Table S1 Plasmids used in this study

Plasmids	Notes	References
pWB980		[1]
pHT-XCR6		[2]
pcrF11		[2]
pWB-manBl	pWB980 with *manBl* gene	This study
pWB-MBM1	pWB980 with ManBl (Y247D/ T272M) gene	This study
pWB-MBM2	pWB980 with ManBl (S96G) gene	This study
pWB-MBM3	pWB980 with ManBl (I91N/ L211I) gene	This study
pWB-DMBM3	pWB-MBM3 vector antibiotics gene is replaced by *dal* gene	This study
pdal1	pcrF11 with *dal* crRNA	This study
pdal2	pdal1 inserted with dal homologous arm at *P*st I and *H*ind III sites	This study

References

1. Wu, S. C. & Wong, S. L. Development of improved pUB110-based vectors for expression and secretion studies in Bacillus subtilis. *J Biotechnol* **72**, 185–195 (1999).
2. Wu, Y. *et al.* CAMERS-B: CRISPR/Cpf1 assisted multiple-genes editing and regulation system for Bacillus subtilis. *Biotechnol Bioeng* **117**, 1817–1825 (2020).

Table S2 Strains used in this study.

Strains	Notes	References
B. subtilis DB104	his, nprR2, nprE18, ΔaprA3	[1]
B. licheniformis KD-1		This study
B. subtilis XCR6	*B. subtilis* DB104 (pHT-XCR6)	This study
B. subtilis DBFG-1	*B. subtilis* DB104 Δ*dal*	This study
B. subtilis DB104 (pWB980)		This study
B. subtilis MTV13	*B. subtilis* DB104 (pWB-manBl)	This study
B. subtilis YM40	*B. subtilis* DB104 (pWB-MBM1)	This study
B. subtilis YM70	*B. subtilis* DB104 (pWB-MBM2)	This study
B. subtilis YM102	*B. subtilis* DB104 (pWB-MBM3)	This study
B. subtilis FGYM102	*B. subtilis* DBFG-2(pWB-DMBM3)	This study

References

1. Kawamura, F. & Doi, R. H. Construction of a Bacillus subtilis double mutant deficient in extracellular alkaline and neutral proteases. *J Bacteriol* **160**, 442–444 (1984).

Table S3 Primers used in this study.

Primers	Sequence 5′-3′
P1	GTTTCTCCGGTGAACCCGAATGCCCA
P2	CGCCGTCCCATATCTCTCCTTTATTCA
P3	TGAATAAAGGAGAGATATGGGACGGCG
P4	TGGGCATTCGGGTTCACCGGAGAAAC
P5	CTGGCACAAAAAGCCGTCTCTGTAC
P6	CTCCATTTAGGTAAGTACGACACTTCCTAGCTTTTATTC
P7	CTAGGAAGTGTCGTACTTACCTAAATGGAGAATTCATAAAAC
P8	CTTTTAGTGTGTCAGTCCACAGTTGG
P9	TGAACTGATTCAAGCAAGCTTGTTCTCACGCCGTCTTTAAATAAG
P10	TCTGCCGTTCGACGATCTGCAGTCTATTTGAGCAACCTGCAAATATGTC
P11	CGCGGATCCTCTATTTGAGCAACCTGCAAATATGTC
P12	GTGAATGGACCAATAATAATGAGCACAAAACCTTTTTACAG
P13	GCTGAACAGATTAATAATAGATTTTAATTGCTTATATTTACCTG
P14	CATTATTATTGGTCCATTCACTATTC
P15	CAGGTAAATATAAGCAATTAAAATCTATTATTAATCTGTTCAGC
P24	CCCAAGCTTGTTCTCACGCCGTCTTTAAATAAG
P16	ATGAGCACAAAACCTTTTTAC
P17	TTAATTGCTTATATTTACCTG
dal-crRNA-F	AGATGATGAAGCGATTTCACTGCGCAA
dal-crRNA-R	AATTTTGCGCAGTGAAATCGCTTCATC

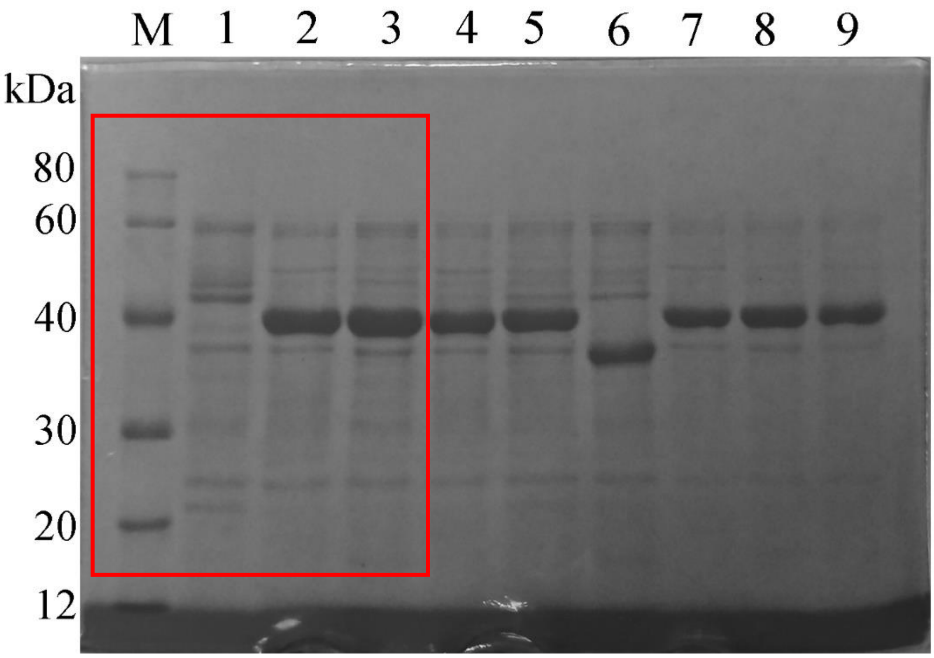

Figure S1: analysis of β-mannanase secreted by *B. subtilis* engineering strains.
M, ProteinRuler I (12-80 kDa). Lane 1, DB104 (pWB980). Lane 2, MTV 13. Lane 3, FGYM102.
Lane4-9, other β-mannanase producing strains not included in this paper.
In this paper, we cropped lane M and lane 1-3 as Fig1 A (as shown in red line).

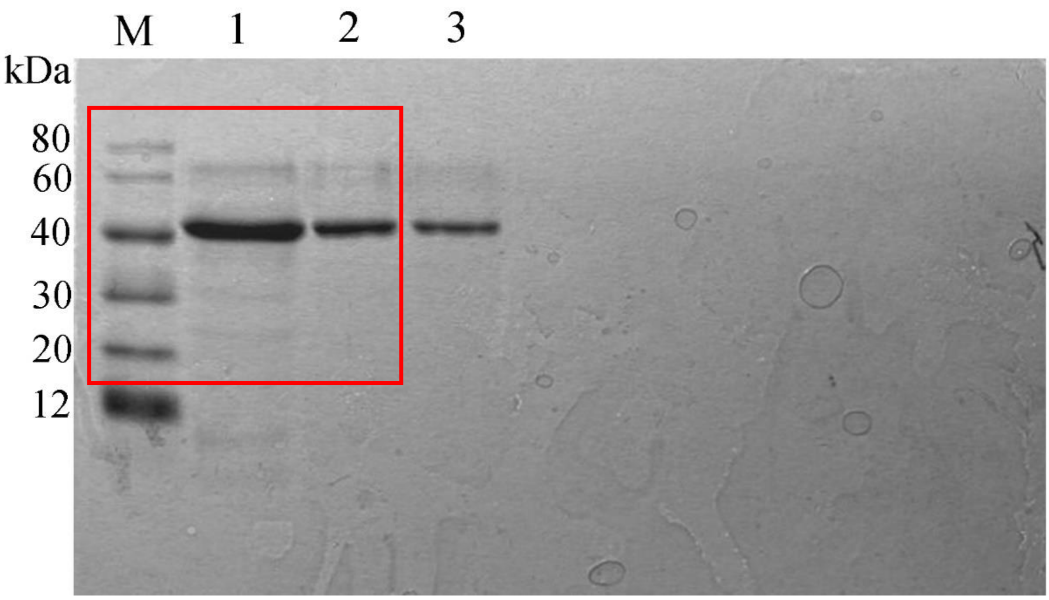

Figure S2: the purified β-mannanase.
M, ProteinRuler I (12-80 kDa). Lane 1, ManBl. Lane 2, ManBl (I91N/L211I). Lane 3, other purified β-mannanase not included in this paper.
In this paper, we cropped lane M and lane 1-2 as Fig1 B (as shown in red line).