Electronic Supplementary Information

High-level expression of nitrile hydratase in *Escherichia coli* for 2-amino-2,3-dimethylbutyramide synthesis

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Materials and Methods

Batch reaction for the hydration of ADBN to ADBA

The HFE-7100/H_2O (10 %, v/v) biphasic system used for hydration of ADBN contained 120 mM ADBN, 30 U/mL of whole-cells in a total volume of 25 mL. The reaction was conducted at 20 ℃ and 200 rpm. Samples were taken at indicated time points and analyzed by GC. After reaction finished, the reaction mixture was centrifuged at 8, 000 rpm for 10 min. The upper aqueous phase was removed, and the HFE-7100 phase and whole-cells were reserved. 375 uL of ADBN plus 22.5 mL of distilled water (pH 7.0) were supplemented for the next batch.

Table S1. Recombinant NHase library conserved in this lab.

Source	GenBank number	Restriction enzymes	Strain /plasmid
Burkholderia phymatum STM815	NC_010623	*Bam*HI/*Hind*III	*E.coli* BL21(DE3)/pET28a
Zymomonas mobilis subsp. mobilis ZM4	AE008692	*Nde*I/*Hind*III	*E.coli* BL21(DE3)/pET28a
Sphingomonas wittichii RW1	YP_001264656	*Nde*I/*Hind*III	*E.coli* BL21(DE3)/pET28a
Rhodopseudomonas palustris CGA009	NC_005296	*Nco*I/*Hind*III	*E.coli* BL21(DE3)/pET24a
Burkholderia graminis C4D1M	NZ_ABLD01000011	*Nde*I/*Hind*III	*E.coli* BL21(DE3)/pET28a
Methylibium petroleiphilum PM1	NC_008825	*Nde*I/*Hind*III	*E.coli* BL21(DE3)/pET28a
Roseobacter sp. GAI101	NZ_DS999213	*Nde*I/*Hind*III	*E.coli* BL21(DE3)/pET28a
Pseudomonas protegens Pf-5	NC_004129	*Nde*I/*Hind*III	*E.coli* BL21(DE3)/pET28a
Burkholderia ambifaria AMMD	NC_008390	*Nde*I/*Hind*III	*E.coli* BL21(DE3)/pET28a
Neosartorya fischeri NRRL 181	XM_001261814	*Nde*I/*Hind*III	*E.coli* BL21(DE3)/pET28a
Aspergillus clavatus NRRL 1	XM_001276026	*Nde*I/*Hind*III	*E.coli* BL21(DE3)/pET28a
Bordetella bronchiseptica RB50	BX640440	*Nde*I/*Hind*III	*E.coli* BL21(DE3)/pET28a
Arthrobacter aurescens TC1	NC_008711	*Nde*I/*Hind*III	*E.coli* BL21(DE3)/pET28a
Bacillus pumilus ATCC 7061	NZ_ABRX01000005	*Nde*I/*Bam*HI	*E.coli* BL21(DE3)/pET28a
Burkholderia cenocepacia J2315	YP_002231697	*Bam*HI/*Hind*III	*E.coli* M15/pQE30
Stappia aggregata IAM 12614	ZP_01549810	*Nde*I/*Bam*HI	*E.coli* BL21(DE3)/pET28a
Bradyrhizobium sp. ORS 278	YP_001206496	*Nde*I/*Hind*III	*E.coli* BL21(DE3)/pET28a
Bradyrhizobium sp. BTAi1	YP_001240698	*Bam*HI/*Hind*III	*E.coli* M15/pQE30
Nocardia farcinica IFM 10152	YP_119480	*Nde*I/*Hind*III	*E.coli* BL21(DE3)/pET28a
Burkholderia xenovorans LB400	NC_007951	*Nde*I/*Hind*III	*E.coli* BL21(DE3)/pET28a
Shewanella pealeana ATCC 700345	NC_009901	*BamHI/NotI*	*E.coli BL21(DE3)/*pET28a
Pseudonocardia thermophile JCM3095	DD029959.1	*Nde*I/*Hind*III	*E.coli* BL21(DE3)/pET21a
Alcaligems faecalis ATCC8750	P20960	*Nde*I/*Hind*III	*E.coli* BL21(DE3)/pET21a
Pseudomonas putida MTCC5110	ABV21758	*Nde*I/*Xho*I	*E.coli* BL21(DE3)/pET21a

Table S2. Effect of different carbon sources on cell growth and NHase expression.

Carbon source	OD_{600}	Activity (U/mL)	Specific activity(U/mg)
Sorbitol	**12.44±0.20**	**0.88±0.017**	**0.46±0.021**
Glycerol	12.18±0.72	0.63±0.011	0.38±0.013
Dextrin	8.34±0.38	0.43±0.018	0.49±0.027
Starch	7.36±0.48	0.43±0.052	0.50±0.017
Sucrose	7.34±0.18	0.42±0.020	0.38±0.018
Glucose	5.42±0.44	0.16±0.0049	0.38±0.023
Lactose	7.72±0.54	0.45±0.038	0.37±0.016

Recombinant strain *E. coli* BL21(DE3)/pET-28a+NHase was cultivated in TY medium. 0.8 % (w/v) of glycerol in TY medium were substituted by 0.8 % (w/v) of sorbitol, dextrin, starch, sucrose, glucose or lactose, respectively. Induction temperature (20 °C), inducer (0.1mM IPTG), 0.5 mM Co^{2+} and cultivation time (24 h).

Table S3. Effect of different nitrogen sources on cell growth and enzyme production.

Nitrogen source	OD_{600}	Enzyme activity (U/mL)	Specific activity (U/mg protein)
Tryptone	**13.82±0.49**	**1.47±0.041**	**0.60±0.016**
Ox bone peptone	5.31±0.59	0.31±0.075	0.37±0.032
Fish peptone	4.66±0.66	0.50±0.060	0.64±0.045
Peanut powder	10.00±0.52	0.57±0.037	0.14±0.0030
Corn steep liquor	9.26±0.65	0.62±0.092	0.44±0.0081

Recombinant strain *E. coli* BL21(DE3)/pET-28a+NHase was cultivated in modified TY medium. 2 % (w/v) of tryptone in TY medium were substituted by 2.0 % (w/v) of Ox bone peptone, fish peptone, peanut powder or corn steep liquor, respectively. Induction temperature (20 °C), inducer (0.1mM IPTG), 0.5 mM Co^{2+} and cultivation time (24 h).

Table S4. Effect of the Angel yeast extract concentration on the growth and NHase expression of recombinant strains.

Angel yeast extract (%, w/v)	OD_{600}	Enzyme activity (U/mL)	Specific activity (U/mg protein)
0	1.70±0.097	0.10±0.0078	0.31±0.021
1	**13.04±0.69**	**0.98±0.052**	**0.41±0.021**
2	13.94±0.64	0.52±0.028	0.19±0.0092
3	13.56±0.65	0.46±0.034	0.17±0.013
4	13.66±0.57	0.43±0.031	0.13±0.0085

Recombinant strain *E. coli* BL21(DE3)/pET-28a+NHase was cultivated in modified TY medium. 1 % (w/v) of Oxoid yeast extract in this modified TY medium were substituted by 0-4.0 % (w/v) of Angel yeast extract. Induction temperature (20 °C), inducer (0.1mM IPTG), 0.5 mM Co^{2+} and cultivation time (24 h).

Table S5. Effects of metal ions on the growth and NHase expression of recombinant strains.

Metal ions (0.2 mM)	OD_{600}	Enzyme activity (U/mL)	Specific activity (U/mg protein)
Li^{+}	13.56±0.92	1.79±0.045	0.70±0.052
Ca^{2+}	11.86±0.55	1.68±0.057	0.68±0.050
Mg^{2+}	12.46±0.31	1.95±0.10	0.69±0.038
Zn^{2+}	13.24±0.71	1.94±0.057	0.77±0.038
Mn^{2+}	12.27±0.66	2.00±0.14	0.87±0.045
Ni^{2+}	10.40±1.02	1.79±0.086	0.74±0.050
Cu^{2+}	14.34±0.49	2.16±0.054	0.80±0.050
Fe^{3+}	14.00±0.24	1.84±0.061	0.59±0.029
Fe^{2+}	12.60±0.40	2.00±0.071	0.81±0.041

Recombinant strain *E. coli* BL21(DE3)/pET-28a+NHase was cultivated in modified TY medium. 0.2 mM of different metal ions were added to the medium. Induction temperature (20 °C), inducer (0.1mM IPTG), 0.5 mM Co^{2+} and cultivation time (24 h).

Table S6. Effect of induction time on cell growth and NHase expression of recombinant strains

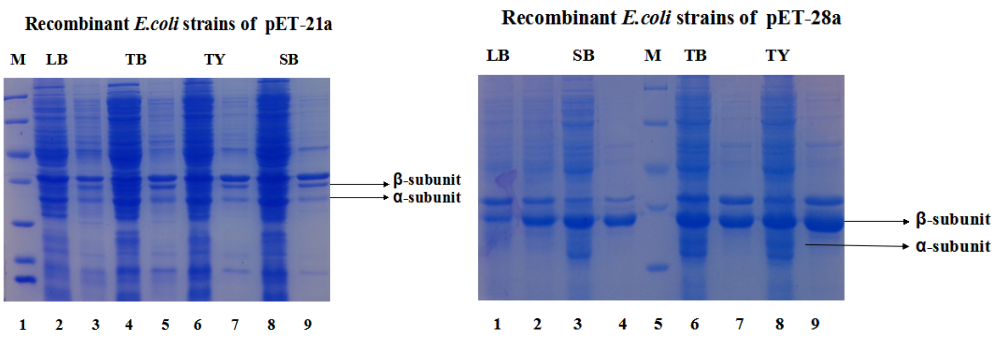
Induction time (OD_{600})	OD_{600}	Enzyme activity (U/mL)	Specific activity (U/mg protein)
0.4	11.50±0.48	1.89±0.083	0.85±0.042
0.6	12.68±0.42	1.98±0.069	0.84±0.037
0.8	**13.58±0.35**	**2.23±0.066**	**0.88±0.049**
1.0	**13.76±0.31**	**2.24±0.082**	**0.91±0.057**
1.2	13.38±0.20	2.00±0.10	0.78±0.042
1.5	12.56±0.57	1.49±0.093	0.76±0.050
2.0	11.24±0.13	1.33±0.070	0.66±0.025

Recombinant strain of *E. coli* BL21(DE3)/pET-28a+NHase was cultivated in modified TY medium and induced by IPTG at different time (OD_{600}). Induction temperature (20 °C), inducer (0.1mM IPTG), 0.5 mM Co^{2+} and cultivation time (24 h).

Table S7. Effect of feeding medium on cell growth and enzyme activity.

Feeding medium		24 h	36 h	48 h
Glycerol	OD_{600}	14.37±0.81	17.40±1.05	15.60±0.89
	Enzyme activity (U/mL)	2.62±0.15	2.57±0.13	1.76±0.081
	Productivity (U/mL/h)	0.11±0.0063	0.07±0.0036	0.04±0.0017
	Specific activity (U/mg protein)	1.09±0.06	1.19±0.07	0.97±0.06
Tryptone	OD_{600}	14.97±0.97	**18.39±0.96**	17.79±1.05
	Enzyme activity (U/mL)	1.95±0.10	**3.33±0.20**	2.27±0.12
	Productivity (U/mL/h)	0.08±0.0041	**0.09±0.0056**	0.05±0.0025
	Specific activity (U/mg protein)	1.04±0.05	**1.13±0.10**	1.02±0.05
Glycerol and tryptone	OD_{600}	14.67±0.81	17.01±1.03	16.83±0.99
	Enzyme activity (U/mL)	1.81±0.11	2.38±0.13	2.26±0.15
	Productivity (U/mL/h)	0.08±0.0043	0.07±0.0037	0.05±0.0032
	Specific activity (U/mg protein)	1.09±0.083	1.12±0.082	0.10±0.071

Recombinant strain *E. coli* BL21(DE3)/pET-28a+NHase was cultivated in the optimized medium. 0.8 % (w/v) glycerol, 1 % (w/v) tryptone and 0.8 % (w/v) glycerol+ 1 % (w/v) tryptone were added to the medium at 8 and 16 h after induction respectively; Conditions: induction temperature (20 °C), inducer (0.1 mM IPTG) and 0.5 mM Co^{2+}.



(a) (b)

Figure S1 Effect of recombinant strains and basic media on protein production. **(a)** Effect of pET-21a on protein production. Lane M (1): protein molecular weight marker, from top to bottom 116, 66.2, 45, 35, 25, 18.4, 14.4 kDa; Lane 2, 4, 6 and 8: soluble fraction of LB, TB, TY, SB; Lane 3, 5, 7 and 9: insoluble fraction of LB, TB, TY, SB; **(b)** Effect of pET-28a on protein production. Lane M (5): protein molecular weight marker, from top to bottom 116, 66.2, 45, 35, 25 kDa; Lane 1, 3, 6 and 8: soluble fraction of LB, SB, TB, TY; Lane 2, 4, 7 and 9: insoluble fraction of LB, SB, TB, TY.