Identification and functional application of a new malonyltransferase NbMaT1 towards diverse aromatic glycosides from *Nicotiana benthamiana*

Yuyu Liu, Xiaohui Wang, Ting Mo, Yaru Yan, Yuelin Song, Yunfang Zhao, Jun Li, Shepo Shi, Xiao Liu, Pengfei Tu

Modern Research Center for Traditional Chinese Medicine, School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing 100029, People’s Republic of China

* fcliuxiao@163.com; † pengfeitu@163.com;

Tel/Fax: (86)-10-82802750
Supplementary Material

Table of Contents

Figure S1. Multiple alignment of the deduced amino acid sequence of NbMaT1 and related enzymes.................................................................3

Figure S2. HPLC-UV spectra of standard malonyl-CoA and enzymatic synthesis of malonyl-CoA by MatB.................................................................4

Figures S3–S18. HPLC-UV and HR-ESI-MS analyses of malonylated product of 1-16..................................................................................5-20

Figure S19. $^1$H NMR spectrum of malonylated product 9a in Methanol-$d_4$..........21

Figure S20. $^{13}$C NMR spectrum of malonylated product 9a in Methanol-$d_4$..........21

Figure S21. HSQC spectrum of malonylated product 9a in Methanol-$d_4$.............22

Figure S22. HMBC spectrum of malonylated product 9a in Methanol-$d_4$.............22

Figure S23. Heterogenous expression and purification of three targeted His6-tag fusion proteins after gene expression.............................................23
Figure S1. Multiple alignment of the deduced amino acid sequence of NbMaT1 and related enzymes.

Black shading shows the identical amino acids in at least four sequences. Motifs 1–3 are the region conserved among BAHD acyltransferases. Abbreviations and GenBank accession numbers are: NbMaT1 (KY563646); NtMaT1, *Nicotiana tabacum* phenolic glucoside-6′-O-malonyltransferase with broad substrate specificity (BAD93691); Gs5AT, hydroxycinnamoyl-CoA: anthocyanin 5-glucoside-6-O-hydroxycinnamoyltransferase from *Gentiana scabra* var. *buergeri* (BAD44688); Sc3MaT, malonyl-coenzyme A: anthocyanidin 3-O-glucoside-6′′′-O-malonyltransferase from *Pericallis cruenta* (AAO38058); Dm3MaT1, anthocyanidin 3-O-glucoside-6′′′-O-malonyltransferase from *Chrysanthemum x morifolium* (AAQ63615); Pf5MaT, anthocyanin 5-O-glucose 6′′′-O-malonyltransferases from *Salvia splendens* (AAL50565).
Figure S2. HPLC-UV spectra of standard malonyl-CoA (A) and enzymatic synthesis of malonyl-CoA by MatB (B).
Figure S3. HPLC-UV/HR-ESI-MS (negative) spectra of malonylated product of 1.
A: HPLC chromatogram and UV spectra of 1 and malonylated product 1a catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); B: HR-ESI-MS (negative) spectrum of 1a.
Figure S4. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 2.

A: HPLC chromatogram and UV spectra of 2 and malonylated product 2a catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); B: HR-ESI-MS (positive) spectrum of 2a.
Figure S5. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 3.

A: HPLC chromatogram and UV spectra of 3 and malonylated product 3a catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); B: HR-ESI-MS (positive) spectrum of 3a.
Figure S6. HPLC-UV/HR-ESI-MS (negative) spectra of malonylated product of 4.
A: HPLC chromatogram and UV spectra of 4 and malonylated product 4a catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); B: HR-ESI-MS (negative) spectrum of 4a.
Figure S7. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 5.

A: HPLC chromatogram and UV spectra of 5 and malonylated product 5a catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); B: HR-ESI-MS (positive) spectrum of 5a.
A:

A1: HPLC chromatogram and UV spectra of 6 and malonylated product 6a catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3);

A2: HR-ESI-MS (positive) spectrum of 6a.

B:

Inten. [x10,000]

Figure S8. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 6.

A: HPLC chromatogram and UV spectra of 6 and malonylated product 6a catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); B: HR-ESI-MS (positive) spectrum of 6a.
Figure S9. HPLC-UV/HR-ESI-MS (negative) spectra of malonylated product of 7.
A: HPLC chromatogram and UV spectra of 7 and malonylated product 7a catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); B: HR-ESI-MS (negative) spectrum of 7a.
Figure S10. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 8.  
A: HPLC chromatogram and UV spectra of 8 and malonylated product 8a catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); B: HR-ESI-MS (positive) spectrum of 8a.
Figure S11. HPLC-UV/HR-ESI-MS (negative) spectra of malonylated product of 9.

A: HPLC chromatogram and UV spectra of 9 and malonylated product 9a catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); B: HR-ESI-MS (negative) spectrum of 9a.
Figure S12. HPLC-UV/HR-ESI-MS (negative) spectra of malonylated product of 10.

A: HPLC chromatogram and UV spectra of 10 and malonylated product 10a catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); B: HR-ESI-MS (negative) spectrum of 10a.
Figure S13. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 11.

A: HPLC chromatogram and UV spectra of 11 and malonylated product 11a catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); B: HR-ESI-MS (positive) spectrum of 11a.
Figure S14. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 12.
A: HPLC chromatogram and UV spectra of 12 and malonylated product 12a catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); B: HR-ESI-MS (positive) spectrum of 12a.
Figure S15. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 13.  
A: HPLC chromatogram and UV spectra of 13 and malonylated product 13a catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); B: HR-ESI-MS (positive) spectrum of 13a.
Figure S16. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 14.

A: HPLC chromatogram and UV spectra of 14 and malonylated product 14a catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); B: HR-ESI-MS (positive) spectrum of 14a.
Figure S17. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 15. 
A: HPLC chromatogram and UV spectra of 15 and malonylated product 15a catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); B: HR-ESI-MS (positive) spectrum of 15a.
Figure S18. HPLC-UV/HR-ESI-MS (positive) spectra of malonylated product of 16.

A: HPLC chromatogram and UV spectra of 16 and malonylated product 16a catalysed by NbMaT1 (A1), one-pot reaction system (A2), fusion protein of MatB-NbMaT1 (A3); B: HR-ESI-MS (positive) spectrum of 16a.
Figures S19. $^1$H NMR spectrum of malonylated product 9a in Methanol-$d_4$.

Figures S20. $^{13}$C NMR spectrum of malonylated product 9a in Methanol-$d_4$. 
Figures S21. HSQC spectrum of malonylated product 9a in Methanol-$d_4$.

Figures S22. HMBC spectrum of malonylated product 9a in Methanol-$d_4$. 
Figure S23. Heterogenous expression and purification of targeted His₆-tag fusion proteins after gene expression.

The 10% (w/v) SDS polyacrylamide gel was stained with Coomassie Brilliant Blue G-250;
Lane M, molecular mass standards;
Lane 1, soluble protein before induction;
Lane 2, soluble protein after IPTG induction;
Lane 3&4, purified His₆ fusion protein of MatB (60.07 KDa) and NbMaT1 (50.91 KDa).