Supplementary Materials

Application Potential of Cyanide Hydratase from *Exidia Glandulosa*: Free Cyanide Removal from Simulated Industrial Effluents

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Contents:

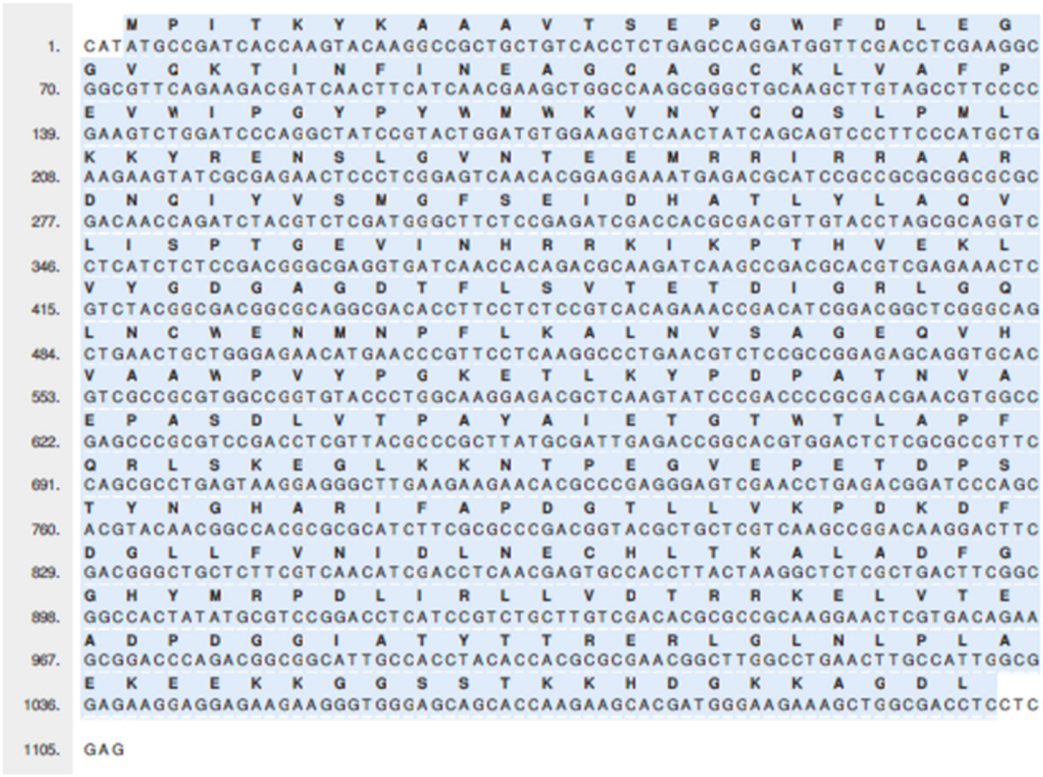

Figure S1. Optimized sequence of *cynH* gene from *Exidia glandulosa.* Gene optimization in terms of *E. coli* codon usage and optimized gene synthesis were carried out by GeneArt (ThermoFisher), Regensburg, Germany.

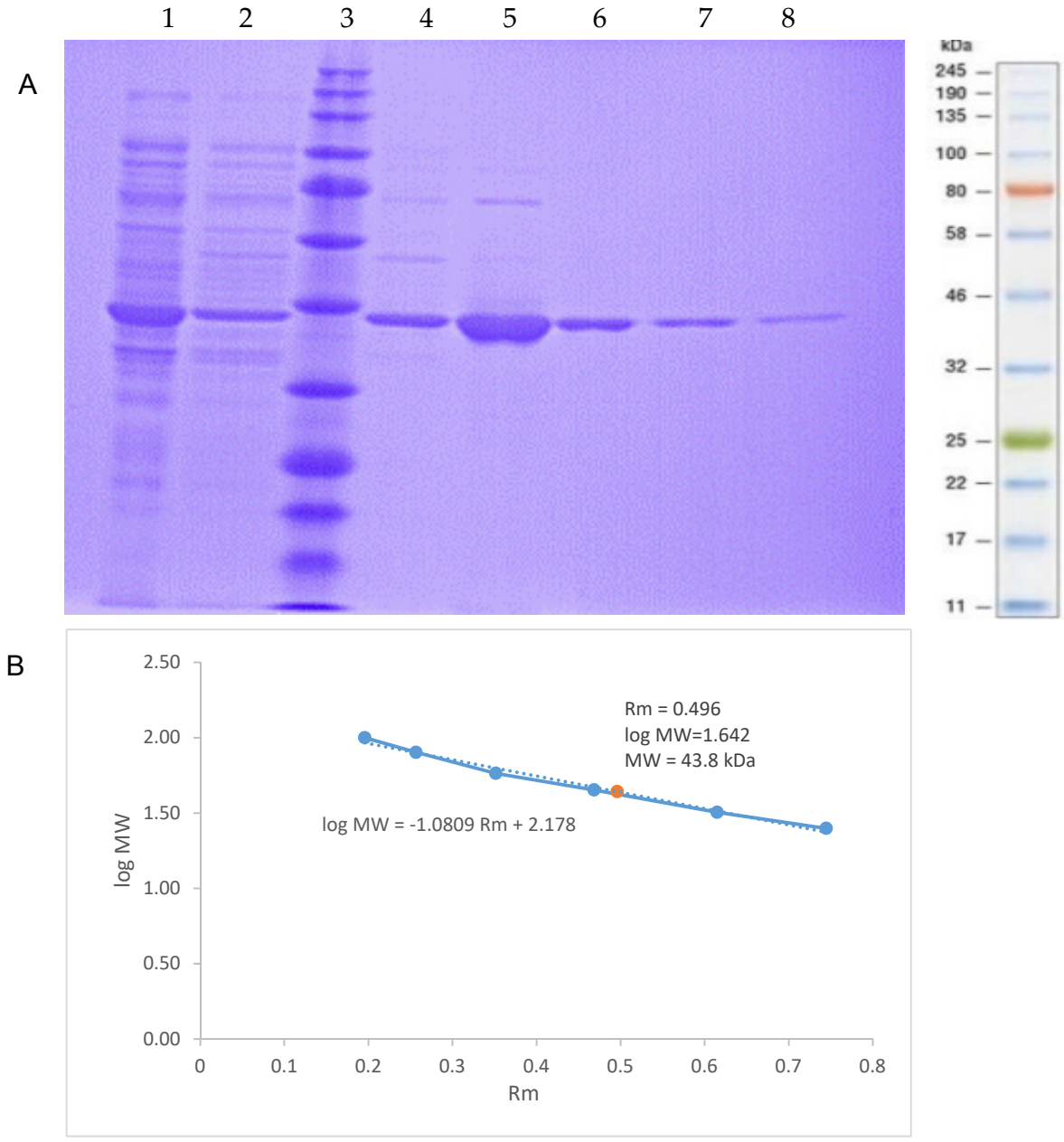

Figure S2. (**A**) Left, SDS-PAGE of NitEg in 10% polyacrylamide gels. (1) Cell-free extract, (2) column wash, (3) marker, (4-8) fractions from cobalt-affinity chromatography; Right, prestained marker; (**B**) Determination of molecular weight of NitEg (log MW = 1.642, MW = 43.85 kDa); MW = molecular weight; Rm, relative mobility = distance traveled by the protein/distance traveled by the dye front). The calibration curve (blue) was constructed for standard proteins within MW range of 25-100 kDa; NitEg in orange.

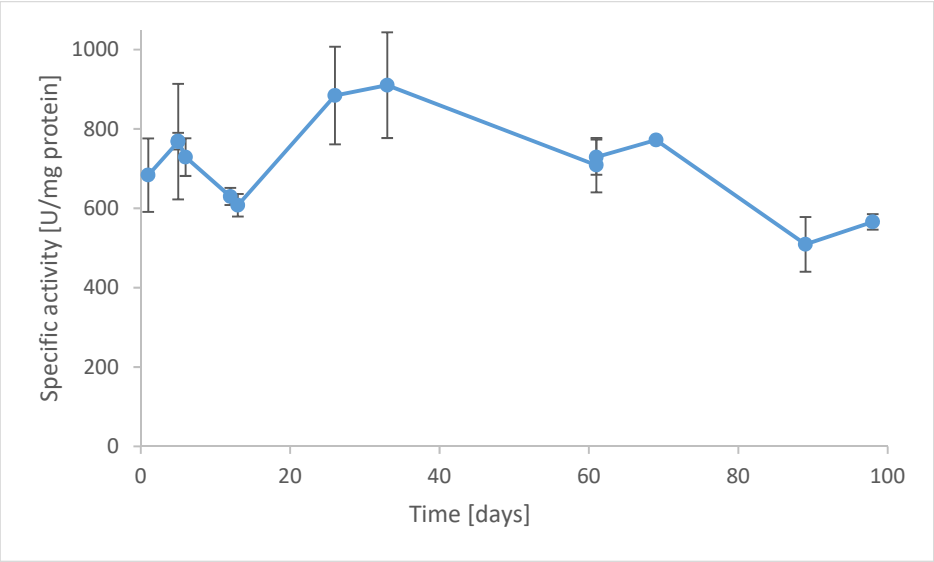

Figure S3. Shelf life of NitEg at pH 8.0 and 4 °C.

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A0A165HZS1 MPITKYKAAAVTSEPGWFDLEGGVQKTINFINEAGQAGCKLVAFPEVWIPGYPYWMWKVN 60
Q7RVT0     MVLTKYKAAAVTSEPCWFDLEGGVRKTIDFINEAGQAGCKLVAFPEVWIPGYPYWMWKVT 60
           * :************ ********:***:******************************.

A0A165HZS1 YQQSLPMLKKYRENSLGVNTEEMRRIRRAARDNQIYVSMGFSEIDHATLYLAQVLISPTG 120
Q7RVT0     YQQSLPMLKKYRENAMAVDSDEFRRIRRAARDNQIYVSLGFAEIDHATLYLAQALIDPTG 120
           **************:::.*:::*:***************:**:***********.**.***

A0A165HZS1 EVINHRRKIKPTHVEKLVYGDGAGDTFLSVTETDIGRLGQLNCWENMNPFLKALNVSAGE 180
Q7RVT0     EVINHRRKIKPTHVEKLVYGDGAGDTFMSVTPTELGRLGQLNCWENMNPFLKSLNVSMGE 180
           ****************************:*** *::*****************:**** **

A0A165HZS1 QVHVAAWPVYPGKETLKYPDPATNVAEPASDLVTPAYAIETGTWTLAPFQRLSKEGLKKN 240
Q7RVT0     QIHIAAWPIYPGKETLKYPDPATNVADPASDLVTPAYAIETGTWTLAPFQRLSVEGLKKN 240
           *:*:****:*****************:************************** ******

A0A165HZS1 TPEGVEPETDPSTYNGHARIFAPDGTLLVKPDKDFDGLLFVNIDLNECHLTKALADFGGH 300
Q7RVT0     TPEGVEPETDPSTYNGHARIYRPDGSLVVRPDKDFDGLLFVDIDLNECHLTKALADFAGH 300
           ********************: ***:*:*:***********:***************.**

A0A165HZS1 YMRPDLIRLLVDTRRKELVTEADPDGGIATYTTRERLGLNLPLAEKEEKKGGSSTKKHDG 360
Q7RVT0     YMRPDLIRLLVDTSRKELVTEVDRNGGIVQYSTRERLGLNTPLENDKEGKK--------- 351
           ************* *******.* :***. *:******** ** :.:* *

A0A165HZS1 KKAGDL 366
Q7RVT0     ------
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Figure S4. Multiple sequence alignment of NitEg (UniProtKB: A0A165HZS1) and its closest characterized homologue from *Neurospora crassa* (UniProtKB: Q7RVT0) using UniProt Align (https://www.uniprot.org/align/). The catalytic residue is Cys (highlighted in red); *identical amino acids; : similar amino acids; . less similar amino acids.

Table S1. Specific activities of cyanide hydratases

Organism	Heterologous expression [1]	Specific activity [2]			Reference [3]
		[U/mg dcw]	[U/mg protein]		
			Cell-free extract	Purified enzyme	
Gloeocercospora sorghi	-	0.044	23.3	555	[22]
Fusarium lateritium	-	102.5	55.9	1109	[23]
	+	≈ 600	n.d.	n.d.	[29]
Fusarium solani	-	1.4	4.6	128	[24]
Fusarium oxysporum	-	n.d.	85.0	840	[25]
Pyrenophora tritici	+	n.d.	n.d.	185	[31]
Botryotinia fuckeliana	+	n.d.	n.d.	100	[31]
Stereum hirsutum	+	153	n.d.	n.d.	[32]
Aspergillus niger	+	385	736	1324	[33]

[1] in *Escherichia coli*

[2] substrate KCN

[3] see main text for references

dcw = dry cell weight; n.d. = not determined

Table S2. Purification of NitEg from 200 mL of culture

Sample	Volume [mL]	Protein [mg/ml]	Total protein [mg]	Specific activity [U/mg protein]	Total activity [U]	Purification (fold)	Yield [%]
Cell-free extract	23	9.3±0.1	214±2	280±14	≈ 59,920	-	-
Purified enzyme	1.2	10.3±0.3	12.4±0.4	697±95[a]	≈ 8,643	≈ 2.5	≈14.4

Activities were determined by picric acid method.

Table S3. Performance of cyanide hydratase and cyanide dihydratase in model mixtures and real effluents

Reaction mixture, fCN concentration, pH	**Biocatalyst**	**Removal (Time)**	**Reference** [1]
Buffer, 0.6 mM, pH 9.0	NitEg[2]	100% (10 min)	This work
Buffer, 4.5 mM, pH 9.0		100% (30 min)	
Buffer, 25 mM, pH 9.0		100% (20 min)	
Buffer, 25 mM, pH 9.5		100% (45 min)	
Buffer, 25 mM, pH 10.0		83% (1 h)	
Buffer, 25 mM, pH 10.5		22% (1 h)	
Buffer, 100 mM, pH 9.0		98% (2 h)	
Buffer, 100 mM, pH 8.0	CynH (*N. crassa*) [2]	100% (1 h)	[3]
Buffer, 10 mM, pH 8.0	CynH (*A. niger*) [2]	100% (5 min)	[4]
Coking effluent, simulated, 0.6 mM, pH 9.0	NitEg[b]	100% (1.5 h)	This work
Mine effluent, 528 mM, pH 11	CynD (*B. pumilus*) immobilized cells	43% (4 h)	[2]
Mine effluent, 17.6 mM, -		98% (4 h)	
Petrochemical effluent, simulated, 4.6 mM, pH 9.1	NitEg [2]	96% (45 min)	This work
Cu-plating effluent, simulated, 100 mM, pH 9.0		96% (2 h)	
Ag-plating effluent, simulated, 100 mM, pH 9.0		98 (2 h)	
Cu-plating effluent, diluted, 100 mM, pH 8.0	CynH (*N. crassa*) [2]	≈ 65% (12 h)	[3]
Ag-plating effluent, diluted, 100 mM, pH 8.0		≈ 90 (2 h)	[3]

[1] see main text for references

[2] purified