Supplementary Material

Bacterial bifunctional chorismate mutase-prephenate dehydratase PheA increases flux into the yeast phenylalanine pathway and improves mandelic acid production

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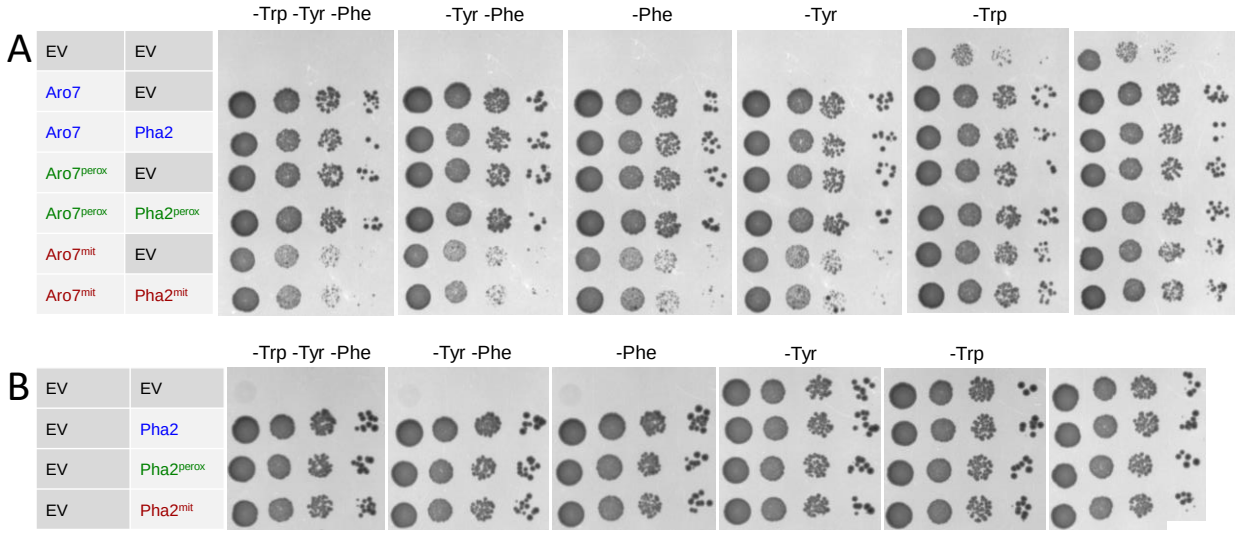

Figure S1. Complementation of *aro7* and *pha2* deletions by *ARO7* and/or *PHA2* fused to mitochondrial or peroxisomal targeting sequences. A) Growth test with MRY36 (CEN.PK2-1C *TRP1* Shik↑ *aro10Δ aro8Δ pdc5Δ aro7Δ*) harboring different plasmid combinations. B) Growth test with MRY15 (CEN.PK2-1C *TRP1* Shik↑ *pha2Δ*) harboring different plasmid combinations. The growth tests were performed on SCD agar plates, without histidine, without uracil, with and without supplementation of aromatic amino acids. Cell dilutions of OD_{600} 1, 10^{-1}, 10^{-2} and 10^{-3} (from left to right, respectively) were used. The pictures were taken after 3 d at 30 °C. EV, empty vector.

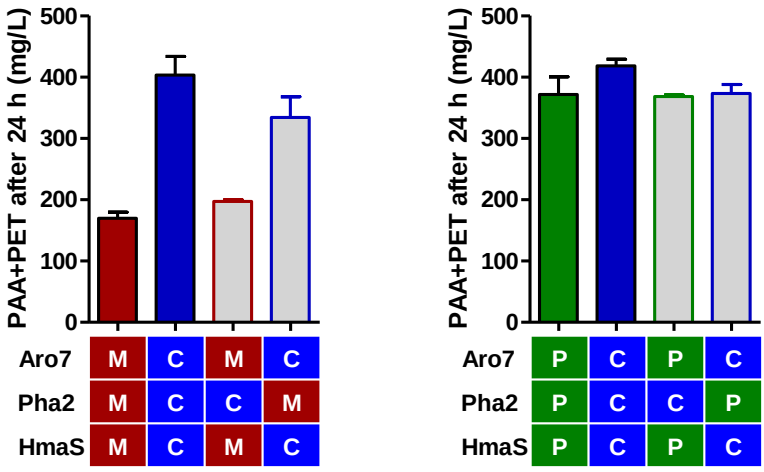

Figure S2. Phenylacetic acid and phenylethanol formation for MA-pathway localized to mitochondria and peroxisomes. A) and B) Phenylacetic acid and phenylethanol (PAA+PET) titers after 24 h of fermentation with MRY25 (CEN.PK2-1C *TRP1* Shik↑ *aro7Δ trp2Δ*) expressing Aro7^{fbr}, Pha2 and *N. uniformis* HmaS targeted to mitochondria (M, red), cytosol (C, blue) or peroxisomes (P, green). Colored bars present samples in which all three consecutive enzymes were localized in one compartment (blue, cytosol; red, mitochondria; green, peroxisomes). The fermentation was performed in SMD (20 g/L glucose) with anthranilate supplementation and a starting OD_{600} of 5. Error bars indicate standard deviation of biological duplicates.

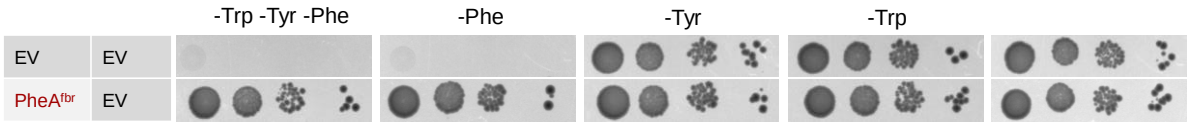

Figure S3. Complementation of a *pha2* deletion by *pheA*. Growth test with MRY15 (CEN.PK2-1C *TRP1* Shik↑ *pha2Δ*) harboring the empty vector p423HXT7 (EV) and either the empty vector p426HXT7 (EV) or the *pheA* vector MBaV4. The growth test was performed on SCD agar plates, without histidine, without uracil, with and without supplementation of aromatic amino acids. Cell dilutions of OD_{600} 1, 10^{-1}, 10^{-2} and 10^{-3} (from left to right, respectively) were used. The pictures were taken after 3 d at 30 °C.

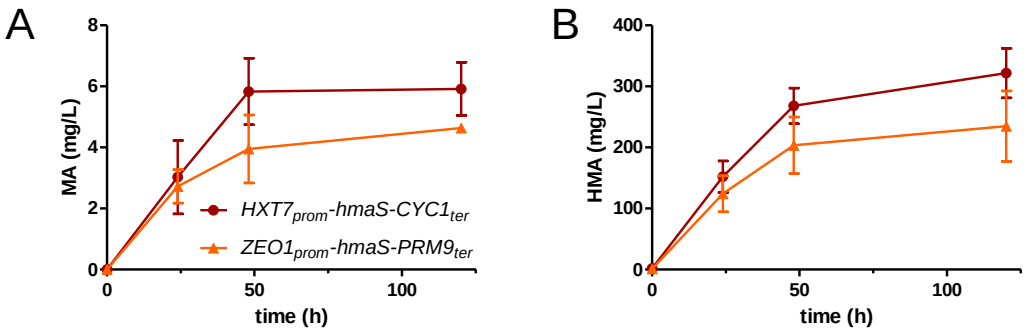

Figure S4. Changes in mandelic acid (MA) and hydroxymandelic acid (HMA) titers after *hmaS* promoter and terminator exchange. A) MA titers and B) HMA titers of Y4 (CEN.PK2-1C *TRP1* Shik↑ *aro10Δ aro8Δ pdc5Δ*, Reifenrath and Boles, 2018) expressing *N. uniformis hmaS* from plasmids MRV104 (red) and MRV143 (orange). The fermentations were performed in SMD (20 g/L glucose) without supplementation aromatic amino acids and with a starting OD_{600} of 5. Error bars indicate standard deviation of biological duplicates.

Table S1. Plasmids used in this study

Plasmids	Characteristics	Reference
Plasmids used for fermentations		
p423HXT7	2µ, *HIS3, Amp^r, HXT7$_{pr}$, CYC1$_{ter}$*	(Hamacher et al., 2002)
p425HXT7	2µ, *LEU2, Amp^r, HXT7$_{pr}$, CYC1$_{ter}$*	(Hamacher et al., 2002)
p426HXT7	2µ, *URA3, Amp^r, HXT7$_{pr}$, CYC1$_{ter}$*	(Hamacher et al., 2002)
p425MET25	2µ, *LEU2, Amp^r, MET25$_{pr}$, CYC1$_{ter}$*	(Mumberg et al., 1995)
MBaV2	p423HXT7 *MTS-PHA2*	This study
MBaV3	p425MET25 *MTS-Nu-hmaS^{opt}*	This study
MBaV4	p426HXT7 *pheA$^{fbr,\ opt}$*	This study
MBaV5	p426HXT7 *MTS-ARO7^{fbr}*	This study
MBaV6	p426HXT7 *MTS-ARO7^{fbr}-sfGFP*	This study
MRV104	p425MET25 *Nu-hmaS^{opt}*	(Reifenrath and Boles, 2018)
MRV108	p423HXT7 *PHA2*	This study
MRV133	p423HXT7 *PHA2-ePTS1*	This study
MRV134	p425MET25 *Nu-hmaS^{opt}-ePTS1*	This study
MRV135	p426HXT7 *ARO7^{fbr}*	This study
MRV137	p426HXT7 *ARO7^{fbr}-ePTS1*	This study
MRV139	p426HXT7 *sfGFP-ARO7^{fbr}-ePTS1*	This study
MRV140	p425MET25 *Nu-hmaS^{opt}* S195C	This study
MRV141	p425MET25 *Nu-hmaS^{opt}* S195M V197M Y333A	This study
MRV142	p425MET25 *Nu-hmaS^{opt}* S195M V197F Y333A	This study
MRV143	p425 ZEO1$_{prom}$ *Nu-hmaS^{opt}-PRM9$_{ter}$*	This study
MRV144	p425 *ZEO1$_{prom}$-Nu-hmaS^{opt}-PRM9$_{ter}$, HXT7$_{prom}$-pheA$^{fbr,\ opt}$-CYC1$_{ter}$*	This study
MRV145	p425HXT7 *pheA$^{fbr,\ opt}$*	This study
CRISPR/Cas9 Plasmids		
pRCC-K	2µ, *kanMX*, *Amp^r, ROX3$_{pr}$-cas9^{opt}-CYC1$_{ter}$, pSNR52-gRNA*	(Generoso et al., 2016)
pRCC-N	2µ, *natMX*, *Amp^r*, *ROX3$_{pr}$-cas9^{opt}-CYC1$_{ter}$, pSNR52-gRNA*	(Generoso et al., 2016)
pRCC-K-*ARO7*	2µ, *kanMX*, *ROX3$_{pr}$-cas9^{opt}-CYC1$_{ter}$, pSNR52-gRNA* for *ARO7* locus	This study

Table S2. Strains used in this study

Strains	Parent strain	Characteristics	Reference
CEN.PK2-1C		*MATa his3D1 leu2-3_112 ura3-52 trp1-289; MAL2-8c SUC2*	(Entian and Kötter, 2007)
Y1		CEN.PK2-1C *TRP1* Shik↑ (*aro4Δ*:: $HXT7_{pr}$-*ARO1* *ura3Δ::*$TPI1_{pr}$-*ARO4fbr_*$HXT7_{pr}$-$ARO3^{fbr,\ opt}$)	(Reifenrath and Boles, 2018)
Y4		CEN.PK2-1C *TRP1* Shik↑ *aro10Δ aro8Δ pdc5Δ*	(Reifenrath and Boles, 2018)
Y5		CEN.PK2-1C *TRP1* Shik↑ *aro10Δ aro8Δ pdc5Δ trp2Δ*	(Reifenrath and Boles, 2018)
MRY15	Y1	CEN.PK2-1C *TRP1* Shik↑ *pha2Δ*	This study
MRY26	Y1	CEN.PK2-1C *TRP1* Shik↑ *aro7Δ*	This study
MRY25	MRY26	CEN.PK2-1C *TRP1* Shik↑ *aro7Δ trp2Δ*	This study
MRY31	Y5	CEN.PK2-1C *TRP1* Shik↑ *aro10Δ aro8Δ pdc5Δ trp2Δ aro7Δ*	This study
MRY36	MRY31	CEN.PK2-1C *TRP1* Shik↑ *aro10Δ aro8Δ pdc5Δ aro7Δ*	This study

Table S3. Primers used in this study

Primer name	Sequence 5‘-3‘	Application
Plasmid construction or sequencing		
SZP107	CTGCGTGTTCTTCTGAGG	Binding in *HXT7*$_{prom}$, Sequencing
MOP289	CAAGAACAAACAAGCTCAAC	Binding in *HXT7*$_{prom}$, Sequencing
MOP291	GCGTCTGTTAGAAAGGAAGTTTTTCC	Binding in *MET25*$_{prom}$, Sequencing
HDP145	CGTGAATGTAAGCGTGAC	Binding in *CYC1*$_{ter}$, Sequencing or amplification of an insert with overhang to the *CYC1*$_{ter}$
MOP290	ACCTAGACTTCAGGTTGTC	Binding in *CYC1*$_{ter}$, Sequencing
MRP169	ACAAAAAGTTTTTTTAATTTTAATCAAAAAATGGCCAGCAAGACTTTGAG	*PHA2* amplification, fwd, overhang to *HXT7*$_{prom}$
MRP170	AGCGTGACATAACTAATTACATGACTCGAGTATTTGTGATAATATCTCTCATTTCTGGG	*PHA2* amplification, rev, overhang to *CYC1*$_{ter}$
MRP233	AGCGTGACATAACTAATTACATGACTCGAGTTACAACTTAGATCTTCTACCTCTACCCAATTTGTGATAATATCTCTCATTTCTGGG	*PHA2* amplification, PTS-introduction, rev, overhang to *CYC1*$_{ter}$
MRP234	AGCGTGACATAACTAATTACATGACTCGAGTTACAACTTAGATCTTCTACCTCTACCCAATCTTTCAGTTCTGTGTCTTTCAGC	*Nu-hmaS*^{opt} amplification, PTS-introduction, rev, overhang to *CYC1*$_{ter}$
MRP172	ACAAAAAGTTTTTTTAATTTTAATCAAAAAATGGATTTCACAAAACCAGAAAC	ARO7 amplification, fwd, overhang to HXT7prom
MGP12	CTAGTGGCAACAGAAGAGAAGTTATTCTTATC	Introducing mutation G141S into *ARO7*, rev
MGP11	GATAAGAATAACTTCTCTTCTGTTGCCACTAG	Introducing mutation G141S into *ARO7*, fwd
MRP173	AGCGTGACATAACTAATTACATGACTCGAGTTACTCTTCCAACCTTCTTAGCAAG	*ARO7* amplification, rev, overhang to *CYC1*$_{ter}$
MRP232	CGTGACATAACTAATTACATGACTCGAGTTACAACTTAGATCTTCTACCTCTACCCAACTCTTCCAACCTTCTTAGCAAG	*ARO7* amplification, PTS-introduction, rev, overhang to *CYC1*$_{ter}$
MRP237	ACAAAAAGTTTTTTTAATTTTAATCAAAAAATGAGCAAAGGAGAAGAACTTTTC	*sfGFP* amplification, fwd, overhang to *HXT7prom*
JTP39	GTGAAATCGGATCCACCAGAACCTTTGTAGAGCTCATCCATGC	*sfGFP* amplification, rev
MRP236	ATGGCATGGATGAGCTCTACAAAGGTTCTGGTGGATCCGATTTCACAAAACCAGAAACTG	*ARO7* amplification, fwd, overhang to *sfGFP*, used together with HDP145
MRP244	AGATTGAACAACCTTACAGTTCATAGCTTGAGCACCAAC	Introducing mutation S195C into *Nu-hmaS*^{opt}, rev
MRP245	CAAGCTATGAACTGTAAGGTTGTTCAATCTACTTCTGG	Introducing mutation S195C into *Nu-hmaS*^{opt}, fwd
MRP242	GATTGAACCATCTTCATGTTCATAGCTTGAGCACCAAC	Introducing mutations S195M and V197M into *Nu-hmaS*^{opt}, rev
MRP243	CAAGCTATGAACATGAAGATGGTTCAATCTACTTCTGGTGCTG	Introducing mutations S195M and V197M into *Nu-hmaS*^{opt}, fwd
MRP240	TCAACAGCTTCAGCCAAAGCCTTGATGTTACCAGAAC	Introducing mutation Y333A into *Nu-hmaS*^{opt}, rev
MRP241	TAACATCAAGGCTTTGGCTGAAGCTGTTGAAGCTGAAAGAC	Introducing mutation Y333A into *Nu-hmaS*^{opt}, fwd
MRP238	GATTGAACGAACTTCATGTTCATAGCTTGAGCACCAAC	Introducing mutations S195M and V197F into *Nu-hmaS*^{opt}, rev
MRP239	CAAGCTATGAACATGAAGTTCGTTCAATCTACTTCTGGTGCTG	Introducing mutations S195M and V197F into *Nu-hmaS*^{opt}, fwd
MBaP3	CAAAAAGTTTTTTTAATTTTAATCAAAAAATGGCTAGTACCAGAGTTTTAGC	*MTS* amplification, fwd, overhang to *HXT7*$_{prom}$
MBaP2	GGAGGAGTAAGCTCTCTTTTG	*MTS* amplification, rev

MBaP4	CAAGCCTTCCAAAAGAGAGCTTACTCCTCCGCCAGCAAGACTTTGAGG	*PHA2* amplification, fwd, overhang to *MTS*, used together with HDP145
MBaP1	CAAGCCTTCCAAAAGAGAGCTTACTCCTCCGATTTCACAAAACCAGAAACTG	*ARO7* amplification, fwd, overhang to *MTS*, used together with HDP145
JTP124	AATTCTATTACCCCCATCCATACTCTAGAAATGGCTAGTACCAGAGTTTTAG	*MTS* amplification, fwd, overhang to $MET25_{prom}$
MBaP5	CAAGCCTTCCAAAAGAGAGCTTACTCCTCCGCTGCTCAAGCTGGTTCTG	$Nu\text{-}hmaS^{opt}$ amplification, fwd, overhang to *MTS*, used together with HDP145
MRP235	GTGGAATACTTGCTAAGAAGGTTGGAAGAGGGTTCTGGTGGATCCAGC	*sfGFP* amplification, fwd, overhang to *ARO7*, used together with HDP145
Amplification of CrisprCas9 Plasmids pRCC-K/N		
WGP234	AACAAAAACCTGCAGGAAACGAAG ATAAATCATTTAAACTGTGAGGACCTTAATAC ATTC	Amplification of donor DNA, binding $TPI1_{prom}$, overhang to $URA3_{prom}$, forward
WGP235	GTTTAGTATACATGCATTTACTTATAATACAGTTTTTTAGGTACCGGCCGCAAATTAAAG	Amplification of donor DNA, binding $CYC1_{ter}$, overhang to $URA3_{ter}$, reverse
Deletion of *ARO7*		
JHP3	TGTATAGCGGGATATCCGATGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG	Amplification of prCC-K
JHP4	CATCGGATATCCCGCTATACAGATCATTTATCTTTCACTGCGGAG	
MRP198	CAAATAGCACTCAGCATCCTGCATAAAATTGGTATAAGATGATTACTCCAGAATATTTGG TAAAAATTTATAAGGAAATT	Donor DNA
JHP16	CAAACTTCGTGTTGCATTTC	Control PCR, *ARO7* locus
JHP19	GCGATGAGGTTATTGGGTTG	
Deletion of *TRP2*		
MRP219	CACGGCGCCCGCATAAACCCCGATCATTTATCTTTCACTGCGGAG	Amplification of prCC
MRP220	GGGGTTTATGCGGGCGCCGTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG	
MRP218	ATAATTAGCACTGATATTCTGATTGGAAAAAAGGCAAAAAAAGGGCATTCTCTGTTT TTCTCCCCTTAGACGACATTTTT	Donor DNA
MRP221	GAATAATCCTTTCAATCGTTGAAGTAG	Control PCR, *TRP2* locus
MRP222	AATTTTATTACAAAGCCATAACTCTTGC	
Reintegrating *TRP2* in MRY31		
MRP221	GAATAATCCTTTCAATCGTTGAAGTAG	Amplification and sequencing of *TRP2* for reintegration, forward

Table S4. Codon optimized sequences used in this study

Name	**Sequence**
pheA^{fbr}, codon optimized	ATGACCTCTGAAAACCCATTGTTGGCTTTGAGAGAAAAGATTTCTGCTTTGGA CGAAAAGTTGTTGGCTTTGTTGGCTGAAAGAAGAGAATTGGCTGTTGAAGTT GGTAAGGCTAAGTTGTTGTCTCACAGACCAGTTAGAGACATTGACAGAGAAA GAGACTTGTTGGAAAGATTGATTACCTTGGGTAAGGCTCACCACTTGGACGC TCACTACATTACCAGATTGTTCCAATTGATTATTGAAGACTCTGTTTTGACCCA ACAAGCTTTGTTGCAACAACACTTGAACAAGATTAACCCACACTCTGCTAGAA TTGCTTTCTTGGGTCCAAAGGGTTCTTACTCTCACTTGGCTGCTAGACAATAC GCTGCTAGACACTTCGAACAATTCATTGAATCTGGTTGTGCTAAGTTCGCTGA CATTTTCAACCAAGTTGAAACCGGTCAAGCTGACTACGCTGTTGTTCCAATTG AAAACACCTCTTCTGGTGCTATTAACGACGTTTACGACTTGTTGCAACACACC TCTTTGTCTATTGTTGGTGAAATGACCTTGACCATTGACCACTGTTTGTTGGT TTCTGGTACCACCGACTTGTCTACCATTAACACCGTTTACTCTCACCCACAAC CATTCCAACAATGTTCTAAGTTCTTGAACAGATACCCACACTGGAAGATTGAA TACACCGAATCTACCTCTGCTGCTATGGAAAAGGTTGCTCAAGCTAAGTCTCC ACACGTTGCTGCTTTGGGTTCTGAAGCTGGTGGTACCTTGTACGGTTTGCAA GTTTTGGAAAGAATTGAAGCTAACCAAAGACAAAACTTCACCAGATTCGTTGT TTTGGCTAGAAAGGCTATTAACGTTTCTGACCAAGTTCCAGCTAAGACCACCT TGTTGATGGCTAAGCAAGCTGGTGCTTTGGTTGAAGCTTTGTTGGTTTTGAG AAACCACAACTTGATTATGACCAGATTGGAATCTAGACCAATTCACGGTAACC CATGGGAAGAAATGTTCTACTTGGACATTCAAGCTAACTTGGAATCTGCTGA AATGCAAAAGGCTTTGAAGGAATTGGGTGAAATTACCAGATCTATGAAGGTT TTGGGTTGTTACCCATCTGAAAACGTTGTTCCAGTTGACCCAACCTAA
MTS (*atp-9*, *N. crassa*), codon optimized	ATGGCTAGTACCAGAGTTTTAGCTTCCAGATTGGCTTCTCAAATGGCTGCTTC AGCAAAGGTAGCAAGACCAGCTGTCAGAGTGGCTCAGGTGTCTAAAAGGAC TATACAAACCGGCAGTCCATTGCAAACTCTAAAGAGAACACAAATGACAAGT ATCGTCAATGCAACAACAAGACAAGCCTTCCAAAAGAGAGCTTACTCCTCC
ePTS1 (DeLoache et al., 2016), codon optimized	TTGGGTAGAGGTAGAAGATCTAAGTTG

Table S5. gRNA sequences used for deletions in this study

Locus	gRNA sequence 5’-3’
ARO7	TGTATAGCGGGATATCCGAT
PHA2	TTATGGCCAGCAAGACTTTG
TRP2	GGGGTTTATGCGGGCGCCGT