

## SUPPLEMENTAL MATERIAL

### **Halolysin SptA, a serine protease, contributes to growth-phase transition of haloarchaeon *Natrinema* sp. J7-2, and its expression involves cooperative action of multiple *cis*-regulatory elements**

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Running title: Function and regulation of halolysin SptA

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**TABLE S1. Strains and plasmids used in this study**

Strain or plasmid	Description	Reference or source
<b>Strains</b>		
<i>E. coli</i>		
DH5 $\alpha$	F <sup>-</sup> $\phi$ 80d/lacZ $\Delta$ M15 $\Delta$ (lacZYA-argF) U169 <i>deoR recA1 hsdR17</i> (rk <sup>-</sup> mk <sup>-</sup> ) <i>phoA supE44</i> $\lambda$ <i>thi-1 gyrA96 relA1</i>	Novagen
JM110	<i>dam dcm supE44 hsdR17 thi leu rpsL1 lacY galK galT ara tonA thr tsx</i> (lac-proAB)/F' [ <i>traD36 proAB</i> <sup>+</sup> lac <sup>F</sup> lacZ $\Delta$ M15]	Novagen
<i>Natrinema</i> sp.		
J7-2	A subculture of <i>Natrinema</i> sp. J7 (CCTCC AB91141) lacking the plasmid pHH205	(1)
$\Delta$ <i>sptA1</i>	J7-2 mutant with a deletion of the core promoter and ORF region (-43 to +1655) of <i>sptA</i>	This study
$\Delta$ <i>sptA2</i>	J7-2 mutant with a deletion of the core promoter and transcript region (-43 to +1925) of <i>sptA</i>	This study
<b>Plasmids</b>		
pNBK07	Haloarchaeal nonreplicative plasmid, Mev <sup>R</sup> , Amp <sup>R</sup>	(2)
pNBK- <i>Aud</i>	Knockout plasmid for the core promoter and ORF region of <i>sptA</i>	This study
pNBK-3'UTR <i>ud</i>	Knockout plasmid for the 3'UTR region of <i>sptA</i>	This study
pYC-SHSmcs	<i>E. coli</i> - <i>Natrinema</i> sp. J7-2 shuttle vector, Mev <sup>R</sup> , Amp <sup>R</sup>	(2)
pSHS- <i>sptA</i>	pYC-SHSmcs-derived vector containing the core promoter and ORF region of <i>sptA</i> (-43 to +1655)	This study
pD355	pYC-SHSmcs-derived vector containing <i>sptA</i> transcript and its 5'-flanking region (-335 to +1925)	This study
pD215	pYC-SHSmcs-derived vector containing <i>sptA</i> transcript and its 5'-flanking region (-215 to +1925)	This study
pD174	pYC-SHSmcs-derived vector containing <i>sptA</i> transcript and its 5'-flanking region (-174 to +1925)	This study

pD142	pYC-SHSmcs-derived vector containing <i>sptA</i> transcript and its 5'-flanking region (-142 to +1925)	This study
pD116	pYC-SHSmcs-derived vector containing <i>sptA</i> transcript and its 5'-flanking region (-116 to +1925)	This study
pD82	pYC-SHSmcs-derived vector containing <i>sptA</i> transcript and its 5'-flanking region (-82 to +1925)	This study
pD63	pYC-SHSmcs-derived vector containing <i>sptA</i> transcript and its 5'-flanking region (-63 to +1925)	This study
pD51	pYC-SHSmcs-derived vector containing <i>sptA</i> transcript and its 5'-flanking region (-51 to +1925)	This study
pD43	pYC-SHSmcs-derived vector containing <i>sptA</i> transcript and its 5'-flanking region (-43 to +1925)	This study
pM1 to pM3-6-11	pD215-derived vectors with mutations introduced into 5'-flanking region of <i>sptA</i>	This study
pST1	A vector containing haloarchaeal <i>bgaH</i> gene, Amp <sup>R</sup> , Nov <sup>R</sup>	(3)
pD215b	pYC-SHSmcs-derived vector containing <i>bgaH</i> ORF and 5'-flanking region (-215 to -1) of <i>sptA</i>	This study
pD142b	pYC-SHSmcs-derived vector containing <i>bgaH</i> ORF and 5'-flanking region (-142 to -1) of <i>sptA</i>	This study
pD116b	pYC-SHSmcs-derived vector containing <i>bgaH</i> ORF and 5'-flanking region (-116 to -1) of <i>sptA</i>	This study
pD63b	pYC-SHSmcs-derived vector containing <i>bgaH</i> ORF and 5'-flanking region (-63 to -1) of <i>sptA</i>	This study
pD43b	pYC-SHSmcs-derived vector containing <i>bgaH</i> ORF and 5'-flanking region (-43 to -1) of <i>sptA</i>	This study

## References

1. Feng J, Liu B, Zhang Z, Ren Y, Li Y, Gan F, Huang Y, Chen X, Shen P, Wang L, Tang B, Tang XF. 2012. The complete genome sequence of *Natrinema* sp. J7-2, a haloarchaeon capable of growth on synthetic media without amino acid supplements. PLoS One 7:e41621.
2. Wang Y, Sima L, Lv J, Huang S, Liu Y, Wang J, Krupovic M, Chen X. 2016. Identification, characterization, and application of the replicon region of the halophilic temperate sphaerolipovirus SNJ1. J Bacteriol 198:1952-1964.
3. Tang W, Wu Y, Li M, Wang J, Mei S, Tang B, Tang XF. 2016. Alternative translation initiation of a haloarchaeal serine protease transcript containing two in-frame start codons. J Bacteriol 198:1892-1901.

**TABLE S2. Primers used in this study**

<b>Primer</b>	<b>Sequence (5'→3')<sup>a</sup></b>
<b>Gene knockout</b>	
SptA-F	ATGTTTAGGAAGAATTTAATAG
SptA-R	CTATCGACCGCGTTCGTCG
SptAus-F	GAAGATCTTCTCAACGAACAACCCGACTG
SptAus-R	GCGGCCGCGCTCGATCAAGAAATACAATAGAT
SptAds-F	ATCTATTGTATTTCTTGATCGAGCGGCGGCCG
SptAds-R	CCCAAGCTTGGGGGAACGACAGTCAGCAAGT
3'UTRus-R	GTGAACTCAGCGGTTTACAAGAAATACAATAGAT
3'UTRds-F	ATCTATTGTATTTCTTGTAACCGCTGAGTTCAC
3'UTRds-R	CCCAAGCTTGGGGGCACGGGCCTCACTGGCCAC
3'UTR-F	CGAACGCGGTTCGATAGATGAGCAGCGGAGGCC
3'UTR-R	GGCTCCGCTGCTCATCTATCGACCGCGTTCG
<b>Gene complementary expression</b>	
SptA-BamHI-F	CGGGATCCCGATGTTTAGGAAGAATTTAATAGCGTG CTGG
SptA-HindIII-R	CCCAAGCTTGGGCTATCGACCGCGTTCGTCGATAGT CATGG
<b>Truncation of 5'-flanking region of <i>sptA</i></b>	
up335-F	CGGGATCCCGACGCGGTCAAACATGGTC
up215-F	CGGGATCCCGGATGGTCCGAATCACCCC
up174-F	CGGGATCCCGAATAAACATTCCCTAAGTTCATTTC
up142-F	CGGGATCCCGTACAATTATTACTATTAC
up116-F	CGGGATCCCGACCCTCATCATGGGTACTION
up82-F	CGGGATCCCGGATTATAATATTAGTGTTTCG
up63-F	CGGGATCCCGGAAATCTATTGTATTTCTTG
up51-F	CGGGATCCCGATTCTTGATGTTTAGGAAG
up43-F	CGGGATCCCGATGTTTAGGAAGAATTTAATAGCGTG CTGG
3'UTR-HindIII-R	CCCAAGCTTGGGATATAAGGTGTGGATATG
<b>Replacement of <i>sptA</i> with <i>bgaH</i></b>	
up-bgaH-R	TAGCAGACACCAACTGTCATAACGCAACCGAAGAA ATTCC
bgaH-HindIII-R	CCCAAGCTTGGGTCACCTCGGACGCGAGTCC

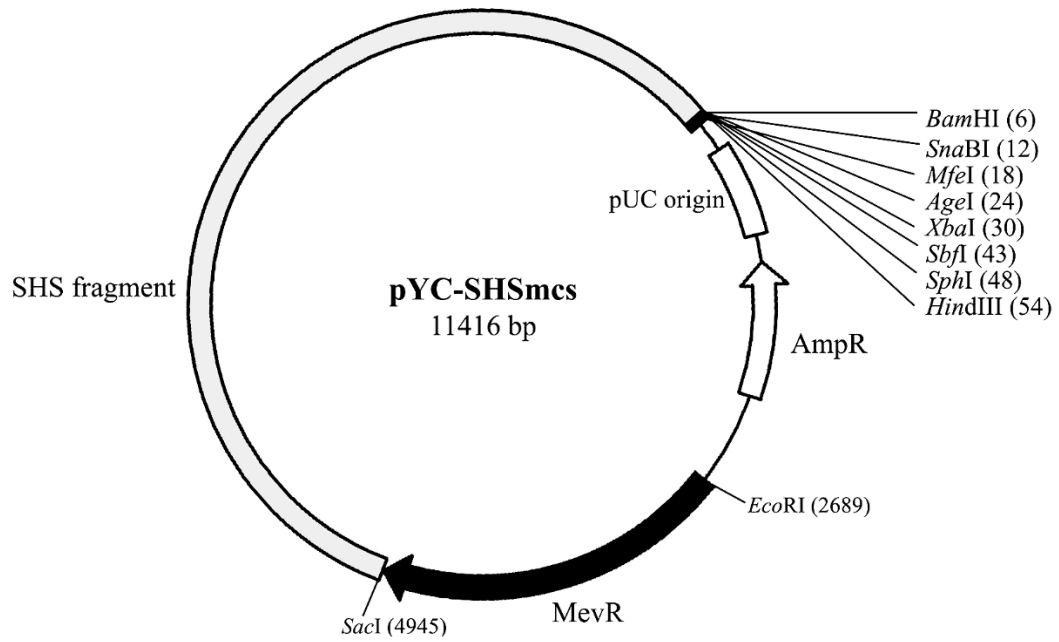
### **Mutation of 5'-flanking region of *sptA***

m1-R	AAATACAATAG <b>CGGG</b> GAGAACTAATA
m2-R	CCTAAACATCAAGAAC <b>CGACA</b> ATAGATTT <b>CGAACA</b>
m3-R	ATTCTTCCTAAACAT <b>CCCGAA</b> ATACAATAG
m4-R	ACGCTATTAA <b>AGGAGG</b> CCCTAAACATCA
m5-R	TCCAGCACGCT <b>CGCC</b> CTTCTTCCTAAA
m6-R	AACCGAAGAA <b>AGGACAG</b> CACGCTAT
m7-R	ATAACGCAACCT <b>CCGAA</b> ATTCCAGC
m8-R	CTTAGGGAAT <b>GGGGCGG</b> CCACA <b>ACTCTT</b>
m9-R	AATGAACTTAG <b>TTCCG</b> TTTTATTCCACA
m10-R	GACTGAAATGAC <b>AGGCTG</b> GAAATGTTTAT
m11-R	GTATGCGACTG <b>CCCGTCA</b> CTTAGGGAAAT
m12-R	ATAATTGTATGAT <b>CATGAA</b> ATGAACTTA
m13-R	GTAATAGTAATAAT <b>GTGCGT</b> CGACTGAAATG
m14-R	GTGTAATAGTAC <b>CGCGT</b> GTATGCGACT
m15-R	ACCAAGTGTAATAT <b>GCATA</b> ATTGTATGCGA
m16-R	GTTACCAAGT <b>GGCCGCG</b> TAATAATTGT

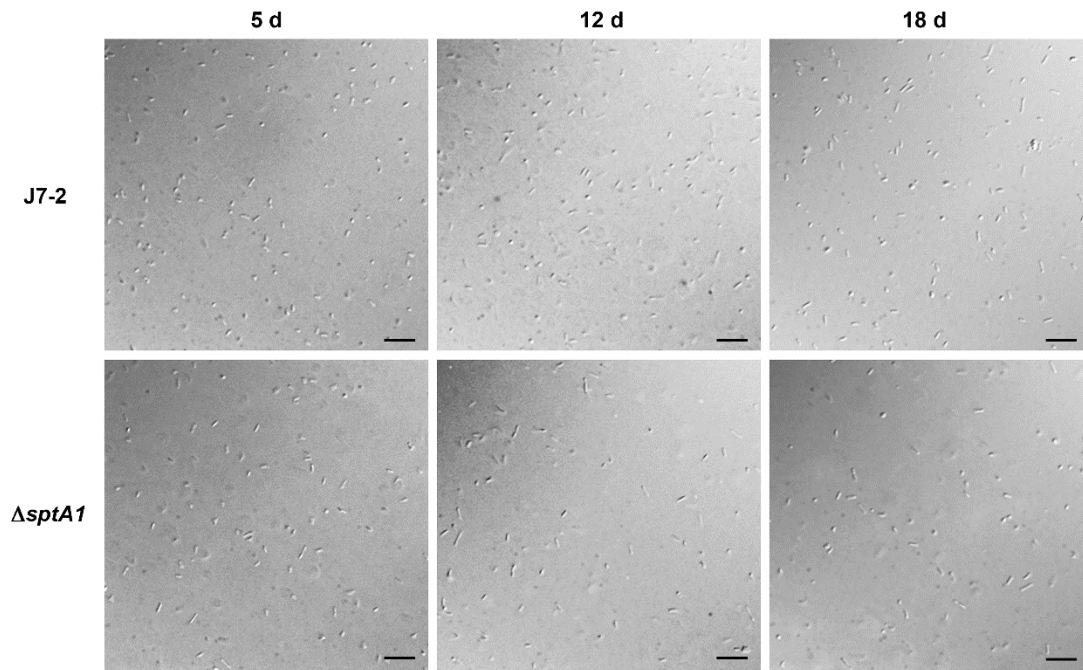
### **qRT-PCR**

SptAQRT-F	CGAACATTGTCCACACGAAC
SptAQRT-R	GGTCACGTTGTCTTCGGAGT
16SrRNAQRT-F	GGTACGGGTTGTGAGAGCAA
16SrRNAQRT-R	CAGTGTAAGGTTTCGCGCC

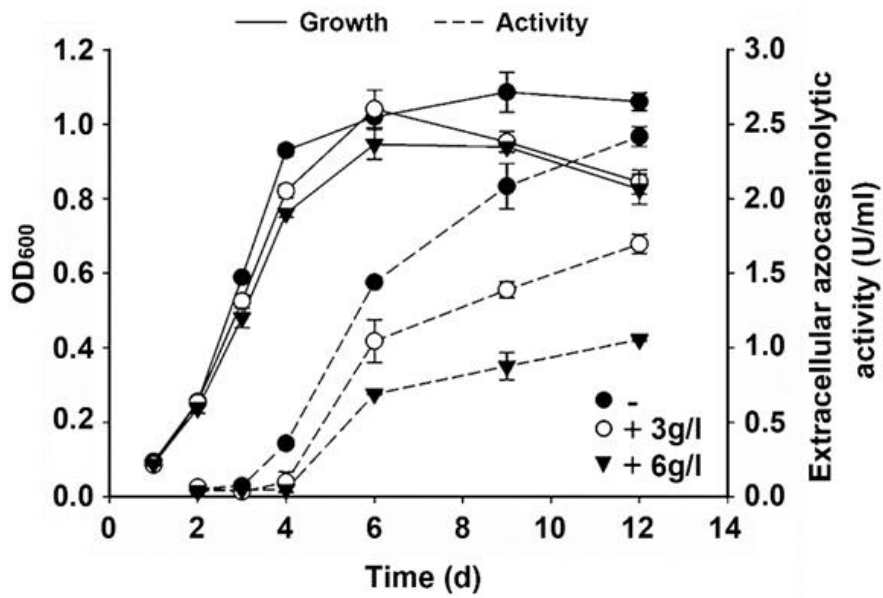
<sup>a</sup> Italicized sections indicate restriction enzyme sites. The mutated nucleotides are in bold.



**FIG S1** A restriction map of the plasmid pYC-SHSmcs. SHS fragment represents the minimal replicon of SNJ1, which is a temperate haloarchaeal virus identified in *Natrinema* sp. J7.



**FIG S2** Phase-contrast microscopy of strain J7-2 and mutant  $\Delta sptA1$  grown in 23% MGM. The late-log (5 d) and death (12 d and 18 d) phase cultures were examined. Bars = 10  $\mu\text{m}$ .



**FIG S3** Effects of ammonium on SptA production in *Natrinema* sp. J7-2. Strain J7-2 was cultivated at 37°C in 23% MGM without (-) or with (+) 3 or 6 g/l of NH<sub>4</sub>Cl. Haloarchaeal growth was monitored by change in OD<sub>600</sub>, and the culture supernatants were subjected to azocaseinolytic activity assay. Values are expressed as the means and standard deviations (error bars) of three independent experiments.