

Bacillus coagulans in Food Industry

Subjects: Food Science & Technology

Contributor: Gözde Konuray

Probiotic microorganisms are generally considered to beneficially affect host health when used in adequate amounts. Although generally used in dairy products, they are also widely used in various commercial food products such as fermented meats, cereals, baby foods, fruit juices, and ice creams. Among lactic acid bacteria, *Lactobacillus* and *Bifidobacterium* are the most commonly used bacteria in probiotic foods, but they are not resistant to heat treatment. Probiotic food diversity is expected to be greater with the use of probiotics, which are resistant to heat treatment and gastrointestinal system conditions. *Bacillus coagulans* (*B. coagulans*) has recently attracted the attention of researchers and food manufacturers, as it exhibits characteristics of both the *Bacillus* and *Lactobacillus* genera. *B. coagulans* is a spore-forming bacterium which is resistant to high temperatures with its probiotic activity.

Keywords: *Bacillus coagulans* ; probiotic ; microbial enzyme

1. Introduction

Nowadays, the interest in probiotic foods is increasing due to the growing consumer demand for safe and functional foods with health-promoting properties and high nutritional value [1]. Probiotics are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” [2]. In order to obtain benefits, probiotic products should contain at least 10^7 – 10^9 cfu/g probiotic microorganism and should survive until the end of shelf life [3]. Probiotic microorganisms, which are naturally found in intestinal microbiota, could protect humans from diseases, modulate and strengthen the immune system, prevent tooth decay, have anticarcinogenic properties, and be effective against coronary heart disease [4][5]. Probiotic microorganisms can produce organic acids (such as lactic and acetic acid), hydrogen peroxide, and bacteriocin [5]. Probiotics have several mechanisms to inhibit pathogen microorganisms. The primary mechanisms are as follows: (1) the lowering of the pH of food through lactic acid production; (2) the production of antimicrobial substances such as microcin, hydrogen peroxide, and compounds like free radicals; (3) competition for food resources by attaching to receptors; and (4) stimulation of the production of secretory IgA (Immunoglobulin A) by the formation of protective mucin (parent substance of the mucus composed of tissue of epithelial or connective origin and a mixture of glycoprotein and mucoprotein) [5].

There are two basic forms of probiotic microorganisms used in foods: the vegetative form and the spore form. The vegetative form is more susceptible to high temperatures, moisture, acidity, shelf life of food, and negative environmental conditions during the manufacture of food than the spore form. However, some probiotic microorganisms do not have spore forms [4]. Fermentation conditions, freezing, thawing, drying, cell protection additives, rehydration of dried probiotics, and microencapsulation applications are factors that affect the survival of probiotic microorganisms during probiotic food production. Food compounds, food additives, oxygen content, redox potential, moisture content/water activity, storage temperature, pH and titration acidity, and packaging conditions are factors that also affect survival of probiotic microorganisms during storage [6]. Gastrointestinal system conditions and stress factors could cause significant loss of viable probiotic cells [7].

Lactic acid bacteria (LAB; for example, *Lactobacillus* and *Bifidobacterium* and some *Saccharomyces* species) are the microorganisms most commonly used in probiotic food production [8][9][10][11]. However, these microorganisms cannot survive heat treatment, for which the cold spot temperature is approximately 75 °C [8][10]. Heat treatment is not applicable for most probiotic foods that contain commercial probiotic microorganisms due to their sensitivity to heat. Nevertheless, it has been stated that this restriction could be overcome by the usage of spore-forming probiotic microorganisms. It is known that some non-pathogenic *Bacillus* species, which are not as well-known as LAB and yeasts, are being used as probiotics [12]. The survival and stability of these bacteria have considerably improved compared to others through their spore-forming abilities. They are identified as an ideal choice in order to development of functional foods by protecting their vitality in high-temperature applications [13][14].

Bacillus coagulans (*B. coagulans*) was firstly isolated from spoiled milk [6]. In 1933, it was identified as *Lactobacillus sporogenes* by Horowitz-Wlassowa and Nowotelnov. Afterwards, it was classified as *B. coagulans* [15].

B. coagulans is a gram-positive, facultative anaerobic, nonpathogenic, spore-forming, lactic acid-producing bacteria [4]. It is resistant to heat; the optimum growth temperature for *B. coagulans* is 35 to 50 °C and the optimum growth pH is 5.5 to 6.5 [4][15]. It has the characteristics of microorganisms used as probiotics [15]. Some strains of *B. coagulans* have been reported as facultative anaerobe, thermophile bacteria able to grow at pH 6.2, 60–65 °C [6][16]. Although *B. coagulans* produces acid, it does not produce gas from maltose, raffinose, mannitol, and sucrose fermentation. It was reported that *B. coagulans* causes deterioration in dairy, fruit, and vegetable products due to acid production. In addition to lactic acid production, some strains also produce thermostable α -amylase [4][17]. For this reason, *B. coagulans* is important from an industrial point of view. *B. coagulans* spores are terminal, while spores of other species are central or subterminal. Furthermore, it differs from other *Bacillus* species due to the absence of cytochrome-C oxidase, and it does not reduce nitrate to nitrite [4]. It was reported that *B. coagulans* could grow at pH 4.5 at 65 °C and was isolated from products containing milk and carbohydrate [18].

B. coagulans has been reported as safe by the US Food and Drug Administration (FDA) and the European Union Food Safety Authority (EFSA) and is on the Generally Recognized As Safe (GRAS) and Qualified Presumption of Safety (QPS) list [19]. In addition, it was reported that genome sequencing can provide information about the overall characterization of the bacterium, for example with respect to its safety as a food supplement [20]. The *B. coagulans* GBI-30, 6086 genome was investigated, and it was found that it did not contain any hazardous genes [21]. Some of the non-pathogenic strains among the 100 known *Bacillus* spp., including *B. coagulans* and *Bacillus subtilis* var. *natto*, were stated as safe for human consumption [22][23].

2. Probiotic Activity of *B. coagulans*

Heat-treated food products are generally not used for probiotic purposes because of the factors affecting their viability and stability [15]. In order to obviate this difficulty, *B. coagulans*, *Bacillus racemilacticus*, and *Bacillus laevolacticus* as well as the *Sporolactobacillus* genus could be used as probiotics due to their heat-resistant spore forms [12][15]. Although there are limited research studies on the use of *Bacillus* spp. in human nutrition, many food products containing *B. coagulans* have been sold in various countries (Table 1). Traditionally, probiotic microorganisms have been used as freeze-dried in probiotic food supplements, in dairy products such as yogurt, and in fermented beverages [24][25][26]. The viability and stability of these bacteria improved considerably compared to others by means of spore formation. It is stated that they are an ideal choice for the development of cereal-based functional products because they can maintain their viability in heat-treated processes such as baking and boiling. In addition, the spores gain a stable state during the food storage [13].

Table 1. Probiotic food supplements containing *Bacillus coagulans*.

Strain	Supplement	Reference
<i>Bacillus coagulans</i> 15B	Nutrition essentials Probiotic	[27]
<i>B. coagulans</i> and <i>Bacillus subtilis</i> (<i>B. subtilis</i>)	NutriCommit	[27]
<i>B. coagulans</i> and <i>Saccharomyces boulardii</i>	Flora3	[27]
<i>B. coagulans</i>	THORNE	[27]
<i>B. coagulans</i>	Sunny Green Cleansing Green	[27]
<i>Bacillus indicus</i> HU36, <i>B. coagulans</i> , <i>Bacillus clausii</i> (<i>B. clausii</i>), <i>Bacillus subtilis</i> HU58	Just Thrive	[27]
<i>Bacillus indicus</i> , <i>B. subtilis</i> , <i>B. coagulans</i> , <i>Bacillus licheniformis</i> , <i>B. clausii</i>	MegaSporeBiotic	[27]

Strain	Supplement	Reference
<i>B. coagulans</i>	Sustenex	[26]
<i>B. coagulans</i>	Neolactoflorene	[26]
<i>B. coagulans</i>	GanedenBC30	[28]

The survival rates of *Lactobacillus* strains are highly affected by the production process, storage, and transportation of food. It is reported that some strains of *B. coagulans* are better able to survive in high-temperature heat treatment and stomach conditions than other commercial probiotic microorganisms. It is suggested that strains which have these properties are likely to survive better in the digestive tract [29].

B. coagulans GBI-30, 6086 is a commercial probiotic mixture also known as GanedenBC³⁰ [13][20]. Many research studies have been conducted and have reported the beneficial effects of *B. coagulans* GBI-30, 6086 on human and animal health [30][31][32]. It has been reported as safe by EFSA, and included in the GRAS and QPS list. It is available in various probiotic foods in markets [13][20].

3. Products of *B. coagulans*

In recent years, biological production of many metabolites (such as ethanol, lactic acid, fumaric acid, xylonix acid and other important products) has attracted greater attention as compared to chemical production with petroleum materials [33]. Various substances produced by *B. coagulans* are shown in **Table 2**.

Table 2. Substances produced by *B. coagulans*.

Strain	Substrate	Product	Reference
<i>Bacillus coagulans</i> DSM 2314	Wheat straw	Lactic acid	[34]
<i>Bacillus coagulans</i> DSM2314	Sugarcane bagasse	Lactic acid	[35]
<i>B. coagulans</i>	Sorghum water	Lactic acid	[36][37]
<i>B. coagulans</i>	Coffee extract	Lactic acid	[38]
<i>Bacillus coagulans</i> IPE 22	Wheat straw	Lactic acid	[39]
<i>Bacillus coagulans</i> LA 204	Corn stover	Lactic acid	[40]
<i>B. coagulans</i>	Corn stover	Lactic acid	[41]
<i>Bacillus coagulans</i> HL-5	Corn flour	Lactic acid	[42]
<i>Bacillus coagulans</i> TB/04	Medium	Lactic acid	[43]
<i>Bacillus coagulans</i> PS5	Medium	Lactic acid	[44]
<i>Bacillus coagulans</i> arr4	Granulated sugar and yeast extract	Lactic acid	[45]

Strain	Substrate	Product	Reference
<i>Bacillus coagulans</i> JI12	Oil palm empty fruit bunch	Lactic acid	[46]
<i>Bacillus coagulans</i> RCS3	Medium	β -galactosidase	[47]
<i>Bacillus coagulans</i> KM-1	Fermented soybean	α -galactosidase	[48]
<i>Bacillus coagulans</i> BL174	Medium	α -galactosidase	[49]
<i>Bacillus coagulans</i> B49	Wheat bran	α -amylase	[50]
<i>Bacillus coagulans</i> BL174	Medium	Lipase	[49]
<i>Bacillus coagulans</i> ZJU318	Medium	Lipase	[51]
<i>B. coagulans</i>	Melon wastes	Lipase	[52]
<i>Bacillus coagulans</i> VKI1	Coconut oil cake	Lipase	[53]

References

- Kesenaş, H.; Kınık, Ö.; Seçkin, K.; Günç Ergönül, P.; Akan, E. Keçi sütünden üretilen sinbiyotik beyaz peynirde *Enterococcus faecium*, *Bifidobacterium longum* ve *Lactobacillus paracasei* ssp. *paracasei* sayılarının değişimi. Ege Üniv. Ziraat Fak. Derg. 2018, 53, 75–81.
- Lebeer, S.; Bron, P.A.; Marco, M.L.; Pijkeren, J.P.V.; Motherway, M.O.; Hill, C.; Pot, B.; Roos, S.; Klaenhammer, T. Identification of probiotic effector molecules: Present state and future perspectives. Curr. Opin. Biotechnol. 2018, 49, 217–223.
- Küçükçetin, A.; Göçer, E.M.Ç.; Ergin, F.; Arslan, A.A. Farklı inkübasyon sıcaklığı ile inkübasyon sonlandırma pH'sının probiyotik yoğurdun fizikokimyasal ve mikrobiyolojik özellikleri üzerine etkisi. Akademik Gıda 2016, 14, 341–350.
- Aşan Özusağlam, M. Importance of *Bacillus coagulans* Bacterium as Probiotic in Animal Nutrition. Süleyman Demirel Üniv. Ziraat Fak. Derg. 2010, 5, 50–57.
- Kalkan, S. Probiyotik laktik asit bakterilerinin *Staphylococcus aureus*'a karşı antimikrobiyel etkilerinin farklı matematiksel modeller ile analizi. Sinop Univ. J. Nat. Sci. 2016, 1, 150–159.
- Kristjansson, J.K. Thermophilic Bacteria; CRC Press: Boca Raton, FL, USA, 1991; pp. 26–29.
- Ünal Turhan, E.; Erginkaya, Z.; Polat, S.; Özer, E.A. Design of probiotic dry fermented sausage (sucuk) production with microencapsulated and free cells of *Lactobacillus rhamnosus*. Turk. J. Vet. Anim. Sci. 2017, 41, 598–603.
- Ruiz, L.; Ruas-Madiedo, P.; Gueimonde, M.; De Los Reyes-Gavilán, C.G.; Margolles, A.; Sánchez, B. How do *Bifidobacteria* counteract environmental challenges? Mechanisms involved and physiological consequences. Genes Nutr. 2011, 6, 307–318.
- Budak Bağdatlı, A.; Kundakçı, A. Fermente et ürünlerinde probiyotik mikroorganizmaların kullanımı. Celal Bayar Üniv. J. Sci. 2013, 9, 31–37.
- Baka, M.; Noriega, E.; Tsakali, E.; Van, I.; Van Impe, J.F.M. Influence of composition and processing of Frankfurter sausages on the growth dynamics of *Listeria monocytogenes* under vacuum. Food Res. Int. 2015, 70, 94–100.
- Garriga, M.; Aymerich, T.; Jofré, A. Probiotic fermented sausages: Myth or reality? Procedia Food Sci. 2015, 5, 133–136.
- Hyronimus, B.; Le Marrec, C.; Sassi, A.H.; Deschamps, A. Acid and bile tolerance of spore-forming lactic acid bacteria. Int. J. Food Microbiol. 2000, 61, 193–197.

13. Fares, C.; Menga, V.; Martina, A.; Pellegrini, N. Nutritional profile and cooking quality of a new functional pasta naturally enriched in phenolic acids, added with β -glucan and *Bacillus coagulans* GBI-30, 6086. *J. Cereal Sci.* 2015, 65, 260–266.
14. Hosseini, H.; Pilevar, Z. Effects of starter cultures on the properties of meat products: A review. *Ann. Res. Rev. Biol.* 2017, 17, 1–17.
15. Karri, S.K.; Majeed, M.; Natarajan, S.; Sivakumar, A.; Ali, F.; Pande, A.; Majeed, S. Evaluation of anti-diarrhoeal activity of *Bacillus coagulans* MTCC 5856 and its effect on gastrointestinal motility in wistar rats. *Int. J. Pharm. Biol. Sci.* 2016, 7, 311–316.
16. Benson, K.F.; Redman, K.A.; Carter, S.G.; Keller, D.; Farmer, S.; Endres, J.R.; Jensen, S.J. Probiotic metabolites from *Bacillus coagulans* GanedenBC30™ support maturation of antigen-presenting cells in vitro. *World J. Gastroenterol.* 2012, 18, 1875–1883.
17. De Clerk, E.; Rodriguez-Diaz, M.; Forsyth, G.; Lebbe, L.; Logan, N.A.; DeVos, P. Polyphasic characterization of *Bacillus coagulans* strains, illustrating heterogeneity within this species, and emended description of the species. *Syst. Appl. Microbiol.* 2004, 27, 50–60.
18. Ercan Akkaya, S.; Kıvanç, M. Termofil bakteriler; sıcak su kaynaklarında yaşayan Gr (+) basillerin izolasyon ve identifikasyon yöntemleri. *AKÜ Fen Bilim. Derg.* 2008, 2, 61–70.
19. EFSA. Scientific Opinion on The Maintenance of the List of QPS Biological Agents Intentionally Added to Food and Feed (2013 update). *EFSA J.* 2013, 11, 3449.
20. Orrù, L.; Salvetti, E.; Cattivelli, L.; Lamontanara, A.; Michelotti, V.; Capozzi, V.; Spano, G.; Keller, D.; Cash, H.; Martina, A.; et al. Draft genome sequence of *Bacillus coagulans* GBI-30, 6086, a widely used spore-forming probiotic strain. *Genome Announc.* 2014, 2.
21. Salvetti, E.; Orrù, L.; Capozzi, V.; Martina, A.; Lamontanara, A.; Keller, D.; Cash, H.; Felis, G.E.; Cattivelli, L.; Torriani, S.; et al. Integrate genome-based assessment of safety for probiotic strains: *Bacillus coagulans* GBI-30, 6086 as a case study. *Appl. Microbiol. Biotechnol.* 2016, 100, 4595–4605.
22. Urdaci, M.C.; Bressollier, P.; Pinchuk, I. *Bacillus clausii* probiotic strains: Antimicrobial and immunomodulatory activities. *J. Clin. Gastroenterol.* 2004, 38, 86–90.
23. Nithya, V.; Halami, P. Evaluation of the probiotic characteristics of *Bacillus* species isolated from different food sources. *Ann. Microbiol.* 2013, 63, 129–137.
24. Gülmez, M.; Güven, A. Probiyotik, prebiyotik ve sinbiyotikler. *Kafkas Üniv. Vet. Fak. Derg.* 2002, 8, 83–89.
25. Uymaz, B. Probiyotikler ve kullanım alanları. *Pamukkale Üniv. Müh. Bilim. Derg.* 2010, 16, 95–104.
26. Cutting, S.M. *Bacillus* probiotics. *Food Microbiol.* 2011, 28, 214–220.
27. Elshagabee, F.M.F.; Rokana, N.; Gulhane, R.D.; Sharma, C.; Panwar, H. *Bacillus* as Potential Probiotics: Status, Concerns, and Future Perspectives. *Frontiers Microbiol.* 2017, 8, 1490.
28. Kalman, D.S.; Schwartz, H.I.; Alvarez, P.; Feldman, S.; Pezzullo, J.C.; Krieger, D.R. A prospective, randomized, double-blind, placebo-controlled parallel-group dual site trial to evaluate the effects of a *Bacillus coagulans*-based product on functional intestinal gas symptoms. *BMC Gastroenterol.* 2009, 9, 85.
29. Endres, J.R.; Clewell, A.; Jade, K.A.; Farber, T.; Hauswirth, J.; Schauss, A.G. Safety assessment of a proprietary preparation of a novel probiotic, *Bacillus coagulans*, as a food ingredient. *Food Chem. Toxicol.* 2009, 47, 1231–1238.
30. Siezen, R.J.; Wilson, G. Probiotics genomics. *Microb. Biotechnol.* 2010, 3, 1–9.
31. Honda, H.; Hoyles, L.; Gibson, G.; Farmer, S.; Keller, D.; McCartney, A.L. Impact of GanedenBC30 (*Bacillus coagulans* GBI-30, 6086) on population dynamics of the human gut microbiota in a continuous culture fermentation system. *Int. J. Probiot. Prebiot.* 2011, 6, 65–72.
32. Jäger, R.; Shields, K.A.; Lowery, R.P.; De Souza, E.O.; Partl, J.M.; Hollmer, C.; Purpura, M.; Wilson, J.M. Probiotic *Bacillus coagulans* GBI-30, 6086 reduces exercise-induced muscle damage and increases recovery. *PeerJ* 2016, 4, e2276.
33. Zhou, J.; Ouyang, J.; Xu, Q.; Zheng, Z. Cost-effective simultaneous saccharification and fermentation of L-lactic acid from bagasse sulfite pulp by *Bacillus coagulans* CC17. *Bioresour. Technol.* 2016, 222, 431–438.
34. Maas, R.H.W.; Bakker, R.R.; Jansen, M.L.A.; Visser, D.; de Jong, E.; Eggink, G.; Weusthuis, R.A. Lactic acid production from lime-treated wheat straw by *Bacillus coagulans*: Neutralization of acid by fed-batch addition of alkaline substrate. *Appl. Microbiol. Biotechnol.* 2008, 78, 751–758.
35. Van der Pol, E.C.; Eggink, G.; Weusthuis, R.A. Production of L-(+)-lactic acid from acid pretreated sugarcane bagasse using *Bacillus coagulans* DSM2314 in a simultaneous saccharification and fermentation strategy. *Biotechnol. Biofuels*

36. Ou, M.S.; Awasthi, D.; Nieves, I.; Wang, L.; Erickson, J.; Vermerris, W.; Ingram, L.O.; Shanmugam, K.T. Sweet sorghum juice and bagasse as feedstocks for the production of optically pure lactic acid by native and engineered *Bacillus coagulans* strains. *Bioenergy. Res.* 2016, 9, 123–131.
37. Wang, Y.; Chen, C.; Cai, D.; Wang, Z.; Qin, P.; Tan, T. The optimization of L-lactic acid production from sweet sorghum juice by mixed fermentation of *Bacillus coagulans* and *Lactobacillus rhamnosus* under unsterile conditions. *Bioresour. Technol.* 2016, 218, 1098–1105.
38. Neu, A.K.; Pleissner, D.; Mehlmann, K.; Schneider, R.; Puerta-Quintero, G.I.; Venus, J. Fermentative utilization of coffee mucilage using *Bacillus coagulans* and investigation of down-stream processing of fermentation broth for optically pure L-(+)-lactic acid production. *Bioresour. Technol.* 2016, 211, 398–405.
39. Zhang, Y.; Chen, X.; Luo, J.; Qi, B.; Wan, Y. An efficient process for lactic acid production from wheat straw by a newly isolated *Bacillus coagulans* strain IPE22. *Bioresour. Technol.* 2014, 158, 396–399.
40. Hu, J.; Zhang, Z.; Lin, Y.; Zhao, S.; Mei, Y.; Liang, Y.; Peng, N. High-titer lactic acid production from NaOH-pretreated corn stover by *Bacillus coagulans* LA204 using fed-batch simultaneous saccharification and fermentation under non-sterile condition. *Bioresour. Technol.* 2015, 182, 251–257.
41. Ma, K.; Hu, G.; Pan, L.; Wang, Z.; Zhou, Y.; Wang, Y.; Ruan, Z.; He, M. Highly efficient production of optically pure L-lactic acid from corn stover hydrolysate by thermophilic *Bacillus coagulans*. *Bioresour. Technol.* 2016, 219, 114–122.
42. Lv, X.; Yu, B.; Tian, X.; Chen, Y.; Wang, Z.; Zhuang, Y.; Wang, Y. Effect of pH, glucoamylase, pullulanase and invertase addition on the degradation of residual sugar in L-lactic acid fermentation by *Bacillus coagulans* HL-5 with corn flour hydrolysate. *J. Taiwan Inst. Chem. Eng.* 2016, 61, 124–131.
43. Payot, T.; Chemaly, Z.; Fick, M. Lactic acid production by *Bacillus coagulans*—Kinetic studies and optimization of culture medium for batch and continuous fermentations. *Enzyme Microbial Technol.* 1999, 24, 191–199.
44. Fan, R.; Ebrahimi, M.; Quitmann, H.; Aden, M.; Czermak, P. An Innovative Optical Sensor for the Online Monitoring and Control of Biomass Concentration in a Membrane Bioreactor System for Lactic Acid Production. *Sensors* 2016, 16, 411.
45. Coelho, L.F.; Beitel, S.M.; Sass, D.C.; Neto, P.M.A.; Contiero, J. High-titer and productivity of L-(+)-lactic acid using exponential fed-batch fermentation with *Bacillus coagulans* arr4, a new thermotolerant bacterial strain. *3 Biotech* 2018, 8, 213.
46. Juturu, V.; Wu, J.C. Production of high concentration of L-lactic acid from oil palm empty fruit bunch by thermophilic *Bacillus coagulans* JI12. *Biotechn. App. Biochem.* 2018, 65, 145–149.
47. Batra, N.; Singh, J.; Banerjee, U.C.; Patnaik, P.R.; Sobti, R.C. Production and characterization of a thermostable β -galactosidase from *Bacillus coagulans* RCS3. *Biotechnol. Appl. Biochem.* 2002, 36, 1–6.
48. Nam, K.H.; Jang, M.S.; Park, H.Y.; Koneva, E. Biochemical characterization of α -galactosidase-producing thermophilic *Bacillus coagulans* KM-1. *Korean J. Fish. Aquat. Sci.* 2014, 47, 516–521.
49. Parkouda, C.; Diawara, B.; Debrah, K. Enzyme profiles of potential starter cultures for the fermentation of baobab seeds. *Afr. J. Food Sci.* 2014, 8, 249–252.
50. Babu, K.R.; Satyanarayana, T. α -amylase production by thermophilic *Bacillus coagulans* in solid state fermentation. *Process Biochem.* 1995, 30, 305–309.
51. Lianghua, T.; Liming, X. Purification and partial characterization of a lipase from *Bacillus coagulans* ZJU318. *Appl. Biochem. Biotechnol.* 2005, 125, 139–146.
52. Alkan, H.; Baysal, Z.; Uyar, F.; Doğru, M. Production of lipase by a newly isolated *Bacillus coagulans* under solid-state fermentation using melon wastes. *Appl. Biochem. Biotechnol.* 2007, 136, 183–192.
53. Gowthami, P.; Muthukumar, K.; Velan, M. Utilization of coconut oil cake for the production of lipase using *Bacillus coagulans* VKI1. *Biocontrol Sci.* 2015, 20, 125–133.