

Supporting Information

Biodegradation of the Allelopathic Chemical, Pterostilbene, by *Sphingobium* sp. from Peanut Rhizosphere

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Supplemental figures: Figure S1 – S6

Supplemental tables: Table S1 –S2

Materials and Methods –Extra LCMS methods for sample analysis

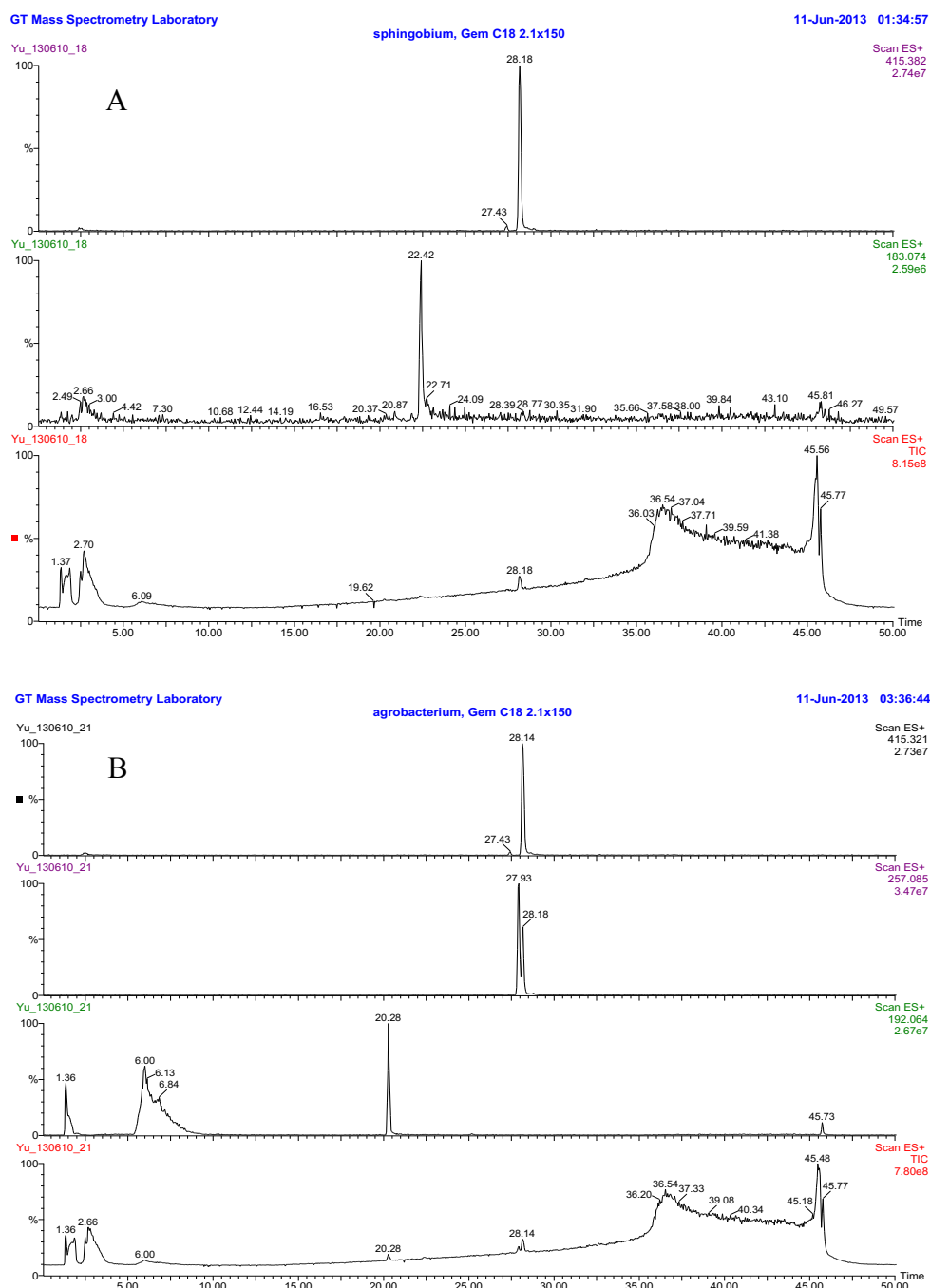


Figure S1. LC-MS analyses of novel products from pterostilbene metabolism by *Sphingobium* sp. JS1018 (**panel A**) at HPLC RT 6.23 min (or RT 28.18 min on LC-MS with an m/z value of 415); and novel products from pterostilbene metabolism by *Agrobacterium tumefaciens* JS1013 (**panel B**) at HPLC RT 6.23 min (or RT 28.14 min on LC-MS with an m/z value of 415) and RT

5.57 min (or RT 20.28 on LC-MS with an m/z value of 192) with pterostilbene as a reference (m/z of 257).

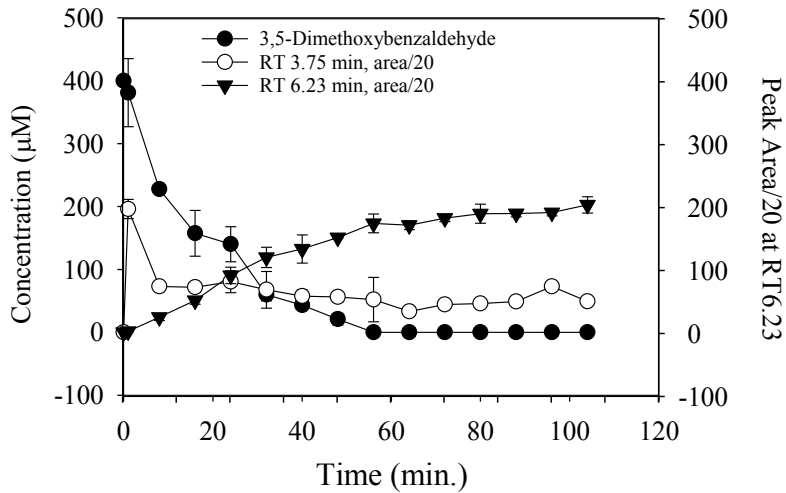


Figure S2. Dimethoxybenzaldehyde metabolism kinetics and novel metabolites produced during enzyme assays with dialyzed crude extracts prepared from 3,5-dimethoxybenzaldehyde-induced cells (grown in 1 mM 3,5-dimethoxybenzaldehyde for 4 days) of *Sphingobium* sp. strain JS1018.

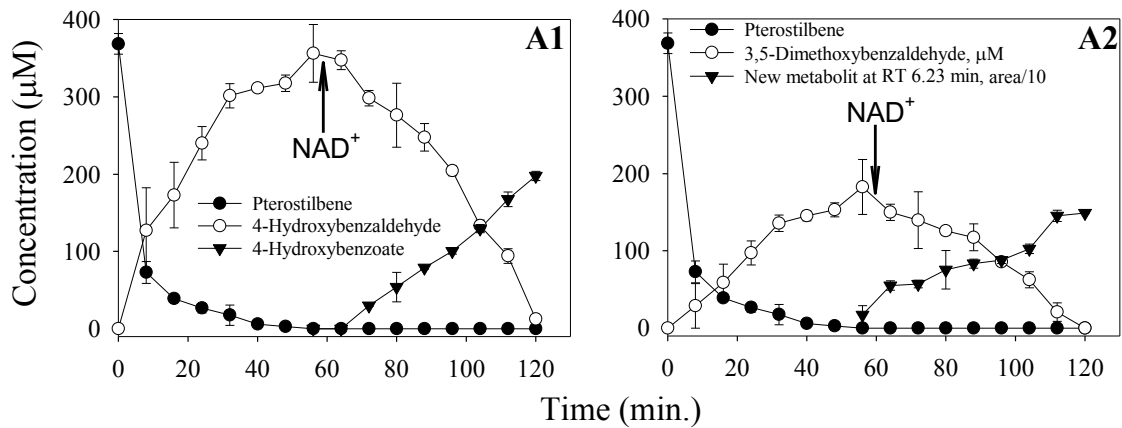


Figure S3. Degradation of pterostilbene (A1 and A2) in dialyzed crude extract of resveratrol-grown JS1018 cells. NAD⁺ (3 mM) was added at 60 min in the enzyme assay.

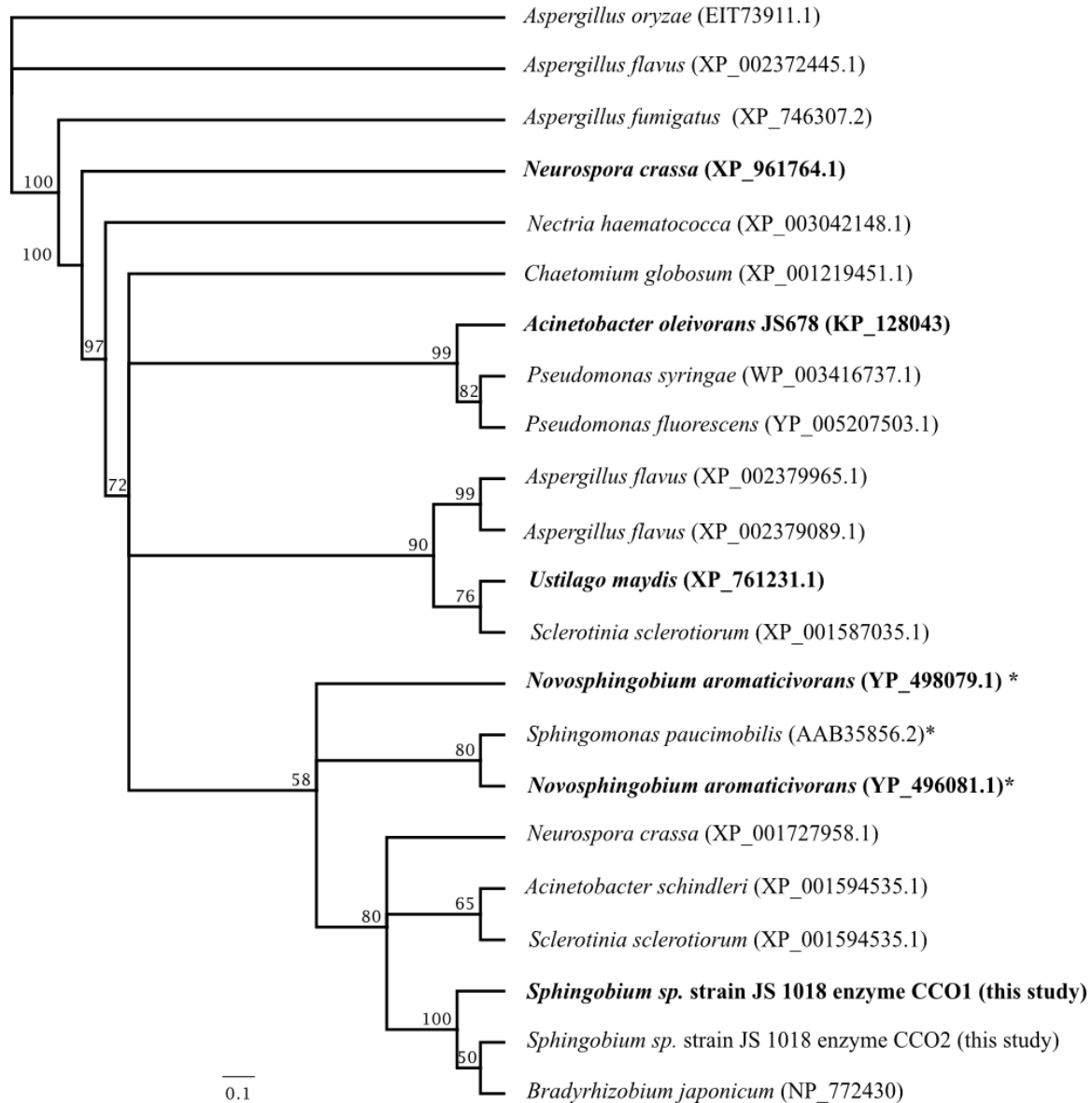


Figure S4. Phylogenetic analysis of functionally characterized carotenoid cleaving oxygenases (1-5). Enzymes that transform a stilbenoid are in bold and enzymes that were originally identified as lignostilbene oxygenases are marked with *. The tree was constructed based on neighbor joining method and bootstrap values are included in nodes.

Sphingomonas paucimobilis
Spingobium sp. JS 1018M
Acinetobacter sp. JS678M
Arabidopsis thaliana
Synechocystis sp. PCC6803M
Novosphingobium aromaticivorans MASLIITTKAMSHHHVLSSTRITTLYSDNSIGDQIQIKTKPQVPHRLPARRIFGVTRAVIN

Sphingomonas paucimobilis MAHFPQTTPGFSGLTRP - - - - - LRIEGDILDIEIEGVEVPPQLNGTFHRVHPDAQFPF
Spingobium sp. JS 1018 TSCFPDDPIYRGFDAP - - - - - GRVEANVFDLEVEGRVPELDGTFFRVAPDPQWPP
Acinetobacter sp. JS678 SFTFPNTSEFTGLYEP - - - - - CRIEADITDLVIEGDIIPSAIKGTFYQVAPDPQYYP
Arabidopsis thaliana MGAFPQTIYFTGANAP - - - - - VGEEPDRLGLKVEGDLPAEVRGFSFYRAIPDPAPFP
Synechocystis sp. PCC6803 VTSPTSSPSQRSYSPQDWLRGYQSQQPEWDYVWEDVEGSIPPDLQGTLYRNGPGLLEIG
Novosphingobium aromaticivorans SAAPSPLPEKEKVEGERRCHVAWTSVQQENWEGELTVQGKIPTWLNGLTYLRNGPGLWNIG
 * : * : * : * : * : *

Sphingomonas paucimobilis RFEDDQFFNGDMVSLFRPH - DGKIDFRQRYAQTDKWKVERKAG - - - - - KSLFGAYRNPLT
Spingobium sp. JS 1018 MLGHDIFPFGDGMVCAFPRFK - DGRVDPTRSYAQTDKFAERQAR - - - - - KALYGAYRNPYT
Acinetobacter sp. JS678 MLGNDIFPFGDGVVTAIELG - EGRVSMKRRYVQTPRLVAQKQAH - - - - - RSLNGVYRNIIYT
Arabidopsis thaliana RFENDHTLSGDGMVSRLSFNGDGTADFIQKYVETARYKAEKAAG - - - - - KALFGKYRNPFT
Synechocystis sp. PCC6803 DRPLKHPFDGDMVTAFAKFPDGRVHFSQKVFVTSQGYVEEQKAGKMIYRGVFGSQPAGGW
Novosphingobium aromaticivorans DHDFRHLFDGYSTLVKLPD - GGRIFAHRFLSDAYKAAKHNRLCYRFSETPKSVII
 : * : * : * : * : * : *

Sphingomonas paucimobilis DDAVQGMIRGT - - - - - ANTNNVMVHAG - - - - - KLYAMKEDSPCLIMDPLTLETEGYT
Spingobium sp. JS 1018 DDPVAVGSIRST - - - - - ANTNNIVHHG - - - - - LLLALKEDSPVAMRPDTLETIGNY
Acinetobacter sp. JS678 NDP - LAAKNNTT - - - - - ANTTVIEHNG - - - - - VLLAMKEDALPWALDLKTLLETIGEW
Arabidopsis thaliana DDPVQGVDRITV - - - - - ANTTVPVHAG - - - - - RMLMAKEDGRPVDRPRLATIGSY
Synechocystis sp. PCC6803 LKTI FDLRLKNI - - - - - ANTNITYWGD - - - - - RLLALWEGQPRLRLEPSNLATIGLD
Novosphingobium aromaticivorans KNKPSFGIEIVRLFSGESLTDNANTGVILKGDGRVMCLTETQKGSILVDHETLETIGKF
 * * * : : * * *

Sphingomonas paucimobilis NFDGKLQS - QTFCAPHPKIDPVT - - - - - GNLCAFAYGAKGLMTLDMAYIEISPTGKLLKEIP
Spingobium sp. JS 1018 RFGDKMMS - EFTTAHPKVDPS - - - - - GELIAFGYSAGKVAATSDLAYVYIDRHGEVVEAW
Acinetobacter sp. JS678 DFNQINS - ATFTAHPKIEPKT - - - - - GNLLCFAYEAKGDGTPDIAYYIEISATGELLKEIW
Arabidopsis thaliana DFGGALKS - ETMTAHPVRIIDAGT - - - - - GELFFYGYEADGQASTKVAYCIVGPDGELKRQW
Synechocystis sp. PCC6803 DLGGILAEQPLSAHPRIDPASTFDGGQPCYVTFISKSLSTLLELDPQKLLRQKT
Novosphingobium aromaticivorans EYDDVLSDHMIQSAHPPIVTE - - - - - MWTLPDLVVKPYRVRMEAGSNKREVVRVRCR
 * * : :

Sphingomonas paucimobilis FQNPYYCMHDFGVTEHYAVFAVMPLLSS - - - - - WDRLEQRLPFPFDDTTLPCYLGLPRN
Spingobium sp. JS 1018 FTAPRAASIHDFAVTENYVVPVGSHEIE - - - - - TERLKAGKPAFVWRPDEVIYGLVPRR
Acinetobacter sp. JS678 FQAPYAAIHDFAVTENYVIFPIIPLTVD - - - - - IERMKKGQHPQWQDLEQLFGLIPRS
Arabidopsis thaliana FDAPYCAMHDFTISENYALFPIYPTAD - - - - - LDRLKAGGEHWHQPELDSWLVMPRY
Synechocystis sp. PCC6803 ETPFGFAIHDFAITPHYALFLQMNVTNLGLFYLFLGRGAGECVQFHDPKPAQIILVPRD
Novosphingobium aromaticivorans SSGSWGPGWHSFAVTENYVVIEMPLRYSVKNLLRAEPTLYKFEWCPQDGAFIHMVMSKL
 * * : * : * : * : * : *

Sphingomonas paucimobilis GDARDLRWFKTGN - CFVGHVMAFNADGT - - - - - KVHIDMPVSRNNSFPFF -
Spingobium sp. JS 1018 GNAEDMRWFTVPTNGFQGHITINAWDDGH - - - - - KVYVDMPLNDNAFWFYP
Acinetobacter sp. JS678 QQAEDIQWFGPKNGFQCHTLNSFEKNG - - - - - KIYVDMPVASGNVYFYP
Arabidopsis thaliana GDVSEIKWFKGPKGCHSYHMMNAWEDADG - - - - - MLHFDACLNNTNAPAFIR
Synechocystis sp. PCC6803 G - - - - - GEIKRIPVQAGFVPHANAFEENG - - - - - KILDSICYNSLPQVDTD
Novosphingobium aromaticivorans TG - - - - - EVVASVEVPAYVTFHFINAYEEDKNGDGKATV IADCEHNADTRLDMRLDLRL
 * * : * : *

Sphingomonas paucimobilis DVHGAFFDFVAGQGLTRWTVDMASNGDSFEKTERLDFRDPFPFRIDERYATRAYERHGW
Spingobium sp. JS 1018 DENGHAHPSTLQTMTRWIFDLSNS - VTPQMDIIPAPMGEPHIDERYATRYRHAFL
Acinetobacter sp. JS678 PAEAPVHSEQITSAIMRWEFDLQATDHHVVKPPIITNKQYPCFPFRCDERFNGLEYSYGFL
Arabidopsis thaliana EPSGIHMQPDIKGLTRWTVDPADG - - - - - GDVVETVIGPPCFPVIPAKLQGRPYKTGWM
Synechocystis sp. PCC6803 GDFRSTNFDNLDPGLNRFTIDFAAAT - - - - - VEKQLMVRCCFPVVFHQVGRPYRYVYM
Novosphingobium aromaticivorans SSHGHVLPDARIGRFRIPLDGSKYGLKLETAVEAEKHGRAMMCSINPLYLGQKYRYVYA
 : : * : * : *

Sphingomonas paucimobilis LILDTEKPYEAPGG - - - - - AFYALTNTLGHIDLATGKSSSWAGPRCAIQEPCFIPRSPD
Spingobium sp. JS 1018 AVIDPTAPYDFQRCGPP - - - - - SVNAFLNGLAHVDMTASRRWLPGPTSTVQEPVFAFRSPE
Acinetobacter sp. JS678 LAFDPLAFDHEHNLG - E - - - - - YPFQFNQLARVMVQTGITETWYFGDKYCFCEPIFIFRSND
Arabidopsis thaliana LSMNPELQGPLFAGPVGVSNLLRLRDGMTPAPQVTGALALPMPAGFNEPVHVP - - AA
Synechocystis sp. PCC6803 GAAHSTG - - - - - NAPLQAILKVDLESGETLRSFAPHGFAGEPIFVFRPFGG
Novosphingobium aromaticivorans CGAQRPCN - - - - - FPNALSVDIVEKKVKNWHEHG - MIPSEPFVFRPGA
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Sphingomonas paucimobilis APEGDGYVIALVDDHVANY - - - - - SDLAIFDAQHVQGP IARAKLPVRI RQGLHGNWADASR
Spingobium sp. JS 1018 SPEGDGYVIALVNRLDEM - - - - - SDLVVLDQAQHIDEGPVATIRLPRLRNLGHNWVPSSA
Acinetobacter sp. JS678 ASEGDGWVASIMNDLLEEK - - - - - SELVILDQTNWEKGP IARVKIPFRLRMSLHGNWSPQEK
Arabidopsis thaliana DPAKDGLVFLVDQVQVGDGQVHEAWVVDAGNIGAGAVAKVHIPTRLRQVHGNWVPQAO
Synechocystis sp. PCC6803 VAEDDGWLLCLLYKADLHR - - - - - SELVILDQAQDITAPAIATLKLKHHPYPLHGSWAQT - -
Novosphingobium aromaticivorans THEDDGVVISIVSEENGSS - - - - - FAILLDGSSFEIARAKFPYGLPYLHGCWIPKD -
 * * : * : : * : * : * : *

Sphingomonas paucimobilis LAVAA - - - -
Spingobium sp. JS 1018 MRSPLPA - - - -
Acinetobacter sp. JS678 - - - - -
Arabidopsis thaliana LDALEGSAA
Synechocystis sp. PCC6803 - - - - -
Novosphingobium aromaticivorans - - - - -

Figure S5. Conserved histidines (red rectangles) and glutamic acids (blue rectangles) of CCO1 and previously characterized carotenoid oxygenases using T-coffee server. The accession numbers of the sequences from top to the bottom used are Q53353, KY888940, Q8VY26, P74334*, KX523172 and Q2G4H8*. *enzymes with established crystal structures.

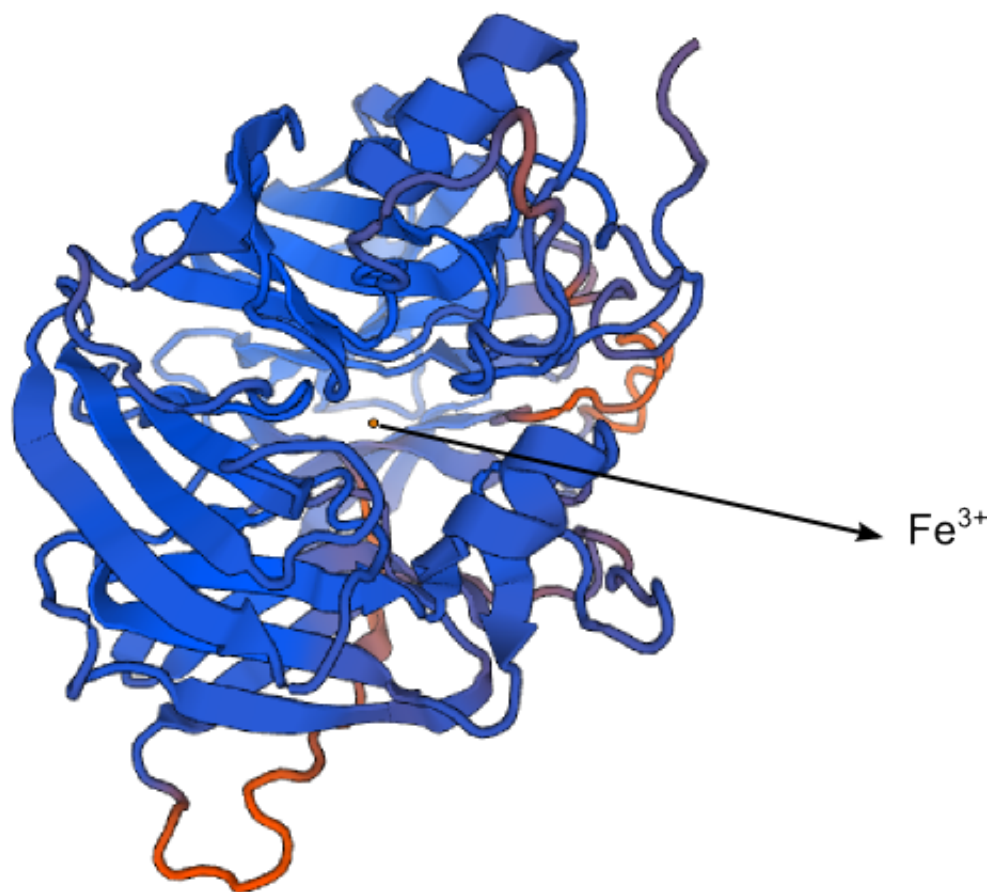


Figure S6. Model using SWISS-MODEL (6-8).

Table S1 Stilbenoid degrading bacteria isolated from peanut plants in Dawson, GA.

Isolates	Most closely related sequence (Accession number)	Max Identifica- -tion (%)	Grow on Res/Ptero (Base pairs)	Potential PGPB species** (see ref.)
Rhizosphere soil				
JS678	<i>Acinetobacter calcoaceticus</i> B9 (JQ579640)	99.9	Res/Ptero (1411)	H(9, 10)
JS679	<i>Pseudomonas fluorescens</i> NBRC 13922 (AB680523)	99.7	Res/Ptero (1409)	H/N(11)
JS680	<i>Pseudomonas fluorescens</i> strain IBFC2012-45 (KC246049)	99.9	Res (1408)	H/N(11)
JS681	<i>Pseudomonas</i> sp. MGR37 (JQ627866)	99.8	Res (1393)	-
JS682	<i>Pseudomonas</i> sp. d6(2010) (HQ166101)	99.8	Res/Ptero (1410)	N
JS683	<i>Burkholderia cenocepacia</i> SR2-07 (KF891406)	99.8	Res/Ptero (1402)	H/N(12)
JS1012 Soil 1 D2	<i>Pseudomonas oryzihabitans</i> (JX067903)	99.8	Res (1407)	H(13)
JS1013 Soil 2 C3	<i>Agrobacterium tumefaciens</i> (KC107786)	100	Res/Ptero (1352)	H/N(14)
JS1014 Soil 3 F5	<i>Pseudomonas putida</i> strain SP2 (GQ200822)	100	Res/Ptero (1402)	H/N(15)
JS1015 Soil 4 B9	<i>Pseudomonas frederiksbergensis</i> strain M60 (KC934887)	99.8	Res/Ptero (1394)	-
JS1016 Soil 5 2ndC3	<i>Pseudomonas putida</i> strain P-1017-1 (HQ324912)	99.6	Res/Ptero (1392)	H/N(15)
JS1017 Soil 6 A1Ptero	<i>Pseudomonas putida</i> strain NB2011 (JF261631)	99.4	Res/Ptero (1401)	H/N(15)
JS1018 Soil 7 C2Ptero	<i>Sphingobium yanoikuyae</i> strain BF-18 (EU307932)	100	Res/Ptero (1361)	-
JS1019 Soil 8 D2Ptero	<i>Sphingobium yanoikuyae</i> strain St16 (JN700070)	99.5	Res/Ptero (1360)	-
JS1020 Soil 9 F3Ptero	<i>Sinorhizobium morelense</i> strain LMG 9954 (AM181735)	99.9	Res/Ptero (1359)	N(16)
JS1021 Soil 10 H3Ptero	<i>Pseudorhodoferax aquiterrae</i> NAFc-7 (NR 108842)	99.0	Res/Pteto (1406)	-
JS1022 Soil 11 B4Ptero	<i>Pseudomonas</i> sp. SJH-007 (KC335141)	100	Res/Ptero (1410)	-
Peanut seeds				
JS1023 Seed 12 A1	<i>Xanthomonas translucens</i> strain P25 (AY994101)	99.7	Res (1419)	N(17)
JS1024 Seed 13 E3	<i>Xanthomonas translucens</i> strain P7 (AY994100)	99.6	Res (1417)	N(17)
JS1025 Seed 14 F3	<i>Xanthomonas</i> sp. Sbr1009a (KC311265)	99.8	Res/Ptero (1405)	N(17)

JS1026 Seed 15_A1	<i>Pandoraea</i> sp. JB1 (DQ167022)	99.8	Res/Ptero (1399)	N(18)
JS1027 Seed 16_B2	<i>Pandoraea</i> sp. CCUG 39680 (AY268171)	99.5	Res/Ptero (1407)	N(18)
JS1028 Seed 17_C1	<i>Pandoraea</i> sp. B-6 (JN128829)	99.5	Res/Ptero (1400)	N(18)
JS1029 Seed 19_B2Ptero	<i>Xanthomonas</i> sp. Era34 (JQ977166)	99.8	Res/Ptero (1401)	N(17)
JS1030 Seed 20_D2Ptero	<i>Xanthomonas translucens</i> NBRC 13559 (AB680445)	99.8	Res/Ptero (1418)	N(17)
Peanut shells				
JS1031 Shell 21_D1	Uncultured <i>Herbaspirillum</i> sp. clone A4H6M9 (GQ206314)	99.8	Res/Ptero (1401)	-
JS1032 Shell 22_E1	<i>Pandoraea</i> sp. LY (AF532595)	99.8	Res/Ptero (1406)	N(18)
JS1033 Shell 23_F1	<i>Burkholderia</i> sp. A45 (KF788025)	99.5	Res/Ptero (1401)	N(12)
JS1034 Shell 24_H1	<i>Pandoraea</i> sp. AU1775 (AY043377)	99.9	Res/Ptero (1401)	N(18)
JS1035 Shell 25_C4	<i>Burkholderia</i> sp. IBP-VNS150 (JQ518349)	99.6	Res/Ptero (1398)	H/N(12)
JS1045 Shell 26_F4Ptero	<i>Pectobacterium cypripedii</i> strain B1 (JF430157)	99.0	Res/Ptero (1409)	N(19)

* Strains were isolated from resveratrol enrichments except for the ones with “ptero” in the designation, which were enriched with pterostilbene”.

** PGPB represents Plant Growth-Promoting Bacteria (including rhizobia) that could enhance plant growth through producing plant hormones (‘H’) and/or improving plant nutrition (‘N’).

Table S2 DNA sequences of two putative carotenoid cleavage oxygenase (*cco*) genes identified from the genome of *Sphingobium yanoikuyae* strain JS1018.

>*cco1*-JS1018 (KX523172)

atgacatcgtgctttcctgacgatccgatctatcgccgattgatgcaccaggtcgagt
gaggcgaatgtttcgcgatcgcaggtcgaaggccgggtcctccggagcttgatggaaca
ttcttcgcgctcgtcctgacccccaatggccaccgatgctggggcatgacattttctc
aacggcgatgggatggtttgtcctccgcttcaaggacggacgtgctgattttacgtcc
agatatgcacagacggacaagttgtggcggaaacggcaggcacgcaaggcgtgtacggc
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ggtccggtcgcgaccatccgtcctccggtgcgactcgcgaatggactgcatggaattgg
gttcttcaagtgccatgaggtcgccattccggcttga

>*cco2*-JS1018 (KX523173)

atggcaactcgttacggcaccgggtgattgatcatgcgcccgctggcggaccgataat
ccgcacctaaccggagcatgggcgcatggacggaagaacgtcagccttcgatgtacc
gtcgaaggcaccattcctgaagatctgtcggcggcctttccggatttcagcaaatccc
cgctttgagcctatcgatctggaaacctaccattggttcgatggagacggcatggtgggt
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aactgggctgatgccgaaagtctggatcaggcggccgctgcttatgcgacgggaaattga

Materials and Methods – Extra LCMS Methods for Sample Analysis

A few of selected samples were also analyzed by a mass spectrometry Thermo Orbitrap XLT (Thermo Sci.). The column was a Supelco Ascentis Express C8, 300 μm \times 15cm, using 2.7 μm particles. The LC system was a Nano ACQUITY UPLC System (Waters Corp.). Solvent A was 5% acetonitrile in water, and solvent B was 95% acetonitrile in water. Both solvents contained 0.1% (v/v) formic acid. The flow rate was 5 $\mu\text{L min}^{-1}$. The gradient began at 100% A and was held for 5 min, then ramped to 100% B at 45 min. It was held there until 55 min and then reset to 100% A until the end of the run at 60 mins. The Orbitrap was scanned in positive ESI mode from 200-2000 Da, at a mass resolution of 30000.

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