Expression of colSR Genes Increased in the rpf Mutants of Xanthomonas oryzae pv. oryzae KACC10859

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The rpf genes and colS\textsubscript{XOO120}/colR\textsubscript{XOO1208} were known to require for virulence of Xanthomonas oryzae pv. oryzae (Xoo). In Xoo KACC10331 genome, two more colS/colR genes, colS\textsubscript{XOO3535} (raxH)/colR\textsubscript{XOO3535} (raxR) and colS\textsubscript{XOO3762}/colR\textsubscript{XOO3763} were annotated. The colS\textsubscript{XOO3534}/colR\textsubscript{XOO3535} were known to control AvrXa21 activity and functions of colS\textsubscript{XOO3762}/colR\textsubscript{XOO3763} were unknown in Xoo. To characterize the relationship between rpf and colS/colR genes, expression of colS/colR genes in Rpf mutants of Xoo were analyzed with quantitative reverse transcription PCR (qRT-PCR). Expressions of all three colS/colR genes increased in the rpf\textsubscript{F} mutant in which DSF synthesis is defective. Expression of colS\textsubscript{XOO120}/colR\textsubscript{XOO1208}, colS\textsubscript{XOO3534}/colR\textsubscript{XOO3535} and colS\textsubscript{XOO3762}/colR\textsubscript{XOO3763} increased 2, 2–7, 3–13 folds respectively. Expression of colS\textsubscript{XOO3534} and colS\textsubscript{XOO3762} also increased 2–4 folds in the rpf\textsubscript{G} mutant in which the signal from DSF is no longer transferred to down-stream. Expression of the other colS/colR genes was not significantly changed in the rpf\textsubscript{G} mutant compared to the wild type. Since Rpf\textsubscript{F} and Rpf\textsubscript{G} are responsible for DSF synthesis and signal transfer from DSF to down-stream to regulate virulence gene expression, these results suggest that the DSF and DSF-mediated signal regulate negatively three colS/colR genes in Xoo.

Keywords: ColS/ColR, Rpf, virulence regulation, Xanthomonas oryzae pv. oryzae

Plant pathogenic bacteria must be virulent and able to suppress host resistance to cause a disease on host plant. As bacterial two-component system (TCS), which perceives outside signals and regulates responses inside bacterial cell, rpf\textsubscript{Cir}/gF and colS\textsubscript{XOO120}/colR\textsubscript{XOO1208} are required for virulence of Xanthomonas oryzae pv. oryzae (Xoo) (Slater et al., 2000; Subramoni et al., 2012). The Rpf (regulation of pathogenicity factors) system is known to regulate virulence by the cell-cell communication in X. campestris pv. campestris (Xcc) (Barber et al., 1997; He et al., 2007; Slater et al., 2000; Tang et al., 1991). Among rpf genes that were identified as a cluster (rpf\textsubscript{A-J}) (Tang et al., 1991), Rpf\textsubscript{F} are responsible for diffusible signal factor (DSF) production (Barber et al., 1997; Wang et al., 2004), and Rpf\textsubscript{C} and Rpf\textsubscript{G} comprise a two-component system, which senses the DSF signal and transfers it to signal cascades involved in virulence (Slater et al., 2000). Virulence factor production and biofilm dispersal are controlled by cyclic di-GMP and Clp, which are on downstream of Rpf\textsubscript{G} (He et al., 2007; Ryan et al., 2007). The rpf genes and functions of the core rpf genes, rpf\textsubscript{B}, rpf\textsubscript{C}, rpf\textsubscript{F}, rpf\textsubscript{G} are well conserved in Xoo (Chatterjee and Sonti, 2002; Jeong et al., 2008).

The colS/colR genes, which encode a two-component system, were originally identified from the root-colonizing bacterium Pseudomonas fluorescens and found to be involved in the capacity of the bacterium to colonize plant roots (Dekkers et al., 1998). Subsequently, colS/colR genes were reported to regulate different biological responses to transposition of transposon and various stresses including phenol and heavy metals (Hu and Zhao, 2007; Kivistik et al., 2006). The colS/colR genes are required for virulence of several important plant pathogens including Xoo (Subramoni et al., 2012; Yan and Wang, 2011; Zhang et al., 2008).

Mutation of colS\textsubscript{XOO120}/colR\textsubscript{XOO1208} decreased virulence of Xoo. These mutations also caused growth defect in iron-limiting condition and deficiency in elicitation of hypersensitive response on non-host tomato (Subramoni et al., 2012). Another colS/colR genes, colS\textsubscript{XOO3534} (raxH)/colR\textsubscript{XOO3535} (raxR), were known to control avrXa21 activity and...
in Xoo pXO99A (Burdman et al., 2004; Lee et al., 2008). These previously published results indicate two virulence regulation systems, Rpf and ColS/ColR, control virulence in Xoo. To characterize the relationship between rpf and colS/colR genes for virulence regulation, expression of colS/colR genes in Rpf mutants of Xoo were analyzed with quantitative reverse transcription PCR (qRT-PCR).

Three colS/colR genes are conserved in the important plant pathogens. In Xoo KACC10331 genome, three colS/colR genes, colS<sub>XOO1207</sub>/colR<sub>XOO1208</sub>, colS<sub>XOO3334</sub>/colR<sub>XOO3555</sub> and colS<sub>XOO3763</sub>/colR<sub>XOO3769</sub>, have been annotated (Lee et al., 2005). Based on literature and homologous gene search, the three colS/colR genes were identified to be well conserved in Xcc 8004 (a black rot pathogen of cabbage) and X. axonopodis pv. citri (Xac) 306 (a citrus canker pathogen of citrus) (Table 1). Nucleotide identity between the colS/colR genes of Xoo KACC10331 and Xac 306 or Xcc 8004 were all more than 90%. E-value obtained by BlastN between the three colS/colR genes in each plant pathogen. Zhang et al. (2008) showed that colS<sub>XC_1059</sub>/colR<sub>XC_1049</sub> was involved in virulence, hypersensitive response and tolerance to various stresses in Xcc 8004. The colS<sub>XC_1059</sub>/colR<sub>XC_1049</sub> positively regulated expression of hrpC and hrpE operons and expression of colS<sub>XC_1059</sub>/colR<sub>XC_1049</sub> were critical for X. citri subsp. citri (Xac) in virulence, growth in planta, biofilm formation, catalase activity, LPS production, and resistance to environmental stress. In Xoo, colS<sub>XOO1207</sub>/colR<sub>XOO1208</sub> were required for virulence and hypersensitive response on non-host plant (Subramoni et al., 2012).

### Table 1. Conservation of colS/colR genes in *Xanthomonas oryzae* pv. *oryzae* KACC10331, *Xanthomonas axonopodis* pv. *citr* 306 and *Xanthomonas campestris* pv. *campestris* 8004

| Xoo KACC10331 | Xac 306 | Xcc 8004 |
|---------------|---------|----------|
| Gene/Gene ID  | Function | Gene/Gene ID  | Function | Gene/Gene ID  | Function |
| colS, XOO1207/colR, XOO1208 | Virulence, HR on non-host, growth in iron-limiting; Subramoni et al. (2012) | colS, XAC3249/colR, XAC3250 | Virulence, biofilm formation, resistance to environmental stress; Yan & Wang (2011) | XC_1050/XC_1049 | Virulence, HR, tolerance to various stress; Zhang et al. (2008) |
| colS (raxH), XOO3534/colR (raxR), XOO3334 | AvrXa21; Burdman et al. (2004), Lee et al. (2008) | colS, XAC1222/colR, XAC1221 | unknown | XC_3125/XC_3126 | unknown |
| colS, XOO3762/colR, XOO3763 | unknown | colS, XAC0835/colR, XAC0834 | unknown | XC_3451/XC_3452 | unknown |

*<sup>n</sup>Nucleotide identity covered regions between the colS/colR genes of Xoo KACC 10331 and Xac 306 or Xcc 8004 were more than 90%. E-values between colS/colR genes of Xoo KACC10331 and corresponding genes in either Xac 306 or Xcc8004 were all 0.0.*
Table 2. Bacterial strains and plasmids used in this study

| Strain/Plasmid | Relevant Characteristics* | Source |
|----------------|---------------------------|--------|
| **Xanthomonas oryzae pv. oryzae** | | |
| KACC10859      | Wild-type, Cp'            | RDA, South Korea |
| CBNUXO005      | rpfF::EZ-Tn5, Km'        | Jeong et al., 2008; He et al., 2010 |
| CBNUXO006      | rpfG::EZ-Tn5, Km'        | Jeong et al., 2008; He et al., 2010 |
| CBNUXO005C     | CBNUXO05/pVSP61-mcs-sp::rpfF, Km', Sp' | This study |
| CBNUXO06C      | CBNUXO06/pVSP61-mcs-sp::rpfG, Km', Sp' | This study |
| pVSP61         | Km'                       | Loper and Lindow, 1994 |
| pVSP61-mcs-sp  | pVSP61::MCS of Puc19::Sp', Sp' | This study |
| pVSP61-mcs-sp::rpfF | pVSP61-mcs-sp::rpfF, Sp' | This study |
| pVSP61-mcs-sp::rpfG | pVSP61-mcs-sp::rpfG, Sp' | This study |
|
| *Cp’: cephalexin resistance, Km’: kanamycin resistance, Sp’: spectinomycin resistance. |

**HindIII-BamHI** sites with PCR products amplified with primers, rpfG-hind3-F: 5’ AGTAAGCTTAAGGACGCGGTTGACGACGACG 3’ and rpfG-bamh1-R: 5’ AGTAAGCTTAAGGACGCGGTTGACGACGACG 3’ (pVSP61-mcs-sp::rpfG). Plasmid pVSP61-mcs-sp::rpfF and pVSP61-mcs-sp::rpfG were transformed into CBNUXO05 (rpfF::EZ-Tn5) and CBNUXO06 (rpfG::EZ-Tn5) with standard electroporation protocol. Mutants and their complementation strains were confirmed by PCR genotyping (supplementary Fig. 1).

RNA was isolated from the bacterial cells cultured in hrp-inducing culture conditions (Seo et al., 2008). Wild type, mutant and complement strains were cultured in PS broth (peptone 10 g, sucrose 10 g, L-glutamic acid 1 g per 1 L, pH 7.0) to OD<sub>600</sub> = 0.2 and the bacterial cells were washed twice with sterilized water and transferred to Xom2 medium (0.18% xylose, 670 µM L-methionine, 10 mM L-glutamic acid, 14.7 mM potassium phosphate (monobasic), 40 µM manganese sulfate, 240 µM Fe(III) EDTA, 5 mM magnesium chloride per 1 L, pH 6.5). After 18 h further culture, bacterial cells were harvested for RNA isolation. Total RNA of each strains was isolated using the RNeasy<sup>®</sup> Mini kit (Qiagen, Valencia, CA), and residual genomic DNA was removed using the RNaive-Free DNase Set (Qiagen, Valencia, CA) and RNeasy<sup>®</sup> MinElute<sup>TM</sup> Cleanup kit (Qiagen, Valencia, CA), according to the manufacturer’s instructions. DNase I-treated total RNA was measured with a Nano Drop 2000 (Thermo scientific, Wilmington, USA) and 1 µg total RNA was used to synthesize cDNA. cDNA was generated using SuperScript<sup>®</sup> III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA), following the manufacturer’s protocol. After the reaction, samples were diluted with 190 µL of distilled water and used as a template cDNA for qRT-PCR. qRT-PCR experiments were performed by Rotor-gene Q (Qiagen, Valencia, CA) and using 2× Rotor-gene<sup>TM</sup> SYBR<sup>®</sup> Green PCR kit (Qiagen, Valencia, CA) containing 1 µL of diluted cDNA. The constitutively expressed rrsA gene coding 16S rRNA was used as an internal control for relative quantification (Subramoni et al., 2012). Gene-specific primers designed and their amplification efficiency was checked with RNA isolated from the wild type strain. Primers with R² > 0.99 and slope 3.2–3.6 were used (Table 3). qRT-PCR condition included initial heating for 10 min at 95°C, followed by 40 cycles of PCR (95°C, 15 s; 58°C, 30 s; 72°C 30 s). To analyze obtained results, ΔΔCt method was used and quantification results were calculated.

**Expression of rpfF and rpfG genes in its mutant and complement strains.** Expression of rpfF and rpfG in its mutants and complement strains was checked to confirm the mutation and complementation works. The rpfF and rpfG was expressed about 11 fold and 100 fold less in its mutant strain, CBNUXO05 (rpfF::EZ-Tn5) and CBNUXO06 (rpfG::EZ-Tn5) than in the wild type strain respectively (Table 4). Expression of rpfF in the complement strain, CBNUXO005C (rpfF::EZ-Tn5/pVSP61-mcs-sp::rpfF) was similar to the wild type strain while rpfG was expressed in the complemented strain, CBNUXO006C (rpfG::EZ-Tn5/pVSP61-mcs-sp::rpfG), was about 4 fold higher than in the wild type (Table 4). Forward and reverse primer of rpfF- and rpfG-specific primers were designed from outside of both end of EZ-Tn5 insertion site. Since EZ-Tn5 contains transcription terminator, expression level of the two genes must be near 0 in the mutant strains. When the PCR products were checked by gel electrophoresis after qRT-PCR, no proper-size band or very faint band were appeared in triplicate lanes. Although results of qRT-PCR showed the low expression level of rpfF and rpfG in its mutant strains, these results indicate that RpfF and RpfG
were non-functional in the respective mutant while those in the complemented strains were similar to the wild-type strain.

**Expression of colS/colR genes was increased in rpfF and rpfG mutants.** Expressions of \( \text{colS}_{\text{XOO1207}} \)/\( \text{colR}_{\text{XOO1208}} \) about 2 fold increased in CBNUXO05 (\( \text{rpfF}^{::}\text{EZ-Tn5} \)), while expression of both genes was not different in CBNUXO006 (\( \text{rpfG}^{::}\text{EZ-Tn5} \)) (Table 4). Although we do not know biological significant of 2 fold increase of this two-component system in the \( \text{rpfF} \) mutant yet, qRT-PCR results suggest that expression \( \text{colS}_{\text{XOO1207}} \)/\( \text{colR}_{\text{XOO1208}} \) is influenced directly by DSF rather than signal through RpfG. In Rpf virulence regulation system, RpfF are responsible for the production of DSF (Barber et al., 1997; Wang et al., 2004) and RpfG transfers signal from RpfC that sense the DSF signal to its downstream (He et al., 2007; Ryan et al., 2007; Slater et al., 2000).

**Table 3. Primers used for quantitative RT-PCR**

| Gene ID/Gene  | Product                             | Sequence (5’ – 3’) | Source                  |
|--------------|-------------------------------------|--------------------|-------------------------|
| \( rrsA \)   | 16S ribosomal RNA                   | F: CACATAGATCGAGGACGGTG<br>R: AGTTGCGGCGGCCGACTTC | Subramoni et al., 2012 |
| \( \text{rrpF}_{\text{XOO2869}} \) | \( \text{RpfF} \)                   | F: GAGCTGCCACACCATCATCG<br>R: GGCGGAGTACAGATTGCCTTCT | This study               |
| \( \text{rrpG}_{\text{XOO2871}} \) | Response regulator                  | F: TTTCATCAAGCTCATCTCTCGTG<br>R: TCTCGAAGCATGTTCATGTGGG | This study               |
| \( \text{colS}_{\text{XOO1207}} \) | Two-component system sensor protein | F: TACAGCGAACAACAGATCG<br>R: TTGTTACGGGGTCCGAATTA | This study               |
| \( \text{colR}_{\text{XOO1208}} \) | Two-component system regulatory protein | F: AGCTTTGTGCTCAGACAGTC<br>R: ATCTGTCCTGATCTACACTT | This study               |
| \( \text{colS}_{\text{XOO3534}} \) | Two-component system sensor protein, \( \text{RaxH} \) | F: GATAGCGGATGCGGATGAT<br>R: ATCTGCAACTGGCTCTGGAG | This study               |
| \( \text{colR}_{\text{XOO3535}} \) | Two-component system regulatory protein, \( \text{RaxR} \) | F: AAGGATCGGGCGCTGATAG<br>R: GCTGGCTGTTCATGGAAGACA | This study               |
| \( \text{colS}_{\text{XOO3762}} \) | Two-component system sensor protein | F: AGCGCTTGGTCAGTGATCATC<br>R: CAATGCCACGCCGGTGATG | This study               |
| \( \text{colR}_{\text{XOO3763}} \) | Two-component system regulatory protein | F: ACGGGCTTGGTCAGTGATCATC<br>R: CAATGCCACGCCGGTGATG | This study               |

**Table 4. Effect of Xanthomonas oryzae pv. oryzae KACC10859 \( \text{rpfF} \) and \( \text{rpfG} \) mutations on expression of expression of \( \text{colS/colR}, \text{rpfF} \) and \( \text{rpfG} \)**

| strain                                | Fold expression change ± standard deviation* |
|---------------------------------------|---------------------------------------------|
|                                       | \( \text{XOO1207} \) | \( \text{XOO1208} \) | \( \text{XOO3534} \) | \( \text{XOO3535} \) | \( \text{XOO3762} \) | \( \text{XOO3763} \) | \( \text{rpfF} \) | \( \text{rpfG} \) |
| CBNUXO05 (\( \text{rpfF}^{::}\text{EZ-Tn5} \)) | 2.11±0.14** | 1.90±0.13** | 7.53±0.87** | 2.95±0.11** | 13.31±0.55** | 3.45±0.20** | 0.09±0.01b | 1.44±0.15b |
| CBNUXO05C (\( \text{rpfF}^{::}\text{EZ-Tn5}/\text{pVSP61-mcs-sp::rpfF} \)) | 1.23±0.04b | 1.06±0.09b | 1.25±0.15b | 1.12±0.15b | 1.58±0.15bc | 1.32±0.23b | 1.06±0.13a | 0.93±0.12c |
| CBNUXO06 (\( \text{rpfG}^{::}\text{EZ-Tn5} \)) | 1.04±0.05b | 1.12±0.13b | 4.24±0.04b | 1.78±0.12b | 2.13±0.13b | 3.14±0.09b | 0.92±0.06a | 0.01±0d |
| CBNUXO06C (\( \text{rpfG}^{::}\text{EZ-Tn5}/\text{pVSP61-mcs-sp::rpfG} \)) | 1.02±0.08b | 1.15±0.02b | 1.33±0.04c | 1.09±0.04c | 1.21±0.04c | 1.03±0.01b | 0.88±0.05a | 4.88±0.19a |

*The fold expression change (mutant or complemented mutant/wild type) was calculated using \( 2^{-\Delta\Delta\text{Ct}} \) with three replicates.

**Means with the same letter are not significantly different by Turkey’s HSD test using SAS 9.2.
(Lee et al., 2008; Burdman et al., 2004), DSF may suppress avirulence activity by suppression of the expression of two genes for promoting virulence.

Expression of colS_{XOO3762}/colR_{XOO3762} was increased about 13 and 3 folds, respectively in CBNUXO05 (rpfF::EZ-Tn5) and expression of colS_{XOO3762} was also increased about 2 folds in CBNUXO06 (rpfG::EZ-Tn5) comparing to wild type strain and expression of its cognate regulator, colR_{XOO3762}, was not changed significantly in CBNUXO06 (rpfG::EZ-Tn5). These results suggest DSF regulates negatively this two-component system in the wild type. Biological function of positive regulation of colS_{XOO3762} is not clear, since function of colS_{XOO3762}/colR_{XOO3762} is not known.

In this study, expression of three two-component system genes, two of them are known to control virulence and avirulence, increased significantly in the rpfF mutant, CBNUXO05 (rpfF::EZ-Tn5), and expression of colS_{XOO3534} (raxH)/colR_{XOO3535} (raxR) and colS_{XOO3762} also increased in the rpfG mutant, CBNUXO06 (rpfG::EZ-Tn5). Overall these results indicate DSF and downstream of DSF signal regulate negatively three colS/colR genes in Xoo. Although biological function of these genes by DSF is unclear yet and further detail work on this area is needed, we think that these regulations may be a part of hierarchal control of pathogenicity, which is needed for pathogen to successfully cause a disease on host plant.

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References

Barber, C. E., Tang, J. L., Feng, J. X., Pan, M. Q., Wilson, T. J. G., Slater, H., Dow, J. M., Williams, P. and Daniels, M. J. 1997. A novel regulatory system required for pathogenicity of Xanthomonas campestris is mediated by a small diffusible signal molecule. Mol. Microbiol. 24:555–566.

Burdman, S., Y. Shen, S.-W. Lee, Q. Xue, and P. Ronald. 2004. RaxH/RaxR: a two-component regulatory system in Xanthomonas oryzae pv. oryzae required for AvrXa21 activity. Mol. Plant-Microbe Interact. 17:602–612.

Chatterjee, S. and Sonti, R. 2002. rpfF mutants of Xanthomonas oryzae pv. oryzae are deficient for virulence and growth under low iron conditions. Mol. Plant-Microbe Interact. 15:463–471.

Dekkers, L., Bloemendaal, C. J., Weger, L., Wijffelman, C., Spaink, H. and Lugtenberg, B. J. 1998. A two-component system plays an important role in the root-colonizing ability of Pseudomonas fluorescens strain WCS365. Mol Plant-Microbe Interact. 11:45–56.

He, Y.-W., Ng, A. Y.-J., Xu, M., Lin, K., Wang, L.-H., Dong, Y.-H. and Zhang, L.-H. 2007. Xanthomonas campestris cell–cell communication involves a putative nucleotide receptor protein Clp and a hierarchical signalling network. Mol. Microbiol. 64:281–292.

He, Y.-W., Wu, J., Cha, J.-S. and Zhang, L.-H. 2010. Rice bacterial blight pathogen Xanthomonas oryzae pv. oryzae produces multiple DSF-family signals in regulation virulence factor production. BMC Microbiol. 10:187.

Hu, N. and Zhao, B. 2007. Key genes involved in heavy-meral resistance in Pseudomonas putida CD2. FEBS Microbiol. Lett. 267:17–22.

Jeong, K. S., Lee, S. E., Han, J. W., Yang, S. U., Lee, B. M., Noh, T. H. and Cha, J. S. 2008. Virulence reduction and differing regulation of virulence genes in rpf mutants of Xanthomonas oryzae pv. oryzae. Plant Pathol. J. 24:143–151.

Kivistik, P. A., Putrins, M., Puvı, K., Ilves, H., Kivsaar, M. and Horak, R. 2006. The ColR two-component system regulates membrane functions and protects Pseudomonas putida against phenol. J. Bacteriol. 188:8109–8117.

Loper, J. E and Lindow, S. E. 1994. A biological sensor for iron availability to bacteria in their habitats in their plant surfaces. Appl. Environ. Microbiol. 60:1934–1941.

Lee, N. M., Park, Y. J., Park, D. S., Kang, H. W., Kim, J. G., Song, E. S., Park, I. C., Yoon, U. H., Hahn, J. H., Koo, B. S., Lee, G. B., Kim, H., Park, H. S., Yoon, K. O., Kim, J. H., Jung, C. H., Koh, N. H., Seo, J. S. and Go, S. J. 2005. The genome sequence of Xanthomonas oryzae pathovar oryzae KACC10331, the bacterial blight pathogen of rice. Nucleic Acids Res. 33:577–586.

Lee, S.-W., Jeong, K.-S., Han, S.-W., Lee, S.-E., Phee, B.-K., Hahn, T.-R. and Ronald, P. 2008. The Xanthomonas oryzae pv. oryzae PhoPQ two-component system is required for AvrXa21 Activity, hrg expression, and virulence. J. Bacteriol. 190:2183–2197.

Ryan, R. P., Fouhy, Y., Lucey, J. F., Jiang, B.-L., He, Y.-Q., Feng, J.-X., Tang, J.-L. and Dow, J. M. 2007. Cyclic di-GMP signalling in the virulence and environmental adaptation of Xanthomonas campestris. Mol. Microbiol. 63:429–442.

Seo, Y.-S., Sirriyanun, M., Wang, L., Pfeiff, J., Phetsom, J., Lin, Y., Jung, K.-H., Chou, H. H., Bogdanove, A. and Ronald, P. 2008. A two-genome microarray for the rice pathogens Xanthomonas oryzae pv. oryzae and X. oryzae pv. oryzicola and its use in the discovery of a difference in their regulation of hrg genes. BMC Microbiol. 8:99.

Slater, H., Alvarez-Morales, A., Barber, C. E., Daniels, M. J. and Dow, J. M. 2000. A two-component system involving an HD-GYP domain protein links cell-cell signalling to pathogenicity gene expression in Xanthomonas campestris. Mol. Microbiol. 38:986–1003.
oryzae is required for virulence and growth in iron-limiting conditions. Mol. Plant Pathol. 13:690–703.

Tang, J. L., Liu, Y. N., Barber, C. E., Dow, J. M., Wootton, J. C. and Daniels, M. J. 1991. Genetic and molecular analysis of a cluster of rpf genes involved in positive regulation of synthesis of extracellular enzymes and polysaccharide in Xanthomonas campestris pathovar campestris. Mol. Gen. Genet. 226:409–417.

Wang, L. H., He, Y. W., Gao, Y. F., Wu, J. E., Dong, Y. H., He, C. Z., et al. 2004. A bacterial cell–cell communication signal with cross-kingdom structural analogues. Mol. Microbiol. 51: 903–912.

Yan, Q. and Wang, N. 2011. The ColR/ColS two-component system plays multiple roles in the pathogenicity of the citrus canker pathogen Xanthomonas citri subsp. citri. J. Bacteriol. 193:1590–1599.

Zhang, S. S., He, Y. Q., Xu, L. M., Chen, B. W., Jiang, B. L., Liao, J., Cao, J. R., Liu, D., Huang, Y. Q., Liang, X. X., Tang, D. J., Lu, G. T. and Tang, J. L. 2008. A putative colR(XC1049)-colS(XC1050) two-component signal transduction system of Xanthomonas campestris positively regulates hrpC and hrpE operons and is involved in virulence, the hypersensitive response and tolerance to various stresses. Res. Microbiol. 159:569–578.