Supporting Information

Reagents — All restriction enzymes and DNA modifying enzymes were obtained from New England BioLabs (Beverly, MA). *Taq* DNA polymerase was from Promega (Madison, WI). QIAprep spin plasmid mini-prep kit, QIAEX II gel purification kit, and QIAquick PCR purification kit were purchased from Qiagen (Valencia, CA). Various oligonucleotide primers were obtained from Integrated DNA Technologies (Coralville, IA). Unless otherwise specified, all chemicals were obtained from Sigma (St. Louis, MO).

Bacterial strains, plasmids and cell growth — *E. coli* BL21 (DE3) and DH5a were obtained from Novagen (Madison, WI) and the Media Preparation Facility of the University of Illinois Biochemistry Department (Urbana, IL), respectively. *Streptomyces wedmorensis* was obtained from ATCC (Manassas, VA) and *Streptomyces luridus* was obtained from the National Center for Agricultural Utilization Research (Peoria, IL). Vectors pET-28a(+) and pET-15b(+) were purchased from Novagen (San Diego, CA) and were used for cloning and expression of N-terminal His₆-tagged FomC and DhpG. When necessary, kanamycin and ampicillin were added at 50 µg/mL and 100 µg/mL, respectively. *S. wedmorensis* and *S. luridus* were grown aerobically at 30 °C in malt-yeast-glucose (MYG) broth (4 g/L yeast extract, 10 g/L malt extract and 4 g/L glucose) with constant shaking (250 rpm). The corresponding solid medium ISP2 was obtained from Difco (Franklin Lakes, NJ).

S. wedmorensis fomC and S. luridus dhpG cloning — S. wedmorensis and S. luridus were grown in 3 mL ISP2 medium for 24 hours at 30 °C and genomic DNA was prepared using Wizard[®] genomic DNA purification kit (Promega, Madison WI). The S. wedmorensis fomC gene was PCR-amplified using a forward primer 5'-ATATGCTACATATGAGTCCGC GCCGTCC-3' (NdeI restriction site is underlined; the sequence corresponding to the first 17 bp of *fomC* is italicized and the start codon GTG is changed to ATG) and a reverse primer 5'-AGTCAGTCAAGCTTTCAGGAACACCTCATGAG-3' (HindIII restriction site is underlined, and the sequence corresponding to the last 18 bp of *fomC* is italicized). A mixture of *Taq* DNA polymerase (Promega, Madison WI) and *pfu* Turbo DNA polymerase (Stratagene, La Jolla, CA) with a unit ratio of 1:1 was used to minimize potential point mutations introduced by PCR under standard conditions. FailsafeTM 2x premix buffer G from EPICENTRE Biotechnologies (Madison, WI) was used due to its advantage in amplifying fragments from a genome with high GC content. The PCR program is 3 min at 98 °C followed by 20 cycles of 10 sec at 98 °C, 1 min at 60 °C and 2 min at 72 °C, and a final elongation of 8 min at 72 °C. The PCR product was digested with NdeI and HindIII and purified using gel purification kit purchased from Qiagen. The purified product was ligated into the NdeI- and HindIII-digested vector pET-28a(+). S. luridus PCR-amplified dhpGwas similarly using primers 5'-GCGGCCGCCATATGAGCCGGCCCCTGGCG-3' (NdeI restriction site is underlined; the sequence corresponding the first 18 of DhpGitalicized) and 5'to bp is GCGGCCGCGGATCCACGTCAGGGGCAGCAC-3' (BamHI restriction site is underlined; the sequence corresponding to the last 16 bp of *dhpG* is italicized). The PCR product was digested by NdeI and BamHI and subjected to the ligation reaction with the digested pET-15b(+) vector. The sequences of the coding regions were confirmed at the Biotechnology Center of University of Illinois using the Big DyeTM Terminator sequencing method and an ABI PRISM 3700 sequencer (Applied Biosystems, Foster City, CA).



Fig. S1. (a) pH dependence of steady state kinetic parameters of FomC, $\log k_{cat}$ (\blacksquare), $\log k_{cat}/K_m$ (\square), for PnAA reduction with PnAA and NADPH as substrates. (b) pH dependence of steady state kinetic parameters of FomC, $\log k_{cat}$ (\blacksquare), $\log k_{cat}/K_m$ (\square), for HEP oxidation with HEP and NADP⁺ as substrates. For assays at different pH values, the reactions were performed in universal buffers. Universal buffer I (25 mM acetate/MES/HEPES/borate plus 200 mM NaCl) with pH 5.0-9.5 was used for determining the kinetic parameters for PnAA reduction. Universal buffer II (25 mM HEPES/glycine plus 100 mM NaCl) with pH 7.5-10.5 was used for determining the kinetic parameters for HEP oxidation. To construct the pH profiles, the kinetic parameters k_{cat} and k_{cat}/K_m for PnAA and HEP were determined at each pH, and the pH dependence of $Y(k_{cat}/K_m)$ was fitted to a bell-shaped curve described by equation 1, where *H* is the proton concentration, K_I and K_2 are the dissociation constants for the groups that ionize at low and high pH, respectively, and Y_H is the pH-independent plateau value of *Y* at intermediate pH. The pH profile for k_{cat} was constructed in a point-to-point manner.

$$\log Y = \log[Y_H / (1 + H / K_1 + K_2 / H)]$$
(Eq.1)



Fig. S2. Determination of the metal identity of PhpC. EDTA was used to chelate the metal from the active enzyme. After removing the free EDTA in excess, different divalent metals were mixed with the inactivated PhpC. The activity of PhpC reconstituted by divalent metals was assayed and compared with that of the holo form PhpC as a control.



Fig. S3. HPLC size exclusion chromatography to determine the quaternary structure. The standard proteins are represented by closed square. The retention time of FomC sample (represented by open square) was fitted to the standard curve.

FomC	LGATRVLVVHGAHSFEASGA
DhpG	MSRPLARGSGPRRDTVLLTGRGSFGRVPGLSEG-RRVLVVASSRALLRAPV
PhpC	MTAVFPGELLLAEGIHEIARVTALLSGPLRRAPRVAQVVGPGFAGRPWA
102D	MGSDKIHHHHHHVWEFYXPTDVFFGEKILEKRGNIIDLLGKRALVVTGKSSSKKNGS
2BL4	RGYQKALIVTDKTLVQCGVV
	* * .
FomC	SRMMESFGRRTLVHRWHDFSPNPAVDDVARGLDAVRELRPDAVVGVGGGSAMDMAKLL
DhpG	AAWLPPDAVTFTAFHPNPTVAQGVEAARLRRASGADLVVGIGGG\$ALDVAKAA
PhpC	PRLTDALRPLDPTVVVHDGPTTPDSVAALARQLRAIRADVAVAIGGGTVMDAAKAA
102D	LDDLKKLLDETEISYEIFDEVEENPSFDNVXKAVERYRNDSFDFVVGLGGG\$PXDFAKAV
2BL4	AKVTDKMDAAGLA-WAIYDGVVPNPTITVVKEGLGVFQNSGADYLIAIGGG\$PQDTCKAI
	.: * :.:***: * .*
FomC	$\label{eq:cvldgvpradled} CVLDGVPRADLEDRVRTGPAADTRRMKLVLAPTTSGSGSEATHFAVVYVGDAK$
DhpG	RVLPGDAGAADEVIAGRRPVPEG-VPELLLVPTTAGTGSEVTQFATLYRGHRK
PhpC	AALADGGPPDADRVRQACAAGPAAGDTPPAVRVVAVPTTAGTGAEATPFATLWDLKHRRK
102D	AVLLKEKDLSVEDLYDREKVKHWLPVVEIPTTAGTGSEVTPYSILTDPEGNKR
2BL4	GIISNNPEFADVRSLEGLSPTNKPSVPILAIPTTAGTAAEVTINYVITDEEKRRK
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FomC	YSVAGAGLLPDAAVLDPALTESGTPHQRATSGVDAIAQGIESLWAVGGDDESRGLARRGL
DhpG	VSLDTPAARADVAVVDPELTETCPADLTWTCAFDTLAHAVESLWSVRSTAESREHAVAAL
PhpC	LSLTGPRVRPSAAVLAPELLAGLGRRALATGILDALOQGAEASWSIRSTPESIRWGTSAV
102D	GCTLXFPVYAFLDPRYTYSXSDELTLSTGVDALSHAVEGYLSRKSTPPSDALAIEAX
2BL4	FVCVDPHDIPQVAFIDADMMDGMPPALKAATGVDALTHAIEGYITTGAWALTDALHIKAI
	. *.: . : .*: : . *. : . : .
FomC	RLLLAGIEDFV-AAPHAGVARSMAVGSHLVGRAINRSKTTGAHALSYRMTSRYGVSHGHA
DhpG	RSSLPVLRCAG-DLPSPAERDALSRAATLAGQAIDLTRTTAAHALSYPLTAHLGIPHGLA
PhpC	TLAAEALDQVQDDAPDAAARLALQRAAHHSGRAIALAQTSSCHAISYPLTLRLGLAHGHA
102D	KIIHRNLPKAIEGNREARKKXFVASCLAGXVIAQTGTTLAHALGYPLTTEKGIKHGKA
2BL4	EIIAGALRGSVAGDKDAGEEMALGQYVAGMGFSNVGLGLVHGMAHPLGAFYNTPHGVA
	: * : *.:.: . ** *
FomC	VALSLGAFLDAHAHADASDLRTSVRPSEYRRALDDIAAVLGAS-GPAESGARFVALGRR
DhpG	CALNLAWLAPLVEAAGPERVTDPRGHAAVREAVGTLRELFGADEQGTDLAGALRALLARR
PhpC	eq:cgvtlgrllrynhavpagdcadprgtghvrrvldalaaplggtparaalrverfita
102D	TGXVLPFVXEVXKEEIPEKVDTVNHIFGGSLLKFLKE
2BL4	NAILLPHVMRYNADFTGEKYRDIARVMGVKVEGMSLEEARNAAVEAVFALNRD
	. * . : :

Fig. S4. Multiple sequence alignment. Key residues are indicated by boxes. 1O2D: alcohol dehydrogenase from *Thermotoga maritime*; 2BL4: 1, 2-propanediol oxidoreductase from *E. coli*; NAD(P)H-preferring residues: Gly35 (FomC), Ser41 (DhpG), Gly40 (PhpC); NAD(P)H recognition residues: Gly91, Gly92, Gly93 (FomC), Gly92, Gly93, Gly94 (DhpG), Gly94, Gly95, Gly96 (PhpC); Metal coordinating residues: Asp189, Gln193, His257, His271 (FomC), Asp189, His193, His257, His271 (DhpG), Asp199, Gln203, His268, His282 (PhpC).

Gene ID	BLAST Homolog	Identity		
Amycolatopsis orientalis				
CZA382.04	serineglyoxylate transaminase (Syntrophomonas wolfei, 383 aa)	37%		
CZA382.05	aminotransferase, class I and class II (Chloroflexus aurantiacus, 386 aa)	36%		
CZA382.06	nucleotidyl transferase (Carboxydothermus hydrogenoformans, 237 aa)	32%		
CZA382.07	N-acetylglutamate synthase and related acetyltransferase	24%		
	(Thermobifida fusca, 269 aa)			
CZA382.08	transposase (Streptomyces coelicolor, 281 aa)	26%		
	Azoarcus sp. BH72			
azo2697	glycosyltransferase (Oceanobacter sp., 346 aa)	32%		
azo2701	CDP-glycerol:poly(glycerophosphate) glycerophosphotransferase	33%		
	(Congregibacter litoralis, 391 aa)			
azo2702	glycosyl transferase, family 2 (Pseudomonas putida, 1162 aa)	33%		
azo2703	TPR repeat (Methylobacillus flagellatus, 700 aa)	36%		
azo2704	flagellin domain protein (Verminephrobacter eiseniae, 501 aa)	55%		
	Frankia alni ACN14a			
FRAAL6372	aldehyde dehydrogenase PhpJ (Streptomyces viridochromogenes, 466 aa)	47%		
FRAAL6373	hypothetical protein			
FRAAL6374	Permeases of the major facilitator superfamily	27%		
	(Brevibacterium linens BL2, 452 aa)			
FRAAL6375	PhpD (Streptomyces viridochromogenes, 443 aa)	51%		
FRAAL6376	putative D-3-phosphoglycerate dehydrogenase PhpE	62%		
	(Streptomyces viridochromogenes, 336 aa)			
Delta proteobacterium MLMS-1				
MIdDRAFT_5199	lic-1 operon protein (Haemophilus influenzae, 267 aa)	19%		
MIdDRAFT_5200	thymidylate kinase (Salinispora tropica, 203 aa)	26%		
MIdDRAFT_5187	transposase, IS5 family (Azoarcus sp., 345 aa)	32%		
MIdDRAFT_5188	transposase, IS5 family (Azoarcus sp., 299 aa)	22%		
MIdDRAFT_5189	transposase, IS204/IS1001/IS1096/IS1165 family protein (Alkalilimnicola	68%		
	ehrlichei, 421 aa)			
Bacteroides fragilis YCH46				
BF2575	glycosyl transferase, family 2 (Geobacter uraniumreducens, 316 aa)	28%		
BF2579	putative cholinephosphotransferase (Bacteroides vulgatus, 428 aa)	32%		
BF2580	putative carbamoylphosphate synthase large subunit short form (Anabaena	38%		
	variabilis, 428 aa)			
BF2581	nucleotide sugar transaminase (Bacteroides thetaiotaomicron, 369 aa)	76%		
BF2582	putative lipopolysaccharide biosynthesis protein (Bacteroides thetaiotaomicron,	43%		
	494 aa)			

Table S1. Open reading frame analysis of the clusters containing PPM, PPD and ADH.