

## Shedding light on the role of photosynthesis in pathogen colonization and host defense

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**T**he role of photosynthesis in plant defense is a fundamental question awaiting further molecular and physiological elucidation. To this end we investigated host responses to infection with the bacterial pathogen *Xanthomonas axonopodis* pv. *citri*, the pathogen responsible for citrus canker. This pathogen encodes a plant-like natriuretic peptide (XacPNP) that is expressed specifically during the infection process and prevents deterioration of the physiological condition of the infected tissue. Proteomic assays of citrus leaves infected with a *XacPNP* deletion mutant ( $\Delta$ XacPNP) resulted in a major reduction in photosynthetic proteins such as Rubisco, Rubisco activase and ATP synthase as compared with infection with wild type bacteria. In contrast, infiltration of citrus leaves with recombinant XacPNP caused an increase in these host proteins and a concomitant increase in photosynthetic efficiency as measured by chlorophyll fluorescence assays. Reversion of the reduction in photosynthetic efficiency in citrus leaves infected with  $\Delta$ XacPNP was achieved by the application of XacPNP or *Citrus sinensis* PNP lending support to a case of molecular mimicry. Finally, given that  $\Delta$ XacPNP infection is less successful than infection with the wild type, it appears that reducing photosynthesis is an effective plant defense mechanism against biotrophic pathogens.

Natriuretic peptides (NPs) are hormones strongly implicated in the regulation of salt and water balance in vertebrates. In higher plants, the heterologous plant NPs (PNPs) elicit a number of responses that contribute to the regulation of homeostasis and growth.<sup>1</sup> PNPs act via rapid and transient increases in cellular cGMP levels<sup>2</sup> and promote tissue specific ion movements,<sup>3</sup> increases in net water uptake into cells as well as stomatal opening.<sup>4-6</sup> PNPs are upregulated under conditions of osmotic stress<sup>7</sup> and K<sup>+</sup> starvation<sup>8</sup> and have been localized in conductive tissue.<sup>9</sup> Furthermore, PNPs have been identified in the apoplastic proteome<sup>10</sup> and biologically active PNP was isolated from xylem exudates.<sup>9</sup>

We found that the citrus canker causing bacteria *Xanthomonas axonopodis* pv. *citri* (Xac), but no other phytopathogen or bacteria, has a PNP-like gene (*XacPNP*)<sup>11</sup> and that this gene is expressed during Xac infection suggesting a role in pathogenicity.<sup>12</sup> We also observed that lesions produced in leaves infected with a *XacPNP* deletion mutant ( $\Delta$ XacPNP) were more necrotic than those infected with the wild type and that the mutant causes the formation of highly necrotic tissue leading to earlier bacterial cell death.<sup>12</sup> We also demonstrated that recombinant XacPNP, much like PNPs, can cause plant responses such as stomatal opening as well as improved tissue hydration.<sup>12</sup> This would suggest that the plant-like bacterial PNP enables the plant pathogen to modify

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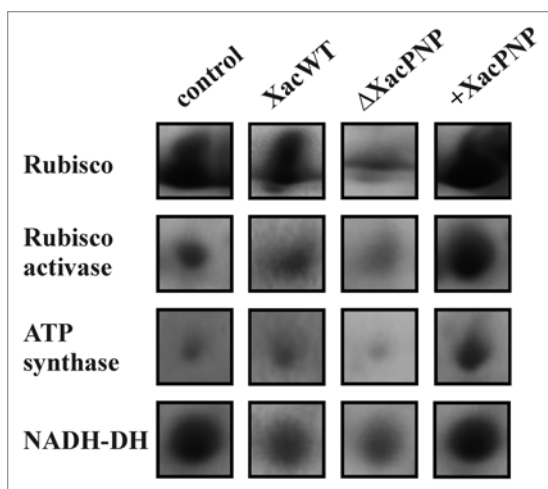
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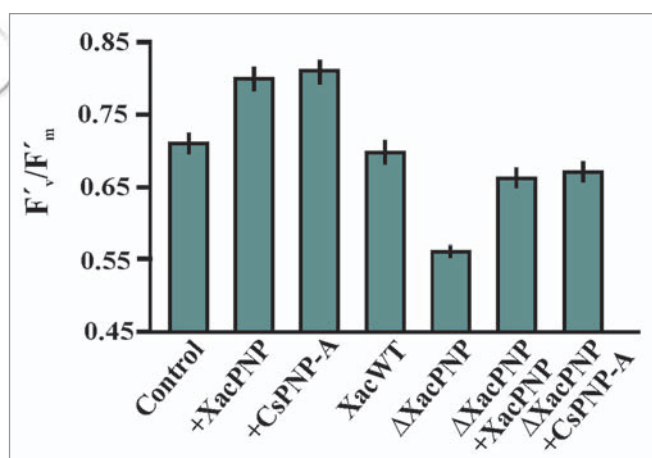
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**Figure 1.** Changes in photosynthetic proteins during bacterial infections and XacPNP treatment. Protein spots from 2-DE SDS-PAGE of proteins from citrus leaves stained with Coomassie blue. Citrus leaves were infiltrated with XacWT, ΔXacPNP ( $10^7$  CFU/ml) and  $5 \mu\text{M}$  XacPNP pure protein (+XacPNP). After 3 days of bacterial infections or 30 minutes after infiltration with recombinant protein, total plant proteins were extracted and subjected to the proteomics analysis. As control, citrus leaves were infiltrated with Tris 50 mM.



**Figure 2.** Effect of PNPs in effective quantum efficiency of photosystem II in host leaves. Chlorophyll fluorescence was measured by an 0.8 s saturating pulse at  $5,000 \text{ mmol m}^{-2} \text{ s}^{-1}$  in leaves infiltrated with  $5 \mu\text{M}$  PNPs, Xac wild type, ΔXacPNP and ΔXacPNP ( $10^7$  CFU/ml) complemented with XacPNP and CsPNP-A. In the control, citrus leaves were infiltrated with Tris 50 mM. The results are the mean of three replicates and error bars represent the standard deviations.

host responses thereby creating conditions favorable to its own survival.<sup>12,13</sup>

With a view to further characterize responses to PNP we undertook proteomics studies on citrus leaves infected with Xac and observed a decrease in the expression of sugar-regulated photosynthetic proteins such as Rubisco and Rubisco activase and also of ATP synthase and an increase in NADH dehydrogenase diagnostic for a reduction in photosynthetic efficiency during citrus canker

(Fig. 1).<sup>14</sup> This indicates that during pathogen attack the biosynthesis of defense-related compounds is a priority for the plant while other (e.g., growth related) cellular activities are reduced thus permitting a reduction in photosynthetic rates until pathogenic growth has been halted.<sup>15,16</sup> Such a reduction in photosynthesis may starve biotrophic pathogens of nutrients thereby benefitting the plant. Further, we analyzed the host proteome after infection with a XacPNP deletion

mutant strain (ΔXacPNP) and observed that the main difference between infections with Xac wild type and ΔXacPNP was in proteins with a key role in carbon metabolism and photosynthesis showing that ΔXacPNP caused a considerably bigger reduction in the expression of photosynthesis genes (Fig. 1).<sup>14</sup> Consistent with this we observed that application of recombinant XacPNP to leaves increases the expression levels of these photosynthetic proteins (Fig. 1).<sup>17</sup>

Since the *XacPNP* gene is only present in *X. axonopodis* pv. *citri* we consider it likely that this gene has been acquired by the bacteria in an ancient lateral gene transfer event and speculated that this might be a case of molecular mimicry where the pathogen modulates photosynthesis and consequently homeostasis to its own advantage. In order to assess this hypothesis we compared how XacPNP and its plant homolog *Citrus sinensis* PNP (CsPNP-A) modify photosynthetic performance by examining chlorophyll fluorescence parameters after 30 minutes of incubation in leaves infiltrated with purified peptides at a concentration of  $5 \mu\text{M}$ . We noted that photosynthetic efficiency is enhanced in the presence of either purified peptides compared with control leaves (Fig. 2). Similar to our previous work<sup>14</sup> we observed a reduced photosynthetic efficiency in wild type infiltrations as compared to control leaves and a larger reduction in ΔXacPNP infiltrated tissue (Fig. 2). We also observed that the reduction in photosynthetic efficiency caused by ΔXacPNP can be complemented by co-infiltration with either recombinant XacPNP or CsPNP-A (Fig. 2) hence adding further strength to the case of molecular mimicry. Given that keeping photosynthesis going is advantageous to the biotrophic pathogen, the converse implies that shutting photosynthesis down must be beneficial to the host and may be an important part of plant defense.

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