

Supplementary Information:

Efficient conversion of spent mushroom substrate into high value-added anticancer drug pentostatin with engineered *Cordyceps militaris*

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Table S1 Oligonucleotides and sequences used in this study.

Name	Sequences (5' -3')
<i>ctcBh1</i> optimized for <i>E. coli</i>	<p>atgatgtataaaaagtttgcggcactagctgctctctgtagcggcgcgctccgcacagcaggcgtgtagctgaccgcggagaaccaccgagcttgacctgaaaa cgctgcacctcaggcggtagctgctccacgggtaagtggcagttaccattgatgcgaactggcgtggaccacaccgtgctggttcgacgaactgctataccg gtaaccagtgggataccctctgtactgacggcaaaagctgcgcacaacctgctgtgacggctgctgattacagctctacctacggcatcaccactccgg cgatagcttgaatctcaagttcgttaccagcaccagctacggcactaacgtcggctcccgtttatctgatggaaaatgacaccaagctaccagatgtttgagctgct gggtaacgagttacccttggatgctgacgtgagcaacctgggctcgggttgatggcgtctgtatttcgtgagcatggatggcggatggcggcatgagcaagtact cgggtaataaggctggcgaatacggcactgggtattgtgacgcgaatgtccgctgatctgaaatcatcaacgggtaagcgaacgtgggcaactggacc cgtgcaccaacgatgcgaacgcgggtttggctgttacggcagttgctgcagcgaatggacgtgtgggaagccaacaacatggcaacggcgtttacccgcac ccgtgtactacgggtggcagagcagatgcgaagcggacacctgggtggcacctactctagcgtacgctacgccggcgtgtgcatccggacggctgagct taacgcgtaccgtcagggtgataaaaccttacggcaagggtatgaccgtgacaccaacaagaaatgaccgtgttaccctcctataaaaactccgcagggt ttctgagcgagatcaaacgcttctatgtgcaggatggcaaatcattgccaatgcggagagcaagatcccgggcaacccgggtaacagcattaccaagaatattg cgacccccaaaagtgtcttcagcaaacaccgacactcaatcgaagggtgggatggcacaatgagcaaggcgtgtagcaggtccgatggtttgtgatgtca gtttgggacgatcattatgcaaatgctgtggctgattccacgtaccgatccagcggcgtgctccgggtgctgagcgcggctgctcccaccacatctg gtgtcccagcggagatcgaagcccaggttccgaattcaacgtgattttcctcaatattcgttttggcgaatggcagcaccgtaccggcctggacggcagcaat ccaggtaaccgactacgacagtggttccgctgcatccaccagcagcagcggctccgactccagcaccagctccccgggtgagcagccaaccggacaaccg gggtgctgcagcaccagaagtgggtaaatgggtgggatcggctacaccggctgcaccaatgctgctggcaccacctgtacgcaactgaaccgtggt attctcagtgctgtaa</p>
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C. militaris

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Ura5R	cccgcattgtacagcatcattgg
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Dens3R	cttgcattgctcaggtcgactctagagagcagcccgccactggacc

Table S2. Composition of spent mushroom (*Hypsizygus marmoreus*) substrate.

Nutrients	Content in SMS (%)	Content in pretreated SMS (%)
Aspartic acid	0.47 ± 0.02	Not tested
Threonine	0.24 ± 0.01	Not tested
Serine	0.22 ± 0.01	Not tested
Glutamate	0.68 ± 0.02	Not tested
Glycine	0.27 ± 0.01	Not tested
Alanine	0.28 ± 0.01	Not tested
Valine	0.27 ± 0.01	Not tested
Methionine	0.29 ± 0.02	Not tested
Isoleucine	0.20 ± 0.01	Not tested
Leucine	0.33 ± 0.03	Not tested
Tyrosine	0.10 ± 0.01	Not tested
Phenylalanine	0.26 ± 0.02	Not tested
Lysine	0.22 ± 0.01	Not tested
Histidine	0.15 ± 0.01	Not tested
Arginine	0.17 ± 0.01	Not tested
Proline	0.08 ± 0.01	Not tested
Total amino acids	4.24 ± 0.17	Not tested
Cellulose	26.34 ± 1.24	38.56 ± 3.54
Hemicellulose	8.93 ± 0.34	12.34 ± 1.34
Ligin	5.03 ± 0.15	1.24 ± 0.02
Crude fiber	39.30 ± 2.14	52.14 ± 5.04
Soluble reducing sugar	1.67 ± 0.03	Not detected
Glucose	1.1 ± 0.02	Not detected

Table S3. Coefficients of factors obtained from regression model analysis of the special cubic model.

Factors	SMS ^a	pSMS ^a	SMS ^b	pSMS ^b
CF	1.06	1.23	1.30	2.98
CBH1	26.75	32.24	39.95	41.84
XynA	16.22	17.37	16.84	19.80
CF*CBH1	41.00	40.63	29.61	36.28
CF*XynA	33.13	36.39	44.50	45.50
CBH1*XynA	29.92	27.11	17.11	27.70
CF*CBH1* XynA	383.25*	543.66*	600.67*	653.88*

^a Hydrolyzed by mixture composed by recombinant CBH1 of *Trichoderma reesei*

^b Hydrolyzed by mixture composed by recombinant CBH1 of *Chaetomium thermophilum*

* The interaction between these factors was statistically significant

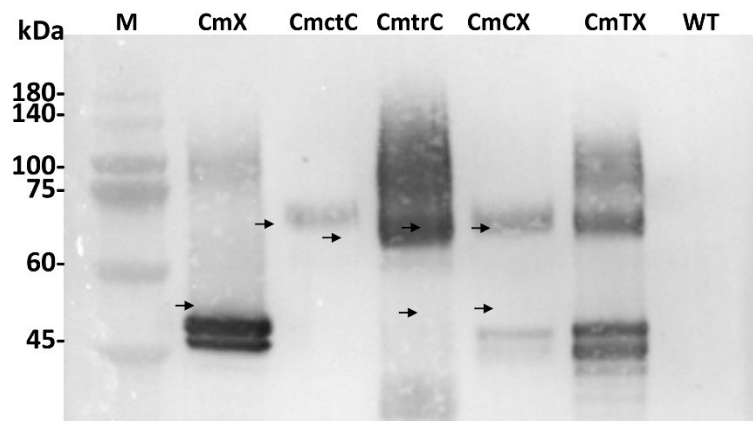


Figure S1 Expression of recombinant cellulases in transformants according to Western Blot. M: marker. Different volumes of 1 μ L (CmX, CmtrC, CmtrCX) or 30 μ L (CmctC, CmCX, WT) of cultrate filtrate were loaded on each lane. The main bands of each recombinant cellulase are indicated by a black arrow.

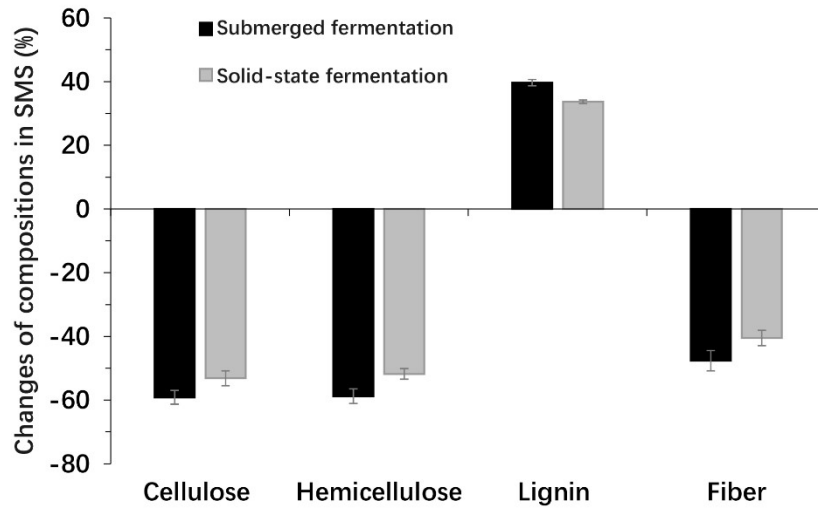


Figure S2. Changes of SMS composition after fermentation with transformant CmTX.