

Fig. S1. Co-transcription of *rnr-trmH* operon. **(A)** RT-PCR analysis of *P. syringae* RNA, showing 338 bp amplified DNA expected for a common transcript of the two genes. **(B)** Schematics of the location of primers on the *rnr-trmH* locus. ATG/TGA overlapping start and stop codons of *trmH* and *rnr* reading frames are shown. The numbers shown below the line depicting *rnr-trmH* encoding region corresponds to nucleotides sequence number in *P. syringae* Lz4W sequence at NCBI (acc no HQ122447). The positions of forward (FP) and reverse (RP) primers shown here is not according to scale.

Supplementary Figure S1

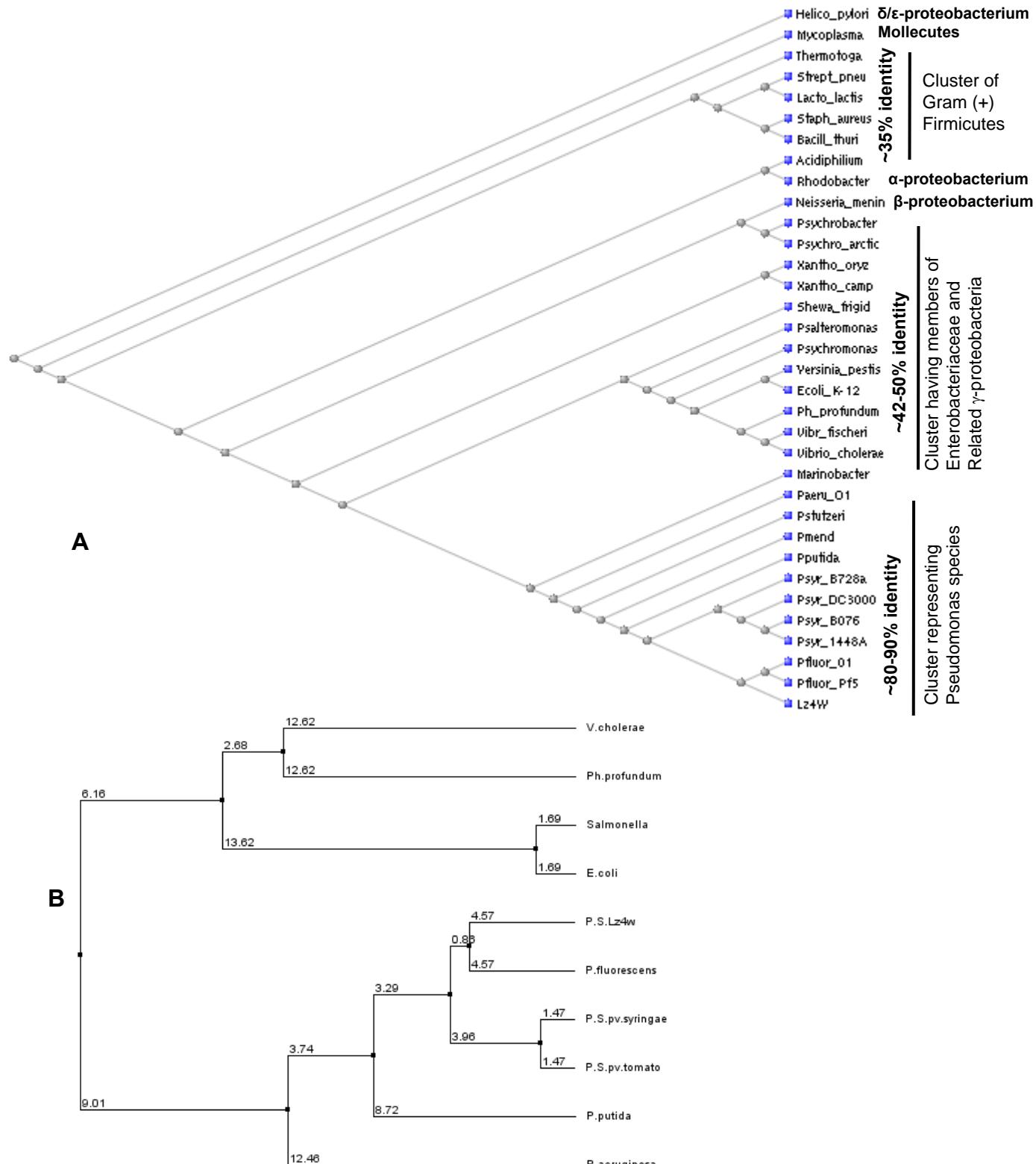


Fig. S2. Phylogenetic tree based on multiple alignments of amino acid sequences of selected RNase R homologues. The tree shown in (A) is based on Cobalt (www.ncbi.nlm.nih.gov) and the tree in (B) is based on ClustalW (www.ebi.ac.uk) using default parameters. Both employ neighbour joining (NJ) method, but use different protein distance matrix, as described in the respective web sites. The distance scales shown between species are based on substitution rates of the sequences along the phylogenograms. The numerical values in (B) represent the distance between the branch and adjacent node estimated from the rate of divergence.

Accession number of sequences used in RNase R phylogram analysis:

1. Lz4W: *Pseudomonas syringae* Lz4W, Acc no HQ122447.
2. Pfluor_Pf5: *Pseudomonas fluorescens* Pf-5 RNase R, gi|68348378|gb|AAV95984.1|
3. Pfluor_01: *Pseudomonas fluorescens* Pf0-1, gi|77456760|ref|YP_346265.1|
4. Psyr_1448A: *Pseudomonas syringae* pv. *phaseolicola* 1448A, gi|71733290|ref|YP_272874.1|
5. Psyr_B076: *Pseudomonas syringae* pv. *glycinea* str. B076, gi|320321876|gb|EFW77972.1|
6. Psyr_DC3000: *Pseudomonas syringae* pv. *tomato* str. DC3000, gi|28872049|ref|NP_794668.1|
7. Psyr_B728a: *Pseudomonas syringae* pv. *syringae* B728a, gi|63254553|gb|AAV35649.1|
8. Pputida: *Pseudomonas putida* KT2440, gi|24986645|gb|AAN70448.1|AE016686_2|
9. Pmend: *Pseudomonas mendocina*, gi|145573879|gb|ABP83411.1|
10. Pstutzeri: *Pseudomonas stutzeri* A1501, gi|146283973|ref|YP_001174126.1|
11. Paeru_O1: *Pseudomonas aeruginosa* PAO1, gi|15600130|ref|NP_253624.1|
12. Marinobacter: *Marinobacter aquaeolei*, gi|120325149|gb|ABM19464.1|
13. Vibrio_cholerae: *Vibrio cholerae*, gi|297535773|gb|EFH74607.1|
14. Vibr_fischeri: *Vibrio fischeri*, gi|59712921|ref|YP_205697.1|
15. Ph_profundum: *Photobacterium profundum* SS9, gi|46914862|emb|CAG21639.1|
16. Ecoli_K-12: *Escherichia coli* str. K-12 substr. MG1655, gi|90111698|ref|NP_418600.4|
17. Yersinia_pestis: *Yersinia pestis*, gi|229842330|ref|ZP_04462485.1|
18. Psychromonas: *Psychromonas ingrahamii*, gi|119865624|gb|ABM05101.1|
19. Psalteromonas: *Pseudoalteromonas atlantica*, gi|109699600|gb|ABG39520.1|
20. Shewa_frigid: *Shewanella frigidimarina*, gi|114335809|gb|ABI73191.1|
21. Xantho_camp: *Xanthomonas campestris* pv. *campestris*, gi|188992142|ref|YP_001904152.1|
22. Xantho_oryz: *Xanthomonas oryzae* pv. *oryzicola*, gi|166711526|ref|ZP_02242733.1|
23. Psychro_arctic: *Psychrobacter arcticus*, gi|71066655|ref|YP_265382.1|
24. Psychrobacter: *Psychrobacter cryohalolentis*, gi|92394922|gb|ABE76197.1|
25. Neisseria_menin: *Neisseria meningitidis*, gi|261392528|emb|CAX50083.1|
26. Rhodobacter: *Rhodobacter sphaeroides*, gi|126105211|gb|ABN77889.1|
27. Acidiphilium: *Acidiphilium cryptum*, gi|146400739|gb|ABQ29266.1|
28. Bacill_thuri: *Bacillus thuringiensis*, gi|49332682|gb|AAT63328.1|
29. Staph_aureus: *Staphylococcus aureus*, gi|147740309|gb|ABQ48607.1|
30. Lacto_lactis: *Lactococcus lactis*, gi|116107413|gb|ABJ72553.1|
31. Strept_pneu: *Streptococcus pneumoniae*, gi|242266981|gb|ACS91342.1|
32. Thermotoga: *Thermotoga petrophila*, gi|147734896|gb|ABQ46236.1|
33. Mycoplasma: *Mycoplasma capricolum*, gi|83283663|gb|ABC01595.1|
34. Helico_pylori: *Helicobacter pylori*, gi|254779791|ref|YP_003057897.1|

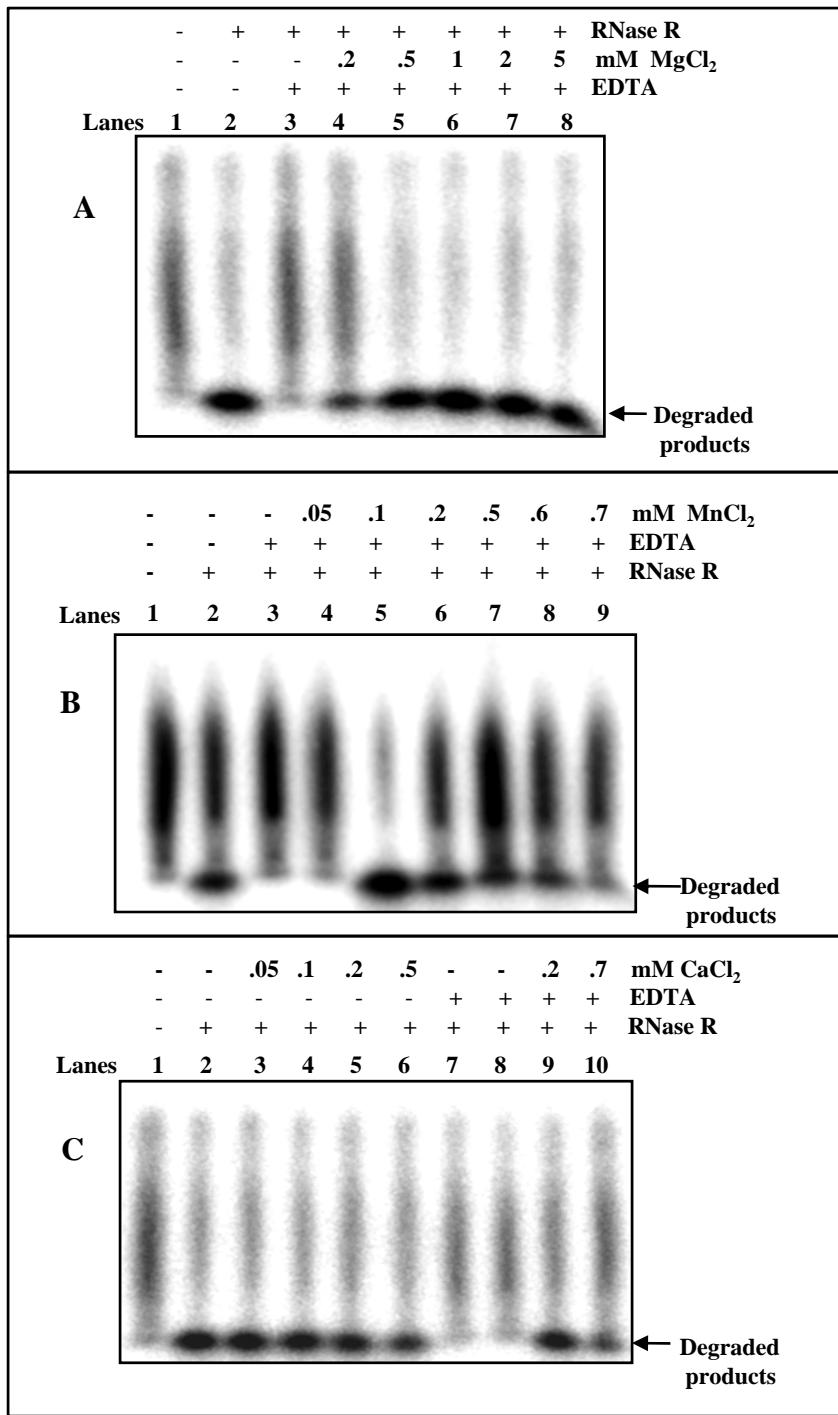


Fig. S3. Divalent cation requirement of RNaseR^{Ps} for RNA degradation. ³²P-end labeled poly(A) were used for the assays in the presence of Mg²⁺ (Panel A), Mn²⁺ (Panel B), and Ca²⁺ (Panel C). The degraded products were analyzed on 8% polyacrylamide – 8M urea gel. Reactions were carried out in the presence of 10 ng enzyme and 10 pmole of poly(A), and stopped after five minutes of incubation. Note that 1 mM EDTA was used to inactivate the initial activity of the enzyme (lane 3) in reaction buffer to which the indicated amount of divalent cations were added. Undigested control for the labeled poly(A) are in lane 1 of all the panels.

Supplementary Figure S3

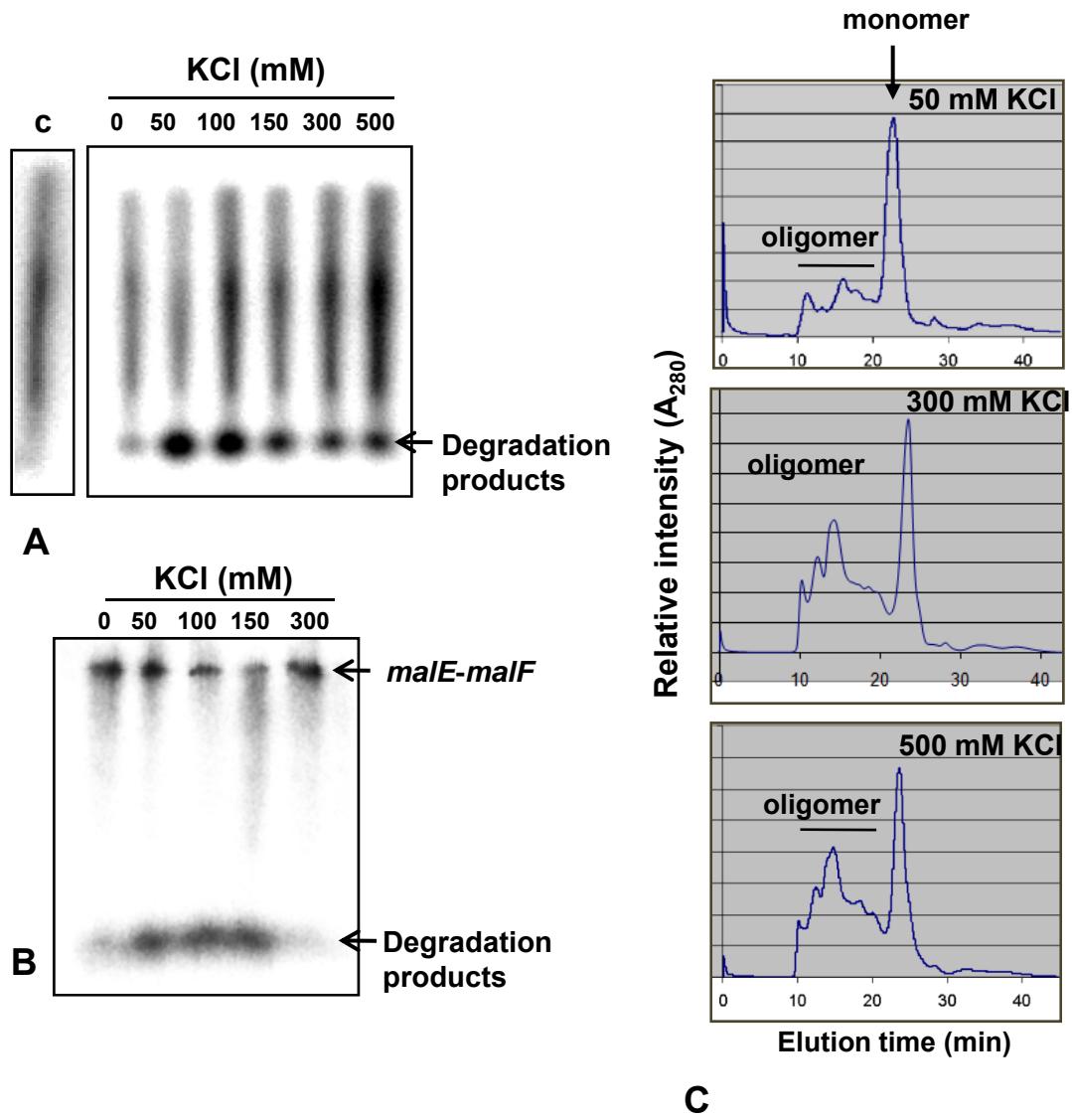


Fig. S4. Analysis of the activity and oligomerization states of RNaseR^{Ps} under different salt concentrations. The gel-filtration analysis of RNaseR^{Ps} shown here was performed in the presence of 50 and 500 mM KCl on Superdex-200. Fractions were checked by SDS-PAGE (not shown) for the presence of enzyme. The activities were measured on (A) ³²P-labeled poly(A), and (B) *malE-malF* RNA. The elution profiles of the enzyme from the gel-filtration column are shown in (C). The elution rates were at the rate of 1 ml min⁻¹.

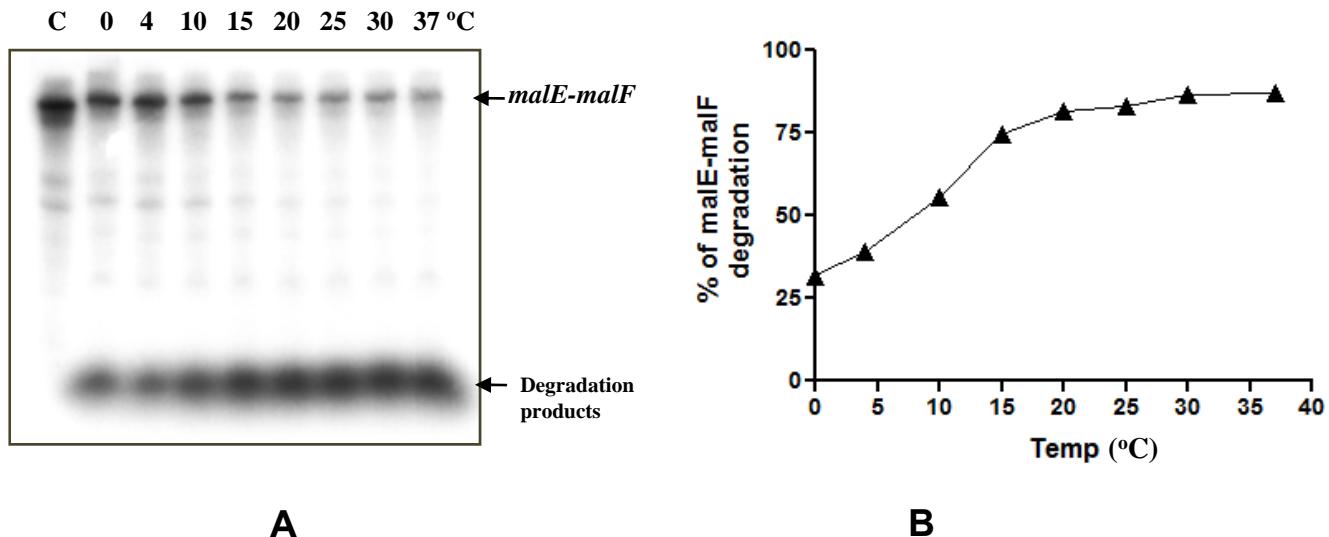


Fig. S5. Temperature dependent degradation of *malE-malF* RNA by *P. syringae* RNase R. Phosphorimage of *malE-malF* degradation (A) and its quantification (B) have been shown. 10 nM protein was added to 32P-internally labeled *malE-malF* RNA, incubated for 30 minutes at the indicated temperatures. Reaction products were separated on denaturing 8 M urea – 8% PAGE and detected by phosphorimager.

Lz_Rnr	MADWQTLDEAAREAEKYENPIPSRELILQHLLAERGSPAAAREQLVEEFGLTTEQIEALRRRLRAMERDAQLIYTRRGTY	80
Ec_Rnr	MSQ----DPFQEREEAKYANPIPSREFILEHLTKREKPASRDELAVELHIEGEEQLEGLRRRLRAMERDGQLVFRQCY	76
Ec_RII	-----MFQDNPLLAQLKQQQLH	16
-CSD1-		
81	APVD K LDL L GRISGH R DGF G FLIPDDGS S DDLFM P SQMRLVFDGD R ALARVSG-LD R RG R REGVIVEVV S RAH E TIVGR	159
77	ALPER L DLV K GT V I G H R D G Y G FLRVEGR K DD L YL S SE Q MKTC I H G D Q V L A Q PL G -ADR K GR R EAR I R V R L V P K T S QIVGR	155
17	SQTPRAE---GVVKATEKG F GLE D A-QKS Y FI P PPQ M KKVMHG D R I I A VI H SE E RESAE E EL V EP F L---TRFVG K	89
-CSD2-		
160	YFEEGGIGFVVADNP K IQ Q EV--LV T PGRNAGAQ V Q G Q V EV V K I H W P T PR F QP-QGD V LEV V GN Y MAP G ME I DIAL R TY D	236
156	Y F TEAGVG F V P DD S RL S FD I --L I PPD Q IM G ARM G V V V V EL T QR P TR R TK A -VG K IVE V L G DN M GT G MA V DIAL R THE	232
90	V Q G K N D R L A I VP D H P LL K DA I PC A R G LN H E F K E GD W AV A EM R R H PL K DR S F Y AE L T Q Y I TF G DD H F V P W WT L AR H N	169
-RNB-		
237	IPHV W PEAV L KE A AK L K P E V E E K D KE R IDL R HL P F V T I D G ED A RF D DA V C E ARP G K L R L F S GG W TLY V AI D V S YY V	316
233	IP I Y W P Q AV E QQ V AG L KE E V P E E A K GR V DL R DL P L V T I D G ED A RF D DA V CE K K R -----GG W R L W V AI D V S YY V	306
170	LE K E A P D G V A E ML-----DE G LV R ED L T A DF V T I D S AS E TD M DD A LF A K L PD-----DK L Q L I V A J AD P T A WI	235
I		
317	KIGS A LD A ES Q VR G NS V Y F PER V IP M L P E Q LS N GL C SL N PK V DR L AM V CE M T I S G EM T D-Y K F Y EA V I H S Q AR L T Y N K	395
307	RP S T P L D R E AR N R G TS V Y F PS Q V I P M L P E V L S NG L CS L N P Q V DR L CM V CE M T V S S K R LT G -Y K F Y EA V M S SH A R L T Y T K	385
236	<u>AEGSKLDKA</u> AK I RAFT N Y L PG F N I P M L P REL S DD L CS L RANE V RP V LAC R MT L SAD G T I E F FA A T I E S K A K L V Y DQ	315
II		
396	V S T I LE Q P K T S EA K QL R SE Y AD V VPH L K Q LY S LY K V L LA R H V R G A I D F E T Q E TR I IF G S E R K I A E I R P T T -R N DA H K L I	474
386	V W H I L Q G D Q D L R E-----Q Y AP L V K H L E E L H N L Y K V L D K ARE E RG G I S FE E EE A K F IF N A E R R I E QT Q -R N DA H K L I	459
316	V S DW L E N T G D W Q P E S EA-----IA E Q V R L LA Q I C Q R GE W R H N H AL V <u>FK</u> D RP D Y R F I LG-E K GE V LD I VA E P R R I AN R I V	389
III		
475	E EC M LA A AN V ATA E FL K K H E I P A LY R V H D G PP R ER L KE L R A FL G EL G LS L H K G D GP S PK D Y Q ALL A SI K D R PD F H L I Q TV	554
460	E EC M IL A NI S AR F VE K AK E PA L F R I H D K P S TE A IT S FR S V L AE L G L E L -PG G N K PE P R D Y A EL E S V AD R P D AE M LT Q TM	538
390	E EA M IA A NI C AR V L R DK L GF G I Y NV H MG F DP AN ADA L A A LL K TH G HL H -DA E EV L TD G F C K L R E LD A Q P T G -FL D SR	467
555	M LR S MS Q AV S AD N Q G H F GL N YE A Y T H F <u>T</u> S P I R R PD L TL H RA I Y L LA Q E G -----HQ G NT T E T GG Y H S ME E ML--Q	634
539	L LR S MX Q AI Y DP E NR G F G LA L Q S Y A H F <u>T</u> S P I R R PD L TL H RA I Y L LA Q E G -----HQ G NT T E T GG Y H S ME E ML--Q	611
468	I RR F Q S FA E I S TE P G P <u>H</u> FL G LE A Y T <u>T</u> S P I R K Y G DM I N H RL L K A V I K G E T AT R -----PQ D E I TV--	529
IV		
-S1-		
635	L G E Q C S M S S E R R ADE---AT R D V T N WL K C E F M K D R V <u>GES</u> --FP G V V T A V T G F GL F VEL T DI V E G LV---H V T A K P GD Y Y H F	707
612	L G Q H C S M S A E R R ADE---AT R D V W D WL K C D F M LD Q V GN V--FK G V I S V T G F G FF V R L DD L F I D G LV---H V S S LD N D Y Y R F	684
530	-----Q M A E RR R RL N R M A <u>E</u> RD V W D LY A FL K D K A G T D TR F AA E I V D I S R GG M R V R L V D NG A I A F I P A P F L H AV R DEL V C F	604
708	D QR H HL A GER T GR S F R L G D T VE V R M V R VL D LD E RF K IDE F E M -A E K T L S API G R--KNRG T D A P A G K N A G K S---G T K A T E K	781
685	D Q V Q RL M GE S SS G Q T Y R LG D R V R V R E A V N M DE R K I D F SL I SS E AP R RV N G K T A E K K G D A G K GG K R R Q V G K V N F E	764
605	Q ENG T V Q I K G E TT--V V K T D V I D V T I A E V R M E T R S II A R P V A -----	644
782	P V E P P A A R R S A P K T A K -S D E T Y R P G D A A K N A E R K S REL K Q A LL A D K N G R R A E S G K S GR G A P K D A G K P K S K H	860
765	P D S A FR G E K K T K P K A AK K D A K K A K P---S A K T Q K I-----	797
861	R K G P K S GT A PA K SG G ARK K P V K S	644
798	-----AA A T K A R AK K K V A E	813
	-----644	

Fig. S6. Alignment of RNase R homologue of *P. syringae* (Lz4w_Rnr) with *E. coli* RNase R (Ec_Rnr) and *E. coli* RNase II (Ec_RII) sequences. N-terminal CSD domains (CSD1 as brown and CSD2 as blue), central RNB domain (shown in green), and C-terminal S1 domain (in purple) have been marked. The sequences of four conserved motifs in RNB domain of proteins have been underlined. Residues in RNB that contact with RNA nucleotides have been highlighted red. (-) denotes gap generated by the alignment tool to show maximum homology.