Supporting Information

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SI Text

The nicR Gene. The *nic* cluster contains a putative regulatory gene (*nicR*) encoding a MarR-like transcriptional regulator (Fig. 1 and Table S1). Whereas disruption of the *nicR* gene accelerated growth of the *P. putida* KT2440d*nicR* mutant strain in 6HNA, it drastically delayed the growth of the strain in NA. Further gene expression analyses confirmed the role of NicR as a transcriptional activator of the *nicAB* operon and as a repressor of the *nicXR* and *nicCDEFTP* operons (J.I.J., unpublished observation).

Biochemical Properties of NicX. The 2,5DHP dioxygenase activity required the addition of 50 μM Fe^{2+} to the reaction assay, and it was inhibited when some iron chelants like α,α'-dipyridyl or EDTA (5 mM) and some oxidizing agents like H_2O_2 (5 mM) were added to the assay. Moreover, replacement of Fe^{2+} by some other divalent cations such as Mg^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Ca^{2+}, Cd^{2+}, and Cu^{2+} did not lead to a detectable 2,5DHP dioxygenase activity, and the addition of Fe^{3+} caused a significant reduction of the enzyme activity [some Fe^{3+} becomes reduced to Fe^{2+} in the presence of DTT as already shown by Gauthier and Rittenberg (1)]. All of these data suggest that Fe^{2+} is essential for catalysis as already reported for other extradiol dioxygenases (2, 3). The enzyme was most active at pH 8.0 and 25°C, and the K_m for 2,5DHP and V_{max} values were 70 μM and 2.3 $\mu M \cdot min^{-1} \cdot mg^{-1}$, respectively. To test the substrate specificity of NicX, several aromatic compounds were checked, but only 2,5DHP was oxidized by the enzyme, which did not use 2,3DHP, 2,4DHP, 2-hydroxypyridine, 3-hydroxypyridine, 4-hydroxypyridine, NA, 6HNA, 2-carboxypyridine, pyridoxamine, pyridoxal, catechol, protocatechuate, gentisate, gallate, resorcinol, hydroquinone, or pyrogallol.

Abbreviations and Accession Codes for the Different Gene Clusters Detailed in Fig. 1. Abbreviations: Ppu, *P. putida*; Bor, *Bordetella* spp.; Bcen, *Burkholderia cenocepacia*; Reut, *Ralstonia eutropha*; Ctest, *Comamonas testosteroni*; Aaven, *Acidovorax avenae*; Dacid, *Delftia acidovorans*; Bxen, *Burkholderia xenovorans*. Accession codes: *Bordetella pertussis* Tohama I, genes from BP1946 to BP1960 (NC_002929). *Bordetella parapertussis* 12822, genes from BPP2320 to BPP2332 (NC_002928). *Bordetella bronchiseptica* RB50, genes from BB1771 to BB1783 (NC_002927). *Burkholderia cenocepacia* PC184, genes from BcenP_01003686 to BcenP_01003695 (AAKX01000106). *Ralstonia eutropha* JMP134, genes from Reut_A2518 to Reut_A2508 (NC_007347). *Comamonas testosteroni* KF-1, genes from CtesDRAFT_2783 to CtesDRAFT_2792 (NZ_AAUJ00000000). *Acidovorax avenae* sbsp. citrulli AAC00–1, genes from Aave_3922 to Aave_3934 (NC_008752). *Delftia acidovorans* SPH-1, genes from DeciDRAFT_2195 to DeciDRAFT_2206 (NC_010002). *Burkholderia xenovorans* LB400, genes Bxe_B0662, Bxe_B0663 and genes from Bxe_C0212 to Bxe_C223 (NC_007952 and NC_007953). *Pseudomonas putida* GB-1, genes from PputGB1DRAFT_2329 to PputGB1DRAFT_2338 (NZ_AAXR01000012). *Pseudomonas putida* W619, genes from PputW619DRAFT_2982 to PputW619DRAFT_2991 (NZ_AAVY01000008). *Pseudomonas putida* F1, genes from Pput_1889 to Pput_1899 (NC_009512). *Pseudomonas putida* KT2440, genes from PP3939 to PP3948 (NC_002947).

Descriptions, Accession Codes, and Conserved Domains for the Different Enzymes Detailed in Fig. 2*B*. HxA, xanthine dehydrogenase from *Emericella nidulans* (Q12553); QoxLMS, quinaldine 4-oxidase from *Arthrobacter ilicis* Ru61a (CAD61045, CAD61046 and CAD61047); QorLMS, quinoline 2-oxidoreductase from *Pseudomonas putida* 86 (CAA66828, CAA66829 and CAA66830); NdhABC, nicotine dehydrogenase from *Arthrobacter nicotinovorans* (CAA53086, CAA53087 and CAA53088); IorAB, isoquinoline 1-oxidoreductase from *Brevundimonas diminuta* 7 (CAA88753 and CAA88754); NdhFSML nicotinate dehydrogenase from *Eubacterium barkeri* (ABC88396, ABC88397, ABC88398 and ABC88399); NicAB, nicotinate hydroxylase from *Pseudomonas putida* KT2440 (PP3947 and PP3948). The conserved domains are: [FeS_1], binding site for the ferredoxin-like [2Fe-2S] cluster; [FeS_2], binding site for the second [2Fe-2S] cluster; FAD, consensus FAD-binding site; CODH, specific FAD-binding site of members of the xanthine dehydrogenase/oxidase family; MPT_1, MPT_2 and MPT_3, are the three motifs for binding to the MCD cofactor (MPT cofactor in xanthine dehydrogenases); $CytC_1$, $CytC_2$ and $CytC_3$, are the three cytochrome *c* binding motives.

SI Materials and Methods

Recombinant Plasmid Constructions. The pNIC plasmid (18.8 kb) was built by cloning a 14-kb NsiI/EcoRI fragment harboring the *nic* genes from cosmid 3n5, a Lawrist 7-derived cosmid from a genomic library of *P. putida* KT2440, into the NsiI/EcoRI double-digested broad-host range plasmid pBBR1MCS-5 (Table S2). The pNicAB plasmid expressing the *nicAB* genes under control of the *Plac* promoter was constructed by cloning firstly the 1.4-kb BamHI/SgrAI PCR-amplified *nicA* gene into pFUSAB plasmid producing plasmid pUCNAB (Table S4). The pFUSAB plasmid is a pUC19 derivative that contains a 4.1-kb ScaI/EcoRI *nicB* fragment from cosmid 3n5 (Table S2). The 4.6-kb HindIII/EcoRI *nicAB* fragment from pUCNAB was then subcloned into pBBR1MCS-5 giving rise to plasmid pNicAB (Table S2). The pIZNicC plasmid expressing the *nicC* gene under *Ptac* was constructed by cloning firstly a 1.5-kb BamHI/XbaI PCR-amplified *nicC* gene into pUC18 generating plasmid pUCNicC (Table S4). The 1.5-kb EcoRI/XbaI *nicC* fragment was then subcloned into pIZ1016 giving rise to plasmid pIZNicC (Table S2). To construct the pETNicX, pETNicD, and pETNicF plasmids overexpressing the *nicX*, *nicD*, and *nicF* genes under control of the P_{T7} promoter and *lac* operator, the 1.1-kb, 0.8-kb, and 0.7-kb NdeI/EcoRI PCR-amplified *nicX*, *nicD*, and *nicF* genes (Table S4), respectively, were cloned into pET-29a(+) (Table S2).

***P. putida* KT2440dnic Mutant Strain Construction.** To construct the *P. putida* KT2440d*nicA*, KT2440d*nicB*, KT2440d*nicC*, KT2440d*nicD*, KT2440d*nicX*, and KT2440d*nicR* mutant strains, an internal fragment (250–700 bp) of the target *nic* gene was PCR-amplified and cloned into the polylinker of pK18*mob* (Table S4), a mobilizable plasmid that does not replicate in *Pseudomonas* (4). To transfer the corresponding pKnic plasmids into *P. putida* KT2440, a triparental filter mating was performed as previously described (4) using *E. coli* DH10B (pKnic) as donor strain, *E. coli* HB101 (pRK600) as helper strain, and *P. putida* KT2440 as recipient strain. *P. putida* KT2440 exconjugants harboring the *nic*-disrupted gene were isolated on MC minimal medium plates containing citrate (which selected for the *Pseudomonas* recipient cells) and kanamycin (which selected for the insertion of the suicide vector) after incubation at 30°C for 16 h. All mutant strains were analyzed by PCR to confirm the disruption of the target genes.

NA Hydroxylase Assay. Resting cells assays were carried out with bacterial cultures grown in 50 ml of citrate-containing M63 minimal medium supplemented with 1 mM NA until they reach an A_{600} of 0.8. Cells were washed and resuspended in 10 ml of M63 medium containing 5 mM NA. Resting cells assays were carried out at 30°C with shaking, and samples (0.1 ml) were taken at different times and centrifuged to remove cells, and the clear supernatants were 1/100 diluted in water and analyzed (20 μl) by HPLC. NA hydroxylase activity in cell extracts was routinely assayed by measuring spectrophotometrically the formation of 6HNA at 295 nm ($\varepsilon = 5{,}200\ M^{-1}{\cdot}cm^{-1}$). The assay mixture (1 ml) contained 20 mM NaH_2PO_4 (pH 7.5), 150–200 μg of total protein from cell extract, 10 mM NA, and 0.4 mM PMS. In some assays PMS was substituted by other electron acceptors such as 2 mM NADP or NAD, 0.2 mM FMN or FAD, 0.1 mM Fe^{3+}, 0.1 mM NO_3^-, and 150 μg of total protein from a crude extract of *P. fluorescens* R2f (pVLTCytC).

6HNA 3-Monooxygenase Assay. The 6HNA 3-monooxygenase (NicC) was assayed using 100 μg of total protein from a crude extract of *P. fluorescens* R2f (pIZNicC) according to ref. 5, and the formation of 2,5DHP was monitored by HPLC.

Overproduction and Purification of NicX. The NicX protein was overproduced in *E. coli* BL21(DE3) (pETNicX) cells grown at 30°C during 12 h in ampicillin-containing LB medium supplemented with 1 mM IPTG to induce gene expression. Cells were harvested and disrupted by passage through a French press (Aminco), and the clear supernatant fluid (cell extract) was applied to a DEAE-cellulose column previously equilibrated with 50 mM NaH_2PO_4 (pH 7.5) buffer. Column was then washed with 20 volumes of buffer containing 0.1 M NaCl, and the NicX protein was eluted with a linear gradient of NaCl ranging from 0.1 to 0.3 M in 20 mM NaH_2PO_4 (pH 7.5) buffer. The protein fractions showing 2,5DHP dioxygenase activity were pooled, dialyzed in 20 mM NaH_2PO_4 (pH 7.5) buffer, loaded onto a hydroxyapatite column, and eluted with a linear gradient (20–100 mM) of NaH_2PO_4 (pH 7.0) buffer. Fractions showing 2,5DHP dioxygenase activity were pooled, dialyzed in 20 mM NaH_2PO_4 (pH 7.5), and concentrated in a Centricon (Amicon). The purified NicX protein was analyzed by 12.5% SDS/PAGE.

Metabolite Detection. NA, 6HNA, 2,5DHP, NFM, and maleamic acid were monitored with a Gilson HPLC equipment using a Phenomenex 5 RP-18 column and a diode array, and 10 mM NaH_2PO_4 (pH 3.0), acetonitrile (98:2, vol/vol) as mobile phase pumped at a flow rate of 1 ml/min. The elution of the compounds was monitored at 210 nm and compared with that of standards of NA, 6HNA, 2,5DHP, and maleamic acid that showed retention times of 4.9 min, 8.1 min, 5.7 min, and 3.0 min, respectively. NMR spectra of 2,5DHP, NFM, and maleamic acid were acquired on an AVANCE 500 MHz spectrometer equipped with a broad-band z-gradient probe (Bruker). NMR samples were prepared in 100% D_2O. Heteronuclear single-quantum coherent spectrometry experiments were performed by using the standard procedures provided by the manufacturer as described (4). Mass spectra of NFM was performed in a LCQ Deca XP plus mass spectrometer (Thermo Instruments) using an ion trap analyzer operated as previously described (4). Additionally, three experiments were performed in negative and full scan mode, with zoom scan of the ions defined by *m/z* 170 and 130. A MS/MS analysis of the ion defined by *m/z* 142 was accomplished. Electrospray ionization with the quadruple analyzer was performed by using a HPLC1100MSD spectrometer (Agilent Technologies) as reported (4).

Three-Dimensional Modeling of NicX and NicD. Initially, PSI-BLAST searches were performed against the NCBI nonredundant protein sequence database to identify homologues of analyzed proteins. For NicX, collected sequences included two related proteins of known structure, i.e., aminopeptidase S (AmpS, 1zjc) from *S. aureus* (6) and aminopeptidase T (AmpT, 2ayi) from *T. thermophilus* (7). Both full NicX sequence as well as single N- and C-terminal domain regions were analyzed with 3D-Jury method (8) to derive reliable sequence-structure mapping using consensus alignment approach and 3D assessment. Final sequence-to-structure alignment was used to build a three-dimensional model of NicX with the MODELLER program (9) using the structure of AmpS as template. The comparison (superposition) of the predicted Fe^{2+}-binding site of NicX and the known catalytic site of the type I extradiol dioxygenase, 2,3-dihydroxybiphenyl 1,2-dioxygenase (BphC, 1han), from the PCB-degrading *Bhurkholderia xenovorans* LB400 strain (10) was accomplished manually by using InsightII (Accelrys). The NicD model was built by homology to other known structures from the α/β-hydrolase family of proteins, using the 3D-JIGSAW Protein Comparative Server (www.bmm.icnet.uk/servers/3djigsaw/). The program automatically assigned as template the α-subunit of γ-lactamase from *Aureobacterium* sp. (1hkh_A) (11).

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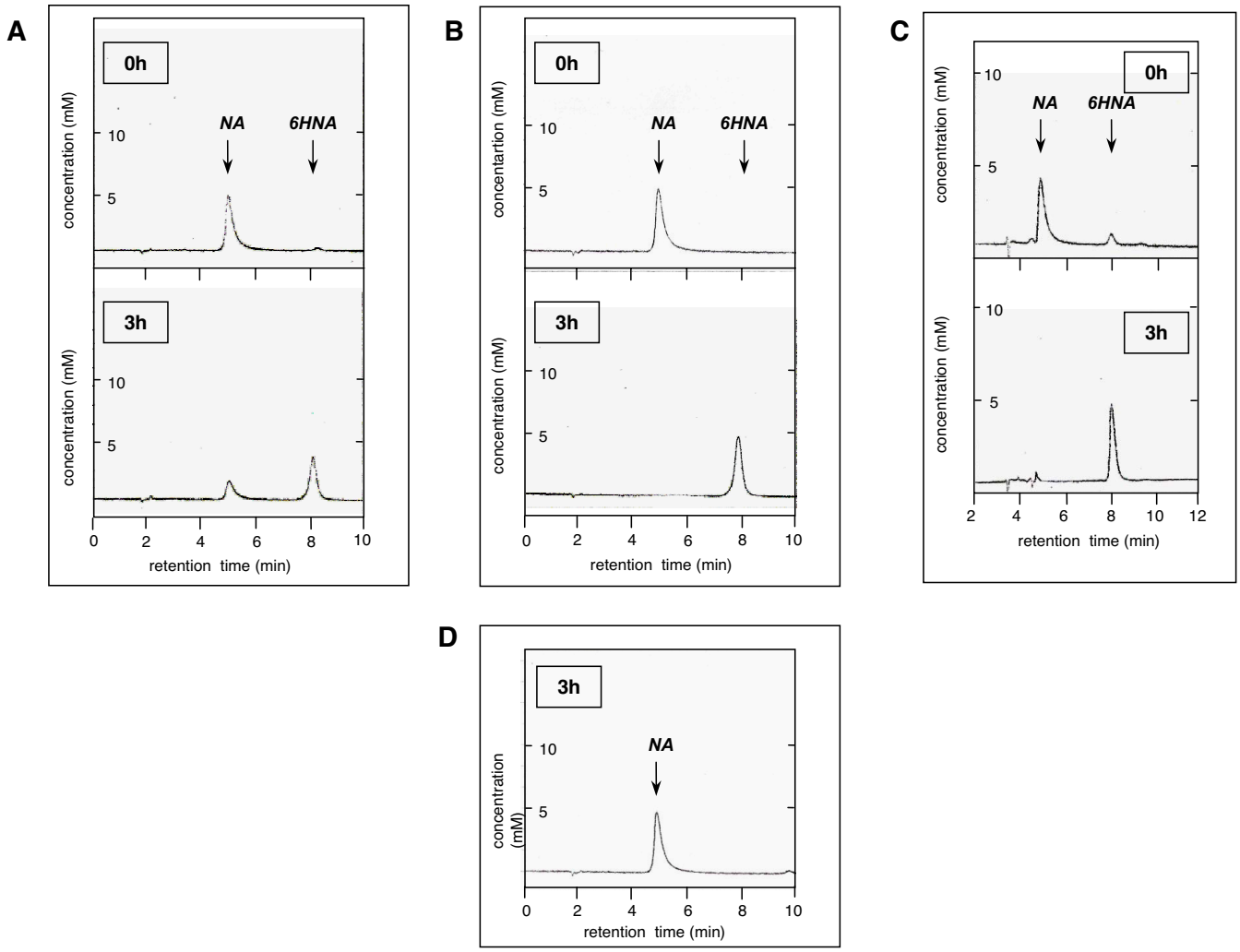

Fig. S1. Hydroxylation of NA to 6HNA by the *nicAB* gene products. Resting cell assays of *P. putida* KT2440d*nicC* (*A*), *P. fluorescens* R2f (pNicAB) (*B*), *Pseudomonas* sp. DSM6412 (pNicAB) (*C*), and the control *P. fluorescens* R2f (pBBR1MCS-5) strain (*D*) in the presence of 5 mM NA were carried out as indicated in *Materials and Methods*. The peaks of NA and 6HNA in the HPLC chromatograms of the culture supernatants after 0-h and 3-h incubation are shown by arrows. Results of one experiment are shown; values were reproducible in three separate experiments.

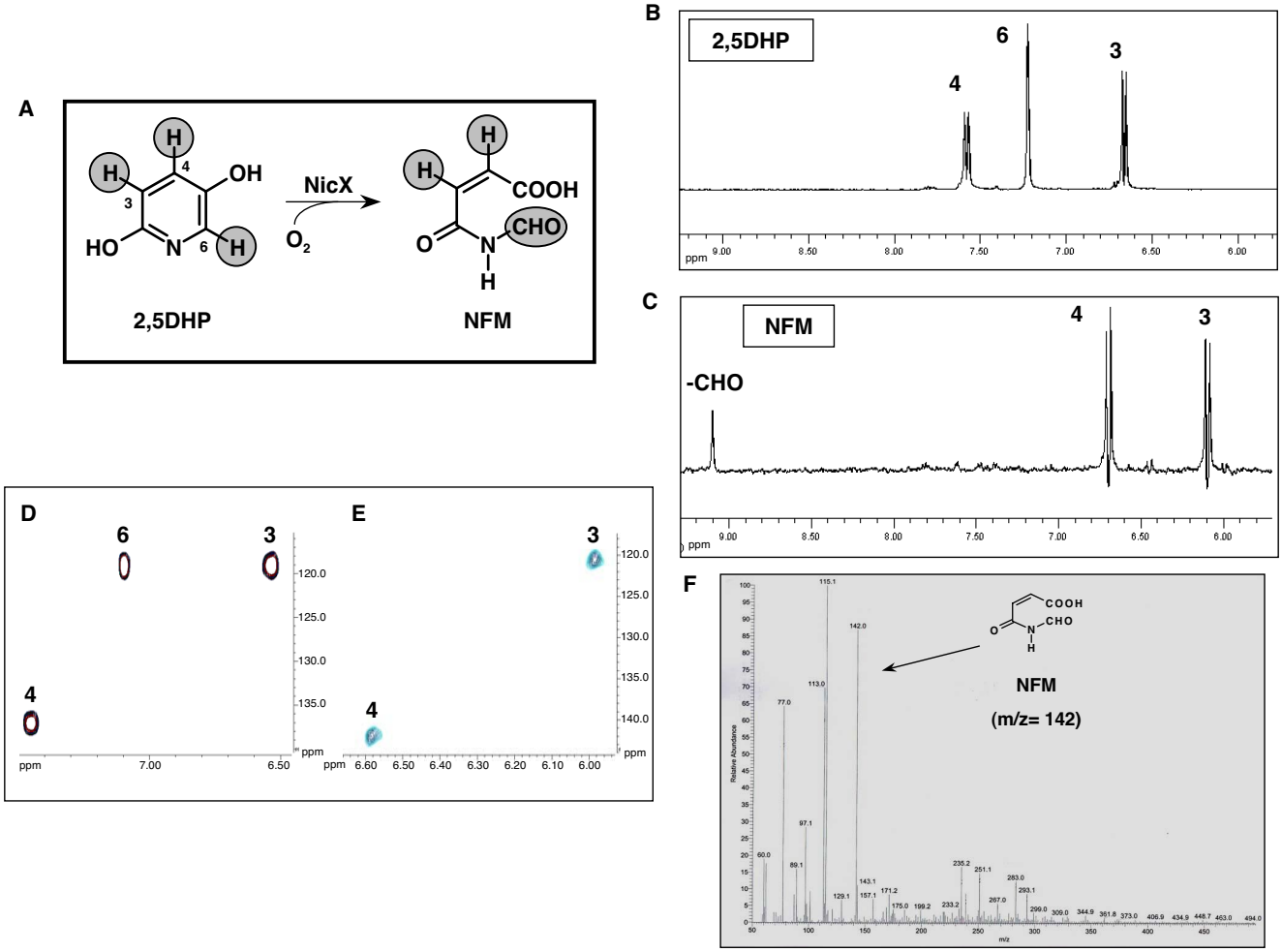

Fig. S2. Structural analysis of the product of the NicX-mediated dioxygenase reaction. (*A*) Scheme of the substrate and product of the reaction catalyzed by NicX. Protons detected by ^{1}H NMR analysis are circled, and numbers correspond to that shown for peaks in *B*–*E*. ^{1}H NMR signals (*B*) and ^{13}C NMR signals (*D*) at reaction time 0 correspond to that of the substrate (2,5DHP). ^{1}H NMR signals (*C*) and ^{13}C NMR signals (*E*) at reaction time 15 min correspond to that of the reaction product (NFM). The ^{1}H NMR spectrum of the reaction product gives two mutually coupled doublet signals at δ 6.18 ppm (proton H-3) and δ 6.74 ppm (proton H-4), and a singlet signal at δ 9.18 ppm (corresponding to a proton of an aldehyde group) that substitutes to the signal provided by proton H-6 in 2,5DHP. Moreover, the ^{13}C NMR signals (HSQC) of the reaction product are consistent with a compound having a *cis*-disubstituted double bond between carbons C3 and C4 (signals at 120.6 ppm and 144.0 ppm, respectively) and an aldehyde moiety (signal at 211.0 ppm) at former carbon C6 of 2,5DHP. The reaction assay and the NMR analyses were performed as described in *Materials and Methods*. (*F*) Mass spectrum analysis of the 2,5DHP ring-cleavage product. The product generated by the NicX-mediated reaction was extracted and analyzed by mass spectrometry as indicated in *Materials and Methods*, and it corresponds to NFM.

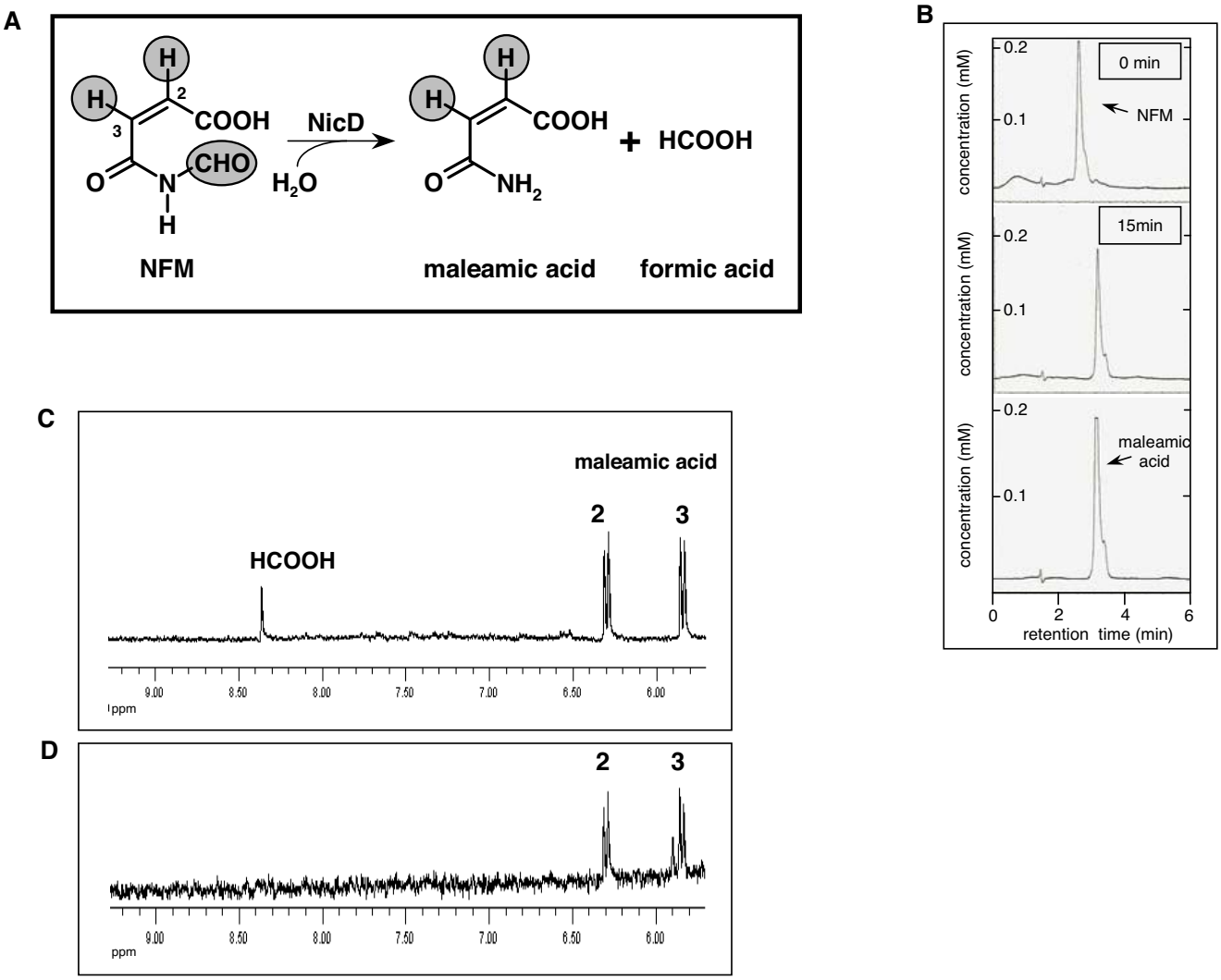

Fig. S3. Structural analysis of the products of the NicD-mediated reaction. (*A*) Scheme of the substrate and products of the reaction catalyzed by NicD. Protons detected by ^{1}H NMR analysis are circled, and numbers correspond to that shown for peaks in *C* and *D*. (*B*) HPLC analysis of the conversion of 0.2 mM NFM to maleamic acid after 0-min and 15-min incubation time with crude extracts of *E. coli* BL21(DE3) (pETNicD). The peaks of NFM and standard maleamic acid in the HPLC chromatograms are shown by arrows. The reaction assay and HPLC analysis were performed as described in *Materials and Methods*. (*C*) ^{1}H NMR signals at reaction time 15 min correspond to that of formic and maleamic acids. Thus, the disappearance of the resonance signal corresponding to the aldehyde moiety of the substrate (NFM) parallels the appearance of a new peak (HCOOH) with a chemical shift of 8.35 ppm, which corresponds to the formic acid released in the reaction. On the other hand, the two coupled doublet signals at δ 6.52 ppm and 6.06 ppm, corresponding to protons of carbons 2 and 3 in a *–cis* double bond of the NFM, shifted to δ 6.30 ppm and 5.83 ppm, respectively. Identical resonance signals at δ 6.30 ppm and 5.83 ppm were observed for the protons of the *cis* double bond of standard maleamic acid (*D*). The NMR analyses were performed as described in *Materials and Methods*.

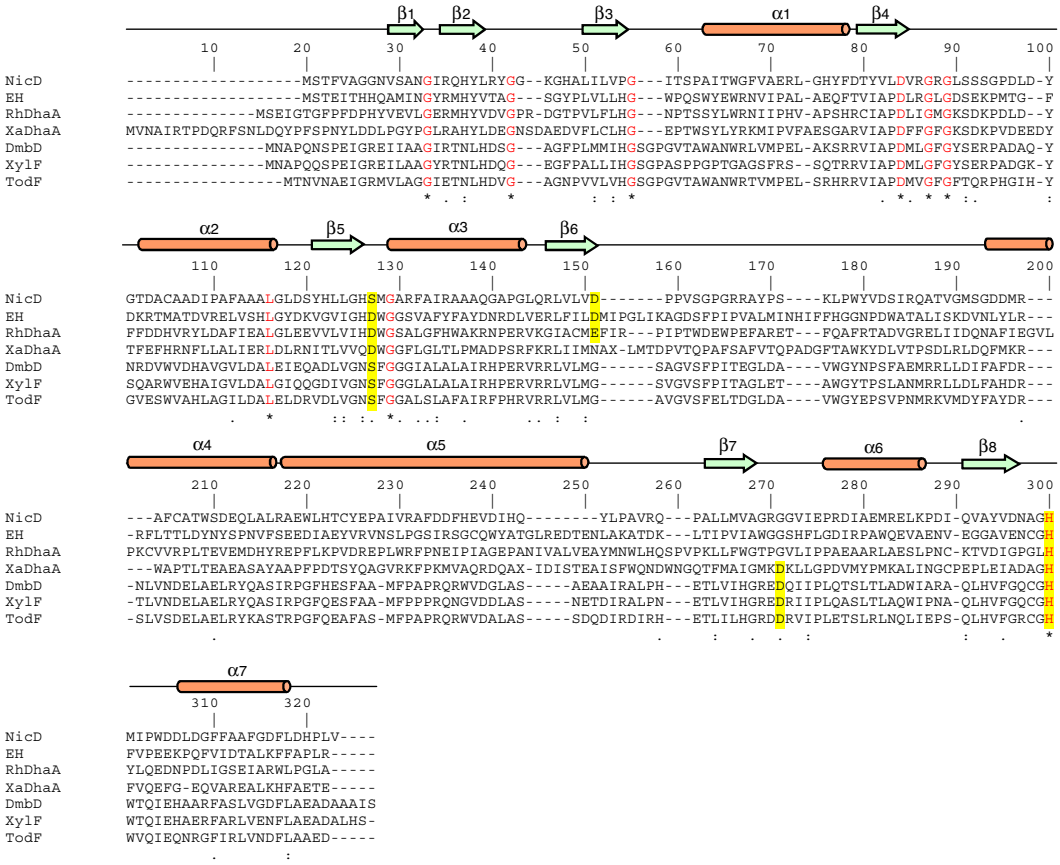

Fig. S4. Multiple sequence alignment of NicD. Alignment of NicD from *P. putida* KT2440 (PP3943) and several members of the α/β hydrolase-fold superfamily. The description and accession numbers of the sequences are as follows: EH, epoxide hydrolase from *Corynebacterium* sp. C12 (O52866); RhDhaA, haloalkane dehalogenase from *Rhodococcus* sp. TDTM0003 (P59336, 1CQW); XaDhaA, haloalkane dehalogenase from *Xanthobacter autotrophicus* GJ10 (1CIJ); DmbD (P19076), XylF (P23105), and TodF (P23133) are 2-hydroxymuconic semialdehyde hydrolases from *Pseudomonas* sp. CF600, *P. putida* mt-2, and *P. putida* F1, respectively. Sequences were aligned using ClustalW program (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_clustalw.html). Identical residues are indicated in red. Residues forming the catalytic triad are shown in yellow. Secondary structure elements predicted for NicD sequence by using the Scratch program (www.igb.uci.edu/tools/scratch/) are drawn at the top of the alignment.

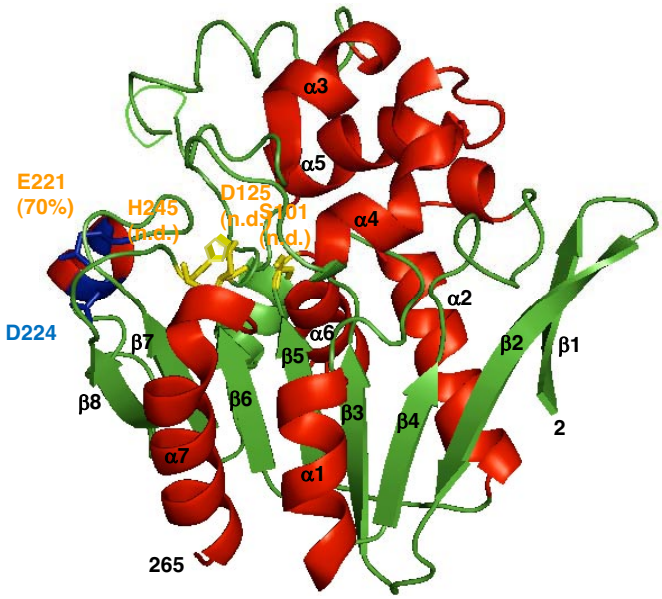

Fig. S5. Three-dimensional structural model of NicD. The α-helices and β-strands are shown in red and green, respectively. The residues that constitute the catalytic triad (S101, D125, and H245) are displayed in yellow, and residues E221 and D224 are shown in blue. Residues that have been substituted by alanine are labeled in orange, and the percentage of specific activity of the corresponding mutant proteins with respect to that of the wild-type enzyme is indicated in brackets. n.d., activity not detected. Figures were generated with Pymol (DeLano Scientific).

Table S1. *nic* genes and their products in *P. putida* KT2440

		Related gene products				
Gene (PP no.)	Gene product (aa)	Name (aa)	Function	Organism	% identity	Accession no.
nicP (PP3939)	NicP (430)	BenF (397)	Specific benzoate porin	*P. putida*	59	AAF63454
nicT (PP3940)	NicT (437)	TtuB (433)	Tartrate transporter	*A. vitis*	41	Q44470
nicF (PP3941)	NicF (213)	CSHase (264)	N-carbamoylsarcosine amidase	*Arthrobacter* sp.	34	AAB23138
		PZase (180)	Nicotinamidase	*P. horikoshii*	28	1ILW
nicE (PP3942)	NicE (250)	MaiA (250)	Maleate *cis/trans* isomerase	*S. marcescens*	82	BAA96747
nicD (PP3943)	NicD (268)	EstA (315)	Lipase/esterase	*P. aeruginosa*	25	AAF89744
		BB1780 (273)	Probable α/β-hydrolase	*B. bronchiseptica*	64	CAE32277
nicC (PP3944)	NicC (382)	6HNA3MO (385)	6-hydroxynicotinic acid 3-monooxygenase	*P. fluorescens*	60	E130001
nicX (PP3945)	NicX (350)	Reut_B3788 (350)	Conserved hypothetical protein	*R. eutropha*	56	AAZ63146
nicR (PP3946)	NicR (158)	MarR (144)	Multiple antibiotic resistance repressor	*E. coli*	27	P27245
nicA (PP3947)	NicA (157)	IorA (152)	Isoquinoline 1-oxidoreductase (A subunit)	*B. diminuta*	54	CAA88753
nicB (PP3948)	NicB (1187)	CoxL (748)	Probable aldehyde dehydrogenase/cytochrome c	*P. aeruginosa*	61	AAG04990
		Mop (907)	Aldehyde oxidoreductase	*D. gigas*	31	Q46509
		IorB (781)	Isoquinoline 1-oxidoreductase (B subunit)	*B. diminuta*	27	CAA88754

Table S2. Bacterial strains and plasmids used in this study

Strain or plasmid	Genotype and/or description	Source
Pseudomonas strains		
P. putida KT2440		1
P. putida KT2440d*nicA*	*P. putida* KT2440 *nicA*::pK18*mob*	This study
P. putida KT2440d*nicB*	*P. putida* KT2440 *nicB*::pK18*mob*	This study
P. putida KT2440d*nicC*	*P. putida* KT2440 *nicC*::pK18*mob*	This study
P. putida KT2440d*nicR*	*P. putida* KT2440 *nicR*::pK18*mob*	This study
P. putida KT2440d*nicX*	*P. putida* KT2440 *nicX*::pK18*mob*	This study
P. putida KT2440d*nicD*	*P. putida* KT2440 *nicD*::pK18*mob*	This study
Pseudomonas sp. MT14		2
P. fluorescens R2f		3
Pseudomonas sp. DSM6412	.	4
E. coli strains		
DH10B	F′, *mcrA* Δ(*mrr hsdRMS-mcrBC*) ϕ80d*lac*ΔM15 Δ*lacX74 deoR recA1 araD139* Δ(*ara-leu*)7697 *galU galK* λ *rpsL endA1 nupG*	Invitrogen
HB101	*supE44 ara14 galK2 leuB lacY1* Δ(*gpt-proA*)62 *rps*L20 *xyl-5 mtl-1 recA13* Δ(*mcrC-mrr*) *hsdS20* (r_B^- m_B^-) Sm^r	5
XL1-Blue	*recA1 endA1 gyrA96 thi-1 hdsR17 supE44 relA1 lac*[F′ $proA^+B^+$ $lacI^q$ Δ(*lacZ*)M15 Tn*10* (Tc^r)]	5
BL21 (DE3)	F^- *ompT* $hsdS_B(r_B^-mB^-)$ *gal dcm* λDE3 (harbouring gen *1* of the RNA polymerase from the phage T7 under the *PlacUV5* promoter	5
Plasmids		
3n5	Lawrist 7 derived cosmid from a genomic library of *P. putida* KT2440 that contains the *nic* genes	D. Stepjandic/J. D. Hoheisel
pUC19/pUC18	Ap^r *ori*ColE1 *lacZ*α^+ *lac* promoter	6
pGEMT-easy	Ap^r *ori*ColE1 *lacZ*α^+ SP6 T7 *lac* promoters, direct cloning of PCR products	Promega
pK18*mob*	Km^r *ori*ColE1 Mob^+ *lacZ*α^+, used for directed insertional disruption	7
pBBR1MCS-5	Gm^r *ori*pBBR1 Mob^+ *lac* promoter *lacZ*α^+, broad-host-range cloning and expression vector	8
pNIC	Gm^r 14332-bp *NsiI*/*Eco*RI containing *nic* genes fragment from 3n5 cosmid cloned into pBBR1MCS-5	This study
pFUSAB	Ap^r pUC19 containing *nicB* and a 5′- truncated *nicA* gene.	This study
pUCNAB	Ap^r pUC19 containing the *nicAB* genes.	This study
pNicAB	Gm^r pBBR1MCS-5 containing the *nicAB* genes under control of the *Plac* promoter	This study
pNicABtr	Gm^r pBBR1MCS-5 containing the *nicA* gene and a truncated *nicB* gene lacking the region that encodes the C-terminal cytochrome *c* domain.	This study
pVLT31	Tc^r RSF1010-$lacI^q$/*Ptac* hybrid broad-host-range cloning and expression vector	9
pVLTCytC	Tc^r pVLT31 containing the cytochrome *c* domain of NicB	This study

Table S2. Continued

Strain or plasmid	Genotype and/or description	Source
pIZ1016	Gm^r *ori*pBBR1 Mob$^+$ *tac* promoter, broad-host-range cloning and expression vector	10
pIZNicC	Gm^r pIZ1016 containing *nicC* gene	This study
pET-29a(+)	Km^r *ori*ColE1 T7 promoter	Novagen
pETNicX	Km^r pET29 containing *nicX* gene	This study
pETNicD	Km^r pET29 containing *nicD* gene	This study
pETDS101A	Km^r pET29 containing *nicD* gene with the substitution S101A	This study
pETDD125A	Km^r pET29 containing *nicD* gene with the substitution D125A	This study
pETDH245A	Km^r pET29 containing *nicD* gene with the substitution H245A	This study
pETDE221A	Km^r pET29 containing *nicD* gene with the substitution E221A	This study
pETNicF	Km^r pET29 containing *nicF* gene	This study

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Table S3. Substrate specificity of NA hydroxylase

Substrate (1 mM)		% activity
NA		100
Pyridine-3-carboxyaldehyde		5
Pyridine-3-methanol		n.d.
Nipecotic acid		8
Pyridine-3-sulfonic acid		4
3-cyanopyridine		4
Methylnicotinate		n.d.
Pyrazine carboxamide		n.d.
Pyrazine-2-carboxylic acid		8
Picolinic acid		7
Quinolinic acid		n.d.
Isonicotinic acid		n.d.

n.d., not detected.

Table S4. Primers designed for PCRs and driven cloning strategy

Primer	Sequence (5′-3′)*	Amplified fragment/cloning strategy	Recombinant plasmid
NicAint5 NicAint3	CGGAATTCGAAGTCAGCGCCATGCC GGGGTCGACGTGGGTGCCGCAGCG	The 394-bp PCR *nicA* internal fragment was double-digested with EcoRI/SalI and cloned into pK18*mob*	pK*nicA*
NicBint5 NicBint3	CGGAATTCGGCCCATCATGTGCAC CGAAGCTTGATGGTGACGCGCATG	The 495-bp PCR *nicB* internal fragment was double-digested with EcoRI/SalI and cloned into pK18*mob*	pK*nicB*
Nih5 Nih3	GCAAGCTTCATCGGGTGGCAGGCG CCGAATTCCGCTGGGCGAGTTTGC	The 644-bp PCR *nicC* internal fragment was double-digested with HindIII/EcoRI and cloned into pK18*mob*	pK*nicC*
NicRint35 NicRint33	GCAAGCTTCCACCCCCAGCAGCGCCC CGGAATTCCTTCAGGCGCTCGACGATG	The 267-bp PCR *nicR* internal fragment was double-digested with HindIII/EcoRI and cloned into pK18*mob*	pK*nicR*
Unkint5 Unkint3	GGGAATTCAAGACCGGCACGCGC GCAAGCTTGAACGGCAGGATGATGTC	The 334-bp PCR *nicX* internal fragment was double digested with HindIII/EcoRI and cloned into pK18*mob*	pK*nicX*
NicD25 NicD23	GCGAATTCAAGCGGCCCCGACCTGG GCAAGCTTCGCCGGCTCGTAGCAGGTG	The 371-bp PCR *nicD* internal fragment was-double digested with HindIII/EcoRI and cloned into pK18*mob*	pK*nicD*
NicA5 SgrAI	GGGGATCCGTAACCGTTGCCGCCACCACCGTATTCG CGCTGTCATGGCCGGCATAGGG	The 1436-bp PCR fragment containing *nicA* was double-digested with BamHI/SgrAI and cloned into pFUSAB	pUCNAB
CytC5 CytC3	GCTCTAGATAGATAGAAGGAGATATACATATGTCAACCAAGCGTTCGAAGTG CGAAGCTTCAAGAATTCTGGCTGCCAGGGTTGGCGCGTAC	The 1291-bp PCR fragment containing the CytC domain of *nicB* was double-digested with XbaI/HindIII and cloned into pVLT31	pVLTCytC
PnicX5 NicC3	GGGGATCCAGTTGTGCATTGCTCACCGG GCTCTAGACGAAGGTGCTCATGCCGC	The 1.5-kb PCR fragment containing *nicC* was double-digested with BamHI/XbaI and cloned into pIZ1016	pIZNicC
NicX5 NicX3	GCCATATGCCGGTGAGCAATGCACAAC GCGAATTCTGGGTGGAAAGAGGCTGGCC	The 1.1-kb PCR fragment containing *nicX* was double digested with NdeI/EcoRI and cloned into pET29	pETNicX
NicD5 NicD3	CGCATATGAGCACCTTCGTCGC GCGAATTCTCGTGCTCCGTCAG	The 829-bp PCR fragment containing *nicD* was double-digested with NdeI/EcoRI and cloned into pET29	pETNicD
DS101A5 DS101A3	CTGCTCGGTCATGCTATGGGGGCGCGTTTTGC GCAAAACGCGCCCCCATAGCATGACCGAGCAGATG	Oligonucleotides used for S101A substitution in NicD	pETDS101A
DD125A5 DD125A3	CTGGTGCTGGTCGCCCCACCGGTGTCC GGACACCGGTGGGGCGACCAGCACCAG	Oligonucleotides used for D125A substitution in NicD	pETDD125A
DE221A5 DE221A3	GGTGGAGTGATCGCGCCCCGTGACATTGCTG CAGCAATGTCACGGGGCGCGATCACTCCACC	Oligonucleotides used for E221A substitution in NicD	pETDE221A
DH245A5 DH245A3	GACAACGCCGGCGCCATGATCCCCTGGG CCCAGGGGATCATGGCGCCGGCGTTGT	Oligonucleotides used for H245A substitution in NicD	pETDH245A
NicF5 NicF3	GAAATCGGGAGAGGCATATGAGCGACGC AAGAATTCCCGCACCGGTCCTTGATTG	The 704-bp PCR fragment containing *nicF* was double-digested with NdeI/EcoRI and cloned into pET29	pETNicF

*Engineered restriction sites are underlined.