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ELECTRONIC SUPPLEMENTARY INFORMATION

Exploring glutathione lyases as biocatalysts: Paving the way for enzymatic lignin depolymerization.

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9_LigG_Loktanella_cinnabarina      --------------MARAIIYHIPVCPFSQRVEILLALKGLTGAVEFRVI 36
11_LigG_Loktanella_hongkongensis   --------------MNKPVIYHIPVCPFSQRVEILLALKGLSDAVAFRVI 36
7_LigG_Rhodobacter_sp_CACIA14H1    --------------MTKPIIHHIPVCPFSQRVEILLALKGLREAVEFRVV 36
2_LigG_Maritimibacter_alkaliphilus -------------MTGRHIVYHIPVCPFSQRLEILLALRGQQDAVEFRVV 37
10_LigG_Ruegeria_conchae           -------------MTGKHIVYHIPVCPFSQRLEILLALRGLTDAVEFRVV 37
13_LigG_Ruegeria_pomeroyi_DSS-3    MIRFRQHDPGAKTLSGKFIIHHIPVCPFSQRIEILLALRSLSDAVEFRVV 50
12_LigG_Kaistia_granuli            -------------MPLKPIVYHIPVCPFSQRIEILLQLKGHADAVEFRVV 37
4_LigG_Thiobacillus_denitrificans  --------------MSHPTVYHIPACPFCQRLDILLTLKGRRDEIDFQVI 36
8_LigG_Thiobacillus_thioparus      --------------MSKPIIYHIPVCPFCQRLDILLTLKGRRDEIDFQVI 36
6_LigG_Thiobacillus_den_ATCC_25259 --------------MTRPTVYHIPVCPFCQRVEILLSLKGRREDVDFRMI 36
5_LigG_Thioalkalivibrio_thio       --------------MSGPTVYHIPVCPFCQRLEILLSLKGKRQQVDFHVV 36
3_LigG_Leeia_oryzae                ---------------MKHTMYHIPVCPFSQRLEILLTLKGCRDQVDFQVI 35
1_LigG_Sphingobium_SYK-6           ------MAEPQE-----LTIYHIPGCPFSERVEIMLELKGLR--MKDVEI 37
14_LigG_Novosphingobium_PP1Y       ------MAWHEEPPPGALRMYHIPGCPFSERVEILLDLKGLSGIMDDHEV 44
                                                      ::*** ***.:*::*:* *:.    :    :

9_LigG_Loktanella_cinnabarina      DITEPRPDWLLEKTGGTTALPLLETETGGILKESLVILRYVEERWAEPRI 86
11_LigG_Loktanella_hongkongensis   DITEPRPGWLLEKTGGTTALPVLETEGGGILKESLVILRYVEERWATPRI 86
7_LigG_Rhodobacter_sp_CACIA14H1    DITRPRDPDLLAKTRGTTALPVLELEDGRILKESLVLMRYFEERFPQVPV 86
2_LigG_Maritimibacter_alkaliphilus DITKPRDPELLAKTRGTTALPVLETRDGRILKESLVILRYLDEALEGPML 87
10_LigG_Ruegeria_conchae           DITKPRDPELLAKTRGTTALPVLETSDGRILKESLVILRYLDEALPGAPL 87
13_LigG_Ruegeria_pomeroyi_DSS-3    DITRPRDPELLAKTRGTTALPVLETPDGRIIKESLVILRYLDEVLPGAPL 100
12_LigG_Kaistia_granuli            DITRPRDAKLLRKTRGTTALPVLETEDGRILKESLVILQYLDETVAGGLK 87
4_LigG_Thiobacillus_denitrificans  DITRPRPDWLLQKTRGTTALPVLETADGHIIKESLVILQYLEDIYPERPV 86
8_LigG_Thiobacillus_thioparus      DITQPRPDWLLKKTRGTTALPVLETADGHIIKESLVILQYLEDLYPEHPV 86
6_LigG_Thiobacillus_den_ATCC_25259 DITAPRPDWLLQKTRGTTALPVLETADGRVIKESLVILRYFEDIYREPQI 86
5_LigG_Thioalkalivibrio_thio       DITRPRPEWLLRKTRGTTVLPVMETADGYIIRESLVILAYLEDIFPELPV 86
3_LigG_Leeia_oryzae                DITKPRPDWLLAKTHGTTSLPVMELPDGRILKESLVLLQYLESIYPARPV 85
1_LigG_Sphingobium_SYK-6           DISKPRPDWLLAKTGGTTALPLLDVENGESLKESMVILRYLEQRYPEPAV 87
14_LigG_Novosphingobium_PP1Y       DISKPRPDWLLSKTRGTTSLPALELENGETLKESMVILRYFEERFPERPV 94
                                   **: **   ** ** *** ** ::   *  ::**:*:: *.:.

9_LigG_Loktanella_cinnabarina      ASADPFERGIERLFITREGPFGMAGYLYVINTDSAKTDEKREALLGHYRW 136
11_LigG_Loktanella_hongkongensis   AREDPFERGIERLFITREGPFGMAGYGFVMNTDRTRTDDKRAALLGHYRW 136
7_LigG_Rhodobacter_sp_CACIA14H1    ARADPYERAVERLFISREGPFGNAGYGYVMNRDRGQAGAKRQALLDQYAW 136
2_LigG_Maritimibacter_alkaliphilus RRSDPLEHAIESMLIAREGPFTMAGYLFVMNQDRARRGEHLDNLLALYRD 137
10_LigG_Ruegeria_conchae           RRSDPTEHAIESMLIAREGPFTMAGYLFVMNQDQARRDEYLNKLLALYRD 137
13_LigG_Ruegeria_pomeroyi_DSS-3    RRIDPAEHAIESMLIAKEGAFVTAGYLYVMNQDRSQREAHLDRLLGLYRG 150
12_LigG_Kaistia_granuli            RRADPFEHAVERMLIAKEGPFTAAGYTFVMNQDRAARRSHEEKLLKLFAQ 137
4_LigG_Thiobacillus_denitrificans  AQRDPYRRAVENMLTRMDGEFFSLGYGWLMNQDSARREVLREGMLKQYAQ 136
8_LigG_Thiobacillus_thioparus      AQRDPYRRAVENMLTRMDADFFSQGYTWLMNQDPTRCAVLREDMLKQYAL 136
6_LigG_Thiobacillus_den_ATCC_25259 AQTDPYRRAVENMLTTMDRDFVAAGYGWLMNQDPKQRDALRENMLKQYAQ 136
5_LigG_Thioalkalivibrio_thio       AQRDPYRRAVENMLTMMEGAFGTQGYLWVMNQDPGRRDALRAAMLKQYER 136
3_LigG_Leeia_oryzae                AQQDPYKHAVESMMARMEGDFCNQGYRFVMNQNPTRQQELREGMLAQYAK 135
1_LigG_Sphingobium_SYK-6           AHPDPFCHAVEGMLAELAGPFSGAGYRMILNREIGKREEMRAAVDAEFGK 137
14_LigG_Novosphingobium_PP1Y       AQRDPFEHAVEAMLCATDGQFTGAGYRMILNRDRAKRDDCKAEVDAQYAR 144
                                      **  :.:* ::      *   **  ::* :          :   :

9_LigG_Loktanella_cinnabarina      LNGMLERHNPDGMWLFDR-FGLPEIVYTPLLMRFWFLEHYEGFTLPDTPE 185
11_LigG_Loktanella_hongkongensis   LGDMLERHNPDGTWLFDR-FGLPEIVYTPLMMRFWFLDHYEGFELPDTPE 185
7_LigG_Rhodobacter_sp_CACIA14H1    LDDMLRHHNPDGVFLFDR-FGWAEAVYTPLMMRFWFLDYYEGFALPDDPA 185
2_LigG_Maritimibacter_alkaliphilus INDFLTDRNPKGTYLFED-FGLAEAVFAPMFKRFWFLDYYEDFELPRGTD 186
10_LigG_Ruegeria_conchae           LDDFLSHYNPTGPFLFED-FGLAEAVFTPMFKRFWFLDYYEGFKLPVGKE 186
13_LigG_Ruegeria_pomeroyi_DSS-3    LDDFLVEHNPKGTWLFDG-FGLAEAVYTPLFKRFWFLDYYEDFALPDDPA 199
12_LigG_Kaistia_granuli            LDDFLREHSPGGEFLFAE-FGLAEAVFTPIFMRFWFLDYYEGFELPAEPE 186
4_LigG_Thiobacillus_denitrificans  LDDFLREHAPVGPFLFGE-FGWAETVFTPFFQRFWLLEYYENFELPGDDR 185
8_LigG_Thiobacillus_thioparus      LNDFLLEHAPSGPFLFET-FGWAEAVFTPFFERFWFLEYYEGFTLPGDDR 185
6_LigG_Thiobacillus_den_ATCC_25259 LDDFLLEHGAPGPFLFET-FGWAETVFTPFFQRFWFLEYYEDFELPTASR 185
5_LigG_Thioalkalivibrio_thio       LNEFLVEHAPRGPFLFDA-FGWAEAVFTPLFMRFWFLEYYENFRLPDGPR 185
3_LigG_Leeia_oryzae                LNDFLLAHSPDGTYLFDD-FGWAETIFTPFFMRFWFLDYYEDFQLPDTPS 184
1_LigG_Sphingobium_SYK-6           VDAFLKRYATGSDFLFDDRFGWAEVAFTPMFKRLWFLDYYEDYEVP--AN 185
14_LigG_Novosphingobium_PP1Y       LDDFLRHYSPDGDYLFAS-FGWAEVVFAPMFKRLWFLEYYEGYEVP--QH 191
                                   :. :*    . . :**   ** .*  ::*:: *:*:*::**.: :*
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9_LigG_Loktanella_cinnabarina      FARVARWRAACLGHEAAQQV-TPDQIVKLYYDYAVGAGNGALPEGRQRSS 234
11_LigG_Loktanella_hongkongensis   FARVRRWRAACLDHEAAQQV-RYDQIVKLYYDYAVGSGNGARPEGRERSS 234
7_LigG_Rhodobacter_sp_CACIA14H1    FARVARWKAACLAHPAAQQV-TVEEIVKLYYDYAVGKGNGALAEGRVRSS 234
2_LigG_Maritimibacter_alkaliphilus YERVRIWRDACMAHPSTHQV-TEEQIVKLYYDYALGAGNGALPEGRNVSS 235
10_LigG_Ruegeria_conchae           YDRVRAWRDACMAHPETSQV-TEEEIVKLYYDYALGAGNGALLDGRSVSS 235
13_LigG_Ruegeria_pomeroyi_DSS-3    YDRVRVWRAACIAHPATDQV-SEEEIVKLYYDYALGAGNGALVDGRTVSS 248
12_LigG_Kaistia_granuli            FDRVRRWRAACLDHPAARQT-SREEIVKLYYDYAFGAGNGSLLPGRTKSS 235
4_LigG_Thiobacillus_denitrificans  YARVRKWIDACVAHPAAQQV-AREEVVKLYYDYAKGAGNGALLPGRKQSS 234
8_LigG_Thiobacillus_thioparus      YARVREWVDACISHPAAQQT-TLEEVVKLYYDYSKGAGNGALPPGRTKSS 234
6_LigG_Thiobacillus_den_ATCC_25259 YARVREWIDACVAHPAAQQT-TREEVIKLYYDYACGAGNGALLPGRSRSS 234
5_LigG_Thioalkalivibrio_thio       YTRVHQWVDACLAHPAAQQV-TREEVIKLYYDYAQGAGNGELLPGRSRSS 234
3_LigG_Leeia_oryzae                YQRVKKWQAACLAHPAAQQV-TREEIVKNYYDYAKGAGNGTLLPGRQKSS 233
1_LigG_Sphingobium_SYK-6           FDRVLRWRAACTAHPAAQYR-SKEELLKLYYDYTQGGGNGRIPEGRSISS 234
14_LigG_Novosphingobium_PP1Y       LARVLRWREACTEHPAVANRHSHRELMTLYYDYSQGGGNGRLPQGRHISS 241
                                     **  *  **  *  .       :::. ****: * ***    **  **

9_LigG_Loktanella_cinnabarina      FAFEPHWRDRPMPPADKYERIASDAELGLLTA---- 266     52
11_LigG_Loktanella_hongkongensis   FAFEPDWRDRPMPPSDKYDRVASDAELGLLVS---- 266     51
7_LigG_Rhodobacter_sp_CACIA14H1    FVFDPHWKGRPMPPKDKYDRIASDAELGLV------ 264     52
2_LigG_Maritimibacter_alkaliphilus FVFEPSWQSRPMPPKDKYAGSATDRDLGLVA----- 266     49
10_LigG_Ruegeria_conchae           FAFEPPWQSRPMPPREKYAVPATDKELGLVA----- 266     50
13_LigG_Ruegeria_pomeroyi_DSS-3    FAFEPDWRSRPWPPRDKYDGTASDAALGLLSV---- 280     48
12_LigG_Kaistia_granuli            FVFEPDFKTRPWPPAEKYGPPASDAALGLAAGSGA- 270     50
4_LigG_Thiobacillus_denitrificans  FAFEPDWRGRPWPPKDKYRHSASDLELGL------- 263     51
8_LigG_Thiobacillus_thioparus      LSPAPDWRARPWPPRNKYAHSATDAELGLL------ 264     52
6_LigG_Thiobacillus_den_ATCC_25259 FALEPDWRARPWPPRSKYRQPAGDTELGL------- 263     50
5_LigG_Thioalkalivibrio_thio       FAFEPDWRLRPWPPKDKYTYSATDAELGL------- 263     50
3_LigG_Leeia_oryzae                FVFEPHWKTRPWPPKDKYNTSATDDELGLNG----- 264     51
1_LigG_Sphingobium_SYK-6           FSPDVDWRTRPMPPRDKWGHAATDAELGLTR----- 265    100
14_LigG_Novosphingobium_PP1Y       FTLDPAWEKRPMPPRDKWGTPATDRELGLVTATVGA 277     66
                                         :     :. ** ** .*:   * *  ***     % Similarity
                                                                            to LigG
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Figure S1. Multiple sequence alignment of known and novel glutathione lyases, discovered by database mining using the amino acid sequence of LigG as query.

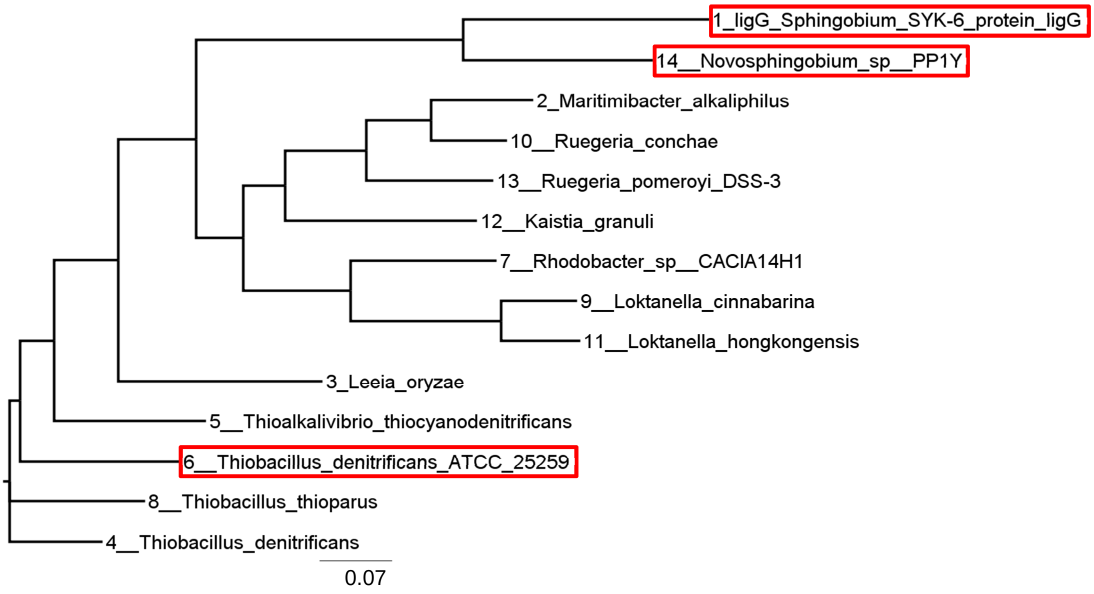

Figure S2. Neighbour-joining phylogenetic representation based on amino acid sequences of LigG from *Sphingobium sp.* SYK6 and their closest homologues identified in the GenBank nr database of NCBI.

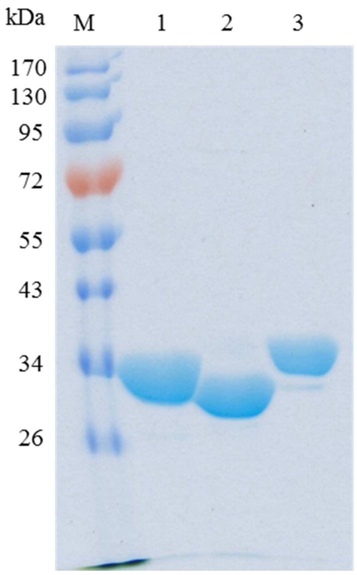

Figure S3. SDS-PAGE analysis of purified glutathione lyases. Lane M: molecular weight marker; Lane 1: LigG; Lane 2: LigG-NS; Lane 3: LigG-TD.

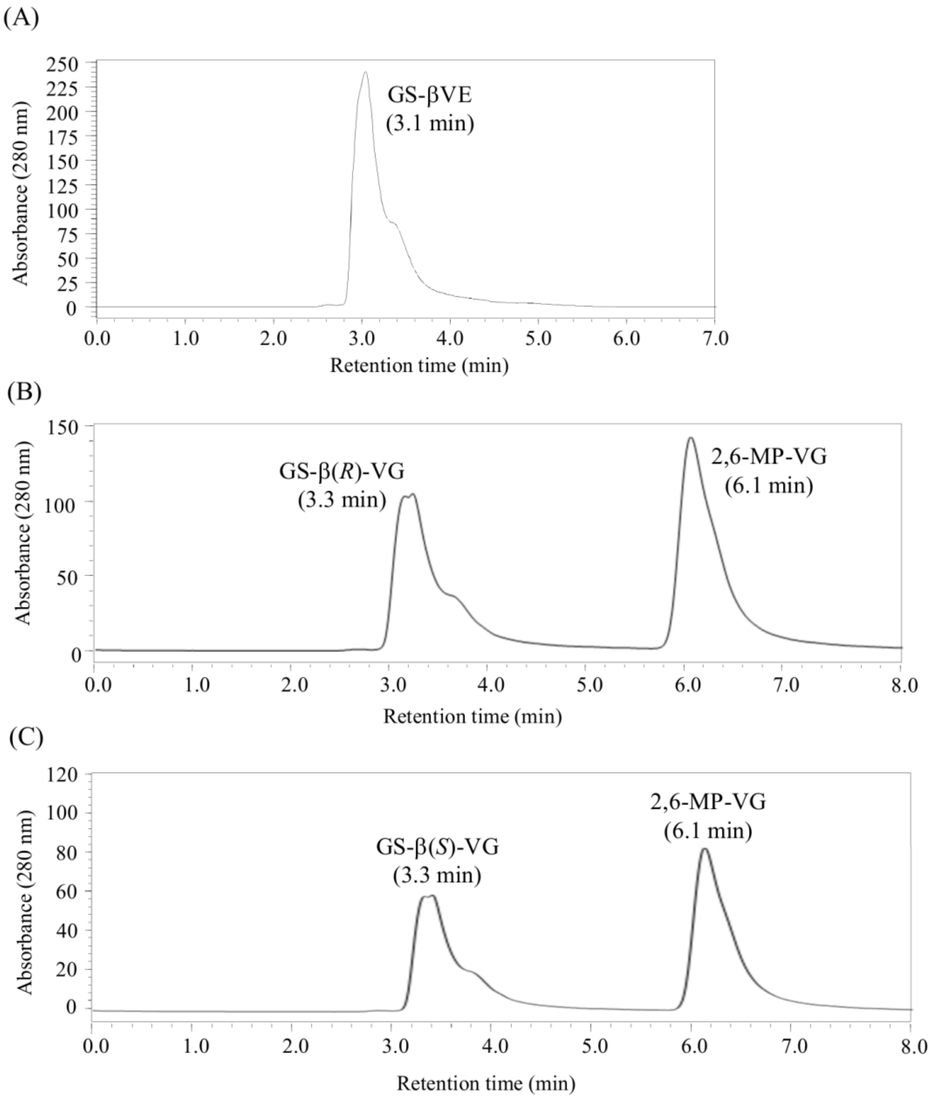

Figure S4. (A) HPLC chromatogram of substrate GS-βVE (**3**) used in our study to determine pH and temperature dependencies as well as kinetics of novel glutathione lyase enzymes. (B+C) HPLC chromatograms of substrates GS-β(*R*)–VG (*R*-**4**; generated by the conversion of 2,6-MP-VG with β-etherase LigF; B) and GS-β(*S*)–VG (*S*-**4**; generated by the conversion of 2,6-MP-VG with β-etherase LigE; C) used to determine the stereoselectivity of the identified enzymes.

Table S1. The sequences of primers used in this study for site-directed mutagenesis of the different glutathione lyase genes.

Primer name	Primer sequence	Mutation
LigG_M167A_FW	5´- TTGCTTTCACCCCGGCGTTCAAGCGTCTGT-3´	**Met167Ala**
LigG_M167A_RV	5´-ACAGACGCTTGAACGCCGGGGTGAAAGCAA-3´	
LigG_S109A_FW	5´-GCGGGTCCATTCGCCGGTGCGGGTTA-3´	**Ser109Ala**
LigG_S109A_RV	5´-TAACCCGCACCGGCGAATGGACCCGC-3´	
LigG_L117A_FW	5´-GGTTACCGTATGATCGCGAACCGTGAGATTGG-3´	**Leu117Ala**
LigG_L117A_RV	5´-CCAATCTCACGGTTCGCGATCATACGGTAACC -3´	
LigG-NS_M173A_FW	5´-TGGTGTTTGCGCCGGCGTTCAAACGTCTGT-3´	**Met173Ala**
LigG-NS_M173A_RV	5´-ACAGACGTTTGAACGCCGGCGCAAACACCA-3´	
LigG-NS_T116A_FW	5´-CGACGGTCAATTCGCCGGTGCGGGTTAC-3´	**Thr116Ala**
LigG-NS_T116A_RV	5´-GTAACCCGCACCGGCGAATTGACCGTCG-3´	
LigG-TD_F165A_FW	5´-CGTTTTCACCCCGGCCTTCCAGCGTTTC-3´	**Phe165Ala**
LigG-TD_F165A_RV	5´-GAAACGCTGGAAGGCCGGGGTGAAAACG-3´	
LigG-TD_F165M_FW	5´-CCGTTTTCACCCCGATGTTCCAGCGTTTCT-3´	**Phe165Met**
LigG-TD_F165M_RV	5´-AGAAACGCTGGAACATCGGGGTGAAAACGG-3´	
LigG-TD_V108A_FW	5´-ACCGTGATTTCGCGGCGGCGGGTTAC-3´	**Val108Ala**
LigG-TD_V108A_RV	5´-GTAACCCGCCGCCGCGAAATCACGGT-3´	
LigG-TD_V11G_FW	5´-TACCACATTCCGGGCTGCCCGTTCTGC-3´	**Val11Gly**
LigG-TD_V11G_RV	5´-GCAGAACGGGCAGCCCGGAATGTGGTA-3´	
LigG-TD_M116A_FW	5´-GTTACGGTTGGCTGGCGAACCAAGATCCGA-3´	**Met116Ala**
LigG-TD_M116A_RV	5´-TCGGATCTTGGTTCGCCAGCCAACCGTAAC -3´	