Metabolic Engineering of *Escherichia coli* for High-Titer De Novo Biosynthesis of Mandelic Acid

Supplementary materials

Supplementary figures and tables

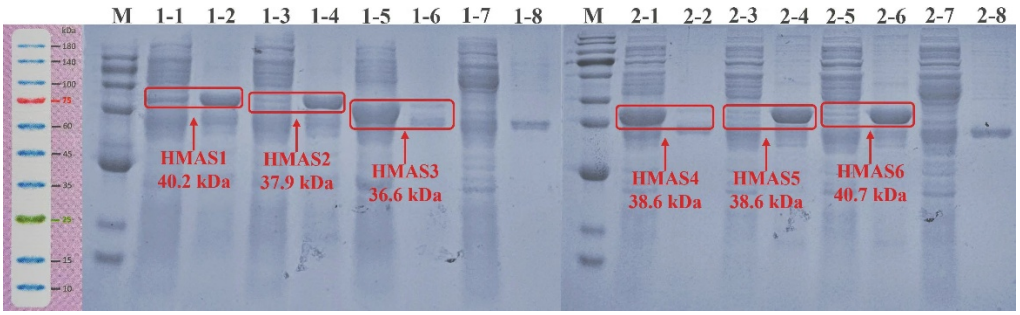


Figure S1 Protein expression analysis of HMAS1–HMAS6. Lanes 1-1 to 1-6 correspond to HMAS1–HMAS3, while lanes 1-7 and 1-8 serve as negative controls. Lanes 2-1 to 2-6 correspond to HMAS4–HMAS6, with lanes 2-7 and 2-8 as negative controls.

Table S1. Amino acid sequences of genes used in this study.

Gene Name	Amino Acid Sequence (N→C)
HMAS1	MLPPFPFLHWRAAMPPSDIAYAELYVADDREASGFLVDSLGF VPLAVAGPATGTHDRRSTVLRSGEVTLVVTQALAPDTPVARY VERHGDSIADLAFGCDDVRSCFDRAVLAGAEALQAPTPSHRA GQDAWFATVSGFGDIRHTLVPAADGDGAGLLPPDRDWALLPA ATGRTGPRPLLDHVAVCLESGTLRSTAEFYEAAFDMPYYSSEY IEVGEQAMDSIVVRNAGGGITFTLIEPDDTRVPGQIDQFLSAH DGPGVQHLAFLVDDIVGSVRSLGDRGVAFLRTPGAYYDLLTE RVGAMADAIEDLRETNVLADRDEWGYLLQIFTRSPYPRGTLF YEYIQRNGARGFGSSNIKALYEAVEREREVAGR*
HMAS2	MSAQESTQNFEIDYVEMYVANLEAAASGWMDKYGFSVTATD RSADHRSVTLRHSAIALVLTEPLSDRHPGATYLQTHGDGVADI ALRTPDVAAAFEAAVKAGAEPVREPEKRADSVVTATVRGFGD VVHTLIQSDAAEETPRGKGGVELGVIDHFAVCLNAGDLGPTV AFYERALGFKQIFEEHIVVGAQAMNSTVVQSTSGAVTLTLIEP DKTADPGQIDDFIKEHHGSGVQHIAFTSPDAVRAVKELSARGV EFLKTPDAYYDLLGERIELETHSLDDLRETKLLADEDHGGQL FQIFTASTHPRKTIFFEIIERQGAGTFGSSNIKALYEAVELERTG QSKLGPVRR*
HMAS3	MAAQAGSVFDGMTLDHTVFYVGDADRAAGELTDKYGLVVL GTSETSSVRSVAVGGGSIRLVFSQAIADDTPAAAYVKVHGDGV ADLALGVADARAAFAEAVRRGARPVAEPTEADGAVLATIMGF GDVVHTFVQRPGGAPGEDPESAGGLRVLDHFAVCLEAGGLEP TVAFYQEVLDFRVVFEEKIVVGAQAMNSKVVQSTSGAVTLTL IEPDTSRKPGQIDDFIKNHGGAGVQHIAFATDGIVDAVRRLRE RGVELLTTPAAYYDLLADRLGPTRYSTAELAELNLLVDEDQD GKLYQIFARSTHPRGTFFFEIIERAGAHTFGSGNIKALYEAVEA ERHRTER*
HMAS4	MTINGIDHLELYVGDARQAAFYFDNAVGFRLCGQGGPETGLD GQRSLLLAQAGIRLLLTTGLSAEHPAATYVHRHGDGIAVVALA VDDAAGAYAELVDRGATALSPPRTFTGADAEVVVAEVGGFG DVVHRLVERRGDPGVFLPGAIEPVTAPDGADTLLAEVDHLAI CVPPGQLGETVAHYEKVFGFADIFTEHIEVAGQAMNSKVVQS PSGKVTFVLLEPDTGARPGQIEDFLRWHAGAGVQHLGLRTDD IVTAVGALAGRGVRFASTPGTYYDGLEQRVGRLDAPVDRLRE LSILVDSDHDGQLLQIFTESMHVRRTLFLELIERRGARTFGSGN IKALYEAKERELAAAGATPAVAAGGVGE*
HMAS5	MEFSRVEHAELFVGDAVLSSYFFCHALGFRMVASGGPETGLP GRRSFVLEQGAARLVVTSALDADSEVAAFVRTHGDGVRDIAL GTPDAHAAFTEAVRRGARPVAEPVTYESHGQRVVKATIAGPG DWVHSFIQRDASSSDVLPGVYLPVEAGPSGGASLFTAVDHFA FALRPHTLMDTVGFYESVLGFEQTHREDVRTEYSGMSSRVVQ SVGGRVCFPLQEPVSGTRRGQLEDFLGAHGGAGVQHIAFLSN

	DIARAVDALRQHGVSLLDAPQGYYESLEARLGTLSPFEREPL QARNILMDRDAWGALLQVFTRSQHARRTLFFEVIQRHQARG FGGANIQALYAAKEQEAVRAAS*
HMAS6	MTSPTSLDTTPQLDDFDYVEFYVGNARQTAHFLRTAFGFKPIA YAGLETGVRDRASILLQQGAIRLIITEALDPESPIADHVKLHGD SIKDIAFTVANVHSAFEAAVKRGARPILEPVTIESPQGSIIKATIG TYGDTTHSLIQRVDLADNAFPQFQPIENPAHVIDGGFSVVDHV AISLEPGRLAEWVDFYINVLGFHQSHEENIVTEYSGMNSRVV QNHAGTIKFPMQEPIQGKRRSQVEEFLTFHHGAGAQHLAILTD DIIHSIQTLRANGIEFVRTPATYYENLQERVGLIDEDIAMLRDL HILVDRDSSGYLLQIFTKPLQSRPTMFFEIIQRKNAIGFGSGNIK ALFAAVEREQALRGNL*
AroG^{fbr}	MNYQNDDLRIKEIKELLPPVALLEKFPATENAANTVAHARKAI HKILKGNDDRLLVVIGPCSIHDPVAAKEYATRLLALREELKDE LEIVMRVYFEKPRTTVGWKGLINDPHMDNSFQINDGLRIARK LLLDINDSGLPAAGEFLNMITLQYLADLMSWGAIGARTTESQ VHRELASGLSCPVGFKNGTDGTIKVAIDAINAAGAPHCFLSVT KWGHSAIVNTSGNGDCHIILRGGKEPNYSAKHVAEVKEGLN KAGLPAQVMIDFSHANSSKQFKKQMDVCADVCQQIAGGEKA IIGVMVESHLVEGNQSLESGEPLAYGKSITDACIGWEDTDALL RQLANAVKARRG*
PheA^{fbr}	MTSENPLLALREKISALDEKLLALLAERRELAVEVGKAKLLS HRPVRDIDRERDLLERLITLGKAHHLDAHYITRLFQLIIEDSVL TQQALLQQHLNKINPHSARIAFLGPKGSYSHLAARQYAARHF EQFIESGCAKFADIFNQVETGQADYAVVPIENTSSGAINDVYD LLQHTSLSIVGEMTLTIDHCLLVSGTTDLSTINTVYSHPQPFQQ CSKFLNRYPHWKIEYTESTSAAMEKVAQAKSPHVAALGSEAG GTLYGLQVLERIEANQRQNFTRFVVLARKAINVSDQVPAKTT LLMATGQQAGALVEALLVLRNHNLIMPRLESRPIHGNPWEEM FYLDIQANLESAEMQKALKELGEITRSMKVLGCYPSENVVPV DPT*
TktA	MSSRKELANAIRALSMDAVQKAKSGHPGAPMGMADIAEVL WRDFLKHNPQNPSWADRDRFVLSNGHGSMLIYSLLHLTGYD LPMEELKNFRQLHSKTPGHPEVGYTAGVETTTGPLGQGIANA VGMAIAEKTLAAQFNRPGHDIVDHYTYAFMGDGCMMEGISH EVCSLAGTLKLGKLIAFYDDNGISIDGHVEGWFTDDTAMRFE AYGWHVIRDIDGHDAASIKRAVEEARAVTDKPSLLMCKTIIGF GSPNKAGTHDSHGAPLGDAEIALTREQLGWKYAPFEIPSEIYA QWDAKEAGQAKESAWNEKFAAYAKAYPQEAAEFTRRMKGE MPSDFDAKAKEFIAKLQANPAKIASRKASQNAIEAFGPLLPEF LGGSADLAPSNLTLWSGSKAINEDAAGNYIHYGVREFGMTAI ANGISLHGGFLPYTSTFLMFVEYARNAVRMAALMKQRQVMV YTHDSIGLGEDGPTHQPVEQVASLRVTPNMSTWRPCDQVESA VAWKYGVERQDGPTALILSRQNLAQQERTEEQLANIARGGYV

	LKDCAGQPELIFIATGSEVELAVAAYEKLTAEGVKARVVSMPS TDAFDKQDAAYRESVLPKAVTARVAVEAGIADYWYKYVGLN GAIVGMTTFGESAPAELLFEEFGFTVDNVVAKAKELL*
PpsA	MSNNGSSPLVLWYNQLGMNDVDRVGGKNASLGEMITNLSG MGVSVPNGFATTADAFNQFLDQSGVNQRIYELLDKTDIDDVT QLAKAGAQIRQWIIDTPFQPELENAIREAYAQLSADDENASFA VRSSATAEDMPDASFAGQQETFLNVQGFDAVLVAVKHVFASL FNDRAISYRVHQGYDHRGVALSAGVQRMVRSDLASSGVMFS IDTESGFDQVVFITSAWGLGEMVVQGAVNPDEFYVHKPTLAA NRPAIVRRTMGSKKIRMVYAPTQEHGKQVKIEDVPQEQRDIFS LTNEEVQELAKQAVQIEKHYGRPMDIEWAKDGHTGKLFIVQA RPETVRSRGQVMERYTLHSQGKIIAEGRAIGHRIGAGPVKVIH DISEMNRIEPGDVLVTDMTDPDWEPIMKKASAIVTNRGGRTC HAAIIARELGIPAVVGCGDATERMKDGENVTVSCAEGDTGYV YAELLEFSVKSSSVETMPDLPLKVMMNVGNPDRAFDFACLPN EGVGLARLEFIINRMIGVHPRALLEFDDQEPQLQNEIREMMK GFDSPREFYVGRLTEGIATLGAAFYPKRVIVRLSDFKSNEYAN LVGGERYEPDEENPMLGFRGAGRYVSDSFRDCFALECEAVKR VRNDMGLTNVEIMIPFVRTVDQAKAVVEELARQGLKRGENG LKIIMMCEIPSNALLAEQFLEYFDGFSIGSNDMTQLALGLDRD SGVVSELFDERNDAVKALLSMAIRAAKKQGKYVGICGQGPS DHEDFAAWLMEEGIDSLSLNPDTVVQTWLSLAELKK*
Xfpk	MTSPVIGTPWKKLNAPVSEEAIEGVDKYWRAANYLSIGQIYL RSNPLMKEPFTREDVRHRLVGHWGTTPGLNFLIGHINRLIAD HQQNTVIIMGPGHGGPAGTAQSYLDGTYTEYFPNITKDEAGL QKFFRQFSYPGGIPSHYAPETPGSIHEGGELGYALSHAYGAVM NNPSLFVPAIVGDGEAETGPLATGWQSNKLINPRTDGIVLPILH LNGYKIANPTILSRISDEELHEFFHGMGYEPYEFVAGFDNEDH LSIHRRFAELFETVFDEICDIKAAAQTDDMTRPFYPMIIFRTPK GWTCPKFIDGKKTEGSWRSHQVPLASARDTEAHFEVLKNWL ESYKPEKLFDENGAVKPEVTAFMPTGELRIGENPNANGGRIRE ELKLPKLEDYEVKEVAEYGHGWGQLEATRRLGVYTRDIIKNN PDSFRIFGPDETASNRLQAAYDVTNKQWDAGYLSAQVDEHM AVTGQVTEQLSEHQMEGFLEGYLLTGRHGIWSSYESFVHVID SMLNQHAKWLEATVREIPWRKPISSMNLLVSSHVWRQDHNG FSHQDPGVTSVLLNKCFNNDHVIGIYFPVDSNMLLAVAEKCY KSTNKINAIIAGKQPAATWLTLDEARAELEKGAAEWKWASNV KSNDEAQIVLAATGDVPTQEIMAAADKLDAMGIKFKVVNVV DLVKLQSAKENNEALSDEEFAELFTEDKPVLFAYHSYARDVR GLIYDRPNHDNFNVHGYEEQGSTTPYDMVRVNNIDRYELQ AEALRMIDADKYADKINELEAFRQEAFQFAVDNGYDHPDYT DWVYSGVNTNKQGAISATAATAGDNE*

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Table S2. MA production from PP by whole-cell catalysts expressing HMAS1–HMAS6.

Time (h)	HMAS1	HMAS2	HMAS3	HMAS4	HMAS5	HMAS6
0	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
2	0.346625	0.177222	5.996128	4.808072	0.000000	0.000000
4	0.438587	0.106110	7.206895	6.174467	0.000000	0.000000
6	0.879035	0.074463	7.467143	5.738858	0.008191	0.121375
8	2.276704	0.022711	7.630962	5.012845	0.000000	0.000000
10	1.339588	0.048773	6.138352	4.072750	0.000000	0.000000
12	1.136304	0.000000	5.821885	3.815481	0.000000	0.000000

Table S3. Effects of individual and combined gene overexpression on MA production.

Strain	Overexpressed Gene(s)	**MA Titer (mM)**
Control	—	0.13 ± 0.00
OE-aroG^{fbr}	*aroG^{fbr}*	0.86 ± 0.03
OE- pheA^{fbr}	*pheA^{fbr}*	3.72 ± 0.02
OE-ppsA	*ppsA*	0.09 ± 0.00
OE-tktA	*tktA*	0.13 ± 0.01
OE-xpfk	*xpfk*	0.19 ± 0.00
OE-aroG^{fbr} + pheA^{fbr}	*aroG^{fbr}*, *pheA^{fbr}*	4.33 ± 0.04
OE- aroG^{fbr} + pheA^{fbr} + xpfk	*aroG^{fbr}*, *pheA^{fbr}*, *xpfk*	5.70 ± 0.25

Table S4. sgRNA spacer sequences used for CRISPRi-mediated gene interference.

Target Gene	sgRNA Spacer Sequence (5′→3′)
csrA	GTTGGTGAGACCCTCATGAT
pykF	AATCTGAAGAGATGTTAGCT
trpA	ATCTCTGTTTGCCCAGTTGA
trpE	CTCGAACTGCTAACCTGCGA
trpR	ATCACCCTATTCAGCAGCGA
tyrA	GAATTTATTAGCGAAGCGTC
tyrR	GCGAATTACTCGATCTACTC

Table S5. Effects of CRISPRi-mediated gene interference on MA production.

Strain (Gene Interfered)	MA Titer (mM)
Control	5.35 ± 0.06
-*trpR*	6.43 ± 0.35
-*trpA*	6.61 ± 0.25
-*csrA*	6.84 ± 0.10
-*tyrR*	8.30 ± 1.00
-*pykF*	9.24 ± 1.78
-*trpE*	8.50 ± 0.38
-*tyrA*	6.22 ± 0.80
-*tyrR-pykF*	10.83 ± 0.12
-*tyrR-pykF-trpE*	14.76 ± 0.14

Table S6. Time-course data of cell growth and MA production during HCDC.

Time (h)	OD600	MA Titer (mM)
0	0.00	—
8	9.30	—
12	27.10	—
16	45.00	0.000000
18	55.10	0.859302
22	67.99	2.481105
26	77.54	3.878030
30	80.70	5.420902
34	93.10	10.52161
38	114.50	12.29904
42	124.20	16.05272
46	146.50	19.75129
50	147.96	24.62489
54	159.50	29.63625
58	159.91	30.41811
62	161.90	34.85238
66	167.50	41.76626
70	170.80	43.51614
74	175.60	49.96463
78	182.70	53.83670
82	183.50	62.66801
86	183.30	62.80576