IDENTIFICATION OF NICOTINAMIDE MONONUCLEOTIDE DEAMIDASE OF THE BACTERIAL PYRIDINE NUCLEOTIDE CYCLE REVEALS A NOVEL BROADLY CONSERVED AMIDOHYDROLASE FAMILY

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Table S1. Primers used for gene cloning and mutant construction

Primer	Sequence	Utilization
cinA_F	5’-TACGGATCCTGATGAAGTTAGAGATGATT-3’	cloning SO0272 (*pncC*) into pET28c
cinA_R	5’-TACAAGCTTTTACTTACTGAAACGCCCA-3’	cloning SO0272 (*pncC*) into pET28c
2342_F-O	5’-GATCTTAAAGAAGGCACCA-3’	SO2342 (*nadA*) deletion testing
2342_R-O	5’-TTACGTTCGATTTCATC-3’	SO2342 (*nadA*) deletion testing
2342_5-O	5’-CGGAACTGTTGCGACGCC-3’	SO2342 (*nadA*) deletion, upstream
2342_5-I	5’-AGACCCATGTTCGGCAATGCGCTCATAACAAATCCA-3’	SO2342 (*nadA*) deletion, upstream
2342_3-I	5’-GCATTGCCGAACATGGGTCTCTAAGCGCTTTGAGC-3’	SO2342 (*nadA*) deletion, downstream
2342_3-O	5’-CAACTGAGGCTCAAAGG-3’	SO2342 (*nadA*) deletion, downstream
0272_F-O	5’-CAGAAGTGAAACGCAATCG-3’	SO0272 (*pncC*) deletion testing
0272_R-O	5’-CAGTGTCAGACTCGGTG-3’	SO0272 (*pncC*) deletion testing
0272_5-O	5’-CCATGTGAGAGTAGGTC-3’	SO0272 (*pncC*) deletion, upstream
0272_5-I	5’-GGAGACCATGAACCGATTCCCTCTAACTTCATCATA-3’	SO0272 (*pncC*) deletion, upstream
0272_3-I	5’-GGAATCGGTTCATGGTCTCCCGTTTCAGTAAGTAA-3’	SO0272 (*pncC*) deletion, downstream
0272_3-O	5’ CAAGTATCAATTACTAATCC-3’	SO0272 (*pncC*) deletion, downstream
1981_F-O	5’-TAATCAGCAGCAATATGC-3’	SO1981 (*nadV*) deletion testing
1981_R-O	5’-AGCAAGCCATTCCGCT -3’	SO1981 (*nadV*) deletion testing
1981_5-O	5’-GTTAGACCGAGTGCAGG-3’	SO1981 (*nadV*) deletion, upstream
1981_5-I	5’-ATTCATGTGCCAGTGCCGTGCGGGATTCAAGTACAT-3’	SO1981 (*nadV*) deletion, upstream
1981_3-I	5’-CACGGCACTGGCACATGAATAGAAGGGGTCTTGTGG-3’	SO1981 (*nadV*) deletion, downstream
1981_3-O	5’-GAAGCACAAATGTGTCAAGG-3’	SO1981 (*nadV*) deletion, downstream

Table S2. List of strains and plasmids used in this work

Strains and plasmids	Phenotypes or characteristics	Reference or source
***Shewanella oneidensis* strains**		
MR-1	Manganese-reducing strain (Lake Oneida, NY)	(1)
Δ*nadA*	SO2342 deletion derivative of MR-1	This work
Δ*pncC*	SO0272 deletion derivative of MR-1	This work
Δ*nadV*	SO1981 deletion derivative of MR-1	This work
Δ*nadA*/Δ*pncC*	SO2342/SO0272 double deletion derivative of MR-1	This work
Δ*nadA*/Δ*nadV*	SO2342/SO1981 double deletion derivative of MR-1	This work
Δ*pncC*/Δ*nadV*	SO0272/SO1981 double deletion derivative of MR-1	This work
Escherichia coli strains		
WM3064 λpir	Host used for construction of pDS3.0 clones and subsequent conjugation with MR-1. *thrB1*004 *pro thi rpsL hsdS lacZdelta*M15 RP4–1360 delta(*araBAD)567 delta dapA*1341::[*erm pir*(wt)]	(2)
Plasmids		
pET28c	Isopropyl-1-thio-β-D-galactopyranoside-inducible expression vector; Km^r	Novagen
pET28c-*cinA*	*cinA* inserted between the *BamH*I and *Hind*III sites of pET-28c	This work
pDS3.0	Suicide vector, Amp^r, Gm^r, $sacB^+$	(3)
pDS3.0Δ*nadA*	pDS3.0 containing sequences that flank *nadA*	This work
pDS3.0Δ*pncC*	pDS3.0 containing sequences that flank *pncC*	This work
pDS3.0Δ*nadV*	pDS3.0 containing sequences that flank *nadV*	This work

Organism	*Taxonomy*	NMN biosynthesis			NAD biosynthesis			Nm/Na Salvage	
GENE PATTERN 1: NMN biosynthesis via NadV		**NMPRT**	**PNUC**	**RNK**	**NAMNAT**	**NMNAT**	**NADS**	**NAM**	**NAPRT**
Clostridium beijerincki	Clostridia; Clostridiales	nadV			nadD		nadE	pncA	pncB
Chromobacterium violaceum	Betaproteobacteria; Neisseriales	nadV		rnk	nadD		nadE	pncA	pncB
Flavobacterium johnsonia	Bacteroidetes; Flavobacteria	nadV	pnuC		nadD		nadE		pncB
Burkholderia xenovorans	Betaproteobacteria; Burkholderiales	nadV			nadD		nadE		pncB
Magnetospirillum magnetotacticum	Alphaproteobacteria; Rhodospirillales	nadV			nadD		nadE	pncA	
Cytophaga hutchinsonii	Bacteroidetes; Sphingobacteria	nadV			nadD		nadE		
Campylobacter fetus	Epsilonproteobacteria; Campylobacterales	nadV			nadD		nadE		
Shewanella spp (4)	Gammaproteobacteria; Alteromonadales	nadV			nadD		nadE		
Mycoplasma gallisepticum R	Mollicutes; Mycoplasmatales	nadV			nadD				
Mycoplasma genitalium G37	Mollicutes; Mycoplasmatales	nadV			nadD		nadE		
Mycoplasma pneumoniae M129	Mollicutes; Mycoplasmatales	nadV			nadD		nadE		
GENE PATTERN 2: NMN biosynthesis via PnuC-RNK		**NMPRT**	**PNUC**	**RNK**	**NAMNAT**	**NMNAT**	**NADS**	**NAM**	**NAPRT**
Gramella forsetii	Bacteroidetes; Flavobacteria		pnuC	rnk	nadD		nadE	pnCA	pncB
Flavobacterium sp. MED217	Bacteroidetes; Flavobacteria		pnuC	rnk	nadD		nadE	pnCA	pncB
Opitutus terrae PB90-1	Chlamydiae; Verrucomicrobia		pnuC	rnk	nadD		nadE	pnCA	pncB
Pseudomonas aeruginosa (5)	Gammaproteobacteria; Pseudomonadales		pnuC	rnk	nadD		nadE	pnCA	pncB
Pseudomonas entomophila	Gammaproteobacteria; Pseudomonadales		pnuC	rnk	nadD		nadE	pnCA	pncB
Pseudomonas fluorescens (2)	Gammaproteobacteria; Pseudomonadales		pnuC	rnk	nadD		nadE	pnCA	pncB
Pseudomonas putida W619	Gammaproteobacteria; Pseudomonadales		pnuC	rnk	nadD		nadE	pnCA	pncB
Pseudomonas syringae (3)	Gammaproteobacteria; Pseudomonadales		pnuC	rnk	nadD		nadE	pnCA	pncB
Janthinobacterium sp. Marseille	Betaproteobacteria; Burkholderiales		pnuC	rnk	nadD		nadE		pncB
Pseudomonas fluorescens SBW25	Gammaproteobacteria; Pseudomonadales		pnuC	rnk	nadD		nadE		pncB
Pseudomonas putida (3)	Gammaproteobacteria; Pseudomonadales		pnuC	rnk	nadD		nadE		pncB
Pseudomonas stutzeri	Gammaproteobacteria; Pseudomonadales		pnuC	rnk	nadD		nadE		pncB
Croceibacter atlanticus	Bacteroidetes; Flavobacteria		pnuC	rnk	nadD		nadE		
Cellulophaga sp. MED134	Bacteroidetes; Flavobacteria		pnuC	rnk	nadD		nadE		
Flavobacterium psychrophilum	Bacteroidetes; Flavobacteria		pnuC	rnk	nadD		nadE		
Tenacibaculum sp. MED152	Bacteroidetes; Flavobacteria		pnuC	rnk	nadD		nadE		
Polaribacter irgensii	Bacteroidetes; Flavobacteria		pnuC	rnk	nadD		nadE		
Robiginitalea biformata	Bacteroidetes; Flavobacteria		pnuC	rnk	nadD		nadE		
Flavobacteriales bacterium	Bacteroidetes; Flavobacteria		pnuC	rnk	nadD		nadE		
Flavobacteria sp. BBFL7	Bacteroidetes; Flavobacteria		pnuC	rnk	nadD		nadE		
Hyphomonas neptunium	Alphaproteobacteria; Rhodobacterales		pnuC	rnk	nadD		nadE		
Novosphingobium aromaticivorans	Alphaproteobacteria; Sphingomonadales		pnuC	rnk	nadD		nadE		
Sphingopyxis alaskensis RB2256	Alphaproteobacteria; Sphingomonadales		pnuC	rnk	nadD		nadE		
Pseudoalteromonas atlantica	Gammaproteobacteria; Alteromonadales		pnuC	rnk	nadD		nadE		
GENE PATTERN 3: NMN biosynthesis via NadV and PnuC-RNK		**NMPRT**	**PNUC**	**RNK**	**NAMNAT**	**NMNAT**	**NADS**	**NAM**	**NAPRT**
Chitinophaga pinensis	Bacteroidetes; Sphingobacteria	nadV	pnuC	rnk	nadD		nadE		

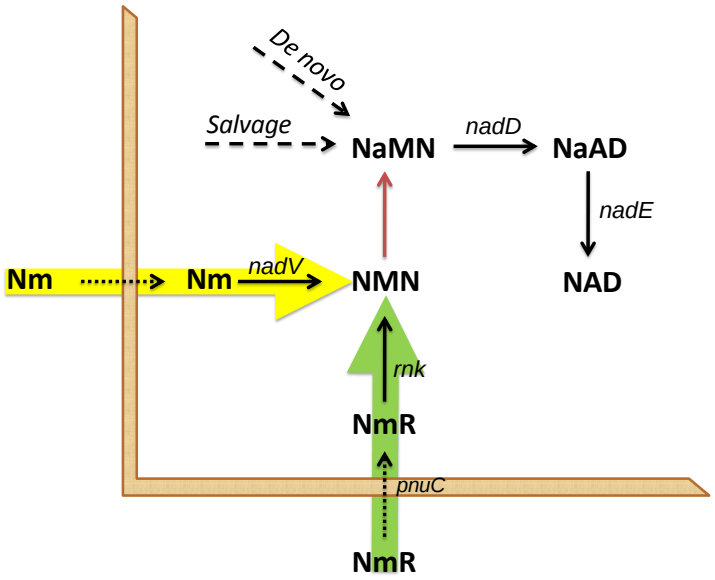

Figure S1. Phylogenomic distribution of genes involved in gapped NMN biosynthesis. Table lists bacterial species where active salvage pathways leading to NMN synthesis occur in the absence of *nadR* or *nadM*, the only genes encoding a NMN adenylyltransferase capable of the subsequent conversion of NMN to NAD. The diagram represents a simplified scheme of NAD biosynthesis in these bacteria. In such context, NMN deamidase activity (represented by the red arrow in the diagram) appears as an indispensable function to feed NMN intermediate into the ubiquitous downstream reactions catalyzed by NadD and NadE family of enzymes. Bacteria are divided into three subgroups according to the different routes of NMN synthesis: a) via NadV (yellow solid arrow) as in *S. oneidensis*, b) via PnuC-Rnk (green arrow) as in *Pseudomonas aeruginosa*, or via both routes as in *Chitinophaga pinensis*. Dotted arrows indicate uptake and dashed arrows indicate *de novo* and other salvage routes leading to NaMN intermediate, that vary from bacterium to bacterium.

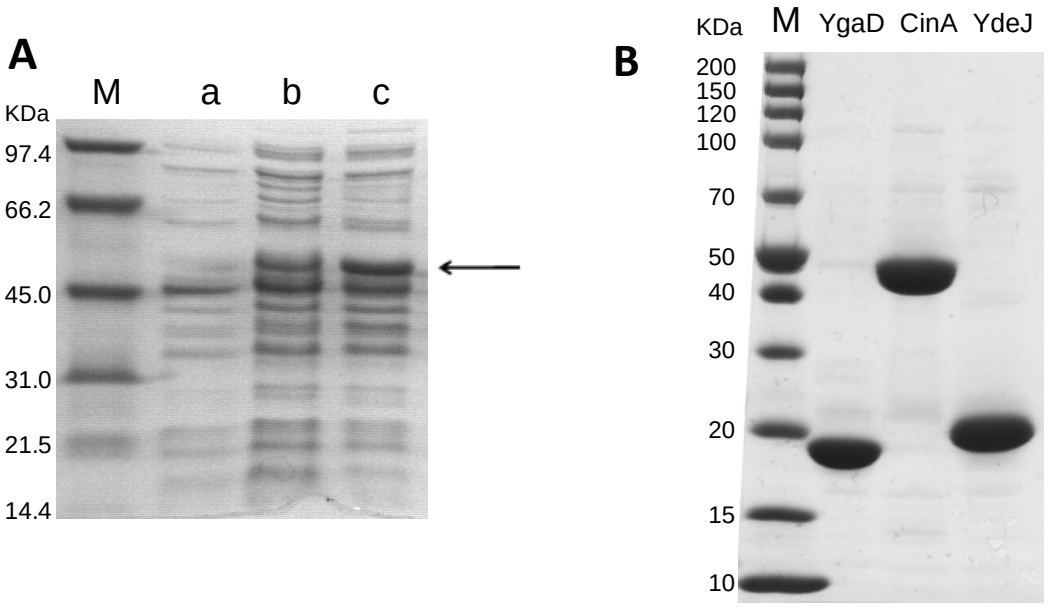

Figure S2. Expression of *Shewanella oneidensis* CinA (A) and *E. coli* YgaD, YfaY and YdeJ (B). (A) SDS-PAGE analysis of BL21(DE3) cell extracts obtained from cells transformed with pET28c harboring the *cinA* gene and collected before (b), and after 3 hours (c) and 12 hours (d) IPGT induction. The arrow indicates the position of the recombinant protein (B) SDS-PAGE analysis of recombinant YgaD, YfaY and YdeJ purified from extracts of overexpressing cells. Lane M: molecular mass standards.

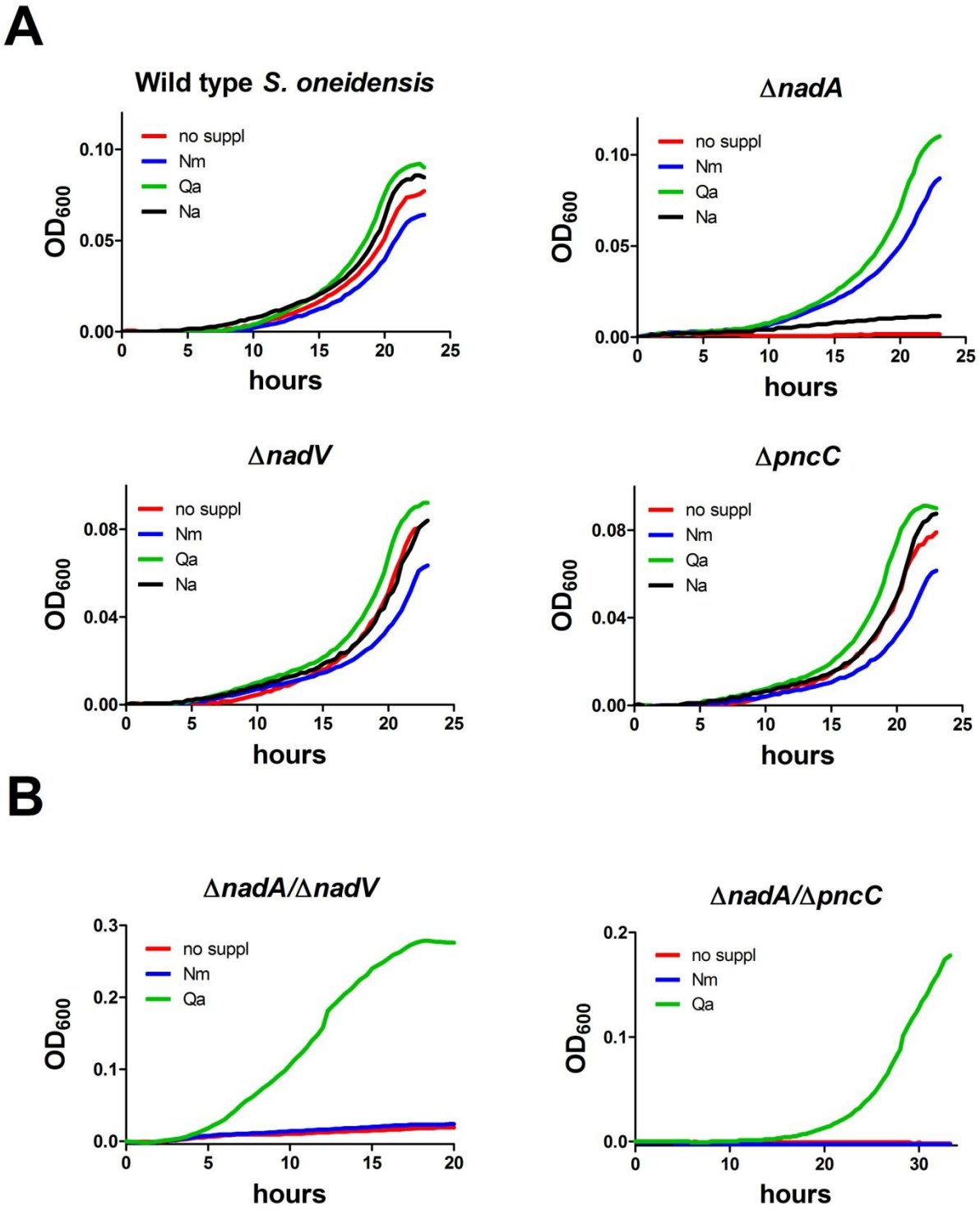

Figure S3. Growth of *S. oneidensis* wild-type and *nadA, nadV, pncC, nadA/nadV, nadA/pncC* gene deletion mutants on various media. Minimal media were supplemented with 200 μM each of the indicated compounds.

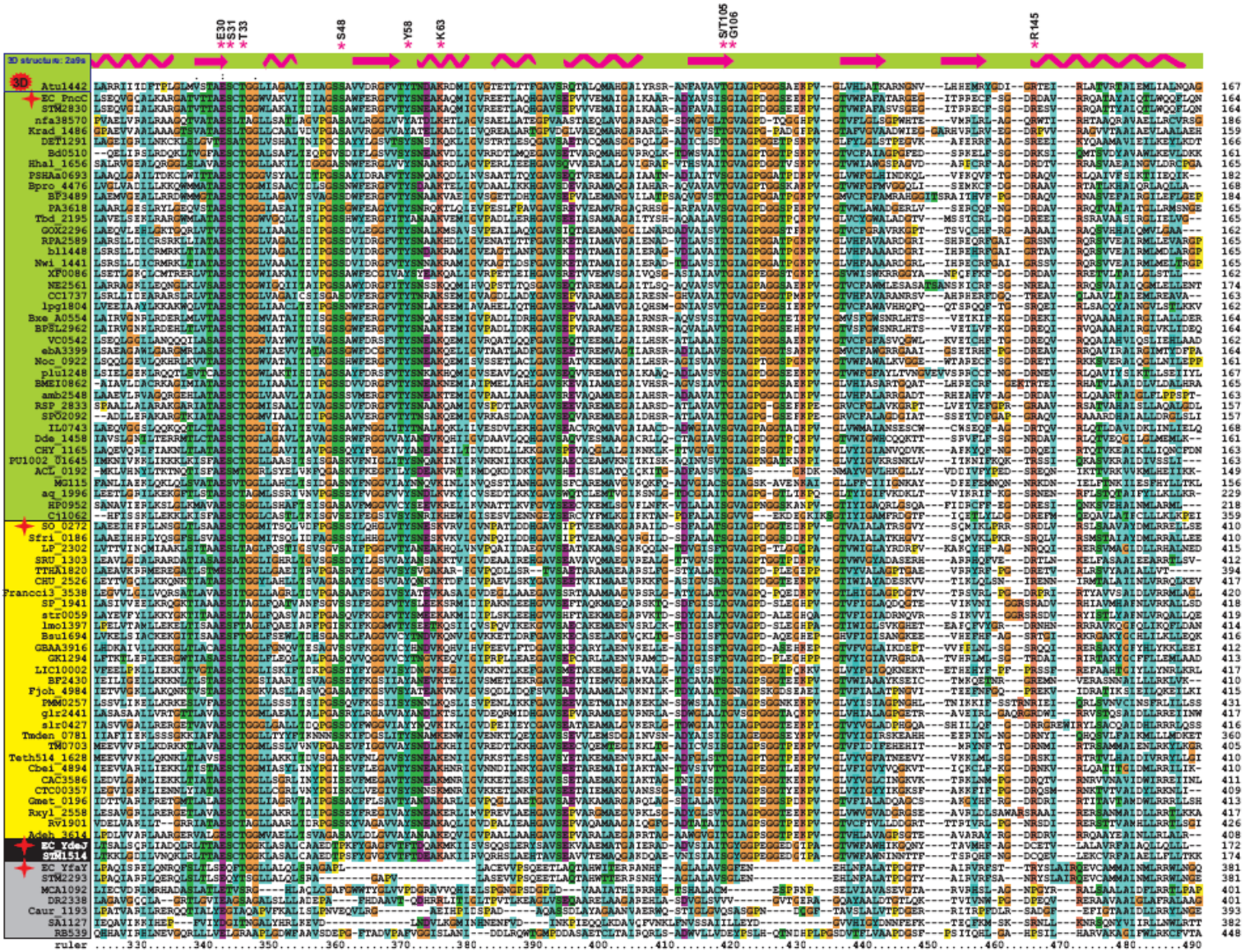

Figure S4. Multiple alignment of PncC domains identified in 86 bacterial genomes.
Secondary structure elements mapped according to the known 3D structure of *A. tumefaciens* are shown by arrows (α-helices) and zigzags (β-strands). PncC domains predicted to be functional are marked by background green (for single-domain proteins) or yellow (for two-domain proteins). Non-functional domains are marked in gray (for two-domain proteins) and black (for single-domain proteins). Proteins experimentally characterized in this work are marked by a red star. Conserved residues predicted to be involved in NMN stabilization are marked by an asterisk. The complete list of analyzed bacterial genomes and gene identificators (locus tags) is provided in Figure 7.

Figure S5. Neighbor-joining phylogenetic tree constructed for PncC domains identified in 86 bacterial genomes. The bootstrapping values with 1000 replicates are shown by numbers on each branch. The tree was constructed using ClustalW. Genome abbreviations are listed in Figure 7. Experimentally characterized proteins are marked by a red star. Color code reflects domain structure and likely enzymatic activity of NMN deamidase: green, single-domain functional enzyme; yellow, two-domain functional enzyme; gray, two-domain non-functional enzyme; black, single-domain non-functional enzyme. Experimentally characterized proteins are marked by a red star.

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