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ORIGINAL PAPER

# Enhanced fed-batch production, partial purification, characterization of jensenin P, and discovery of a new bacteriocin-like substance produced by *Propionibacterium jensenii* B1264

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**Abstract** *Propionibacterium jensenii* B1264 was discovered to inhibit various *Propionibacterium*, *Lactobacilli*, and *Lactococcus* strains based on a deferred agar spot-on-lawn detection method in previous research, in which *Lactobacillus delbrueckii* ssp. *lactis* ATCC 4797 was the most susceptible one. The antimicrobial substance(s) was named jensenin P and *L. delbrueckii* ssp. *lactis* ATCC 4797 was thereafter used as the indicator strain in subsequent research. In current study, production of jensenin P was significantly enhanced via an improved fed-batch fermentation method and was partially purified using reverse phase silica gel (C18). This partially purified jensenin P was inactivated by trypsin, pronase, and proteinase K. The molecular weight of jensenin P was determined to be between 4.5 and 5 kDa. Jensenin P was characterized as being pH stable with partial inactivation at 100 °C. Jensenin P was determined to be bactericidal, causing 2.38 log reduction within 60 min at room temperature. A new bacteriocin-like inhibitory substance (BLIS) produced by *P. jensenii* B1264 was discovered in this study. The new BLIS inhibited the growth of *Propionibacterium acnes* ATCC 6919 but not the previously used indicator strain *L. lactis* ATCC 4797. The new discovered BLIS was recovered from the 30 % acetonitrile

elution fraction of C18 resin silica column purification process. This new BLIS might present new potential application in the pharmaceutical industry for acne treatment.

**Keywords** Bacteriocin · Fed-batch fermentation · Purification · Stability · Bactericidal · Anti-acne

## Introduction

Bacteriocins are ribosomally encoded peptides or proteins that usually exhibit antimicrobial activity against species genetically closely related to the producer strains [1]. Various bacteriocins produced by Gram-positive bacteria, especially lactic acid bacteria, have been purified and characterized [2], and most of the characterized bacteriocins thus far show relatively narrow spectrum of inhibition [3]. The most intensively studied bacteriocins are produced by lactic acid bacteria since they are categorized as “Generally Recognized As Safe” (GRAS) and have the most immediate potential in being applied in the food industry.

Based on their habitats, the genus *Propionibacterium* is divided into classical and cutaneous species [4]. Classical propionibacteria, typically including *P. freudenreichii*, *Propionibacterium jensenii*, *P. thoenii*, *P. acidipropionici*, and *P. cyclohexanicum* [5], are known as “food grade” microorganisms since they have been widely used in dairy products and olive fermentations for a longtime [6]. As starter cultures, their presence contributes to the characteristic flavors and desirable textures for fermented foods. Classical propionibacteria are responsible for the typical “eyes” in Swiss cheese. They are also used in industry in the production of vitamin B<sub>12</sub>, propionic acid, acetic acid, and bacteriocins [5]. Several bacteriocins produced by classical propionibacteria have been described including jensenin G

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[7], jensenin P [8], a precursor protein of an antimicrobial peptide (PAMP) [2], propionin PLG1 [9], propionin T1 [3], propionin SM1 and SM2 [10], and propionin F [1]. Cutaneous propionibacteria are among the most common anaerobic microorganisms proliferating on human skin. This group typically includes *Propionibacterium acnes*, *P. avidum*, *P. granulosum*, and *P. lymphophilum* [11], in which *P. acnes* is the major pathogen that causes inflammation in acne vulgaris development [12]. Very few bacteriocins or bacteriocin-like inhibitory substances (BLIS) have been reported to inhibit *P. acnes* to date [13, 14]. However, these types of BLIS(s) attract great interest in the pharmaceutical industry for acne treatment due to the emergence of antibiotic resistant microorganisms [14, 15].

In 1993, Prince discovered that *P. jensenii* B1264 inhibited the growth of several species of dairy *Propionibacterium*, *Lactobacilli*, and *Lactococcus* by the deferred agar spot-on-lawn detection method. The inhibitory agent(s) was defined to be a bacteriocin and was named jensenin P. However, based on this previous study, it was not clear whether one or more bacteriocins were responsible for the antimicrobial activity against the above reported species because original assays were conducted using filtered and concentrated spent culture medium. *Lactobacillus delbrueckii* ssp. *lactis* ATCC 4797 was the most sensitive strain of all strains tested and has been used as the indicator strain to assess jensenin P activity afterward [16]. In subsequent studies, Ratnam partially purified jensenin P by anion exchange chromatography and the crude jensenin P was discovered to be also antagonistic against many clinical isolates of *P. acnes* [17]. In 2000, Wallin produced jensenin P in batch and fed-batch cultures recording the maximum activity in the fed-batch system at 400 AU/ml [18].

The objective of this research is to develop more efficient growth conditions to produce a sufficient amount of jensenin P and a better purification system to purify the bacteriocin for subsequent stability and characterization studies. In addition, purification of the bacteriocin will help to determine whether more than one bacteriocin(s) exists in the partially purified supernatant.

## Materials and methods

### Bacterial strains, chemicals, and growth conditions

Jensenin P producer strain *P. jensenii* B1264 and *L. delbrueckii* ssp. *lactis* ATCC 4797 were obtained from Dr. Susan Barefoot, Clemson University, South Carolina. *P. acnes* ATCC 6919 was purchased from American Type Culture Collection (ATCC, Manassas, VA). *L. delbrueckii* ssp. *lactis* ATCC 4797 was cultivated at 35 °C with 5 % CO<sub>2</sub> in lactobacilli MRS broth or MRS agar. *P. acnes* and

*P. jensenii* were incubated at 37 and 32 °C, respectively, in sodium lactate broth (NLB) consisting of 1 % tryptic soy broth without dextrose, 1 % yeast extract, and 1 % sodium lactate (syrup, 60 % w/w). Sodium lactate agar (NLA) plates and soft NLA overlay were prepared by adding 1.5 or 0.75 % agar to NLB, respectively. The anaerobic growth environment for propionibacterial cultures grown on NLA plates, or in small volume, NLB was generated by the addition of an anaerobic gas mixture (H<sub>2</sub> 9.5 %, CO<sub>2</sub> 10.5 %, N 80 %) using the Anoxoma AN2CTS system (Spiral Bio-Tech, Holland). Larger volume of *P. jensenii* cultures were grown in a 2 l bioreactor (BioFlo 110 Fermentor/Bioreactor, New Brunswick Scientific, Connecticut USA) under conditions of reduced oxygen by sparging with nitrogen gas during incubation.

### Fed-batch fermentation

Fed-batch fermentation was carried out in a 2 l bioreactor with slow agitation (100 rpm). *P. jensenii* B1264 was refreshed from frozen culture (−80 °C), subcultured two times on NLA plates, and subsequently transferred to NLB. A 1 % inoculum of a 4-day broth culture of *P. jensenii* B1264 was inoculated to the 2 l NLB in the bioreactor and incubated at 32 °C for 18 days with constant slow agitation. Sodium lactate (syrup, 60 %, w/w) was added to the fermenter twice daily to provide a 0.3 % feeding by volume. Samples (5 ml) of cultures were taken daily for bacteriocin activity assay against *L. delbrueckii* ssp. *lactis* ATCC 4797, turbidity (OD<sub>600</sub>), viable cells count (CFU/ml), and pH measurements. Viable cell counts (CFU/ml) were determined by making appropriate dilutions of daily culture samples to the surface of NLA plates in triplicate and incubating the plates anaerobically. In preliminary batch of fermentation, the culture was fermented in the same condition for 33 days to determine a time point of maximum jensenin P production. In the experimental batch of fermentation, cultures were harvested at day 18 for subsequent purification.

### Partial purification of jensenin P

Supernatant (2 L) was collected by centrifugation (15 min, 4 °C, 10,000g) and filtered (polyethersulfone membrane 0.45 μm) and was dialyzed (3500MWCO membrane) twice against 12 l of 50 mM sodium phosphate buffer overnight. The supernatant was concentrated by freeze drying using Virtis DBT Benchtop 7.0 l Freeze Dryer (SP Industries; Warminster PA, USA) and was resuspended in 50 ml sodium phosphate buffer (50 mM). Concentrated sample was partially purified by reversed phase chromatography. In brief, 0.5 g C18 reverse phase silica gel (Cat # 360130500, Acros Organics) was placed in a plastic disposable column and was successively eluted by 5 ml of 5 % acetonitrile

solution plus 0.1 % trifluoroacetic acid (TFA), 99.9 % acetonitrile plus 0.1 % TFA, and 5 % acetonitrile solution plus 0.1 % TFA to equilibrate the column. Second, 1 ml dialyzed sample was loaded onto the resin and incubated by shaking on an ice bed for 1 h. Third, the resin was successively eluted with 5 ml of 50 mM sodium phosphate buffer (pH 6.4), 10, 20, 30, 40, 50, and 100 % acetonitrile solution plus 0.1 % TFA. Elutes from each fraction were separately collected and freeze-dried to completion and resuspended in one milliliter buffer. Bacteriocin activity of each fraction was measured by the microtiter plate method, and the active fraction was used in subsequent characterization studies.

#### SDS-PAGE and overlay assay

The partially purified jenseniin P (5,120 AU/ml) was loaded onto Tris-Tricine SDS-PAGE (18 % acrylamide) in duplicate. Low-Range Rainbow Molecular Weight Marker (Amersham Biosciences, RPN 755) was loaded in a separate lane. The gel was run at a constant voltage of 100 V for 45 min. Half of the gel was stained by silver staining, while other half was washed in 200 ml of 2.5 % Triton X-100 for 1 h, followed by 200 ml of sterile distilled water for 4 h with gentle shaking [10]. The washed gel was placed on a MRS plate and overlaid with 8 ml 0.75 % soft MRS agar seeded with 50  $\mu$ l *L. delbrueckii* ssp. *lactis* ATCC 4797 and incubated as previously described. Bands exhibited on the stained gel and the clear inhibitory zones exhibited on the overlay were compared.

#### Bactericidal effect

An overnight culture of *L. delbrueckii* ssp. *lactis* ATCC 4797 was centrifuged and resuspended in 50 mM phosphate buffer (PBS) with OD<sub>600</sub>1.0 (~10<sup>7</sup> CFU/ml). The washed cell suspension (500  $\mu$ l) was mixed with 500  $\mu$ l of partially purified jenseniin P (5,120 AU/ml) in PBS and held at 37 °C for 60 min with gentle shaking. Aliquots (50  $\mu$ l) taken in 10 min intervals were plated to determine viable cell counts (CFU/ml) by making appropriate dilutions of daily cultures to the surface of MRS agar plates and incubating at 37 °C overnight. Three experiments in triplicates were performed.

#### Enzyme treatment

Aqueous solution (25  $\mu$ l each) of trypsin, proteinase K, pronase, lysozyme, and catalase (10 mg/ml) were loaded onto sterilized disks (7 mm in diameter) on top of MRS agar plates. At a 1 mm distance, 25  $\mu$ l of partially purified jenseniin P (5120 AU/ml) was loaded onto identical disks. The close distance allowed the enzymes to partially diffuse to the jenseniin P disk. After 1 h of incubation at 37 °C,

the plate was overlaid with *L. delbrueckii* ssp. *lactis* ATCC 4797 in MRS soft agar and incubated at 37 °C for overnight. The shapes of clear inhibitory zones around the disks loaded with jenseniin P were examined, to evaluate if the diffusion of the enzymes in the neighboring disk have partially deactivated the bacteriocin activity.

#### Thermal stability

Partially purified Jenseniin P (5,120 AU/ml) was held in boiling water in Eppendorf tubes for 90 min. Samples (100  $\mu$ l) were removed and placed on ice every 10 min. The activity of each sample was measured using the microtiter plate method and was examined on Tris-Tricine SDS-PAGE (18 % acrylamide).

#### pH stability

HCl and NaOH were added into sterile distilled water to prepare the standard solution at different pHs (1, 2, 7, 12, and 13). Partially purified jenseniin P (20  $\mu$ l at 5,120 AU/ml) were mixed with 300  $\mu$ l of each standard solution. The mixtures were then incubated at room temperature for 1 h, neutralized with PBS buffer, and the remaining activity was quantified using the microtiter plate method as described above. Three experiments in triplicates were performed.

#### New BLIS against *P. acnes* ATCC 6919

The inhibitory activity of each eluate (10, 20, 30, 40, 50, and 100 % acetonitrile solution) from C18 silica gel was separately tested against *L. delbrueckii* ssp. *lactis* ATCC 4797 and *P. acnes* ATCC 6919. In brief, to test the bacteriocin activity against *L. delbrueckii* ssp. *lactis* ATCC 4797, 10  $\mu$ l drops of each fraction were first spotted in duplicate on the top of MRS plate and allowed to dry in room temperature. 20  $\mu$ l of overnight culture was mixed with 5 ml soft MRS agar and overlaid on top of the MRS plate and the plates were incubated at 37 °C overnight. To test bacteriocin activity against *P. acnes* ATCC 6919, 10  $\mu$ l drops of each fraction was spotted onto the top of NLA plate in duplicate and allowed to dry in room temperature. 5 ml soft NLA agar inoculated with 50  $\mu$ l 5 days fresh culture was overlaid on top of NLA plates, and the plate was incubated at 37 °C anaerobically for 5 days. Clear inhibitory zones were examined after incubation.

#### Bacteriocin activity assay

The antimicrobial activity of the bacteriocin in liquid was quantified in a microtiter plate assay with slight modifications [2]. In brief, each well of the microtiter plate was filled with 50  $\mu$ l of serially diluted sample (twofold

dilution) and 150  $\mu$ l of diluted overnight culture of *L. delbrueckii* ssp. *lactis* ATCC 4797 in MRS broth (1 % final inoculation). The plates were incubated at 37 °C for 12 h, and the absorbance at 620 nm was measured with a microtiter plate reader. By definition, one unit of antimicrobial activity (AU) causes 50 % growth inhibition (50 % of the turbidity of a control culture). The total antimicrobial unit was defined as the reciprocal of the highest dilution showing 50 % inhibition of the indicator strain and was expressed in activity unit (AU/ml).

#### Total protein BCA assay

Total protein was measured by Thermo Scientific Micro BCA™ Protein Assay Kit (Mfr. No. 23252) according to the standard protocol with slight modifications. The absorbance was measured at 570 nm.

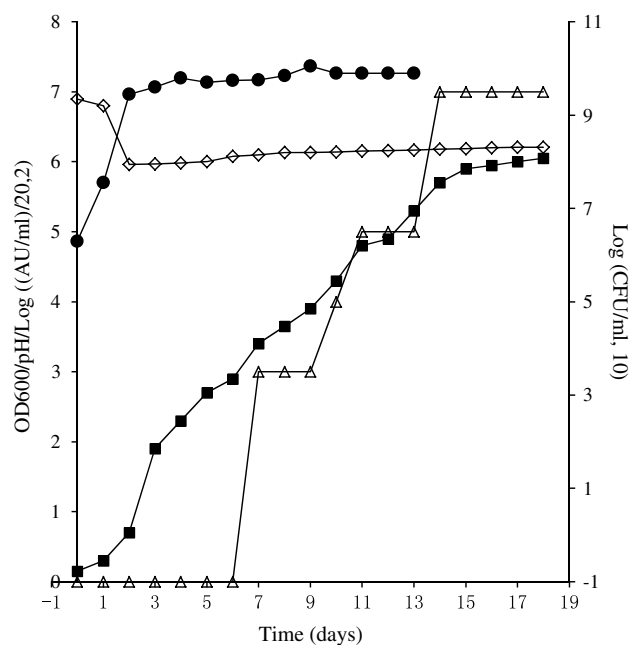
## Results and discussions

### Production of jenseniiin P in fed-batch fermentation

Several batches of fermentation were conducted in jenseniiin P production. Preliminary studies monitoring jenseniiin P production for 33 days showed that bioactivity could be detected in the middle exponential phase (day 7), reached highest activity, kept stable in the early stationary phase (day 12–18), and drastically decreased from the middle to late stationary phases (day 19–31). The OD<sub>600</sub> increased to 6 till day 17 and kept stable from day 18 to day 33. The pH was relatively stable between 6 and 7 (data not shown).

In order to harvest the maximum amount of jenseniiin P in the current batch, the supernatant was collected on day 18. In the first 18 days, the trend of bacteriocin activity, turbidity (OD<sub>600</sub>), and pH (15 days) were generally consistent with the previous 33-day studies. Viable cell numbers reached 10<sup>7</sup>CFU/ml and kept consistent through day 5 to day 18, while the turbidity (OD<sub>600</sub>) increased to 6 until day 14 and remained stable through day 18 (Fig. 1). The increase in turbidity from day 6 to day 14 was not caused by viable cells increase, and it might be caused by cell morphology changes [19] or production of polysaccharide, which is similar with the fed-batch fermentation of *P. thoenii* P126 in producing jenseniiin G [20]. Based on the report that the initial high concentration of sodium lactate was shown to be toxic to *P. jensenii*, sodium lactate syrup was fed into the media daily to provide a sufficient carbon source [18].

The highest antimicrobial activity was obtained at early stationary phase, which is consistent with bacteriocin production by other propionibacteria: propionicin T1 [3], propionicin SM1 [10], and propionicin F [1]. This indicates that the production of jenseniiin P is controlled by an inducible



**Fig. 1** *P. jensenii* B1264 growth and jenseniiin P fermentation. Cultures were grown at 30 °C for 18 days with constant slow agitation anaerobically. 60 % sodium lactate syrup was added to the fermenter twice daily to provide a constant 0.3 % feeding by volume. Bacteriocin activity, turbidity (OD<sub>600</sub>), viable cells count (CFU/ml), and pH were analyzed daily as indicated in materials and method. Filled square OD<sub>600</sub>; Open diamond pH; Open triangle bacteriocin activity; Log [(AU/ml)/20, 2]; Filled circle log (CFU/ml, 10)

promoter and the production of this bacteriocin might be initiated by environmental stress as a secondary metabolite, such as high cell density or nutrient depletion [21].

Jenseniiin P has been previously produced using the same NLB medium either by batch or fed-batch fermentation by Prince [16], Graves [19], Ratnam [8], and Wallin [18]. The activity of jenseniiin P produced by the fed-batch method was generally higher than in batch fermentations. However, the highest activity obtained in previous experiments was 400 AU/ml by Wallin using the spot-on-lawn assay method at a single day 14 [22]. To compare the results obtained in our current study, bacteriocin activity of our daily samples were also quantified using the same spot-on-lawn method for consistence. The result showed that the maximum bacteriocin activity obtained in our study was 1600 AU/ml which lasted for 5 days (days 14–18).

The increased production of jenseniiin P in this study could be due to the use of a 4-day inoculum culture instead of the previously used 2-day culture, since the 4-day culture has a higher cell density and possibly better quorum sensing between cells. It is also possible that the harvest time in this study was accurately controlled based on time-course studies, since the accumulation of jenseniiin P in the supernatant decreases after the maximum level is attained.

**Table 1** Partial purification summary of jenseiniin P

	Bioactivity (AU/ml)	Total protein (mg/ml)	Specific activity (AU/mg)	Purification factor (fold)
Supernatant of day 18	2,560 ± 0	6.6 ± 0.8	387 ± 42	1.0
Dialyzed supernatant	37,540 ± 8,360	12 ± 2.3	2,919 ± 1042 <sup>a</sup>	7.5
50 % elution fraction	5,120 ± 0	0.7 ± 0.1	7,445 ± 498 <sup>a</sup>	19.2

<sup>a</sup> The specific activity of dialyzed supernatant and 50% elution fraction is significantly higher than the specific activity of supernatant of day 18 ( $p < 0.05$ ),  $n = 6$

### Partial purification of jenseiniin P

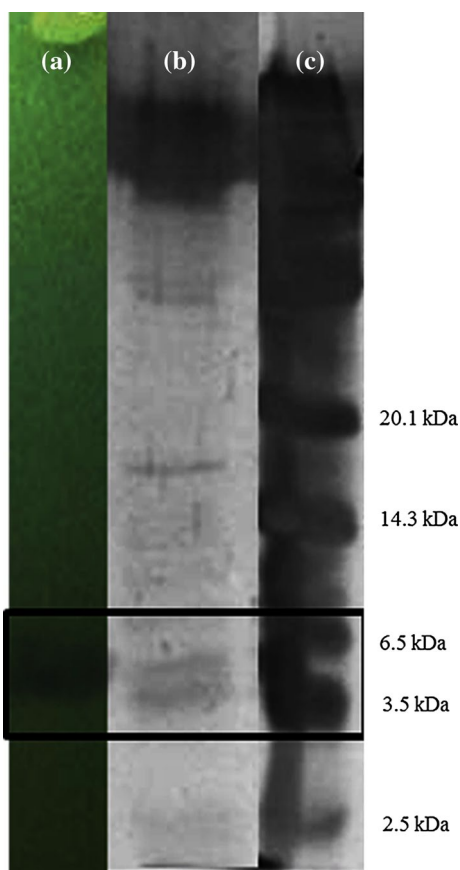
Jenseiniin P was mainly purified by C 18 resin silica gel column from 18 days supernatant after removing the cells and concentration by lyophilization. After C18 purification, bacteriocin activity of each individual fraction was measured against *L. delbrueckii* ssp. *lactis* ATCC 4797 and jenseiniin P activity was mainly in the 50 % acetonitrile elution fraction and very low in 40 % fraction. The bioactivity

after each purification step is shown (Table 1). Dialysis removed peptides smaller than 3.5 kDa and purified 7.81-fold. After purifying with C18, the 50 % acetonitrile elution fraction had higher than 90 % of the total activity against *L. delbrueckii* ssp. *lactis* ATCC 4797 and the specific activity increased 19.22-fold in the 50 % acetonitrile elution fraction after C18 resin silica column.

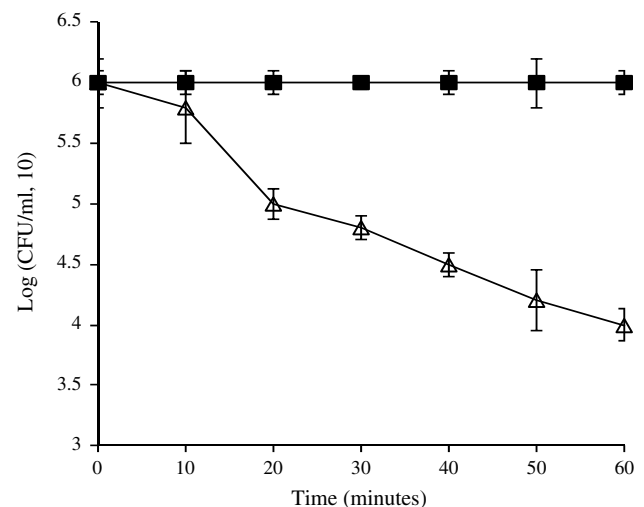
In our preliminary studies, ammonium sulfate protein precipitation was also attempted to separate and concentrate jenseiniin P. The activity was not precipitated out by lower than 60 % ammonium sulfate, but due to high loss of total activity, ammonium sulfate precipitation was not adapted in this purification process. However, the result of ammonium sulfate precipitation indicated that jenseiniin P is more polar than propionicin T1 and propionicin F, since those two were mainly precipitated by 40 % ammonium sulfate [1, 3].

### Molecular weight determination

Two prominent bands with molecular weight ranging from 3.5 to 6.5 kDa were observed on Tris-Tricine SDS-PAGE

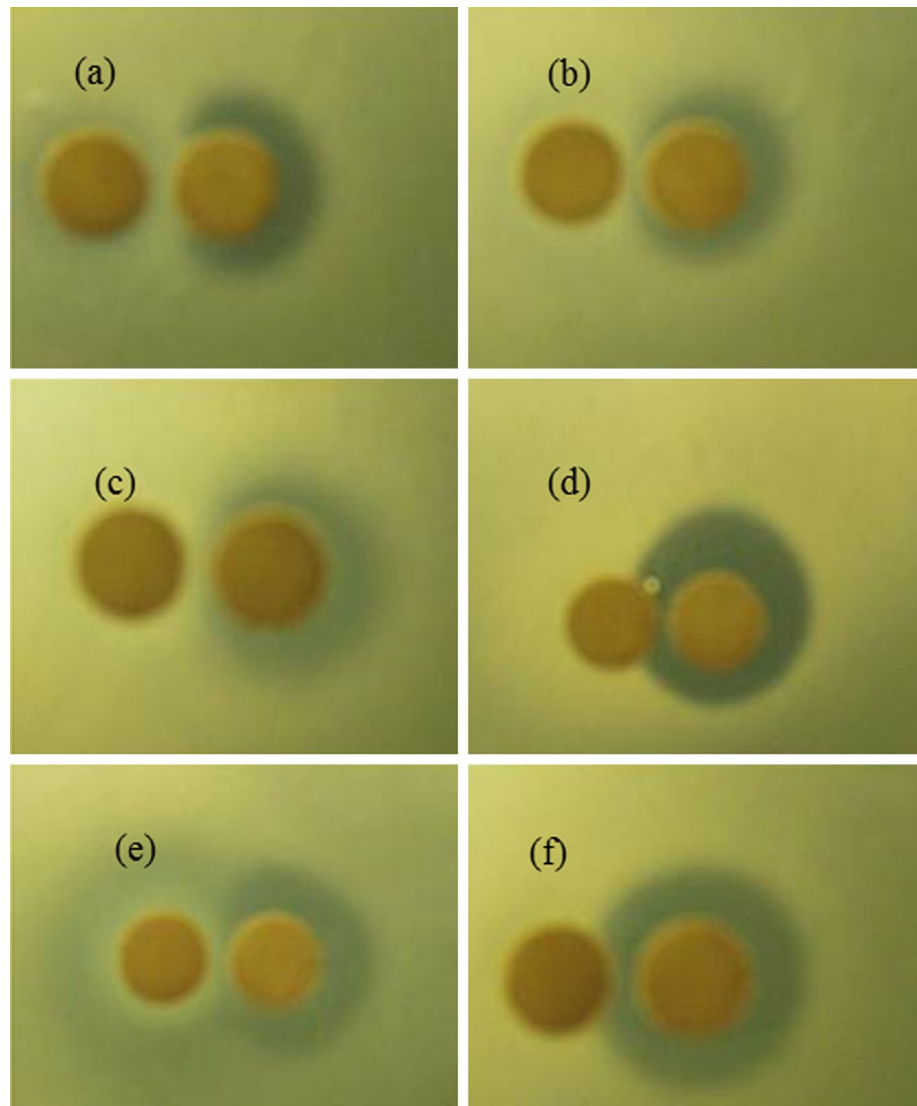


**Fig. 2** SDS-PAGE and overlay assay of partially purified jenseiniin P (5,120 AU/ml). **a** 18 % polyacrylamide gel after renaturation step with Triton X-100, on a MRS agar plate, overlaid with MRS soft agar (0.75 %) inoculated with *L. delbrueckii* ssp. *lactis* ATCC 4797; **b** Silver-stained protein gel; **c** Silver-stained low-range rainbow molecular weight marker



**Fig. 3** Bacterial activity of partially purified jenseiniin P (5,120 AU/ml) against *L. delbrueckii* ssp. *lactis* ATCC 4797. CFU/ml was determined by three independent experiments in triplicates. Mean and standard deviation were presented in the figure. *Open triangle* jenseiniin P (5,120 AU/ml); *Filled square* buffer control

**Fig. 4** Effect of enzyme treatment on further purified jenseiniin P (5,120 AU/ml)  
*Left disks: a* trypsin; *b* protease K; *c* pronase; *d* catalase; *e* lysozyme. *f* MRS broth. *Right disks:* partial purified jenseiniin P (5,120 AU/ml)



using the C-18 resin silica gel partially purified jenseiniin P sample. A clear zone was formed on the duplicate gel in the corresponding position. The two bands were cut separately according to the location of the marker, and each band was washed with Triton X-100 and water and overlaid with the indicator strain. The lower band was determined to inhibit the growth of the indicator culture (data not shown). The molecular weight of jenseiniin P was narrowed down to 4.5–5 kDa based on comparing the center of the inhibition zone and the lower band with the markers (Fig. 2).

#### Mode of action of jenseiniin P

Treatment of *L. delbrueckii* ssp. *lactis* ATCC 4797 with jenseiniin P caused a 2.38 log reduction in the population in 60 min. This result indicated that jenseiniin P has a bactericidal mode of action instead of bacteriostatic (Fig. 3). Most bacteriocins produced by LAB have a bactericidal mode of action [23].

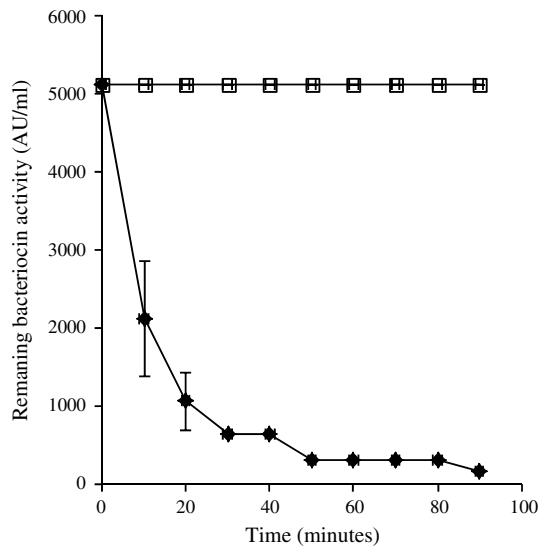
The fast acting mode of action indicated that jenseiniin P might act on the membrane level against indicator strains.

#### Enzyme treatment

In this present work, activity of jenseiniin P was inactivated by trypsin, proteinase K, and pronase E, as indicated by altered clear zones around the disk. Activity of jenseiniin P was not affected by lysozyme and catalase (Fig. 4). Jenseiniin P showed proteinaceous nature by being inactivated by the non-specific enzymes proteinase K and pronase. It indicated that jenseiniin P contains lysine or arginine residues, since it was also inactivated by trypsin [24].

#### Heat stability

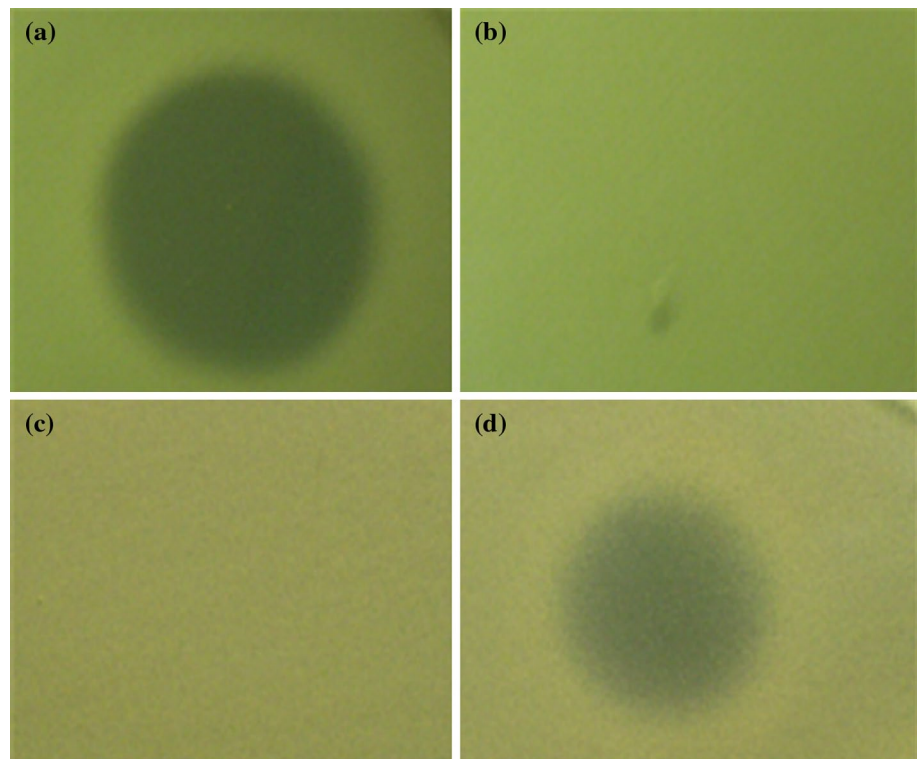
Jenseiniin P was partially inactivated by boiling water treatment for 90 min, showing a 2-log reduction in activity



**Fig. 5** Heat treatment of partially purified jenseiniin P (5,120 AU/ml). Activity against *L. delbrueckii* ssp. *lactis* ATCC 4797 was determined by three independent experiments in triplicates. *Open square* 4 °C, control; *Filled diamonds* 100 °C

(Fig. 5). High temperatures were reported to affect the activity of some bacteriocins: propionicin PLG-1 loses activity [25] while jenseiniin G remains stable [7]. As indicated by SDS-PAGE, the jenseiniin P band was more dominant compared to other contaminant proteins after heat treatment (data not shown).

**Fig. 6** Spot-on-lawn assay of 50 % acetonitrile eluent and 30 % acetonitrile eluent. **a** 50 % acetonitrile eluent on *L. delbrueckii*; **b** 30 % acetonitrile eluent on *L. delbrueckii* ssp. *lactis* ATCC 4797; **c** 50 % acetonitrile eluent on *P. acnes* 6919; **d** 30 % acetonitrile eluent on *P. acnes* 6919



## pH stability

After the samples were mixed with different pH standards ranging from 2 to 13, the pH of different mixtures changed slightly, from 1.9 to 12.8. Under different acidic or basic conditions, jenseiniin P exhibited the same activity (320 AU/ml) after being treated for 1 h, which indicated that the activity of jenseiniin P was not affected by moderate to extreme acidic or basic conditions. The stability of jenseiniin P under extreme pH conditions indicated its potential as a food preservative or starter culture. Some bacteriocins are pH-dependent since their net charge was affected in different pH environments. For example, some lantibiotic and non-lanthionine-containing bacteriocins show higher activity at lower pH (pH 5 and below) than at physiological pH, because their adsorption to the cell surface of Gram-positive bacteria, including the producing cells is pH-dependent [22].

## Discovery of a new bacteriocin-like substance

The 30 % acetonitrile elution fraction inhibited the growth of *P. acnes* ATCC 6919, but did not inhibit the growth of *L. delbrueckii* ssp. *lactis* ATCC 4797. The 50 % acetonitrile elution fraction inhibited the growth of *L. delbrueckii* ssp. *lactis* ATCC 4797, but did not inhibit the growth of *P. acnes* ATCC 6919 (Fig. 6). 40 % fraction only slightly inhibits the *L. delbrueckii* ssp. *lactis* ATCC 4797 (data not shown).

10, 20, and 100 % acetonitrile elution fractions inhibited none of the indicator strains. It is highly possible that the inhibitory agent in the 30 % fraction is a new BLIS based on the fact that this BLIS was neither removed by dialysis with a cutoff of 3.5 kDa, nor eluted by up to 20 % acetonitrile wash on C18 silicon resin. However, the proteinaceous nature needs to be further determined by enzyme digestion assays. In fact, since the purity of both 30 and 50 % acetonitrile elution fractions were not confirmed, more than one BLIS are possible to exist in each fraction. This BLIS against *P. acne* has the potential to be an alternative acne vulgaris treatment since antibiotic resistance is an emerging problem in acne treatment [12].

## Conclusion

This research improved the fed-batch fermentation method to produce jensenin P in a new cultivation system. The partially purified jensenin P was determined to be heat labile and pH stable. Results in this work also indicated that more than one bacteriocin or BLIS might be produced by *P. jensenii* B1264. Further purification and analyses are needed in the future to accurately evaluate their potential in both food and pharmaceutical industries.

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**Conflict of interest** None.

**Compliance with Ethics Requirements** This article does not contain any studies with human or animal subjects.

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