Supplementary Information for

RNase Z oxidative degradation impedes tRNA maturation and is involved in streptococcal translation regulation in response to oxidative stress

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Figures S1 to S11

Table S1

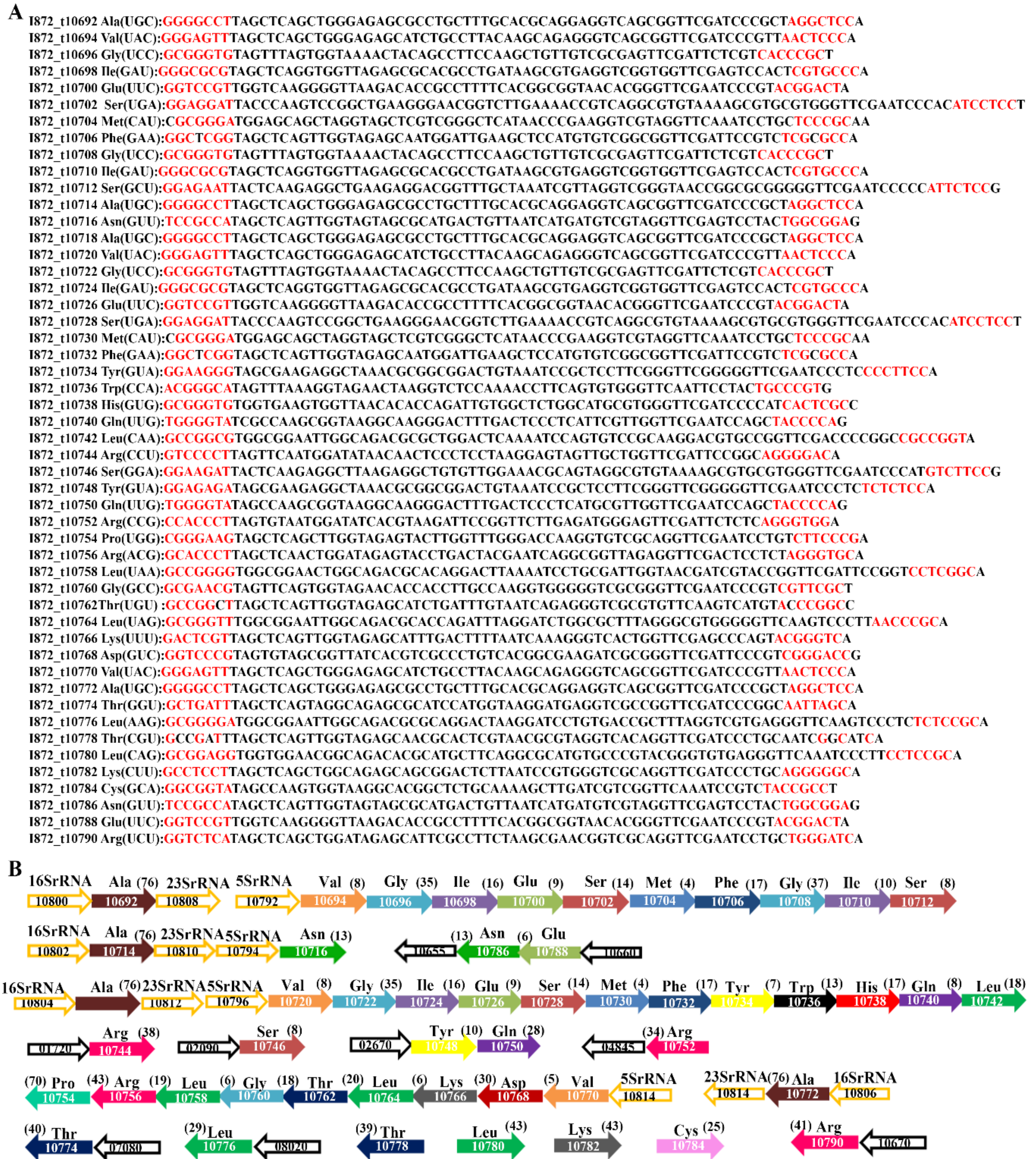

Fig. S1. Sequences (A) and the genomic organizations (B) of the 50 *S. oligofermentans* tRNA genes. A. Sequences of the 50 tRNA genes of *S. oligofermentans* are retrieved from KEGG database, and the accession numbers and amino acid trigrams are provided. Nucleotides in parentheses are the anticodons and those in red are reversely complemented to form the acceptor stem. The last one in each

21 sequence is the discriminator nucleotide. B. Schematics show the genomic
22 organizations of the *S. oligofermentans* tRNA genes (solid bullets) with other genes
23 (empty bullets). The amino acid trigram represents its carrier tRNA gene with the
24 accession numbers shown. Number inside parenthesis at the 3' end of each tRNA gene
25 represents the 3' trailer length of the tRNA precursor. The tRNA genes shown in the
26 same colored bullets carry the same amino acid.

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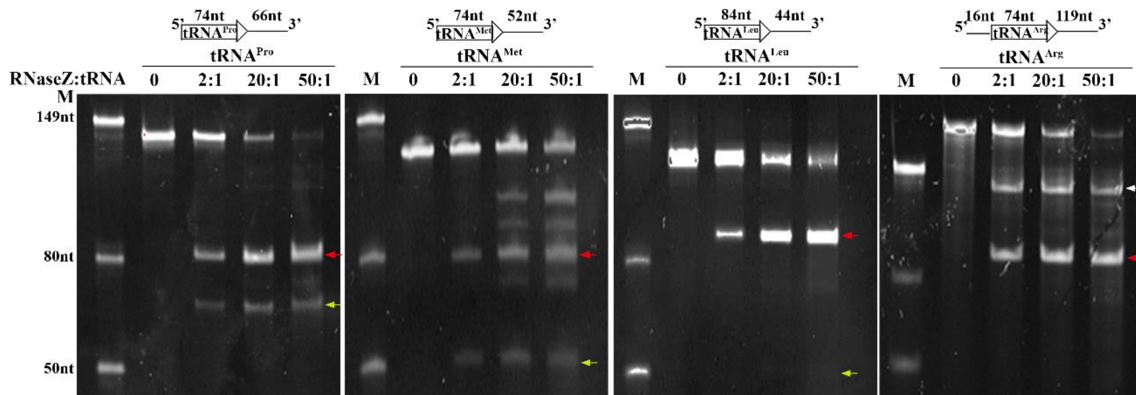

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29 **Fig. S2. Urea-PAGE analysis of So-RNaseZ cleaving four tRNA precursors.** The

30 140 nt $tRNA^{Pro}$ (I872_t10754), 126 nt $tRNA^{Met}$ (I872_10730), 128 nt $tRNA^{Leu}$

31 (I872_t10780) and 209 nt $tRNA^{Arg}$ (I872_t10790) precursor fragments were obtained

32 by *in vitro* transcription, and 50 ng of each tRNA precursor was used as the nucleolytic

33 substrates. Nucleolytic assay was initiated by addition of So-RNaseZ protein in a

34 gradient increased molar ratio as indicated, and incubated at 37ºC for 30 min as

35 described in "Materials and Methods". Nucleolytic products were separated on 10%

36 Urea PAGE gel. Schematics at top show the lengths of 5' extension, tRNA gene and 3'

37 trailer in each tRNA precursor. Red and yellow arrows indicate the cleaving products

38 comprising of tRNA gene or 5' extension and tRNA gene for $tRNA^{Arg}$ precursor, and

39 3' trailer, respectively. M, RNA molecular weight marker is shown at the left.

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41 **Fig. S3. Mass spectrometry analysis of So-RNaseZ protein and the disulfide**

42 **linkages.** A. Ten microgram recombinant So-RNase Z protein in 500 μL PBS was

43 reduced with 10 mM DTT, and the molecular weight was identified by MALDI-TOF

44 analysis. One main peak with molecular mass of 35.461 kDa was identified

45 corresponding to the predicted molecular mass of recombinant So-RNaseZ plus 6

46 Histidine tag (35.15 kDa). B. The So-RNaseZ protein bands named "o", "dd" and "id"

47 in Figure 2A were sliced and trypsin digested as described in "Materials and Methods",

48 and then subject to LC-MS/MS identification. The upper panel shows the LC-MS/MS
49 spectrum of a 4-charged peptide fragment with the precursor mass of 3468.7258 that
50 was identified as Cys149-Cys149 linked peptide as shown in insert. The lower panel
51 shows the LC-MS/MS spectrum of a 6-charged peptide fragment with precursor mass
52 of 4766.3212 that was identified as an intra-molecular Cys38-Cys149 linked peptide
53 (insert).
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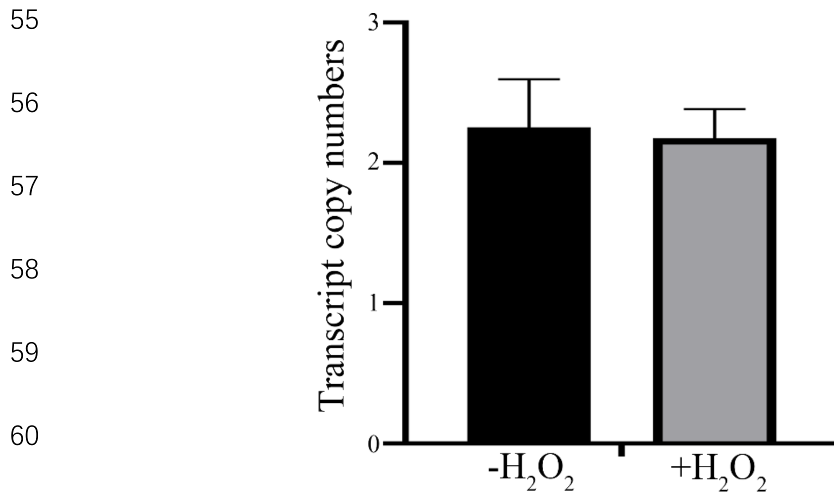

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62 **Fig. S4. qPCR determines the So-*rnaseZ* transcript levels in anaerobically grown**
63 ***S. oligofermentans* treated (+) with or without (-) H_2O_2.** Total RNAs were extracted
64 from the mid-exponential cells using TRIzol reagent. After quality confirmation on 1%
65 agarose gel, cDNAs were generated from 2 μg of total RNA with random primers using
66 Moloney murine leukemia virus reverse transcriptase, and then qPCR was implemented.
67 Experiments were repeated three times on triplicate samples. Transcript copies were
68 calculated as per 1000 16S rRNA copies, and the averages ± SD of three independent
69 experiments are shown.

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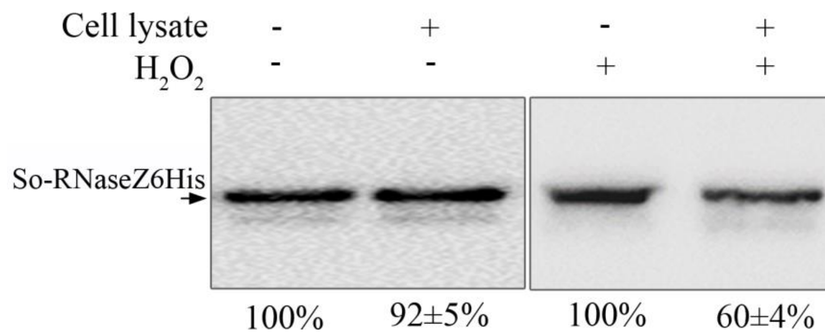

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77 **Fig. S5. Determination of H_2O_2 oxidized So-RNaseZ degradation by the *S.*

78 *oligofermentans* cell lysate.** The recombinant So-RNaseZ6His protein (10 μg) was first

79 reduced with 10 mM DTT at 37ºC for 1 h, and then super-filtrated to remove DTT. An

80 aliquot of So-RNaseZ protein was treated with 500 μM H_2O_2 for 30 min, and leaving

81 another aliquot untreated as a control. After 30 min co-incubation with or without the

82 cell lysate of *S. oligofermentans* at 37°C, So-RNaseZ6His protein was run on 12%

83 SDS-PAGE gel and the abundance was determined by western blot using the anti-His

84 antibody. Band intensities were measured using Image J, and expressed as the

85 percentiles of the protein treated with and without H_2O_2 among their respective

86 abundance in non-cell lysate treatments. Experiments were repeated three times, and

87 the averages ± SD of three experiments are shown beneath the representative gel.

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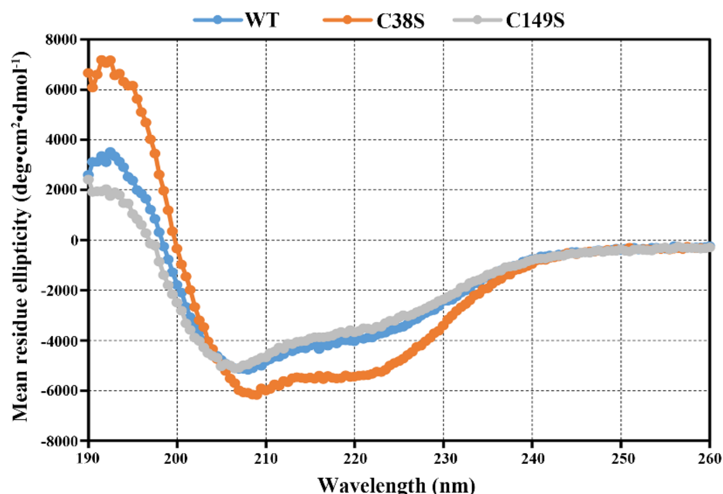

	Helix1	Helix2	Strand1	Strand2	Turns	Unordered	Total
WT	0.037	0.097	0.204	0.124	0.131	0.408	1.001
C38S	0.065	0.102	0.198	0.117	0.128	0.390	1
C149S	0.031	0.096	0.203	0.124	0.132	0.414	1

Fig. S6. Circular dichroism (CD) spectra of the wild-type and cysteine mutated So-RNaseZ. The So-RNaseZ6His (WT) Cys38 and Cys149 were each mutated into serine to construct So-RNaseZC38S6His (C38S) and So-RNaseZC149S6His (C149S), respectively. The three proteins were diluted into 200 µl CD buffer (10 mM K_3PO_4, 100 mM NaF, pH 7.5) to make a final concentration of 5 µM. CD spectra were collected in 0.1 cm quartz cuvettes from wave length of 190 to 260 nm at 0.5 nm interval with a 60 nm/min scan rate at Chirascan circular dichroism spectrometer (Applied photophysics Ltd) under a constant nitrogen purge. Data were acquired at 25 °C, and processed using the pro-Data Viewer 4.2.15 (Spectra Manager software) (Jasco, USA). Each spectrum represents the average of three scans of a sample. Protein secondary structures were analyzed via website Dichroweb (http://dichroweb.cryst.bbk.ac.uk), the output results were shown below the figure.

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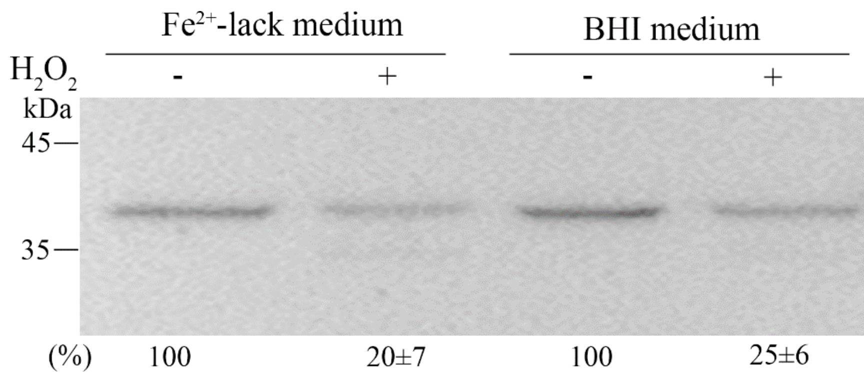

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117 **Fig. S7. Assay of H_2O_2 or Fenton chemistry-generated hydroxyl radical caused the**

118 **cellular So-RNaseZ degradation.** The *S. oligofermentans* RNaseZ-6His strain was

119 anaerobically grown in Fe^{2+}-lack chemically defined FMC medium and BHI broth,

120 respectively. Until OD_{600} of ~ 0.3-0.4, an aliquot of the culture was 30 min treated with

121 500 μM H_2O_2, and leaving another aliquot untreated. Cells were then sonicated and the

122 lysates were run on 12% SDS-PAGE gels. The cellular So-RNaseZ abundance was

123 examined by western blot using anti-His antibody. Band intensities were measured

124 using Image J, and expressed as the percentiles of the So-RNaseZ protein in the

125 bacterium grown in the corresponding medium not treated with H_2O_2. Molecular weight

126 marker is shown at the left. Experiments were repeated three times, and the averages ±

127 SD of three experiments are shown beneath the representative gel.

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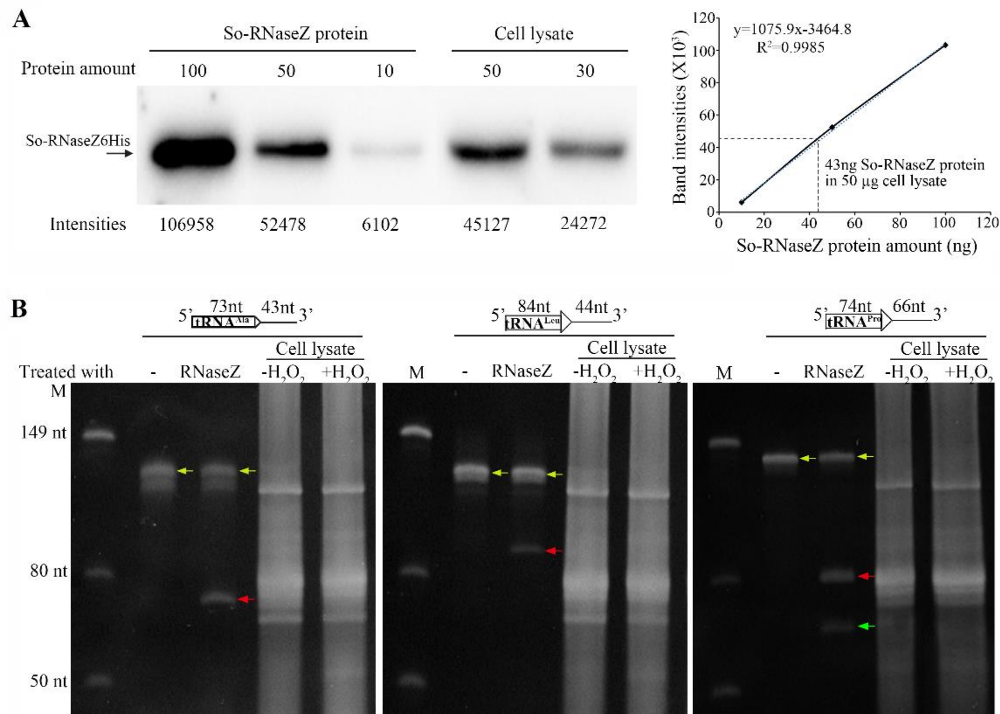

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131 **Fig. S8. Determination of tRNA precursor degradation by *S. oligofermentans* cell**
132 **lysates.** A. Western blot assayed the cellular So-RNaseZ protein content. Various
133 amounts of purified So-RNaseZ protein (ng) and cell lysates (µg) of the RNaseZ-6His
134 strain were run on 12% SDS-PAGE gel and So-RNaseZ protein was detected using
135 anti-His antibody (left panel). Band intensities were measured using Image J. Band
136 intensities of purified So-RNaseZ protein were plotted against So-RNaseZ protein
137 amount (right panel). Referenced to the linear regression equation, 43 ng of So-RNaseZ
138 protein was calculated in 50 µg cell lysate. B. The 116 nt $tRNA^{Ala}$ (I872_t10692), 128
139 nt $tRNA^{Leu}$ (I872_t10780) and 140 nt $tRNA^{Pro}$ (I872_t10754) precursor fragments were
140 obtained by *in vitro* transcription. Four aliquots of 50 ng of each tRNA precursor were
141 prepared; one aliquot was untreated as a control, two aliquots were incubated with 10

142 μg cell lysate of anaerobically cultured *S. oligofermentans* that were 30 min treated
143 with (+H_2O_2) or without 0.5 mM H_2O_2 (-H_2O_2), respectively, and the other one was
144 incubated with 15 ng So-RNaseZ protein (RNaseZ), by referenced to the So-RNaseZ
145 abundance in 10 μg cell lysate estimated in A. After 30 min incubation, the samples
146 were separated on 10% Urea PAGE gel and stained with SYBR Gold Nucleic Acid Gel
147 Stain. Schematics at top show the lengths of the tRNA gene and 3' trailer in each
148 precursor. Yellow arrow indicates the tRNA precursors, red and green arrows indicate
149 the cleaving products comprising of tRNA gene and 3' trailer, respectively. M, RNA
150 molecular weight marker with length shown at the gel left.
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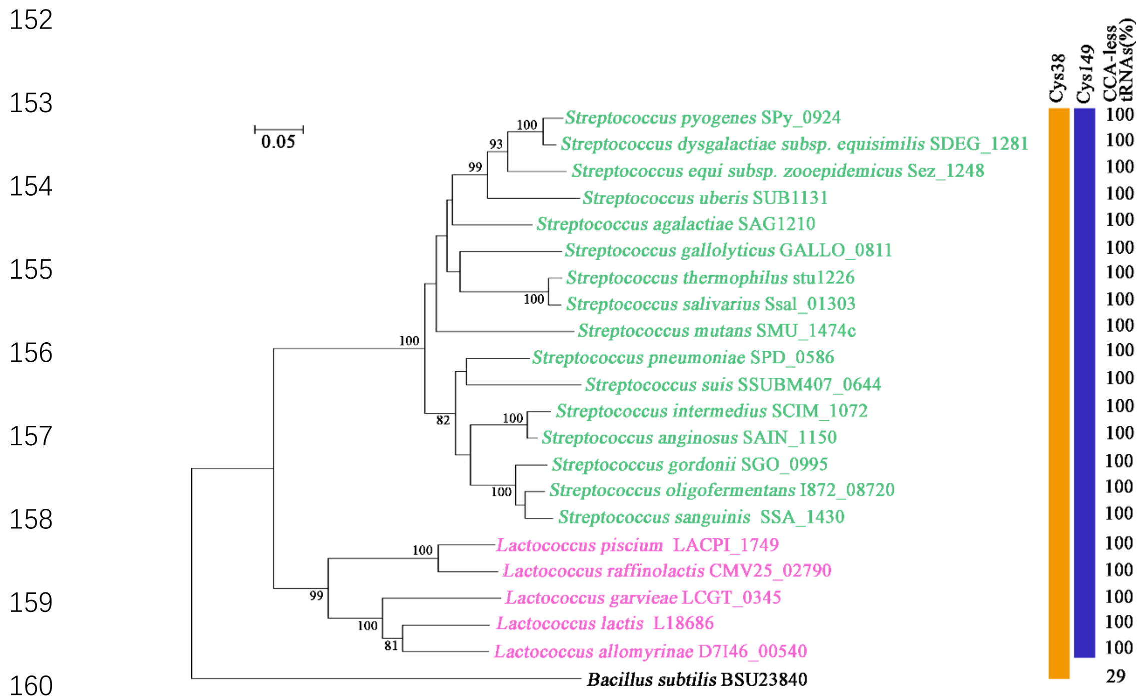

Fig. S9. Phylogenetic analysis of the So-RNaseZ orthologs and its Cys38/Cys149 in Streptococcaceae that encode CCA-less tRNAs. Sequences of So-RNaseZ protein and its orthologs from all species of Streptococcaceae were obtained from KEGG database, and aligned using ClustalW. The phylogenetic tree was constructed using the Neighbor-joining method with bootstrap values of 1000 replicates. The bar of 0.05 represents the evolution distance. Orange and blue bars at right indicate the presence of Cys38 and Cys149 in the RNase Z orthologs, respectively; and numbers represent the percentiles of CCA-less tRNAs in the respective species.

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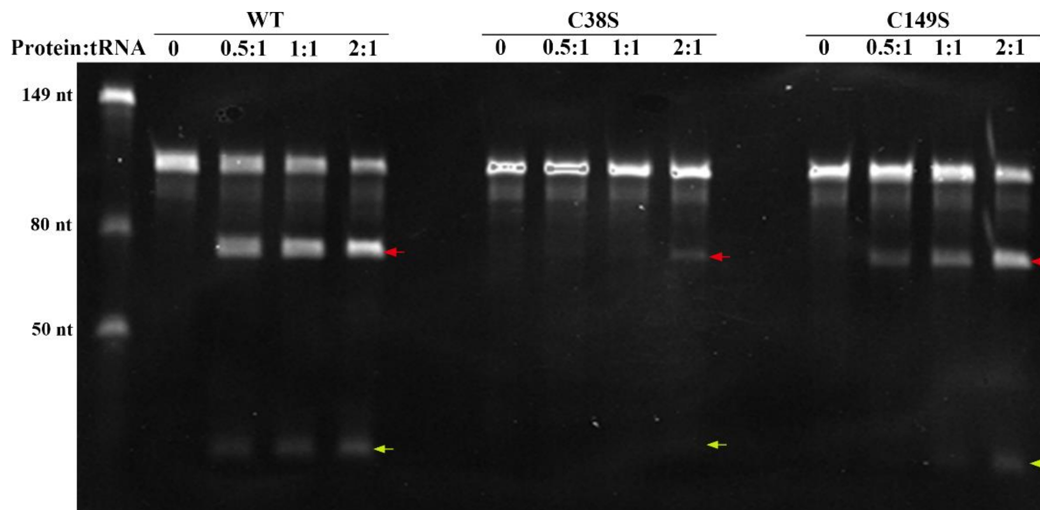

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172 **Fig. S10. Comparison of the wild-type and cysteine mutated So-RNaseZ activities.**

173 The 104 nt tRNA^{Arg} (I872_t10790) precursor fragment, comprising of 74 nt mature

174 tRNA^{Arg} and 30 nt 3' trailer, was obtained by *in vitro* transcription, and 50 ng was used

175 as the nucleolytic substrates. Nucleolytic assay was initiated by addition of a gradient

176 of increasing molar ratio of wild-type So-RNaseZ6His protein (WT) and Cys38 and

177 Cys149 serine substituted So-RNaseZC38S6His (C38S) and So-RNaseZC149S6His

178 (C149S), respectively, and incubated at 37℃ for 30 min as described in "Materials and

179 Methods". Nucleolytic products were separated on 10% Urea PAGE gel. Red and

180 yellow arrows indicate the cleaving products of mature tRNA gene and 3' trailer,

181 respectively. RNA molecular weight marker is shown at the gel left.

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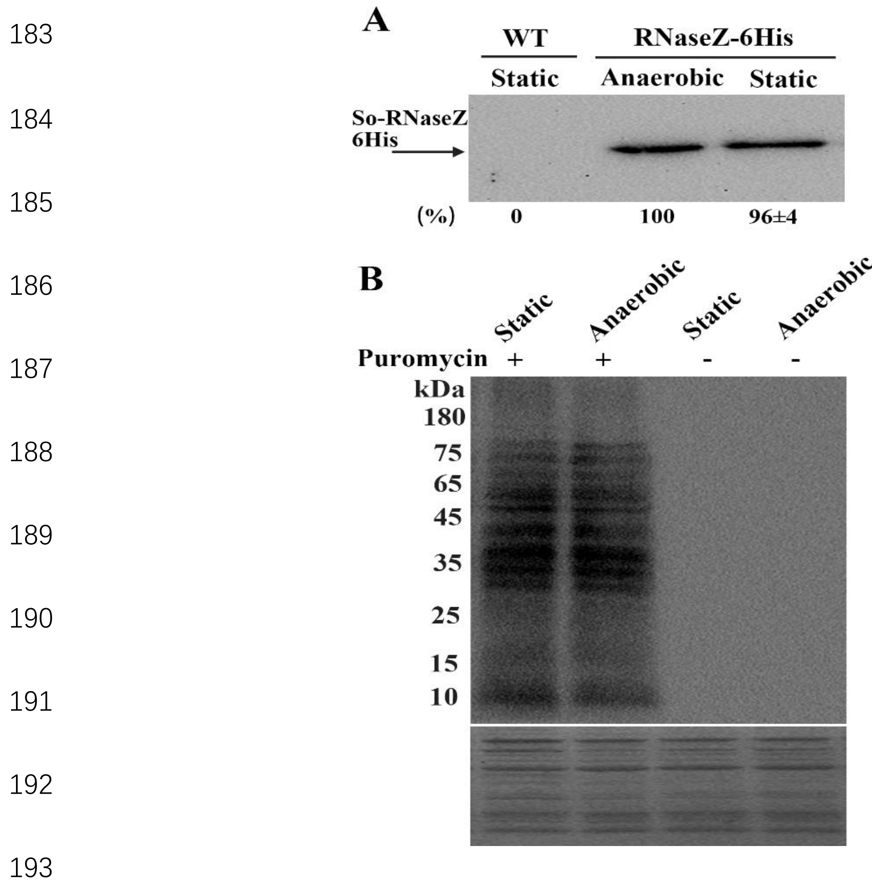

194 **Fig. S11. Comparison of So-RNaseZ abundance (A) and protein synthesis (B) of**
195 **the statically and anaerobically grown *S. oligofermentans*.** A. Western blot assayed
196 the So-RNaseZ abundance in RNaseZ-6His cells. Early mid-exponential cells of
197 statically or anaerobically grown RNaseZ-6His were collected and lysed. Same
198 amounts of total cell protein were loaded on 12% SDS-PAGE gels, and the So-RNaseZ
199 abundance was examined by western blot using anti-His antibody. Cell lysate from
200 statically grown wild-type (WT) cells was included as a control. Western blot signal
201 intensities of the So-RNaseZ protein were measured using Image J, and expressed as
202 the percentages of anaerobic culture. Triplicate experiments were performed, and
203 averages ± SD are shown below one representative image. B. Strain RNaseZ-6His was
204 cultured as described in A, and puromycin was added 10 min before cell collection.

205 Western blot assayed puromycin integration using the anti-puromycin antibody (upper
206 panel), and same contents of total proteins were run on 12% SDS-PAGE gels and
207 stained with Coomassie brilliant blue (lower panel). Molecular weight marker is shown
208 at each gel left. Triplicate experiments were performed, and one representative result is
209 shown.
210

211 **Table S1. Strains, plasmids and primers used in this study***

Strain, plasmid or primer	Characteristics and description	Reference or sources
Strain		
E. coli		
DH5α	*supE44 lacU169* (80 *lacZ*M15) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1 luxS*	TransGen Biotech, Beijing, China
BL21 (DE3)	F- *ompT hsdS* (rBB - mB -) gal dcm (DE3)	TransGen Biotech, Beijing, China
S. oligofermentans		
Wild-type	WT, Kan^s Sp^s	1
RNaseZ-6His	WT RNaseZ::6His; Kan^r; wild-type with RNaseZ fused with 6×His tag at C-terminus	This study
So-RNaseZcom	WT RNaseZ::Kan; pDL278-SoRNaseZ6His; Kan^r; Sp^r; wild-type with So-*rnaseZ* deletion and carrying pDL278-SoRNaseZ6His plasmid	This study
So-RNaseZC38Scom	WT RNaseZ::Kan; pDL278-SoRNaseZC38S6His; Kan^r; Sp^r; wild-type with So-*rnaseZ* deletion and carrying pDL278-SoRNaseZC38S6His plasmid	This study
So-RNaseZC149Scom	WT RNaseZ::Kan; pDL278-SoRNaseZC149S6His; Kan^r; Sp^r; wild-type with So-*rnaseZ* deletion and carrying pDL278-SoRNaseZC149S6His plasmid	This study
So-RNaseZover	WT pDL278-P*ldh*-SoRNaseZ6His; Sp^r; wild-type harboring pDL278 plasmid with So-RNaseZ6His driven by lactate dehydrogenase promoter	This study
pDL278RNaseZ-6His	WT RNaseZ::6His; Kan^r; RNaseZ-6His strain carrying pDL278 plasmid	This study
Plasmid		
pALH124	Kan^r	2
pDL278	Sp^r	3

pDL278-SoRNaseZ6His	pDL278 carrying SoRNaseZ6His; Sp^r	This study
pDL278-SoRNaseZC38S6His	pDL278 carrying SoRNaseZC38S6His; Sp^r	This study
pDL278-SoRNaseZC149S6His	pDL278 carrying SoRNaseZC149S6His; Sp^r	This study
Primer	**Sequence (5'-3')**	**Purpose**
RNaseZ-6His upF	CTGGTTCCCGTCTGCCTTATC	RNaseZ-6His construction
RNaseZ-6His upR	CTAA*GGATCC*CTAGTGGTGGTGGTGGTGTAACTCCACCTCTTCCAAG	RNaseZ-6His construction
RNaseZ-6His dnF	CTAA*GGATCC*TTTTCCAATTTAGGACAGTTTC	RNaseZ-6His construction
RNaseZ-6His dnR	GCTCCTTCCAACCAAAATCAGTCGG	RNaseZ-6His construction
GipDL278F	CACCACCACTAGTCTAGAGTCGACCTGCAGG	So-RNaseZcom construction
GipDL278R	CAGAAAAGGAGAAATCACCTGGCCGTCGTTTTAC	So-RNaseZcom construction
PoperonF	CGACGGCCAGGTGATTTCTCCTTTTCTGAACTTTTA	So-RNaseZcom construction
PoperonR	GTAATTGCATCTTTTTTCCTTGAAAAAATTTTAATTTTTC	So-RNaseZcom construction
rnz-comF	AGGAAAAAAGATGCAATTACAATTTTTAGGAACCG	So-RNaseZcom construction
rnz-comR	GGTCGACTCTAGACTAGTGGTGGTGGTGGTGGTGTAACTCCACCTCTTC	So-RNaseZcom construction
rnz-deleteupF	GATTTGGCCCTGAAATACGGCGG	So-RNaseZcom construction
rnz-deleteupR	CCTA*GGATCC*GTAATTGCATGTTATTCTTTCTAAATG	So-RNaseZcom construction
rnz-deletedownF	GGA*GGATCC*GGAGTTATAGTTTTCCAATTTAG	So-RNaseZcom construction
rnz-deletedownR	CAGTCGGTCATGGGGCAAGAGC	So-RNaseZcom construction
*rnz*C38SF	GGTCTGGATGTTTGATTCCGGTGAAGGTACCCAG	So-RNaseZC38Scom construction
*rnz*C38SR	CTGGGTACCTTCACCGGAATCAAACATCCAGACC	So-RNaseZC38Scom construction
*rnz*C149SF	GCTAGACCACACGATTTTCTCTGTGGGTTATCGGGTCATGC	So-RNaseZC149Sco

		m construction
*rnz*C149SR	GCATGACCCGATAACCCACAGAGAAAATCGTGTGGTCTAGC	So-RNaseZC149Scom construction
PLDHF	CCG*GAATTC*TAAAAAATGCCTCGTTTCAATATTAAATC	So-RNaseZover construction
PLDHR	CCGGTTCCTAAAAATTGTAATTGCATTTCTAAACATCTCCTTATTATTTTTAG	So-RNaseZover construction
Pldh-*rnz*overF	CTAAAAATAATAAGGAGATGTTTAGAAATGCAATTACAATTTTTAGGAACCGG	So-RNaseZover construction
Pldh-*rnz*overR	ACGC*GTCGAC*CTAGTGGTGGTGGTGGTGGTGTAACTCCACCTCTTCC	So-RNaseZover construction
TransArgF	TAATACGACTCACTATAGGGAGAATGAAAAGGTATCGGTCTC	*In vitro* transcription for RNaseZ activity
TransArgR	CTTACAAGATCTATTATAAATAAAATTTC	*In vitro* transcription for RNaseZ activity
TransAlaF	TAATACGACTCACTATAGGGGGGCCTTAGCTCAG	*In vitro* transcription for RNaseZ activity and Northern blot
TransAlaR	CAATTTTCAATGGACAAGTTTAGAATAGTC	In vitro transcription for RNaseZ activity
Northern AlaR	GTTTATTTTCTTGTTACTATTTGATATAGTTATTC	In vitro transcription for Northern blot
TransProF	TAATACGACTCACTATAGGGCGGGAAGTAGCTCAG	In vitro transcription for RNaseZ activity
TransProR	CAAAAAAACGGCTCCAGCCGTTATTTAAATAC	In vitro transcription for RNaseZ activity
TransLeuF	TAATACGACTCACTATAGGGGCGGAGGTGGTGGAACGG	In vitro transcription for RNaseZ activity
TransLeuR	CAAAAGAAAAACTCTTGTCAACTTG	In vitro transcription for RNaseZ activity
TransMetF	TAATACGACTCACTATAGGGCGCGGATGGAGCAGCTAG	In vitro transcription for RNaseZ activity

TransMetR	GACACATGGAGCTTCAATCC	In vitro transcription for RNaseZ activity
Ala probe	5'-biotin-CGAACCGCTGACCTCCTGCGTGCAAAGCAGGCGCTCTCCCAG	Mature $tRNA^{Ala}$ Northern blot
Pro probe	5'-biotin-CCTGCGACACCTTGGTCCCAAACCAAGTACTCTACCAAGCTGAGCTACTTCCCG	Mature $tRNA^{Pro}$ Northern blot
Leu probe	5'-biotin-GATTTGAACCCTCACACCCGTACGGGCACATGCGCCTGAAGCATGCGTGTCT	Mature $tRNA^{Leu}$ Northern blot
$pretRNA^{Ala}$ probe	5'-biotin-CTATTTGATATAGTTATTCAATTTTCAATGGACAAGTTTAGAATAG	$tRNA^{Ala}$ precursor Northern blot
$pretRNA^{Pro}$ probe	5'-biotin-AAAAAAACGGCTCCAGCCGTTATTTAAATACAGTAATGATTAGAAAATTCTAAACCAACAATATA	$tRNA^{Pro}$ precursor Northern blot
$pretRNA^{Leu}$ probe	5'-biotin-CATAATTTTTCAAAAGAAAAACTCTTGTCAACTTGGACAAGAGTTCCTGTTCGA	$tRNA^{Leu}$ precursor Northern blot
5S rRNA probe	5'-biotin-CACAGGGGGCAACCCCCAACTACTTCCGGCGTTCTAGGGCTTAACTTCTGTGTTCGGCATGGGTACAGGTGTAT	5S rRNA Northern blot
Ala3RACEF	GGGGCCTTAGCTCAGCTGGG	$tRNA^{Ala}$ 3' race
Ala3RACER	ATTGATGGTGCCTACAG	$tRNA^{Ala}$ 3' race
Ala5RACEF	CAGACTGGATCCGTCCTC	$tRNA^{Ala}$ 5' race
Ala5RACER	CAATTTTCAATGGACAAGTTTAGAATAGTC	$tRNA^{Ala}$ 5' race

212 *: Kan, kanamycin; Sp, spectinomycin; r, resistant; s, sensitive.

213 Italic nucleotide bases indicate restriction enzyme digestion sites.

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215 **References**

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217 oral isolate from caries-free humans. *Int J Syst Evol Microbiol* 53:1101-1104.
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