Supporting Information

Rational identification of catalytically promiscuous nitrilase by predicting a unique catalytic triad motif feature through an *in-silico* strategy

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Table S1 Information on sequences used in phylogenetic analysis.

	UniProt ID	Submitted name	Organism	Length
CHT	P32964	Cyanide hydratase	*Microdochium sorghi*	368
	A9QXE0	Cyanide hydratase	*Aspergillus niger*	356
	P32963	Cyanide hydratase	*Gibberella baccata*	355
	C8V9R7	Cyanide hydratase	*Emericella nidulans*	364
	Q7RVT0	Cyanide hydratase	*Neurospora crassa*	351
	Q96UG7	Cyanide hydratase	*Fusarium solani*	363
	P9WEU5	Cyanide hydratase	*Stereum hirsutum*	371
	E3RV84	Cyanide hydratase	*Pyrenophora teres f. teres*	366
	G2XQT1	Cyanide hydratase	*Botryotinia fuckeliana*	371
	I1RP53	Cyanide hydratase	*Gibberella zeae*	355
	B6HCY4	Cyanide hydratase	*Penicillium rubens*	358
	A0A2H3E4G0	Cyanide hydratase	*Armillaria gallica*	356
	Q7Z8M6	Cyanide hydratase	*Alternaria brassicicola*	363
	Q9P8V3	Cyanide hydratase	*Leptosphaeria maculans*	356
CP-NLase	V5IPE4	Arylacetonitrilase	*Neurospora crassa*	327
	C7YS90	Arylacetonitrilase	*Fusarium vanettenii*	339
	P9WEU6	Arylacetonitrilase	*Trametes versicolor*	320
	G9AIU0	Aliphatic nitrilase	*Rhizobium fredii*	308
	Q19A54	Nitrilase	*Gibberella moniliformis*	320
	G7XTA8	Aromatic nitrilase	*Aspergillus kawachii*	320
	Q5EG61	NitA	*Pseudomonas fluorescens*	350
	Q55949	Nitrilase	*Synechocystis sp.*	341
	C5XY71	Nitrilase	*Sorghum bicolor*	361
NLase	A2R6M7	Arylacetonitrilase	*Aspergillus niger*	337
	D4B1Q8	Arylacetonitrilase	*Arthroderma benhamiae*	335
	K2QXC4	Arylacetonitrilase	*Macrophomina phaseolina*	344
	G7X8S6	Arylacetonitrilase	*Aspergillus kawachii*	328
	A0A0P1DJE3	Arylacetonitrilase	*Auricularia subglabra*	331
	Q2U4D6	Arylacetonitrilase	*Aspergillus oryzae*	333
	A2RA31	Arylacetonitrilase	*Aspergillus niger*	328
	G9N4E3	Arylacetonitrilase	*Hypocrea virens*	329
	Q9UYV8	Nitrilase	*Pyrococcus abyssi*	262
	Q89GE3	Nitrilase bll6402	*Bradyrhizobium diazoefficiens*	334
	P20960	Nitrilase, arylacetone-specific	*Alcaligenes faecalis*	356
	Q500U1	Nitrilase	*Pseudomonas syringae pv. Syringae*	336
	B6Q5I3	Nitrilase	*Talaromyces marneffei*	322
	B6HVR6	Nitrilase	*Penicillium rubens*	328
	A5DNJ4	Nitrilase	*Meyerozyma guilliermondii*	317
	Q89PT3	Nitrilase blr3397	*Bradyrhizobium diazoefficiens*	321
	Q02068	Aliphatic nitrilase	*Rhodococcus rhodochrous*	383

Table S2 Information on reported crystal structures used in this study.

UniProt ID	PDB code	Organism	Length	Resolution (Å)	Method
Q9UYV8	3ivz	*Pyrococcus abyssi GE5*	262	1.57	X-RAY DIFFRACTION
P46011	6i00	*Arabidopsis thaliana*	361	3.4	ELECTRON MICROSCOPY
Q55949	3wuy	*Synechocystis sp. PCC 6803 substr. Kazusa*	349	3.1	X-RAY DIFFRACTION
Q5EG61	6zby	*Pseudomonas fluorescens*	350	3.1	ELECTRON MICROSCOPY

Table S3 Information on potential CP-NLase candidates.

UniProt ID	Submitted name	Organism	Length
A0A1W2TCV9	Putative aliphatic nitrilase	*Rosellinia necatrix*	323
A0A378TBT1	Nitrilase 2	*Mycolicibacterium tokaiense*	316
A0A182BSR4	Nitrilase	*Gordonia terrae*	366
A0A653FFS7	Nitrilase	*Mycolicibacterium smegmatis*	341
A0A385ZDY9	Nitrilase	*Streptomyces griseorubiginosus*	339
Q1W2L4	Nitrilase	*Acidovorax facilis*	369
S5PQ64	Nitrilase	*Gordonia terrae*	366
H9NN54	Nitrilase	*Nocardia globerula*	366
G1FTR4	Nitrilase	*Rhodococcus sp. BX2*	366
F6GDZ2	Nitrilase	*Lacinutrix sp.*	316
F4CZ44	Nitrilase	*Pseudonocardia dioxanivorans*	349
A0A085F6G6	Nitrilase	*Bosea sp. LC85*	323
C7CG56	Putative nitrilase	*Methylorubrum extorquens*	305
B6R8T8	Nitrilase, putative	*Pseudovibrio sp. JE062*	331
B6R8T7	Nitrilase	*Pseudovibrio sp. JE062*	332
A0A0U1TDG4	Regioselective nitrilase	*Acidovorax facilis*	372
A0A7Y9DS80	Nitrilase	*Actinomycetospora corticicola*	346
A0A839SAC7	Nitrilase	*Mucilaginibacter gotjawali*	318
G0LAG6	Nitrilase	*Zobellia galactanivorans*	316
A0A099KNW3	Nitrilase	*Thalassotalea sp. ND16A*	324
A0A517WTX7	Nitrilase	*Gimesia aquarii*	347
A0A7W8A8A8	Nitrilase	*Nonomuraea endophytica*	306
A0R378	Nitrilase	*Mycolicibacterium smegmatis*	341
A0A7W0CD04	Nitrilase	*Nonomuraea soli*	303
F0RCY1	Nitrilase	*Cellulophaga lytica*	316
Q0AQN1	Nitrilase	*Maricaulis maris*	310

E6X4J4	Nitrilase	*Cellulophaga algicola*	316
A0A517W1X2	Nitrilase	*Gimesia aquarii*	357
A0A2Z4LQF7	Nitrilase	*Muricauda aurantiaca*	317
L2G5Z2	Arylacetonitrilase	*Colletotrichum fructicola gloeosporioides*	372
A0A2N6NA38	Arylacetonitrilase	*Beauveria bassiana*	320
A0A329T4D1	Arylacetonitrilase	*Phytophthora cactorum*	341
A0A4T0WFD2	Arylacetonitrilase	*Colletotrichum higginsianum*	371
A0A6A4AXL9	Arylacetonitrilase	*Phytophthora rubi*	309
A0A6A3GCP7	Arylacetonitrilase	*Phytophthora rubi*	289
A0A6A3GEF2	Arylacetonitrilase	*Phytophthora rubi*	309
A0A177DUN2	Arylacetonitrilase	*Alternaria alternata*	342
A0A4Q4S236	Arylacetonitrilase	*Alternaria tenuissima*	343
A0A420QY53	Arylacetonitrilase	*Fusarium oxysporum*	326
A0A420MER3	Arylacetonitrilase	*Fusarium oxysporum*	326
A0A3L6MVK0	Arylacetonitrilase	*Fusarium oxysporum f. sp. Cepae*	326
A0A6V8R3E7	Arylacetonitrilase	*Trichoderma asperellum*	309

Table S4 The sequence similarity of putative CP-arylacetonitrilase candidates.

	UniProt ID	1	2	3	4	5	6	7	8	9	10	11	12	13
1	**L2G5Z2**		91.4	24.73	25	21.77	25	25	27.69	14.78	27.69	27.69	26.88	22.04
2	**A0A4T0WFD2**	91.4		24.19	24.19	22.04	24.73	24.73	27.69	16.13	27.69	27.69	27.96	23.12
3	**A0A4Q4S236**	24.73	24.19		97.58	30.91	28.76	29.03	27.42	27.96	27.96	27.96	24.19	27.42
4	**A0A177DUN2**	25	24.19	97.58		30.91	29.03	29.3	27.69	28.23	28.23	28.23	24.46	27.69
5	**A0A329T4D1**	21.77	22.04	30.91	30.91		29.57	29.57	26.34	25.27	26.34	26.34	25	42.74
6	**A0A6A3GEF2**	25	24.73	28.76	29.03	29.57		99.46	34.68	31.99	34.41	34.41	35.75	48.39
7	**A0A6A4AXL9**	25	24.73	29.03	29.3	29.57	99.46		34.41	31.99	34.14	34.14	35.75	48.12
8	**A0A420QY53**	27.69	27.69	27.42	27.69	26.34	34.68	34.41		30.65	99.46	99.46	56.72	32.26
9	**A0A6A3GCP7**	14.78	16.13	27.96	28.23	25.27	31.99	31.99	30.65		30.65	30.65	35.22	31.72
10	**A0A3L6MVK0**	27.69	27.69	27.96	28.23	26.34	34.41	34.14	99.46	30.65		100	56.45	31.72
11	**A0A420MER3**	27.69	27.69	27.96	28.23	26.34	34.41	34.14	99.46	30.65	100			31.72
12	**A0A6V8R3E7**	26.88	27.96	24.19	24.46	25	35.75	35.75	56.72	35.22	56.45	56.45	100	30.91
13	**A0A2N6NA38**	22.04	23.12	27.42	27.69	42.74	48.39	48.12	32.26	31.72	31.72	31.72	30.91	

Table S5 The enantioselectivity of reported NLase or NHase toward mandelonitrile.

Enzyme	Organisms	ee (%)	References
Nitrile hydratase	*Rhodococcus rhodochrous* ATCC BAA-87	*R*, 81	[1]
Nitrile hydratase	*Klebsiella oxytoca* 38.1.2	*S*, 89	[2]
Nitrile hydratase	*Rhodococcus rhodochrous* J1	*S*, 52.6	[3]
Mutant of nitrile hydratase	*Rhodococcus rhodochrous* J1	*S*, 96.8	[3]

NLase	*Neurospora crassa* OR74A	*S*, 51 (amide ratio, 15%)	[4]
NLase	*P. fluorescens* EBC191	*S*, 84 (amide ratio, 19%)	[5]
NitFO	*Fusarium oxysporum*	*R*, 90.3 (amide ratio, 16%)	This study
NitAT	*Alternaria tenuissima*	*S*, 96.5 (amide ratio, 28%)	This study

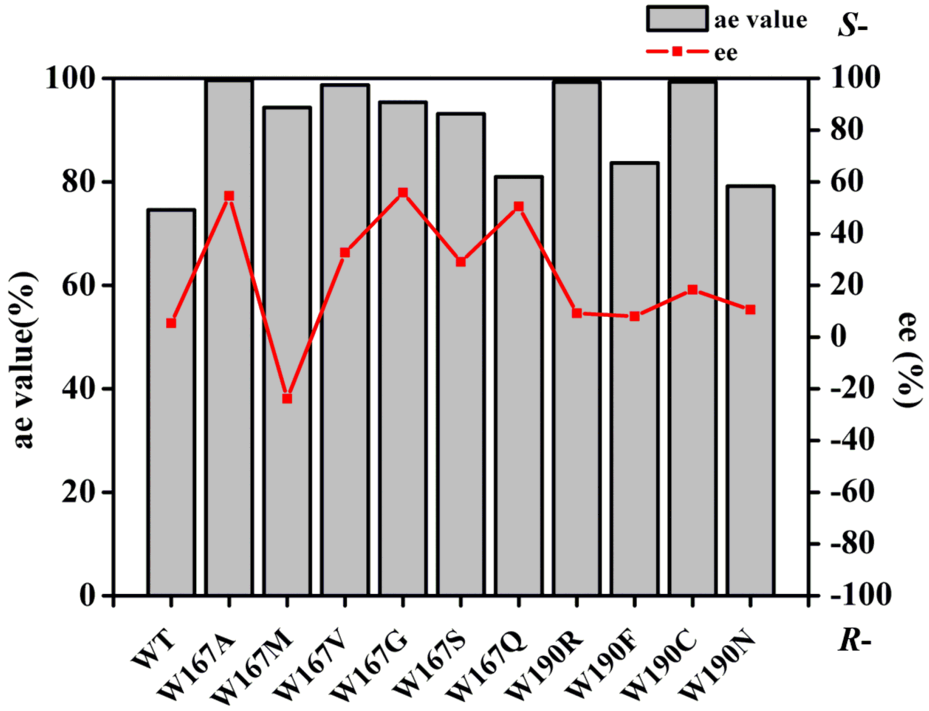

Fig. S1 The enantioselectivity of beneficial mutants.

Table S6 The substrate tolerance of NitPC-W167A toward mandelonitrile.

Substrate (mM)	Reaction time (h)	Yield (%)
50	4	100
100	16	100
150	24	10.2
200	24	4.3

References

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