

Supplementary Information

Enantioselective sulfoxidation using *Streptomyces glaucescens* GLA.0

Sara Salama¹, Tarek Dishisha², Mohamed H. Habib³, Ahmed Z. Abdelazem¹, Walid Bakeer²,
Mahmoud Abdel-Latif⁴, and Yasser Gaber^{2,5*}

¹Biotechnology and Life Sciences Department, Faculty of Postgraduate Studies for Advanced Sciences, Beni-Suef University, Beni-Suef, 62517, Egypt

²Department of Pharmaceutical Microbiology and Immunology, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, 62511, Egypt

³Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, Cairo, 11562, Egypt

⁴Immunity Division, Zoology Department, Faculty of Science, Beni-Suef University, Beni-Suef, 62511, Egypt.

⁵Department of Pharmaceutics and Pharmaceutical Technology, College of Pharmacy, Mutah University, Al-Karak, 61710, Jordan

***Corresponding author:**

E-mail:

Tel.

Telefax.

	ORCID	Email
Sara Salama	0000-0002-8722-8627	sarasalama@psas.edu.eg
Tarek Dishisha	0000-0001-8644-2640	tarek.dishisha@pharm.bsu.edu.eg
Mohamed H. Habib	0000-0002-3732-5963	mohamed.habib@pharma.cu.edu.eg
Ahmed Z. Abdelazem	0000-0002-2417-6073	aznagi@psas.bsu.edu.eg
Walid Bakeer	0000-0002-6079-8072	walid.bakeer@yahoo.com
Mahmoud Abdel-Latif	0000-0001-5060-0475	mahmoudsayed@science.bsu.edu.eg
Yasser Gaber	0000-0003-2244-4406	yasser.gaber@pharm.bsu.edu.eg

Table of Contents

Fig. S1. Multiple sequence alignment of a putative BVMO enzyme in the genome of <i>Streptomyces glaucescens</i> GLA.0.....	3
Fig. S2. Homology model of the putative BVMO identified in the genome of <i>S. glaucescens</i> GLA.0.	4
Fig. S3. Zoom-in view of the active site of the putative BVMO in <i>S. glaucescens</i> GLA.0.	5
Fig. S4. GC chromatogram of phenyl methyl sulfide at the beginning of the reaction.	6
Fig. S5. GC chromatogram of phenyl methyl sulfide after a substrate control reaction.....	7
Fig. S6. Full scan GC chromatogram of phenyl methyl sulfide showing the effect of agitation on the biotransformation process.	8
Fig. S7. IR spectrum of the chemically synthesized phenyl methyl sulfoxide.	9
Fig. S8. IR spectrum of the chemically synthesized phenyl methyl sulfone.	10
Fig. S9. GC chromatogram showing the formation of phenyl methyl sulfoxide after 15 h.....	11
Fig. S10. GC chromatogram showing the formation of phenyl methyl sulfoxide after 6 h	12
Fig. S11. The effect of cosolvent addition on the product (PMSO) and by-product (sulfone).....	13
Fig. S11.A. GC chromatograms showing products formed under conditions of reaction A.	13
Fig. S11.B. GC chromatogram showing products formed under conditions of reaction B.	14
Fig. S14. Chiral GC chromatograms showing oxidized products formed from PMS biooxidation with the whole cells of <i>Streptomyces glaucescens</i> GLA.0 under conditions of reaction (I).....	17
Fig. S16. Chiral GC chromatograms showing oxidized products formed from PMS biooxidation with the whole cells of <i>Streptomyces glaucescens</i> GLA.0 under conditions of reaction (K).	19
Fig. S17. Chiral GC chromatogram showing <i>R</i> -PMSO produced from enzymatic oxidation of PMS using phenyl acetone monooxygenase (PAMO).....	20
Fig. S18. Chiral GC chromatogram showing <i>R</i> -PMSO produced from the enzymatic oxidation of PMS using cyclohexanone monooxygenase (CHMO).....	21
Fig. S19. Chiral GC chromatogram showing standard phenyl methyl sulfone.....	22
Table S1. Preliminary biotransformation experiments of PMS biooxidation using <i>S. glaucescens</i> GLA.0 and their effects on PMS biotransformation.	23
Table S2. Optimization of the biotransformation experiments with whole growing cells of <i>S. glaucescens</i> GLA.0.....	24

Annotation	A0A089YZ45_STRGA	1	-----MAEHEHVRVAVIGSGFGGLGAAVRLRREGVTFDFVVLERADS	41
<input checked="" type="checkbox"/> Nucleotide binding	BVMO2_STRCO	1	-----MAEHEQVHEHVRVAVIGSGFGGLGAAVRLRREGITDFVVLERAGS	45
<input type="checkbox"/> Chain	BVMO_PSEAE	1	MYTFANNHNRSLAMSTQPTPAARHCKVAIIGTGFSLGMAIRLRQEGEDDFLIFEKDG	60
<input type="checkbox"/> Transmembrane	B8N653_ASPFN	1	-----MNGTQASNGVLHLDALIIGSGFSGIYLLHKLRLDELKLVKVFEEESD	47
<input type="checkbox"/> Region	A0A089YZ45_STRGA	42	VGGTWRDINSYPGCACDVPVSHLYSFSFAP---NPDWPRAFSGQEHIRAYLERVADVFGLRP	98
<input checked="" type="checkbox"/> Site	BVMO2_STRCO	46	VGGTWRDINSYPGCACVPSHLYSFSFAP---NPEWPRTFSGQEHIRAYLEHVADTFGLRP	102
<input checked="" type="checkbox"/> Binding site	BVMO_PSEAE	61	VGGTWRVNNYPGCACVQVSHVYSFSFEA---NPEWTRMFARQFEIRAYLEKWEKYLRLQE	117
	B8N653_ASPFN	48	IGGTWNNNRYPGARVDCFPVPHYAYSLPEVWQSWNTELYPNQKEIKSYFDHVRDRLDVRK	107
			:****. * ***. * .*:*: . : * : * * : : : : : : : : : :	
	A0A089YZ45_STRGA	99	HLRFGEVVKLMTWDPHELRWDIETGSSGR-LTADLVVSATGPLSDPKIPDIPGLDTFFPGKV	157
	BVMO2_STRCO	103	HLRFDSEVKRMAWDTEQLRWEIETVGRGT-LTADVVVSATGPLSDPKVPDIPGLDTFFPGKV	161
	BVMO_PSEAE	118	KTLLENTEIGKLAWDERQSLWHLHDAQGNHYTANAVVSGMGGLSTPAYPRLDGLENFQGV	177
	B8N653_ASPFN	108	DCLFHSRVNEGTFDEATGRVVTWTDGKVATAKYLLVAVGFASKSLPDKWGLDSFKGTI	167
			. : : : . : : : : : * : * : * : : : * : : : : * : * : : * : * : : :	
	A0A089YZ45_STRGA	158	FHSARWDHD--YDLKGRVAVMVGTSASAIQVPAIQPRVGRITLFFORTPPVVMRMDRAI	215
	BVMO2_STRCO	162	FHSARWDHD--YDLAGQRVAMIGTGASAIQIVPSIQPKVDRLTLFQRTPAWVMRVDRAI	219
	BVMO_PSEAE	178	FHSQWWDHD--YDLKGRVAVIGTGASAIQFVPEIQPLVAALDLYQRTPPWILPKPDRAI	235
	B8N653_ASPFN	168	YHSAHWPEAEETSVKGGKVAVIGTGSTGQIQFQEWAREAEAEFLFQRTPNLCLPMRQQL	227
			:** : * . : : * : : : : : : : : : : * : * * * : * : : : :	
	A0A089YZ45_STRGA	216	SGAERWLHQ---RFPVTTQ-----ARRGLLWGIREFLQVQAFTHK	251
	BVMO2_STRCO	220	SGAERALHR---ALPATTK-----LRGLLWGIREFLQVQAFTHK	255
	BVMO_PSEAE	236	SETERRRFR---RFPLVQK-----LWRGGLYSLLEGRVGLGFTFA	271
	B8N653_ASPFN	228	HAGYQVKDKGEYADYLAEALTFGGLEYQQTPKNTFDASEEEREAEWEDL-YQMGGFREW	286
			: : : : : : : : : : * : : : : : : : : : : : : : : :	
	A0A089YZ45_STRGA	252	PGQLG-FV-----EQAKRNMARAIKDP---ALRAKLTPDYRIGCKRILLSSEYYP	298
	BVMO2_STRCO	256	PNELG-FV-----EQIAKRNMGAAIKDP---ALRAKLTPDYRIGCKRILLSSTYYP	302
	BVMO_PSEAE	272	FQVMK-LV-----QRLAIRHIHKQIKDP---ELRRKVTPTYRIGCKRILMSHNYYP	318
	B8N653_ASPFN	287	QNNYQDLSLTDANREAYNFWARTRARIQDPKRDLLAPLEPPYPFQTRPSLEQDFYE	346
			: : : : : : * : * : * : * : * : * * : * * : * : : * :	
	A0A089YZ45_STRGA	299	ALARPNDVVDVAS---GLAEVRGSLVAADGSEAEADAIVFGTGFHVIDM-PIAERVVGAD	354
	BVMO2_STRCO	303	ALAKPNVDVVAS---GLSEVRGSLVAADGTEAEADAIVFGTGFHVIDM-PIAERVVGAD	358
	BVMO_PSEAE	319	ALAAANSTVITE---GIRAVTANGIVDNGREREVDALIFGTGFTANDP-IPRGVVFGRD	374
	B8N653_ASPFN	347	QFNKSNVHIVDTKSOPIVGVVPTGIVTADKEVHEVDIIAVATGFDAVTTGGLRLGLKDVN	406
			: * : : : : * : * : * : * : * : * : * : * : * : : * :	
	A0A089YZ45_STRGA	355	GRTLAEVWKGMEALRGASAAAGFPNWMNTIIGPNTGLGNSSMILMIESQLNLYLADFIQLD	414
	BVMO2_STRCO	359	GRTLAEVWKGMEALRGGTAAGFPNFMNTIIGPNTGLGNSSMILMIESQLNLYLADFIQLD	418
	BVMO_PSEAE	375	GRDLLDSWSKGPPEAYKGTTFAGFPNLFLLMGPNLTGLGHNSMVMYIESQIAVLDALKLMK	434
	B8N653_ASPFN	407	GVGLDERWKGDMSTYLGMAISGFPNMFPLYSLOAPTAFANGPTLIEIQGDWITSLIRKME	466
			* * : * . * : : * : : * * : : . : : . : . : * * * : : : : : :	
	A0A089YZ45_STRGA	415	VLGGRVALDARPGAVGANNRRVOERMKRTVWNTGGCTSWSYLDG-N-GRNT--TIWPGTTA	470
	BVMO2_STRCO	419	VLGGRTALDPRPAAVRNWNRVOERMKRTVWNTGGCTSWSYLDG-S-GRNT--TWWPGTTA	474
	BVMO_PSEAE	435	RR-ELLSLEVKAIPVQERYNEYLQRLDRSVWSVGGCKSWYLPVVS-GRNC--TWWPGFTW	490
	B8N653_ASPFN	467	ME-NVQSVTATPHAESAWNDDEVNMIANKTLLP--LTDWSYMGSNIPGKVPQSLNYLGGLP	523
			: : : : . : * : * : : : : : : : : : : : : * * * : * : * :	
	A0A089YZ45_STRGA	471	EFRRATR-RVDLMEYEVLRPFAAKAGGDAPESGKHSAAAGTEAAL--	514
	BVMO2_STRCO	475	EFRRETR-RVDLAEYQVLRPAPAQVGAKEADTGA-DTGADAEVSA	519
	BVMO_PSEAE	491	RFRALTR-CFDASAYHLTTTFLAALSNEARQQAEGVPA-----	527
	B8N653_ASPFN	524	TYRERCAKVLDEDFPFKA-----	543
			: * : * : * :	

Fig. S1. Multiple sequence alignment of a putative BVMO enzyme in the genome of *Streptomyces glaucescens* GLA.0 (A0A089Y245_STRGA). The Uniprot Accession number is A0A089YZ45 and Gene Bank Accession number is CP009438.1. The alignment shows sequence identity with SCO3172BVMO, a Baeyer-Villiger monooxygenase from *Streptomyces coelicolor* A3(2) (Gene Bank Accession No. CAB55657.1), PA1538BVMO, a BVMO from *Pseudomonas aeruginosa* PAO1 (Gene Bank Accession No. AAG04927.1) and BVMOAFL838, a BVMO from *Aspergillus flavus*.

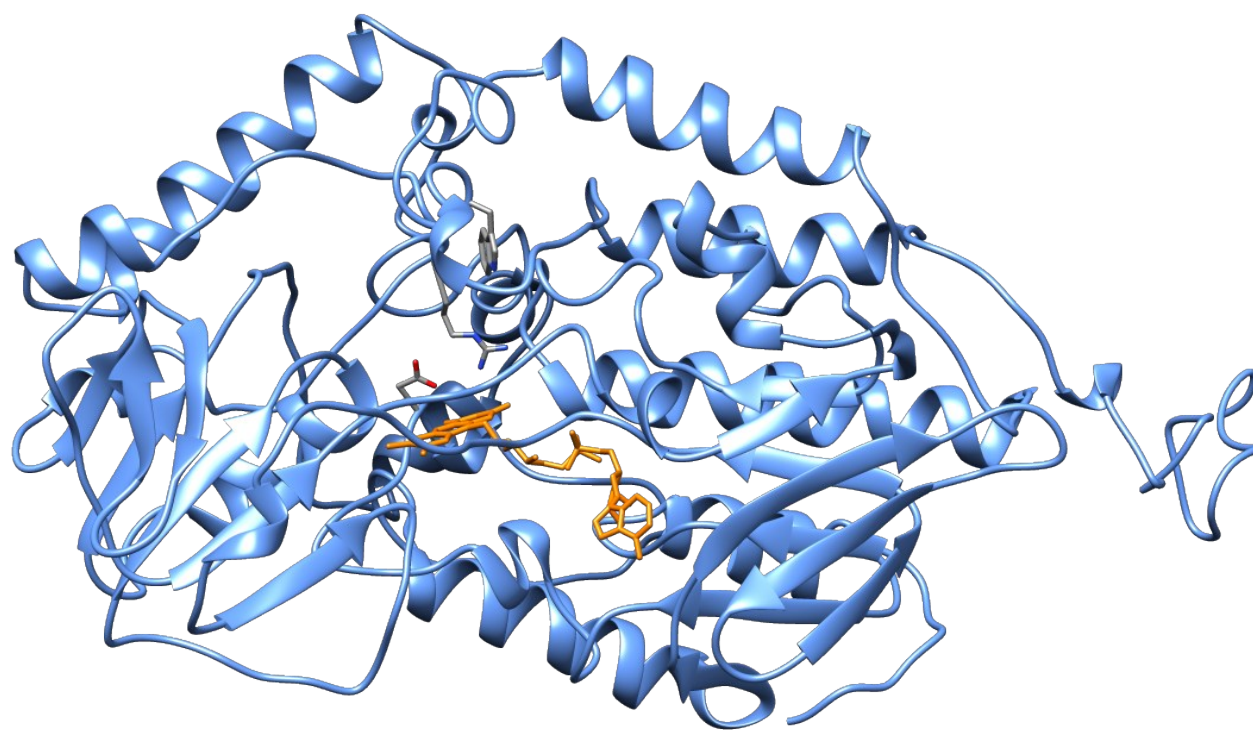


Fig. S2. A homology model of the putative BVMO identified in the genome of *S. glaucescens* GLA.0. The model was constructed using I-Tasser server.

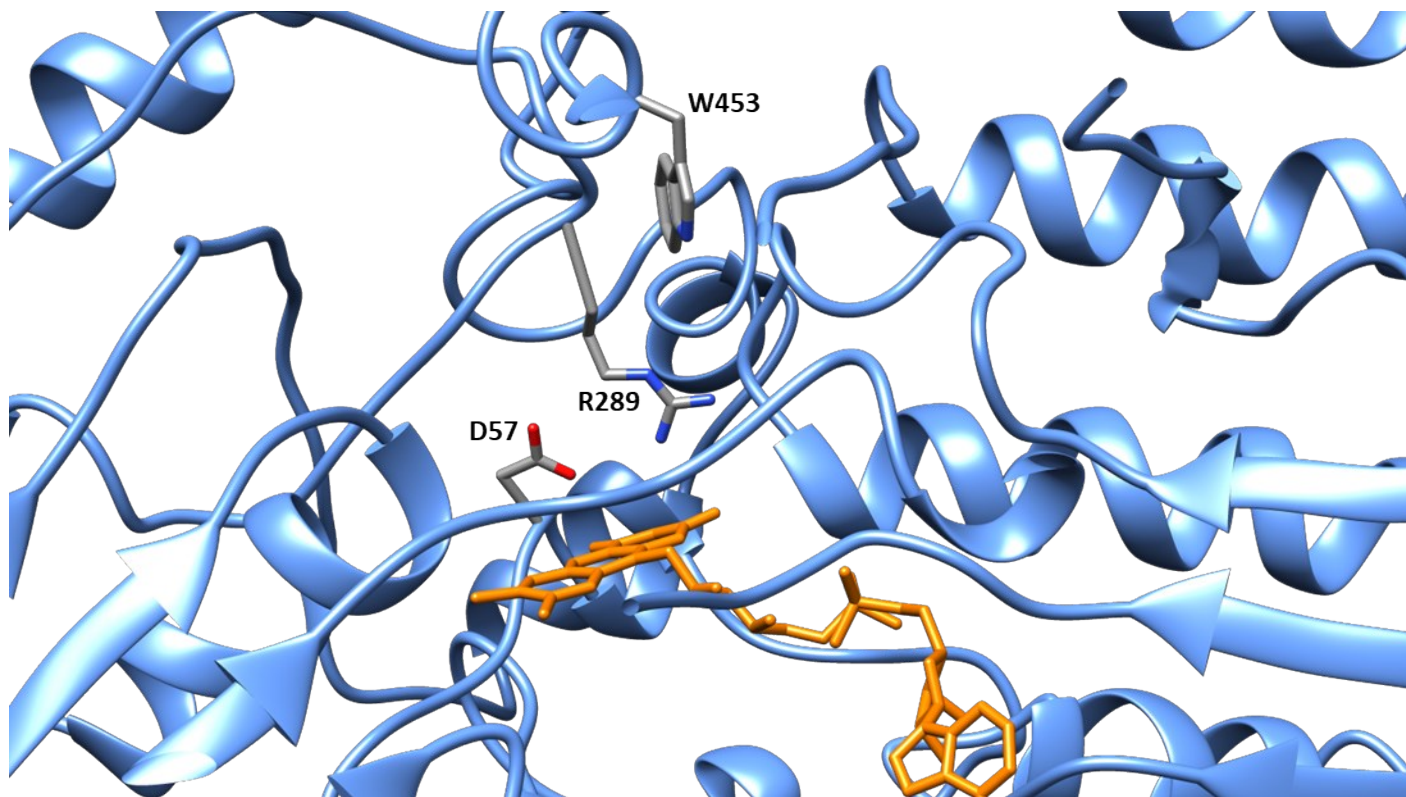
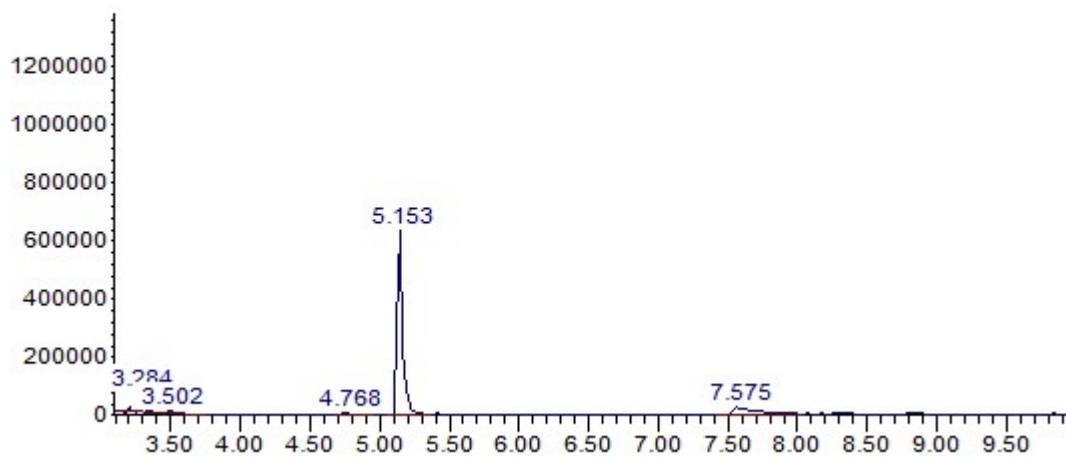


Fig. S3. Zoom-in view of the active site of the putative BVMO in *S. glaucescens* GLA.0. The residues Arg289 and Asp57 are conserved residues that contribute to substrate binding.

Abundance



Time-->

Fig. S4. GC chromatogram of phenyl methyl sulfide at the beginning of the reaction. PMS was added in an amount to form a 1 mM concentration in Medium 2. The retention time of PMS at zero time is 5.1 min.

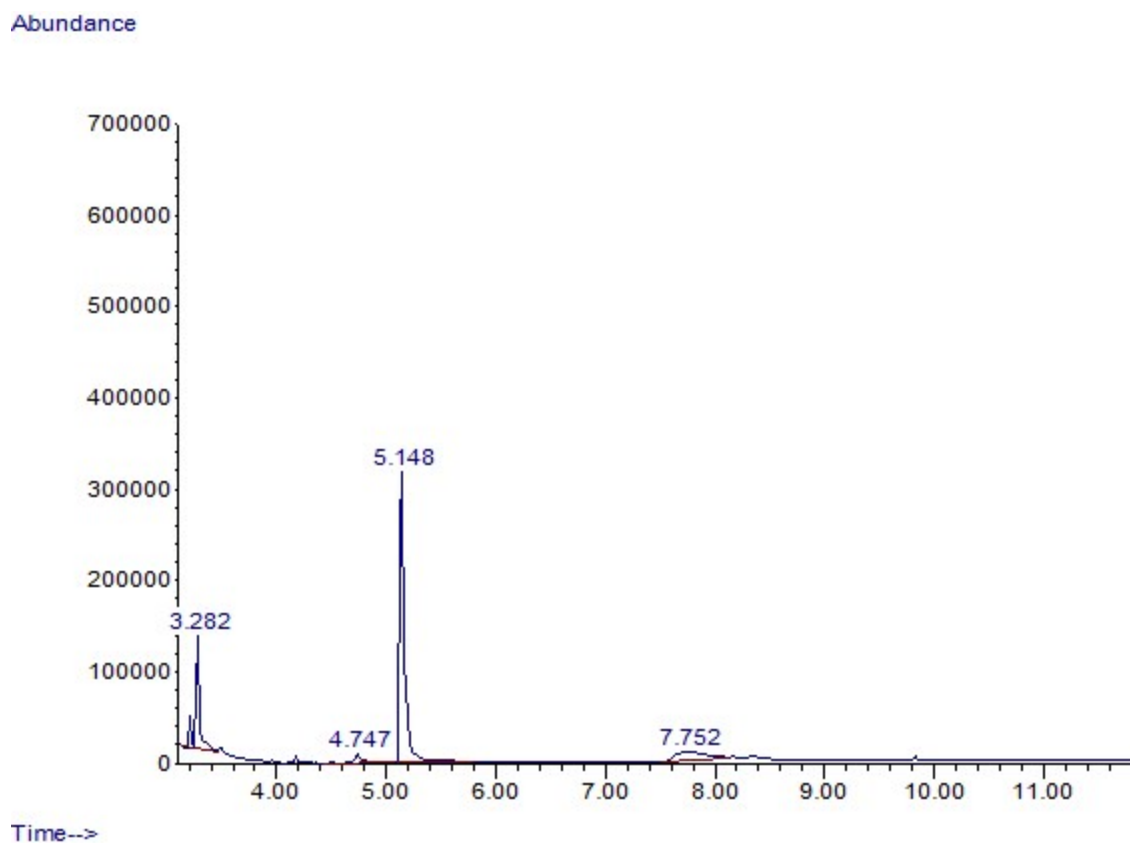


Fig. S5. GC chromatogram of phenyl methyl sulfide in a control reaction. PMS was added at an initial concentration of 1 mM to Medium 2. The reading was taken after 72 h from the beginning of the reaction and the experiment was done without the addition of bacterial culture. PMS was eluted at a retention time of 5.1 min.

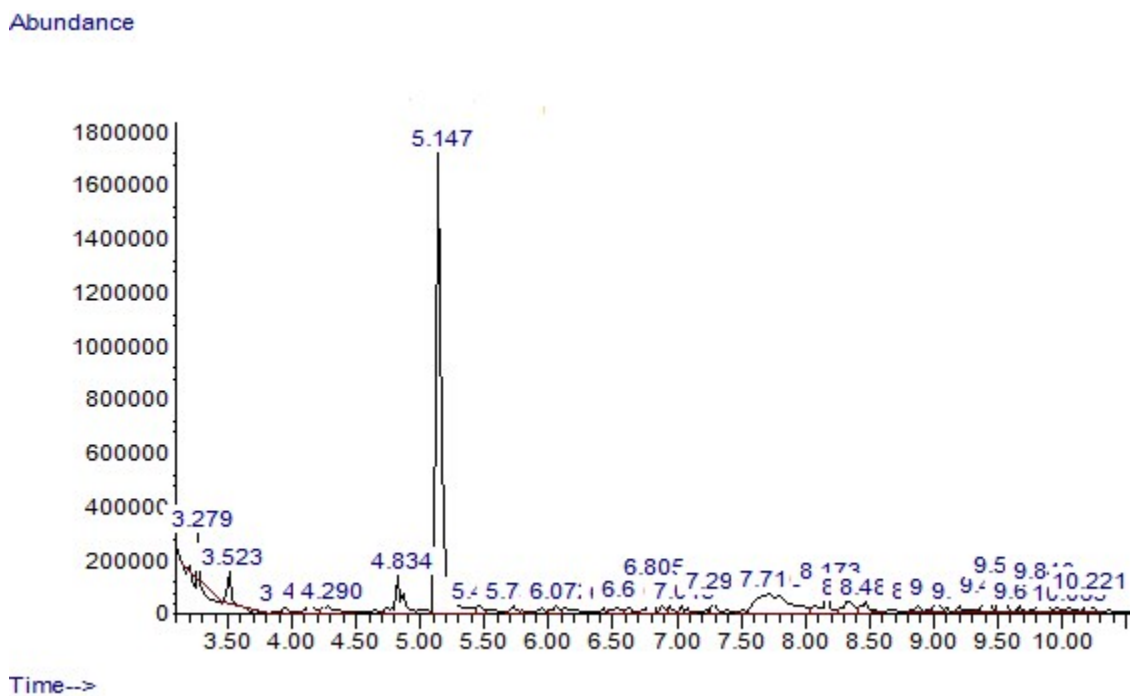


Fig. S6. Full scan GC chromatogram of phenyl methyl sulfide showing the effect of agitation on the biotransformation process. PMS was added at an initial concentration of 1 mM to Medium 3. The sample was taken after 72 h from the beginning of the reaction to give a PMS retention time of 5.1 min and a very small amount of PMSO at 7.7 min. The experiment was performed without agitation.

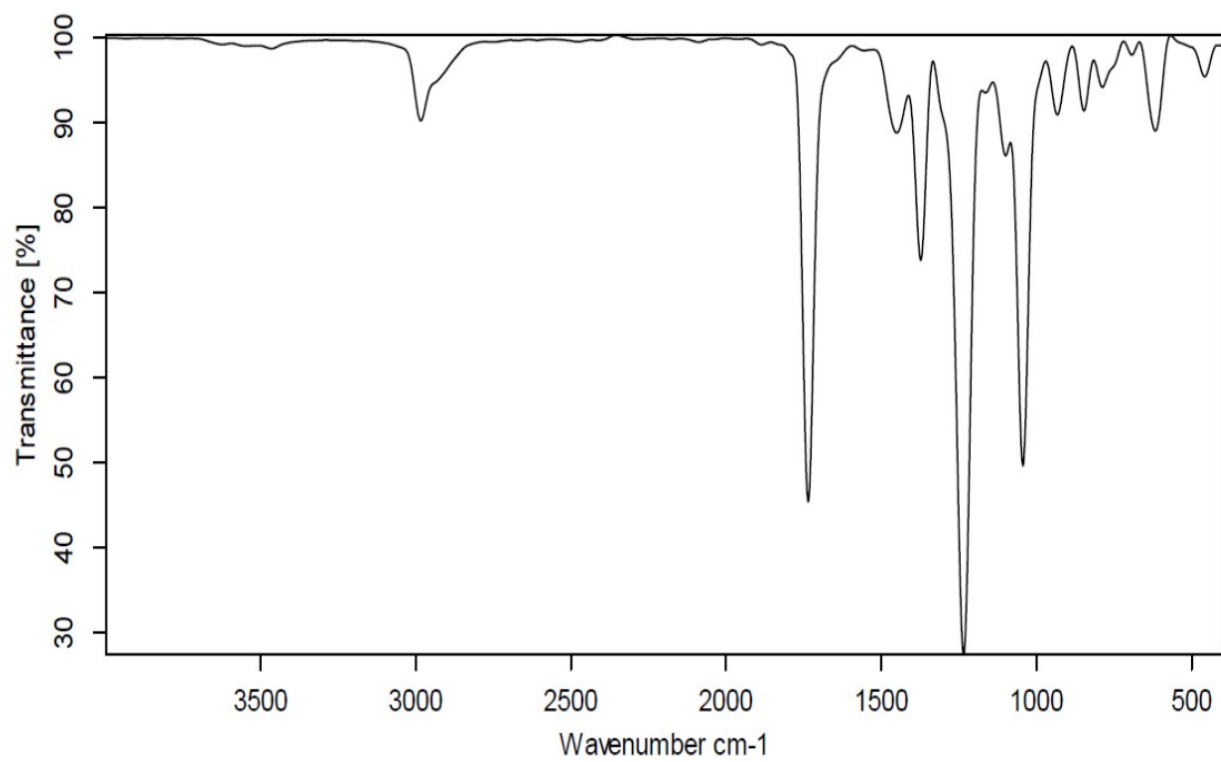


Fig. S7. IR spectrum of the chemically synthesized phenyl methyl sulfoxide.

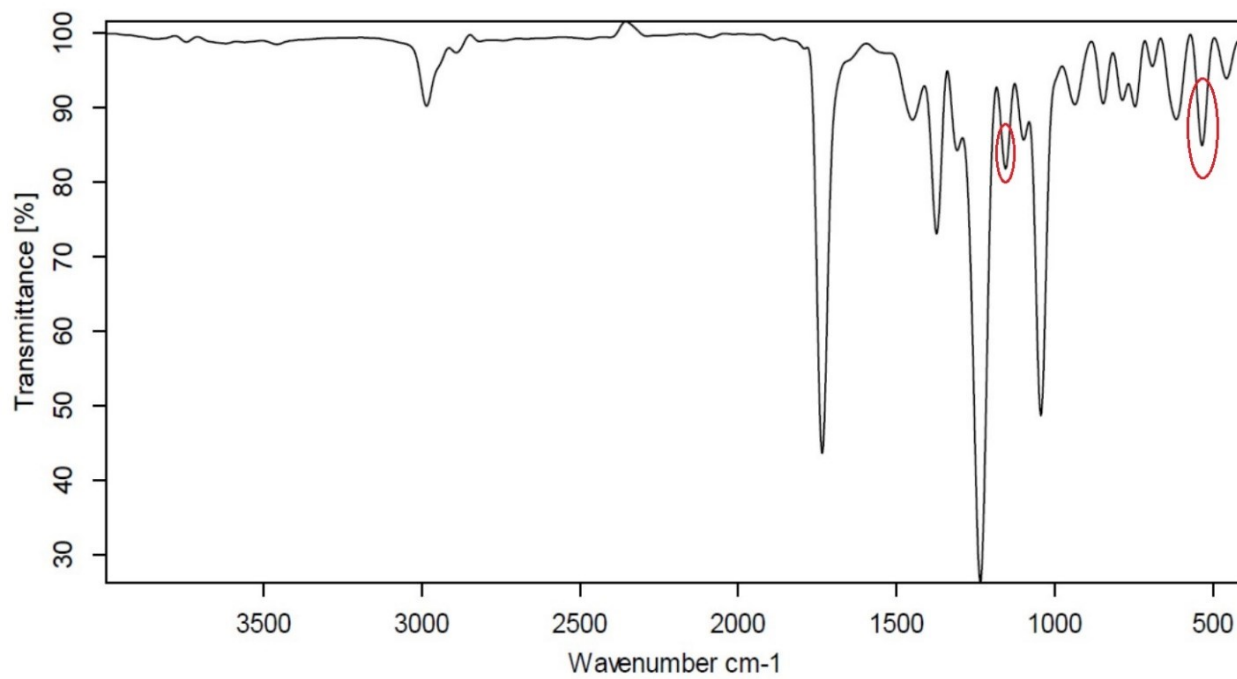
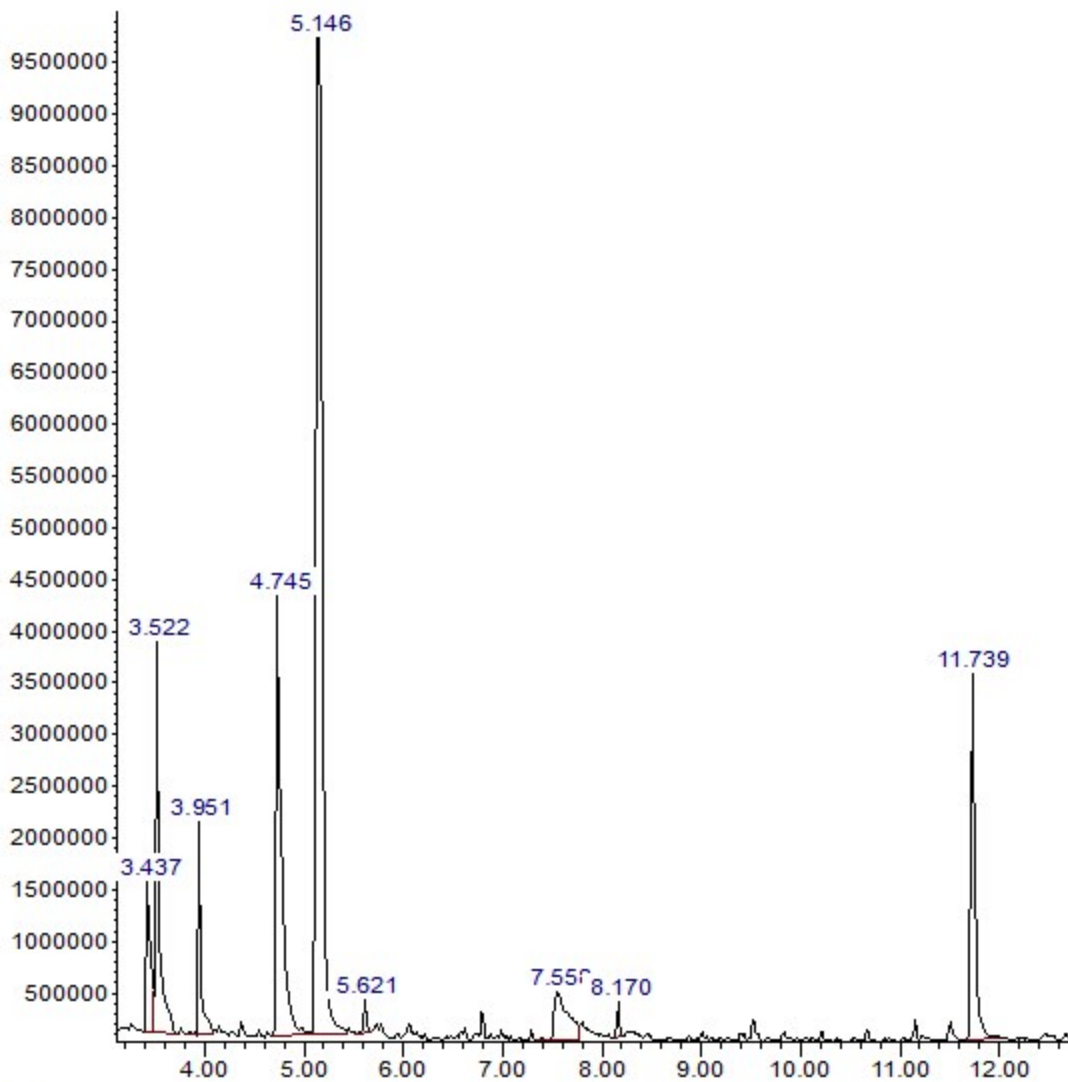


Fig. S8. IR spectrum of the chemically synthesized phenyl methyl sulfone.

Abundance



Time-->

Fig. S9. Full scan GC chromatogram showing the formation of PMSO ($R_t = 7.5$ min) after 15 h from the beginning of the reaction. This chromatogram was taken from a sample withdrawn after 15 h from the beginning of the biotransformation of phenyl methyl sulfide (added at an initial concentration of 3 mM) by growing cells of *Streptomyces glaucescens* GLA0.

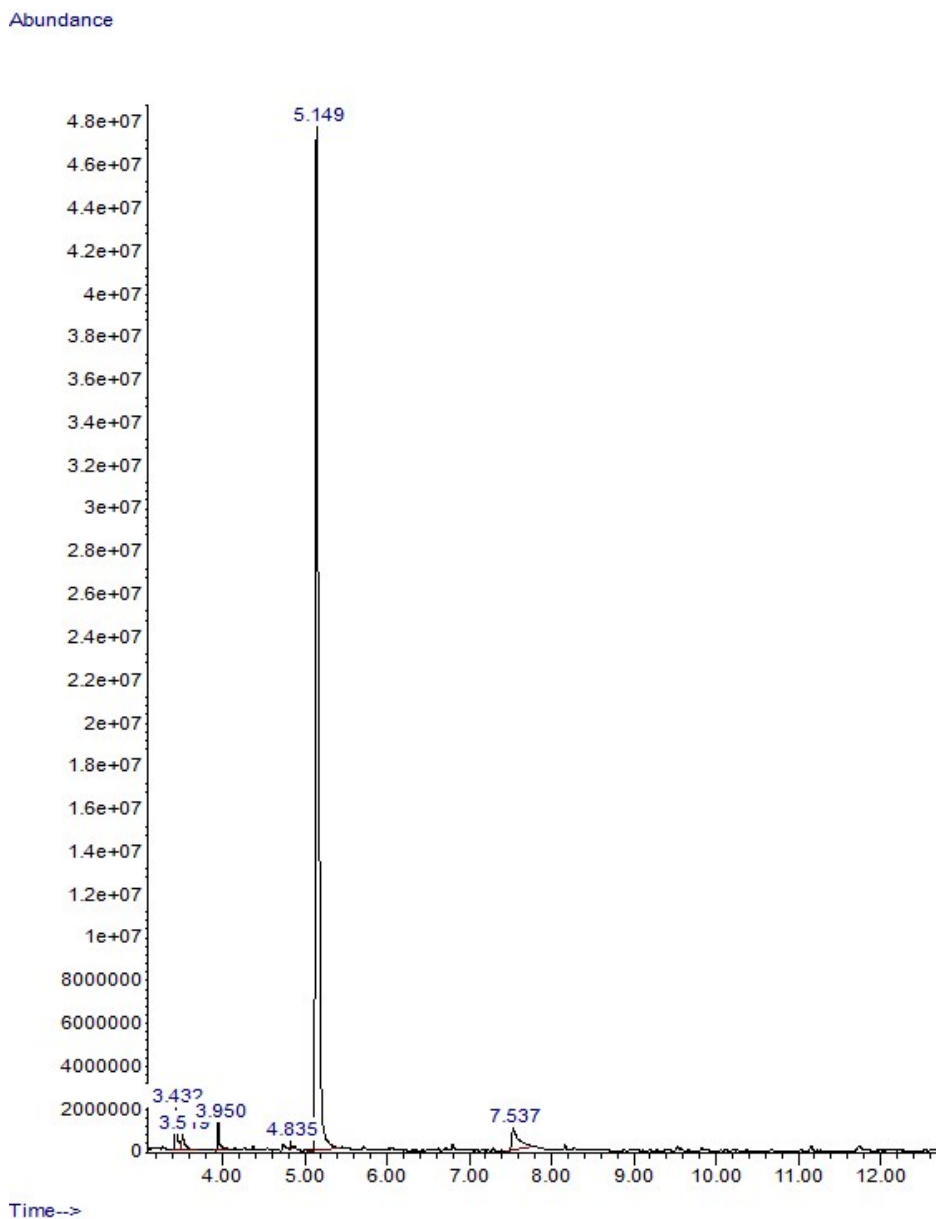
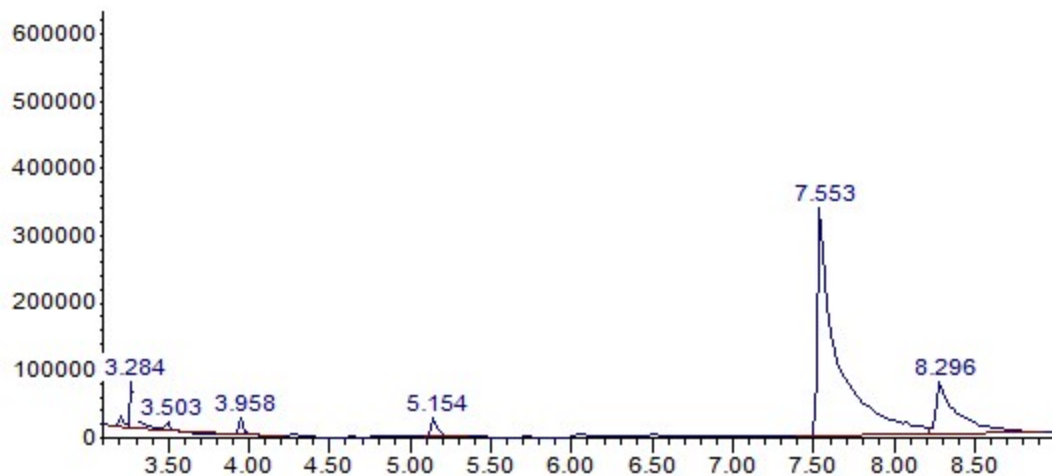


Fig. S10. Full scan GC chromatogram showing the formation of PMSO ($R_t = 7.5$ min) after 6 h from the beginning of the reaction. This chromatogram was taken from a sample withdrawn after 6 h from the beginning of the biotransformation of phenyl methyl sulfide (added at an initial concentration of 5 mM) by growing cells of *Streptomyces glaucescens* GLA0.

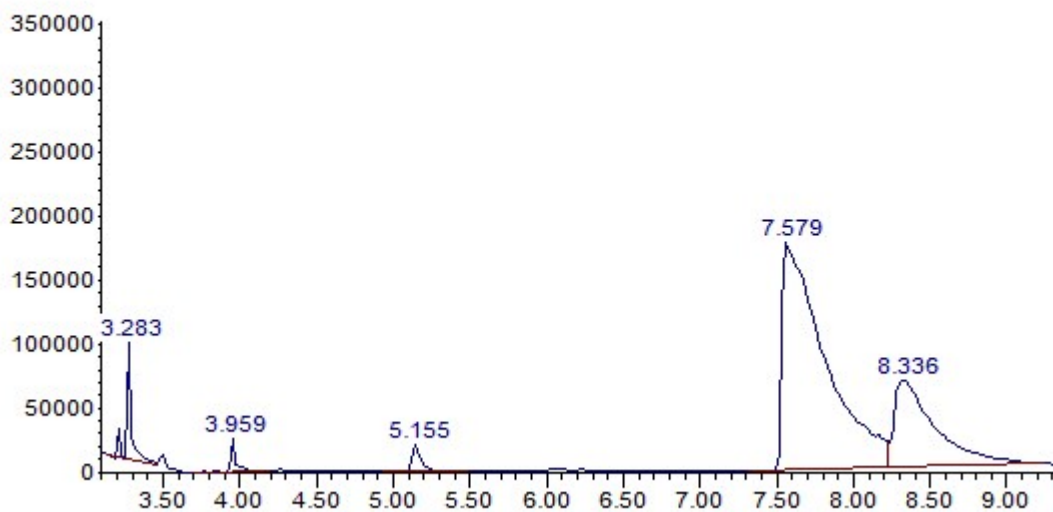
Abundance



Time-->

Fig. S11.A The effect of co-solvent addition on PMSO and sulfone formation. The GC chromatogram shows PMSO ($R_t = 7.5$ min) and phenyl methyl sulfone ($R_t = 8.3$ min) formed under conditions of reaction **A** (see **Table 1**). The sample was taken after 96 h from the beginning of the reaction.

Abundance



Time-->

Fig. S11.B The effect of co-solvent addition on PMSO and sulfone formation. The GC chromatogram shows PMSO ($R_t = 7.5$ min) and phenyl methyl sulfone ($R_t = 8.3$ min) formed under conditions of reaction **B** (see **Table 1**). The sample was taken after 96 h from the beginning of the reaction.

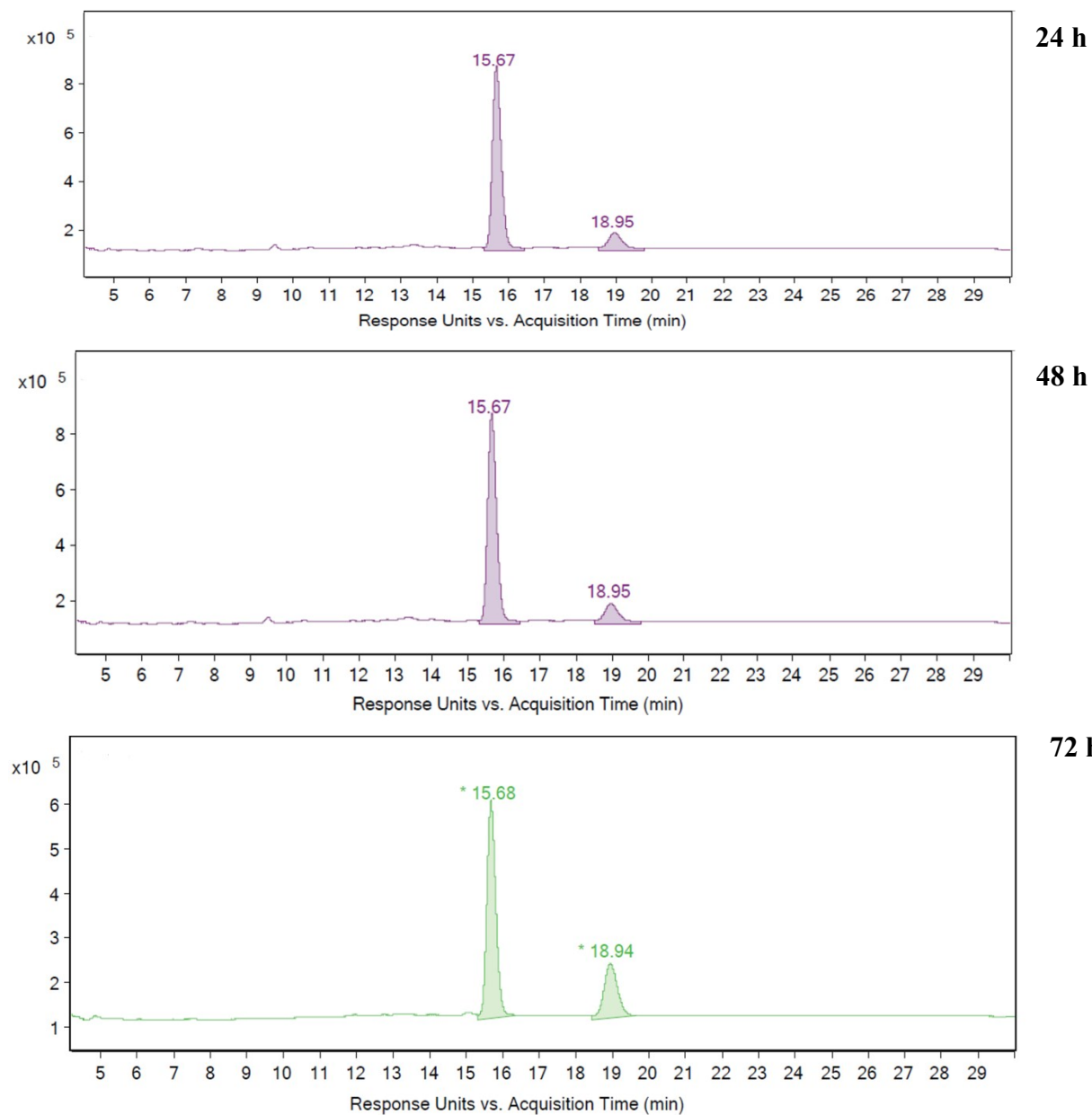


Fig. S12. Chiral GC chromatograms showing oxidized products formed from PMS biooxidation by the whole cells of *Streptomyces glaucescens* GLA.0 under conditions of reaction (C). Samples were taken after 24 h, 48 h, and 72 h, respectively. Retention times of *R*-PMSO and phenyl methyl sulfone were 15.6 min and 18.9 min, respectively.

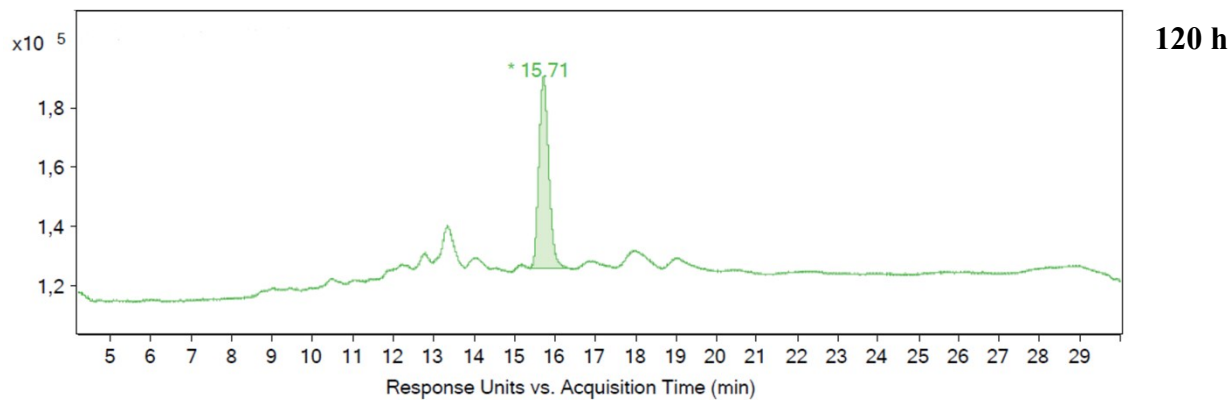
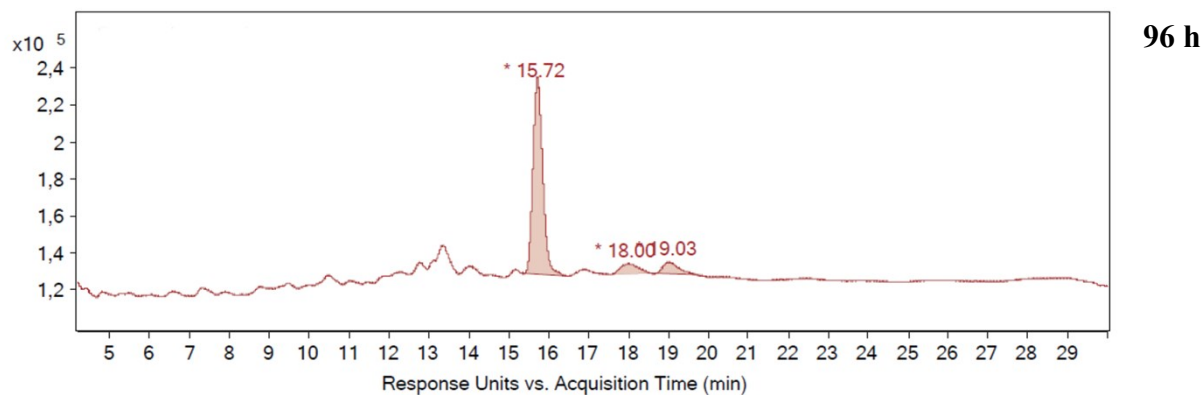
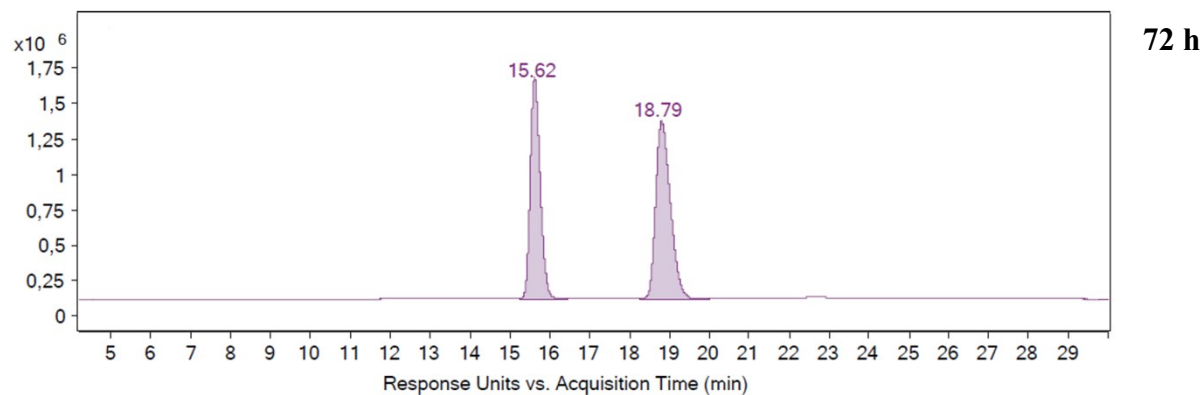


Fig. S13. Chiral GC chromatograms showing oxidized products formed from PMS biooxidation by the whole cells of *Streptomyces glaucescens* GLA.0 under conditions of reaction (D). Samples were taken after 72 h, 96 h and 120 h, respectively. Retention times of *R*-PMSO, *S*-PMSO and phenyl methyl sulfone were 15.6 min, 18-18.7 min and 19 min, respectively.

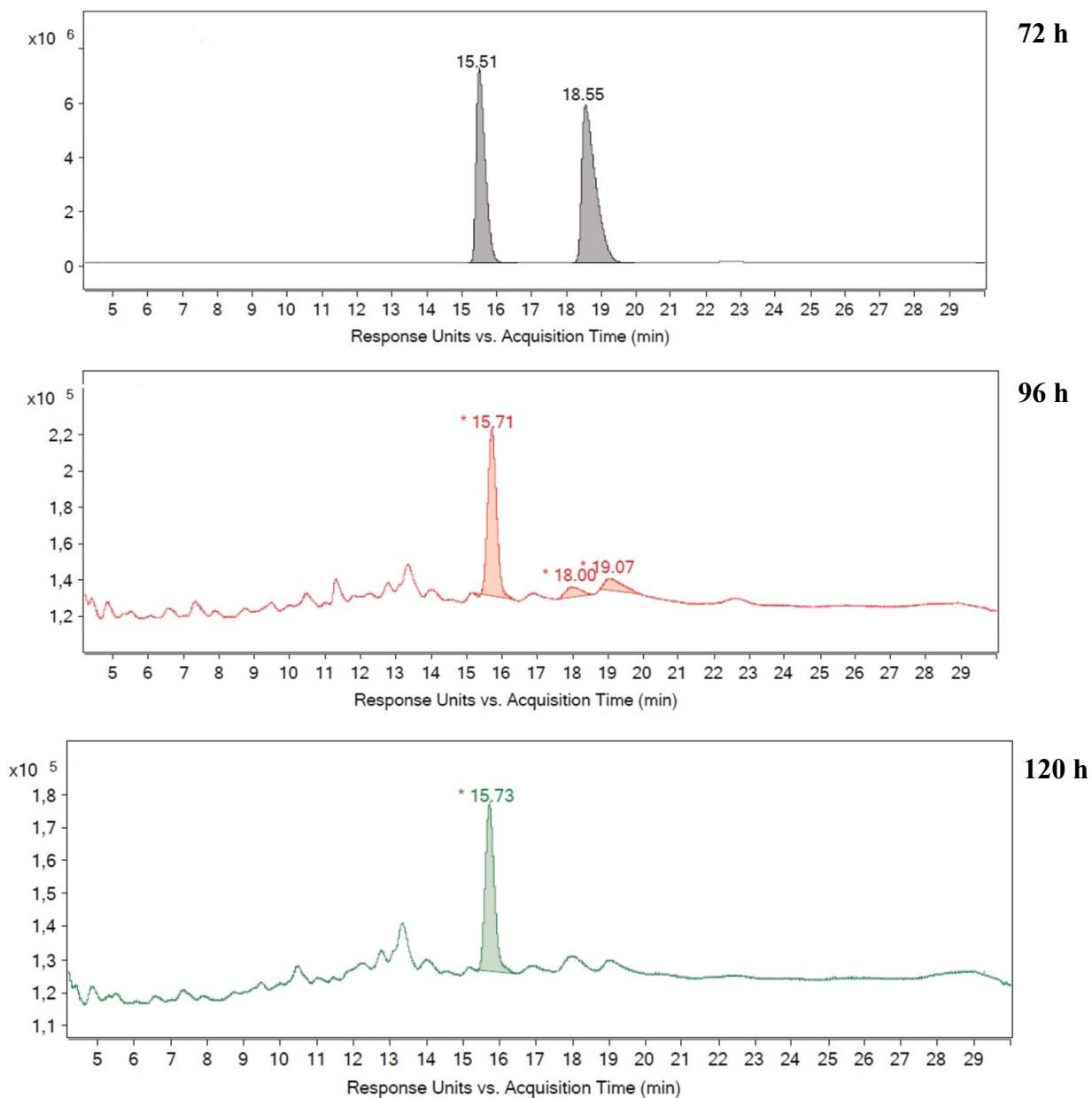


Fig. S14. Chiral GC chromatograms showing oxidized products formed from PMS biooxidation by the whole cells of *Streptomyces glaucescens* GLA.0 under conditions of reaction (I). Samples were taken after 72 h, 96 h and 120 h, respectively. Retention times of *R*-PMSO, *S*-PMSO and phenyl methyl sulfone were 15.5-15.7 min, 18-18.5 min and 19 min, respectively.

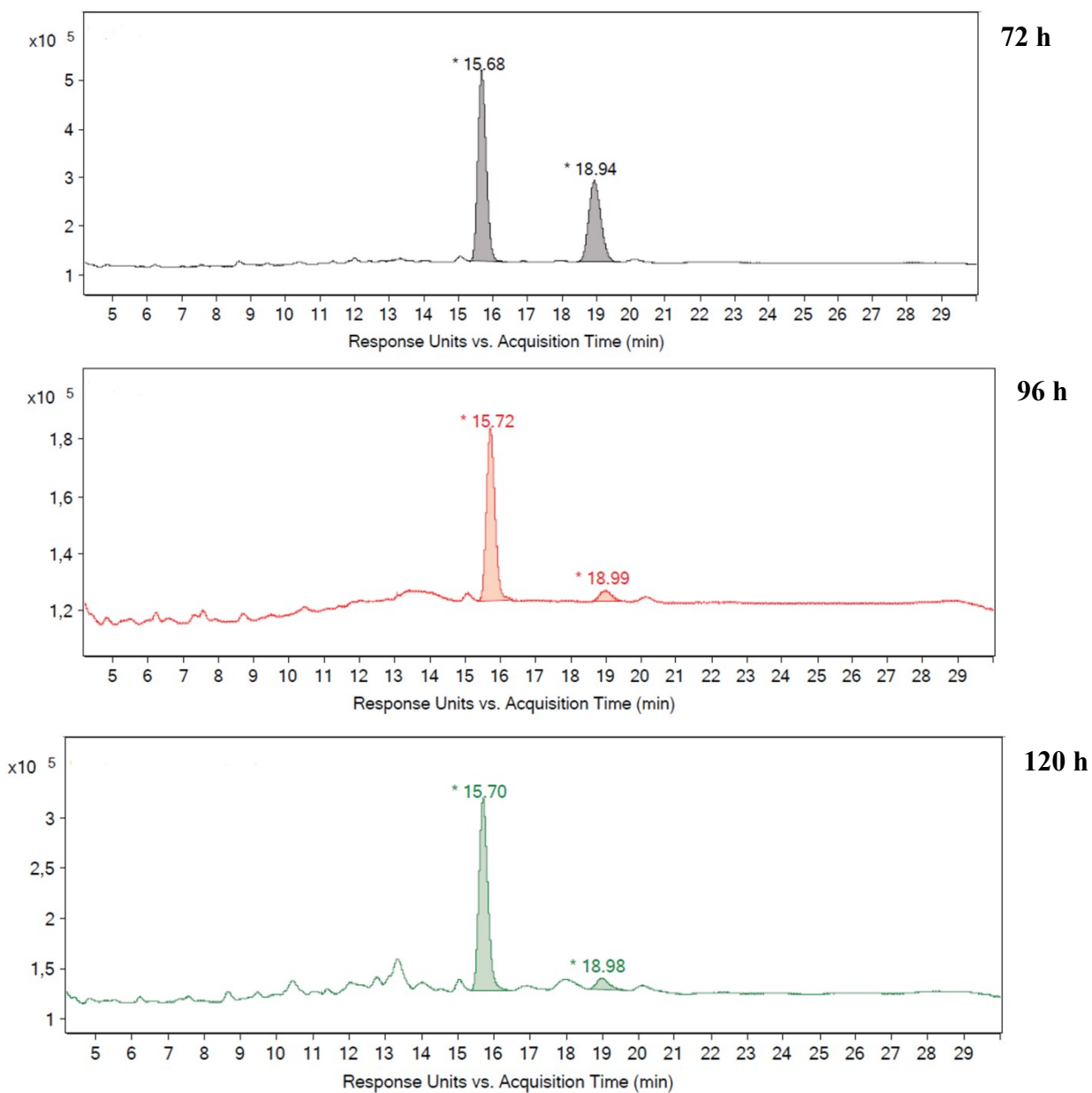


Fig. S15. Chiral GC chromatograms showing oxidized products formed from PMS biooxidation by the whole cells of *Streptomyces glaucescens* GLA.0 under conditions of reaction (J). Samples were taken after 72 h, 96 h and 120 h, respectively. Retention times of *R*-PMSO and phenyl methyl sulfone were 15.6-15.7 min and 18.9 min, respectively.

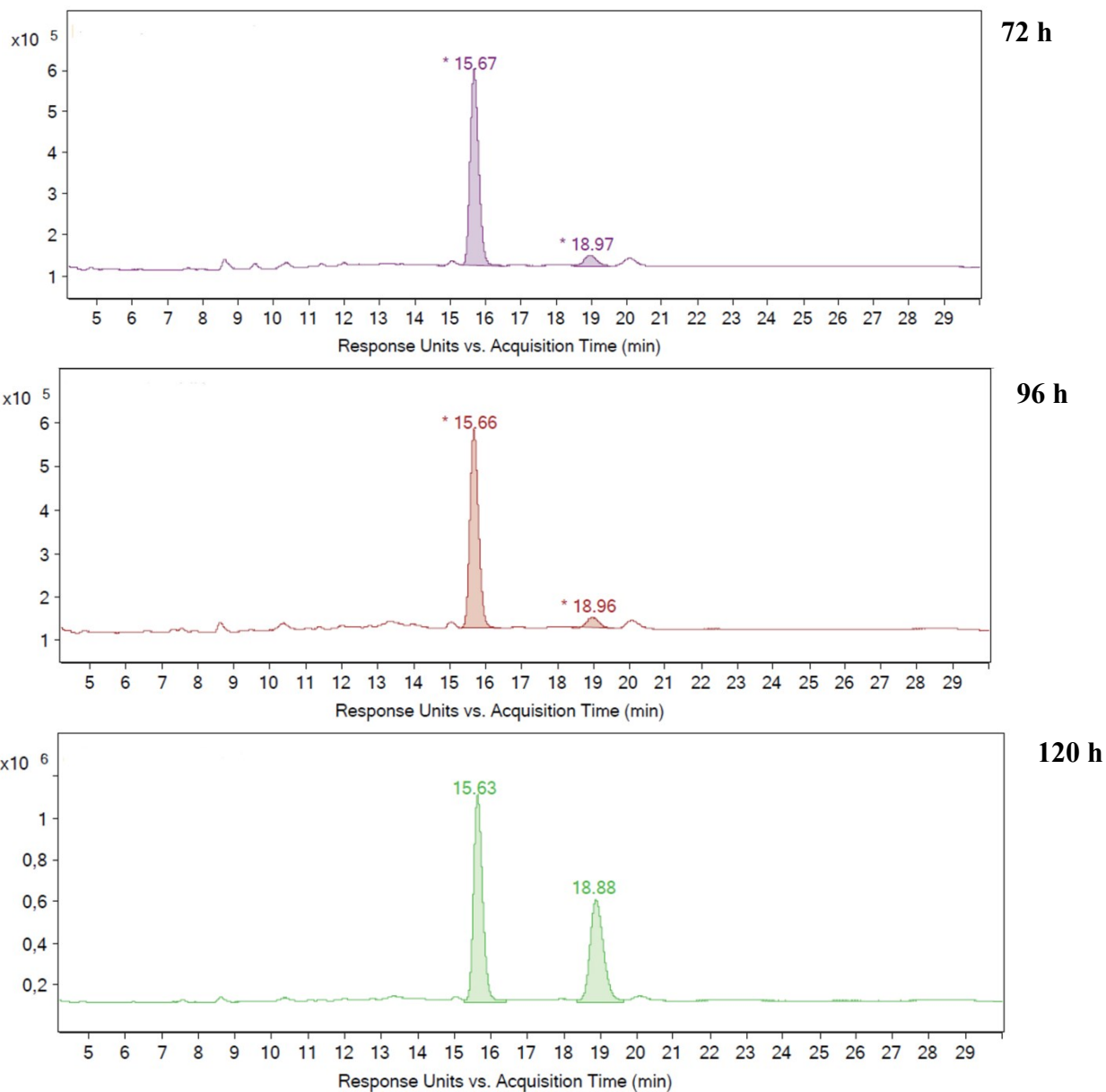


Fig. S16. Chiral GC chromatograms showing oxidized products formed from PMS biooxidation by the whole cells of *Streptomyces glaucescens* GLA.0 under conditions of reaction (K). Samples were taken after 72 h, 96 h and 120 h, respectively. Retention times of *R*-PMSO and phenyl methyl sulfone were 15.6 min and 18.8-18.9 min, respectively.

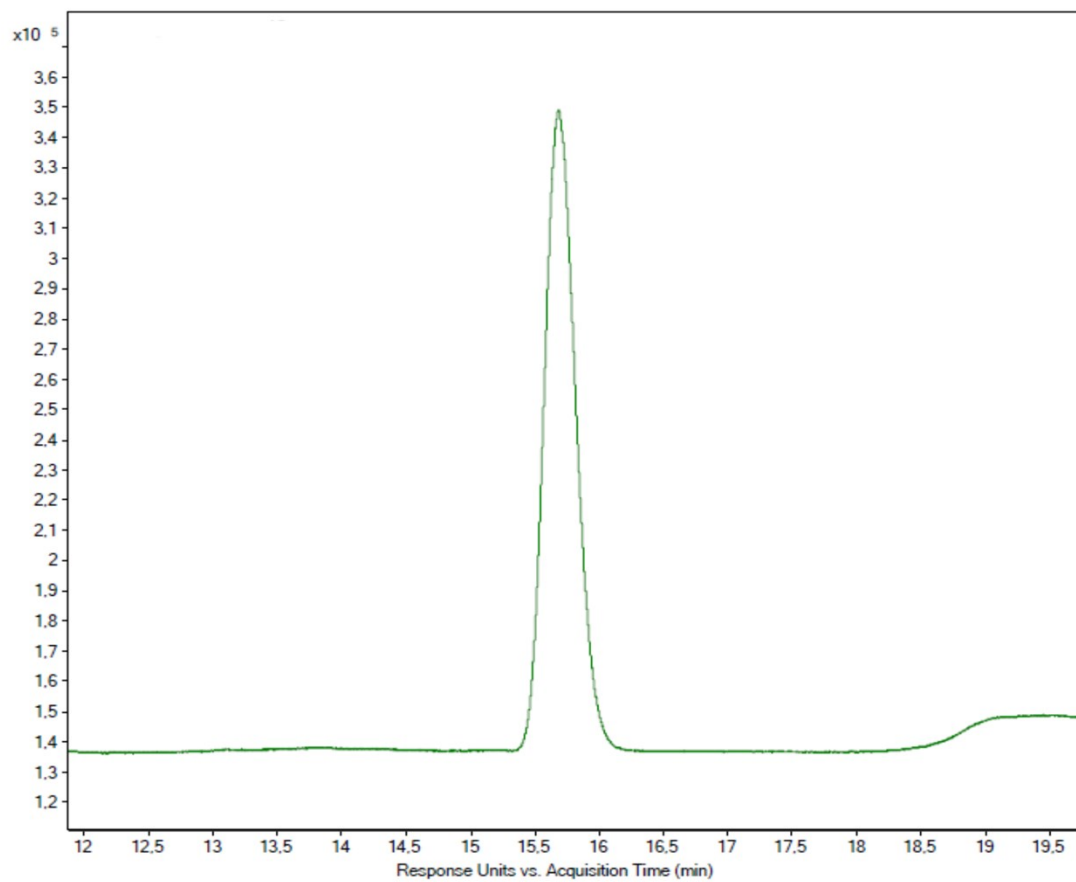


Fig. S17. Chiral GC chromatogram showing *R*-PMSO ($R_t = 15.5$ min) produced from enzymatic oxidation of PMS using phenyl acetone monooxygenase (PAMO).

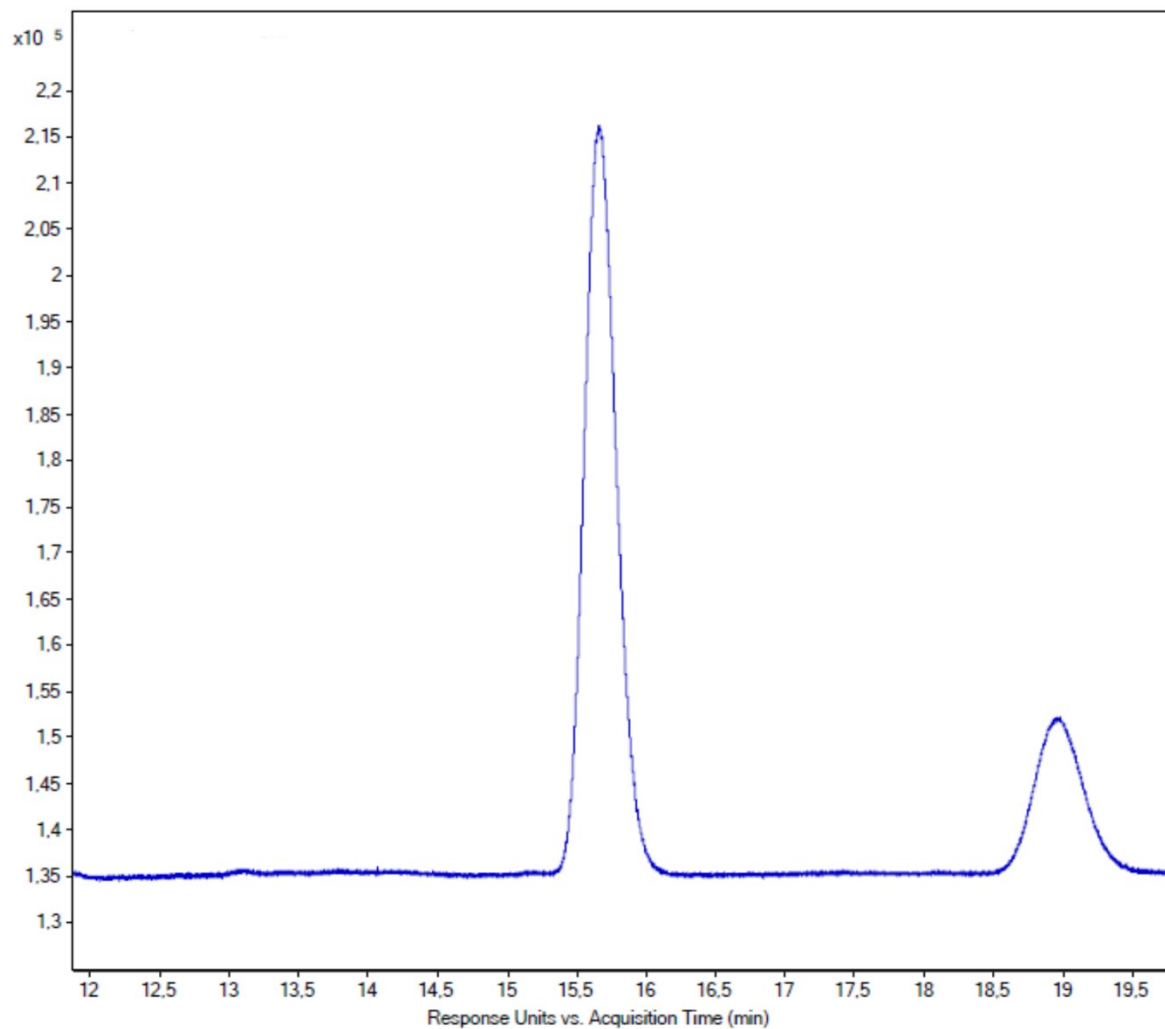


Fig. S18. Chiral GC chromatogram showing *R*-PMSO ($R_t=15.5$ min) produced from the enzymatic oxidation of PMS using cyclohexanone monooxygenase (CHMO).

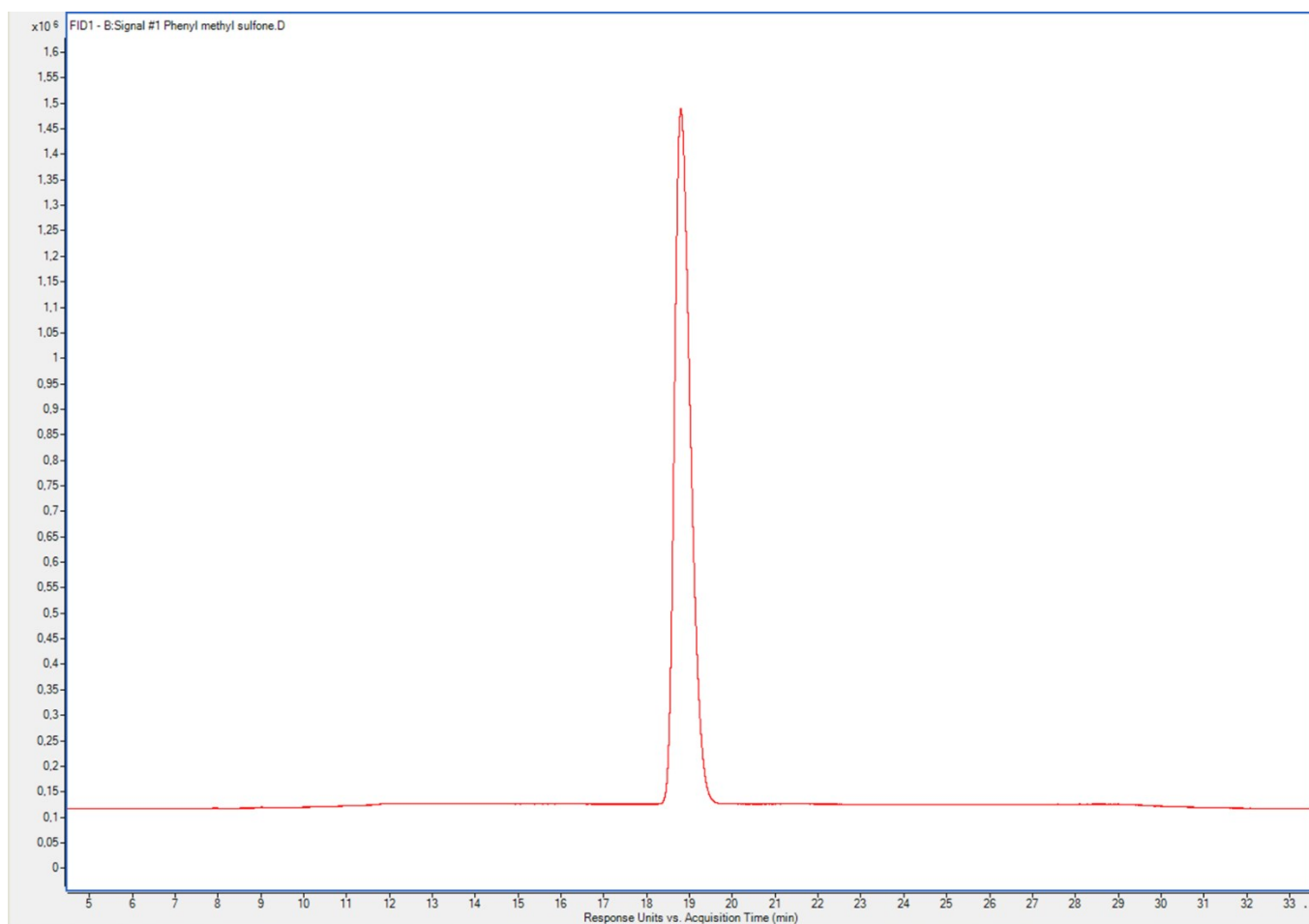


Fig. S19. Chiral GC chromatogram showing standard phenyl methyl sulfone ($R_t = 18.8$ min).

Table S1. Preliminary biotransformation experiments of PMS biooxidation using *S. glaucescens* GLA0 and their effects on PMS biotransformation.

Reaction	Substrate Conc.	Co solvent (%v/v)	Time of substrate addition	Reaction medium	RPM	Sampling Time ^[1]	PMS C% ^[2]	PMSO C% ^[2]	Sulfone C% ^[2]	Configuration ^[3]	ee% ^[3]
A	1.0 mM	IPA (0.2%)	3 rd day	Medium 3	150	120	19	71	10	ND	ND
						144	7	77	16		
						168	2	78	20		
B	1.0 mM	None	3 rd day	Medium 3	150	120	39	57	4	ND	ND
						144	8	74	18		
						168	1	75	24		
C	1.0 mM	None	3 rd day	Medium 3	180	96	1	56	43	<i>R</i>	>99
						120	1	83	17	<i>R</i>	>99
						144	1	50	49	<i>R</i>	>99
D	1.0 mM	None	Zero	Medium 3	180	72	7	80	13	<i>S</i>	9
						96	3	82	15	<i>R</i>	83
						120	2	83	15	<i>R</i>	>99

[1] Addition of the substrate at zero time of inoculation or at the 3rd day. Sampling time was calculated from the beginning of the experiment.

[2] Relative amount of phenyl methyl sulfide, phenyl methyl sulfoxide and phenyl methyl sulfone in reaction samples as calculated by GC-MS.

[3] Configurations and (*ee* %) enantiomeric excess values were analyzed by chiral GC-FID,

ND – not determined

Table S2. Optimization of the biotransformation experiments using the whole growing cells of *S. glaucescens* GLA0.

Reaction	Substrate Conc.	IPA Conc. (% v/v)	Reaction medium	RPM	Sampling Time ^[1]	PMS C% ^[2]	PMSO C% ^[2]	Sulfone C% ^[2]	Configuration ^[3]	ee% ^[3]
E	1.0 mM	0.2%	Medium 2	150	72	9	79	12	ND	ND
					120	9	70	21		
					144	12	65	23		
					168	7	63	30		
F	1.0 mM	0.2%	Medium 3	150	72	45	55	0	ND	ND
					120	15	68	17		
					144	5	71	24		
					168	1	61	38		
G	5.0 mM	1%	Medium 3	150	72	10	76	14	ND	ND
					120	5	73	22		
					144	6	61	33		
					168	3	54	43		
H	5.0 mM	1%	Medium 2	150	72	4	83	13	ND	ND
					120	5	83	12		
					144	7	79	14		
					168	7	80	13		
I	1.0 mM	0.2%	Medium 3	180	72	2	43	55	<i>S</i>	11
					96	2	63	35	<i>R</i>	82
					120	0	81	19	<i>R</i>	>99
J	1.0 mM	0.2 %	Medium 2	180	72	18	71	11	<i>R</i>	>99
					96	6	90	4	<i>R</i>	>99
					120	2	90	8	<i>R</i>	>99
K	5.0 mM	1%	Medium 2	180	72	4	82	14	<i>R</i>	>99
					96	1	85	14	<i>R</i>	>99
					120	1	89	10	<i>R</i>	>99

[1] Addition of substrate at zero time of inoculation.

[2] Relative amount of phenyl methyl sulfide, phenyl methyl sulfoxide and phenyl methyl sulfone in reaction samples_ calculated by GC-MS.

[3] Configurations and (*ee* %) enantiomeric excess values were analyzed by chiral GC-FID.

ND – not determined
