

Supporting Information

Transformation of phytosterols to pregnatetraenedione by a combined microbial and chemical process

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Table S1 primers used in this work

Primers		
Gene deletion in mycobacteria		
pQC-ltp2-U-F&R	TATActgcagACCAGGCTCGCCATCAACGGGGTC/ GACCaagcttATCCACGGCATGAACGGGATCGCC	This study
pQC-ltp2-D-F&R	GCGCaagcttGTCCATCGTGAAGGTGACCAGACC/ TATAgcggccgcGAGATGGACTGGCGCATCATGAAG	This study
pQC-igr-U-F&R	GCACaagcttCGCGTTCTCGTCGACGTTTCGGCCAC/ TATAgaattcCCGTCGCGCGGTCCACCGGCATCGG	This study
pQC-igr-D-F&R	GCGCgaattcCATGTTGAGCAGGACGGCCTTCTGC/ ATCGg gatccCGTCGAGGTGGTAGTAGGTCTCGGC	This study
pQC-E1E2-U-F&R	GGCAggtaccCACCGTAGATCTGCAGTTCGGGCA/ GCTAgaattcCAGTCCGAGCCCGTCACCACCGAG	This study
pQC-E1E2-D-F&R	TAGCgaattcCTCGGGCACCGCCAGCGCCGCCAC/ TATAgcggccgcATCCCGGTCTGGCCGCAGGAGATG	This study
pQC-H1H2-U-F&R	TATActgcagTGCCGCAGAAGCCGAGCTCCTCGA/ GCGCaagcttGACACCATCACCTTCAGTGGTGAG	This study
pQC-H1H2-D-F&R	TAGCaagcttGACGATACCCGGATGACCTGCGGC/ TATAgcggccgcCGATCGAGCAGCAGATCTTCATCA	This study
Gene augmentation in mycobacteria		

H1H2- F&R	ATATgaattcGTGAGCGAACTGCAGGCGGGTATCG/ AATTatcgatTCACTTGTCCCCAGGGTGAGCGTC	This study
Ltp2- F&R	AATTggatccGGTGACATCGTCCGGAAAGGCCGG/ AATTaagcttTCAGCCGAGGATCAGACCCGATGTCTG	This study
Cyp125- F&R	AATTgaattcGTGGTACTAGAAGGAATTGTCTCGGCA CCCGTCG/ AATTaagcttCTAGCTCGAGGCGCCGGCGC	This study
A1-F&R	TGCagaattcGTGACTACCGAGACAGCCGGCA/ AGTAaagcttTCAGCTCGGCTGAGCCGGTT	This study
4A-F&R	TAGCctgcagAAATGAACGACAACCCGATCGACCTGT / GCGCaagcttTCAAGAACCCATGAGCTCAGTTGCG	This study
A5-F&R	AATTggatccAATGGGTAATCCTGTCATCGTCGAAGC C/ AATTgaattcTTAGATCCGCTCGATGATGGTGCCCG	This study
A14A-F&R	CTCAGCCGAGCTGAGAATTCGAGAAGGAGATATA ATGAACGACAACCCGATC/ GTTAACTACGTCGACATCGATATCAAGAACCCATG AGCTCAGTTGCGGAG	This study
E1E2-F&R	CGGATCCAGCTGCAGAATTCGTGGACTTCACGCCG AAGCCCG/ CGTCGACATCGATAAGCTTTCACCGGGTCACCCTC GGCAC	This study
A1E1E2-F&R	CTAAaagcttGAGAAGGAGATATAGTGACTACCGAG ACAGCCGGC/ AATTgtaacTCAGCTCGGCTGAGCCGGTTC	This study

4AE1E2-F&R	GTGAaagcttGAGAAGGAGATATAATGAACGACAAC CCGATC/ AATTgtaacTCAAGAACCCATGAGCTCAGTTGCGGA G	This study
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Note: all sequences of primers were showed in 5'-3'; the lowercase letters indicated the restriction sites.

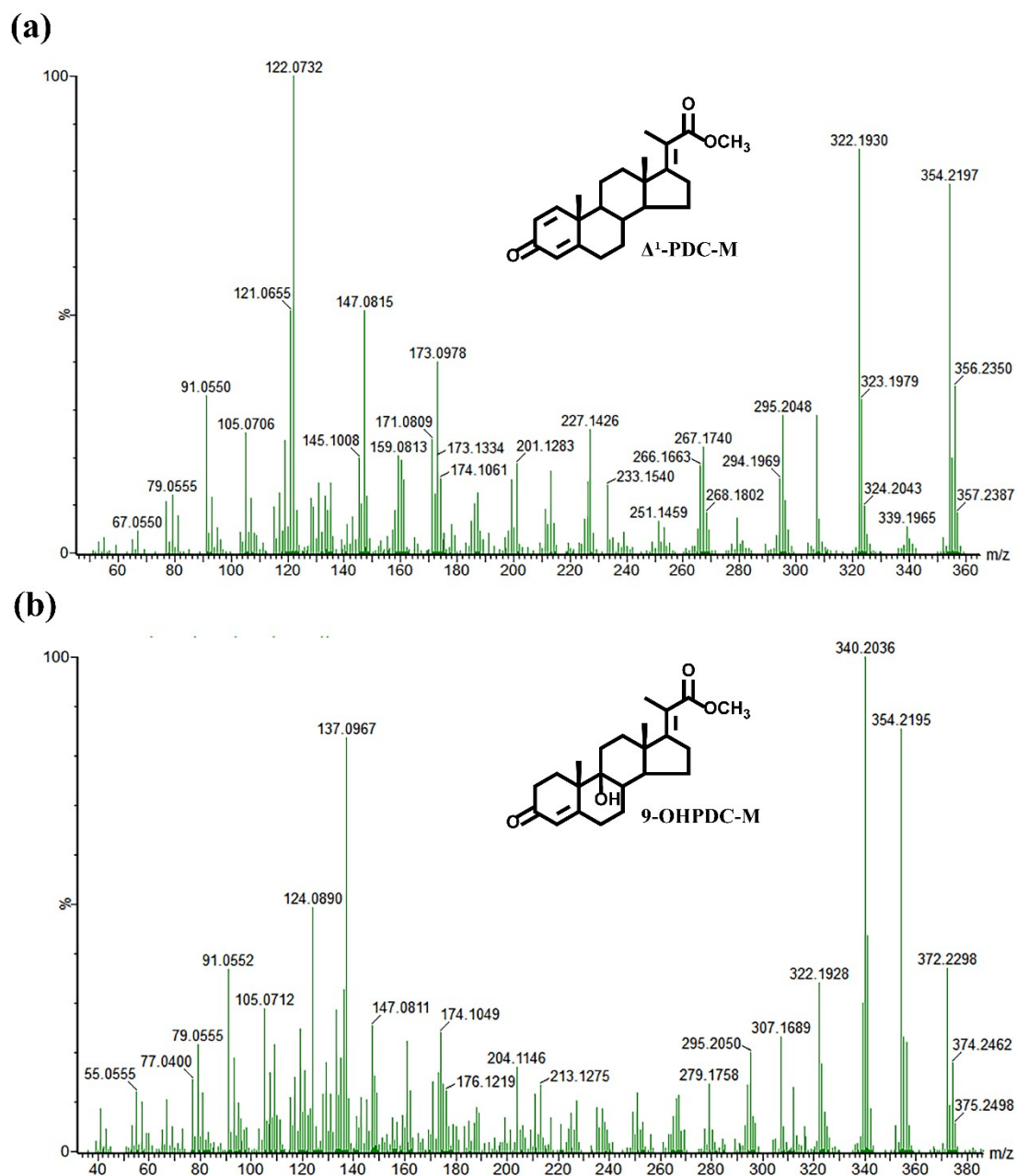
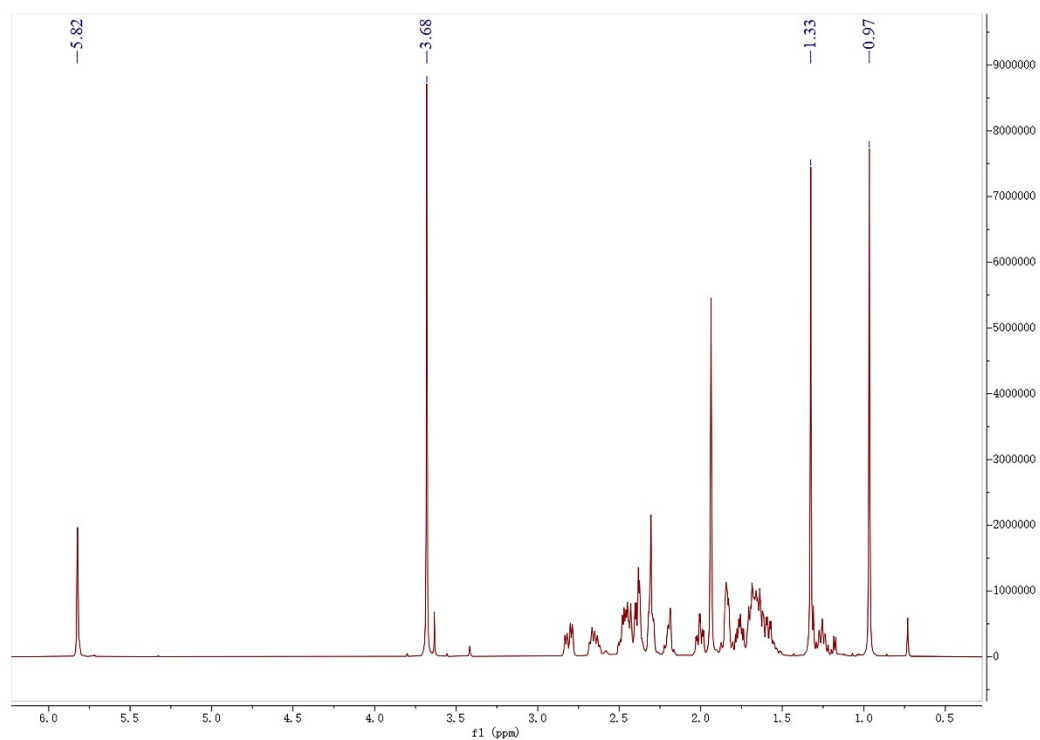


Fig. S1 The mass spectrometry data of Δ^1 -PDC-M and 9-OHPDC-M. (a) MS data of Δ^1 -PDC-M, EI: 354.2197[M]⁺; (b) MS data of 9-OHPDC-M, EI: 372.2298[M]⁺.

(a)



(b)

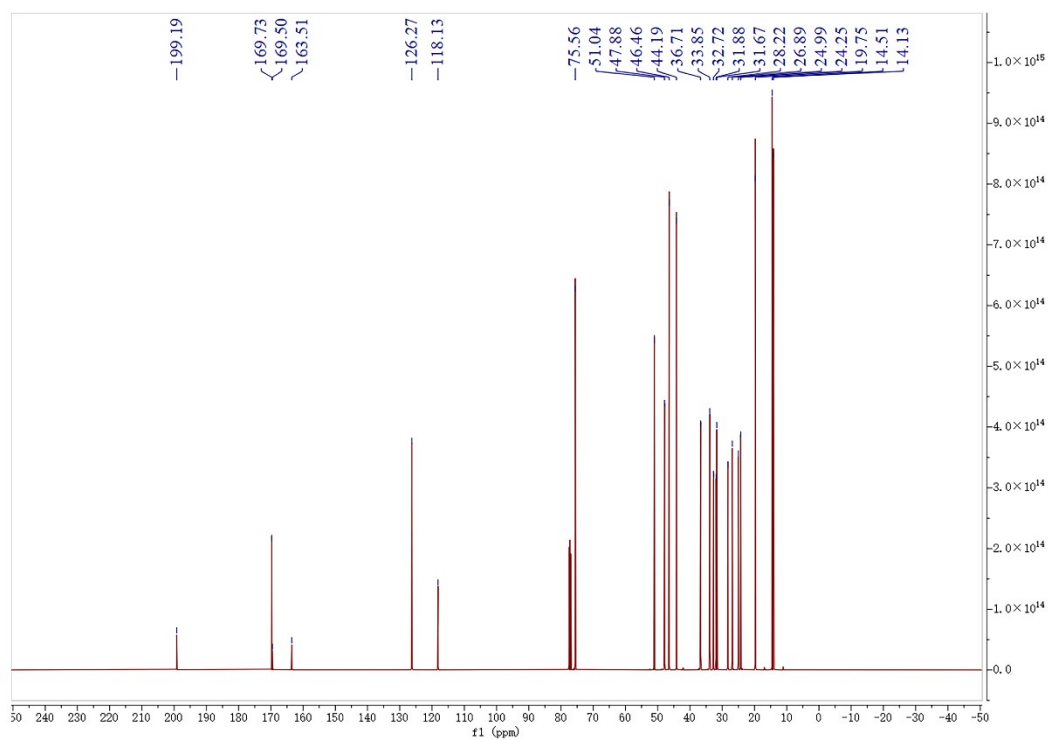


Fig. S2 NMR data of 9-OHPDC-M. (a) ^1H NMR of 9-OHPDC-M; (b) ^{13}C NMR of 9-OHPDC-M.

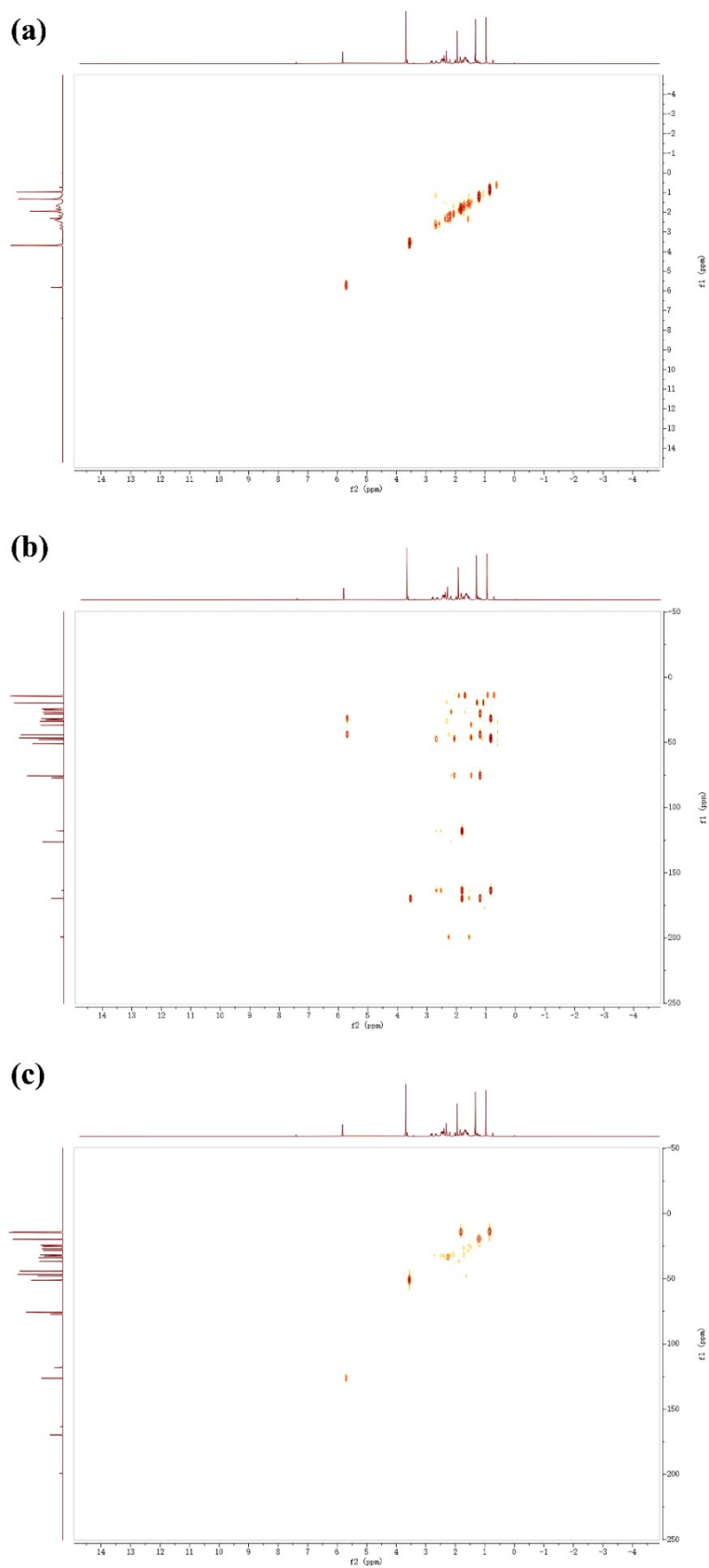


Fig. S3 2D NMR spectra data of 9-OHPDC-M. (a) COSY of 9-OHPDC-M; (b) HMBC of 9-OHPDC-M; (c) HMQC of 9-OHPDC-M.

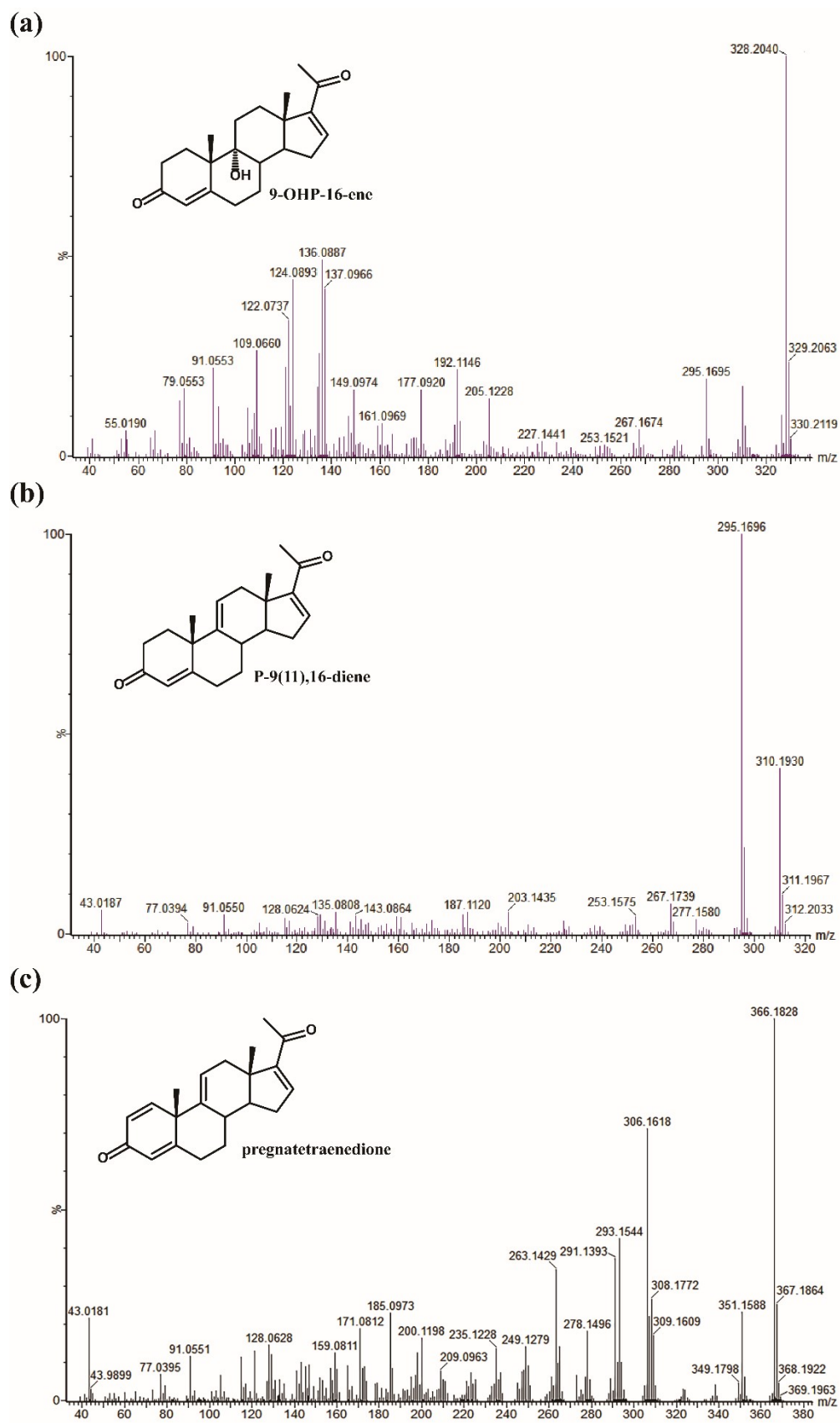
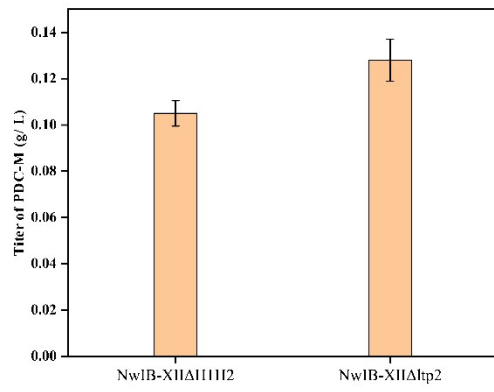


Fig. S4 Mass spectrometry data of 9-OHP-16-ene, P-9(11),16-diene and Pregnatetraenedione.

(a)



(b)

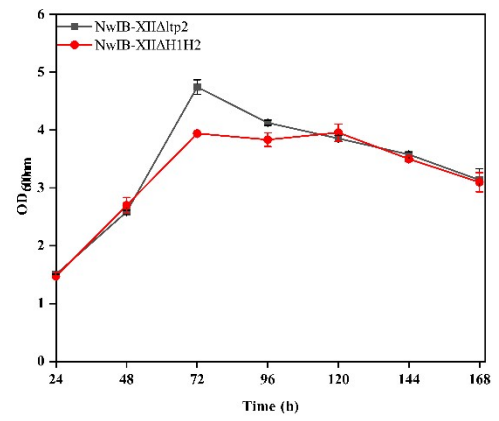


Fig. S5 Comparison of NwIB-XIIΔtp2 and NwIB-XIIΔH1-H2 in conversion of phytosterols in MYC/02.

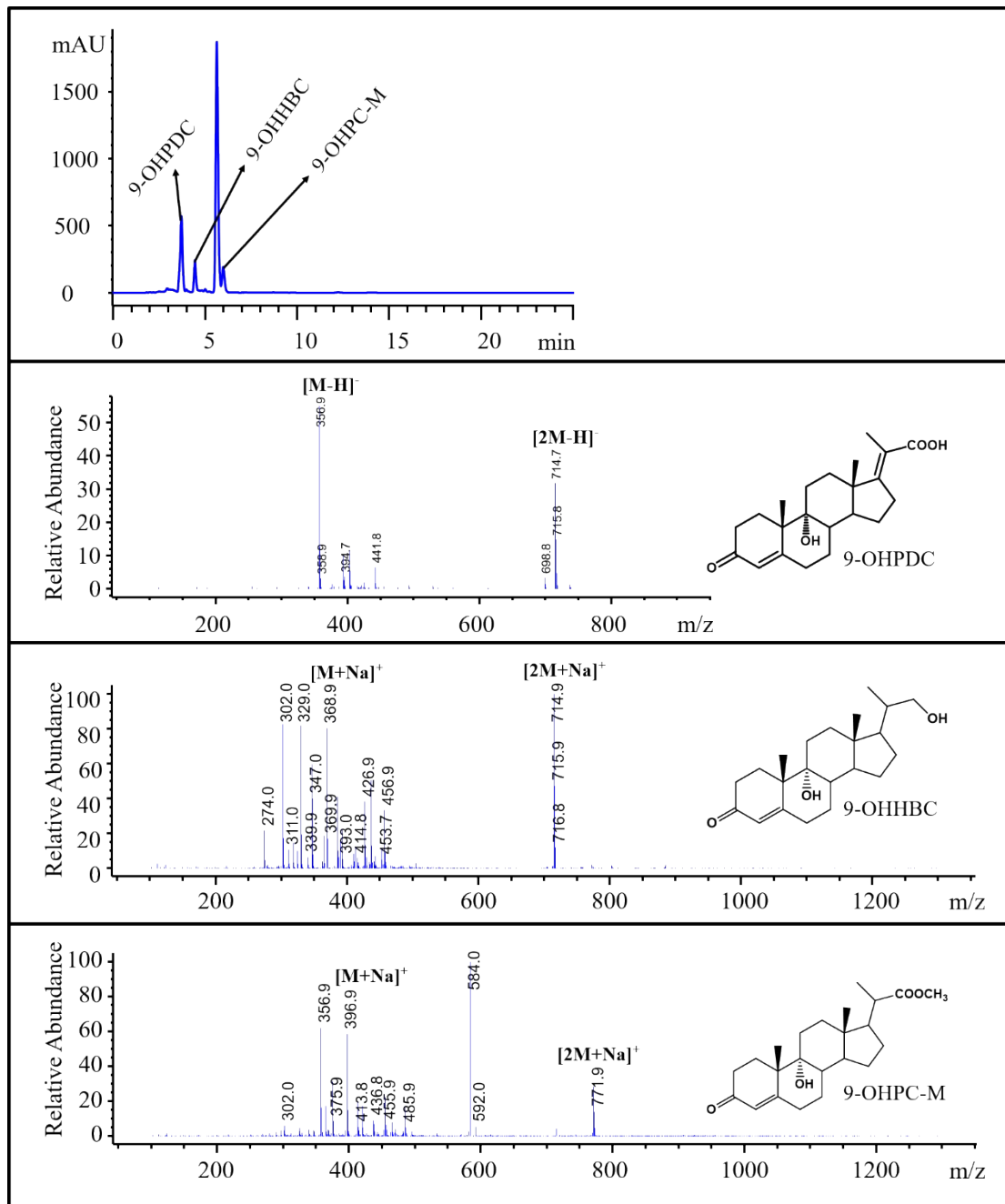


Fig. S6 LC-MS data of the minor metabolites resulted from NwIB- Δ ltp2 in the MYC/02 with phytosterols. The molecular weight of 9-OHPDC, 9-OHHBC and 9-OHPC-M was determined as m/z 357.9, m/z 345.9 and m/z 374.5, respectively. 9-OHPDC: 9 α -hydroxy-3-oxopregna-4,17(20)-diene-20-carboxylic acid; 9-OHHBC: 9,22-dihydroxy-23,24-bisnorchol-4-ene-3-one; 9-OHPC-M: 3-oxopregn-4-ene-20-carboxylic acid methyl ester.