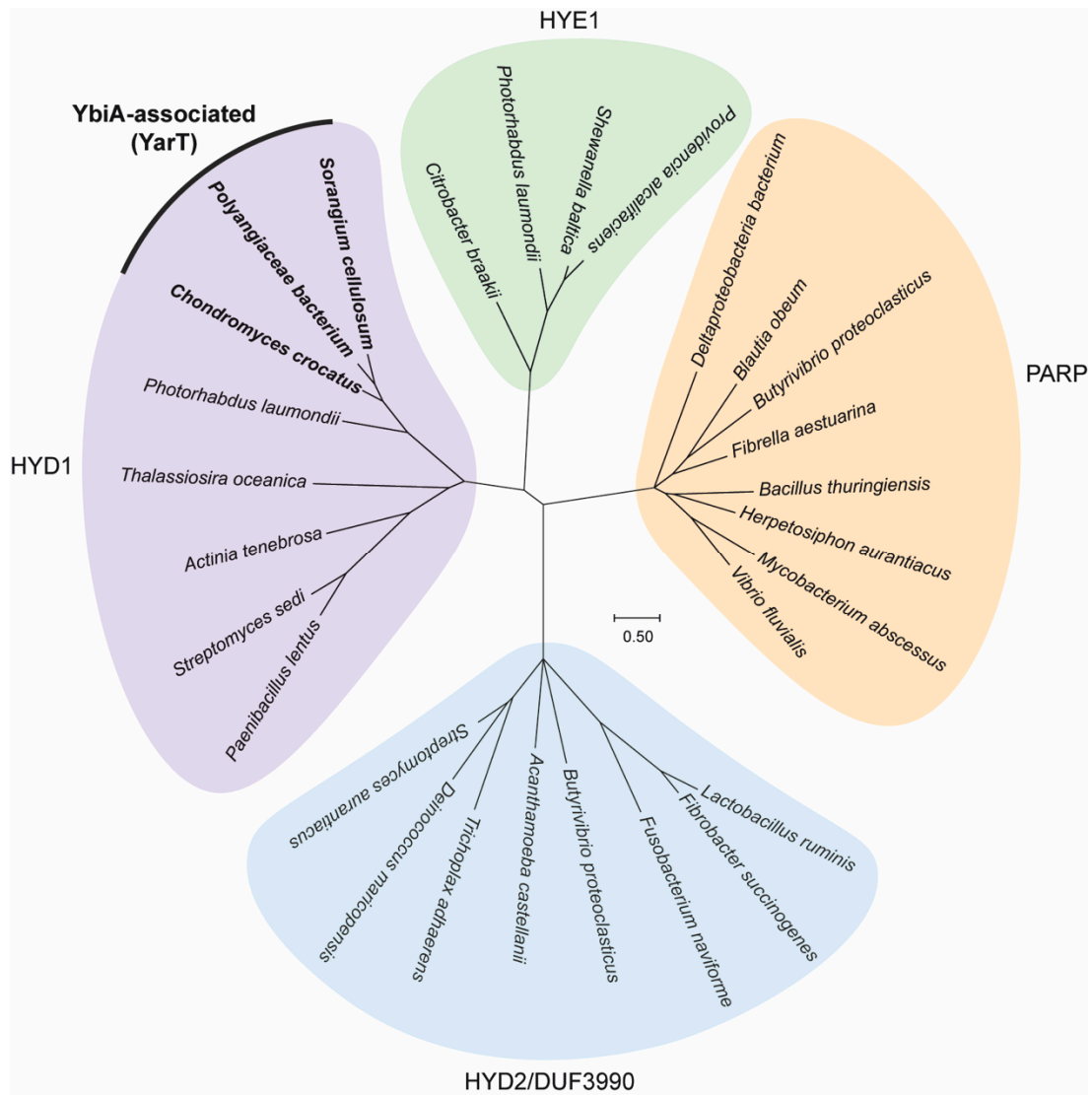


# Specificity of DNA ADP-Ribosylation Reversal by NADARs



**Figure S1. Phylogenetic tree of bacterial ART toxins.** The YbiA-associated YarT ARTs cluster within the HYD1 subfamily. NCBI accession IDs are provided in Table S3.

**Table S1. NCBI accession IDs related to Figure 1.**

Species	Accession number
<b>Bacterial YbiA-like</b>	
<i>Chondromyces crocatus</i>	WP_179955513.1
<i>Escherichia coli</i>	WP_001145126.1
<i>Salmonella enterica</i>	WP_140040215.1
<i>Listeria monocytogenes</i>	HAA3934926.1
<b>Bacterial DarT-associated</b>	
<i>Escherichia coli</i>	WP_032219797.1
<i>Geobacter lovleyi</i>	WP_012470628.1
<i>Sinorhizobium fredii</i>	WP_014330845.1
<b>Bacterial stand-alone</b>	
<i>Escherichia coli</i>	WP_001183948.1
<i>Pseudomonas aeruginosa</i>	WP_116806626.1
<i>Streptomyces coelicolor</i>	BDD75137.1
<b>Phage</b>	
<i>Escherichia coli</i> T4 phage	NP_049816.1
<i>Klebsiella</i> phage vB KpnM KpV477	YP_009288818.1
<i>Rhizobium</i> phage RL38J1	QGZ13929.1
<b>Fungi</b>	
<i>Fomitopsis pinicola</i>	EPS93933.1
<i>Fomitopsis betulina</i>	KAI0715550.1
<b>Plant</b>	
<i>Arabidopsis thaliana</i>	VYS59636.1
<i>Theobroma cacao</i>	EOY09810.1
<i>Cannabis sativa</i>	KAF4360218.1
<b>Archaea</b>	
<i>Methanobrevibacter ruminantium</i>	WP_012956765.1
<i>Candidatus Lokiarchaeota</i>	MBD3226909.1
<i>Thermoplasmata</i>	MBE6519942.1
<b>Nematode</b>	
<i>Ancylostoma caninum</i>	RCN47812.1
<i>Loa loa</i>	XP_003139559.1
<i>Trichinella papuae</i>	KRZ69666.1
<b>Cephalochordate</b>	
<i>Brachiostoma floridae</i>	XP_035698547
<i>Brachiostoma belcheri</i>	XP_019631451
<b>Echinoderm</b>	
<i>Purple sea urchin</i>	XP_793312
<i>Painted urchin</i>	XP_054764016
<i>Crown-of-thorns starfish</i>	XP_022087852
<b>Insect</b>	
<i>Bradysia coprophila</i>	XP_037037289
<i>Seven-spotted ladybird</i>	XP_044764571

<i>Folsomia candida</i>	XP_021960032
<b>Crustacea</b>	
<i>Eurytemora affinis</i>	XP_023334262
<b>Mollusca</b>	
<i>Golden apple snail</i>	XP_025111217
<i>Northern quahog</i>	XP_045159950
<i>Peltospirid snail</i>	XP_041358894
<b>Cnidaria</b>	
<i>Dendronephthya gigantea</i>	XP_028391651
<b>Porifera</b>	
<i>Amphimedon queenslandica</i>	XP_019853895

**Table S2. NCBI accession IDs related to Figure 3.**

Species	Accession number
<b>YarT-associated YbiA proteins</b>	
<i>Chondromyces crocatus</i>	WP_179955513.1
<i>Polyangiaceae bacterium</i>	NUQ75852.1
<i>Sorangium cellulosum</i>	WP_020736121.1
<b>YbiA proteins</b>	
<i>Escherichia coli</i>	WP_001145126.1
<i>Salmonella enterica</i>	WP_140040215.1
<i>Listeria monocytogenes</i>	HAA3934926.1
<i>Streptomyces griseiscabiei</i>	WP_086756638.1
<i>Gimesia chilikensis</i>	QDT22974.1
<b>DarT-associated NADAR proteins</b>	
<i>Escherichia coli</i>	WP_001183948.1
<i>Geobacter lovleyi</i>	WP_012470628.1
<i>Sinorhizobium fredii</i>	WP_014330845.1
<i>Vibrio cholerae</i>	WP_172778105.1
<i>Achinetobacter baumannii</i>	WP_001129309.1
<b>Stand-alone NADAR proteins</b>	
<i>Escherichia coli</i>	WP_032219797.1
<i>Pseudomonas aeruginosa</i>	WP_116806626.1
<i>Streptomyces coelicolor</i>	BDD75137.1
<i>Methylococcaceae bacterium</i>	NOQ36470.1
<i>Mycobacterium tuberculosis</i>	CNF61934.1
<b>Phage NADAR proteins</b>	
<i>Escherichia coli</i> T4 phage	NP_049816.1
<i>Klebsiella</i> phage vB KpnM KpV477	YP_009288818.1
<i>Rhizobium</i> phage RL38J1	QGZ13929.1

**Table S3. NCBI accession IDs related to Figure S1.**

Species	Accession number
<b>HYD1</b>	
<i>Chondromyces crocatus</i>	WP_050429593.1
<i>Polyangiaceae bacterium</i>	NUQ75853 .1
<i>Sorangium cellulosum</i>	WP_020736122 .1
<i>Paenibacillus lentus</i>	AZK48993.1
<i>Streptomyces sedi</i>	WP_139640642.1
<i>Photorhabdus laumondii</i>	WP_011144745.1
<i>Thalassiosira oceanica</i>	EJK58845.1
<i>Actinia tenebrosa</i>	XP_031550746.1
<b>HYD2</b>	
<i>Deinococcus maricopensis</i>	WP_148234928.1
<i>Streptomyces aurantiacus</i>	WP_190851997.1
<i>Fusobacterium naviforme</i>	KAB0576670.1
<b>DUF3990</b>	
<i>Lactobacillus ruminis</i>	WP_014073068.1
<i>Butyrivibrio proteoclasticus</i>	WP_013281589.1
<i>Fibrobacter succinogenes</i>	WP_014546709.1
<i>Trichoplax adhaerens</i>	XP_002114834.1
<i>Acanthamoeba castellanii</i>	XP_004358258.1
<b>HYE1</b>	
<i>Shewanella baltica</i>	WP_006083171.1
<i>Citrobacter braakii</i>	WP_149330609.1
<i>Photorhabdus laumondii</i>	WP_011145957.1
<i>Providencia alcalifaciens</i>	WP_207910800.1
<b>PARP</b>	
<i>Bacillus thuringiensis</i>	EEM25277.1
<i>Fibrella aestuarina</i>	WP_041257497.1
<i>Mycobacterium abscessus</i>	WP_271960408.1
<i>Vibrio fluvialis</i>	WP_020332344.1
<i>Deltaproteobacteria bacterium</i>	MBI1947919.1
<i>Herpetosiphon aurantiacus</i>	ABX07394.1
<i>Blautia obeum</i>	WP_055060497.1
<i>Butyrivibrio proteoclasticus</i>	WP_013282825.1

**Table S4. Oligonucleotides used in this study.**

Oligo	Sequence (5'-3')	Purpose
PolyT-G	TTTTTTGTTTTTTTTTTTTT	ADP-ribosylation activity assay
DarT-27mer-3	CACGACACGAGCAGGCATGTCCACGTG	ADP-ribosylation activity assay
GJ-P22	GAGCTGTACAAGTCAGATCTCGAGCTC	ADP-ribosylation activity assay
Protein X_fwd	ctagcgaattcgagctcatgGGCAGCAGCCATCATCATC	Cloning of <i>C. crocatus</i>
Protein X_rev	ccgcaaaacagccaagcttTTAGAACGGGTTCGGGCC	Protein X into

pBAD33_fwd	AAGCTTGGCTGTTTTGGC	pBAD33 by Gibson Assembly
pBAD33_rev	CATGAGCTCGAATTCGCTAG	Vector amplification for Gibson Assembly

**Table S5. Strains and plasmids used in this study.**

Strain	Description	Source
DH5α	<i>huA2 a(argF-lacZ)U169 phoA glnV44 a80a(lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17</i>	NEB
BL21 (DE3)	<i>fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS λ DE3 = λ sBamHI ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5</i>	NEB
Rosetta™ BL21 (DE3)	<i>F-ompT hsdSB(rB- mB-) gal dcm (DE3) pRARE (cam<sup>R</sup>)</i>	Novagen
<b>Plasmid</b>		
pET28a	Medium copy plasmid containing the IPTG-inducible promoter; kan <sup>R</sup>	Novagen
pBAD33	Medium copy plasmid with an arabinose-inducible promoter; cam <sup>R</sup>	Guzman et al., 1995 [62]
pBAD33_Taq_darT2	pBAD33 carrying <i>T. aquaticus darT</i> full-length; cam <sup>R</sup>	Jankevicius et al., 2016 [18]
pET28_Taq_darG_macro	pET28a carrying <i>T. aquaticus darG</i> macrodomain (aa 1-155); kan <sup>R</sup>	Jankevicius et al., 2016 [18]
pET28_SC_SCO5461	pET28a carrying <i>S. coelicolor scarp (SCO5461)</i> full-length; kan <sup>R</sup>	Lalić, J. et al., 2016 [19]
pET28_EPEC_nadar	pET28a carrying EPEC <i>nadar</i> full-length; kan <sup>R</sup>	This study
pET28_Ecoli_nadar	pET28a carrying <i>E. coli</i> C7 <i>nadar</i> full-length; kan <sup>R</sup>	Schuller et al., 2023 [33]
pET28_T4_nadar	pET28a carrying <i>E. coli</i> T4 phage (gp30.3) <i>nadar</i> full-length; kan <sup>R</sup>	This study
pET_Ecoli_ybia	pET28a carrying <i>E. coli</i> K-12 <i>ybiA</i> full-length; kan <sup>R</sup>	This study
pBAD33_Ecoli_darT1	pBAD33 carrying <i>E. coli</i> C7 <i>darT1</i> full-length; cam <sup>R</sup>	Schuller et al., 2023 [33]
pBAD33_Croc_nadar	pBAD33 carrying <i>C. crocatus nadar</i> full-length; cam <sup>R</sup>	This study
pBAD33_Croc_yarT	pBAD33 carrying <i>C. crocatus yarT</i> full-length; cam <sup>R</sup>	This study