Understanding the antimicrobial role of the fungal effector AvrStb6

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What is AvrStb6?

AvrStb6 is an effector of the wheat pathogen *Zymoseptoria tritici* that localizes to the leaf apoplastic space, accumulates in substomatal cavities, and triggers immune responses in wheat (1, 2). It is recognized by Stb6, a wall-associated kinase that mediates resistance against *Z. tritici* isolates carrying the native *AvrStb6* allele (2, 3, 4).

However, despite the strong selective pressure imposed by Stb6 recognition, *Z. tritici* retains *AvrStb6* in field populations, as evidenced by the presence of diverse *AvrStb6* allelic variants in wheat crops (2, 5). This persistence suggests that *AvrStb6* might play a crucial role in fungal virulence beyond its interaction with plant immunity.
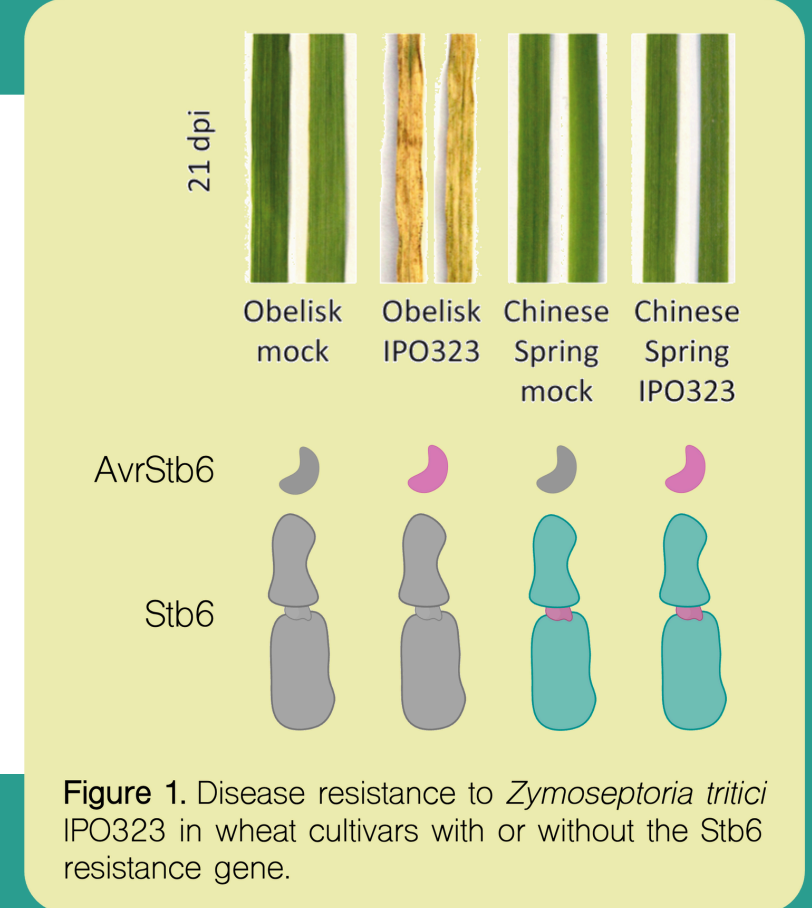

Figure 1. Disease resistance to *Zymoseptoria tritici* IPO323 in wheat cultivars with or without the Stb6 resistance gene.

AvrStb6 *in silico* is predicted to be an antimicrobial peptide

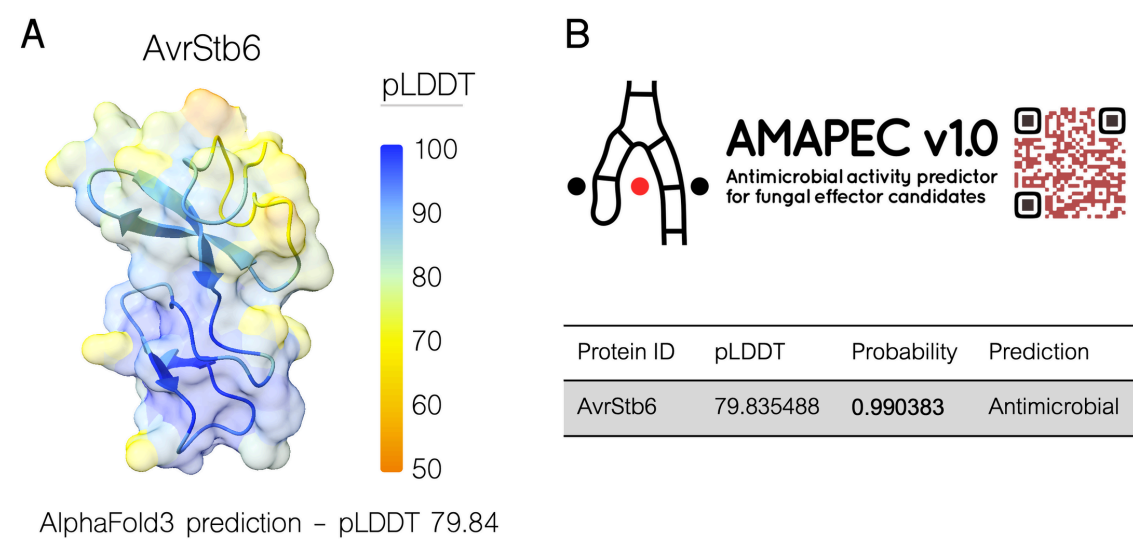
AlphaFold3 prediction – pLDDT 79.84

Figure 2. AvrStb6 *in silico* is predicted to be an antimicrobial fungal effector. (A) AvrStb6 structural prediction by AlphaFold3 (www.alphafoldserver.com). AF3 model confidence in AvrStb6 regions, following pLDDT–predicted local distance difference test– colour scale. AvrStb6 has an average pLDDT score of 79.84. (B) AvrStb6 is predicted to be an antimicrobial effector, when submitted to the AMAPEC antimicrobial activity predictor software for fungal effectors (Mesny & Thomma, 2024).

Protein ID	pLDDT	Probability	Prediction
AvrStb6	79.835488	0.990383	Antimicrobial

AvrStb6 is structurally similar to proteins from predatory bacteria
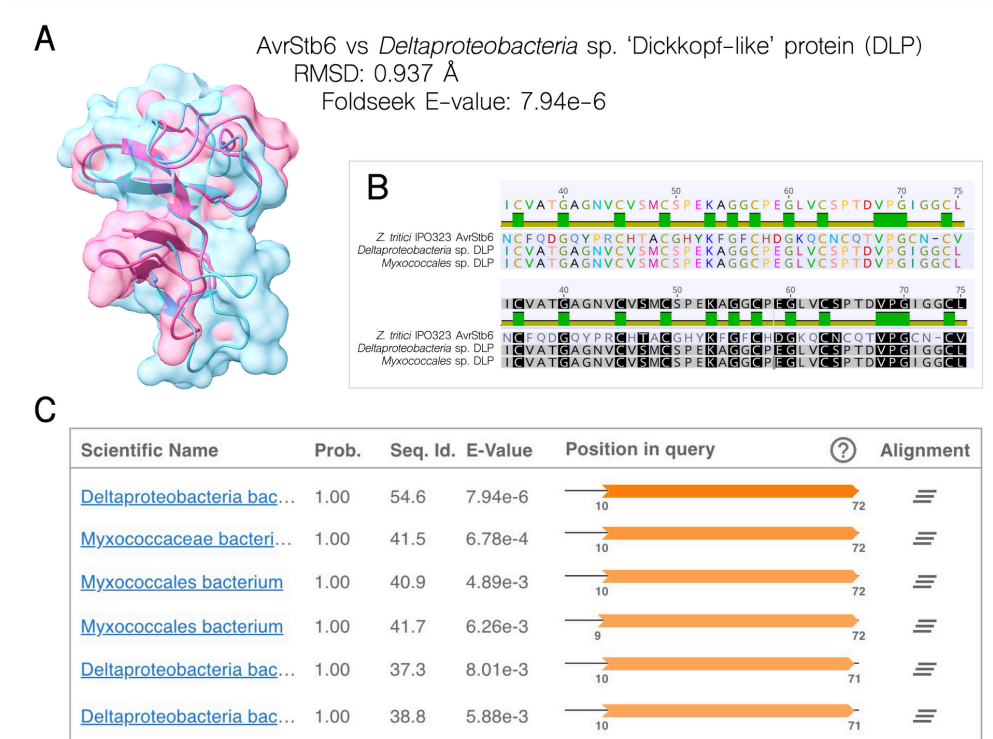

Figure 3. AvrStb6 shares structural similarity with proteins from predatory bacteria. (A) AvrStb6 is structurally similar to 'Dickkopf-like' proteins (DLPs) found in predatory bacteria, including *Deltaproteobacteria* sp. and *Myxococcales* sp. RMSD: root-mean-square-deviation. (B) Amino acid sequence alignment of AvrStb6 and bacterial DLPs reveals conserved cysteine residues in the C-terminal region, despite low overall sequence similarity (26.76%). (C) Foldseek (foldseek.com/search) best protein hits to AvrStb6. E-values between 10^{-7} to 10^{-5} represent significant hits (van Kempen et al, 2022).

AvrStb6-associated changes in the wheat apoplast microbiome profile
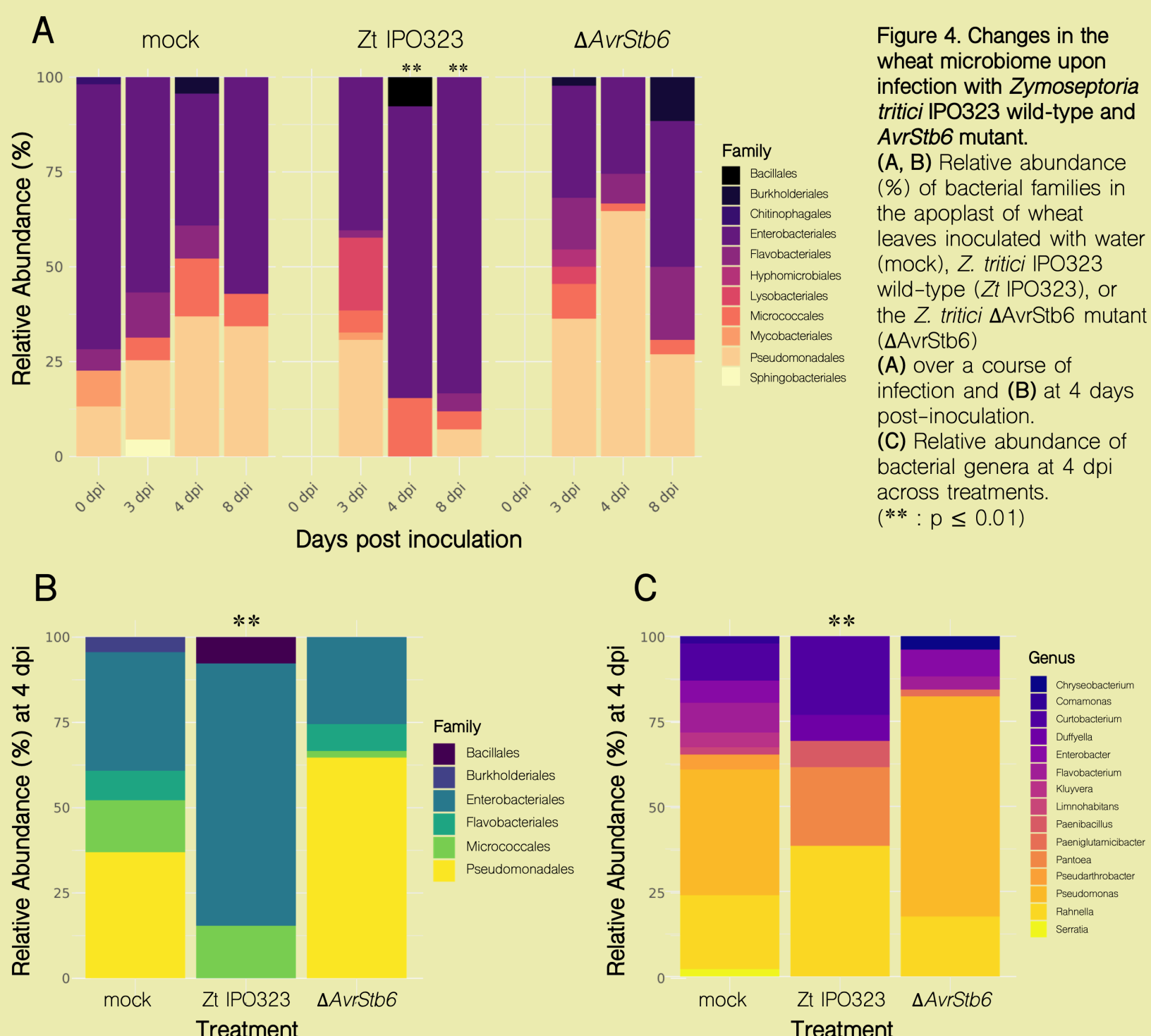

Figure 4. Changes in the wheat microbiome upon infection with *Zymoseptoria tritici* IPO323 wild-type and *AvrStb6* mutant. (A, B) Relative abundance (%) of bacterial families in the apoplast of wheat leaves inoculated with water (mock), *Z. tritici* IPO323 wild-type (*Zt* IPO323), or the *Z. tritici* ΔAvrStb6 mutant (ΔAvrStb6) (A) over a course of infection and (B) at 4 days post-inoculation. (C) Relative abundance of bacterial genera at 4 dpi across treatments. (** : $p \leq 0.01$)

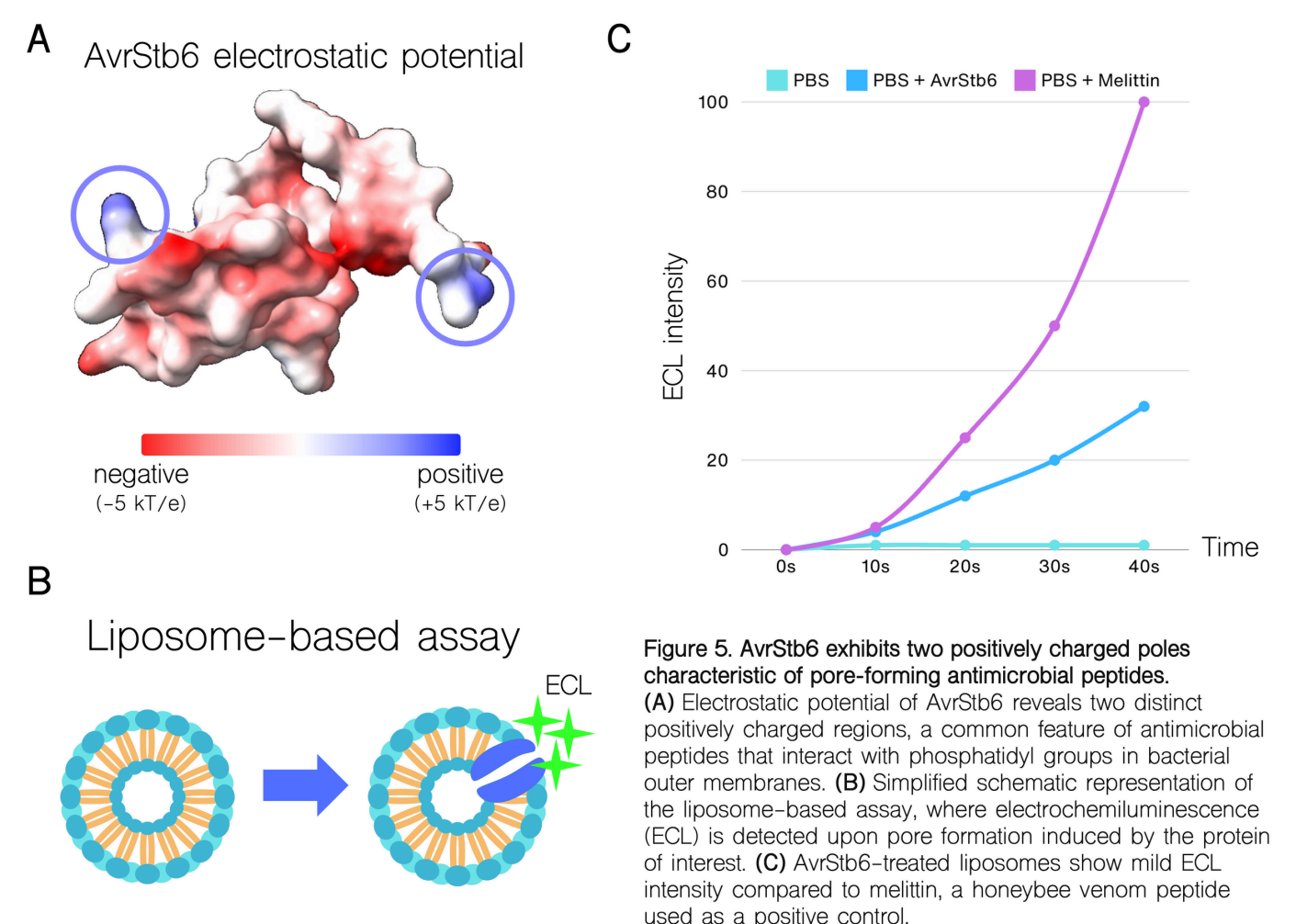## Pore-formation as AvrStb6 putative antimicrobial mechanism of action

Figure 5. AvrStb6 exhibits two positively charged poles characteristic of pore-forming antimicrobial peptides. (A) Electrostatic potential of AvrStb6 reveals two distinct positively charged regions, a common feature of antimicrobial peptides that interact with phosphatidyl groups in bacterial outer membranes. (B) Simplified schematic representation of the liposome-based assay, where electrochemiluminescence (ECL) is detected upon pore formation induced by the protein of interest. (C) AvrStb6-treated liposomes show mild ECL intensity compared to melittin, a honeybee venom peptide used as a positive control.

References:
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