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The Analysis and Application of a Recombinant Monooxygenase Library as a Biocatalyst for the Baeyer-Villiger Reaction

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Abstract Because of their selectivity and catalytic efficiency, BVMOs are highly valuable biocatalysts for the chemoenzymatic synthesis of a broad range of useful compounds. In this study, we investigated the microbial Baeyer-Villiger oxidation and sulfoxidation of thioanisole and bicyclo[3.2.0]hept-2-en-6-one using whole *Escherichia coli* cells that recombined with each of the Baeyer-Villiger monooxygenases originated from *Pseudomonas aeruginosa* PAO1 and two from *Streptomyces coelicolor* A3(2). The three BVMOs were identified in the microbial genome database by a recently described protein sequence motif; *e.g.*, BVMO motif (FXGXXXHXXXW). The reaction products were identified as (R)-/(S)-sulfoxide and 2-oxabicyclo/3-oxabicyclo[3.3.0]oct-6-en-2-one by GC-MS analysis. Consequently, this study demonstrated that the three enzymes can indeed catalyze the Baeyer-Villiger reaction as a biocatalyst, and effective annotation tools can be efficiently exploited as a source of novel BVMOs.

Keywords: Baeyer-Villiger oxidation, sulfide oxidation, monooxygenase, biocatalysis, GC-MS

Biocatalysis is well recognized as a powerful tool for the organic synthesis of optically active compounds [18, 24, 28]. In recent years, the use of biocatalysts in the food, fine chemicals, agrochemicals, and pharmaceutical industries has become an increasingly attractive alternative to conventional chemical methods [5, 11, 32]. Furthermore, the application of biocatalysts in the mediation of oxidative transformations is an area of particular academic and industrial interest because of their high chemo-, regio-, and stereoselectivities [15, 25, 26, 30]. One of the most promising enzyme groups in oxidative enzymes is the Baeyer-Villiger monooxygenases (BVMOs) (EC 1.14.13.x).

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BVMOs are flavoprotein monooxygenases capable of using molecular oxygen to mediate the nucleophilic oxygenation of a wide range of linear or cyclic ketones, yielding the corresponding esters or lactones, respectively [29]. The BVMO-catalyzed reaction is similar to the conventional Baeyer-Villiger oxidation using peroxyacids that were characterized over a century ago [1], but as a result of the exquisite enantio- and regioselectivities of the enzymatic catalysts, the use of BVMOs gives access to a wide range of optical pure esters and lactones that are not attainable by chemical approaches [20, 23, 26, 29]. In addition, BVMOs can also catalyze the electrophilic oxygenation of various heteroatoms; *e.g.*, sulfur, selenium, nitrogen, and phosphorous [2, 4, 16]. It has been known that BVMOs are produced by numerous bacteria (*e.g.*, the genera *Acinetobacter*, *Arthrobacter*, *Nocardia*, *Pseudomonas*, *Rhodococcus*, *Streptomyces*, and *Xanthobacter*) and fungi (*e.g.*, the genera *Curvularia*, *Aspergillus*, *Cunninghamella*, and *Cylindrocarpon*) and they are involved in oxidative degradation processes and the biosynthesis of secondary metabolites such as aflatoxin [27, 31]. Since their discovery, extensive research has been performed to explore the biocatalytic properties of BVMOs, using either whole cells or isolated enzymes [3, 19, 33]. At present, only a few types of BVMOs have been cloned and overexpressed, such as CHMO (EC 1.14.13.22) from *A. calcoaceticus* NCIMB 9871 [6–8, 29], SMO (EC 1.14.13.54) from *R. rhodochrous*, HAPMO (EC 1.14.13.x) from *P. fluorescens* ACB, CDMO (EC 1.14.13.x) from *R. ruber* SC1, and CPMO (EC 1.14.13.16) from *Comamonas* sp. NCIMB 9872 [12–14, 17, 21]. However, so far, only a scarce number of BVMO genes have been cloned and overexpressed, owing to low activity, limited stability, and need of an expensive cofactor-NAD(P)H, which led to the limited application of these types of biocatalysts for industrial processes.

In order to find novel BVMOs that can be used for biocatalysis, first of all, we retrieved three putative

```
chnB      ---------------------------------------------------------MSQ
PA1538    ---------------------------------VYTPANNHNRSLAMS-----TQPTPAA
SCO0300   MAHAQELTPEALAGLRERYRRERERRVRPDGTRQYLGADAEFGFYAADPWAGESDVREPV
SCO3172   -----------------------------------------------------MAEHEQV

                    GxGxxG                          GG
chnB      KMDFDAIVIGGGFGGLYAVKKLRDELELKVQAFDKATDVAGTWYWNRYPGALTDTETHLY
PA1538    ARHCKVAIIGTGFSGLGMAIRLRQEGEDDFLIFEKDAGVGGTWRVNNYPGCACDVQSHVY
SCO0300   RDRVDVAVVGGGFGGVLAGARLRQQGVARVRVVEKGGDFGGTWYWNRYPGIHCDIEAHVY
SCO3172   HEHVRVAVIGSGFGGLGAAVRLRREGITDFVVLERAGSVGGTWRDNSYPGCACDVPSHLY
                 . ::* **.*:    :** :     .  .::  ...***  * ***   *  :*:*

chnB      CYSWDKELLQS---LEIKKKYVQGPDVRKYLQQVAEKHDLKKSYQFNTAVQSAHYNEADA
PA1538    SFSFEA----N---PEWTRMFARQPEIRAYLEKCWEKYRLQEKTLLNTEIGKLAWDERQS
SCO0300   LPMLDE----TGYVPEWK--YAPGEEIRRHAMRIAETFDLYTDVLFSTAVTSLSWDDTTG
SCO3172   SFSFAP----N---PEWPRTFSGQEHIRAYLEHVADTFGLRPHLRFDSEVKRMAWDTEQL
                .     *    :     .:* :  :  :.. *     :.: :      ::
                          ATG                FxGxxxHxxxW
chnB      LWEVTTEYGDKYTARFLITALGLLSAPNLPNIKGINQFKGELHHTSRWPDDV--------
PA1538    LWHLHDAQGNHYTANAVVSGMGGLSTPAYPRLDGLENFQGKVFHSQQWDHDY--------
SCO0300   EWIVETDRHDAFRATYVITATGVLSELKLPGIPGIERFKGHTFHTSRWDYAYTGGGPDGG
SCO3172   RWEIETVRGT-LTADVVVSATGPLSDPKVPDIPGLDTFPGKVFHSARWDHDY--------
           * :         *  :::. * **      * : *:: * *. .*: :*

                 GxGxxG
chnB      --SFEGKRVGVIGTGSTGVQVITAVAPLAKHLTVFQRSAQYSVPIGNDPLSEEDVKKIK-
PA1538    --DLKGKRVAVIGTGASAIQFVPEIQPLVAALDLYQRTPPWILPKPDRAISETERRRFRR
SCO0300   LTGLADKRVGVVGTGATGVQVIPKLAEDAGQLHVFQRTPSSVDVRANRRTTARDVGADRA
SCO3172   --DLAGQRVAMIGTGASAIQIVPSIQPKVDRLTLFQRTPAWVMPRVDRAISGAERALHRA
            .: .:**.::***::.:*.:. :    .   * ::**:.        :   :  :   :

chnB      -DNYDKIWDGVWNSALAFGLNESTVPAMSVSAEERKAVFEKAWQTGGGFRFMFETFGD--
PA1538    FPLVQKLWRG-----GLYSLLEGRVL----------------------------GFTF--
SCO0300   GWA--------------SERRDNFLRVVSGEAVEEDLVADRWTATAGLLEKLLPSFRRPD
SCO3172   LPATTKLRRG-----LLWGIRELQVQ----------------------------AFTK--
                             :  :                              *

chnB      -----IATNMEANIEAQNFIKGKIAEIVKDPAIAQKLMPQ-DLYAKRPLCDSGYYNTFNR
PA1538    -------APQVMKLV--QRLAIRHIHKQIKDPELRRKVTPDYTIGCKRILMSHNYYPALAA
SCO0300   DLAAFEAAYEVADAARMNDIRARVDDLVTDPATADRLKPWYRYACKRPTFSDLYLQAFNR
SCO3172   ------HPNELGFV--EQIAKRNMGAAIKDPALRAKLTPDYRIGCKRILLSSTYYPALAK
                 .       .: :.**    :: *     .**   .  *  ::
                                          DxxxxATG
chnB      DNVRLEDVK-ANPIVEITENGVKLENGDFVELDMLICATGFDAVD-GNYV-RMDIQGKNG
PA1538    ANS---TVI-TEGIRAVTANGIVDGNGREREVDAIIFGTGFTAND-P-IP-RGVVFGRDG
SCO0300   DNVTLVDTADTHGIERMNERGVVVGD-TEYPLDCLVFATGFSVGVSGVHSGRLPVRGRGG
SCO3172   PNV---DVV-ASGLSEVRGSTLVAADGTEAEADAIVFGTGFHVTD-M-PI-AERVVGADG
           *    .  :  :  :    :   :        * :: .*** .           : * .*

chnB      LAMKDYWK-EGPSSYMGVTVNNYPNMFMVLGPNGPFTN--LPPSIESQVEWISDTI-QYT
PA1538    RDLLDSWS-KGPEAYKGTTTAGFPNLFFLMGPNTGLGHNSMVYMIESQIAYVLDALKLMK
SCO0300   VRLRDAWSARGPRTLHGLTSNGFPNLIQLGGVQSASSVNH--THVLDEHAVHGAAL-VAA
SCO3172   RTLAETWK-GGMEALRGGTAAGFPNFMTVIGPNTGLGNSSMILMIESQLNYLADYLRQLN
            : : *.  *  :  * *  .:**:: : * :                : .:     :

chnB      VENNVESIEATKEAEEQWTQTCANIAEMTLFPKA--QSWIFGANIPGKKNTVY-----FY
PA1538    -RRELLSLEVKAPVQERYNEYLQRKLDRSVWSVGGCKSWYLH-PVSGRNCTLWPGFTWRF
SCO0300   AEAKGAVVEPTREAEDAWIATLAEHAPDHAWFHAECTPGYYN--AEGRGRPNGP------
SCO3172   VLGGRTALDPRPAAVRNWNHRVQERMKRTVWNTGGCTSWYLD--ASGRNTTVWPGTTAEF
                 ::     .    :     .          :    .    .       *:    .

chnB      LGGLKEYRSALANCKNHAYEGFDIQLQR---SDIKQPANA----
PA1538    RALTRQFDASAYHLTTTPLAALSNEARQQAEGVPA---------
SCO0300   ----------TAYPHGAAAFHELLRRWREESMDELLAPRARVRAC
SCO3172   RRETRRVDLAEYQVLRPAPAQVGAKAAEADTGADTGADAEVSA-
                :     .    .    .    .    .
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Fig. 1. Multiple sequence alignment of Baeyer-Villiger monooxygenases (BVMOs) presented in microorganisms.

chB is cyclohexanone monooxygenase from *Acinetobacter calcoaceticus* (GeneBank Accession No. BAA86293), PA1538 is a probable flavin-containing monooxygenase from *Pseudomonas aeruginosa* PAO1 (MO. 57, GeneBank Accession No. AAG04927), SCO0300 is a putative monooxygenase from *Streptomyces coelicolor* A3(2) (MO. 96, GeneBank Accession No. CAB55657), and SCO3172 is a putative monooxygenase from *Streptomyces coelicolor* A3(2) (MO. 103, GeneBank Accession No. CAB59668). Sequences were aligned with the ClustalW program. Asterisks (*) indicate identical amino acids. Common BVMO sequence motifs are indicated in the top line.

monooxygenase sequences that contain several protein sequence motifs. These motifs are indicative of the presence of two Rossman fold domains (GXGXXG, GG, ATG) involved in FAD and NADPH binding and BVMO activity (FXGXXXHXXXW) from the TIGR-CMR database by sequence alignment analysis using a public database (Fig. 1) [10]. In addition, the enzymes were obtained from recombinant *E. coli* strains. Using each newly cloned enzyme, we investigated possible biocatalytic systems that are capable of recycling the cofactor in whole-cell catalyzing Baeyer-Villiger reactions.

MATERIALS AND METHODS

Materials

Restriction enzymes used in cloning procedures were obtained from Roche. *E. coli* Rosetta gami (DE3) and plasmid pGEX-KG were used for cloning and gene expression. Thioanisole and Bicyclo[3.2.0]hept-2-en-6-one were purchased from Aldrich and Merck-Schuchardt. Stock substrates (Thioanisole and Bicyclo[3.2.0]hept-2-en-6-one) solutions were prepared in DMSO (Dimethylsulfoxide; Amresco). All of the other chemicals and reagents used in the enzyme assay were commercially available analytical grade.

Screening of BVMOs Using the Effective Annotation Tools

By using the keyword "monooxygenase" *via* the TIGR-CMR database, we screened all available microbial genomes that can be probed for the occurrence of BVMO genes. As a result of screening, we have constructed a list of 120 putative monooxygenases from various sequencing-finished genomes (Table 1). The putative monooxygenases can be classified into several groups through sequence-based analysis (conserved domain, protein sequence motif, activity site analysis) using a public database. In total, three putative monooxygenase sequences were retrieved from the current databases as a family of BVMOs. Fig. 2 indicates the process of monooxygenase list construction and monooxygenase classification. The BLAST program at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/BLAST/) was used to search for proteins showing sequence similarity. Multiple sequence alignments were made with the ClustalW program at the European Bioinformatics Institute (www.ebi.ac.uk/clustalw).

Cloning and Expression of Recombinant BVMOs

Three monooxygenase genes were amplified using chromosomal DNA as a template from diverse microbial sources. The primers used for PCR to amplify the ORF of the PA1538 (MO.57), SCO0300 (MO.96), and SCO3172 (MO.103) contained proper restriction enzyme sites. The forward primers contained EcoRI restriction sites just right before the start codon, and the reverse primers contained XhoI or HindIII restriction sites at the C-terminal end of the open reading frames. PCR was performed for 30 cycles using a PFU DNA polymerase. Each cycle consisted of the following three steps: 95°C for 1 min, 60°C for 45 sec, and 72°C for 1 min. The resulting PCR products were purified with an AccuPrep Gel Purification Kit (Bioneer, Korea). Purified PCR products were cut with a proper restriction

Table 1. Occurrence of BVMO in microbial genomes from the TIGR-CMR database.

Organism	Total number of genes	Number of putative BVMO genes
Agrobacterium tumefaciens C58 Cereon	10	
Bacillus halodurans C-125	1	
Bacillus subtilis 168	5	
Caulobacter crescentus CB15	3	
Clostridium perfringens 13	2	
Corynebacterium glutamicum ATCC 13032	6	
Deinococcus radiodurans R1	1	
Escherichia coli K12-MG1655	1	
Halobacterium sp. NRC-1	2	
Listeria monocytogenes EGD-e	1	
Mesorhizobium loti MAFF303099	16	
Mycobacterium tuberculosis H37Rv (lab strain)	4	
Neisseria meningitidis serogroup A Z2491	1	
Nostoc sp. PCC 7120	2	
Pseudomonas aeruginosa PAO1	6	1 (MO. 57)
Pseudomonas putida KT2440	7	
Pseudomonas syringae DC3000	1	
Pyrococcus furiosus DSM 3638	2	
Ralstonia solanacearum GMI1000	1	
Salmonella typhimurium LT2 SGSC1412	3	
Sinorhizobium meliloti 1021	6	
Staphylococcus aureus Mu50	1	
Streptococcus pyogenes MGAS8232	1	
Streptomyces coelicolor A3(2)	27	2 (MO. 96, 103)
Sulfolobus solfataricus P2	4	
Vibrio cholerae El Tor N16961	1	
Xanthomonas axonopodis pv. citri 306	4	
Xylella fastidiosa 9a5c	1	
Total	120	3

enzyme pair and then ligated into the plasmid pGEX-KG expression vector that was previously digested with the same restriction enzymes, generating fusion constructs that give an N-terminal GST fusion protein for acquirement of soluble proteins. For expression, the monooxygenase vectors were transformed into *E. coli* Rosetta gami (DE3) competent cells. Transformed cells grown in 10 ml of LB broth containing ampicillin (100 μg/ml) were incubated at 37°C overnight, and 5 ml of the seed culture was used to inoculate 100 ml of LB broth (ampicillin, 100 μg/ml), which was incubated at 37°C with shaking at 200 rpm to an A_{600} of 0.5±0.1. At this point, sterile isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.25 mM and then the cultures were incubated overnight at 21°C with shaking at 200 rpm. Cultured cells were collected by centrifugation at 7,000 rpm for 15 min, resuspended in 5 ml of 50 mM potassium phosphate buffer, pH 7.0, containing 150 mM NaCl, and lysed by ultrasonication (Sonifier 250; Brason). The soluble and insoluble fractions of the cell lysates were obtained by centrifugation at 9,000 rpm for 30 min. Crude extracts were analyzed using a 12% SDS-polyacrylamide gel electrophoresis and visualized by Coomasie brilliant blue staining (Fig. 3).

Analytical Methods

For product identification, the sample of biotransformation mixtures were centrifuged at 6,000 rpm for 10 min and the supernatant extracted with an equal volume of ethyl acetate. Extracts were dried by speed Vac, and analyzed using gas chromatography coupled to mass spectrometry (GC-MS, Agilent 6890N and 5973N). For GC-MS analysis, the dried extracts were redissolved at a concentration of 2 mg/ml in ethyl acetate. Compounds were separated on an HP-1MS (30 m×0.25 mm×0.25 μm) capillary column. The injector temperature was operated at 250°C and the oven was programmed from an initial temperature of 50°C (3 min) to 300°C (10 min) at a rate of 10°C/min. In addition, the mass spectrometer was operated at an ionization energy of 70 eV with an ionization temperature of 230°C.

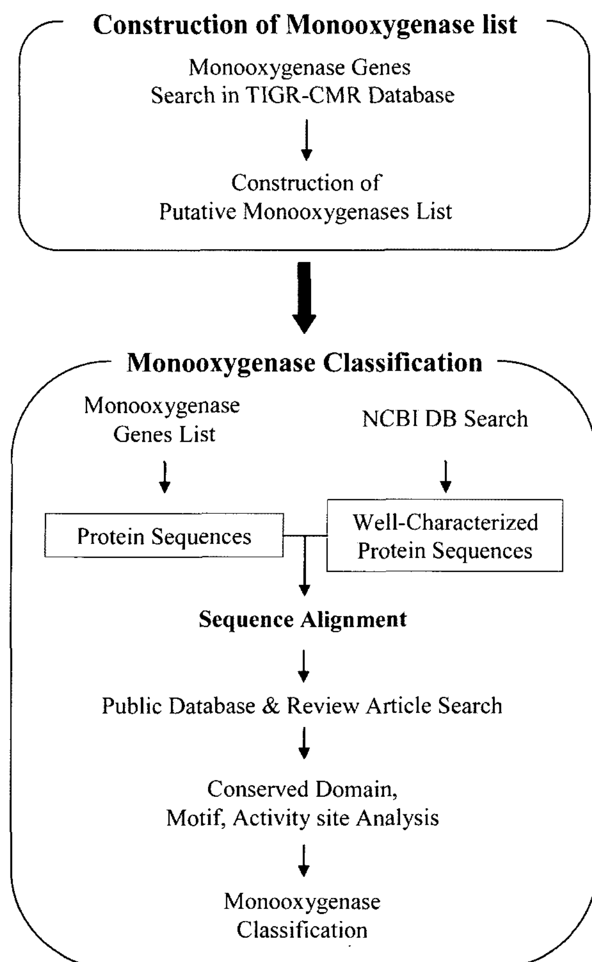


Fig. 2. Process of monooxygenase list construction and monooxygenase classification.

RESULTS AND DISCUSSION

Baeyer-Villiger monooxygenases (BVMOs) are extremely useful enzymes for eco-friendly synthesis of compounds, whereas some of these products are difficult to obtain by other strategies. The recent characterization of several new BVMOs has expanded the range of enzymatic Baeyer-Villiger reactions. Moreover, the recent rationale to discover new Baeyer-Villiger biocatalysts is that the identified BVMO-specific sequence motif is a powerful tool to find novel BVMOs in the rapidly growing collection of sequenced genomes [9]. As a result, a large number of putative BVMOs can be annotated in microbial genomes.

Using the genetic information, 120 putative monooxygenases were classified into several different monooxygenases, as follows: $FMNH_2$-utilizing monooxygenase, FAD-dependent monooxygenase, BVMO, cytochrome P450 monooxygenase, multicomponent phenol 2-hydroxylase, flavin-NAD(P)H reductase, N-hydroxylating monooxygenase, *etc.* As a result of this classification, three monooxygenases were classified as BVMOs. Three putative BVMO genes found in the genomes of *P. aeruginosa* PAO1 (MO.57) and *S. coelicolor* A3(2) (MO. 96,103) contained two dinucleotide, binding sequence motifs (GXGXXG/A) that are involved in binding of the cofactor FAD and the coenzyme NAD(P)H, and a strictly conserved sequence motif (FXGXXXHXXXW(P/D)) that is not present in members from the other two subfamilies (flavin-monooxygenases [FMOs], amine-hydroxylating monooxygenases [NMOs]) [9] (Fig. 1). As hinted by the presence of these motifs, we have been able to demonstrate that three putative monooxygenase genes represent a BVMO. Alignment with the known BVMOs showed that MO.57 from *P. aeruginosa* PAO1 (Accession No. AAG04927) possesses 26%, 34%, and 36% sequence identity with cyclohexanone monooxygenase (CHMO) from *Acinetobacter* sp. (accession BAA86293), 4-hydroxyacetophenone monooxygenase (HAPMO) from *P. fluorescens* ACB (Accession No. AAK54073), and steroid monooxygenase (SMO) from *R. rhodochrous* (Accession No. BAA24454), respectively. In addition, the MO.96 from *S. coelicolor* A3(2) (Accession No. CAB55657) shows 31% sequence identity with CHMO (Accession No. BAA86293) and 36% with SMO (Accession No. BAA24454). On the other hand, the MO.103 from *S. coelicolor* A3(2) (Accession No. CAB59668) shows 28% sequence identity with CHMO (Accession No. BAA86293) and 35% with SMO (Accession No. BAA24454).

Most biocatalytic studies with BVMOs have been performed with whole cells or isolated enzymes. Although the reactions with isolated enzymes have certain advantages,

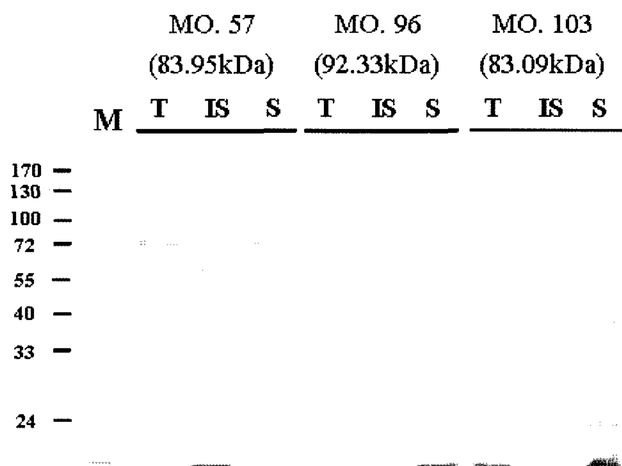
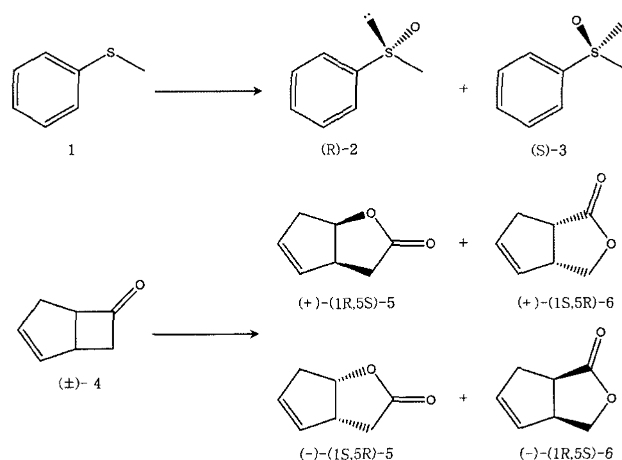


Fig. 3. SDS-PAGE analysis of recombinant *E. coli* strain (Cloning pGEX-KG expression vector in *E. coli* Rosetta Gami (DE3): N-terminal GST fusion protein [26 kDa]) that overexpresses three putative monooxygenases: one from *Pseudomonas aeruginosa* PAO1 (MO. 57) and two from *Streptomyces coelicolor* A3(2) (MO. 96, 103).
Lane M, molecular marker (kDa); lane T, total fraction from cells induced under IPTG (isopropyl-β-D-thiogalactopyranoside); lanes IS and S, insoluble and soluble fractions from cells induced under IPTG.

whole-cell conversions with oxygenase are more attractive for several reasons. One major advantage is the efficient intracellular coenzyme regeneration. The utilization of living cells circumvents the obstacle to recycle the cofactors (NADH or NADPH) consumed by BVMOs. However, the whole-cell approach involves a multitude of additional enzymes, which increases the complexity of the system. The construction of artificial regeneration cycles can be avoided by simplifying the application of such biocatalysts in synthetic chemistry. In addition, potent overexpression systems minimize the chance of unwanted side reactions caused by competing enzymes with overlapping substrate acceptance in a living cell. Accordingly, to express the three monooxygenases in *E. coli*, each of the DNA fragments containing the monooxygenase gene was amplified and subcloned into the pGEX-KG, as described in the Materials and Methods. The pGEX-KG expression vector, generating fusion constructs that give an N-terminal GST fusion protein, was transformed into *E. coli* competent cells and protein expression was induced by the presence of IPTG (0.25 mM) in the medium. These conditions were required



Scheme 1. Baeyer-Villiger reaction of thioanisole (**1**, methyl phenyl sulfide) to (R)-methyl phenyl sulfoxide (**2**) and (S)-methyl phenyl sulfoxide (**3**) and of bicyclo[3.2.0]hept-2-en-6-one (**4**) to lactone 2-oxabicyclo[3.3.0] oct-6-en-3-one (**5**) and 3-oxabicyclo[3.3.0]oct-6-en-2-one (**6**) by whole-cell preparation of *Pseudomonas aeruginosa* PAO1 and two *Streptomyces coelicolor* A3(2).

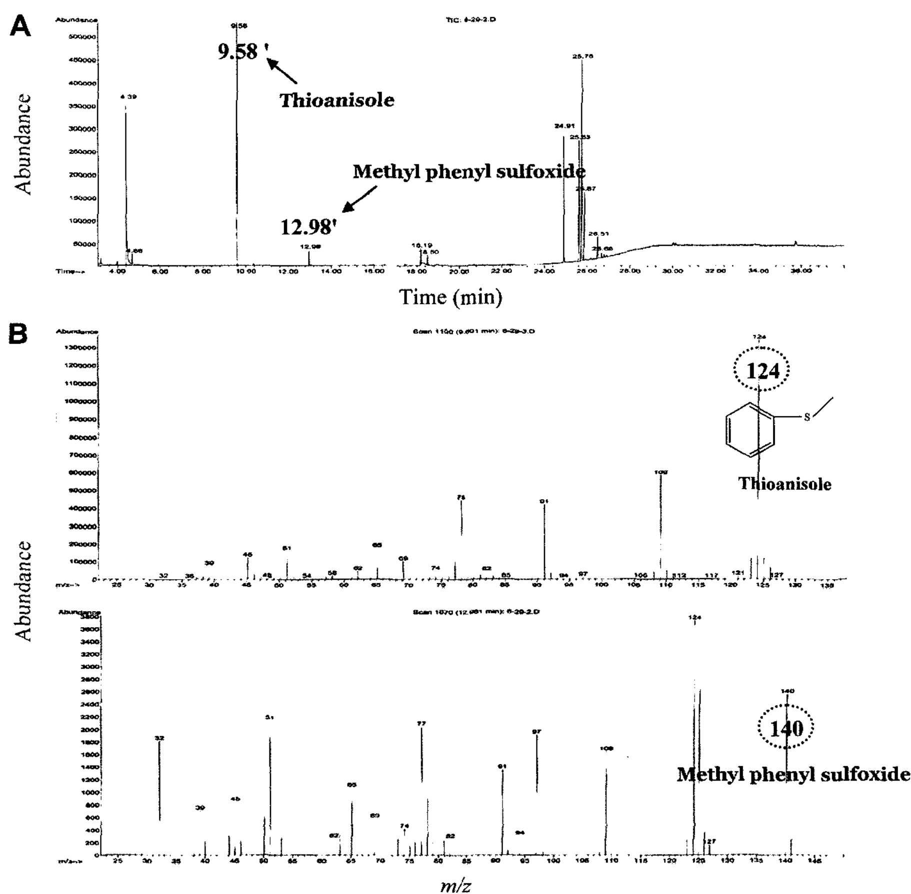


Fig. 4. GC/MS analysis of the products derived from the reaction mixture with monooxygenases. Gas chromatogram (**A**) and mass spectral data (**B**) of the sulfoxidation products of thioanisole, catalyzed by *Streptomyces coelicolor* A3(2) (MO. 103).

A. Gas chromatogram: tr (thioanisole)=9.58 min and t_r (methyl phenyl sulfoxide)=12.98 min. **B.** Mass spectra data: *m/z* (thioanisole)=124 and *m/z* (methyl phenyl sulfoxide)=140.

to obtain good yields of soluble proteins. As a result, the three overexpressed monooxygenases were identified by SDS-PAGE analysis, and they corresponded to apparent molecular masses of ≈83.95 (MO.57), 92.33 (MO.96), and 83.09 (MO.103) kDa (Fig. 3).

Using whole cells of the recombinant *E. coli* strain without requiring the tedious process of enzyme purification or cofactor regeneration, the biotransformations of the following racemic mixtures were examined by GC/MS: bicyclo[3.2.0]hept-2-en-6-one **4** to the corresponding 2-oxabicyclo[3.3.0]oct-6-en-3-one **5** (which are valuable intermediates for synthesis of prostaglandins and nucleosides) or 3-oxabicyclo[3.3.0]oct-6-en-2-one **6**; and thioanisole to the corresponding (R)-sulfoxide **2** (which is a versatile and convenient chiral auxiliary) or (S)-sulfoxide **3**. Likewise, the Baeyer-Villiger biocatalytic system using whole recombinant cells accomplished the sulfoxidation of methyl phenyl sulfide (thioanisole). The sulfide was found to be readily converted by three putative monooxygenases: one from *P. aeruginosa* PAO1 (MO.57) and two from *S. coelicolor* A3(2) (MO. 96 and MO.103) (Fig. 4). Fig. 4A shows the gas chromatogram of the sulfoxidation products of thioanisole, catalyzed by MO.103 (t_r (thioanisole)=9.58 min and t_r (methyl phenyl sulfoxide)=12.98 min). Furthermore, the mass spectrum (Fig. 4B) was identical with that of thioanisole with a peak of *m/z* 124 and methyl phenyl sulfoxide with a peak of *m/z* 140 (which are addition of one atom of oxygen (16) to thioanisole). Furthermore, the Baeyer-Villiger reaction of thioanisole, catalyzed by MO.57, and MO.96, produced the same results as the MO.103-catalyzed sulfoxidation (data not shown). Therefore, three enzymes in the Baeyer-Villiger biocatalytic system converted bicycle[3.2.0]hept-2-en-6-one into its oxidative products and they were also analyzed by GC/MS [bicyclo[3.2.0]hept-2-en-6-one (t_r=7.01 min, *m/z*=108) to the corresponding 2-oxabicyclo[3.3.0]oct-6-en-3-one or 3-oxabicyclo[3.3.0]oct-6-en-2-one (t_r=10.86, *m/z*=124) (data not shown)].

In conclusion, this study revealed that three putative monooxygenases expressed in heterologous systems were able to perform the Baeyer-Villiger reaction on thioanisole and bicyclo[3.2.0]hep-2-en-6-one. Furthermore, this study validated the value and expediency of the genome mining by systematic gene annotation and also demonstrated that mining of available genes can be an efficient approach to obtain new biocatalysts.

Acknowledgments

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