (Bressuire-Isoard et al., 2018). After the sporulation process, the structure of the spores of *B. coagulans* is distinctly different from the vegetative cells. Mature *B. coagulans* spores are composed of a core, spore membrane, cortex and spore coat. In contrast to *Bacillus cereus* or *Clostridium sporogenes*, at no stage in the development of the spore can a membrane with the loose flexible characteristics of the exosporium be observed (Ohye & Murrell, 1962).

Spores of *Bacillus* strains can survive for years in their dormant state, but if given the proper stimulus, they can rapidly germinate (Setlow, 2014). Similar to other *Bacillus* strains, the germination of *B. coagulans* can be triggered by factors such as nutrients, non-nutrient agents and physical treatments (Madan et al., 2002; Setlow, Cowan, & Setlow, 2010). For example, in the case of *B. subtilis*, some amino acids (L-alanine, L-valine and L-asparagine) showed the ability to trigger the germination of its spores (Atluri, Ragkousi, Cortezzo, & Setlow, 2006; Gould & Hurst, 1969). In addition, specific amino acid changes in GR subunits can alter either the specificity or concentration dependence of a GR's response to a nutrient (Christie, Götzke, & Lowe, 2010; Mongkolthanaruk, Cooper, Mawer, Allan, & Moir, 2011). Therefore, as a digestive organ where it is filled with nutrients including sugar, purine nucleosides and amino acids, the small intestine is probably the main site for the germination of *B. coagulans* spores in the human body.

2.2. The life cycle of B. coagulans in humans

Animal studies *in vivo* have demonstrated that *Bacillus* spores can germinate and proliferate in the GIT of mice. For instance, the abundance of spores of *B. subtilis* excreted in the feces of mice was six times

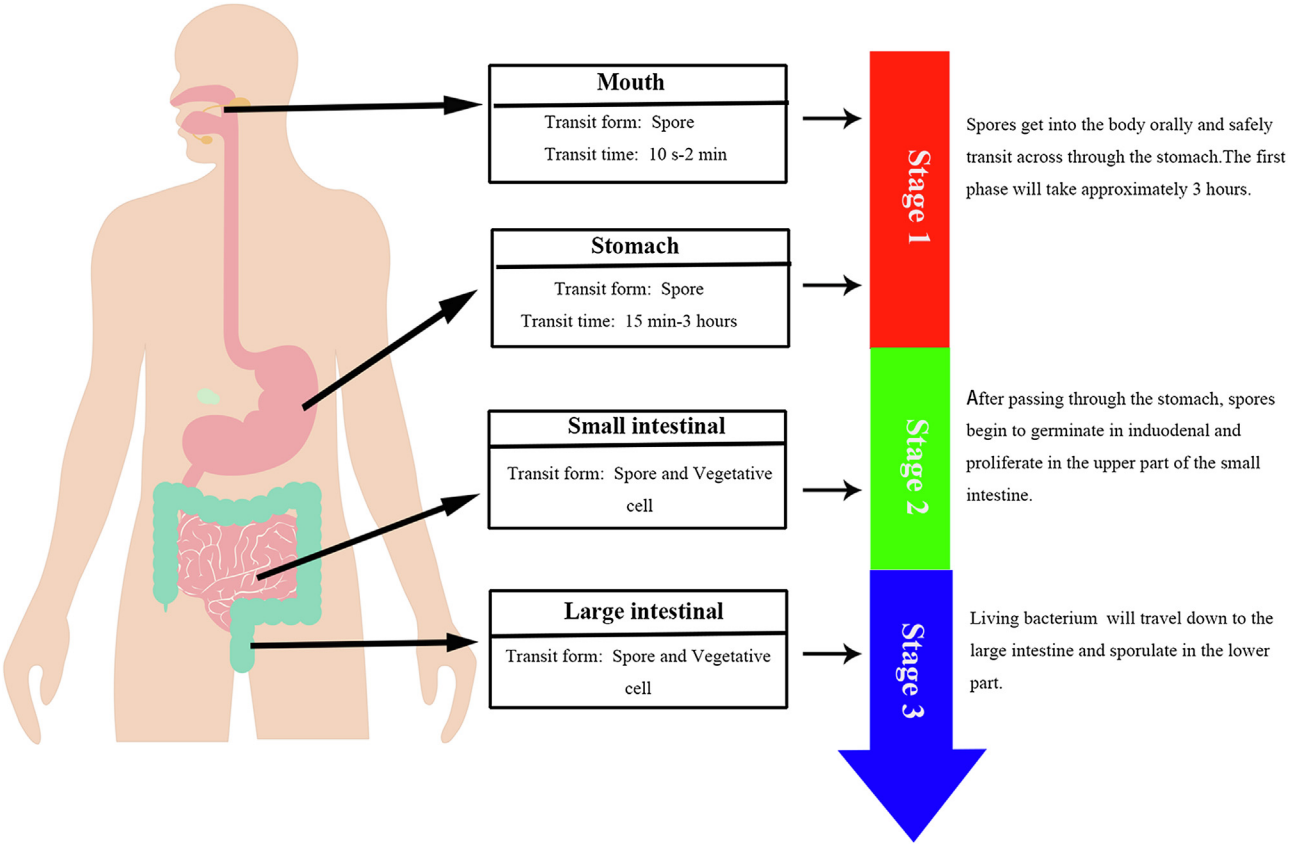

Fig. 1. Life cycle of *B. coagulans* in the human digestive tract.

higher than in the original inoculum, indicating that spores can germinate, grow and then resporulate in the GIT (Casula & Cutting, 2002; Hoa et al., 2001). In *in vitro* models, although species of *Bacillus*, including *B. coagulans*, *B. subtilis* and *B. cereus,* showed a similar life cycle in artificial GIT models, the germination ratios of spores varied (Duc et al., 2004; Hatanaka et al., 2012; Keller et al., 2019; Maathuis et al., 2010). The germination ratio of spores of *B. coagulans* PTA-6086 (93%) in the GIT model was much higher than that of *B. subtilis* C-3102 (8%) (Hatanaka et al., 2012; Keller et al., 2019). Differences in the strain and models studied may be the main reasons for the huge differences in these results.

Based on the previous studies, in this review, we have constructed a life cycle of spore germinationin in humans that probably suitable for most *B. coagulans* strains (Fig. 1) (Bernardeau, Lehtinen, Forssten, & Nurminen, 2017; Keller et al., 2019). In stage 1, spores enter the body orally and safely transit to the stomach. Mastication is the first step of this stage and has an important influence on the overall digestive process, particularly on the gastric emptying rate (Woda et al., 2010). Following mechanical and enzymatic degradation in the mouth, spores are transported through the esophagus to the stomach by peristalsis (Guerra et al., 2012). The presence of gastric juices and bile in the stomach make germination of the *B. coagulans* spore difficult (Fakhry, Sorrentini, Ricca, De Felice, & Baccigalupi, 2010; Minekus, Marteau, & Havenaar, 1995; Spinosa et al., 2000). In an adult, the first phase will take approximately three hours. In stage 2, after passing through the stomach, the spores begin to germinate in the duodenum and proliferate in the upper part of the small intestine (Casula & Cutting, 2002). A nutrient-rich environment with low microbial stress is a major factor in spore germination. Usually, the residence time of *B. coagulans* in the small intestine ranges from 2 to 5 h. Lastly, in stage 3, live *B. coagulans* will travel down to the large intestine and sporulate in the lower part of the colon (Tam et al., 2006). The chance of germination in the large intestine is very low due to the nutrient-deficient environment and the increased sensitivity of vegetative cells in the exponential phase (Bernardeau et al., 2017; Tam et al., 2006).

3. The beneficial effects of *B. coagulans* on host health

3.1. Effects of *B. coagulans* on host digestive system and body metabolism

As noted above, spores of *B. coagulans* can germinate in the upper part of the small intestine. In co-operation with gut microbiota, *B. coagulans* has been shown to increase gut nutrient absorption and availability to aid digestion (Maathuis et al., 2010). *B. coagulans* is capable of improving the effective utilization of consumed foods, mainly due to its ability to produce a variety of enzymes (Table 1). For example, *B. coagulans* RCS3 can secrete β-galactosidase during growth, which can degrade lactose, found in milk, into glucose and galactose, improving the digestibility of milk and effectively alleviating lactose intolerance (Batra, Singh, Banerjee, Patnaik, & Sobti, 2011). Studies *in vitro* and *in vivo* have also found that *B. coagulans* PTA-6086 is capable of promoting the digestion of protein and carbohydrates (Keller et al.,

Table 1
Enzymes produced by *B. coagulans*.

Strain	Product	Citation
RCS3	β-galactosidase	(Batra et al., 2011)
KM-1	α-galactosidase	(Nam, Jang, Park, & Koneva, 2014)
BL174	α-galactosidase	(Parkouda, Diawaraa, & Ehima, 2014)
B49	α-amylase	(Babu & Satyanarayana, 1995)
–	Lipase	(Alkan, Baysal, Uyar, & Dogru, 2007)
BL174	Lipase	(Parkouda et al., 2014)
ZJU318	Lipase	(Lianghua & Liming, 2005)
VKl1	Lipase	(Gowthami, Muthukumar, & Velan, 2015)
–	Alkaline proteases	(Asokan & Jayanthi, 2010)
PTA-6086	Alkaline proteases	(Keller, Dinter, Cash, Farmer, & Venema, 2017)

2019; Maathuis et al., 2010). This *B. coagulans* strain has been demonstrated to produce various enzymes to degrade proteins and carbohydrates into smaller peptide molecules and free amino acids, thereby promoting metabolism in the upper part of the small intestine, improving the intestinal environment of the colon, and reducing toxic metabolites (Gibson, 2009; Keller et al., 2019; Maathuis et al., 2010). Digestive enzymes produced by *B. coagulans* were known to be active in the gut, and its proteases was found to regulate amino acid metabolism elsewhere in the body (Minevich et al., 2015). For example, a study has reported that consumption of *B. coagulans* PTA-6086 could increase absorption of specific amino acids to athletes, such as branched-chain amino acids (leucine, isoleucine, valine) and amino acids involved in blood flow regulation (citrulline) or recovery (glutamine) (Jäger et al., 2017).

In addition to the production of digestive enzymes, *B. coagulans* can also produce metabolites such as diacetyl, short-chain fatty acids (SCFAs) and vitamins. *B. coagulans* can also stimulate intestinal peristalsis, reduce the production of harmful substances such as amines and improve the intestinal metabolic environment, thereby promoting healthy bowel movements and avoiding accumulation of toxins in the body (Nyangale, Farmer, Keller, Chernoff, & Gibson, 2014). A study on the effects of *B. coagulans* lilac-01 on intestinal movements in human patients showed that the administration of *B. coagulans* could effectively relieve constipation, ameliorate the sensation of fecal retention and improve the intestinal environment compared with the control group (Kimiko, Mie, Kazunori, & Jun, 2015). *B. coagulans* SANK 70258 has also been demonstrated to improve bowel movement frequency, improve stool shape and color, reduce intestinal ammonia, reduce sputum and p-cresol and improve fecal odor (Ara et al., 2002).

Furthermore, a study has shown administration of *B. coagulans* PTA-6086 can significantly enhance the health of the cells of the gut lining by decreasing inflammation, thereby improving nutrient absorption through optimum development of the absorptive area of the villi (Kimmel, Keller, Farmer, & Warrino, 2010). Moreover, *B. coagulans* can also mediate digestion and metabolism in humans through other mechanisms. For example, in an animal model, *B. coagulans* was found to reduce cholesterol levels through degrading cholesterol in blood serum (Aminlari et al., 2018). Additionally, a combination of soya pulp and *B. coagulans* lilac-01 successfully suppressed the increased production of secondary bile acid in cholic acid-fed rats compared with soya pulp alone, thereby improving intestinal bile acid metabolism (Lee et al., 2016).

3.2. Effects of B. coagulans on host microbiota regulation

Emerging evidence suggests that the host microbiota playing an important role in maintaining human health (David et al., 2014; Rampelli et al., 2016). Many diseases have been associated with an imbalance of the microbiota, whereas restoration of the microbiota has been demonstrated to maintain health and treat disease (Scott, Jean-Michel, Midtvedt, & Hemert, 2015). *B. coagulans* is able to influence the diversity, composition and metabolic function of the intestinal and vaginal microbiota.

3.2.1. Effects of B. coagulans on gut microbiota

According to the results of recent studies, *B. coagulans* has been reported to have a good regulatory effect on dysbacteriosis caused by various factors. For instance, one study has assessed the regulation effect of *B. coagulans* SANK 70258 on enteric dysbacteriosis caused by a high-protein diet. The results indicated that this strain of *B. coagulans* was able to ameliorate the effects of a persistently aggravated intestinal environment while remodeling the gut microbiota in both humans and rats (Ara et al., 2002). Increasing age can also lead to disturbances in the intestinal flora. In aging populations (> 65 years old), the number of bifidobacteria and *Firmicutes* decreases in the gut, while the number of *Proteobacteria* and several opportunistic pathogens increases (Duncan & Flint, 2013). In the elderly, *B. coagulans* PTA-6086 administered with prebiotics significantly increased populations of microbial groups known to elicit some beneficial effects. Meanwhile, the concentrations of butyrate, acetate and propionate also increased. Showing that the increase in probiotic populations and subsequent prebiotic supplementation may pose another route for improving gut health of older (Nyangale et al., 2014, 2015). Furthermore, antibiotic abuse appeared to play an important role in the pathogenesis of several disorders associated with microbiota impairment, such as *Clostridium difficile* infection or metabolic disorders (Ianiro, Tilg, & Gasbarrini, 2016). *B. coagulans* has been found to be capable of preventing the microbial imbalance and related diseases caused by the abuse of antibiotics (Rosa et al., 2003; Susanne et al., 2012). Consumption of *B. coagulans* was able to increase populations of specific probiotic microorganisms including *Lactobacillus* and *Bifidobacteria* in the gut (Ara et al., 2002). On the other hand, *B. coagulans* can competitively outcompete several opportunistic pathogens, such as vancomycin-resistant enterococci and *Escherichia coli*, thereby maintaining homeostasis with the intestinal microbiota (Donskey et al., 2001; Lopamudra & Gandhi, 2016).

With research ongoing into the relationship between gut microbiota and *B. coagulans*, our understanding of the mechanisms by which *B. coagulans* promotes a healthy gut microbiome is constantly changing. In this review, several basic mechanisms are proposed to explain the beneficial effects of *B. coagulans* on the gut microbiota.

Firstly, *B. coagulans* can help create an anaerobic and acidic intestinal environment, which is not hospitable to various pathogens, thereby promoting the growth of some probiotics. As facultative anaerobic bacteria, *B. coagulans* strains can consume free oxygen in the intestine and stomach and reduce redox reactions. This has been proved to be beneficial to the growth of anaerobic microorganisms such as *Lactobacillus* and *Bifidobacterium* (Abhari et al., 2015; Kodali & Sen, 2008).

Secondly, therapeutic benefits of *B. coagulans* may be related to its ability to produce antimicrobial compounds, thereby hindering pathogenic bacterial growth and balancing microbiota populations (Honda, Gibson, Farmer, Keller, & Mccartney, 2011). Among these compounds, few studies have been reported that some *B. coagulans* strains can secrete bacteriocin, a well-known antibacterial substance (Abada, 2008; Hyronimus, Le, & Urdaci, 1998). *B. coagulans* I_4 was the first strain of *B. coagulans* found to produce a bacteriocin-like inhibitory substance (BLIS) named coagulin (Hyronimus et al., 1998). Further studies found that the bacteriocin produced by *B. coagulans* I_4 was a plasmid-linked antimicrobial peptide, consisting of 44 residues. This peptide has an amino acid sequence similar to pediocins produced by different *Pediococcus acidilactici* strains. Coagulin and pediocin differ only by a single amino acid at their C terminus (Marrec, Hyronimus, Bressollier, Verneuil, & Urdaci, 2000). As an anionic antibacterial substance, the bacteriocin produced by *B. coagulans* is a broad spectrum antibacterial agent against Gram-positive bacteria, which are implicated in transmission of foodborne disease (Abdhul et al., 2015; Fu et al., 2018). Moreover, it has also shown antifungal effects on *Botrytis cinerea, Fusarium pallidoroseum*, and *Fusarium moniliforme* (De Senna & Lathrop, 2017). The mechanism of action of bacteriocins relates to their ability to penetrate the surface of pathogenic bacteria, causing amino acids and inorganic salts to leak from the cells, thereby inhibiting growth (Riazi, Dover, & Chikindas, 2012; Riazi, Wirawan, Badmaev, & Chikindas, 2009). In addition to bacteriocins, *B. coagulans* can also secrete other antimicrobial substances such as lactic acid and acetic acid. Lactic acid is an important antimicrobial substance produced by *B. coagulans* in the human gut. Many *in vitro* studies have focused on the lactic acid fermentation characteristics of *B. coagulans*. Their results demonstrate that *B. coagulans* can utilize various carbon sources to produce lactic acid in anaerobic conditions at temperatures under 50 °C, indicating the possibility that *B. coagulans* can produce lactic acid and its derivatives in the human GIT (Glaser & Venus, 2018; Jiang et al., 2016). In addition to its antimicrobial properties, lactic acid and its

derivatives have also been shown to accelerate intestinal stem cell-mediated epithelial development in rats (Lee et al., 2018). Therefore, considering the high yield of lactic acid, *B. coagulans* may possess the ability to promote gut epithelial development and repair (Ou et al., 2016; Pol, Eggink, & Weusthuis, 2016; Pol, Springer, Vriesendorp, Weusthuis, & Eggink, 2016).

3.2.2. Effects of B. coagulans on vaginal microbiota

The vaginal microbiome is dominated by *Lactobacillus* in healthy women (Onderdonk, Delaney, & Fichorova, 2016). However, some pathogenic strains, such as *Gardnerella*, *Mobiluncus* and *Atopobium* can alter the balance of the vaginal flora, leading to bacterial vaginosis (BV). A study has found that *B. coagulans* can alleviate the symptoms of non-specific vaginitis (Shirodkar et al., 1980). A similar observation suggested that *B. coagulans* PTA-11748 might be an effective adjunct to antimicrobial treatment, improving recovery. Following antibiotic treatment, 80% of the probiotic-treated subjects showed a significant reduction in vaginosis symptoms compared to the control group, in which only 45% subjects exhibited a reduction in symptoms. Interesting, a study reported that lactosporin, an antimicrobial protein produced by *B. coagulans* ATCC 7050 potentially exerts its antimicrobial activity by selective dissipation of ΔpH and/or by causing leakage of ions from the *Micrococcus luteus* sensitive cells (Riazi et al., 2012).

3.3. Effects of B. coagulans on host immune system

A growing body of evidence suggests that probiotics play an important role in maintaining human immunologic homeostasis. In recent years, *B. coagulans* has attracted great interest in this field due to its strong modulatory effects on host microbiota and immune responses with almost no safety concerns (Table 2). One study has revealed that *B. coagulans* is able to normalize both the quantitative parameters of the immune system (the number of splenic lymphocytes, macrophages and T-lymphocytes) and the cells' functional activity to promote host immune system function (Bomko, Nosalskaya, Kabluchko, Lisnyak, & Martynov, 2017). However, the exact relationship between *B. coagulans* and the human immune system requires further elucidation.

One aspect of immunological research associated with *B. coagulans* is determining the mechanism by which *B. coagulans* activates immune cells. Several reports have documented the ability of probiotics to influence host cells in several ways, including via specific bacterial cell wall components and interactions of secreted metabolites with Toll-like receptors (TLRs), thereby inducing innate immune defense mechanisms (Delcenserie, Martel, Amiot, Boutin, & Roy, 2008; Neish, 2009). Some studies had a investigation on *B. coagulans in vitro*. The results indicated that both cell wall components and metabolites from *B. coagulans* PTA-6086 could increase the activity of various kinds of human immune cells, including inhibition of reactive oxygen species (ROS) formation induced by polymorphonuclear (PMN) cells, increasing phagocytic activity of PMN cells and maturation of mononuclear phagocytes toward macrophage and dendritic cell phenotypes. Furthermore, the induction of the CD69 early activation marker on $CD3^+$ $CD56^-$ T lymphocytes, $CD3^+$ $CD56^+$ natural killer T (NKT) cells, $CD3^-$ $CD56^+$ natural killer (NK) cells and some cells within a $CD3^-CD56^-$ non-T non-NK cell subset were also reported (Jensen, Benson, Carter, & Endres, 2010; Jensen, Cash, Farmer, & Keller, 2017; Kathleen, Kimberlee, Steve, David, & Sean, 2012). The direct regulation of immune cells by *B. coagulans* is an immediate way to maintain immunologic homeostasis. However, considering the highly strain-dependent effects of *B. coagulans*, the universality of this mechanism requires further investigation (Clerck et al., 2004).

Following activation of host immune cells, the consumption of *B. coagulans* can also strengthen the host immune system and relieve inflammation through cell-derived mediators. *B. coagulans* can alter the production of immune activating, anti-inflammatory cytokines, chemokines and growth factors. As shown in Table 2, certain strains of *B. coagulans* can act as an adjuvant in the treatment of inflammatory diseases, the underlying mechanism of which is thought to be through Th1\Th2 cell mediated immune responses. For example, in some cases (Table 2), the consumption of *B. coagulans* can significantly down-regulate inflammatory immune factors, such as interferon-gamma (IFN-gamma), interleukin (IL) and tumor necrosis factor (TNF), without altering normal regulatory cytokines. In parallel to the increases in immune-activating and pro-inflammatory cytokines, robust increases in anti-inflammatory cytokines are also triggered by some strains of *B. coagulans*.

4. *B. coagulans* attenuates human diseases

4.1. Gastrointestinal diseases

Probiotic therapy offers a promising route for treating various gastrointestinal diseases such as diarrhea, indigestion, nutrient malabsorption, small intestinal bacterial overgrowth (SIBO), IBS, inflammatory bowel disease (IBD), pouchitis, ulcerative colitis and Crohn's disease, without a risk of spreading antibiotic resistance (Gibson, Pesesky, & Gautam, 2014). Many studies have reported that some strains of *B. coagulans* are effective in the treatment of various GI disorders (Table 3). *B. coagulans* has been widely used in the treatment of IBS (Table 3). IBS is a chronic functional gastrointestinal disorder characterized by intestinal symptoms including recurrent abdominal pain or discomfort and a change in bowel habits (Müller, 2006). As an intestinal disorder whose mechanism is not yet clear, common IBS symptoms comprise a variety of phenotypes, including abdominal pain, flatulence and bloating (Houghton et al., 2006). Although the mechanism of action is still unknown, several clinical trials have showed that the admistraion of *B. coagulans* can improve the quality of life of patients who suffered from IBS. Participants in these clinical trials have predominantly been adults and the elderly. Only one study showed that *B. coagulans* PTA-11748 could ameliorate IBS symptoms in children.

Besides, as shown in Table 3, some clinical trials have showed the therapeutic potential of *B. coagulans* in alleviating diarrhea caused by various factors. However, it is noteworthy that a study in India has reported that *B. coagulans*, as an adjunct to oral rehydration salts, had no therapeutic impact on the management of acute dehydrating diarrhea of diverse etiology including rotavirus-associated diarrhea, which contradicts previous studies (Dutta et al., 2011). The failure of this study is likely to be related to various factors, such as strain-dependent effects, the final formulation, dosages of supplementary medication and different underlying medical conditions (Konuray & Erginkaya, 2018). However, these conflicting results suggest that attention should be given to such factors in probiotics research. The ineffectiveness of probiotics might be due to strain-specific differences or because microorganisms already in the digestive tract of the study population might have inhibited the probiotic effect of the drug being studied. Probiotic preparations should be selected on the basis of convincing, locally generated efficacy data.

4.2. Metabolic diseases

Metabolic diseases, such as obesity, diabetes and cardiovascular diseases represent the global epidemics of the modern era (Harakeh et al., 2016; Nascimento, Pecoitsfilho, Lindholm, Riella, & Stenvinkel, 2002). Recently studies have demonstrated that *B. coagulans* could probably be an excellent therapy agent for several metabolic diseases, ncluding overweight and high cholesterol. For instance, a study has reported that the administration of *B. coagulans* and galactomannans can improve the clinical outcomes of morbidly obese patients undergoing laparoscopic sleeve gastrectomy (Kazzi et al., 2018). Moreover, an animal study showed that *B. coagulans* and *Lactobacillus plantarum* could significantly reduce the levels of triglycerides, cholesterol, low-density lipoproteins, very low-density lipoprotein and the atherogenic

Table 2
B. coagulans-mediated host immune system under various conditions.

Condition	Subject	Strain	Result	Citation
Clostridium difficile (*C. difficile)*-induced colitis	C57BL/6 mice	PTA-6086	*B. coagulans* can alleviate colon pathology caused by *C. difficile* (glandular fossa damage, edema, white blood cell index, etc.) and inhibit the secretion of inflammatory factor, such as NF-kB P65	(Fitzpatrick, Small, Greene, Karpa, & Keller, 2011)
C. difficile-induced colitis following vancomycin withdrawal	C57BL/6 mice	PTA-6086	In PTA-6086-treated mice, there was evidence of better stool consistency, as well as improved biochemical and histological indices of colitis, following initial treatment of animals with vancomycin	(Fitzpatrick, Small, Greene, Karpa, & Keller, 2012)
Cyclophosphamide induced immunosuppression and streptomycin-associated diarrhea	Non-inbred male mice	–	*B. coagulans* proved to be an effective immune adjuvant to improve the immunity of mice, with a significant reduction in IL-2 and TNF-α production	(Bomko et al., 2017)
Rheumatoid arthritis (RA)	Wistar mice	–	A significant decrease in the production of pro-inflammatory cytokines, such as TNF-α. Furthermore, no significant anti-inflammatory effects were observed following different treatments using α_1 *AGp* as an RA indicator	(Abhari et al., 2016)
Colon cancer	COLO 205, HeLA, and HEK 293T cells	PTA-11748	*B. coagulans* can be effective in inducing apoptosis of colon cancer cells indicating its potential use as an adjuvant therapy in the treatment of colon carcinoma	(Madempudi & Kalle, 2017)
LPS-induced inflammation	Mouse macrophage cells, RAW 264.7 cells	PTA-11748	*B. coagulans* inhibits COX-2 in inflammatory cells, thereby inhibiting RelA protein and inhibiting the expression of pro-inflammatory factors IL-12, TNF-α and IFN-γ	(Sudha & Arunasree, 2015)
LPS-induced interleukin 8 in adenocarcinoma cell line HT-29	HT-29 intestinal epithelial cells	–	In the pre-treatment assay, a significant decrease in IL-8, at both protein and mRNA levels, was measured for *B. coagulans* spores after the addition of LPS	(Azimirad, Alebouyeh, & Naji, 2017)
Lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cells (PBMCs)	PBMCs	PTA-6086	LPS-stimulated PBMCs show 0.2 ng/mL increase in the anti-inflammatory cytokine IL-10 28 d after consumption of PTA-6086($p < 0.05$), whereas the placebo did not affect IL-10.	(Nyangale et al., 2015)
PBMCs and polymorphonuclear (PMN) cells stimulated *in vitro* by cell wall components and metabolites of *B. coagulans*	PBMCs and PMNs	PTA-6086	*B. coagulans* induced the production of Th2 cytokines IL-4, IL-6, IL-10 and inhibited IL-2. These effects led to the stimulation of B lymphocyte proliferation and differentiation, and a resultant anti-inflammatory effect. In addition, the probiotic significantly increased the γ-interferon production	(Jensen et al., 2010)
PBMCs stimulated *in vitro* by inactivated *B. coagulans*	PBMCs	PTA-6086	*B. coagulans* showed robust increases in the immune-activating cytokines IL-1β, IL-6, IL-17A, and TNF-α. IFN-γ levels were increased, along with three chemokines, MCP-1, MIP-1α, and MIP-1β. The two anti-inflammatory cytokines IL-1ra and IL-10 showed increases, as well as the G-CSF growth factor involved in tissue repair and stem cell biology. In contrast, GM-CSF levels showed a mild decrease, showing a highly selective growth factor response	(Jensen et al., 2017)
Antiretroviral drug-suppressed chronic HIV-1 infection	Human	PTA-6086	None of the biomarkers showed significant changes following probiotic treatment. However, some biomarkers showed significant correlations to each other, particularly D-dimer with CRP and sCD14 with TNF-α, indicating this probiotic could be beneficial in reducing residual inflammation in treated HIV-1 infection.	(Yang, Kelesidis, Cordova, & Khanlou, 2014)
T-cell exposure to adenovirus and influenza A	Human	PTA-6086	*B. coagulans* PTA-6086 can induce the production of large amounts of TNF-α by human T cells in response to adenovirus exposure and influenza A (H3N2 Texas strain) exposure, but it did not have a significant effect on the response to other strains of influenza	(Baron, 2009)

Table 3
Clinical researches of *B. coagulans* on GI disorders.

Target	Strain	Subjects	Duration	Result	Citation
IBS	PTA-6086	Adults	8 weeks	Improvements from baseline abdominal pain and bloating scores in the *B. coagulans* GBI-30, PTA-6086 group were statistically significant for all 7 weekly comparisons.	(Hun, 2009)
IBS	PTA-6086	Adults	8 weeks	*B. coagulans* GBI-30, PTA-6086 is safe and effective for reducing daily bowel movements in patients with IBS-D.	(Dolin, 2009)
IBS	–	Adults	12 weeks	*B. coagulans* improves abdominal pain and diarrhea in IBS patients.	(Rogha, Esfahani, & Zargarzadeh, 2014)
IBS	MTCC 5856	Adults	90 days	A significant decrease in clinical symptoms such as bloating, vomiting, diarrhea, abdominal pain and stool frequency was observed.	(Majeed, Nagabhushanam, et al., 2016)
IBS	PTA-11748	Children	8 weeks	A significant reduction in pain intensity and improved bowel habit satisfaction along with global assessment of relief was observed in the probiotic treated group.	(Sudha et al., 2018)
Constipation	lilac-01	Adults	12 weeks	In the test group of functionally constipated subjects, the changes in the average scores of self-reported fecal size, sensation of incomplete evacuation, defecation frequency and fecal color and odor	(Kimiko et al., 2015)
Constipation	SANK 70258	Adults	2 weeks	Improvement of fecal shape, change of fecal color from dark brown to yellowish brown, decrease of fecal odor and fecal pH and an increase in defecation frequency was seen.	(Ara et al., 2002)
Acute diarrhea	PTA-11748	Adults	10 days	Diarrhea time decreased by 80%, defecation times also showed a decline trend	(Sudha & Bhonagiri, 2012)
Acute diarrhea	LBSC	Adults	< 7 days	The LAB was effective in recovering from acute diarrhea with abdominal pain and discomfort, and exhibited improved quality of life.	(Maity & Kumar, 2019)
Rotavirus-associated diarrhea	–	Infants	5 days	*B. coagulans*, as an adjunct to oral rehydration salts, had no therapeutic impact on management of acute dehydrating diarrhea of diverse etiology including rotavirus associated diarrhea in children.	(Dutta et al., 2011)
Rotavirus-associated diarrhea	–	Newborns infants	12 months	The prophylactic feeding of *Lactobacillus sporogenes* has a preventive effect on the incidence and duration of acute rotavirus diarrhea.	(Chandra, 2002)
Antibiotic-induced diarrhea	–	Infants	10 days	Prophylaxis with *Lactobacillus* sporogens, associated to FOS, significantly reduced the number of days and duration of events in children with antibiotic-induced diarrhea	(Rosa et al., 2003)
Necrotizing enterocolitis(NEC)	–	Infants	16.3 ± 9.4 days	*B. coagulans* supplementation is not effective in reducing the incidence of death or NEC in very low birth weight infants; however, it could improve the feeding tolerance.	(Sari et al., 2011)
Functional abdominal pain (FAP)	PTA-11748	Children	8 weeks	Response rate was higher with symbiotic treatment than placebo after medication, indicating the symbiotic containing *B. coagulans* and FOS seems to be effective in the treatment of childhood FAP. However, there was no difference between the two groups at week 12.	(Saneian, Pourmoghaddas, Roohafza, & Gholamrezaei, 2015)
Gastrointestinal flatulence-related symptoms	PTA-6086	Adults	4 weeks	*B. coagulans*-based product was effective in improving quality of life and reducing gastrointestinal symptoms in adults with post-prandial intestinal gas-related symptoms and no GI diagnoses.	(Kalman et al., 2009)

index in the serum of hypercholesterolemic animals. The body weight of mice administered with these two strains was considerably reduced (Aminlari et al., 2018). Similar results were obtained from *B. coagulans* MTCC 5856, since the results indicated that the administration of *B. coagulans* MTCC 5856 can reduce the high cholesterol levels of mice (Majeed et al., 2019).

4.3. Immune diseases

The immune system is an important line of defense for the health of the host. Studies have shown that there is a relationship between gastrointestinal microbiota and the mucosal and systemic immune responses. As an immune adjuvant, many clinical studies have shown that *B. coagulans* can be beneficial in the treatment of several immune disorders caused by different inflammatory factors, such as human immunodeficiency virus (HIV)-1 infection and rheumatoid arthritis (RA) (Table 2). For example, a double-blind clinical experiment demonstrated that *B. coagulans* PTA-6086 is an effective therapeutic adjuvant in the treatment of RA, which is an autoimmune disorder (Smolen & Günter, 2003). The association was further established when *B. coagulans* PTA-11748 was used to treat RA in rats (Table 2). The results indicated that *B. coagulans* can regulate pro-inflammatory cytokines in mice, thereby leading to an anti-inflammatory effect that alleviates RA symptoms (Kiran & Deane, 2008; Rodríguez-Cabezas et al., 2008).

Additionally, as shown in Table 2, the anticancer (anti-proliferative) effects of *B. coagulans* have been evaluated in human cancer cells. The cytotoxicity assay clearly demonstrated a decrease in cell proliferation of COLO 205, HeLa, and K562 cells. The underlying molecular mechanism of apoptosis was analyzed by immunoblot and indicated that the strain of *B. coagulans* can downregulate Bcl2 expression significantly in COLO 205 cells with a concomitant increase in Bax levels, suggesting that the induction of apoptosis occurs due to high Bax/Bcl2 ratio (Madempudi & Kalle, 2017).

4.4. Major depressive disorder

Major depressive disorder (MDD) is a psychiatric disease which is characterized by numerous factors, including a preoccupation with mortality, feelings of guilt, low mood, reduced quality of life, and disturbed sleep or appetite (Rudolf, Payne, Barbara, & Perlis, 2014). In recent years, emerging evidence has shown that patients suffering from IBS are more prone to depression compared to healthy individuals (Ford et al., 2014; Lovell & Ford, 2012). Significant changes were found indicating that *B. coagulans* MTCC 5856 can alleviate MDD induced by IBS in mice. Furthermore, serum myeloperoxidase, an inflammatory biomarker, was also significantly reduced ($p < 0.01$) when compared with the baseline at the end of the study, indicating that alterations in the balance between anti- and pro-inflammatory cytokines may play an important role in the pathogenesis of depression (Majeed, Nagabhushanam, Arumugam, Majeed, & Ali, 2018; Vlainiä‡, J, Vlainiä‡, & Vukorep, 2016). However, larger-scale trials with extended follow-up durations are warranted to establish the underlying mechanism, as well as a detailed assessment of the therapeutic effects of *B. coagulans* MTCC 5856 supplementation in managing major depressive disorder in IBS patients.

5. Conclusions

Due to its unique GI life cycle and probiotic effects, *B. coagulans* has shown great potential in the treatment of various human diseases. For example, through direct regulation or indirect modulation via the host microbiota, *B. coagulans* plays an impressive role in eliminating infections and attenuating both GI diseases and diseases in remote tissues. Furthermore, the ability of *B. coagulans* to maintain host microbiota homeostasis can also aid the digestion of daily meals and promote gut health. Therefore, beneficial effects of *B. coagulans* result from comprehensive and coordinated processes, involving the host microbiome, metabolism and immune system. However, there are multiple *B. coagulans* strains with different host origins, and many of the probiotic functions of *B. coagulans* are strain-dependent. Therefore, it may be advantageous to combine different strains of *B. coagulans* to maximize their beneficial effects in the future.

Ethics statements

The review did not include any human subjects and animal experiments.

Contributors

Jiang Cao and Zhiming Yu contributed to literature search and writing the manuscript. Qixiao Zhai and Wei Chen reviewed and revised the manuscript. Wenyin Liu prepared the figures and tables. Hao Zhang and Jianxin Zhao guided and advised the topic of this article.

Declaration of Competing Interest

Authors declare that they do not have a conflict of interest in any capacity including competing or financial.

Acknowledgements

This work was supported by the National Natural Science Foundation of China Program (No. 31820103010 and No. 31530056), the Natural Science Foundation of Jiangsu Province of China (BK20141098 and BK20160175), National First-Class Discipline Program of Food Science and Technology (JUFSTR20180102), the BBSRC Newton Fund Joint Centre Award, and Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu Province.

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