VIRULENCE
2016, VOL. 7, NO. 8, 853–855
http://dx.doi.org/10.1080/21505594.2016.1244596

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EDITORIAL

More wrinkles to Bt susceptibility

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ARTICLE HISTORY Received 28 September 2016; Accepted 28 September 2016

Often it is the case that it is far easier to identify a pathogenic microbe than to understand the mechanisms that lead to host susceptibility or resistance. This is particularly the case for the common soil-dwelling Gram-positive bacterium *B. thuringiensis* (Bt). While studies have constructed a general understanding of the mechanism of Bt activity, there remain many unknowns. Briefly, *B.* thuringiensis produces toxins (*δ*-endotoxins) during sporulation that have specific toxicity to many insect species. The toxin is produced as an inactive crystal protein, but following ingestion by susceptible insects, is solubilized in the gut and further processed by host digestive enzymes.[1,2] The activated toxin then binds to specific receptors on gut cells, which results in either cell membrane pores leading to cell lysis or the activation of intracellular signaling pathways resulting in ischemic cell death.[3,4]

The selective activity of Bt toxin has led it to become the most widely used biological insecticide worldwide. Since the discovery of its insecticidal activity over 100 y ago, Bt spore and crystal preparations have been used to control crop pests and vectors of human and animal diseases. More recently, Bt toxin genes have been expressed in transgenic crops. Given the wide spread use of Bt microbial preparations and genetically modified plants, the evolution of resistance is a concern, and has been reported for some pest species. Yet, despite over a century of research, there remains a great deal unknown about this widely used entomopathogen, and the mechanism of Bt killing remains controversial as disparate processes that lead to host/pest resistance have been identified.[5]

In this issue of *Virulence*, the authors of the article "Immuno-physiological adaptations confer wax moth *Galleria mellonella* resistance to *Bacillus thuringiensis*" utilized an artificial selection experiment to identify traits that lead to Bt resistance, focusing on several characteristics previously implicated in toxin resistance.[6] Following selection for Bt resistance over 20 generations, Dubovskiy *et al.* first measured the expression of 15 genes with roles in immune, stress, and inflammation responses in both the midgut (site of toxin binding) and the fat body, which is an important metabolic and homeostatic organ in the body cavity that produces many stress and immune effectors. Comparison between larvae from susceptible and resistant lines showed that resistant larvae had significantly higher expression levels of an inducible metalloprotease inhibitor, which inhibits proteases that contribute to Bt toxin activation, 2 growth factors in the midgut, and slightly elevated expression of additional immune and stress response genes. Similar elevated expression levels of stress and immune responses were also observed in the fat body of uninfected resistant larvae, including the same inducible metalloprotease inhibitor (IMP1), as well as several antimicrobial peptides. In general, Bt infection induces many of these immune and stress genes in susceptible larvae, which are only further elevated by infection in resistant larvae. Dubovskiy *et al.* hypothesize that these elevated basal responses "prime" the larvae, such that they have a faster response to Bt ingestion and management of damage that the toxin may cause.

In addition to an elevated immune and stress responsive state, Dubovskiy *et al.* show that resistant larvae also express lower levels of 2 midgut Bt toxin receptors, alkaline phosphatase and aminopeptidase-N. This suggests that there may be fewer binding sites for the toxin in the midgut, which when coupled with increased metalloprotease inhibitor levels (suggesting less activated toxin) could greatly contribute to resistance in these larvae.

Lastly, given previous studies showing that gut microbiota can impact Bt susceptibility, the authors use high

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Comment on: Dubovskiy IM, et al. Immuno-physiological adaptations confer wax moth Galleria mellonella resistance to Bacillus thuringiensis. Virulence 2016; 7(8): 860-870; PMID:27029421; http://dx.doi.org/10.1080/21505594.2016.1164367

throughput sequencing to compare the gut microbial communities of larvae of their selected and non-selected lines. Both resistant and sensitive larvae were associated with only a few dominant phyla, with 4 phyla representing greater than 99% of the community. Ingestion of Bt led to changes in composition for both susceptible and resistant larvae, with dominance shifting from Firmicutes to Proteobacteria. However, there were notable differences between the selected and non-selected lines; while Bt ingestion increased the relative abundance of *Pseudomonas* in susceptible larvae, populations of this genus were no longer detectable in resistant larvae following Bt infection. In addition, community richness and abundance were significantly reduced by Bt ingestion in resistant larvae, an effect not observed in susceptible larvae. The authors speculate that the reduced diversity and notable loss of *Pseudomonas* sp., which can be highly pathogenic to many insects, could also contribute to increased survival of the resistant line, as this might reduce midgut microbiota members that would cross the gut and lead to death by sepsis.[7]

Altogether, the data suggest that increased expression of genes that contribute to defense and tissue repair protect larvae from Bt toxin. In this manner, resistant larvae may be in a 'primed' state, which improves the timing of the response to the toxin and associated damage, including the breech of bacteria from the gut to the hemocoel. Another possibility is that given the lower expression of receptors and higher expression of protease inhibitors in the resistant line larvae incur less damage following ingestion of the toxin. Moreover, the enhanced basal immune activity in the resistant line could explain why microbiome diversity is significantly reduced in these larvae following additional immune challenge following ingestion of Bt. Overall, these results are in agreement with an emerging theme from a number of host-pathogen models; that the key in host survival following intestinal damage is the balancing of repair mechanisms (recovery) with defense mechanisms that eliminate microbial threats, including the indigenous microbiota.[8,9] A strength of this study is the comparison between the gut versus hemocoel response, which provide insight to which tissues and mechanisms to target for future studies and comparison to other systems. What remains to be determined from these studies is what host signaling pathways are involved across the breadth of larval responses to Bt and how the host host coordinates the local (gut) and systemic (hemocoel) response. Similarly, the causal role of the shift in community composition and abundance observed in resistant larvae will require further study. Another unexpected results of this study was the positive trade-off of resistance selection on host physiology, specifically the larger pupal size and higher fecundity of the resistant line.

All the same, this study is in agreement with observations that interactions between the host immunity and the gut microbial community underlie mechanisms of susceptibility and resistance to Bt toxin. Nearly a decade ago we presented a new model proposing that in in some, but not all, lepidopteran species Bt and its insecticidal toxin acted in concert with enteric bacteria to account for the final death of insect larvae, and that alteration of the host innate immune responses might contribute to this linkage.[10-12] This model launched a heated debate in the field, as others showed that while elimination of the gut microbiota by antibiotic feeding could reduce susceptibility, the mechanism of gut microbiota suppression was due to a direct effect of antibiotics on the Bt toxin.[13-15] More recently, a number of studies, including this issues' paper and previous work from Dubovskiy and colleagues[16] have described impacts of Bt on the microbiome and contributions of microbiota and the immune response to host susceptibility. Similarly, a recent paper from Caccia *et al.*[17] reported on a related mechanism of Bt-induced lethality in which septicemia caused by the midgut bacteria of another lepidopteran host, *Spodoptera littoralis*, was more pronounced in immunocompromised hosts following RNAi knockdown of a specific immune gene.

Perhaps the most important lesson from these collective studies is that microbiota can have diverse effects on hosts and should be viewed as a component of host physiology and homeostatic state, and thus influence susceptibility and resistance to pathogens often through their impacts on host immunity. In addition, these host states are known to feedback on the microbiome. There is a great deal we still need to learn about these associations and the signals and feedback mechanisms that lead to susceptibility and resistance. In the specific case of lepidopteran larvae and Bt toxin these interactions are likely to be species dependent and dictated by many factors (e.g. health state, age, nutritional status), all of which will need to be taken into consideration when studying the mechanism of action. As evidenced by the Dubovskiy *et al.* and Caccia *et al.* studies advances in tools such as in vivo RNA interference, targeted genome editing with CRISPR/CAS9 in non-model hosts, and inexpensive high throughput sequencing costs will further advance our understanding of these complex interactions.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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