

Supporting Information

Boosting expression of a selective targeted antimicrobial peptide K in *Pichia pastoris* by employing a 2A self-cleaving peptide-based expression system and optimizing the fermentation conditions.

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| Item | Title |
|--------------------|---|
| Figure. S1. | Screening of positive recombinant GS115 on MM plates. |
| Figure. S2. | Blast between gene sequencing and gene synthesis for transformants. |
| Figure. S3 | WB for recombinant peptide in supernatant. |
| Table. S1. | The nucleic acid sequence of inserted fragment. |

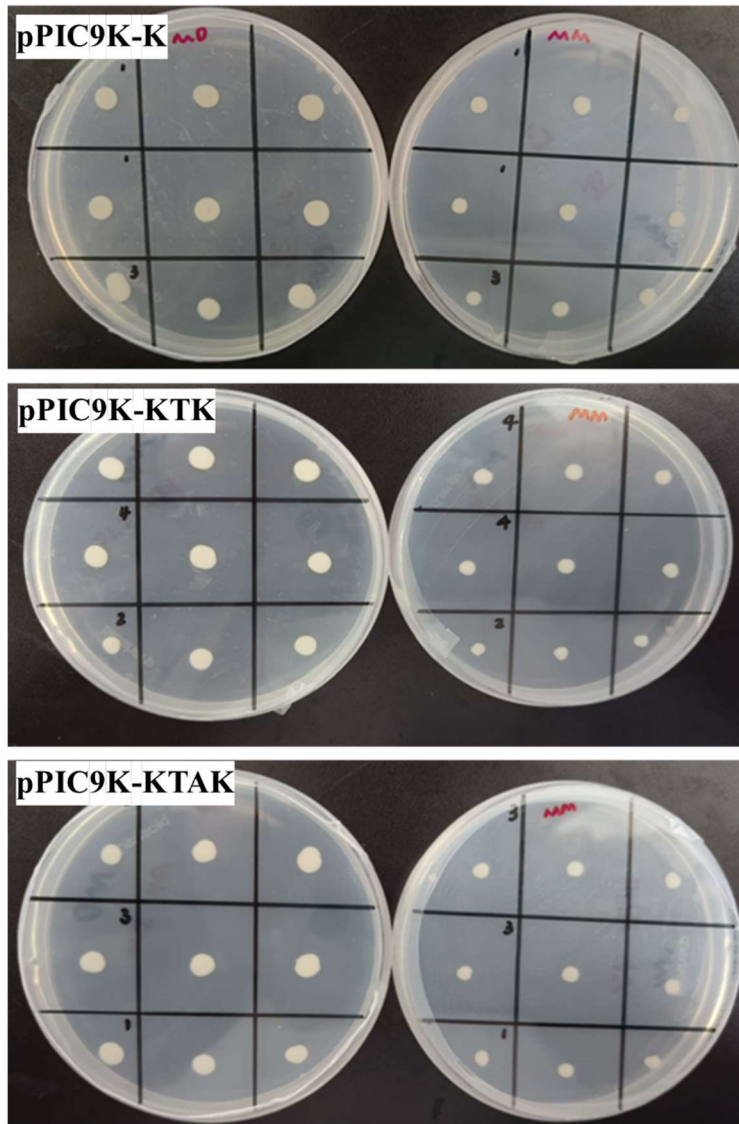


Figure. S1. Screening of positive recombinant GS115 on MM plates.

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| Score | Excerpt | Identities | Gaps | Strand |
|----------------|---|---------------|-----------|------------|
| 1452 bits(786) | 0.0 | 786/786(100%) | 0/786(0%) | Plus/Minus |
| Query 50 | GATCCAAAGCATGAGATTTCCTCAATTTTATCGACGTTTTATCGCAGCATCTCCGC | | | 109 |
| Sbjct 1 | GATCCAAAGCATGAGATTTCCTCAATTTTATCGACGTTTTATCGCAGCATCTCCGC | | | 60 |
| Query 110 | ATTAGCTGCTCCGAGCAACACTACACGAAGATGAAGCGGCAAAATTCGGCTGAAGC | | | 169 |
| Sbjct 61 | ATTAGCTGCTCAGTCAACACTACACGAAGATGAAGCGGCAAAATTCGGCTGAAGC | | | 120 |
| Query 170 | TGTCATCGGTACTCAGATTAGAAGGGGATTTCGATGTTCGTGTTTGGCCATTTC | | | 229 |
| Sbjct 121 | TGTCATCGGTACTCAGATTAGAAGGGGATTTCGATGTTCGTGTTTGGCCATTTC | | | 180 |
| Query 230 | CAGCACAATACCGGGTTATGTTTATAAATCTACTATGCCAGCATGCTGCTGAAGA | | | 289 |
| Sbjct 181 | CAGCACAATACCGGGTTATGTTTATAAATCTACTATGCCAGCATGCTGCTGAAGA | | | 240 |
| Query 290 | AGAGGGGGTATCTCTCGAAGAAAGAGAGCGTGAAGCTTAGCGAATTCTTGGAGAAAG | | | 349 |
| Sbjct 241 | AGAGGGGGTATCTCTCGAAGAAAGAGAGCGTGAAGCTTAGCGAATTCTTGGAGAAAG | | | 300 |
| Query 350 | TTGGAAGCCAGGTATTAAAGAAGTGATTAAAGGTGGTGTGCTCAAGAGCTCC | | | 409 |
| Sbjct 301 | TTGGAAGCCAGGTATTAAAGAAGTGATTAAAGGTGGTGTGCTCAAGAGCTCC | | | 360 |
| Query 410 | ATTGCTGCTGCTGTTCTGGTGAAGTAGAGGTTCTTTGTGACTGTGGTATGTTGA | | | 469 |
| Sbjct 361 | ATTGCTGCTGCTGTTCTGGTGAAGTAGAGGTTCTTTGTGACTGTGGTATGTTGA | | | 420 |
| Query 470 | AAACCCCGGCTACCATGAGATTTCATCTATTTTACCGCGTTGTTTGGCCGCTCTC | | | 529 |
| Sbjct 421 | AAACCCCGGCTACCATGAGATTTCATCTATTTTACCGCGTTGTTTGGCCGCTCTC | | | 480 |
| Query 530 | TGCTTGGCTGCTCAGATTAACTACTACTGAAAGTGAAGTCTGAGTTCAGCTGA | | | 589 |
| Sbjct 481 | TGCTTGGCTGCTCAGATTAACTACTACTGAAAGTGAAGTCTGAGTTCAGCTGA | | | 540 |
| Query 590 | AGCTGTTATTGGTTACTCGATTGGAAGGAGTTTTCGATGTTCGGTTTGGCAATTTC | | | 649 |
| Sbjct 541 | AGCTGTTATTGGTTACTCGATTGGAAGGAGTTTTCGATGTTCGGTTTGGCAATTTC | | | 600 |
| Query 650 | TAACCTATGCCACAACCGCTGTGTGTTGCAACACCATATGCGCTCTGAGTGTCTAA | | | 709 |
| Sbjct 601 | TAACCTATGCCACAACCGCTGTGTGTTTATACACCATATGCGCTCTATGCTGTCTAA | | | 660 |
| Query 710 | GGAGGAAGGTTGTTCTTTGGAAGAAAGGAGGCGAGCTGTGGAAGAAGATTGGAAGC | | | 769 |
| Sbjct 661 | GGAGGAAGGTTGTTCTTTGGAAGAAAGGAGGCGAGCTGTGGAAGAAGATTGGAAGC | | | 720 |
| Query 770 | AGGTATTAAAGATGGATTAAAGGTTGGTGTCAAAAAGACCAAGATAGATTGTCTGC | | | 829 |
| Sbjct 721 | AGGTATTAAAGATGGATTAAAGGTTGGTGTCAAAAAGACCAAGATAGATTGTCTGC | | | 780 |
| Query 830 | TTAAGC 835 | | | |
| Sbjct 781 | TTAAGC 786 | | | |

Figure. S2. Blast between gene sequencing and gene synthesis for transformants. **A.** pPIC9K-K; **B.** pPIC9K-KTK, **C.** pPIC9K-KTAK.

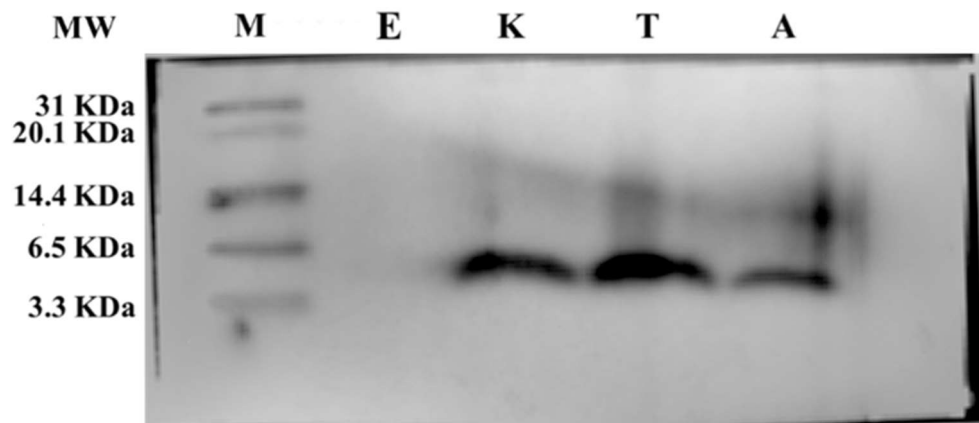


Figure. S3. WB for recombinant peptide. M: marker; E: empty vector; T: KTK; A: KTAK

Table S1. The nucleic acid sequence of inserted fragment.

| Items | Nucleic acid sequence |
|-------|---|
| K | TGGAAGAAGATTTGGAAGCCAGGTATTAAGAAGTGGATTAAGG GTGGTGGTCAAAAAGACCAAGAGTTAGATTGTCTGCT TGGAAGAAGATTTGGAAGCCAGGTATTAAGAAGTGGATTAAGG GTGGTGGTCAAAAGCGTCCAAGAGTTAGATTGTCTGCTGGTTCT |
| KTK | GGTGAAGGTAGAGGTTCTTTGTTGACTTGTGGTGATGTTGAAGA AAACCCAGGTCCATGGAAGAAGATTTGGAAGCCAGGTATTAAG AAGTGGATTAAGGGTGGTGGTCAAAAAGACCAAGAGTTAGAT TGTCTGCT GGAAGAAGATTTGGAAGCCAGGTATTAAGAAGTGGATTAAGGG TGGTGGTCAAAAGCGTCCAAGAGTTAGATTGTCTGCTGGTTCTG GTGAAGGTAGAGGTTCTTTGTTGACTTGTGGTGATGTTGAAGAA AACCCCGGTCCAATGAGATTTCCATCTATTTTACCGCCGTTTTG TTCGCCGCCTCTTCTGCTTTGGCTGCTCCAGTTAACACTACTACT GAAGATGAAACTGCTCAGATTCCAGCTGAAGCTGTTATTGGTTA |
| KTAK | CTCTGATTTGGAAGGTGATTTTCGATGTTGCCGTTTTGCCATTTTC TAACTCTACCAACAACGGCTTGTTGTTTCATTAAACACCACTATCG CCTCTATCGCTGCTAAGGAAGAAGGTGTTTCTTTGGAAAAGAGA GAGGCCGAAGCTTGGAAGAAGATTTGGAAGCCAGGTATTAAGA AGTGGATTAAGGGTGGTGGTCAAAAAGACCAAGAGTTAGATT GTCTGCTT |