Supporting Information

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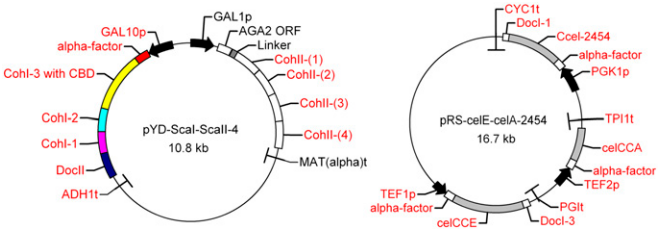

Fig. S1. Recombinant plasmids pYD-ScaI-ScaII-4 and pRS-celE-celA-2454 used in this study. The structures of pYD-ScaI-ScaII-1, pYD-ScaI-ScaII-2, and pYD-ScaI-ScaII-3 were similar to pYD-ScaI-ScaII-4. The only difference was that they had one, two, and three repeating CohIIs, respectively. Plasmid pYD-ScaII-2 did not have miniscaffoldin I expression cassette, and its miniscaffoldin II had two repeating CohIIs. The structures of plasmids pRS-celE, pRS-celA, pRS-2454, pRS-celE-celA, pRS-celE-2454, and pRS-celA-2454 could be derived from pRS-celE-celA-2454 by removing proper gene expression cassettes. pET28-ScaI, pET28-celA, and pET28-2454 were constructed based on pET28a. They were used for expression of miniscaffoldin I, celCCA, and CcelI_2454 in *E. coli*. pETduet-celE was constructed based on pETduet-1 and for *E. coli* expression of celCCE. All of the plasmids pET28-ScaI, pET28-celA, pET28-2454, and pETduet-celE used T7 promoter and T7 terminator, and α-factor was not fused with genes. Yeast constitutive promoters (PGK1p, 750 bp; TEF1p, 412 bp; TEF2p, 560 bp) and the corresponding terminators (CYC1t, 249 bp; PGlt, 401 bp; TPI1t, 401 bp) were PCR-amplified from the genomic DNA isolated from *S. cerevisiae* EBY100. Galactose-inducible promoter (GAL10p, 491 bp) and the corresponding terminator (ADH1t, 324 bp) were amplified from pYES2 (Invitrogen). Yeast-secretion signal (α-factor, 270 bp) was amplified from pPICZαA (Invitrogen). Cellulases [celCCE without native dockerin, 2,403 bp; celCCA with native dockerin (DocI-2), 1,350 bp; CcelI_2454, 2,133 bp] and CohI-2 (438 bp) were cloned from the genomic DNA isolated from *C. cellulolyticum* DSM 5812. DocI-3 (246 bp), CohI-3 with CBD (1,131 bp), DocII (492 bp), and CohII (522 bp) were amplified from the genomic DNA isolated from *C. thermocellum* ATCC 27405. DocI-1 (174 bp) and CohI-1 (414 bp) were amplified from the genomic DNA of *C. cellulovorans* DSM 3052. All of the primers are listed in Table S2–S7.

Table S1. Summary of the recombinant *S. cerevisiae* EBY100 and *E. coli* BL21 (DE3) used in this study

Strain	Plasmid(s)	Phenotype
EBY (control)	pYD1 and pRS425	No surface display (negative control)
EBY (EA2-1)	pYD-ScaI-ScaII-1 and pRS-celE-celA-2454	Displays trifunctional minicellulosome with one CohII on scaffoldin II
EBY (EA2-2)	pYD-ScaI-ScaII-2 and pRS-celE-celA-2454	Displays trifunctional minicellulosome with two CohIIs on scaffoldin II
EBY (EA2-3)	pYD-ScaI-ScaII-3 and pRS-celE-celA-2454	Displays trifunctional minicellulosome with three CohIIs on scaffoldin II
EBY (EA2-4)	pYD-ScaI-ScaII-4 and pRS-celE-celA-2454	Displays trifunctional minicellulosome with four CohIIs on scaffoldin II
EBY (E-2)	pYD-ScaI-ScaII-2 and pRS-celE	Displays unifunctional minicellulosome with CBH activity and with two CohIIs on scaffoldin II
EBY (A-2)	pYD-ScaI-ScaII-2 and pRS-celA	Displays unifunctional minicellulosome with EG activity and with two CohIIs on scaffoldin II
EBY (2454-2)	pYD-ScaI-ScaII-2 and pRS-2454	Displays unifunctional minicellulosome with BGL activity and with two CohIIs on scaffoldin II
EBY (EA-2)	pYD-ScaI-ScaII-2 and pRS-celE-celA	Displays bifunctional minicellulosome with CBH and EG activities and with two CohIIs on scaffoldin II
EBY (E2-2)	pYD-ScaI-ScaII-2 and pRS-celE-2454	Displays bifunctional minicellulosome with CBH and BGL activities and with two CohIIs on scaffoldin II
EBY (A2-2)	pYD-ScaI-ScaII-2 and pRS-celA-2454	Displays bifunctional minicellulosome with EG and BGL activities and with two CohIIs on scaffoldin II
EBY (C_4doc-2)	pYD-ScaI-ScaII-2	Displays scaffoldin I and scaffoldin II with two CohIIs
EBY [EA2-2 (–)]	pYD-ScaII-2 and pRS-celE-celA-2454	Displays trifunctional minicellulosome with two CohIIs on scaffoldin II and without scaffoldin I
E. coli (C_4doc)	pET28-ScaI	Expression of miniscaffoldin I in *E. coli* BL21 (DE3)
E. coli (E)	pETduet-celE	Expression of CBH in *E. coli* BL21 (DE3)
E. coli (A)	pET28-celA	Expression of EG in *E. coli* BL21 (DE3)
E. coli (2454)	pET28-2454	Expression of BGL in *E. coli* BL21 (DE3)

Table S2. Primers used for constructing cellulase gene expression cassettes in pUC19

Name	Sequence
α-factor-F	CGGAATTCCGATGAGATTTCCTTCAATT
α-factor-R	CCGAGCTCGAGCTTCAGCCTCTCTTTTC
PGK1p-F	CGGAATTCACGCACAGATATTATAACATC
PGK1p-R	CGGAATTCTGTTTTATATTTGTTGTAAAAAGTAG
TEF1p-F	CGGAATTCATAGCTTCAAAATGTTTCTACTC
TEF1p-R	CGGAATTCTTTGTAATTAAAACTTAGATTAG
TEF2p-F	CGGAATTCGGGCCGTATACTTACATATAG
TEF2p-R	CGGAATTCGTTTAGTTAATTATAGTTCGTTG
CYC1t-F	ACGCGTCGACATCATGTAATTAGTTATGTCACGC
CYC1t-R	CCCAAGCTTGCAAATTAAAGCCTTCGAG
PGIt-F	ACGCGTCGACAACAAATCGCTCTTAAATATATACC
PGIt-R	CCCAAGCTTGGTATACTGGAGGCTTCATGAGTTATG
TPI1t-F	GCTCTAGAGATTAATATAATTATATAAAAATATTATC
TPI1t-R	ACGCGTCGACCTATATAACAGTTGAAATTTGG
celCCA-pUC19-F	CGGAGCTCCGCATCATCACCATCACCATTATGATGCTTCACTTATTCCGAATC
celCCA-pUC19-R	TGCTCTAGATTAGTTGCTTGGAAGCTTACTTACC
celCCE-pUC19-F	TCCCCCGGGGCATCATCACCATCACCATATAGGACAAGCATTTGCCCTT
celCCE-pUC19-R	ACGCGTCGACAGTTGGAGGAGTCACTGACCC
Ccel_2454-pUC19-F	CGGAGCTCCGCATCATCACCATCACCATATGCAATACGATCAGATAGATAA
Ccel_2454-pUC19-R	ACGCGTCGACCAGAGCAAGAGCTATAGCTATCGGAA
DocI-1-F	ACGCGTCGACTTAAAAGGTGATGTTAACTCTGATGC
DocI-1-R	ACGCGTCGACTTAAGCAAGAAGTGCTTTCTTTAA
DocI-3-F	ACGCGTCGACttcccggatatgacatataaag
DocI-3-R	ACGCGTCGACTTAGTTCTTGTACGGCAATG

F, forward; R, reverse.

Table S3. Primers used for shifting cellulase gene expression cassettes into pRS425

Name	Sequence
M3-NotI-F	ATAAGAATGCGGCCGCGTAAAACGACGGCCAGT
M3-NotI-R	ATAAGAATGCGGCCGCAGATCTCAGGAAACAGCTATGAC
M3-BglII-F	GAAGATCTGTAAAACGACGGCCAGT
M3-BglII-R	GAAGATCTGCATGCCAGGAAACAGCTATGAC
M3-SphI-F	ACATGCATGCGTAAAACGACGGCCAGT
M3-SphI-R	ACATGCATGCCAGGAAACAGCTATGAC

Table S4. Primers for constructing miniscaffoldin I expression cassette in pUC19

Name	Sequence
GAL10p-F	CGGAATTCCTGATTAATTACCCCAGAAATAAGGC
GAL10p-R	CGGAATTCTTATATTGAATTTTCAAAAATTCTTACTT
ADH1t-F	ACGCGTCGACTGGACTTCTTCGCCAGAGGTTTGG
ADH1t-R	CCCAAGCTTGCATGCCGGTAGAGGTGTGGTC
α-factor-F	See Table S1
α-factor-R	See Table S1
CohI-1-F	GCTCTAGAACACCAGTTGAAGCTGTAACAGCTA
CohI-1-R	GCTCTAGATGATAGTTACTGTTCCTGGGTTAACTGC
CohI-2-F	CGGGATCCGGCGATTCTCTTAAAGTT
CohI-2-R	CGGGATCCTTGAGTACCAGGATCTAT
CohI-3-CBD-F	CGAGCTCGGTGTGGTAGTAGAAATTGGCAAAG
CohI-3-CBD-R	CGGGATCCCGGATCATCTGACGGCGGTATT
DocII-F	AACTGCAGaataaacctgtaatagaagg
DocII-R	CCCAAGCTTTTACTGTGCGTCGTAATCAC

F, forward; R, reverse.

Table S5. Primers used for shifting miniscaffoldin I expression cassette into pYD1 or pET28a

Name	Sequence
pYD1-mutation-F	GCGTCGACACTAGTATTACGCCAAGCTCGGAATT
pYD1-mutation-R	GCGTCGACAGATCTCATGGTCATAGCTGTTTCCTG
Scaff I-pYD1-F	GGACTAGTCTGATTAATTACCCCAGAAATAAGGC
Scaff I-pYD1-R	GAAGATCTGCATGCCGGTAGAGGTGTGGTC
Scaff I-pET28-F	GGAATTCCATATGGGTGTGGTAGTAGAAATTGGC
Scaff I-pET28-R	CCGCTCGAGTTACTGTGCGTCGTAATCAC

F, forward; R, reverse.

Table S6. Primers for construction of miniscaffoldin IIs in pET22b (+), and for shifting miniscaffoldin IIs into pYD1

Name	Sequence
CohII-(1)-F	GGAATTCCATATGGAAGCAACTCCAAGTATTG
CohII-(1)-R	CATGCCATGGCTGCCTCTACAACTATAAGATC
CohII-(2)-F	CATGCCATGGATGAAGCAACTCCAAGTATTGAAATGG
CohII-(2)-R	CGGGATCCGAGTCTTTTAACGGTTCTGCCTCT
CohII-(3)-F	CGGGATCCGGAAGCAACTCCAAGTATTGAAATGG
CohII-(3)-R	CGGAATTCGGGTCTTTTAACGGTTCTGCCTCT
CohII-(4)-F	CGGAATTCGGAAGCAACTCCAAGTATTGAAATGG
CohII-(4)-R	GCGTCGACGTCTTTTAACGGTTCTGCCTCT
Miniscaffoldin II-F	CTAGCTAGCATACATATGGAAGCAACTCCAA
Miniscaffoldin II-R	CTCAGTGGTGGTGGTGGTGCTCGAG

F, forward; R, reverse.

Table S7. Primers used for expression of cellulases in *E. coli* based on pET28a or pETduet-1

Name	Sequence
celCCA-pET28-F	GGAATTCCATATGTATGATGCTTCACTTATTCCGAATC
celCCA-pET28-R	CCGCTCGAGTTAGTTGCTTGGAAGCTTACTTACC
celCCEdoc-pETduet-F	GAAGATCTCCATCATCACCATCACCATATAGG
celCCEdoc-pETduet-R	CCGCTCGAGTTAGTTCTTGTACGGCAATG
Ccel_2454-pET28-F	GGAATTCCATATGCAATACGATCAGATAGATAA
Ccel_2454- pET28-R	CCGCTCGAGTTAAGCAAGAAGTGCTTTCTTTAA

F, forward; R, reverse.