http://dx.doi.org/10.22037/afb.v%vi%i.23958

Review Article

APPLIED FOOD BIOTECHNOLOGY, 2019, 6 (2):91-100
Journal homepage: www.journals.sbmu.ac.ir/afb

pISSN: 2345-5357
eISSN: 2423-4214

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.

Article Information

Article history:
Received 8 Jan 2019
Revised 20 Feb 2019
Accepted 3 Mar 2019

Keywords:
- *Bacillus coagulans*
- Functional foods
- Probiotics
- Safety assessment
- Stress tolerance
- Therapeutic aspect

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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 K_2 [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 *B. coagulans* GBI-30, 6086 consumption [38,39]. *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 *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 [33].

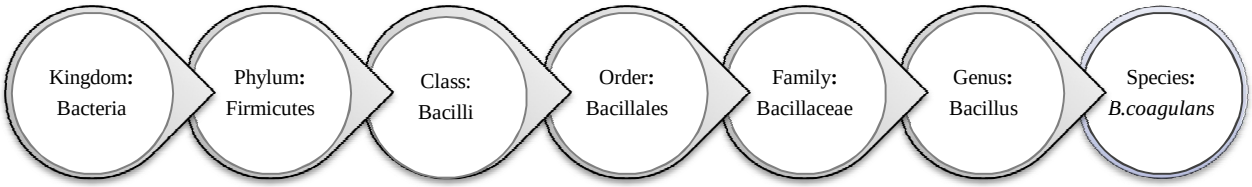

Figure 1. Scientific classification of *Bacillus coagulans*

Table 1. Various probiotic strains of *Bacillus coagulans*

Bacteria	Reference
B. coagulans GBI-30, 6086	[38]
B. coagulans MTCC 5856	[33]
B. coagulans Unique IS-2 (MTCC 5260)	[49]
B. coagulans S-lac	[65]
B. coagulans 2-6	[65]
B. coagulans CSIL1	[65]
B. coagulans 36D1	[66]
B. coagulans HM-06	[65]
B. coagulans H1	[65]
B. coagulans HM-08	[67]

B. coagulans= Bacillus coagulans

Table 2. Examples of probiotic food products and nutritional supplements containing *B. coagulans* available in global markets

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

*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 (a_w). 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 microorganism [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 2×10^9 CFU of *B. coagulans* Unique IS2 two times a day for ten days. Efficacy was assessed by recording the duration of diarrhea, frequency of 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 symptom scores in probiotic group were statistically significant for all comparisons during the study. Changes in abdominal pain scores at Weeks 6 and 8 showed statistical significance in the placebo group. Due to no treatment-linked adverse effects during the study, primitive results suggested that this spore-forming probiotic might be a safe and impressive choice for the reduction of symptoms in patients with IBS [59].

Generally, decreased benefits of probiotic bacteria (e.g. *Bifidobacterium* spp.) and aspects of inherent immune functions are linked to advanced life age. Nyangale et al. investigated possible effects of daily oral administration of a spore-forming probiotic [*B. coagulans* GBI-30, 6086 (BC30); GanedenBC30] on improving immune and gut functions in elderly men and women aged 65-80 years [60]. A total number of 36 participants were randomly assigned to receive a microcrystalline cellulose capsule (placebo) or a capsule containing 1×10^9 CFU of BC30 probiotic (placebo or probiotic) a day for 28 days, followed by 21 days of washout before shifting to other treatments. Blood and fecal samples were collected before and after each treatment and bacteria were enumerated in the fecal samples. Populations of *Faecalibacterium prausnitzii* significantly increased by consumption of BC30, compared to consumption of the placebo. Populations of *Bacillus* spp. increased significantly by 0.5 $\log^{10}$ cells ml^{-1} from the 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* spp. 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 1.36×10^{11} CFU g^{-1} since the suggested human daily dose included 100×10^6 to 3×10^9 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} (5×10^9 *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} (5×10^9 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 vaginoses 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

The authors acknowledge funds from the Research Institute of Food Science and Technology (RIFST), Mashhad, Iran.

9. Conflict of Interest

The authors declare no conflict of interest.

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http://dx.doi.org/10.22037/afb.v%vi%i.23958

Review Article

APPLIED FOOD BIOTECHNOLOGY, 2019, 6 (2): 91-100
Journal homepage: www.journals.sbmu.ac.ir/afb

pISSN: 2345-5357
eISSN: 2423-4214

مروری بر *باسیلوس کواگولانس* به عنوان یک پروبیوتیک هاگزا

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چکیده

سابقه و هدف: رشد پروبیوتیک‌های مفید به تحمل آنها در برابر عوامل استرس نسبت داده می‌شود، در حالی که اکثر پروبیوتیک‌ها عمر مفید نسبتاً کوتاه و ناپایدار دارند. *باسیلوس کواگولانس*، به‌عنوان گونه‌های هاگزای مهم به لحاظ اقتصادی، در زمینه پروبیوتیک‌ها، به طور فزاینده‌ای به منظور کاهش اثرات زیان‌آور شرایط فراوری مواد غذایی و محیط زیست بر زنده‌مانی سلول‌های باکتری‌ها و اطمینان از عملکرد آنها در بدن انسان مورد توجه قرار گرفته‌اند. هدف از این مطالعه مروری، بررسی تحقیقات علمی انجام شده روی خواص درمانی، عملکردی و ایمنی زیستی *باسیلوس کواگولانس* به عنوان یک پروبیوتیک جدید بوده است.

یافته‌ها و نتیجه‌گیری: مطالعات بسیاری در زمینه سلامت ترویج به‌کارگیری هاگ‌های باسیلوس در مواد غذایی به چاپ رسیده است. *باسیلوس کواگولانس* اغلب مزایای سلامتی بخش پروبیوتیک‌های غیرهاگزا را دارند و قادر به تحمل حرارت و شرایط استرس‌زای فراوری مواد غذایی و نیز شرایط مجرای معده‌ای-روده‌ای می‌باشند. با توجه به مشخصات ویژه پروبیوتیک‌های هاگزا، *باسیلوس کواگولانس* پتانسیل امیدوارکننده‌ای در تولید مواد غذایی پروبیوتیک نشان می‌دهد.

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

تاریخچه مقاله

دریافت 8 ژانویه 2019
داوری 20 فوریه 2019
پذیرش 3 مارس 2019

واژگان کلیدی

- *باسیلوس کواگولانس*
- مواد غذایی فراسودمند
- پروبیوتیک
- ارزیابی ایمنی
- تحمل تنش
- جنبه درمانی

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