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**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

<b>Gene</b>	<b>Size</b>	<b>Vector</b>	<b>Oligonucleotides 5'→3'</b>
<i>Af1</i>	2718	pET200	Fw: CACCATGACCAGAATACGTCCAGAC Rev_EcoRI: <u>CGGAATTCT</u> CAATGCCCATTAGTAGTAACGG
<i>Af1 truncated</i>	1614	pCRE2	Fw: ACTCGAGATCTGCAGCTGGTATGACCAGAATACGTCCAG Rev: GTTCGGGCCCAAGCTTTAACGTGTAAAGCTCATA
<i>Af2</i>	1461	pET200	Fw_Nde I: <u>CACCCATATG</u> GATTACGATATTATCATTGTTGG Rev_Hind III: GCG <u>AAGCTT</u> CTATTGCTGCTTCTTCCAGCC
		pCRE2	Fw: ACTCGAGATCTGCAGCTGGTATGGATTACGATATTATCA Rev: GTTCGGGCCCAAGCTTTATTGCTGCTTCTTCCAG
<i>Af3</i>	1806	pET200	Fw_Nde I: <u>CACCCATATG</u> CGTTGCATACCCTGCC Rev_Eco RI: GCG <u>AATTCT</u> TATAGAAGCGGCCGCGGC
<i>Af3 truncated</i>	1632	pCRE2	Fw: ACTCGAGATCTGCAGCTGGTATGTCAGAACACTACCTCG Rev: GTTCGGGCCCAAGCTTTATAGAAGCGGCCGCGGC

## 2. Expression conditions

**Table S2**

All the expression conditions assayed are listed below

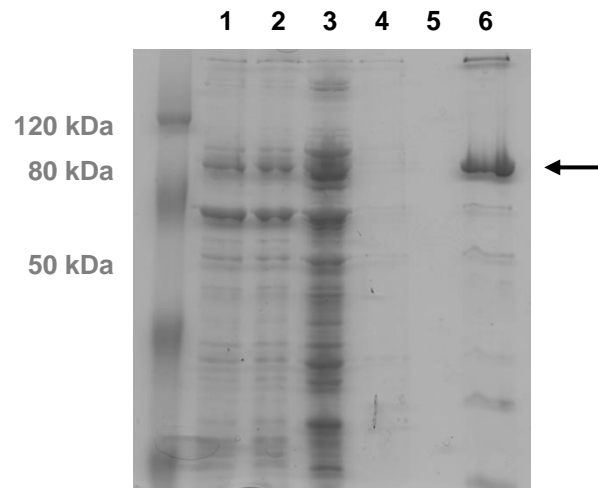
<i>Vector</i>	<i>Cells</i>	<i>Growing T<sup>a</sup></i>	<i>Inductor</i>	<i>Induction T</i>	<i>Induction time</i>
pET-Af1 pET-Af2 pET-Af3	BL21	37 °C (OD <sub>600</sub> =0.5)	IPTG 0.5 mM	17°C 24°C 30°C 37°C	6 h
pET-Af1 pET-Af2 pET-Af3	BL21	37 °C (OD <sub>600</sub> =0.5)	IPTG 1 mM	17°C 24°C 30°C 37°C	6 h
pCRE-Af1 pCRE-Af2 pCRE-Af3	TOP 10	-	Arabinose 0.002% w/v	17°C 24°C 30°C 37°C	48 h 36 h 24 h 12 h
pCRE-Af1 pCRE-Af2 pCRE-Af3	TOP 10	-	Arabinose 0.02% w/v	17°C 24°C 30°C 37°C	48 h 36 h 24 h 12 h
pCRE-Af1 pCRE-Af2 pCRE-Af3	TOP 10	-	Arabinose 0.2% w/v	17°C 24°C 30°C 37°C	48 h 36 h 24 h 12 h

<sup>a</sup> For pCRE2 based constructs, no pre-culture is needed, growing is directly in the presence of inducer

3. Expression of BVMO<sub>Af1</sub>

**Figure S1**

SDS-PAGE analyses for BVMO<sub>Af1</sub> 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<sub>Af1</sub>



#### 4. Substrates screening

**Table S3**

BVMO<sub>Af1</sub> substrates profile

<b>Entry</b>	<b>Substrate</b>	<b>BVMO<sub>Af1</sub><sup>a</sup></b>
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	

<sup>a</sup> 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  $\mu$ m, 12.2 psi N<sub>2</sub>); column B: Hewlett Packard HP-1 (30m x 0.32 mm x 0.25 $\mu$ m, 12.2 psi N<sub>2</sub>) and C: Chirasil Dex CB (30 m x 0.25 mm x 0.25  $\mu$ m, 12 psi N<sub>2</sub>). For all the analyses, the injector temperature was 200°C and the FID temperature was 250°C

**Table S4**

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

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

<sup>a</sup> Program: initial T (°C)/ time (min)/ slope (°C/min)/T (°C)/ time (min)/ slope (°C/min)/T (°C)/ time (min).

Bicyclo[3.2.0]hepten-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<sub>Af1</sub> (XP\_747160), BVMO<sub>Af2</sub> (XP\_746949), BVMO<sub>Af3</sub> (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 *Cylindrocarpum radicolica*. The two Rossmann folds (GxGxxG) and the

BVMO fingerprint (FxGxxxHxxxWP/D) are in bolds

