Supporting Information

Heterologous synthesis of Monacolin J by reconstructing its biosynthetic gene cluster in *Aspergillus niger*

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Supplemental Table S1. The plasmid construction strategy used in this study

Fragments assembled by overlapping PCR (Corresponding primers)			Generated plasmid via in-fusion cloning
Fragment A	**Fragment B**	**Fragment C**	
*Up$_{pyrG}$-P$_{gpdA}$ (Up$_{pyrG}$-F/R, P$_{gpdA}$-F/R)	*LovC* (*lovC*-F/R)	T$_{tef}$-*Dn$_{pyrG}$ (T$_{tef}$-1-F/R, Dn$_{pyrG}$-F/R)	pM18T-Up$_{pyrG}$-P$_{gpdA}$-*lovC*-T$_{tef}$-Dn$_{pyrG}$
Up$_{aamA}$-P$_{tef}$ (Up$_{aamA}$-F/R, P$_{tef}$-1-F/R)	*LovG* (*lovG*-F/R)	T$_{tef}$-*pyrG*$_{An}$-Dn$_{aamA}$ (T$_{tef}$-2-F/R, *pyrG*$_{An}$-F/R, Dn$_{aamA}$-F/R)	pM18T-Up$_{aamA}$-P$_{tef}$-*lovG*-T$_{tef}$-*pyrG*$_{An}$-Dn$_{aamA}$
Up$_{amyA}$-P$_{tef}$ (Up$_{amyA}$-F/R, P$_{tef}$-2-F/R)	*lovA* (*lovA*-F/R)	T$_{tef}$-Dn$_{amyA}$ (T$_{tef}$-3-F/R, Dn$_{amyA}$-F/R)	pM18T-Up$_{amyA}$-P$_{tef}$-*lovA*-T$_{tef}$-Dn$_{amyA}$

*Up$_{geneA}$: represents the upstream region of gene A.

Dn$_{geneA}$: represents the downstream region of gene A.

Supplemental Table S2. Construction and identification primers used in this study

Primer	Sequences	Function description
lovB-up-F	ACTGAGAGCCTGAGCTTCATCCCCAGCATC ATTACACCTCAGCATCTAGAATGGCTCAAT CTATGTATCCTAATG	Amplification of the upstream fragment of *lovB* ORF
lovB-up-R	GCCGGTCCCTGCTCCAATCTCCAGAATAT CCATTGACTGATAGCGATGGGCGATCTGC GCCACCAATTCCCGGGCGTAGT	Amplification of the upstream fragment of *lovB* ORF
lovB-dn-F	ACTACGCCCGGGAATTGGTGGCGCAGATC GCCCATCGCTATCAGTCAATGGATATTCTG GAGATTGGAGCAG	Amplification of the downstream fragment of *lovB* ORF
lovB-dn-R	TCGTAAAGGCTTTTTTAAGGAAGTCATAA CGGCATAAATCGAATGTCCGCTCATGCCA GCTTCAGGGCGG	Amplification of the downstream fragment of *lovB* ORF
Up_{pyrG}-F	<u>ACCCGGGGATCCTCTAGAGA</u>CTGGTGGC AGGCGTCAAGT	Amplification of the upstream fragment of $pyrG_{An}$
Up_{pyrG}-R	<u>CTCTTGGGTCTCTCCCGTCA</u>GATGTTAAA GGGTTGGGATGG	Amplification of the upstream fragment of $pyrG_{An}$
P_{gpdA}-F	<u>CATCCCAACCCTTTAACATCTGACGGGAG</u> AGACCCAAGAG	Amplification of the fragment P_{gpdA}
P_{gpdA}-R	<u>ATGAATGGCTGGTCGCCCATT</u>GTTTAGATG TGTCTATGTGGCG	Amplification of the fragment P_{gpdA}
lovC-F	<u>CACATAGACACATCTAAACA</u>ATGGG CGACCAGCCATTCATT	Amplification of the whole *lovC* ORF
lovC-R	<u>TAGCGAAATGGATTGATTGTTTACGG</u> CCCCTCGAGCCGA	Amplification of the whole *lovC* ORF
T_{tef}-1-F	<u>TTCGGCTCGAGGGGCCGTAA</u>GCGGACATT CGATTTATGCCGTT	Amplification of the fragment T_{tef}
T_{tef}-1-R	<u>GACGTTGGTTTTCTTCTCCT</u>GTATTGGGAT GAATTTTGTATGCAC	Amplification of the fragment T_{tef}
Dn_{pyrG}-F	<u>TACAAAATTCATCCCAATAC</u>AGGAGAAG AAAACCAACGTCAC	Amplification of the downstream fragment of $pyrG_{An}$
Dn_{pyrG}-R	<u>CTTGCATGCCTGCAGGTCGA</u>CGATAACTC AACCGAGCGTGCCGT	Amplification of the downstream fragment of $pyrG_{An}$
Up_{aamA}-F	<u>ACCCGGGGATCCTCTAGAGA</u>GCGTT GTTTCTTACCCCC	Amplification of the upstream fragment of Up_{aamA}
Up_{aamA}-R	<u>GCGGTGATTCTGCTGTCTCG</u>GAATTG TCCGTCCTACCGAA	Amplification of the upstream fragment of Up_{aamA}

Primer	Sequence	Description
P_{tef}-1-F	<u>TTCGGTAGGACGGACAATTCC</u>GAGACAGCAGAATCACCGC	Amplification of the fragment P_{tef}
P_{tef}-1-R	<u>GGAGATGCTTGGTAACGCATG</u>TGAAGGTTGTGTTATGTTTTGTG	Amplification of the fragment P_{tef}
$lovG$-F	<u>AAACATAACACAACCTTCACATGCG</u>TTACCAAGCATCTCCA	Amplification of the whole $lovG$ ORF
$lovG$-R	<u>GGCATAAATCGAATGTCCGCC</u>TACTCCAATGTCTGGGCCG	Amplification of the whole $lovG$ ORF
T_{tef}-2-F	<u>CGGCCCAGACATTGGAGTAG</u>GCGGACATTCGATTTATGCCGTT	Amplification of the fragment T_{tef}
T_{tef}-2-R	<u>CGCGTTCTCGAGGAAGTTGCG</u>TATTGGGATGAATTTTGTATGCA	Amplification of the fragment T_{tef}
$pyrG_{An}$-F	<u>TACAAAATTCATCCCAATACGCAACT</u>TCCTCGAGAACGCG	Amplification of the fragment $pyrG_{An}$
$pyrG_{An}$-R	<u>CCAACGTACTTGGTCACCCGCCCTTT</u>TAGTCAATACCGTTAC	Amplification of the fragment $pyrG_{An}$
Dn_{aamA}-F	<u>AACGGTATTGACTAAAAGGGCGGGT</u>GACCAAGTACGTTG	Amplification of the downstream fragment of $aamA$
Dn_{aamA}-R	<u>ATGCCTGCAGGTCGACGATA</u>GCCCTGGTACAGTTAGCG	Amplification of the downstream fragment of $aamA$
Up_{amyA}-F	<u>ACCCGGGGATCCTCTAGAG</u>CAATCCATTTATTTCCTTCTC	Amplification of the upstream fragment of Up_{amyA}
Up_{amyA}-R	<u>GCGGTGATTCTGCTGTCTCG</u>GAGCGCCTTCAAGTCATCT	Amplification of the upstream fragment of Up_{amyA}
P_{tef}-2-F	<u>CAGATGACTTGAAGGCGCTCC</u>GAGACAGCAGAATCACCGC	Amplification of the fragment P_{tef}
P_{tef}-2-R	<u>TGAGCGCGTCGACAGTCATG</u>GTGAAGGTTGTGTTATGTTTTGTG	Amplification of the fragment P_{tef}
$lovA$-F	<u>AACATAACACAACCTTCACC</u>ATGACTGTCGACGCGCTCACA	Amplification of the whole $lovA$ ORF
$lovA$-R	<u>GGCATAAATCGAATGTCCGCC</u>TATAGTGAACCAGGAAGGCGG	Amplification of the whole $lovA$ ORF
T_{tef}-3-F	<u>GCCTTCCTGGTTCACTATAGGCGGAC</u>ATTCGATTTATGCCGTT	Amplification of the fragment T_{tef}
T_{tef}-3-R	<u>CGACCATAAGATACATCCCCGTATTG</u>GGATGAATTTTGTATGCA	Amplification of the fragment T_{tef}
Dn_{amyA}-F	<u>TACAAAATTCATCCCAATACGGGGAT</u>GTATCTTATGGTCG	Amplification of the downstream fragment of $amyA$
Dn_{amyA}-R	<u>ATGCCTGCAGGTCGACGATA</u>CGGAT	Amplification of the

	CACCGTCGAGCAC	downstream fragment of *amyA*
LovB-YZ1-F	CGTACCTGGCGACCTATGACTA	Yeast transformation verification primers
LovB-YZ1-R	GTAGGTAGCCTTGATGAGTGCC	Yeast transformation verification primers
LovB-YZ2-F	AGGGAGACTACTTGAGCGGTGA	Yeast transformation verification primers
LovB-YZ2-R	TGAACACTCTCAGGCACAAACC	Yeast transformation verification primers
LovB-YZ3-F	ACTGTCTCTCGGGCAAGAATAC	Yeast transformation verification primers
LovB-YZ3-R	TCAACTCTCCCCTCTGTGCTAA	Yeast transformation verification primers
LovB-JD-1-F	GAGGATTGCCTGAACATTGACA	Location of *lovB* in the *glaA* (An03g06550) locus
LovB-JD-1-R	ACTCACGCCTCTGGCATAGC	Location of *lovB* in the *glaA* (An03g06550) locus
LovB-JD-2-F	TAACATCTAAACTCTTCTCCATCG	Location of *lovB* in the *glaA* (An03g06550) locus
LovB-JD-2-R	ACGGTCTGGTTCTGTAGTTCC	Location of *lovB* in the *glaA* (An03g06550) locus
LovC-JD-1-F	CGGAAAGTGGAACGATTGGGGG	Location of *lovC* in the *pyrG~An~* locus
LovC-JD-1-R	TGGCAAAGGCAGCCCAAGCAAC	Location of *lovC* in the *pyrG~An~* locus
LovC-JD-2-F	GCAGTGAGGAAGAGCGGCAG	Location of *lovC* in the *pyrG~An~* locus
LovC-JD-2-R	ATTGTCGATCTGCCCACTTTCT	Location of *lovC* in the *pyrG~An~* locus
LovG-JD-1-F	CGTCCTTCGGTCGGAATGATG	Location of *lovG* in the *aamA* (An11g03340) locus
LovG-JD-1-R	CGCTCACGGAGGGTCCATTAT	Location of *lovG* in the *aamA* (An11g03340) locus
LovG-JD-2-F	GGAACGAAGTGATGAAGAACCA	Location of *lovG* in the *aamA* (An11g03340) locus
LovG-JD-2-R	CATACGAGGCGAAACGGGGA	Location of *LovG* in the *aamA* (An11g03340) locus
LovA-JD-1-F	ATATCAGGCCGCGCACGAAAG	Location of *lovA* in the *amyA* (An05g02100) locus
LovA-JD-1-R	CCTCATGGTCGTCAGTTCCC	Location of *lovA* in the *amyA* (An05g02100) locus
LovA-JD-2-F	TTCGGTTGGCATCCACGGGC	Location of *lovA* in the *amyA* (An05g02100) locus

LovA-JD-2-R	TTAACAGTTTGCGGGAGATGCA	Location of *lovA* in the *amyA* (An05g02100) locus
RT-*lovB*–F	GCGTCCAACCGCATCTCG	Semi-quantitative primers for *lovB* gene
RT-*lovB*–R	AGATTCGCACCCGCAGCAA	Semi-quantitative primers for *lovB* gene
RT-*lovC*–F	ACCGCTGGATTGGCGATGA	Semi-quantitative primers for *lovC* gene
RT-*LovC*–R	CGATAGTCAAAGACCTCCTC	Semi-quantitative primers for *lovC* gene
RT-*lovG*–F	CGACAACAATCATAATGGACCC	Semi-quantitative primers for *lovG* gene
RT-*lovG*–R	CTTGCCTCGTCTTGATACCAG	Semi-quantitative primers for *lovG* gene
RT-*lovA*–F	CTGGTTCTCGCAAAATGATAAG	Semi-quantitative primers for *lovA* gene
RT-*lovA*–R	GTGACTTCCTTGAATCCATCG	Semi-quantitative primers for *lovA* gene
RT-*gpdA*-F	CTCTGCTCCTTCCGCTGAT	Semi-quantitative primers of internal reference gene *gpda* (An16g01830)
RT-*gpdA*-R	ACCCTCAACGATGCCGAAC	Semi-quantitative primers of internal reference gene *gpda* (An16g01830)

Supplemental Table S3. CRISPR/Cas9 plasmids used in this study

Plasmids	Description/derivation	Reference
PFC332-*pyrGAn*-*hygB*	PFC332 derivate with gRNA scaffold of *pyrGAn* under the control of promoter of *A. oryzae* U6 and *A. fumigatus* U6-2	This work
PFC332-*aamA-hygB*	PFC332 derivate with gRNA scaffold of *aamA* under the control of promoter of *A. oryzae* U6 and *A. fumigatus* U6-2	This work

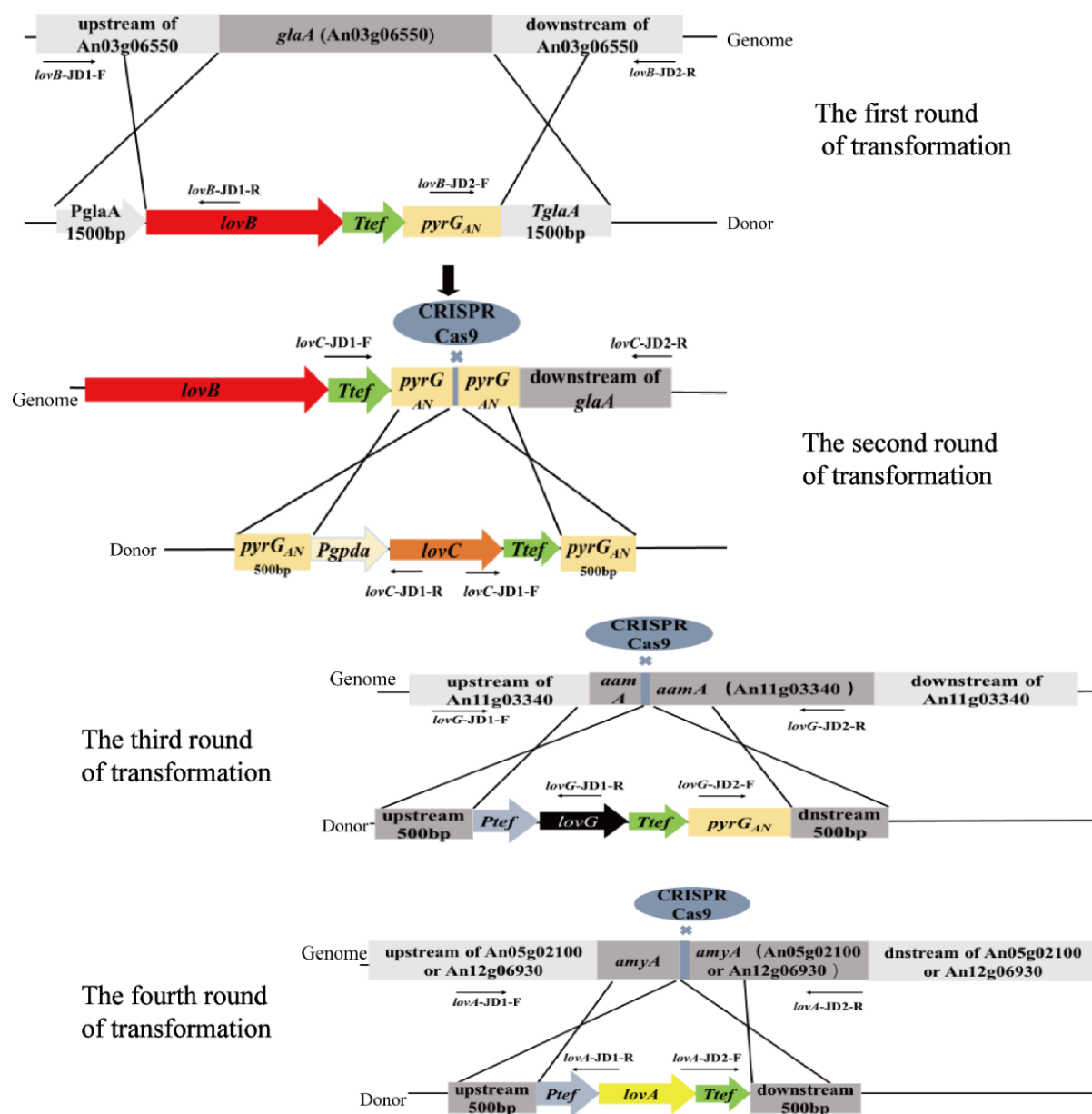| PFC332-*aamA*-*amyA*-*hygB* | PFC332 derivate with gRNA scaffold of *aamA* and *amyA* under the control of promoter of *A. oryzae* U6 and *A. fumigatus* U6-2 | This work |

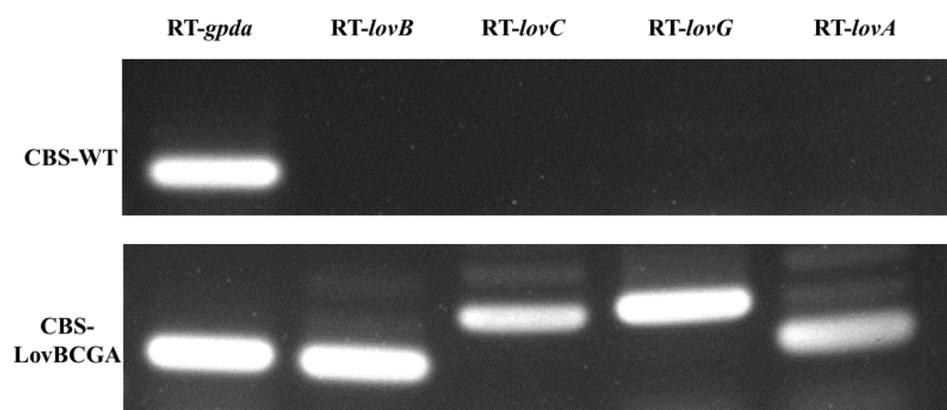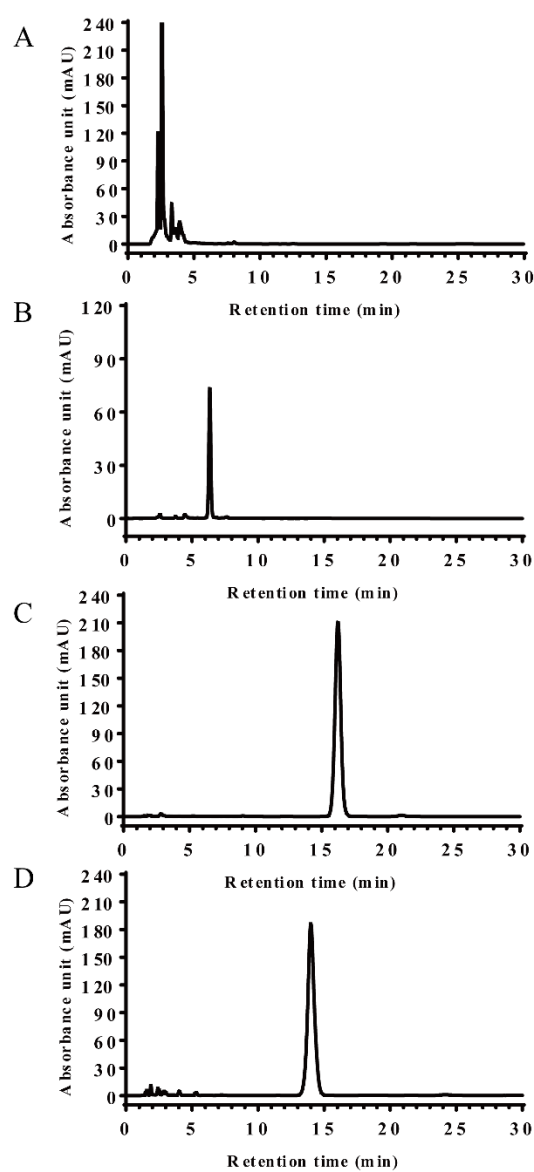**Figure S1**. Schematic gene integration in *A. niger*.

Figure S2. RT-PCR analysis of the expression of MJ genes in *A. niger* CBS-WT and CBS-LovBCGA strain. *gpda* was used as the internal reference gene.

Figure S3. HPLC identification of *A. niger* strain fermentation product and standard products at 237 nm wavelength. (A) *A. niger* strain CBS513.88(Δ*kusA*, Δ*pyrG*) (B) Monacolin J acid standard, retention time 6.473 min (C) Lovastatin lactone standard, retention time 16.238 min (D) Lovastatin acid standard, retention time 13.999 min.
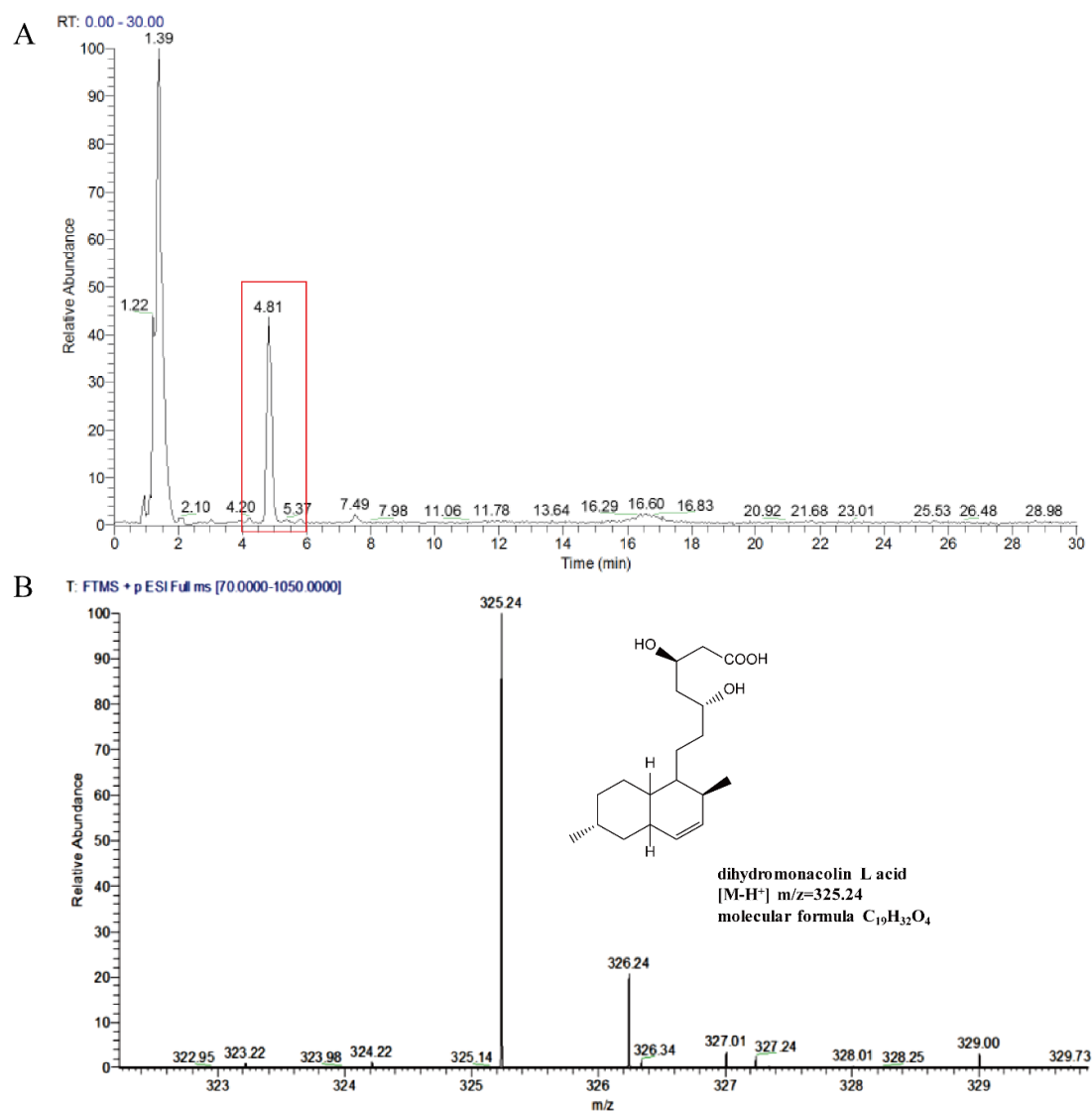
Figure S4. LC-MS identification DML acid of *A. niger* transformated strain CBS-LovBCG fermentation product (A) DML acid, retention time 4.81 min with the red square. (B) LC-MS identification of DML acid from *A. niger* strain CBS-LovBCG.
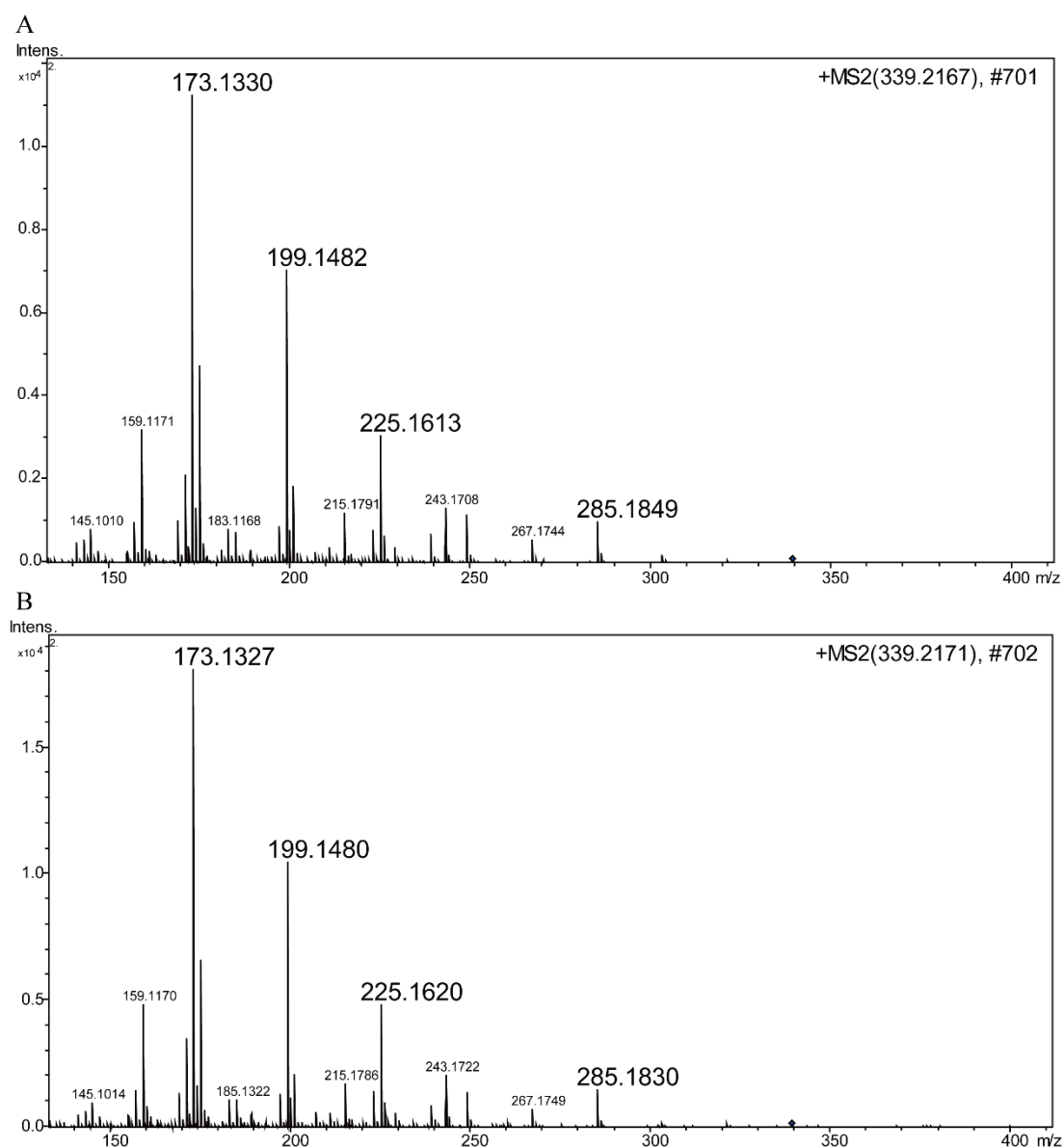
Figure S5. Tandem LC-MS/MS analysis of the mass spectra fragmentation of MJ standard (A) and MJ produced by *A. niger* strain CBS-LovBCGA (B).
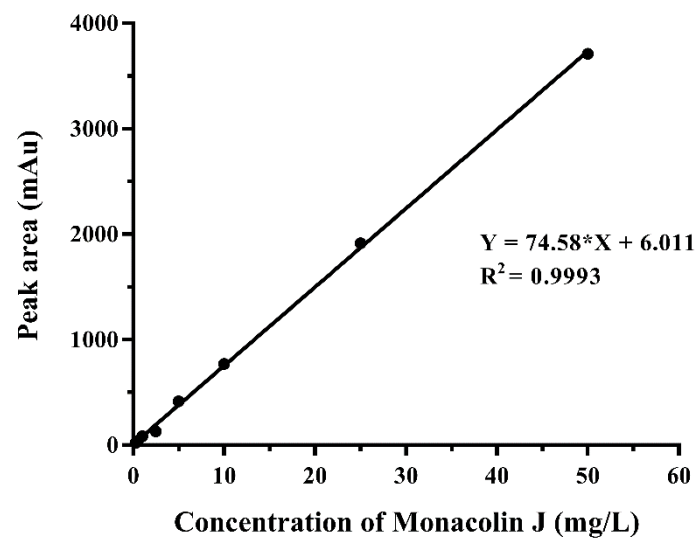
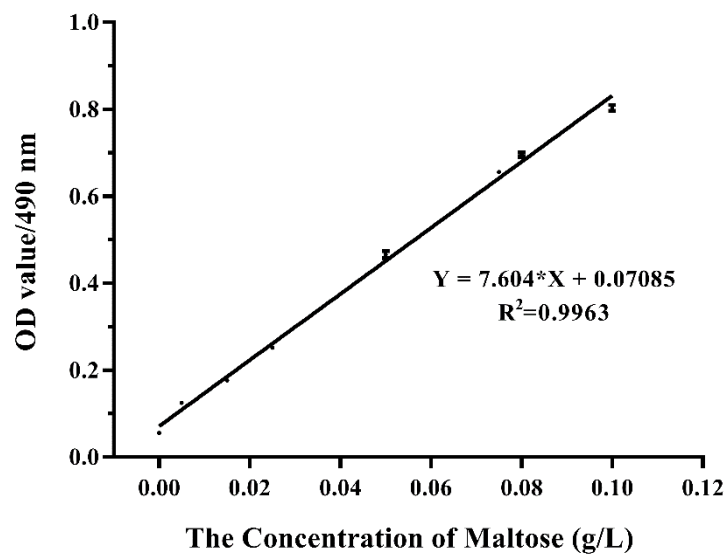**Figure S6.** Calibration curves of Monacolin J developed by HPLC

Figure S7. Calibration curves of the concentration of Maltose