Exoribonuclease RNase R protects Antarctic *Pseudomonas syringae* Lz4W from DNA damage and oxidative stress

Running Title: A cryptic Role of RNase R in protecting against DNA damage and oxidative stress.

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Abstract

RNase R is a highly processive, 3' - 5' exoribonuclease involved in RNA degradation, maturation, and processing in bacteria. In *Pseudomonas syringae* Lz4W, RNase R interacts with the RNase E to form the RNA degradosome complex and is essential for growth at low temperature. RNase R is also implicated in general stress response in many bacteria. We show here that *rnr* gene (encoding RNase R) deletion mutant of *P. syringae* is highly sensitive to various DNA damaging agents and oxidative stress. RNase R is a multidomain protein comprised of CSD, RNB and S1 domains. We investigated the role of each domain of RNase R and its catalytic activity in nucleic acid damage and oxidative stress response. Our results revealed that RNB domain alone without its exoribonuclease activity is sufficient to protect against DNA damage and oxidative stress. We also show that association of RNase R with degradosome complex is not required for this function. Finally, based on homology modelling of RNB domain of RNase R of *P. syringae*, we propose that the presence of a tri-helix wedge domain may be involved in protection against DNA damage and oxidative stress in *P. syringae* through a putative helicase activity. Our study for the first time has discovered a hitherto unknown role of RNase R in protecting *P. syringae* Lz4W against DNA damage and oxidative stress.

Importance

Bacterial exoribonucleases play a crucial role in RNA maturation, degradation, quality control and its turnover. In this study, we have uncovered a previously unknown role of 3'- 5' exoribonuclease RNase R of *P. syringae* Lz4W in DNA damage and oxidative stress response. Here, we show that neither the catalytic exoribonuclease function of RNase R, nor its association with the RNA degradosome is essential for this function. Nevertheless, a putative helicase function of RNase R appears to have an important role in protecting against DNA damage and oxidative stress. Interestingly, in *P. syringae* Lz4W, the hydrolytic RNase R exhibits physiological roles similar to phosphorolytic 3'- 5' exoribonuclease PNPase of *E. coli*. Our data suggests that during the course of evolution, mesophilic *E. coli* and psychrotrophic *P. syringae* have apparently swapped these exoribonucleases to adapt to their respective environmental growth conditions.

Key words: *Pseudomonas syringae*, RNase R, DNA damage, Oxidative stress, RNA degradation, and RNA unwinding.

Introduction

Exoribonucleases play an important role in determining the levels of RNA in the cells (1). Some of the exoribonucleases also associate with degradosome, a multi-protein complex involved in degradation and processing of cellular RNA (2, 3). The RNR superfamily of enzymes that includes proteins like RNase II, RNase R, and the eukaryotic Rrp44/Dis3 are present in all domains of life (4). RNase R, encoded by *rnr* gene, is a 3´ to 5´ highly processive exoribonuclease involved in degradation, maturation and processing of RNA in bacteria (5-8). RNase R plays an important role in RNA quality control, such as degradation of defective tRNA and removal of aberrant fragments of the 16S and 23S rRNAs (7, 9). RNase R is capable of processing dsRNA without the help of a helicase, hence implicated in removal of mRNAs with stable stem loops, including repetitive extragenic palindromic (REPs) elements (6, 10) It is also involved in processing of tmRNA (transfer-messenger RNA) and is required for the process of trans-translation (11). Structurally, RNase R is a multidomain protein consists of RNA binding domains cold-shock domains (CSD) and S1, and the catalytic RNB domain (12). The catalytic RNB domain contains four highly conserved Aspartate residues that help to coordinate Mg^{2+} ions in the catalytic center to facilitate ribonucleolytic activity (12-14).

In *Pseudomonas syringae* Lz4W, RNase R associates with endoribonuclease RNase E, along with DEAD box helicase RhlE to form the degradosome complex (15). The *rnr* gene *P. syringae* Lz4W and *P. putida* is implicated in survival at low temperature (7, 16-18). The *P. syringae* Lz4W cells lacking RNase R accumulate unprocessed 3' ends of 16S rRNAs in polysomes, making the translation machinery inefficient at low temperature, causing cold sensitivity (7). RNase R is also involved in general cellular stress responses. In *E. coli*, RNase R levels increase under cold shock, stationary phase, and heat shock (19, 20).

In a quest to understand the role of RNase R of *P. syringae* Lz4W (RNase R^{Ps}) in general cellular stress response, we subjected the *rnr* mutant to various types of stresses and found that *rnr* mutant cells are sensitive to oxidative stress and DNA damaging agents. We show that this role of RNase R is independent of its association with the degradosome complex in *P. syringae* Lz4W. Using a series of domain deletion constructs and mutating catalytically important residues, we discovered that the catalytic RNB domain alone without its exoribonuclease function is sufficient to protect *rnr* mutant of *P. syringae* from DNA damage and oxidative stress. Our data further indicates that a putative helicase function of RNB domain is likely to play a role in protecting *P. syringae* Lz4W cells from DNA damage and oxidative stress.

Results

P. syringae rnr mutant cells are sensitive to oxidative stress and DNA damaging agents

In an attempt to understand the role of RNase R in general stress response in *P. syringae*, the *rnr* mutant was subjected to various conditions that include low and high temperatures (4 and 37 ºC), osmotic stress (1M NaCl), envelope stress (10% SDS, 100 mg/ml Lysozyme with 0.5 mM EDTA), antibiotics treatment (100 μg/ml Rifampicin and Spectinomycin), oxidative (0.25 mM Hydrogen peroxide, 15 μM Paraquat) and DNA damage stress (24 J/m^2.s ultraviolet radiation, 0.25 mM Cisplatin, 1 μg/ml Mitomycin C and 2.5 mM Hydroxyurea). As shown in Table 1, we observed that the sensitivity of *rnr* mutant against osmotic, envelope and antibiotic stresses is comparable to that of wild-type (*wt*) cells. We confirmed that the *rnr* mutant cells are cold sensitive, as shown earlier (7). Nevertheless, we found that *rnr* mutant is also sensitive to high temperature stress (Table 1).

Interestingly, we discovered that the *rnr* mutant cells are highly sensitive to the oxidative stress induced by H_2O_2 and Paraquat; and to DNA damage induced by ultraviolet (UV) radiation, Mitomycin C (MMC) and Hydroxyurea (HU) (Table 1). H_2O_2 and Paraquat generate reactive oxygen species (ROS), such as hydroxyl and superoxide radicals that can easily react with biological macromolecules including DNA, RNA, proteins, and lipids (21). We quantified the effect of these agents on the growth and viability of *rnr* mutant in comparison to the *wt* cells. We assessed the colony-forming ability of cells upon treatment with various doses of H_2O_2, Paraquat, UV radiation, MMC and HU. The *rnr* mutant displayed more than 100-fold higher sensitivity compared to *wt* cells to all the agents tested, suggesting a cryptic role of RNase R in protecting *P. syringae* cells from DNA damage and oxidative stress (Figure 1A). Spot assays on ABM plates containing the indicated amounts of H_2O_2, Paraquat, MMC, HU and UV irradiation, further confirmed that *rnr* mutant of *P. syringae* is highly sensitive to all the agents (Figure 1C). In contrast, we observed that the mesophilic *E. coli* cells lacking RNase R (Δ*rnr*) are refractory to oxidative stress and DNA damaging agents (Figure S1) suggesting that the protective role of RNase R against DNA damage is specific to *P. syringae*.

Furthermore, introducing the *rnr* gene encoding RNase R^{Ps} on a low copy plasmid (pGL10::*rnr*^{Ps}) rescued the growth defects of *rnr* mutant caused by DNA damage/oxidative stress confirming that the functional loss of RNase R causes the sensitivity (Figure 1A & 1C). Interestingly, when RNase R of *E. coli* (pGL*rnr*^{Ec}) is overexpressed in *P. syringae* Lz4W, it also rescued the growth defect of *rnr* mutant of *P. syringae* (Figure 1A & 1C). In *E. coli*, another

126 exoribonuclease PNPase has been shown to protect cells against oxidative stress and DNA
127 damage (22-25). We examined if PNPase of *P. syringae* has similar functions and could
128 complement the loss of *rnr* gene against oxidative and DNA damage stress. However, we
129 observed that *rnr* mutants over-expressing PNPase could not rescue the sensitivity of *rnr*
130 mutant to oxidative and DNA damage stress (Figure S2).

131 RNase R is known to upregulate under different stress conditions in *E. coli* (19, 20),
132 hence we examined the levels of RNase R in *P. syringae* Lz4W cells by Western blotting with
133 polyclonal anti-RNase R antibodies (15). The levels of RNase R remained unchanged after
134 treating with stress causing agents (Figure 1B). As a control, the expression of DNA repair
135 protein RecA was induced quickly upon treatment with UV irradiation as shown earlier (26). This
136 is in line with a previous report that RNase R of *P. syringae* Lz4W is not induced under cold
137 stress and in stationary phase (7). This suggests that unlike *E. coli*, the RNase R^{Ps} is not a
138 stress inducible protein.

139 **RNase R protects *P. syringae* cells against oxidative stress and DNA damage**
140 **independent of RNA degradosome complex**

141 RNase R associates with endonuclease RNase E and RNA helicase RhlE to form the
142 RNA degradosome complex in *P. syringae* Lz4W (15). The C-terminal domain of RNase E acts
143 as a scaffold for the formation of degradosome complex with RNase R and RhlE in *P. syringae*
144 (15). Thus, we constructed a C-terminal deletion mutant of RNase E ($rne^{\Delta 595\text{-}1074}$) to investigate
145 the role of RNase R associated degradosome complex in oxidative stress and DNA damage.
146 The generation of C-terminal deleted *rne* mutant ($rne^{\Delta 595\text{-}1074}$) was carried out as described in
147 materials and methods. The $rne^{\Delta 595\text{-}1074}$ strain was subjected to oxidative stress and DNA
148 damage causing agents. Unlike the *rnr* mutant, the $rne^{\Delta 595\text{-}1074}$ strain is not sensitive to the DNA
149 damage and oxidative stress causing agents and showed resistance similar to *wt* (Figure 2).
150 The results suggest that the requirement of RNase R in protecting *P. syringae* cells against
151 oxidative stress and DNA damage is an intrinsic property of RNase R and is independent of its
152 association with the RNA degradosome complex.

153 **The RNB domain of RNase R is sufficient in protecting *P. syringae* Lz4W against**
154 **oxidative stress and DNA damage**

155 The RNase R^{Ps} is a multidomain protein comprising of N-terminal RNA binding cold
156 shock domain CSD (1 – 225 aa), a central nuclease domain RNB (226 – 665 aa) and the RNA
157 binding domain S1 at the C-terminus (666 – 885 aa) (Figure 3A). To understand the role of each

158 domain in protecting *P. syringae* Lz4W cells against oxidative stress and DNA damage, we
159 constructed a series of domain deletion mutants of RNase R: i) a mutant lacking CSD domain
160 (ΔCSD), ii) a mutant lacking S1 domain (ΔS1), and iii) a mutant lacking both CSD and S1
161 domains (RNB) (Figure 3A). In our viability assays, the *rnr* mutants complemented with the
162 domain deleted mutants of RNase R showed resistance to the oxidative stress and DNA
163 damaging agents similar to *wt* cells (Figure 3B & C). This suggests that the RNB domain of
164 RNase R alone, without the RNA binding CSD and S1 domains is sufficient to protect *P.*
165 *syringae* Lz4W against oxidative stress and DNA damage.

166 **The exoribonuclease activity of RNase R is not required for protecting *P. syringae* Lz4W**
167 **against oxidative stress and DNA damage**

168 The catalytic site of RNase R^{Ps} has four conserved aspartate residues in the active site
169 located at positions 276, 282, 284 and 285 (Figure 4A). D276 and D285 have been proposed to
170 be important for coordinating with Mg^{2+} ion, D282 for holding the RNA in place while D284 is
171 known for being the residue that cleaves the phosphodiester bond (27). It was shown in *E. coli*
172 that D280 (equivalent to the D284 residue of *P. syringae*) is the only crucial residue for the
173 catalytic activity of RNase R without affecting binding of the RNA (14). This residue is also
174 shown to play a crucial role in the activity of RNase II of *E. coli* and RNase R of *Legionella*
175 *pneumophila* (28). Hence, we mutated the equivalent Asp284 in RNase R to alanine,
176 overexpressed and purified the mutant protein ($RNaseR^{D284A}$) as described under materials and
177 methods. SDS-PAGE profile of $RNaseR^{D284A}$ is shown in Fig 4B(i). RNase R^{D284A} show double
178 protein bands on a SDS-polyacrylamide gel. The lower band is a product of C-terminally
179 degraded RNase R protein as observed earlier (10). We couldn't separate these two bands due
180 to marginal difference in their molecular weights. We analyzed the exoribonucleolytic activity of
181 $RNaseR^{D284A}$ protein on Poly(A) and *malE-malF* substrates and compared with the wild-type.
182 Wild-type RNase R degraded both poly(A) and *malE-malF* substrates within 10 mins. However,
183 despite using high protein concentration of $RNaseR^{D284A}$ mutant (0.5 μM vs 15 μM) it failed to
184 degrade both substrates even after prolonged incubation (Fig 4B (ii & iii)). This shows that the
185 D284A mutation leads to complete loss of exoribonuclease activity of RNase R^{Ps}.

186 In our viability assays, all four single amino acid mutants protected Δ*rnr* mutant cells
187 from oxidative and DNA damage stress, similar to *wt* cells (Figure 5). However, the D282A,
188 D284A and D285A mutants showed marginal sensitivity to stress induced by hydroxyurea (HU)
189 suggesting that the functional role of these residues in the activity of RNase R have a plausible

190 role in protecting particularly against HU induced stress. In general, the data suggest that the
191 role of RNase R in protecting cells against oxidative stress and DNA damage is independent of
192 exoribonuclease activity of RNase R.

193 To further substantiate our finding, we also combined the following mutations on full
194 length RNase R: D284A + D285A; D276A + D284A; D276A + D284A + D285A. We examined
195 their role in the function of RNase R in protecting against stress (Supplementary Figure 3). All
196 the double and triple point mutants also protected Δ*rnr* cells against oxidative stress and DNA
197 damage similar to *wt* cells. This further confirms that the exoribonuclease activity of RNase R is
198 not essential for the protection against oxidative stress and DNA damage.

199 **The RNB domain alone with D284A mutation can protect *P. syringae* Lz4W cells against**
200 **oxidative stress and DNA damage.**

201 RNB is the main catalytic domain responsible for the exoribonuclease activity of RNase
202 R. As mentioned above the four conserved Aspartate residues important for the catalytic activity
203 of RNase R are located in the motif-1 of the RNB domain (Figure 4A(ii)). To further corroborate
204 our finding that the exoribonuclease function of RNase R^{Ps} is not required for protection against
205 DNA damage and oxidative stress; we mutated the D284 and D284+D285 residues on catalytic
206 RNB domain alone to alanine. RNB domain with D284A and D284A+D285A mutations was
207 expressed in Δ*rnr* strain. The viability assays for the RNB domain with mutations show that the
208 mutants moderately protect cells against oxidative stress and DNA damage. However, the point
209 mutations slightly affected the ability of RNB domain to protect cells against Micromycin C,
210 Paraquat and Hydroxyurea (Figure 5A).

211 In *E. coli*, RNB domain is also suggested to play an important role in RNA unwinding. It
212 has been recently suggested that a triple helix wedge region in the RNB domain of *E. coli*
213 RNase R is responsible for unwinding the duplex-RNA substrates (29). Our homology modelling
214 of RNase R^{Ps} based on the crystal structure of *E. coli* RNase R, also shows the presence of a
215 tri-helix wedge region in the RNB domain of RNase R^{Ps} (**Figure 5B**). Although we haven't
216 examined the role of tri-helix wedge of RNase R^{Ps} in RNA unwinding function, it is possible that
217 it may exhibit helicase activity similar to the tri-helix wedge region in the RNB domain of *E. coli*
218 RNase R.

219

220 ## Discussion

221 Ribonucleases are important modulators involved in various cellular processes by virtue
222 of their RNA processing and degradation activity in maintaining the dynamic transcript levels in
223 bacterial cells. Exoribonucleases like RNase R, PNPase and RNaseJ1 are multifunctional
224 enzymes and are implicated in various biological processes such as stress tolerance, protection
225 from DNA damage and oxidative stress apart from their role in RNA metabolism (19, 20, 22, 24,
226 30, 31). RNase R is important for the RNA quality control and implicated in various stress
227 conditions in bacteria (5, 20). In this study, we have uncovered a novel role of RNase R in
228 protection of *P. syringae* Lz4W from oxidative stress and DNA damaging agents such as H_2O_2,
229 Paraquat, MMC, Hydroxyurea and UV radiation.

230 PNPase is a phosphorolytic 3'- 5' exoribonuclease an important component of *E. coli*
231 RNA degradosome complex. However, in *P. syringae*, instead of PNPase, the hydrolytic 3'-5'
232 exoribonuclease RNase R interacts with the RNA degradosome complex (15). Interestingly, *pnp*
233 mutant of *E. coli* and the *rnr* mutant of *P. syringae* exhibit similar phenotypic behaviors. The *pnp*
234 mutant of *E. coli* and the *rnr* mutant of *P. syringae* are both sensitive to cold and both enzymes
235 are shown to be involved in maturation of 3' end of 16S rRNA (32-35). RNase R of
236 *Pseudomonas putida* is also implicated in survival at low temperature (7, 16-18). PNPase is also
237 implicated in protecting *E. coli* cells against oxidative damage (31) and in DNA repair process by
238 virtue of its 3'→5' DNase activity in *B. subtilis* and *E. coli* (22-25).

239 We show here that the RNase R is involved in oxidative damage and DNA repair
240 process in *P. syringae* Lz4W. Unlike *P. syringae* RNase R, the *rnr* mutant of *E. coli* has no
241 discernable phenotype and is refractory to these stress conditions. This implies that the
242 protective role of RNase R is either specific to *P. syringae* Lz4W or possibly specific to
243 *Pseudomonas* species. It is intriguing that although RNase R of *E. coli* is not involved in DNA
244 damage and oxidative stress response in *E. coli*, yet it protects *P. syringae* Lz4W from DNA
245 damage and oxidative stress. It is possible that the $PNPase^{Ec}$ and RNase R^{Ps} have
246 evolutionarily swapped their functions with respect to their roles in the cellular milieu of these
247 two bacteria. Although, we don't know why RNase R has been chosen over PNPase (unlike *E.*
248 *coli)* to protect *P. syringae* Lz4W from oxidative stress and DNA damage, we speculate that the
249 highly processive, hydrolytic RNase R is selected over the less processive, phosphorolytic
250 PNPase to conserve the energy at low temperature.

251 RNA degradosome is a multi-enzyme complex that significantly contributes to the
252 steady-state profiles of RNA transcripts in bacteria (36). Our laboratory has previously reported
253 a novel degradosome complex comprising of the RNase E, the exoribonuclease RNase R and a
254 DEAD-box RNA helicase RhlE in *P. syringae* Lz4W (15). By deleting of C-terminal domain of
255 RNase E that is responsible for the assembly of degradosome complex, we showed that the
256 association of RNase R with the degradosome complex is not needed to protect against
257 oxidative and DNA damage stress, thus suggesting that the DNA damage resistance phenotype
258 is an intrinsic function of RNase R.

259 We further investigated the possible role of each domain of multi-domain RNase R in
260 protecting *P. syringae* cells from oxidative and DNA damage stress. Interestingly, we found the
261 catalytic RNB domain of RNase R^{Ps} alone is sufficient for protecting *P. syringae* cells from
262 oxidative and DNA damage stress, while the CSD and S1 domains are dispensable. In *E. coli*,
263 RNB domain alone degrades structured RNA (12). Therefore, we envisaged that the
264 degradation of structured RNAs either by full-length RNase R^{Ps} or by the catalytic RNB domain
265 itself could play a protective role against oxidative and DNA damage stress in *P. syringae*
266 Lz4W. We replaced the highly conserved catalytically important aspartate residues of RNase
267 R^{Ps} with alanine. These mutants showed no apparent sensitivity to oxidative stress and DNA
268 damaging agents, indicating the RNA degradation function of RNase R is not required. Further,
269 the double- and triple-point mutations of the aspartate residues in combinations also resulted in
270 similar results, confirming that the ribonuclease function of RNase R^{Ps} is not essential. Thus, the
271 ability of RNase R to degrade highly stabilized RNA secondary structures does not have a role
272 in protecting *P. syrin*gae cells against DNA damage and oxidative stress. Intriguingly, our data
273 shows that the catalytically inactive RNase R^{Ps} and RNB domain itself can protect cells from
274 oxidative and DNA damage stress, implying the importance of RNB domain alone with no
275 exoribonuclease activity in this scenario.

276 If the association of RNase R with degradosome complex and its exoribonuclease
277 activity is not essential, then how does RNase R protect *P. syringae* cells from DNA damage
278 and oxidative stress? How does a catalytically inactive RNB domain itself without CSD and S1
279 domains could rescue *P. syringae* cells from oxidative and DNA damage stress? However, our
280 data suggests that the RNB domain without catalytic function has a cryptic functional role which
281 helps in protecting Lz4W cells from these stress conditions.

282 RNase R is known to process structured dsRNA without the help of a helicase and is
283 implicated in removal of mRNAs with stable stem loops (6). It was earlier shown that cold
284 sensitivity of *csdA* mutant of *E. coli* can be complemented by overexpressing RNase R, possibly
285 through its helicase function (37). Importantly, the RNB domain of RNase R is also shown to
286 possess helicase activity in *E. coli* (32, 38, 39). A recent study showed the presence of a winged
287 tri-helix domain in RNB domain of *E. coli* that is responsible for the helicase function (29). This
288 prompted us to investigate the presence of a similar tri- helix wedged domain in RNase R of *P.*
289 *syringae*. Our homology modelling of *P. syringae* RNase R based on the RNase R of *E. coli*,
290 shows a similar tri-helix wedge in RNB domain. Hence, we hypothesize that RNase R could
291 participate in protection of *P. syringae* Lz4W against oxidative and DNA damage stress via its
292 helicase activity. This is further supported by a recent observation in *P. syringae* Lz4W that the
293 DEAD box helicases CsdA and SrmB are involved in protection against DNA damage and
294 oxidative stress (40).

295 This study uncovered a hitherto unknown role of RNase R in the physiology of cold
296 adapted bacterium *P. syringae* Lz4W. Here, we report a novel role of the RNase R in protecting
297 *P. syringae* Lz4W against DNA damage and oxidative stress. Our study indicate that the
298 protective role of RNase R is apparently specific to *P. syringae* Lz4W. Our structural homology
299 modelling provides an insight into the presence of a tri-helix wedge region in the RNB domain of
300 RNase R. which might play a role in protecting against oxidative and DNA damage stress by
301 virtue of its putative helicase activity. Although our data biochemically doesn't not establish the
302 helicase activity of RNB domain of RNase R, the genetic evidence provided here is
303 comprehendible. In future it would be of relevance to elucidate the mechanism by which RNase
304 R protects *P. syringae* Lz4W cells from DNA damage and oxidative stress.

305 Materials and Methods

306 Bacterial strains, plasmids and growth conditions

307 Bacterial strains and plasmids used in this study are described in Table 2. *Pseudomonas*
308 *syrinage* Lz4W cells were grown in Antarctic Bacterial medium (ABM: 5g liter^{-1} peptone and 2g
309 liter^{-1} yeast extract) at both 22ºC and 4ºC. *E. coli* strains were routinely grown in Luria-Bertani
310 medium at 37ºC. The growth media, when required, were supplemented with Antibiotics as
311 follows: 100 µg ml^{-1} Ampicillin, 50 µg ml^{-1} Kanamycin, 20µg ml^{-1} tetracycline, and 400 µg ml^{-1}
312 rifampicin. For complementation studies, plasmids were mobilized into *P. syringae* strain by
313 conjugation using *E. coli* S17-1 helper strain. For growth analysis, cultures were grown in ABM

314 overnight and were inoculated in a fresh media in 1:100 dilutions and OD_{600} (O.D at 600 nm)
315 was measured at various time intervals as indicated.

316 **Spot assays and quantitation of sensitivity to DNA damaging agents**

317 Cells were grown overnight at 22 ºC in AB media containing appropriate antibiotics and
318 inoculated into fresh AB media with 1:100 dilutions. To measure the sensitivity to NaCl (1M),
319 SDS (10% w/v), Lysozyme (100 mg/ml) + EDTA (500 mM), Ampicillin (100 µg), Rifampicin (100
320 µg), Spectinomycin (100 µg), Paraquat (20 µM), Mitomycin C (1000 ng), H_2O_2 (0.2 mM),
321 Hydroxyurea (2.5 mM), Cisplatin (0.2 mM); up to 5-fold serial dilutions of exponentially grown
322 cells at 22 ºC were made and spotted on ABM agar plates containing stress inducing agents as
323 indicated. Plates were incubated at 22 ºC for 48 h. For UV sensitivity, up to 5-fold serially diluted
324 exponentially grown cultures were spotted on ABM plates and exposed to UV radiations at
325 indicated doses at the rate of 3 J/m^2/sec and plates were incubated in dark at 22 ºC for 48 h.

326 For quantitative analysis, exponentially growing cells were incubated for 30 mins with
327 oxidative stress/DNA damaging agents at indicated concentrations, washed, then serially
328 diluted, and plated on ABM-Agar plates or LB agar plates (for *E. coli* strains). Plates were
329 incubated at 22 ºC for 48 h and the colony forming units (cfu) were scored. The percentage of
330 cell survival was calculated by considering the cfu from untreated cells as 100%.

331 **Reagents and general DNA recombinant methods**

332 Routine molecular biology techniques like Genomic DNA isolation, restriction enzyme
333 digestion, PCR, ligation, transformation, conjugation, Southern hybridization, Western analysis
334 and RNA isolation were performed as described earlier (41). All restriction enzymes, T4 DNA
335 ligase, T4 Polynucleotide kinase were purchased from New England Biolabs. Oligos used in the
336 study were from Bioserve India (Hyderabad, India). PCR was carried out using proof reading
337 Pfx DNA polymerase from Invitrogen (San Deigo, CA, USA). DNA sequencing was performed
338 using ABI Prism dye terminator cycle sequencing method (Perkin-Elmer) and analyzed on an
339 automated DNA sequencer (ABI model 3700; Applied Biosystems). Antibodies were purchased
340 from Santa Cruz Biotechnology.

341 For Southern hybridization, genomic DNA was isolated from WT and *ΔC-rne* mutant
342 cells, digested with PstI enzyme. The products of restriction digest were separated on a 1% w/v
343 Agarose gel and transferred onto Hybond N+ membrane (Amersham Biosciences). For the
344 probe, PCR amplicon of full-length *rne* gene was labeled with [α-^{32}P] dATP using random primer

345 labeling kit (Jonaki, BARC, India). Radioactive signals were detected and quantified by
346 phosphorimager (Fuji FLA 3000).

347 **Construction of mutants of RNase R and RNB by site directed mutagenesis and genetic**
348 **complementation studies**

349 The *rnr*^{Ec}, *rnr*^{Ps} genes and the domain deletion mutants of *rnr*^{Ps} were amplified from the
350 genomic DNA of Lz4W using the primers listed in Table 3. All constructs contain 6x-Histidine tag
351 at the N-terminal end of the protein. For D276A, D282A, D284A and D285A point-mutations in
352 RNase R^{Ps}, site-directed mutagenesis of pGL*rnr* (7) was carried out using self-complementary
353 primers harboring indicated mutations (listed in Table 3). Subsequently, the double D284A +
354 D285A, D276A + D284 and triple D276A+D284A+D285A mutations were generated by using
355 respective complementary primers using pGLrnr plasmid with preexisting mutations. Similarly, the
356 D284A and D284A+D285A mutations were introduced into pGLRNB vector. Mutations and
357 integrity of full-length genes were confirmed by DNA sequencing.

358 **Purification of RNase R** D284A **mutant and biochemical assays**

359 The wild-type RNase R and D284A mutant derivatives were purified by expressing these
360 proteins in *rnr* null mutant of *P. syringae* Lz4W as escribed earlier (10). RNase R activity was
361 assayed *in vitro* by its ability to degrade ssRNA substrate Poly(A) and structured RNA substrate
362 *malE-malF* as described earlier (10). Briefly, Poly(A) was labelled at 5'-end using [γ^{32}P] and the
363 *malE-malF* substrate was internally labelled using (α^{32}P). Assays were carried out in 20-µl
364 reaction mixtures containing 25 mM Tris-HCl (pH 7.0), 100 mM KCl, 0.25 mM $MgCl_2$, 5 mM
365 DTT. 10 pmol of labelled substrate was treated with 0.5 µM RNase R^{WT} and 15 µM RNase
366 R^{D284A} at the indicated time-points at room temperature, and stopped by adding 10mM EDTA,
367 0.2% SDS and 1 mg/ml of proteinase K. RNA degradation products were resolved on 8%
368 denaturing 8 M urea PAGE and degradation products were analysed by Phosphoimager (Fuji).

369 **Western analysis**

370 For examining the induction of RNase R, the exponentially grown wild-type *P. syringae*
371 cells were incubated with DNA damaging agents. 1 ml aliquot was collected before treating cells
372 with DNA damaging agents. 5 ml of cells were treated separately with Paraquat (15 µM), MMC
373 (1000 ng), UV (18 J/m^2), and incubated at 22ºC. 1 ml of aliquot was collected at 30, 60 and 120
374 min intervals, Cells were further lysed by sonication in presence of Protease inhibitor cocktail
375 (Roche, USA). Protein concentration from cell extracts was measured using Bio-rad Protein

376 Assay kit, also by the absorbance at 280 nm (A_{280}) using the Nanodrop spectrophotometer.
377 Equal amount of protein was loaded and resolved on a 10 % w/v SDS-PAGE. The resolved
378 proteins were later electro-blotted onto Hybond C-Extra membrane (Amersham Biosciences)
379 and immunodetection was carried with polyclonal rabbit Anti-RNase R antibodies as described
380 earlier (15). For detecting the levels of RecA expression, cells were treated with UV (18 J/m^2)
381 and incubated at 22 ºC in dark. Culture aliquots were collected after 30 minutes of treatment
382 and immuno-detected by polyclonal anti-RecA rabbit antibodies as described earlier (26).

383 **Generation of C-terminal deletion of *rne* (RNase E gene)**

384 For generating C-terminal deletion of *rne* gene, a 784 bp fragment from N-terminal of *rne*
385 gene was first amplified using primer set Rne_Nterm_FP_BamHI and Rne_Nterm_RP_HindIII
386 having the restriction enzyme sites for BamHI and HindIII respectively **(Table 2).** A second
387 fragment from C-terminal of *rne* gene was PCR amplified using the primer set
388 Rne_Cterm_FP_HindIII and Rne_Nterm_RP_SalI having the sites for restriction enzymes
389 HindIII and SalI (Table 2). Both fragments were initially digested with HindIII and ligated. The
390 ligated product was further digested with BamHI and SalI and cloned into pBlueScript-KS
391 vector. The resultant plasmid pBS(N+C)*rne* was linearized by HindIII, and a tetracycline
392 resistance gene was cloned to obtain the pBS(N+C)*rne*-tet plasmid. The tetracycline resistance
393 gene (*tet*) cassette (~2.4 KB) was obtained from the $pMOS^{tet}$ (42) as a HindIII fragment. Plasmid
394 construct was confirmed by sequencing and PCR analysis. Plasmid pBS(N+C)*rne*-tet plasmid
395 was alkaline denatured as described earlier (42). Alkali denatured plasmid DNA was
396 electroporated into the wild-type *P. syringae* cells. Recombinants were selected on ABM plates
397 containing tetracycline and the knock-out was confirmed by Southern and Western analysis.

398 **Modeling of RNase R^{Ps} protein**

399 The amino acid sequence of Lz4W RNase R was taken from NCBI (GenBank:
400 AUB77091.1). A model of RNase R^{Ps} was constructed using the SWISS-MODEL (43). The
401 closest template identified was PDB: 5xgu.1, which refers to the RNase R structure from
402 *Escherichia coli*. The RMSD between these two structures was then calculated using the
403 Matchmaker tool of Chimera (44). The RMSD value was calculated between 613 pruned atom
404 pairs is 0.248 angstroms; (across all 625 pairs: 0.930). The two structures were superimposed
405 as shown in the figure 5B.

406 **Statistical analysis**

407 Data was calculated and analyzed by using Prism 5 (GraphPad Software Inc, USA).

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544 ## Figure legends

545 **Figure 1 *S*ensitivity of *P. syringae* of Δ*rnr* and complemented strains to DNA damage and**
546 **oxidative stress causing agents. A,** Percentage cell survival of WT, Δ*rnr*-pGL10, Δ*rnr*-
547 pGL*rnr*^{Ps} and Δ*rnr*- pGL*rnr*^{Ec} upon treating with DNA damage and oxidative stress causing
548 agents at indicated concentrations. Data are from the three independent experiments with mean
549 ± SE. **B,** Expression levels of RNase R of *P. syringae* upon treating with DNA damage and
550 oxidative stress causing agents, determined by Western analysis using polyclonal RNase R
551 specific antibodies. The bottom panel shown the expression level of RecA protein upon UV
552 irradiation as a positive control. **C,** Qualitative assessment of cell viability by spot assays. WT,
553 Δ*rnr*-pGL10, Δ*rnr*- pGL*rnr*^{Ps} and Δ*rnr*- pGL*rnr*^{Ec} treated with DNA damage and oxidative stress
554 causing agents at indicated concentrations.

555 **Figure 2 *S*ensitivity of C-terminal domain deletion mutant of *rne* gene (*rne* $^{\Delta 595 - 1074}$)**
556 **encoding RNase E compared to Δ*rnr P. syringae* cells.** Qualitative assessment of cell
557 viability by spot assays. WT, Δ*rnr* and Δ*rne* $^{\Delta 595 - 1074}$ were exposed to DNA damage and
558 oxidative stress causing agents at indicated concentrations.

559 **Figure 3 *S*ensitivity of Δ*rnr P. syringae* strain complemented with domain/s deleted**
560 **mutants of RNase R. A**, Schematic representation of structural domains of *P. syringae* RNase
561 R. The domain/s deleted RNase R constructs; cold shock domain (CSD, 1 – 225 aa), catalytic
562 RNB domain (226 – 665 aa), and S1 domain (666 – 885 aa) are shown. **B**, Percentage of cell
563 survival of WT, Δ*rnr*-pGL10, Δ*rnr*- pGL*rnr-ΔCSD*, Δ*rnr*- pGL*rnr-ΔS1* and Δ*rnr*- pGLRNB*(ΔCSD*
564 *+ΔS1*) upon treating with DNA damage and oxidative stress causing agents at indicated
565 concentrations. Data are from the three independent experiments with mean ± SE. **C**,
566 Qualitative assessment of cell viability by spot assays. WT, Δ*rnr*-pGL10, Δ*rnr*- pGL*rnr-ΔCSD*,
567 Δ*rnr*- pGL*rnr-ΔS1* and Δ*rnr*- pGLRNB*(ΔCSD +ΔS1*) were treated with DNA damage and
568 oxidative stress causing agents at indicated concentrations.

569 **Figure 4 *S*ensitivity of Δ*rnr P. syringae* strain complemented with catalytic site mutants**
570 **of full-length RNase R to DNA damage and oxidative stress. A, (i)** The multiple sequence
571 alignment of the catalytic region of *P. syringae* RNase R with RNase R of closely related
572 bacteria including *E. coli*. Boxed and highlighted regions indicate the highly conserved
573 catalytically important aspartate residues in motif-I of catalytic RNB domain of RNase R. **(ii)**
574 Schematic representation of structural domains of *P. syringae* RNase Rand the motif-I of RNB

with catalytically important residues are shown. **B.** Purification of RNase R^{D284A} and its exoribonuclease activity on single-stranded and structured RNA substrates. (i), SDS-PAGE analysis of purified RNase R^{D284A}, '*' indicate the degraded products of purified RNase R^{D284A} protein. (ii), shows the time-dependent exoribonuclease activity of wild-type RNase R^{WT} and RNase R^{D284A} on single stranded Poly(A) and (iii), *malE-malF* structured RNA substrate at indicated time-points. The end products of size 4-5 mer from degradation of Poly(A), and NMPs (nucleotide monophosphate) from *malE-malF* substrates are indicated. **C**, Qualitative assessment of cell viability by spot assays. The WT, Δ*rnr*-pGL10, Δ*rnr*- pGL*rnr*-D276A, *rnr*-pGL*rnr*-D282A, *rnr*- pGL*rnr*-D284A and *rnr*- pGL*rnr*-D285A were exposed to indicated concentrations of DNA damage and oxidative stress causing agents.

Figure 5 *S*ensitivity of Δ*rnr P. syringae* strain complemented with catalytic site mutants of RNB domain of RNase R. A, Qualitative assessment of cell viability by spot assays. WT, Δ*rnr*-pGL10, Δ*rnr*- pGLRNB, Δ*rnr*- pGLRNB-D284A and Δ*rnr*- pGLRNB-D284+D285A were treated with DNA damage and oxidative stress causing agents at indicated concentrations. **B,** Structural model of *P. syringae* RNase R constructed by superimposing on the structure of *E. coli* RNase R (PDB:5xgu.1) using SWISS-MODEL. The tri-helix wedge region of RNB domain of *P. syringae* RNase R is shown in black color.

Figure S1 *S*ensitivity of Δ*rnr E. coli* strain to DNA damage and oxidative stress causing agents. Qualitative assessment of cell viability by spot assays of WT, Δ*rnr* and Δ*recB* mutant of *E. coli*. RecB mutant was used as a positive control against DNA damaging agents. Cells were exposed to DNA damage and oxidative stress causing agents at indicated concentrations.

Figure S2 *S*ensitivity of Δ*rnr P. syringae* strain complemented with PNPase of *P. syringae*. Qualitative assessment of cell viability by spot assays. WT, Δ*rnr*-pGL10 and Δ*rnr*-pGL*pnp* were exposed to DNA damage and oxidative stress causing agents at indicated concentrations.

Figure S3 *S*ensitivity of Δ*rnr P. syringae* strain complemented with double- and triple-mutants of catalytic site residues of RNase R. Qualitative assessment of cell viability by spot assays. WT, Δ*rnr*-pGL10, *rnr*- pGL*rnr*-(D276A+D284A), *rnr*- pGL*rnr*-(D276A+D285A) and *rnr*-pGL*rnr*-(D276A+D284A+D285A) treated with DNA damage and oxidative stress causing agents at indicated concentrations.

Table 1

Type of stress	Condition/Chemicals tested	Sensitivity levels	
		Δ*rnr*	WT
Temperature	High temperature (37 °C)	+++	+
	Low temperature (4 °C)	+++	-
Osmotic	NaCl (1M)	++	++
Envelope	SDS (10% w/v)	+	+
	Lysozyme (100 mg/ml) + EDTA (0.5 M)	+++	+++
DNA damage	UV light (24 J/m2)	+++	+
	Cisplatin (0.25 mM)	+++	+
	Mitomycin C (1 µg/ml)	+++	+
	Hydroxyurea (2.5 mM)	+++	+
Oxidative	Hydrogen peroxide (0.25 mM)	+++	+
	Paraquat (15 µM)	+++	+
Antibiotics	Ampicillin (100 µg/ml)	+	-
	Rifampicin (100 µg/ml)	++	++
	Spectinomycin (100 µg/ml)	++	++

Qualitative measurement of sensitivity of Δ*rnr* mutant and wild-type *P. syringae* cells to various stress causing agents. Sensitivity levels are indicated as follows: - not sensitive, + marginally sensitive, ++ moderately sensitive, and +++ very sensitive.

Table 2. Bacterial strains and plasmids used in the study

Bacterial strains:		
Bacterial strain	**Description**	**Source / reference**
P. syringae Lz4W	Wild-type, Antarctic isolate	(45)
Δrnr	*P. syringae* Lz4W but *Δrnr::tet*^{r}	(15)
ΔC-rne	*P. syringae* Lz4W but ΔC-*rne::tet*^{r}	This study
S17-1	*F*$^{-}$ *pro recA1 (r*$^{-}$ *m*$^{-}$*) RP4-2* integrated *(Tc::Mu) (Km::Tn7) [Smr Tpr] E. coli* strain: used as plasmid mobilizing strain	(46)
Plasmids:		
Plasmid	**Description**	**Source**
pGL10	Broad-host-range vector *with IncP* replicon	(47)
pGL*rnr*$^{Ps\text{-}His}$	pGL10 vector expressing His-tagged RNase R of *P. syringae* Lz4W	(7)
pGL*rnr*$^{Ec\text{-}His}$	pGL10 vector expressing His-tagged RNase R of *E. coli*	This study
pGL*ΔCSD*$^{-His}$	pGL10 vector expressing His-tagged RNase R without CSD domain	This study
pGL*ΔS1*$^{-His}$	pGL10 vector expressing His-tagged RNase R without S1 domain	This study
pGL*RNB*$^{-His}$	pGL10 vector expressing His-tagged RNB domain of RNase R without CSD and S1 domains	This study
pGL*rnr*$^{D276A\text{-}His}$	pGL10 vector expressing His-tagged RNase R with D276A mutation	This study
pGL*rnr*$^{D282A\text{-}His}$	pGL10 vector expressing His-tagged RNase R	This study

	with D282A mutation	
pGL*rnr*$^{D284A\text{-}His}$	pGL10 vector expressing His-tagged RNase R with D284A mutation	This study
pGL*rnr*$^{D285A\text{-}His}$	pGL10 vector expressing His-tagged RNase R with D285A mutation	This study
pGL*rnr*$^{D276A + D284A\text{-}His}$	pGL10 vector expressing His-tagged RNase R with D276A and D284A mutations	This study
pGL*rnr*$^{D284A + D285A\text{-}His}$	pGL10 vector expressing His-tagged RNase R with D284A and D285A mutations	This study
pGL*rnr*$^{D276A + D284A + D285A\text{-}His}$	pGL10 vector expressing His-tagged RNase R with D276A, D284A and D285A mutations	This study
pGLRNB$^{D284A\text{-}His}$	pGL10 vector expressing His-tagged RNB domain of RNase R with D284A mutation	This study
pGLRNB$^{D284A + D285A\text{-}His}$	pGL10 vector expressing His-tagged RNB domain of RNase R with D284A and D285A mutations	This study
pGLpnp$^{-\ His}$	pGL10 vector expressing PNPase of *P. syringae* Lz4W	(7)

Table 3. Primers and oligonucleotides used in the study

Primer	Sequence (5' - 3')	Description
D276 A_FP2	CGTTCGTCACCATTGCCGGCGA AGATGCCCGC	Forward primer for generating D276A mutation
D276A_RP2	GCGGGCATCTTCGCCGGCAATG GTGACGAACG	Reverse primer for generating D276A mutation
D282AFP	GGCGAAGATGCCCGCGCCTTCG ATGACGCTGTTTAC	Forward primer for mutation of D282A
D282ARP	GTAAACAGCGTCATCGAAGGCG CGGGCATCTTCGCC	Reverse primer for mutation of D282A
D284A_FP	GAAGATGCCCGCGACTTCGCTG ACGCTGTTTACTG	Forward primer for generating D284A mutation
D284A_RP	CAGTAAACAGCGTCAGCGAAGT CGCGGGCATCTTC	Reverse primer for generating D284A mutation
D285AFP	GCC CGC GAC TTC GAT TAC GCT GTT TAC TGC GAA GCC	Forward primer for generating D285A mutation
D285ARP	GGC TTC GCA GTA AAC AGC GTA ATC GAA GTC GCG GGC	Reverse primer for generating D285A mutation
D284-285A_FP2	GATGCCCGCGACTTCGCTGCCG CTGTTTACTGCG	Forward primer for generating D284A and D285A mutation
D284-285A_RP2	CGCAGTAAACAGCGGCAGCGAA GTCGCGGGCATC	Reverse primer for generating D284A and D285A mutation
Rne_Nterm_FP _BamHI	CGCGAGTGCCTGGATCCAGACC GTGCCCGC	Forward primer for amplifying the N-terminal region of *rne* gene for C-terminal deletion
Rne_Nterm_R P_HindIII	CGCTCTTCGCGAAGCTTGCGCT CTTCATCG	Reverse primer for amplifying the N-terminal region of *rne* gene for C-terminal deletion
Rne_Cterm_FP _HindIII	CGCTCCTGCTGAAGCTTAGGCC AATCAACAG	Forward primer for amplifying C-terminal region of r*ne* gene for C-terminal deletion

Rne_Nterm_RP_SalI	CGCGTAGTCGACTCAGACGAGGGGTTTAGGCTC	Reverse primer for amplifying C-terminal of *rne* gene for C-terminal deletion
RP885	CTC GAG TGA CTT GAC CTT AGG TTT ACG CGC TCC	Reverse primer for deletion of CSD domain region.
FP1	ACC ATG GCG GAA AAA TAC GAA AAC CCT ATC	Forward primer deletion of S1 domain region.
FP225	ACC ATG GAA ATC GAC ATT GCC CTG C	Forward primer the deletion of CSD and S1 domains
RP665	CTC GAG ACG ATC CTT CAT GAA CTC GCA CTT GAG	Reverse primer for deletion of CSD and S1 domains

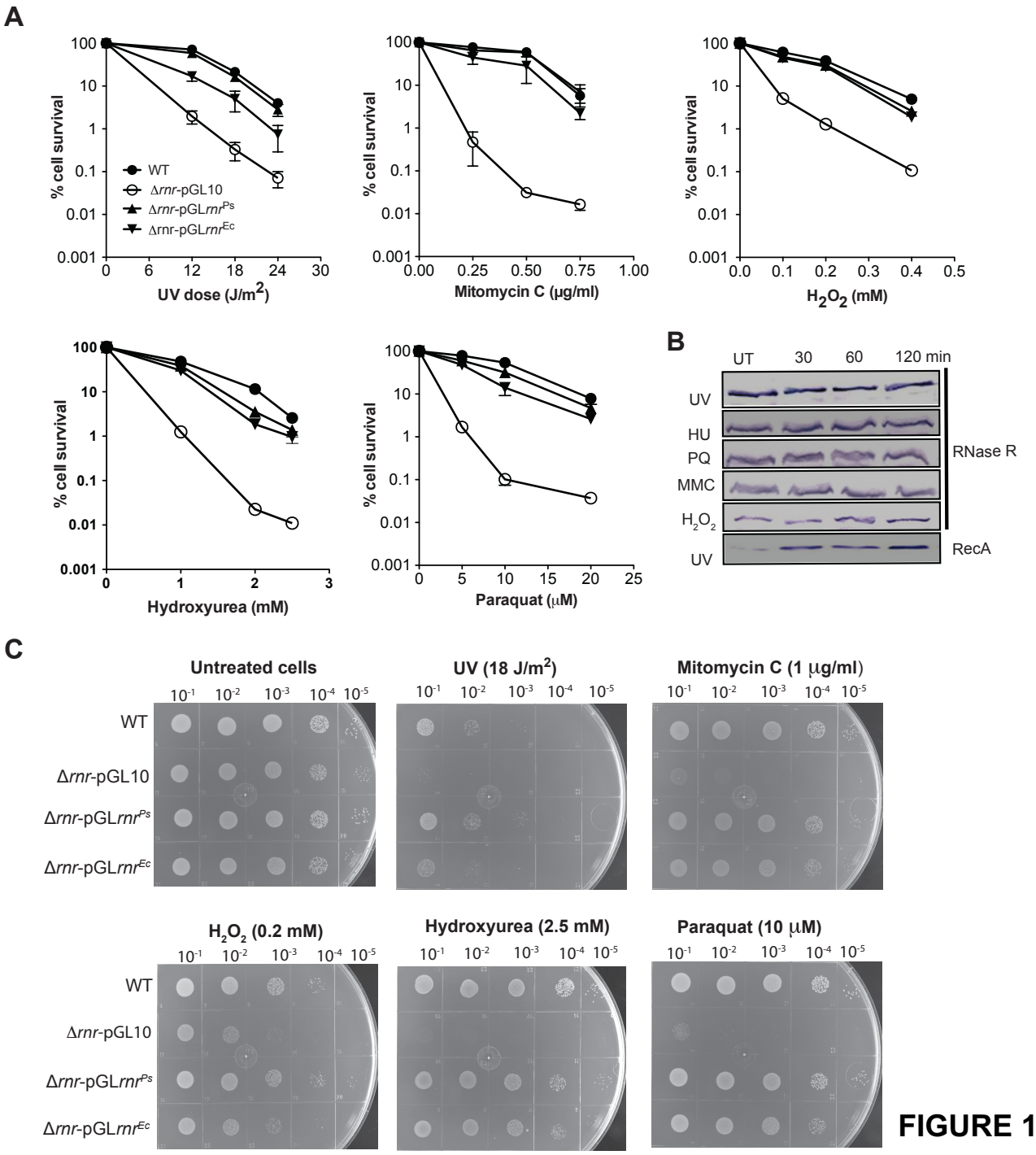


FIGURE 1

Figure 1 *Sensitivity of P. syringae* of Δ*rnr* and complemented strains to DNA damage and oxidative stress causing agents. A, Percentage cell survival of WT, Δ*rnr*-pGL10, Δ*rnr*-pGL*rnr*^{Ps} and Δ*rnr*- pGL*rnr*^{Ec} upon treating with DNA damage and oxidative stress causing agents at indicated concentrations. Data are from the three independent experiments with mean ± SE. **B,** Expression levels of RNase R of *P. syringae* upon treating with DNA damage and oxidative stress causing agents, determined by Western analysis using polyclonal RNase R specific antibodies. The bottom panel shown the expression level of RecA protein upon UV irradiation as a positive control. **C,** Qualitative assessment of cell viability by spot assays. WT, Δ*rnr*-pGL10, Δ*rnr*- pGL*rnr*^{Ps} and Δ*rnr*- pGL*rnr*^{Ec} treated with DNA damage and oxidative stress causing agents at indicated concentrations.

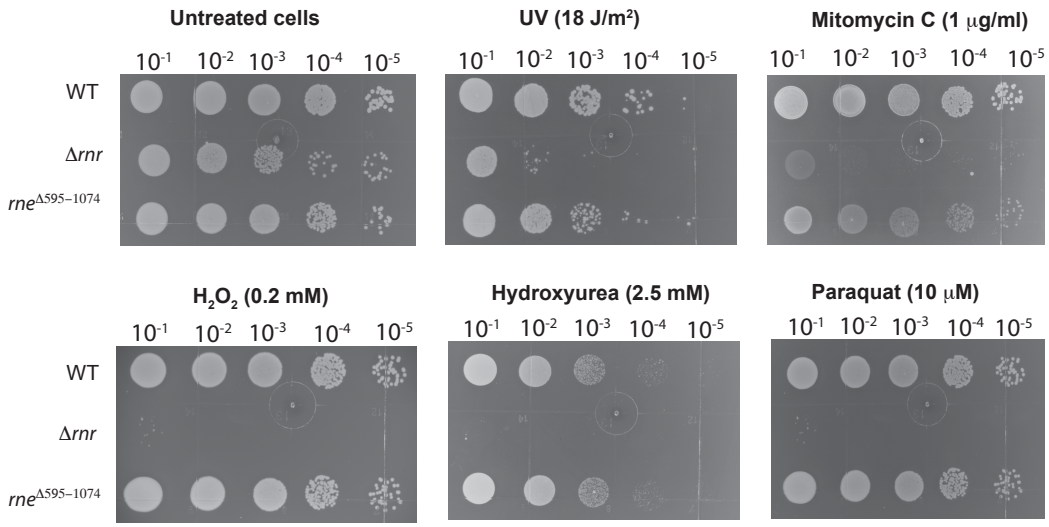


FIGURE 2

Figure 2 *S*ensitivity of C-terminal domain deletion mutant of *rne* gene (*rne* $^{\Delta 595 - 1074}$) encoding RNase E compared to Δ*rnr P. syringae* cells. Qualitative assessment of cell viability by spot assays. WT, Δ*rnr* and Δ*rne* $^{\Delta 595 - 1074}$ were exposed to DNA damage and oxidative stress causing agents at indicated concentrations.

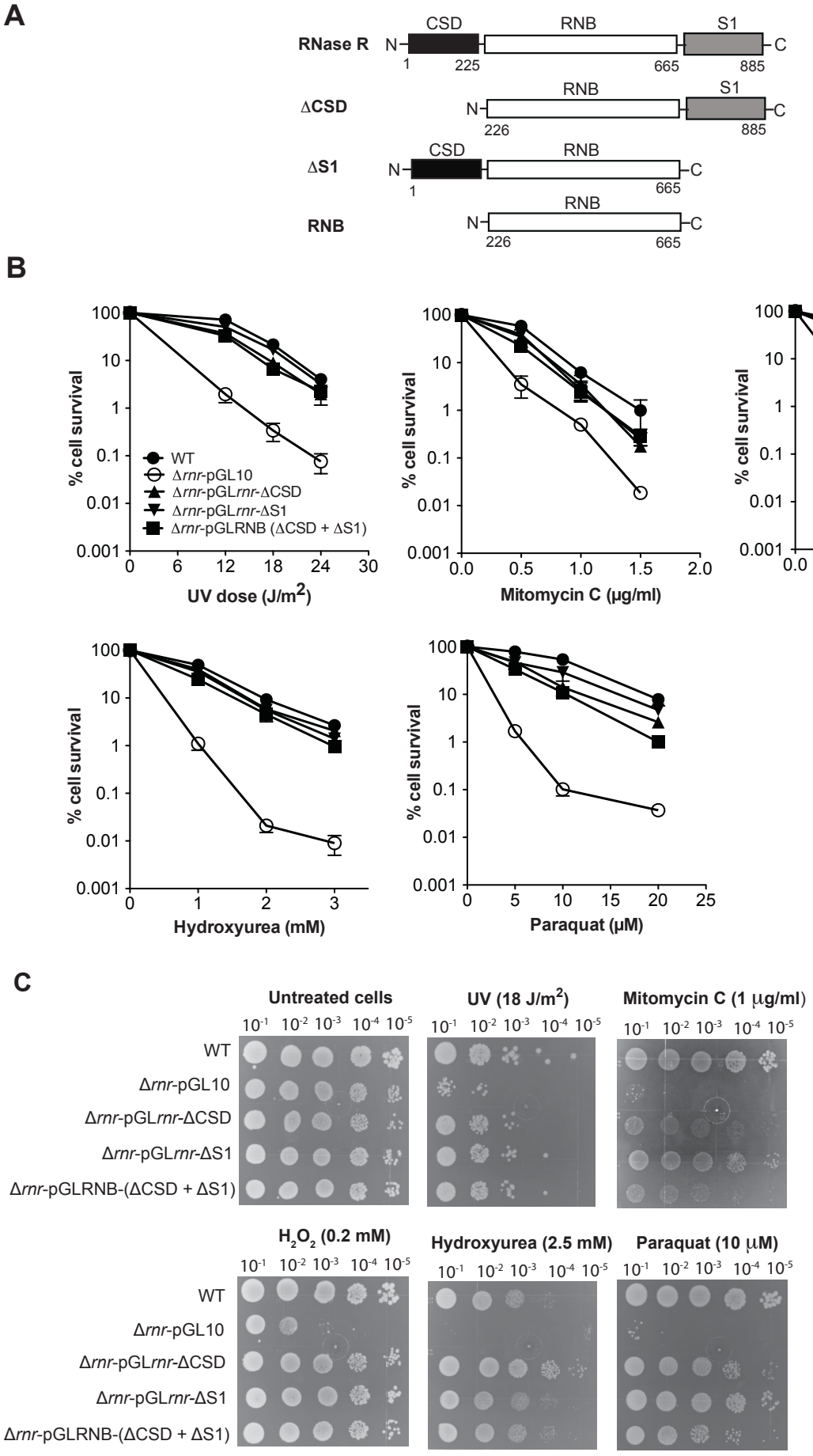


Figure 3 *S*ensitivity of Δ*rnr P. syringae* strain complemented with domain/s deleted mutants of RNase R. A, Schematic representation of structural domains of *P. syringae* RNase R. The domain/s deleted RNase R constructs; cold shock domain (CSD, 1 – 225 aa), catalytic RNB domain (226 – 665 aa), and S1 domain (666 – 885 aa) are shown. **B**, Percentage of cell survival of WT, Δ*rnr*-pGL10, Δ*rnr*- pGL*rnr-ΔCSD*, Δ*rnr*- pGL*rnr-ΔS1* and Δ*rnr*-pGLRNB*(ΔCSD +ΔS1*) upon treating with DNA damage and oxidative stress causing agents at indicated concentrations. Data are from the three independent experiments with mean ± SE. **C**, Qualitative assessment of cell viability by spot assays. WT, Δ*rnr*-pGL10, Δ*rnr*- pGL*rnr-ΔCSD*, Δ*rnr*-pGL*rnr-ΔS1* and Δ*rnr*-pGLRNB*(ΔCSD +ΔS1*) were treated with DNA damage and oxidative stress causing agents at indicated concentrations.

FIGURE 3

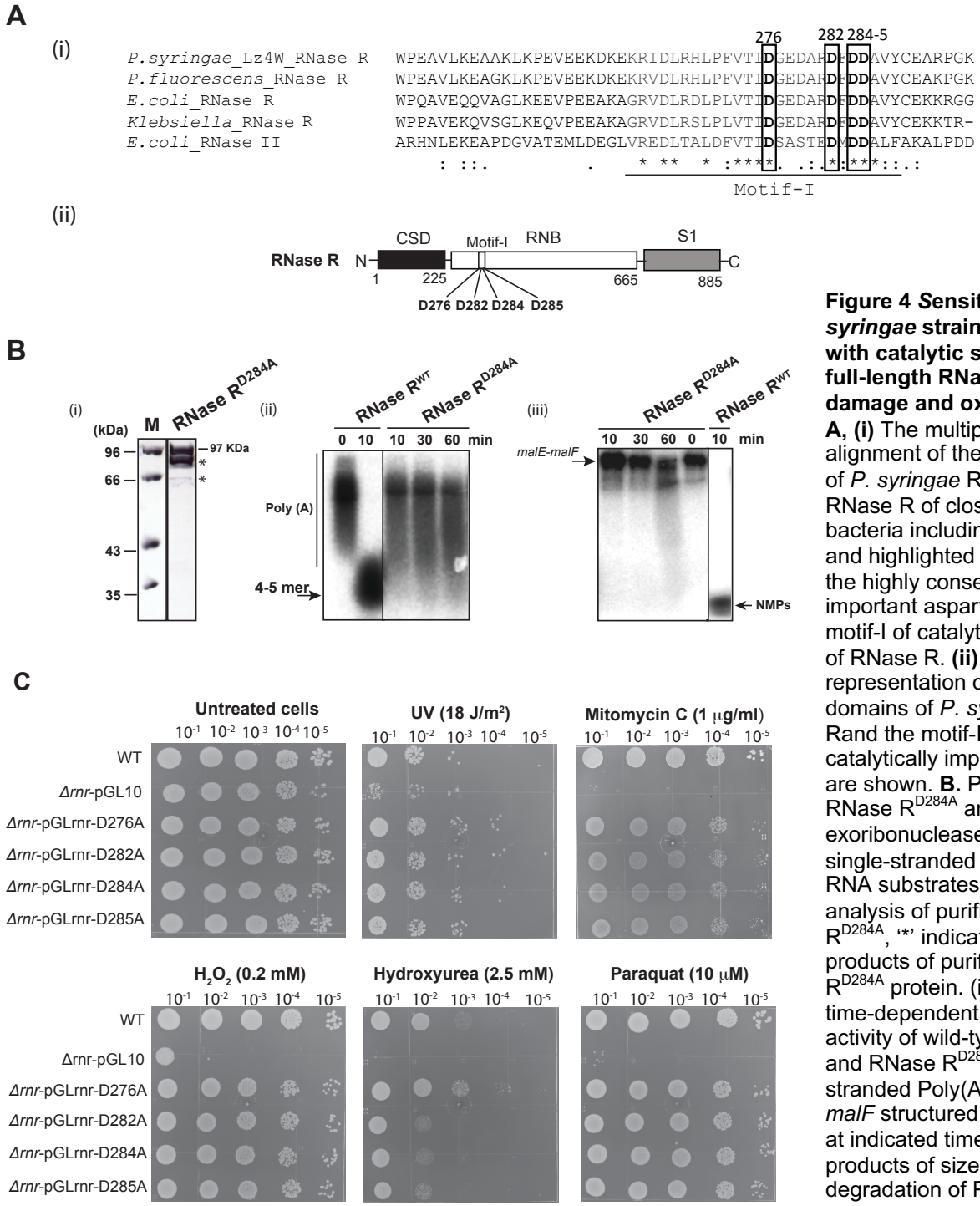
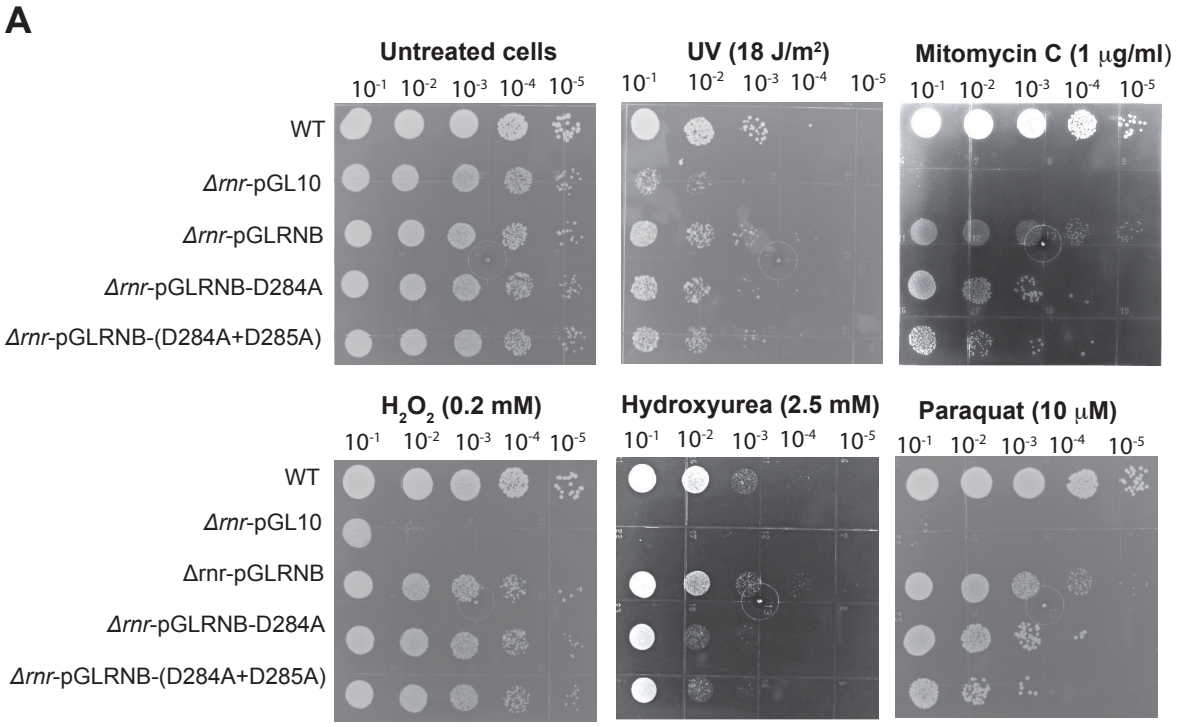


Figure 4 *Sensitivity of Δrnr P. syringae* strain complemented with catalytic site mutants of full-length RNase R to DNA damage and oxidative stress. A, (i) The multiple sequence alignment of the catalytic region of *P. syringae* RNase R with RNase R of closely related bacteria including *E. coli*. Boxed and highlighted regions indicate the highly conserved catalytically important aspartate residues in motif-I of catalytic RNB domain of RNase R. **(ii)** Schematic representation of structural domains of *P. syringae* RNase Rand the motif-I of RNB with catalytically important residues are shown. **B.** Purification of RNase R^{D284A} and its exoribonuclease activity on single-stranded and structured RNA substrates. (i), SDS-PAGE analysis of purified RNase R^{D284A}, '*' indicate the degraded products of purified RNase R^{D284A} protein. (ii), shows the time-dependent exoribonuclease activity of wild-type RNase R^{WT} and RNase R^{D284A} on single stranded Poly(A) and (iii), *malE-malF* structured RNA substrate at indicated time-points. The end products of size 4-5 mer from degradation of Poly(A), and NMPs (nucleotide monophosphate) from *malE-malF* substrates are indicated. **C**, Qualitative assessment of cell viability by spot assays. The WT, Δ*rnr*-pGL10, Δ*rnr*- pGL*rnr*-D276A, *rnr*- pGL*rnr*-D282A, *rnr*-pGL*rnr*-D284A and *rnr*- pGL*rnr*-D285A were exposed to indicated concentrations of DNA damage and oxidative stress causing agents.



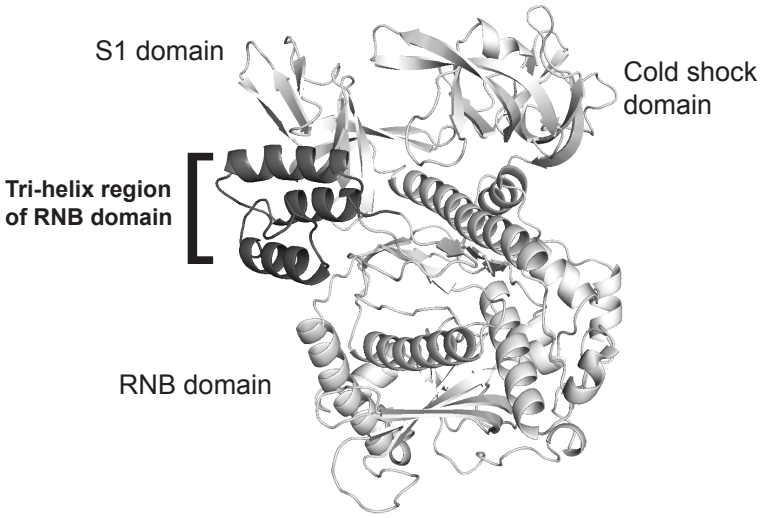


FIGURE 5

Figure 5 *S*ensitivity of Δ*rnr P. syringae* strain complemented with catalytic site mutants of RNB domain of RNase R. A, Qualitative assessment of cell viability by spot assays. WT, Δ*rnr*-pGL10, Δ*rnr*- pGLRNB, Δ*rnr*- pGLRNB-D284A and Δ*rnr*- pGLRNB-D284+D285A were treated with DNA damage and oxidative stress causing agents at indicated concentrations. **B,** Structural model of *P. syringae* RNase R constructed by superimposing on the structure of *E. coli* RNase R (PDB:5xgu.1) using SWISS-MODEL. The tri-helix wedge region of RNB domain of *P. syringae* RNase R is shown in black color.