Supplement Information

Bacillus subtilis RNase HII is inefficient at processing guanosine monophosphate and damaged ribonucleotides

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Short title: RNase HII resolves canonical and mismatched ribonucleotides

Keywords: RNase HII, *Bacillus subtilis*, DNA replication, DNA damage, ribonucleotides, mismatches

Supporting Table S1. Strains used in this study.

Strain ID	Species	Strain	Genotype/Plasmid	Reference
FCL1	*E. coli*	MC1061	pFLC1	Lab stock
FCL70	*E. coli*	MC1061	pFCL25	Lab stock
FCL71	*E. coli*	BL21(DE3)	pFCL25	Lab stock
FCL72	*E. coli*	MC1061	pFCL26	Lab stock
FCL73	*E. coli*	BL21(DE3)	pFCL26	Lab stock
JRC5	*E. coli*	BL21(DE3)		Lab stock
JRC6	*E. coli*	MC1061		Lab stock
JRC9	*B. subtilis*	PY79	Prototroph Spβ°	(Youngman *et al.*, 1984)
JRC20	*E. coli*	MC1061	pJC10	This work
JRC21	*E. coli*	BL21(DE3)	pJC10	This work
JRC22	*E. coli*	MC1061	pJC11	This work
JRC23	*E. coli*	BL21(DE3)	pJC11	This work

Supporting Table S2. Plasmids used in this study.

Name	Genotype	Reference
pFCL1	His_6-SUMO-*B. subtilis fenA*	This work
pFCL25	His_6-SUMO-*B. subtilis rnhB*	This work
pFCL26	His_6-SUMO-*B. subtilis rnhB* D78A E79A	This work
pJC10	His_6-SUMO-*E. coli rnhB*	This work
pJC11	His_6-SUMO-*E. coli rnhB* D16A E17A	This work

Supporting Table S3. Oligonucleotides used as primers in this study.

Oligo	Sequence
oJR46	tcgagcaccaccaccaccaccactgag
oJR47	acctccaatctgttcgcggtgagcctcaataatatcg
oJR88	ccgcgaacagattggaggtgtgaatacattaaccgtaaaggacattaaagacc
oJR89	tggtggtggtggtgctcgattatctgaaagattgaacaggagcg
oJR92	gtgaaatacttgggctgacagactc
oJR94	gttgccgcggtcggccg
oJR95	gaccgcggcaacacctgcaatc
prFCL25	cctttcgggctttgttagcagcc
prJC36	accgcgaacagattggaggtatgatcgaatttgtttatcc
prJC40	gtggtggtggtggtgctcgatcaggacgcaagtc
prJC41	tgtggctgcagtcggacgcgggccgttagttg
prJC42	ccgactgcagccacacccgcaaccagctgcg
prJC44	acctccaatctgttcgcggtg
prJC45	tcgagcaccaccaccaccac
prJC51	tccggcgtagaggatcgagatctcg
prJC52	cgggctttgttagcagccggatctc

Primers were used for PCR amplification, mutagenesis, Gibson assembly, and colony PCR.

Supporting Table S4. Oligonucleotides used as substrates for RNase HII.

Oligo	Sequence	Label
oJC1	ggcttatacagcatcgagctcagga	5' IR800CWN
oJC2	tcctgagctcgatgctgtataagcc	n/a
oJC3	ggcttatacagcrAtcgagctcagga	5' IR800CWN
oJC4	ggcttatacagcrGtcgagctcagga	5' IR800CWN
oJC5	tcctgagctcgacgctgtataagcc	n/a
oJC6	ggcttatacagcrCtcgagctcagga	5' IR800CWN
oJC7	tcctgagctcgaggctgtataagcc	n/a
oJC8	ggcttatacagcrUtcgagctcagga	5' IR800CWN
oJC9	tcctgagctcgaagctgtataagcc	n/a
oJC10	ggcttatacagcrOHtcgagctcagga	5' IR800CWN
oJC11	ggcttatacagcr8-oGtcgagctcagga	5' IR800CWN

Oligos were used to assemble double-stranded substrates for RNase HII endonuclease assays. All oligos are 25 nucleotides in length. The single rNMP modification has been underlined for clarity.

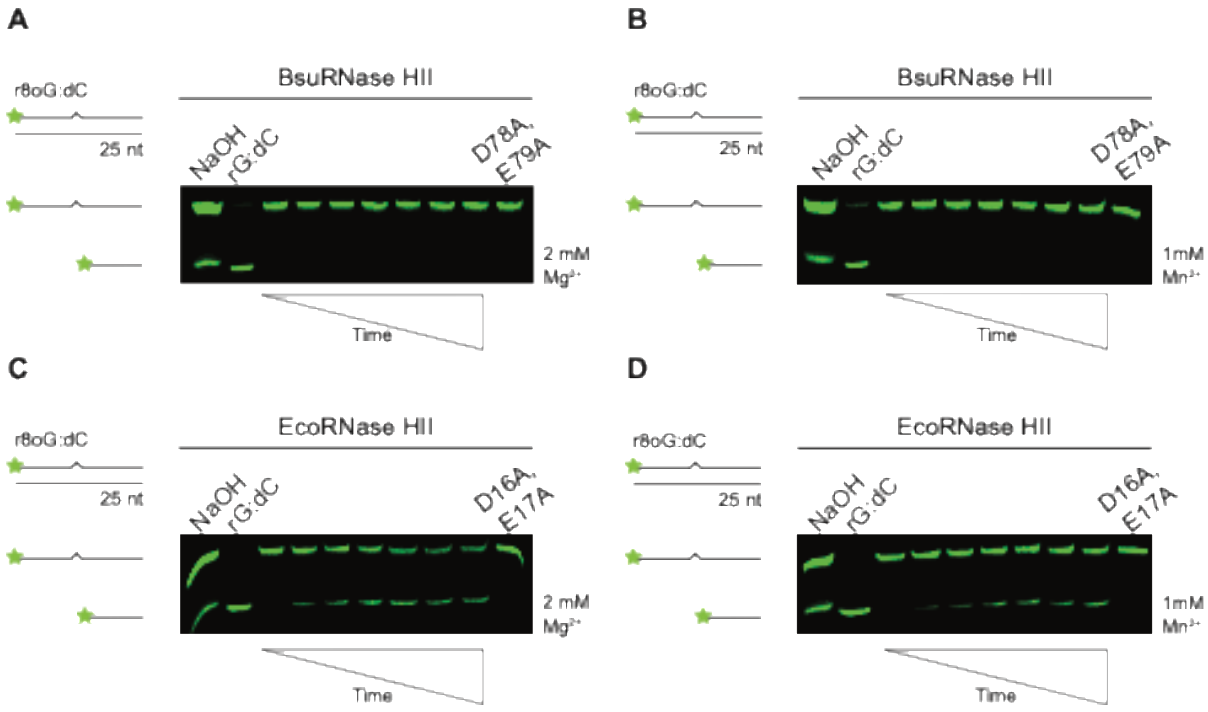

Figure S1. **Changing metal and Mg^{2+} concentration does not affect BsuRNase HII nuclease activity on r8oG:dC.** Shown are representative urea-PAGE assays performed over a 160-minute time course at 37°C with (A) 50 nM *B. subtilis* RNase HII and 100 nM r8oG:dC, (B) 50 nM *B. subtilis* RNase HII and 100 nM r8oG:dC, (C) 6.25 nM *E. coli* RNase HII and 100 nM r8oG:dC, and (D) 6.25 nM *E. coli* RNase HII and 100 nM r8oG:dC. Assays shown in (A) and (C) were performed with 2 mM $MgCl_2$, whereas (B) and (D) were performed with 1 mM $MnCl_2$. Each substrate is composed of a 25-oligonucleotide dsDNA with a single rNMP, represented by a zigzag. Each gel contains a canonical base pair (rG:dC) and catalytic impaired RNase HII controls. An alkaline ladder was prepared by incubating each substrate with 200 nM NaOH. rOH:dC was prepared with oJC10 and oJC5, and r8oG:dC was prepared with oJC11 and oJC5.

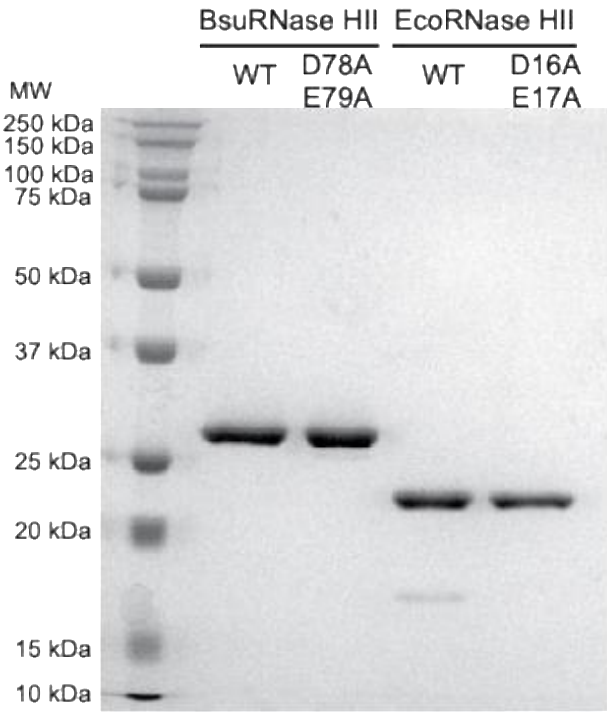

Figure S2. Purified BsuRNase HII and EcoRNase HII. Shown is an SDS-PAGE. Lane 1 contains a protein dual color standards with the corresponding molecular weight (MW) indicated. Lanes 2-5 each contain 1 µg of *B. subtilis* RNase HII (28.4 kDa), *B. subtilis* RNase HII D78A E79A (28.3 kDa), *E. coli* RNase HII (21.5 kDa), and *E. coli* RNase HII D16A E17A (21.4 kDa), respectively.

REFERENCES

Youngman, P., Perkins, J.B., and Losick, R. (1984) Construction of a cloning site near one end of Tn917 into which foreign DNA may be inserted without affecting transposition in Bacillus subtilis or expression of the transposon-borne erm gene. *Plasmid* **12**: 1–9.