

Supporting Information

Total Heterologous Biosynthesis of Fungal Natural Products in *Aspergillus nidulans*

This review is dedicated to Professor Yueh-Hsiung Kuo on the occasion of his 80th birthday.

Yi-Ming Chiang,^{†, ‡,*} Tzu-Shyang Lin,[†] and Clay C.C. Wang,^{†, §,*}

[†]Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, California 90089, USA

[‡]Department of Pharmacy, Chia Nan University of Pharmacy and Science, Tainan 71710, Taiwan

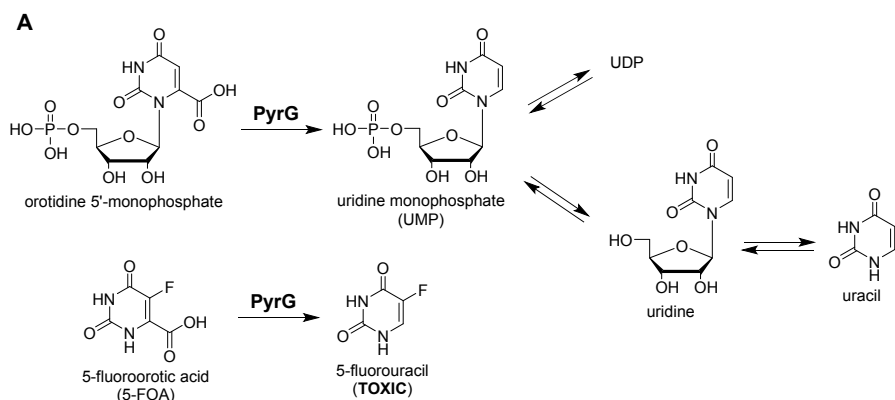
[§]Department of Chemistry, Dornsife College of Letters, Arts, and Sciences, University of Southern California, Los Angeles, California 90089, USA

Present address for Y.-M Chiang: Hexagon Bio, Inc. Menlo Park, California 94025, USA

*Corresponding authors. ymchiang@mail.cnu.edu.tw (Y.-M. Chiang) and clayw@usc.edu (C. C. C. Wang)

Table of Contents

Figure S1. Selection principle of the auxotrophic markers <i>pyrG</i> , <i>riboB</i> , and <i>pyroA</i> .	S3
Figure S2. <i>AMA1</i> -based episomal expression vectors.	S4
Table S1. Vectors used for <i>cgh</i> expression and their corresponding chemical products.	S5
Table S2. Vectors used for <i>vir</i> expression and their corresponding chemical products.	S6
Table S3. Vectors used for <i>dcs</i> expression and their corresponding chemical products.	S7
Table S4A. Vectors used for <i>hql</i> expression and their corresponding chemical products.	S8
Table S4B. Feeding of different substrates to the HqlC – F expression strain and the products identified.	S8
Table S5. Vectors used for <i>frz</i> expression and their corresponding chemical products.	S9
Table S6. Vectors used for <i>qlt</i> expression and their corresponding chemical products.	S10
Table S7. Vectors used for <i>pvh</i> expression and their corresponding chemical products.	S11
Table S8. Vectors used for <i>lep</i> expression and their corresponding chemical products.	S12
Table S9. Vectors used for <i>icc</i> expression and their corresponding chemical products.	S13
Table S10. Vectors used for <i>har</i> expression and their corresponding chemical products.	S14
Table S11. Vectors used for <i>pfp</i> expression and their corresponding chemical products.	S15
Table S12. Vectors used for <i>fub</i> expression and their corresponding chemical products.	S16
Table S13. Vectors used for <i>thm</i> expression and their corresponding chemical products.	S17
Table S14. Vectors used for <i>cm3</i> expression and their corresponding chemical products.	S18
Table S15. Vectors used for <i>Pgmpa</i> expression and their corresponding chemical products.	S19
Table S16A. Vectors used for <i>flv</i> expression and their corresponding chemical products.	S20
Table S16B. Feeding of different substrates to the various Flv expression strains and the products identified.	S20
Table S17. Vectors used for <i>elc</i> expression and their corresponding chemical products.	S21
Table S18. Vectors used for <i>vdt</i> expression and their corresponding chemical products.	S22
Table S19. Vectors used for <i>sth</i> expression and their corresponding chemical products.	S23
Table S20. Vectors used for <i>nan</i> expression and their corresponding chemical products.	S24
Table S21. Vectors used for <i>buA</i> expression used and their corresponding chemical products.	S25
Table S22. Different combinations of <i>Ateafo</i> genes and their corresponding chemical products.	S26
Table S23. Different combinations of <i>ctv</i> genes and their corresponding chemical products.	S27



medium	genotype	<i>pyrG</i> ⁺	<i>pyrG</i> ⁻
no supplements		growth	no growth
uridine/uracil + 5-FOA		no growth	growth

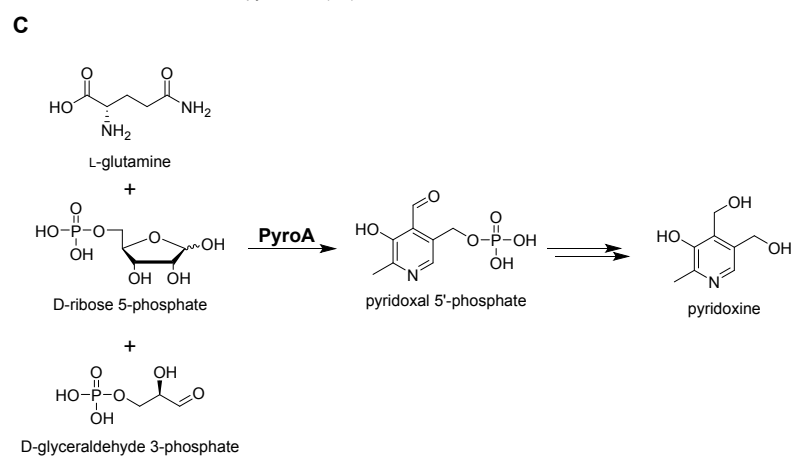
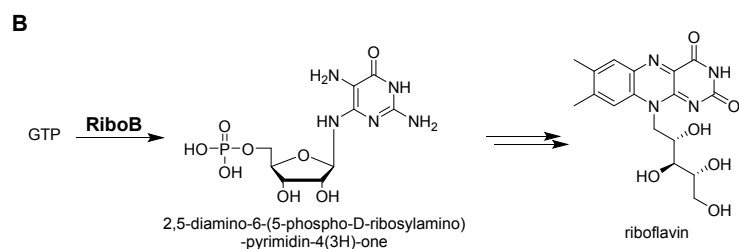


Figure S1. Selection principle of the auxotrophic markers *pyrG*, *riboB*, and *pyroA*. (A) *PyrG* catalyzes the conversion of orotidine 5'-monophosphate to UMP. Therefore, fungal cells with *pyrG*⁻ require uridine/uracil to grow. *PyrG* also catalyzes the conversion of 5-FOA to 5-fluorouracil, which is toxic to fungal cells. Only fungal cells with *pyrG*⁻ can grow in the presence of uridine/uracil and 5-FOA (counter-selection). (B) *RiboB* catalyzed the conversion of GTP to 2,5-diamino-6-(5-phospho-D-ribosylamino)-pyrimidin-4(3H)-one, a precursor of riboflavin. (C) *PyroA* catalyzed the conversion of L-glutamine, D-ribose 5-phosphate, and D-glyceraldehyde 3-phosphate to pyridoxal 5'-phosphate, a precursor of pyridoxine. Gene functions of *pyrG*, *riboB*, and *pyroA* were curated from FungiDB (<https://fungidb.org>).

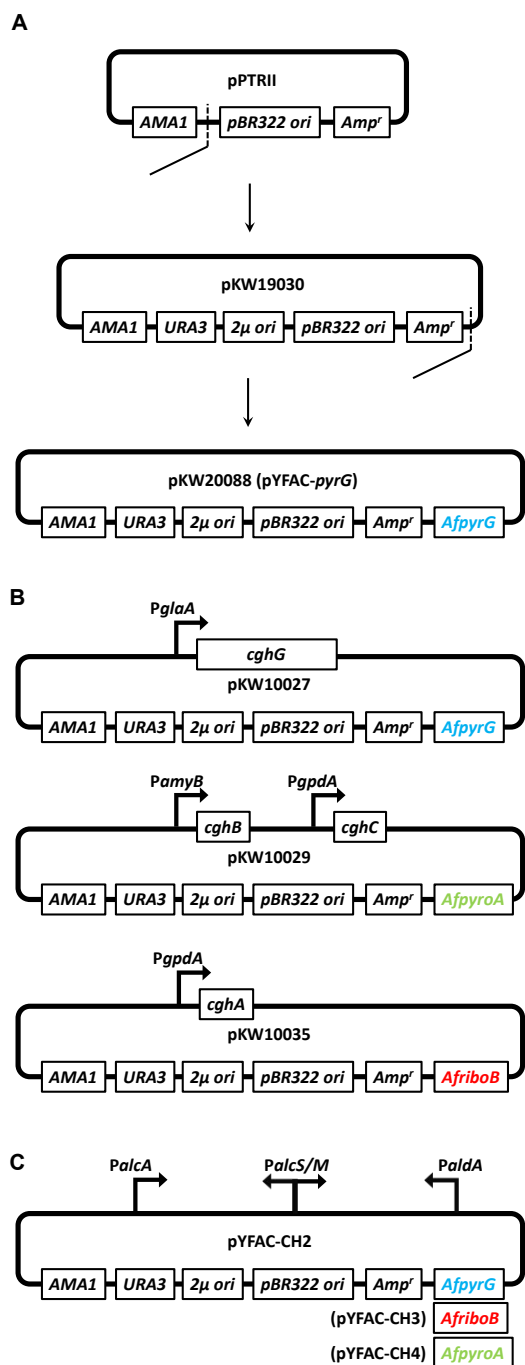


Figure S2. *AMA1*-based episomal expression vectors. (A) pKW20088 is derived from pPTRII, which contains the *AMA-1* replicator for extrachromosomal vector maintenance in *Aspergillus* spp. as well as pBR322 *ori* and *Amp^r* for plasmid maintenance and selection in *E. coli*. Insertion of *2μ ori* and *URA3* allows plasmid maintenance and selection in *S. cerevisiae* (pKW19030). Addition of *AfpyrG* enables selection of successful transformants in *Aspergillus* (pKW20088). (B) Expression vectors used to reconstitute the biosynthesis of Sch 210972 (**1**) (see Figure 3). (C) Yeast fungal artificial chromosome (YFAC) expression vectors developed by the Chooi group allow the expression of four GOIs per vector.

Table S1. Vectors used for *cgh* expression and their corresponding chemical products.

Expression cassettes	Enzymes expressed	Products
1. <i>PglaA-cghG_AfpYrG</i> 2. <i>PgpdA-cghA_AfriboB</i> 3. <i>PamyB-cghB_PgpdA-cghC_AfpyroA</i>	CghA/B/C/G	1
1. <i>PglaA-cghG_AfpYrG</i> 2. <i>PamyB-cghB_PgpdA-cghC_AfpyroA</i>	CghB/C/G	1 + 4

Table S2. Vectors used for *vir* expression and their corresponding chemical products.

Expression cassettes	Enzymes expressed	Products
1. <i>PglaA-virI_PgpdA-virE_PamyB-virD_PtrpC-virB_AfpyrG</i> 2. <i>PgpdA-virK_PamyB-virJ_PglaA-virG_PtrpC-virF_AfriboB</i> 3. <i>PamyB-virL_PtrpC-virH_PglaA-virC_PgpdA-virA_AfpyroA</i>	VirA – L	7 (1.2 mg/L) + 8 (0.3 mg/L) + 9 (0.4 mg/L) + 10 (0.9 mg/L)
1. <i>PgpdA-virA_AfpyrG</i>	VirA	11 (1.5 mg/L)
1. <i>PgpdA-virA_AfpyrG</i> 2. <i>PgpdA-virC_AfriboB</i>	VirA/C	11 (> 1.5 mg/L)
1. <i>PgpdA-virA_AfpyrG</i> 2. <i>PgpdA-virC_PPEgpdA-virD_AfriboB</i>	VirA/C/D	11
1. <i>PgpdA-virA_PPOgpdA-virB_AfpyrG</i>	VirA/B	12 (1.7 mg/L)
1. <i>PgpdA-virA_PPOgpdA-virB_AfpyrG</i> 2. <i>PPEgpdA-virD_AfriboB</i>	VirA/B/D	9 (trace) + 10
1. <i>PgpdA-virA_PPOgpdA-virB_AfpyrG</i> 2. <i>PgpdA-virC_PPEgpdA-virD_AfriboB</i>	VirA – D	9 (trace) + 10
1. <i>PgpdA-virE_PamyB-virD_PtrpC-virB_AfpyrG</i> 2. <i>PglaA-virC_PgpdA-virA_AfpyroA</i>	VirA – E	8 (trace) + 9 (trace) + 10
1. <i>PamyB-virD_PtrpC-virB_AfpyrG</i> 2. <i>PglaA-virG_AfriboB</i> 3. <i>PglaA-virC_PgpdA-virA_AfpyroA</i>	VirA – D/G	9
1. <i>PgpdA-virE_PamyB-virD_PtrpC-virB_AfpyrG</i> 2. <i>PglaA-virG_AfriboB</i> 3. <i>PglaA-virC_PgpdA-virA_AfpyroA</i>	VirA – E/G	8 + 9
1. <i>PglaA-virI_PgpdA-virE_PamyB-virD_PtrpC-virB_AfpyrG</i> 2. <i>PglaA-virG_PtrpC-virF_AfriboB</i> 3. <i>PtrpC-virH_PglaA-virC_PgpdA-virA_AfpyroA</i>	VirA – I	9 + 10 (trace) + 14 (trace)
1. <i>PglaA-virI_PgpdA-virE_PamyB-virD_PtrpC-virB_AfpyrG</i> 2. <i>PamyB-virJ_PglaA-virG_PtrpC-virF_AfriboB</i> 3. <i>PtrpC-virH_PglaA-virC_PgpdA-virA_AfpyroA</i>	VirA – J	9 + 10 (trace) + 14 (trace)
1. <i>PglaA-virI_PgpdA-virE_PamyB-virD_PtrpC-virB_AfpyrG</i> 2. <i>PgpdA-virK_PglaA-virG_PtrpC-virF_AfriboB</i> 3. <i>PamyB-virL_PtrpC-virH_PglaA-virC_PgpdA-virA_AfpyroA</i>	VirA – I/K/L	7 + 8

Table S3. Vectors used for *dcs* expression and their corresponding chemical products.

Expression cassettes	Enzymes expressed	Products
1. <i>PamyB-dcsA_PgpdA-dcsB_AfpyroA</i>	DcsA/B	16 (~250 mg/L)
1. <i>PglaA-dcsC_AfpyrG</i> 2. <i>PamyB-dcsA_PgpdA-dcsB_AfpyroA</i>	DcsA – C	no product
1. <i>PgpdA-dcsD_AfriboB</i> 2. <i>PamyB-dcsA_PgpdA-dcsB_AfpyroA</i>	DcsA/B/D	16
1. <i>PglaA-dcsD_PgpdA-dcsC_AfpyrG</i> 2. <i>PamyB-dcsA_PgpdA-dcsB_AfpyroA</i>	DcsA – D	15

Table S4A. Vectors used for *hql* expression and their corresponding chemical products.

Expression cassettes	Enzymes expressed	Products
1. <i>PgpdA-hqID_PglaA-hqIF_AfpyrG</i> 2. <i>PgpdA-hqIA_PamyB-hqIB_AfriboB</i> 3. <i>PgpdA-hqIC_PamyB-hqIE_AfpyroA</i>	HqIA – F	18 + 19
1. <i>PgpdA-hqIA_PamyB-hqIB_AfriboB</i> 2. <i>PgpdA-hqIC_AfpyroA</i>	HqIA/B	20
1. <i>PgpdA-hqIA_PamyB-hqIB_AfriboB</i> 2. <i>PgpdA-hqIC_AfpyroA</i>	HqIA – C	20 + 21
1. <i>PglaA-hqIF_AfpyrG</i> 2. <i>PgpdA-hqIA_PamyB-hqIB_AfriboB</i> 3. <i>PgpdA-hqIC_AfpyroA</i>	HqIA – C/F	20 + 23 + 24 + 25
1. <i>PglaA-hqIF_AfpyrG</i> 2. <i>PgpdA-hqIA_PamyB-hqIB_AfriboB</i> 3. <i>PgpdA-hqIC_PamyB-hqIE_AfpyroA</i>	HqIA – C/E/F	18 + 19

Table S4B. Feeding of different substrates to the HqIC – F expression strain and the products identified.

Substrates	Expression cassettes	Enzymes expressed	Products
20	1. <i>PgpdA-hqID_PglaA-hqIF_AfpyrG</i> 2. <i>PgpdA-hqIC_PamyB-hqIE_AfpyroA</i>	HqIC – F	18 + 19
21	1. <i>PgpdA-hqID_PglaA-hqIF_AfpyrG</i> 2. <i>PgpdA-hqIC_PamyB-hqIE_AfpyroA</i>	HqIC – F	no new product
23	1. <i>PgpdA-hqID_PglaA-hqIF_AfpyrG</i> 2. <i>PgpdA-hqIC_PamyB-hqIE_AfpyroA</i>	HqIC – F	18 + 19 + 24
24	1. <i>PgpdA-hqID_PglaA-hqIF_AfpyrG</i> 2. <i>PgpdA-hqIC_PamyB-hqIE_AfpyroA</i>	HqIC – F	no new product

Table S5. Vectors used for *frz* expression and their corresponding chemical products.

Expression cassettes	Enzymes expressed	Products
1. <i>PgpdA-frzA_AfriboB</i> 2. <i>PglaA-frzB_AfpyroA</i>	FrzA/B	30 (10 mg/L)
1. <i>PgpdA-frzA_PglaA-frzC_AfriboB</i> 2. <i>PglaA-frzB_AfpyroA</i>	FrzA – C	30 + 31
1. <i>PglaA-frzD_PgpdA-frzC_AfpyrG</i> 2. <i>PgpdA-frzA_PglaA-frzC_AfriboB</i> 3. <i>PglaA-frzB_AfpyroA</i>	FrzA – D	30 + 31 + 32
1. <i>PglaA-frzD_PgpdA-frzC_AfpyrG</i> 2. <i>PgpdA-frzA_PglaA-frzC_AfriboB</i> 3. <i>PamyB-frzE_PglaA-frzB_AfpyroA</i>	FrzA – E	<i>N</i> -methyl- 32
1. <i>PglaA-frzD_PgpdA-frzC_PamyB-frzF_AfpyrG</i> 2. <i>PgpdA-frzA_PglaA-frzC_AfriboB</i> 3. <i>PglaA-frzB_AfpyroA</i>	FrzA – D/F	<i>O</i> -methyl- 32
1. <i>PglaA-frzD_PgpdA-frzC_PamyB-frzF_AfpyrG</i> 2. <i>PgpdA-frzA_PglaA-frzC_AfriboB</i> 3. <i>PamyB-frzE_PglaA-frzB_AfpyroA</i>	FrzA – F	30 + 33
1. <i>PglaA-frzD_PgpdA-frzC_PamyB-frzF_AfpyrG</i> 2. <i>PgpdA-frzA_PglaA-frzG_AfriboB</i> 3. <i>PamyB-frzE_PglaA-frzB_AfpyroA</i>	FrzA – G	36
1. <i>PglaA-frzD_PgpdA-frzC_PamyB-frzF_AfpyrG</i> 2. <i>PgpdA-frzA_PglaA-frzG_AfriboB</i> 3. <i>PamyB-frzE_PPOgpdA-frzH_PglaA-frzB_AfpyroA</i>	FrzA – H	36 + 37
1. <i>PglaA-frzD_PgpdA-frzC_PamyB-frzF_AfpyrG</i> 2. <i>PgpdA-frzA_PglaA-frzI_PamyB-frzG_AfriboB</i> 3. <i>PamyB-frzE_PPOgpdA-frzH_PglaA-frzB_AfpyroA</i>	FrzA – I	36 + 38 (3 mg/L)
1. <i>PglaA-frzD_PgpdA-frzC_PamyB-frzF_AfpyrG</i> 2. <i>PgpdA-frzA_PglaA-frzI_PamyB-frzG_AfriboB</i> 3. <i>PamyB-frzE_PPOgpdA-frzH_PgpdA-frzJ_PglaA-frzB_AfpyroA</i>	FrzA – J	no detectable 28 , 36 , 37 , or 38
1. <i>PglaA-frzD_PgpdA-frzC_PamyB-frzF_PPOgpdA-frzK_AfpyrG</i> 2. <i>PgpdA-frzA_PglaA-frzI_PamyB-frzG_PPOgpdA-frzJ_AfriboB</i> 3. <i>PamyB-frzE_PPOgpdA-frzH_PglaA-frzB_AfpyroA</i>	FrzA – K	28 (1 mg/L) + 36 + 38
1. <i>PglaA-frzD_PgpdA-frzC_PamyB-frzF_PPOgpdA-frzK_AfpyrG</i> 2. <i>PgpdA-frzA_PglaA-frzI_PamyB-frzG_PPOgpdA-frzJ_AfriboB</i> 3. <i>PamyB-frzE_PPOgpdA-frzH_PgpdA-frzL_PglaA-frzB_AfpyroA</i>	FrzA – L	28 + 36 + 38

Table S6. Vectors used for *qlt* expression and their corresponding chemical products.

Expression cassettes	Enzymes expressed	Products
1. <i>PgpdA-qltA_AfpyrG</i> 2. <i>PgpdA-qltB_AfriboB</i> 3. <i>PgpdA-qltD_PPOgpdA-qltC_PPEgpdA-qltE_AfpyroA</i>	QltA – E	39 (5 mg/L)
1. <i>PgpdA-qltA_AfpyrG</i> 2. <i>PgpdA-qltB_AfriboB</i> 3. <i>PgpdA-qltD_PPEgpdA-qltE_AfpyroA</i>	QltA/B/D/E	39 (~5 mg/L)
1. <i>PgpdA-qltA_AfpyrG</i> 2. <i>PgpdA-qltB_AfriboB</i> 3. <i>PgpdA-qltD_PPOgpdA-qltC_AfpyroA</i>	QltA – D	no product
1. <i>PgpdA-qltA_AfpyrG</i> 2. <i>PgpdA-qltB_PPEgpdA-qltE_AfriboB</i> 3. <i>PgpdA-qltC_AfpyroA</i>	QltA – C/E	no product
1. <i>PgpdA-qltD_PPOgpdA-qltC_PPEgpdA-qltE_AfpyroA</i>	QltC – E	no product

Table S7. Vectors used for *pvh* expression and their corresponding chemical products.

Expression cassettes	Enzymes expressed	Products
1. <i>PglaA-pvhC_AfpyrG</i> 2. <i>PgpdA-pvhA_AfriboB</i> 3. <i>PamyB-pvhB_PgpdA-pvhE_PglaA-pvhD_AfpyroA</i>	PvhA – E	44
1. <i>PglaA-pvhC_AfpyrG</i> 2. <i>PgpdA-pvhA_AfriboB</i> 3. <i>PamyB-pvhB_PgpdA-pvhE_AfpyroA</i>	PvhA – C/E	45
1. <i>PglaA-pvhC_AfpyrG</i> 2. <i>PgpdA-pvhA_AfriboB</i>	PvhA/C	46 + 47 + 48
1. <i>PglaA-pvhC_AfpyrG</i> 2. <i>PgpdA-pvhA_AfriboB</i> 3. <i>PamyB-pvhB_AfpyroA</i>	PvhA – C	46 + 47 + 48
1. <i>PglaA-pvhC_AfpyrG</i> 2. <i>PgpdA-pvhA_AfriboB</i> 3. <i>PgpdA-pvhE_AfpyroA</i>	PvhA/C/E	49
1. <i>PglaA-pvhC_AfpyrG</i> 2. <i>PgpdA-pvhA_AfriboB</i> 3. <i>PgpdA-pvhE_PglaA-pvhD_AfpyroA</i>	PvhA/C/D/E	50

Table S8. Vectors used for *lep* expression and their corresponding chemical products.

Expression cassettes	Enzymes expressed	Products
1. <i>PglaA-lepA_AfpyrG</i> 2. <i>PgpdA-lepG_AfriboB</i> 3. <i>PamyB-lepH_AfpyroA</i>	LepA/G/H	52
1. <i>PglaA-lepA_AfpyrG</i> 2. <i>PgpdA-lepG_PamyB-lepF_AfriboB</i> 3. <i>PamyB-lepH_AfpyroA</i>	LepA/F/G/H	54 (1.2 mg/L) + 55 (1.8 mg/L) + 56 (4.2 mg/L) + 57 (4.3 mg/L) + 58 (5.4 mg/L)
1. <i>PglaA-lepA_AfpyrG</i> 2. <i>PgpdA-lepG_PamyB-lepF_AfriboB</i> 3. <i>PglaA-lepI_PamyB-lepH_AfpyroA</i>	LepA/F/G/H/I	54
1. <i>PglaA-lepA_AfpyrG</i> 2. <i>PgpdA-lepG_PamyB-lepF_AfriboB</i> 3. <i>PglaA-lepI_PamyB-lepH_PgpdA-lepD_AfpyroA</i>	LepA/D/F/G/H/I	51

Table S9. Vectors used for *icc* expression and their corresponding chemical products.

Expression cassettes	Enzymes expressed	Products
1. <i>PglaA-iccA_PgpdA-iccB_AfpYrG</i>	<i>IccA/B</i>	61 (20 mg/L)
1. <i>PglaA-iccA_PgpdA-iccB_AfpYrG</i> 2. <i>PgpdA-iccC_AfriboB</i>	<i>IccA – C</i>	61 + 62 (20 mg/L) + 63 (trace)
1. <i>PglaA-iccA_PgpdA-iccB_AfpYrG</i> 2. <i>PgpdA-iccC_PgpdA-cyb5_PgpdA-iccD_AfriboB</i>	<i>IccA – D</i>	61 + 63 (10 mg/L)
1. <i>PglaA-iccA_PgpdA-iccB_AfpYrG</i> 2. <i>PgpdA-iccC_PgpdA-cyb5_PgpdA-iccD_PglaA-iccE_AfriboB</i>	<i>IccA – E</i>	60 (10 mg/L) + 61

cyb5 encodes for transmembrane cytochrome P450 reductase (CPR). Fungal class II P450s microsomal transmembrane enzymes are often associated with CPR partnering enzymes, and together they modulate electron transfer from NADPH.

Table S10. Vectors used for *har* expression and their corresponding chemical products.

Expression cassettes	Enzymes expressed	Products
1. <i>PglaA-harA_AfpyrG</i> 2. <i>PamyB-harC_PgpdA-harE_PglaA-harF_AfriboB</i> 3. <i>PglaA-harB_PgpdA-harD_PamyB-harG_AfpyroA</i>	HarA – G	65 (0.5 mg/L) + 68 (5.0 mg/L) + 69 (2.5 mg/L) + 70 (3.0 mg/L)
1. <i>PglaA-harA_AfpyrG</i> 2. <i>PgpdA-harE_AfriboB</i>	HarA/E	71 (7.5 mg/L)
1. <i>PglaA-harA_AfpyrG</i> 2. <i>PgpdA-harE_AfriboB</i> 3. <i>PamyB-harG_AfpyroA</i>	HarA/E/G	68 + 71 + 72 (7.5 mg/L)
1. <i>PglaA-harA_AfpyrG</i> 2. <i>PgpdA-harE_AfriboB</i> 3. <i>PglaA-harB_PamyB-harG_AfpyroA</i>	HarA/B/E/G	68 + 71 + 72
1. <i>PglaA-harA_AfpyrG</i> 2. <i>PglaA-harC_PgpdA-harE_AfriboB</i> 3. <i>PamyB-harG_AfpyroA</i>	HarA/C/E/G	68 + 71 + 72
1. <i>PglaA-harA_AfpyrG</i> 2. <i>PgpdA-harE_AfriboB</i> 3. <i>PgpdA-harD_PamyB-harG_AfpyroA</i>	HarA/D/E/G	68 + 71 + 72 + 74 (0.5 mg/L)
1. <i>PglaA-harA_AfpyrG</i> 2. <i>PgpdA-harE_PglaA-harF_AfriboB</i> 3. <i>PamyB-harG_AfpyroA</i>	HarA/E/F/G	68 + 71 + 72
1. <i>PglaA-harA_AfpyrG</i> 2. <i>PgpdA-harE_AfriboB</i> 3. <i>PglaA-harB_PgpdA-harD_PamyB-harG_AfpyroA</i>	HarA/B/D/E/G	68 + 69 + 70
1. <i>PglaA-harA_AfpyrG</i> 2. <i>PglaA-harC_PgpdA-harE_AfriboB</i> 3. <i>PgpdA-harD_PamyB-harG_AfpyroA</i>	HarA/C/D/E/G	68 + 71 + 72 + 74
1. <i>PglaA-harA_AfpyrG</i> 2. <i>PgpdA-harE_PglaA-harF_AfriboB</i> 3. <i>PgpdA-harD_PamyB-harG_AfpyroA</i>	HarA/D/E/F/G	68 + 71 + 72 + 74
1. <i>PglaA-harA_AfpyrG</i> 2. <i>PglaA-harC_PgpdA-harE_AfriboB</i> 3. <i>PglaA-harB_PgpdA-harD_PamyB-harG_AfpyroA</i>	HarA – E & G	65 + 68 + 69 + 70
1. <i>PglaA-harA_AfpyrG</i> 2. <i>PgpdA-harE_PglaA-harF_AfriboB</i> 3. <i>PglaA-harB_PgpdA-harD_PamyB-harG_AfpyroA</i>	HarA/B/D/E/F/G	68 + 69 + 70

Table S11. Vectors used for *pfp* expression and their corresponding chemical products.

Expression cassettes	Enzymes expressed	Products
1. <i>PglaA-pfpA_AfpyrG</i>	PfpA	76 (30 mg/L)
1. <i>PglaA-pfpA_AfpyrG</i> 2. <i>PgpdA-pfpB_AfriboB</i>	PfpA/B	76 + 78 (15 mg/L)
1. <i>PglaA-pfpA_PgpdA-pfpC_AfpyrG</i> 2. <i>PgpdA-pfpB_AfriboB</i>	PfpA – C	76 + 78 + 79 (8 mg/L) + 80 (10 mg/L)
1. <i>PglaA-pfpA_PgpdA-pfpC_AfpyrG</i> 2. <i>PgpdA-pfpB_PPOgpdA-pfpD_AfriboB</i>	PfpA – D	76 + 78 + 79 + 80 + 85 + 86
1. <i>PglaA-pfpA_PgpdA-pfpC_AfpyrG</i> 2. <i>PgpdA-pfpB_PPOgpdA-pfpD_AfriboB</i> 3. <i>PgpdA-pfpE_AfpyroA</i>	PfpA – E	75/75' (0.8 mg/L) + 76 + 78 + 79 + 80 + 85 + 86

Table S12. Vectors used for *fub* expression and their corresponding chemical products.

Expression cassettes	Enzymes expressed	Products
1. PgpdA- fub1 _PglaA- fub4 _Afp _{yr} G 2. PgpdA- fub7 _PglaA- fub6 _PamyB- fub8 _Afr _{ibo} B	Fub1/4/6/7/8	88
1. PgpdA- fub1 _PglaA- fub4 _Afp _{yr} G 2. PgpdA- fub7 _PglaA- fub6 _PamyB- fub8 _Afr _{ibo} B 3. PamyB- fub9 _Afp _{yr} oA	Fub1/4/6/7/8/9	87

Table S13. Vectors used for *thm* expression and their corresponding chemical products.

Expression cassettes	Enzymes expressed	Products
1. <i>PglaA-thmA_AfpyrG</i> 2. <i>PgpdA-thmB_PglaA-thmF_PamyB-thmG_AfriboB</i> 3. <i>PamyB-thmC_PglaA-thmD_PgpdA-thmE_AfpyroA</i>	ThmA – G	94 + 96 + 99 + 100
1. <i>PglaA-thmA_AfpyrG</i> 2. <i>PgpdA-thmB_AfriboB</i>	ThmA/B	101 + 102
1. <i>PglaA-thmA_AfpyrG</i> 2. <i>PgpdA-thmB_AfriboB</i> 3. <i>PamyB-thmC_AfpyroA</i>	ThmA – C	97 + 98
1. <i>PglaA-thmA_AfpyrG</i> 2. <i>PgpdA-thmB_PglaA-thmE_AfriboB</i>	ThmA/B/E	103 + 104 + 105 + 106
1. <i>PglaA-thmA_AfpyrG</i> 2. <i>PgpdA-thmB_PamyB-thmC_PglaA-thmE_AfriboB</i>	ThmA – C/E	94 + 96 + 99 + 100

Table S14. Vectors used for *cm3* expression and their corresponding chemical products.

Expression cassettes	Enzymes expressed	Products
1. <i>PglaA-cm3A_AfpyrG</i> 2. <i>PgpdA-cm3C_PglaA-cm3D_Afribob</i> 3. <i>PamyB-cm3B_AfpyroA</i>	Cm3A – D	107 (13 mg/L) + 108 (18 mg/L)

Table S15. Vectors used for *Pgmpa* expression and their corresponding chemical products.

Expression cassettes	Enzymes expressed	Products
1. <i>PglaA-PgmpaC_AfpyrG</i> 2. <i>PgpdA-PgmpaDE_PglaA-PgmpaA_AfriboB</i>	PgMpaA/C/DE	110 + 111
1. <i>PglaA-PgmpaC_AfpyrG</i> 2. <i>PgpdA-PgmpaDE_PglaA-PgmpaA_PamyB-PgmpaB_AfriboB</i> 3. <i>PamyB-PgmpaG_AfpyroA</i>	PgMpaA – C/DE/G	109 + 110 + 112 (trace)

Table S16A. Vectors used for *flv* expression and their corresponding chemical products.

Expression cassettes ^a	Enzymes expressed	Products
1. PPEgpdA- <i>flvD</i> _Pgpda- <i>flvE</i> _PPOgpdA- <i>flvF</i> _AfpYrG 2. Pgpda- <i>flvC</i> _PPOgpdA- <i>flvG</i> _PPEgpdA- <i>flvH</i> _AfriboB 3. Pgpda- <i>flvA</i> _PPOgpdA- <i>flvB</i> _PPEgpdA- <i>flvI</i> _AfpYrOA	FlvA – I	116 (1.2 mg/L)
1. PPEgpdA- <i>flvD</i> _Pgpda- <i>flvE</i> _PPOgpdA- <i>flvF</i> _AfpYrG 2. Pgpda- <i>flvC</i> _PPOgpdA- <i>flvG</i> _PPEgpdA- <i>flvH</i> _AfriboB 3. Pgpda- <i>flvA</i> _PPOgpdA- <i>flvB</i> _AfpYrOA	FlvA – H	117 (1.2 mg/L) + 118 (0.9 mg/L)
1. PPEgpdA- <i>flvD</i> _Pgpda- <i>flvE</i> _PPOgpdA- <i>flvF</i> _AfpYrG 2. Pgpda- <i>flvC</i> _PPOgpdA- <i>flvG</i> _PPEgpdA- <i>flvH</i> _AfriboB	FlvC – H	118
1. Pgpda- <i>flvA</i> _PPOgpdA- <i>flvB</i> _AfpYrOA	FlvA/B	117
1. PPEgpdA- <i>flvD</i> _PPOgpdA- <i>flvF</i> _AfpYrG 2. Pgpda- <i>flvC</i> _PPOgpdA- <i>flvG</i> _PPEgpdA- <i>flvH</i> _AfriboB	FlvC/D/F/G/H	no product
1. PPEgpdA- <i>flvD</i> _Pgpda- <i>flvE</i> _AfpYrG 2. Pgpda- <i>flvC</i> _PPOgpdA- <i>flvG</i> _PPEgpdA- <i>flvH</i> _AfriboB	FlvC – E/G/H	120a/120b
1. PPEgpdA- <i>flvD</i> _Pgpda- <i>flvE</i> _AfpYrG 2. Pgpda- <i>flvC</i> _AfriboB	FlvC – E	120a/120b
1. PPEgpdA- <i>flvD</i> _Pgpda- <i>flvE</i> _AfpYrG	FlvD/E	121a/121b
1. PPEgpdA- <i>flvD</i> _Pgpda- <i>flvE</i> _PPOgpdA- <i>flvF</i> _AfpYrG 2. PPOgpdA- <i>flvG</i> _PPEgpdA- <i>flvH</i> _AfriboB	FlvD – H	122
1. PPEgpdA- <i>flvD</i> _Pgpda- <i>flvE</i> _PPOgpdA- <i>flvF</i> _AfpYrG 2. Pgpda- <i>flvC</i> _AfriboB 3. PPOgpdA- <i>flvG</i> _AfpYrOA	FlvC – G	118 (trace)
1. PPEgpdA- <i>flvD</i> _Pgpda- <i>flvE</i> _PPOgpdA- <i>flvF</i> _AfpYrG 2. Pgpda- <i>flvC</i> _AfriboB	FlvC – F	120a/120b

^aPPEgpdA and PPOgpdA are *gpdA* promoters from *Penicillium expansum* and *Penicillium oxalicum*, respectively.

Table S16B. Feeding of different substrates to the various Flv expression strains and the products identified.

Substrates	Expression cassettes ^a	Enzymes expressed	Products
117 + 118	1. PPEgpdA- <i>flvI</i> _AfpYrOA	FlvI	116 + 117
118	1. Pgpda- <i>flvA</i> _PPOgpdA- <i>flvB</i> _PPEgpdA- <i>flvI</i> _AfpYrOA	FlvA/B/I	116 + 117
119	1. PPEgpdA- <i>flvD</i> _PPOgpdA- <i>flvF</i> _AfpYrG 2. PPOgpdA- <i>flvG</i> _PPEgpdA- <i>flvH</i> _AfriboB	FlvD/F/G/H	122
121a/121b	1. PPEgpdA- <i>flvD</i> _PPOgpdA- <i>flvF</i> _AfpYrG 2. PPOgpdA- <i>flvG</i> _PPEgpdA- <i>flvH</i> _AfriboB	FlvD/F/G/H	no product
124	1. PPEgpdA- <i>flvD</i> _Pgpda- <i>flvE</i> _AfpYrG	FlvD/E	121a/121b
124	1. PPEgpdA- <i>flvD</i> _Pgpda- <i>flvE</i> _PPOgpdA- <i>flvF</i> _AfpYrG	FlvD – F	122
125	1. PPEgpdA- <i>flvD</i> _Pgpda- <i>flvE</i> _PPOgpdA- <i>flvF</i> _AfpYrG 2. Pgpda- <i>flvC</i> _AfriboB 3. PPOgpdA- <i>flvG</i> _AfpYrOA	FlvC – G	118
125	1. PPEgpdA- <i>flvD</i> _Pgpda- <i>flvE</i> _PPOgpdA- <i>flvF</i> _AfpYrG 2. Pgpda- <i>flvC</i> _AfriboB	FlvC – F	120a/120b
124	1. PPEgpdA- <i>flvD</i> _Pgpda- <i>flvE</i> _PPOgpdA- <i>flvF</i> _AfpYrG 2. Pgpda- <i>flvC</i> _AfriboB	FlvC – F	118

^aPPEgpdA and PPOgpdA are *gpdA* promoters from *Penicillium expansum* and *Penicillium oxalicum*, respectively.

Table S17. Vectors used for *elc* expression and their corresponding chemical products.

Expression cassettes	Enzymes expressed	Products
1. <i>PalcA-elcA_AfpyrG</i>	ElcA	127 + unknown
1. <i>PalcA-elcA_AfpyrG</i> 2. <i>PalcM-elcB-MT_AfriboB</i>	ElcA/B-MT	128
1. <i>PalcA-elcA_AfpyrG</i> 2. <i>PalcM-elcB_AfriboB</i>	ElcA/B	129 + 130 + 131
1. <i>PalcA-elcA_AfpyrG</i> 2. <i>PalcM-elcB_AfriboB</i> 3. <i>PalcS-elcD_AfpyroA</i>	ElcA/B/D	133
1. <i>PalcA-elcA_AfpyrG</i> 2. <i>PalcS-elcB_PalcM-elcE_AfriboB</i> 3. <i>PalcS-elcD_AfpyroA</i>	ElcA/B/D/E	129 (trace) + 131 (trace) + 133
1. <i>PalcA-elcA_AfpyrG</i> 2. <i>PalcS-elcB_PalcM-elcE_AfriboB</i> 3. <i>PalcS-elcD_PalcM-elcF_PalcA-elcG_AfpyroA</i>	ElcA/B/D/E/F/G	129 + 131 + 133 + (P)-135 + (P)-136

Table S18. Vectors used for *vdt* expression and their corresponding chemical products.

Expression cassettes	Enzymes expressed	Products
1. <i>PalcA-vdtA_AfpYrG</i>	VdtA	139
1. <i>PalcA-vdtA_AfpYrG</i> 2. <i>PalcM-vdtC_AfpYrA</i>	VdtA/C	140
1. <i>PalcA-vdtA_AfpYrG</i> 2. <i>PalcM-vdtC_PaldA-vdtF_AfpYrA</i>	VdtA/C/F	141
1. <i>PalcA-vdtA_AfpYrG</i> 2. <i>PalcA-vdtE_PalcM-vdtC_AfpYrA</i>	VdtA/C/E	142 + 143 (major)
1. <i>PalcA-vdtA_AfpYrG</i> 2. <i>PalcA-vdtE_PalcM-vdtC_PaldA-vdtF_AfpYrA</i>	VdtA/C/E/F	144 + 145
1. <i>PalcA-vdtA_AfpYrG</i> 2. <i>PalcA-vdtE_PalcS-vdtD_PalcM-vdtC_AfpYrA</i>	VdtA/C/D/E	142 (major) + 143
1. <i>PalcA-vdtA_AfpYrG</i> 2. <i>PalcA-vdtE_PalcS-vdtD_PalcM-vdtC_PaldA-vdtF_AfpYrA</i>	VdtA/C/D/E/F	144
1. <i>PalcA-vdtA_AfpYrG</i> 2. <i>PalcA-vdtB_AfriboB</i> 2. <i>PalcA-vdtE_PalcM-vdtC_PaldA-vdtF_AfpYrA</i>	VdtA – C/E/F	(<i>M</i>)- 138 ^a (trace) + (<i>P</i>)- 138 ^a (trace) + (<i>M</i>)- 146 ^a + (<i>P</i>)- 146 ^a + (<i>P</i>)- 147 + (<i>P</i>)- 148
1. <i>PalcA-vdtA_AfpYrG</i> 2. <i>PalcA-vdtB_AfriboB</i> 3. <i>PalcA-vdtE_PalcS-vdtD_PalcM-vdtC_PaldA-vdtF_AfpYrA</i>	VdtA – F	(<i>M</i>)- 138 ^b + (<i>P</i>)- 138 ^b + 144
1. <i>PalcA-vdtA_AfpYrG</i> 2. <i>PalcA-vdtB_PalcM-orf1_AfriboB</i> 3. <i>PalcA-vdtE_PalcS-vdtD_PalcM-vdtC_PaldA-vdtF_AfpYrA</i>	VdtA – F/ORF1	(<i>M</i>)- 138 ^b + (<i>P</i>)- 138 ^b + 144

^a: The ratio of (*M*)-**138**:(*P*)-**138** and (*M*)-**146**:(*P*)-**146** is 1:2. ^b: The ratio of (*M*)-**138**:(*P*)-**138** is 20:1.

Table S19. Vectors used for *sth* expression and their corresponding chemical products.

Expression cassettes	Enzymes expressed	Products
1. <i>PalcA-sthA_AfpyrG</i> 2. <i>PalcA-sthE_AfriboB</i>	SthA/E	153
1. <i>PalcA-sthA_AfpyrG</i> 2. <i>PalcA-sthE_PalcM-sthC_AfriboB</i>	SthA/C/E	154
1. <i>PalcA-sthA_AfpyrG</i> 2. <i>PalcA-sthE_PalcS-sthF_AfriboB</i>	SthA/E/F	150 (trace) + 151 (trace) + 153 + 155
1. <i>PalcA-sthA_AfpyrG</i> 2. <i>PalcA-sthE_PalcS-sthF_PalcM-sthC_AfriboB</i>	SthA/C/E/F	150
1. <i>PalcA-sthA_AfpyrG</i> 2. <i>PalcA-sthE_PalcS-sthF_PalcM-sthB_AfriboB</i>	SthA/B/E/F	151 + 153 + 155 + 156
1. <i>PalcA-sthA_AfpyrG</i> 2. <i>PalcA-sthE_PalcS-sthF_AfriboB</i> 3. <i>PalcA-sthD_PalcM-sthB_AfpyroA</i>	SthA/B/D/E/F	149 + 153 (trace) + 156 (trace)
1. <i>PalcA-sthA_AfpyrG</i> 2. <i>PalcA-sthE_PalcS-sthF_AfriboB</i> 3. <i>PalcA-sthD_AfpyroA</i>	SthA/D/E/F	151 (trace) + 153 + 155 (trace) + 157

Table S20. Vectors used for *nan* expression and their corresponding chemical products.

Expression cassettes	Enzymes expressed	Products
1. <i>PalcA-nanA_AfpyrG</i>	NanA	no product
1. <i>PalcA-nanA_AfpyrG</i> 2. <i>PalcA-nanC_AfriboB</i>	NanA/C	159
1. <i>PalcA-nanA_AfpyrG</i> 2. <i>PalcA-nanC_PaldA-nanD_AfriboB</i>	NanA/C/D	160
1. <i>PalcA-nanA_AfpyrG</i> 2. <i>PalcA-nanC_AfriboB</i> 3. <i>PalcA-nanE_AfpyroA</i>	NanA/C/E	159 + 161
1. <i>PalcA-nanA_AfpyrG</i> 2. <i>PalcA-nanC_PalcS-nanF_AfriboB</i>	NanA/C/F	159
1. <i>PalcA-nanA_AfpyrG</i> 2. <i>PalcA-nanC_PaldA-nanD_AfriboB</i> 3. <i>PalcA-nanE_AfpyroA</i>	NanA/C/D/E	159 + 161 + 162
1. <i>PalcA-nanA_AfpyrG</i> 2. <i>PalcA-nanC_PalcS-nanF_PalcM-nanB_AfriboB</i>	NanA – C/F	159
1. <i>PalcA-nanA_AfpyrG</i> 2. <i>PalcA-nanC_PalcS-nanF_PaldA-nanD_AfriboB</i>	NanA/C/D/F	159 (trace) + 160 + 163 ^a
1. <i>PalcA-nanA_AfpyrG</i> 2. <i>PalcA-nanC_PalcS-nanF_PaldA-nanD_AfriboB</i> 3. <i>PalcA-nanE_AfpyroA</i>	NanA/C/D/E/F	159 (trace) + 160 + 161 + 162 (trace) + 163 ^a + 164 ^a
1. <i>PalcA-nanA_AfpyrG</i> 2. <i>PalcA-nanC_PalcS-nanF_PalcM-nanB_PaldA-nanD_AfriboB</i>	NanA – D/F	165
1. <i>PalcA-nanA_AfpyrG</i> 2. <i>PalcA-nanC_PalcS-nanF_PalcM-nanB_PaldA-nanD_AfriboB</i> 3. <i>PalcA-nanE_AfpyroA</i>	NanA – F	158 + 159 (trace) + 160 (trace) + 161 (trace) + 162 (trace)

^a: Purification and characterization of the compound was unsuccessful.

Table S21. Vectors used for *buA* expression used and their corresponding chemical products.

Expression cassettes	Enzymes expressed	Products
1. <i>PalcA-buA_AfpyrG</i> 2. <i>PalcA-buAC_AfriboB</i>	BuA/C	no product
1. <i>PalcA-buA_AfpyrG</i> 2. <i>PalcA-buAC_PalcS-buAE_AfriboB</i>	BuA/C/E	no product
1. <i>PalcA-buA_AfpyrG</i> 2. <i>PalcA-buAC_PalcS-buAE_PaldA-buAG_AfriboB</i> 3. <i>PalcA-buAB_PaldA-buAF_AfpyroA</i>	BuA – C/E/F/G	168 + 170 + 171
1. <i>PalcA-buA_AfpyrG</i> 2. <i>PalcA-buAC_PalcS-buAE_PaldA-buAG_AfriboB</i> 3. <i>PalcA-buAB_PalcS-buD_PaldA-buAF_AfpyroA</i>	BuA – G	168 + 170 + 171

Table S22. Different combinations of *Ateafo* genes and their corresponding chemical products.

Expression cassettes	Enzymes expressed	Products
<i>AfpyrG_PalCA-AteafoG_PalCA-AteafoE_AfpyroA</i>	AteAfoE/G	174 (~ 95 mg/L) + 177 (trace) + 178 (trace) + 179 (trace) + 180 (trace)
<i>AfpyrG_PalCA-AteafoG_PalCA-AteafoE_PalCA-AteafoF_AfriboB</i>	AteAfoE/F/G	174 (~ 95 mg/L) + 177 (trace) + 178 (trace) + 179 (trace) + 180 (trace)
<i>AfpyrG_PalCA-AteafoG_PalCA-AteafoE_PalCA-AteafoD_AfriboB</i>	AteAfoD/E/G	179 (trace) + 175 + 176 (trace) + 180 (trace) + 181 (trace)
<i>AfpyrG_PalCA-AteafoG_PalCA-AteafoE_PalCA-AteafoC_AfriboB</i>	AteAfoC/E/G	174 (~ 300 mg/L) + 177 + 178 + 179 (trace) + 180 (trace) + 181 (trace)
<i>AfpyrG_PalCA-AteafoG_PalCA-AteafoC_AfpyroA</i>	AteAfoC/G	179 (~ 1 mg/L)
<i>AfpyrG_PalCA-AteafoG_PalCA-AteafoE_PalCA-AteafoC_PalCA-AteafoD_AfpyroA</i>	AteAfoC/D/E/G	179 (trace) + 175 + 176 + 180 (trace) + 181 (trace)
<i>AfpyrG_PalCA-AteafoG_PalCA-AteafoE_PalCA-AteafoC_PalCA-AteafoD_PalCA-AteafoC_AfriboB</i>	AteAfoC/D/E/G	179 (~2 mg/L) + 175 (~10 mg/L) + 17 (~6 mg/L) + 180 (~3 mg/L) + 181 (~1 mg/L)
<i>AfpyrG_PalCA-AteafoG_PalCA-AteafoE_PalCA-AteafoC_PalCA-AteafoD_PalCA-AteafoF_AfriboB</i>	AteAfoC – G	173 + 179 (trace) + 175 + 176 (trace) + 181 (trace)
<i>AfpyrG_PalCA-AteafoG_PalCA-AteafoE_PalCA-AteafoF_PalCA-AteafoD_PalCA-AteafoC_AfriboB</i>	AteAfoC – G	173 + 179 (trace) + 175 + 176 (trace) + 181 (trace)
<i>AfpyrG_PalCA-AteafoG_PalCA-AteafoE_PalCA-AteafoF_PalCA-AteafoC_PalCA-AteafoD_AfriboB</i>	AteAfoC – G	173 + 179 (trace) + 175 + 176 (trace) + 181 (trace)
<i>AfpyrG_PalCA-AteafoG_PalCA-AteafoE_PalCA-AteafoF_PalCA-AteafoD_PalCA-AteafoC_PalCA-AteafoB_AfpyroA</i>	AteAfoB – G	173 + 179 (trace) + 175 + 176 (trace) + 181 (trace)

Table S23. Different combinations of *ctv* genes and their corresponding chemical products.

Expression cassettes	Enzymes expressed	Products
<i>AfriboB_PalcA-ctvA_AfpyrG</i>	CtvA	185 (~10 mg/L)
<i>AfriboB_PalcA-ctvA_PalcA-ctvB_AfpyroA</i>	CtvA/B	186 (~5 mg/L)
<i>AfriboB_PalcA-ctvA_PalcA-ctvC_AfpyroA</i>	CtvA/C	185 (~10 mg/L)
<i>AfriboB_PalcA-ctvA_PalcA-ctvB_PalcA-ctvC_AfpyrG</i>	CtvA – C	184 (trace) + unknowns
<i>AfriboB_PalcA-ctvA_PalcA-ctvB_PalcA-ctvC_PalcA-ctvD_AfpyroA</i>	CtvA – D	184 (~3 mg/L)