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One Pathway, Many Compounds: Heterologous Expression of a Fungal Biosynthetic Pathway Reveals its Intrinsic Potential for Diversity.

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Electronic Supplementary Information

Table S1. Primers used during this study

Gene	Primer Name	DNA Sequence (5' -----> 3')	Template
tenA	**adh/asc-F**	ACGTGAACCATCACCCAAATCAAGTTTTTTAAGTGCTTATGCGCCATCGT	pTAYAargPageTenellin
	trpOrf1-R	TTCAGTGTCGAAAGATCCACTAGAGTAAATCCGTCTTTGTGAAGGACAGT	
P_{adh}	**adh/asc-F**	ACGTGAACCATCACCCAAATCAAGTTTTTTAAGTGCTTATGCGCCATCGT	*A. oryzae* gDNA
	adh/asc-R	GTAAATCTG**GGCGCGCC**CTTACAGGCATCATTTGACTTTGGGATCTTGTG	
P_{gpdA}	**newgpdA-F**	TGATGCCTGTAA**GGGCGCGCC**CAGATTTACTCTAGTGGATCTTTCGACAC	*A. oryzae* gDNA
	newgpdA-R	GCGTAGTTA**GGCGCGCC**ATATACCTCTTACGGTGATGTCTGCTCAAGCGG	
P_{eno}	**eno/asc-Page-F**	GTAAGAGGTATAT**GGCGCGCC**TAACTACGCGTCTCATTACTAGTCTACTA	*A. oryzae* gDNA
	eno/asc-Page-R	ATACGTCAAAGCAACCATAGTA**GGCGCGCC**TTTGACGAGCTGCGGAATTG	
*apd*E	**adh-apdE-F**	TTTCTTTCAACACAAGATCCCAAAGTCAAAATGAGTCTGGCCAGCACGCT	*A. nidulans* gDNA
	adh-apdE-R	ACGTATTTCAGTGTCGAAAGATCCACTAGAAGCTCAGTAAGCTCTCTTGA	
*apd*C	**gpdA-apdC-F**	AACAGCTACCCCGCTTGAGCAGACATCACCATGATCCCTCCCAAGCAACA	*A. nidulans* gDNA
	gpdA-apdC-R	ACAGAGTTACTAGTAGACTAGTAATGAGACCGCTGCTCGGGAAATACGTT	
*apd*B	**eno-apdB-F**	GTCGACTGACCAATTCCGCAGCTCGTCAAAATGGGTTTCGTTAATACTCT	*A. nidulans* gDNA
	eno-apdB-R	TTCACACCGCATACGTCAAAGCAACCATAGTGAATGACACATTTGCAGAG	
P_{trpC}+bar	**YA-TrpC-F**	GCTGAAGATCAGTTGGGCCTCATTTGACTACAGAAGATGATATTGAAGGA	pCB1530-G
	Bar-YA-R	CTATATATTTAAATGGAAGCCTGGTTATATAGCTTACCTAAATCTCGGTG	
ble	**Ble-YA-R**	CTATATATTTAAATGGAAGCCTGGTTATATTCAGTCCTGCTCCTCGGCCA	pBleamyBGSA2
	trpC-Ble-R	CTACCCAAGCGCTTCGATTAGGAAGTAACCATGGCCAAGTTGACCAGTGC	
*apd*G	**apdG-F**	AACAGCTACCCCGCTTGAGCAGACATCACCATGGCCGAGATTTCCAGCGT	*A. nidulans* gDNA
	apdG-R	ACAGAGTTACTAGTAGACTAGTAATGAGACAACTGATAAAGCGGCCGACC	
*apd*D	**apdD-F**	GTCGACTGACCAATTCCGCAGCTCGTCAAAATGCCTACCGAAAGCCTCAA	*A. nidulans* gDNA
	apdD-R	TTCACACCGCATACGTCAAAGCAACCATAGAGTGTTTATTTGGATGAGGT	

apdA	**apdA1-F**	TTGTACAAAAAAGCAGGCTCCGCGGCCGCCATGCAGGACTTGATCGCCAT	*A. nidulans* gDNA
	apdA1-R	ATGGGATGTTGTCCACTGCG	
	apdA2-F	GTTTCTGAAGTGACCAGCCG	
	apdA2-R	TCTGGAGATCCCAAACTCGA	
	apdA3-F	GGAAATCTTCCTGCTTCACG	
	apdA3-R	GGTGAACAGCTCCTCGCCCTTGCTCACCATAAGAGACCCCGCTTCCCCCA	

qRT-PCR Primers			
apdA	**egfp/Q-F**	TACCTGAGCACCCAGTCCGC	*A. oryzae* cDNA
	P1P2egfp rev	TTAATTAATTACTTGTACAGCTCGTCCA	
*apd*B	**apdB-Q-F**	CCTGTCGTTATAGACGCCAA	*A. oryzae* cDNA
	apdB-Q-R	GAAGGTGGGATATCCCGAAA	
*apd*C	**apdC-Q-F**	TTTAAACGGGATGCGTGTCC	*A. oryzae* cDNA
	apdC-Q-R	TTCACATCCGATGTATGCGG	
*apd*E	**apdE-Q-F**	GATGTCGTCCAGGTCAACAA	*A. oryzae* cDNA
	apdE-Q-R	TTCCAGTAGGGCCGCAAATT	
eGFP	**egfp/Q-F**	TACCTGAGCACCCAGTCCGC	*A. oryzae* cDNA
	P1P2egfp rev	TTAATTAATTACTTGTACAGCTCGTCCA	
β-tubulin	**AObeta-tub-F**	AGCCCTACAACGCCACTCTT	*A. oryzae* cDNA
	AObeta-tub-R	GTGCGCATGCAAATGTCATA	

qRT-PCR

In order to confirm all the genes in the pTAYAargASP were expressed, qRT-PCR was performed on one of the transformant with the primers shown in the Table. RevertAid H Minus First Strand cDNA Synthesis Kit and SYBR green PCR mix (Fermentas) was used to synthesise cDNA and to perform qRT-PCR respectively according to manufacturer's protocol. PCR was carried out in a Mx3005 Real-Time PCR System (Stratagene) using program: 95 °C for 10 min, (1 cycle) 95 °C for 30 sec, 55 °C for 30 sec and 72 °C for 60 sec (40 cycles). Table S2 shows the activity of each promoter compared to the control gene. The results showed that *apd*A expressed at the highest level by P*amy*B, followed by *apd*E (P*adh*), *apd*C (P*gpd*A) and *apd*B (P*eno*) respectively.

Table S2: Results of the qRT-PCR experiments on the pTAYAargASP transformant

Sample	Gene (promoter)	ΔCt[a]	ΔΔCt[b]	Fold difference[c]
pTAYAargASP	*apd*A (P_{amyB})	14.55	-5.63	49.52
	*apd*B (P_{eno})	19.13	-1.05	2.07
	*apd*C (P_{gpdA})	17.35	-2.83	7.11
	*apd*E (P_{adh})	16.46	-3.72	13.18
	β-tubulin	20.18		

[a] The ΔC_t was determined by the average Ct cycle.

[b] The ΔΔCt value was determined by subtracting the ΔCt value of control gene from the ΔCt value of a sample.

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[c] Assuming the efficiency is 100%, the $\Delta\Delta C_t$ was described as $2^{-\Delta\Delta Ct}$.

Media

50 gram of Czapek Dox agar was dissolved in 1 litre of deionized water. The solution was made with constant stirring and divided in three 500 ml Erlenmeyer flasks, stoppered with a foam bung and covered with aluminium foil before sterilization. 25 ml of melted Czapek Dox agar was poured in plates and cooled to solidify.

The spores of *A. oryza*e transformants were grown on plates in DPY medium (dextrin-peptone-yeast extract, 2% (w/v) dextrin, 1% (w/v) polypeptone, 0.5% (w/v) yeast extract, 0.5% (w/v) potassium dihydrogen phosphate, 0.05% (w/v) magnesium sulphate, 2.5% (w/v) agar.

The liquid media used for production of metabolites was CMP medium (3.5 % Czapek Dox broth, 2% maltose and 1% poypeptone). 1 litre liquid media was divided in ten 250 ml baffled Erlenmeyer flasks each containing 100 ml of the CMP media. The Erlenmeyer flasks were stoppered with a foam bung and covered with aluminium foil before sterilization.

Structures of compounds used for $^3J_{CH}$ modelling