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Biocatalyst assessment of recombinant whole-cells expressing the Baeyer-Villiger monooxygenase from *Xanthobacter* sp. ZL5

Daniela V. Rial [a], Petra Cernuchova [a], Jan B. van Beilen [b], Marko D. Mihovilovic [a,*]

[a] *Vienna University of Technology, Institute of Applied Synthetic Chemistry, Getreidemarkt 9/163-OC, A-1060 Vienna, Austria*
[b] *ETH Zürich, Institute of Molecular Systems Biology, 8093 Zürich, Switzerland*

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Dedicated to Professor Dr. Vicente Gotor Santamaría on the occasion of his 60th birthday.

Abstract

An *Escherichia coli*-based expression system for the Baeyer-Villiger monooxygenase (BVMO) from *Xanthobacter* sp. ZL5 was screened for whole-cell-mediated biotransformations. Biooxidation studies included kinetic resolutions and regiodivergent conversions of structurally diverse cycloketones. An extended phylogenetic analysis of the BVMOs currently available as recombinant systems with experimentally determined Baeyer-Villigerase activity showed that the enzyme originating from *Xanthobacter* sp. ZL5 clusters together with the sequences of bacterial CHMO-type BVMOs. The regio- and enantiopreferences experimentally observed for this enzyme are clearly similar to the biocatalytic performance of cyclohexanone monooxygenase from *Acinetobacter* as prototype for this group of BVMOs and support our previously reported family grouping.

Keywords: Baeyer-Villiger oxidation; Biocatalysis; Biotransformation; Oxygenation; Substrate acceptance

1. Introduction

During the last decades the Baeyer-Villiger monooxygenase family has emerged as one of the most outstanding protein families used as powerful biocatalysts in organic chemistry. Due to its versatility and high stereoselectivity the enzymatic Baeyer-Villiger oxidation allows access to chiral lactones of great importance for the subsequent synthesis of bioactive compounds and natural products and for the production of enantiomerically pure intermediates for industrial applications [1–4].

Baeyer-Villiger monooygenases (BVMOs) are flavoenzymes that catalyze the Baeyer-Villiger reaction by inserting an oxygen atom next to a keto function thus converting ketones to the corresponding esters or lactones (Scheme 1). They are dependent on non-covalently but firmly bound flavin nucleotides as cofactors and on NADPH or NADH as reducing agents. The flavin moiety and a C4a-flavin-peroxo intermediate in particular were proposed [5] and later confirmed by stopped-flow techniques as the critical active species for the oxygenation process [6]. In addition to the above reactivity, flavoenzymes are capable to perform other oxygenation reactions, and a comprehensive comparison of their structure and mechanism of catalysis has been published recently [7].

BVMOs in particular have been classified into two groups depending on the flavin cofactor (FMN or FAD) and the source of electrons (NADH or NADPH) required. Type I BVMOs require FAD and NADPH for activity while type II BVMOs contain FMN and use NADH as electron source [8]. This concept is consistent with the general classification of flavoproteins and the above two types are identical to class B and C flavin-containing monooxygenases [7]. Over the years, several organisms have been reported to express enzymes capable of catalyzing Baeyer-Villiger oxidations on non-natural substrates of large structural diversity [3]. However, the monooxygenase MtmOIV from the mithramycin biosynthetic pathway in *Streptomyces argillaceus* is the only enzyme of this superfamily identified as such with its natural substrate premithramycin B [9].

Amongst BVMOs, cyclohexanone monooxygenase (CHMO) from *Acinetobacter* sp NCIMB 9871 is the best studied enzyme so far, and it has been the most widely used in organic synthesis

Abbreviations: BVMO, Baeyer-Villiger monooxygenase; CHMO, cyclohexanone monooxygenase; CPMO, cyclopentanone monooxygenase; PAMO, phenylacetone monooxygenase; STMO, steroid monooxygenase; HAPMO, 4-hydroxyacetophenone monooxygenase; CDMO, cyclododecanone monooxygenase; CPDMO, cyclopentadecanone monooxygenase.

* Corresponding author. Tel.: +43 1 58801 15420; fax: +43 1 58801 15499.
E-mail address: mmihovil@pop.tuwien.ac.at (M.D. Mihovilovic).

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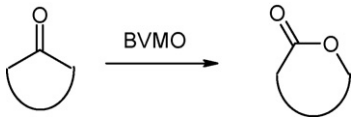


Scheme 1. Biocatalytical Baeyer-Villiger oxidation.

due to its broad substrate acceptance profile and excellent stereoselectivity [3,10]. In facilitating applications of such biocatalysts on laboratory scale, particular progress was made by employing recombinant whole-cells bearing strong promoters [11–13] to overexpress the BVMO inside prokaryotic or eukaryotic hosts as a solution to circumvent cofactor recycling and limited enzyme stability as well as to minimize the potential of unwanted enzymatic side-reactions. The identification, cloning and crystallization of phenylacetone monooxygenase (PAMO) originating from the moderately thermophilic organism *Thermobifida fusca* [14,15] broadened the perspectives to overcome the thermoinstability drawback faced by BVMOs and provided the first crystallographic structure of an enzyme of the type I family. Nowadays, as a result of genome mining projects and successful cloning strategies the ever-growing availability of recombinant organisms bearing appropriate overexpression systems for BVMOs [14,16–28] not only enlarge the collection of enzymes feasible for being used as whole-cell biocatalysts but also offers the possibility to purify them, thus facilitating and complementing their study and characterization [29].

Previous multiple sequence alignments and phylogenetic studies have distinguished defined groups of BVMOs based on their high degree of identity at primary structure level [30,31]. Furthermore, following a similar approach the protein sequences of eight BVMOs with well-defined substrate profiles were aligned, the phylogenetic relationships between them were traced and the results were correlated with their biocatalytic performances and stereoselectivities determined experimentally [32]. Based on complementary stereopreferences for the biooxidation of cycloketone substrates, the clustering of cycloketone converting BVMOs into CHMO- or CPMO-type enzymes was proposed by us. Based on these results the possibility to predict the performance of a novel BVMO became an option and represented the first link between protein sequence and biocatalytic behaviour in this field.

In 2003, highly degenerated oligonucleotides were designed based on two short amino acid sequences conserved between two known and one putative BVMO in an effort to clone new type I BVMO genes [31]. With the aim of amplifying internal fragments of type I BVMO genes by PCR, these primers were used together with total DNA isolated from strains able to grow on alicyclic compounds. In this way internal fragments of 12 type I BVMO genes from Gram+ and Gram− bacteria were cloned. All these fragments were found to encode peptides homologous to published BVMO sequences. Three of these fragments were used as probes to clone the complete BVMO genes and flanking DNA regions from *Xanthobacter* sp. ZL5, *Comamonas* sp. NCIMB 9872 and *Rhodococcus rhodochrous* DSM 11097. In the first case, the nature and activity of the cloned enzyme were confirmed by heterologous expression from a pET11a derived-vector and by BVMO assays where the recombinant enzyme converted cyclohexanone to the corresponding lactone [31].

In this work we report a more detailed analysis of the substrate profile, stereo- and regioselectivity of recombinant whole-cells expressing the BVMO from *Xanthobacter* sp. ZL5 (GenBank Accession number CAD10801). The phylogenetic analysis of the BVMOs currently available as recombinant systems with experimentally determined BVMO activity showed that the BVMO from *Xanthobacter* clusters together with the CHMO-type sequences. Consequently, the biocatalytic performance of the system was expected to be similar to CHMO from *Acinetobacter*, which was tested in kinetic resolutions and regiodivergent biooxidations.

2. Experimental

2.1. General

Chemical reagents and microbial growth media were purchased from commercial suppliers and used without further purification, unless otherwise specified. Solvents were distilled before use. Substrates used in this study were either commercially available or previously synthesized in our lab. Wherever possible, chemical Baeyer-Villiger oxidation of ketones utilizing *m*-chloroperbenzoic acid was carried out for reference purposes.

2.2. Strains and plasmids

The BVMO from *Xanthobacter* sp. was expressed in *Escherichia coli* from plasmid p11X5.1. The construction of this pET11a derived-vector was reported previously [31]. BL21 (DE3) cells were used as hosts for the expression of the BVMO from *Xanthobacter* sp. under the control of the *T7/lac* promoter using plasmid p11X5.1. These cells carry a chromosomal copy of the T7 RNA polymerase gene under the control of a *lacUV5* promoter. DH5α cells were used for propagation and production of plasmid.

Plasmid was isolated using the Wizard *Plus* SV Minipreps DNA Purification System (Promega) and BL21 (DE3) bacteria were freshly transformed before screening and biotransformations. BL21 (DE3) cells were made competent by treatment with $CaCl_2$ and transformed with plasmid DNA by incubation on ice and heat shock at 42 °C according to general procedures [33].

2.3. Phylogenetic relationships

We selected 18 sequences that represent the majority of BVMOs available as recombinant systems in *E. coli* to our knowledge and that have been employed in biotransformation studies. Clustal X v. 1.83 [34] was used to generate multiple sequence alignment of the full-length sequences as well as for phylogenetic tree reconstruction and bootstrapping. The tree was visualized using TreeView v. 1.4 [35].

2.4. Typical procedure for screening of whole-cells using multi-well plates

Precultures were prepared by inoculating 10 mL of LB medium supplemented with ampicillin (200 μg/mL) with a single colony of the corresponding strain and were grown overnight at 30 °C. Cultivation of recombinant *E. coli* BL21 (DE3) bacteria for the expression of the BVMO from *Xanthobacter* sp. was carried out in baffled Erlenmeyer flasks containing LB medium supplemented with ampicillin (200 μg/mL) and inoculated with 1% (v/v) of the overnight grown-preculture. Cultures were shaken at 120 rpm and 30 °C until they reached an OD_{590} of about 0.7. Then, IPTG was added to a final concentration of 0.1 mM, the culture was homogenized and distributed into 24-well plate dishes. Aliquots of 1 mL were loaded onto each well and 0.3–0.5 mg of the appropriate ketone substrate (as a solution in dioxane) was added. The plate was shaken at 24 °C and 120 rpm for 24 h. After this fermentation time samples of 700 μL were taken, centrifuged and the supernatant was extracted with 700 μL of ethylacetate containing 1 mM of methyl benzoate as internal standard. The organic phase was dried over anhydrous Na_2SO_4 and analyzed by chiral phase GC (ThermoFinnigan Trace GC 2000 or Focus GC with a BGB 173 or BGB 175 column, 30 m × 0.25 mm i.d., 0.25 μm film) or GC–MS.

To monitor the time-dependent conversion of 2-methyl-, 2-allyl-, 2-phenyl- and 2-benzyl-cyclohexanones, the same procedure was carried out but samples were taken at different time points after addition of substrate (between 0.5 and 24 h).

Assignments of enantiomers were performed according to the literature. Data were compared to biooxidations using a recombinant expression system of CHMO from *Acinetobacter* (whenever possible) [36,37] or to data from other BVMO-mediated biotransformations.

2.5. Typical procedure for screening of whole-cells using shaking flasks

As a general procedure, 25 mL of fresh LB-ampicillin medium (200 μg/mL) in baffled Erlenmeyer flasks were inoculated with 1% (v/v) of an overnight grown-preculture of BL21 (DE3) bacteria transformed with p11X5.1. The culture was shaken at 120 rpm at 30 °C until OD_{590} was about 0.6–0.7 and IPTG was added to a final concentration of 0.1 mM together with 10 mg of the corresponding substrate. The culture was shaken at 120 rpm and 24 °C during 24 h. After this fermentation time, 1.5 mL samples were taken, centrifuged and the supernatant was extracted with 700 μL of ethylacetate containing 1 mM of methyl benzoate as internal standard. The organic phase was dried over anhydrous Na_2SO_4 and analyzed by chiral phase GC.

3. Results and discussion

3.1. Phylogenetic relationships among confirmed BVMOs

With the aim to include the BVMO from *Xanthobacter* sp. ZL5 into the current scenario of available BVMOs, we selected 18 BVMO sequences that fulfil the following two requirements: (i) they correspond to BVMOs that had been cloned and expressed in *E. coli* and (ii) their activity had been confirmed experimentally by biooxidation of ketones to lactones or esters. Using Clustal X v. 1.83 [34] we aligned their primary sequences and constructed the unrooted phylogenetic tree as shown in Fig. 1. It contains well-defined groups for the CHMO-type and the CPMO-type cycloketone-converting BVMOs as reported previously [30–32] together with other groups of distinctly different biocatalytic profiles. The BVMO from *Xanthobacter* sp. ZL5 was initially localized in the branch corresponding to cycloalkanone monooxygenases [31].

By expanding the phylogenetic analysis, we found that it clusters together with CHMO-type BVMOs. This group also includes CHMOs from *Acinetobacter* sp. NCIMB 9871, *Rhodococcus* Phi1, *Rhodococcus* Phi2, *Arthrobacter* BP2, *Brachymonas petroleovorans*, while CPMO from *Comamonas* sp. NCIMB 9872 and CHMO 2 from *Brevibacterium* HCU compose the CPMO-type cluster. The CHMO 1 from *Brevibacterium* HCU is located at the borderline of these two clusters, which have been reported to produce enantiocomplementary chiral lactones for several substrates. PAMO and STMO define a clade between the CHMO- and CPMO-family, however with a clearly distinct substrate profile. CDMO clusters together with CPDMO; while HAPMO and the sequences for BVMOs from *Mycobacterium* and *Pseudomonas fluorescens* are localized more distantly from the classical cycloalkanone monooxygenases.

3.2. Substrate profiling of the BVMO from Xanthobacter sp. ZL5

To investigate the potential of the BVMO from *Xanthobacter* sp. ZL5 to catalyze enzyme-mediated Baeyer-Villiger oxidations of ketones with different structural demands, a large collection of monosubstituted cyclic ketones, fused bicyclobutanones and terpenone derivatives was screened for substrate acceptance in order to confirm the predicted similar behaviour to the representatives of the CHMO-type cluster. As described in Section 2, BL21 (DE3) cells freshly transformed with p11X5.1 were grown until appropriate OD at 30 °C and protein expression was induced with 0.1 mM IPTG followed by addition of the corresponding substrate. Biotransformations were carried out for 24 h at 24 °C and 120 rpm and were analyzed by chiral phase GC or GC–MS.

3.3. Kinetic resolution of 2-substituted cyclohexanones

Since previous reports indicated that the CHMO from *Acinetobacter* can be used for the kinetic resolution of 2-monosubstituted cyclohexanones (Scheme 2) [38–41], the time course of the bioconversion of this class of racemic cyclohexanones was also monitored for the BVMO from *Xanthobacter*. Both aliphatic (methyl and allyl) and aromatic (phenyl and benzyl) substituents were analyzed. Experiments were carried out using 24-well dishes. Both conversions and enantiomeric compositions of remaining substrate and lactone were determined by chiral phase GC during the time course of the reaction. To calcu-

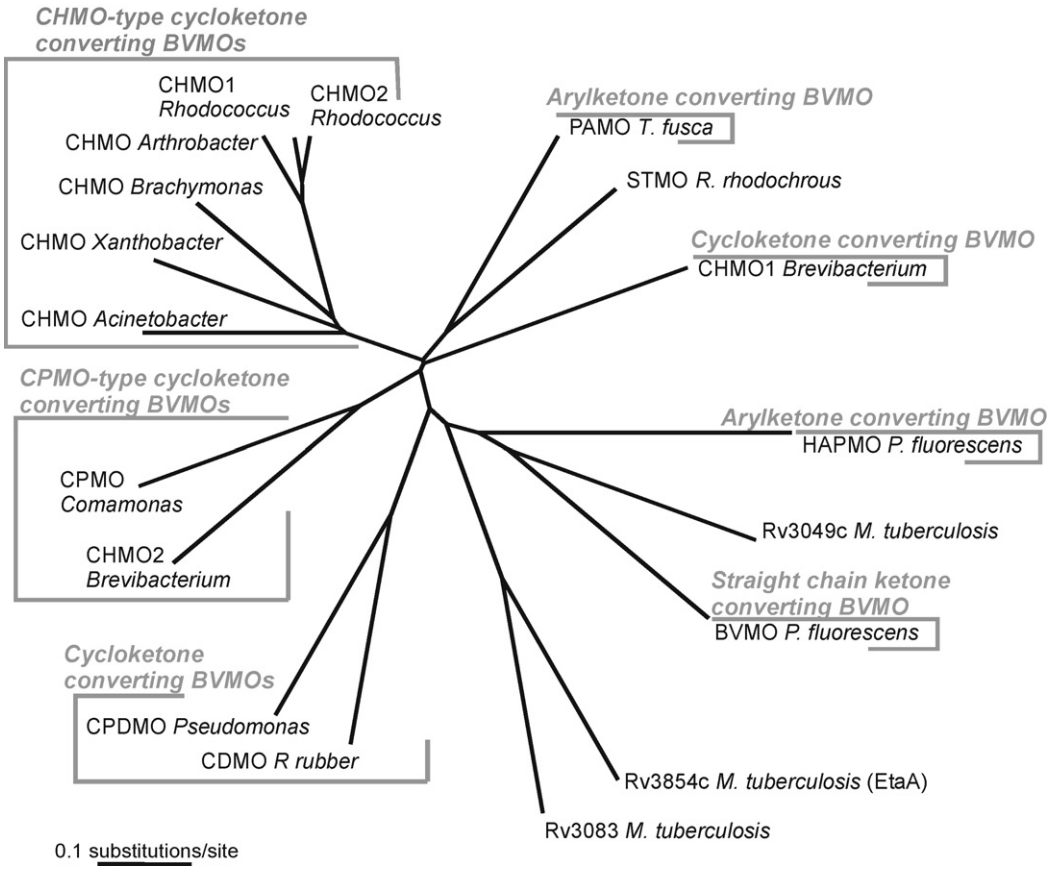
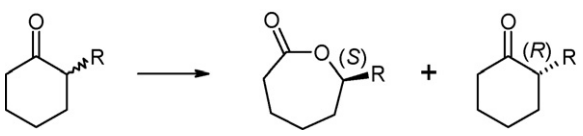


Fig. 1. Phylogenetic relationships within BVMOs. The sequences of 18 proteins with confirmed BVMO activity were aligned and an unrooted phylogenetic tree was calculated using Clustal X v. 1.83 and TreeView v. 1.4 for visualization as described in Section 2. Abbreviations and GenBank accession numbers of protein sequences: CHMO *Acinetobacter*: CHMO *Acinetobacter* sp. NCIMB 9871: BAA86293; CHMO2 *Brevibacterium*: CHMO 2 *Brevibacterium* sp. HCU: AAG01290; CHMO *Arthrobacter*: CHMO *Arthrobacter* sp. BP2: AAN37479; CHMO *Brachymonas*: CHMO *Brachymonas petroleovorans*: AAR99068; CHMO 1 *Brevibacterium*: CHMO1 *Brevibacterium* sp. HCU: AAG01289; CHMO1 *Rhodococcus*: CHMO *Rhodococcus* sp. Phi1: AAN37494; CHMO2 *Rhodococcus*: CHMO *Rhodococcus* sp. Phi2: AAN37491; CHMO *Xanthobacter*: BVMO *Xanthobacter* sp. ZL5: CAD10801; CPMO *Comamonas*: cyclopentanone monooxygenase *Comamonas* sp. NCIMB 9872: BAC22652; PAMO *T. fusca*: phenylacetone monooxygenase *Thermobifida fusca*: 1W4X_A; HAPMO *P. fluorescens*: 4-hydroxyacetophenone monooxygenase *Pseudomonas fluorescens*: AAK54073; STMO *R. rhodochrous*: steroid monooxygenase *Rhodococcus rhodochrous*: BAA24454; CDMO *R. rubber*: cyclododecanone monooxygenase *Rhodococcus rubber*: AAL14233; Rv3854c *M. tuberculosis*: ETaA *Mycobacterium tuberculosis* H37Rv: CAB06212; CPDMO *Pseudomonas*: cyclopentadecanone monooxygenase *Pseudomonas* sp. HI-70: BAE93346; Rv3049c *M. tuberculosis:* BVMO from *Mycobacterium tuberculosis* H37Rv: CAA16134; Rv3083 *M. tuberculosis:* BVMO from *Mycobacterium tuberculosis* H37Rv: CAA16141; BVMO *P. fluorescens*: BVMO from *Pseudomonas fluorescens*: AAC36351.

late the enantioselectivity of the enzymatic process – represented by the Enantiomeric Ratio E – GC data was fitted to a mathematical equation relating the conversion of the reaction with the enantiomeric excess of the remaining substrate and the product using the *Selectivity* software [42] and results are compiled in Table 1. In all four cases oxygen insertion and migration was observed at the more substituted center to give the expected normal regioisomers of lactones. Reactions were usually completed within 6–12 h, except in the case of 2-phenyl-cyclohexanone **3** where only the (+)-enantiomer of the ketone is converted to the (+)-lactone. With the exception of substrate **1**, the reactions proceeded in excellent enantioselectivity ($E > 200$ for 2-phenyl and 2-benzylcyclohexanones, and $E = 99$ for 2-allylcyclohexanone). Similar behaviours were observed for the bioconversions of these racemic ketones catalyzed by CHMO from *Acinetobacter* as summarized in Table 1 and reported previously [38–40].

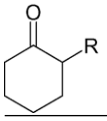


Scheme 2. Kinetic resolution of 2-substituted cyclohexanones.

3.4. Regiodivergent biooxidations

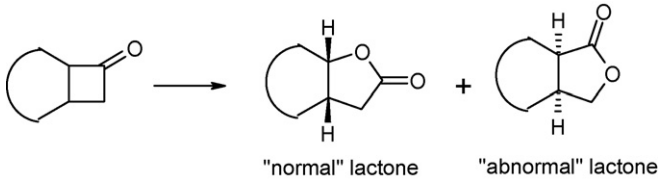
Some fused cyclobutanones and terpenone precursors were screened to thoroughly study the acceptance capability and regiopreference of the BVMO from *Xanthobacter*. Previous studies with CHMO-type enzymes indicated that such racemic ketones are converted in a regiodivergent fashion: each substrate enantiomer is oxidized to regioisomeric lactones; if migration occurs to the more substituted carbon atom the "normal" lactone is generated while in the opposite case the "abnormal" lactone is formed [43]. This behaviour was previously described for other BVMOs and available data are shown as references [44–48].

Table 1
Kinetic resolution of racemic 2-substituted cyclohexanones

Ketone	R	No.	BVMO *Xanthobacter* sp. ZL5			Reference biotransformation using CHMO *Acinetobacter* as biocatalyst			
			Conversions (%)[a]	e.e.$_P$ (%)[a] e.e.$_S$ (%)[a]	E[b]	Yield (%)	e.e.$_P$ (%) e.e.$_S$ (%)	E[b]	References
	Me	**1**	24	62 (−), *29* (+)	6	35	61 (−), *35* (+)	6	[38]
	Allyl	**2**	59	90 (−), *>99* (−)	99	30	>98 (−), *>98* (−)	>200	[39,40]
	Ph	**3**	48	>99 (+), *76* (−)	>200	40	>98 (+), *86* (−)	>100	[38]
	Bn	**4**	45	>99 (−), *71* (−)	>200	22	>96 (−), *78* (−)	>100	[38]

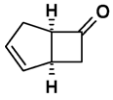
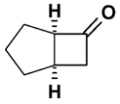
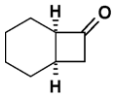
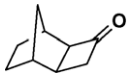
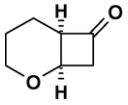
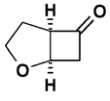
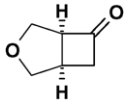
[a] Conversions and e.e. for remaining substrate (e.e.$_S$) and product lactone (e.e.$_P$) obtained were determined by chiral phase gas chromatography. Data for lactones in normal font, data for ketones in italics. Sign of specific rotation is given in parenthesis.
[b] Enantioselectivity values (E) were calculated using the Selectivity software developed by Faber [42].

Table 2 shows the regioselective nature of the *Xanthobacter* BVMO-mediated-biooxidation of fused ketones containing a cyclobutanone structural motif (substrates **5**–**11**, Scheme 3). The enzyme performs similarly to CHMO *Acinetobacter* and other CHMO-type monooxygenases both in conversion rates and regiopreference. For this class of substrates, the BVMO from *Xanthobacter* allowed access to approximately equivalent amounts of "normal" and "abnormal" lactones in very good to excellent enantioselectivities for both regioisomers. A distinctly different observation was made for CPMO-type biocatalysts,



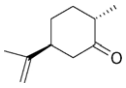
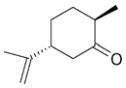
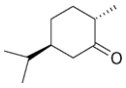
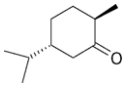
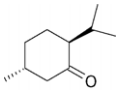
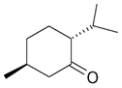
Scheme 3. Regiodivergent biooxidations of fused bicyclobutanones.

Table 2
Regiodivergent biooxidation of fused cyclobutanones

Ketone	No.	BVMO *Xanthobacter* sp. ZL5		Reference biotransformation using CHMO *Acinetobacter* as biocatalyst		
		Consumption (%)[a]/ratio[b]	e.e. (%)[c]	Yield (%)/ratio[b]	e.e. (%)[c]	References
	**5**	+++/52:48	91, *>99*	86/51:49	>95, *>95*	[44]
	**6**	+++/51:49	97, *>99*	67/54:46	>95, *>95*	[44]
	**7**	+++/55:45	84, *>99*	80/65:35	60, *>95*	[44]
	**8**	+++/57:43	75, *>99*			
	**9**	+++/58:42	84, *>99*	78/77:23	33, *>98*	[48]
	**10**	+++/56:44	99, *>99*	74/44:55	>98, *>98*	[48]
	**11**	+++/55:45	84, *97*	70/50:50	97, *>98*	[48]

[a] Consumption of starting material according to gas chromatography: +++: >90%, ++: 50–90%, +: <50%.
[b] Ratio of "normal":"abnormal" lactones.
[c] Data for "normal" lactones in normal font, data for "abnormal" lactones in italics.

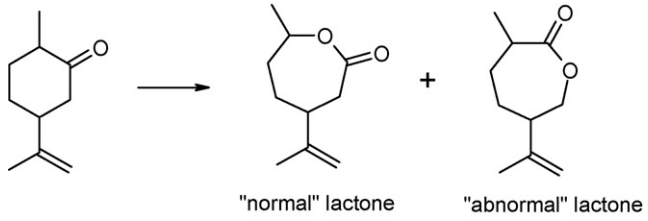
Table 3
Regiodivergent biooxidation of terpenone derivatives

Ketone	No.	BVMO *Xanthobacter* sp. ZL5		Reference biotransformation using CHMO *Acinetobacter* as biocatalyst		
		Consumption (%)[a]/ratio[b]	Specific rotation[c,d]	Consumption (%)/ratio[b]	Specific rotation[c,d]	References
	**12** (−)	+++/100:0	(+)	+++/100:0	(+)	[46,47]
	**12** (+)	+++/0:100	*(−)*	+++/0:100	*(−)*	[46,47]
	**13** (−)	+/100:0	(+)	++/100:0	(+)	[47]
	**13** (+)	+++/0:100	*(−)*	++/0:100	*(−)*	[47]
	**14** (−)	n.c.		n.c.		[46,47]
	**14** (+)	n.c.		+++/100:0	(+)	[46,47]

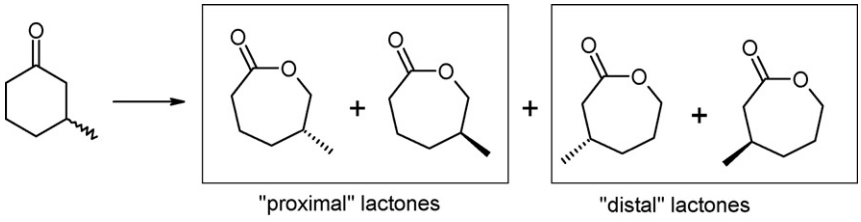
n.c.: no conversion.
[a] Consumption of starting material according to gas chromatography: +++: >90%, ++: 50–90%, +: <50%.
[b] Ratio of "normal":"abnormal" lactones.
[c] Data for "normal" lactones in normal font, data for "abnormal" lactones in italics.
[d] Experiments on terpenone derivatives were performed using pure enantiomers as substrates. The sign of specific rotation is shown in parentheses and has been assigned according to the literature.

as these enzymes predominantly generate "normal" lactones, however, in low stereoselectivity [45]. A BVMO from *Mycobacterium* was recently reported to display kinetic resolutions of such substrates to form "abnormal" lactones, preferably [49].

The general correlation between regiodivergence and family clustering described above for the fused cycloketones is also applicable to pure terpenone derivatives (Table 3, Scheme 4). Due to their high volatility the screenings of terpenone derivatives were carried out in shaking flasks as described in Section 2. The *Xanthobacter* BVMO oxidized (−)-*trans*-dihydrocarvone (−)-**12** to the normal lactone and its (+)-enantiomer (+)-**12** to the abnormal lactone, in agreement to previously reported biooxidations by the CHMO from *Acinetobacter* [46,47]. A similar trend was observed for the biotransformation of carvomenthone **13**. The only difference in substrate acceptance for the BVMO from *Xanthobacter* sp. was encountered in the case of the (±)-menthone **14**. Although (−)-menthone (−)-**14** was biooxidized by none of these two biocatalysts, the CHMO from *Acinetobacter* converted (+)-menthone (+)-**14** to the corresponding normal lactone while cells expressing the BVMO from *Xanthobacter* sp. were unable to oxidize this substrate.



Scheme 4. Baeyer-Villiger biooxidations of terpenone derivatives.



Scheme 5. Biooxidation of 3-methylcyclohexanone to regioisomeric products.

The enzyme from *Xanthobacter* sp. also gave a clear regiodivergent oxidation of racemic 3-methylcyclohexanone (**15**) to equal amounts of proximal and distal lactones (50:50) in excellent enantioselectivities (>99% e.e. in both cases) as it was described for CHMO from *Acinetobacter* when starting from pure enantiomers of 3-methylcyclohexanone (Scheme 5) [50]. Again, this is in marked contrast to CPMO-type enzymes, which usually yield proximal lactones, only.

4. Conclusions

In this work we have exposed the BVMO from *Xanthobacter* sp. ZL5 – as a recombinant whole-cell biocatalyst – to diverse sets of racemic substrates of different sizes, substituents, functional groups and structural features to underscore the remarkably broad substrate acceptance of this enzyme. According to a phylogenetic comparison to other biocatalysts of this class with determined substrate profiles and stereoselectivities, this novel enzyme is a member of the group of CHMO-type cycloketone converting BVMOs. It should be emphasized that – for the substrates described in this work – the BVMO from *Xanthobacter* sp. follows the classical trend of a CHMO-type enzyme both in substrate acceptance as well as in stereo- and regiopreference. Taking into account that the enzyme was cloned from a cyclohexane degrading strain [31] it deserves the more appropriate designation of "CHMO *Xanthobacter*". Consequently, our previous concept to link biocatalyst performance with sequence information was further supported by confirming the predictions for the biotransformation characteristics of a novel BVMO based on sequence alignment in key biooxidation experiments.

Presently we are extending our profiling efforts to desymmetrization reactions using CHMO *Xanthobacter* in order to identify unique properties of this enzyme within the group of CHMO-type BVMOs and to determine the efficiency of the enzyme in preparative scale biotransformations.

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