BACTERIAL TOXINS

The touch of death

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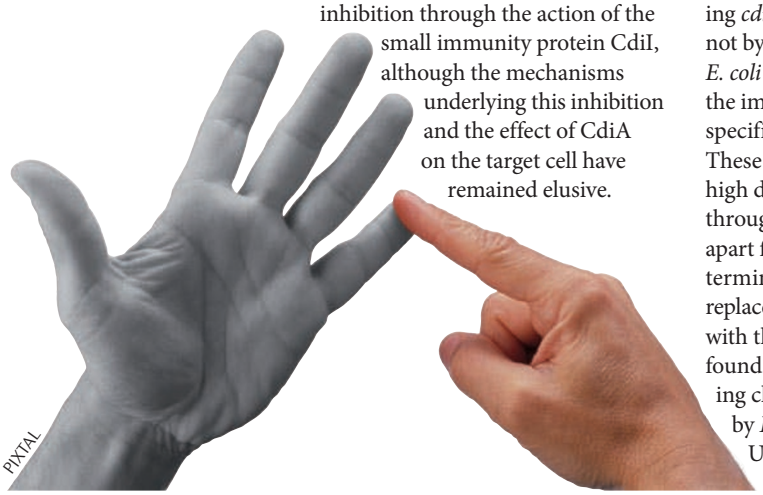
PIXTAL

On direct cell-to-cell contact, *Escherichia coli* str. EC93 inhibits the growth of other *E. coli* strains. However, although the components of this contact-dependent growth inhibition (CDI) system have been identified, little is known about the mechanisms involved. Writing in *Nature*, Aoki *et al.* now show that CDI systems are widespread among Gram-negative bacteria, display distinct mechanisms of action and have roles in intraspecies competition.

CDI is mediated by a two-partner secretion system in which CdiB facilitates the delivery of CdiA, the inhibitory toxin, to the cell surface. CDI$^+$ cells are protected from auto-inhibition through the action of the small immunity protein CdiI, although the mechanisms underlying this inhibition and the effect of CdiA on the target cell have remained elusive.

Bioinformatic analyses by Aoki *et al.* revealed that CDI systems are widespread among Gram-negative bacteria but are present in only certain strains of any given species. Furthermore, some bacterial strains were found to encode multiple CDI systems.

Uropathogenic *E. coli* (UPEC) str. 536 encodes homologues of *cdiB* and *cdiA* but no obvious *cdiI* homologue. Close inspection of the *cdi* locus in UPEC str. 536 revealed a small ORF that was in the same relative location as *cdiI* from *E. coli* str. EC93 but lacked significant sequence identity. Expression of this ORF protected cells from growth inhibition mediated by cells expressing *cdiA* from UPEC str. 536 but not by cells expressing *cdiA* from *E. coli* str. EC93, suggesting that the immunity conferred by CdiI is specific to its cognate CDI system. These two CdiA proteins have a high degree of sequence identity throughout most of the protein, apart from divergent carboxyl termini (CdiA-CTs). The authors replaced the UPEC str. 536 CdiA-CT with that from *E. coli* str. EC93 and found that immunity to the resulting chimeric protein was provided by *E. coli* str. EC93 CdiI but not UPEC str. 536 CdiI, indicating that this divergent region provides the specificity for CDI immunity. The authors speculated that CdiI might function by binding to its cognate CdiA-CT. To test this idea, they used an affinity pulldown approach as well as a bacterial two-hybrid system to show that CdiA-CT and CdiI from UPEC str. 536, as well as the region 2 CdiA-CT and CdiI from *Dickeya dadantii* str. 3937, interact both *in vitro* and *in vivo*.

How, then, does CdiA inhibit growth in target cells? Purified *D. dadantii* str. 3937 region 2 CdiA-CT exhibited DNase activity, digesting linear and supercoiled plasmid DNA, and although UPEC str. 536 CdiA-CT could not digest DNA, it could cleave tRNA, suggesting that distinct CDI mechanisms exist in different bacteria. In both cases, the addition of the cognate CdiI blocked nuclease activity.

Finally, the authors carried out growth competition experiments both in culture with *E. coli* str. EC93 and on chicory plants with *D. dadantii* str. 3937. In both cases, they observed that the CDI$^+$ strains outcompeted strains in which the CDI systems had been disrupted, suggesting that the primary role for CDI systems is likely to be in intraspecies competition during colonization of a niche.

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ORIGINAL RESEARCH PAPER Aoki, S. K. *et al.* A widespread family of polymorphic contact-dependent toxin delivery systems in bacteria. *Nature* **468**, 439–442 (2010)