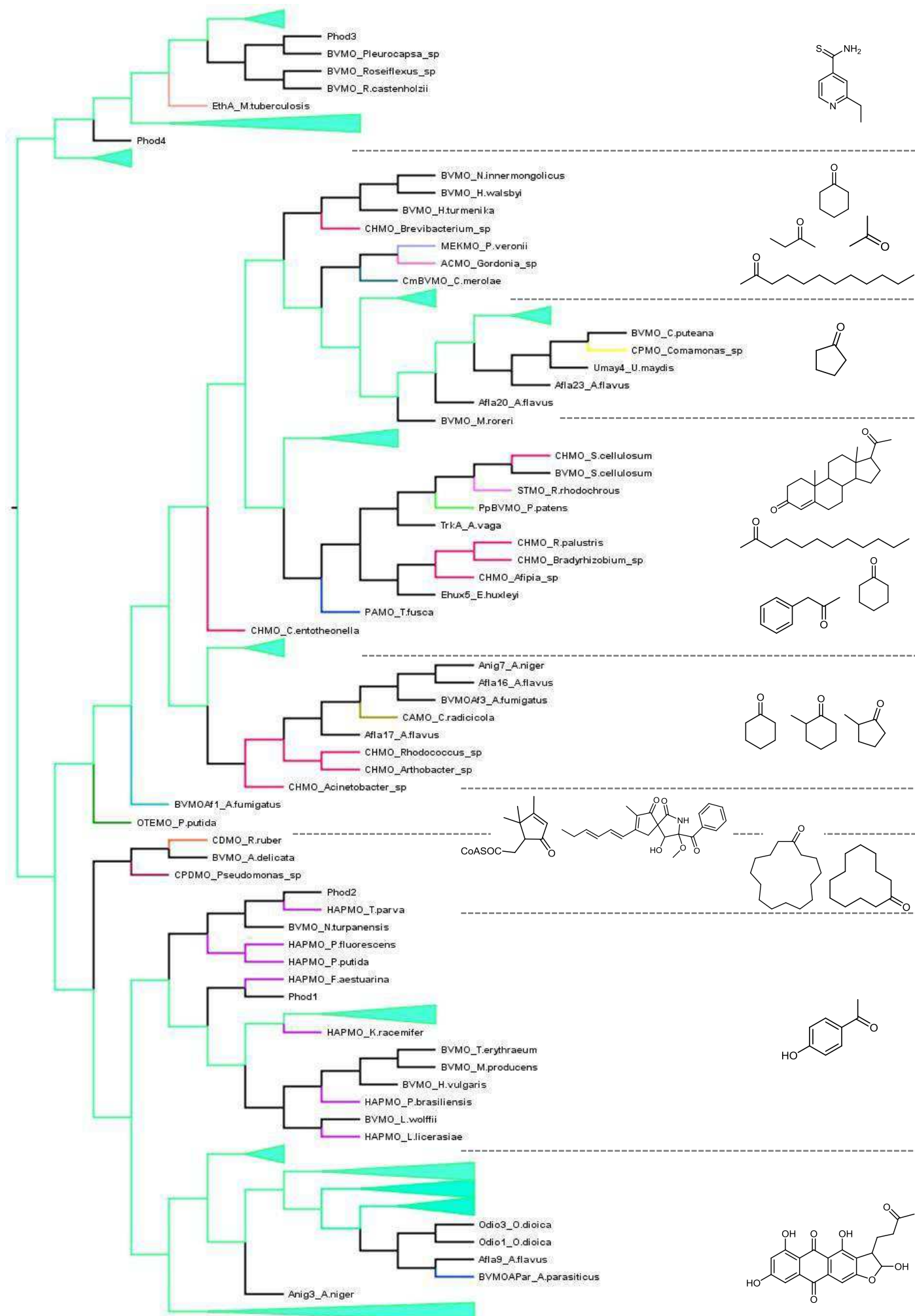


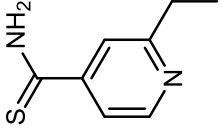
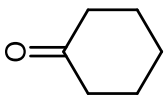
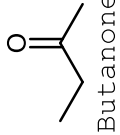
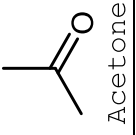
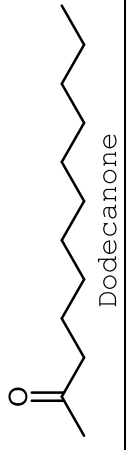
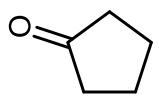
Analysis of the phylogenetic distribution of BVMOs and its substrate specificity

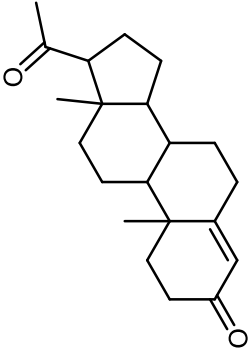
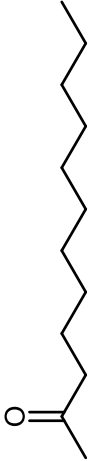
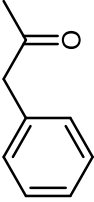
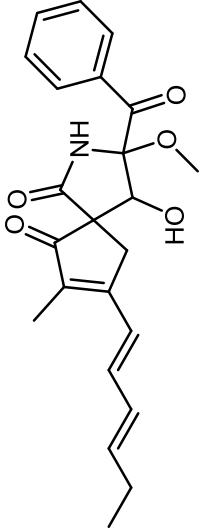
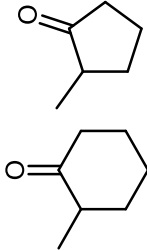
In the literature it has been largely stated that BVMOs cluster together according to its substrate specificity [1, 2]. To test this hypothesis, we analyzed the distribution of the already characterized BVMOs in our phylogenetic tree (Fig. 2 in the manuscript). To do this, we generated a tree in which branches displaying: (i) BVMO encoding sequences identified for the first time in this work and (ii) BVMO sequences with non-natural substrates identified, are collapsed (Fig. below). Branches were colored according to BVMOs substrate specificity and the structure of the substrate was placed on the right (a full list of BVMO sequences with its natural substrates and the specific references is provided in a table below).

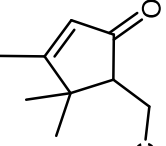
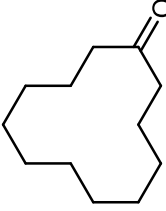
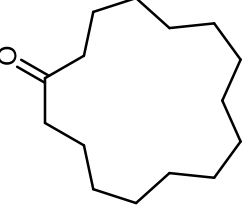
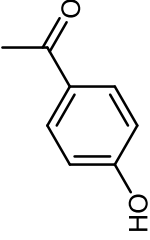
This analysis suggests that BVMOs do not cluster according to its substrate specificity. However it is important to highlight that BVMOs have been deeply characterized using non-natural substrates, mainly with biotechnological purposes. Therefore, there is very limited information about their physiological substrates.

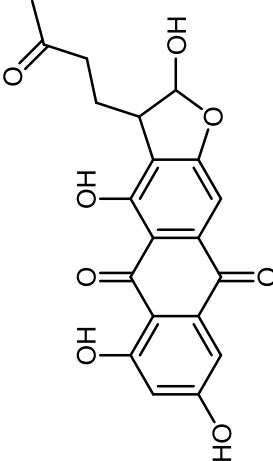
-
1. Mihovilovic MD, Rudroff F, Grotzl B, Kapitan P, Snajdrova R, Rydz J, et al. Family clustering of Baeyer-Villiger monooxygenases based on protein sequence and stereopreference. *Angew Chem Int Ed Engl.* 2005;44(23):3609-13. Epub 2005/04/30. doi: 10.1002/anie.200462964. PubMed PMID: 15861400.
 2. Fink MJ, Fischer TC, Rudroff F, Dudek H, Fraaije MW, Mihovilovic MD. Extensive substrate profiling of cyclopentadecanone monooxygenase as Baeyer–Villiger biocatalyst reveals novel regiodivergent oxidations. *Journal of Molecular Catalysis B: Enzymatic.* 2011;73(1–4):9-16. doi: <http://dx.doi.org/10.1016/j.molcatb.2011.07.003>.



BVMO	Organism	Substrate	Reference
EthA	<i>Mycobacterium tuberculosis</i>	 Ethionamide	[1]
CHMO	<i>Acinetobacter</i> NCIMB 9871	 Cyclohexanone	[2]
MEKMO	<i>Pseudomonas veronii</i> MEK700	 Butanone	[3]
ACMO	<i>Gordonia</i> sp TY-5	 Acetone	[4]
CmBVMO	<i>Cyanidioschyzon merolae</i>	 Dodecanone	[5]
CPMO	<i>Comamonas</i> sp NCIMB 9872	 Cyclopentanone	[6]

STMO	<i>Rhodococcus rhodochrous</i>	 <p>Dehydro-<i>epi</i>-androsterone</p>	[7]
PpBVO	<i>Physcomitrella patens</i>	 <p>Dodecanone</p>	[5]
PAMO	<i>Thermobifida fusca</i>	 <p>Phenylacetone</p>	[8]
BVMO_{Af1}	<i>Aspergillus fumigatus</i>	 <p>Deepoxy-synerazole</p>	[9]
CAMO	<i>Cylindrocarpon radiculicola</i>	 <p>2-methylcyclohexanone, 2-methylcyclopentanone</p>	[10]

OTEMO	<i>Pseudomonas putida</i> ATCC 17453	<div><p>2-oxo-Delta(3)-4,5,5-trimethylcyclopentenylacetyl-Coenzyme A</p></div>	[11]
CDMO	<i>Rhodococcus ruber</i> SC1	<div><p>Cyclododecanone</p></div>	[12]
CPDMO	<i>Pseudomonas</i> sp	<div><p>Cyclopentadecanone</p></div>	[13]
HAPMO	<i>Pseudomonas fluoresces</i> ACB	<div><p>4-hydroxyacetophenone</p></div>	[14]

BVMO_{Apar}	<i>Aspergillus parasiticus</i>	 <p>Hydroxyversicolorone</p>	[15]
----------------------------	--------------------------------	--	------

References

1. Fraaije MW, Kamerbeek NM, Heidekamp AJ, Fortin R, Janssen DB. The prodrug activator EtaA from *Mycobacterium tuberculosis* is a Baeyer-Villiger monooxygenase. *J Biol Chem.* 2004;279(5):3354-60. Epub 2003/11/12. doi: 10.1074/jbc.M307770200 PMID: 14610090.
2. Trudgill PW. Cyclohexanone 1,2-monooxygenase from *Acinetobacter* NCIMB 9871. *Methods Enzymol.* 1990;188:70-7. PubMed PMID: 2177829.
3. Volker A, Kirschner A, Bornscheuer UT, Altenbuchner J. Functional expression, purification, and characterization of the recombinant Baeyer-Villiger monooxygenase MekA from *Pseudomonas veronii* MEK700. *Appl Microbiol Biotechnol.* 2008;77(6):1251-60. doi: 10.1007/s00253-007-1264-6. PubMed PMID: 18034235.
4. Kotani T, Yurimoto H, Kato N, Sakai Y. Novel acetone metabolism in a propane-utilizing bacterium, *Gordonia* sp. strain TY-5. *J Bacteriol.* 2007;189(3):886-93. doi: 10.1128/JB.01054-06. PubMed PMID: 17071761; PubMed Central PMCID: PMC1797311.
5. Beneventi E, Niero M, Motterle R, Fraaije M, Bergantino E. Discovery of Baeyer-Villiger monooxygenases from photosynthetic eukaryotes. *Journal of Molecular Catalysis B: Enzymatic.* 2013;98(0):145-54. doi: <http://dx.doi.org/10.1016/j.molcatb.2013.10.006>.

6. Iwaki H, Hasegawa Y, Wang S, Kayser MM, Lau PC. Cloning and characterization of a gene cluster involved in cyclopentanol metabolism in *Comamonas* sp. strain NCIMB 9872 and biotransformations effected by *Escherichia coli*-expressed cyclopentanone 1,2-monooxygenase. *Appl Environ Microbiol*. 2002;68(11):5671-84. PubMed PMID: 12406764; PubMed Central PMCID: PMC129933.
7. Franceschini S, van Beek HL, Pennetta A, Martinoli C, Fraaije MW, Mattevi A. Exploring the structural basis of substrate preferences in Baeyer-Villiger monooxygenases: insight from steroid monooxygenase. *J Biol Chem*. 2012;287(27):22626-34. doi: 10.1074/jbc.M112.372177. PubMed PMID: 22605340; PubMed Central PMCID: PMC3391149.
8. Fraaije MW, Wu J, Heuts DP, van Hellemond EW, Spelberg JH, Janssen DB. Discovery of a thermostable Baeyer-Villiger monooxygenase by genome mining. *Appl Microbiol Biotechnol*. 2005;66(4):393-400. doi: 10.1007/s00253-004-1749-5. PubMed PMID: 15599520.
9. Wiemann P, Guo CJ, Palmer JM, Sekonyela R, Wang CC, Keller NP. Prototype of an intertwined secondary-metabolite supercluster. *Proc Natl Acad Sci U S A*. 2013;110(42):17065-70. Epub 2013/10/02. doi: 1313258110 [pii] 10.1073/pnas.1313258110. PubMed PMID: 24082142; PubMed Central PMCID: PMC3801025.
10. Leipold F, Wardenga R, Bornscheuer UT. Cloning, expression and characterization of a eukaryotic cycloalkanone monooxygenase from *Cylindrocarpum radicicola* ATCC 11011. *Appl Microbiol Biotechnol*. 2012;94(3):705-17. doi: 10.1007/s00253-011-3670-z. PubMed PMID: 22075635.
11. Leisch H, Shi R, Grosse S, Morley K, Bergeron H, Cygler M, et al. Cloning, Baeyer-Villiger biooxidations, and structures of the camphor pathway 2-oxo-Delta(3)-4,5,5-trimethylcyclopentenylacetyl-coenzyme A monooxygenase of *Pseudomonas putida* ATCC 17453. *Appl Environ Microbiol*. 2012;78(7):2200-12. Epub 2012/01/24. doi: AEM.07694-11 [pii] 10.1128/AEM.07694-11. PubMed PMID: 22267661; PubMed Central PMCID: PMC3302634.

12. Kostichka K, Thomas SM, Gibson KJ, Nagarajan V, Cheng Q. Cloning and characterization of a gene cluster for cyclododecanone oxidation in *Rhodococcus ruber* SC1. *J Bacteriol.* 2001;183(21):6478-86. doi: 10.1128/JB.183.21.6478-6486.2001. PubMed PMID: 11591693; PubMed Central PMCID: PMC100144.
13. Iwaki H, Wang S, Grosse S, Bergeron H, Nagahashi A, Lertvorachon J, et al. *Pseudomonas* cyclopentadecanone monooxygenase displaying an uncommon spectrum of Baeyer-Villiger oxidations of cyclic ketones. *Appl Environ Microbiol.* 2006;72(4):2707-20. doi: 10.1128/AEM.72.4.2707-2720.2006. PubMed PMID: 16597975; PubMed Central PMCID: PMC1449013.
14. Kamerbeek NM, Moonen MJ, Van Der Ven JG, Van Berkel WJ, Fraaije MW, Janssen DB. 4-Hydroxyacetophenone monooxygenase from *Pseudomonas fluorescens* ACB. A novel flavoprotein catalyzing Baeyer-Villiger oxidation of aromatic compounds. *Eur J Biochem.* 2001;268(9):2547-57. PubMed PMID: 11322873.
15. Wen Y, Hatabayashi H, Arai H, Kitamoto HK, Yabe K. Function of the *cypX* and *moxY* genes in aflatoxin biosynthesis in *Aspergillus parasiticus*. *Appl Environ Microbiol.* 2005;71(6):3192-8. doi: 10.1128/AEM.71.6.3192-3198.2005. PubMed PMID: 15933021; PubMed Central PMCID: PMC1151844.