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RecA protein mediates fitness, virulence and DNA damage response (SOS response) in Riemerella anatipestifer

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Research article

Keywords: Riemerella anatipestifer, RecA gene, fitness, virulence, SOS response

Posted Date: June 22nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-32460/v1

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Abstract

Background: DNA damage response (SOS response) refers to an important protective mechanism for bacteria to cope with genomic DNA damage. The RecA protein plays a key regulatory role in the induction of SOS responses in many bacteria, and its homologous proteins are widely present in various organisms. However, the biological functions of RecA protein of *Riemerella anatipestifer* have never been explored.In this work, we constructed the deletion strain RA-GDΔ*RecA* and complemented strain RA-GD△*RecA* pCPRA::*RecA*, and the growth ability, drug sensitivity, virulence and SOS response of the parental RA-GD, RA-GDΔ*RecA* and RA-GD△*RecA* pCPRA::*RecA* strains were detected.

Results: The results showed that the RecA protein reduced the fitness of RA-GD strain, and decreased the resistance of cells to three antibiotics (tetracycline, oxytetracycline and gentamicin sulfate) and the virulence of the strain to ducklings. Within a certain range of ultraviolet irradiation (UV), the transcription level of *RecA* gene increased significantly with the prolongation of UV time. Furthermore, the deletion of *RecA* gene reduced significantly the integrity of genomic DNA of RA-GD, indicating that the *RecA* gene may play a positive regulate role in the SOS response of *R. anatipestifer*. However, Western blotting showed that the levels of RecA protein in parent strain had no significant change under the condition of UV treatment.

Conclusion: This experiment demonstrated for the first time that *RecA* gene participated in the fitness, resistance, virulence and DNA SOS response in *R. anatipestifer*.

Background

DNA damage response (SOS response) is a global regulatory network present in most bacteria and targets at addressing DNA damage. As with many other genetic pathways, the SOS response was first identified and then thoroughly studied in *Escherichia coli* (*E. coli*)[1]. The SOS response is induced through the activation of the RecA protein [2], RecA is found in all free-living bacteria, and is the most slowly evolving gene involved in DNA metabolism, with an average sequence conservation of 60~70% across the entire bacterial domain of life [3]. *E. coli* RecA is a 38 KD single subunit protein consisting of 352 amino acid residues, which is the first protein to be identified that involves in recombinant repair of DNA damage [3,4]. In uninduced cells, it is present at less than 10,000 monomers per cell [5] and its homologous proteins are widely present in various organisms. The homologous proteins of RecA in structure and function are ubiquitous in all classes of life. The archaea homolog is called RadA [6-8]. In eukaryotes, there are two RecA homologs, the Rad51 and Dmc1 proteins [9-11]. For prokaryotes, RecA is a central molecule in the homologous recombination, and RecA of *Escherichia coli* (*E. coli*).

RecA participates in SOS response, and SOS response dependent of RecA regulates the transcription of the corresponding SOS genes through the universal intracellular DNA signaling pathway [12]. This system is regulated by both the LexA transcriptional repressor and the RecA recombinase. SOS response plays a role in homologous recombination repair and control of the expression of many DNA repair proteins in

most bacteria. The bacterial SOS response was first identified in response to UV light [13], but it is also intrinsically linked to the natural response against many DNA-damaging agents, both physical (ionizing radiation, antibiotics, acid-base stimulation, heavy metal damage) and chemical (alkylating, crosslinking, oxidizing agents, etc.) damage factors [14].

The bacterial SOS response regulates DNA repair and restarts the stagnant replication fork, affecting bacterial adaptation to a variety of stresses. SOS responses can also produce resistance by inducing genetic recombination and promoting the horizontal transfer of resistance genes [15]. In addition, the SOS response regulates the bacterial virulence. Koomey et al found that *RecA* deficiency significantly reduced the mutation rate of *Neisseria gonorrhoeae,* indicating that the SOS response plays a role in antigenic variation [16]. Secondly, the relationship between SOS response and pathogenic virulence is mostly focused on the regulation of the expression and transmission of phage-encoded virulence genes, such as Shiga toxin encoded by prophage in *enterohemorrhagic E. coli* (EHEC) [17] and the type III secretion system of pathogenic *E. coli* [18]. The SOS response also has a certain influence on the invasiveness of bacteria. Studies have shown that the expression of the genes related to *Escherichia coli* type I pili *(flcG, focA, sfaD, fimC)* is down-regulated more than 2 times under the action of enrofloxacin, related to colonization and adhesion of *E. coli* [14]. These findings have irrevocably transformed the image of the SOS response from a DNA-repair textbook paradigm into a key component in the fight against antibiotic resistance and virulence.

R. anatipestifer RecA gene is 1032 bp, encoding approximately a 37.7 KD protein. By amino acid sequences analysis, RecA protein of *anatipestifer* shares 60.5% identity with that of *E. coli*, but the biological functions of this protein have not been reported at present. This work studied RecA-mediated SOS response, resistance, fitness and virulence in *R. anatipestifer* for the first time, and obtained information to help to increase understanding of the biological functions of *R. anatipestifer* RecA protein.

Results

Growth curve and Competition Experiments *in Vitro*

Compared with RA-GD strain, deletion strain RA-GDΔ*RecA* decreased significantly growth rate based on the growth curves. The growth rate of the complemented strain was restored by pCPRA::*RecA* plasmid (Fig. 1). It suggested that the *RecA* gene affected the growth characteristic of *R. anatipestifer.*

The competition experiments *in vitro* revealed that the CFUs for RA-GD and RA-GD△*RecA* were 3.63×10^{10} (4.95×10^{10}–1.32×10^{10}) CFU/ml and 1.32×10^{10} CFU/ml, respectively. *In vitro* CI of the RA-GD/RA-GD△*RecA* was 2.75 ($3.63\times10^{10}/1.32\times10^{10}$). In addition, the CI between RA-GD△*RecA* pCPRA::*RecA* and RA-GD△*RecA* was 2.4 ($2.78\times10^{10}/1.16\times10^{10}$), which was consistent with the CI of RA-GD/RA-GD△*RecA*.

Drug susceptibility test

The RA-GD, RA-GD△*RecA* and RA-GD△*RecA* pCPRA::*RecA* strains were tested for susceptibility to twenty-four antimicrobial agents belonging to ten classes with dissimilar structures. The results showed that RA-GD△*RecA* strain increased significantly resistance to roxithromycin (2048-fold) and erythromycin (2048-fold) belonging to macrolide antibiotics, because the strain carried erythromycin resistant cassette. Comparison to RA-GD strain, RA-GD△*RecA* strain increased susceptibility to tetracycline (four-fold), oxytetracycline (four-fold) and gentamicin (four-fold), but the MICs of other antimicrobial agents for RA-GD and RA-GD△*RecA* had no change. RA-GD△*RecA* pCPRA::*RecA* strain complemented with *RecA* gene completely or partly restored the resistance to tetracycline, oxytetracycline and gentamicin (Table 3). These results displayed that RecA protein could play a positive role in the resistance of *R. anatipestifer* to tetracycline, oxytetracycline and gentamicin resistance.

Duckling virulence test

The virulence experiment showed that LD_{50} for RA-GD and RA-GD△*RecA* pCPRA::*RecA* was 6.75×10^7 CFU and 6.87×10^7 CFU, respectively, and significant difference was not obtained between the two strains. It was 8.85×10^8 CFU for RA-GD△*RecA* strain. The virulence of RA-GD△*RecA* strain reduced 13.1 fold compared to that of RA-GD strain. On the other hand, the survival percentages of ducklings were compared along the three groups of animals infected with RA-GD, RA-GD△*RecA* pCPRA::*RecA* and RA-GDΔ*RecA* with 3.2×10^8 CFU/animal for each strain. The survival percentages of the animals infected with deletion strain RA-GDΔ*RecA* increased significantly with those of the ducklings infected with the parent strain and complemented strains (Fig. 2). These results revealed that RecA protein mediated the virulence of *R. anatipestifer.* All the control ducklings given PBS survived.

Transcription and expression of *RecA* gene

No significant difference was observed in *RIA_1470* gene transcription between RA-GD and RA-GD△*RecA* strains, which indicated that erythromycin resistant cassette had not a polar effect on the transcription of adjacent genes.

Under the condition of UV treatment, the transcriptional level of *RecA* gene of RA-GD strain increased significantly with the prolongation of UV within a certain period of time (0–45 min) (Fig. 3). In addition, Western blot assay showed no significant difference was observed among the levels of RecA protein in the parent strain at different time point after UV treatment, which revealed that UV damage factor did not affect *RecA* expression in parent strain. The same results were observed at 0, 15 and 30 min in the complemented strain. However, the levels of RecA in the complemented strain significantly decreased at 45 min after UV treatment (Fig. 4). The difference between parent strain and complemented strain may be derived from the different promoters.

DNA damage test

The breakage of bacterial double-stranded DNA (dsDNA) in damage factors (such as UV, X-ray, chemical factors) could be specifically measured by detecting fluorescent signals (Accomando et al., 1991). The

relative fluorescence signal was stronger, and the integrity of dsDNA was more. For the parent strain and deletion strain with UV treatment for 0, 15, 30 and 45 min, the fluorescence signals of dsDNA of parent RA-GD and complemented RA-GD△*RecA* pCPRA::*RecA* strains were significantly stronger than those of deletion strain RA-GDΔ*RecA* strain, which reflected that UV could damage the genomic dsDNA and the deletion of *RecA* gene hindered the SOS response in *R. anatipestifer* (Fig. 5). Compared with RA-GD and complemented strain RA-GD△*RecA* pCPRA::*RecA*, CFUs of RA-GD△*RecA* declined significantly under the condition of radiation treatment during 45 min (Table 4). The results revealed that the presence of *RecA* gene played a positive regulatory role, which offset the killing of the strain by UV. In addition, the comparison between the complemented strain and the deleted strain under the condition of UV damage showed that the *RecA* gene exerted and played some its functions after *RecA* gene complementation.

Discussion

The SOS response triggers a myriad of bacterial cell responses, including DNA repair, elongation of bacterial cells, induction of error-prone DNA polymerases, induction of latent bacteriophage, and inhibition of cell division [19]. Two proteins, LexA and RecA, play key roles in the regulation of the SOS response. During normal growth, the LexA dimer acts as a transcriptional repressor for genes belonging to the SOS regulon by binding to a specific operator sequence in their promoter region [20]. RecA acts as a co-protease to stimulate self-cleavage of LexA as well as other related proteins. *R. anatipestifer* RecA is a 37 KD protein, which shares 60.5% identity with that of *E. coli*, but the biological functions of this protein have not been reported until now. This work studied the above issue, which will help to enhance understanding of the RecA-mediated functions in bacteria.

A study in *Bacteroides fragilis* (*B. fragilis*) [21]compared with the normal wild and RecA mutant strain, and the growth of mutants under normal conditions was not significantly prolonged, indicating there was no obvious correlation between the *RecA* gene and the growth of *B. fragilis.* However, the deletion of *R. anatipestifer RecA* gene significantly affected the cellular growth characteristics.

Bacterial RecA proteins are involved in the mechanism of bacterial resistance to antibiotics as the activator of a bacterial SOS-response. Inhibition of bacterial SOS response, bacterial genomes are more susceptible to mutations, increasing the incidence of durg-resistant mutations. A report demonstrated the combined inhibition of the RecA inhibitor N6-(1-Naphtyhl)-ADP and enrofloxacin [21]. Overexpression of *RecA* gene in *B. fragilis* produced resistance to metronidazole [22], and *E.coli* lacking *RecA* gene is more sensitive to bactericidal antibiotics [23]. In this work, *RecA* gene deletion strain of *R.anatipestifer* increased susceptibility to tetracycline, oxytetracycline and gentamicin, which displayed that RecA protein also participated in *R.anatipestifer* resistance to some certain antimicrobial drugs.

In vitro studies have investigated the effects of SOS responses on virulence-related functions. In *staphylococcus aureus*, the SOS response also induced pathogenicity islands encoding virulence factors [24]. RecA can promoted the cleavage of λphage CI inhibitory protein, induce *Escherichia coli* phage into the bacterial cycle, and promote the expression of shiga-like toxin (STX) encoded by prophage [25]. In this

experiment, compared with the *R.anatipestifer* RA-GD strain, the deletion of *RecA* gene significantly reduced the lethality rate of RA-GDΔ*RecA* in ducklings. It speculated that the *RecA* gene may be related to the expression of virulence genes. The specific mechanism needs further clarification.

Expression of *RecA* RNA and RecA protein are early, measurable, and reliable indicators of the SOS response. We initially measured the transcription and expression of *R. anatipestifer RecA* by qRT-PCR and Western blot assays in response to UV light. Under UV treatment, the transcriptional levels of *RecA* gene significantly up-regulated, but the expressional levels of *RecA* gene had no significant difference in parent strain. It was speculated that the DNA damage factor UV initiated the DNA SOS response, affecting the transcription level. However, the expression levels of *RecA* gene were relatively stable in comparison with the transcription levels under the condition of UV treatment. For the complemented strain, the levels of RecA protein had no significant difference from 0 to 30 min after UV treatment, but a significant decrease was observed at 45 min. This difference may be due to different promoters for *RecA* gene in parent strain and complemented strain. After all, the expression conditions of the *RecA* gene in the plasmid could not be exactly the same as those of *RecA* in the parent strain.

In *E. coli*, cell survival and genomic stability after UV depends on repair mechanisms induced as part of the SOS response to DNA damage. In this study, the deletion of *RecA* gene significantly reduced the integrity of genomic DNA, which revealed that RecA protein involved in the SOS response of *R. anatipestifer*.

The SOS response is a pressure response system that generates mutations by activating mismatched DNA polymerases. The early phase of the SOS response is mostly dominated by accurate DNA repair, while the later phase is characterized with elevated mutation levels caused by error-prone DNA replication. SOS response is inhibited by knocking out the *RecA* gene and mutating the *LexA* gene [25], elucidating the mechanism by which the SOS response promotes drug resistance, and theoretically demonstrates that the inhibition of SOS response is used to slow the improvement of drug resistance is feasible.

Conclusion

This work explored the biological functions of RecA protein of *R. anatipestifer* RA-GD strain. The results confirmed that the RecA protein affected the growth ability of *R. anatipestifer* and participated in cells resistance to tetracycline, oxytetracycline and gentamicin. The deletion of RecA protein significantly decreased the bacterial virulence and the integrity of genomic DNA, which confirmed that RecA protein contributed to the virulence and SOS response in *R. anatipestifer*. The specific mechanism needs further clarification.

Methods

Plasmids, Bacterial Strains, Growth, Media and Animals

All the bacterial strains and plasmids used in this study, and their relevant characteristics and sources are described in Table 1. *R. anatipestifer* RA-GD and LJW-2 strains are cultured in tryptic soybean broth (TSB, Oxoid) or tryptic soy agar at 37℃ (TSA, Oxoid) with 5% calf serum in 5% CO_2. *E. coli* X7213 is grown at 37℃ in Luria-bertani (LB, Oxoid) broth or on LB agar with 2,6-diaminoheptanedioic acid (DPA) of 50 μg/ml. 8-day-old Cerasus pseudocerasus ducklings were purchased from Sichuan Mian Ying Duck Co. Ltd (Chengdou, China). Animals are kept in clean buildings and the temperature of the room is maintained at 28℃–30℃. In addition, the room should be ventilated twice a day. All animal studies complied with the guidelines of Lanzhou Veterinary Research Institute Animal Care and Use Committee.

Construction of RA-GDΔ*RecA*

All primers needed in this work were shown in Table 2. RA-GDΔ*RecA* mutant with deletion of *RecA* gene were constructed as described previously [26]. Briefly, the loci of *RecA* gene in the RA-GDΔ*RecA* mutant were replaced with erythromycin (ErmF) resistant cassette which was amplified with the primers ErmF-F/ErmF-R, using the genomic DNA of LJW-2 strain as template. The primers Up-F/Up-R and Dp-F/Dp-R were used to amplify the upstream (600 bp) and downstream (600 bp) homologous arm regions of *RecA* gene, respectively, using the genomic DNA of RA-GD as template. Then, pRE112 plasmid was digested with *Sac*I and *Sph*I restriction endonucleases to obtain vector fragment. Each segment of three PCR fragments (upstream homologous region, ErmF resistant cassette and downstream homologous region) and pRE112 plasmid fragment were integrated with Unique Clone™ Plus Muti One Step Cloning Kit (Novogene, Tianjin, China) to generate the recombinant plasmid pRE112::*ErmF-600H*. The X7213 strains carrying pRE112::*ErmF-600H* and *R. anatipestifer* RA-GD strain were co-cultured and pRE112::*ErmF-600H* plasmids were introduced into RA-GD strain by conjugation. RA-GDΔ*RecA* mutants were selected with erythromycin of 2 μg/ml and confirmed with conserved primers RA-OmpA-F/RA-OmpA-R and the identifying primers RecA-F/RecA-R and ErmF-F/ErmF-R.

Construction of RA-GD△*RecA* pCPRA::*RecA*

Complementation experiments were performed as described previously [26]. Briefly, the *RecA* gene was amplified with pCP-RecA-F/pCP-RecA-R primers. *RecA* and pCPRA plasmid digested with *Pst*I and *Sph*I endonucleases were ligated using Unique Clone™ One Step Cloning Kit (Novogene, Tianjin, China) to generate the complemented plasmid pCPRA::*RecA*. The X7213 strains carrying pCPRA::*RecA* were selected with ampicillin of 100 μg/ml. pCPRA::*RecA* plasmid from X7213 was introduced into RA-GD△*RecA* by conjugation. The complemented strains RA-GD△*RecA* pCPRA::*RecA* were selected with erythromycin of 2 μg/ml and cefoxitin of 1 μg/ml. Further, the strains were confirmed with conserved primers RA-OmpA-F/RA-OmpA-R and the identifying primers RecA-F/RecA-R.

Growth curve and Competition Experiments *in Vitro*

The growth curves for RA-GD, RA-GD△*RecA* and RA-GD△*RecA* pCPRA::*RecA* strains were monitored under non-competitive conditions, based on OD_{600} values at different time points. Namely, 10^8 colony-

forming unit (CFU) of each strain was inoculated into 5 ml TSB containing 5% calf serum and cultured at 37℃ with 200 rpm. The concentrations of bacteria solution under the 600nm wave length were measured from the first hour until the twelveth hour with an interval of one hour. The growth curve for each strain was drawn according to the OD_{600} values.

In vitro competition experiments were performed for RA-GD, RA-GD△*RecA* and RA-GD△*RecA* pCPRA::*RecA* strains as the reference [26]. The competition index (CI) was calculated as the ratio between RA-GD CFU and RA-GD△*RecA* CFU. Similarly, CI between RA-GD△*RecA* pCPRA::*RecA* and RA-GD△*RecA* was calculated.

Drug sensitivity test

According to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2016), the minimal inhibitory concentrations (MICs) of antimicrobial agents for RA-GD, RA-△*RecA* and RA-GD△*RecA* pCPRA::*RecA* strains were determined with 2-fold serial broth microdilution method. The procedure was carried out according to the references [26, 27]. The concentrations of antimicrobial agents ranged from 512 to 0.25μg/ml. *E. coli* ATCC 25922 was used as control. All tests were performed in triplicates. The MIC was recognized as the minimum concentration of the antimicrobial agent that could inhibit visible growth of bacteria (CLSI, 2016).

Duckling toxicity test

This experiment was performed as our previous work [26]. The virulence of RA-GD, RA-GD△*RecA* and RA-GD△*RecA* pCPRA::*RecA* strains was defined according to the median lethal dose (LD_{50}) and percent survival of the ducklings [26, 28]. After this work, the survivals were narcotized by pectoral muscle injection of pentobarbital sodium in 20 mg/kg of body weight. Then, the ducklings with deep narcosis were euthanized by cervical dislocation.

Transcription and expression of *RecA* gene

The parent strain RA-GD, deletion strain RA-GD△*RecA* and complemented strain RA-GD△*RecA* pCPRA::*RecA* were subjected to UV treatment. When the fresh bacterial solution reached to OD_{600} value of 1.0, the above three strains were irradiated with a UV dose of 60 μW/cm^2. We inflicted the UV damage factor on the strains at 0, 15, 30 and 45 min, respectively, thereby acquiring a series of treated samples.

The transcriptional level of *RecA* gene was tested with the primers RecA-qRT-F/ RecA-qRT-R by qRT-PCR assay. 16S gene amplified with the primer pairs of 16S-F/16S-R was used as an internal control. In addition, the transcriptional levels of downstream *RIA_1470* genes of RA-GD and RA-GD△*RecA* strains were detected with RIA-1470-F/RIA-1470-R primers to exclude the possibility that erythromycin resistant cassette had a polar effect on the transcription of adjacent genes. The amplification procedure was the same as the reference [26]. The relative gene expression levels were quantified according to the comparative $2^{-\triangle\triangle}$CT method [29].

The complete *RecA* gene was amplified with the primers pET-RecA-F/pET-RecA-R, using the genomic DNA of RA-GD strain as the templates. *RecA* gene was cloned into pET-32a vector with *BamH*I and *Xho*I to generate recombinant plasmid pET-32a-*RecA*, and this plasmid was introduced into *E. coli* BL21(DE3). RecA protein carrying Trx-tag and His-tag was expressed in BL21(DE3) with 1 mM/L IPTG induction. The RecA protein, purified with nickel-nitrilotriacetic acid (NTA) column (TransGen, Beijing) according to the maufacture's manual, was used as antigen to immunize rabbit and generate polyclonal antibody. The expression level of RecA protein of *R. anatipestifer* was detected by Western blotting using rabbit polyclonal antibody and HRP-labeled goat anti-rabbit secondary antibody (Sigama, USA). These tests could determine the effect of UV on the transcription and expression levels of RecA.

DNA damage test

The experiment was carried out to measure the genome integrity of cells to analyze the damage condition of DNA, according to the reference [30] with appropriate modification. Briefly, for each sample, 500 μl of cell suspension (100 cells/μl) were irradiated with a UV dose of 60 μW/cm^2 for 45 min in a 24-well plate. Then, genomic DNA was extracted from each cell sample with Universal Genomic DNA Extraction Kit (TakaRa, China). 55 μl of 0.2 M NaOH was added to 50 μl of DNA solution, and a pH value of 12.5 was reached under these conditions. Then, 10^5 μl of DNA solution for each sample was transferred to the 96-well plate. Thereafter the temperature setting was shifted to 30°C and kept constant for 60 min in order to allow alkaline unwinding of the DNA. Prior to the addition of 70 μl of neutralization solution (14 mM β-mercaptoethanol; 1 M glucose) at a rate of 200 μl/s, the temperature was shifted to 22°C. For the positive control sample, an internal standard representing cells with 100% double-stranded DNA, 70 μl of neutralization solution was added prior to 55 μl of 0.2 M NaOH. The negative control without any double-stranded DNA only consisted of 50 μl of DNA elution buffer, 55 μl of 0.2 M NaOH and 70 μl of neutralization solution. After dispensing 78 μl diluted SybrGreen (1:8333 in H_2O), respectively, the samples were mixed by pipetting a volume of 253 μl up and down once at a rate of 100 μl/s. At last, samples were analysed in a 96-well-plate fluorescence reader at 492 nm excitation/520 nm emission immediately after SybrGreen addition. The relative fluorescence signals for each sample was calculated as follows: fluorescence signals (%) =Sample-Negative/Sample-Positive. Statistical significance was evaluated by using Student's *t-test*. In addition, in order to compare the changes of parental strain, deletion strain and complementary strain in different UV durations, we calculated and compared the colonies of the above three strains at different time durations of UV for 0 min, 15 min, 30 min and 45 min.

Statistical analysis

Statistical analysis was carried out using GraphPad Prism version 6.0 Windows software. Student's t-test was used to ascertain the significance of the between-group differences. A value of $^*P < 0.05$ was considered significant difference, and $^{**}P < 0.01$ and $^{***}P < 0.001$ were all considered highly significant.

Abbreviations

E. Coli：*Escherichia coli*；EHEC：Enterohemorrhagic *Escherichia coli*；*B. fragilis*：*Bacteroides fragilis*；STX：Shiga-like toxin；CFU：Colony-formingunit；CI：Competition index；MICs：Minimal inhibitory concentrations；LD_{50}：The median lethal dose；NTA：Nickel-nitrilotriacetic acid

Declarations

Ethics approval and consent to participate

Animal protocols were approved by the Lanzhou Veterinary Research Institute Animal Care and Use Committee and met the standards for humane animal care and use.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

The authors acknowledge National Natural Science Foundation of China (No. 31572532) for providing grant to Fuying Zheng and Bacterial Disease in Grazing animal Team for providing grants to Xiaowei Gong, Qiwei Chen, Yongsheng Liu and Fuying Zheng. The funding body has no role in study design, collection, analysis, data interpretation and in writing the manuscript.

Authors' contributions

GJ performed the experimental work, data collection & analysis and prepared the manuscript. XG and QC have designed the work plan. FZ and YL has conceived the plan and supervised the whole study. All the authors have read and approved the final version of the manuscript.

Acknowledgments

The authors would like to acknowledge State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences for providing infrastructure facility.

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Tables

Due to technical limitations the Tables are available as a download in the Supplementary Files.

Figures

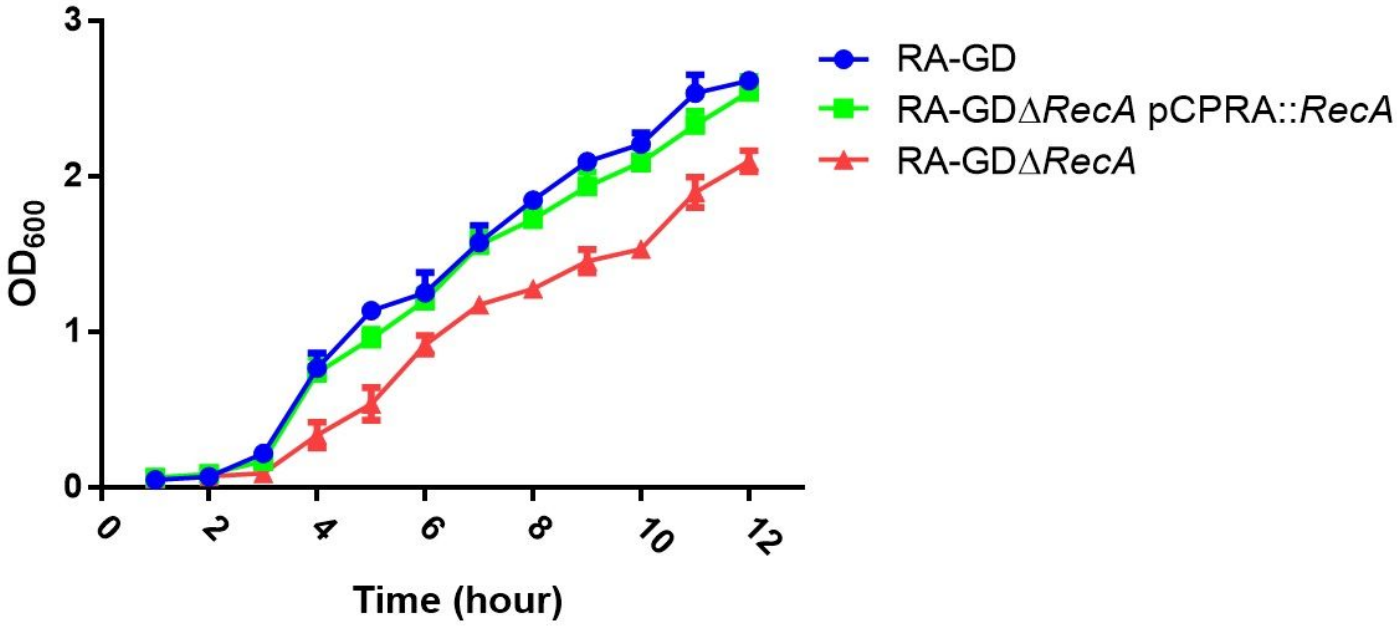

Figure 1

Comparison of growth curves of parental strain RA-GD, deletion mutant RA-GD△RecA and complemented strain RA-GD△RecA pCPRA::RecA.

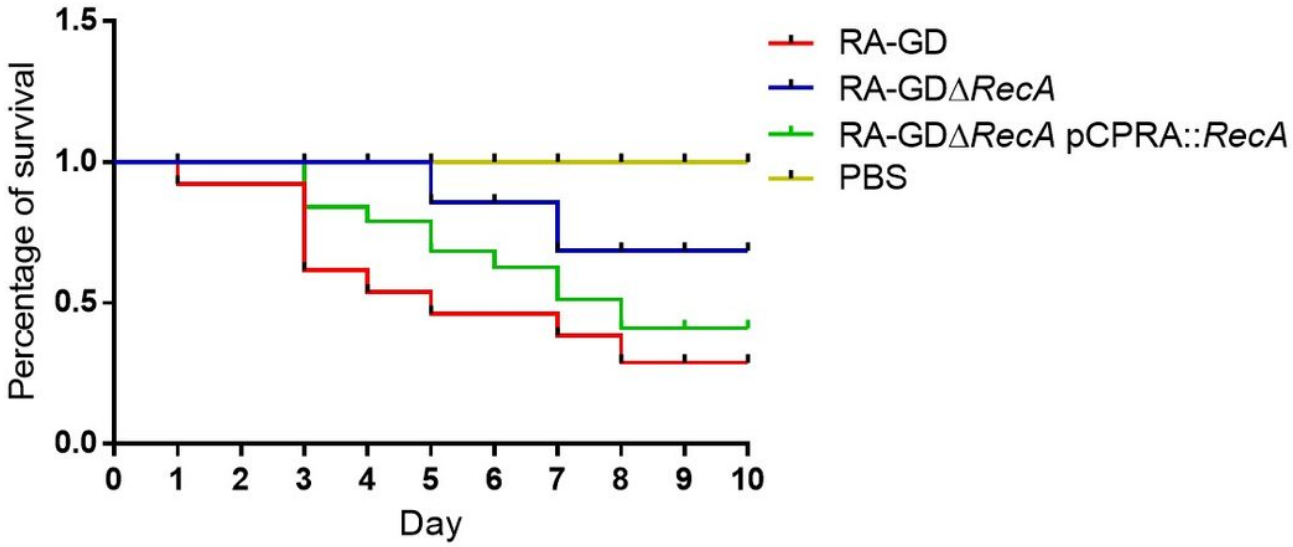

Figure 2

The dose of challenge is 3.2×108 CFU/animal for duckling survival curve.

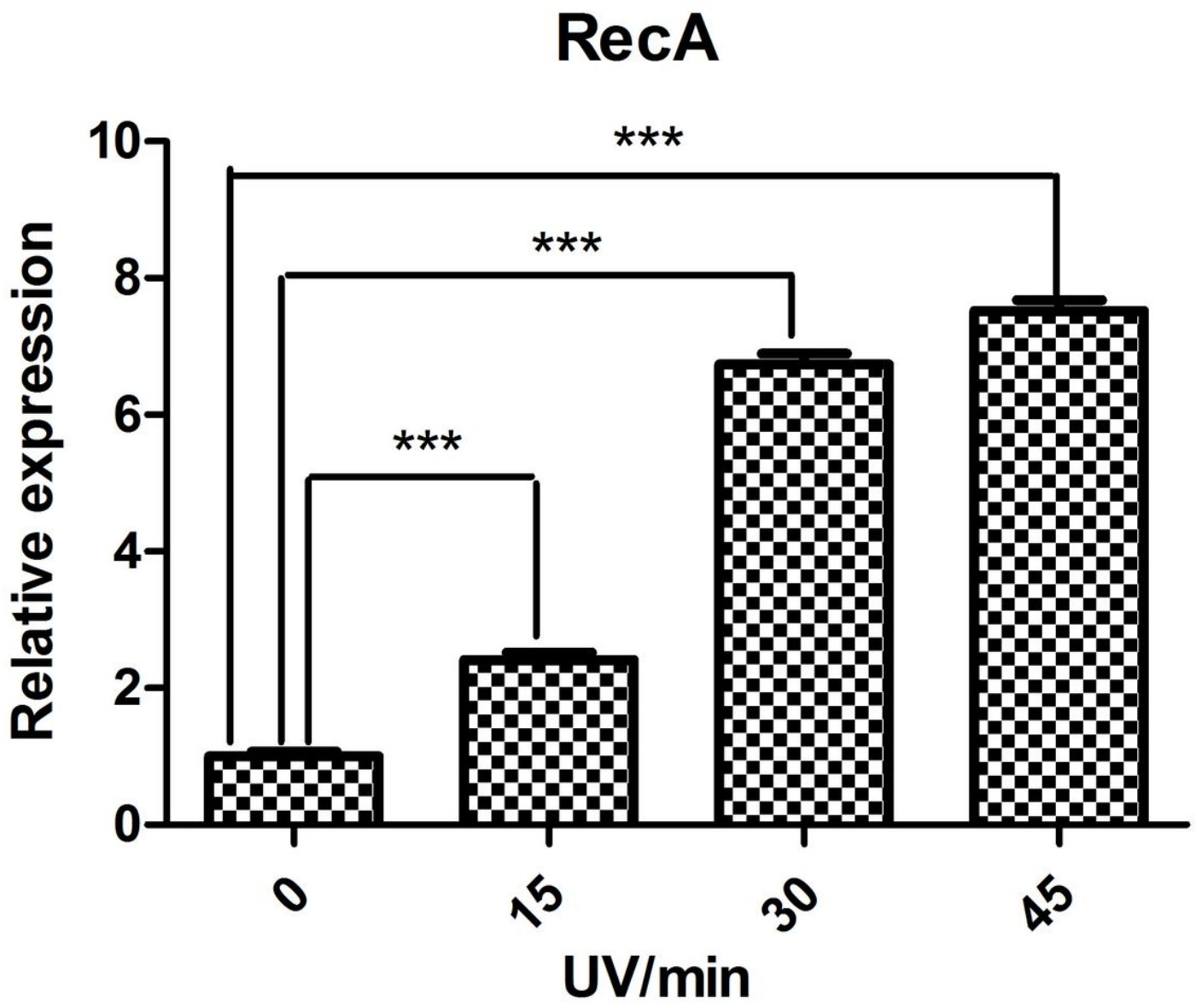

Figure 3

Transcriptional levels of RecA gene of RA-GD strain with UV irradiation treatment for different time.

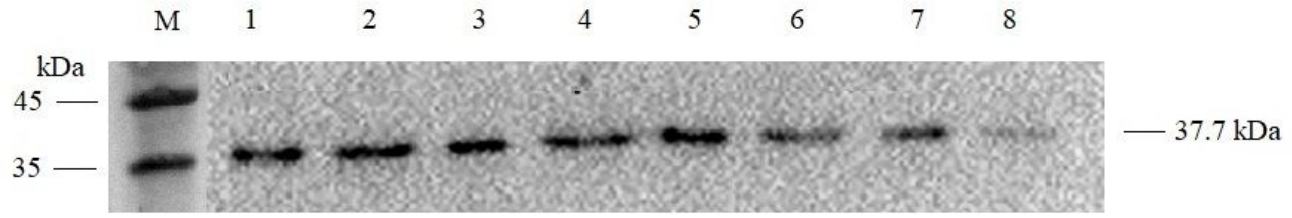

Figure 4

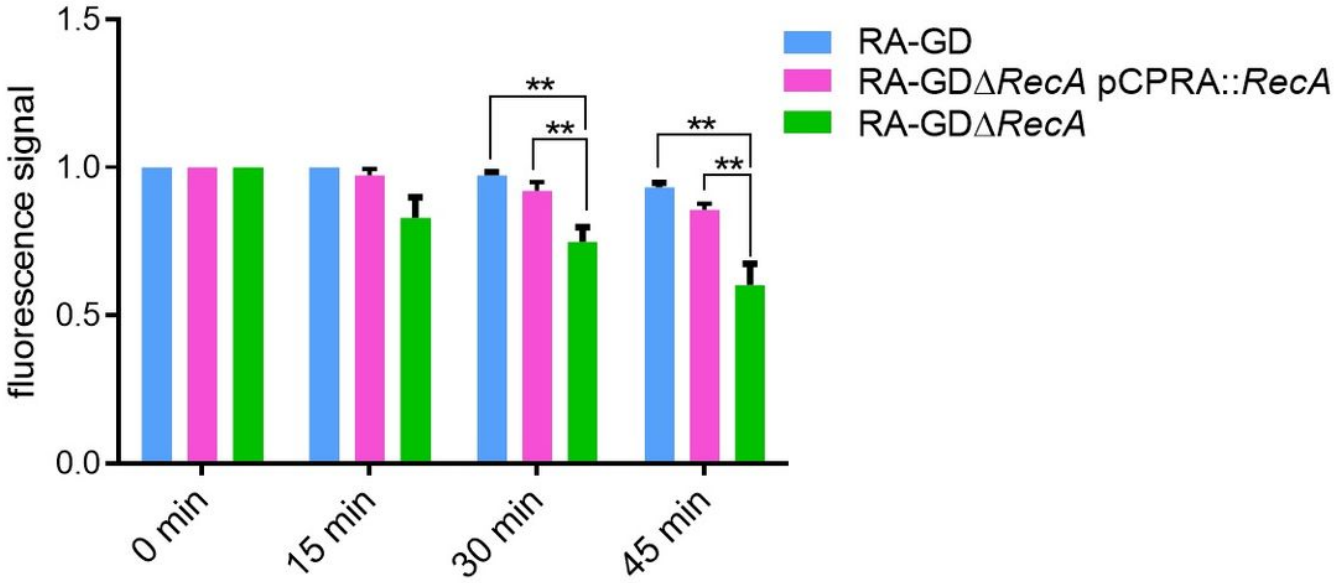

Figure 5

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- NC3RsARRIVEGuidelinesChecklist2014RecA.docx
- Additionalfile.docx
- Tables.pdf