A review on *Bacillus coagulans* as a Spore-Forming Probiotic

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**Abstract**

**Background and objective:** Growth of beneficial probiotics is attributed to their tolerance to stress factors while most probiotics have a relatively short and unstable shelf life. *Bacillus coagulans*, an economically important spore-forming species, is becoming increasingly remarkable in field of probiotics for the decrease of harmful effects of processing and environment conditions on the survival of bacterial cells and to assure their functionality in the human body. The aim of this review was to explore scientific research on therapeutic, functional and biosafety properties of *Bacillus coagulans* as a novel probiotic.

**Results and conclusion:** Many scientific literatures have been published on the use of health promoting Bacillus spores in foods. *Bacillus coagulans* mostly includes several health benefits of non-spore forming probiotics and is able to tolerate heat and stressful requirements of food processing as well as gastrointestinal tract conditions. Considering specific characteristics of spore-forming probiotics, *Bacillus coagulans* demonstrates a promising potential in production of probiotic foods.

**Conflict of interest:** The authors declare no conflict of interest.

**1. Introduction**

Probiotics are available in various forms of functional foods and dietary supplements for human use. Dairy products are the most commonly sold carriers of probiotic bacteria. Furthermore, capsules and tablets containing lyophilized probiotic bacteria are available in health food stores [1]. E-commerce platforms provide medications, which can be used to treat gastrointestinal tract (GIT) diseases. Many of these products contain lactobacilli and bifidobacteria as key members of probiotic group [2]. A large number of scientific reports have focused on these probiotic species, investigating various aspects such as industrial applications, therapeutic properties and biosafeties [1,3]. Lactobacilli and bifidobacteria are sensitive to physiological conditions such as pH of the stomach and bile salts. Moreover, bioavailability of these bacteria is affected by various conditions of production, storage and transportation [4,5]. Use of spore-forming bacteria includes advantages compared to that use of lactobacilli does because products containing these bacteria can be preserved in dry forms at room temperature without any negative effects on the survival of spores. Another advantage of using spores is linked to their ability to tolerate low pH and successfully passing the GIT. Bacterial spores include a greater resistance to thermal lethal effects, drying, freezing, toxic chemicals and radiation compared to that vegetative cells do [6,7].

In recent years, a number of *Bacillus* spp. have been reported as novel probiotics [8]. Spore-forming bacilli have been used for many years for food production and preservation. The importance of bacilli bacteria in food chains is associated to their innate production of an extensive number of enzymes, vitamins, antimicrobials and protein compounds as well as organic pigments such as carotenoids. In fact, *Bacillus* spp. become popular in human health and functional food research primarily due to their enhanced tolerance and survivability under the...
antagonistic environment of GIT [9,10]. Furthermore, stability of Bacillus microflora during food processing and storage makes them ideally functional candidates for health boosting formulations [7]. A challenge currently exists as most probiotic products failed to receive much credentials in public view despite of their scientific advancements and evidence that show effectiveness of commercial probiotic supplements and functional foods containing Bacillus species. One of the important reasons for this is linked to ongoing controversy between the probiotic and pathogenic aspects of Bacillus spp. Therefore, the current review aimed to provide up-to-date scientific information on Bacillus species as spore-forming probiotics, particularly Bacillus (B.) coagulans.

2. Spore-forming bacillus as probiotics

Use of Bacillus species in food products has been documented for more than 50 years. This species was introduced to European markets as Enterogermina®, first introduced and registered in Italy as a medicinal supplement in 1958 [4,11]. Traditional use of spore-forming bacteria that produce lactic acid (LA) has been documented as well [12,13]. The most comprehensively investigated species of spore-forming Bacillus include B. licheniformis, B. clausii, B. coagulans, B. cereus and B. subtilis [14]. Although the most common probiotic species are those producing LA (Lactobacillus spp.), no universal classes of probiotics exist currently. These bacteria are found normally in GIT of humans and animals; however, obscure reports show that use of indigenous microorganisms somehow restitutes the natural microflora of GIT [15,16]. The second class of probiotics includes those not normally found in GIT. A member of this group of probiotics is Bacillus genus, which typically produces spores to survive harsh environmental conditions such as heat, drought, freezing, radiation and rising oxygen levels [17,18]. Relatively, material coating and encapsulation are suggested to enhance the survival of probiotics exposed to simulated GIT conditions [19]. Most recently, certified spore-forming Bacillus spp. are used in dry probiotic products, meaning that they can be stored indefinitely on the shelf [20,21]. However, use of products containing spore-forming bacteria raises a number of questions. One of these questions mainly includes the fact that since germination, proliferation and resporulation (when food is restricted) comprise of the spore life cycle, how Bacillus probiotics exert beneficial effects within such a life cycle? Based on the suggested performance models for probiotic bacteria, probiotics demonstrate functionalities of live bacteria within the GIT [12].

Based on the medical importance of probiotic microorganisms, food and drug producers are interested in formulating products with novel and further resistant probiotic strains [22]. Bacterial endospore structures include a condensed inactive chromosome and extra layers such as a peptidoglycan-rich cortex and proteinaceous materials. These structures preserve bacteria in harsh conditions such as those in GIT [23-25]. A specified dose of Bacillus spores can be stored indefinitely outside of refrigerators and almost an entire dose of the intake bacteria reaches the small intestine intact. Rapidly after exposure to nutrients and moistures, spores shift from inactive form to vegetative form [26-28]. Spores can survive in industrial conditions and hence provide long-term survivals of the bacteria [29]. However, it is important to provide appropriate conditions such as heat shock and optimized culture media for the activation and recovery of spores to assess the probiotic viability during shelf life or exposure to GIT conditions [30-32]. A limited number of spore-forming bacilli are considered as probiotics in following food categories of dairy products, baked goods, fruit juices, confections, grain products, pastas, beverages, chewing gums, breakfast cereals, hard candies, seasonings, nut products, processed vegetables, coffees, teas, blends, extracts, flavorings and several other wet and dry food products [33].

3. Bacillus coagulans in health foods and dietary supplements

Nowadays, many Bacillus products are sold online as novel foods and dietary supplements with claimed health benefits such as restoring the natural microflora of the GIT. However, some of these products carry poorly defined or invalid probiotic species [6,12]. Of hundreds of known Bacillus spp., only B. coagulans (Figure 1) and B. subtilis var. natto have generally been accepted as admissible probiotics for human consumption. Natto is a Japanese food product made by fermenting cooked soybeans with B. subtilis var. natto. This food product has been proven to include probiotic attributes since B. subtilis var. natto is believed to stimulate the immune system, pose anti-cancer properties and produce vitamin K2 [34-37]. The wide use of Natto in Japan and public belief in its beneficial and functional properties seem to support the concept of probiotics. To date, a number of B. coagulans strains are described as probiotics (Table 1) and used in production of food products and dietary supplements (Table 2). In a recent report, B. coagulans GBI-30, 6086 was described as an aid for the absorption and utilization of proteins. The bacteria can tolerate acidic conditions of the stomach to arrive in the intestine (where it can germinate), and subsequently help in digestion of carbohydrates and proteins. Consumption of B. coagulans GBI-30, 6086 with proteins has been demonstrated to increase protein absorption, boosting efficiency of dietary protein supplementation. Therefore, health improvement of muscles in different populations and reduced quality
differences between proteins from different sources (e.g., plant proteins) are resulted from \textit{B. coagulans} GBI-30, 6086 consumption \cite{38,39}. \textit{B. coagulans} MTCC 5856 has been studied in functional foods to show its stability during processing and storage. A number of functional food products with various nutritional profiles (e.g., banana muffins, waffles, chocolate fudge frostings, peanut butters, fruit preserves and vegetable oils) have been studied to investigate the stability of \textit{B. coagulans} MTCC 5856. The bacteria was reportedly stable during baking, brewing, refrigerating and freezing of the highlighted functional products for up to 24 m \cite{33}.

![Figure 1. Scientific classification of Bacillus coagulans](image)

### Table 1. Various probiotic strains of Bacillus coagulans

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{B. coagulans} GBI-30, 6086</td>
<td>[38]</td>
</tr>
<tr>
<td>\textit{B. coagulans} MTCC 5856</td>
<td>[33]</td>
</tr>
<tr>
<td>\textit{B. coagulans} Unique IS-2 (MTCC 5260)</td>
<td>[49]</td>
</tr>
<tr>
<td>\textit{B. coagulans} S-lac</td>
<td>[65]</td>
</tr>
<tr>
<td>\textit{B. coagulans} 2-6</td>
<td>[65]</td>
</tr>
<tr>
<td>\textit{B. coagulans} CSIL1</td>
<td>[65]</td>
</tr>
<tr>
<td>\textit{B. coagulans} 36D1</td>
<td>[66]</td>
</tr>
<tr>
<td>\textit{B. coagulans} HM-06</td>
<td>[65]</td>
</tr>
<tr>
<td>\textit{B. coagulans} H1</td>
<td>[65]</td>
</tr>
<tr>
<td>\textit{B. coagulans} HM-08</td>
<td>[67]</td>
</tr>
</tbody>
</table>

*\textit{B. coagulans} = Bacillus coagulans*

### Table 2. Examples of probiotic food products and nutritional supplements containing \textit{B. coagulans} available in global markets

<table>
<thead>
<tr>
<th>Product brand</th>
<th>Product type</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>THRONE</td>
<td>NS</td>
<td>United States</td>
</tr>
<tr>
<td>Harvest Soul</td>
<td>Fruit and vegetable juices</td>
<td>United States</td>
</tr>
<tr>
<td>Nature’s Bounty</td>
<td>Gummies</td>
<td>United States</td>
</tr>
<tr>
<td></td>
<td>Dark chocolate</td>
<td></td>
</tr>
<tr>
<td>Solaray</td>
<td>NS</td>
<td>United States</td>
</tr>
<tr>
<td>Nature Made</td>
<td>Gummies</td>
<td>United States</td>
</tr>
<tr>
<td>Attune</td>
<td>Dark chocolate</td>
<td>United States</td>
</tr>
<tr>
<td>Linwoods</td>
<td>Ground flaxseed</td>
<td>United States</td>
</tr>
<tr>
<td>Purely Elizabeth</td>
<td>Chocolate sea salt</td>
<td>United States</td>
</tr>
<tr>
<td>Udi’s</td>
<td>Granola bar</td>
<td>United States</td>
</tr>
<tr>
<td>Bigelow</td>
<td>Lemon ginger tea</td>
<td>United States</td>
</tr>
<tr>
<td>Megasporebiotic</td>
<td>NS</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>Betty Lou’s</td>
<td>Dark chocolate</td>
<td>United States</td>
</tr>
<tr>
<td>COOPER MOON</td>
<td>Cappuccino</td>
<td>United States</td>
</tr>
<tr>
<td>ProDURA</td>
<td>NS</td>
<td>United States</td>
</tr>
<tr>
<td>Happy Yo Pro™</td>
<td>Yogurt</td>
<td>United States</td>
</tr>
</tbody>
</table>

NS, nutritional supplement
4. Bacillus species in therapeutic products

Potential advantages of Bacillus species include improved nutritional values and growth, boosted immunity levels and prevented GIT diseases such as diarrhea, irritable bowel syndrome (IBS) and inflammatory bowel disease, ulcerative skin disorders, bacterial vaginoses and cancers [40]. These originally soil based organisms exhibit typical characteristics of Lactobacillus and Bacillus genera. They are spore-forming (terminal spore), Gram positive, catalase-positive, metabolically thermotolerant, rod-shaped, facultative anaerobes, which produce only L-lactic acid but do not produce gas from the fermentation of various sugars such as maltose, mannitol, raffinose, sucrose and trehalose. B. coagulans was first isolated from spoiled milk in 1915 when an outbreak of flat-sours (coagulation) occurred in evaporated milk packed by an Iowa condenser and wrongly classified as a L. sporogenes [41,42]. This microorganism was reclassified in Bergey's Manual of Determinative Bacteriology as B. sporogenes since 1957. According to 8th edition of this book, B. sporogenes shares the same characteristics of B. coagulans and has therefore been re-categorized in B. coagulans group. Terminal position of the endospore in cells, lack of cytochrome C oxidase and inability to reduce nitrate to nitrite discriminate these bacteria from other bacteria within Bacillus genus.

Based on the Patent Cooperation Treaty, Kibow Biotech Inc. is developing B. coagulans for curing GIT infections. The bacterial therapeutic effect is due to coagulin (the bacteriocin secreted from B. coagulans), which includes activities against extended-spectrums of enteric microbes [43]. Documented evidence have demonstrated useful effects of Bacillus probiotics on urinary tract infections. Bacillus probiotics cause a significant depletion in frequency of urinary tract infections episodes in patients treated with Bacillus spores and antibiotics [44]. Manipur Ngari is a fermented fish food that is made by B. coagulans BDU3B. This strain produces a novel bacteriocin that shows antimicrobial activities against a wide range of pathogenic microorganisms. This bacteriocin, which is the smallest bacteriocin isolated from B. coagulans, can be used as a bio-preservative in foods [45]. B. coagulans is considered as an economically significant species based on a spore-like protein coat that protects the bacteria from acidic conditions of the stomach [46]. This Bacillus produces optically pure LA [47], coagulin and thermostable enzymes [46]. The optimum temperature and pH for the growth of these bacteria include 35-50°C and 5.5-6.5, respectively. Studies have shown that the presence of diluted HCL or NaOH solutions promotes germination of spores [30,48]. A recent study has investigated in vitro anti-proliferative effects of B. coagulans Unique IS2 on three types of cancers, including human colon cancer, cervical cancer and chronic myeloid leukemia. A human embryonic kidney cell line was used as noncancerous control. Results have shown that the heat-killed culture supernatant of this strain is effective in induction of programmed cell death (apoptosis) of the colon cancer. This can be considered for adjuvant therapy in treatment of colon carcinoma [49]. Bacillus probiotics are developed for topical and oral treatments of uremia. This bacterial genus is able to produce antimicrobial compounds and small effector molecules that interact with the host milieu, resulting in bacterial biotherapeutic potentials [7].

5. In vivo and human studies on Bacillus coagulans as a probiotic

Published scientific reports have described significant roles of B. coagulans as a novel probiotic in hosts (humans and animals), compared to existing documented uses of probiotic Lactobacillus spp. [7,50]. As discussed earlier, B. coagulans is used as a probiotic in liquid food products with low water activities (aw). Following oral administration, spores of B. coagulans pass through the stomach and, upon arrival in the first part of the intestine (duodenum), germination and multiplication occurs rapidly [26,48]. Assessments suggest that the average time between oral dosing and germination includes four hours [48,51]. Germinated B. coagulans cells produce LA once they become metabolically active as a part of the facultative anaerobes in small intestine and colon. These cells are slowly excreted through feces over approximately seven days after discontinuation of consumption [52,53]. B. coagulans has been asserted to improve GIT ecology by restoring the quantity of favorable obligatory bacteria and antagonizing pathogenic microorganisms [33]. Effects of B. coagulans Unique IS2 on serum cholesterol levels were investigated in hypercholesterolemic patients. At the end of the studies, total cholesterol and LDL were slightly decreased, while HDL cholesterol was increased. Furthermore, no adverse effects of B. coagulans Unique IS2 administration were observed [54,55]. In another study, effects of oral supplementation of B. coagulans Unique IS2 were assessed in subjects with bacterial vaginosis. Forty Indian women suffering from bacterial vaginosis were participated in this study. Participants received probiotic treatments in three major groups. Results from the study showed an 80% reduction in vaginosis symptoms in patients treated with the probiotics, compared to the control group [56]. Sudha et al. investigated effects and safety of B. coagulans Unique IS2 in treatment of acute diarrhea [57]. A total of 28 patients
(at various ages) suffering from acute diarrhea were
participated in a clinical trial. All participants received
capsules containing at least $2 \times 10^9$ CFU of *B. coagulans*
Unique IS2 two times a day for ten days. Efficacy was
assessed by recording the duration of diarrhea, frequency
defecation, consistency of feces and abdominal pain.
Results of therapy with *B. coagulans* Unique IS2
demonstrated decreases in abdominal pain, duration of
diarrhea and frequency of defecation.

Effects of oral consumption of two spore-forming probiotics on population of fecal coliforms and other gut
microbiota have been investigated in rat models. Totally,
36 adult male albino rats were divided into four major
groups of nine members and used in an experiment for 49
days with seven days of adaptation. Group 1 was fed on a
basal diet in addition to sterile skim milk for 28 days.
Groups 2 and 3 received the basal diet and skim milk
inoculated with at least $8 \log$ CFU ml$^{-1}$ of *B. coagulans*
B37 and *B. pumilus* B9 spores, respectively. The control
group was fed with the basal diet and clean water. Fecal
sampling was carried out at 7-day intervals and
enumerated for Bacillus spores, coliforms and lactobacilli.
Fecal coliform counts decreased but Bacillus spores and
lactobacilli counts increased in rats fed with either *B.
pumilus* or *B. coagulans* B37, compared to control group.
This study showed that oral administration of *B. coagulans*
B37 and *B. pumilus* B9 strains might be useful in control
of coliform bacteria and in increase of lactobacilli counts
in intestinal microflora of rats [58]. Patients who suffer
from IBS are mostly unsatisfied with current medications.
Probiotics may alleviate this lifelong disease and relieve its
unfavorable symptoms. In 2009, a study was carried out to
investigate possible effects of *B. coagulans* GBI-30, 6086
on IBS symptoms. Totally, 44 patients participating in that
clinical trial study received placebo and *B. coagulans* GBI-
30, 6086 every day for eight weeks and the severity of IBS
symptoms was recorded. Improvements from baseline in probiotic group. Results of the study indicated
that daily consumption of BC30 by elderly adults could
increase populations of beneficial bacteria in human gut and
potentially increases production of anti-inflammatory
cytokines.

6. Safety assessment of *Bacillus coagulans* as a spore-forming probiotic

Strains of *B. coagulans* can survive in harsh conditions
(heat, stomach acid and bile salts); to which, generally
consumed probiotics are sensitive. Although strains of *B.
coagulans* have widely been consumed around the world
for decades, a few studies on toxicology of *B. coagulans*
are available. Contrary to other LA producing bacteria, *B.
coagulans* is not commonly considered as a part of the GIT
microflora. Moreover, limited studies have been carried out
to assess the ability of spore-forming probiotic *Bacillus*
ssp. to bond to the intestinal epithelium cells. Scientists
have reported that *B. coagulans* is a transient microorganism because it is completely absent in fecal
samples only within a few days after the end of consumption [61]. Ripamonti et al. [62] assessed adhesion of
*B. coagulans* to a human embryonic intestine cell line. They showed that *B. coagulans* could adhere conspicuously to INT407 cells. Endres et al. [63]
completely assessed safety of long oral consumption of *B.
coagulans* (GanedenBC30™), including in vitro and in vivo toxicology studies of mutagenicity assays, mouse
micronucleus tests, chromosomal aberration assays, rabbit
acute eye and skin irritation assays and acute oral toxicity
tests in laboratory animals as well as 90-day sub-chronic
oral toxicity tests in Wistar rats. Results of the
toxicological safety studies showed that GanedenBC30™
did not include mutagenic, clastogenic or genotoxic
effects. Concentrations of the probiotic biomass used in
90-day studies included $1.36 \times 10^{11}$ CFU g$^{-1}$ since the
suggested human daily dose included $100 \times 10^9$ to $3 \times 10^9$
CFU.

To further assess the safety of long-term *B. coagulans*
consumption, a study was carried out in 2011 on one-year
oral chronic toxicity of the bacteria combined with a single
one-generation reproduction. Feeding *B. coagulans*
GanedenBC30™ to male and female Wistar rats revealed no symptoms of toxicity at the highest doses tested in parental generation and F1 offspring [64]. B. coagulans Unique IS-2 (MTCC-5260) is another spore-forming probiotic; for which, acute and sub-acute oral toxicity assessments were carried out in Sprague Dawley rats. Doses of 3250 and 6500 mg kg$^{-1}$ body weight day$^{-1}$ ($5 \times 10^7$ B. coagulans Unique IS-2 spores g$^{-1}$) were fed orally to the animals in acute toxicity assessment. Experimental animals were fed with doses of 130, 650, 1300 mg kg$^{-1}$ body weight day$^{-1}$ ($5 \times 10^7$ spores g$^{-1}$) for 14 consecutive days in sub-acute toxicity assessment. Control animals received water alone. Half of the rats were sacrificed after 14 and the remaining after 28 days of beginning of the experiment. The toxicity study indicated that clinical symptoms, clinical chemistry, body weight, food intake, urinalysis, hematological examinations, gross pathology and histopathology included no treatment-linked changes after 14 and 28 days. Based on the results of the study, B. coagulans Unique IS-2 strain could be considered as non-pathogenic and safe for the human consumption [51].

7. Conclusion

Various strains of B. coagulans are used as probiotics to improve and preserve ecological balances within the intestinal microflora. Although these probiotics are not native to human GIT, they can be used as beneficial bacteria; similar to other probiotic strains. In literatures, a limited number of reports on in vitro sub-chronic and chronic toxicity assessments, in vivo genotoxicity and human clinical trials are available. All of these studies approve safety of B. coagulans use at recommended doses. No enough information are available on how B. coagulans acts in medical applications. However, people consume these microorganisms to treat diseases such as diarrheas, IBS, inflammatory bowel disease, skin disorders, bacterial vaginosis and cancers. Appropriate doses of B. coagulans depend on several factors such as consumer age and health. In recent years, numerous clinical studies have been carried out on use of health promoting Bacillus spores in foods. Considering specific characteristics of spore-forming probiotics, B. coagulans has demonstrated promising health potentials in probiotic food products.

8. Acknowledgements

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9. Conflict of Interest

The authors declare no conflict of interest.

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Bacillus coagulans a novel spore-forming probiotic


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مروری بر باسیلوس کواگولانس به عنوان یک پروپیتیک هاگزا

حسنی نژاد، نیکی یادنمود، امیر بهشتی، مهدی ایران

چکیده

نقطه سایه و هدف: رشد پروپیتیک‌های منفی به حمل آنها در برابر عوامل استرس نسبت داده می‌شود، در حالی که اکثر پروپیتیک‌های عمل منفی نسبتاً کوتاه و تاییدنی دارند باسیلوس کواگولانس به عنوان گونه‌های هاگزای مهمی به لحاظ اقتصادی در زمینه پروپیتیک‌ها به طور معناداری به عنوان کاهش اثرات زیان‌آور شرایط فراوری مواد غذایی و محیط زیست بر زندگی سلول‌های باکتری‌ها و اطمینان از عملکرد آنها در بدن انسان مورد توجه قرار گرفته‌اند.

هدف از این مطالعه موردنی بررسی تحقیقات علمی انجام شده روی خواص درمانی، عملکردی و ایمنی زیستی باسیلوس کواگولانس به عنوان یک پروپیتیک جدید است.

یافته‌ها و نتیجه‌گیری: مطالعات بسیاری در زمینه سلامتی ترویجی بهتر کیفری هاگهای باسیلوس در مواد غذایی به چالیده، باسیلوس کواگولانس اغلب مزایای سلامتی بخش پروپیتیک‌های غیرهزاها را دارد و قادر به تحلیل حرارت و شرایط استرس‌زا و فراوری مواد غذایی و نیز شرایط محرک مردمی را می‌پذیرد. در این مطالعه توجه به مسئولیت ویژه پروپیتیک‌های هاگزا باسیلوس کواگولانس پتانسیل امیدوار کننده‌ای در تویید مواد غذایی پروپیتیک

نگاه میده.

تغییر منافع: نویسندگان اعلام می‌کنند که هیچ نویس تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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