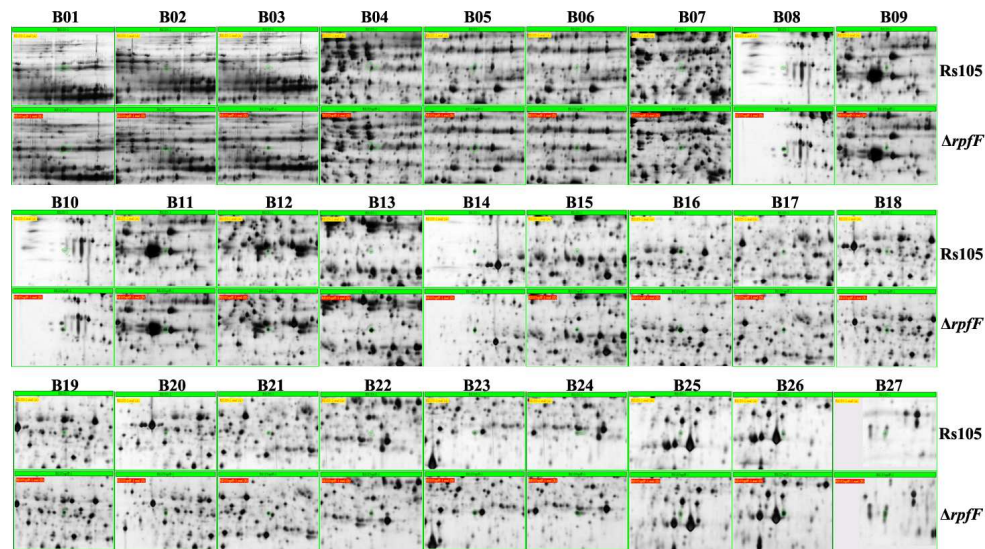
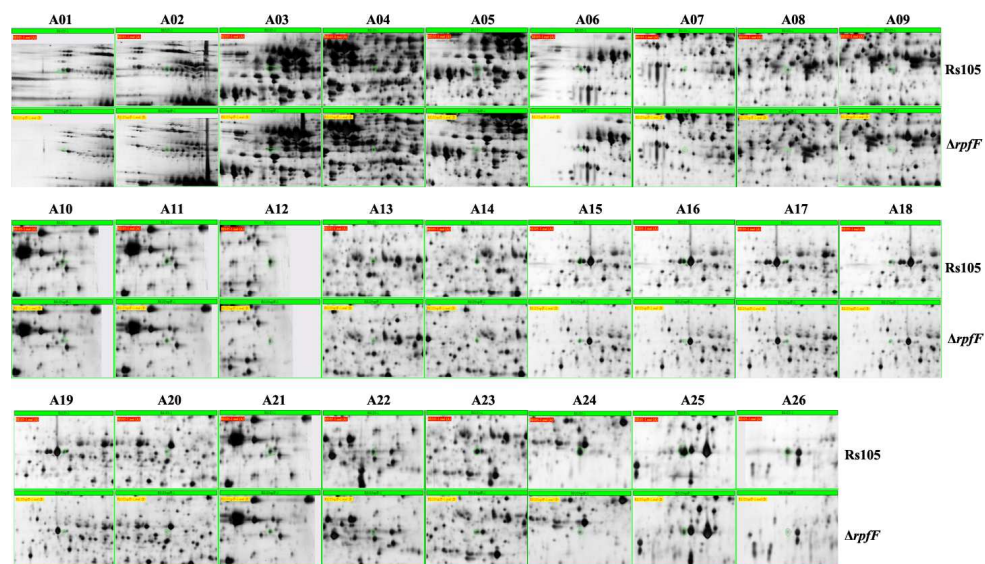


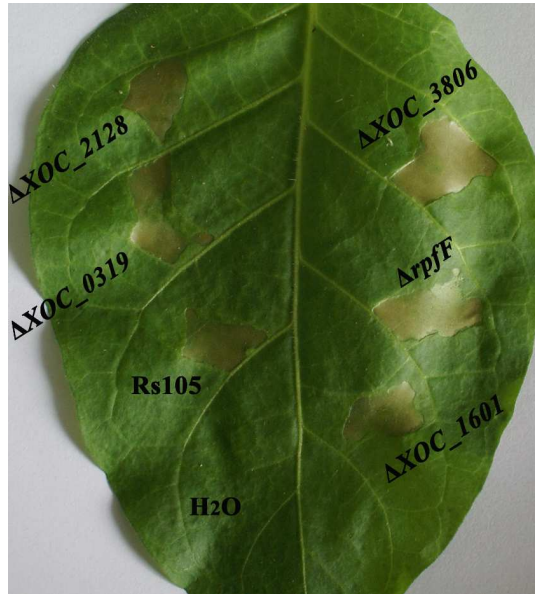
**Figure S1. Comparison of the growth of the wild-type *Xanthomonas oryzae* pv. *oryzicola* (Xoc) strain Rs105 and the *rpfF* mutant in nutrient-rich broth (NB).** The point highlighted with a red line represented the time-point for extraction of extracellular proteins from the supernatants of wild-type strain and *rpfF* mutant. At that time, the cell density (OD<sub>600nm</sub>) of wild-type strain and *rpfF* mutant was 2.5 and 2.9, respectively. Rs105, wild-type strain of Xoc; *ΔrpfF*, the diffusible signal factor (DSF)-deficient mutant of Xoc (11). Three replicates for each treatment were used, and the experiment was repeated three times.



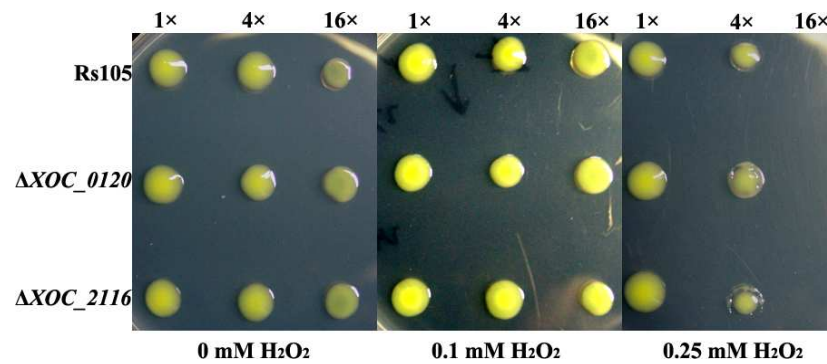
**Figure S2. Identification of 27 extracellular protein spots negatively regulated by diffusible signal factor (DSF) in *Xanthomonas oryzae* pv. *oryzicola* (Xoc).** These 27 protein spots exhibited remarkably increased expression level ( $> 1.5$  fold) in *rpfF* mutant compared to wild-type strain. Protein identifications are provided in Table 2. Rs105, the wild-type strain of Xoc;  $\Delta rpfF$ , the diffusible signal factor (DSF)-deficient mutant of Xoc (11). Three replicates for each treatment were used, and the experiment was repeated three times.



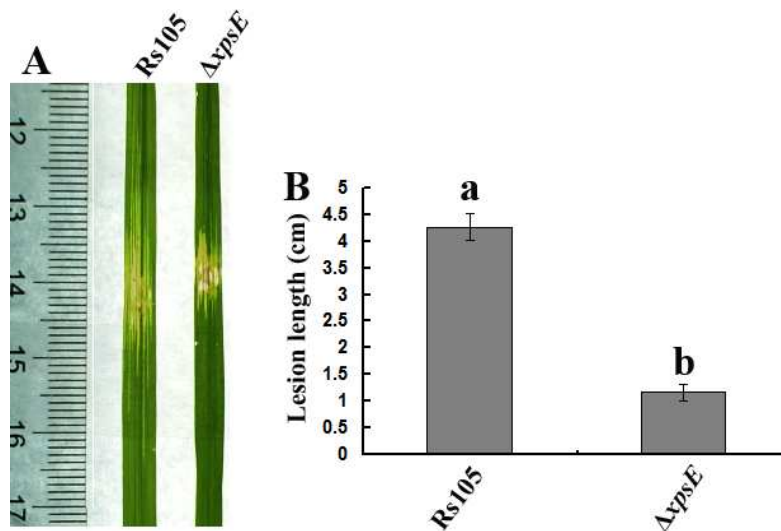
**Figure S3. Identification of 26 extracellular protein spots positively regulated by diffusible signal factor (DSF) in *Xanthomonas oryzae* pv. *oryzicola* (Xoc).** These 26 protein spots exhibited significantly decreased expression level ( $> 1.5$  fold) in *rpfF* mutant compared to wild-type strain. Protein identifications are provided in Table 2. Rs105, the wild-type strain of Xoc; *ΔrpfF*, the diffusible signal factor (DSF)-deficient mutant of Xoc (11). Three replicates for each treatment were used, and the experiment was repeated three times.



**Figure S4. All the four selected genes of *Xanthomonas oryzae* pv. *oryzicola* (Xoc) were not required for triggering hypersensitive response (HR) in non-host plant (tobacco).** The phenotype of HR, a programmed cell death, was observed around the inoculation sites in tobacco leaves after 24 h of infiltration with the wild-type and mutants. Rs105, the wild-type strain of Xoc;  $\Delta XOC\_0319$ , the deletion mutant of *XOC\_0319*, which encodes a Ax21 (activator of XA21-mediated immunity)-like protein;  $\Delta XOC\_3806$ , the deletion mutant of *XOC\_3806*, which encodes a serine protease;  $\Delta XOC\_1601$ , the deletion mutant of *XOC\_1601*, which encodes a cysteine protease;  $\Delta XOC\_2128$ , the deletion mutant of *XOC\_2128*, which encodes a polygalacturonase.  $\Delta rpfF$ , the diffusible signal factor (DSF)-deficient mutant of Xoc (11); H<sub>2</sub>O, a negative control. Three replicates for each treatment were used, and the experiment was repeated three times.

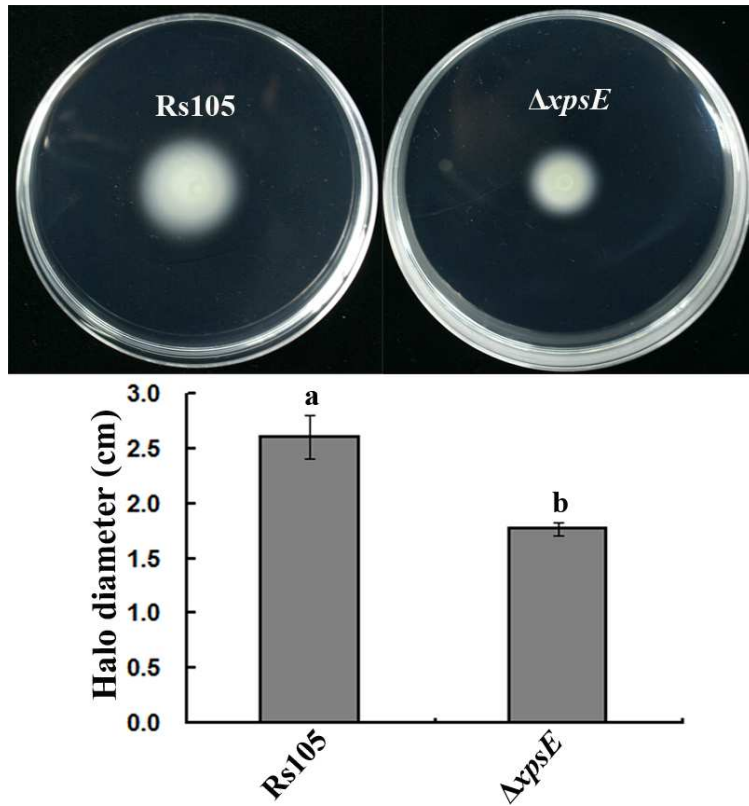


**Figure S5. Mutation of *XOC\_0120* or *XOC\_2116* did not impair the resistance ability to hydrogen peroxide ( $H_2O_2$ ) in *Xanthomonas oryzae* pv. *oryzzicola* (Xoc).** Xoc strains were cultured to mid-logarithmic phase ( $OD_{600nm} = 1.0$ ) in NB medium (1 x), and then four-fold (4 x) and sixteen-fold dilutions (16 x) were made, and grown on NB agar plates with 0, 0.1, or 0.25 mM  $H_2O_2$ . Rs105, the wild-type strain of Xoc;  $\Delta XOC_0120$ , the deletion mutant of *XOC\_0120*, which encodes a glutathione peroxidase;  $\Delta XOC_2116$ , the deletion mutant of *XOC\_2116*, which encodes a superoxidase dismutase. Three replicates for each treatment were used, and the experiment was repeated three times.

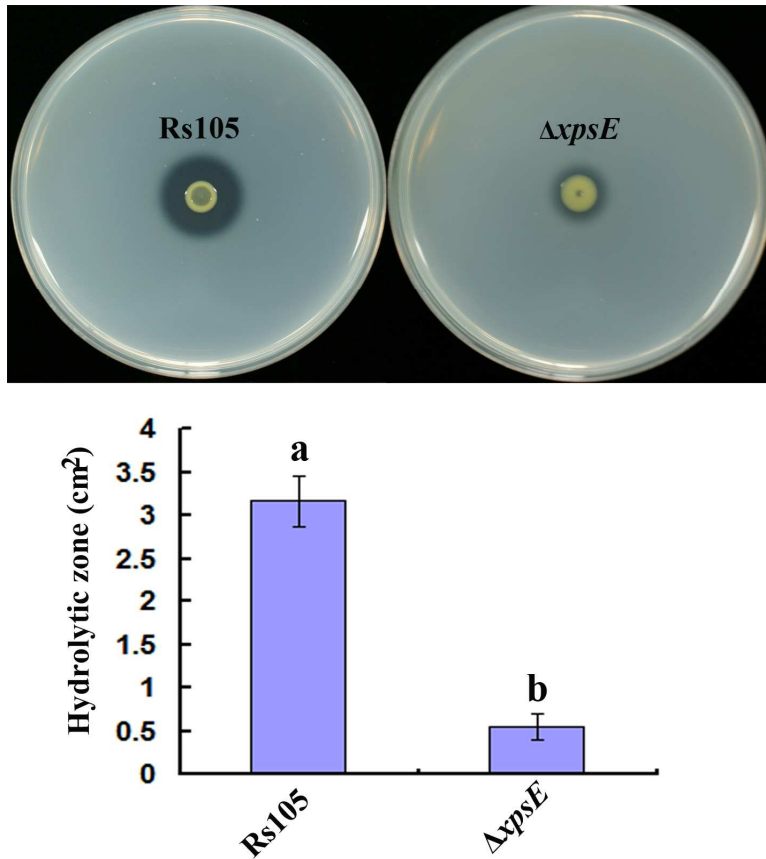


**Figure S6. Mutation of *xpsE* impaired the virulence of *Xanthomonas oryzae* pv. *oryzae* (Xoc) on host rice.**

(A) Representative result of water-soaking lesion lengths on the rice seedling leaves (cv. Shanyou63, 2-week old) by infiltration with wild-type strain and selected four mutants. (B) Calculated data of water-soaking lesion lengths on the leaves of rice seedling leaves. Rs105, wild-type strain of Xoc;  $\Delta xpsE$ , the deletion mutant of *xpsE* (*XOC\_3805*). The product of *xpsE* is a component of the *xps* cluster responsible for T2SS constitution. Three replicates were used for each treatment, and the experiment was repeated three times. Vertical bars represent standard errors. Different letters above data bars indicate a significant difference between the wild-type strain and tested mutants ( $P < 0.05$ ; *t*-test).

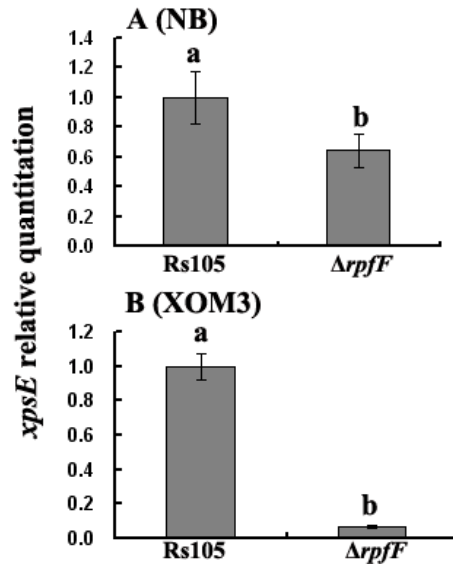


**Figure S7. Mutation of *xpsE* impaired cell motility in *Xanthomonas oryzae* pv. *Oryzicola* (Xoc).** (A) Representative photograph of motility halos formed by wild-type strain and the four selected mutants on XOM3 semi-solid motility medium. (B) Quantitative determination of motility halos of the wild-type strains and the four selected mutants. Rs105, the wild-type strain of Xoc; Δ*xpsE*, the deletion mutant of *xpsE* (*XOC\_3805*). The product of *xpsE* is a component of the *xps* cluster responsible for T2SS constitution. Three replicates were used for each treatment, and the experiment was repeated three times. Vertical bars represent standard errors. Different letters above data bars indicate a significant difference between the wild-type strain and the tested mutants ( $P < 0.05$ ; *t*-test).

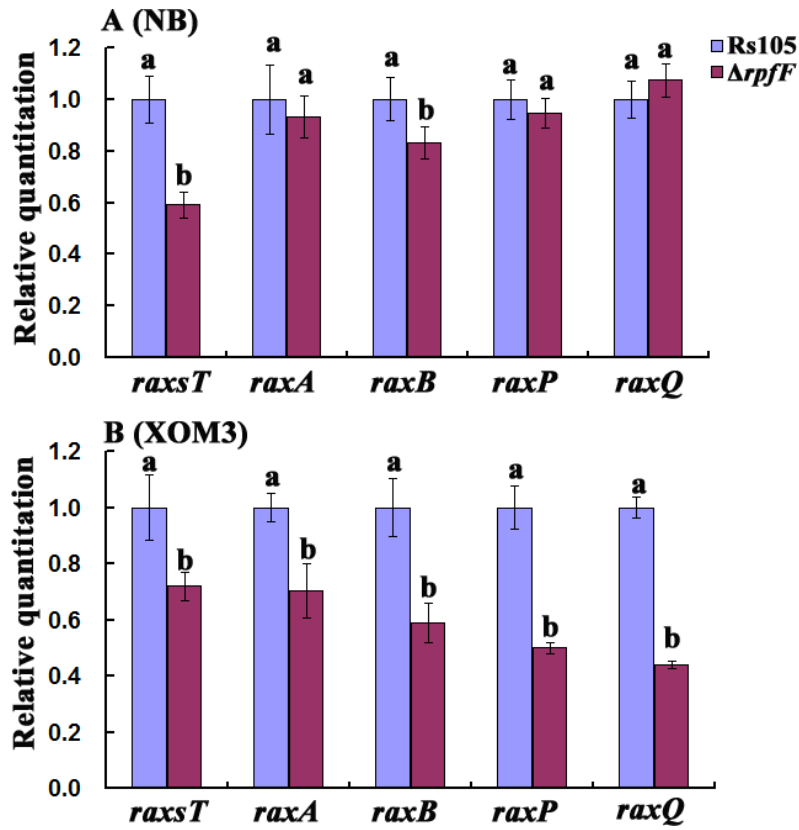


**Figure S8. Mutation of *xpsE* impaired extracellular protease activity in *Xanthomonas oryzae* pv. *oryzicola* (Xoc).** (A) Representative photograph of hydrolytic zone formed by the wild-type strain and the *xpsE* mutant on selective medium. (B) Quantitative determination of hydrolytic zone of the wild-type strains and *xpsE* mutant. Rs105, the wild-type strain of Xoc; Δ*xpsE*, the deletion mutant of *xpsE* (XOC\_3805). The product of *xpsE* is a component of the *xps* cluster responsible for T2SS constitution. Three replicates were used for each treatment, and the experiment was repeated three times. Vertical bars represent standard errors. Different letters above data bars indicate a significant difference between the wild-type strain and tested mutants ( $P < 0.05$ ; *t*-test).



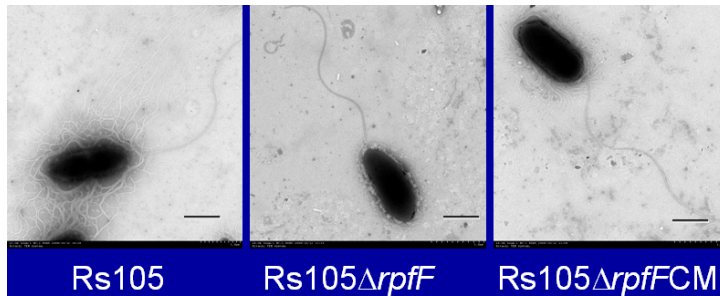


**Figure S9.** *xpsE* transcription was positively regulated by diffusible signal factor (DSF) in *Xanthomonas oryzae* pv. *oryzicola* (Xoc). (A) Comparison of transcriptional expression of *xpsE* between the *rpfF*-deletion mutant and the wild-type strain Rs105 in nutrient-rich broth (NB). Total RNA was extracted from the Rs105 and  $\Delta rpfF$  at the mid-stage of growth ( $OD_{600nm} = 1.6$ ). (B) Comparison of transcriptional expression of *xpsE* between the *rpfF*-deletion mutant and the wild-type Rs105 in plant-cell mimicking broth (XOM3). The Xoc strains were pre-incubated in NB medium overnight, re-suspended at  $OD_{600} = 2.0$  in XOM3 medium, and washed twice. 2 ml of the bacterial suspension was then inoculated into 50 ml of XOM3 plant-cell mimicking broth (pH 6.5) at 28 °C for 16 h.<sup>69</sup> Total RNA was then extracted from the Rs105 and  $\Delta rpfF$ . Three replicates for each treatment were used, and the experiment was repeated three times. Vertical bars represent standard errors. Different letters above bars indicate a significant difference between the wild-type strain and *rpfF*-deletion mutant ( $P < 0.05$ ; *t*-test).



**Figure S10. Transcriptional expression of the five *rax* genes of *Xanthomonas oryzae* pv. *oryzicola* (Xoc) in the *rpfF*-deletion mutant and wild-type strain. (A) Comparison of transcriptional expression of the five *rax* genes (required for Ax21) between the *rpfF*-deletion mutant and the wild-type Rs105 in nutrient-rich broth (NB). Total RNA was extracted from the Rs105 and  $\Delta rpfF$  at the mid-stage of growth ( $OD_{600nm} = 1.6$ ). (B) Comparison of transcriptional expression of the five *rax* genes between the *rpfF*-deletion mutant and the wild-type Rs105 in plant-cell mimicking broth (XOM3). The Xoc strains were pre-incubated in NB medium overnight, re-suspended at  $OD_{600nm} = 2.0$  in XOM3 medium, and washed twice. 2 ml of the bacterial suspension was then inoculated into 50 ml of XOM3 plant-cell mimicking broth (pH 6.5) at 28 °C for 16 h.<sup>69</sup> Total RNA was then extracted from the Rs105 and  $\Delta rpfF$ . Ax21, activator of XA21-mediated immunity. Three replicates for each treatment were used, and**

the experiment was repeated three times. Vertical bars represent standard errors. Different letters above bars indicate a significant difference between the wild-type strain and the *rpfF*-deletion mutant ( $P < 0.05$ ; *t*-test).



**Figure S11. Effect of the *rpfF* mutation on flagella and cell shapes in *Xanthomonas oryzae* pv. *oryzicola* (Xoc).**

Cells were negatively stained with 2% potassium phosphotungstate. Flagella and cells were detected under a Hitachi transmission electron microscope at 80 kilovolts. Rs105, wild-type strain of Xoc;  $\Delta rpfF$ , the diffusible signal factor (DSF)-deficient mutant of Xoc (11).  $\Delta rpfFCM$ , the corresponding complemented strain of  $\Delta rpfF$ . Bar represents 1  $\mu\text{m}$ ; three replicates for each treatment were used, and the experiment was repeated three times.