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Different Cell Wall-Degradation Ability Leads to Tissue-Specificity between Xanthomonas oryzae pv. oryzae and Xanthomonas oryzae pv. oryzicola

Jianbo Cao 1,2,* , Chuanliang Chu 1, Meng Zhang 1, Limin He 2, Lihong Qin 2, Xianghua Li 1 and Meng Yuan 1,3

1 National Key Laboratory of Crop Genetic Improvement, National Center of Plant Gene Research (Wuhan), Huazhong Agricultural University, Wuhan 430070, China; cch@mail.hzau.edu.cn (C.C.); mengzhang@webmail.hzau.edu.cn (M.Z.); xhl@mail.hzau.edu.cn (X.L.)
2 Public Laboratory of Electron Microscopy, Huazhong Agricultural University, Wuhan 430070, China; liminhe@whu.edu.cn (L.H.); qlh@mail.hzau.edu.cn (L.Q.)
* Correspondence: myuan@mail.hzau.edu.cn (M.Y.); caojb@mail.hzau.edu.cn (J.C.)

Received: 29 January 2020; Accepted: 3 March 2020; Published: 4 March 2020

Abstract: Xanthomonas oryzae pv. oryzae (Xoo) and Xanthomonas oryzae pv. oryzicola (Xoc) lead to the devastating rice bacterial diseases and have a very close genetic relationship. There are tissue-specificity differences between Xoo and Xoc, i.e., Xoo only proliferating in xylem vessels and Xoc spreading in intercellular space of mesophyll cell. But there is little known about the determinants of tissue-specificity between Xoo and Xoc. Here we show that Xoc can spread in the intercellular spaces of mesophyll cells to form streak lesions. But Xoo is restricted to growth in the intercellular spaces of mesophyll cells on the inoculation sites. In vivo, Xoc largely breaks the surface and inner structures of cell wall in mesophyll cells in comparison with Xoo. In vitro, Xoc strongly damages the cellulose filter paper in comparison with Xoo. These results suggest that the stronger cell wall-degradation ability of Xoc than that of Xoo may be directly determining the tissue-specificity.

Keywords: Xanthomonas oryzae pv. oryzae; Xanthomonas oryzae pv. oryzicola; rice; cell wall-degradation; tissue-specificity

1. Introduction

Xanthomonas oryzae pv. oryzae (Xoo) and Xanthomonas oryzae pv. oryzicola (Xoc) lead to bacterial leaf blight and bacterial leaf streak in rice (Oryza sativa L.), respectively [1]. Bacterial leaf blight and bacterial leaf streak are devastating bacterial disease of rice worldwide [1,2]. Xoo and Xoc both belong to Xanthomonas species in the Gammaproteobacteria and have a very close genetic relationship [1,2]. However, Xoo enters rice leaf through hydathodes or wounds then multiplies in xylem vessels to cause leaf blight [1]. In contrast, Xoc gains access to leaf by stomata or wounds and multiplies in the sub-stomatal cavity [1]. Xoc can spread in the intercellular spaces of mesophyll cells to form streak lesions between the veins [1]. But the determinants of Xoo and Xoc parasitic tissue-specificity are still largely unknown [3].

Many factors possibly determine the tissue-specificity of Xanthomonas species among vascular and non-vascular pathogens. The minimal distinguishing differences, such as the subter difference of amino acid and non-coding nucleotide polymorphisms, maybe determine the tissue-specificity between vascular and non-vascular pathogens [4]. The four positions of amino acid residue from HpaA and XpsD proteins correlate with tissue-specificity among Xoc strain BLS256, Xoo strain KACC100331 and MAFF311018, and other vascular or non-vascular Xanthomonas strains [5]. HpaA is a secreted and translocated protein as the type III secretion system (T3SS) components that affect the secretion of effectors and translocators [5,6]. XpsD is an outer membrane protein that serves as a
gatekeeper for the type II secretion system (T2SS) and directs the secretion of different extracellular proteins in species for functioning in specific tissues [5,7].

T2SS and T3SS play important roles in the pathogenesis and the virulence factor secretion of Xoo and Xoc. The T2SS in *Xanthomonas* consists of at least 12 membrane proteins on the outer membrane of the bacterial cell [8]. T2SS is responsible for the extracellular secretion of toxins, proteases, cellulases and lipases, which are required for virulence and pathogenesis in *Xanthomonas* [8,9]. Mutations in genes of the T2SS components, cAMP regulatory protein and extracellular protease A of Xoc strain RS105 can impair the extracellular protease activity and reduce the virulence of Xoc bacteria in rice [8]. Furthermore, the ecpA*<sub>Xoo</sub>* from PXO99<sup>a</sup> with a frame shift, which is also different from the EcpA<sub>Xoc</sub> of RS105 in C-terminal residues, may cause the loss of extracellular protease A (EcpA) activity [8]. It is suggested that the EcpA<sub>Xoc</sub> is a tissue-specific virulence factor for Xoc [8]. The T3SS is responsible for the delivery of transcription activator-like (TAL) effectors into plant cell nuclei [10]. Many TAL effectors of Xoo activate the transcript of rice susceptibility or resistant genes [2,11]. However, except for Tal2a effector eliciting dose-dependent resistance, most TAL effectors of Xoc suppress the innate immunity response of rice against Xoc [11]. It is implied that the TAL effectors secreted by T3SS may determine the tissue-specificity between Xoo and Xoc.

The *feoABC* (Ferrous iron transporter) system may regulate Xoo tissue-specific adaption to rice xylem vessels according to the following evidences [12]. Firstly, the *feo* genes of Xoo are only induced by xylem tissues of infected rice and no expression of *xss* (*Xanthomonas* siderophore synthesis) operon could be detected in xylem tissues [13]. Secondly, the *xsu-xss* (*Xanthomonas* siderophore utilization) operon of Xoc is expressed during Xoc infecting the rice mesophyll tissue [14]. Thirdly, the xylem vessels contain enough Fe<sup>3+</sup>-citrate complex which is sufficient for bacterial growth but mesophyll tissue may have little Fe<sup>3+</sup> or Fe<sup>2+</sup> ions [15].

The cell wall-degradation enzymes (CWDEs) such as lipase, cellulase, xylanase, and endoglucanase secreted by the T2SS of Xoo contribute to disease symptoms [8,16]. There are few reports about the function of CWDEs secreted by Xoc [8]. Only the ecpA mutant of Xoc can cause no xylanase or cellulase activities of bacteria and is avirulent to rice [8]. Whether CWDEs are correlated with the tissue-specificity of Xoo and Xoc should be analyzed.

To further investigate the direct determinants of tissue-specificity between Xoo and Xoc, we compared the structural differences of cell walls, which are in direct contact with Xoo and Xoc by infiltration inoculation methods [17,18], in mesophyll cells of susceptible rice lines. We found that the cell wall-degradation ability of Xoc was stronger than that of Xoo in in vivo and in vitro conditions. These findings imply that the difference of cell wall-degradation ability in Xoo and Xoc contributes to the tissue-specificity between Xoo and Xoc.

2. Materials and Methods

2.1. Bacterial Strains and Rice Cultivar

Xoo strain PXO99<sup>a</sup> and Xoc strain RH3 were obtained from China General Microbiological Culture Collection Center and International Rice Research Institute [18,19]. The susceptible cultivar IR24 belongs to the *indica* (*Oryza sativa* ssp. *indica*) subgroup of Asian cultivated rice [18].

2.2. Pathogen Inoculation and Filter Paper in Vitro Assay

Rice plants at 4-leaf stage were inoculated by infiltrating leaves with 10<sup>4</sup> cells ml<sup>-1</sup> of Xoo and Xoc bacterial suspension by using a needleless syringe [17]. The sterile cellulose filter paper strips (2 mm × 3 cm) embedded in Xoo and Xoc bacterial exudes on potato dextrose agar (PDA) for 5 days. At the 5<sup>th</sup> day, the filter paper strips were observed by using normal field-emission scanning electron microscope. All the inoculation of plants and filter paper treatment with Xoo and Xoc were biologically carried out at least twice with similar results, and one replicate was shown.

2.3. Normal and Cryo Field-Emission Scanning Electron Microscopy
On the corresponding day, the rice leaves at the inoculation sites were longitudinally cut for exposing cell wall and were then cut into 2 mm × 2 mm blocks with new razors and the filter paper strips were cut into 2 mm × 2 mm blocks, then the leaf blocks and the small strips were immediately immersed in 2.5% (v/v) glutaraldehyde in sodium phosphate buffer (0.1 M, pH 7.2) at 4 ℃ for 12 h. The sample blocks were dehydrated in a gradient series of ethanol, then dried in the critical point equipment (HCP-2, Hitachi, Tokyo, Japan), sputter-coated with platinum in sputtering apparatus (MCIOO, Hitachi, Tokyo, Japan), and observed with a field-emission scanning electron microscope (SU8010, Hitachi, Tokyo, Japan). To quantify the images of cell wall with cellulose microfibrils at high magnification and the images with interstitial cavity between fibers or cellulose microfibrils on filter paper, 8–14 images were obtained from three plants in two independent inoculations and 4–15 images were obtained from three filter papers in one treatment.

For in vivo analyzing the growth of bacteria on rice leaf, the fresh rice leaves with inoculation sites and non-inoculation sites were rapidly frozen in solid liquid-nitrogen, sublimated, and sputter-coated with platinum in cryo-scanning electron microscopy transfer system (PP3010T, Quorum, London, England). The coated leaves were observed under a field-emission scanning electron microscope (SU8010, Hitachi, Tokyo, Japan) at 1 kV and a working distance of 8.3 mm.

2.4. Transmission Electron Microscopy

The leaf tissues at the inoculation site and non-inoculation site (adjacent to inoculation site) were cut into 2 mm × 1 mm blocks which were then fixed in 2.5% (v/v) glutaraldehyde in sodium phosphate buffer (0.1 M, pH 7.2) at 4 ℃ overnight, and washed for 30 min with the same buffer three times. The leaf blocks were post-fixed in 1% (w/v) osmium tetroxide for 2 h, washed 30 min with the same buffer three times, and dehydrated in a series of acetone concentration. Dehydrated blocks were progressively infiltrated and embedded in Spurr’s resin (SPI, SPI Chem, West Chester, United States), then polymerized at 65 ℃ for 48 h. The samples were cut into ultrathin sections (60–70 nm thick) with diamond knife, stained with 2% uranyl acetate, and observed with a Hitachi transmission electron microscope (H-7650, Hitachi, Japan) at 80 kV.

2.5. Cellulose Synthase Gene Expression Analysis

Total RNA of rice leaves isolated from the 3-cm leaf fragments including inoculations sites, was extracted by using Trizol reagent (Invitrogen, Carlsbad, CA, USA). An aliquot (5 μg) of total RNA was firstly treated with RNase-free DNase I (Invitrogen, Carlsbad, CA, USA) to remove potentially contaminating DNA, and then first-strand cDNA was reverse transcribed with oligo(dT)18 primer using M-MLV reverse transcriptase (Promega, Madison, WI, USA) according to the manufacturer’s protocols. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was carried out by using cellulose synthase gene (CESA) specific primers (Supplementary Table S1), as described previously [20]. The transcript level of rice actin gene was used to standardize the RNA sample of each RT-PCR, and the expression level relative to that of IR24 inoculated by PXO99A at 0 h was calculated. Each qRT-PCR assay was biologically carried out twice with similar results, within each repetition having three technical replicates and only one biological replicate was presented.

2.6. Statistical Analysis

The significant differences among lesion length, percentage of images with cellulose microfibril, percentage of image with interstitial cavity between fibers or cellulose microfibrils on filter paper and relative expression level of CESA genes were assessed using pairwise Student's t-test in Excel (Microsoft, http://www.microsoftstore.com).

3. Results

3.1. The Leaf Tissue Morphology of Susceptible Rice Infected with Xoo and Xoc
Leaf is a major infection site of Xoo and Xoc [1]. To study whether Xoo can grow and multiply in the extracellular spaces of mesophyll cells, we firstly analyzed the leaf phenotypes of susceptible rice IR24 inoculated with Xoo strain PXO99A and Xoc strain RH3. On the inoculation sites, the leaves inoculated with Xoo represented water-soaked symptom with little brown color around inoculation sites and the leaves inoculated with Xoc were yellow colored at 5 days after infection (DAI) (Figure 1A). There were many bacteria around mesophyll cells in leaf inoculated by Xoo and Xoc on inoculation sites (Figure 1B-1,C-3). In the non-inoculation sites (adjacent to the inoculation site), only rice leaves inoculated with Xoc represented yellow streak lesions with yellow exudates on leaf surface (Figure 1A) and contained many bacteria around the mesophyll cells (Figure 1C-4); but there was no bacterium around the mesophyll cells in rice leaf inoculated by Xoo (Figure 1B-2). At 5 DAI, some bacterial exudates showed on stoma of inoculation site for both leaves inoculated with Xoo and Xoc (Supplementary Figure S1); but there were many bacterial exudates on stoma of non-inoculation site (adjacent to the inoculation site) leaf inoculated with Xoc and no bacterial exudates on stoma of non-inoculation site leaf inoculated with Xoo (Supplementary Figure S1). At 14 DAI, the lesion length of the rice leaf inoculated by Xoo strain PXO99A was only 0.5 cm (the diameter of inoculation syringe was 0.5 cm); the lesion length of rice leaf inoculated by Xoc strain RH3 exceeded 2 cm (Figure 1D).

Figure 1. Xoo only multiplied around mesophyll cells at inoculation site, but Xoc spread through mesophyll cells. DAI, days after inoculation; B, Xoo or Xoc bacterium; Ch, Chloroplast; CW, cell wall; N, nucleus; The inside area of dotted red arc, the area of inoculation site; Red square frame 1 and 3, inoculation site; Red square frame 2 and 4, non-inoculation site. (A) Leaf response of
susceptible rice IR24 inoculated Xoo strain PXO99A and Xoc strain RH3. (B, C) Ultrastructural feature of mesophyll cells in inoculation sites (1,3) and non-inoculation sites (2,4). (D) Lesion length of IR24 infected with Xoo strain PXO99A and Xoc RH3 at 14 DAI. Bar represent mean (5 to 12 leaves from three plants) ± standard deviation (SD). Double asterisks (**) stand for the significant difference between IR24 inoculated with RH3 and IR24 inoculated with PXO99A at p < 0.01.

3.2. The Cell Wall Surface Structures of Mesophyll Cells in Rice Infected with Xoo and Xoc

Plant cell wall is the first physical barrier against pathogens in plant-pathogen interaction [21]. Therefore, we analyzed the cell wall structures of susceptible rice IR24 leaves infected with Xoo and Xoc. In leaves infected with Xoo strain PXO99A, the cell wall surface of the mesophyll cells was still flat (low magnification images) and represented many cellulose microfibrils (black arrow) (high magnification images) at 3 and 5 DAI which were almost similar to a lot of cellulose microfibrils on the flat surface of cell wall at 0 DAI (Figure 2A). In rice leaves infected with Xoc strain RH3, the cell wall surface of mesophyll cells had more pits (low magnification images), especially on the sites of cell wall in contact with bacterium, and fewer cellulose microfibrils (high magnification images) at 3 and 5 DAI in comparison with the flat cell wall surface and many cellulose microfibrils at 0 DAI (Figure 2B). At 5 DAI, the number of images with cellulose microfibrils (high magnification image) from leaves inoculated with Xoo strain PXO99A were significantly (p < 0.01) more than the number of images with cellulose microfibrils from leaves inoculated with Xoc strain RH3 (Figure 2C).

Figure 2. The intact cell wall of mesophyll cells in rice infected with Xoo, but the broken cell wall in rice infected with Xoc. B, bacterium; black arrow, cellulose microfibril. (A) The intact surface structures of cell wall in mesophyll cells of rice leaves inoculated with Xoo strain PXO99A represented by low magnification images (down) and high magnification images (upper) at 0, 3, and 5 day after inoculation (DAI). (B) The broken surface structures of cell wall in mesophyll cells of rice leaves infected with Xoc strain RH3 at 3 and 5 DAI in comparison with plants at 0 DAI represented by low magnification images (down) and high magnification images (upper). (C) The percentage of images with cellulose microfibril in total observed images (%) at 5 DAI. Data represent mean (at least six mesophyll cells were observed from six different plants in two independent inoculation) ± standard deviation (SD). Double asterisks (**) stand for the
significant difference between percentage of image with cellulose microfibril in rice inoculated with PXO99A and percentage of image with cellulose microfibril in rice inoculated with RH3 at $p < 0.01$. n, the total number of observed high magnification images.

3.3. The Cell Wall Inner Structure of Mesophyll Cells in Rice Infected with Xoo and Xoc

Under transmission electron microscopy (TEM) analyzing rice mesophyll cells, the normal cell wall represented low electron-density (transparent) layer structure; the cell wall broken by pathogens represented high electron-density (opaque) layer structure because of osmiophilic material deposition in cell wall [22,23]. To further investigate the cell wall inner structure, we observed the cell wall of the mesophyll cell in susceptible rice IR24 inoculated by Xoo strain PXO99A and Xoc strain RH3 under TEM. There was slightly higher electron density of cell wall (shown by black arrow head) and distinct layers of cell wall in mesophyll cells of leaves inoculated with Xoo at 3, 5 DAI in comparison with the electron-density of cell wall at 0 DAI (Figure 3A). However, there was dramatically higher electron-density of cell wall (black arrow head) and non-layers of cell wall in mesophyll cells of leaves inoculated with Xoc at 3, 5 DAI in comparison with the electron density of cell wall at 0 DAI (Figure 3B). At the same time, the out layer of the cell wall contacting with the bacterium consisted of many electron-dense (osmiophilic) particles in mesophyll cell of leaves inoculated with Xoc at 3 DAI (Figure 3B). Cellulose as the major component of plant cell wall is synthesized by cellulose synthase genes (CESA) [21]. To further analyze the cell wall inner structure, we found that the relative expression levels of CESA4/7/9 significantly reduced in IR24 rice leaves inoculated both with Xoo strain PXO99A and with Xoc strain RH3 (Supplementary Figure S2). However, the expression of CESA4/7/9 in rice leaves inoculated by Xoc were significantly ($p < 0.01$ and $p < 0.05$) induced to lower levels than in rice leaves inoculated by Xoo at 2, 4, 12, and 24 hours after inoculation (Supplementary Figure S2).

![Figure 3](image_url)

**Figure 3.** The lower electron-density of cell wall degraded by Xoo in comparison with the cell wall degraded by Xoc. CW, cell wall; B, Xoo or Xoc bacterium; Ch, chloroplast; black arrow head, the cell wall of mesophyll. (A) The normal electron-density layers of cell wall in mesophyll cells of rice IR24 leaves inoculated with Xoo strain PXO99A at 3, 5 DAI in comparison with cell wall of rice leaves at 0 DAI. (B) The higher electron-density layers of cell wall with electron-dense particles in mesophyll cells of rice IR24 leaves inoculated with Xoc strain RH3 at 3, 5 DAI in comparison with cell wall of rice leaves at 0 DAI.

3.4. The Cellulose Filter Papers Less Damaged by Xoo than Xoc
The qualitative filter paper (Grade 1, GE Whatman, Hangzhou, China), which mostly consisted of cellulose, hemicellulose, and lignin (www.gelifesciences.com), were treated with bacteria to in vitro analyze the cell wall-degradation ability of Xoo strain PXO99A and Xoc strain RH3. After 5 days, the surface of filter paper treated by Xoo showed few interstitial cavities between big fibers in low magnification image and no interstitial cavities between the densely packing microfibrils in high magnification image (Figure 4A); however, the surface of filter paper treated by Xoc showed many interstitial cavities between big fibers in low magnification image and many interstitial cavities between microfibrils in high magnification image (Figure 4B). Meanwhile, the surface of filter paper treated by H2O (negative control) also showed few interstitial cavities between big fibers or microfibrils after 5 days treatment (Figure 4C). The number of images with interstitial cavities between fibers or microfibrils in filter paper treated by Xoc strain RH3 was 3-fold higher than the number of images with interstitial cavities between fibers or microfibrils in filter paper treated by Xoo strain PXO99A or H2O (Figure 4D).

Figure 4. In vitro lower degradation ability of Xoo than that of Xoc. (A) The fibers densely packing and few interstitial cavities between microfibrils on surface of filter paper treated by Xoo strain PXO99A at the 5th day. (B) Many interstitial cavities between big fibers and microfibrils on surface of filter paper treated by Xoc strain RH3 at the 5th day. (C) The fibers densely packing and few interstitial cavities between microfibrils on surface of filter paper treated by H2O (negative control) at the 5th day. (D) The percentage of image with interstitial cavity between fibers in total observed images from filter papers treated by Xoo strain PXO99A, Xoc strain RH3 and H2O at the 5th day. Data
represent mean (at least three images were observed from three different filter papers) ± standard deviation (SD). Double asterisk (**) stand for the significant differences between percentage of images with interstitial cavities among fibers or microfibrils between infilter paper treated by PXO99A and infilter paper treated by RH3, between infilter paper treated by RH3 and infilter paper treated by H2O (negative control) at p < 0.01. n, the total number of observed images.

4. Discussion

In rice leaf tissue, Xoo proliferates in xylem vessel, while Xoc multiplies in intercellular spaces of mesophyll tissue [1]. However, many wild type Xoo strains and Xoc strains, which are infiltrated into mesophyll tissue after 3–7 days, can both cause water-soaked lesions on leaves of susceptible rice varieties [17,24,25]. At the 5th day after infiltration inoculation, Xoo and Xoc proliferated in the intercellular space between mesophyll cells, which resulted in yellow lesions and bacterial exudate formations on inoculation sites of leaves (Figure 1B-1,C-3; Supplementary Figure S1). These evidences suggest that Xoo and Xoc can both grow in intercellular spaces between mesophyll cells. But at the non-inoculation sites (adjacent to inoculation site) of leaves inoculated by Xoo, no Xoo bacteria were observed in the intercellular spaces between mesophyll cells following by no lesions and bacterial exudate formations (Figure 1A,B-2; Supplementary Figure S1A). However, many bacteria of Xoc were growing in the intercellular spaces between mesophyll cells which caused streak lesions and bacterial exudates formations on the leaves of non-inoculation sites (Figure 1A,C-4; Supplementary Figure S1B). Almost all the wild Xoc strains can infect indica and japonica rice leaves to form streak lesions along the veins [1,2,8,11]. The mesophyll tissue of rice leaf is composed of the lobed mesophyll cells that are joined to one another by the tight fusion cell wall of the lobes [26]. So, Xoc bacteria must break the cell wall junction between the lobes of mesophyll cell then spread to the neighbor intercellular spaces between mesophyll cells in rice leaf. These evidences indicate that Xoo and Xoc can both grow in the intercellular spaces between mesophyll cells. But only Xoc can break the cell wall junction between mesophyll cells to spread in the extracellular spaces and form streak lesions.

The cell wall is consisted of cellulose microfibrils embedded in gel-like matrix of hemicellulose (xylan, glucuronoxylan, xyloglucan, arabinoxylan, mixed linkage glucan, or glucomannan), pectic polysaccharides, and minor amounts of structural proteins [27]. The T2SS of Xoo can secrete CWDEs such as cellulase, polygalacturonase, xylanase, lipase, and endoglucanase as virulence factors facilitating Xoo invading rice [8,16,28]. The T2SS-deficiency or the single endoglucanase gene (eglXoB) loss of Xoo are severely virulence-deficient in rice [28,29]. However, the single mutation of cellulose or lipase gene causes only a partial loss of Xoo virulence [16]. The purified cellulase, endoglucanase and lipase of Xoo can induce rice defense response which are suppressed by the T3SS of Xoo [16]. These evidences suggest that the Xoo growing in intercellular of mesophyll cells or multiplying in xylem vessels are dependent on the T3SS but independent on the most CWDEs secreted by T2SS [16]. The T2SS-deficient mutant of Xoc is also severely virulence-deficient in rice [8]. The T2SS component-XpsD protein and the extracellular protease A (EcpA) secreted by T2SS are tissue-specific virulence factors between Xoc strain BLS256 and Xoo strains [5,8]. Many genes of cellulase, xylanase and lipase are analyzed in the genome of Xoc strains [4,12]. The gene mutants of CWDEs are not screened in Xoc strain BLS256 Tn5-mutant library [8] which is possibly because of the CWDEs keeping the housekeeping role in Xoc growth [30]. Furthermore, the cell wall of mesophyll cell and the cellulose filter papers were more severely damaged by Xoc than that of Xoo (Figures 2–4). Meanwhile, Xoc can break the cell wall of lobes in mesophyll cells to form streak lesions (Figure 1). Therefore, in comparison with Xoo, Xoc has the stronger cell wall-degradation ability by breaking cell wall linkage of mesophyll cell lobes to facilitate tissue-specific spread in the intercellular spaces between mesophyll cells. The strong cell wall degradation ability of Xoc, which may directly determine the tissue-specificity of Xoc, should be determined by the CWDEs in T2SS signal pathways.

A lot of cell wall-related genes of rice are involved in rice-Xoo/Xoc interactions. Rice major resistance (MR) genes Xa4 and Xa27 against Xoo are associated with the up-regulated expression of
cellulose synthase genes and the secondary cell-wall thickening in vascular bundle elements [31,32]. Rice lines with overexpression of EXPA1/5/10, which encoding cell wall-loosening proteins, show increased susceptibility to Xoo and Xoc [33]. These evidences imply that Xoo and Xoc may both impair the cell wall mediated resistance in rice. However, no MR gene against Xoc is identified in rice until now [2], only some cell wall-related genes are associated with rice resistance to Xoc. The overexpression of polygalacturonase-inhibiting protein 1/4 (OsPGIP1/4) and small heat shock protein (OsHsp18.0-Cl) genes enhance resistance to bacterial leaf streak in rice [34–36]. The polygalacturonase-inhibiting protein functions in inhibiting the activity of enzymes which secreted by bacteria to degrade cell wall component-pectic polysaccharides [34]. The expression of nucleotide-binding site-leucine rich repeats (NB-LRR) maize MR gene Rxo1, which mediates rice resistance to Xoc, can induce lignin deposition on the inoculation sites [37]. Furthermore, The T3SS defective strain of Xoc strain GX01 induces the up-regulated expression of cellulose synthesis enzyme genes and the down-regulated expression of expansin genes encoding cell-wall loosening proteins in japonica rice [38]. The expression of cellulose synthase 4/7/9 genes of susceptible rice leaves were induced by Xoc to slightly lower levels than control leaves and rice leaves inoculated by Xoo (Supplementary Figure S2). The evidences suggest that the cell wall-degradation ability is possibly reinforced by the T3SS effectors of Xoc.

**Supplementary Materials:** The following are available online at www.mdpi.com/2076-0817/9/3/187/s1, Figure S1: No Xoo bacterial exudate but many Xoc bacterial exudates on leaf of non-inoculation sites, Figure S2: Expression levels of cellulose synthase gene (CESA) in IR24 leaves inoculated by PXO99a and RH3, Table S1: PCR primers used for quantitative RT-PCR assays.

**Author Contributions:** Writing, Supervision, Funding acquisition, J.C. and M.Y.; Investigation, C.C. and M.Z.; Methodology, H.M. and L.Q.; Resources, X.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the National Natural Science Foundation of China grant numbers 31500977, 31821005 and 31822042.

**Conflicts of Interest:** The authors declare no conflict of interest.

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