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# The fungal effector AvrStb6 regulates the wheat pathobiome

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## What is AvrStb6?

AvrStb6 is an effector secreted by the wheat pathogen *Zymoseptoria tritici* that localizes to the leaf apoplast, accumulates in substomatal cavities, and activates immune responses in wheat<sup>1,2</sup>. It is recognized by the immune receptor Stb6, a wall-associated kinase that mediates resistance against *Z. tritici* isolates carrying the native *AvrStb6* allele<sup>2,3,4</sup>.

Despite strong selective pressure from Stb6, *Z. tritici* retains *AvrStb6* in field populations, with diverse *AvrStb6* allelic variants detected in wheat crops<sup>2,5</sup>. This persistence suggests that *AvrStb6* might serve a function beyond host recognition. Given increasing evidence that fungal pathogens can modulate, and be modulated by, the host microbiome during infection<sup>6</sup>, we hypothesize that AvrStb6 plays a role in shaping the wheat apoplastic microbiome involved in disease progression, the pathobiome.

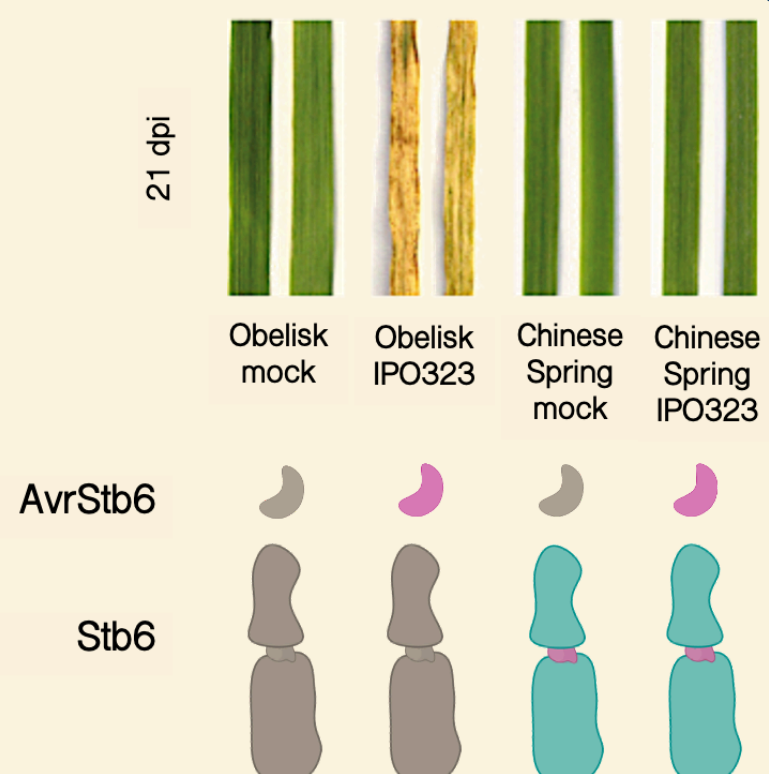


Figure 1. Disease resistance to *Zymoseptoria tritici* IPO323 wild-type in wheat cultivars with or without the Stb6 resistance gene.

## AvrStb6 is not involved in pycnidia formation or necrosis in wheat

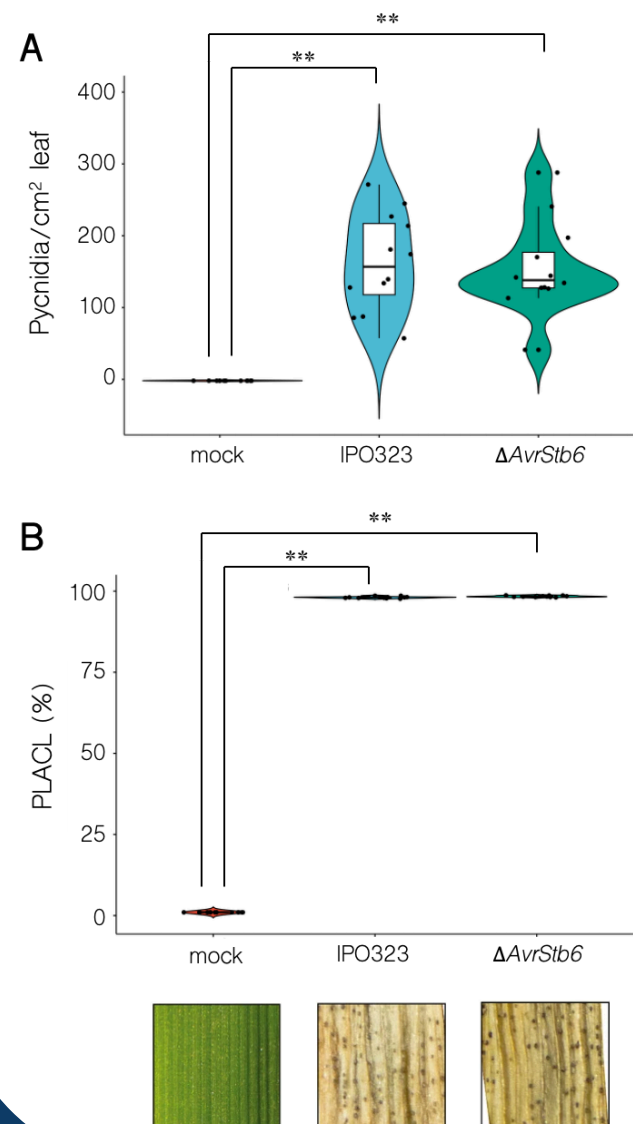


Figure 2. AvrStb6 is not involved in pycnidia formation or host necrosis.

(A) Pycnidia density (pycnidia/cm<sup>2</sup> leaf) and (B) percentage leaf area covered by lesions (PLACL%) are not significantly different ( $p > 0.05$ ) between *Z. tritici* IPO323 wild-type and the  $\Delta$ AvrStb6 mutant on the wheat cultivar Obelisk. Median and interquartile ranges are indicated. Individual data points shown ( $n = 12$ ). Obelisk does not carry the Stb6 resistance gene.

## AvrStb6 in silico

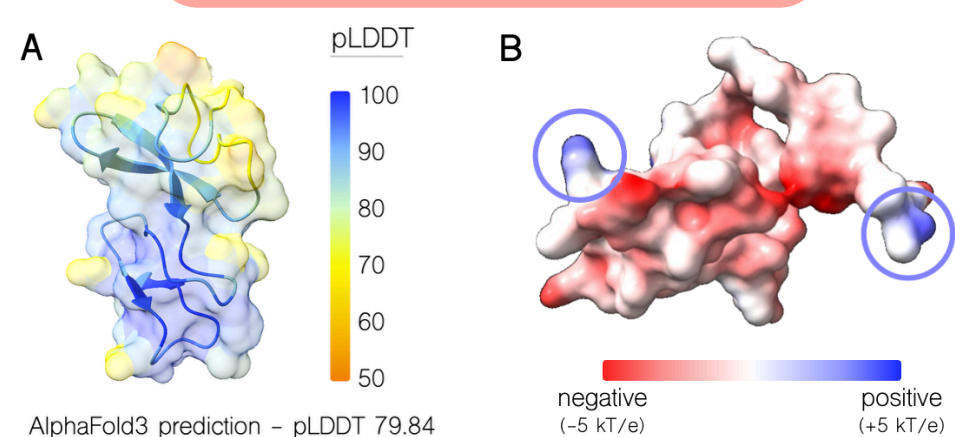


Figure 3. *In silico* analysis suggests that AvrStb6 interacts with the bacteria cell membrane. (A) AvrStb6 structural prediction by AlphaFold3 ([www.alphafoldserver.com](http://www.alphafoldserver.com)). (B) Electrostatic potential of AvrStb6 reveals two distinct positively charged regions, a common feature of peptides that interact with phosphatidyl groups in bacterial outer membranes.

## AvrStb6 influences Z. tritici-Pseudomonas interaction dynamics in vitro.

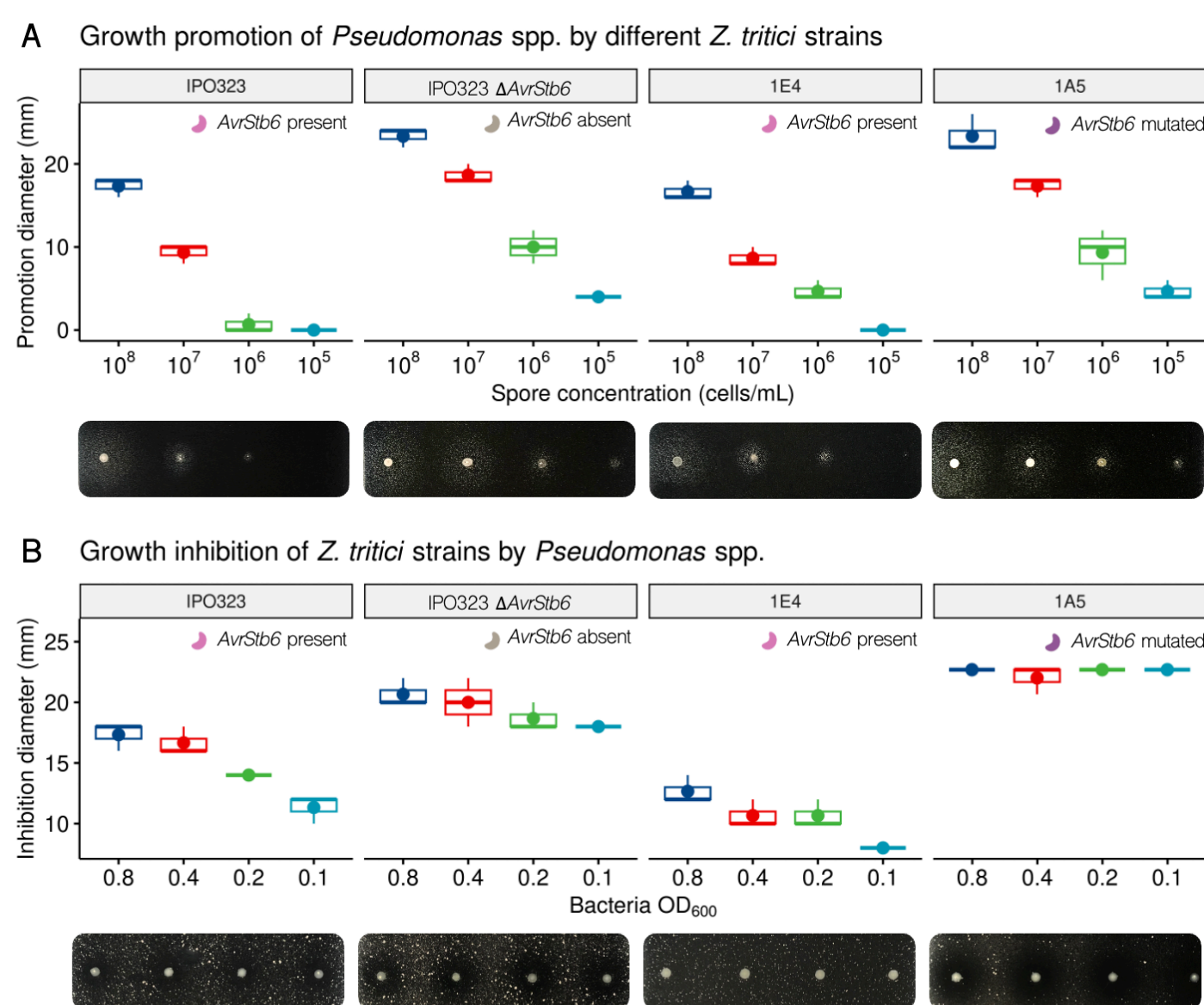


Figure 5. AvrStb6 influence on reciprocal *in vitro* interactions between *Z. tritici* strains and *Pseudomonas* spp. isolated from the wheat apoplast. (A) Growth promotion of *Pseudomonas* spp. by different *Z. tritici* strains: IPO323,  $\Delta$ AvrStb6, 1E4 (field isolate with a native *AvrStb6* allele), and 1A5 (field isolate with a mutated *AvrStb6* allele). (B) Growth inhibition of *Z. tritici* strains by *Pseudomonas* spp. All interactions were assessed *in vitro* using co-culture confrontation assays on agar plates.

## AvrStb6-associated changes in the wheat microbiome

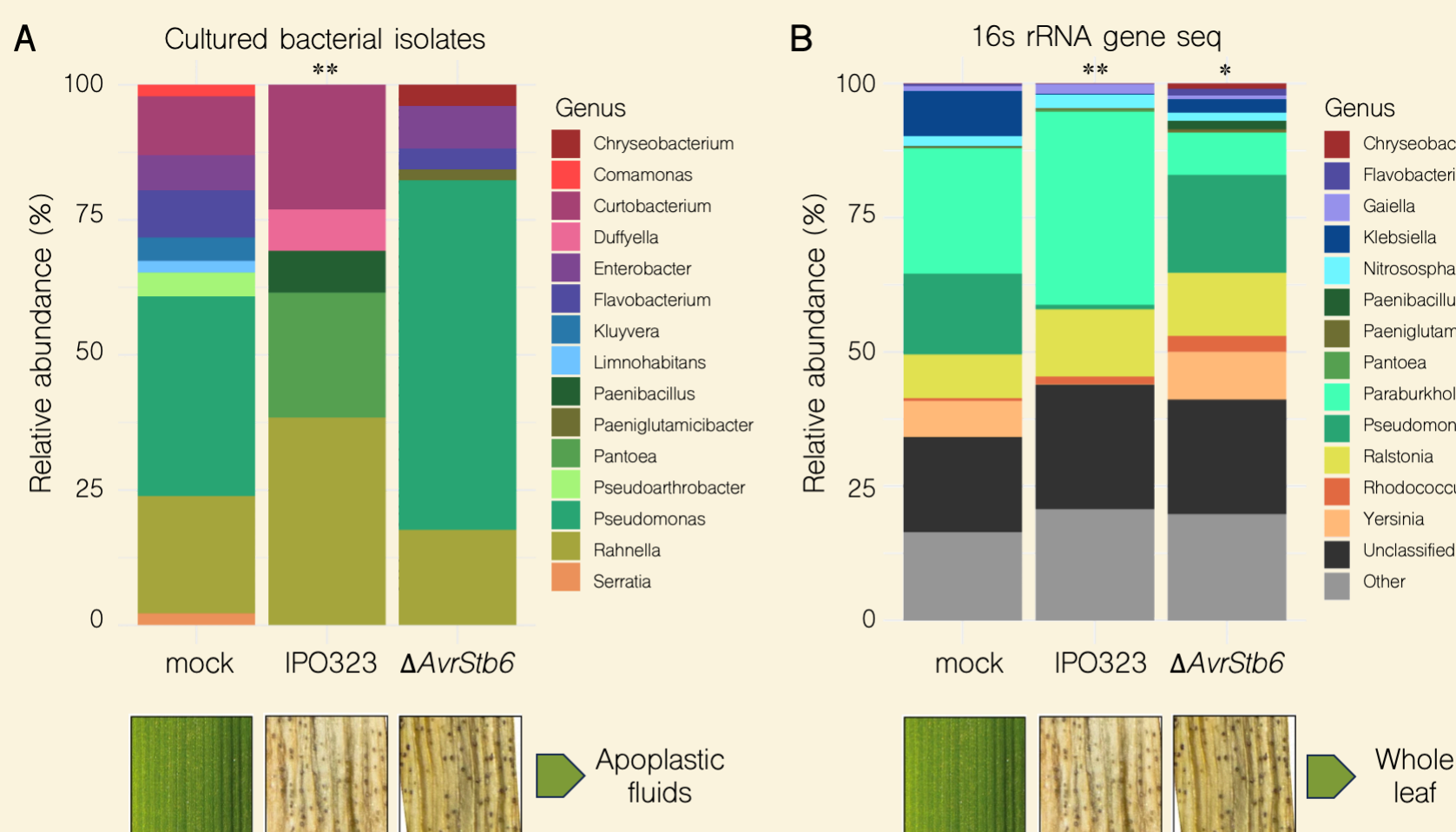


Figure 4. Changes in the wheat microbiome correlate with the presence of AvrStb6. (A) Relative abundance (%) of culturable bacteria isolated from wheat apoplastic fluid. (B) Bacterial community composition based on 16S rRNA amplicon sequencing of whole wheat leaves. Plants were inoculated with water (mock), *Z. tritici* IPO323 wild-type (IPO323), or the  $\Delta$ AvrStb6 mutant ( $\Delta$ AvrStb6). \*\* :  $p \leq 0.01$ , \* :  $p \leq 0.05$ .

## References:

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## Concluding remarks

- AvrStb6 modulates *Pseudomonas* spp. abundance in both the wheat apoplast and the whole-leaf microbiome.
- *In vitro*, wheat-apoplast *Pseudomonas* spp. strongly inhibit *Z. tritici* strains lacking AvrStb6 ( $\Delta$ AvrStb6) or carrying a mutated allele (1A5) than those carrying the native allele (IPO323, 1E4).
- Similarly, *Z. tritici*  $\Delta$ AvrStb6 and 1A5 strains strongly promote the growth of *Pseudomonas* spp. compared to IPO323 and 1E4.
- Overall, AvrStb6 seems to shape a reciprocal interaction between *Z. tritici* and wheat-apoplast *Pseudomonas* spp. where AvrStb6 moderates the enhancement of this bacteria genera likely aiming for a balanced pathobiome that benefits *Z. tritici*. Further protein assays are currently undergoing.