Supporting Information

Discovery of a short-chain ε-poly-L-lysine and its highly efficient production via synthetase swap strategy

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Supplementary methods:

H nuclear magnetic resonance (NMR) spectra was recorded using a Bruker AVANCE AV-500 NMR spectrometer (Bruker Group, Fällanden, Switzerland) operated at 500 MHz. The purified oligomer samples were prepared at 10 mg/ml in D_2O, and chemical shifts were measured at 25 °C in 5 mm diameter tue.

Supplementary Table S1. Sequences of primer pairs used in this study[a]

Primer name	Primer sequences (5′-3′)
P1-F	TTCGACGCSTCSTGYGAGGAGATG
P1-R	CGGTCGTCGAASARRTGSGACTG
P2663D-1	CCTGCTGACCGCGGCGGCGCTGTC
S2772D-2	CTCGGCCGCCGCCAAGTGGCTGCTG
S2870D-3	CGTGGAGATGCTGGCGGTGCCGTG
S354U-1	GTCCAGCGGCAGGCCGATCCGCACC
S245U-2	GTGACCAGGCGCTGCACCAGCTCG
S137U-3	GTGGAGACCACGGTGATCTCCTGC
A-domain-F	Atgggtcgcggatcc**GAATTC**ATGACAGCTGAACCGAGCCA(***EcoR*** Ⅰ)
A-domain-R	ctcgagtgcggccgc**AAGCTT**CTCCAGCTCGGGCAGCGG (***Hind*** Ⅲ)
Up-F	acgacggccagtgcc**AAGCTT**CCTCGGTGAGCAGGCCCA
Up-R	tattcgccttgatccGGTAGGTGAGCGCCTCGG
Tsr-F	ctaccGGATCAAGGCGAATACTTCATATG
Tsr-R	atcagcatgctgGAGGAACAGAGGCGCTTATCG
Down-F	tgttcctcCAGCATGCTGATGCCCGG
Down-F	ctatgacatgattac**GAATTC**CAGCTCAGCGAGGAGTTCACG (***EcoR*** Ⅰ)
Check-F	AGGCGATCGCCCTGGACACGG

Check-R	GTTCGGCGCCCTGGCCGACGACG
PlsII-F	gggctgcaggtcgac**<u>TCTAGA</u>**GTGGTCCGCAAGGAGACGC (***Xba*I**)
PlsII-R	ctatgacatgattac**<u>GAATTC</u>**AGGTGGTCACGGCGTGCT (***EcoR* Ⅰ)
*PermE**-F	gggctgcaggtcgac**<u>TCTAGA</u>**TCTAGTATGCATGCGAGTGTCCG (***Xba*I**)
*PermE**-R	ctcggttcagctgtcatCATATGTGGATCCTACCAACCGG
P_{plsI}-F	gggctgcaggtcgac**<u>TCTAGA</u>**GCTGATGCTGGTGCAGTCG (***Xba*I**)
P_{plsI}-R	ctcggttcagctgtcatCGATATGCCTCTGTTCGGTGC
PlsII-F-ORF	ATGACAGCTGAACCGAGCCA
PlsII-R-ORF	ctatgacatgattac**<u>GAATTC</u>**AGGTGGTCACGGCGTGCT (***EcoR* Ⅰ)

[a]The restriction sites are underlined.

Figure S1

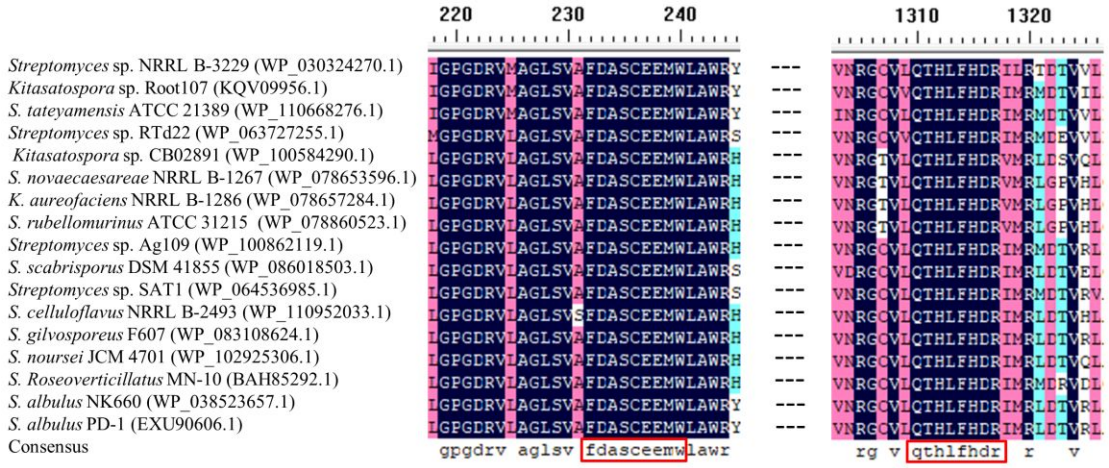

Figure S1. Two highly conserved regions, FDASCEEMW and QTHLFHDR, were identified based on an alignment of the known Pls and its homologous amino acid sequences (an overall similarity ranging from 99.7 % [between EXU90606.1 and WP_038523657.1] to <54.94 % [between EXU90606.1 and WP_030324270.1], Black shadowing: 100% conserved; Figure S1).

Figure S2

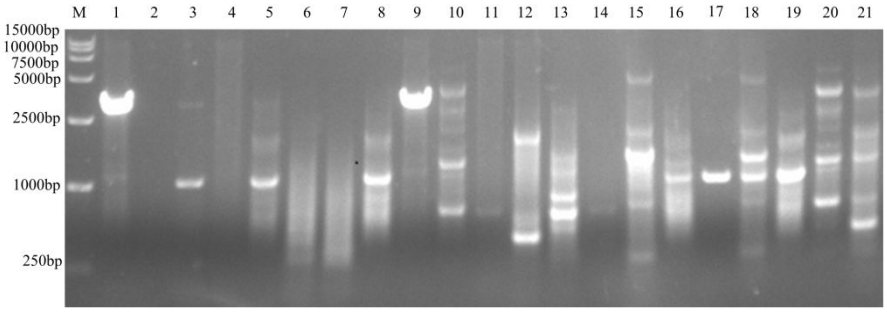

Figure S2. Amplification of the *pls* partial gene using degenerate primers from different gDNA; Lane 1: the positive control (gDNA from *S. albulus* PD-1), Lane 2: the negative control, Lane 9: the gDNA from *K. aureofaciens* PL-1, Lane 3-8 and 10-21: the gDNA from other Actinomycetes

Figure S3

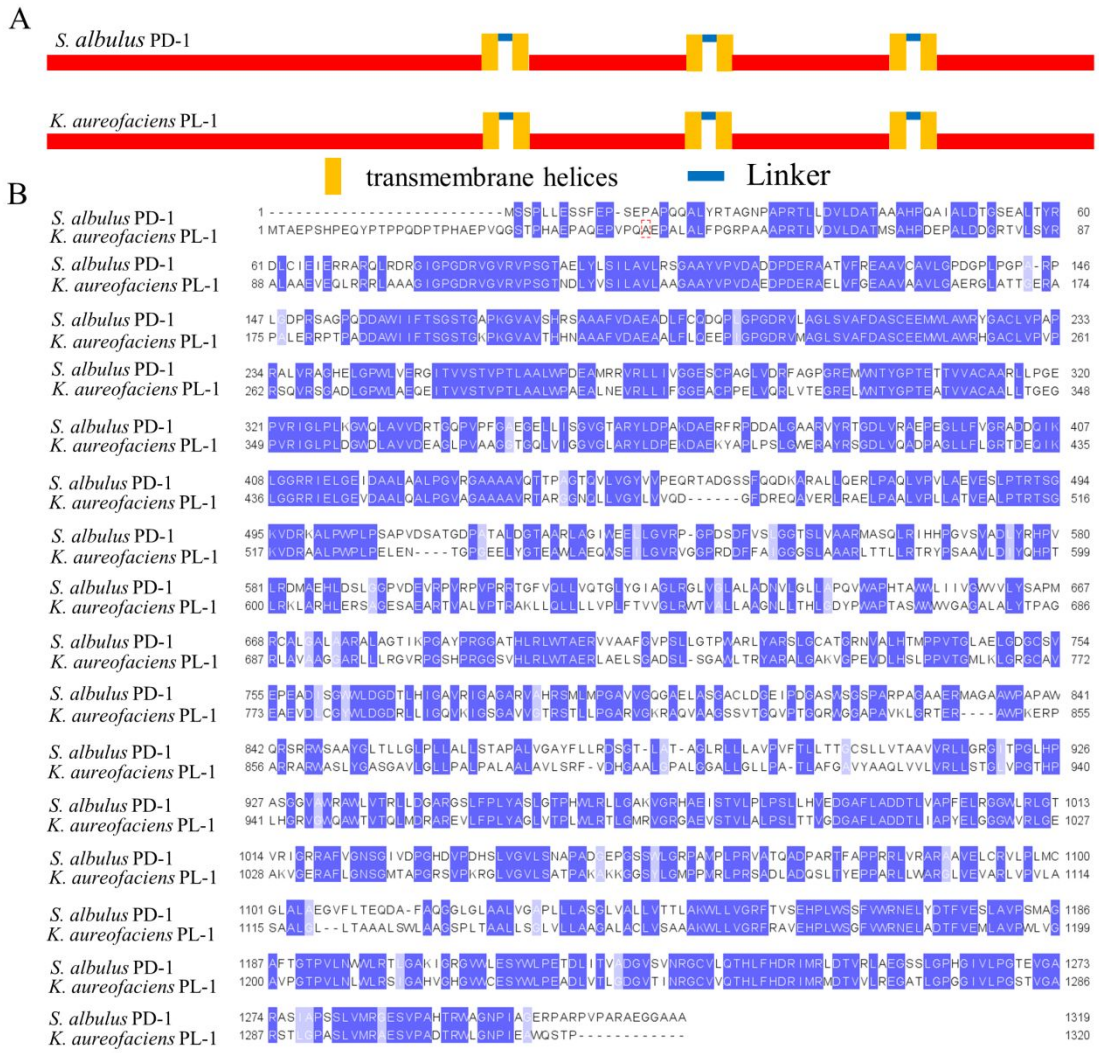

Figure S3. (A) Proposed transmembrane structure of Pls Ⅰ and Pls Ⅱ from *S. albulus* PD-1 and *K. aureofaciens* PL-1, respectively. (B) Amino acid sequences alignment of Pls Ⅰ and PlsII. Residues are colored according to conservation in sequence identity (dark blue: 100% conserved)

Figure S4

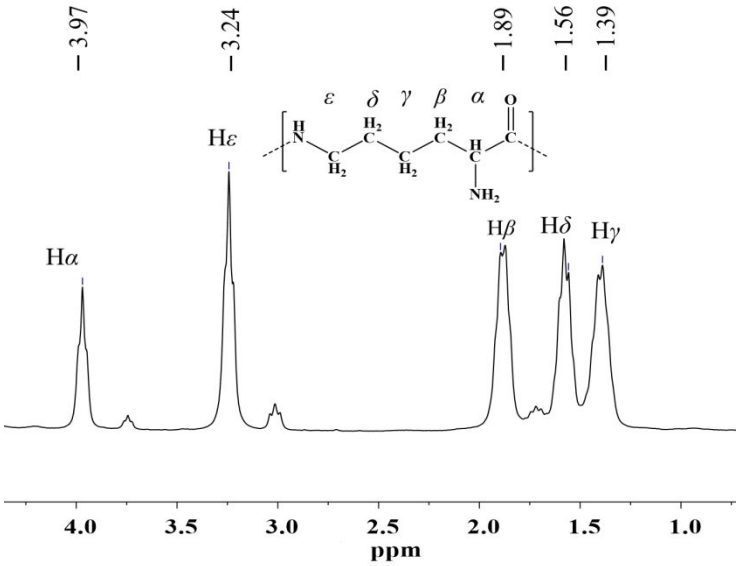

Figure S4. ^{1}H NMR spectrum of ε-PL produced by *K. aureofaciens* PL-1.