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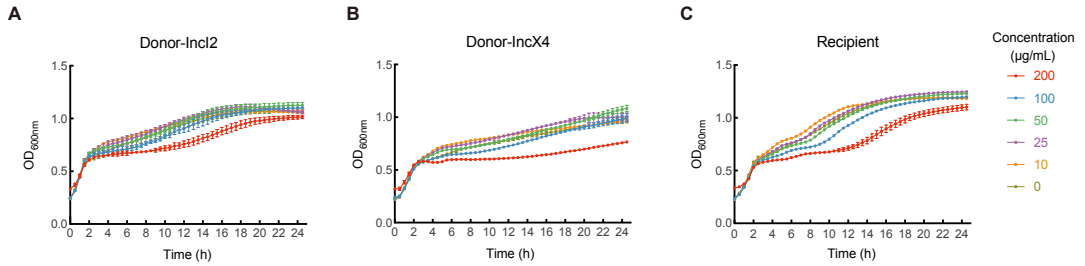
Supplementary Materials

Dihydroartemisinin inhibits plasmid transfer in drug-resistant *Escherichia coli* via limiting energy supply

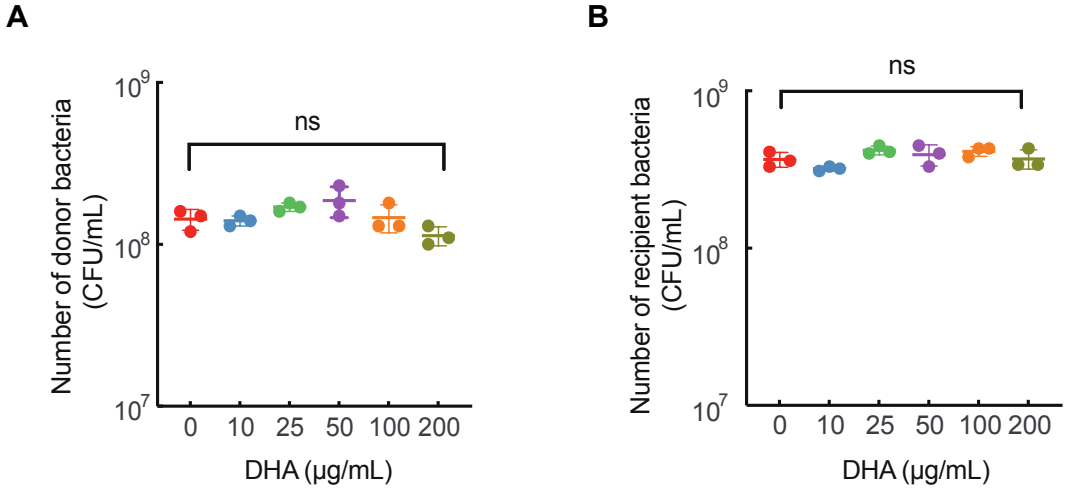
Xue-Yang Wang[1,2], Huang-Wei Song[1,2], Tian Yi[1,2], Ying-Bo Shen[1,2], Chong-Shan Dai[1,2], Cheng-Tao Sun[1,2], De-Jun Liu[1,2], Jian-Zhong Shen[1,2], Cong-Ming Wu[1,2], Yang Wang[1,2,*]

[1] *National Key Laboratory of Veterinary Public Health Security, College of Veterinary Medicine, China Agricultural University, Beijing* 100193*, China*
[2] *Guangdong Laboratory for Lingnan Modern Agriculture, Guangzhou, Guangdong* 510642*, China*

[*]Corresponding author, E-mail: wangyang@cau.edu.cn

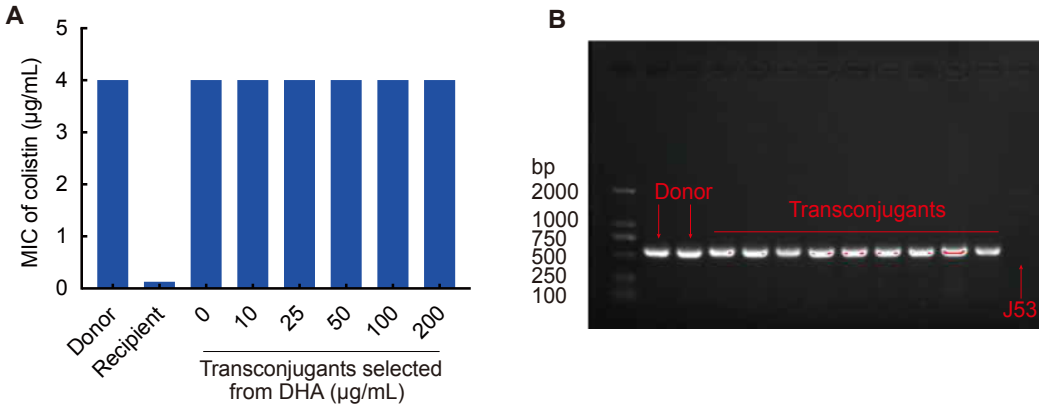


Supplementary Figure S1 Growth curve of donor (A and B) and recipient (C) bacteria under DHA exposure at different concentrations



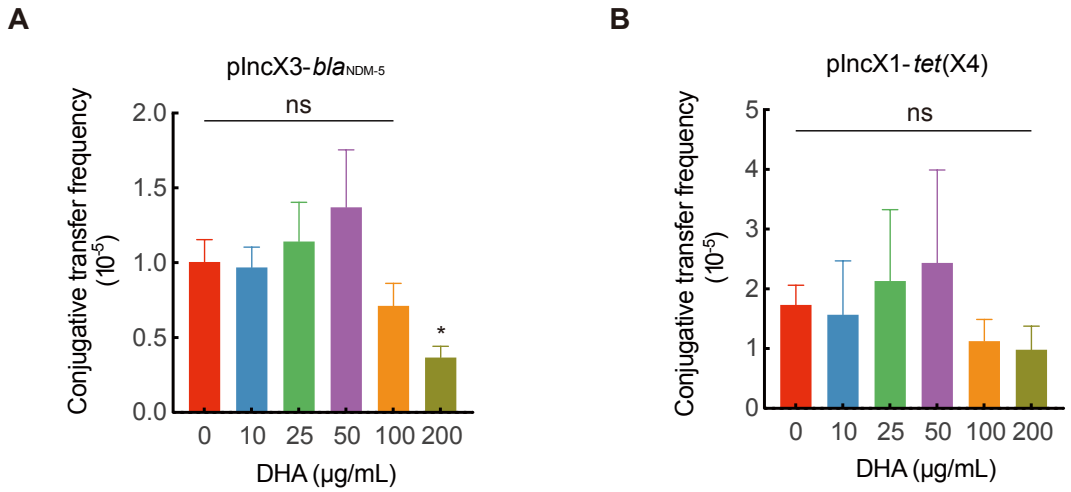
Supplementary Figure S2 Number of donor (A) and recipient bacteria (B) in response to DHA exposure at different concentrations

Error bars represent standard deviations of triplicate tests. ns: no significance.



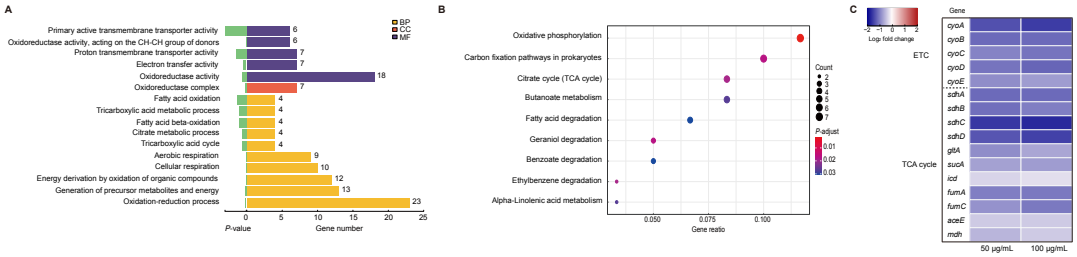
Supplementary Figure S3 MIC determination and PCR verification of donor (*E. coli* ZJ807 and ZJ28), recipient (*E. coli* J53), and transconjugant bacteria under different DHA concentrations

A: MIC was determined according to EUCAST clinical breakpoint standards. All MIC experiments were conducted with biological triplicates. B: PCR analysis of plasmid-carrying *mcr-1*, followed by agarose gel electrophoresis verification.



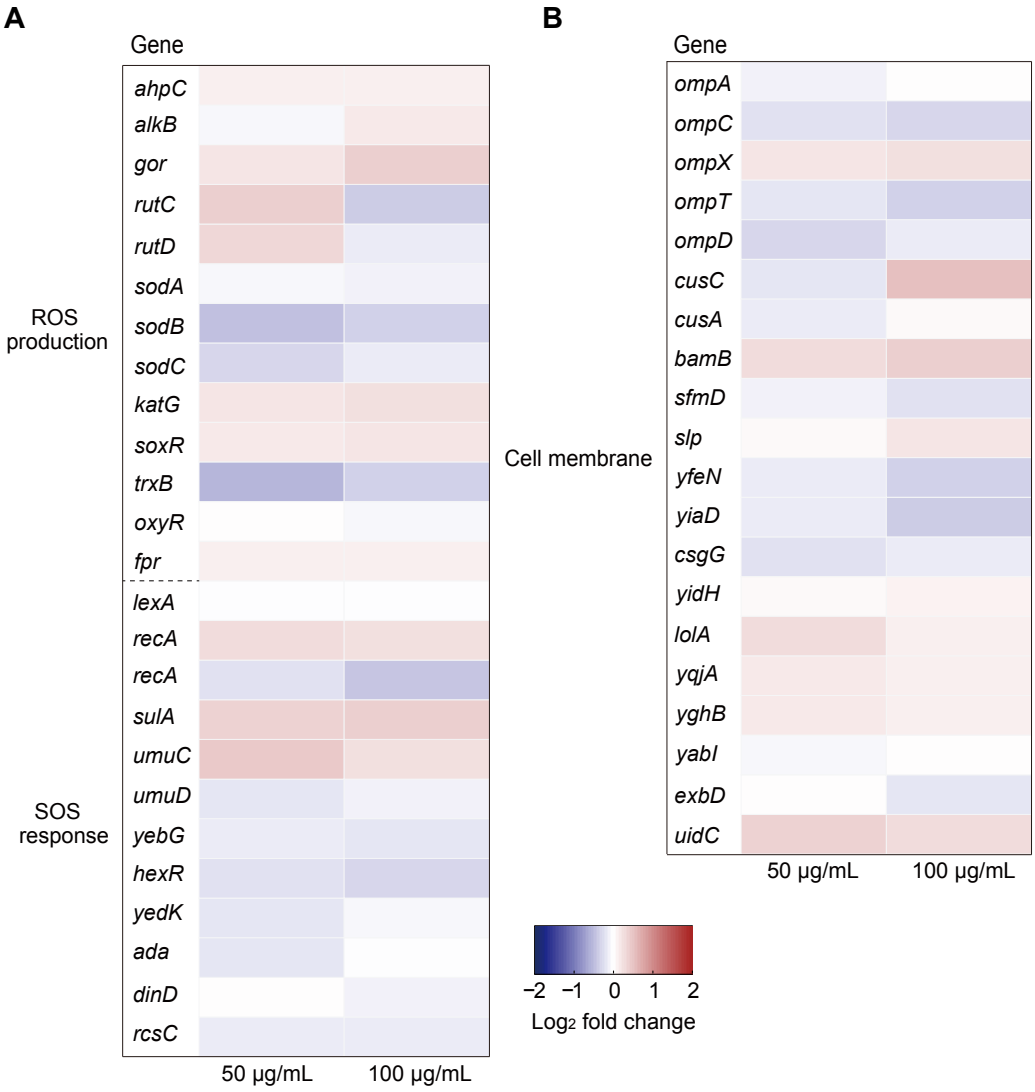
Supplementary Figure S4 Effects of DHA on conjugative transfer of pIncX3-*bla*$_{NDM-5}$ and pIncX1-*tet*(X4)

A: Fold-change in conjugative transfer frequency of pIncX3-*bla*$_{NDM-5}$. B: Fold-change in conjugative transfer frequency of pIncX1-*tet*(X4). Error bars represent standard deviations of triplicate tests. *P*-value was detected using one-way ANOVA and corrected using Dunnett's method. ns: No significance; *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$;****: $P<0.0001$.



Supplementary Figure S5 Transcriptomic analysis of DHA in recipient bacteria

A: GO enrichment analyses of down-regulated genes in recipient bacteria treated with 50 μg/mL DHA. B: KEGG pathway enrichment analyses of down-regulated genes in recipient bacteria treated with 50 μg/mL DHA. C: Fold-change in expression of core cellular respiratory-related and TCA cycle-related genes in recipient bacteria.



Supplementary Figure S6 Effects of DHA on ROS production, SOS response, and cell membrane permeability

A: Expression levels of core genes related to ROS production and SOS response in recipient bacteria. B: Expression levels of core genes related to inner and outer cell membranes in recipient bacteria.

Supplementary Table S1 Genes relevant to energy metabolism in donor bacteria after exposure of DHA

Gene	Log_2 (fold change of FPKM)	
	50 μg/mL	100 μg/mL
cyoA	-1.3214769	-1.482544
cyoB	-1.1350783	-1.1212972
cyoC	-0.9131818	-1.0366636
cyoD	-1.0343372	-1.1576017
cyoE	-0.8766587	-0.7226277
sdhA	-1.1161921	-1.10652
sdhB	-1.083699	-0.9505919
sdhC	-1.5112313	-1.6349842
sdhD	-1.2860142	-1.4286996
gltA	-0.8245646	-0.5977435
sucA	-0.6822118	-0.7241386
icd	-0.2784845	-0.1966236
fumA	-0.9424553	-1.0053067
fumC	-0.7827804	-0.9862871
aceE	-0.3450416	-0.3693557
mdh	-0.5282449	-0.3448885

Supplementary Table S2 Genes relevant to energy metabolism in recipient bacteria after exposure of DHA

Gene	Log_2 (fold change of FPKM)	
	50 μg/mL	100 μg/mL
cyoA	-1.3411675	-1.5031993
cyoB	-1.154324	-1.1420094
cyoC	-0.9331464	-1.0572875
cyoD	-1.0538136	-1.1775065
cyoE	-0.8744058	-0.7295005
sdhA	-1.1361748	-1.1275417
sdhB	-1.0955306	-0.9650844
sdhC	-1.5294695	-1.6555857
sdhD	-1.3063952	-1.4517718
gltA	-0.8609289	-0.6442822
sucA	-0.7006729	-0.7444457
icd	-0.3004027	-0.220026
fumA	-0.9624256	-1.0271693
fumC	-0.800729	-1.0051329
aceE	-0.3656067	-0.3909831
mdh	-0.5485306	-0.3667156

Supplementary Table S3 Genes relevant to conjugation in donor bacteria after exposure of DHA

Gene	Log_2 (fold change of FPKM)	
	50 μg/mL	100 μg/mL
virB1	-0.5499623	-0.4664265
virB2	-0.5499623	-0.4664265
virB3	-0.5869453	-0.2921883
virB4	-0.3106221	-0.1062003
virB5	-1.0193773	-0.790576
virB6	-1.0717194	-0.8105867
virB8	-1.1773491	-1.2639981
virB9	0.02326698	-0.574893
virB10	-0.4033263	-0.0507795
virB11	-0.4192312	0.01658776
virD4	-0.1604902	-0.1855251
nikB	-0.4092347	-0.337808
traC	-0.6801713	-0.6706121
pilV	-0.3743468	-0.2329932
pilU	-0.7038774	-0.7385857
pilT	-0.1608604	-0.2119744
pilS	-0.4933056	-0.5731384
pilR	-0.5658594	-0.5503596
pilM	-0.7404352	-0.6124419
pilL	-0.5886762	-0.4961183

Supplementary Table S4 Genes relevant to adhesive-pilus generation in donor bacteria after exposure of DHA

Gene	Log_2 (fold change of FPKM)	
	50 μg/mL	100 μg/mL
fimB	-0.5672759	-0.5605237
fimZ	-2.2217894	-1.9638449
ybgD	-1.1805742	-1.2718673
glgS	-1.3134639	-1.2579198
yehB	-1.5512041	-2.3970815
yehD	-1.1496575	-1.5088246
yehC	-1.2897711	-1.8317782
yefM	-1.307206	-1.1831413
ychH	-1.1245639	-1.1418967
pgaD	-1.2006745	-0.8877224
bssR	-1.6414332	-1.4252362

Supplementary Table S5 Genes relevant to ROS and SOS in donor bacteria after exposure of DHA

Gene	Log_2 (fold change of FPKM)	
	50 μg/mL	100 μg/mL
ahpC	0.17833269	0.18049857
alkB	-0.0531156	0.20318432
gor	0.24941857	0.42536859
rutC	0.45696322	-0.3397857
rutD	0.36931735	-0.1184388
sodA	-0.0510928	-0.0798461
sodB	-0.4503983	-0.3202134
sodC	-0.2848488	-0.1112166
katG	0.25531206	0.29907486
soxR	0.18143659	0.2512086
trxB	0.02053468	-0.0326841
oxyR	0.17872276	0.14753655
fpr	-0.0661386	0.06410546
lexA	0.00456751	0.00039398
recA	0.3131698	0.28945752
recX	-0.1510492	-0.3475076
sulA	0.38348894	0.42423087
umuC	0.46717361	0.29204285
umuD	-0.1416002	-0.0873607
yebG	-0.1285369	-0.1480257
hexR	-0.1886593	-0.2977824
yedK	-0.1566261	-0.0436815
ada	-0.1767421	0.00739258
dinD	0.00287048	-0.118018
rcsC	-0.1196769	-0.1052797

Supplementary Table S6 Genes relevant to ROS and SOS in recipient bacteria after exposure of DHA

Gene	Log_2 (fold change of FPKM)	
	50 μg/mL	100 μg/mL
ahpC	0.15581819	0.15513168
alkB	-0.0694274	0.18012844
gor	0.23011298	0.4041422
rutC	0.43599454	-0.363275
rutD	0.3477025	-0.1414605
sodA	-0.0701035	-0.1018817
sodB	-0.4708764	-0.3414671
sodC	-0.3036039	-0.133569
katG	0.23514938	0.27746657
soxR	0.16078656	0.22069757

trxB	-0.5408121	-0.3362043
oxyR	0.00102534	-0.0542221
fpr	0.15732283	0.12489162
lexA	-0.0157753	-0.0208175
recA	0.29264241	0.26330946
recX	-0.2203318	-0.4243544
sulA	0.36684122	0.40231557
umuC	0.44654593	0.26005871
umuD	-0.1778917	-0.1093361
yebG	-0.1486888	-0.1706538
hexR	-0.2064729	-0.3160918
yedK	-0.1847237	-0.0739366
ada	-0.1966268	-0.0136527
dinD	0.00179372	-0.0921959
rcsC	-0.134468	-0.1205069

Supplementary Table S7 Genes relevant to cell membrane in donor bacteria after exposure of DHA

Gene	Log_2 (fold change of FPKM)	
	50 μg/mL	100 μg/mL
ompA	-0.0942885	0.03489998
ompC	-0.2245623	-0.2669793
ompX	0.22633431	0.27639849
ompT	-0.0117972	-0.1309031
ompD	-0.338345	-0.1541804
cusC	-0.157498	0.48795729
cusA	-0.1249563	0.07923411
bamB	0.29981091	0.42249118
sfmD	-0.1107216	-0.1790589
slp	0.07094354	0.22287414
yfeN	-0.1374295	-0.301433
yiaD	-0.1105066	-0.3607525
csgG	-0.2083577	-0.0966964
yidH	0.10047215	0.11238365
lolA	0.30441922	0.16783759
yqjA	0.17954129	0.17931106
yghB	0.18558639	0.18029139
yabI	-0.0295269	0.04392754
exbD	0.04738678	-0.1489572
uidC	0.17663569	0.20398896

Supplementary Table S8 Genes relevant to cell membrane in recipient bacteria after exposure of DHA

Gene	Log_2 (fold change of FPKM)	
	50 μg/mL	100 μg/mL
ompA	-0.1146515	0.01295625
ompC	-0.234189	-0.2801182
ompX	0.2056149	0.25346471
ompT	-0.1923355	-0.3446067
ompD	-0.2819706	-0.1347471
cusC	-0.1712964	0.54376219
cusA	-0.1374787	0.05669071
bamB	0.28113724	0.40025375
sfmD	-0.1127266	-0.2023649
slp	0.05053065	0.20020218
yfeN	-0.1590753	-0.3252483
yiaD	-0.1298713	-0.3816429
csgG	-0.2208468	-0.1204668
yidH	0.07989575	0.08536977
lolA	0.28256586	0.1472096
yqjA	0.16172989	0.15801935
yghB	0.16444497	0.15669806
yabI	-0.074396	0.03227627
exbD	0.02869241	-0.1702976
uidC	0.39309796	0.3183796