CLONING, OVEREXPRESSION AND BIOCATALYTIC EXPLORATION OF A NOVEL BAEYER-VILLIGER MONOOXYGENASE FROM *ASPERGILLUS FUMIGATUS* AF293

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Electronic Supplementary Material

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1. PCR protocols: Primers & Vectors

Table S1

Cloned genes, expression vectors and primers used for genomic DNA amplification. Restriction sites are underlined

Gene	***Size***	***Vector***	***Oligonucleotides 5'→3'***
Af1	2718	pET200	Fw: CACCATGACCAGAATACGTCCAGAC Rev_EcoRI: CGGAATTCTCAATGCCCATTAGTAGTAACGG
Af1 truncated	1614	pCRE2	Fw: ACTCGAGATCTGCAGCTGGTATGACCAGAATACGTCCAG Rev: GTTCGGGCCCAAGCTTTAACGTGTAAAGCTCATA
Af2	1461	pET200	Fw_Nde I: CACCCATATGGATTACGATATTATCATTGTTGG Rev_Hind III: GCGAAGCTTCTATTGCTGCTTCTTCCAGCC
		pCRE2	Fw: ACTCGAGATCTGCAGCTGGTATGGATTACGATATTATCA Rev: GTTCGGGCCCAAGCTTTATTGCTGCTTCTTCCAG
Af3	1806	pET200	Fw_Nde I: CACCCATATGCGTTGCATACCCTGCC Rev_Eco RI: GCGGAATTCTTATAGAAGCGGCCGCGGC
Af3 truncated	1632	pCRE2	Fw: ACTCGAGATCTGCAGCTGGTATGTCAGAACACTACCTCG Rev: GTTCGGGCCCAAGCTTTATAGAAGCGGCCGCGGC

2. Expression conditions

Table S2

All the expression conditions assayed are listed below

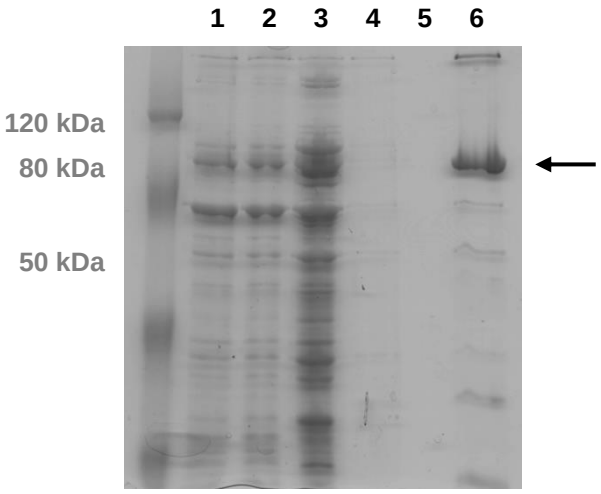
Vector	***Cells***	***Growing T***[a]	***Inductor***	***Induction T***	***Induction time***
pET-*Af1* pET-*Af2* pET-*Af3*	BL21	37 °C (OD_{600}=0.5)	IPTG 0.5 mM	17°C 24°C 30°C 37°C	6 h
pET-*Af1* pET-*Af2* pET-*Af3*	BL21	37 °C (OD_{600}=0.5)	IPTG 1 mM	17°C 24°C 30°C 37°C	6 h
pCRE-*Af1* pCRE-*Af2* pCRE-*Af3*	TOP 10	-	Arabinose 0.002% *w/v*	17°C	48 h
				24°C	36 h
				30°C	24 h
				37°C	12 h
pCRE-*Af1* pCRE-*Af2* pCRE-*Af3*	TOP 10	-	Arabinose 0.02% *w/v*	17°C	48 h
				24°C	36 h
				30°C	24 h
				37°C	12 h
pCRE-*Af1* pCRE-*Af2* pCRE-*Af3*	TOP 10	-	Arabinose 0.2% *w/v*	17°C	48 h
				24°C	36 h
				30°C	24 h
				37°C	12 h

[a] For pCRE2 based constructs, no pre-culture is needed, growing is directly in the presence of inducer

3. Expression of $BVMO_{Af1}$

Figure S1

SDS-PAGE analyses for $BVMO_{Af1}$ purification fractions. *lane 1:* crude extract, lane *2:* flow through, lanes *3, 4,* and *5:* sequential washes with imidazole increasing concentrations*, lane 6:* yellow, active fraction. The arrow refers to purified $BVMO_{Af1}$



4. Substrates screening

Table S3

$BVMO_{Af1}$ substrates profile

Entry	***Substrate***	***$BVMO_{Af1}$[a]***
1	2-propanone	
2	2-butanone	
3	3-buten-2-one	
4	2-octanone	
5	3-octanone	
6	4-octanone	
7	2-decanone	
8	butyl levulinate	
9	3-methyl-2,4-pentanedione	
10	cyclobutanone	
11	cyclopentanone	
12	cyclohexanone	
13	cyclopentadecanone	
14	2-oxocyclohexanecarbonitrile	++
15	4-methyl cyclohexanone	
16	2-propyl cyclohexanone	
17	dehydrocarvone	
18	cyclopropyl methyl ketone	
19	norcamphor	
20	bicyclo[3.2.0]hept-2-en-6-one	+++
21	progesterone	
22	androstenedione	
23	4-dimethylamino benzaldehyde	
24	nicotine	
25	thioanisole	+

26	benzyl ethyl sulfide	+++
27	benzyl phenyl sulfide	+
28	ethionamide	+
29	diphenylmethylthioacetamide	
30	thiacetazone	
31	indole	
32	3-acetyl indole	
33	5-methyl furfural	
34	benzaldehyde	
35	acetophenone	
36	4-hydroxyacetophenone	
37	2,6-dihydroxy acetophenone	
38	3-phenylpentane-2,4-dione	+++
39	phenylacetone	
40	4-(4-hydroxyphenyl)-2-butanone	
41	2-phenyl cyclohexanone	
42	benzoin	
43	phenindione	
44	2-indanone	
45	1-indanone	

[a] Activity was measured employing a colorimetric (phosphate- based detection) screening assay, previously reported for BVMOs substrate screening (Riebel et al. 2012). The activity is indicated as +, ++ or +++ representing 1.2-, 2- or 5-fold phosphate formation (substrate conversion) respectively, when comparing with the blanks

5. GC analyses

The following columns were used for the determination of conversions and enantiomeric excesses: Column A: Alltech GT-A (30 m x 0.25 mm x 0.25 µm, 12.2 psi N_2); column B: Hewlett Packard HP-1 (30m x 0.32 mm x 0.25µm, 12.2 psi N_2) and C: Chirasil Dex CB (30 m x 0.25 mm x 0.25 µm, 12 psi N_2). For all the analyses, the injector temperature was 200ºC and the FID temperature was 250ºC

Table S4

GC employed conditions and retention times (t_R) of substrates and products

compound	***program*[a]**	***column***	***t_R (min) substrates***	***t_R (min) products***
1	130°C isotherm	C	9 (1*S*,5*R*) 9.2 (1*R*,5*S*)	16.5 Abnormal (1*R*,5*S*) 16.9 Normal (1*R*,5*S*) 17.2 Abnormal (1*S*,5*R*) 17.4 Normal (1*S*,5*R*)
2	70/0/5/200/0	B	9.63	13.4 sulfoxide 14.2 sulfone
	100/0/10/160/8	A	6.9	13.5 (*R*) 14.9 (*S*)
3	70/5/5/200/5	B	12.5	16.5 sulfoxide 16.9 sulfone
	40/0/10/160/8	A	8.8	14.4 (*R*) 15.5 (*S*)

[a] Program: initial T (ºC)/ time (min)/ slope (ºC/min)/T (ºC)/ time (min)/ slope (ºC/min)/T (ºC)/ time (min).

Bicyclo[3.2.0]6hepten-2-one (**1**), thioanisol (**2**), benzyl ethyl sulfide (**3**)

6. *Aspergillus fumigatus* Af293 BVMO encoding genes

Figure S2

Multiple sequence alignment of *A. fumigatus* Af293 BVMOs sequences. Sequences are: $BVMO_{Af1}$ (XP_747160), $BVMO_{Af2}$ (XP_746949), $BVMO_{Af3}$ (XP_755274), XP_751302, XP_747774, XP_754119, XP_752204, XP_756084 from *A. fumigatus* Af293, PAMO (YP_289549) from *Thermobifida fusca*, CHMO (AAG10021) from *Acinetobacter sp*., and CAMO (AET80001.1) from *Cylindrocarpon radicicola*. The two Rossmann folds (GxGxxG) and the BVMO fingerprint (FxGxxxHxxxWP/D) are in bolds

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                      61                                                  120
BVMOAf1_Af293     (5) RPDYDAIVIGAGFSGVRSLWELR----RLGLTARCFDAGSDVGGTWWWNRYPGCRTDGEA
BVMOAf2_Af293     (1) -MDYDIIIVGAGISGINAAYRIQS--QLPSHRYAILEARNAIGGTWDLFKYPGIRSDSDL
BVMOAf3_Af293    (61) EHYLDALVVGTGFSGIYALQSLL----KLNLKVKAIDAASDVGGTWYWSRYPGAMSDSWS
          PAMO   (14) PEEVDVLVVGAGFSGLYALYRLR----ELGRSVHVIETAGDVGGVWYWNRYPGARCDIES
          CAMO    (7) VLNVDALVVGAGVAGIYSTYRLS----RAGLNVQCIDTAGDVGGTWYWNTYPGAMSDTET
          CHMO    (4) KMDFDAIVIGGGFGGLYAVKKLRD---ELELKVQAFDKATDVAGTWYWNRYPGALSDTET
    XP_751302    (51) LPKKRVAVIGAGLTGVSSAAHCIG----HGFDVQLFEARP---------KDKGLGGIWSV
    XP_747774    (32) ARPLRVVIIGSGISGIIASIRFRQR--IPNVDLCVYEKNEDIGGTWLENRYPGCACDIPA
    XP_754119    (54) VRQIKVGVIGAGLSGITAGVLLPAK--LPGLDLRIYDKNADVGGTWFENTYPGVRCDIPA
    XP_751255    (29) PRKLRVVCIGAGFSGLILAYKLKHERPIDFVDYTIYEKNPEVGGTWYENVYPGVGCDIPA
    XP_752204    (19) HTYSPVLVIGAGVSGIATGCRLKEA--LGFDQFRIFERQSGIGGTWWINRYPGAACDVPA
    XP_756084    (37) FTRATAVIIGAGISGICTAIDLIKR--SKCHNFIILEKSSGVGGTWNDNRYPGSCCDVWS
                          GXGXXG
                      121                                                 180
BVMOAf1_Af293    (61) WVYALKFLPELLEEWDFTERYPPQEEIQWYLSRVLDRYDLRKDIEFNTEVKSAHYSDHDS
BVMOAf2_Af293    (58) FTFGFSWNPWN-----QDTPIAEGASISKYMRDTAAQYGIDKHIHFQHRLLAADWSSADN
BVMOAf3_Af293   (117) HLYRYSFDYE----YPLYRRYVSQPEMLAYLRHVVEKYDLRGHMQFNTDMTSAVWDEGTS
          PAMO   (70) IEYCYSFSEEVLQEWNWTERYASQPEILRYINFVADKFDLRSGITFHTTVTAAAFDEATN
          CAMO   (63) YLYRYSWDKEDLRSYPWSNHYVYQPEILQYLRHVVERHDLRKYIRFDTEMQKAVWDDQRS
          CHMO   (61) HLYCYSWDKELLQSLEIKKKYVQGPDVRKYLQQVAEKHDLKKSYQFNTAVQSAHYNEADA
    XP_751302    (98) HSIMYRFHPS----VKYTTAYPSQQEIRDQIIDVWKRYGLQKRTAFDTPVTSVKQTK-DG
    XP_747774    (90) HTYQATFEPN----KEWSTFYAAAPEIHAYWKRVAEKYGCMKYIKLKQAVVEAVWDDSKS
    XP_754119   (112) HVYQSGFEPN----TQWTEEFAQGHEIREYWQRVARKYDVYKYLRPRQKVQKVVWRPEEA
    XP_751255    (89) HSYVFPFEPN----PNWSKFYVSGPEIQDYIVKTTDKYGLRDKITFNTKLLQVAWDEGDG
    XP_752204    (77) LLYSFSFAPK----KDWTTLHPPGPEIVQYLADVCEKYQIVDKIQLNTAVKEMRWLEDAE
    XP_756084    (95) SLYSYSFEQK----ADWTREYPGQEEILDYLIGVAQKYGLYRYIRFNSAVEEARWDEADL

                      241                                                 300
BVMOAf1_Af293   (151) PFPGLQSFKGEVYQTSTWPAHEIEFENKRIGVIGTGSTGIQVITKLAP---VAEQLIVFQ
BVMOAf2_Af293   (148) DVPGLSQFQGQVIHPQFWPQD-LDYTDKKVVIIGSGATAVTLLPKMAE---KAAKVTMLQ
BVMOAf3_Af293   (203) DLPGLQTFRGEIRHTSAWDTD-LDLKGKRVGLVGVGSSGVQLVPAVAD---TVQSLHVFI
          PAMO  (160) NFPGLKDFAGNLYHTGNWPHEPVDFSGQRVGVIGTGSSGIQVSPQIAK---QAAELFVFQ
          CAMO  (153) DIKGISSFAGDLIHTAKWDHS-VELQGKTVGIIGNGSTGVQVMTAIAP---KVSRLVSFQ
          CHMO  (151) NIKGINQFKGELHHTSRWPDD-VSFEGKRVGVIGTGSTGVQVITAVAP---LAKHLTVFQ
    XP_751302   (183) PLPDQGRFKGKIFHSSELDGK--DVEGKKVLIIGGGASAIEALEFAVKS--KARAIDVLS
    XP_747774   (181) DIPGLHDFKGKLQHSARWDES-YDYTGKRAAVIGNGSSGIQIVPGMLP---KVAHIDHYI
    XP_754119   (203) DYEGINDYQGDLFHSSNWNHN-VELKGKRVALIGNGASGLQVLPSIQP---IAAHVDHYA
    XP_751255   (178) DVEGLNLFQGKLLHSARWDPD-YDWTGKGIAVIGNGSSALQIVPELQP---KAAKIVNYI
    XP_752204   (193) TVPGIETFEGEVVHTARWKSD-LDLCGKDVVIIGSGCSATQVVSELTKPEYKIRSITQLM
    XP_756084   (191) DIPGLDDYKGKLMHSARWDWS-FDWTGKRVAVIGNGATAAQIIPEIAK---TAAHVSVYQ
                          FXGXXXHXXXW(P/D)         GXGXX(G/A)
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