[Supplementary materials]

Development of a new platform for secretory production of recombinant proteins in *Corynebacterium glutamicum*

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Table S1. List of primers used in PCR experiments.

Primer name	Primer sequence (5' to 3')[a]
MCS-F	ATTAAT**GGTACC**GGGCCCCCCCTCGAGGTCGACTCTAGAGGCCCAGCCGGCC ATTATAATTAGGCCTCGGGGGCC**GCGGCCGC**ATTAAT
MCF-R	ATTAATGCGGCCGCGG
rrnBTer-F	GA**GCGGCCGC**TGCCTGGCGGCAGTA
rrnBTer-R	GA**GCGGCCGC**AAAAGGCCATCCGTCAGGAT
Cg1514-F	ATTAAT**GGTACC**AGCCTGACTAGCGGTGTTTAAG
Cg1514-R	TAAT**GCGGCCGC**TTAATGATGGTGATGGTGATGTTTTAGAGTTTTTAGTCTA CCGAGATTTGTCCA
Cg1514-R2	ATTAAT**TCTAGA**TTTTAGAGTTTTTAGTCTACCGAGATTTGTCC
Cg1514ss-R	ATTAAT**TCTAGA**GCTTTGTGCAGTGGAAGTAGG
Cg1514P-R	AAAGAGCTCCTGATCATGTAGGTG
H36-F1	ATTAAT**GGTACC**TCTATCTGGTGCCCTAAACGGGGGAATATTAACGGGCCCA GGGTGGTCGCACCTT
H36-cg1514-F2	CCAGGGTGGTCGCACCTTGGTTGGTAGGAGTAGCATGGGATCCTTGTTAAAC AGAGTCAGTCGTATTGC
H36-cg1514ATG-F2	CCAGGGTGGTCGCACCTTGGTTGGTAGGAGTAGCATGGGATCCATGTTAAAC AGAGTCAGTCGTATTGC
aaGFP-F	ATTAAT**GGCCCAGCCGGCC**AAAGTAAAGGAGAAGAACTTTTCACTGGAGTT G
GFP-R	ATTAAT**GGCCCCCGAGGCC**TTATTTGTCATCGTCATCTTTATAATCGTCGAC CTTGGATAGTTCATCCA
aaXynA-F	ATTAAT**GGCCCAGCCGGCC**AAGCCGAGAGCACGCTCGGCGCCGCGGCGG
XynA-R	ATTAAT**GGCCCCCGAGGCC**CTATTAATGATGGTGATGGTGATGGGTGCGGG TCCAGCGTTGGTTGCT
aaM18-F	ATTA**GGCCCAGCCGGCC**AAGACATTCAGATGACCCAGACC
M18-R	TAAT**GGCCCCCGAGGCC**TTATCACTTATCATCGTCGTCCTTGTA
aaAmyA-F	ATTAAT**GGCCCAGCCGGCC**AAGATGAACAAGTGTCAATGAAAGATGGTAC
AmyA-R	ATTAAT**GGCCCCCGAGGCC**CTATTAATGATGGTGATGGTGATGTTTTAGCCC ATCTTTATTATAGTTTCCAGATTTTACAAGG
aacAbHuL22-F	ATGCATGC**TCTAGA**CAGGTCCAACTGCAAGAAAGCGGT
cAbHuL22-R	ATGCATGC**GCGGCCGC**TCAGTGATGGTGATGATGATGTGAAGAGAC

[a] Restriction enzyme sites are shown in bold.

[b]Stratagene Cloning System, La Jolla, CA.

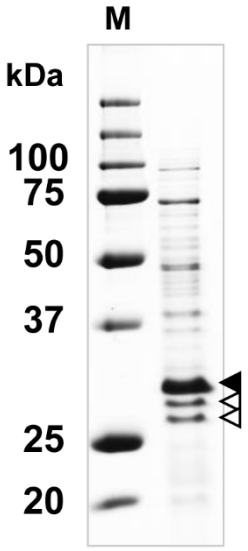

Figure S1. SDS-PAGE analysis of culture supernatant from the fed-batch cultivation of *C. glutamicum* harboring pH36M2 (Yim et al., 2014). Arrows indicate the most overexpressed protein bands (Identified as Cg1514 (black arrow), and Cg2052 (white arrows)).

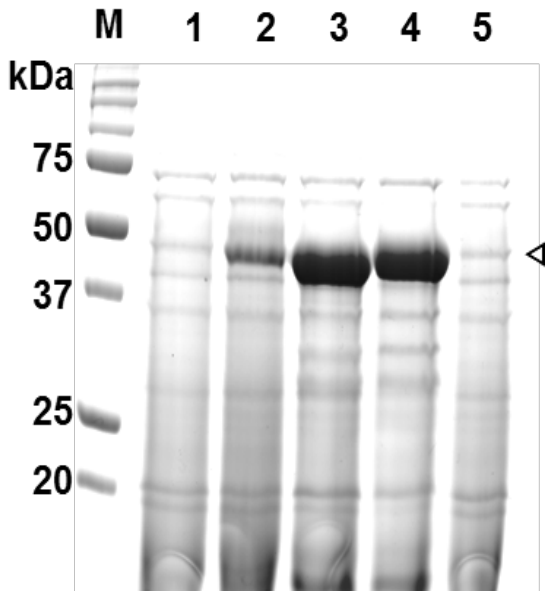

Figure S2. Comparison of four different signal peptides for secretion of endoxylanase. SDS-PAGE analysis of culture supernatant. Lane 1, pCES208; Lane 2, pH36-cspBss-XynA; Lane 3, pCG-H36A-XynA (H36-cg1514ss-XynA); Lane 4, pCES-H36-XynA (H36-porBss-XynA); Lane 5, pH36-torAss-XynA. Same volume (10 μL) of 30 times concentrated culture supernatant was loaded on each lane. Arrowhead indicates XynA (~48 kDa)

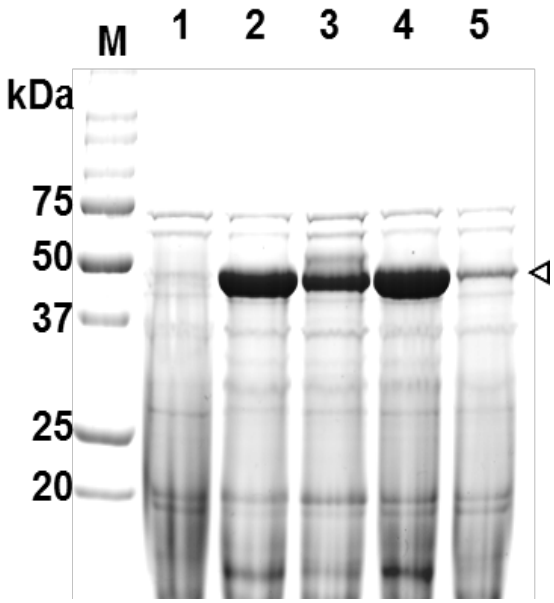

Figure S3. SDS-PAGE analysis of culture supernatant in the secretory production of endoxylanase under four different promoters. Lane 1, pCES208; Lane 2, pCG-H36A-XynA (H36-cg1514ss-XynA); Lane 3, P_{Sod}-cg1514ss-XynA; Lane 4, pCG-S-XynA (P_{cg1514}-cg1514ss-XynA); Lane 5, P_{Tuf}-cg1514ss-XynA. Same volume (10 μl) of 30 times concentrated culture supernatant was loaded on each lane. The arrowhead indicates XynA (~48 kDa).

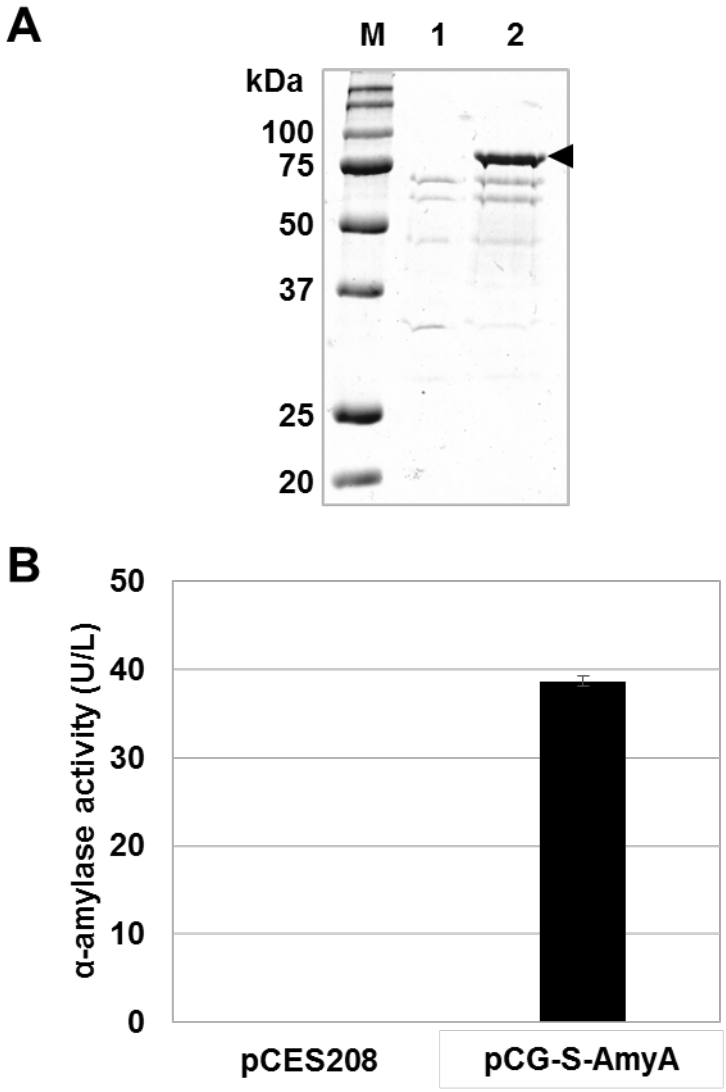

Figure S4. Secretory production of α-amylase (AmyA). A: SDS-PAGE analysis of extracellular proteins by Cg1514-based AmyA secretion system in the flask cultivation. Black arrow indicates AmyA. Lane 1, *C. glutamicum* harboring pCES208 (negative control); Lane 2, pCG-S-AmyA. Same volume (10 μL) of 30 times concentrated culture supernatant was loaded on each lane. B: Secreted α-amylase volume activity in the culture supernatant. One unit of activity was defined as the amount of enzyme required to release 1 μmol of glucose from starch per minute at 30˚C.

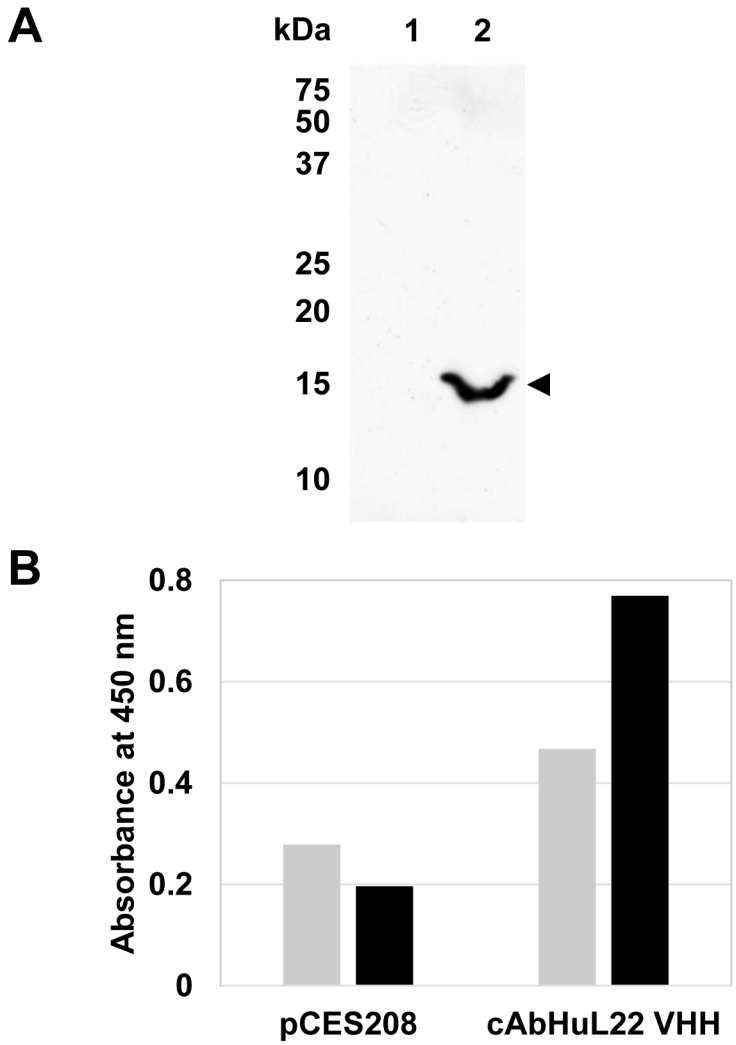

Figure S5. Secretory production of cAbHuL22 VHH. A: Western blot analysis of extracellular proteins by Cg1514-based cAbHuL22 VHH secretion system in the flask cultivation. Black arrow indicates cAbHuL22 VHH. Lane 1, *C. glutamicum* harboring pCES208 (negative control); Lane 2, pCG-S-cAb. Same volume (10 μL) of 30 times concentrated culture supernatant was loaded on each lane. B: ELISA of culture supernatant. Gray bar (signal from negative control (Bovine serum albumin, BSA) coated well), Black bar (signal from target antigen (Human lysozyme)).

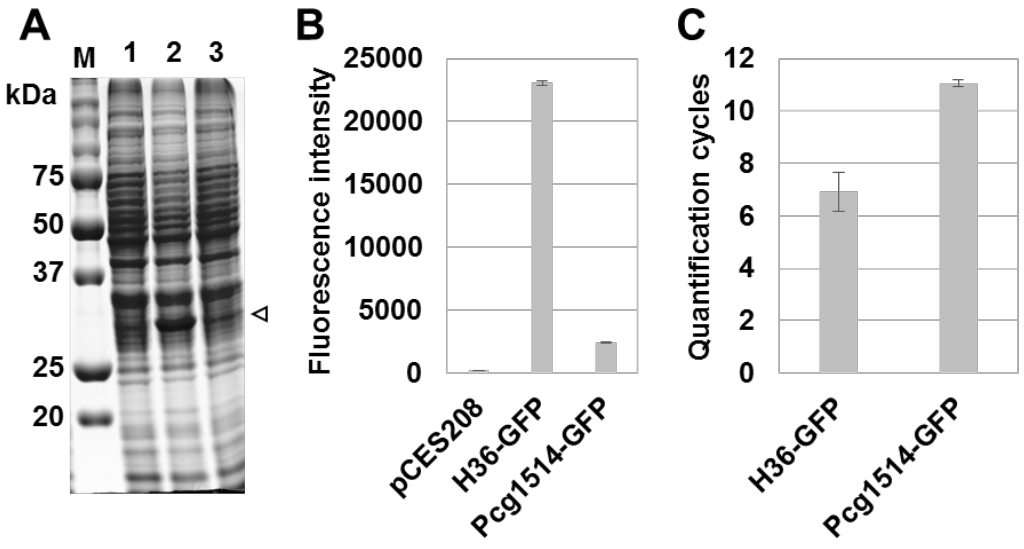

Figure S6. Comparison of promoter strength of H36 synthetic promoter and *cg1514* promoter. A) SDS-PAGE analysis of whole cell lysate. Lane 1, pCES208; Lane 2, pCES-H36-GFP (H36-GFP); Lane 3, pCg1514-GFP (P_{cg1514}-GFP). Arrowhead indicates GFP (~28 kDa). B) Analysis of fluorescence intensity by fluorometer. C) Quantification cycle value from qRT-PCR experiment.