Heterologous Expression of Pediocin/Papa in *Bacillus Subtilis*

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Research Article

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Abstract

Listeria moniocytogenes is food-borne pathogen. Pediocin is group II α bacteriocin with anti-listeria activity, naturally produced by Pediococuus acidilactic and Lactobacillus plantarum. Gene pepA/papA encode for pediocin. Expression and secretion of active papA was relayed on transporter papC and accessary protein papD on the same operon in native host. The excretion machines were also necessary for pediocin protein expression in heterologous host of E. coli, Lactobacillus lactis, and Corynebacterium glutamicum. In this study, two vector carrying codon sequence of papA mature peptide was constructed, with or without His tag. Both fragments were inserted into plasmid pHT43 and transformed Bacillus subtilis WB800N. The strains were induced with IPTG to secrete recombination protein PA1 and PA2 respectively. Supernatant from both recombination strains can inhibit Listeria monocytogenes ATCC54003 directly. The fused protein possesses inhibition activity as a whole, exempting from cleavage of leading peptide. Protein PA1 can be purified by nickel-nitrilotriacetic acid (Ni-NTA) metal affinity chromatography. This is the first report for active pediocin expression without assistance of papCD in heterogenous host.

Introduction

Bacteriocin are ribosomally synthesized peptide that usually exhibit antimicrobial activity against microbial closely related to the producing bacteria. Pediocin (PA-1) belongs to class Ⅱα bacteriocins as heat-stable, unmodified peptide, and produced by lactic acid bacteria [1]. They possess ability of anti-*Listeria*, a food-borne pathogenic bacteria [2]. Pediocin-like group Ⅱα bacteriocin is located in an operon of four genes necessary for bacteriocin production and secretion in native host [3]. The first gene is structural pre-bacteriocin gene *pepA,* followed by the immunity gene *pepB* encoding an immunity protein that protects the bacteriocin producer from its own bacteriocin; gene *pepC* encoding an ABC transporter (ATP-binding cassette) necessary for secretion; and gene *pepD* encoding an accessory protein with chaperone-like activity and ensure the formation of correct disulfide bond in bacteriocin [4]. The whole pre-bacteriocin peptide papA contains an N-terminal leading peptide and a C-terminal mature peptide. The leading peptide is site-specific cleavage from the mature peptide by ABC transport protein papC. The mature peptide is then secreted and displayed bacteriocin activity. *Lactobacillus plantarum* Zhang-LL is a strain isolated from fermentation rice [5]. The structure and sequence of *papABCD* are nearly identical to that of *pedABCD* in *Pediococcus acidilactici, Pediococcus pentosaceus,* and *Pediococcus parvulus*. *pedABCD/ papABCD* operon is often located in Mega-plasmid and horizontally transfers between strains.

Pediocin gene has been introduced to *lactococcus lactis* [6], and *E. coli*[7, 8], where the ABC transporter protein and secretion apparatus-genes or other secretion apparatus were necessary for active peptide. To overcome this shortage, *pedA* gene was fused with dihydrogolate reductase (DHFR) gene [9], thioredoxin (Trx) gene [10], green fluorescent protein (GFP) gene [11] on the 5´ side. The fused protein had no biological activity after IPTG induction. Peciocin had to be cleaved from the fused peptide to obtain activity of anti-listeria. Recently, Goldbeck et al heterologous expressed pepA (papA) in *Corynebacterium glutamicum*. Gene *pepACD* had to be inserted into vector to ensure an active pepA peptide, which means

pepCD peptides are also essential for pepA/papA in *C. glutamicum* [12]. Protein of pepC (papC) and pepD (pepD) are 174 and 724 amino acids respectively, which are both larger than pepA/papA mature peptide (44 aa) itself. A large non-function sequence has to be inserted, which was a burden both for vector and for the host. So, it's necessary to establish a simple and rational expression system for papA/pepA. *B. subtilis* is general regarded as safe (GRAS) by FDA. *B. subtilis* is also a promising microbial cell factory with the developing of genetic manipulation tools. In this study, papA/pepA was introduced to *B. subtilis.* Intact fused protein was demonstrated anti-listeria activity directly without any further process.

Material And Method

Bacterial strains, plasmids, and oligonucleotide

Bacterial strains, plasmids, and oligonucleotides used in this study were listed in Table 1. *L. plantarum* Zhang-LL was grown at 37 °C under static condition for 24 h using MRS-lactobacilli broth. *L. monocytogenes* ATCC54003 was grown at 37 °C for 16 to 24 h in TSBYE medium (Hopebio, China). *B. subtilis* WB800N was grown at 37 °C in 2YT medium. The upper three strains and vector pHT43 were generous gifts of Dr. Yuanhong Xie in Beijing University of Agriculture. *E. coli* Trans1 (TransGen biotech) was grown at 37 °C in LB medium for appropriate time. Ampicillin and chloramphenicol was added at 100 μg/ml and 5μg/ml respectively when necessarily.

Construction of plasmid

Two plasmid was constructed to express papA mature peptide in *B. subtilis* WB800N. *E. coli-B. subtilis* shuttle vector pHT43 was used for construction of recombination vector and expression. Fragment of papA1 was synthesized according to sequence of codon sequence from papA mature peptide. Sequence of His tag and thrombin reorganization site was added before *papA* sequence. The whole fragment papA1 was optimized according to codon usage frequency and synthesized in Genewiz (China), which was then inserted into vector pHT43 between site of *Bam*H I and *Xba* I. The vector pHT43-papA1 was verified by sequence with primers p43F and p43R. In another plasmid pHT43-papA2, sequence encoded papA mature peptide was amplified with primers PAF+PAR from genome of *L. platntarum* Zhang-LL and used as template. The PCR product was digested with *Bam*H I and *Xba* I, and ligated into pHT43 with the same enzyme-digestion site.

Preparation of competent *B. subtilis* WB800N and transformation

Transformation of *B. subtilis* WB800N was according to the protocol of Spizizen [13]. In brief, one colony of *B. subtilis* WB800N was picked from petri dish and inoculated in grow medium I (GM I, 1 ▯minimal mineral salt (g/L, K_2HPO_4, 14; KH_2PO_4, 6, $(NH_4)_2SO_4$, 2; trisodium citrate·$2H_2O$, 1; $MgSO_4·7H_2O$) 95 mL, 50% glucose 1 mL, 5% acid hydrolyzed casein 0.08 mL, 10% yeast juice 1 mL, L-Trp (2 mg/mL) 2.5 mL) at 30°C , 100 rpm for 16 h. 2 mL o/n culture was inoculated in 18 mL fresh GM▯ (10%) and incubated at 37°C , 200 rpm for 3 h. 10 ml above mentioned culture was inoculated in 90 mL GM II (1 ▯minimal mineral salt 97.5 ml, 50% glucose 1 mL, 5% acid hydrolyzed casein 0.08 mL, 10% yeast juice 0.04 mL, L-Trp (2

mg/mL) 0.5 mL, 500 mmol/L $MgCl_2$ 0.5 mL, 100 mmol/L $Cacl_2$ 0.5 mL) and grown at 37°C , 100 rpm for 90 min. Bacteria was collect by centrifuge at 5000 × g for 10 min. The sediment was suspended in 10 mL GM II, which was competent cell of WB800N. 500 ng plasmid was added to 500 μL competent cell and mixed thorough. The system was incubated at 37 °C, 80 rpm for 30 min. the culture was spreaded on LB/CM agar and incubated at 37 °C for 12-16 h until colony appeared. Positive transformants was confirmed by colony PCR.

Inducible expression and purification of papA protein

Single colony of positive recombination strain was inoculated in 2TY/CM liquid medium and cultured at 37 °C, 200 rpm overnight. The broth was inoculated in fresh 2TY/CM liquid medium and cultured for another 3 h. IPTG was added to broth to induce protein expression at 30 °C, 200 rpm. Samples were withdrawed at 0, 2, 4 h. Samples were centrifuged at 12000 rpm for 5 min and stored at 4 °C until further assay. Fused protein was purified using Ni-NTA Rsein (Thermo scientific) according to user's guide.

Antimicriobial activity assay

Bacteriocin activity of fused proteins was assessed by agar diffuse test and standard microtiter plate assay. *L. monocytogenes* ATCC 54003 was grown in TSB-YE medium overnight at 37 °C and diluted 1:25 in fresh TSB-YE prior to the assay. Mueller-Hinton (MH) soft agar was added with appropriate volume *L. monocytogenes* ATCC 54003 solutions to cell concentration of 10^5 CFU/mL. The medium was plated on petri dish with Oxdord cup set beforehand. 100 μL of supernatant of expressed protein was added in the hole for agar diffuse. *L. plantarum* Zhang-LL was incubated at 37 °C statically for 24 h. The culture was centrifuge and supernatant were used for agar diffuse test. The petri dishes were incubated at 37 °C for 16-20 h and antibacterial activity was observed as a halo of inhibition in the bacterial lawn formed around the sample. For microtiter plate assay, two-fold dilution series of sample (100μl) were mixed with 100μl indicator strain in sterilized 96-well plates. The plates were incubated at 37℃ for 5-6 h or 21 h. Growth was monitored by measuring OD_{600} using Epoch plate reader (Bi-Tek). Bacteriocin activity in supernatants was determined in a semi-quantitative manner according to Holo [14]. According to this method, one Unit of bacteriocin activity (BU) were defined as the highest dilution showing at least 50% inhibition of the indicator strain.

Cultivation in bioreactors

Fed-batch cultivation was carried out with *B. subtilis* DBN-SKL-PA2 in 30 L bioreactor (Baoxin, Shanghai) stirred tank bioreactor with an initial working volume of 15 L. Single colony was grown in 2YT medium supplemented with 5 μg/mL chloramphenicol in Erlenmeyer flasks with aeration at 37℃, 200rpm overnight to obtain primary seed culture, which was then inoculated in 300 mL 2YT/CM liuquid medium and grown for 8 h. The preculture was inoculated the 15 L 2YT/CM batch culture in 30 L bioreactor, stirred at 200 rpm, 37℃. IPTG was added at3 h to a final concentration 0.1 mM, and the temperature was lowered and kept at 30℃. The initial pH was 5.8, and 20% phosphoric acid was addd

automatically to adjusted pH to blow 7. Dissolved oxygen was controlled by adaption of stirrer speed and aeration rate of 200~300 rpm. The culture was fed with 100 g/L glucose and double concentration 2YT from 10 h, and IPTG was added at the beginning of fed batch to a final concentration 0.1 mM. Total 1.6 L medium was fed. Dissolved oxygen and pH were recorded from the control panel of bioreactor. OD and glucose concentration were assayed respectively. Sample was centrifuged and the supernatant was stored at 4 °C for strain inhibition. Fermentation was conducted for 24 h. The broth was collected by centrifuge, and the supernatant was stored at 4 °C. To assay the dry material content in final broth, 10 g supernatant of 24 h fermentation broth was subjected to moisture measure on Moisture Analyzer (METTLER TOLEDO, HE53). The remaining material was redisolved in sterilized PBS (Thermo Fisher).

Scan electronic microscope

L. monocytogenes ATCC 54003 was grown in TSB-YE medium at 37 °C, 180 rpm overnight. Culture was inoculated into fresh TSB-YE and grew to OD_{600} of 0.6. Supernatant of papA fused protein was sterilized by filter and added to broth. The mixture was incubated at 37 °C for 3 h. Culture of *L. monocytogenes* ATCC 54003 without papA protein was grown simultaneously as control. Cells were harvested and washed with PBD, and fixed with 2.5% (V/V) glutaraldehyde and incubated at 4 °C o/n. After dehydrated by gradient alcohol solutions, samples were freeze-dried and coated with gold. The specimens were examined using scanning electronic microscope (SV8010, HITACHI).

SDS-PAGE and coomassie brilliant blue staining

SDS-PAGE electrophoresis was performed with Tris-Tricine 10-20% (Thermo Scientific) according to the manufacturer's protocol. Samples were mixed with sample buffer and heated to 70 °C for 10 min before they were loading onto the gels. After electrophoresis, gels were soaked in fast staining solution (Tiangen, Beijing), boiled for 1 min, and incubated at room temperature for 30 min. The gels were then washed with ddH_2O, boiled for 1 min, and incubated at room temperature for 30 min. The washing step was repeated until clear bands were shown.

Strain access number

Strain *B. subtilis* DBN-SKL-PA1 and *B. subtilis* DBN-SKL-PA2 were deposited in China General Microbiological Culture Collection Center (CGMCC) as CGMCC No. 23101 and CGMCC No. 23102.

Results

Introduction of gene *papA* to *B. subtilis*

Mature papA/PA-1 peptide harbors 44 amino acid. Expression vector pHT43 has a signal peptide of α-amylase, which led the fused protein out of the host cell. In consideration of the activity of fused protein, a His-tag plus thrombin digestion site was inserted between sequence of signal peptide and papA, which resulted in a fused protein PA1. The sequence was optimized according to codon usage preference in *B.*

subtilis. The result plasmid pHT43-PA1 was confirmed by sequence and transformed to competent *B. subtilis* WB800N using Spizizen protocol [15]. Colony PCR was carried out to confirm the insertion of *papA* gene in recombination strain. The positive clone was named *B. subtilis* DBN-SKL-PA1 and subjected to induction for protein expression by addition of IPTG. The fused protein was expected to excrete out of host cell, due to the signal peptide of α-amylase. The fused protein, named PA1, contains signal peptide of α-amylase, His-tag, thrombin digestion site and papA mature peptide. Pure papA mature peptide can be released from the fused protein when necessary. Supernatant was obtained by centrifuged and tested for Listericidal activity.

Codon sequence of papA mature peptide was also amplified from genome of *L. plantarum* Zhang-LL. Vector pHT43-PA2 was constructed by inserted this fragment into pHT43 between *Bam*H I and *Xba* I site. Plasmid pHT43-PA2 was introduced into WB800N after sequence confirmation. The positive colony was confirmed by colony PCR and named as *B. subtilis* DBN-SKL-PA2.

Induction for papA expression in *B. subtilis*

To induce gene *papA* expression in recombination strain, 0.01~0.5 mM IPTG was added to 2YT medium. The leading peptide α-amylase of the vector is secretion type, the fused protein was produced with induction and transferred outside of the host cell. The broth was collected four hours after induction. Supernatant was obtained by centrifuge. *B. subtilis* DBN-SKL-PA1 produced a fused protein, which consisted leading peptide α-amylase, His tag, thrombin digestion site, and mature peptide of papA. This protein was purified using NI-NTA resin and electrophoresis on SDS-PAGE (Fig. 1). Pure papA mature peptide can be obtained by cleavage of thrombin on the Ni column. *B. subtilis* DBN-SKL-PA2 produce a protein consisted of leading peptide of α-amylase and mature peptide of papA. Supernatant of induction was stored at 4°C until further assay.

Inhibition of *L. monocytogenes*

Gene *papA*/*pediocin* is located in an operon of harboring four gene *papABCD*/*pepABCD*. The other three gene is necessary for expression of *pepA*/*papA*. It seemed that active papA can't be produced in *E. coli* or *L. lactis* without secretory apparatus [8]. In this study, supernatant of induced recombination strains directly inhibited growth of *L. monocytogenesis* ATCC54003, both from strain DBN-SKL-PA1 and strain DBN-SKL-PA2 (Fig. 2). This result demonstrated that the fused protein PA1 and PA2 expressed in *B. subtilis* were both active. Neither papC/pepC nor papD/pepD was necessary for active papA/PA-1 in *B. subtilis*. The result also illustrated that the leading peptide didn't have to be removed from the fused peptide to result a pure papA peptide, which was quite different from papA fused protein expressed in *E. coli*.

Fused protein PA1 harbor a His tag, which was purified using Ni-NTA column. Thrombin was also added to Ni-NTA column to remove the leading peptide. Purified papA protein was eluted from column. The elution was subjected to agar diffuse. Both elution harbored papA peptide showed anti-listeria activity. Fused protein of PA2 were not distinguished on SDS-PAGE (data not shown). Since both of the two

proteins can be used for anti-listeria directly, removing the leading peptide was not necessary for an active protein. DNA sequence was optimized for vector pHT43-pap1, but not for pHT43-pap2. However, inhibition activity to *L. monocytogenes* of PA2 fused protein was stronger than that of PA1, which can be illustrated from the inhibition zone (Fig. 2). DBN-SKL-PA2 was thus used for further assay. Various IPTG concentration was tried. PA2 can be expressed even with 0.005 mM IPTG. The inhibition activity reached its maximum at 0.1 mM, which was applied for batch fermentation.

Batch fermentation of DBN-SKL-PA2 and inhibition effect

Batch fermentation was conducted with DBN-SKL-PA2 to demonstrate feasibility of recombinant pediocin on a large scale. The start culture conditions were similar to those of the shake flask. IPTG was added at 3 h to a final concentration of 0.1 mM. The culture was fed with 100 g/L glucose and double concentration 2YT from 10 h, and IPTG was added at the beginning of fed batch. Total 1.6 L fed medium was supplied. pH was adjusted by automatically addition of Phosphoric acid to blow 7. OD600, pH, glucose concentration, and dissolved oxygen was recorded and shown in Fig. 3.

The broth was withdrawed according to the time of Fig. 3. Samples were stored at 4 °C after centrifuge. Moisture was 96.80% for supernatant of 24 h fermentation broth, which resulted in 0.32 g dry matter. The remaining powder was redissolved in sterilized PBS to a final concentration 0.1 g/mL, i.e., 3.2 mL PBS, which resulted in nearly 3 times concentration than the original supernatant. The drying duration was nearly 40 minutes under 105 °C. The rehydration PA2 was also subjected to microtiter plate assay to test if heating disrupt activity of PA2.

Supernatant of 18, 21, 24 h and rehydration PA2 dry powder (105 °C) were subjected to microtiter plate assay. The cells were grown at 37 °C for 6 h. The result illustrated that inhibition activity was improved as prolonging fermentation time (Fig.4A). Most impressing, rehydration of PA2 demonstrated the best activity. Though the rehydration volume is nearly one third to the original supernatant, the activity was nearly eight times to its original one. Pediocin/papA was stable by heating at 80°C for 1 h, and at 100°C for 10 min [3] [16]. The result in this study implied activity of PA2 can be kept at 105 °C for 40 min, which is more tolerance than previous reports.

24 h filtrate was obtained by sterilization of 24 h supernatant using Millipore filter of 0.22μm. Supernatant of 24 h (24h), 24 h filtrate, and 105 °C were subjected to microtiter plate assay. The microtiter plate was incubated at 37 °C for 21 h. Inhibition activity of 24 h filtrate was similar with that of 24 h supernatant. For 105 °C drying, similar trend was found as incubated of cells for 6 h. Inhibition effect of 105 °C drying was nearly 8 times to that of 24 h and 24 h filtrate (Fig.4B). 24 h filtrate was applied for scan electronic microscope.

Scan electronic microscope of *L. monocytogenesis* ATCC54003

L. monocytogenesis ATCC54003 was grown to exponential phase. 24 h filtrate of PA2 was added to the culture according to microtiter plate assay and incubated for another 3 h. Cells were collected and

subjected to SEM. The untreated cells were intact and smooth under SEM. By contrast, cell undergone severe damage with co-culture of PA2. Biofilm was disrupted, and the cell collapsed due to outflow of cytoplasm (Fig.5). At the same time, the apparent unbroken cells were elongated. This implied abnormal division of L. monocytogenesis ATCC54003 under PA2. Cell division can be seen on untreatment group, while no signal of division was found for treated cells, even the length was 5.69 μm, which was more than two times longer than normal (2.46 μm) (Fig. S1). The destination of this cells should be broken, due to disordered cell cycle.

Discussion

In this study, codon sequence of pediocin papA mature peptide was introduced into *B. subtilis* WB800N. After induction, supernatant with anti-listeria active was obtained. This was an advance, because it was the first time for an active secreting pediocin peptide in heterogenous host. Pediocin was a secretion type peptide in native host. Gene of pediocin was naturally located in an operon, which consists of three additional genes of *papBCD*/*pepBCD*. In native host, protein of papCD are responsible for cleavage leading papA peptide and excretion of mature peptide of papA. Excretion system was also indispensable for active papA protein in *L. lactis* [6], *E. coli* [8], and *C. glutamicum* [12]. The sum of pepC/papC and pepC/papC protein are 20 times larger than pepA/papA mature peptide, which implied that 20 times redundant sequence had to be introduced for active peiodiocin pepA/papA. This is a burden both for vector and heterogenous expression host. In this study, an *E. coli-B. subtilis* shuttle vector pHT43 was applied, which harbors a signal peptide of α-amylase. This signal peptide was secreted out of cells, together with the peptide followed. Activity was remained in the fused peptide.

In *E. coli*, papA in fused protein was inactive, which had to be cut from the fused protein to get pure papA mature peptide [9, 11, 17]. The pure papA peptide possessed anti-listeria active. In this study, His tag and thrombin digestion site were inserted between signal peptide and papA mature peptide firstly, in case the signal peptide had to be removed to release an active papA. The fused peptide PA1 can effectively inhibit *L. monocytogenesis* directly, which illustrated that the signal peptide didn't hamper the function of papA. Then, a "simple" plasmid was constructed, where only pure codon sequence of papA mature peptide was inserted into pHT43 to get pHT43-papA2. The expressed fused protein PA2 contained signal peptide of α-amylase and papA mature peptide. To our surprise, inhibition activity of PA2 was superior to that of PA1, though DNA sequence for PA1 was optimized according to codon usage preference of *B. subtilis*. Strain DBN-SKL-PA2 was easily to be induced by IPTG at various concentration. The supernatant possessed anti-listeria activity, even with 0.005 mM IPTG. This result implied that the difference of codon use frequency did not influence expression level in *B. subtilis*, at least for papA DNA sequence. On the other hand, it was yet to be explore if the His fragment influenced the anti-listeria activity. In addition, the lower activity of PA1 may be attributed to low protein expression, which might be boosted by improved condition. Activity of PA2 was stronger and the supernatant can be used directly, PA2 was employed for the latter assay in this study. Induction condition of PA1 was not exploited fully. Nevertheless, pure papA mature peptide can be obtained from PA1 fused peptide by His-Ni affinity chromatography and thrombin digestion. Induction elements should be optimized further when necessary.

Pediocin/papA was stable during storage in refrigerator, by heating at 80°C for 1 h, and at 100°C for 10 min [3] [16]. In this study, the fused protein was subjected to 105°C for 40 minutes to measure the moisture content of supernatant. The activity was reserved in the heated remaining powder, which can inhibit *L. monocytogenes* after rehydration. This result demonstrated that papA protein was thermo-tolerant, like carotene. Furthermore, the activity seemed to be boosted. The original content was 10 g (nearly 10 ml) for supernatant. The remaining powder was 0.32 g, which was dissolved in 3.2 mL PBS to a final concentration of 0.1 mg/mL. The concentration was nearly 3 times to the original supernatant, while the inhibition activity was nearly 8 times. This result implied 2 times more activity enhanced after heating under 105 °C. Supernatant of WB800N with pHT43 empty-vector was subjected to the same treatment. The control re-dissolved solution did not show inhibition activity to *L. monocytogenes*. So, this inhibition activity was from PA2 protein. This result demonstrated the stability and special activity of PA2 protein, while the specific reason for improved activity still needs to be explored. The structure of papA peptide might be modified, which resulted in more competent molecular. Further study should focus on what happened to papA peptide under heating.

Conclusion

Two recombination strains DBN-SKL-PA1 and DBN-SKL-PA2 were constructed to express pediocin papA in *B. subtilis*. The strains can be induced to excrete papA fused protein to supernatant. The supernatant possessed anti-listeria activity. The fused protein was thermotolerant.

Declarations

Ethics approval and consent to participate: Not applicable

Competing interests: The authors declared that they have no competing interest.

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Author contribution: G. W., Z. G., and X. L. designed the project and wrote the manuscript. G. W., Z. G., and X. Z. constructed the recombination strains and performed induction expression. H. W., X. B., R. H., and C. H. performed inhibition assay. H. Z., and Y. P., performed SEM; H. W., Z. G., L.Y., L.Z., and C. P. performed batch fermentation. All authors reviewed the manuscript.

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Tables

Table 1 Strains, plasmid, and primers used in this study

Strain and plasmid	description	source
Strain		
E. coli Trans10		Transgen
L. plantarum Zhang-LL		Jin (Jin, Jie et al. 2020)
B. sbutilis WB800N	*nprE aprE epr bpr mpr::ble nprB::bsr Δvpr wprA::hyg cm::neo; NeoR*	Dr Xie
B. sbutilis DBN-SKL-PA1	*B. sbutilis* WB800N with plasmid pHT43-papA1	This study
B. sbutilis DBN-SKL-PA2	*B. sbutilis* WB800N with plasmid pHT43-papA2	This study
L. monocytogenes ATCC 54003		Dr Xie
Plasmid		
pHT43	Cm for *B.subtilis*, Amp for *E.coli*, P*grac01* promoter, modified signal peptide of α-amylase	Dr Xie
pHT43-papA1	pHT43 with His + Xa site + papA mature peptide cds	This study
pHT43-papA2	pHT43 with papA mature peptide cds	This study
Primer		
PAF	5´-CGGGATCCAAATACTACGGTAATGGGGT-3´	
PAR	5´- GCTCTAGATTGTTTAATATGTTCCGACT-3´	
P43F	5´- CGGACAGTTTCGTTCAGA-´3	
P43R	5´- TGAGTATCTTCTTCCGTGAT-´3	

Figures

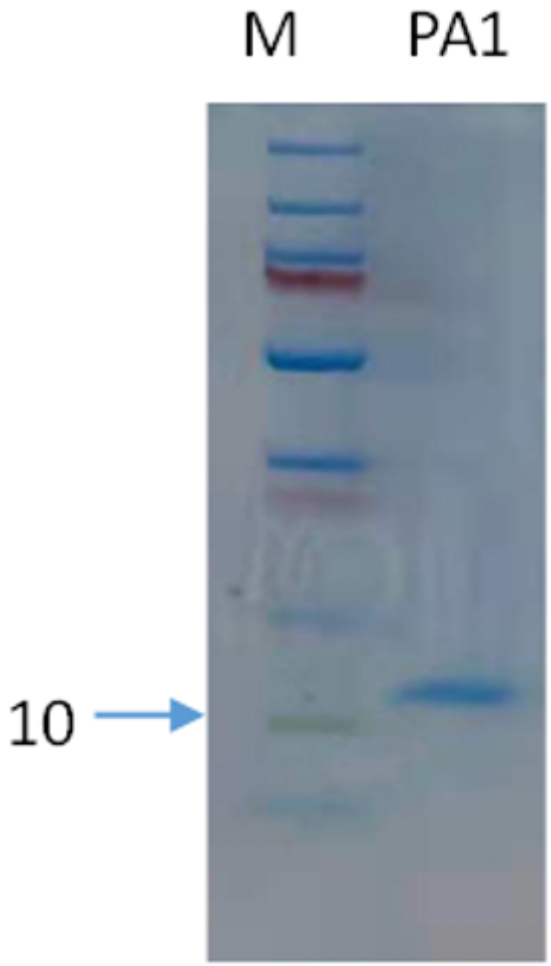


Figure 1

SDS-PAGE of PA1 purified protein.

Figure 2

Inhibition of papA protein to L. monocytogenes ATCC54003.

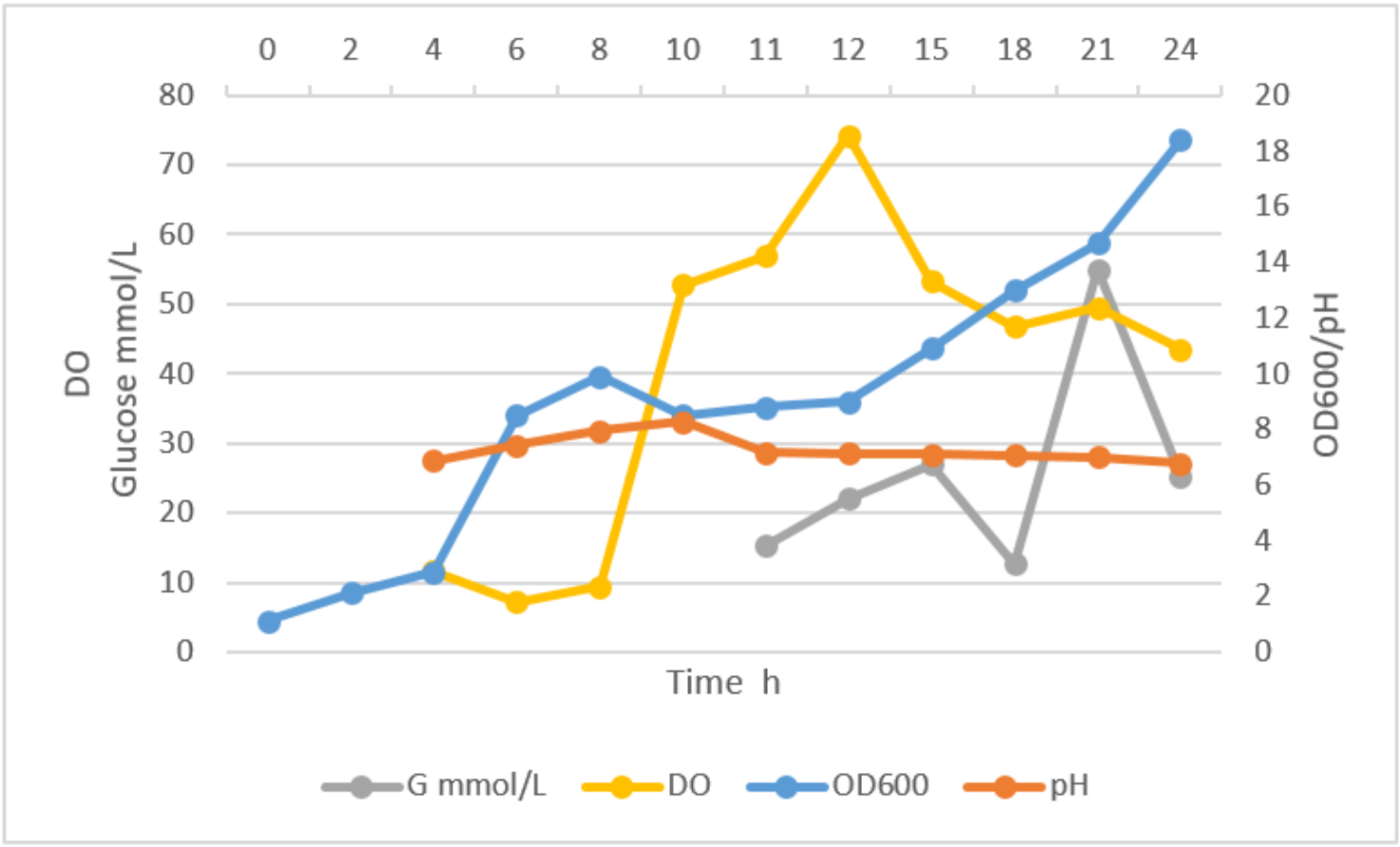
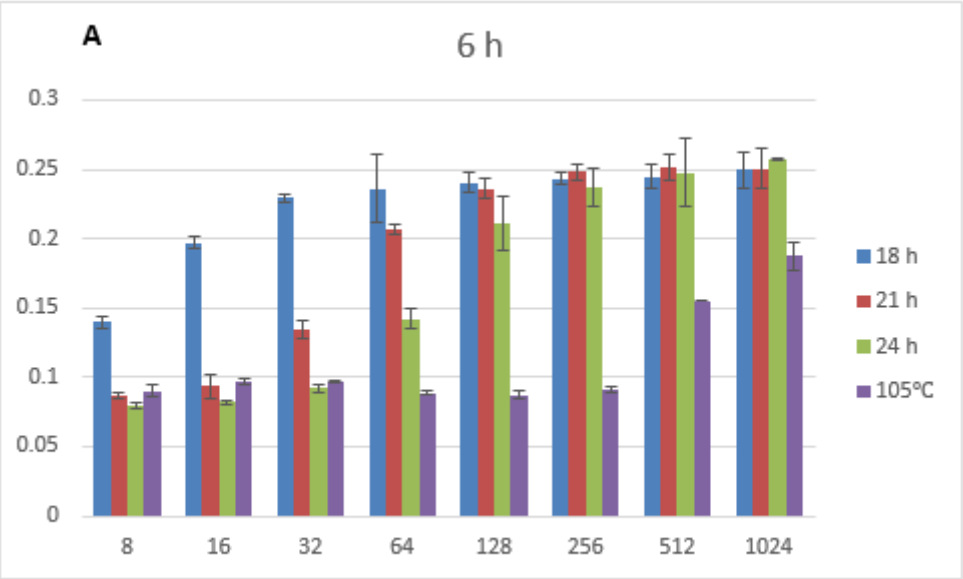


Figure 3

Substrate, dissolved oxygen, OD600, and pH during batch fermentation of DBN-SKL-PA2.



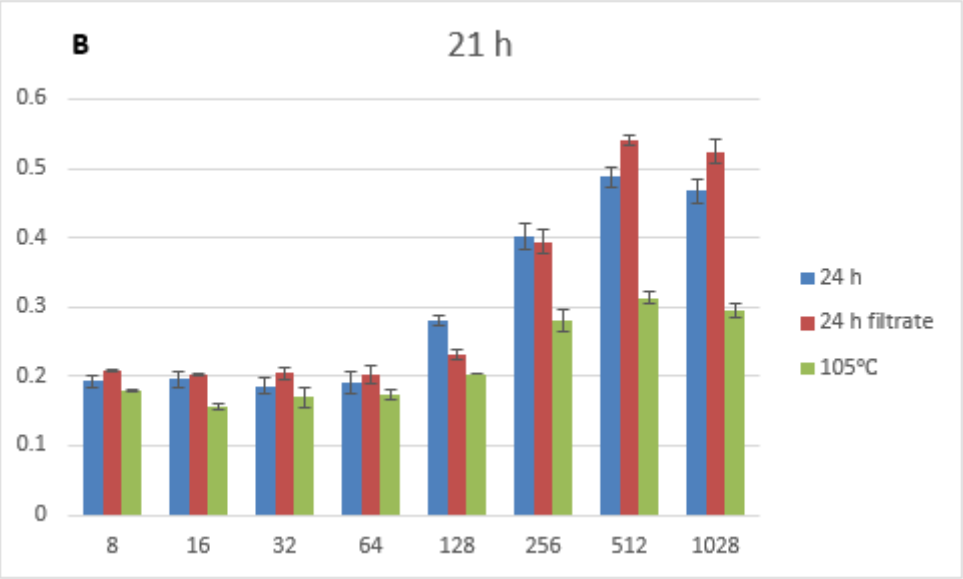


Figure 4

Growth inhibition of L. monocytogenesis ATCC54003 by supernatant of DBN-SKL-PA2.

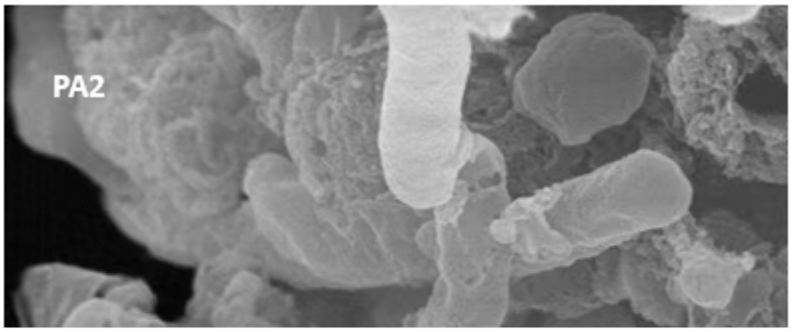


Figure 5

SEM of L. monocytogenes ATCC54003.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryofpapA.docx