

To sense or not-to-sense: Expression of *Blumeria* effector repertoires on barley loss-of-function mutant hosts

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The interaction of barley, *Hordeum vulgare* L., with the biotrophic powdery mildew fungus, *Blumeria graminis* f. sp. *hordei* (*Bgh*), is an ideal model to address fundamental questions in host resistance and susceptibility. Effector proteins secreted by *Bgh* suppress or induce host processes to promote nutrient acquisition and colonization. The 130-Mb *Blumeria* genome harbors ca. 540 predicted secreted effectors, designated BECs (*Blumeria* Effector Candidates) or CSEPs (Candidate Secreted Effector Proteins). Large-scale RNA-Seq of *Bgh*-infected barley resistance-signaling mutants indicates that distinct subsets of effector candidates are differentially expressed at penetration, or during haustorial formation. This suggests that *Bgh* is able to sense compromised resistance functions in the various isogenic mutant hosts and modify expression of its effector repertoire accordingly.

BEC1019, a predicted single-copy metalloprotease, is differentially expressed in haustoria among all barley loss-of-function mutants. *Barley stripe mosaic virus*-mediated gene silencing of *BEC1019* in *planta* significantly reduces fungal colonization of barley epidermal cells, demonstrating that BEC1019 plays a central role in virulence. In addition, delivery of BEC1019 to the host cell cytoplasm via *Xanthomonas* type III secretion suppresses cultivar non-specific hypersensitive reaction (HR) induced by *Xanthomonas oryzae* pv. *oryzicola*, as well as cultivar-specific HR triggered by the AvrPphB effector from *Pseudomonas syringae* pv. *phaseolicola*. *BEC1019* homologs are present in 96 of 241 sequenced fungal genomes, including plant pathogens, animal pathogens, and free-living non-pathogens. Comparative analysis revealed variation at several amino acid positions that correlate with fungal lifestyle, and several highly conserved, non-correlated motifs. Site-directed mutagenesis of one of these, ETVIC, compromises the HR suppressing activity of BEC1019. We postulate that BEC1019 represents an ancient, broadly important fungal protein family, members of which have evolved to function as effectors in plant and animal hosts.

Supported by NSF-PGRP grants 09-22746 to RPW, AJB, DN, and JAD; 13-39348 to RPW, AJB, DN, and RWI; and NIH grant R01 GM046451 to RWI.