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MEETING REVIEW

Diversity of Contact-Dependent Growth Inhibition Systems of *Pseudomonas aeruginosa*

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ABSTRACT Contact-dependent growth inhibition (CDI) systems are used in bacterial competition to hinder the growth of neighboring microbes. These systems utilize a two-partner secretion mechanism to display the CdiA exoprotein at the bacterial cell surface. CdiA forms a long filamentous stalk that facilitates binding to a target cell and delivery of a C-terminal toxin (CT) domain. This CT domain is processed and delivered into the cytoplasm of a target cell upon contact. CDI systems also encode a cognate immunity protein (CdiI) that protects siblings and resistant targeted cells from intoxication by high-affinity binding to the CT. CdiA CT domains vary among strains within a species, and many alleles encode enzymatic functions that target nucleic acids. This variation is thought to help drive diversity and adaptation within a species. CdiA diversity is well studied in *Escherichia coli* and several other bacteria, but little is known about the extent of this diversity in *Pseudomonas aeruginosa*. The purpose of this review is to highlight the variability that exists in CDI systems of *P. aeruginosa*. We show that this diversity is apparent even among strains isolated from a single geographical region, suggesting that CDI systems play an important role in the ecology of *P. aeruginosa*.

KEYWORDS contact-dependent inhibition, *Pseudomonas aeruginosa*

Bacteria are complex social organisms that live in mixed communities where they must compete for limited space and resources (1). As a result, they have evolved multiple systems to scavenge nutrients and interfere with the growth of other microbes (2). Multiple transport proteins facilitate uptake of crucial nutrients (3), while siderophores scavenge key metals (4). Secreted antibiotics and bacteriocins act to inhibit or kill competitors beyond arm's reach (5–7). Bacteria can also intoxicate neighboring cells upon direct cell contact in several ways, including by outer membrane exchange (8) and type VI secretion and contact-dependent growth inhibition systems (9, 10). These contact-mediated competition systems are capable of bombarding competitors with an assorted array of antimicrobial effectors. Advances in whole-genome sequencing have rapidly increased our appreciation of the vast effector repertoire found throughout these bactericidal systems (11).

Contact-dependent growth inhibition (CDI) systems consist of an outer membrane Omp85-TpsB superfamily β-barrel protein (CdiB) that facilitates secretion of a large exoprotein (CdiA) from the periplasm onto the bacterial cell surface in a type V_b (i.e., two-partner) secretion process (12, 13). CdiA then facilitates delivery of its own C-terminal toxin domain into a neighboring bacterium to kill or prevent the growth of susceptible target strains (14). Siblings are protected from intoxication because they produce an immunity protein (CdiI) to neutralize the toxin domain. CdiA is a polymorphic toxin, meaning that different bacterial strains within a species contain CdiA proteins with different toxin domains (15). Many of these target nucleic acids for degradation (16, 17), while others have been shown to form pores in the inner membrane (18). This

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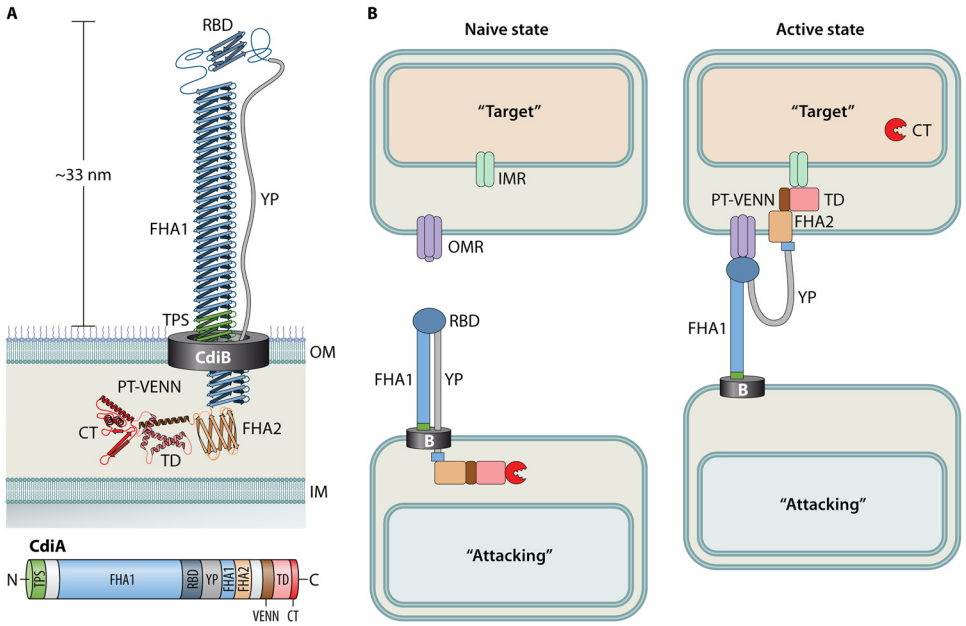

FIG 1 Current model of CDI intoxication. (A) A cartoon diagram of CdiA in its native state at the cell surface of an attacking bacterium, as modeled by Ruhe et al. (25). The corresponding CdiA protein domains are illustrated to scale below the cartoon. See the text for details. (B) CdiA-dependent intoxication of a target bacterium. Upon binding of the CdiA-RBD to an outer membrane receptor (OMR) on a target bacterium, the periplasmic domains are released from the attacking bacterium to deliver the CT into the cytosol of the target bacterium. This is thought to occur through a stepwise process whereby the FHA2 domain delivers the TD and CT into the periplasm of a target cell and the TD binds an inner membrane receptor (IMR) that facilitates translocation of the CT into the cytosol through an unknown mechanism.

diversity is thought to drive important interactions that help shape bacterial populations (19).

A considerable amount of effort has been devoted to understanding variation in the CDI system of *Escherichia coli* and several other bacterial species (15, 20, 21), but this system has been less thoroughly examined in *Pseudomonas aeruginosa*. Here, we review what can be inferred about the *P. aeruginosa* CDI system by comparing it to the CDI system of *E. coli*, and we describe its genetic organization and diversity.

CURRENT MODEL OF CdiA-DEPENDENT INTOXICATION

As with other bacteria, the key component of the *P. aeruginosa* CDI system is the CdiA exoprotein. Little experimental work has been done on *P. aeruginosa* CdiA, but its mechanism can be inferred from what is known about CdiA of *E. coli*. CdiA is a large modular protein containing several domains with distinct functions. Some domains are highly conserved within a species, while others appear to rearrange in various combinations to generate functional diversity (22, 23). All CdiA proteins contain a conserved two-partner secretion (TPS) domain (Pfam identifier [ID] PF05860) and an FHA1 domain (Pfam ID PF05594) of variable length (Fig. 1A). The FHA1 domain is composed of dozens of β-strands that presumably stack to form a rigid extracellular filament called a β-helix, as described in the filamentous hemagglutinin protein of *Bordetella pertussis* (24). In *E. coli*, this long extracellular filament is anchored at the cell surface and presents a receptor binding domain (RBD) at its distal end (Fig. 1A) (25). The RBD is required for adherence to neighboring bacteria during CDI (Fig. 1B) (22, 25). Adjacent to the RBD is a stretch of amino acids with an abundance of tyrosine and proline residues that is referred to as the YP domain. This region is thought to act as an extended linker between the RBD and the following FHA2 domain (Pfam ID PF13332). In its native conformation, the FHA2 and remaining C-terminal domains are sequestered in the periplasm of the attacking cell until contact of the RBD with its cognate receptor on a target bacterium (25). The signal of receptor binding is then likely transduced through

the YP domain to release the sequestered domains and continue the intoxication process (Fig. 1B).

In *E. coli*, the FHA2 domain was shown to localize with the outer membrane of target cells following receptor binding, and labeling experiments suggest that this domain facilitates translocation of the remaining C-terminal domains across the target outer membrane into the periplasm (Fig. 1B) (25). The final destination of a C-terminal toxin (CT) domain depends upon its mechanism of action. CTs that disrupt membrane potential by forming pores can function from within the periplasm by attacking the periplasmic face of the inner membrane (18). In contrast, nucleic acid-degrading CTs must be further translocated to the cytosol to reach their target. This is thought to be accomplished by specific translocation domains (TD), sandwiched between the FHA2 and CT domains, which interact with specific inner membrane receptors (IMRs) to facilitate translocation of the CT across the inner membrane into the target cell cytosol (Fig. 1B) (23). IMRs that have been identified in *E. coli* function as various metabolite permeases, but the inherent transport function of these proteins is not required for CDI intoxication. Rather, it was proposed that the inner membrane-associated AAA+ superfamily protein transporter FtsH may be responsible for the actual translocation of the CT across the inner membrane and that TD binding to a cognate IMR facilitates an interaction with FtsH. This type of interaction has since been modeled with the secreted bacteriocin colicin D (26). Residues 410 to 437 of the colicin D central domain constitute a binding site for interaction with the inner membrane type I signal peptidase LepB. This interaction is required for FtsH-dependent import of the colicin D C-terminal RNase domain (CRD) and release of the CRD into the target cell cytoplasm (27). Likewise, it is thought that the CdiA CT separates from the TD during translocation into the target cell (28). Some have proposed the *E. coli* PT-VENN domain (Pfam ID PF04829) immediately preceding the TD domain may facilitate cleavage of the CT from the filament for proper delivery to the target bacterium (29), but it is also possible that FtsH-dependent cleavage of the CT occurs during import, as occurs with the nuclease colicins (27, 30).

GENETIC ORGANIZATION OF *cdi* LOCI IN *P. AERUGINOSA*

CDI systems are widespread among proteobacteria (15), and individual strains within these different groups may possess multiple *cdi* loci (31). An analysis of 104 completed *P. aeruginosa* genomes representing strains from four different continents (NCBI strains [see Table S1 in the supplemental material]) revealed that all strains contain a *cdi* homolog in the same genetic location upstream of the gene encoding the type III secretion system effector ExoT (Fig. 2A). In addition, 81% of strains were found to contain a second *cdi* locus downstream of the ferrioxamine receptor gene (*foxA*) (Fig. 2A). In *P. aeruginosa* strain PAO1, the *cdiA* genes located in proximity to *exoT* and *foxA* were previously designated *cdiA1* (PA0041) and *cdiA2* (PA2464), respectively (Fig. 2A) (32, 33). A single promoter upstream of *cdiB* is thought to drive expression of the entire *cdiBAI* operon at each locus (Fig. 2A). This genetic arrangement is similar to that observed in *E. coli* and many other Gram-negative bacteria (14, 16). In contrast to the high proportion of *P. aeruginosa* strains containing multiple *cdi* loci, only approximately 15% of sequenced *E. coli* strains contain any *cdi* genes (19), suggesting an important role for CDI systems in *P. aeruginosa* biology.

Variations in both *cdiA1* and *cdiA2* suggest that the 3′ end of *cdiA* undergoes diversification through homologous recombination (Fig. S3), but how such genetic material would spread by horizontal gene transfer in different *P. aeruginosa* populations is unclear. Immediately downstream of the *cdi2* locus on the complementary strand is another polymorphic gene encoding a predicted outer membrane protein with numerous Rhs repeats (Pfam ID PF05593) (Fig. 2B). This gene also contains 3′ termini that differ between strains (data not shown). Polymorphic genes encoding Rhs repeat proteins have been associated with type VI secretion systems (34); however, the function of these specific genes in *P. aeruginosa* is unknown. It is intriguing that two polymorphic genes with variations in their respective 3′ ends share an intergenic region, and the question remains as to whether there is a shared mechanism of 3′

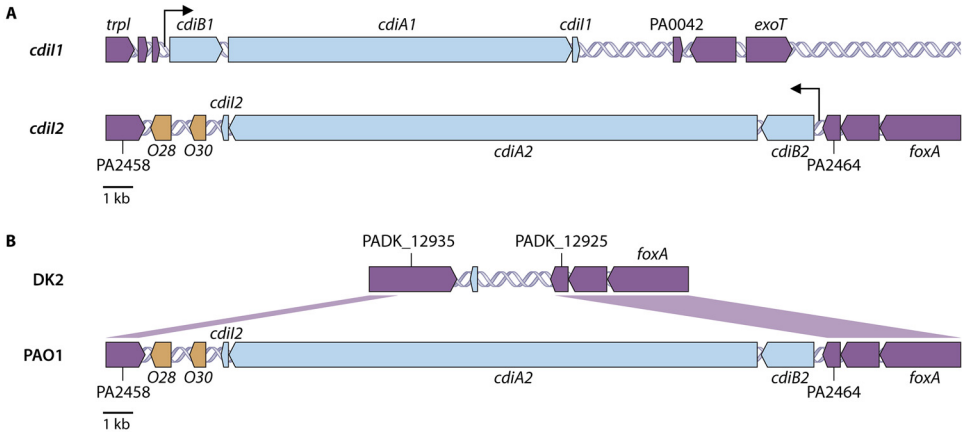

FIG 2 Organization of *P. aeruginosa* CDI systems. (A) The *cdi1* and *cdi2* loci are displayed as blue arrows in their genetic orientation within *P. aeruginosa* strain PAO1. Core genes outside the *cdi* loci are colored purple. O28 and O30, colored orange, represent orphan immunity genes located in the intergenic region downstream of the functional *cdiBAI* cluster. Black arrows indicate the predicted promoter sites. (B) Alignment of the *P. aeruginosa* strain PAO1 *cdi2* locus and flanking regions to the same genetic region in strain DK2, which does not contain a *cdi2* locus.

diversification through some common mobile genetic element. Of note, this Rhs repeat protein is truncated in *P. aeruginosa* strain PAO1 (PA2458) (Fig. 2B), but this not the case for most *cdiA2*-containing strains. All other *cdi2* flanking regions appear intact, with no obvious genetic signatures to suggest a mechanism for horizontal transfer. *P. aeruginosa* CdiA1$^{UCBPP\text{-}PA14}$ was found on an isolated contig from *Enterobacter cloacae* strain e403 (contig ERS380557SCcontig000071), suggesting that horizontal transfer of *cdiA* may occur; however, no additional *P. aeruginosa cdiA* alleles have been identified outside the genus *Pseudomonas*. CDI systems have been observed within mobile genetic elements in other organisms. *E. coli* and *Burkholderia pseudomallei* carry *cdi* gene clusters on different pathogenicity islands (35), and *Xenorhabdus doucetiae* contains a *cdi* locus on an integrative conjugative element (36). *Burkholderia thailandensis* E264 carries a *cdi* locus on a novel 210-kb composite transposon that can exist as an extrachromosomal circular double-stranded DNA (dsDNA) intermediate referred to as a "megacircle" (37). Since *P. aeruginosa* is an environmental bacterium, it is possible that the reservoirs of mobile elements that facilitate horizontal transfer of *cdiA* genes in *P. aeruginosa* exist in this niche and not in the clinical setting, where most strains are obtained for study (38). Additional sampling from natural environments where this type of diversification likely occurs may help answer some of these questions.

THE AMINO-TERMINAL HALF OF CdiA IS CONSERVED

There is substantial similarity in the overall domain structure between the CdiA proteins of *E. coli* and *P. aeruginosa*, suggesting that they likely adopt the same general architecture (Fig. 3). *P. aeruginosa* CdiA proteins contain longer FHA1 domains than those of *E. coli*, with the FHA1 domain of CdiA2 having over twice the length (Fig. 3). In *E. coli* strain EC93, the CdiA filament was shown by cryo-electron tomography to be approximately 33 nm in length (25). If the model of CdiA in its native conformation is correct, the RBD would be the most distal domain on the filament (Fig. 1A). The length of the primary amino acid sequence to the end of the RBD in *E. coli* EC93 is approximately 1,635 residues. Based upon the ratio of this number of residues over the measured length by tomography in *E. coli*, the *P. aeruginosa* CdiA1 and CdiA2 filaments are predicted to extend approximately 45 nm and 84 nm, respectively. Consequently, the YP domain of CdiA2 is larger than that of CdiA1, which may accommodate the longer β-helical filament of CdiA2. The consequences of these size differences are unknown, as both systems promote bacterial competition (32, 33).

The only region of sequence divergence in the amino half of CdiA occurs in the

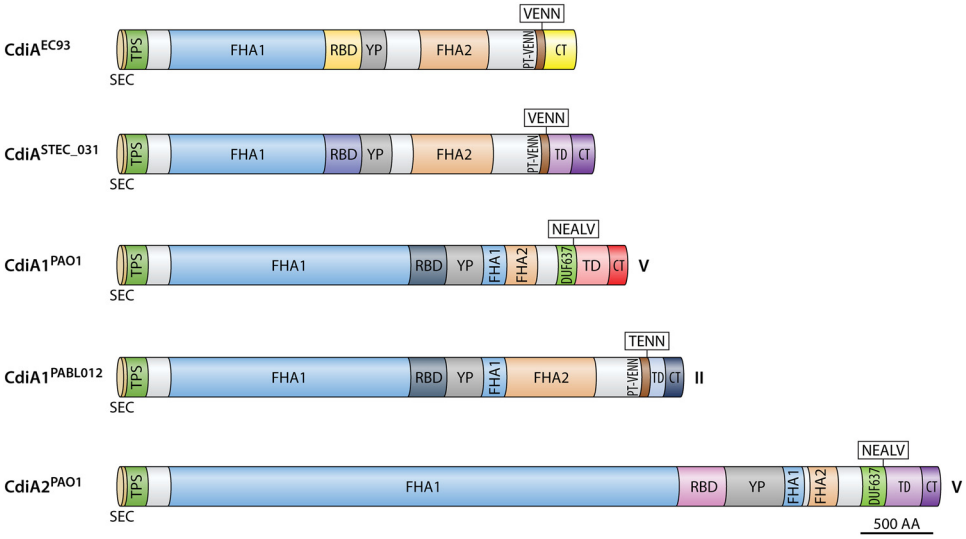

FIG 3 CdiA protein diversity. CdiA protein domain organization of *E. coli* CdiA^{EC93} and CdiA^{STEC_031} based upon the work of Ruhe et al. (24) and of CdiA1^{PAO1}, CdiA1^{BL012}, and CdiA2^{PAO1} from this work. Roman numerals indicate the *P. aeruginosa* CdiA class type (20). Colors reflect the different predicted protein domains from sequence alignments, domain searches, and domain descriptions in *E. coli*. Sec, general secretory signal.

receptor-binding domain (RBD). Four separate CdiA-RBDs have been identified in *E. coli* (22, 39, 40), and 5 have been identified in *P. aeruginosa* (denoted *rbdA* through *rbdE*) based upon sequence alignments (see Fig. S1 in the supplemental material). The primary amino acid sequences of the respective RBDs from *P. aeruginosa* and *E. coli* do not share any similarity and therefore do not provide direct insight into the specific cellular receptors. Interestingly, all *P. aeruginosa* CdiA1 proteins contain the same RBD, which was not observed in any of the CdiA2 proteins. This suggests that recombination between this portion of the two *cdi* loci does not occur or is extremely rare. Of the 4 identified RBDs from *E. coli*, 3 have respective target cell receptors that have been identified. The RBD from CdiA^{EC93} binds to BamA, a component of the outer membrane β-barrel protein assembly complex, through specific interactions that target extracellular loops L6 and L7 (39, 41). CdiA^{EC536} interacts with extracellular loops L4 and L5 of osmoporin OmpC (40), and CdiA^{STEC031} interacts with the outer membrane nucleoside transporter Tsx (22). Bacteriocins from *E. coli* are known to hijack these same outer membrane protein transporters to mediate their toxic effects. For example, the *E. coli* colicin N was shown to use OmpF as a receptor for binding and translocation (42) while colicin 10 uses Tsx (43). Unlike bacteriocins, which typically require energy from the Ton or Tol systems for toxin uptake by the receptor (5), CDI systems do not have this same energy dependency for intoxication, and the interaction of the CdiA-RBD with its cognate receptor is thought only to promote intercellular binding and not outer membrane translocation of CT into the periplasm (44). The receptor overlap between bacteriocins and CdiA proteins in *E. coli* suggests a similar relationship in *P. aeruginosa*. The *Pseudomonas* bacteriocin Pyocin S2 binds the ferripyoverdine receptor FpvA (45, 46), while pyocin S5 uses the ferripyochelin receptor FptA for uptake (47). In addition, the lectin-like bacteriocin LlpA interacts with specific residues on extracellular loop 6 of BamA to facilitate intoxication (48), and the colicin M-like bacteriocin PaeM4 uses the heme receptor HxuC (49). These proteins are possible candidates for CdiA receptors in *P. aeruginosa*; however, further work is needed to determine the exact receptors used by different *P. aeruginosa* CdiA proteins.

THE CARBOXY-TERMINAL HALF OF CdiA IS DIVERSE

CDI systems among *Pseudomonas* species can be divided into 5 classes based upon a set of defined protein motifs immediately preceding the translocation domain (TD) that demarcate the highly variable carboxy terminus of CdiA (32). Similar pretoxin

TABLE 1 Putative activity and distribution of CTs

Function	No. of unique CTs	Frequency of CTs[a]
Unknown	18	39.2
Deaminase	5	16.6
Methyltransferase	1	0.3
Nuclease	7	15.0
Peptidase	2	1.1
RNase	2	7.1
tRNase	4	20.7

[a]Among all *P. aeruginosa* strains analyzed.

motifs have been described for *E. coli* (VENN) and *Burkholderia* species (NXXLYN) (15). In *Pseudomonas* species, these motifs include WVHN (class I), TENN (class II), LYVT (class III), DAMV (class IV), and NEALV (class V), of which *P. aeruginosa* CdiA proteins are restricted to the class II and class V groups. Class II CdiA proteins of *P. aeruginosa* contain a large FHA2 domain followed by a PT-VENN domain (Pfam ID PF04829) and a TENN motif (Fig. 3). Class V CdiA proteins contain a smaller FHA2 domain followed by a DUF637 domain (Pfam ID PF04830) and a NEALV motif (Fig. 3). As stated, the FHA2 domain was proposed to facilitate translocation of the final CdiA C-terminal domains across the target cell outer membrane in *E. coli* (25). It is unclear whether the smaller class V FHA2 domain could also facilitate outer membrane translocation of the C terminus in *P. aeruginosa*.

The greatest diversity in CdiA proteins occurs at their carboxy termini (Fig. 3). Studies in *E. coli* have shown these regions to be highly modular, with various pairings of different TD and CT domains (23). An analysis of CdiA proteins from 104 *P. aeruginosa* NCBI strains uncovered a total of 9 different TDs and 37 different CTs that were arranged in different combinations (see Fig. S3 in the supplemental material). Eighteen of the 37 CTs had no known function based upon peptide sequence, and most CTs with a predicted function had enzymatic activities that target nucleic acids (Table 1). CTs containing a MafB19-like deaminase domain (Pfam ID PF14437) or an EndoU RNase domain (PF14436) occurred the most (Fig. 4); however, the primary amino acid sequences within these predicted groups were highly diverse (Table S3). This sequence diversity was also mirrored in the respective CdiI proteins. Both MafB19-like deaminase domains and EndoU RNase domains are common among protein systems involved in bacterial competition (11, 50), and such sequence diversity has been shown to dictate substrate specificity for EndoU toxins in other bacterial species (51). Overall, *P. aeruginosa* CDI systems contain substantial C-terminal diversity on par with that observed in CDIs from other Gram-negative bacteria.

REGIONAL AND GLOBAL CdiA DIVERSITY

It is conceivable that CDI diversity in *P. aeruginosa* occurs on a global scale but that only a few distinct CTs are found in any one geographic location. Alternatively, *P. aeruginosa* bacteria encoding many different CTs may coexist in the same region. To examine this question, we determined the extent of CdiA diversity among *P. aeruginosa* isolates within a restricted geographical area. Sequences from a published collection of 100 clinical *P. aeruginosa* bloodstream (PABL) isolates from Northwestern Memorial Hospital in Chicago, IL, were analyzed for their CdiA diversity (see Table S2 in the supplemental material) (52). As with the NCBI genomes, CdiA homologs of the PABL strains were highly diverse. All 9 TDs and 5 RBDs were observed within this small collection, along with 27 CTs, including 1 new CT. The proportions of each TD and RBD were equivalent between the NCBI and PABL strains, but the proportions of individual CTs varied between the two collections (Fig. 4).

Sequence divergence in the CdiA carboxy terminus suggests that the TD and CT domains recombine as modular units to generate substantial diversity within the species; however, distribution of the different TD and CT domains suggests some biological limitation to the extent of this recombination in *P. aeruginosa*. Of the 9 TDs

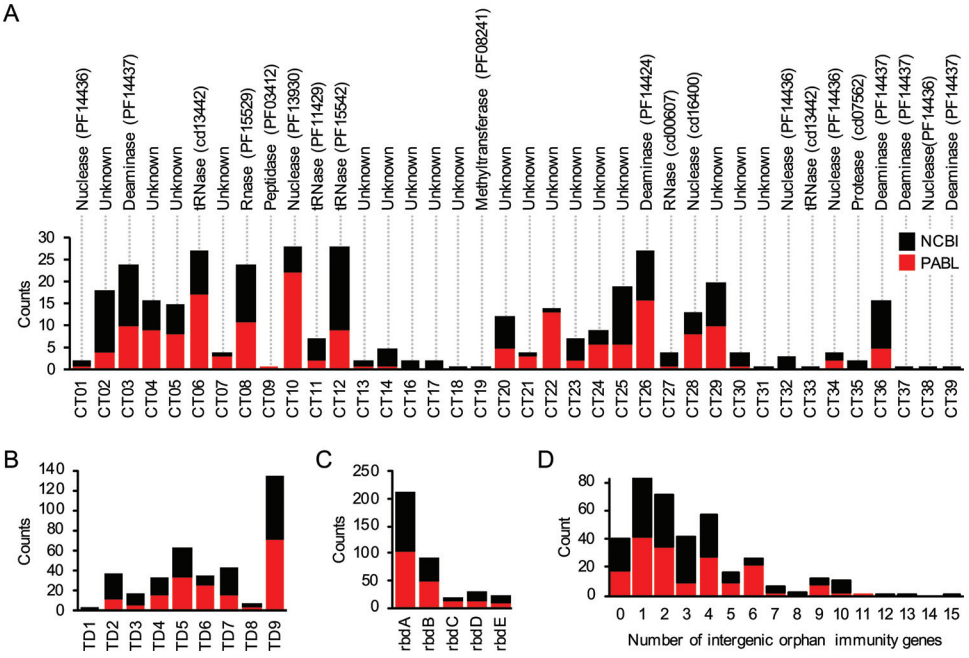

FIG 4 Distribution of CdiA domains in the PABL and NCBI strain collections. (A to C) The occurrence of each CT (A), TD (B), and RBD (C) variant was determined for the *P. aeruginosa* PABL and NCBI strain collections. Each bar represents the number of strains that contain the indicated domain variant, which are labeled based on designations in Fig. S3. Notations above the CT graph reflect predicted enzymatic functions determined by a conserved domain search (https://www.ncbi.nlm.nih.gov/cdd). (D) Counts represent the number of intergenic orphan *cdiI* genes at a *cdi* locus for each strain collection.

observed in *P. aeruginosa*, 6 were unique to the *cdi1* locus, 2 were unique to the *cdi2* locus, and only a single TD was shared between the two loci (Fig. 5). Except for two instances, each of the CTs was always associated with a specific TD. Furthermore, the distribution of CT domains was found to be evenly split between the two *cdi* loci with no overlap. This distribution suggests that there is little recombination between the two *cdi* loci; however, recombination must help drive *cdiA* diversity within a given locus. The tight association of CTs with specific TDs also suggests that CTs may have evolved preferred translocation pathways that are the most efficient for a given CT (23).

Analysis of the CdiA phylogenetic distribution revealed that strains within the same clonal lineage tended to share the same *cdiA* allele, but a given *cdiA* allele could be found in several unrelated clonal lineages. Likewise, different clades typically contained CdiA proteins with different combinations of RBD, TD, and CTs (Fig. 6). This suggests that substantial recombination of CdiA C terminus alleles occurs in *P. aeruginosa* and, as a consequence, may facilitate the evolution of clonal lineages, even within isolates from a single type of infection at one hospital.

ORPHAN IMMUNITY GENES

cdi loci often contain orphan 3′ *cdiA* fragments consisting of partial CT genes and their cognate immunity genes in the intergenic region downstream of the functional *cdiA* gene (Fig. 1A) (16). While these orphan CT gene fragments no longer facilitate bacterial competition, the orphan immunity genes are functional and may protect bacteria from assault by strains expressing the corresponding CdiA CTs (16). Some investigated *P. aeruginosa* strains had no orphan genes at either *cdi* locus, while others had up to 15 orphan immunity genes (Fig. 4D). On average, an individual *P. aeruginosa* strain was observed to contain 2 and 4 orphan immunity genes at the *cdi1* and *cdi2* loci, respectively. Of particular interest was a unique orphan that encompassed an entire class V carboxy terminus, including the FHA2 domain, with a unique CT (CT15). The coding sequence for this unusually large orphan motif occurred immediately downstream of a functional class II *cdiA*1 gene in *P. aeruginosa* NCGM2 and closely related

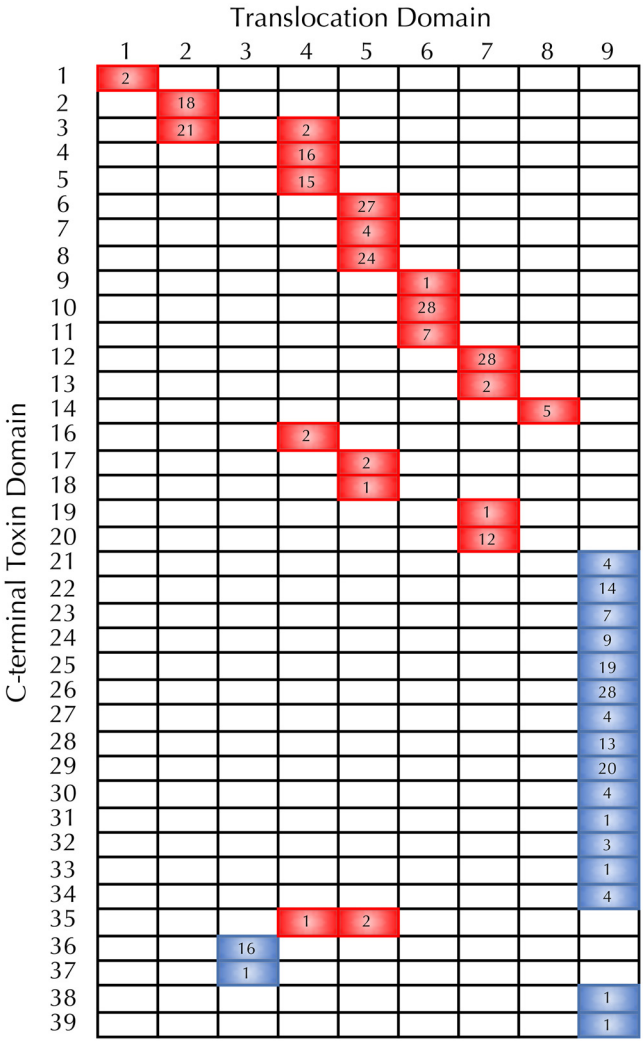

FIG 5 *P. aeruginosa* CdiA C-terminal diversity. Pairwise comparison of CdiA translocation domains (TD) and respective C-terminal toxin (CT) domains were performed for the NCBI and PABL strain collections. Cells colored red indicate that the TD-CT combination was observed at the CDI1 locus, and blue indicates the CDI2 locus. Numbers indicate the number of strains with that observed TD-CT combination.

strains. This orphan CT was predicted to contain a ParB-like nuclease domain (smart00470) and was never observed as a functional CdiA molecule in either the NCBI or PABL collections. Further investigations revealed that all class II *cdiA* genes encode a class V *cdiA* C terminus in the immediate downstream intergenic region. Often, this orphan class V C terminus was the actual class V CdiA C terminus of the closest phylogenetic neighbor (Fig. 6). As an example, the *cdiA1* class V C-terminal allele of PABL001 can be found just downstream of the *cdiA1* class II C-terminal allele of the nearest phylogenetic neighbor, PABL012 (Fig. 6; see Fig. S4 in the supplemental material). Thus, it appears that a class II *cdiA* C terminus coding sequence can insert into an established class V *cdiA1* gene, effectively shifting the original class V C terminus coding sequence into the downstream intergenic region (Fig. 7). This insertion occurs just upstream of the region encoding the FHA2 domain. Neither the mechanism of this insertion nor the source of the class II *cdiA* C terminus is known. These data suggest that the class V alleles were ancestral at the *cdi1* locus and that class II alleles were later horizontally acquired and inserted. Moreover, this phenomenon was only observed at the *cdi1* locus, as *cdiA* genes at the *cdi2* locus encoded only class V C termini (Fig. 6). Overall, this may indicate that separate mobile elements encoding CdiA C termini

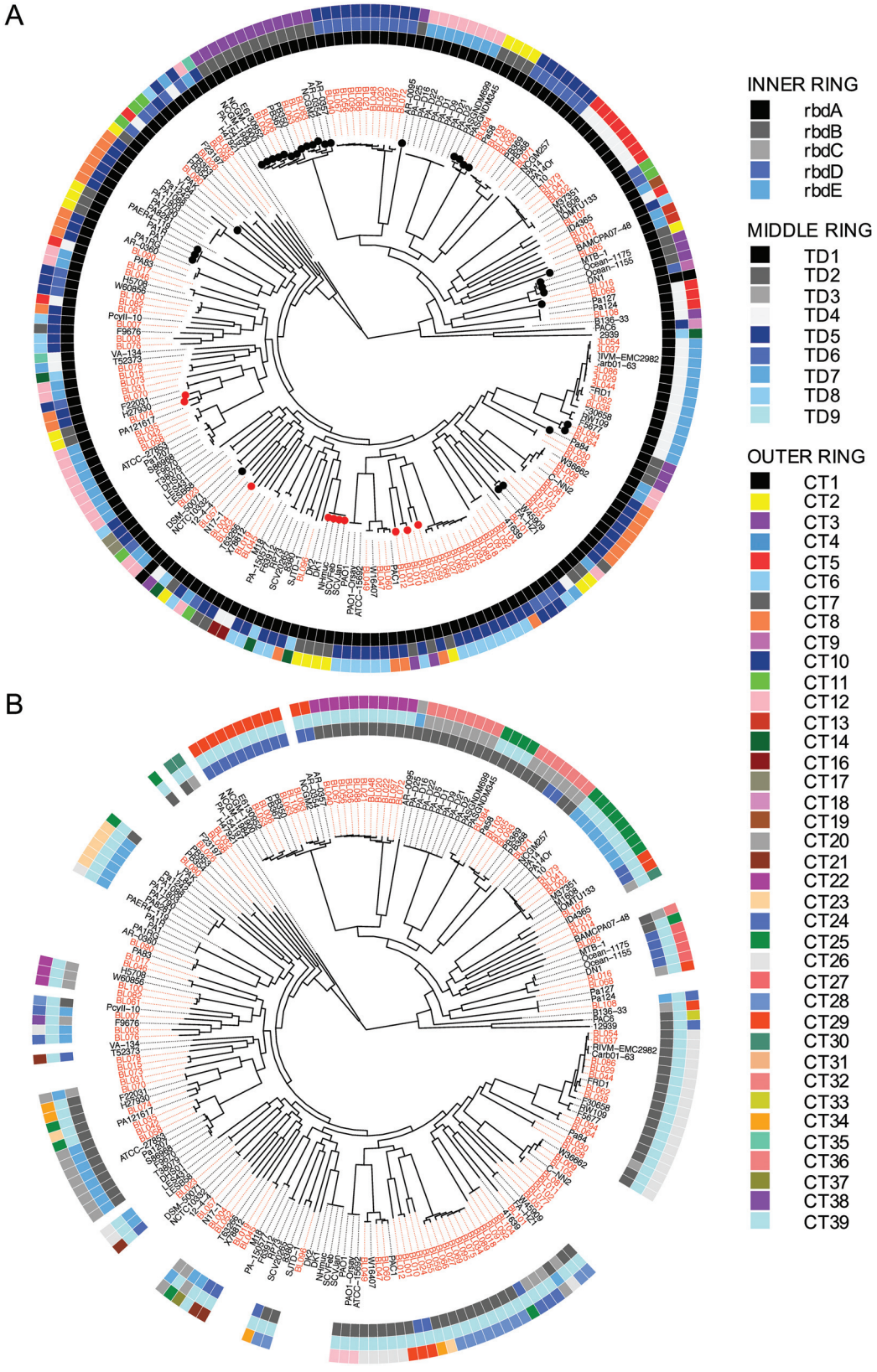

FIG 6 Phylogenetic distribution of *cdiA* alleles. The RBDs (inner ring), TDs (middle ring), and CTs (outer ring) for CdiA1 (A) and CdiA2 (B) were mapped onto maximum likelihood core genome trees of the PABL (red) and NCBI (black) strains. Ring colors represent different variants of the respective domains as noted in Fig. S1 and S3. Branches with dots at the tips indicate a class II C terminus. A red circle means the downstream orphan class V CdiA C terminus is the same as the functional class V CdiA C terminus of the closest phylogenetic neighbor.

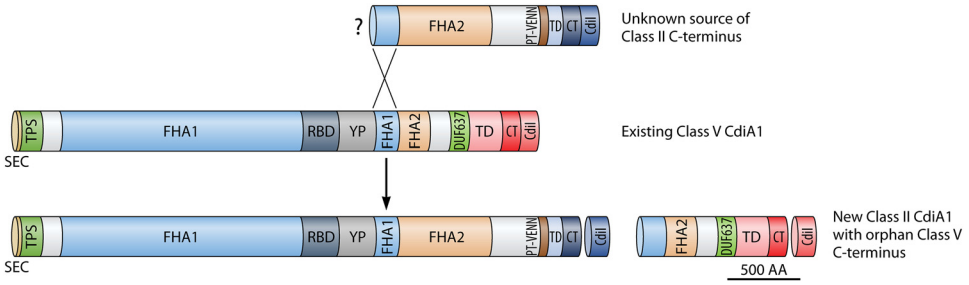

FIG 7 Illustration of *cdiA1* C-terminal class switching. Alignments from Fig. S4 indicate that a *cdiA* class II C terminus can recombine with a region of conserved sequence immediately upstream of the FHA2 domain, shifting the original class V C terminus into the downstream intergenic region. The source of the class II C terminus is unknown.

recombine with the *cdi1* and *cdi2* loci, but more work is needed for a complete understanding of CdiA diversification in *P. aeruginosa*.

CONCLUSIONS

This report reveals the breadth of *P. aeruginosa* CDI diversity and suggests that these systems play an important role in shaping populations within the species. Studies have shown that the majority of bacterial proteins are modular, created by genetically recombining functional domains in various combinations (53). This is especially true for many proteins involved in bacterial competition (11). Such diversity is thought to drive competition within a particular niche (2) and may lead to coordinated or symbiotic actions that promote survival or niche expansion (54).

Understanding the diverse biology of CDI systems may have implications outside bacterial competition. In some organisms, CDI systems have been shown to mediate behaviors independent from their bacterial growth inhibition function. CDI systems in *Burkholderia thailandensis* promote biofilm growth in a process termed "contact-dependent signaling" (CDS) (55). Aggregation phenotypes associated with CDI systems in *Erwinia chrysanthemi* EC16, *Xylella fastidiosa*, and *Xanthomonas axonopodis*, are required for bacterial virulence on plant hosts (56–58). In *Neisseria meningitidis*, mutants of the *cdiA* homolog *hrpA* were attenuated for intracellular survival in HeLa cells (59), and *Pseudomonas aeruginosa* PAO1 *cdiA* mutants had decreased virulence on lettuce and in *Caenorhabditis elegans* infection models (33). For many of these examples, it is not known whether these phenotypes can be attributed to the specific CT or if they are a general function of the CdiA protein. Thus, understanding the function and diversity of *cdiA* alleles in *P. aeruginosa* and other microbes may help elucidate how these systems aid in colonization and virulence. Ultimately, it is clear that CDI systems play an important role in the biology of many Gram-negative bacteria.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JB.00776-18.

SUPPLEMENTAL FILE 1, PDF file, 1.5 MB.

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