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THE GROWTH AND PROBIOTIC CHARACTERISTICS OF *Bacillus velezensis* BS IN SOYBEAN MEAL USED AS SYNBIOTIC-LIKE PREPARATIONS FOR *Litopenaeus vannamei* CULTURE

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SUMMARY

Soybean meal (SBM) is the by-product of soybean oil extraction. It is well known to contain at least 47% protein and more than 6% crude fiber. This study showed that *Bacillus velezensis* BS grew well in medium containing 1% SBM and gained the prebiotic index and activity score of 15.40 ± 4.10 and 0.85 ± 0.22, respectively. In this medium, *B. velezensis* BS showed various probiotic characteristics, such as digestive enzyme secretion (α-amylase, cellulase, and protease), anti-*Vibrio parahaemolyticus*, biofilm formation on the solid surface after 24 h of interaction and the ability to grow in a broad range of environmental conditions (pH 6-9, temperature of 20-45ºC, salinity of 4%). In addition, SBM fermented with *B. velezensis* BS significantly reduced 79.7 ± 1.9% of the trypsin inhibitor content. Dietary supplement of the fermented product in *Litopenaeus vannamei* culture improved the specific growth rate of shrimp by approximately 7 times and concomitantly decreased the density of *V. parahaemolitycus* pathogen by 10 times compared to the group without synbiotic-like fed. Hence, the *B. velezensis* BS and its SBM fermented product were suggested as a potential feed additive in aquaculture applications.

Keywords: Bacillus velezensis, soybean meal, digestive enzymes secretion, trypsin inhibitor degradation, Vibrio parahaemolyticus resistant, synbiotic-like preparations

INTRODUCTION

Currently, the intensive farm area is expanding rapidly to meet the increasing human demand for seafood. The application of a high feed per unit area of land might lead to organic pollution and consequently the occurrence of disease outbreaks in these intensive farms (Anh *et al.*, 2010). Therefore, to sustainably develop the aquacultural industry, biotechnology is widely applied to environmentally friendly enhance the growth and pathogen-resistant ability of aquatics animals (Hardi *et al.*, 2022; Huynh *et al.*, 2017; Mugwanya *et al.*, 2022). Probiotics are live microorganisms that can secrete digestive enzymes such as proteases, α-amylase, and cellulases that help to improve digestion and hence increase the feed conversion rate of host organisms, while the prebiotic is a nondigestible food ingredient but can selectively stimulate the growth of beneficial bacteria. Studies showed that the supplement of the synbiotics which contained prebiotics such as MOS (mannooligosaccharides), FOS (fructooligosaccharide), and GOS (galactooligosaccharide) with probiotics such as

Enterococcus faecalis, Pediococcus acidilactici, and *Lactobacillus* significantly improved the intestinal microflora, and immune responses of animals (Firouzbakhsh *et al.,* 2014; Hoseinifar *et al.,* 2015). However, due to the high production cost, the addition of customary prebiotics in large-scale aquaculture farms is still limited. On the other hand, a study by Mandalari *et al.* showed that agricultural by-products such as nuts contain many polysaccharides, prebiotics, and vitamins (Mandalari *et al.,* 2007). Therefore, these agricultural by-products can be considered natural sources of prebiotics that can be used to supplement food production. Soybean meal (SBM) is a by-product of the soybean oil extraction process whose prebiotics content accounted for approximately 6% of dried weight (Choct *et al.,* 2010). SBM also contains the protease inhibitors like trypsin inhibitors (TI) that can interfere with digestion activity (Kårlund *et al.,* 2021). The common protease enzymes found in the hepatopancreas of white leg shrimp include trypsin, chymotrypsin, and elastase (Senphan *et al.*, 2015). When ingested by shrimp, the TI will form competitive bindings that reduce proteolytic activity and chymotrypsin enzyme activity by 65%. The existence of TI in the shrimp gastrointestinal tract from 2 to 4 hours also inhibited 40% of trypsin enzyme activity (Maytorena-Verdugo *et al.,* 2017). However, trypsin inhibitors are highly stable and difficult to degrade during heat treatment to create animal feed. Interestingly, Gao et al. proved that about 57.1% of the trypsin inhibitor (TI) could be removed by fermentation of SBM with probiotics *L. brevis* (Gao *et al.,* 2013). Therefore, the investigation of using soybean meal for promoting the growth of probiotics and the production of synbiotic-like perparations should be carried out to evaluate its prebiotic potential.

In this study, we evaluated whether bacteria can utilize the prebiotic present in soybean meal and determine the prebiotic index and activity score of the soybean meal by the newly isolated probiotic, *Bacillus velezensis* BS. The effect of soybean meal-containing medium on the growth, digestive enzyme secretion, and pathogenic resistance of the *B. velezensis* BS were also examined. In addition, a high biofilm formation ability of the bacteria would facilitate the adhesion of bacteria to SBM during the fermentation, thus having an advantage on the ability of beneficial bacteria to adhere to the surface of the intestinal tract of aquatic animals. Solid-state fermentation of BS strain with soybean meal was conducted to form synbiotic-like preparations. An *in vivo* experiment was carried out to access the effect of the fermented SBM dietary supplement on the growth performance of white-leg shrimp *L. vannamei* and the spread of *Vibrio* spp. pathogen. Based on the obtained results, the applicability of the isolated probiotic and its fermented product with SBM in aquatic feed is discussed.

MATERIALS AND METHODS

Growth of *Bacillus velezensis* BS in the SBM-containing medium

The *B. velezensis* BS strain was previously isolated from the shrimp farming water sample in Bac Lieu province, Vietnam, and stored at the Environmental Bioremediation Lab., Institute of Biotechnology until further use. To test the effect of SBM addition on the growth of *B. velezensis* BS, various SBM-containing media were prepared by adding different SBM concentrations of 0.1, 0.3, 0.5, 0.7, and 1% into phosphate-buffered saline (PBS). The MPA media used for the culture of isolate consisted of g/L: meat extract (5), peptone (10), NaCl (5), pH ~7. The overnight cultured isolate in the MPA medium was centrifuged, washed, and resuspended in 0.9% sterilized saline solution before being supplemented in the SBM-containing medium. The inoculum ratio was set at 1%. The bacterial growth was regularly monitored for 24 h. Concurrently, a positive control in which the isolate grew in MPA media was also carried out.

Prebiotic index and activity score of soybean meal

B. velezensis BS was used to estimate the prebiotic index (I_{preb}) of soybean meal based on

the method of Figueroa-gonzález et al., 2019. The bacteria biomass was collected by centrifuging at 8,000 rpm for 5 min. The pellet was resuspended in 0.9% sterilized saline solution and transferred separately into the M_1 (PBS + 1% SBM) and M_2 (PBS + 1% glucose) media with the inoculation ratio of 1% v/v. The incubation condition was set at 35ºC, 150 rpm. Then, the bacteria density (CFU/mL) of the two cultures was accessed at 0 and after 24 h of incubation. The I_{preb} was calculated by the following equation:

$$I_{preb} = \frac{Probiotic\ density\ \left(\frac{CFU}{mL}\right) in\ M_1}{Probiotic\ density\ \left(\frac{CFU}{ml}\right) in\ M_2}$$

The prebiotic activity score (A_{preb}) of SBM was also detected by the method of Figueroa-gonzález *et al., 2019*. The enteric *E. coli* strain that was gifted by the IBT, was also cultured in M_1 and M_2 medium under the same conditions. The *E. coli* density was counted at 0 and after 24 h of incubation. Then, the A_{preb} was calculated based on the below equation:

$$A_{preb} = \frac{(LogP_{24} - LogP_0)_{M_1}}{(LogP_{24} - LogP_0)_{M_2}} - \frac{(LogE_{24} - LogE_0)_{M_1}}{(LogE_{24} - LogE_0)_{M_2}}$$

Where Log P is the log of probiotic concentration (CFU/mL) at 0 h (P_0) and 24 h (P_{24}) on M_1 and M_2. Log E indicates the log of growth of *E.coli* (CFU/mL) at 0 h (E_0) and 24 h (E_{24}) in M_1 and M_2 medium.

Probiotic characteristics of *B. velezensis* grew in the SBM-containing medium

Digestive enzyme secretion

The activity of α-amylase secreted by the isolate was checked by the agar diffusion method (Möttönen, 1970). The agar plate which contained starch (1%) and agar (1%) was used. The overnight culture of the *B. velezensis* BSin an SBM-containing medium was first centrifuged (10,000 rpm, for 5 min), then pipetted 100 µL into a 5 mm-well of the agar plate. The negative control well was added with sterilized water, while the positive control well was filled with 100 µL of the α-amylase enzyme (20 U/mL) (Sigma-Aldrich, US). To determine the existence of cellulase and protease activities, a similar procedure was carried out, but the starch content was replaced by carboxymethyl cellulose (CMC, 1%) or casein (0.1%), respectively. The positive controls used in CMC and casein-containing plates were cellulase (1 U/mL) (Sigma-Aldrich, US) and protease enzymes (20 U/mL) (Sigma-Aldrich, US). After 24 h of incubation at 25ºC, the starch and CMC-containing plate were over-layered with Lugol solution (5% I_2, 10% KI), while the Folin's reagent (Merck, German) was filled in the casein-contained plate. Next, the diameter of the clearance zone that indicates the hydrolytic activity of the enzymes was observed and recorded.

Anti-Vibrio parahaemolyticus activity

The *V. parahaemolyticus* pathogenic was provided by the Institute of Biotechnology (IBT), VAST. The 10^5 CFU/mL of *V. parahaemolyticus* was spread on the TCBS agar (Hi media, India) plate. Consequently, a 5 mm-well was made using a cork borer in the agar plate and filled with 100 µL centrifuged *B. velezensis* BS culture which was previously prepared in the SBM-containing medium (1% w/v). The plate was then left to stay inside the incubator at 35ºC overnight before the inhibitor zone was observed and measured.

Biofilm formation

The biofilm formation of the isolate was estimated through the solid-surface-associated biofilm formation experiment (Morikawa *et al.*, 2006). First, the overnight culture of the isolate was diluted to obtain an OD_{600} value of 0.3, and 3 µL of the diluted isolate culture was added to 297 µL of SBM-containing medium (1% w/v) in an Eppendorf tube. The procedure was done in triplicate. Then, the tubes were kept at 35ºC, after each 24 h, one tube was taken out for the biofilm staining step. Briefly, the culture was carefully

removed, and the tube was rinsed twice with distilled water. Next, the remaining cells and matrices were stained with 1% crystal violet solution for 10 min at room temperature. After washing twice with distilled water, the crystal violet trapped inside the biofilm was solubilized with 300 µL of acetic acid 33% and measured its absorbance at 570 nm.

Growth ability in different environmental conditions

The growth of the isolate at different pH (2, 4, 6, 7, 8, 9, 10), temperature (10, 15, 20, 25, 30, 35, 40, 45^{o}C), and NaCl concentration (0, 1, 2, 3, 4‰) was determined by adding 100 µL aliquot of *B. velezensis* BS in 9.9 mL of SBM-containing medium (1% w/v) followed by the incubation at 35^{o}C, 150 rpm for 24 h. The cell growth was estimated by measuring the medium optical density at 600 nm on UV-1601 Spectrophotometer, Shimadzu, Japan. Experiments were done in triplicate. The Student's t-test was used to identify the statistical differences in the collected data, $p< 0.05$ was considered an indicator of statistical significance.

Degradation of SBM trypsin inhibitor fermented with *B. velezensis* BS

The probiotic biomass (10^8 CFU/mL) was diluted in 0.9% sterilized saline solution (ratio of 1:1), then mixed with the SBM to reach the humidity of 40%, and incubated at 35^{o}C for 48 h. The trypsin inhibitor (TI) content in fermented SBM was measured according to the American Oil Chemists' Society (AOCS) (AOCS, 2017). The crude protein content of SBM was also calculated by multiplying the amount of total nitrogen in SBM with the traditional conversion factor of 6.25, in which the total nitrogen content was determined by the Vietnamese standards no. TCVN 10034:2013 (TCVN, 2013). Additionally, probiotic density in SBM was measured at 0 h and after 48 h of incubation. The attachment of bacterial cells onto the SBM was observed by a scanning electron microscope (SEM) (Hitachi S-4800 High-Resolution Scanning Electron Microscope).

Effect of the *B. velezensis* BS-fermented SBM on survival rate and growth performance of *L. vannamei*

An *in vivo* feeding trial was set up, and the healthy *L. vannamei* shrimps (average body weight of 2-3) were cultured separately in six composite tanks (100 L, three tanks per treatment, 15 shrimps per tank). In the treatment tanks, the shrimp diet was supplemented with 1×10^7 CFU of probiotic in fermented SBM/g of diet, while only commercial feed (Nasa 2203-S, C.P Vietnam Corporation) was used in the control tanks. All shrimps were fed at 5% of body weight, three times per day. During the experiment, water parameters were kept at 28 ± 1.0^{o}C, pH 7 - 8, salinity 15 - 20‰, and DO 4 - 6 mg/L. Besides, some crucial water quality parameters such as NH_4^+/NH_3 and H_2S concentrations were monitored regularly according to the Phenate and methylene blue methods, respectively (Le & Boyd, 2012, Eaton et al., 2014). Importantly, the mortality, shrimp weight, and amount of consumed feed were monitored regularly to estimate the difference in survival rate, weight gain, and feed conversion ratio between the treatment and control tank according to Hong *et al.*, 2022. In addition, the water samples were collected to estimate the total *Vibrio* spp. cell count. The water was serially diluted and spread on TCBS agar (Hi media, India), and incubated at 35°C for 24 hours. Afterward, colonies formed with shiny yellow color and smooth were used in the viable count of *Vibrio* spp. (Hosen *et al.*, 2021).

RESULTS AND DISCUSSIONS

Growth of *B. velezensis* BS in SBM-containing medium

The growth of BS strain on the medium that contained different SBM concentrations (0.1, 0.3, 0.5, 0.7, 1.0%) was accessed in the present study. As shown in Figure 1a, the higher the SBM content, the better the bacteria growth was. The bacteria optical density on 0.7% and 1.0% SBM-containing media were respectively about 2 and 3 times higher than in 0.1% SBM media

after 24 h of incubation. Notably, the bacteria growth in 1.0% SBM-containing media was 2.9 ± 0.1 close to the value of 3.0 ± 0.2 in MPA broth. Therefore, the SBM concentration of 1% was selected as the optimal growth condition for the BS strain in the further experiment.

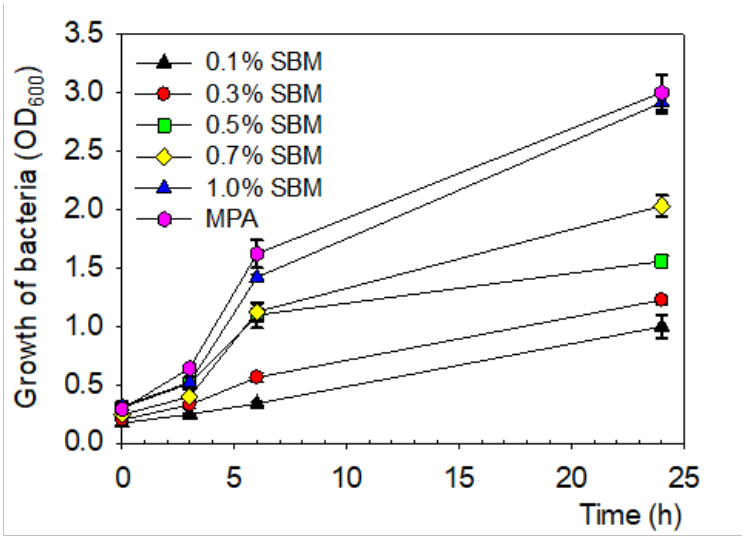

Figure 1. Effect of SBM concentration on the growth of *B. velezensis* BS.

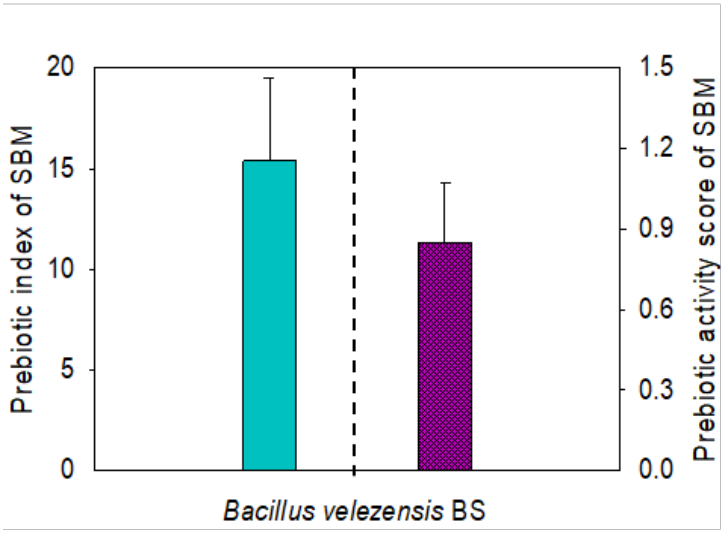

Figure 2. Prebiotic index and activity score of SBM.

Prebiotic index and activity score of SBM tested with *B. velezensis* BS

In this study, to estimate the prebiotic index and prebiotic activity score of SBM, the bacterial density of the probiotic strain grew in a 1.0% SBM-containing media compared to that in a 1.0% glucose-containing media. A prebiotic index greater than 1 indicates the stimulation effect on the growth of probiotics while a positive prebiotic activity score shows that the growth of probiotics in the prebiotic-containing environment is better than that of pathogenic bacteria (Figueroa-González *et al.*, 2019). A similar method was also applied to evaluate the I_{preb} and A_{preb} of processed foods containing

prebiotics (Moore *et al.*, 2001). Using the *B. velezensis* BS strain representing probiotic bacteria and the *E. coli* strain representing pathogenic bacteria, the I_{preb} and A_{preb} of soybean meal were calculated to reach 15.40 ± 4.10 and 0.85 ± 0.22, respectively. These results demonstrated that soybean meal could be utilized as a natural source of prebiotics to stimulate the growth of *B. velezensis*. However, the extraction of prebiotics from soybean meal using a variety of solvents yielded low efficiency as the final product contained only about 3% of oligosaccharides (Refstie *et al.*, 1999). Therefore, this study aimed to utilize the prebiotic component in soybean meal without the extraction process, to direct ferment it with probiotics to create a synbiotic-like product to reduce the production cost of feed supplements in aquaculture.

Probiotic characteristics of *B. velezensis* grew in the SBM-containing medium

When grown in the SBM-containing medium, the BS strain could produce all three digestive enzymes, including α-amylase, cellulase, and protease with clear hydrolytic zones (Figure 3). Moreover, BS was also able to inhibit the growth of the *V. parahaemolyticus* pathogen with an inhibition zone of approximately 30 ± 1.63 mm. These are critical properties that decide the effect of the probiotic in promoting feed conversion rate, and the resistance of animals to infectious diseases like vibriosis.

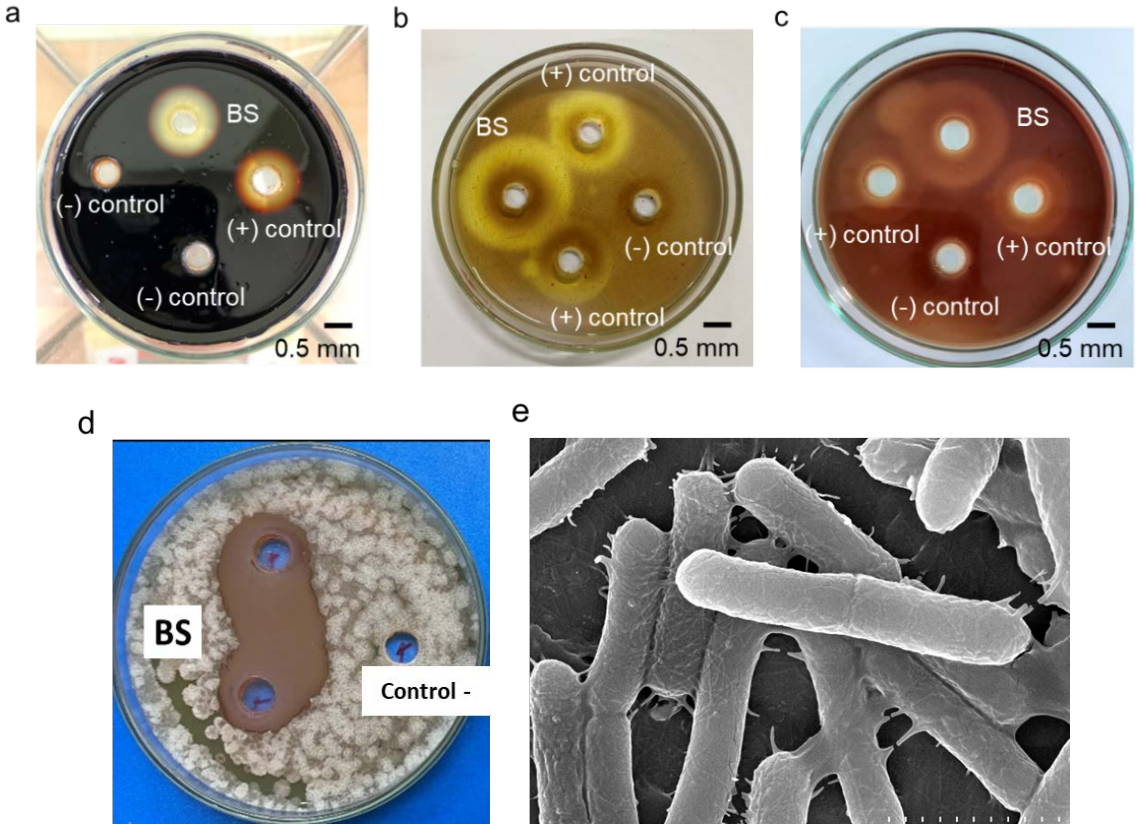

Figure 3. Digestive enzyme secretion (**a:** protease; **b:** α-amylase; **c:** cellulase) and *V. parahaemolyticus* inhibition ability of *B. velezensis* BS strain (**e:** SEM images of pure-cultured *B. velezensis* BS strain).

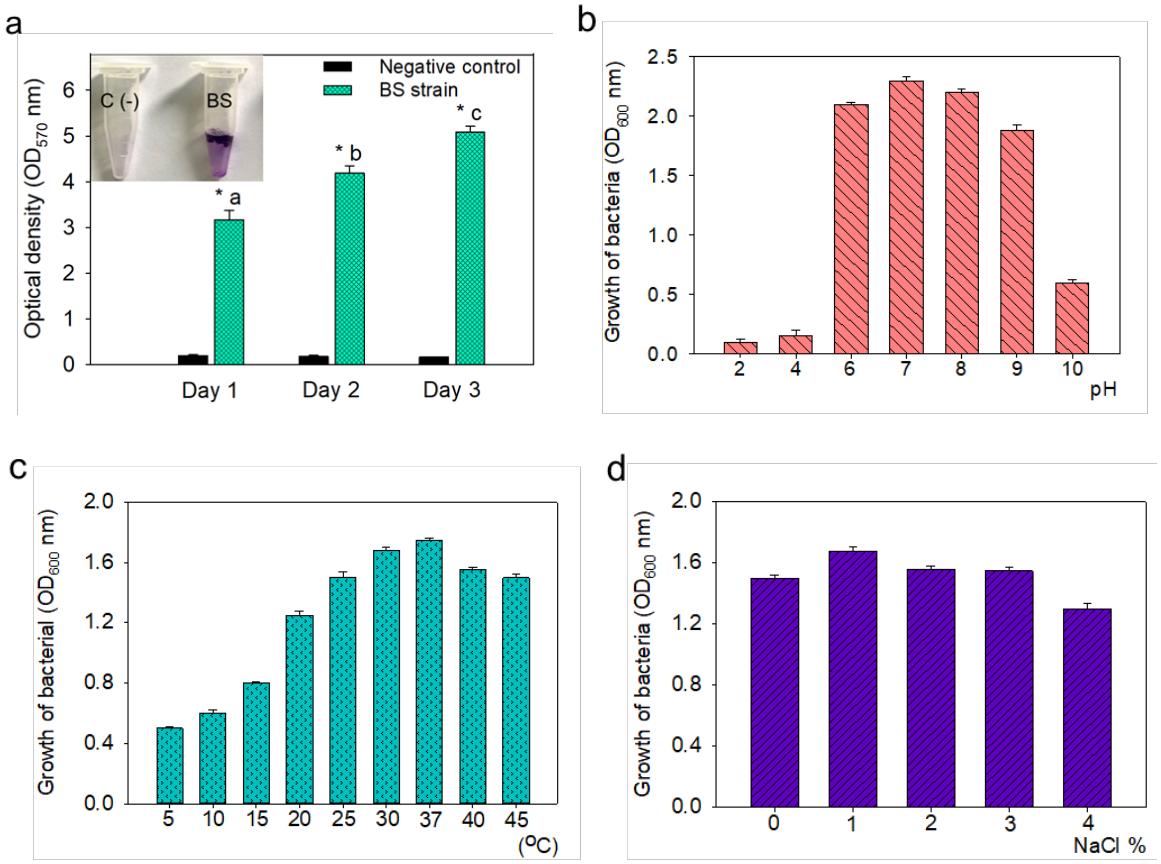

Figure 4. Biofilm growth (**a**) of *B. velezensis* BS strain according to time and tolerance of *B. velezensis* BS strain in adverse environmental conditions: **b:** pH; **c:** temperature; **d:** salinity (NaCl %). All values are presented as the means ± SD (n=3). For the (a) panel, **p*<0.05 versus control and value with different letters are significant differences among samples.

The gut bacterial biofilm is considered a physical barrier to enhance the host's defense capacity against pathogenic bacteria (Deng et al., 2020). The invasion of probiotics in the intestinal tract prevents entry of pathogenic bacteria, toxins, and other foreign unwanted compounds (Raheem *et al.*, 2021). Figure 4a indicated that the BS could form biofilm on the solid surface after 24 h of interaction. The OD_{570} value at 24 h was 3.2 ± 0.2 which was close to the value of 3.14 ± 0.60 of the *B. velezensis* LPL-K103 (Elegbeleye, Buys, 2020). Besides checking for bacterial adherence ability, the effect of various environmental factors on the growth of BS strain was also carried out in this study. As depicted in Figure 4b, the BS grew well in a wide pH range from 6 to 9. Additionally, the preferred growth temperature of BS was from 20 to 45°C, and the optimal temperature was 35°C. Furthermore, challenging BS at NaCl of 4% only slightly decreased the optical density to 1.3 ± 0.04, and this reduction was not significant. Therefore, these obtained results revealed that the *B. velezensis* BS survived and grew well in various adverse environmental conditions.

Reduction of trypsin inhibitor in SBM by the solid stage fermentation with *B. velezensis*BS strain

The fermentation experiment was set up, in which bacterial biomass was mixed with SBM and let ferment at 35°C. After 48 h of incubation, the bacteria density increased significantly from (72 ± 5) $\times 10^6$ CFU/g to (68 ± 3) $\times 10^9$ CFU/g

along with the rise of the crude protein content of fermented SBM from 42.6 ± 1.6 to 56.3 ± 2.2%. Interestingly, a high TI content of 41.1 ± 0.4 TUI/mg was detected in SBM before the fermentation process but declined to the final concentration of 8.4 ± 0.7 TUI/mg in the fermented product. This reduction was equal to the TI degradation efficiency of 79.7 ± 1.9 % (Figure 5). According to Adeyemo et al., the anti-nutrient factor could be degraded by the activity of generated protease during fermentation, hence, preventing the adverse effects of this inhibitor on protein digestion. Adeyemo et al. also reported a TI removal efficiency of about 99.17% when the soybean was fermented with probiotics *L. plantarum* (Adeyemo & Onilude, 2013). Often, the TI concentration in soybeans might vary from 17 to 48 mg/g, but the value increased to 28-52 mg/g when defatted (Gilani et al., 2005). Thus, it might contribute to the lower TI removal efficiency in soybean meal by the BS strain compared to Adeyemo's study. Thus, fermented products could be added to shrimp/fish/poultry and livestock feeds without causing indigestion due to the removal of nutritional inhibitors.

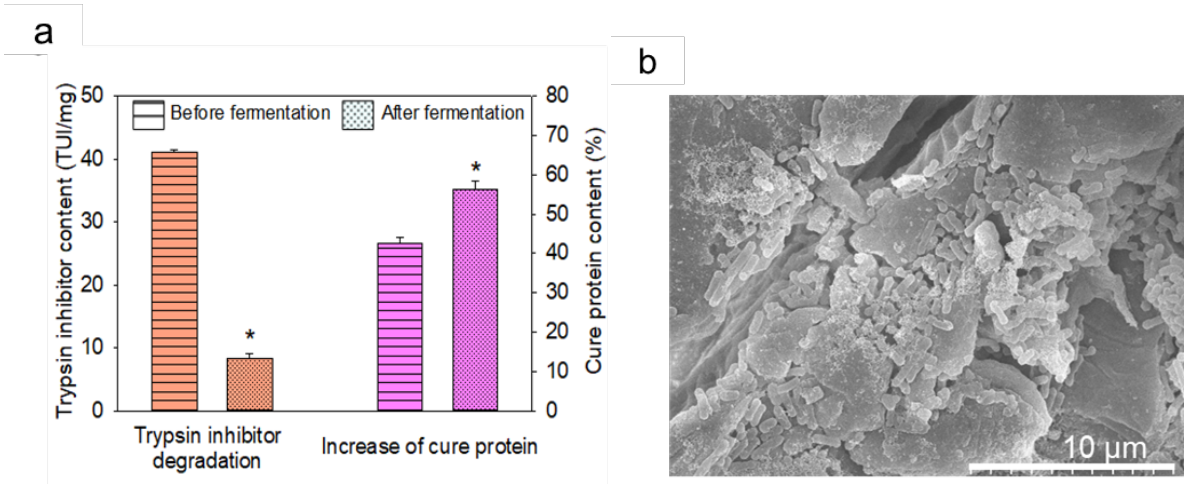

Figure 5. The changes of trypsin inhibitor and crude protein contents in SBM after fermentation with BS (**a**) and SEM images of SBM fermented with BS strain (**b**). All values are presented as the means ± SD (n=3). *$p < 0.05$ versus the before fermentation sample.

Effect of the fermented SBM on growth performance of *L. vannamei* and control *Vibrio spp.* density

The prebiotic potential of soybean meal has been verified in this study. SBM is a nutritious aquatic-feed ingredient that is highly available in Vietnam. However, without the fermentation process, the post-consumption of SBM can cause gastrointestinal problems and metabolic disturbances in animals (Liu, 1997). Aimed at solving this problem, the SBM was fermented with *B. velezensis* BS to significantly reduce the anti-nutrient, trypsin inhibitor content. To verify the safety and beneficial effect of the fermented SBM on the white-leg shrimp *L. vannamei* culture, an *in vivo* feeding trial was set up. The results showed that, during the 3 weeks of the feeding trial, no significant differences in water quality parameters were observed between the treatment and control groups. The total nitrogen (0.08 – 0.13 mg/L), DO (4.36 – 4.43 mg/L), alkalinity (113.37 – 116.35 mg/L), salinity (19.31 – 19.40‰), pH (7.61 – 7.67) and temperature (28 ± 1.0) were within acceptable ranges for the growth of *L. vannamei* shrimp (Table 1). Figure 6a also indicated there was no statistical change in shrimp survival rate as well as the feed conversion ratio of the two groups ($p > 0.05$). However, the supplementation of fermented SBM significantly enhanced the shrimp growth performance (Figure 7). Indeed,

the treatment shrimp reached a weight gain of 48.11 ± 13.95% and a specific growth rate of 1.85 ± 0.44% which were significantly higher than the control group, respectively (Figure 6b). Moreover, with the fermented product supplement, *Vibrio* spp. the population decreased significantly from 1.25 × 10^3 to 0.13 × 10^3 CFU/mL (Table 1). Thus, this fermented product was recommended as a promising dietary supplement for aquatic animals.

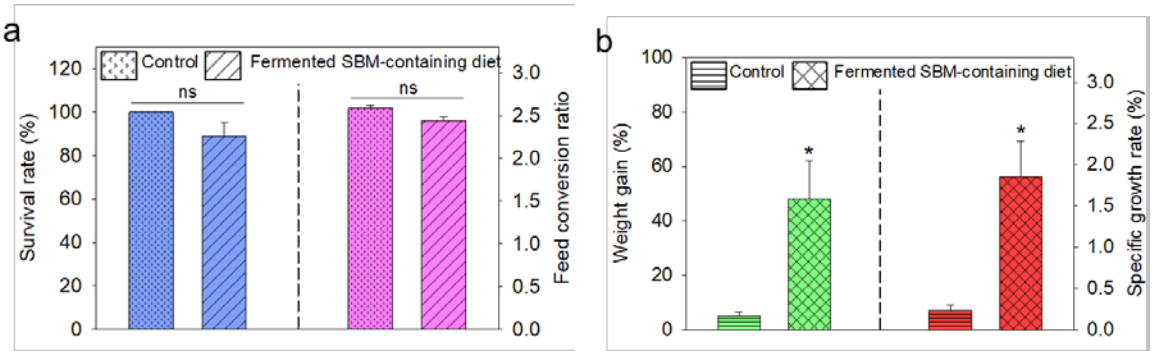

Figure 6. Effect of fermented SBM administration on the survival rate, feed conversion ratio (**a**) and on weight gain, specific growth rate (**b**) of whiteleg shrimp *L. vannamei*. All values are presented as the means ± SD (n=3). *p <0.05 verus control; ns means not significant.

Table 1. Water quality parameters and *Vibrio* spp. count in whiteleg shrimp *L. vannamei* culture supplemented with fermented SBM. Data were presented as the mean ± SD (n=3).

	Fermented SBM-added diet	**Control**
DO (mg/L)	4.43 ± 0.34	4.36 ± 0.34
NH_4^+/NH_3 (mg/L)	0.08 ± 0.19	0.13 ± 0.22
Alkalinity (mg/L)	113.37 ± 15.21	116.35±13.67
Salinity (S‰)	19.31 ± 0.71	19.40 ± 0.73
pH	7.61 ± 0.13	7.67 ± 0.18
Temperature	28 ± 1.0	28 ± 1.0
Vibrio spp. (CFU/mL)	0.13 × 10^3	1.23 × 10^3

Figure 7. The appearance of *Litopananeus vannamei* shrimp in control (left) and synbiotic-like supplemented pond (right).

CONCLUSION

Bacillus velezensis BS could grow in the medium containing 1% SBM as the same in MPA medium. *B. velezensis* BS used prebiotics in SBM with a prebiotic index of 15.40 ± 4.10 and a prebiotic activity score of 0.85 ± 0.22. When grown in the SBM-containing medium, the bacteria grew well at pH 6 - 9, temperate 20 - 45^{o}C, NaCl 0 - 4%, and produced α-amylase, proteinase, cellulase. In particular, the bacteria inhibited the *V.parahaemolyticus* pathogen growth with an inhibited zone of about 30 ± 1.63 mm. The use of BS to ferment soybean meal also effectively reduced 79.7 ± 1.9% of the trypsin inhibitor content, while concurrently increasing the crude protein content from 42.6 ± 1.6 to 56.3 ± 2.2%. Moreover, the administration of fermented SBM increased shrimp weight gain and specific growth rate compared to the control. Therefore, the isolate BS as well as its fermented product with SBM were suggested as the potential dietary supplement for aquatic animals.

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REFERENCES

Adeyemo SM, Onilude AA (2013) Enzymatic reduction of anti-nutritional factors in fermenting soybeans by *Lactobacillus plantarum* isolates from fermenting cereals. *Niger. Food J.* 31(2): 84–90.

AOCS (2017) *Official methods and recommended practices, 7th ed. Method Ba 12-75. Trypsin inhibitor activity* (First appr). Champaingn, IL.

Anh PT, Kroeze C, Bush SR, MolAPJ (2010) Water pollution by intensive brackish shrimp farming in south-east Vietnam: Causes and options for control. *Agric. Water Manag.* 97(6): 872–882.

Choct M, Dersjant-Li Y, McLeish J, Peisker M (2010) Soy oligosaccharides and soluble non-starch polysaccharides: A review of digestion, nutritive and anti-nutritive effects in pigs and poultry. *Asian-Australas. J. Anim. Sci.* 23(10): 1386–1398.

Deng Z, Luo XM, Liu J, WangH (2020) Quorum sensing, biofilm, and intestinal mucosal barrier: Involvement the role of probiotic. *Front. Cell. Infect. Microbiol.*, 10, 504.

Eaton AD, Clesceri LS, Greenberg AE (2014) *Standard Methods : For the examination of water and wastewater*.

Elegbeleye JA, Buys EM (2020) Molecular characterization and biofilm formation potential of *Bacillus subtilis* and *Bacillus velezensis* in extended shelf-life milk processing line. *J. Dairy Sci.*, 103(6): 4991–5002.

Figueroa-González I, Rodríguez-Serrano G, Gómez-RuizL, García-Garibay M, Cruz-Guerrero A (2019) Prebiotic effect of commercial saccharides on probiotic bacteria isolated from commercial products. *J. Food Sci. Technol.* 39(3): 747–753.

Firouzbakhsh F, Mehrabi Z, Heydari M, Khalesi MK, TajickMA (2014) Protective effects of a synbiotic against experimental *Saprolegnia parasitica* infection in rainbow trout (*Oncorhynchus mykiss*). *Aquac. Res.* 45(4): 609–618.

Gao YL, Wang CS, Zhu QH, Qian GY (2013) Optimization of solid-state fermentation with *Lactobacillus brevis* and *Aspergillus oryzae* for trypsin inhibitor degradation in soybean meal. *J. Integr. Agric.* 12(5): 869–876.

Gilani GS, Cockell KA, Sepehr E (2005) Effects of antinutritional factors on protein digestibility and amino acid availability in foods. *J. AOAC Int.* 88(3): 967–987.

Hardi EH, Nugroho RA, Rostika R, Mardliyaha CM, Sukarti K, Rahayu W, Supriansyah A, Saptiani G (2022) Synbiotic application to enhance growth, immune system, and disease resistance toward bacterial infection in catfish (*Clarias gariepinus*). *Aquaculture 549*, 737794.

Hong NTX, Linh NTH, Baruah K, Thuy DTB, Phuoc NN (2022) The combined use of *Pediococcus pentosaceus* and fructooligosaccharideimproves growth performance, immune response, and

resistance of whiteleg shrimp *Litopenaeus vannamei*against *Vibrio parahaemolyticus*. *Front Microbiol* 13, 168.

Hoseinifar SH, Esteban MÁ, CuestaA, Sun YZ (2015) Prebiotics and fish immune response: A Review of current knowledge and future perspectives. *Rev. Fish. Sci. Aquac.* 23(4): 315–328.

Hosen MA, Ovi FK, Rashid H, Hasan MH, Khalek MA, Hasan M, Easmin F, Rumi NA, Islam MS (2021) Characterization of *Vibrio* spp. in environmental water samples collected from flood prone areas of Bangladesh and their antibiotic resistance profile. *AIMS Microbiol.* 7(4), 471.

Huynh TG, Shiu YL, Nguyen TP, Truong QP, Chen JC, Liu CH (2017) Current applications, selection, and possible mechanisms of actions of synbiotics in improving the growth and health status in aquaculture: A review. *Fish Shellfish Immunol.* 64: 367–382.

Kårlund A, Paukkonen I, Gómez-Gallego C, Kolehmainen M (2021) Intestinal exposure to food-derived protease inhibitors: Digestion physiology- and gut health-related effects. *Healthcare* 9(8).

Le PTT, Boyd CE (2012) Comparison of phenate and salicylate methods for determination of total ammonia nitrogen in freshwater and saline water. *J World Aquac Soc*. 43(6): 885–889.

Liu K (1997) Chemistry and nutritional value of soybean components *in Soybeans. Springer New York, NY,* 25–113.

Mandalari G, Nueno Palop C, Tuohy K, Gibson GR, Bennett RN, Waldron KW, Bisignano G, Narbad A, Faulds CB (2007) *In vitro* evaluation of the prebiotic activity of a pectic oligosaccharide-rich extract enzymatically derived from bergamot peel. *Appl. Microbiol. Biotechnol.* 73: 1173–1179.

Maytorena-Verdugo CI, Córdova-Murueta JH, García-Carreño FL (2017) Peptidase compensation in the digestive system of whiteleg shrimp *Penaeus vannamei* against dietary Kunitz-type soybean trypsin inhibitor. Aquac. Nutr. 23(5): 1095-1103.

Mehrabi Z, Firouzbakhsh F, Jafarpour A (2012) Effects of dietary supplementation of synbiotic on growth performance, serum biochemical parameters and carcass composition in rainbow trout (*Oncorhynchus mykiss*) fingerlings. *J. Anim. Physiol. Anim. Nutr.* 96(3): 474–481.

Moore KE (2011) Biological analysis of prebiotics in various processed food matrices. *Master Thesis*, University of Nebraska-Lincoln, America.

Morikawa M, Kagihiro S, Haruki M, Takano K, Branda S, Kolter R, Kanaya S (2006) Biofilm formation by a *Bacillus subtilis* strain that produces gamma-polyglutamate. *Microbiology* 152(9): 2801–2807.

Möttönen K (1970) Comparison of the agar-plate diffusion method for α-Amylase assay with the colorimetric stationary-stage method. *J. Sci. Food Agric.* 21(5): 261–272.

Mugwanya M, Dawood MAO, Kimera F, Sewilam H (2022) Updating the role of probiotics, prebiotics, and synbiotics for Tilapia aquaculture as leading candidates for food sustainability: a review. *Probiotics Antimicrob. Proteins* 14(1): 130–157.

Raheem A, Liang L, Zhang G, Cui S (2021) Modulatory effects of probiotics during pathogenic infections with emphasis on immune regulation. *Front. Immunol. 12*, 571.

Refstie S, Svihus B, Shearer KD, Storebakken T (1999) Nutrient digestibility in Atlantic salmon and broiler chickens related to viscosity and non-starch polysaccharide content in different soyabean products. *Anim. Feed Sci. Technol.* 79:331–345.

Rodriguez-Estrada U, Satoh S, Haga Y, Fushimi H, Sweetman J (2013) Effects of inactivated *Enterococcus faecalis* and mannan oligosaccharide and their combination on growth, immunity, and disease protection in rainbow trout. *N. Am. J. Aquac.* 75(3): 416–428.

Senphan T, Benjakul S, Kishimura H (2015) Purification and characterization of trypsin from hepatopancreas of Pacific white shrimp. *J. Food Biochem.* 39(4): 388-397.

TCVN 10034:2013 National standard for food and animal feeding stuffs - General guidelines for the determination of nitrogen content by the Kjeldahl method.