Supporting Information

Purification, characterization, mode of action, and application of jileicin, a novel antimicrobial from *Paenibacillus jilinensis* YPG26

Ke Ma[a†], Wei Chen[a†], Shi-Qing Yan[a], Zhen-Zhen Liu[a], Xiao-Qi Lin[a], Jia-Bao Zhang[a], Yu Gao[a], Tao Wang[a], Jian-Gang Zhang[a], Yong-Jun Yang[a*]

[a]Key Laboratory of Zoonosis Research, Ministry of Education, College of Veterinary Medicine, Jilin University, Changchun 130062, China.

***Corresponding Author:** Yong-Jun Yang, Email: youngjune@jlu.edu.cn; No. 5333 Xi'an Road, Changchun 130062, China; Tel: 86-431-87836424.

†K.M. and W.C. contributed equally to this paper.

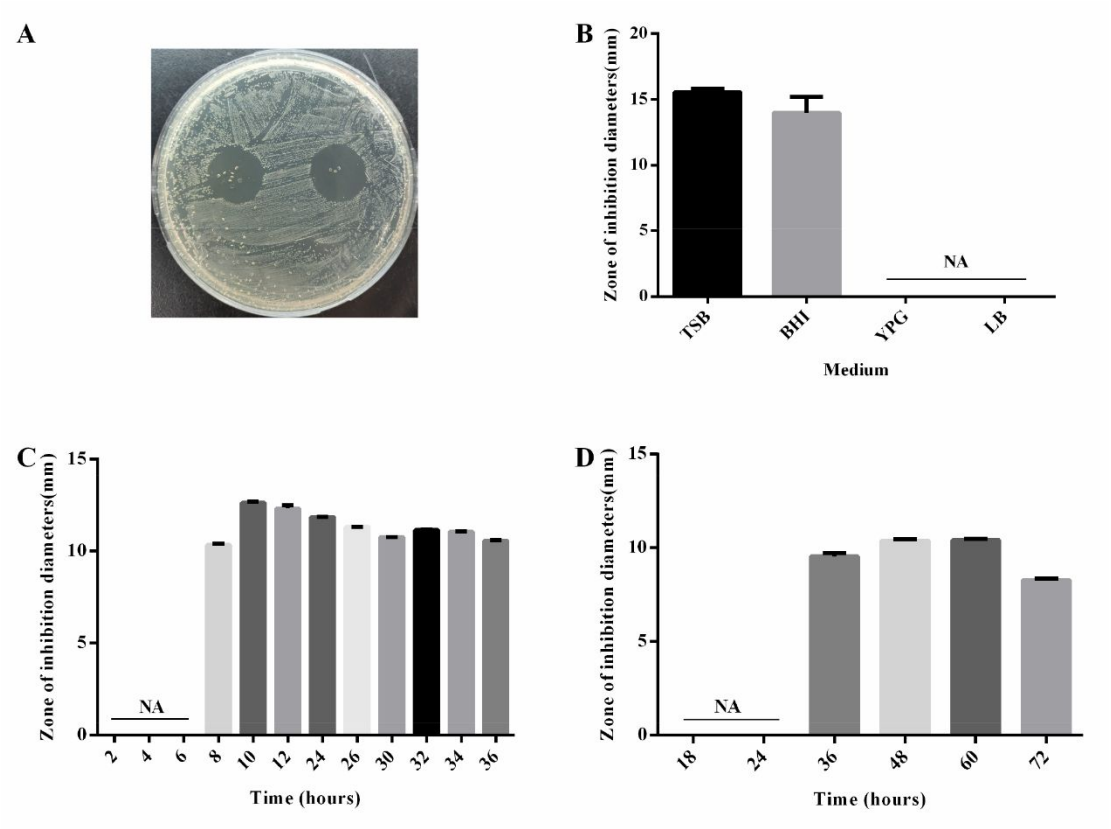

Figure S1. Optimization of partial fermentation conditions. (A) The antibacterial activity of cell-free supernatant (CFS) of *P. jilinensis* YPG26 against *E. faecium*; (B) Antibacterial activity of CFS against *E. faecium* in different medium; (C) Antibacterial activity of CFS against *E. faecium* in different time under aerobic conditions; (D) Antibacterial activity of CFS against *E. faecium* in different time under anaerobic conditions.

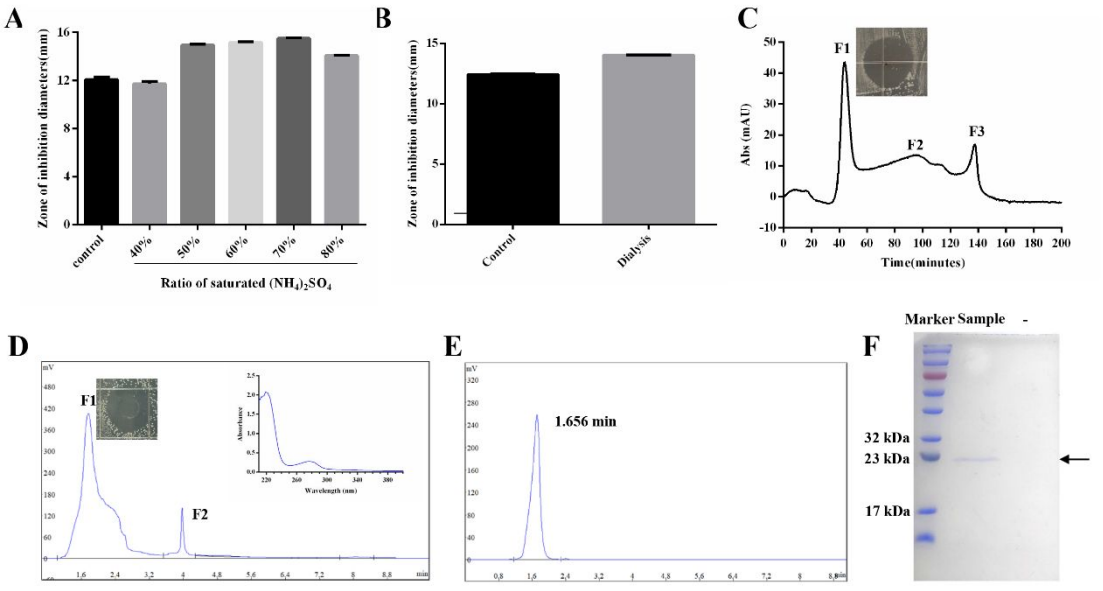

Figure S2. The purification of jileicin. (A) Purification by ammonium sulfate precipitation; (B) Dialysis desalination; (C) Purification by Sephadex G-75 gel filtration; (D) Purification by preparative RP-HPLC purification, and inlay depicts UV-visible absorbance spectrum of the partially purified jileicin; (E); RP-HPLC analysis of purified jileicin (with retention time of 1.656min); (F) SDS-PAGE analysis of purified jileicin (molecular mass approximately 23kDa).

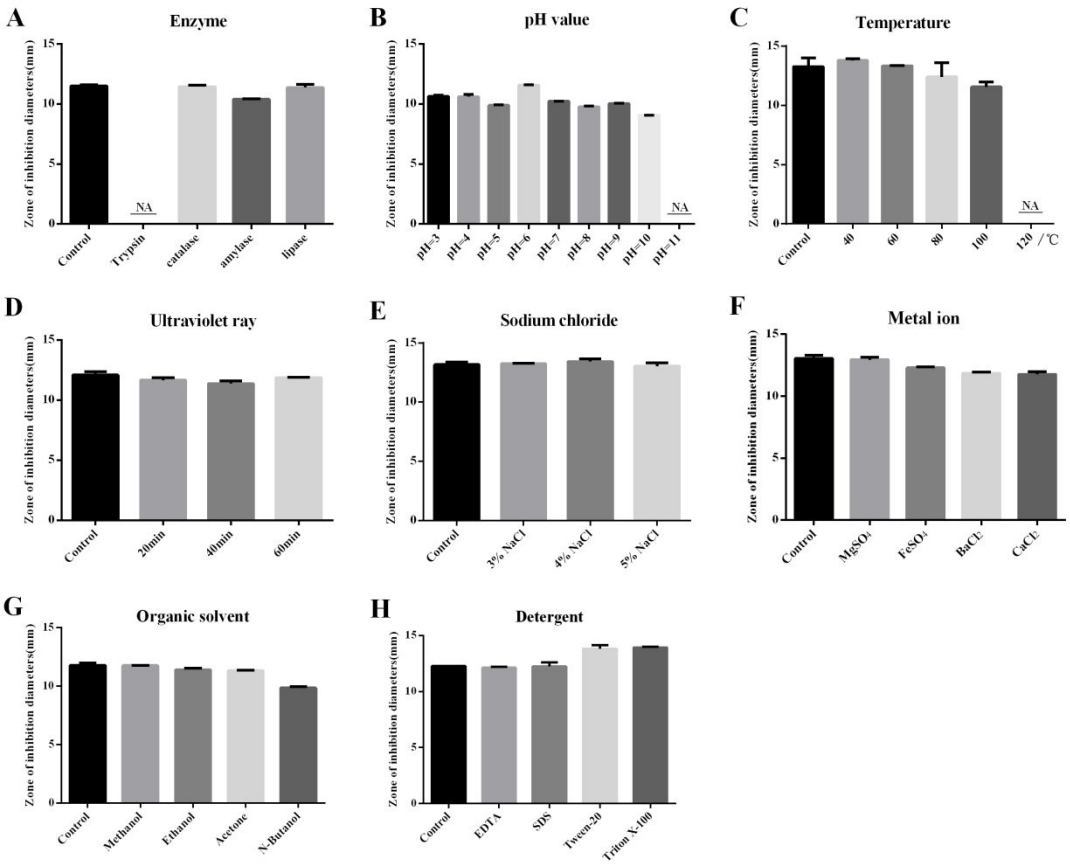

Figure S3. Stability assays of jileicin. The antimicrobial activity of jileicin after enzymes (A), pH (B), temperature (C), ultraviolet rays (D), sodium chloride (E), metal ions (F), organic solvents (G), and detergents (H) treatment.

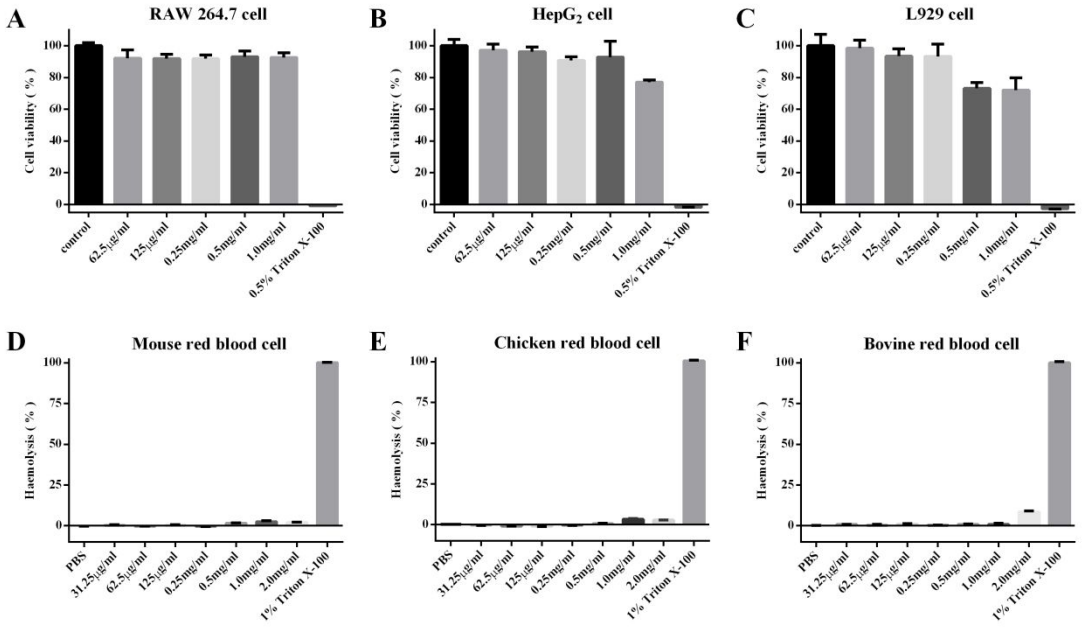

Figure S4. Safety assays of jileicin. (A-C) Cytotoxicity of jileicin against RAW 264.7 cells (A), HepG2 cells (B), L929 cells (C); (D-F) Hemolytic activity of jileicin to the mouse (D), chicken (E), bovine (F) red blood cells.

Table S1. Peptide fragments of jileicin identified by MALDI-TOF/TOF

No.	Peptide fragment
1	NVVNVVGNGEISIKPDIAYLTIGVEAQAATAQGAQK
2	ATAAQIAK
3	ATAAQIAKLTNLLK
4	LTNLLK
5	LTNLLKNTWK
6	NTWKLDAADIQTASFYVQPNYTYSDKEGQK
7	LDAADIQTASFYVQPNYTYSDK
8	LDAADIQTASFYVQPNYTYSDKEGQK
9	LDAADIQTASFYVQPNYTYSDKEGQKLK
10	LKGYNASHSLQIK
11	GYNASHSLQIK
12	GYNASHSLQIKYR
13	YRQLDK
14	YRQLDKVGQLLDDAAK
15	QLDKVGQLLDDAAK
16	QLDKVGQLLDDAAKNGANR
17	VGQLLDDAAK
18	VGQLLDDAAKNGANR
19	VGQLLDDAAKNGANRIDNVR
20	NGANRIDNVR
21	IDNVRFTVENPDQFQEQVINK
22	FTVENPDQFQEQVINK
23	FTVENPDQFQEQVINKALANAELK
24	ALANAELK
25	ALANAELKAGIIAK
26	RGLGTVLSVSQGGISSAPVFEQNYLTMDK
27	RGLGTVLSVSQGGISSAPVFEQNYLTMDKAASSESAPGSSVEPGEIK
28	GLGTVLSVSQGGISSAPVFEQNYLTMDK
29	GLGTVLSVSQGGISSAPVFEQNYLTMDKAASSESAPGSSVEPGEIK
30	AASSESAPGSSVEPGEIK
31	LTTSLSVQYELK