Ebb and flow of vascular pathogenesis through gain and loss of a molecular switch

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Introduction

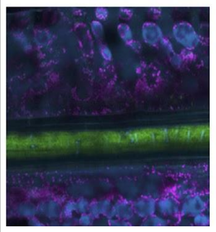
- **Vascular pathogens** of plants and animals move long distances through host veins, leading to **systemic infection**
- **Non-vascular pathogens** remain restricted to the site of infection, triggering **localized symptom development**
- **Differences between these modes of infection have important ecological and economic consequences,** yet their genetic and biological bases are unclear.
- **We aimed to determine the origins of vascular pathogenesis in *Xanthomonas***, a diverse genus of Gram-negative bacteria that cause vascular and non-vascular diseases in over 200 monocot and dicot plants.

Methods

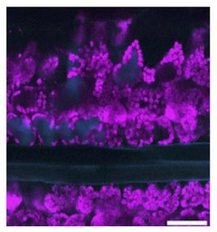
1. **We downloaded 86 complete genomes** of beta- and gamma- proteobacteria, focusing on the Xanthamonadaceae
2. **We tested for associations** between vascular lifestyle and gene family (i.e., orthogroup) presence using BayesTraits
3. **We determined the genomic contexts** of genes in the most significant orthogroup using bespoke bioinformatic scripts
4. **We identified duplications, losses, and horizontal gene transfers (HGTs)** affecting the most significant orthogroup using phylogenetic tree reconciliation and hypothesis testing
5. **We evaluated pathogenicity** of two non-vascular pathogens transformed with genes from the top orthogroup that originated from vascular pathogens

Results

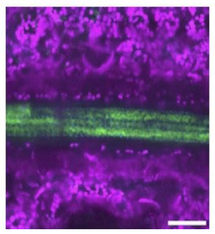
- ***celA* is a cellobiohydrolase** that is significantly associated with transitions to vascular lifestyles
- ***celA* is conserved in vascular pathogens** from three distinct genera: *Xanthomonas*, *Xylella* and *Ralstonia*.
- ***celA* is found in 4 distinct genomic contexts**
- ***celA* has been transferred at least 4 times** to what are now vascular pathogens through recombination-mediated HGT
- ***celA* has been lost at least 10 times** in non-vascular pathogens through TE insertions and sequence deletions
- **Heterologous expression of CelA changed tissue-specificity**, converting two non-vascular *Xanthomonas* strains to xylem-colonizing vascular pathogens



X. translucens pv. translucens (**vascular**)



X. translucens pv. undulosa (**non-vascular**)



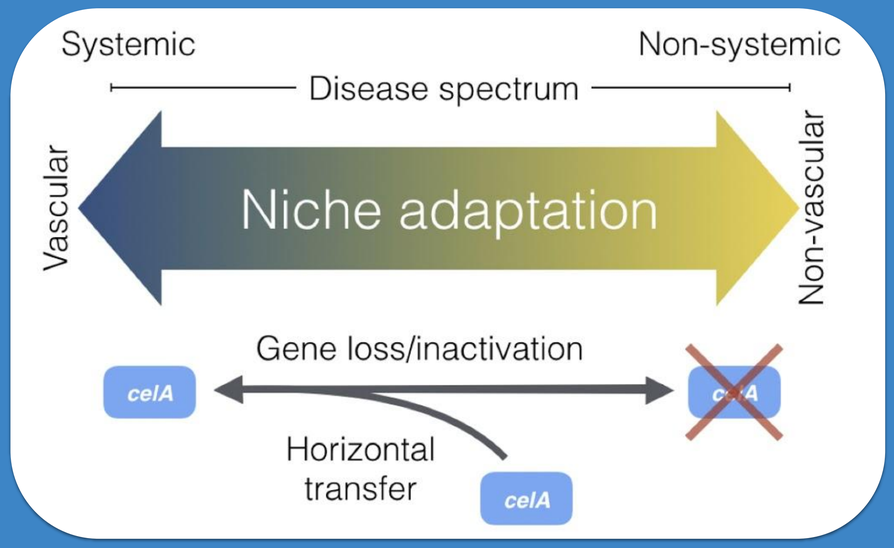
X. translucens pv. undulosa + ***celA*** (**vascular**)

Figure 1: Addition of xylem pathogen-conserved *celA* homologs to non-vascular *X. translucens* pv. undulosa permits tissue-specific vascular pathogenesis. 14 day-old barley (cv. Morex) leaves were clipped with water (control) or water-based inoculum (OD_{600} 0.5) of GFP-expressing vascular *X. translucens* pv. translucens UPB 886 (left), non-vascular *X. translucens* pv. undulosa UPB 513 (middle) and UPB 513 Tn7::$celA_{Xoo}$ (right). Leaf images focus on a vascular bundle downstream of the leaf lesion 12 days post-inoculation. Green: bacterial cells expressing GFP. Magenta: chlorophyll autofluorescence to outline non-vascular mesophyll cells.

Discussion

Rather than representing evolutionary endpoints, our results suggest that Xanthomonadaceae **rapidly modulate between vascular and non-vascular lifestyles through gene gain and loss**

A single, **horizontally transferred** gene enables bacterial infections of plant **vascular systems**



Check out Poster 192 “A single cellobiosidase is required for barley hydathode and xylem colonization by *Xanthomonas translucens*” presented by Jonathan M. Jacobs for more on *celA*

Supporting Data

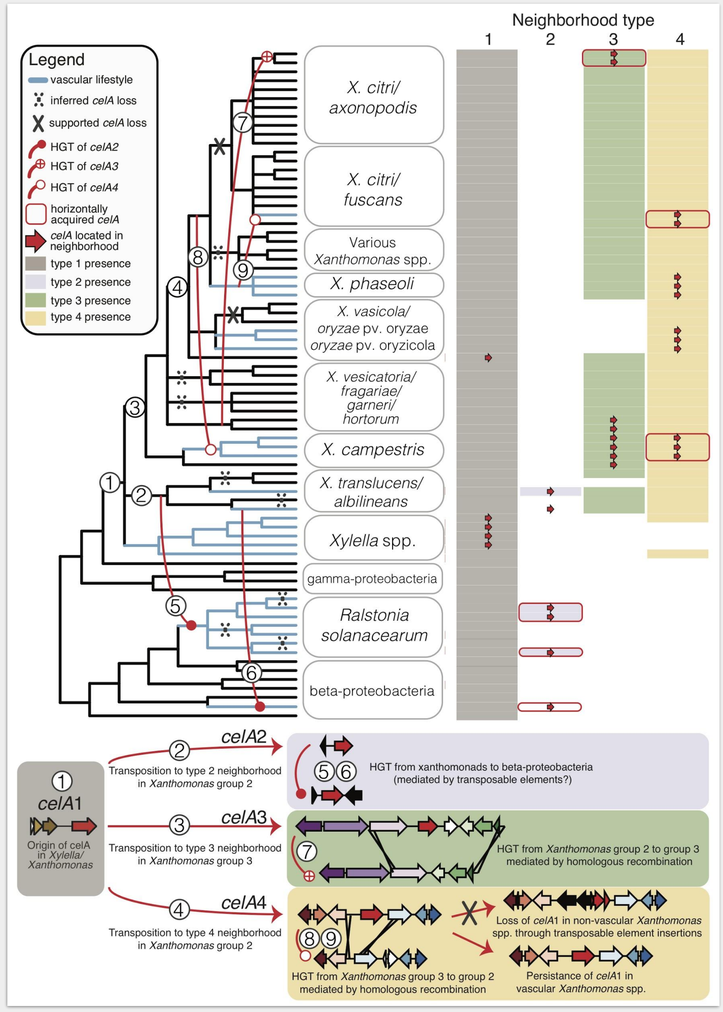


Figure 2. Horizontal gene transfer and loss drive the distribution of *celA* in vascular and non-vascular bacterial pathogens. Shown to the left is a majority rule consensus tree based on 81 maximum likelihood trees of single copy orthologs that summarizes species relationships among bacteria included in this study. To the right of the tree is a graphic summarizing the presence/absence of the four distinct neighborhoods in which *celA* is found across each genome, in all cases whether *celA* is present or not. Transpositions, gains, and losses are numbered and summarized below.

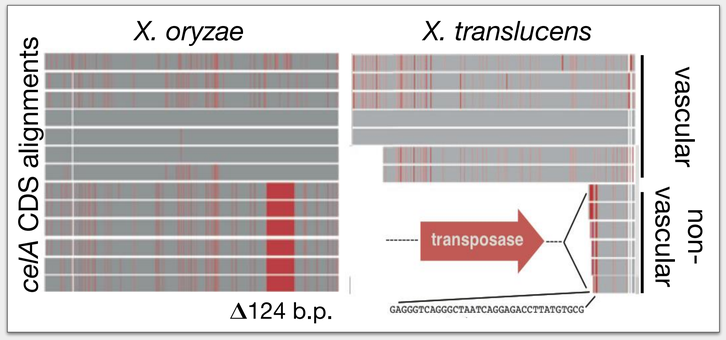


Figure 3: Convergent inactivation of *celA* in non-vascular pathogens through sequence deletions and transposable element (TE) insertions. Multiple sequence alignment of *celA* CDS from vascular and non-vascular *X. oryzae* strains (left) and *X. translucens* strains (right). SNPs shown in red.

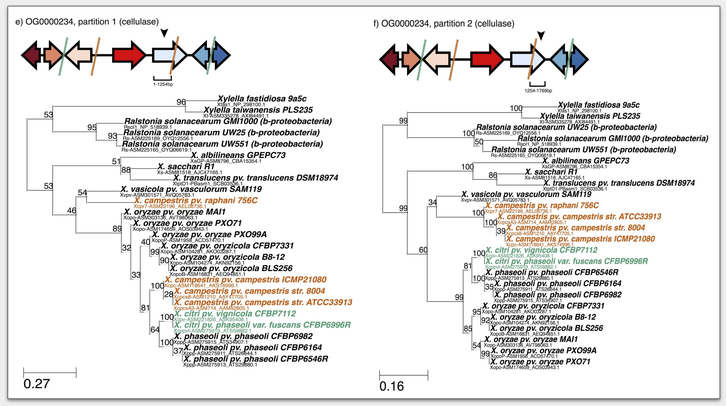


Figure 4: Homologous recombination mediates the insertion of horizontally-acquired *celA* sequences in vascular pathogen genomes. Shown above are two maximum likelihood trees with bootstrap support depicting the phylogenetic relationships of sequence flanking an intragenic recombination breakpoint in *X. campestris* p.v. campestris (vascular)

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