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Supporting Information

for

Activity assays of NnlA homologs suggest the natural product *N*-nitroglycine is degraded by diverse bacteria

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Additional Figures and Tables

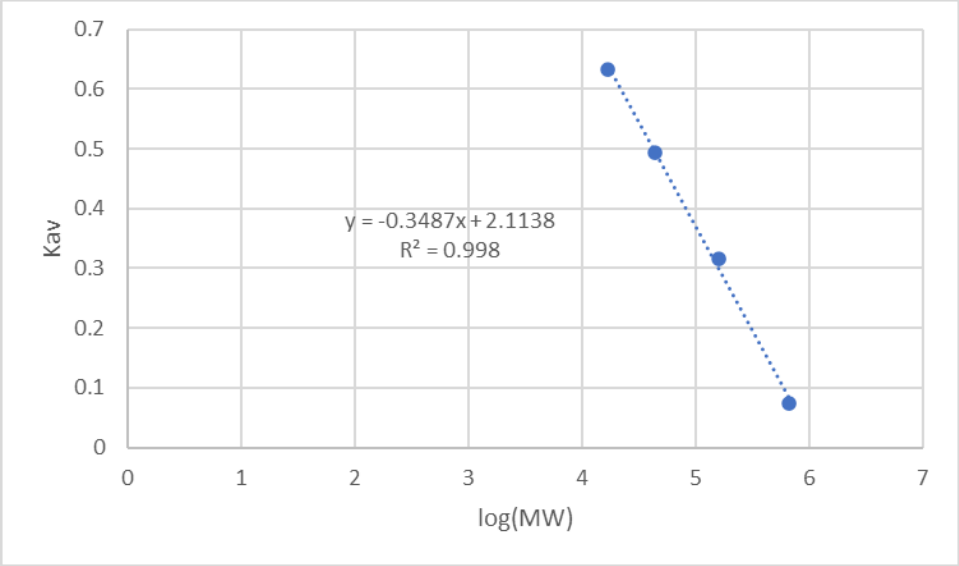

Figure S1: Analytical size exclusion chromatography calibration curve of standards. Flow rate 0.75 mL/min, 100 mM tricine and 100 mM NaCl buffer pH 7.5.

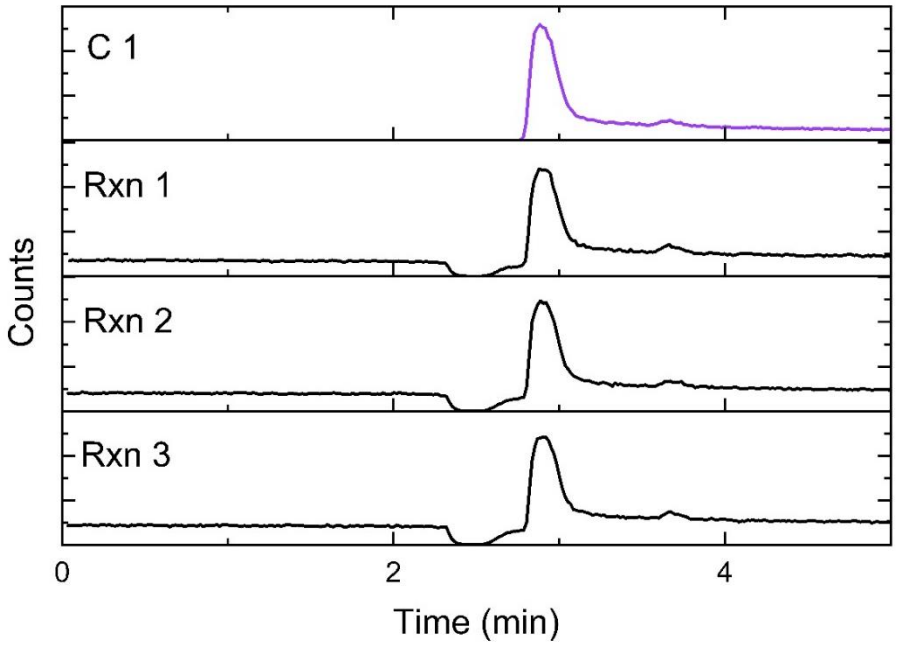

Figure S2: Representative LC–MS EICs monitoring molecular anion of 2-NAE (*m/z* 105.03) in samples containing 2 mM 2-NAE, excess titanium citrate, and either no *Vs* NnlA for the control sample or 20 μM reduced *Vs* NnlA (Fe^{II}-NnlA) for the reaction samples. Samples were incubated overnight at room temperature in deoxygenated 23.3 mM tricine buffer, pH 7.5.

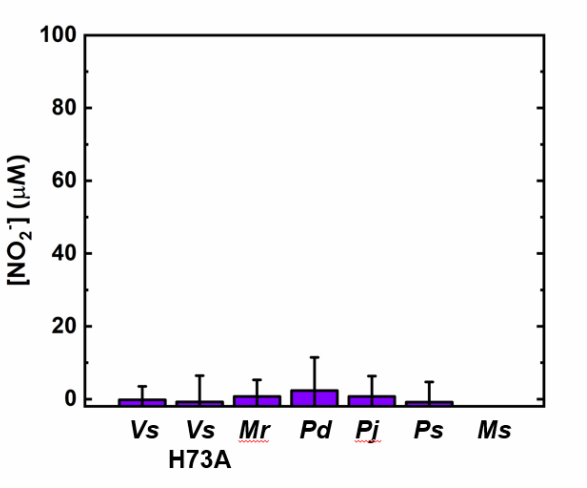

Figure S3: Nitrite concentrations observed in overnight cultures of *E. coli* transformed with NnlA homologs or variants grown in the presence of 2-NAE. Cells were incubated overnight in diluted LB containing IPTG and 3 mM NNG and incubated overnight at 37 °C.

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MBW2064617          --------------MAGNGDKRLTELIRLAMECMGVAVTIIDPQGTLLYYNKQAEKILDR    46
MCK4988321          -------------MNENERKTKLGELVNLAMDCLGVAVTIIDTKGTLLYYNQHSAKILDR    47
WP_189438608        MSQNQHSAFRKQVADRTLDGWELEGCAEWLIDQQGVGVSIIDTEGRLLFYNQWADNKMPR    60
WP_054784913        -MTDNNNELPEVTDQRILEAWKLSGWADRLLEEAGIGVTIIDKDGKLLYYNKWASENLDR    59
WP_282531508        ----MDEKLPEVTQQRVLPGWTVSQWAGGLIEHAGVGVTILDREGRVMFYNQWAANRLDR    56
NnlA_(OUM02170)     MNQVNTEELPEVVDQRILAGWRLSEWADRILEYAGVGVTLVDRLGRCVYYNQWAKDHLDR    60
WP_066989343        --MTTHADLTEVFEHRIVADWALGEWADRLLEQAGLGVTIVDRHGVVMYYNKWAAEHLDR    58
WP_051342206        ---MTQAILPEVTDARILDGWQLSGWADRLLEQAGVGVTIVDRTGRVLYYNKWADEHLDR    57
WP_030511367        --MTSQAEPAEAAESRIATDWGLDEWADRLIDQAGVGVTILNRHGTVMYYNKWASEHLDR    58
WP_191054027        --MSSQVELAEVAESRIATDWGLDQWADRILEQAGFGVTVLDRHGTVMYYNKWASEHLDR    58
WP_195903080        --MSSQVELAEVAESRIATDWGLDQWADRILEQAGVGVTILDRHGTVMYYNKWASEHLDR    58
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MBW2064617          KPEYIGKDVHSHHKRAASNKKLDMMLEDFQ-KGRTEPFHYQARPYGE-TILVILSPIFED   104
MCK4988321          KPEYIGTDIHSHHKEAAINKKVDLMLKEFE-GGRKDHFHYEAKPYGK-IIFVTLAPIIKN   105
WP_189438608        EPEYLGQKVQEHHRKQITNVRFEAMLDLFRKEGRTEAVKYVAKPYEGLTIIVIVTPIIVE   120
WP_054784913        QPRHIGHNVKENHRRSITNPRFDAMLQLFR-DGRKDPVRYVANPYGTTTILVTVSPIHID   118
WP_282531508        KPEYIGKDVRNHHRRKITNPRFDAMLKLFE-EGRTDPVHYVARPYGKITILVTVSPIKVD   115
NnlA_(OUM02170)     KPGYIGDEIHNRHRRAITNPRFDAMLKLFE-EGRMEPVRYVARPYGKTTILVTVSPIYVE   119
WP_066989343        QPGYLGHSVHERHHRKITNPRFDAMLKLFV-DGRIEPVQYVARPYGKTTILVTVSPIRIG   117
WP_051342206        KPEYIGNDVRDRHRQPITNPRFDAMIALFE-EGRVEPVRYVARPYGKTIILVTVSPIWVD   116
WP_030511367        RPEYIGNDVRKRHRRAVTNPRFDAMLKLFE-DGRVEPVRYVARPYGKTTILVTVSPIRVD   117
WP_191054027        KPEYIGNDVRKRHRRAVTNPRFDAMLRLFE-EGRVEPVRYVARPYGRTTILVTVSPIRVD   117
WP_195903080        MPEYIGNDVRKHHRRAVTNPRFDAMLRLFE-EGRVEPVSYVARPYGKITILVTVSPIRVN   117
                     * ::* .::..*:.   * :.: *:  *   ** : . * *.**    *:* ::**

MBW2064617          AKFVGCVQCVRLKDDTESR-----------------------------------------   123
MCK4988321          GEFLGCVQTVRLKNTVSANQ---------------------------------------   125
WP_189438608        GELVAFCQTVLDKDEIQGMCETFDESGNITFQRDILPGSEPG-----------------   162
WP_054784913        EELVGFSQFVLLKEEVQELCCLFDQHGRDPFEKDMLPNGPPT-----------------   160
WP_282531508        GELVGYSQIVLMKDEIQELFRRFDESGRESFEKDMLPAWPFSGND--------------   160
NnlA_(OUM02170)     GELVGYSQIVLLKDEVEALCQRFNASGRESFEREMLPDSTPSNDD--------------   164
WP_066989343        GELVGLAQLVLLKDEVQELFSRFDDSGRESFERDMLPDGYPGA----------------   160
WP_051342206        GELVGFSQIVLLKNEVQELCERFDASGRESFEREMLPNGATGY-------LTYKKNT   166
WP_030511367        GELVGFSQIVLLKDEVQELCARFDESGRESFEREMLPNGPPAT----------------   160
WP_191054027        GELVGFSQVVLLKDEIQELCARFDESGRESFEREMLPDTPAVARDPAAGQCSSRRS-   173
WP_195903080        GELVGFSQIVLLKDEVQELFALFDESGRESFEREMLPNGLPTA----------------   160
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Figure S4: Amino sequence alignment of NnlA homologs shown in Figure 6 of main text. Conserved basic residues are colored red.

Figure S5: Gene neighborhoods of NnlA homologs. NnlA homologs in each neighborhood is color coded red in the middle of the figure.

Table S1: Elution times for standard by size exclusion chromatography.[a]

Standards	MW	Elution volumes	Gel phase distribution coefficient Kav
Thyroglobulin	670000	10.19	0.073
γ-globulin	158000	13.82	0.317
Ovalbumin	44000	16.45	0.493
Myoglobin	17000	18.54	0.634

[a]Flow rate 0.75 mL/min, 100 mM tricine and 100 mM NaCl buffer pH 7.5.

Table S2: NnlA homologs analytical size exclusion values.[a]

Protein sample	MW	Elution volume	Gel phase distribution coefficient Kav
Pd NnlA	49,000	16.23	0.478
Ps NnlA	38,200	16.79	0.516
Ms NnlA	36,400	16.90	0.523
Mr NnlA	35,900	16.93	0.526
Oligomer *Vs* NnlA	397,000	11.51	0.162
Dimer *Vs* NnlA	41,400	16.61	0.504

[a]Flow rate 0.75 mL/min, 100 mM tricine and 100 mM NaCl buffer pH 7.5.

Table S3: Nitrogen mass balance resulting from NnlA reaction with NNG

NnlA[a]	**Reduced NnlA $[NO_2^-]_{final}$ (μM)**	**As Isolated NnlA $[NO_2^-]_{final}$ (μM)**
Mr	250 ± 10	10.6 ± 1.7
Pd	260 ± 10	51.3 ± 5.3
Ps	250 ± 10	31.0 ± 6.7
Ms	250 ± 20	22.2 ± 8.5

[a]Reaction conditions: 5 μM NnlA, 10 μM sodium dithionite for reduced NnlA and no reducing agent for as isolated NnlA, 350 μM NNG in 30 mM tricine buffer at pH 7.5 and room temperature in anaerobic glovebox.

Table S4: Test of 2-NAE degradation *Vs* NnlA (m/z 105.03).[a]

Sample	$[NO_2^-]_{final}$ (μM)	Area of Integration
Control samples	-5.5 ± 1.4	$2.4 \pm 0.4 \times 10^6$
Reaction samples	1.8 ± 11.0	$2.0 \pm 0.5 \times 10^6$

[a]Samples containing 2 mM 2-NAE, excess titanium citrate, and either no *Vs* NnlA for the control samples or 20 μM reduced NnlA (Fe^{II}-NnlA) for the reaction samples. Samples were incubated overnight at room temperature in deoxygenated 23.3 mM tricine buffer pH 7.5

Table S5: Expected NNG degrading bacteria based on this study.

Species	Bacterial class	Location isolated	Ref.
Variovorax sp. Strain *JS 1663*	betaproteobacteria	USA: activated sludge from Ammunition Plant	[1]
Pseudovibrio denitrificans JCM 12308	alphaproteobacteria	Taiwan: seawater	[2]
Pseudovibrio japonicus strain KCTC 12861	alphaproteobacteria	Japan: seawater	[3]
Pseudonocardia spinosispora DSM 44797	actinomycetia	S. Korea: soil	[4]
Mycobacterium sp. 1465703.0	actinomycetia	Mozambique: Host cultures	J. Craig Venter Institute Genome Center for Infectious Diseases.Accession: PRJNA305922
Microbispora rosea subsp. nonnitritogenes strain NRRL B-2631	actinomycetia	Unknown: acidic volcanic ash	Ref. [5]

References

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4. Lee, S. D., Kim, E. S., Kang, S. O., Hah, Y. C. Pseudonocardia spinosispora sp. nov., isolated from Korean soil. *International journal of systematic and evolutionary microbiology*, **2002**, *52*(5), 1603-1608.
5. Nonomura, H. Distribution of actinomycetes in the soil. IV. Isolation and taxonomy of the genus Microbispora. *J. Ferment. Technol.* **1960**, *38*, 401-405.