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Obtaining High Yields of *Bacillus* species During Solid-State Fermentation of Plant Raw Materials for Use as a Feed Additive

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ABSTRACT

Bacillus spp. are a natural alternative to antibiotic therapy and are most suitable for use as probiotic feed additives to improve the growth and productivity of farm animals. The present study aimed to establish culture conditions suitable for the maximal production of probiotic bacteria spores and enzymes of *Bacillus subtilis* IMB-73 and *Bacillus amyloliquefaciens* IMB-79 in solid-state fermentation (SSF) of wheat bran, soybean flour, and cilantro. The tested lignocellulosic materials ensured rapid and abundant growth of bacilli; however, wheat bran proved to be the most suitable growth substrate, yielding the highest spore counts of 5.1×10^{11}/g and 5.5×10^{11}/g in the cultivation of *B. amyloliquefaciens* IMB-79 and *B. subtilis* IMB-73, respectively. Supplementation of an additional nitrogen source to the wheat bran medium resulted in a significant increase in spore productivity. In particular, peptone at a concentration of 67 mg/g substrate provided the maximum spore yield in the fermented product. Both strains secreted high endoglucanase and xylanase activities in the SSF of wheat bran. Additional nitrogen sources slightly suppressed the secretion of enzymes during the SSF of wheat bran by *B. subtilis* IMB-73 but increased cellulase activity in cilantro SSF by approximately 2-5 times. The ease of production and the high spore yield achieved by scaling up production in polypropylene bags demonstrated the feasibility of using the developed technology for commercialization on local agricultural farms.

Keywords: *Bacillus*, Cellulase, Lignocellulose fermentation, Probiotic, Spore production

INTRODUCTION

One of the primary challenges in agriculture in Georgia is the use of antibiotics to prevent diseases in farm animals and poultry. Other challenges facing the economy and agriculture include the lack of local production of probiotics and dependence of the agri-food industry on imports, and the generation of large quantities of by-products and waste by the food industry, which are mostly dumped in open areas, leading to environmental problems.

Research in recent years has shown that *Bacillus* spp. bacteria can prevent gastrointestinal infections and are a natural alternative to antibiotic therapy for use in animal and poultry farms to enhance their growth, productivity, and all performance characteristics (Popov et al., 2021; Herrmann et al., 2023; Liao et al., 2023). These spore-forming bacteria are the most suitable for use as probiotic feed additives. In their spore form, *Bacillus* spp. can withstand harsh physical and chemical environmental conditions during industrial processes, storage, and application. In their vegetative form, the bacteria manifest immunomodulatory properties and the ability to synthesize a wide range of biologically active compounds that provide high efficacy against pathogens.

Given these circumstances, there is a need to develop a simple, low-cost, and highly effective technology for obtaining probiotics using the biosynthetic potential of individual bacilli and their ability to utilize lignocellulose (Mahariawan et al., 2021; Herrmann et al., 2023). The

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nutrient medium should generally support rapid growth and high cell density and ensure their subsequent sporulation. For example, under submerged fermentation conditions, mandarin peel at a concentration of 40 g/L was found to be the best substrate among ten lignocellulosic materials for maximum production of *Bacillus subtilis* KATMIRA 1933 spores (Khardziani et al., 2017). Mahariawan et al. (2021) proved that wheat flour is a promising growth substrate for submerged fermentation to produce spores of *Bacillus megaterium*. Grigs et al. (2023) developed a low-cost medium using locally available broad bean flour and molasses and achieved high spore yields of *Bacillus subtilis* MSCL 897 with a sporulation efficiency of 80-90%.

Compared with submerged fermentation, solid-state fermentation (SSF) of plant materials is known to have several advantages, such as relatively high volumetric productivity and product concentration, simpler downstream processing, less wastewater generation, and the need for simple fermentation equipment (Rodríguez-Couto, 2019). Besides, the SSF is the most appropriate for organizing on-site tailor-made probiotics production, when the whole fermented products are directly used as feed additives (Siqueira et al., 2020). Of course, to achieve the maximum yield of spores, it is necessary to optimize the nutrient medium and cultivation conditions to ensure the maximum formation of bacterial biomass and spores.

Recent studies have shown that *B. subtilis* IMB-73 and *B. amyloliquefaciens* IMB-79 spores stimulate broiler growth and improve feed conversion (Chistyakov et al., 2015; Chkuaseli et al., 2021). Moreover, toxicogenic and mutagenic tests, as well as the ability to coaggregate with *Escherichia coli* and *Pseudomonas aeruginosa*, tolerance to incubation in 0.3% bile salts and pH 2.0-3.0, showed that these strains are promising for food and medical (Algburi et al., 2016).

This study aimed to elucidate the cultivation conditions suitable for the growth and probiotic spore production by *B. subtilis* IMB-73 and *B. amyloliquefaciens* IMB-79 during SSF of abundantly available plant materials to develop a simple technology that could be used and commercialized in local agricultural farms.

MATERIALS AND METHODS

Strains and inoculum preparation

Bacillus subtilis IMB-73 (former strain KATMIRA, 1933) and *B. amyloliquefaciens* IMB-79 (former strain B-1895) obtained from the Russian National Collection of Industrial Microorganisms (Moscow, Russia) were used for the probiotic bacteria production. For inoculum preparation, the bacteria were grown for 24 hours on a rotary shaker at 160 rpm, 37°C in the nutrient medium of the following composition (g/L): Glucose (2.0), yeast extract (2.0), peptone (2.0), KH_2PO_4 (1.0), $MgSO_4$ (0.5). The cells were then harvested, resuspended in sterile physiological saline, and served as the inoculum for SSF.

Lignocellulosic materials

Wheat bran and soybean meal were obtained from the feed company Nutrimax (Georgia), which is interested in enriching these materials with probiotics. Coriander residue was obtained from the essential oil company AromaAmbra (Georgia), which is interested in valorizing this by-product. This material was tested for the first time as a substrate for bacterial growth. These plant residues were oven-dried at 50°C, ground, and sieved through standard-mesh sieves to obtain particles ranging from 0.5 mm to 3 mm.

Cultivation conditions

The SSF of selected plant materials was carried out in 100 mL flasks containing 4 g of substrates moistened with 13 mL of the medium (g/L): KH_2PO_4 (1.0), $MgSO_4 \cdot 7H_2O$ (0.5), peptone (10.0), NaCl (2.0). Three inorganic (physiologically base salt KNO_3, physiologically acid salt $(NH_4)_2SO_4$, and NH_4NO_3) and three organic (peptone, yeast extract, and casein hydrolysate) nitrogen sources were tested for the optimum spore production. Control lacking an additional nitrogen source was run in parallel. The initial pH of all media for cultivating bacteria was adjusted to 7.0 before sterilization at 121^{o}C for 30 minutes. One mL of properly diluted bacterial suspension (to have in inoculated medium 1-2 × 10^6 colony-forming units [CFU]/g substrate) was used to inoculate each flask. The contents of the flasks were well mixed and incubated at 37^{o}C for 4 days.

After 3 and 4 days of SSF, the total fermented biomass was weighed and divided into three parts. One part was used for spore count; for this purpose, 1 g of the biomass was placed into sterile 100 mL flasks, mixed with 10 mL of sterile physiological saline supplemented with 0.1% Tween 80, and homogenized by vigorous vortexing for 1-2 minutes. The second part was utilized for the measurement of the enzymatic activity; specifically, 2 g of the biomasses were extracted twice with 15 mL of distilled water (total volume 30 mL), the solids were separated by centrifugation at 10,000 g for 5 minutes at 4^{o}C, and the supernatants were analyzed for pH, reducing sugars (RS) and cellulase activity. The remaining biomass was

weighed and dried at 105°C to determine the biomass's dry weight.

To scale up the probiotic production, 0.5 kg of milled wheat bran or cilantro was soaked in the optimized nutrient medium to 75% saturation and placed in polypropylene gas-permeable bags, Microsac PPB75/BEU6/X33-57 (SACO2, EKE, Belgium) for sterilization by autoclaving at 121°C for 1 hour. After cooling, the bags were inoculated with bacterial suspension to receive 1×10^6 CFU/g substrate and well mixed to distribute the inoculum evenly. Inoculated bags were incubated for 4 days in the dark in a climate camera at 32°C.

Spore count

The flasks with suspended biomass were heated at 80°C for 10 minutes to kill the remaining vegetative cells. Then 100 µL samples were taken from the 10^7-10^9 diluted suspensions and spread plated onto the sterile agar medium of the following composition (g/L): glucose (2.0), KH_2PO_4 (1.0), $MgSO_4$ (0.5), peptone (2.0), yeast extract (2.0), agar (17.0), pH (7.0). The plated samples were incubated at 37°C for 24 hours and 48 hours, and the grown colonies were counted. The average values from 3 independent plates were used in all experiments to calculate the number of spores per g of dry fermented biomass.

Cellulase and xylanase activity assay

The supernatants obtained after biomass separation were analyzed for carboxymethyl cellulase (CMCase, EC 3.2.1.4) activity according to the IUPAC recommendations, with 1% of low viscosity carboxymethyl cellulose in 50 mM citrate buffer (pH 5.0) at 50°C for 10 minutes (Ghose, 1987). Xylanase (EC 3.2.1.8) activity was determined in the same conditions using birchwood xylan (Roth 7500, 1% w/v) as an enzyme substrate (Bailey et al., 1992). Glucose and xylose standard curves were used to calculate cellulase and xylanase activities. The release of reducing sugars was measured using the dinitrosalicylic acid reagent method. One unit of CMCase or xylanase activity was defined as the amount of enzyme required to release 1 µmol of glucose or xylose, respectively, per minute. Activities were referred to per biomass dry weight (U/g).

Statistical analysis

All experiments were performed twice in three replicates. The results obtained are expressed as the mean ± standard deviation. The mean values and standard deviations were calculated using Excel. Student's t-test was used to perform a statistical analysis of the differences among the experimental groups, and p-values < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Effect of lignocellulosic growth substrate

At the initial stage of the study, *B. subtilis* IMB-73 and *B. amyloliquefaciens* IMB-79 spore yield and enzyme activity in the fermented products were assessed during the SSF of selected materials. Unlike wheat bran and soybean meal, which are common components of culture media for microorganism cultivation and animal feed formulations, cilantro was used as a growth substrate for spore-forming bacteria cultivation for the first time.

The results depicted in Table 1 allow for drawing several important conclusions. Firstly, the tested lignocellulosic materials are suitable growth substrates, ensuring rapid and abundant visual growth of bacilli and the formation of many spores. Secondly, these findings are consistent with previous studies indicating that the spore yield significantly depends on the growth substrate available in the nutrient medium (Ren et al., 2018; Elisashvili et al., 2019). In particular, the wheat bran was the most appropriate growth substrate, providing the highest yield of spores, 5.1×10^{11}/g and 5.5×10^{11}/g in the cultivation of *B. amyloliquefaciens* IMB-79 and *B. subtilis* IMB-73, respectively. Conversely, cilantro and especially soybean meal appeared to be poorer growth substrates for spore formation by both bacterial species. It is likely that, compared to wheat bran, both substrates are richer in nutrients that remain available at the end of bacterial cultivation; therefore, the transition of vegetative cells to sporulation is delayed. Thirdly, all fermented products contain low concentrations of reducing sugars after four days of SSF lignocellulose. Low sugar concentrations are a prerequisite for the initiation of abundant sporulation by most *Bacillus* species studied, including *B. amyloliquefaciens* IMB-79 and *B. subtilis* IMB-73 (Posada-Uribe et al., 2015; Elisashvili et al., 2019). It should be noted that regardless of the bacterial species and the type of growth substrate, the maximum spore yield was observed after 4 days of SSF. This is probably explained by the fact that during the cultivation of bacteria, the availability of nutrients in the medium decreased, and only on the fourth day did the vegetative cells go into starvation and sporulation.

It is known that several *Bacillus* species, among them *B. amyloliquefaciens* and *B. subtilis*, are capable of

producing a high amount of industrially important enzymes (Yadav et al., 2020; My et al., 2022; Mushtaq et al., 2024). The results obtained show that both bacteria produce cellulase and xylanase during the SSF process. This ability of the bacilli allows them to hydrolyze lignocellulosic polysaccharides into metabolizable sugars and use them as carbon sources. Interestingly, during fermentation, the sugar content remains low enough to prevent catabolite repression of cellulase and xylanase synthesis by the bacteria. The results depicted in Table 1 indicated that compared to *B. amyloliquefaciens* IMB-79, the enzymatic activity of *B. subtilis* IMB-73 was less dependent on the lignocellulosic material in the medium, but in both bacterial cultures, xylanase activity was 4-12 times higher than endoglucanase activity. Interestingly, Mazanko et al. (2018) showed that feeding soy products fermented with *B. subtilis* KATMIRA1933 and *B. amyloliquefaciens* B-1895 to laying hens and roosters resulted in increased sperm production, egg production, quality, and hatchability. It is suggested that the hydrolases produced by the bacilli contributed to increased nutritional value and better feed digestibility.

Table 1. *Bacillus subtilis* IMB-73 and *Bacillus amyloliquefaciens* IMB-79 spore production and enzyme activity in solid-state fermentation of lignocellulosic growth substrates

Growth substrate	Reducing sugars (mg/g)	Spores (Number × 10^{11}/g)	CMCase (U/g)	Xylanase (U/g)
B. amyloliquefaciens IMB-79				
Cilantro	0.5 ± 0.2^{4}	$0.3 \pm 0^{4*}$	0.2 ± 0^{3}	2.2 ± 0.3^{4}
Wheat bran	1.7 ± 0.2^{4}	5.1 ± 0.2^{4}	0.5 ± 0.1^{4}	6.1 ± 0.5^{4}
Soybean meal	1.8 ± 0.2^{4}	0.2 ± 0.1^{4}	0.5 ± 0.1^{3}	3.7 ± 0.4^{4}
B. subtilis IMB-73				
Cilantro	0.4 ± 0.1^{4}	0.6 ± 0.1^{4}	0.6 ± 0.1^{3}	2.7 ± 0.3^{4}
Wheat bran	1.8 ± 0.2^{4}	5.6 ± 0.2^{4}	0.6 ± 0.1^{4}	2.8 ± 0.2^{4}
Soybean meal	1.3 ± 0.1^{4}	0.3 ± 0.1^{4}	0.4 ± 0^{3}	2.0 ± 0.2^{4}

* The numbers indicate the days of the maximum values. CMCase: Carboxymethyl cellulase

Effect of nitrogen source

The nature and concentration of nitrogen sources are important nutritional factors influencing both *Bacillus* growth and spore formation (Elisashvili et al., 2019). For example, a significant increase in spore productivity was observed during submerged fermentation of broad bean flour and molasses with *B. subtilis* MSCL 897 and the enrichment of the medium with yeast or corn extract (Grigs et al., 2023). It was indicated that all organic nitrogen sources favored spore production in the submerged fermentation of mandarin peels by *B. subtilis* KATMIRA1933 (now IMB-73), and peptone ensured almost a three-fold increase in the spore count as compared with the control medium (Khardziani et al., 2017). Therefore, several inorganic salts and organic compounds in the concentration of 40 mM as nitrogen were tested as nitrogen sources in addition to the nitrogen present in cilantro and wheat bran to determine their effect on spore production by *B. subtilis* IMB-73. The data revealed several general features (Tables 2 and 3). Firstly, adding organic nitrogen sources to the control medium resulted in an increase in the medium pH at the end of fermentation. Secondly, compared to cilantro, wheat bran without an additional nitrogen source is an excellent growth substrate, accumulating up to 1×10^{11} spores/g of fermented product. It is possible that processing cilantro seeds for essential oil extraction results in a reduction in the nutritional value of the residue or the formation of bacterial growth inhibitors. Thirdly, although spore yields varied significantly when bacilli were grown in the presence of nitrogen sources, all supplements were favorable for sporulation. Among them, inorganic salts increased the spore yield by 2.4-3 times, whereas organic nitrogen sources ensured at least a 5-fold increase in the spore count compared with the control medium. Fourthly, the effect of an additional nitrogen source on cellulase and xylanase production significantly depended on the lignocellulosic growth substrate in the nutrient medium. Adding any nitrogen source to the control medium containing wheat bran suppressed the secretion of enzymes, especially CMCase, by *B. subtilis* IMB-73. On the contrary, in the SSF of cilantro, the cellulase productivity of the same bacterial strain increased 2-5 times with supplementation of the control medium with additional nitrogen sources. It should be noted that although the xylanase activity of *B. subtilis* IMB-73 varied depending on the chemical nature of the nitrogenous compound, compared to endoglucanase activity, its

activity was less dependent on the presence of an additional nitrogen source in the nutrient medium. The data obtained showed that compared to the medium based on soybean meal and cilantro, *B. subtilis* IMB-73 performs better in the wheat bran-containing medium in the presence of peptone. Therefore, it was important to establish the concentration of this nitrogen source that is optimal for the maximum formation of probiotic spores. The results showed that adding peptone to the wheat bran medium at any concentration used can significantly increase ($p \leq 0.05$) spore productivity (Figure 1). The maximum spore yield was observed when the concentration of peptone in the nutrient medium was 100 mM nitrogen (specifically, 67 mg peptone/g substrate). A further twofold increase in the peptone content in the medium reduced the concentration of spores in the fermented product. It is known that sporulation generally occurs in unfavorable environments, such as nutrient starvation (Monteiro et al., 2014; Elisashvili et al., 2019). It is possible that increasing the peptone concentration beyond a certain point (100 mM) prevents nitrogen depletion and thus hinders sporulation, reducing the productivity of the culture. Undoubtedly, depletion of nutrients in the medium is necessary for effective sporulation (Monteiro et al., 2014).

A different response of the bacterial culture was revealed when measuring the enzymatic activity of *B. subtilis* IMB-73 depending on the nitrogen concentration. In this case, the maximum activity of endoglucanase and xylanase was detected in the control medium (Figure 1). The activity of both enzymes in the fermented product decreased as the concentration of the additional nitrogen source in the nutrient medium increased. Notably, the concentration of reducing sugars in the fermented products decreased from 5.8 in the control to 1.4-1.6 mg/g at a concentration of 100-200 mM nitrogen. Therefore, catabolite repression by sugars cannot be the reason for the decrease in cellulase and xylanase secretion during bacterial growth in the presence of high nitrogen concentrations. It is possible that the presence of excess peptone as a nitrogen source caused metabolic stress in vegetative cells and suppressed their biosynthetic activity (Mahariawan et al., 2021). This phenomenon requires further research.

Table 2. Effect of nitrogen sources on the *Bacillus subtilis* IMB-73 spore production and enzyme activity in solid-state fermentation of cilantro

Nitrogen source	Final pH	Reducing sugars (mg/g)	Spores (Number × 10^{11}/g)	CMCase (U/g)	Xylanase (U/g)
Control	7.3 ± 0.1	0.5 ± 0.1[3]	0.1 ± 0.03[4]	0.3 ± 0[3]	2.4 ± 0.6[3]
KNO_3	7.5 ± 0.1	0.2 ± 0[3]	0.3 ± 0.05[4]	0.7 ± 0.4[4]	2.3 ± 0.3[3]
NH_4NO_3	7.3 ± 0.1	0.2 ± 0.1[4]	0.3 ± 0.02[4]	1.6 ± 0.2[3]	3.2 ± 0.5[3]
$(NH_4)_2SO_4$	7.1 ± 0.1	0.2 ± 0.1[4]	0.3 ± 0.03[4]	0.7 ± 0.4[4]	2.7 ± 0.4[4]
Peptone	7.9 ± 0.1	0.4 ± 0.1[3]	0.6 ± 0.07[4]	0.7 ± 0.2[4]	2.6 ± 0.3[3]
Casein hydrolysate	7.7 ± 0.1	0.4 ± 0.1[3]	0.5 ± 0.07[4]	0.5 ± 0.2[4]	2.5 ± 0.2[4]
Yeast extract	7.8 ± 0.1	0.3 ± 0.1[3]	0.6 ± 0.09[4]	1.3 ± 0.4[3]	2.9 ± 0.2[3]

[3-4] The numbers indicate the days of the maximum values. CMCase: Carboxymethyl cellulase

Table 3. Effect of nitrogen sources on the *Bacillus subtilis* IMB-73 spore production and enzyme activity in solid-state fermentation of wheat bran

Nitrogen source	Final pH	Reducing sugars (mg/g)	Spores (Number × 10^{11}/g)	CMCase (U/g)	Xylanase (U/g)
Control	7.8 ± 0.1	6.1 ± 0.4[4]	1.0 ± 0.2[4]	1.1 ± 0.2[3]	3.7 ± 0.3[3]
KNO_3	7.7 ± 0.1	2.0 ± 0.2[3]	2.4 ± 0.2[4]	0.8 ± 0.1[3]	4.3 ± 0.2[4]
NH_4NO_3	7.8 ± 0.1	3.2 ± 0.3[4]	2.9 ± 0.3[4]	0.7 ± 0.1[4]	2.8 ± 0.2[4]
$(NH_4)_2SO_4$	7.6 ± 0.1	1.7 ± 0.2[4]	2.6 ± 0.2[4]	0.9 ± 0.2[3]	2.9 ± 0.2[4]
Peptone	8.2 ± 0.1	1.6 ± 0.2[4]	5.4 ± 0.2[4]	0.7 ± 0.1[3]	2.5 ± 0.2[3]
Casein hydrolysate	8.4 ± 0.1	2.9 ± 0.3[4]	4.7 ± 0.3[4]	0.8 ± 0.1[3]	4.7 ± 0.4[4]
Yeast extract	8.3 ± 0.1	1.7 ± 0.3[3]	5.3 ± 0.2[4]	1.0 ± 0.1[3]	2.9 ± 0.1[4]

[3-4] The numbers indicate the days of the maximum values. CMCase: Carboxymethyl cellulase

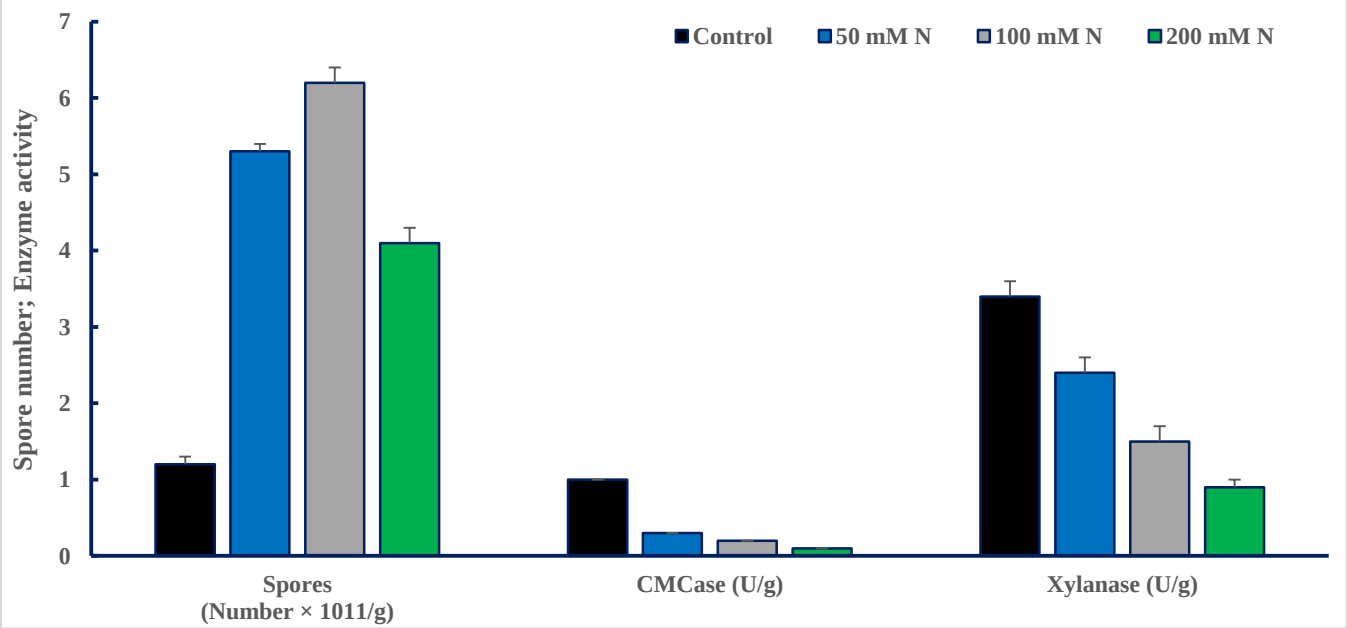

Figure 1. Effect of the peptone concentration on the *Bacillus subtilis* IMB-73 spore production and enzyme activity in solid-state fermentation of wheat bran

At the final stage, to test the feasibility of the conducted in-flask SSF process for pilot scale, cultivation conditions optimized for maximum probiotic spore production by *B. subtilis* IMB-73 were applied to both wheat bran and cilantro SSF in polypropylene bags. Analyses of the spore count after 4 days of culturing the bacteria revealed that the spore yield in the fermented product reached 5.5×10^{11}/g and 0.6×10^{11}/g of dry biomass, respectively, during fermentation of bran and cilantro. Thus, the mass production experiment confirmed the results obtained when culturing bacteria in flasks. These promising results indicate that SSF of locally available renewable feedstock supplemented with appropriate carbon and nitrogen sources provides high yields of *B. subtilis* IMB-73 spores and is the most suitable and low-cost method for establishing on-site probiotic production without requiring significant capital investment. If produced on site, poultry and livestock farms can directly use the entire fermented product as a feed additive.

CONCLUSION

The findings presented in this study provide new knowledge on the physiology of *B. amyloliquefaciens* IMB-79 and *B. subtilis* IMB-73 in the SSF of plant raw materials. Specifically, various lignocellulosic materials may be successfully exploited as growth substrates for cultivating spore-forming bacteria. Nevertheless, a strain-specific growth substrate should be revealed to ensure the highest spore productivity. During the fermentation of lignocellulose, *B. amyloliquefaciens* IMB-79 and *B. subtilis* IMB-73 secrete cellulase and xylanase activity to ensure hydrolysis of polysaccharides into metabolizable sugars to provide the bacterial cultures with the carbon and energy sources. Supplementing the nutrient medium with lignocellulosic growth substrate with an additional nitrogen source at an optimal concentration favors spore production with high yield. Further studies are needed to evaluate the effects of these probiotic bacteria on egg production parameters of laying hens and on growth, productivity, and feed conversion efficiency of broiler chickens.

DECLARATIONS

Authors' contributions

Tamar Khardziani and Eka Metreveli conducted the experiments and formalized and analyzed the data. Vladimir Elisashvili supervised the study and prepared the manuscript. All authors read and approved the last edition of the manuscript.

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Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

All the data and materials are available on request from the corresponding author.

Ethical considerations

The authors affirm that all ethical issues have been addressed, including plagiarism, consent to publish, misconduct, double publication and/or submission, and redundancy.

REFERENCES

Algburi A, Volski A, Cugini C, Walsh EM, Chistyakov VA, Mazanko MS, Bren AB, Dicks LMT, and Chikindas ML (2016). Safety properties and probiotic potential of *Bacillus subtilis* KATMIRA1933 and *Bacillus amyloliquefaciens* B-1895. Advances in Microbiology, 6(6): 432-452. DOI: http://www.doi.org/10.4236/aim.2016.66043

Bailey MJ, Biely P, and Poutanen K (1992). Interlaboratory testing of methods for assay of xylanase activity. Journal of Biotechnology, 23(3): 257-270. DOI: https://www.doi.org/10.1016/0168-1656(92)90074-J

Chistyakov V, Melnikov V, Chikindas ML, Khutsishvili M, Chagelishvili A, Bren A, Kostina N, Cavera V, and Elisashvili V (2015). Poultry-beneficial solid-state *Bacillus amyloliquefaciens* B-1895 fermented soybean formulation. Bioscience of Microbiota, Food and Health, 34(1): 25-28. DOI: https://www.doi.org/10.12938/bmfh.2014-012

Chkuaseli A, Chagelishvili A, Khutsishvili-Maisuradze M, Elisashvili V, Khardziani T, Lashkarashvili T, Chagelishvili G, Lapachi R, Samkurashvili K, and Berikashvili V (2021). New probiotic produced from spore forming *Bacillus* cultivated on local agro-industrial wastes substituting antibiotic in broiler nutrition. Annals of Agrarian Science, 19: 313-323. Available at: https://journals.org.ge/index.php/aans/article/view/287/213

Elisashvili V, Kachlishvili E, and Chikindas ML (2019). Recent advances in the physiology of spore formation for *Bacillus* probiotic production. Probiotics and Antimicrobial Proteins, 11(3): 731-747. DOI: https://www.doi.org/10.1007/s12602-018-9492-x

Ghose TK (1987). Measurement of cellulase activities. Pure and Applied Chemistry, 59(2): 257-268. DOI: http://www.doi.org/10.1351/pac198759020257

Grigs O, Didrihsone E, and Bolmanis E (2023). Investigation of a broad-bean based low-cost medium formulation for *Bacillus subtilis* MSCL 897 spore production. Fermentation, 9(4): 390. DOI: https://www.doi.org/10.3390/fermentation9040390

Herrmann LW, Letti LAJ, Penha RDO, Soccol VT, Rodrigues C, Soccol CR (2023). *Bacillus* genus industrial applications and innovation: First steps towards a circular bioeconomy. Biotechnology Advances, 70: 108300. DOI: https://www.doi.org/10.1016/j.biotechadv.2023.108300

Khardziani T, Kachlishvili E, Sokhadze K, Elisashvili V, Weeks R, Chikindas ML, and Chistyakov V (2017). Elucidation of *Bacillus subtilis* KATMIRA 1933 potential for spore production in submerged fermentation of plant raw materials. Probiotics and Antimicrobial Proteins, 9: 435-443. DOI: https://www.doi.org/10.1007/s12602-017-9303-9

Liao Z, Liu Y, Wei H, He X, Wang Z, Zhuang Z, Zhao W, Masagounder K, He J, and Niu J (2023). Effects of dietary supplementation of *Bacillus subtilis* DSM 32315 on growth, immune response and acute ammonia stress tolerance of Nile tilapia (*Oreochromis niloticus*) fed with high or low protein diets. Animal Nutrition, 15: 375-85. DOI: https://www.doi.org/10.1016/j.aninu.2023.05.016

Mahariawan IMD, Kusuma WE, Yuniarti A, Beltran MAG, Hariati AM (2021). Application of wheat flour (*Triticum aestivum*) on spore density and sporulation efficiency of *Bacillus megaterium* isolated from *Litopenaeus vannamei* gastrointestinal tract. Biodiversitas, 22(9): 3709-3715. DOI: https://www.doi.org/10.13057/biodiv/d220914

Mazanko MS, Gorlov IF, Prazdnova EV, Makarenko MS, Usatov AV, Bren AB, Chistyakov VA, Tutelyan AV, Komarova ZB, Mosolova NI, Pilipenko DN, Krotova OE, Struk AN, Lin A, and Chikindas ML (2018). *Bacillus* probiotic supplementations improve laying performance, egg quality, hatching of laying hens, and sperm quality of roosters. Probiotics and Antimicrobial Proteins, 10: 367-373. DOI: https://www.doi.org/10.1007/s12602-017-9369-4

Monteiro SMS, Clemente JJ, Carrondo MJT, and Cunha AE (2014). Enhanced spore production of *Bacillus subtilis* grown in a chemically defined medium. Advances in Microbiology, 4(8): 444-454. DOI: http://www.doi.org/10.4236/aim.2014.48049

Mushtaq Q, Ishtiaq U, Joly N, Qazi JI, and Martin P (2024). Amylase and cellulase production from newly isolated *Bacillus subtilis* using acid treated potato peel waste. Microorganisms, 12(6): 1106. DOI: https://www.doi.org/10.3390/microorganisms12061106

My NTH, Loan TT, and Thuoc DV (2022). High amylase production by a novel strain of *Bacillus amyloliquefaciens* M37 isolated from Can Gio mangrove forest, Vietnam. Biointerface Research in Applied Chemistry, 12(4): 4675-4685. DOI: https://www.doi.org/10.33263/BRIAC124.46754685

Popov IV, Algburi A, Prazdnova EV, Mazanko MS, Elisashvili V, Bren AB, Chistyakov VA, Tkacheva EV, Trukhachev VI, Donnik IM et al. (2021). A Review of the effects and production of spore-forming probiotics for poultry. Animals, 11(7): 1941. DOI: https://www.doi.org/10.3390/ani11071941

Posada-Uribe LF, Romero-Tabarez M, and Villegas-Escobar V (2015). Effect of medium components and culture conditions in *Bacillus subtilis* EA-CB0575 spore production. Bioprocess and Biosystems Engineering, 38: 1879-1888. DOI: https://www.doi.org/10.1007/s00449-015-1428-1

Ren H, Su Y, and Guo X (2018). Rapid optimization of spore production from *Bacillus amyloliquefaciens* in submerged cultures based on dipicolinic acid fluorimetry assay. AMB

Express, 8: 21. DOI: https://www.doi.org/10.1186/s13568-018-0555-x

Rodríguez-Couto S (2019). Current trends in the production of ligninolytic enzymes. In: S. Saran, V. Babu, and A. Chaubey (Editors), High value fermentation products. Scrivener Publishing LLC, Vol. II, pp. 81-106. DOI: https://www.doi.org/10.1002/9781119555384.ch4

Siqueira JGW, Rodrigues C, de Souza Vandenberghe LP, Woiciechowski AL, and Soccol C (2020). Current advances in onsitecellulase production and application on lignocellulosic biomass conversion to biofuels: A review. Biomass and Bioenergy, 132: 105419. DOI: https://www.doi.org/10.1016/j.biombioe.2019.105419

Yadav A, Mahaboob Ali AA, Ingawale M, Raychaudhuri S, Gantayet LM, and Pandit A (2020). Enhanced co-production of pectinase, cellulase and xylanase enzymes from *Bacillus subtilis* ABDR01 upon ultrasonic irradiation. Process Biochemistry, 92: 197-201. DOI: https://www.doi.org/10.1016/j.procbio.2020.01.011