Supplemental Data

Location of CYP genes

N. aromaticivorans contains two plasmids, pNL1 (180 kbp) and pNL2 (480 kbp), alongside its chromosomal genomic DNA (3.56 Mbp). CYP101C1, CYP101D1, CYP101D2, CYP111A2, ArR and Arx are located on the chromosomal DNA while CYP101B1 is located on pNL2. No *CYP* genes are located on pNL1 (1).

CYP101B1	Saro_3533	pNL2
CYP101C1	Saro_2249	chromosomal
CYP101D1	Saro_0514	chromosomal
CYP101D2	Saro_1478	chromosomal
CYP111A2	Saro_0759	chromosomal
ArR	Saro_0216	chromosomal
Arx	Saro_1477	chromosomal

The organisation of the genes in *Sphingomonas* bacteria are complex in that the genes necessary for degradation of one type of compound may be distributed into multiple operons that also possess genes for the degradation of other compounds. The lateral transfer of genes between *Sphingomonas* and *Pseudomonas* species has previously been proposed (1). Full details on the cloning of the genes have been reported elsewhere (2,3).

Product formation

After all of the NADH had been consumed in an incubation reaction, 10 μ l of a 25 mM stock of 9-fluorenol was added to the mixture to act as an internal standard for the extraction and GC analysis. 1 ml of the turnover was extracted with 400 μ l ethyl acetate and the organic phase was removed and analysed via gas chromatography. Analyses were performed on a ThermoFinnegan Trace GC instrument equipped with an auto-sampler and a DB-1 fused silica column (7 m \times 0.25 mm) using helium as the carrier gas and flame ionization detection. The injector and flame

ionization detector were held at 200°C and 250°C, respectively. The temperature of the DB-1 fused silica column (7m x 0.25m) was held at 60 °C for 1 min and then increased at 15 °C/min up to 150 °C. The retention times of substrate and products were as follows: camphor; 3.78 min, 5*exo*-hydroxycamphor; 5.74 min, 6-*exo*-hydroxycamphor; 5.50 min, 5-*endo*-hydroxycamphor; 5.96 min, 9-hydroxycamphor; 6.14 min, β -ionone; 6.65 min, 4-hydroxy- β -ionone; 8.39 min, 3-hydroxy- β -ionone; 8.71 min, 4-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]hept-2-yl)-3-buten-2-one (α -ionone-epoxide); 7.05 min, linalool; 3.38 min, 8-hydroxylinalool; 5.67 min, 2-adamantanone; 5.30 min, 5-hydroxy-2-adamantanone; 6.52 min, unidentified minor 2-adamantanone oxidation product; 6.62 min and 9-hydroxyflourenol; 9.35 min.

The product concentration in incubation mixtures was calculated by calibrating the concentration response of the GC detector to an authentic sample or a structural isomer (e.g. 2-hydroxy-3-pinanone for 5-*exo*-hydroxycamphor). Mixtures containing different concentrations of the product and the internal standard were extracted and analyzed as for normal incubations. The plot of the ratio of the peak area for the product to the 9-fluorenol internal standard against the product concentration gave a calibration plot from which the absolute concentration of products produced by enzymatic turnover in an incubation mixture could be determined. Where more than one product was produced the detector response was assumed to be equal, e. g. 4-hydroxy- β -ionone and 3-hydroxy- β -ionone. The coupling efficiency was the percentage of NADH consumed that led to product formation.

The NAD-binding site in ArR

In ArR the solvent accessible cavity above the *re* side of the isoalloxazine ring is believed to accommodate the nicotinamide ring of NAD (Fig. S6). The structure of BphA4, a related ONFR protein, with NAD bound has been determined. The position of the loops that constitute the NAD binding site in both proteins are similar (Fig. S6). The conserved acidic residues Glu165 and Glu301 form hydrogen bonds with the carboxamide group of the nicotinamide ring and ribose (δ -ring) oxygen, respectively. Another conserved acidic residue Glu181 may stabilize the adenine ribose through hydrogen bonds with the ribose O2 and O3 atoms. In BphA4, residues Gln177 and Ser182 also form hydrogen bonds with these adenine ribose oxygens. However, the corresponding

residues in ArR are nonpolar Leu183 and Ala188 and thus NAD binding might be weakened. Another potentially significant difference between ArR and BphA4 is the orientation of Arg189 (Arg183 in BphA4). Arg183 in BphA4 may act a wall in the channel of NAD binding site and stabilize the ribose and pyrophosphate group but Arg189 in ArR is located at the surface of the NAD binding site and may form hydrogen bonds with the adenine ribose.

The C-terminal arm of Arx

The hydrogen-bonding network at the C-terminus of Arx is different from other vertebrate-type ferredoxins due to Arg8 which forms hydrogen bonds with the carbonyl and side chain oxygens of Glu34, the carbonyl oxygen of Ala101, and two water molecules (Wat20 and Wat11), as well as a salt bridge with Glu103. The water molecules bridge Arg8 and the carbonyl oxygens of Thr47 and His49 and the main chain amide nitrogen atoms of Leu36 and His49 (Supplemental Fig. S10c). This network of hydrogen bonds and salt bridges around Arg8 links the N-terminus, C-terminus and the cluster loop of Arx. The corresponding residue to Arg8 in other ferredoxins is a histidine whose side-chain points towards the enzyme surface.

The overall structure of CYP101D1

In the native form, one 1,4-dioxane molecule, six phosphate ions, 466 water molecules, and one unidentified density were found. In the camphor-bound form, two camphor molecules, 817 water molecules and four phosphate ions were identified.

Figure S1. SDS page gels of ArR, Arx and CYP101D1.



Figure S2. (a) The effect of KCl on the NADH consumption rate of the ArR/Arx/CYP101D2 system (b) The effect of pH on the NADH consumption rate of the ArR/Arx/CYP101D2 system. The coupling of product formation to NADH consumption (\geq 95%) was unaffected by the presence of \leq 500 mM KCl or the pH values studied. The optimal activity was found at pH 7.4.



Figure	S3 .	Protein	sequence	alignments	of th	e ONFR	proteins	PdR,	PuR,	ArR,	BphA4
and Ad	R.										

PdR PuR ArR BphA4 AdR	10 20 30 40 50 60
PdR PuR ArR BphA4 AdR	70 80 90 100 110 120
PdR PuR ArR BphA4 AdR	130 140 150 160 170 180 DGRALDYDRIVLATGGRPRPIPVASGAVGKANNFRYLKTLEDAECIRROLIADNRLV SGTAIEYGHLVLATGGRPRPIPVASGAVGKANNFRYLKTLEDAECIRROLIADNRLV SGTAIEYGHLVLATGGRPRPIPVASGAVGKANNFRYLKTLEDAECIRROLIANKHVV DGSAIEYGHLVLATGGAPRRLSCVGADLAGVHAVRTKEDADRIMAELDAGAKNAV DGRTLPYGTLVLATGAAPRALPTLQGATMPVHTLRTLEDARRIQAGLRPQSRLL ELREAYHAVVLSYGAEDHRALEIPGEELPGVCSARAFVGWYNGLPENQELEPDLSCDTAV
PdR PuR ArR BphA4 AdR	190 200 210 220 230 240 VIGGGYIGLEVAATAIKANMHVTILLDTAARVLERVTAPPVSA VIGGGYIGLEFAATARAKGLEVDVVBLAPRVMARVVTP
PdR PuR ArR BphA4 AdR	250 260 270 280 290 300 SYEHLHREAGVDIRTGTQVCGFEMSTDQQKVTAVLCEDGTRLPADLVIA YFHDRHSGAGIRMHYGVRATEIAAEGDRVTGVVLSDGNTLPCDLVVV SYQAEHRAHGVDLRTGAAMDCIEGDGTKVTGVRMQDGSVIPADIVIV FVARYHAAQGVDLRFERSVTGSVDGVVLLDDGTRIAADMVVV AFTIKELREMIQLPGARPILDPVDFLGLQDKIKEVPRPRKRLTELLTRTATEKPGPAEAA
PdR PuR ArR BphA4 AdR	310 320 330 340 350 360 GIGLIENCELAS AAGLQVDNGIVINEHMQTSDELIMAVGDCARFHSQLYD GVGVIENVEIAAAAGLPTAAGIIVDQQLLTSDEHISAIGDCALFESVRFG GIGIVPCVGALISAGASGGNGVDVDEFCRTSLTDVYAIGDCAAHANDFAD GIGVLANDALARAGLACDDGIFVDAYGRTTCEDVYALGDVTRQRNPLSG RQASASRAWGLRFFRSPQQVLPSPDGRRAAGVRLAVTRLEGVDEATRAVPTGDMEDLPCG
PdR PuR ArR BphA4 AdR	370 380 390 400 410 420 -RWVRIESVPNALEQARKIAAIICCKVPRDEAAPWFWSDQYEIGFKMVGLSEGYDRI -ETMRVESVONATDQARCVAARLICCAKPYDGYPWFWSDQCDDKLQIVGLTAGFDQV GAVIRLESVONANDMATAAAKDICCAPVPYKATPWFWSNQYDLKLQTVGLSTGHDNA -RFERTETWSNAQNQGIAVARHIVDPTAPGYAELPWYWSDQGALRIQVAGIASG-DEE LVLSSTGYKSRPVDPSVPFDSKIGVIPNVEGRVMDVPGLYCSGWVKRGPTGVIATTMTDS
PdR PuR ArR BphA4 AdR	430 440 450 460 470 480 IVRGSLAQPDFSVFYLQ-GDRVLAVDTVNRPVEFNQSKQIITDRLPVEPNLLGDESVP VIRGSVAERSFSAFCYK-ACKLIGIESVNRAADHVFGRKILPLDKSVTPEQAADLSFD VLRGDPATRSFSVVYLK-GCKVVALDCVNMVKDYVQGKKLVEARAQIAPEQLADAGVP IVRGEVSLDAPKFTLIELQ-KCRIVGATCVNNARDFAPLRRLLAVGAKPDRAALADPATD FLTCQMLLQDLKAGLLPSGPRPGYAAIQALLSSRGVRPVSFSDWEKLDAEEVARGQGTGK



Sequence alignments were performed using ClustalW (http://www.ebi.ac.uk/clustalw/) and annotated using BioEdit (4,5).

Figure S4 a-c) Overall structure comparison of PdR (a), BphA4 (b) and PuR (c) colored in cyan, pink and orange, respectively. The FAD molecules are shown in yellow stick representation. Differences in the secondary structures are colored in red.



Figure S5. The surface of PdR (a) and PuR (b) at the top of the adenine ribose moiety, showing the more open conformations of these enzymes compared to ArR (Fig. 2c).



Figure S6. Overlay of the NAD-binding domain of the NAD⁺ (in yellow) complex of BphA4 (in cyan) with ArR (in orange) without NAD⁺ bound. The structurally conserved loops (I, II and III) involved in cofactor binding and the location of potential key residues such as Glu165, Glu181 and Glu301 are shown.



Figure S7. The electrostatic potentials of the FAD *si* side surface of PdR (a) and PuR (b) compared to ArR (Fig. 3a). Negatively and positively charged surface areas are colored in red and blue, respectively. Residues that contribute to the different surface potential distributions and that may be involved in ferredoxin binding are labeled.



(a)

Figure S8. (a) and (b) The electrostatic potential surfaces of the Arx pentamers. Positively charged regions are colored in blue and negatively charged regions in red. The more neutral surface is the interaction region in the back-to-back packing of pentamers in the crystals. The intermolecular disulfide bonds are also located within this neutral region.



(a)



Figure S10. (a) Stereoview of the electron density and model for the [2Fe-2S] cluster in Arx. The electron density of [2Fe-2S] cluster ($2F_o$ - F_c contoured at 1 σ) and the side chains of the four cysteine residues are colored in marine and grey, respectively.



(b) The location of two non-ligand cysteines (Cys42 and Cys43) in the cluster binding loop of Arx. The [2Fe-2S] cluster, the secondary structure of the core domain (green) and the interaction domain (red), and the side chains of the residues (grey) surrounding Cys43 are shown.



(c) The hydrogen bond network around His49 that connects the Arx C-terminal arm, N-terminal and the cluster binding loop. The relevant residues (green), the hydrogen bonds interactions (black dashed lines) and the water molecules (red) are shown.



Figure S11. The electrostatic surface potentials of the interaction face of Pdx (a) and Adx (b) surrounding the [2Fe-2S] cluster regions compared to Arx (Fig. 3b). Negatively and positively charged surface areas are colored in red and blue, respectively. The electrostatic potential surfaces of the cluster binding loop in Arx and Adx are more neutral than that of Pdx. Residues that contribute to the different surface potential distributions and that may be involved in protein recognition are labeled.



Figure S12. Gel filtration of CYP101D1 (Superdex-200)



Figure S13. Structure of (a) CYP101D1 (green) and (b) CYP101A1 (P450cam, cyan). The more extended N-terminus of the G helix and the different position of the F-G loop in CYP101D1 are shown in red. Other differences were found in the N-terminal helical region, the β 2 sheets and the C-terminal β sheets and are also highlighted in red.



Figure S14. The electrostatic surface potentials of the proximal face of CYP101A1 (a), CYP199A2 from Rhodopseudomonas palustris CGA009 (b) and CYP24A1 from rat mitochondria (c) compared to CYP101D1 (Fig. 3c). Negatively and positively charged surface areas are colored in red and blue, respectively. Residues that contribute to the different surface potential distributions and that may be involved in ferredoxin binding are labeled.





Figure S15. Protein sequence alignments of the CYP101 and CYP111 family proteins from *P. putida*, *P. putida* strain PpG777 and *N. aromaticivorans*.

	10	20	30	40	50	60
			.			
CYP101A1	MTTETIQSNANL	APLPPHVPE	HLVFDFDMYNP	SN-LSAGVQ	EAWAVLQESN	VPDIV 55
CYP101C1		-MIPAHWPA	DRVVDFDIFNP	PG-VEQDYF	'AAWKTLLDG-	- PGLV 40
CYP101D2	-LATNFDEAVRAKV	-ERPANVPE	DRVYEIDMYAL	NG-IEDCYH	IE <mark>AW</mark> KKVQHPG	IPDLI 55
CYP101D1	MNAQTSTATQKHRV	-APPPHVPG	HLIREIDAYDL	DG-LEQGFH	IE <mark>AW</mark> KRVQQPD	TPPLV 56
CYP101B1	MLPHDRGQNSTRRITA	MEAPA <mark>HWP</mark> A	DRVVDIDIYMP	PGLAEHGFH	KAWSDLS-AG	NPAVV 59
CYP111A1	MERPDLKNPDLYT	QQVPHDIFA	RURRFEPVYWN	PESDGS	GFWAVLRHKD	IIEVS 54
CYP111A2	MLDLKNPDTYQ	GGVPYAALQ	DIRAFGPVHWN	PESDGA	GFWAVLGHDE	IVAVS 52
	70	80	90	100	110	120
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CYP101A1	WT <mark>RCN<mark>GGHWIA</mark>TR<mark>G</mark>QL</mark>	IREAYEDYR	H <mark>FSS</mark> ECPFIPR	EA <mark>GEA</mark> YDFI	PT SMDPPPQR	QF <mark>R</mark> AL 115
CYP101C1	WSTAN <mark>GGHWIA</mark> AR <mark>G</mark> DV	VRELWGDAE	RLSSQCLAVTP	GL <mark>GKVMQF</mark> I	PLQQDGADHK	AFRTP 100
CYP101D2	WTPFTGGHWIATNGDT	VKEVYSDPT	RFSSEVIFLPK	EA <mark>GD</mark> KYQMV	PTKMDPPEHT	PYRKA 115
CYP101D1	WTP <mark>FT</mark> GGHWIATRGTL	IDDIYRSPE	RFSSRVIWVPR	EA <mark>CEA</mark> YDMV	PTKLDPPEHT	PYRK A 116
CYP101B1	WTPRNE <mark>GHWIA</mark> LG <mark>G</mark> EA	LQEVQSDPE	RFSS <mark>RIIVLP</mark> K	SV <mark>GD</mark> MHGLI	PTTIDPPEH <mark>R</mark>	RPYRQL 119
CYP111A1	RQPLLFSSAYENGG	HR	IFNENEVGLTN.	AGEA <mark>A</mark> VGVE	FISLDPPVHT	QYRK V 105
CYP111A2	RQPDLFSSAFENGG	HR	IFNENQVGLTG	AGES <mark>A</mark> IGIF	FISRDPPSHT	Qyrk f 103
	130	140	150	160	170	180
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CYP101A1	ANQVVGMPVVDK	IQEL <mark>A</mark> CSLI	ESLRPQ <mark>G</mark> QCNF	TEDY <mark>AE</mark> PFF	IRIFMLL <mark>AG</mark> I	PEEDI 175
CYP101C1	VMKG <mark>L</mark> ASRFVVA <mark>LD</mark> PK	VQAVARKIM	DSLRPRGSCDF	VSDF <mark>AE</mark> ILF	LNIELTLIDV	Pledr 160
CYP101D2	LDKG <mark>LNLA</mark> KIRKVEDK	VREVASSLI	DSFAARGECDF	AAEY <mark>AE</mark> LFE	VHVEMALADI	PLEDI 175
CYP101D1	IDKG <mark>LNLÆ</mark> IRK <mark>LE</mark> DQ	IRTIAVEII	FGFADRGHCEF	GSEFSTVFF	VRVELALAGI	PVEDA 176
CYP101B1	LNAH <mark>I</mark> NPGAIRG <mark>I</mark> SES	IRQTAVDLI	egfaaq <mark>g</mark> hCnf	TAQY <mark>AE</mark> QFE	IRVEMALVGI	EASEA 179
CYP111A1	IMPALSPARLGD I DQR	TRVRAEALT	PRIPLGEEVDL	VPLLSAPL	LLTLAE <mark>LLG</mark> I	DPDCW 165
CYP111A2	VMPALSPARLQGIEER	TAKRVER	AQVPLGETVNI	LPLLTVPL	LLTLAE <mark>LLG</mark> V	Padlw 163
	190	200	210	220	230	240
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CYP101A1	PHIKYLTDQMTRPDG-	SMTF	'AEAKEALYDYL	IPITEQRRC	KPGTDAISIV	ANGQV 229
CYP101C1	PRLRQLGVQLTRPDG-	SMTV	EQLKQAADDYL	WPFTEKRMA	QPGDDLFSRI	:LSEPV 214
CYP101D2	PVLSEYARQMTRPEGN	TPEEMATDL	EAGNNGEYAYV	DPITRARVG	GDGDLITLM	IVNSEI 235
CYP101D1	TKIGLLANEMTRPSCN	TPEEQGRSL	EAANKGFFEYV	APITAARRO	GSGTDLITRI	INVEI 236
CYP101B1	ERIRHWAECMTRPG	MDMTF	DEAKAVEFDYV	GPLVDARRE	TPGEDMISAM	IINADL 233
CYP111A1	YELYNWTNAFVGEDDP	EFRKSPEDM	AKVLGE <mark>F</mark> MGFC	QELFESRRA	NPGPDIATLI	ANADI 225
CYP111A2	PDIHRWTDAFVGEDDP	DFRQSPEAM	QAVLAEFMGFA	TALFEDRRA	NPGPDIASLI	ANTEI 223
	250	260	270	280	290	300
			.			
CYPIUIA1	N-GREITSDEAKRMCG	ппплеспри	VVNFILSESMEF	HAKSPEHRC	<u>ELIERPERIP</u>	288
CYP101C1	G-CRPWTVDEARRMCR	NLLFGGLDT	VAAMIGMVALH	LARHPPDQF	LLRERPDLIP	273
CYP101D2	N-GERIAHDKAQGLIS	LLLLGGLDT	VVNFLSEFMIH	LARHEELVA	ELRSDPLKLM	IRGAED 294
CYP101D1	D-GKPMPDDRALGLVS	LILLGGLDT	VVNFIGEMMIY	SRHPPTVA	EMRREPLKLQ	RGVED 295
CYP101B1	GDERRLTRDEALSVVT	QVIIIAGLDT	VVNVLGEIMRE	GNEALRA	DURQRGADII	PVVH 293
CYP111A1	N-CQPVALRDFIGNLT	LTLVGGNET	TRINSISHTIVT	SQQEDQWD	ILRORPELLK	TATAD 284
CYP111A2	R-GEPAPLRDFIANLI	LALVGGNET	TRNSINHTMIA	LAENPGQWD	ILRADPSLMT	282 AAVKD

	310)	320	330	340	350	360	
			.					
CYP101A1	LLRRFS-LVAI	DGRILTSD	YEFHGVQ	LKKGD <mark>Q</mark> ILLP	QMLS <mark>GLD</mark> ERE	NACPMHVDFS	rQK 34	5
CYP101C1	LMRRYP-TVAV	75 RNAVAD	VDAD <mark>GV</mark> T	IR <mark>KGD</mark> LVYLP	SVIII: NIID PAS	FEAPEEVRFD	rglap 33	2
CYP101D2	MFRRFP-VVSI	EARMVAKD	QEYK GV F	lk <mark>rgdmil</mark> lp	TA <mark>LHGLD</mark> DA7	NPEPWKLDFS	rrs 35	1
CYP101D1	LFRRFA-VVSI	DARYVVSD	MEFHGTM	LKEGDLILLP	TA <mark>LHGLD</mark> DRI	IHDDPMTVDLS	rrd 35	2
CYP101B1	LFRRFG-LVS:	I AREVRRD	IEFHGVH	lik <mark>agdmi</mark> aip	IQV <mark>HGLD</mark> PRV	NPDPLAIDPS	rkr 35	0
CYP111A1	MVRHASPVLH	IRRTAME D	TEIGGQA	IAKGDKVVLW	YASGNRDESV	FSDADRFDVT	RTG 34	2
CYP111A2	MVRFASPVIH	IRRTAMRD	TQLGQQA	IC <mark>KGD</mark> KVVIF	YPAGNR <mark>D</mark> PAV	FENEDRFEIT	rpv 34	0
	37()	380	390	400	410	420	
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CYP101A1	VSHTTFGHGS	ILCLGQHL	ARREIIV	TIKEWITRIP	DESIAPGAQI	QHKSGIVSGV	QALPL 40	5
CYP101C1	IRHTTMGVGA	IRCVGAGL	ARMEVIV	FURDWIGGMP	EFALAPDKAV	TMKGGNVGAC	TALPL 39	2
CYP101D2	ISHSTFGGGP	IRCACMHI	ARMEVIV	TIEEWIKRIP	EFSFKEGETI	PIYHSGIVAAV	ENVEL 41	1
CYP101D1	VTHSTFAQGP	IRCACMHI	ARIEVIV	MIQEWIARTP	EFRLKDRAVI	PIYHSGIVAAV	ENIPL 41	2
CYP101B1	ARHSTIFGSGP	MCPGQEL	ARKEVAI	TIEEWIRRIP	DFALGPNSDI	SPVP <mark>GIV</mark> CAL	RRVEL 41	0
CYP111A1	VQHVGFGSGQ	IVCVGSRL	AEMQLRV	VFEILSTRVK	RFELCS-KSF	RFRSNFLNGL	KNTNV 40	1
CYP111A2	RQHLAFGSGA	IVCVGSRL	AEMQLRL	AFADMARHVR.	ADEVVG-EPS	RVRSNFINGF	KRIEV 39	9
	43(٦						
		5						
CYP101A1	VWDPATTKAV	415						
CYP101C1	VŴRA	396						
CYP101D2	VWPIAR	417						
CYP101D1	EWEPQRVSA-	421						
CYP101B1		414						
CYP111A1	VLVPK	406						
CYP111A2	RLLV	403						

Sequence alignments were performed using ClustalW (http://www.ebi.ac.uk/clustalw/) and annotated using BioEdit (4,5).

Table S1. Steady state NADH turnover activity of CYP101D2 and CYP101A1 with camphor in the presence or absence of 200 mM KCl, with different electron transfer proteins from Pseudomonas putida and Novosphingobium aromaticivorans. Turnover rates are given in nmol.(nmol CYP)⁻¹.min⁻¹ and the data are reported as mean \pm S.D. $(n \ge 3)$. Product formation rates and coupling were estimated using the 5-exohydroxycamphor analogue 2-hydroxy-3-pinanone as a standard and adjusted assuming 100% coupling for the PdR/Pdx/CYP101A1 turnover with camphor. Leak rates were obtained in the absence of substrate and were 25.7 ± 2.7 for ArR/Arx and 11.2 \pm 0.9 for PdR/Pdx. ArR: Aromaticivoransredoxin reductase; Arx: Aromaticivoransredoxin; PdR: putidaredoxin reductase; Pdx: putidaredoxin.

		CYP101D2	
Electron transfer chain	NADH consumption rate	Product formation rate	Coupling %
ArR/Arx (200mM KCl)	745±46	741±28	99
ArR/Arx	2020±78	2010±100	99
PdR/Arx (200mM KCl)	156±4.6	153±4.5	98
PdR/Arx	279±5.6	259±23	93
PdR/Pdx (200mM KCl)	16.0±4.7	11.1±3.4	70
		CYP101A1	
Electron transfer chain	NADH consumption rate	Product formation rate	Coupling %
ArR/Arx (200 mM KCl)	24.6±3.1	0.7±0.1	3
ArR/Pdx (200 mM KCl)	17.9±0.9	6.3±0.3	35
PdR/Pdx (200 mM KCl)	753±16	753±16	100

Table S2. Comparison of the active site residues of CYP101D1 and CYP101A1.Differences in CYP101D1 are underlined.

P. putida	N. aromaticivorans
CYP101A1	CYP101D1
E84	<u>R85</u>
F87	<u>W88</u>
G93	G94
E94	E95
Y96	Y97
F98	<u>M99</u>
T101	T102
T185	T186
L244	L251
V247	<u>L254</u>
V295	V302
D297	D304
I395	I402
V396	V403

 Table S3. Protein-camphor interactions in CYP101D1.

0	Tyr97(2.6 Å)
C2	Leu $251C_{\delta 1}(4.0 \text{ Å})$, Trp $88C_{H2}(4.0 \text{ Å})$
C3	Leu251C _{$\delta 1$} (4.1 Å), Thr102C _{$\gamma 2$} (4.0 Å)
C5	Leu251C _{$\delta 1$} (4.0 Å), Gly255C _{α} (4.0 Å)
C6	Leu251C _{$\delta 1$} (4.2 Å), Gly255C _{α} (3.5 Å), Leu254C _{γ} (4.2 Å)
C8	Val302C _{$\gamma 1$} (3.7 Å)
C9	Val302C _{$\gamma 2$} (3.6 Å), Thr259C _{$\gamma 2$} (3.6 Å),
C10	Leu254C _{γ} (3.9 Å), Leu254C _{$\delta 1$} (3.8 Å), Thr186C _{$\gamma 2$} (3.7 Å), Val403C _{$\gamma 2$} (3.9 Å)

Arx	Adx	Pdx
Leu38	Ala45	Asp38
Gly65	Asp72	Glu65
Asp66	Glu73	Arg66
Asp69	Asp76	Gly69
Asp72	Asp79	Glu72
Asp75	Tyr82	Thr75

Table S4. A comparison of selected residues in Adx, Arx and Pdx.

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