Properties of a fibrinolytic enzyme secreted by *Bacillus subtilis* JS2 isolated from saeu (small shrimp) *jeotgal*

Zhuang Yao¹ · Jeong A Kim¹ · Jeong Hwan Kim^{1,2}

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Abstract *Bacillus* species were screened to be used as starters for *jeotgals*, salted and fermented Korean sea foods. A strain, JS2, showing strong fibrinolytic activity was isolated from saeu (small shrimp) *jeotgal*, and identified as *Bacillus subtilis*. *Bacillus subtilis* JS2 grew well at 20% (w/v) NaCl concentration. SDS-PAGE of culture supernatant from JS2 showed 3 major bands of 27, 29, and 60 kDa in size. Fibrin zymography showed that the 27 kDa band was the major fibrinolytic protein. The gene, *aprEJS2*, was cloned and introduced into *B. subtilis* WB600 using pHY300PLK. A *B. subtilis* transformant harboring pHYJS2 showed higher fibrinolytic activity than *B. subtilis* JS2. *aprEJS2* was overexpressed in *Escherichia coli* BL21 (DE3). The optimum pH and temperature for AprEJS2 were pH 8.0 and 40 °C, respectively. K_m and V_{max} values were determined. AprEJS2 has strong α -fibrinogenase activity and moderate β -fibrinogenase activity.

Keywords *Bacillus subtilis* · Fibrinolytic activity · Saeu *jeotgal* · Fibrinolytic enzymes · Starter

Introduction

Jeotgals are salted and fermented Korean seafoods prepared from many different types of fishes, fish eggs, fish intestines, and shellfishes [1]. Most *jeotgals* are prepared

simply by mixing raw materials with salt (15–30%, w/w), and storing at room temperature for several months or years for some cases [2]. Microorganisms naturally present in raw materials carry out fermentation by secreting digestive enzymes and endogenous enzymes in the raw materials also contribute [3]. Diverse microorganisms have been isolated from various *jeotgals* by cultural methods. Recently, metagenomics approaches have been used to obtain more thorough profiles of microbial communities of different *jeotgals* because many species do not grow on culture media [4–7].

The most serious problem of traditional *jeotgal* preparation method is long time required for the completion of fermentation. It usually takes several months or years in some cases, and high salinity of *jeotgals* is the main reason of slow fermentation. Another problem is difficulty in quality control of *jeotgals*. Only halophilic or halotolerant organisms can grow actively under high salinity of most *jeotgals*, but roles or importance of them for *jeotgal* fermentation are poorly understood. The quality of a *jeotgal* is affected greatly by microbial communities in that *jeotgal* during fermentation. With current knowledge on microbial communities of *jeotgals*, it is not possible to predict accurately the progress of fermentation and the final quality. One way to alleviate these problems is use of starters for *jeotgal* fermentation. An ideal starter should grow well at high salt concentrations and accelerate fermentation by hydrolyzing proteins from raw materials. Microbial proteases are important for the quality of *jeotgals* by producing peptides and amino acids from proteins, which improve taste and flavor of *jeotgals* [8]. *Tetragenococcus halophilus*, a halophilic lactic acid bacteria, is a promising candidate as a starter [6, 8–10]. *Bacillus* sp. with salt tolerance can be a candidate, too because bacilli secrete active proteases. In this work, a *B. subtilis* strain

✉ Jeong Hwan Kim
jeonghkm@gnu.ac.kr

¹ Division of Applied Life Science (BK21 Plus), Graduate School, Gyeongsang National University, Jinju 52828, Korea

² Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 52828, Korea

with strong fibrinolytic activity and salt tolerance was isolated from saeu (small shrimp) *jeotgal*, one of the most popular *jeotgals* in Korea. Its major fibrinolytic enzyme was characterized. The isolate seems promising candidate as a starter for various *jeotgals*. Highly fibrinolytic *Bacillus* strains can accelerate fermentation of *jeotgals* since they usually possess high proteolytic activities. In addition, fibrinolytic enzymes from bacilli have potentials as therapeutic agents for preventing cardiovascular diseases caused by fibrin accumulation in the blood vessels [11, 12]. Nattokinase from *B. subtilis* strains is the most well-known enzyme, but fibrinolytic enzymes from other bacilli also possess the same potentials.

Materials and methods

Isolation and identification of JS2

Bacilli with fibrinolytic activities were isolated from saeu (shrimp, *Acetes chinensis*) *jeotgal* (SJ) purchased at a local market at Jinju, Gyeongnam, Republic of Korea. Ten gram of SJ was mixed with 90 ml of 0.1% peptone water, homogenized by Stomacher 80 (Seward, Worthing, UK), and diluted serially. Diluted samples were spread on tryptic soy broth (TSB, Becton, Dickinson and Company, Sparks, MD, USA) agar plates with NaCl (10%, w/v), and plates were incubated for 48 h at 37 °C. Well-separated colonies were spotted onto Luria-Bertani (LB, tryptone 10 g, yeast extract 5 g, NaCl 5 g, per l, pH 7.0) agar plates with skim milk (1%, w/v), and plates were incubated for 2 days. Isolates showing proteolytic activities were tested for fibrinolytic activities by spotting onto a fibrin plate (see below).

For identification of an isolate, 16S rRNA gene was amplified using primers: bac-F (5'-CGGCGTGCCTAATCATGCAAG-3') and bac-R (5'-GGCATGCTG ATCCGC ATTAC TA -3') [13]. A partial *recA* gene was amplified as described previously [13]. Randomly amplified polymorphic DNA (RAPD)-PCR was done using S30 primer (5'-GTGATCGCAG-3') as described previously [13]. Sequencing was done at Cosmogenetech (Seoul, Korea) and the sequences were analyzed by BLAST (NCBI, Bethesda, MD, USA).

Growth, fibrinolytic activity, and salt tolerance of *B. subtilis* JS2

Effect of media on the growth and fibrinolytic activity of *B. subtilis* JS2 was examined using 4 different media: LB broth, brain heart infusion (BHI, Becton, Dickinson and Company) broth, nutrient broth (NB, Becton, Dickinson and Company), and TSB. Growth was monitored by

measuring OD_{600 nm} values of cultures. For measuring fibrinolytic activity, aliquot of culture was centrifuged at 4000×g for 10 min at 4 °C, and the supernatant was filtered using a 0.45 µm filter (Sartorius Stedim, Goettingen, Germany). Filtered culture supernatant (FS) was used as the sample for fibrinolytic activity measurement by fibrin plate method as described previously [14]. Salt tolerance of *B. subtilis* JS2 was examined by inoculating overnight culture in LB (1%, v/v) into LB broth with NaCl at different concentrations (15–25%, w/v). Inoculated cultures were incubated for 72 h with shaking at 37 °C, and growth was checked at 12 h intervals. All measurements were repeated 3 times and the means are shown with SD (standard deviations) values.

SDS-PAGE and fibrin zymography

Bacillus subtilis JS2 was grown in LB for 96 h at 37 °C. At intervals, FSs were prepared, and analyzed by SDS-PAGE and fibrin zymography using 10% acrylamide gels. A polyacrylamide gel containing fibrin was prepared by mixing fibrinogen (0.12%, w/v) and 100 µl of thrombin (10 NIH units/ml) with acrylamide solution. Electrophoresis was done at a constant current of 10 mA. After electrophoresis, the gel was soaked in 50 mM Tris-HCl buffer (pH 7.4) containing 2.5% Triton X-100 for 30 min at room temperature. The gel was washed with distilled water for 30 min and soaked in zymogram reaction buffer (30 mM Tris-HCl, pH 7.4, 0.02% of NaN₃) for 12 h at 37 °C [15]. The gel was stained with coomassie blue R-250 for 1 h, and destained for 4 h.

Cloning and overexpression of *aprEJS2* gene

An *aprEJS2* gene was amplified from *B. subtilis* JS2 genome by using primers: CH51-F (5'-AGGATCCCAAGAGAGCG ATTGCGGCTGTGTAC-3', *Bam*HI site underlined) and CH51-R (5'-AGAATTCTTCAGAGGGAGCCACCCGTCG ATCA-3', *Eco*RI site underlined) [16]. Amplified fragment was ligated with pHY300PLK (4.87 kb, Tc^R) (Takara, Shiga, Japan) after digested with *Bam*HI and *Eco*RI. *Bacillus subtilis* WB600 competent cells were transformed with the ligation mixture by electroporation. *Bacillus subtilis* transformants (TF) harboring the recombinant plasmid were selected on LB agar plates containing tetracycline (15 µg/ml).

An *aprEJS2* gene without its own signal sequence (pro-*aprEJS2*) was amplified using primer pairs: pETJS2-F (5'-AGAGGATCCGATGGCAGGGAAATC-3' *Bam*HI site underlined) and pETJS2-R (5'-AGACTCGAGCTGAGCT GCCGCTG-3' *Xho*I site underlined). Amplified pro-*aprEJS2* gene was inserted into pET26b(+) (Merck Milipore, Darmstadt, Germany), and the ligation mixture was introduced into *E. coli* BL21 (DE3) by electroporation.

A TF harboring pETJS2 (pET26b(+)) with pro-*aprEJS2* was cultivated in LB (250 ml) containing Km (30 µg/ml) until the OD₆₀₀ reached 0.8. IPTG (isopropyl β-D-1-thiogalactopyranoside) was added at a final concentration of 1 mM, and culture was incubated for additional 20 h at 30 °C. Soluble and insoluble fraction from induced cells were prepared, and AprEJS2 was purified as described previously [14].

Properties of AprEJS2

The effect of pH on the fibrinolytic activity of AprEJS2 was examined by using different buffer systems (50 mM): citrate-NaOH, pH 3–5; sodium phosphate, pH 6–8; and Tris-HCl, pH 9–11. One µg of AprEJS2 in each buffer was incubated for 1 h at 40 °C, and then the activities were measured by the fibrin plate method. For measuring pH stability, 1 µg of AprEJS2 was incubated up to 6 h at 40 °C, and the activities were measured at 1, 3, and 6 h time points. One µg of AprEJS2 in sodium phosphate buffer (pH 8) was incubated for 30 min at different temperature (37–60 °C), and the activities were measured. For testing thermal stability, 1 µg of AprEJS2 was incubated for 3 h at 37–60 °C, and the remaining activities were measured. The effect of metal ions (5 mM) or inhibitors (1 mM) was examined by treating for 30 min at 40 °C and pH 8, and the remaining activities were measured.

Amidolytic activity

The amidolytic activity of AprEJS2 was measured by using N-succinyl-ala-ala-pro-phe-*p*-nitroanilide (S7388, Sigma) as the substrate. Fifty µl of 10 mM substrate in sodium phosphate buffer (pH 7.0) was mixed with 10 µl of AprEJS2 (1 µg) and 440 µl of sodium phosphate buffer. The mixture was incubated for 20 min at 40 °C, and 500 µl of citrate-NaOH buffer (pH 3.0) was added. The mixture was centrifuged for 5 min at 12,000×*g* and the supernatant was measured for its absorbance at 410 nm. The degree of hydrolysis was calculated from the absorbance value and the molar extinction coefficient value of *p*-nitroanilide (8800 M⁻¹ cm⁻¹). V_{max} and K_m values were determined by using the same substrate.

Hydrolysis of fibrinogen

Purified AprEJS2 (50 ng) was mixed with 1 mg of fibrinogen (bovine, MP Biochemicals, Illkirch, France), and the mixture (1 ml of 20 mM Tris-HCl, pH 8) was incubated at 40 °C up to 12 h. Aliquots at intervals were analyzed by SDS-PAGE using a 10% acrylamide gel after boiled for 5 min in 1 × SDS sample buffer.

Results and discussion

Isolation and identification of JS2

JS2 was the strain showing the strongest fibrinolytic activity among total 350 presumptive bacilli isolated from SJ. It was Gram +, rod-typed, and the colony showed a typical *Bacillus* morphology on LB agar plates. JS2 had 94% identity with *Bacillus lentus* when API CHB kit (bioMérieux, Marcy l'Etoile, France) was used. 16S rRNA genes were amplified and sequenced (1236 nucleotides, MF461321). BLAST showed that the gene had 99% identity to those of *B. pumilus* strains. A partial *recA* gene was sequenced (771 nucleotides, MF461326), and the sequence had 99% identity to *recA* genes of *B. subtilis* strains. To confirm the species, RAPD-PCR was done, and the profile of JS2 was identical with those of *B. subtilis* reference strains. A 0.5 kb band, specific to *B. subtilis* strains, was observed from JS2 (results not shown). JS2 was finally identified as *B. subtilis*.

Growth, fibrinolytic activity, and salt tolerance of *B. subtilis* JS2

Bacillus subtilis JS2 grew well in all 4 media tested, and OD₆₀₀ values were 1.4–1.8 at 24 h (results not shown). Cultures in BHI and TSB showed higher OD₆₀₀ values than those in LB and NB. Culture in NB at 24 h showed the highest fibrinolytic activity (307.72 mU/µl), but the activity decreased rapidly, and decreased to basal level after 36 h. Culture in LB showed steady increase in the activity until 48 h, and then higher activities (235.90–251.84 mU/µl) were maintained until 96 h (results not shown). Fibrinolytic activity of *B. subtilis* JS2 was affected by growth medium, but the exact reason was not known. The same phenomenon was observed for other fibrinolytic enzymes [16, 17]. LB was the best medium for the growth and fibrinolytic activity of *B. subtilis* JS2 and used for further experiments.

Bacillus subtilis JS2 grew well in LB with 20% NaCl (w/v), and the OD₆₀₀ was above 1.2 after 48 h (Fig. 1). *Bacillus subtilis* JS2 grew slowly at 22% NaCl, and the OD₆₀₀ was 0.3 at 48 h. *Bacillus subtilis* JS2 can be used as a starter for *jeotgals* and soy sauce where NaCl concentration does not exceed 20%. *Bacillus subtilis* JS2 possesses strong fibrinolytic activity, and this is a desirable property. *Bacillus subtilis* JS2 was tested as a starter for oyster *jeotgal* (23% NaCl, w/w) where *B. subtilis* JS2 was inoculated at 10⁶ CFU/g oyster. Bacilli, suspected to be *B. subtilis* JS2, were counted in the range of 10²–10⁴ CFU/g oyster during 23 weeks of fermentation at 15 °C (unpublished results). Fukui et al. [3] examined the microbial

Fig. 1 Growth of *B. subtilis* JS2 at different NaCl concentrations. Filled circle, 15%; open circle, 16%; down triangle, 17%; open triangle, 18%; filled square, 19%; open square, 20%; filled diamond, 22%; open diamond, 25%; filled triangle, 30%. *Bacillus subtilis* JS2 was cultivated in LB broth with different NaCl concentrations (w/v)

community of a fish sauce (15% NaCl, w/w) from deep sea smelt (*Glossanodon semifasciatus*) by culture-dependent and -independent methods. *Tetragenococcus halophilus* was the most dominant species after 6 weeks, growing on marine agar plates with 12.5% NaCl. *T. halophilus* was also the major species after 6 weeks as examined by a culture-independent method, sequencing of 16S rRNA gene clones. But *B. subtilis* was the most dominant species among non-halophilic organisms after 6 weeks, growing on plate count agar plates without salt. *Bacillus subtilis* was detected at 10^6 CFU/g until the end of fermentation, 32 weeks [3]. These results together with our study support that *Bacillus* species could grow under high salinity of *jeotgals*. Studies on the performance of *Bacillus* species as starters should be done in the future.

SDS-PAGE and fibrin zymography

When FS from *B. subtilis* JS2 was analyzed by SDS-PAGE, 3 major bands of 25, 27, and 60 kDa in size were observed in addition to minor bands [Fig. 2(A)]. Three major bands of 27, 68, and 80 kDa in size and minor bands (32, 48 kDa) were observed on a fibrin zymogram [Fig. 2(B)]. The 27 kDa band appeared at 24 h and the band intensity increased further after 60 h [Fig. 2(B), lanes 6–8]. Fibrin zymogram results agreed with the fibrinolytic activity measurements of cultures. Cultures at 60–96 h showed higher fibrinolytic activities than cultures at earlier stages. The 27 kDa protein is the major fibrinolytic enzyme. A *Bacillus* culture often shows high fibrinolytic activity when the culture enters into stationary growth

Fig. 2 Coomassie blue stained gel (A) and fibrin zymogram (B) of culture supernatant from *B. subtilis* JS2. M, Dokdo-Marker Broad-range (EBM-1034, Elpis-Biotech., Daejeon, Korea). *Bacillus subtilis*

JS2 was grown in LB broth at 37 °C up to 96 h. 1, 12 h; 2, 24 h; 3, 36 h; 4, 48 h; 5, 60 h; 6, 72 h; 7, 84 h; 8, 96 h

phase. Production of proteases such as AprE (subtilisin) and Bpr (bacillopeptidase) is increased when cells enter into stationary phase [18]. Activated proteases seem to help cells utilize available nitrogen sources more efficiently under adverse conditions where nitrogen sources are limited.

Cloning and overexpression of *aprEJS2*

A 1.4 kb fragment containing *aprEJS2* was cloned into pHY300PLK, resulting in pHYJS2 (6.2 kb). BLAST analysis of the sequence (1478 nucleotides, MF677779)

confirmed that the cloned gene was a homolog of *aprE* genes from *Bacillus* sp. An ORF of 1149 bp in size, capable of encoding a protein of 382 amino acids, was located. The first 30 amino acids corresponded to a signal peptide as judged by SignalP 4.1 Server (Technical University of Denmark), and the following 77 amino acids corresponded to a pro-sequence. pI and molecular weight of mature AprEJS2 were 6.65 and 27,502.64 Da, respectively, which matched well with the 27 kDa band observed on a coomassie-blue stained gel (Fig. 2). *aprEJS2* showed 99% nucleotide sequence identity to other gene from *Bacillus amyloliquefaciens* MJ5-41 (JF739176, 1135/1149) [17],

Fig. 3 Growth (A) and fibrinolytic activities (B) of *Bacillus subtilis* TF. Cells were cultivated for 96 h at 37 °C in LB broth and the absorbance (600 nm) and fibrinolytic activities were measured at each time interval. Filled circle: *B. subtilis* JS2; open circle: *B. subtilis* WB600 [pHYJS2]; down triangle: *B. subtilis* WB600 [pHY300PLK]

Fig. 4 Overexpression of *aprEJS2* in *E. coli* BL21(DE3) and purification. **(A)** M, DokDo-marker broad-range; lanes 1–4, soluble fraction from induced cells grown at 30 °C after induction for 2 h (1), 4 h (2), 10 h (3), 20 h (4); lanes 5–8, insoluble fraction from induced cells grown at 30 °C after induction for 2 h (5), 4 h (6), 10 h (7), 20 h

(8); lane 9, insoluble fraction from *E. coli* BL21 [pET26b(+)] grown for 20 h at 30 °C after induction. **(B)** Purified AprEJS2 from insoluble fraction. M, DokDo-Marker broad-range; 1, insoluble fraction; 2, AprEJS2 eluted from column

and 98% identities to genes from *B. amyloliquefaciens* CH51 (EU414203, 1129/1149) [16], and *B. subtilis* HK176 (KJ572414, 1129/1149) [19]. AprEJS2 showed high similarities with other fibrinolytic enzymes: 99.4% with AprE5-41 from *B. amyloliquefaciens* MJ5-41 (AEE81297), and AprE51 from *B. amyloliquefaciens* CH51 (ACA34903), and 98.4% with AprE176 from *B. subtilis* HK176 (AHN52401). Amino acids consisting of catalytic triad (Asp32, His64, and Ser221) are conserved in AprEJS2 and other similar enzymes [20].

Bacillus subtilis WB600 harboring pHYJS2 showed the same growth curve with *B. subtilis* WB600 [pHY300PLK] (control) [Fig. 3(A)]. TF showed the highest fibrinolytic activity (408.2 U/ml) at 96 h whereas control did not show any significant activity [Fig. 3(B)]. *Bacillus subtilis* WB600 [pHYJS2] showed 1.56 fold higher activity than *B. subtilis* JS2.

A pro-*aprEJS2* gene without its own pre-sequence was amplified and inserted into pET26b(+), resulting in pETJS2. In pETJS2, pro-*aprEJS2* has additional six His codons at the 3' end after the last sense codon (Gln), which facilitates purification of recombinant AprEJS2. When soluble and insoluble fractions from induced *E. coli* BL21 (DE3) cells were analyzed by SDS-PAGE, 27 kDa and was observed from both fractions [Fig. 4(A)]. Recombinant AprEJS2 was purified from insoluble fraction by using a HiTrap IMAC FF column, and AprEJS2 was eluted at the imidazol concentration of 250 mM. Eluted AprEJS2 was 27 kDa in size as expected from the calculated size of mature AprEJS2 [Fig. 4(B)].

Properties of purified AprEJS2

AprEJS2 maintained activity at pH 7–10 and the optimum pH was pH 8 [Fig. 5(A)]. The relative activity was 83.6, 100, 94.1, and 71.9%, at pH 7, 8, 9, and 10, respectively. AprEJS2 had no activity at pH 5 and below, and the activity decreased rapidly at pH 6 and 11. The activity decreased rapidly within first hour and then became stable up to 6 h at pH 7, 8, 9 and 11 [Fig. 5(B)]. At pH 6 and 10, the activity decreased within first hour and completely disappeared at 3 h. Rapid inactivation occurred at pH 5 and below. The optimum temperature was 40 °C at pH 8 [Fig. 5(C)]. If temperature increased to 55 °C and above, no activity remained after 30 min. When AprEJS2 was incubated for 3 h at 45 or 50 °C, the activity decreased rapidly within first hour and then became stable [Fig. 5(D)]. The activity decreased gradually during 3 h exposure at 37 and 40 °C. The optimum temperature of AprEJS2 was similar to that of *B. subtilis* HK176 [19] but lower than that of *Bacillus* sp. strain CK11-4 (70 °C) [21].

K⁺ enhanced the fibrinolytic activity by 14.2%, and Na⁺ slightly enhanced (6.8%) but the activity was inhibited by Mn²⁺ (31.6% inhibition), Mg²⁺ (45.7% inhibition), and Zn²⁺ (22.7% inhibition). The activity was completely destroyed by PMSF (phenylmethylsulfonyl fluoride). But EDTA (ethylenediaminetetraacetic acid) and EGTA (ethylene glycol tetraacetic acid) decreased the activity by 22.5 and 35.3%, respectively. The results indicated that AprEJS2 is a serine protease.

Fig. 5 The effect of pH and temperature on the fibrinolytic activity of recombinant AprEJS2. The optimum pH (A), stability against pH variation (B): filled circle, pH 3; open circle, pH 4; down triangle, pH 5; open triangle, pH 6; filled square, pH 7; open square, pH 8; filled

diamond, pH 9; open diamond, pH 10; filled triangle, pH 11. The optimum temperature (C), and the stability against temperature (D): filled circle, 37 °C; open circle, 40 °C; down triangle, 45 °C; open triangle, 50 °C; filled square, 55 °C; open square, 60 °C

Fig. 6 Fibrinogen hydrolysis by AprEJS2. M, DokDo-Marker broad-range; 1, control (no enzyme treatment); 2, 5 min; 3, 10 min; 4, 20 min; 5, 30 min; 6, 1 h; 7, 3 h; 8, 6 h; 9, 12 h. A 10% acrylamide gel was used

Amidolytic activity measurements

K_m and V_{max} values of purified AprEJS2 were 0.09 mM and 16.71 $\mu\text{M/l/min}$, respectively. The K_{cat} was 7.66 S⁻¹, and K_{cat}/K_m was 8.51×10^4 S⁻¹ M⁻¹. The values were similar to those of some other fibrinolytic enzymes reported. The K_{cat}/K_m values were 6.7×10^4 , 6.7×10^4 , 6.4×10^4 , 1.76×10^5 , and 5.66×10^4 S⁻¹ M⁻¹ for a fibrinolytic enzyme from *B. subtilis* IMRNK1 [22], a *B. subtilis* strain [23], *Bacillus* sp. nov. SK006 [24], *B. subtilis* DC33 [25], and *B. amyloliquefaciens* RSB34 [14].

Hydrolysis of fibrinogen by AprEJS2

AprEJS2 quickly degraded A α and B β chains of fibrinogen. The A α chains were the most quickly hydrolyzed in 10 min (Fig. 6). Most B β chains were degraded in 1 h, and completely degraded in 3 h. But the γ -chains were not degraded

even after 12 h (Fig. 6). This indicated that AprEJS2 has strong α -fibrinogenase and moderate β -fibrinogenase activities. The degradation pattern was similar to those observed in other fibrinolytic enzymes such as AprE176 from *B. subtilis* HK176 [19].

Considering its significant salt tolerance and strong fibrinolytic activity, *B. subtilis* JS2 seems promising as a starter for *jeotgals* and soy sauce. *Jeotgals* prepared by traditional methods suffer from some problems, especially long time required for completion of fermentation. Under high salinity, growth of most microorganisms is inhibited except some halophiles or halo-tolerant organisms. Addition of *B. subtilis* JS2 could accelerate the fermentation process, reducing production time for *jeotgals*. The strain could improve the flavor and taste of *jeotgals*. Future studies are necessary on the growth and fibrinolytic activities of *B. subtilis* JS2 at different salt concentrations of different *jeotgals*. Due to its high fibrinolytic activity, *jeotgals* fermented with *B. subtilis* JS2 is expected to have additional functionality.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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