Resveratrol as a growth substrate for bacteria from the rhizosphere.

Zohre Kurt, Marco Minoia and Jim C. Spain*

Supplementary figures and tables



Figure S1. Phylogenetic tree of functionally characterized carotenoid cleaving oxygenases from fungi and bacteria (bold)(1-6). Enzymes that transform resveratrol are marked as * and enzymes that were tested but could not transform resveratrol are marked

as #. The tree was constructed based on neighbor joining method and bootstrap values are included in nodes and the alignment for the tree was made with ClustalW2.

Arabidopsis thaliana Synechocystis sp. PCC6803 Novosphingobium aromaticivorans Sphingomonas paucimobilis Acinetobacter sp. JS678	NKNPFSGIGEIVRLFSGESLTDNANTGVIKLGDGRVMCLTE FQKGSILVDHETLETIGKF -SQPAG - GWLKTIFD - LRLKNIANTNITYWG - DRLLALWE GGQPHRLEPSNLATIGLD -DDPEV Q - GVDRTVANTTPVWHA - GRMLMAKE DGRPYRVDPRTLATIGSY -DDASV Q - GMIRGTANTNVMVHA - GKLYAMKE DSPCLIMDPLTLETEGYT -NDPLA AKNNTTANTTVIEHN - GVLLAMKE DALPWALDLKTLETIGEW .: *** .: * :: .* *
Arabidopsis thaliana Synechocystis sp. PCC6803 Novosphingobium aromaticivorans Sphingomonas paucimobilis Acinetobacter sp. JS678	EYDDVLSDHMIQSAIVTETEMWTLIPDLVKPGYRVVRMEA-GSNKREVVGRVRCR-DLGGILAEGQPLSAPRIDPASTFDG-GQPCYVTFSIKSSLSSTLTLLELDPQGKLLRQKDFGGALK-SETMTAHVRIDAGTGELFFYGYEADGQASTKVAYCIVGPDGELKREQNFDGKLQ-SQTFCAHPKIDPVTGNLCAFAYGAKGLMTLDMAYIEISPTGKLLKEIDFNGQIN-SATFTAHPKIEPKTGNLLCFAYEAKGDGTPDIAYYEISATGELLKEI: : ** : : : : : *: : : : : : : : : :
Arabidopsis thaliana Synechocystis sp. PCC6803 Novosphingobium aromaticivorans Sphingomonas paucimobilis Acinetobacter sp. JS678	- SGSWGPGWVHSFAVTENYVVIPEMPLRYSVKNLLRAEPTPLYKFEWCPQDGAFIHVMSK TETFPGFAFIH FAITPHYAIFLQNNVTLNGLPYLFGLRGAGECVQFHPDKPAQIILVPR WFDAPYCAMH FTISENYALFPIYPTTADLDRLKAGGEHWHHQPELDSWLGVMPR PFQNPYYCMH FGVTEDYAVFAVMPLLSSWDRLEQRLPFFGFDTTLPCYLGILPR WFQAPYAAMIH FAVTENYVIFPIIPLTVDIERMKKGQHFQWQPDLEQLFGILPR :*.* :: .*.:: : : : : : : : : : : : : :
Arabidopsis thaliana Synechocystis sp. PCC6803 Novosphingobium aromaticivorans Sphingomonas paucimobilis Acinetobacter sp. JS678	LTGEVV - ASVEVPAYVTEHFINAYEEDKNGDGKATVIIADCCEHNADTRILDMLRLDTL DGGEI - KRIP - VQAGFVEH HANAFEENG KIILDSICYNSLPQ VDTD YGDVSEIKWFKGPKGCHSYH MNAWEDAD GMLHFDACLNNTNA FAFI NGDARDLRWFK - TGNCFVGH VMNAFNDG TKVHIDMPVSRNNS FPFF SGQAEDIQWFYGPKNGFQCHTLNSFEKN GKIYVDMPVASGNV FYFF * *:::. : : : : : : : : : : : : : : : :
Arabidopsis thaliana Synechocystis sp. PCC6803 Novosphingobium aromaticivorans Sphingomonas paucimobilis Acinetobacter sp. JS678	RSSHGHDV LPDARIGRFRIPLDGSKYGKLETAVEAEKHGRAMDMCSINPLYLGQKYR GDFRSTNFD - NLDPGQLWRFTIDPAA ATVEKQ LMVSRCC EFPVNHPQQVGRPYR REPSGIHMGPQDIKGALTRWTVDPRADGGDVVE TVIGPPC FPVIPAKLQGRPYK - DVHGAPFDPVAGQGFLTRWTVDMASNGDSFEKT - ERLFDRPL EFPRIDERYATRAYR - PPAEAPVHSEQITSALMRWEFDLQATDHHVKPQPITNKQYPC EFPRCDERFNGLEYS : *: : *: *
Arabidopsis thaliana Synechocystis sp. PCC6803 Novosphingobium aromaticivorans Sphingomonas paucimobilis Acinetobacter sp. JS678	YVYACGAQRPCNFPNALSKVDIVEKK-V-KNWHEHGMIPSEPFFVP YVYMGAAHHSTGNAPLQAILKVDL-ESGTETLRSFAPHGFAGEPIFVP TGWMLSMNPELQGPPLFAGPVGVSFNLLLRLDGMDTPAPQVTGALALPPMAGFNEPVHVP HGWMLILDTEKPYEAPGGAFYALTNTLGHIDL-ATGKSSSWWAGPRCAIGEPCFIP YGFLLAFDPDLAFDHENLGEYPFQFFNQLARVNV-QTGITETWYPGDKYCFQEPIFIP : . : : ** .:*
Arabidopsis thaliana Synechocystis sp. PCC6803 Novosphingobium aromaticivorans Sphingomonas paucimobilis Acinetobacter sp. JS678	RPGATHEDDGVVISIVSEENGGSFAILLDGSSFEEIARAKFPYGLPYGIHGCWI RPGGVAEDDGWLLCLIYKADLHRSELVILDAQDITAPAIATLKLKHHIPYPIHGSWA AADPAKDGWLVFLVDQQVGDNQFVHEAWVVDAGNIGAGAVAKVHIPTRLRPQVHGWVV RSPDAPEGDGYVIALVDDHVANYSDLAIFDAQHVDQGPIARAKLPVRIRQGIHGNWA RSNDASEGDGWVASIMNDLLEEKSELVILDTQNWEKGPIARVKIPFRLRMSIHGNWS **:::::::::::::::::::::::::::::::::::

Figure S2. Alignment of Rzo with previously characterized carotenoid oxygenases using

ClustalW2. Marked rectangles represent the conserved histidines (red) and glutamic acids

(green) obtained via T-Coffee server. The accession numbers of the sequences from top

to the bottom used are Q8VY26, P74334*, Q2G4H8*, Q53353 and KY888940.

* crystal structure of this enzyme has been established



Figure S3. Model of Rzo using SWISS-MODEL (7-9).

Table S1. Strains isolated from peanut, identified by their 16S rDNA sequences using 8F

 and 1492R primers (10) and their closest relative.

Strain	Fragment length	Strain	% Identity
JS678	1400 bp	Acinetobacter sp. DR1	99 %
JS679	1386 bp	Pseudomonas sp. 130(2zx)	99 %
JS680	1381 bp	Pseudomonas putida strain AS90	98 %
JS681	1391 bp	Pseudomonas syringae pv. phaseolicola 1448A	98 %
JS682	1387 bp	<i>Pseudomonas syringae</i> pv. <i>aesculi</i> str. 0893_23	99 %
JS683	1385 bp	Burkholderia cenocepacia HI2424	100 %

Table S2. Key genes encoding putative enzymes of the resveratrol degradation pathway

 in the JS678 genome. The sequences of JS678 were specified based on the contig

 numbers they were annotated in the draft genome (SUB1068911).

Genes	Amino acid	Contig	Primers used for RT-PCR
	similarity to nearest homolog ^a		
16S rRNA gene	100 % to 16S rRNA from <i>Acinetobacter</i> sp. DR1 (CP002080.1)	401	5' GGTTGGCGCTAATATCTGGA 3' & 5' TCTGAATCAACGCTGTCTGG 3'
Resveratrol oxygenase (<i>rzo</i>)	73 % to dioxygenase from <i>Marinomonas</i> <i>profundimaris</i> (WP_024022327.1)	2366	5' GGGTGAAGGCCGTGTTAGTA 3' & 5' GATCCAATGCCCATGGTAAG 3'
4-Hydroxybenzaldehyde dehydrogenase (<i>pchA</i>)	97 % to aldehyde dehydrogenase from <i>Acinetobacter baumannii</i> Naval-82 (WP_002123484.1)	2302	5' CTTGGAATTGGCCATTTGTT 3' & 5' GAACCTGCGGATGTTCAGTT 3'
4-Hydroxybenzoate hydroxylase (<i>pobA</i>)	92 % to 4- hydroxybenzoate 3- monooxygenase from <i>Acinetobacter oleivorans</i> JCM 1667 (YP_003732665.1)	5608	5' TGAATTCCGAATAGCCGAAC 3' & 5' GGTTTAGAGGGGGCAACTTCC 3'
Protocatechuate 3,4- dioxygenase (<i>pcd</i>)	85% to protocatechuate 3,4-dioxygenase from <i>Acinetobacter</i> <i>calcoaceticus</i> ANC 3811 (WP_016137964.1)	4965	5' GTGCTTACGCCCAACGTAAT 3' & 5' GGGCCAAACAAATTTGAAGA 3'
3,5- Dihydroxybenzaldehyde dehydrogenase (<i>ken6</i>)	39 % to benzaldehyde dehydrogenase from <i>Streptomyces</i> <i>violaceoruber</i> (CAO52616.1)	1739	5' CCTTGCTTTAGCCAACCAAA 3' & 5' GAGATCCAGTGGTCGGTTGT 3'

^a The similarity was compared using UniProt database

References

- Brefort T, Scherzinger D, Carmen Limon M, Estrada AF, Trautmann D, Mengel C, Avalos J, Al-Babili S. 2011. Cleavage of resveratrol in fungi: Characterization of the enzyme Rco1 from *Ustilago maydis*. *Fung. Genet. Biol.* 48:132-143.
- Kamoda S, Saburi Y. 1993. Structural and enzymatic comparison of lignostilbene-*alpha, beta*-dioxygenase isozymes, I, II, and III, from *Pseudomonas paucimobilis* TMY1009. *Biosci. Biotechnol. Biochem.* 57:931-934.
- Kamoda S, Saburi Y. 1993. Cloning, expression, and sequence analysis of a lignostilbene-*alpha, beta*-dioxygenase gene from *Pseudomonas paucimobilis* TMY 1009. *Biosci. Biotechnol. Biochem.* 57:926-930.
- Marasco EK, Schmidt-Dannert C. 2008. Identification of bacterial carotenoid cleavage dioxygenase homologues that cleave the interphenyl *alpha, beta* double bond of stilbene derivatives via a monooxygenase reaction. *Chem. Biochem.* 9:1450-1461.
- Masai E, Katayama Y, Nishikawa S, Fukuda M. 1999. Characterization of Sphingomonas paucimobilis SYK-6 genes involved in degradation of ligninrelated compounds. J. Ind. Microbiol. Biotechn. 23:364-373.
- Hoffmann J, Bóna Lovász J, Beuttler H, Altenbuchner J. 2012. In vivo and in vitro studies on the carotenoid cleavage oxygenases from Sphingopyxis alaskensis RB2256 and Plesiocystis pacifica SIR - 1 revealed their substrate specificities and non - retinal - forming cleavage activities. FEBS Journal 279:3911-3924.

- 7. Biasini M, Bienert S, Waterhouse A, Arnold K, Studer G, Schmidt T, Kiefer F, Cassarino TG, Bertoni M, Bordoli L, Schwede T. 2014. SWISS-MODEL: modelling protein tertiary and quaternary structure using evolutionary information. Nucleic Acids Research 42:252-258.
- Arnold K, Bordoli L, Kopp J, Schwede T. 2006. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. Bioinformatics 22:195-201.
- 9. Benkert P, Biasini M, Schwede T. 2011. Toward the estimation of the absolute quality of individual protein structure models. Bioinformatics 27:343-350.
- Baker GC, Smith JJ, Cowan DA. 2003. Review and reanalysis of domain specific 16S primers. J. Microbiol. Methods 55:541-555.