

1 Supplemental material

		$\alpha 1$																																	
ORF3	1:	MTTSAPITAPTVEPTRTSQTASLGESVAWLPVEGAEP	TGTQSAGSVL	ETTILRKAF	GCFP	60																													
HpaC_St	1:	-----	-----	MAEVIKS	IMRKFP	13																													
HpaC_Tt	1:	-----	-----	MKEAFKEAL	ARFA	13																													
PheA2	1:	-----	-----	MDDR	LFERNAMGKFA	14																													
TftC	1:	-----	MHAGEAVQQLK	KAFETVA	SFDFRDALSKA	30																													
CodR	1:	-----	MQTVNNII	SSVSTVES	KAYRDAMSHYA	27																													
		$\beta 1$	$\beta 2$	$\beta 3$	$\beta 4$	$\beta 5$	$\alpha 2$	$\beta 6$																											
ORF3	61:	SGVAALCALDPGDEDR	PAGIAASSFTS	VSLDPALVSV	CIHTSTTW	PRLAGLARV	GVSVL	120																											
HpaC_St	14:	LGVAIVTTNWK	GEL---	VGMTVNTFNSLS	LNPLVSV	FFADRMKGN	DI	PYKESKYFV	VNF-	69																									
HpaC_Tt	14:	SGVTVVAARL	GEEE---	RGMTATAFMS	LSLEPPLVALAV	SERAKLLP	VLEGAG	AFTVSL	70																										
PheA2	15:	TGVTVITTE	LNGAV---	HGMTANAFMS	VSLNPKLV	LSIGEKAK	MLEKIQ	SKKYAV	NIL	71																									
TftC	31:	TEVTTVAT	NGPFGL---	AGLTC	SAVCSVCDR	PPTVLLC	INRKS	YAAGI	IKSNGVLSV	NWL	87																								
CodR	28:	GAVQIVTT	AGAAGR---	RGLTLTA	ACSVSDN	NPTILIC	LQK	HEENR	IFIENG	VFAINTL	84																								
		*	*	*	*	*	*	*	*	*	*	*																							
		$\alpha 3$	$\alpha 4$	$\beta 7$	$\beta 8$	$\beta 9$																													
ORF3	121:	AEHHEEVASAL	ASK---	RADRF	DGIDWAHSAS	GAVFVHG	STLWLECA	IDRTV	RAGD	HDI	176																								
HpaC_St	70:	TDNEELFN	IFALK---	PVKE	RFREIKYKE	GIGGCP	ILYDSY	AYIEAK	LYDTI	DVGD	HSI	125																							
HpaC_Tt	71:	REGQEA	VSEHF	AGR---	PKEG	IALLEEG	-----	RVK	GALAV	LRCRL	HALY	PGGD	HRI	118																					
PheA2	72:	SQDQK	VLSMNF	AGQLE	KPV	DVQFEELG	-----	GLP	VIK	DALAQ	ISCQ	VVNEV	QAGD	HTL	125																				
TftC	88:	AAGQAV	ISQTF	AGVGS	VPMEE	RFADK	GWQ	T	IATG	APYR	MDAA	V	FDCT	IANIV	DVGS	HSV	147																		
CodR	85:	AGPHQQL	ADAF	SGRIG	LTD	ERFELAA	WEI	LATG	APV	LK	GALAA	FDCR	VV	SVQD	HST	HHV	144																		
		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*																		
		$\beta 10$	$\beta 11$	$\beta 12$																															
ORF3	177:	VLLRI	I	H-	LRS	NP	DVAP	MV	FHG	SS	FK	RL	TTA	-----	206																				
HpaC_St	126:	IVGEV	IDG	YQ	IRD	N	FTPL	V	Y	M	N	R	K	Y	KL	SSL	-----	156																	
HpaC_Tt	119:	VVGLV	EE-	VEL	GEG	GP	LV	F	Q	R	G	Y	R	L	V	W	PS	-----	149																
PheA2	126:	F	I	G	E	V	T	D-	I	K	I	T	E	Q	D-	P	L	F	F	S	G	K	Y	H	L	A	Q	N	E	K	V	E	T	S	161
TftC	148:	I	F	A	E	V	V	A-	R	N	H	A	E	E	C	T	P	L	I	H	R	R	Q	Y	A	T	T	R	S	L	A	E	-----	179	
CodR	145:	L	F	G	E	V	V	G-	L	S	S	H	A	E	E	E	A	L	I	Y	L	N	R	R	Y	H	K	L	E	L	-----	173			

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3 FIG. S1 Comparison of the deduced amino acid sequences of ORF3 and other flavin
 4 reductases. ORF3 = putative flavin reductase from *Nocardioiodes* sp. PD653; HpaC_St =
 5 flavin reductase component of 4-hydroxyphenylacetate 3-monooxygenase from *Sulfolobus*
 6 *tokodaii* 7 (gi 15621737) (1); HpaC_Tt = flavin reductase component of
 7 4-hydroxyphenylacetate 3-monooxygenase from *Thermus thermophilus* HB8 (gi 55772343)
 8 (2); PheA2 = reductase component of phenol hydroxylase from *Geobacillus*
 9 *thermoglucosidasius* A7 (gi 15621737) (3); TftC = chlorophenol 4-monooxygenase
 10 component 1 from *Burkholderia cepacia* (gi 3220029) (4); CodR = corrin reductase from
 11 *Brucella melitensis* (gi 169791887) (5). Identical residues are marked with asterisks, and
 12 conserved residues are marked with dots. Residues involved in cofactor binding are labeled in
 13 bold. Secondary structural elements are highlighted green for β -sheets and red for α -helices.

14 **Supplemental references**

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16 1. Okai M, Kudo N, Lee WC, Kamo M, Nagata K, Tanokura M. 2006. Crystal structures of
17 the short-chain flavin reductase HpaC from *Sulfolobus tokodaii* strain 7 in its three states:
18 NAD(P)⁺-free, NAD⁺-bound, and NADP⁺-bound. *Biochemistry* 45:5103-5110.
19 <http://dx.doi.org/10.1021/bi052313i>

20 2. Kim S-H, Hisano T, Takeda K, Iwasaki W, Ebihara A, Miki K. 2007. Crystal structure of
21 the oxygenase component (HpaB) of the 4-hydroxyphenylacetate 3-monooxygenase from
22 *Thermus thermophilus* HB8. *J Biol Chem* 282:33107-33117.
23 <http://dx.doi.org/10.1074/jbc.M703440200>

24 3. van den Heuvel RHH, Westphal AH, Heck AJR, Walsh MA, Rovida S, van Berkel WJH,
25 Mattevi A. 2003. Structural studies on flavin reductase PheA2 reveal binding of NAD in an
26 unusual folded conformation and support novel mechanism of action. *J Biol Chem*
27 279:12860-12867. <http://dx.doi.org/10.1074/jbc.M313765200>

28 4. Webb BN, Ballinger JW, Kim E, Belchik SM, Lam K-S, Youn B, Nissen MS, Xun L, Kang
29 C. 2009. Characterization of chlorophenol 4-monooxygenase (TftD) and NADH:FAD
30 oxidoreductase (TftC) of *Burkholderia cepacia* AC1100. *J Biol Chem* 285:2014-2027.
31 <http://dx.doi.org/10.1074/jbc.M109.056135>

32 5. Lawrence AD, Taylor SL, Scott A, Rowe ML, Johnson CM, Rigby SEJ, Geeves MA,
33 Pickersgill RW, Howard MJ, Warren MJ. 2014. FAD binding, cobinamide binding and
34 active site communication in the corrin reductase (CobR). *Biosci Rep* 34:345-355.
35 <http://dx.doi.org/10.1042/BSR20140060>

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