Supplementary Material For:

Extracellular production of *Streptomyces ladakanum* transglutaminase in a food-grade strain, *Bacillus subtilis*

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Table S1 Primers used in this study.

| Name | Oligonucleotides (5′ to 3′) |
|------|-----------------------------|
| TG-F* | CGGTATCCCGGGGACCCACCGCATGGCATGGG |
| TG-R* | CTCTAGAGATCGAGCGGCAGCCCTTGTGGTACCT |
| ProTG-F* | CCTCGAGCTGACAGCGGCCACCGGACATGGCATGGG |
| ProTG-R | CAGCCTCTCTGAGCTAAGTACTAGACGCAGCGCACAGCC |
| CD-F | AGGAGCCGTCAAGCTAGTACGAGCGGCCAAGGAGAGG |
| CD-R* | AAGCTTAGCTTCAGAGCTACGCAGCAGCGCACAGCC |
| SacB-F | CGTGATCGAGCTAAGTACTAGACGCAGCGCACAGCC |
| SacB-R | GCTCAGTACGAGCTAAGTACTAGACGCAGCGCACAGCC |
| DSD-F | GTCCTTCAGGGCCGCAGCAGCAGCGCACAGCC |
| DSD-R# | GCGGGAGGAGTCACCGGCTCAGCGCACAGCC |
| △PS-F | CTTCAAGCGCGTTCGCCGGGAC |
| △PS-R | TCGGGGAGCCGGGAAACCGGAGTCGAG |
| PS-F* | AGGACCCGAGTACGAGCGGCCAAGGAGAGG |
| PS-R# | GGGCCGGAATGCTGACGCGAGAGGC |
| SGS-F# | GCCAGTACGAGCGAGCAGCGAGCAGCGC |
| SGS-R* | CCGGGTCTCGCCCGGCTCGGCAG |
| PD-F# | GCCACCGGCCAGTACGAGCGGCCAAGGAGAGG |
| PD-R# | CTCTCCGCCAGTACGAGCGGCCAAGGAGAGG |
| WapA-F* | TGAACGATGAACATCGGATCGATGAAAGGAGAGG |
| WapA-R* | TGGCCTGGGCAGCGACGCGACGCGACG |
| EpR-F* | CATCTGAGATCCCATGAAACACATGTCTGGGAAAC |
| EpR-R* | TGGCCTGGGCAGCGACGCGACGCGACG |
| NucB-F* | GGCAGTACGAGCGAGCAGCGAGCAGCGC |
| NucB-R* | CTCTTGGGCGACGCGAGCAGCGAGCAGCGC |
| Ync-M-F | GCCACCGGCTCGAAGGGCTCTGCCG |
| Ync-M-R# | CTTCTGAGATCCCATGAAACACATGTCTGGGAAAC |
| YhcR-F* | TGGCCTGGGCAGCGACGCGACGCGACG |
| YhcR-R* | CTCTTGGGCGACGCGAGCAGCGAGCAGCGC |
| WprA-F* | TGGCCTGGGCAGCGACGCGACGCGACG |
| WprA-R* | CTCTTGGGCGACGCGAGCAGCGAGCAGCGC |
| AmyE-F* | TGGCCTGGGCAGCGACGCGACGCGACG |
| AmyE-R* | TGGCCTGGGCAGCGACGCGACGCGACG |
| LytD-F* | CTCTTGGGCGACGCGAGCAGCGAGCAGCGC |
| LytD-R* | CTCTTGGGCGACGCGAGCAGCGAGCAGCGC |
| PenP-F* | TGGCCTGGGCAGCGACGCGACGCGACG |
| PenP-R* | TGGCCTGGGCAGCGACGCGACGCGACG |
AbnA-F* TCTGGATCCCAATGAAAAAGAAAAAACATGG
AbnA-R* GCCCCCGGGTGCGGGAGCAGCAAGATGAT
NprB-F* ACATCGATCCATGCGCAAATTGACCAAAGAC
NprB-R* GCCCCCGGGAGCTGAGGCAATGTTTACAAAAAC
BglS-F* TCTGGATCCAAATGCTTATCTGAAACGAGTG
BglS-R* TGGCCCGGAGCTGAGGCAATGCTGACTG
MotB-F* TCTGGATCCAAATGGCAGAAAAAAAGAAGAGA
MotB-R* GTCGCCCGGGACCTTCTCGCTGACAGCAAAAAAC
LipB-F* GGTGGCCCGGAGCTTCTGTCGCCCGAGACGGCG
LipB-R* GGTGGCCCGGAGCTTCTGTCGCCCGAGACGGCG
LipA-F* ATCTGGATCCCAATGAAAAATTTGATAAAAGAAGG
LipA-R* GGCAGGGGAGCTTCTGACGGCTGCAACGC

* Restriction sites and mutagenesis sites are underlined.
Table S2 Sequences of signal peptides.

| Name | Oligonucleotides (5’ to 3’) |
|------|-----------------------------|
| SacB | ATGAACATCAAAAAGTTTGCAAAACAAGCAACAGTATTAACCTTTTACTACC CGACCTGCTGGCAGGAGGCGCAACTCAAGCTACTTTGTC | |
| WapA | ATGAAGAAGAAAGGAAGACCAGAACTTTTTAAAAAGTTGATTGATGCGACTTATTACTGAGCTTACGTGCTCTCATTCGCCAGAGCCGCTTTGTC | |
| EpR  | ATGAACATGTCATTGCAAAACTTTTGTATCAGTCACCTCTGGTTTTTCAAGTTT TCTCATCGACCAGCTTTGCTACGCTTTTCAAGCTACTTTGTC | |
| NucB | ATGAAAATGAGATGCAGGCGCCGCTTCTTGCTAACAGTACTTTTGTGACTGCTTATGAGGCGGTACGGTAAACGCTATTAACCTCTTTATGAGCTCAAGCTACTTTGTC | |
| YncM | ATGGAAGCAAGAAATTCAGCTCGGTTGTGGCAGTGCTTATTTTTGCA CTGA TTTTCAGCCTTTTTCTCCGGGAACCAAAGCTGCAGCG | |
| YhcR | ATGCTGTCTGTCGAAA TGA TAAGCAGACAAAATCGTTGTCA TTA TGTGTATA AGGGAGGAAATATGATGAGGCGTATTCTGCA TA TTGTGTTGATCACGGCATT AA TGTTCTTAAATGTAATACGTTCGAAGCTC | |
| WprA | ATGAAACGCAGAAAAATTCAGCTCGGTTGTGGCAGTGCTTATTTTTGCA CTGA TTTTCAGCCTTTTTCTCCGGGAACCAAAGCTGCAGCG | |
| AmyE | ATGTTTGCAAACAGATTTCAAAAAACCTTTTTACTGCGGTTATTTTGCAA TATTGCTGTATTCTTTGCTAGGACCGGCGGCTGGCAGAGCTCC | |
| LytD | ATGAAAAGAGAACTATCGCAGCTATGCTCTATCCGCCGCGTTCCCTTGCCCT TTTTGGCCATGCTGTAGGTTTAGCATTCATCGGAGGCGGCTGGCAGAGCTCC | |
| PenP | ATGAAAGTTGAAAGACTAAAGCGTCAATAAAAATTTGTTGTTGGGCTT ATGGGTTTAAGCA TTACTGGTTTCACACCTTTGCAAACACTCAACATCGCCG | |
| AbnA | ATGAAAGAAAAACATGGGAACGCTTTCTACATCTTTTGAGTGCGACG TCTTGCTGCAGGTTTTGTAATATTCATCCTTCCTCCTGTCGAGCCGCA | |
| NprB | ATGCGCAACTTGCAACAGACATCTCTATATCTTGCGGGGCTTTATGCGACAGCG GCCGCAATGGTTTTTTGAACATCAGCATTACGCTCAGCT | |
| BglS | ATGCGACTTATCGAAGGAGGAGGGACTTGTGTTGTGCTTCTTGTCACTGGAATTTTATGA GTTGTGTTCGACGATCTGACTTGCTCCTCAGCT | |
| MotB | ATGCGAGGAAAAAGAAAGGAAGACATGAGGCGACAGTGTGAGGATGATAT CATGCGCTGCTCTTACGCCGACATCTCTTACTCTCTCTCCGAGATTGTTTATT | |
| LipB | ATGAAAGAGATCATTGAGGACATTTCATTTTGGTTTATCAGGTGATTCTACTG TTTAGGCGCTCGGGGGCTTGCAGGCAAAAAGCTC | |
| LipA | ATGAAATTTGTAAGGAGAGACATGTCATTGATTCAAAATTTTTGATGCTGCTTTTGCACTGACAGCGGCGTACGCGTACGCGGGAACCAAAGCTC | |
Fig. S1 Construction of plasmids carrying different signal peptides.
**Fig. S2** PCR amplification of the encoding gene of *S. ladakanum* TG (A). Lane 1, Marker; Lane 2, encoding gene of *S. ladakanum* TG. SDS-PAGE analysis of *S. ladakanum* TG (B). M, Marker; Lane 1, intracellular fraction of *B. subtilis* WB600 carrying plasmid pWB<sub>sacB-LTG</sub>; Lane 2, extracellular fraction of *B. subtilis* WB600 carrying plasmid pWB<sub>sacB-LTG</sub>; Lane 3, extracellular fraction of *B. subtilis* WB600 carrying plasmid pWB<sub>sacB-LTG</sub>. 