Supplementary materials

Secretory expression fine-tuning and directed evolution of diacetylchitobiose deacetylase by *Bacillus subtilis*

Running title: diacetylchitobiose deacetylase

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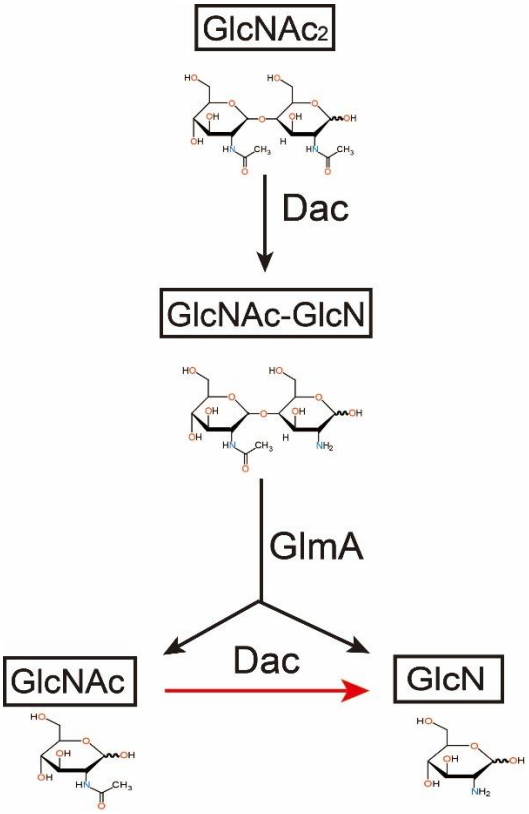

(Figure. S1)

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201 CTACGTTTGC ATGACAGACG GCTATATGGG CACAACAGAC GAAAGCCTGT CAGGACACGA ATTAGCAGCA ATCCGCCGCA AAGAAGAAGA AGAAAGCGCA
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701 CGTTCCTGAG AACAATCGCG ATGTTCTACG GCGAAAAAAT CGGCGTTCGC TACGGAGAAG GCTTTAGAAT TATGCCGGGC CTGTTCTACC ACATCACACC
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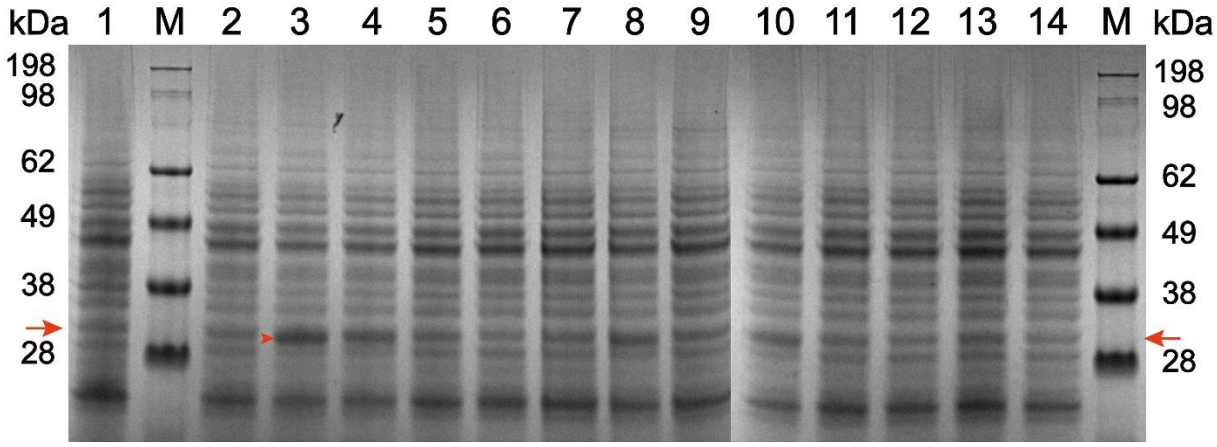
(Figure. S2)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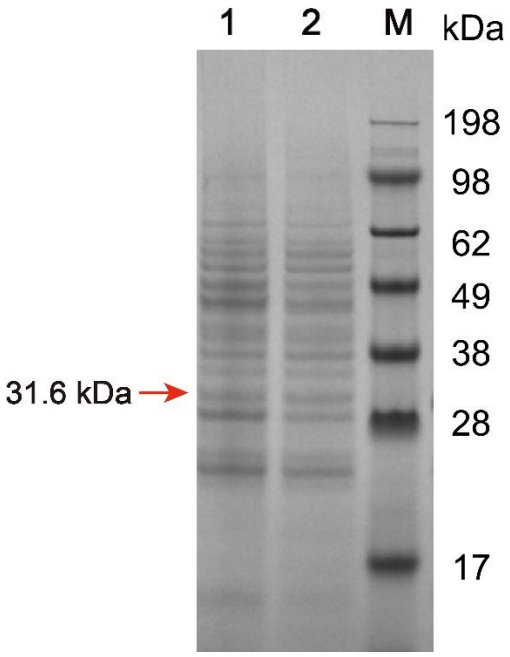
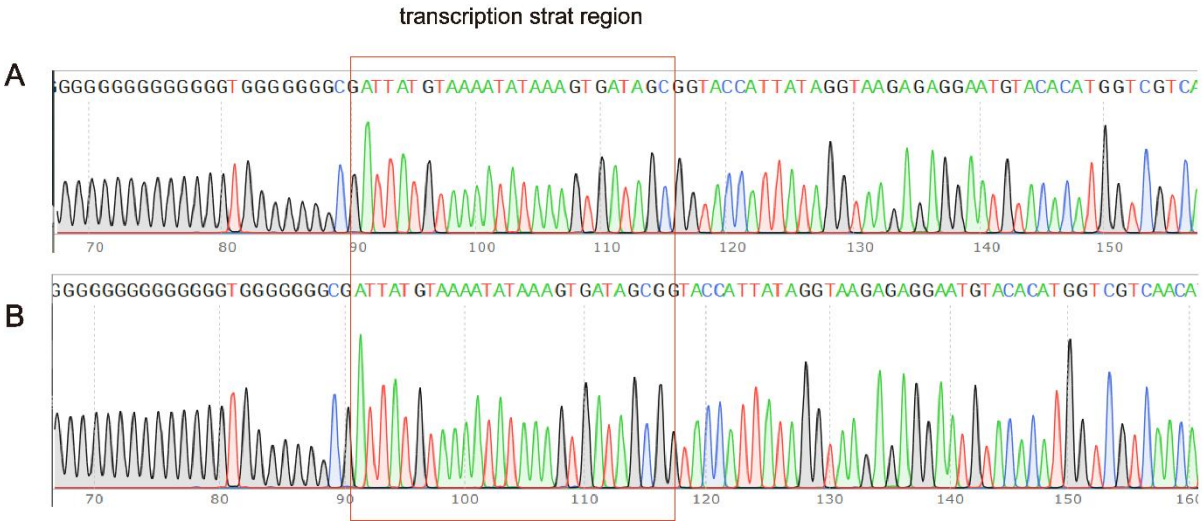
28

(Figure. S3)



(Figure. S4)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B
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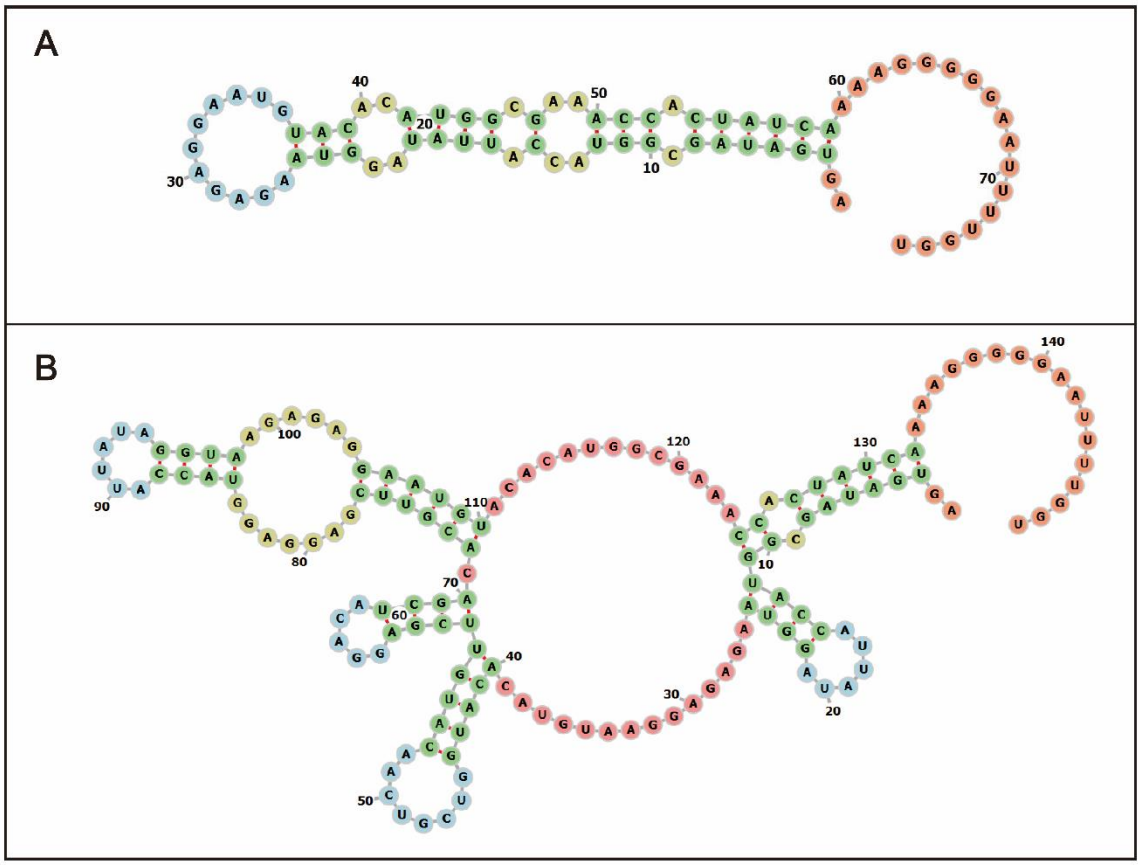
(Figure. S5)



(Figure. S6)

Figure legends

Fig. S1 The catalysis process of $GlcNAc_2$ by Dac.

Fig. S2 Nucleotide sequences of Dac after codon optimization.

Fig. S3 SDS-PAGE analysis of extracellular Dac of the recombinant strains. M: protein marker; 1–14 represent *B. subtilis* WB600 expressing different plasmid respectively. 1: pMA0911 (without Dac gene); 2: pMA0911NS (expression Dac without signal peptide); 3: pMA0911J (Dac with YncM), 4: pMA0911C (Dac with Bpr); 5: pMA0911M (Dac with YwbN); 6: pMA0911Z (Dac with AnsZ); 7: pMA0911O (Dac with YvgO); 8: pMA0911A (Dac with AmyE); 9: pMA0911F (Dac with OppA); 10: pMA0911G (Dac with Vpr); 11: pMA0911L (Dac with LipA); 12: pMA0911H (Dac with WapA); 13: pMA0911E (Dac with Epr); 14: pMA0911I (Dac with YclQ).

The Dac bands are indicated by arrows. The recombinant strains were grown in fermentation medium (see the “Materials and methods” section) containing 10 mg/L of kanamycin in 250-mL flask and incubated at 37°C and 220 rpm for 60 h.

Fig. S4 SDS-PAGE analysis of the recombinant Dac in the pellet of cell lysate. M: protein marker; 1: Control group, *B. subtilis* WB600 harboring pMA0911 plasmid (without *Dac*); 2: *B. subtilis* WB600 harboring pMA0911-YncM-Dac plasmid. The recombinant strains in this work were collected and broken by ultrasonic cell disruption system.

Fig. S5 The 5’-terminal sequencing map after RACE experiments in different strains. A: The 5’-terminal sequencing map in recombinant strain WB-NMK/Dac. B: The 5’-terminal sequencing map in recombinant strain WB-NMKmut/Dac.

Fig. S6 The secondary structure formed by mRNA of 5'-UTR in different strains. A: The secondary structure of 5'-UTR in recombinant strain WB-NMK/Dac. B: The secondary structure of 5'-UTR in recombinant strain WB-NMKmut/Dac.