Electronic Supplementary Material (ESI) for Molecular BioSystems

Supporting information

In vivo investigation of the substrate recognition capability and activity affecting amino acid residues of glycosyltransferase FscMI in biosynthesis of candicidin

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Table S1. Primers used in this study. Restriction sites and mutation sites are underlined.

Primers	Sequences (5′-3′)
Apr-KpnIF	GGTACCCGGGGATCCGTCGACC
Apr-KpnIR	GGTACCGTAGGCTGGAGCTGCTTC
LX1rightF	GGTACCAGTCGTCGGTGGTCTACATG
LX1rightR	AAGCTTTGCATGGCCGTCATCCGGTA
LX1leftF	GAATTCTTCTCGTCGGAGCCCACGA
LX1leftR	GGTACCGCCTTGAAGCGGGAGGACT
fscMI-testF	GACGACGAGGTGTACCGCGAGGTCA
fscMI-testR	CCTGGTCGAAGCAGTCCACCCACA
fscMIF2	CATATGGACTCCGCCGCCCGG
fscMIR2	GGATCCTCATGCCGCCGGGAGCAGG
amphDIF2	CATATGGGTGGACGCGAGGCGTG
amphDIR2	TCAGTCGTTTGCCAGGACGGGGA
nysDIF	CATATGACCCTTCCTTCCGGCAA
nysDIR	TCAGTCGGTTGCCAGGGCGGGAA
pimKF	CATATGGAATCCGCCCGACGGCC
pimKR	TTACGCCAGGGCCGGGAGCGAGA
DH11P1	TGTCGCGGGCGTCATCAAGATGGTC
DH11P2	CGCGCACCGTGTACACGGCGAGCCA
DH11P3	TGGCTCGCCGTGTACACGGTGCGCG
DH11P4	CGGCGAGGGAGGAGAACACCACGAA

DH11testF	ACACGGCACGGGGCTCTTCCTCG
DH11testR	GTGACGTCCTGCCAGGCGAACGG
31-F	GCCGGGGAGCTTGCCGCCCGCGGTGTGCCGGAC
31-R	GTCCGGCACACCGCGGGCGGCAAGCTCCCCGGC
346-F	GAGAGCTGGGTGCCCGCCCAGCTCGATGTGCTG
346-R	CAGCACATCGAGCTGGGCGGGCACCCAGCTCTC
361-F	GTCTCCGTCTTCTTCGTCCACGGCGGCGGCAAC
361-R	GTTGCCGCCGCCGTGGACGAAGAAGACGGAGAC
362-F	TCCGTCTTCTTCTCGGCCGGCGGCGGCAACGCC
362-R	GGCGTTGCCGCCGCCGGCCGAGAAGAAGACGGA
370-F	GGCAACGCCTACCACGCCGGCGTCTACTTCGGT
370-R	ACCGAAGTAGACGCCGGCGTGGTAGGCGTTGCC
387-F	CCCCTGTGGGTGGACGCCTTCGACCAGGCGGTC
387-R	GACCGCCTGGTCGAAGGCGTCCACCCACAGGGG

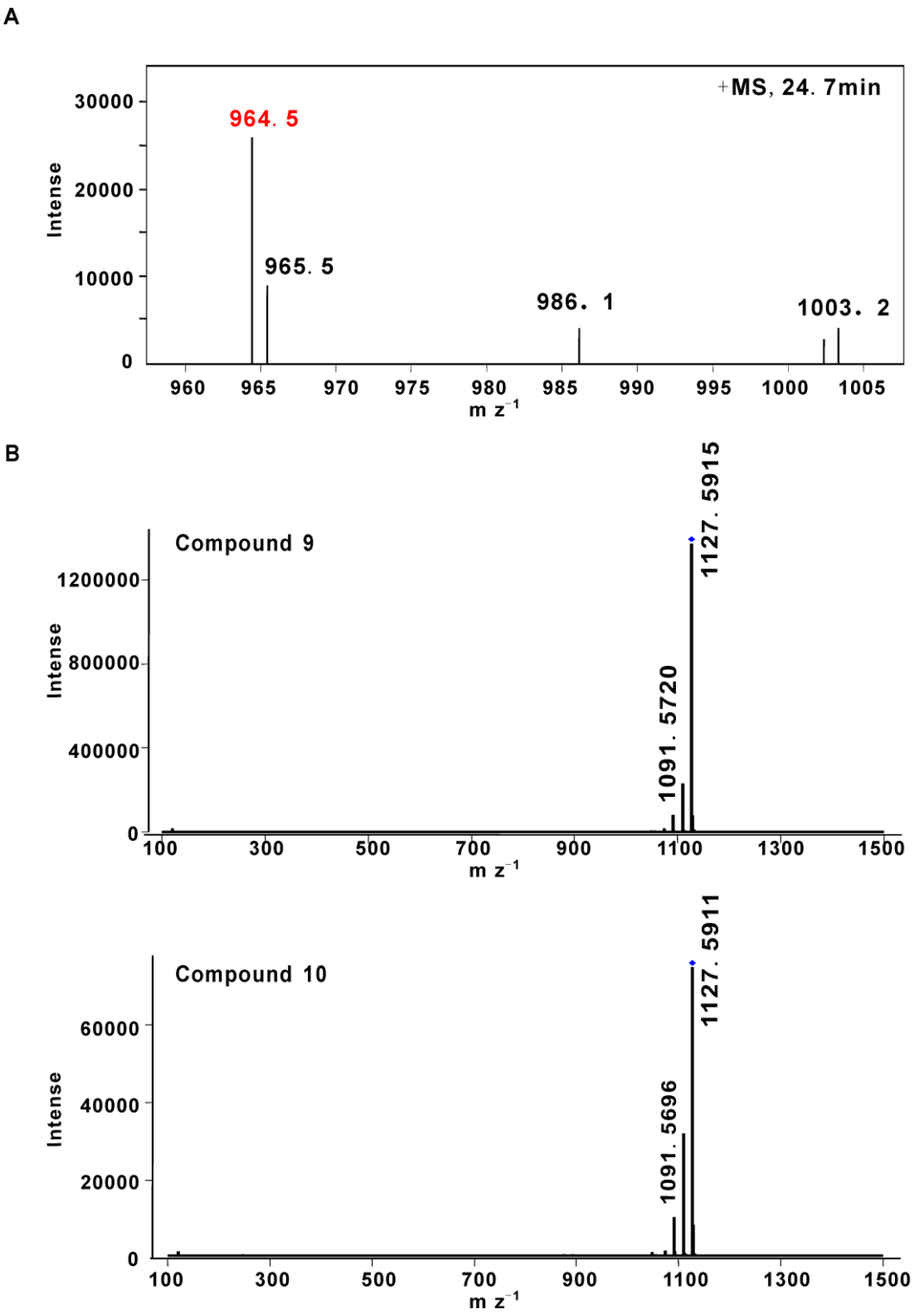

Fig. S1. Further analysis of the fermentation culture extracts of LX1 and LX8 by LC-MS or Q-TOF. (A) LC-MS profile of compound 5, the product of LX1. (B) Q-TOF analysis of compound 9 and 10.

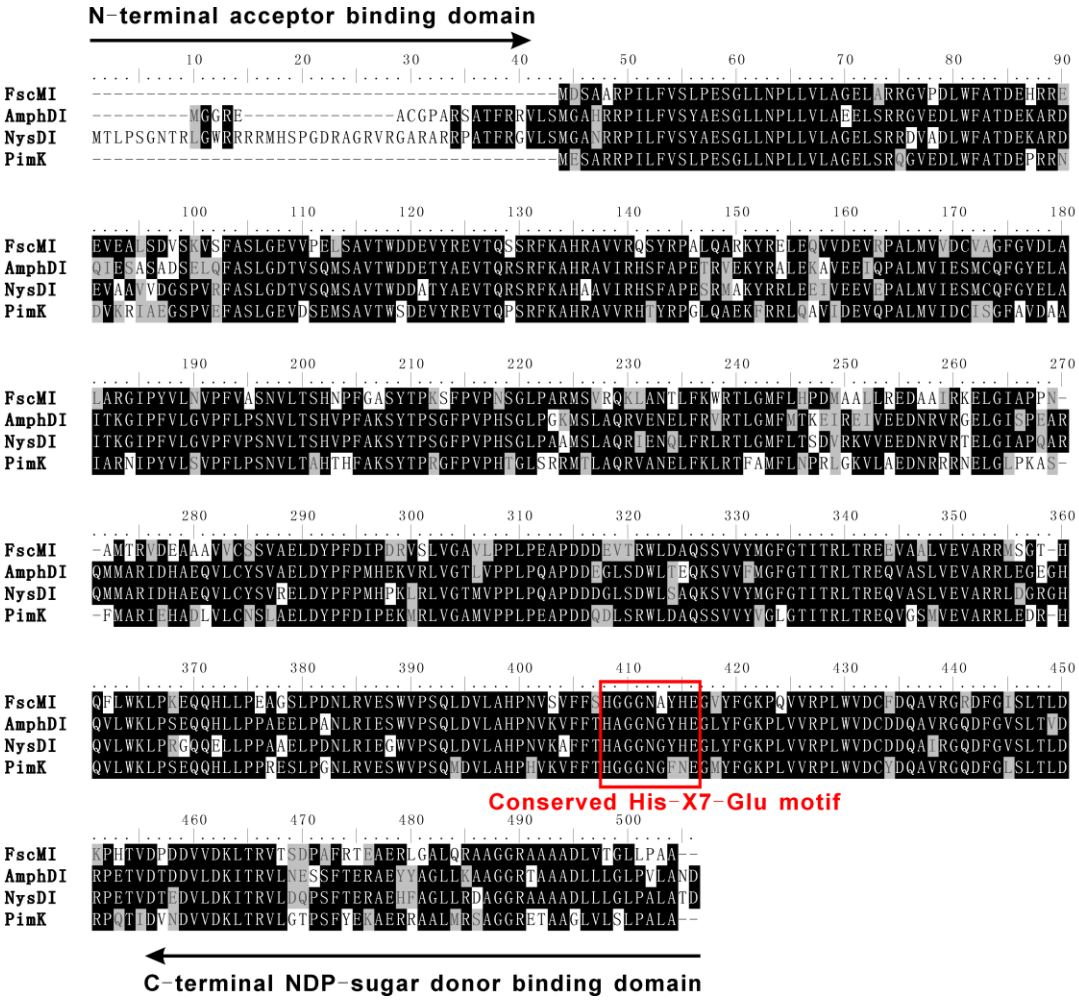

Fig. S2. Alignment of amino acid sequence of FscMI with other three homologous polyene GTs. The N-terminal domain provides the acceptor binding site, whereas the C-terminal domain is responsible for binding the NDP-sugar donor. The conserved His-X7-Glu motif shared by many GT-B GTs is highlighted in the red box.

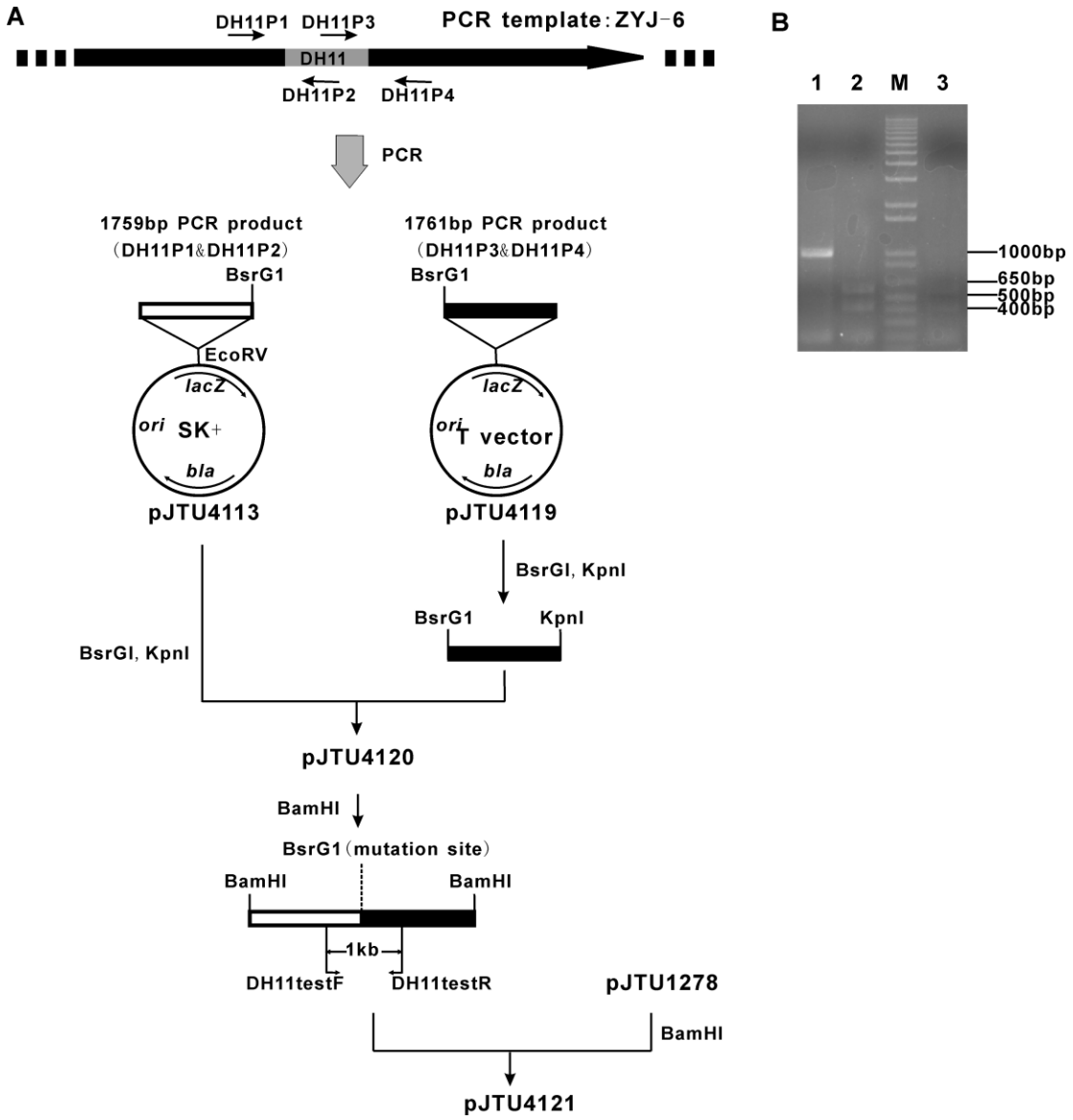

Fig. S3. Construction of the plasmid for point mutation of DH11 and verification of DH11 mutant. (A) Schematic diagram of the plasmid construction procedure for point mutation of DH11 in FscD. (B) Verification of DH11 mutant. PCR products from ZYJ-6 and LX8 were digested by BsrGI. M, 1 kb DNA ladder; 1, PCR product of ZYJ-6; 2, 3, PCR products of mutants LX8. The 1,002 bp PCR product from LX8 was digested into 419 bp and 583 bp DNA fragments by BsrGI.

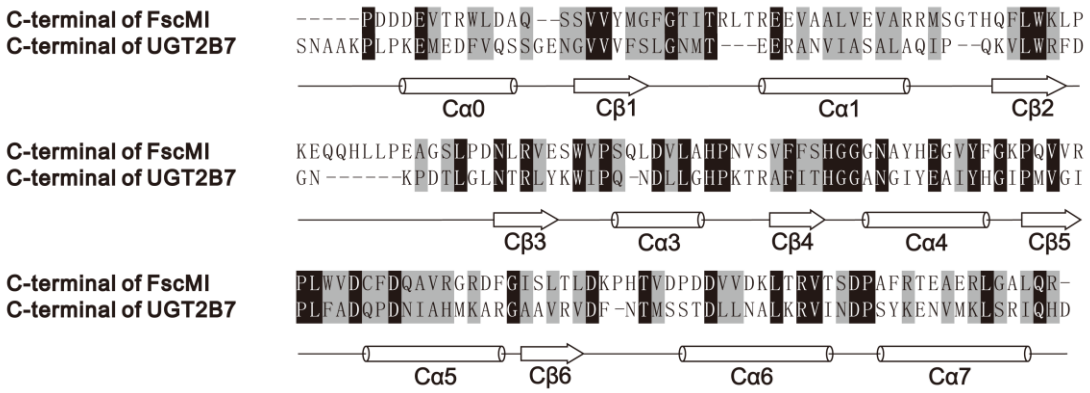

Fig. S4. Alignment of the secondary structures of C-terminal domains of FscMI and UGT2B7. FscMI and UGT2B7 have the same secondary structure in the C-terminal domain. Helices and β sheets are represented by cylinders and arrows respectively.