CLONING, OVEREXPRESSION AND BIOCATALYTIC EXPLORATION OF A NOVEL BAIZER-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: CACCATGACCAGAAATACGTCACAGAC                              |
|              |      |        | Rev_EcoRI: CGGAATTCTCAATGCCCATTTAGTAGTAACGG                  |
| **Af1 truncated** | 1614 | pCRE2  | Fw: ACTCGAGATCTGCAGCTGTATGACCAGAAATACGTCACAG                |
|              |      |        | Rev: GTCGGGCCCCCAAGCTTTAACGTGTAAGCTCATA                     |
| **Af2**      | 1461 | pET200 | Fw_Nde I: CACCATATGGATTACGATATTATCATATTGTTGG                |
|              |      |        | Rev_Hind III: GCGAAGCTTCTATTGCTGCTTCTTCAGGCC               |
|              |      | pCRE2  | Fw: ACTCGAGATCTGCAGCTGTATGACCAGATATTATCATATTGTTGG          |
|              |      |        | Rev: GTCGGGCCCCCAAGCTTTAACGTGTAAGCTCATA                     |
| **Af3**      | 1806 | pET200 | Fw_Nde I: CACCATATGGTTGCTACCCTGCC                          |
|              |      |        | Rev_Eco RI: GCGGAATTCTTATAGAAGCGGCGCGCGGC                   |
| **Af3 truncated** | 1632 | pCRE2  | Fw: ACTCGAGATCTGCAGCTGTATGTCAGAAACTACCTCG                   |
|              |      |        | Rev: GTCGGGCCCCCAAGCTTTATAGAAGCGGCGCGCGGC                   |
2. Expression conditions

**Table S2**

All the expression conditions assayed are listed below

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

* For pCRE2 based constructs, no pre-culture is needed, growing is directly in the presence of inducer
3. Expression of BVMO$_{A1}$

Figure S1

SDS-PAGE analyses for BVMO$_{A1}$ 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$_{A1}$
### 4. Substrates screening

**Table S3**

BVMO<sub>Ap1</sub> substrates profile

| Entry | Substrate                          | BVMO<sub>Ap1</sub><sup>a</sup> |
|-------|------------------------------------|---------------------------------|
| 1     | 2-propanone                        |                                 |
| 2     | 2-butanoone                        |                                 |
| 3     | 3-buten-2-one                      |                                 |
| 4     | 2-octanoone                        |                                 |
| 5     | 3-octanoone                        |                                 |
| 6     | 4-octanoone                        |                                 |
| 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                         | +                               |

<sup>a</sup> Activity is indicated by: 
- +: low activity 
- ++: medium activity 
- +++: high activity
| No. | Compound                          | Activity |
|-----|----------------------------------|----------|
| 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 N₂); column B: Hewlett Packard HP-1 (30m x 0.32 mm x 0.25µm, 12.2 psi N₂) and C: Chirasil Dex CB (30 m x 0.25 mm x 0.25 µm, 12 psi N₂). 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 | column | $t_R$ (min) substrates | $t_R$ (min) products |
|----------|---------|--------|------------------------|---------------------|
| 1        | 130°C isotherm | C       | 9 (1S,5R)              | 16.5 Abnormal (1R,5S) |
| 2        | 70/0/5/200/0   | B       | 9.63                   | 16.9 Normal (1R,5S)   |
|          |          |        |                        |                     |
| 1        | 130°C isotherm | C       | 9.2 (1R,5S)            | 17.2 Abnormal (1S,5R) |
| 2        | 70/0/5/200/0   | B       | 9.63                   | 17.4 Normal (1S,5R)   |
| 3        | 70/5/5/200/5   | B       | 12.5                   | 16.5 sulfoxide        |
| 4        | 40/0/10/160/8  | A       | 8.8                    | 16.9 sulfone          |

Program: initial T (°C)/ time (min)/ slope (°C/min)/T (°C)/ time (min)/ slope (°C/min)/T (°C)/ time (min).

Bicyclo[3.2.0]6hepten-2-one (1), thioanisol (2), benzyl ethyl sulfide (3)
6. Aspergillus fumigatus Af293 BVO encoding genes

Figure S2

Multiple sequence alignment of A. fumigatus Af293 BVOs sequences. Sequences are:
BVOAf1 (XP_747160), BVOAf2 (XP_746949), BVOAf3 (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
BVOMO fingerprint (FxGxxxHxxxWP/D) are in bolds

| BVOAf1 Af293 | (5) RPDYANIHPGVI VRSLHRER----RLGLTPCFDAGS WGVTHMTY YGRSRTAS | 120 |
| BVOAf2 Af293 | (1) -MDYILIITLPLTNNAYTLSQG-QLPSHRYAIALAINTGYDIFPRPIGPEIRGRLD | |
| BVOAf3 Af293 | (61) EHYLVIYMGTMNLQ----KLNKLVKADAS WGVTHNRYSMRVCAMWS | |
| PAMO | (14) PEYVMDYATYSGLYRER----ELGRSVDVHTYGDCWVTHNRYGRAPNTES | |
| CAMO | (7) VLNVAVLTVNGYTVKDR---ELELVQIADFQATRSLVTHNRYGALPEGN | |
| CHMO | (4) KMDHAVIYMGTMNLQ----ELELVQIADFQATRSLVTHNRYGALPEGN | |
| XP_751302 | (51) LPKKAVMTLLSSAARGC----HGPQDVLIPAR-------KDRLLGIV | |
| XP_747774 | (32) ARPLMVIGMVTSNLIRFQRRAIPNVLFRVYKNE---GTVLRHYECAIP | |
| XP_754119 | (54) VVRQKQPLAFSGVAVGTVVPAK---LPLGDLRITKNAVGVTFEPDLLVYGRPVTA | |
| XP_752204 | (29) PRKLAVIYMGTMNLQ----KLNKLVKADAS WGVTHNRYSMRVCAMWS | |
| XP_755104 | (19) HTYSMYIYMGTMNLQ----KLNKLVKADAS WGVTHNRYSMRVCAMWS | |
| XP_755105 | (37) FTRAYVIYMGTMNLQ----KLNKLVKADAS WGVTHNRYSMRVCAMWS | |
| GPXGXX | | |

| BVOAf1 Af293 | (61) WVKALKGLEELLEDKPEPPEECKTSRPILDIVKPEFNEKSKHYBDHS | 121 |
| BVOAf2 Af293 | (58) PTIGFNIWNNN------QDQIFIAGAEKSKMRDLAPDKGKH---QHLRAFVSANN | |
| BVOAf3 Af293 | (117) HLYMDYDIYD-----YPLRYVRSPPLMLARKRHYVDGERGCMKNDMTSWGS | |
| PAMO | (70) IEVCHSHEEVLQENWTKASNLKPRRLNHYVRH--kRHRHAFAT | |
| CAMO | (63) YLRYHEDKEDLRSPGRNHYVPTEDLRHARVHNTLARHDSMQRVYDONS | |
| CHMO | (61) NLKMDKKGQAPPSTLYVQKPKVVRQLRQ---YGCMAEKNHYTA | |
| XP_751302 | (98) HSNIQHRS------WVYPAKMRPIPRPIGPEIRGRLD | |
| XP_747774 | (79) NRPLMVIGMVTSNLIRFQRRAIPNVLFRVYKNE---GTVLRHYECAIP | |
| XP_754119 | (112) HLYMDYDIYD-----YPLRYVRSPPLMLARKRHYVDGERGCMKNDMTSWGS | |
| XP_752204 | (89) IEVCHSHEEVLQENWTKASNLKPRRLNHYVRH--kRHRHAFAT | |
| XP_755104 | (77) LLYMDYDIYD-----YPLRYVRSPPLMLARKRHYVDGERGCMKNDMTSWGS | |
| XP_755105 | (95) SLRSLSEOKK------ADKPRGPEELDHLHSYTRXGTRHNSAPXRH | |

| BVOAf1 Af293 | (151) FPFGQQLSYTVVI VRSLHRER----RLGLTPCFDAGS WGVTHMTY YGRSRTAS | 241 |
| BVOAf2 Af293 | (148) IFPGQQLSYTVVI VRSLHRER----RLGLTPCFDAGS WGVTHMTY YGRSRTAS | |
| BVOAf3 Af293 | (203) FPFGQQLSYTVVI VRSLHRER----RLGLTPCFDAGS WGVTHMTY YGRSRTAS | |
| PAMO | (160) FPFGQQLSYTVVI VRSLHRER----RLGLTPCFDAGS WGVTHMTY YGRSRTAS | |
| CAMO | (153) IEPFPQKQLSYTVVI VRSLHRER----RLGLTPCFDAGS WGVTHMTY YGRSRTAS | |
| CHMO | (150) IEPFPQKQLSYTVVI VRSLHRER----RLGLTPCFDAGS WGVTHMTY YGRSRTAS | |
| XP_751302 | (183) PLPGQQLSYTVVI VRSLHRER----RLGLTPCFDAGS WGVTHMTY YGRSRTAS | |
| XP_747774 | (181) IEPFPQKQLSYTVVI VRSLHRER----RLGLTPCFDAGS WGVTHMTY YGRSRTAS | |
| XP_754119 | (203) IEPFPQKQLSYTVVI VRSLHRER----RLGLTPCFDAGS WGVTHMTY YGRSRTAS | |
| XP_752204 | (178) IEPFPQKQLSYTVVI VRSLHRER----RLGLTPCFDAGS WGVTHMTY YGRSRTAS | |
| XP_755104 | (191) IEPFPQKQLSYTVVI VRSLHRER----RLGLTPCFDAGS WGVTHMTY YGRSRTAS | |

ESM8

FXGXXXHXXX (P/D) GXGXX (G/A)