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Investigating the role of *Helicobacter pylori* PriA Protein

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Abstract

Background: In bacteria, PriA protein, a conserved DEXH-type DNA helicase, plays a central role in replication restart at stalled replication forks. Its unique DNA binding property allows it to recognize and stabilize stalled forks and the structures derived from them. PriA plays a very critical role in replication fork stabilization and DNA repair in *E. coli* and *N. gonorrhoeae*. In our *in vivo* expression technology screen, *pri*A gene was induced *in vivo* when *Helicobacter pylori* infects mouse stomach.

Materials and Methods: We decided to elucidate the role of *H. pylori* PriA protein in survival in mouse stomach, survival in gastric epithelial cells and macrophage cells, DNA repair, acid stress and oxidative stress.

Results: The *priA* null mutant strain was unable to colonize mice stomach mucosa after long-term infections. Mouse colonization was observed after one week of infection but the levels were much lower than the wild type HpSS1 strain. PriA protein was found to be important for intracellular survival of epithelial cell / macrophage cell ingested *H. pylori*. Also, a *priA* null mutant was more sensitive to DNA damaging agents and was much more sensitive to acid and oxidative stress as compared to the wild type strain.

Conclusions: These data suggest that the PriA protein is needed for survival and persistence of *H. pylori* in mice stomach mucosa.

Keywords

Helicobacter pylori ; DNA helicase; replication fork stabilization

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Introduction

Helicobacter pylori (*H. pylori*) is a highly successful human pathogen that colonizes roughly one half of the world's population. It is typically transmitted orally within families during early childhood and can persist for decades in its preferred niche, the gastric mucosa, despite triggering vigorous innate and adaptive immune responses. *H. pylori* infection causes chronic gastritis, which is asymptomatic in the majority of carriers but is considered a major risk factor for the development of gastric and duodenal ulcers and the two gastric malignancies, mucosa-associated lymphoid tissue lymphoma and gastric adenocarcinoma (1). In addition to its tight association with cancer, *H. pylori* stands out among other gram negative bacterial pathogens in its ability to persist and establish chronic infection. The capacity of *H. pylori* to colonize the human stomach can be attributed to the production of specific bacterial products. Numerous *H. pylori* components have been designated colonization factors based on the demonstration that null mutant strains defective in the production of these factors are impaired in the ability to colonize the stomach in animal models. For example, *H. pylori* null mutant strains defective in production of urease or flagella are unable to colonize animal models (2, 3). Urease hydrolyzes urea to yield ammonium ions and thereby contributes to the acid resistance of *H. pylori* (4). Flagella confer the property of motility and enable *H. pylori* to penetrate the gastric mucus layer. In a signature- tagged mutagenesis analysis, 47 *H. pylori* genes were found to be essential for colonization of the Mongolian gerbil stomach but not essential for growth of *H. pylori* in vitro (5). Probably many other *H. pylori* factors are also required for colonization of the stomach.

In vivo expression technology (IVET) was designed to identify genes of pathogens that are preferentially expressed during infection and has been used extensively (6, 7). IVET is a promoter-trapping technique that selects microbial promoters active in a specified niche during the interaction of a microorganism with its host. Using IVET we were able to identify several *H. pylori* genes which were induced *in vivo* during infection of mice stomachs. Thirty one genes were identified. These include genes responsible for a broad and varied group of cellular structures and functions: virulence, cell envelope structures, motility, oxidative stress, nucleic acid and sugar metabolism, translation, protein synthesis, type IV secretion system and a few conserved and hypothetical proteins. Our IVET screening revealed the host-induced expression of several genes involved in nucleic acid metabolism, including *hsd*M/R, *hsd*M, *rec*G, *pri*A and *tnp*B. This class of host-induced genes is involved in DNA synthesis and modification.

PriA is a single-stranded DNA-dependent ATPase, and a 3' to 5' DNA translocase/ helicase that was discovered originally because of its *in vitro* requirement for the conversion of bacteriophage phiX174 viral DNA to the duplex replicative form (8, 9). In *Escherichia coli* this protein, at the crossroads of DNA replication and recombination, plays a central role in origin- independent, replication restart of collapsed or arrested DNA replication forks and is also involved in DNA recombination (10–12). In *Neisseria gonorrhoeae*, *priA* plays a critical role in DNA repair and is important for resisting killing by oxidative damaging agents (13). These activities rely on the ability of PriA to load replication forks at a D loop, an intermediate that forms during homologous recombination, double-strand break-repair,

and stable DNA replication. Since the *priA* gene was induced in our IVET screen (14), and it plays a very critical role in replication fork stabilization and DNA repair in *E. coli* and *N. gonorrhoeae*, we decided to elucidate its role in *H. pylori* virulence.

Materials and Methods

Bacterial strains and growth media.

The *H. pylori* strain used in this study was Sydney Strain 1, SS1 (15). The strains were grown for 16 to 18 hours at 37°C in a microaerophilic atmosphere in bisulfiteless Brucella broth (BLBB) (16) containing 5% fetal bovine serum (Hyclone, Logan, UT). For BLBB solid medium, 1.7% agar was added. Unless stated otherwise, the antibiotics used in BLBB solid or liquid medium were: kanamycin (kan) 15 μg/ml, chloramphenicol (chl) 12.5 μg/ml, Glaxo Selective Supplement A or GSSA (5 μg /ml of Amphotericin-B, 20 μg /ml of Bacitracin, 1.07 μg /ml of Nalidixic acid, 0.33 μg /ml of Polymyxin-B, and 10 μg /ml of Vancomycin) (17). *E. coli* strain DH5 α was grown in Luria broth (LB) medium (18).

Isolation of DNA from bacterial strains.

Plasmid DNA was isolated from *E. coli* DH5 α using the QIAprep Miniprep or QIAfilter plasmid maxi kit (QIAGEN, Valencia, CA) in accordance with manufacturer's recommended protocols. Genomic DNA was extracted from *H. pylori* strain SS1 using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI) as described by the manufacturer.

Construction of *priA* gene knockout strain:

The full length *priA* gene (1,860 bp) was cloned in pUC19. A *pri*A gene null mutation was generated by insertion mutagenesis. A Kanamycin resistance gene along with its own promoter was inserted at the unique Nsi restriction enzyme site (nucleotide 435) of *pri*A. The resulting plasmid, ppriA::Kan, was used to transform *H. pylori* strain SS1 strain by electroporation. Kanamycin-resistant transformants for *pri*A disruption in *H. pylori* SS1 were verified by PCR analysis. The strain was named *priAKO*.

Generation of a *priA* gene complemented strain:

The *priA* gene along with its own promoter (3,300bp) was cloned between KpnI and the BglII sites of the pHel2 shuttle vector (19). This plasmid named ppriA-prom was used to transform strain *priAKO*. Kanamycin and chloramphenicol resistant transformants were verified for the presence of the ppriA-prom plasmid. This strain was named *priAKOComp*. Also in an independent experiment, the *priA* promoter sequence was cloned upstream of a promoterless chloramphenicol (*cat*) gene to check if it can drive the expression of the *cat* gene. The promoter sequence was found to be capable of driving the expression of the promoter less *cat* gene.

Animal housing and diet.

Mice were maintained in a National Institutes of Health (NIH) animal facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care

International (Rockville, MD). They were maintained in a specific-pathogen-free animal care holding room and were confirmed to be free of the following microorganisms: cilium-associated respiratory bacillus, ectromelia, mouse rotavirus, mouse encephalomyelitis virus, lymphocytic choriomeningitis virus, murine cytomegalovirus, mouse hepatitis virus, mouse adenovirus, minute virus of mice, *Mycoplasma pulmonis*, parvovirus, polyomavirus, pneumonia virus of mice, reovirus, and Sendai virus. Mice were housed in 7.5- by 11.5- by 5-in. sterilized ventilated Thoren cages (Thoren Caging System, Inc., Hazleton, PA) on Tek Fresh bedding (Harlan Teklad, Madison, WI). Cages were changed weekly. The animal holding room was maintained under environmental conditions of 20°C, 40 to 70% relative humidity, 15 air changes/h and a 12-h–12-h light-dark cycle. Mice were fed an autoclaved pelleted rodent diet (rodent NIH-31 autoclavable NA; Zeigler Brothers,Inc., Gardners, PA) ad libitum and provided sterilized individual water bottles for an ad libitum water source. Upon arrival, the mice were acclimated for a minimum of 7 days prior to being used in the experiments. This study was reviewed and approved by the NIH Institutional Animal Care and Use Committee. All procedures and use of animals were in compliance with the Public Health Service Guide for the Care and Use of Laboratory Animals (20).

Inoculation of mice with *H. pylori* strains:

We did a comparative analysis of Wild type HpSS1 (WT), *priA* null mutant (*priAKO)* and *priA* gene complemented (*priAKOComp)* strains by inducing mice infections with all three strains. Six week old C57BL/6 mice were inoculated intragastrically with 0.1 ml of 16–18 hours grown cultures of *H. pylori* once a day every other day for a total of three inoculations. Mouse stomachs were harvested at 4 weeks and 8 weeks of infection to determine the colonization levels. Stomach homogenates were plated on BLBB plates at 10^0–10^3 dilutions.

Invasion and intracellular survival assays with gastric epithelial cells:

The day before the assay was performed, GSM06 gastric epithelial cells were seeded in 6-well tissue culture plates to 2×10^5 cells per well in DMEM/Ham F-12 medium (21, 22). An invasion assay and an intracellular bacterial viability assay were performed as described previously (23). GSMO6 cells were infected with 16–18 hours grown cultures of WT, *priA KO* and *priA priAKOComp* strains at a multiplicity of infection (MOI) of 100 for 4 and 24 hrs. The monolayers were centrifuged for 5 min at 600 g to synchronize bacterial contact with the monolayer. After 2 hours of incubation at 33°C in a humidified incubator, the monolayers were washed thrice with PBS and then incubated with 2 ml of DMEM/Ham F-12 containing 100 μg/ml gentamicin for 2 hours to kill extracellular bacteria. This was followed by washing the monolayers thrice with PBS and lysis in 1.0 ml sterile water per well (4hours time point) or overnight incubation in DMEM/Ham F-12 containing 1 μg gentamicin/ml (24 hours time point). Next day, the monolayers were washed, lysed and the lysates were plated on BLBB plates at 10^0–10^3 dilutions.

Invasion and intracellular survival assays with macrophage cells:

The day before the assay was performed, RAW 264.7 cells were seeded in 6-well tissue culture plates to 2×10^5 cells per well in DMEM medium (24). The protocol was similar to the invasion and intracellular survival assays with gastric epithelial cells (23).

UV Survival Assay:

WT, *priA KO* and *priAKOComp* strains were grown on BLBB plates for 18–36 hours. All the strains were grown in liquid culture and were resuspended to an OD600 of 0·2 in BLBB medium. Dilutions (10^0–10^5) were performed in PBS and plated on BLBB plates. The plates were exposed to UV fluence ranging from 10, 15, 20, 30 × 100 mj/cm^2 in a UV Stratalinker. After UV exposure, the plates were incubated under microaerophilic conditions.

Ciprofloxacin Sensitivity Assay:

WT, *priA KO* and *priAKOComp* strains were grown on BLBB plates for 18–36 hours. All the strains were grown in liquid culture containing no ciprofloxacin, 0.0032, 0.0064 and 0.0128 μg/ml of ciprofloxacin for 16–18 hrs. Samples were taken and the absorbance was measured at OD600.

Acid Survival Assay:

WT, *priA KO* and *priAKOComp* strains were grown on BLBB plates for 18–36 hours. All the strains were grown from the plates in liquid culture for 16–18 hrs. pH 4 acidic medium supplemented with 0.5 mM urea, which promotes *H. pylori* survival in acid (25, 26), was prepared by adding 4.8 M HCl to standard growth media and filter sterilized. *H. pylori* strains were resuspended to an OD600 of 0.35 in pH 4 acidic medium. Incubation was performed at 37°C under microaerophilic conditions and samples were removed for analysis at 0, 2, 4 and 24 hours. Dilutions (10^0–10^4) were performed in PBS and plated on BLBB plates.

Oxidative Stress Assay:

WT, *priA KO* and *priAKOComp* strains were resuspended to an OD600 of 0.35 in PBS containing fresh unopened 50 mM H_2O_2 (27). Incubation was performed at 37°C under microaerophilic conditions and samples were removed for analysis at 0, 4 and 24 hours. Dilutions (10^0–10^4) were performed in PBS and plated on BLBB plates.

Statistical analysis:

Data are presented as mean ± SE from three independent experiments. The Student's T-test was used for statistical analysis between values from WT and *priA KO* strains. The threshold significance level for the statistically significant mean differences between these groups was $P < 0.05$.

Results

H. pylori priA mutant is impaired in its ability to colonize mice stomach mucosa.

Using pathogen-free C57BL/6 mice we performed a comparative analysis of WT, *priAKO* and *priAKOComp* strain colonization at 4 weeks and 8 weeks after infection. Unlike the WT and priAKO Comp strains, the HpSS1 *priAKO* strain did not colonize the mouse stomachs at either time point (Figure 1). In addition, the restored colonization ability of the HpSS1 *priAKOComp* strain was significantly lower than the WT HpSS1 strain colonization of mouse stomach at both 4 and 8 weeks.

priA is important for intracellular survival of *H. pylori*.

WT, *priAKO* and *priAKOComp* strains were used to infect GSM06 gastric epithelial cells as described in the materials and methods section. To determine whether the *priAKO* mutant strain was invasion defective, which could contribute to the observed reduced stomach colonization, we determined the numbers of CFU/ml in infected GSM06 cells at several time points. We did not observe any difference in the CFU/ml after 4 hours of infection for the *priAKO* strain and the WT strain, which indicates that the *priAKO* stain was fully capable of invading the gastric epithelial cells. However, the level of survival of the *priAKO strain* was significantly lower than the levels of survival of the WT and *priAKO Comp* strains at 24 hours after infection (Figure 2). Similarly, as shown in figure 3, *priAKO* strain was fully capable of invading RAW 264.7 macrophages after 4 hours of infection. However, the level of survival of the *priAKO* strain was significantly lower than both WT and *priAKOComp* strains at 24 hours after infection in RAW 264.7 cells (Figure 3).

priA is involved in DNA repair in *H. pylori*.

In *E. coli* and in *N. gonorrhoeae, priA* mutants display increased sensitivities to UV light (13, 28). To test whether *H. pylori priA* plays a role in DNA repair, *H. pylori* strains were exposed to increasing doses of UV light. We observed that the *priAKO* strain was more sensitive to UV light than the WT strain at all levels of UV exposure (Figure 4A). This repair defect was restored in the *priAKOComp* strain. We also tested whether inactivation of the *priA* gene in *H. pylori* increased the sensitivity of the bacteria to the DNA gyrase inhibitor ciprofloxacin. Ciprofloxacin induces double strand (ds) breaks in the DNA (29, 30). We found that the *priA* KO strain displayed a reduction in the ability to repair ds breaks as compared to the WT strain (Figure 4B). Survival after ciprofloxacin exposure was restored in *priAKOComp* strain.

H. pylori priA mutant is more sensitive to acid challenge.

WT*, priAKO* and *priAKOComp* strains were incubated in pH 4 acidic medium and samples were taken at 0, 2, 4 and 24 hours and plated on BLBB plates. These results show that the *priAKO* strain was capable of surviving the acid challenge at 4 and 24 hours of incubation; however it was somewhat more susceptible to acid than the WT strain. (Figure 5). At each time point, the *priAKOComp* strain was capable of surviving the acid challenge comparable to the WT strain.

H. pylori priA mutant is more sensitive to oxidative damage.

WT*, priAKO* and *priAKOComp* strains were incubated in PBS containing 50mM H_2O_2. Assessment of viability after exposure to hydrogen peroxide demonstrated that the *priAKO* strain was much more sensitive to oxidative stress when compared to the WT strain (Figure 6). The phenotype could be complemented back to WT levels in the *priAKOComp* strain.

Discussion

During the past 20 years, *H. pylori* has emerged as an important example of a persistent bacterial pathogen. Not only does this bacterium successfully colonize the hostile environment of the human stomach, but the infection regularly persists for the lifetime of

the host in the face of a constant, vigorous innate and adaptive immune response. In most infected people, *H. pylori* infection causes superficial chronic gastritis, which is usually clinically asymptomatic, although histologically apparent. However, significant subsets of infected individuals are at risk of the subsequent development of duodenal and peptic ulcers, and 1% of those that are infected will develop adenocarcinoma or lymphoma of the stomach (31, 32). Most basic research in the *Helicobacter* field has focused on the study of bacterial virulence determinants particularly in the context of their association with severe gastrointestinal sequelae of infection. Many different techniques have been developed to study bacterial genes that are expressed during growth in specific niches (33–36). A useful tool for identifying genes involved in virulence is *in vivo* expression technology (IVET) (37, 38). Using IVET we were able to identify several *H. pylori* genes that were induced *in vivo* in infected mice stomachs. Some of these genes are known virulence factors. *priA* gene was one of the genes identified using IVET (14).

PriA was originally discovered as a factor essential for the conversion of single-stranded circular DNA (SS) to the replicative-form DNA (RF, double-stranded circular DNA) of fX174single- stranded phage *in vitro*. It binds to a specific hairpin DNA structure on the fX174 genome, hydrolyzes ATP and triggers the assembly of a primosome responsible for the primer RNA synthesis on the single-stranded DNA genome (39). PriA is a DEXH-type DNA helicase containing a unique zinc-finger structure inserted among the helicase conserved motifs. It is highly conserved in eubacteria, suggesting its functional conserved roles in bacterial physiology. *priA* null mutants are viable, but grow very poorly (28, 40). SOS responses (an inducible DNA repair system that allows bacteria to survive sudden increases in DNA damage) are chronically activated in portions of the cell population, resulting in filamentous morphology (28, 40). Most notably, *priA* null cells are highly sensitive to genotoxic agents, such as UV (28, 41) and mitomycin C (42), which block ongoing replication. In *Neisseria gonorrhoeae*, *priA* was shown to play a role in DNA repair and DNA transformation processes and another study on *Neisseria meningitidis*, provides evidence for a role of *priA* in preventing both oxidative and nitrosative injury, and in intracellular meningococcal replication (13, 43).

Since the *priA* gene was induced in our *in vivo* screen, and it plays a very critical role in replication fork stabilization and DNA repair in *E. coli* and *N. gonorrhoeae*, we decided to elucidate its role in *H. pylori*. In order to understand the role of *priA* in *H. pylori*, the *priA* gene was disrupted, and assessed for its role in the survival of *H. pylori* in the gastric mucosa. To determine whether a deficiency in PriA activity is important for *H. pylori* host colonization, the relative abilities of WT, *priAKO* and *priA* gene complemented (*priAKOComp)* strains to colonize the stomach were evaluated in a mouse infection model. The *priAKO* strain could colonize mice stomach after one week of infection but at much lower levels than the WT strain (data not shown). Four and eight weeks post infections (p.i.), 5 infected mice from each group were sacrificed and *H. pylori* survival in the gastric tissue was determined. Four weeks p.i., WT infected animals had good colonization whereas the *priA KO* infected mice had no colonization. The bacterial load in the WT population increased from 0.31×10^6 CFU/gm of stomach tissue to 0.54×10^6 CFU/gm of stomach tissue at eight weeks p.i. However, no *H. pylori* was detected in *priAKO* infected mice during the same period. These data suggest that the PriA protein is needed for survival

during mouse stomach colonization. To rule out the possibility that cells lacking PriA protein had an intrinsic impaired growth capability, the *in vitro* growth rate of *priAKO* strain was measured for 1 week. *priAKO* strain showed some reduction in the growth rate but it wasn't significant when compared to the WT strain (data not shown).

H. pylori is able to invade epithelial cells, survive within large, late endosomal vacuoles, modify the molecular make-up of these vacuoles and later egress from this intracellular niche (44). It has been observed that viable *H. pylori* bacteria in large cytoplasmic vacuoles can repopulate the extracellular environment in parallel with the disappearance of intravacuolar bacteria (44). This intravacuolar niche is a place for the release of the bacteria and the place for evasion of the hostile microenvironment of the host. Another study reported that the plasma membranes are the site for *H. pylori* to replicate (45). A recent study found that *H. pylori* can multiply not only in macrophages and bone marrow-derived dendritic cells (46, 47) but also in epithelial cells (48). This finding has several implications for the biological life cycle of *H. pylori* in the host. *H. pylori* can be considered as an intracellular microorganism because it can invade cells, undergo replication within cells, and leave the infected cells for further spread. *H. pylori* invasion and intracellular survival assays of WT, *priAKO* and *priAKOComp* strains were performed with the murine gastric epithelial cell line GSM06 (21). The mouse gastric surface cell line GSM06 has been established from a primary culture of gastric mucosal cells of transgenic mice harboring a temperature-sensitive simian virus 40 (tsSV40) large T-antigen gene (22). GSM06 cells have been shown to retain many of the characteristics of normal gastric surface mucus cells (21). It's an *in vitro* model that closely resembles *in vivo* conditions leading to infection by *H. pylori*. In our assays we observed that the invasion capability of the *priAKO* strain was very similar to the WT strain after 4 hours of infection. However, intracellular survival of the *priAKO* strain was significantly reduced after 24 hours of infection. In these assays gentamicin is used after 2 hours to kill extracellular bacteria. Consequently, the CFU of surviving bacteria represent intracellular bacteria that invaded the GSM06 cells. Our data demonstrated that the ability of the *priAKO* strain to invade epithelial cells appears to be normal. However, the ability of the *priAKO* strain to survive in the epithelial cells is defective. Consistent with these results, microscopic imaging of live *H. pylori* in cells showed that while the numbers of intracellular bacteria were comparable for the WT, *priAKO* and *priAKOComp* strains at 4 hours after infection, the numbers of *priAKO* bacteria were reduced after 24 hours compared to the numbers of WT and *priAKOComp* strains (data not shown). These data suggest a role of PriA protein in the intracellular survival of epithelial cell ingested *H. pylori*.

H. pylori has developed multiple mechanisms to evade elimination by host innate immune responses, including macrophages, which may account for the persistence of this bacterium in the host (49, 50). Once inside macrophages, *H. pylori* interferes with phagosome maturation, which leads to the formation of large phagosomes called megasomes (51–53). *H. pylori* resides in these megasomes during phagocytosis (51–53). The megasomes apparently do not fuse with the lysosomes in macrophages, thus protecting the bacteria from elimination. The number of *priAKO* strain colonies recovered from *H. pylori* infected RAW264.7 macrophages was essentially the same as the numbers of WT and *priAKOComp* strains colonies recovered 4 hours after infection. However, the level of survival of the *priAKO* strain in RAW264.7 cells was significantly lower than the survival of the WT

and *priAKOComp* strains 24 hours after infection. Fluorescence microscopy experiments yielded similar findings which indicate that the priAKO strain has impaired survival 24 hours after infection in macrophages (data not shown). Collectively, these data indicate a role for PriA protein in the intracellular survival of *H. pylori* in both epithelial cells and macrophages. Additionally, our results suggest that the PriA protein plays an important role in the mechanisms used by *H. pylori* for bacterial evasion of elimination in macrophages.

As observed in *E. coli* and *N. gonorrhoeae*, the *priAKO* strain displayed increased sensitivity to UV irradiation. The sensitivity of the *priAKO* strain is not as severe as that of a *H. pylori recA* mutant strain (54); however, it is very similar to that seen in *E. coli* and *N. gonorrhoeae priA* mutants (13, 28, 41). It is likely that efficient UV repair systems can repair UV-mediated DNA lesions, and that only a subset of lesions that interfere with replication fork progression require PriA activity after fork collapse. Our investigations also suggest a role for *H. pylori priA* gene in protection against the DNA gyrase inhibitor ciprofloxacin (29, 30). Ciprofloxacin induces ds breaks in the DNA, a target for AddAB-mediated repair. *H. pylori* AddAB nuclease and helicase plays a very important role in DNA repair (55). As compared to the WT strain, the *priA*KO strain displayed a reduction in the ability to repair ds breaks as compared to the WT strain. These data support the importance of PriA protein in maintaining *H. pylori* viability by facilitating DNA repair following host-inflicted DNA damage.

The ability of *H. pylori* to survive in an acidic environment is critical to its ability to chronically infect the human stomach. Acid stress is likely encountered by *H. pylori* during transmission between hosts and transit through the gastric lumen before the bacteria can situate themselves in the neutral pH environment of the gastric mucosa (25). To check acid survival ability *in vitro*, WT*, priAKO* and *priAKOComp* strains were grown in pH 4.0 growth medium supplemented with 0.5 mM urea for 2, 4 and 24 hours. During the first 2 hours of acidic exposure all strains were relatively unaffected by the treatment. After 4 hours, however, the *priAKO* strain exhibited a decrease in viability relative to the WT strain. At 24 hours of incubation, the *priAKO* strain was significantly more susceptible to acid relative to the WT and *priAKOComp* strains, which indicates that the PriA protein is important for survival of *H. pylori* in an acidic environment.

In its physiological environment, *H. pylori* frequently suffers both acid and oxidative stress, leading to DNA damage. *H. pylori* has developed a battery of antioxidant proteins such as superoxide dismutase, catalase, and peroxiredoxins that enable it to avoid oxidative destruction (56). Urease hydrolyzes urea to yield ammonium ions and thereby contributes to the acid resistance of *H. pylori* (4). In addition to diverse oxidant detoxification enzymes and potent acid avoidance mechanisms, efficient DNA repair systems are required for *H. pylori* to survive in the host (56, 57). To repair DNA double strand breaks and blocked replication forks, *H. pylori* is equipped with an efficient system of DNA recombinational repair. To determine whether *priA* is involved in protection against oxidative stress, WT*, priAKO* and *priAKOComp* strains were exposed to hydrogen peroxide in broth culture and viable counts were taken at 4 and 24 hours. The data shows that the WT strain survived significantly better than the *priAKO* strain under these conditions. The decreased survival of *priA* mutant to oxidative damaging agents indicates that replication restart serves as an additional defense

for countering the effects of toxic oxygen species. These findings provide an evidence for a role of *priA* and replication restart in resistance to oxidative damage.

In this study, we provide evidence that *priA*, whose expression increases in mice stomach mucosa plays a key role in the colonization of mice stomach mucosa, is important for intracellular survival of epithelial cell ingested and macrophage cell ingested *H. pylori* and protects *H. pylori* from DNA damage, acid stress and oxidative stress. These results show that *H. pylori priA* provides a bacterial defense against the host response.

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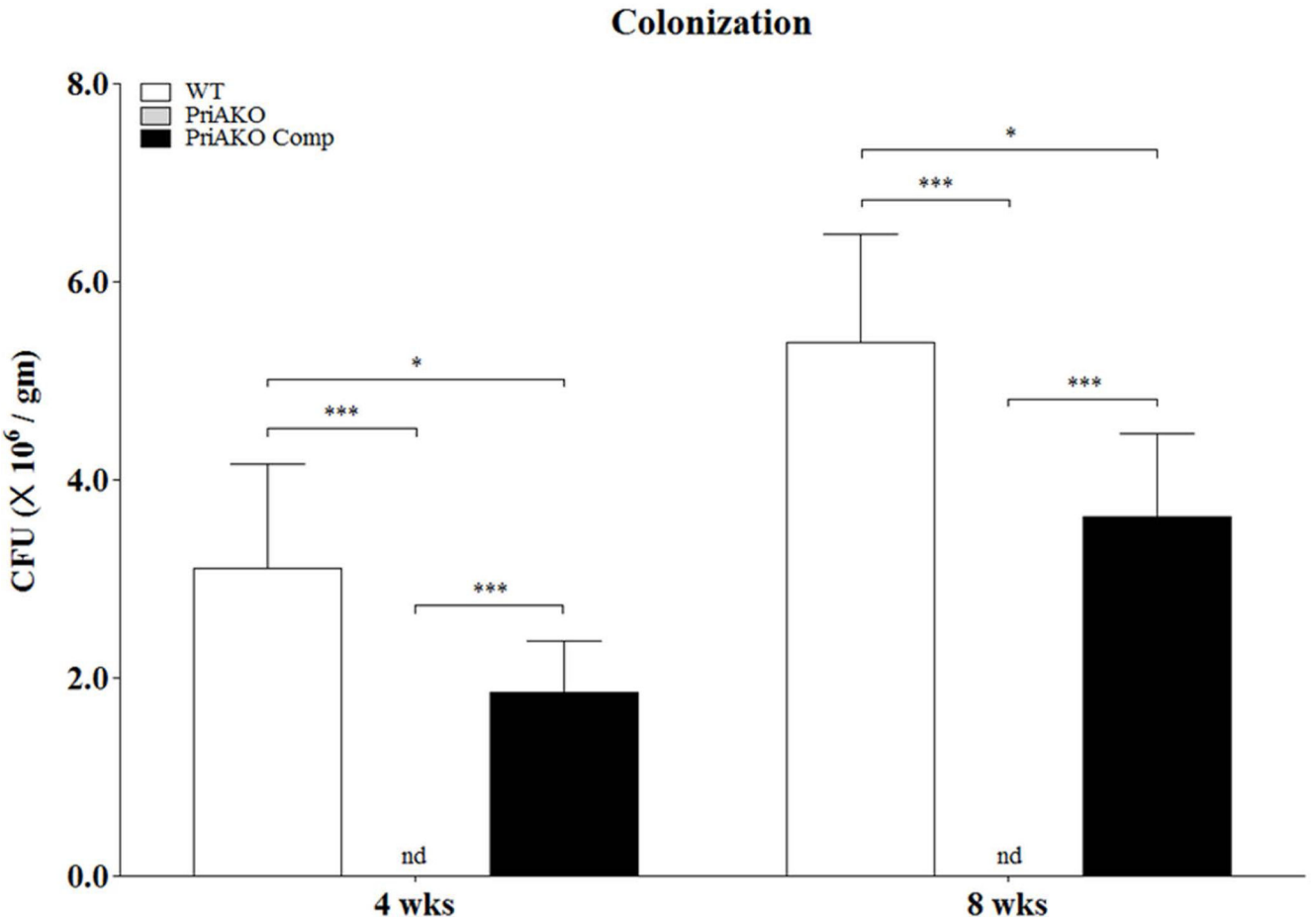

Figure 1: *H.pylori priA* mutant is impaired in colonizing mice stomach mucosa.

Mice infections were done with WT, *priAKO* and *priAKOComp* strains and the stomachs (n=5 for each group) were harvested at 4 weeks and 8 weeks of infection. Colony forming units (CFU) were determined. Error bars represent the standard error for each experimental group. Statistically significant differences determined by the Student's T-test are indicated by * for a p-value of <0.05 and *** for a p-value of <0.001. nd represents no detection of bacteria.

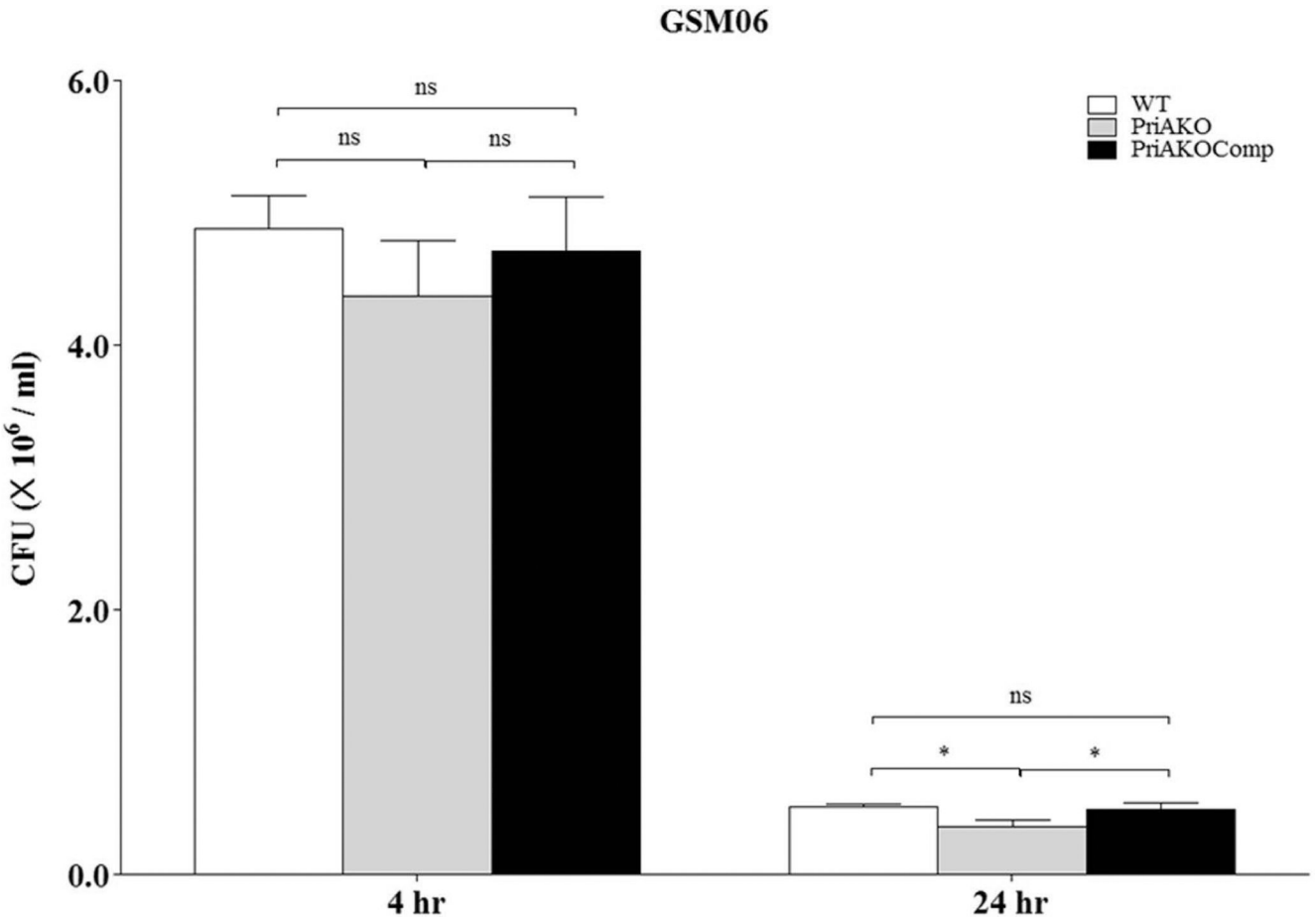

Figure 2: *priA* is important for intracellular survival of *H. pylori*.

GSMO6 cells (2×10^{5}/well) were infected with WT, *priAKO* and *priAKOComp* strains at an MOI of 100, treated with gentamicin and incubated for the indicated times. After lysis, colony forming units (CFU) from the intracellular bacteria were scored. Error bars represent the standard error of the mean of three experiments performed in duplicate. Statistically significant differences determined by the Student's T-test are indicated by * for a p-value of <0.05 and ns represents no significant difference.

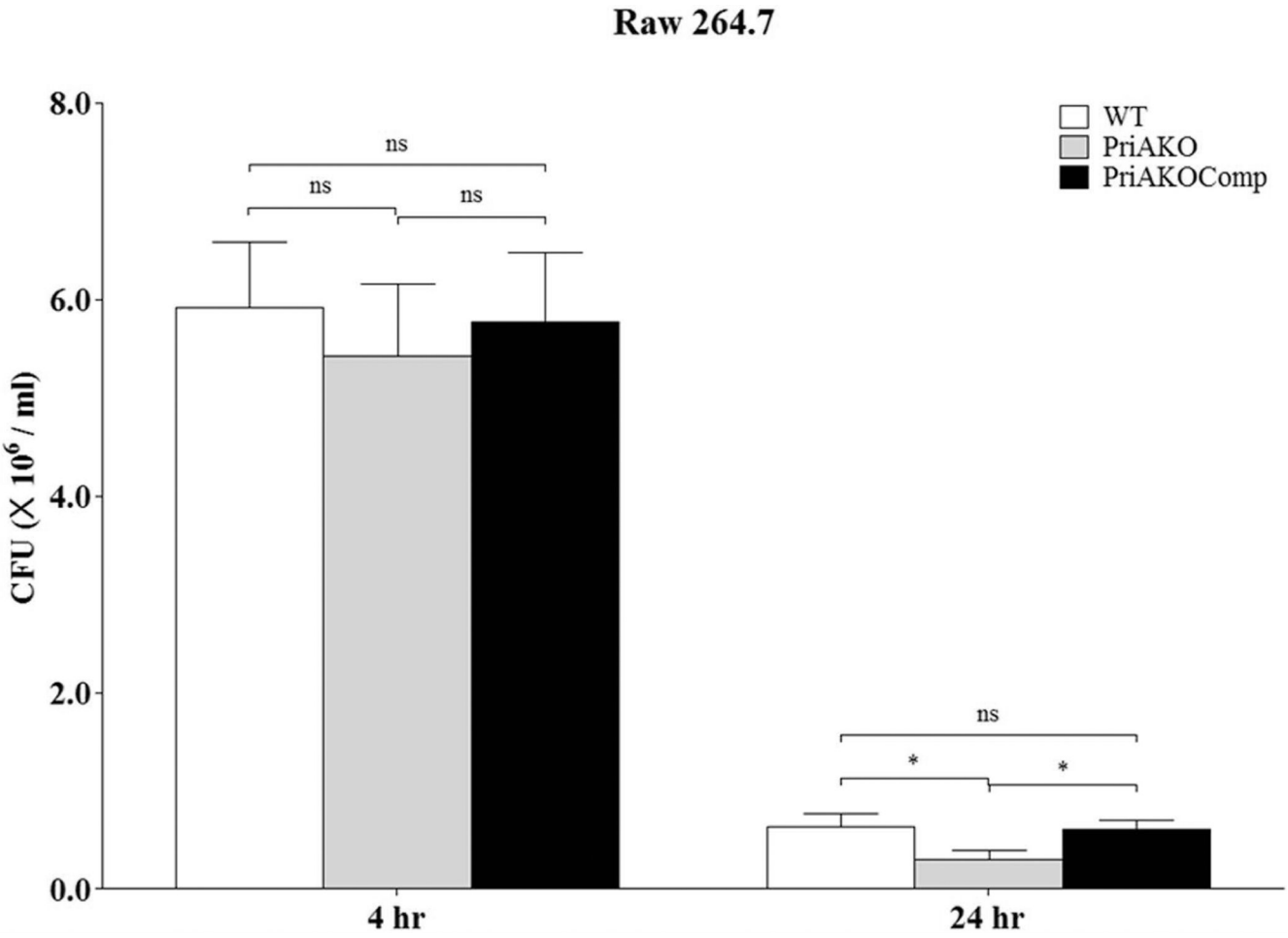

Figure 3: *priA* is important for intracellular survival of *H. pylori*.

RAW264.7 cells (2×10^{5}/well) were infected with WT, *priAKO* and *priAKOComp* strains at an MOI of 100, treated with gentamicin and incubated for the indicated times. After lysis, colony forming units (CFU) from the intracellular bacteria were scored. Error bars represent the standard error of the mean of three experiments performed in duplicate. Statistically significant differences determined by the Student's T-test are indicated by * for a p-value of <0.05 and ns represents no significant difference.

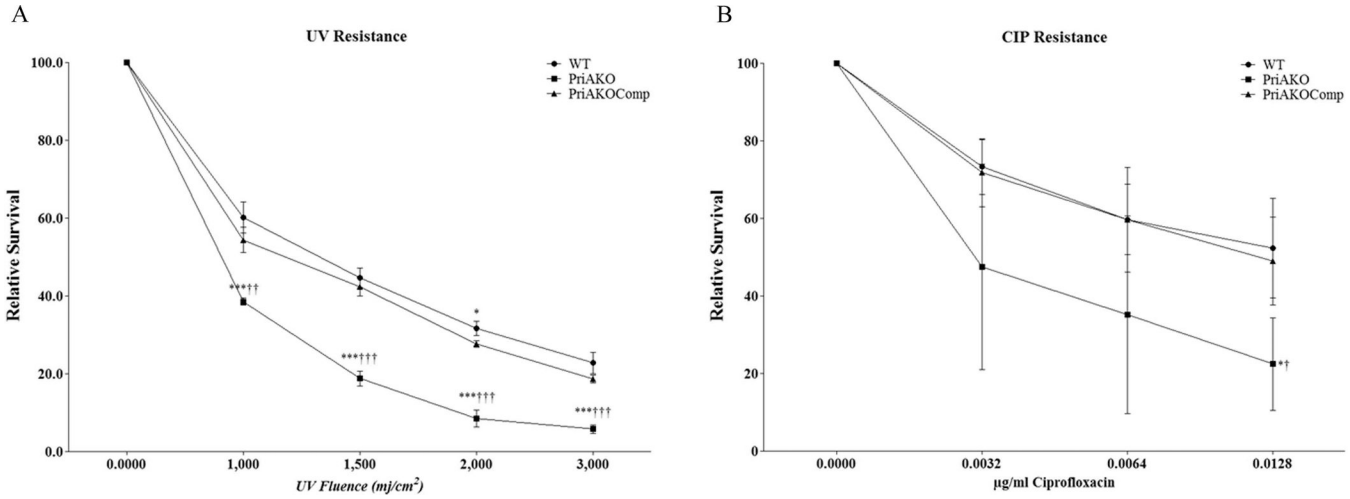

Figure 4: *priA* is involved in DNA repair in *H. pylori*.

Relative survival of WT, *priAKO* and *priAKOComp* strains after irradiation with UV light (A) and double strand breaks induced by ciprofloxacin (B) were determined. Error bars represent the standard error of the mean of three experiments performed in duplicate. * denotes a comparison between WT and *priAKO* and † denotes a comparison between *priAKOComp* and *priAKO*. Statistically significant differences determined by the Student's T-test are indicated by */† for a p-value of <0.05, **/†† for a p-value of <0.01 and ***/††† for a p-value of <0.0001.

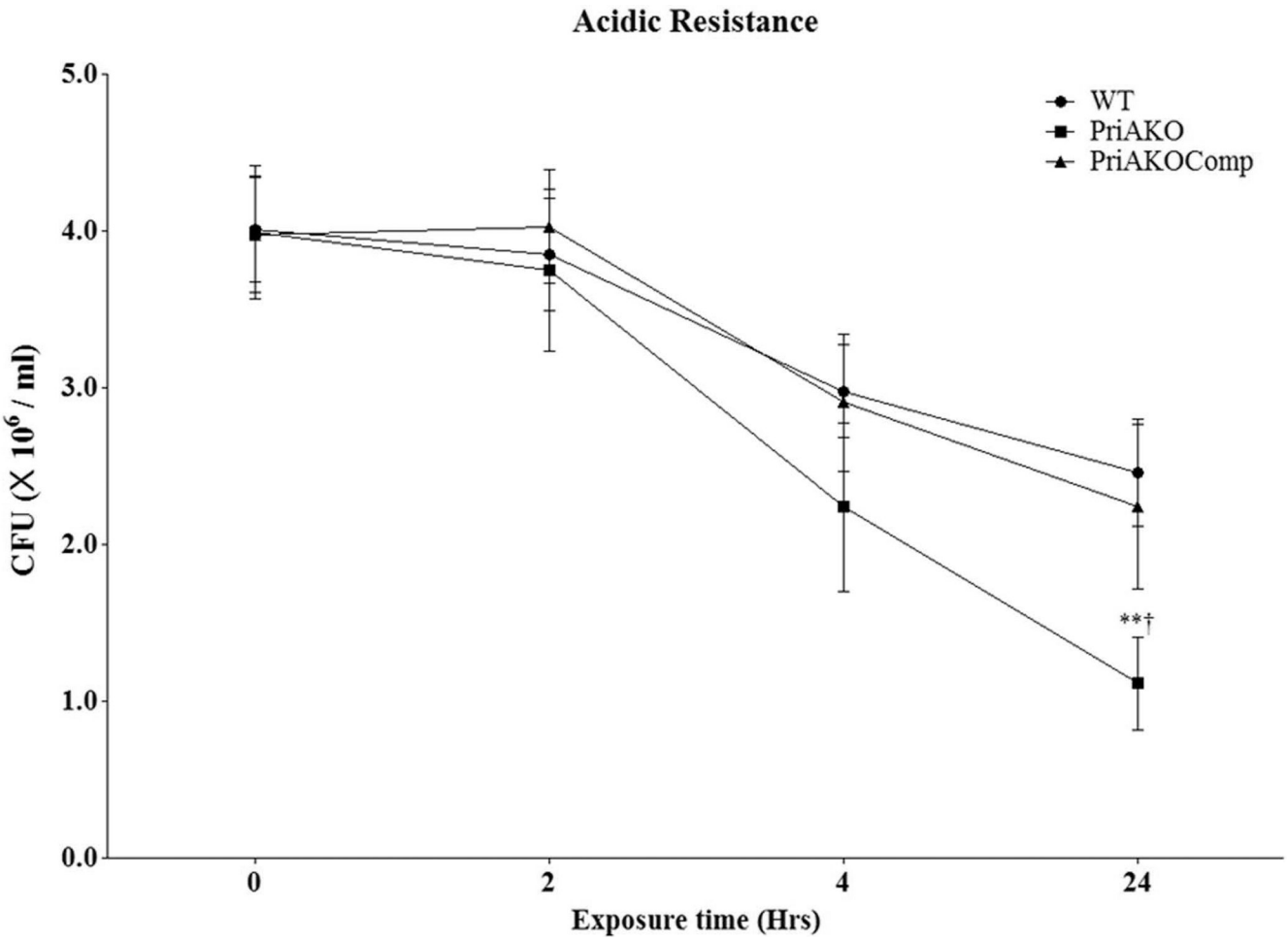

Figure 5: *H. pylori priA* mutant is more susceptible to acid challenge.

WT, *priAKO* and *priAKOComp* strains were grown in pH 4.0 acidic medium. Samples were collected at the indicated time points and the colony forming units (CFU) were determined. Error bars represent the standard error of the mean of three experiments. * denotes a comparison between WT and *priAKO* and † denotes a comparison between *priAKOComp* and *priAKO*. Statistically significant differences determined by the Student's T-test are indicated by ** for a p-value of <0.001 and † for a p-value of <0.05.

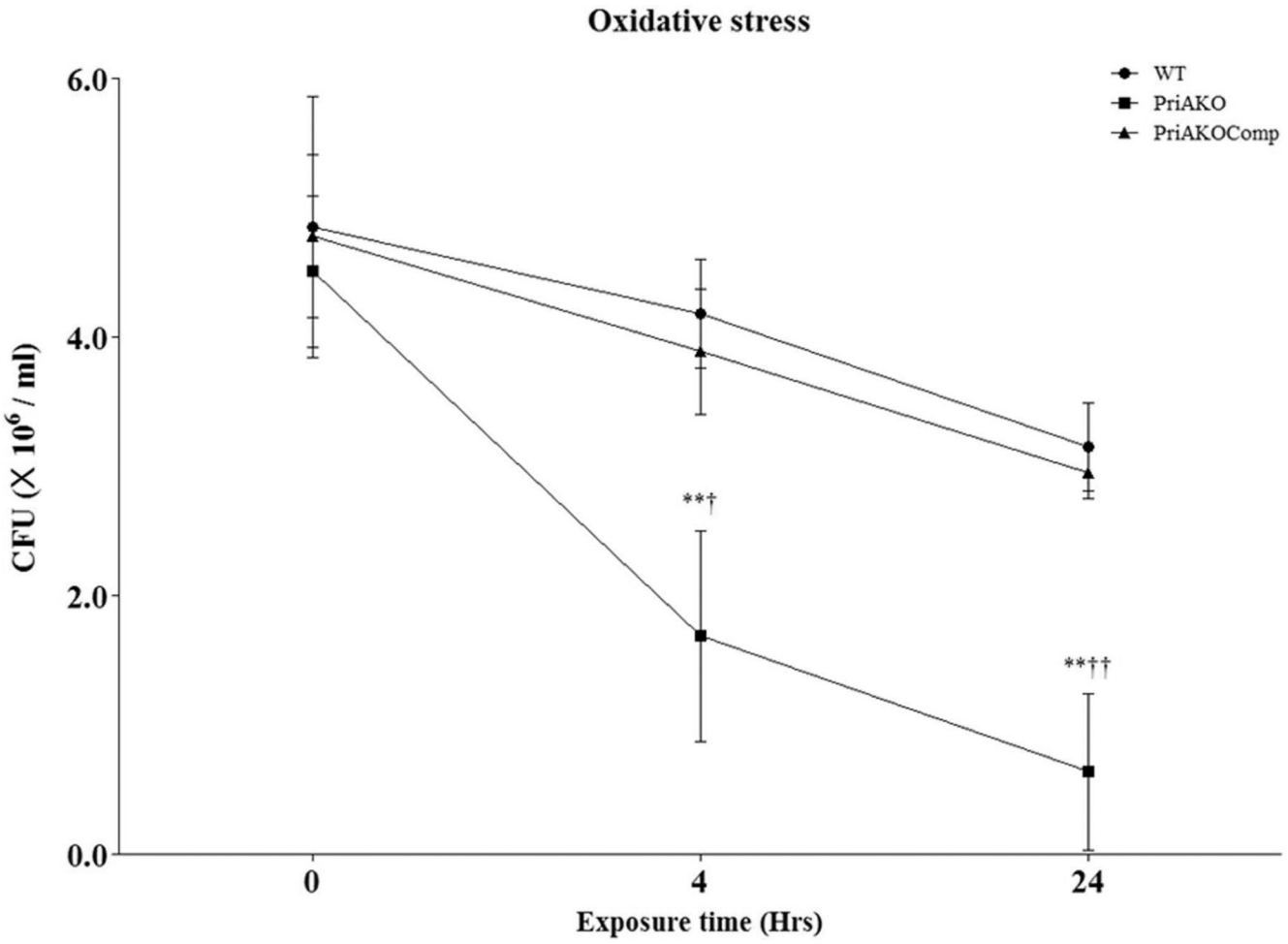

Figure 6: *H. pylori priA* mutant is more sensitive to oxidative damage.

WT, *priAKO* and *priAKOComp* strains were exposed to 50 mM H_2O_2. Samples were collected at the indicated time points and the colony forming units (CFU) were determined. Error bars represent the standard error of the mean of three experiments. * denotes a comparison between WT and *priAKO* and † denotes a comparison between *priAKOComp* and *priAKO*. Statistically significant differences determined by the Student's T-test are indicated by **/†† for a p-value of <0.001 and † for a p-value of <0.05.