Supplementary Materials for

Efficient Microbial Synthesis of Key Steroidal Intermediates from Bio-Renewable Phytosterols by Genetically Modified *Mycobacterium fortuitum* Strains

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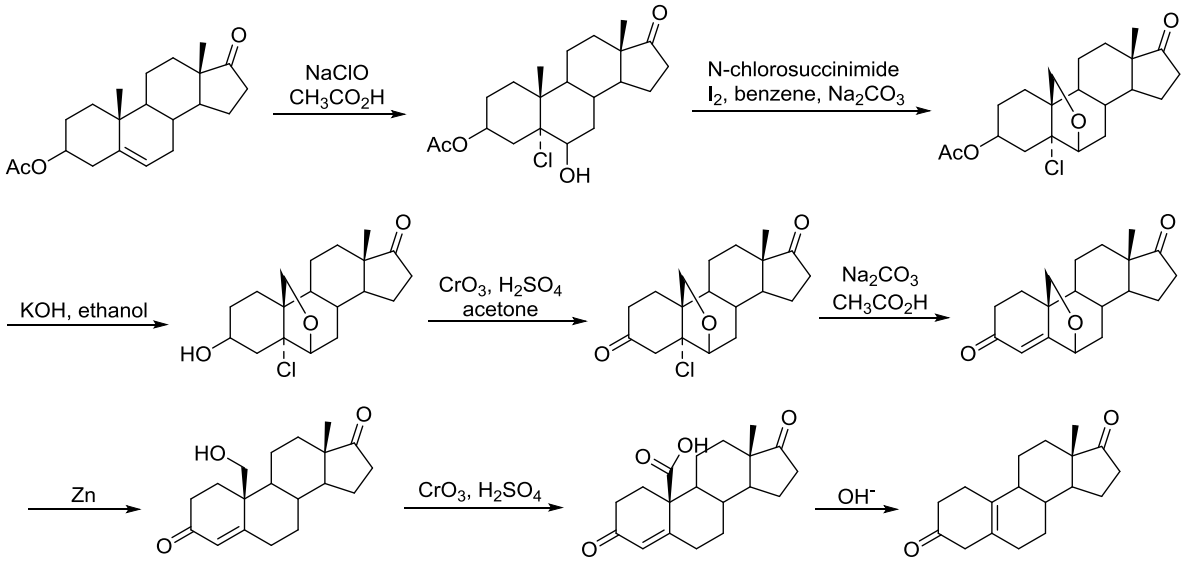
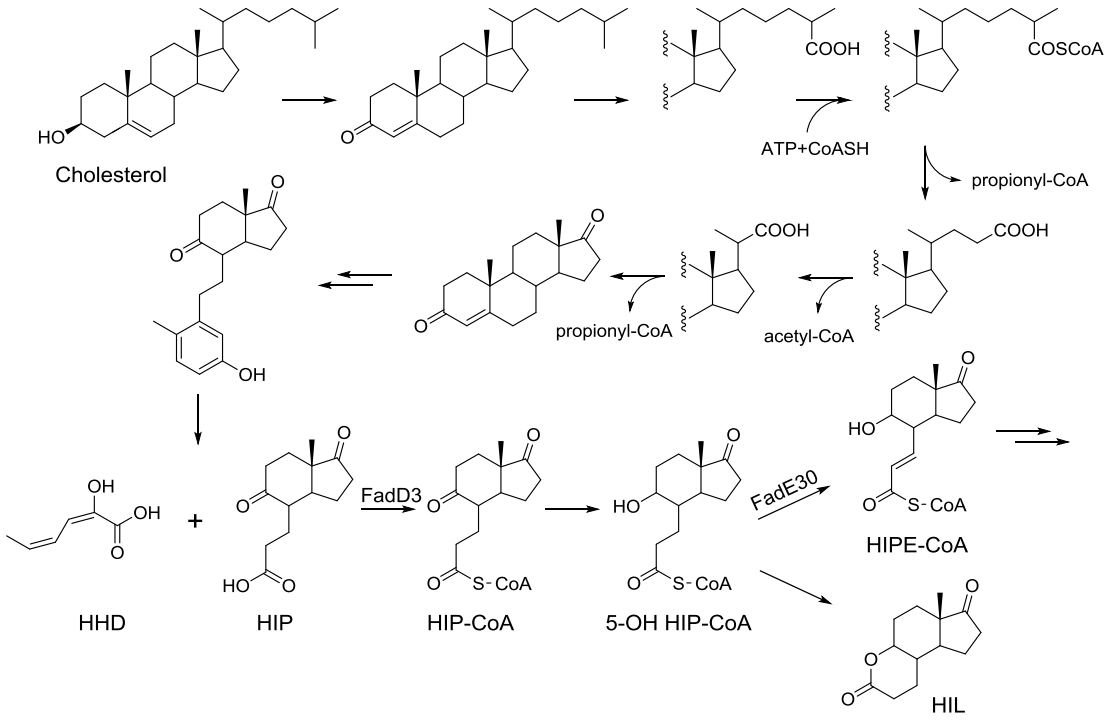


Scheme S1 The elimination of the C-19 methyl group by chemical synthesis.[1-3]



Scheme S2 Overview of the cholesterol degradation pathway.[4-7] The side chain and AB-ring of cholesterol are degraded to produce HIP and 2-hydroxyhexa-2,4-dienoic acid (HHD). The thioesterification of HIP, which is catalyzed by an acyl-CoA synthase FadD3, initiates the catabolism of CD-ring. FadE30, an acyl-CoA dehydrogenase, catalyzes the further degradation of metabolite 5-OH HIP-CoA.

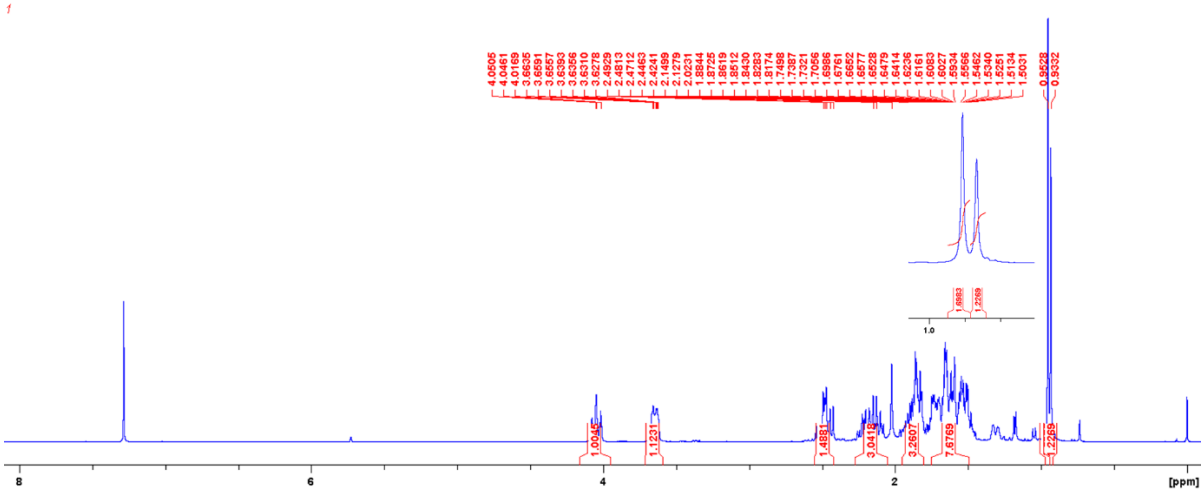


Fig. S1 ^{1}H NMR spectrum of HIK.

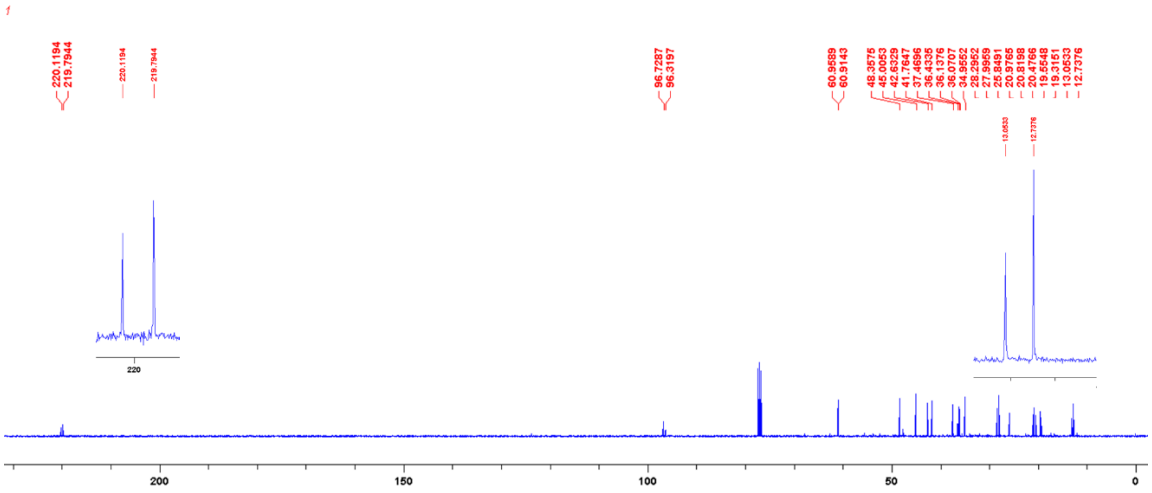


Fig. S2 ^{13}C NMR spectrum of HIK.

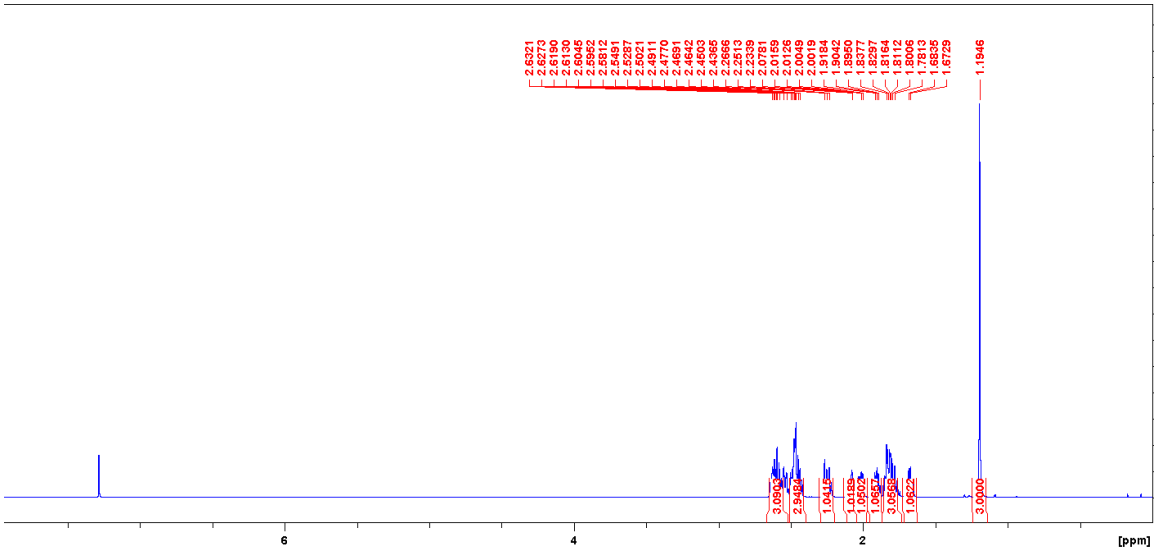


Fig. S3 ^{1}H NMR spectrum of HIP.

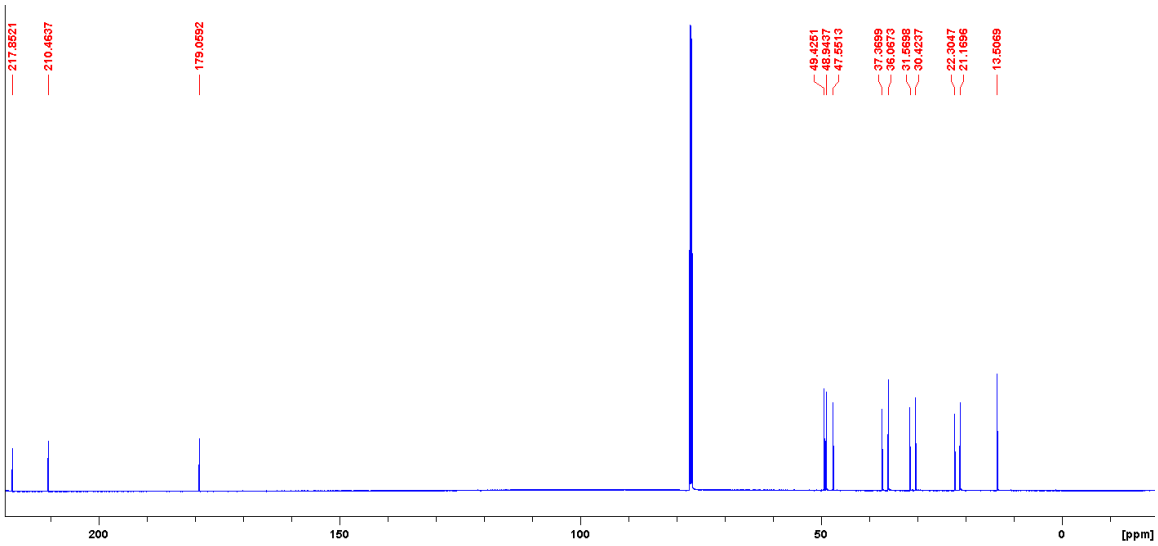


Fig. S4 ^{13}C NMR spectrum of HIP.

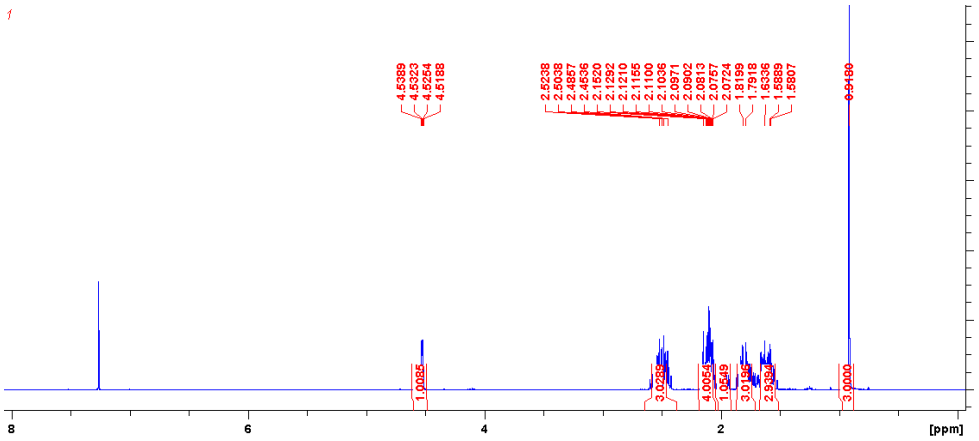


Fig. S5 ^{1}H NMR spectrum of HIL.

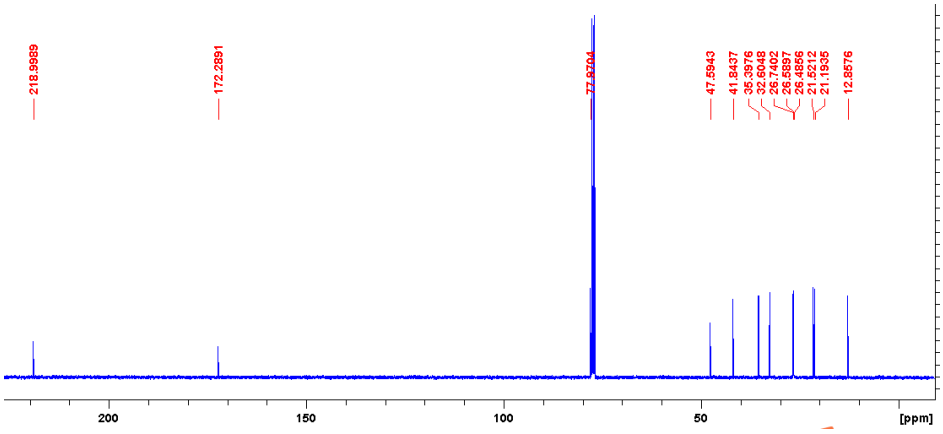


Fig. S6 ^{13}C NMR spectrum of HIL.

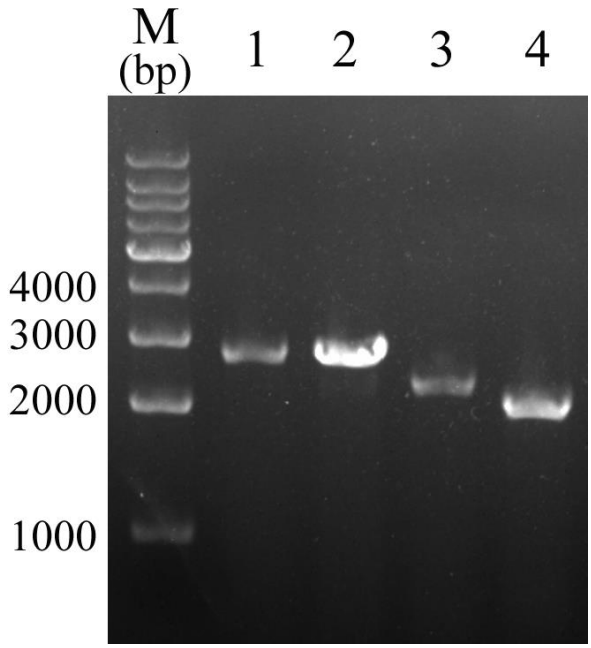


Fig. S7 Identification of *fadD3* and *fadE30* gene deleted mutants of ATCC 6841 by PCR. Lanes: (M) DNA markers; (1) The PCR products of *fadD3* using ATCC 6841 as the control; (2) The PCR products of *fadE30* using ATCC 6841 as the control; (3) The PCR products of *fadD3* with a shortened size (~ 2300 bp) represented a successful deletion; (4) The PCR products of *fadE30* with a shortened size (~ 1900 bp) represented a successful deletion.

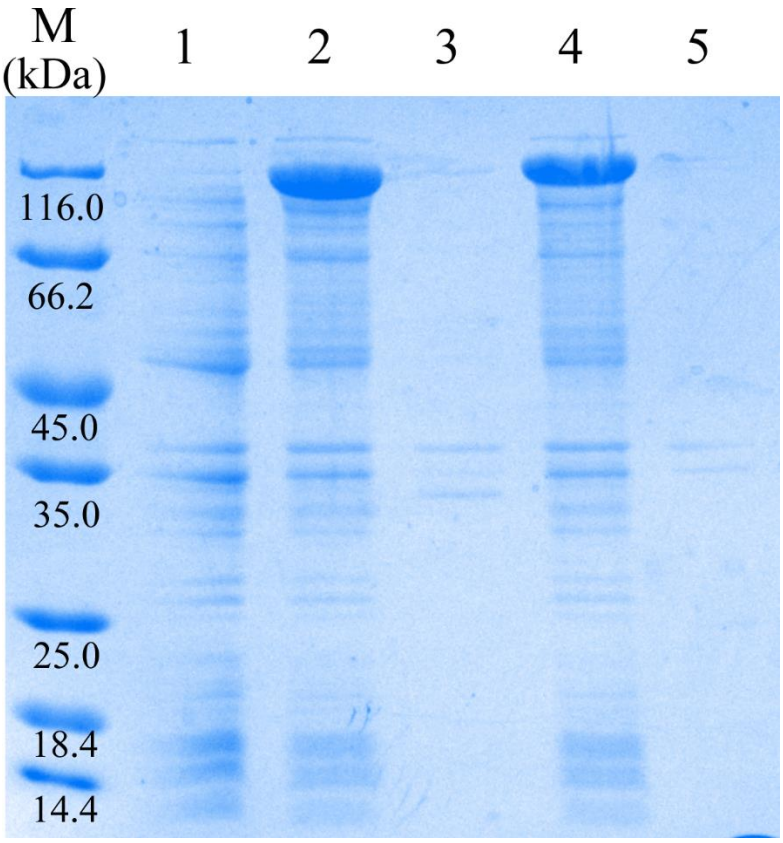


Fig. S8 SDS-PAGE analysis of heterologous expression of CAR1 and CAR2 from ATCC 6841 in *E.coli* BL21(DE3) host cells. Lanes: (1) total cell extracts of *E.coli* BL21(DE3) harboring blank pET28a(+) as the control; (2) supernatant fraction of $CAR1_{E.coli}$; (3) precipitant fraction of $CAR1_{E.coli}$; (4) supernatant fraction of $CAR2_{E.coli}$; (5) precipitant fraction of $CAR2_{E.coli}$; (M) protein markers.

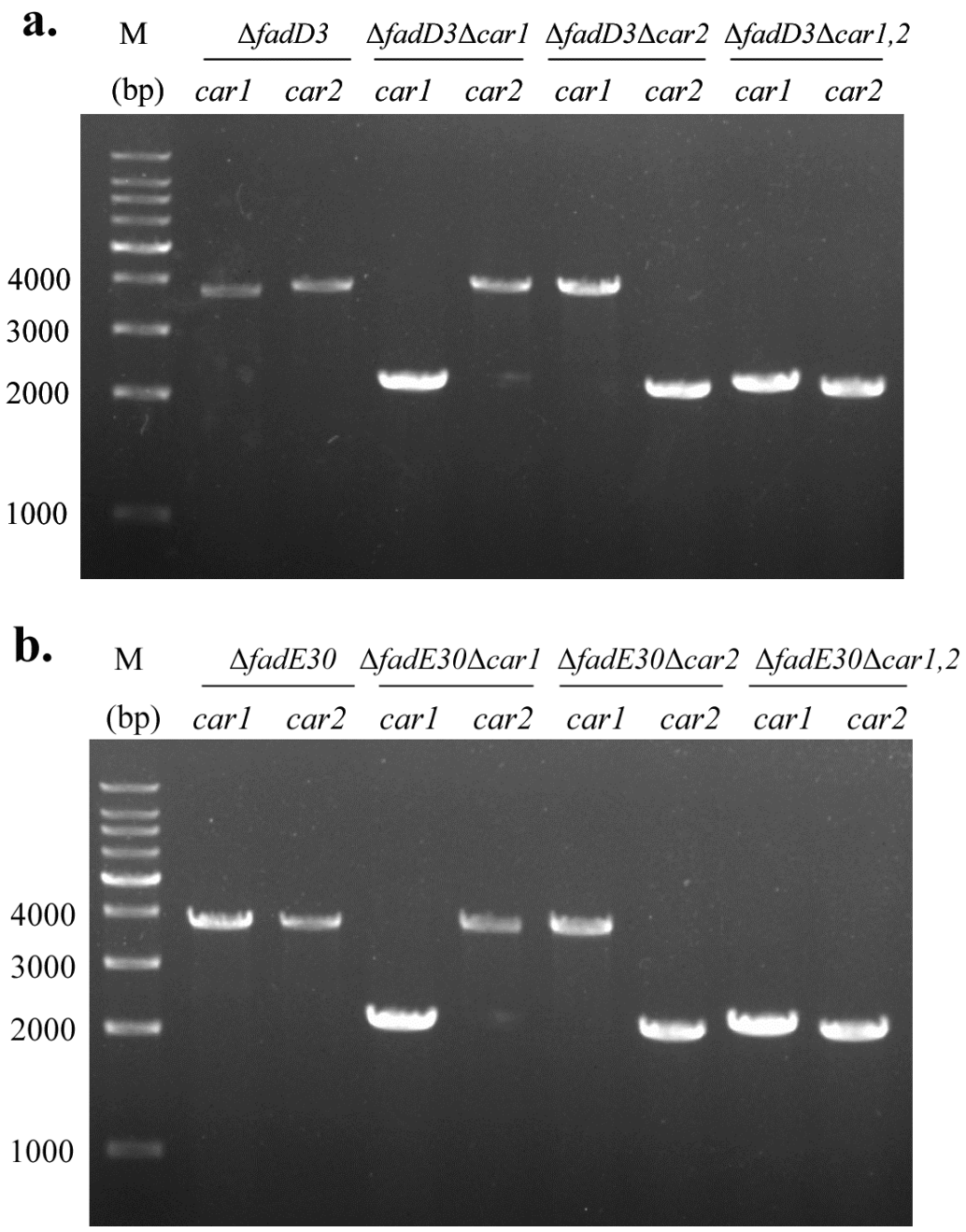


Fig. S9 Identification of *car1* and *car2* gene deleted strains by PCR. The PCR products of *car1* and *car2* with a shortened size (2100bp and 2000 bp) represented a successful deletion. (a) Identification of *car1* and *car2* deleted strains with Δ*fadD3* background. (b) Identification of *car1* and *car2* deleted strains with Δ*fadE30* background.

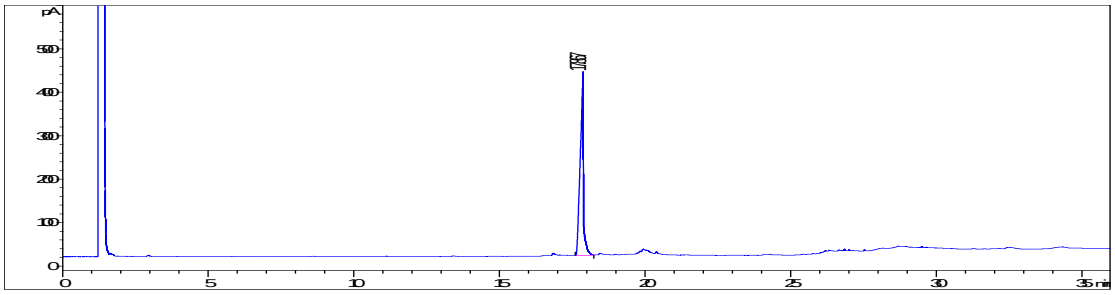


Fig. S10 The biotransformation of phytosterols by strain Δ*fadD3*Δ*car1,2*. The product HIP (17.9 min) was detected from the extract of fermentation supernatant by GC.

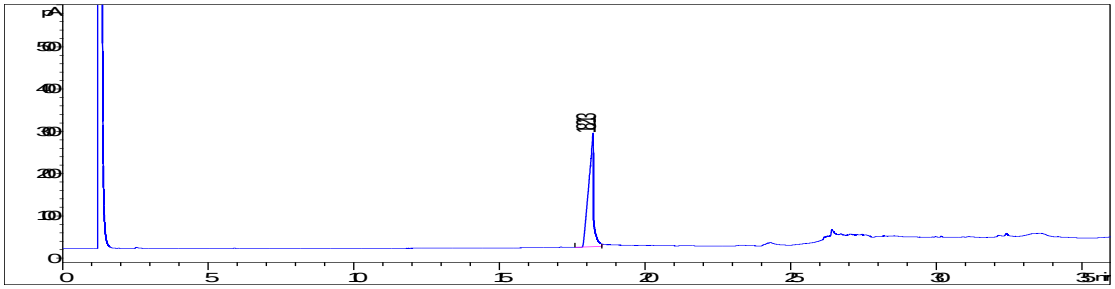


Fig. S11 The biotransformation of phytosterols by strain Δ*fadE30*Δ*car1,2*. The product HIL (18.2 min) was detected from the extract of fermentation supernatant by GC.

Table S1 Strains used in this study.

Name	Description	Source
ATCC 6841	Wild type strain	ATCC
Δ*fadD3*	*fadD3*-deleted strain of 6841	This study
Δ*fadD3*Δ*car1*	*car1*-deleted strain with Δ*fadD3* background	This study
Δ*fadD3*Δ*car2*	*car2*-deleted strain with Δ*fadD3* background	This study
Δ*fadD3*Δ*car1,2*	*car1*and *car2*-deleted strain with Δ*fadD3* background	This study
Δ*fadE30*	*fadE30*-deleted strain of 6841	This study
Δ*fadE30*Δ*car1*	*car1*-deleted strain with Δ*fadE30* background	This study
Δ*fadE30*Δ*car2*	*car2*-deleted strain with Δ*fadE30* background	This study
Δ*fadE30*Δ*car1,2*	*car1* and *car2*-deleted strain with Δ*fadE30* background	This study
pET28a$_{E.coli}$	*E.coli* BL21(DE3) harboring pET28a(+)	Novagen
CAR1$_{E.coli}$	*E.coli* BL21(DE3) harboring pET28a(+)-*car1*	This study
CAR2$_{E.coli}$	*E.coli* BL21(DE3) harboring pET28a(+)-*car2*	This study

Table S2 Primers and plasmids used in this study.

Name	Description	Source/reference
Primers		
fadE30$_{del}$-U-F	TGTTGCCATTG**CTGCAG**GCACCGGGTACAGAGTCG	This study
fadE30$_{del}$-U-R	TGATTCCACGCCCGACGCTCTTC	This study
fadE30$_{del}$-D-F	CGTCGGGCGTGGAATCACCGCTGATCCGCCAAAAG	This study
fadE30$_{del}$-D-R	GTACCGCGGCCGC**TTAATTAA**GCCGAACGCCTCGTCTGA	This study
fadD3$_{del}$-U-F	ACGTTGTTGCCATTG**CTGCAG**CGGTGACGTCGAGGATCTTG	This study
fadD3$_{del}$-U-R	GGTAGCCGAGCATGACATTGGGTTGATGCACAGATAGCGGTCG	This study
fadD3$_{del}$-D-F	CGACCGCTATCTGTGCATCAACCCAATGTCATGCTCGGCTACC	This study
fadD3$_{del}$-D-R	GTACCGCGGCCGC**TTAATTAA**CTCGTTGCTCGACGTGCTCATG	This study
car1$_{del}$-U-F	TGTTGCCATTG**CTGCAG**GCATCTCGCACCATCAGC	This study

Name	Description	Source/reference
car1$_{del}$-U-R	AGGTCACTTGGTCGAGCCAGCGCCGCCTGATTCTC	This study
car1$_{del}$-D-F	CAGCGCCGCCTGATTCTCGCTCGACCAAGTGACCTG	This study
car1$_{del}$-D-R	CGCGGCCGC**TTAATTAA**CGATCGGCTTGCTCTAGG	This study
car2$_{del}$-U-F	TGTTGCCATTG**CTGCAG**GATCAGACTCACAGCACATTG	This study
car2$_{del}$-U-R	GATCTTCGTGGTGAGCGCGGCGAGCGAGGCATACAG	This study
car2$_{del}$-D-F	CTGTATGCCTCGCTCGCCGCGCTCACCACGAAGATC	This study
car2$_{del}$-D-R	CGCGGCCGC**TTAATTAA**GGTTCCCCTGAGCAAATC	This study
car1-F	CGCGGCAGC**CATATG**ACCACCGAAACGC	This study
car1-R	GAGCTCGAATTC**GGATCC**TTACAGCAATCCGAGCAG	This study
car2-F	CGCGGCAGC**CATATG**TCGTTTGATACTCGC	This study
car2-R	GAGCTCGAATTC**GGATCC**CTAGAGCAGGCCGAGCTG	This study

Name	Description	Source/reference
Plasmids		
p2NIL	Gene manipulation vector, Kan^R	Parish and Stoker [8]
pGOAL19	*Hyg* Pag_{85}-*lacZ* P_{hsp60}-*sac*B, *Pac*I cassette vector, Amp^R	Parish and Stoker [8]
pET28a(+)	E. *coli* expression vector, Kan^R	Novagen
pKH_{del}-*fadE30*	p2NIL carrying the alleles of *fadE30* and the selection cassette from pGOAL19, Kan^R, Hyg^R	This study
pKH_{del}- *fadD3*	p2NIL carrying the alleles of *fadD3* and the selection cassette from pGOAL19, Kan^R, Hyg^R	This study
pKH_{del}- *car1*	p2NIL carrying the alleles of *car1* and the selection cassette from pGOAL19, Kan^R, Hyg^R	This study
pKH_{del}- *car2*	p2NIL carrying the alleles of *car2* and the selection cassette from pGOAL19, Kan^R, Hyg^R	This study
pET28a(+)- *car1*	pET-28a(+) harboring the *car1* gene, Kan^R	This study
pET28a(+)- *car2*	pET-28a(+) harboring the *car2* gene, Kan^R	This study

Notes: Kan^R kanamycin-resistant, Amp^R ampicillin-resistant, Hyg^R hygromycin-resistant, the restriction enzyme cutting sites were in bold, and the homologous sequence were underlined.

References

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