Pterin-Dependent Mono-oxidation for the Microbial Synthesis of a Modified Monoterpene Indole Alkaloid

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Supporting Information

Abbreviations: GTPCH- GTP cyclohydrolase I; PTPS- pyruvoyl tetrahydropterin synthase; SR- sepiapterin reductase; PCD- pterin-4-alpha-carbinolamine dehydratase; DHPR- dihydropteridine reductase; TH- tyrosine hydroxylase; TPH- tryptophan hydroxylase; DDC- aromatic-L-amino-acid decarboxylase; STR- strictosidine synthase

Organisms: *Saccharomyces cerevisiae, Escherichia coli, Mortierella alpina, Homo sapiens, Salmo salar, Salinibacter ruber, Phycisphaera mikurensis, Thalassiosira pseudonana, Sus scrofa, Mus musculus, Ophiorrhiza pumila*

Materials and Methods

Reagents. Tetrahydrobiopterin, dihydrobiopterin, and biopterin were purchased from Cayman Chemical (81880, 81882, and 10007662). Dopamine and vanillin were purchased from Alfa Aesar (A11136 and A11169). L-DOPA and serotonin were purchased from TCI America (D0600 and S0370). Secologanin and tryptophan were purchased from Sigma-Aldrich (50741-5MG-F and T0254). 5-chlorotryptamine was purchased from Ark Pharm, Inc. (AK-32281).

Construction of W303-Ade2$^+$ strain. *S. cerevisiae* W303 were transformed with AME245 and AME246 via an adapted electroporation protocol. Transformed cells were plated and subsequently patched on synthetic complete media with 2% glucose lacking adenine (SD (Ade$^-$)). To confirm the presence of a functional *Ade2*, genomic DNA from multiple patches was isolated, the mutation was amplified by PCR using primers AME128/AME247, and the PCR product sequenced with AME247.

Construction of multi-copy vectors expressing the BH_4 synthetic pathway. To construct pAME18, 20, 22-26, and 28, genes were amplified from plasmids carrying codon-optimized nucleotide sequences of *M. alpina* GTPCH, *H. sapiens* GTPCH, *M. alpina* PTPS, *S. salar* PTPS, *S. ruber* PTPS, *P. mikurensis* PTPS, *M. alpina* SR, and *T. pseudonana* SR with primers AME143/AME144, AME141/AME139, AME149/AME150, AME147/AME148, AME151/AME152, AME153/AME154, AME165/AME166, or AME165/AME167, respectively, and cloned into pESC-Leu2 at *BamHI*/*HindIII* (pAME18, 20), pESC-Trp1 at *BamHI*/*SacII* (pAME22-25), or pESC-His3 at *BamHI/SacII* (pAME26, 28). To construct pAME17, *E. coli* GTPCH was amplified from the *E. coli* DH10B genome with primers AME163/164. The gene product was re-amplified with primers AME135/140 and cloned into pESC-Leu2 at *BamHI*/*HindIII*. To construct pAME19, *S. cerevisiae* GTPCH was amplified from *S. cerevisiae* W303 genome with primers AME161/162, and re-amplified with primers AME137/142. The gene product was cloned into pESC-Leu2 at *BamHI*/*HindIII*. To construct pAME27, *S. cerevisiae* SR was amplified from *S. cerevisiae* W303 genome with primers AME168/169, and re-amplified with primers AME180/183. The gene product was cloned into pESC-His3 at *BamHI*/*SacII*. To construct pAME3, 29-30, green fluorescent protein (GFP) was amplified from pEGFP with primers AME123/124 and cloned into pESC-Leu2, pESC-Trp1, or pESC-His3, respectively, at *BamHI*/*HindIII* (Leu2) or *BamHI*/*SacII* (Trp1, His3). Constructs were sequence verified using primers AME104 and AME105.

Construction of single-copy vectors expressing BH_4 synthetic pathway. To construct pAME53-55, the region between terminators T_{ADH1} and T_{CYC1} was amplified from pAME26, 22, or 17 using primers AME184/AME185 and cloned into pRS413, pRS414, or pRS415, respectively, at *BamHI*/*HindIII*. Constructs were sequence verified using primers MH100 and MH101.

Construction of multi-copy vectors expressing BH_4 recycling pathway. To construct pAME22PCD and pAME26DHPR, PCD and DHPR genes were amplified from plasmids carrying the codon optimized genes with primers SS152/SS153 or AME241/AME242**,** respectively, and cloned into pAME22 or pAME26, respectively, at *NotI*/*SacI*. Constructs were sequence verified using primers AME229 and AME104.

Construction of multicopy vectors expressing alkaloid pathway enzymes from inducible promoters. To construct pSS61, the STR gene was amplified from pSS42 with primers SS159/SS160 and cloned into pESC-Ura3 at *BamHI*/*HindIII*. To construct pSS66, the DDC gene was amplified from pSS62 with primers SS157/SS158 and cloned into pAME17 at *NotI*/*SacI*. To construct pSS68, the TH gene was amplified from pSS64 with primers SS179/SS180 and cloned into pESC-Ura3 at *NotI*/*SacI*. To construct pSS70, the TPH gene was amplified from pSS44 with primers SS207/SS208 and cloned into pESC-Ura3 at *BamHI*/*HindIII*. To construct pSS71, the TPH gene was amplified from pSS44 with primers SS177/SS178 and cloned into pSS61 at *NotI*/ *SacI*. Constructs were sequence verified using primer SS112. To construct pAME63, the DDC gene was amplified from pSS62 with primers SS157/SS158 and cloned into pESC-Leu2 at *NotI*/*SacI*. To construct pAME64, the STR gene was amplified from pSS42 with primers SS159/AME406 and cloned into pESC-Ura3 at *BamHI*/*HindIII*. Constructs were sequence verified using primers AME229/AME230 (pAME63) or AME104/AME105 (pAME64).

Construction of multicopy vectors expressing alkaloid pathway enzymes from constitutive promoters. To construct pAME56-58, assembly similar to sewing PCR was utilized. Fragments were amplified from template plasmids using primers as follows (fragment-primer/primer/template):

SR-AME373/AME374/pAME26;
P_{TEF1}_P_{HXT7}-AME365/AME366/pSS102;
PTPS-AME363/AME364/pAME22;
T_{HXT7}-AME369/AME370/pSS102
P_{ADH1g}-AME371/AME372/pSS43;
P_{ADH1ng}-AME371/AME383/pSS43;
GTPCH-AME367/AME368/pAME17;
PCD-AME375/AME376/pAME22PCD;
DHPR-AME377/AME378/pAME26DHPR;
DDC-AME384/AME385/pSS66;
TPH-AME386/AME387/pSS70;
STR-AME388/AME389/pSS61;
vector-AME394/AME395/pAME26DHPR,pAME22PCD,pSS67.

After amplification, PCR products were gel purified. To create pAME56-58, fragments were sewn together using primers AME384/AME389 (P_{TEF1}_P_{HXT7}, T_{HXT7}, P_{ADH1g}, DDC, TPH, STR), AME363/AME374 (P_{TEF1}_P_{HXT7}, T_{HXT7}, P_{ADH1g}, GTPCH, PTPS, SR), and AME375/AME383 (P_{TEF1}_P_{HXT7}, T_{HXT7}, P_{ADH1ng}, PCD, DHPR), respectively, using a typical PCR protocol and equimolar amounts of fragments. Resulting products were gel purified and combined with respective vector fragments (from pAME22PCD, pAME26DHPR, pSS67, respectively) via Gibson assembly[1]. Sequencing was obtained using primers AME105/AME229/AME396/AME397/AME369/AME370/AME372.

Yeast transformation. A modified electroporation method[2] was utilized to transform *S. cerevisiae* W303 or W303-Ade2^{+}. Modifications included no DNA precipitation step and immediately after electroporation, cells were rescued with YPD and left at room temperature overnight before plating on selection media plates.

Yeast cell lysis for intracellular biopterin determination. After 136 h of microbial production, cultures were centrifuged at 3230*g* for 5 min. The supernatant was removed and filtered with a 0.2µm filter. The pellet was frozen at -80°C, thawed, washed with 1mL water, and resuspended in 250µL water. 250µL 0.2M NaOH was mixed in and the cells remained at room temperature for 10 minutes. The lysate was centrifuged and filtered. Both supernatant and lysate were analyzed using liquid chromatography/ mass spectrometry (LC−MS).

***Statistical analysis*.** Two-tailed, paired T-tests were performed in Microsoft Excel.

Determining SR open reading frame from T. pseudonana. As only a portion of the amino acid sequence is known for the predicted SR from *T. pseudonana*, we searched upstream and downstream of the sequence in the genome to obtain a complete open reading frame.

Amino acid limiting experiments. Overnight cultures of strain PPY649 and PPY646 in synthetic media containing 2% glucose and lacking histidine, leucine, uracil, and tryptophan (SD (HWUL-)) was used to inoculate 5mL of synthetic media containing 2% galactose and lacking histidine, leucine, uracil, and tryptophan (SCgal (HWUL$^-$)) to OD_{600}=0.1. Tryptophan was added to strain PPY649 (final concentrations 0-640mg/L) and tyrosine was added to strain PPY646 (final concentrations 30-960mg/L). Cultures were incubated for 136 hours at 30^0C (250 rpm). After incubation, cultures were centrifuged for 5 min at 3230*g*. Supernatant was removed, filtered and analyzed via LC−MS analysis. 5-chlorotryptamine was used as an internal standard.

Determination of GTPCH, PTPS, and SR mRNA levels. Overnight cultures of strain PPY949-950 in synthetic complete media with 2% glucose lacking leucine (SD (L$^-$)) was used to inoculate 5mL of synthetic complete media with 2% galactose lacking leucine (SCgal (L$^-$)) to OD_{600}=0.1 and incubated overnight at 30^0C (250 rpm). Overnight cultures of strain PPY951-952 in synthetic complete media with 2% glucose lacking tryptophan (SD (W$^-$)) was used to inoculate 5mL of synthetic complete media with 2% galactose lacking tryptophan (SCgal (W$^-$)) to OD_{600}=0.1 and incubated overnight at 30^0C (250 rpm). Overnight cultures of strain PPY953-954 in synthetic complete media with 2% glucose lacking histidine (SD (H$^-$)) was used to inoculate 5mL of synthetic complete media with 2% galactose lacking histidine (SCgal (H$^-$)) to OD_{600}=0.1 and incubated overnight at 30^0C (250 rpm). Total RNA for all cultures was extracted using a RNeasy Mini Kit (Qiagen) following the manufacturer's protocol for isolation from yeast using $3x10^7$ cells per culture. RNA quantity was measured using a NanoDrop Lite. 1 µg of total RNA was taken from each strain and converted into cDNA using QuantiTect® reverse transcription kit (Qiagen) using manufacturer's instructions. Relative expression levels of GFP were quantified using QuantiTect® SYBR Green PCR kit (Qiagen) using manufacturer's instructions for LightCyclers 1.x and 2.0 with 150ng cDNA per reaction. Duplicate reactions were set up for each strain. Quantification was completed using a StepOnePlus Real-time PCR system (Applied Biosystems) with primers AME443/AME444 (GTPCH), AME441/AME442 (PTPS), AME445/AME446 (SR), and ACT-F/ACT-R. Cycling conditions: 15 min activation at 95°C followed by 40 cycles of 15 sec 95°C, 15 sec 57°C, and 15 sec 72°C. ACT1, a gene that encodes actin, was used to normalize the amount of the mRNA for the gene of interest in all samples.

SI Table1. Table of strains

Strain #	Strain name	Description	Source
PPY11	W303	*S. cerevisiae* MATa ade2-1 ura3-1 his3-11 trp1-1 leu2-3 leu2-112 can1-100	ATCC® 20835
PPY568	W303-Ade2$^+$	*S. cerevisiae* W303 with a T190G mutation in *Ade2* gene	This study
PPY752	W303-172226	W303 transformed with pAME17, pAME22, and pAME26	This study
PPY753	W303-172227	W303 transformed with pAME17, pAME22, and pAME27	This study
PPY754	W303-172228	W303 transformed with pAME17, pAME22, and pAME28	This study
PPY755	W303-172326	W303 transformed with pAME17, pAME23, and pAME26	This study
PPY756	W303-172327	W303 transformed with pAME17, pAME23, and pAME27	This study
PPY757	W303-172328	W303 transformed with pAME17, pAME23, and pAME28	This study
PPY758	W303-172426	W303 transformed with pAME17, pAME24, and pAME26	This study
PPY759	W303-172427	W303 transformed with pAME17, pAME24, and pAME27	This study
PPY760	W303-172428	W303 transformed with pAME17, pAME24, and pAME28	This study
PPY761	W303-172526	W303 transformed with pAME17, pAME25, and pAME26	This study
PPY762	W303-172527	W303 transformed with pAME17, pAME25, and pAME27	This study
PPY763	W303-172528	W303 transformed with pAME17, pAME25, and pAME28	This study
PPY764	W303-182226	W303 transformed with pAME18, pAME22, and pAME26	This study
PPY765	W303-182227	W303 transformed with pAME18, pAME22, and pAME27	This study
PPY766	W303-182228	W303 transformed with pAME18, pAME22, and pAME28	This study

PPY767	W303-182326	W303 transformed with pAME18, pAME23, and pAME26	This study
PPY768	W303-182327	W303 transformed with pAME18, pAME23, and pAME27	This study
PPY797	W303-182328	W303 transformed with pAME18, pAME23, and pAME28	This study
PPY798	W303-182426	W303 transformed with pAME18, pAME24, and pAME26	This study
PPY799	W303-182427	W303 transformed with pAME18, pAME24, and pAME27	This study
PPY800	W303-182428	W303 transformed with pAME18, pAME24, and pAME28	This study
PPY801	W303-182526	W303 transformed with pAME18, pAME25, and pAME26	This study
PPY802	W303-182527	W303 transformed with pAME18, pAME25, and pAME27	This study
PPY769	W303-182528	W303 transformed with pAME18, pAME25, and pAME28	This study
PPY803	W303-192226	W303 transformed with pAME19, pAME22, and pAME26	This study
PPY804	W303-192227	W303 transformed with pAME19, pAME22, and pAME27	This study
PPY805	W303-192228	W303 transformed with pAME19, pAME22, and pAME28	This study
PPY806	W303-192326	W303 transformed with pAME19, pAME23, and pAME26	This study
PPY807	W303-192327	W303 transformed with pAME19, pAME23, and pAME27	This study
PPY808	W303-192328	W303 transformed with pAME19, pAME23, and pAME28	This study
PPY809	W303-192426	W303 transformed with pAME19, pAME24, and pAME26	This study
PPY770	W303-192427	W303 transformed with pAME19, pAME24, and pAME27	This study
PPY771	W303-192428	W303 transformed with pAME19, pAME24, and pAME28	This study
PPY772	W303-192526	W303 transformed with pAME19, pAME25, and pAME26	This study
PPY773	W303-192527	W303 transformed with pAME19, pAME25, and pAME27	This study
PPY774	W303-192528	W303 transformed with pAME19, pAME25, and pAME28	This study
PPY775	W303-202226	W303 transformed with pAME20, pAME22, and pAME26	This study
PPY776	W303-202227	W303 transformed with pAME20, pAME22, and pAME27	This study
PPY777	W303-202228	W303 transformed with pAME20, pAME22, and pAME28	This study
PPY778	W303-202326	W303 transformed with pAME20, pAME23, and pAME26	This study
PPY779	W303-202327	W303 transformed with pAME20, pAME23, and pAME27	This study
PPY780	W303-202328	W303 transformed with pAME20, pAME23, and pAME28	This study
PPY781	W303-202426	W303 transformed with pAME20, pAME24, and pAME26	This study
PPY782	W303-202427	W303 transformed with pAME20, pAME24, and pAME27	This study
PPY783	W303-202428	W303 transformed with pAME20, pAME24, and pAME28	This study
PPY784	W303-202526	W303 transformed with pAME20, pAME25, and pAME26	This study
PPY785	W303-202527	W303 transformed with pAME20, pAME25, and pAME27	This study
PPY786	W303-202528	W303 transformed with pAME20, pAME25, and pAME28	This study
PPY810	W303-032930	W303 transformed with pAME3, pAME29, and pAME30	This study
PPY751	W303-2226	W303 transformed with pAME22, and pAME26	This study
PPY787	W303A-172226	W303-Ade2^{+} transformed with pAME17, pAME22, and pAME26	This study
PPY749	W303A-57	W303-Ade2^{+} transformed with pAME57	This study
PPY788	W303A-172252	W303-Ade2^{+} transformed with pAME17, pAME22, and pAME52	This study
PPY789	W303A-175426	W303-Ade2^{+} transformed with pAME17, pAME54, and pAME26	This study
PPY790	W303A-552226	W303-Ade2^{+} transformed with pAME55, pAME22, and pAME26	This study
PPY793	W303A-175453	W303-Ade2^{+} transformed with pAME17, pAME54, and pAME53	This study
PPY792	W303A-552253	W303-Ade2^{+} transformed with pAME55, pAME22, and pAME53	This study
PPY791	W303A-555426	W303-Ade2^{+} transformed with pAME55, pAME54, and pAME26	This study
PPY750	W303A-555453	W303-Ade2^{+} transformed with pAME55, pAME54, and pAME53	This study
PPY949	W303A-17	W303-Ade2+ expressing pAME17	This study
PPY950	W303A-55	W303-Ade2+ expressing pAME55	This study
PPY951	W303A-22	W303-Ade2+ expressing pAME22	This study
PPY952	W303A-54	W303-Ade2+ expressing pAME54	This study
PPY953	W303A-26	W303-Ade2+ expressing pAME26	This study
PPY954	W303A-53	W303-Ade2+ expressing pAME53	This study
PPY946	W303A-946	W303-Ade2^{+} transformed with pSS68, pESC-Leu2, pESC-His3, and pESC-Trp1	This study
PPY646	W303A-646	W303-Ade2^{+} transformed with pAME17, pAME22PCD,	This study

		pAME26DHPR, and pSS68	
PPY679	W303A-679	W303-Ade2^{+} transformed with pAME17, pAME22, pAME26, and pSS68	This study
PPY947	W303A-947	W303 Ade2^{+} transformed with pAME63, pSS68, pESC-His3, and pESC-Trp1	This study
PPY658	W303A-658	W303-Ade2^{+} transformed with pSS66, pAME22PCD, pAME26DHPR, and pSS68	This study
PPY743	W303A-743	W303 Ade2^{+} transformed with pSS66, pAME22, pAME26, and pSS68	This study
PPY948	W303A-948	W303 Ade2^{+} transformed with pAME63, pSS70, pESC-His3, and pESC-Trp1	This study
PPY649	W303A-649	W303-Ade2^{+} transformed with pSS66, pAME22PCD, pAME26DHPR, and pSS70	This study
PPY741	W303A-741	W303-Ade2^{+} transformed with pSS66, pAME22, pAME26, and pSS70	This study
PPY650	W303A-650	W303-Ade2^{+} transformed with pSS66, pAME22PCD, pAME26DHPR, and pSS71	This study
PPY955	W303A-955	W303-Ade2^{+} transformed with pSS66, pAME22, pAME26, and pSS71	This study
PPY744	W303A-744	W303 Ade2^{+} transformed with pAME56, pAME57, and pAME58	This study
PPY748	W303A-748	W303Ade2^{+} transformed with pAME56, pAME57, and pESC-Ura3	This study
PPY740	W303A-740	W303 Ade2^{+} transformed with pAME56 and pAME57	This study
PPY827	W303A-64	W303 Ade2^{+} transformed with pAME64	This study
PPY828	W303A-ura	W303 Ade2^{+} transformed with pESC-Ura3	This study
PPY835	W303-835	W303 transformed with pAME22, pAME26, and pSS68	This study
PPY836	W303-836	W303 transformed with pAME17, pAME22, pAME26, and pSS68	This study

SI Table 2. Table of plasmids

Strain #	Plasmid	Description	Source
PPY34	pESC-His3	Yeast shuttle vector with divergent Gal1/Gal10 promoter	Agilent #217451
PPY35	pESC-Ura3	Yeast shuttle vector with divergent Gal1/Gal10 promoter	Agilent #217454
PPY36	pESC-Trp1	Yeast shuttle vector with divergent Gal1/Gal10 promoter	Agilent #217453
PPY39	pESC-Leu2	Yeast shuttle vector with divergent Gal1/Gal10 promoter	Agilent #217452
PPY13	pRS413	YC-type (centromeric) shuttle vector	ATCC® 87518
PPY14	pRS414	YC-type (centromeric) shuttle vector	ATCC® 87519
PPY15	pRS415	YC-type (centromeric) shuttle vector	ATCC® 87520
PPY154	pCR2.1_HGTPCH	Codon optimized* GTPCH from *H. sapiens*	This study
PPY156	pCR2.1_MaGTPCH	Codon optimized* GTPCH from *M.alpinas*	This study
PPY171	pCR2.1_MaPTS	Codon optimized* PTPS from *M. alpinas*	This study
PPY172	pCR2.1_SPTS	Codon optimized* PTPS from *S. salar*	This study
PPY173	pCR2.1_RubPTS	Codon optimized* PTPS from *S. ruber*	This study
PPY174	pCR2.1_PmPTS	Codon optimized* PTPS from *P. mikurensis*	This study
PPY181	pCR2.1_MaSR	Codon optimized* SR from *M. alpina* with N-terminal His_6-tag	This study
PPY182	pCR2.1_PseudoSR	Codon optimized* SR from *T. pseudonana* with N-terminal His_6-tag	This study
PPY435	pCR2.1_DHPR	Codon optimized* DHPR from *H. sapiens*	This study
PPY465	pSS48	Codon optimized* PCD from *H. sapiens*	This study
PPY539	pSS62	Codon optimized* DDC from *S. scrofa*	This study
PPY563	pSS64	Codon optimized* TH from *M. musculus*	This study

PPY444	pSS44	Codon optimized* TPH from *H. sapiens*	This study
PPY442	pSS42	Codon optimized (for *E. coli)* STR from *O. pumila*	Commercially synthesized for this study. Sequence from Bernhardt et al.
PPY38	pEGFP	Enhanced green fluorescent protein	F. Storici lab
PPY40	pAME3	pESC-Leu2-P_{GAL1}-eGFP	This study
PPY242	pAME29	pESC-Trp1-P_{GAL1}-eGFP	This study
PPY243	pAME30	pESC-His3-P_{GAL1}-eGFP	This study
PPY183	pAME17	pESC-Leu2-P_{GAL1}-His_6-*E.coli*_GTPCH	This study
PPY168	pAME18	pESC-Leu2-P_{GAL1}-His_6-*M.alpina*_GTPCH	This study
PPY184	pAME19	pESC-Leu2-P_{GAL1}-His_6-*S.cerevisiae*_GTPCH	This study
PPY166	pAME20	pESC-Leu2-P_{GAL1}-His_6-*H.sapiens*_GTPCH	This study
PPY186	pAME22	pESC-Trp1-P_{GAL1}-His_6-*M.alpina*_PTPS	This study
PPY187	pAME23	pESC-Trp1-P_{GAL1}-His_6-*S.salar*_PTPS	This study
PPY188	pAME24	pESC-Trp1-P_{GAL1}-His_6-*S.ruber*_PTPS	This study
PPY189	pAME25	pESC-Trp1-P_{GAL1}-His_6-*P.mikurensis* _PTPS	This study
PPY190	pAME26	pESC-His3-P_{GAL1}-His_6-*M.alpina*_SR	This study
PPY241	pAME27	pESC-His3-P_{GAL1}-His_6-*S.cerevisiae*_SR	This study
PPY191	pAME28	pESC-His3-P_{GAL1}-His_6-*T.pseudonana*_SR	This study
PPY670	pAME53	pRS413-His3-P_{GAL1}-His_6-*M.alpina*_SR	This study
PPY667	pAME54	pRS414-Trp1-P_{GAL1}-His_6-*M.alpina*_PTPS	This study
PPY668	pAME55	pRS415-Leu2-P_{GAL1}-His_6-*E.coli*_GTPCH	This study
PPY520	pAME22PCD	pESC-Trp1-P_{GAL1}-His_6-*M.alpina*_PTPS-P_{GAL10}-*H.sapiens*_PCD	This study
PPY555	pAME26DHPR	pESC-His3-P_{GAL1}-His_6-*M.alpina*_SR- P_{GAL10}-*H.sapiens*_DHPR	This study
PPY538	pSS61	pESC-Ura3-P_{GAL1}-STR	This study
PPY572	pSS66	pESC-Leu2-P_{GAL1}-His_6-*E.coli*_GTPCH-P_{GAL10}-DDC	This study
PPY574	pSS68	pESC-Ura3-P_{GAL10}-TH	This study
PPY630	pSS70	pESC-Ura3-P_{GAL1}-TPH	This study
PPY631	pSS71	pESC-Ura3-P_{GAL1}-STR- P_{GAL10}-TPH	This study
PPY700	pAME56	pESC-Trp1-P_{HXT7}-DDC- P_{TEF1}-TPH- P_{ADH1}-STR	This study
PPY704	pAME57	pESC-His3-P_{HXT7}-PTPS-P_{TEF1}-GTPCH-P_{ADH1}-SR	This study
PPY701	pAME58	pESC-Ura3-P_{HXT7}-PCD- P_{TEF1}-DHPR	This study
PPY723	pAME63	pESC-Leu2-P_{GAL10}-DDC	This study
PPY338	pSS102	pESC-Ura3-P_{HXT7}/ P_{TEF1}	This study
PPY443	pSS43	pESC-Trp1-P_{TEF1}/P_{ADH1}	This study
PPY573	pSS67	pESC-Ura3-P_{GAL10}-TH-P_{GAL1}-NCS	This study
PPY822	pAME64	pESC-Ura3-P_{GAL1}-STR-His_6	This study

*"Codon optimized" references commercial codon optimization for *S. cerevisiae* unless otherwise noted (Operon)

SI Table 3. Table of primers

Name	Sequence (5'→3')
AME104	CACTTTAACTAATACTTTCAAC
AME105	TAAATAACGTTCTTAATACTAAC
AME123	CGTCAAGGAGAAAAAACCCCGGATCCATCACGTGCACCATGGTGAGCAAGGGCGAG
AME124	TCTTAGCTAGCCGCGGTACCAAGCTTTTACTTGTACAGCTCGTCC
AME128	CTGGAGAAGGGTAAATTTTTA

AME135	TCTTAGCTAGCCGCGGTACCAAGCTTTTAGTTGTGATGACGCACAGC
AME137	TCTTAGCTAGCCGCGGTACCAAGCTTTTAAATACTTCTTCTTCCTAAAAG
AME139	TCTTAGCTAGCCGCGGTACCAAGCTTTTAAGATCTAATCAAAGTCAAG
AME140	CGTCAAGGAGAAAAAACCCCGGATCCATCACGTGCACCATGCATCACCATCACCATCAC CCATCACTCAGTAAAGAAGC
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SS152	GGCGAAGAATTGTTAATTAATTAAGTCATGGAGACAGC
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SS157	TGGCGAAGAATTGTTAATTAATTAAGATTTAATTTCAGCTTTACCTTC
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ACT1-F	TTGGATTCCGGTGATGGTGT
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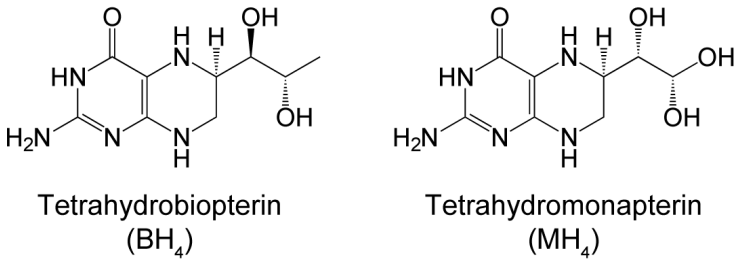
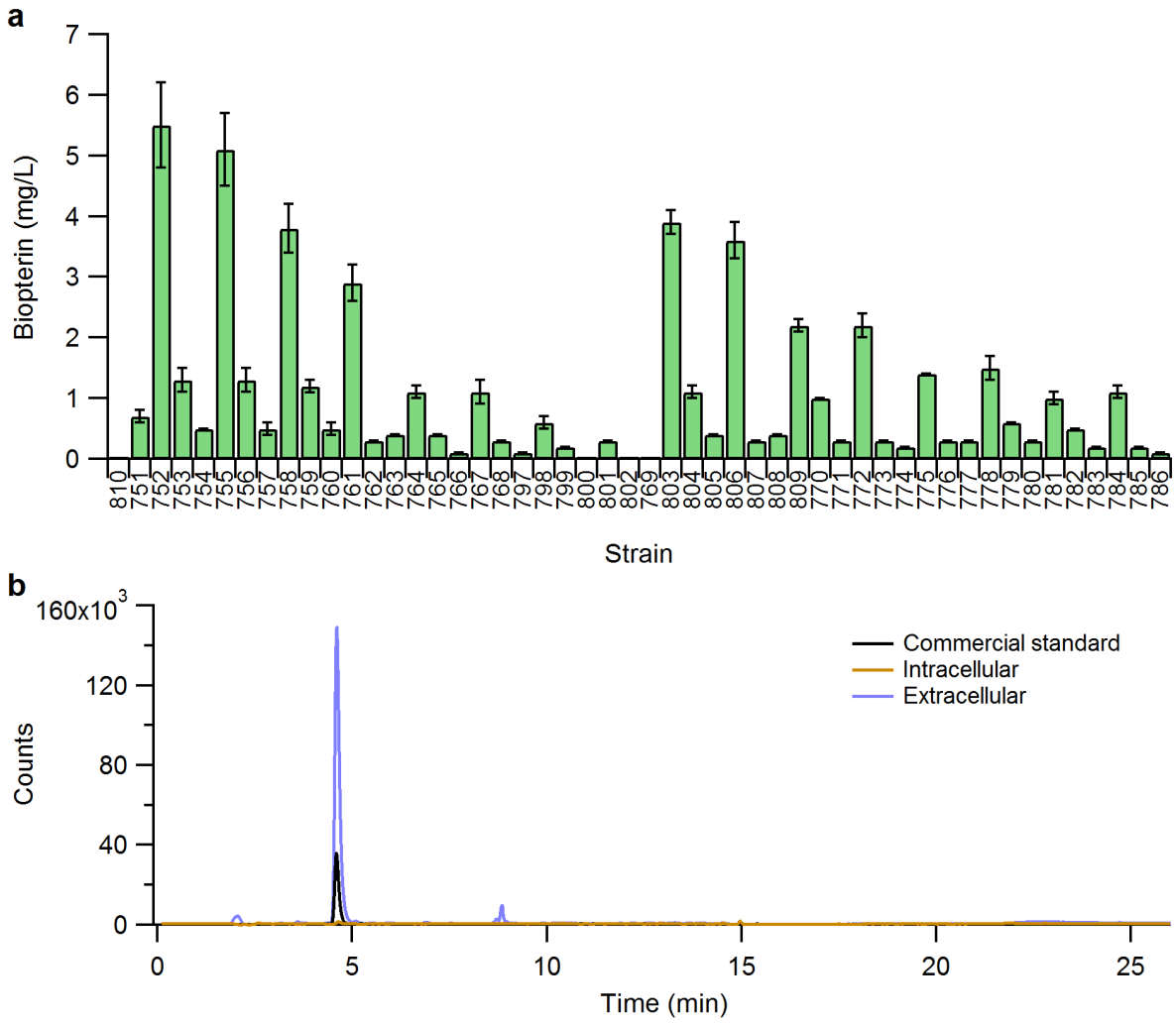
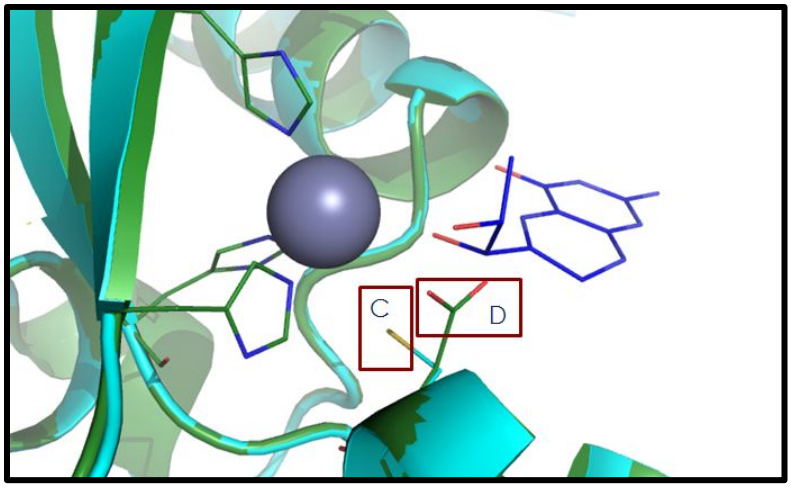


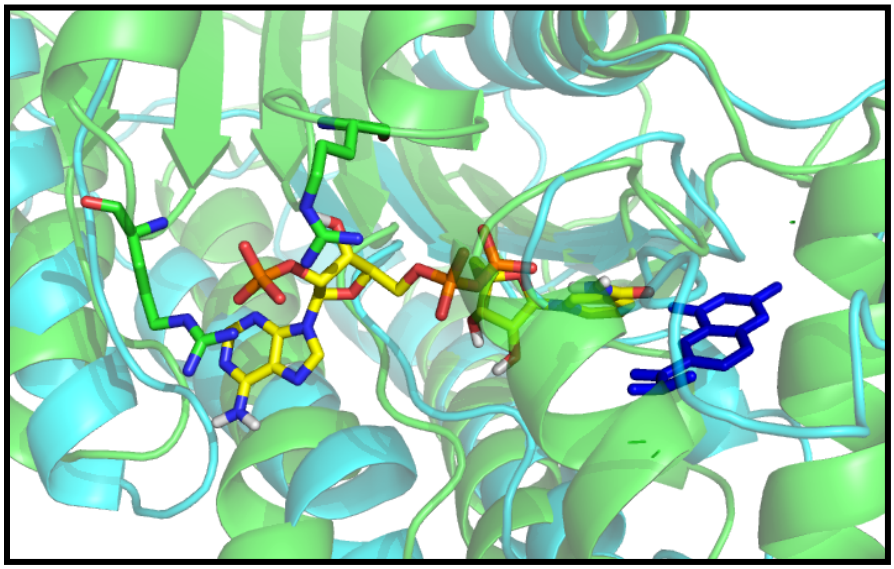
SI Figure 1. Stereochemistry of pterin co-factors. Chemical structures of tetrahydrobiopterin (BH_4), the natural amino acid mono-oxygenase co-factor, and tetrahydromonapterin (MH_4), the BH_4 analogue found in *E. coli*. BH_4 and MH_4 vary in stereochemistry and composition.



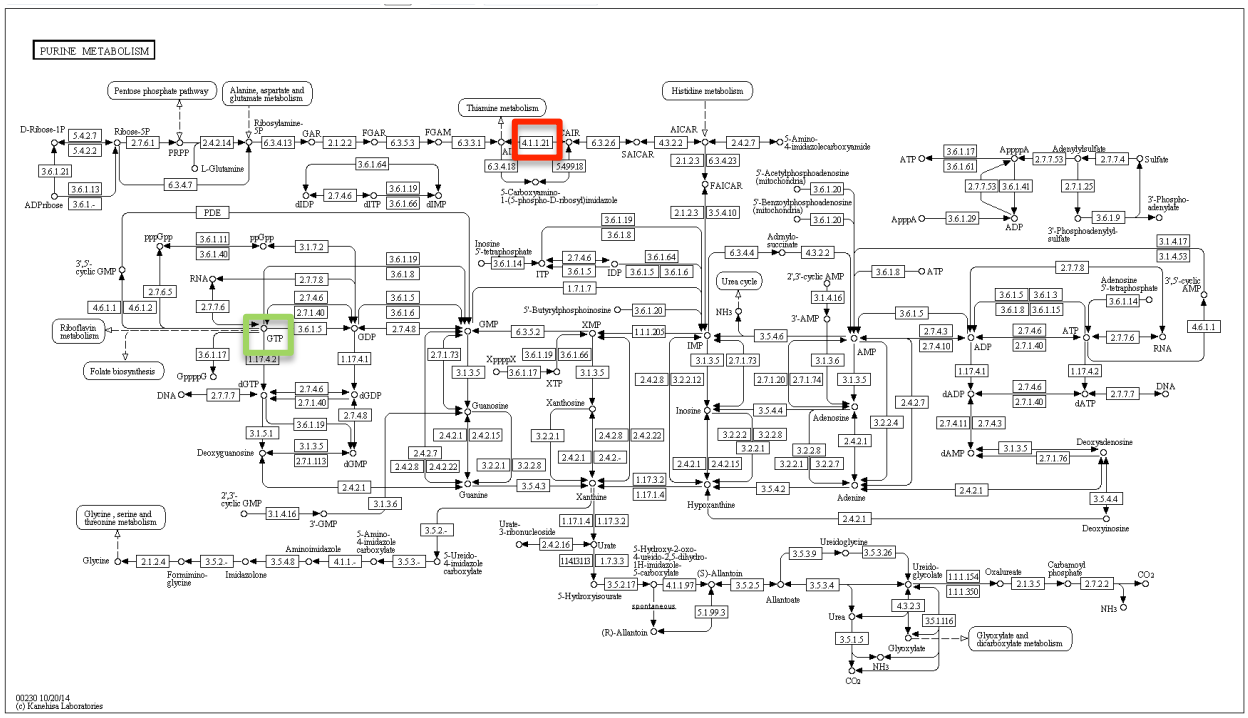
SI Figure 2. Combinatorial production of biopterin. (a) Production levels of biopterin were quantified using LC−MS. Production levels reported as 0.00 were either too low to quantify or undetectable. Strain PPY810 represents a control strain expressing green fluorescent protein in a three-plasmid system. The experiments were run in triplicate and shown are the mean and standard deviation. (b) Full window of LC traces representing biopterin production in Figure 3c.



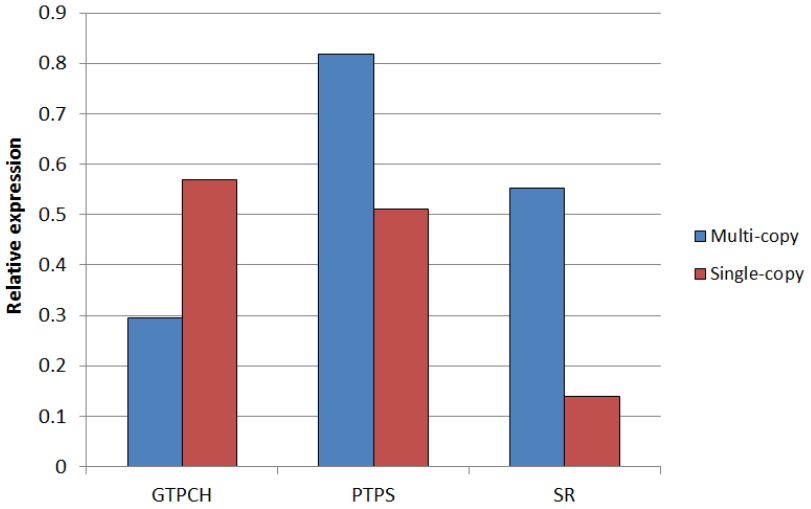
SI Figure 3. Structural alignment of *Salinibacter ruber* and *Salmo salar* pyruvoyl tetrahydropterin synthase (PTPS)**.** Structural alignment of homology models of *S. salar* PTPS (cyan) and *S. ruber* (green) PTPS obtained via structural homology to rat PTPS (PDB:1B66) using SWISS-MODEL[3-5]. Presented is a monomer of the active site of PTPS (which is composed of three monomers) showing the catalytic cysteine residue of *S. salar* PTPS and corresponding aspartate residue of *S. ruber* PTPS. Biopterin (blue) and Zn(II) (purple) were obtained from the crystal structure from rat. Alignment was completed with PyMOL.



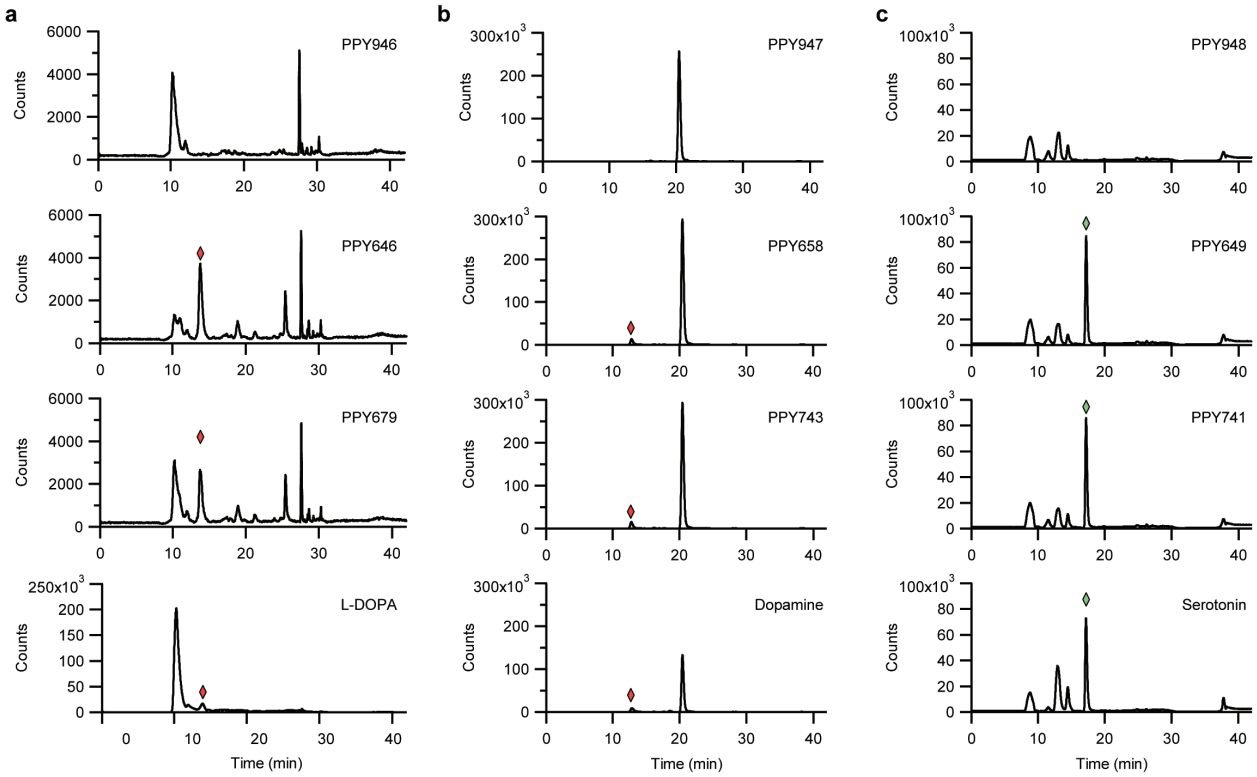
SI Figure 4. Structural alignment of *Mortirella alpina* and *Thalassiosira pseudonana* sepiapterin reductase (SR) Structural alignment of homology models of *M. alpina* SR (green) and *T. pseudonana* (cyan) SR obtained via structural homology to PDB:1Z6Z and 3ICC, respectively, using SWISS-MODEL[3-5]. NADPH (yellow) and biopterin (dark blue) were obtained from the crystal structure of mouse SR (PDB:1SEP). While arginine residues are present in the *M. alpina* structure to stabilize the phosphate group of NADPH, there are no stabilizing residues present in the *T. pseudonana* structure. Alignment was completed with PyMOL.



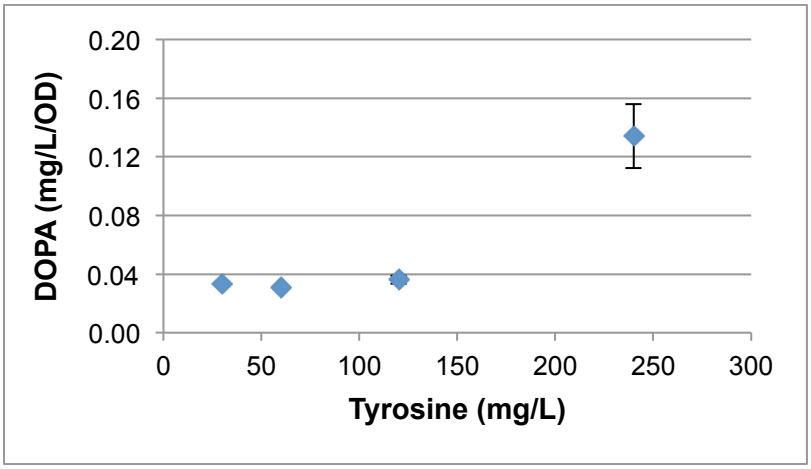
SI Figure 5. Purine biosynthetic pathway from the Kyoto Encyclopedia of Genes and Genomes (KEGG)[6]. The enzyme encoded by *Ade2* is boxed in red. GTP is boxed in green.



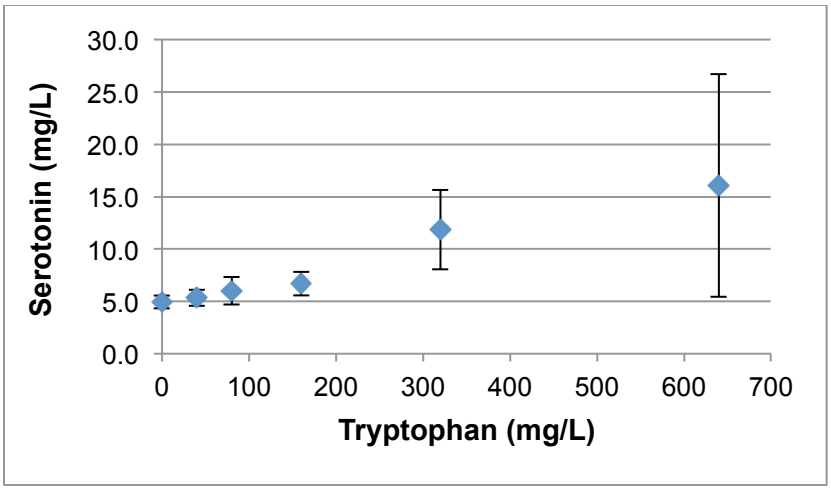
SI Figure 6. GTPCH, PTPS and SR mRNA levels. Multi-copy: multi-copy plasmid. Single-copy: single-copy plasmid. Values represent the mean of two reactions.



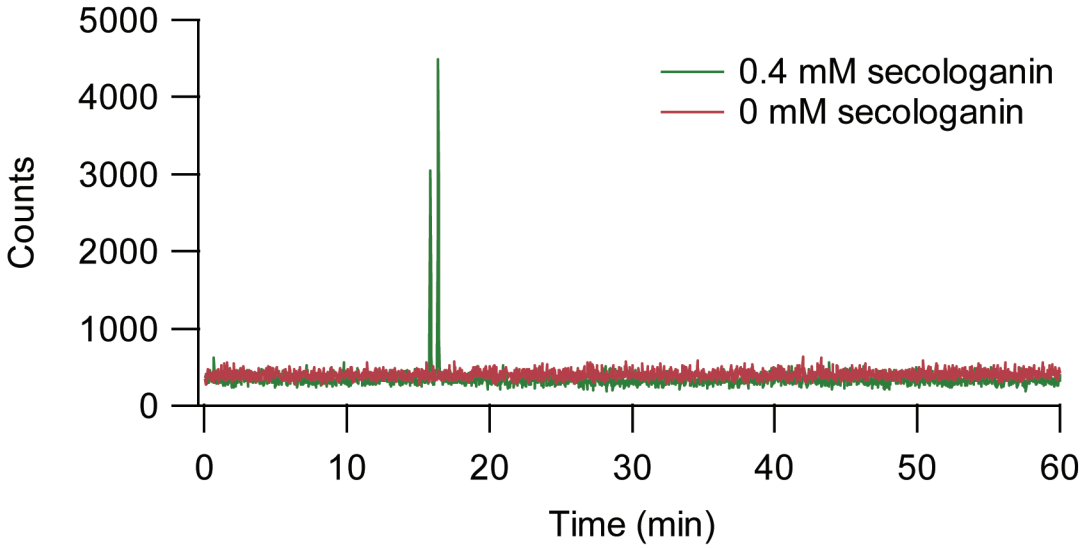
SI Figure 7 Full windows of LC traces in Figures 4b, 5b, 5f. L-DOPA, dopamine and serotonin highlighted with a pink (L-DOPA, dopamine) or green diamond (serotonin). (**a**) L-DOPA (13.8 min), (**b**) dopamine (12.8 min), and (**c**) serotonin (17.2 min).



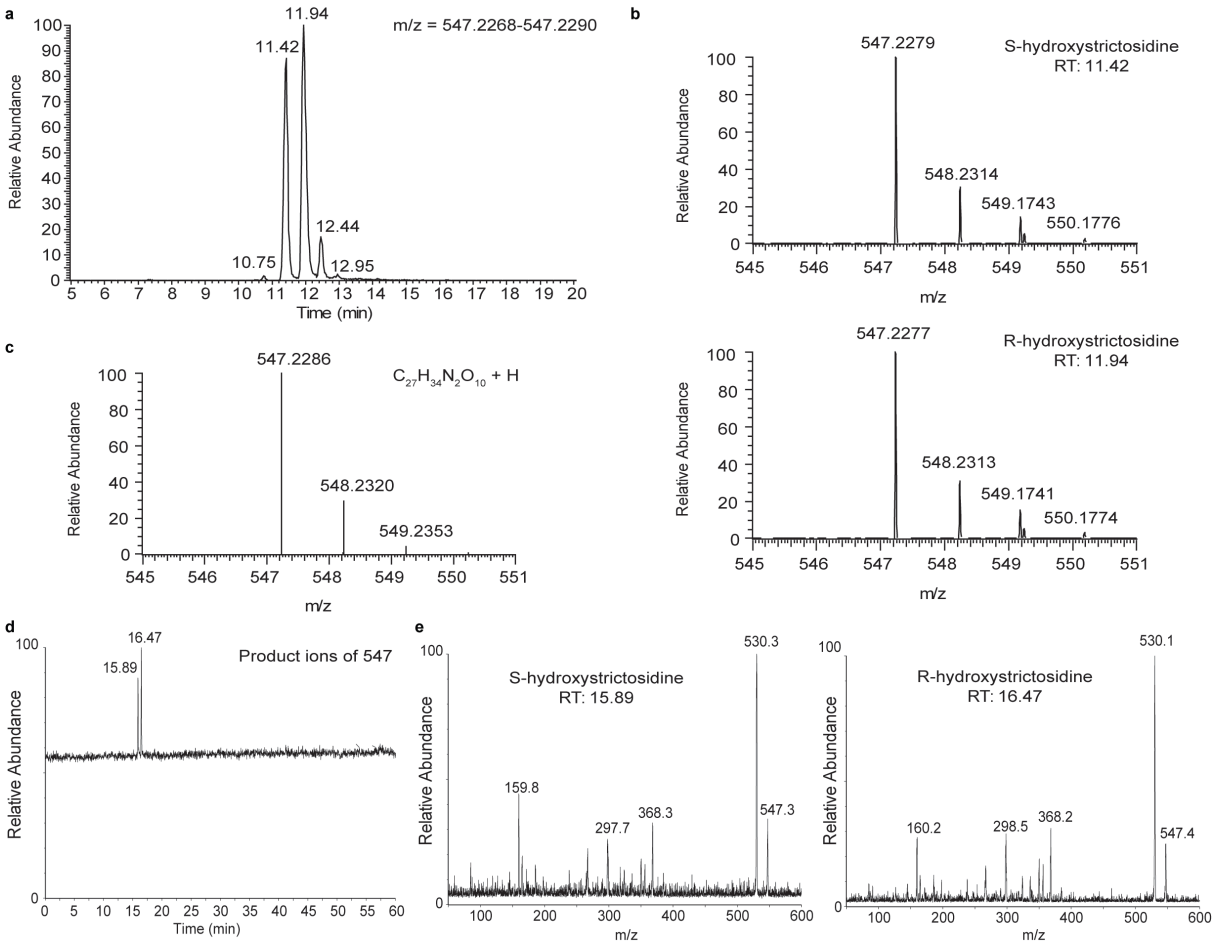
SI Figure 8. Effect of tyrosine on L-DOPA production. In our experiments, 30 mg/L of tyrosine is present when producing L-DOPA or dopamine.



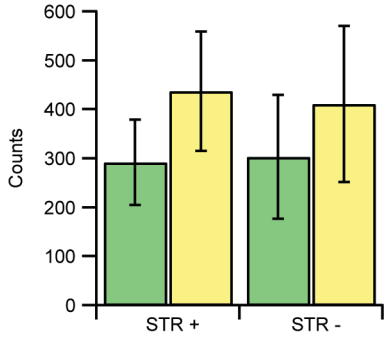
SI Figure 9. Effect of tryptophan on serotonin production. In our experiments, tryptophan is not supplemented when producing serotonin or hydroxystrictosidine.



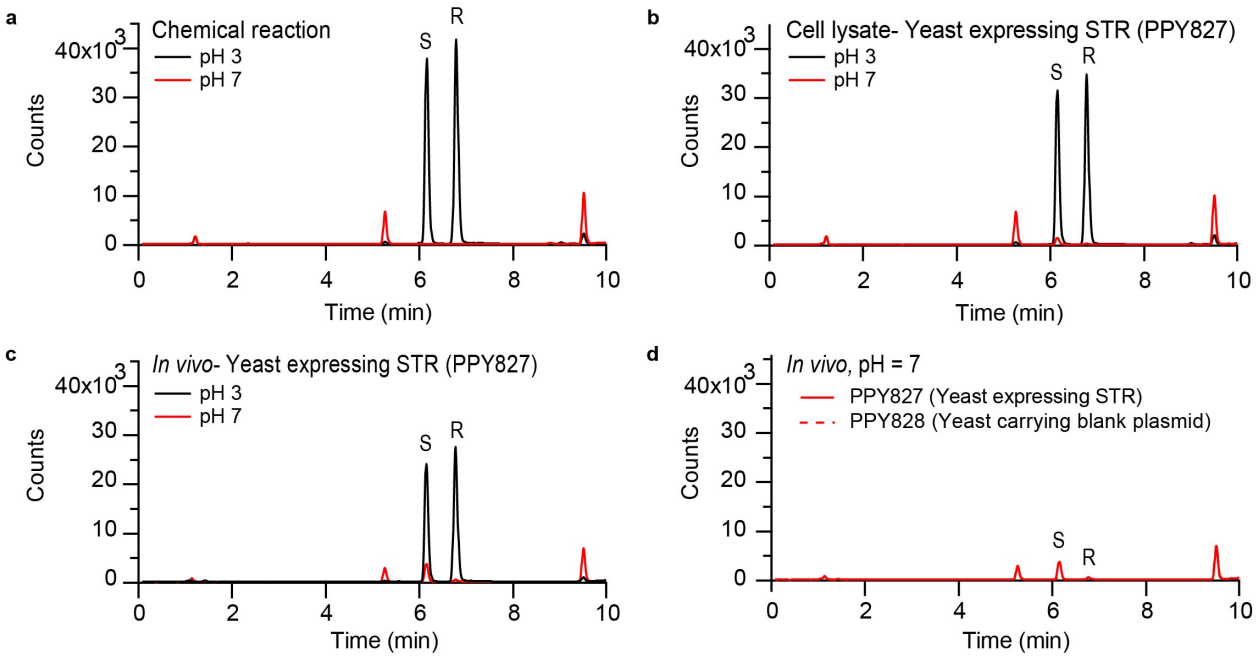
SI Figure 10. Full window of multiple reaction monitoring for Figure 6b. Shown hydroxystrictosidine transition: 547.60 → 530.00 transition.



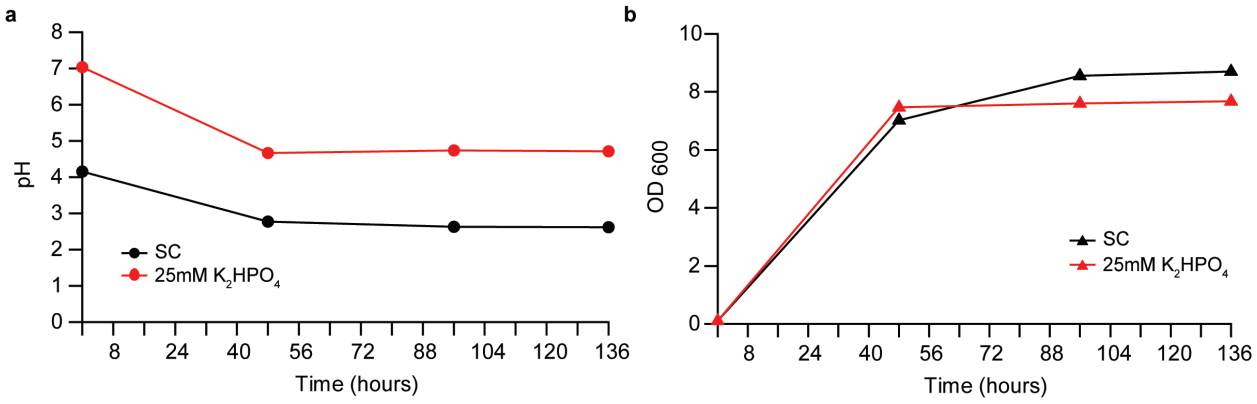
SI Figure 11. Mass spectral characterization of hydroxystrictosidine isomers. (**a**) LC trace (extracted ion chromatogram corresponding to hydroxystrictosidine, *m/z* 547, extracted from full scan data) from high resolution mass spectrometry analysis. (**b**) High-resolution mass spectrum of microbially-produced *S*-hydroxystrictosidine and *R*-hydroxystrictosidine. (**c**) Theoretical high-resolution mass spectrum of hydroxystrictosidine. (**d**) LC trace for product ions of *m/z* 547 from tandem mass spectrometry analysis. (**e**) Tandem mass spectra of microbially-produced *S*-hydroxystrictosidine and *R*-hydroxystrictosidine.



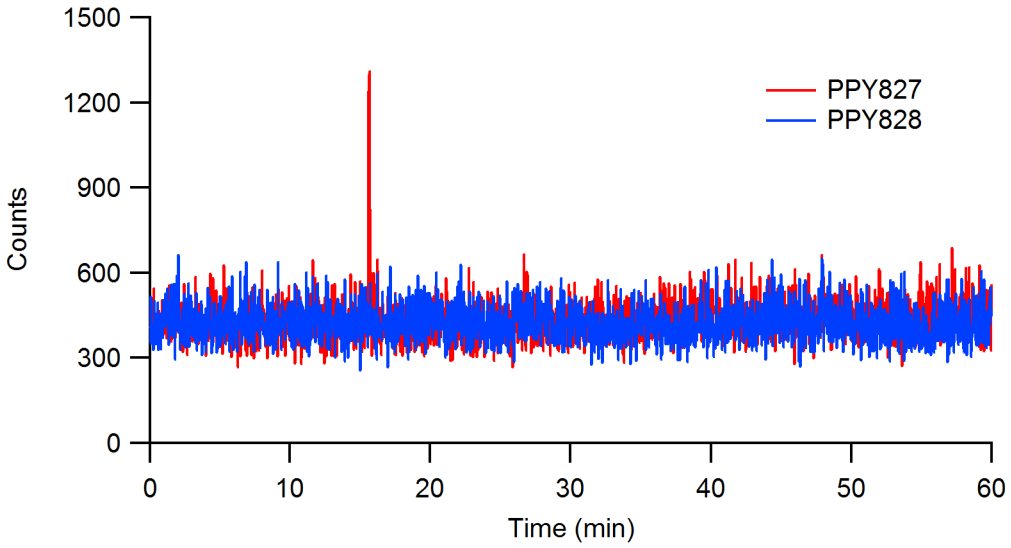
SI Figure 12. Isomer ratios produced in the presence (STR +, PPY650) or absence (STR -, PPY649) of strictosidine synthase using yeast synthetic media (pH=5-3). Green: *S*-hydroxystrictosidine; Yellow: *R*-hydroxystrictosidine.



SI Figure 13. Full windows of LC traces in Figure 7 (extracted ion chromatograms for hydroxystrictosidine, *m/z* 547). *S*-hydroxystrictosidine, rt =6.1 min. *R*-hydroxystrictosidine, rt =6.8 min. Isomer identification was determined due to the fact that strictosidine synthase is known to form only the S-isomer. A single isomer is formed *in vivo* in buffered synthetic media (pH=7 → 5).



SI Figure 14. pH of media over time. (**a**) pH of wild-type W303 yeast grown in synthetic complete media (black) or buffered synthetic complete media (25mM K_2HPO_4, red). (**b**) Cell growth monitored by absorption at OD_{600}.



SI Figure 15. Full window for multiple reaction monitoring (MRM) analysis for Figure 7d. Only one peak can be found in the MRM spectrum corresponding to the characteristic hydroxystrictosidine transition 547.60 → 530.00. All other peaks in the Figure 7d (and SI Figure 13d) LC trace have *m/z* value of 547, but do not have the characteristic hydroxystrictosidine transition 547.60→530.

Gene sequences used in this study (His$_6$ tag underlined)

Escherichia coli GTP cyclohydrolase I (UniProtKB- P0A6T5)

ATGCATCACCATCACCATCACCCATCACTCAGTAAAGAAGCGGCCCTGGTTCATGAAGCGTTAGTTGCGC
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Mortierella alpina GTP cyclohydrolase I (UniProtKB- G3FNL6)

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Saccharomyces cerevisiae GTP cyclohydrolase I (UniProtKB- P51601）

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Homo sapiens GTP cyclohydrolase I (UniProtKB- P30793-1)

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A

Mortierella alpina 6-pyruvoyl tetrahydrobiopterin synthase (UniProtKB- G3FNL7)

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Salmo salar 6-pyruvoyl tetrahydrobiopterin synthase (UniProtKB- B5XE18)

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Salinibacter ruber 6-carboxy-5,6,7,8-tetrahydropterin synthase (UniProtKB- Q2RYU6)

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AGAGGTGAATAA

Phycisphaera mikurensis Putative 6-pyruvoyl tetrahydrobiopterin synthase (UniProtKB- I0IIJ5)
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Mortierella alpina Sepiapterin reductase (UniProtKB- G3FNL8)
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TTGGTCATTCCGTTGCATTGGATTACATAAGACATTCAGGTGCTCATGCTGTTTCCTTTGTTTTGGTTGG
TAGAACTCAACATTCCTTGGAACAAGTTTTGACTGAATTGCATGAAGCTGCATCTCATGCTGGTGTTGTC
TTCAAGGGTGTCGTTGTCTCCGAAGTTGATTTGGCTCATTTGAACTCTTTGGATTCTAATTTGGCTAGAA
TACAATCTGCTGCAGCTGATTTGAGAGACGAAGCTGCACAATCTACCAGAACTATTACTAAGTCTGTTTT
GTTTAATAACGCTGGTTCATTGGGTGATTTGTCCAAGACTGTTAAGGAATTTACCTGGCAAGAAGCTAGA
TCCTACTTGGATTTCAACGTCGTTTCCTTAGTTGGTTTGTGTTCCATGTTCTTGAAGGATACCTTGGAAG
CATTTCCAAAGGAACAATATCCAGATCATAGAACTGTTGTCGTTTCCATTTCTTCCTTGTTAGCTGTTCA
AGCATTCCCAAATTGGGGTTTGTACGCTGCAGGTAAGGCAGCTAGAGATAGATTGTTAGGTGTTATTGCT
TTGGAAGAAGCAGCTAATAACGTTAAGACCTTGAATTACGCTCCAGGTCCATTGGATAACGAAATGCAAG
CTGATGTTAGAAGAACCTTGGGTGATAAAGAACAATTGAAGATTTACGATGATATGCATAAGTCTGGTTC
CTTGGTTAAGATGGAAGATTCCTCTAGAAAGTTGATTCATTTGTTAAAGGCTGATACCTTCACCTCCGGT
GGTCATATTGATTTCTACGATGAATAA

Saccharomyces cerevisiae Putative cytoplasmic short-chain dehydrogenase/reductase (UniProtKB-P40579)
ATGCATCACCATCACCATCACGGTAAAGTTATTTTAGTTACAGGTGTTTCCAGAGGTATCGGTAAGTCCA
TCGTGGATGTTCTTTTCAGTTTGGACAAGGACACGGTTGTTTACGGTGTAGCCAGGTCTGAGGCACCCTT
GAAGAAGTTGAAAGAGAAGTATGGCGACAGGTTTTTTTACGTTGTCGGTGATATTACCGAGGATTCCGTG
TTGAAGCAGTTGGTTAACGCTGCTGTTAAGGGCCACGGCAAGATCGACTCCTTGGTTGCCAACGCTGGTG
TCCTAGAGCCCGTGCAAAATGTCAACGAGATTGATGTCAACGCTTGGAAGAAGCTGTATGACATCAACTT
CTTCAGCATTGTTTCCTTGGTTGGCATTGCGTTACCTGAATTGAAGAAGACCAACGGTAACGTGGTATTC
GTCAGTTCGGACGCCTGTAACATGTACTTCAGCAGTTGGGGAGCTTACGGTTCTTCAAAAGCCGCTCTGA
ACCACTTCGCCATGACTCTGGCCAACGAGGAAAGGCAAGTGAAAGCCATTGCCGTCGCCCCAGGTATTGT
GGACACAGATATGCAAGTTAACATTAGGGAGAACGTGGGGCCTTCCTCCATGAGTGCAGAGCAATTGAAG
ATGTTTAGAGGTTTAAAGGAGAATAACCAGTTGCTGGATAGCTCTGTGCCAGCTACAGTTTATGCCAAAT
TGGCCCTTCATGGTATTCCTGACGGTGTTAATGGACAGTACTTGAGCTATAATGACCCTGCCTTGGCGGA
CTTTATGCCTTAA

Thalassiosira pseudonana Sepiapterin reductase (UniProtKB- B8BVR3)
ATGCATCACCATCACCATCACCAAAACAAGGAAAACGATGAAACCTCCATTGTTGTCGATATTCATGAAA
TGGATTTGTCCGATTTGGATATTTTGGCTGTTAACATGAAGTTGTTGTTTGAATTCTACACCAAGGTTAC

CAAGTACAATCAATGTTGGTTGTTCAACAATGCTGGTTCCTTGGGTCCATTGGGTCCAACCTTGTCCTTG
TGTAACGGTGATCCATTGAGATTAATGCAAGATTTGAAGAAAGCTGTTGATTTGAACGTTACCTCCGCTA
CCTGGATTTCCTCACAATTCGTTTCCACCTTTGGTTCCTCTCATAAGGACGATACTCCACCATTGGTTAG
AATTGTTAACATTTCTTCCTTGTGTGCTATTGAACCATTCCAAACTATGGCTGTTTACTGTATGGGTAAG
GCTGCAAGAGATATGTACCATTTGGTTTTGGCTAAAGAACATAAGGATTCCGATACTATGAAAGTTTTGA
ACTACGCTCCAGGTCCTTGTGATACTGAAATGACTGATGTTTTGGCTGGTTCTGCTGTTTTGGATTGGGA
TTTGCATCAATATTACGCTACATCCAAGAGAGATCAAAAGTTGGTTGATCCTTTGGATTCTGCTAAGAAA
TTGATTGAATTGTTAGAAAAGGATGAATTCACCACAGGTTCCCATGTTGATTACTTCGATGTTTAA

Homo sapiens Pterin-4-alpha-carbinolamine dehydratase (UniProtKB- P61457)
ATGGCTGGTAAAGCTCATAGATTGTCTGCTGAAGAAAGAGATCAATTGTTGCCAAACTTGAGAGCTGTTG
GTTGGAACGAATTGGAAGGTAGAGATGCTATTTTCAAGCAATTCCATTTCAAAGATTTCAATAGAGCCTT
CGGTTTCATGACTAGAGTTGCCTTGCAAGCTGAAAAGTTAGATCATCATCCAGAATGGTTCAACGTCTAC
AATAAGGTCCATATTACCTTGTCCACTCATGAATGTGCTGGTTTGTCTGAAAGAGATATTAACTTGGCAT
CCTTCATTGAACAAGTCGCTGTCTCCATGACTTAA

Homo sapiens Dihydropteridine reductase (UniProtKB- P09417-1)
ATGGCAGCTGCTGCAGCCGCTGGTGAAGCTAGAAGAGTTTTGGTTTACGGTGGTAGAGGTGCTTTGGGTT
CTAGATGTGTCCAAGCATTCAGAGCTAGAAATTGGTGGGTTGCTTCTGTTGATGTCGTTGAAAACGAAGA
AGCATCTGCTTCTATTATTGTTAAAATGACTGATTCTTTTACTGAACAAGCTGATCAAGTTACTGCTGAA
GTTGGTAAATTGTTAGGTGAAGAGAAAGTTGATGCTATTTTGTGTGTTGCTGGTGGTTGGGCTGGTGGTA
ACGCTAAATCTAAATCTTTGTTTAAGAATTGTGATTTGATGTGGAAACAATCTATTTGGACTTCTACTAT
TTCTTCTCATTTGGCTACTAAACATTTGAAAGAAGGTGGTTTGTTAACTTTGGCAGGTGCTAAAGCTGCT
TTGGATGGTACTCCAGGTATGATTGGTTACGGTATGGCTAAAGGTGCAGTTCATCAATTGTGTCAATCTT
TGGCTGGTAAGAACTCTGGTATGCCACCTGGTGCAGCTGCTATTGCTGTTTTGCCAGTTACTTTGGATAC
ACCAATGAATAGAAAATCTATGCCAGAAGCTGATTTCTCTTCTTGGACTCCATTGGAATTCTTGGTTGAA
ACTTTTCATGATTGGATTACTGGAAAGAATAGACCATCTTCTGGTTCTTTGATTCAAGTTGTTACTACTG
AAGGTAGAACTGAATTGACTCCAGCTTATTTCTAA

Mus musculus Tyrosine 3-monooxygenase (UniProtKB- P24529)
ATGCCAACTCCATCCGCTTCCTCCCCACAACCAAAGGGTTTCAGACGCGCTGTGTCTGAACAAGATACTA
AGCAAGCTGAAGCTGTTACTTCCCCAAGATTCATCGGTAGAAGACAATCTTTGATTGAAGATGCTAGAAA
GGAAAGAGAAGCTGCAGCTGCAGCCGCTGCAGCCGCTGTTGCTTCTGCTGAACCAGGTAATCCATTGGAA
GCTGTTGTCTTCGAAGAAAGAGATGGTAATGCTGTTTTGAATTTGTTGTTCTCTTTGAGAGGTACTAAGC
CATCTTCCTTGTCTAGAGCTCTAAAGGTATTCGAAACTTTCGAAGCTAAGATTCATCATTTGGAAACTAG
ACCTGCACAAAGACCATTGGCTGGTTCCCCACATTTGGAATACTTCGTTAGATTTGAAGTTCCATCCGGT
GATTTGGCTGCTTTGTTGTCTTCCGTTAGAAGAGTTTCTGATGATGTTAGATCCGCTAGAGAAGATAAGG
TTCCTTGGTTTCCAAGAAAGGTTTCTGAATTGGATAAGTGTCATCATTTGGTTACTAAGTTTGATCCAGA
TTTGGATTTGGATCATCCAGGTTTCTCCGATCAAGCATACAGACAAAGAAGAAAGTTGATTGCTGAAATT
GCTTTCCAATACAAGCAAGGTGAACCAATTCCACATGTTGAATACACTAAGGAAGAAATTGCTACTTGGA
AGGAAGTTTACGCTACTTTGAAGGGTTTGTACGCTACTCATGCTTGTAGAGAACATTTGGAAGCATTTCA
ATTGTTGGAAAGATACTGTGGTTACAGAGAAGATTCTATTCCACAATTGGAAGATGTTTCTCATTTCTTG
AAGGAAAGAACTGGTTTCCAATTGAGACCAGTTGCTGGTTTGTTGTCCGCTAGAGATTTCTTGGCTTCCT
TGGCTTTCAGAGTTTTCCAATGTACTCAATACATTAGACATGCTTCCTCCCCAATGCATTCTCCAGAACC
AGATTGTTGTCATGAATTGTTGGGTCATGTTCCAATGTTGGCTGATAGAACTTTCGCTCAATTCTCTCAA
GATATTGGTTTGGCTTCTTTGGGTGCTTCTGATGAAGAAATTGAAAAGTTGTCCACTGTTTACTGGTTTA
CTGTTGAATTTGGTTTGTGTAAGCAAAATGGTGAATTGAAGGCTTACGGTGCCGGATTGTTGTCCTCTTA
CGGTGAATTGTTGCATTCTTTGTCTGAAGAACCAGAAGTTAGAGCTTTCGATCCAGATACTGCTGCTGTT
CAACCATACCAAGATCAAACTTACCAACCAGTTTACTTCGTTTCTGAATCTTTCTCTGATGCTAAGGATA
AGTTGAGAAATTACGCTTCTAGAATCCAAAGACCATTCTCTGTTAAGTTTGATCCATACACTTTGGCTAT

TGATGTCTTGGATTCTCCACATACTATTAGAAGATCTTTGGAAGGTGTTCAAGATGAATTGCATACTTTG
ACTCAAGCATTGTCTGCTATTTCTTAA

Homo sapiens Tryptophan-5-hydroxylase 2 isoform 1 AA145-460 (UniProtKB- Q8IWU9-1)
ATGGAAGAATTGGAAGATGTTCCTTGGTTCCCAAGAAAGATTTCCGAATTGGATAAGTGTTCCCATAGAG
TTTTGATGTATGGTTCCGAATTGGATGCTGATCATCCAGGTTTCAAGGATAATGTTTACAGACAAAGAAG
AAAGTACTTCGTTGATGTTGCTATGGGTTACAAGTACGGTCAACCAATTCCAAGAGTTGAATACACTGAA
GAAGAAACTAAGACTTGGGGCGTTGTGTTCAGAGAATTGTCCAAGTTGTACCCAACTCATGCTTGTAGAG
AATACTTGAAGAATTTCCCATTGTTGACTAAGTACTGTGGTTACAGAGAAGATAATGTTCCACAATTGGA
AGATGTTTCCATGTTCTTGAAGGAAAGATCCGGTTTCACTGTTAGACCAGTTGCTGGTTACTTGTCCCCA
AGAGATTTCTTGGCTGGTTTGGCTTACAGAGTCTTCCATTGTACTCAATACATTAGACATGGTTCCGATC
CATTGTACACTCCAGAACCAGATACTTGTCATGAATTGTTGGGTCATGTTCCATTGTTGGCTGATCCAAA
GTTCGCTCAATTCTCCCAAGAAATTGGTTTGGCTTCCTTGGGTGCTTCCGATGAAGATGTTCAAAAGTTG
GCTACTTGTTACTTCTTCACTATTGAATTCGGTTTGTGTAAGCAAGAAGGTCAATTGAGAGCTTACGGTG
CTGGTTTGTTATCCTCTATTGGTGAATTGAAGCACGCTTTGTCCGATAAGGCTTGTGTTAAGGCTTTCGA
CCCAAAGACTACTTGTTTGCAAGAATGTTTGATTACTACTTTCCAAGAAGCATACTTCGTTTCCGAATCC
TTCGAAGAAGCTAAGGAGAAGATGAGAGATTTCGCTAAGTCCATTACTAGACCATTCTCCGTTTACTTCA
ATCCATACACTCAATCCATTGAAATTTTGAAGGATACTTAA

Sus scrofa Aromatic-L-amino-acid decarboxylase (UniProtKB- P80041)
ATGAATGCTTCTGATTTTAGAAGGAGAGGTAAAGAAATGGTTGACTACATGGCTGATTACTTGGAAGGTA
TTGAAGGTAGACAAGTTTACCCAGATGTTCAACCAGGTTACTTGAGACCATTGATTCCAGCTACTGCTCC
ACAAGAACCAGATACTTTTGAAGATATTTTGCAAGATGTTGAGAAGATTATTATGCCAGGTGTCACACAT
TGGCACTCGCCATACTTCTTTGCTTACTTCCCAACTGCTTCCTCCTACCCAGCTATGTTGGCTGATATGT
TGTGTGGTGCTATTGGTTGTATTGGTTTCTCCTGGGCTGCTTCCCCAGCTTGTACTGAATTGGAAACTGT
TATGATGGATTGGTTGGGTAAAATGTTGCAATTGCCAGAAGCCTTCTTGGCTGGTGAAGCTGGTGAAGGT
GGTGGTGTTATTCAAGGTTCCGCTTCCGAAGCTACTTTGGTTGCTTTGTTGGCTGCTAGAACTAAAGTTA
CTAGAAGATTGCAAGCTGCTTCTCCAGGTTTGACTCAAGGTGCTGTTTTGGAGAAGTTGGTTGCTTACGC
CTCCGACCAAGCTCATTCCTCCGTTGAAAGAGCTGGTTTGATTGGTGGTGTTAAATTGAAAGCTATTCCA
TCCGATGGTAAATTTGCTATGAGAGCTTCCGCTTTGCAAGAAGCCTTGGAAAGAGATAAAGCTGCTGGTT
TGATTCCATTCTTCGTTGTTGCTACTTTGGGTACTACTTCCTGTTGTTCCTTTGATAATTTGTTGGAAGT
TGGTCCAATTTGTCATGAAGAAGATATTTGGTTGCATGTTGATGCTGCTTACGCTGGTTCCGCTTTCATT
TGTCCAGAATTTAGACATTTGTTGAATGGTGTTGAATTTGCTGATTCCTTTAATTTCAATCCACATAAAT
GGTTGTTGGTTAATTTTGATTGTTCCGCTATGTGGGTTAAAAGAAGAACTGATTTGACTGGTGCTTTTAA
ATTGGACCCAGTTTACTTGAAACATTCCCATCAAGGTTCCGGTTTGATTACTGATTACAGACATTGGCAA
TTGCCATTGGGTAGAAGATTTAGATCCTTGAAAATGTGGTTTGTCTTCAGAATGTACGGTGTTAAAGGTT
TGCAAGCCTACATTAGAAAGCATGTTCAATTGTCCCATGAATTTGAAGCCTTTGTTTTGCAAGATCCAAG
ATTTGAAGTTTGTGCTGAAGTTACTTTGGGTTTGGTTTGTTTTAGATTGAAAGGTTCCGATGGTTTGAAT
GAGGCTTTGTTGGAAAGAATTAATTCCGCTAGAAAGATTCATTTGGTTCCATGTAGATTGAGAGGTCAAT
TTGTTTTGAGATTTGCTATTTGTTCCAGAAAAGTTGAATCCGGTCATGTTAGATTGGCTTGGGAACATAT
TAGAGGTTTGGCTGCTGAATTGTTGGCTGCTGAAGAAGGTAAAGCTGAAATTAAATCTTAA

Ophiorrhiza pumila Strictosidine synthase AA26-350, His_6 only included in pAME64 (UniProtKB- Q94LW9)[7]
ATGGGCTCTCCTGAGTTTTTCGAATTTATTGAAGCACCGTCTTATGGTCCAAATGCGTATGCGTTCGACA
GCGACGGCGAGTTGTATGCGAGCGTGGAAGACGGTCGTATTATCAAGTACGACAAGCCTTCTAACAAATT
CCTGACTCATGCTGTTGCCAGCCCGATCTGGAACAATGCCCTGTGTGAGAATAATACCAACCAAGACCTG
AAGCCGCTGTGCGGTCGCGTCTACGACTTTGGTTTTCATTATGAAACGCAGCGCCTGTACATTGCAGATT
GCTACTTCGGCTTGGGCTTTGTTGGTCCGGACGGCGGTCACGCGATTCAACTGGCAACCTCCGGTGATGG
CGTTGAGTTCAAGTGGCTGTACGCGTTGGCGATCGACCAACAGGCAGGCTTCGTCTACGTGACGGACGTT
TCTACTAAGTACGATGATCGTGGTGTTCAGGACATTATTCGCATTAATGATACCACGGGTCGCCTGATTA

AGTATGACCCTTCGACCGAAGAGGTGACCGTGCTGATGAAAGGCCTGAATATTCCGGGCGGTACCGAGGT
TAGCAAAGACGGTAGCTTTGTGCTGGTTGGTGAGTTCGCGTCGCATCGTATCCTGAAGTACTGGCTGAAG
GGTCCGAAGGCCAATACCAGCGAGTTTCTGCTGAAGGTGCGCGGTCCAGGTAATATCAAACGTACCAAAG
ATGGTGATTTCTGGGTTGCGTCCAGCGATAACAACGGCATCACGGTGACGCCACGTGGTATCCGCTTCGA
TGAGTTTGGCAACATTCTGGAGGTCGTTGCTATTCCGCTGCCGTATAAAGGTGAACATATCGAGCAGGTC
CAAGAACACGACGGCGCCCTGTTCGTGGGTAGCCTGTTTCATGAGTTCGTCGGCATCCTGCATAACTATA
AGAGCAGCGTTGACCATCATCAGGAAAAGAACTCGGGTGGTCTGAACGCGAGCTTCAAGGAGTTCTCTTC
GTTTGGATCTCATCACCATCACCATCACTAG

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