results from metabolic reconstruction, which suggests that an unusual growth factor may play a role in the ecology of this organism. 

*P. ubique* has taken a tack in evolution that is distinctly different from that of all other heterotrophic marine bacteria for which genome sequences are available. Evolution has divested it of all but the most fundamental cellular systems such that it replicates under limiting nutrient resources as efficiently as possible, with the outcome that it has become the dominant clade in the ocean.

**References and Notes**

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**Supporting Online Material**

www.sciencemag.org/cgi/content/full/309/5738/1242/DC1

Materials and Methods

Tables S1 to S3

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References

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**Contact-Dependent Inhibition of Growth in Escherichia coli**

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Bacteria have developed mechanisms to communicate and compete with each other for limited environmental resources. We found that certain *Escherichia coli*, including uropathogenic strains, contained a bacterial growth-inhibition system that uses direct cell-to-cell contact. Inhibition was conditional, dependent upon the growth state of the inhibitory cell and the pili expression state of the target cell. Both a large cell-surface protein designated Contact-dependent inhibitor A (CdIA) and two-partner secretion family member CdIB were required for growth inhibition. The CdAB system may function to regulate the growth of specific cells within a differentiated bacterial population.

Bacteria communicate with each other in multiple ways, including the secretion of signaling molecules that enable a cell population to determine when it has reached a certain density or that a potential partner is present for conjugation (1, 2). Cellular communication can also occur through contact between cells, as has been shown for *Myxococcus xanthus*, which undergoes a complex developmental pathway (3, 4). Here we describe a different type of intercellular interaction in which bacterial growth is regulated by direct cell-to-cell contact.

*Wild-type Escherichia coli* isolate EC93 inhibited the growth of laboratory *E. coli* K-12

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strains, such as MG1655, when the bacteria were mixed together in shaking liquid culture (Fig. 1A). In contrast, E. coli K-12 strains in general (e.g., EPI100) did not express growth-inhibitory activity (Fig. 1A). We isolated genes from strain EC93, cdiA and cdiB, that when expressed in E. coli K-12 conferred a growth-inhibitory phenotype (Fig. 1A). Growth inhibition was dependent upon the growth state of the inhibitory cells, occurring in logarithmic but not stationary phase. Target cells, however, were inhibited regardless of their growth phase (fig. S1). Protein synthesis also appeared to be required for inhibition, because cdiA+B+E. coli inhibitor cells pre-treated for 2 hours with chloramphenicol did not have measurable inhibitory activity (fig. S2). Experiments measuring the inhibitor-to-target ratio over time (fig. S3) indicate that one cdiA+B+E. coli cell inhibited the growth of multiple target cells. Starting at an initial ratio of 1 inhibitor to 10 target cells, after 1 hour this ratio increased more than a thousandfold (fig. S3). In contrast, a control experiment with cdiAB-negative cells in place of cdiA+B+E. coli inhibitory cells showed that the control-to-target ratio was not altered over the 3-hour time course (fig. S3).

The growth-inhibitory activity did not appear to be a colicin, which is a secreted antimicrobial peptide, because supernatant solutions from logarithmic phase EC93 cultures lacked inhibitory activity even when a known inducer of colicin synthesis, mitomycin C, was present (5, 6). To test the possibility that induction of inhibitory activity might occur only when target cells are present, we prepared conditioned medium from a mixed culture of cdiA+B+E. coli inhibitor cells and E. coli K-12 target cells (with a 10:1 inhibitor-to-target ratio). Viability counts for the top and bottom chambers were measured after incubation (hours). Data are means ± SEM; n = 2.

Fig. 1. Contact-dependent inhibition of bacterial growth. (A) Target E. coli MG1655 tetR cells were mixed with the following test strains: E. coli EC93 inhibitory cells (squares); E. coli K-12 EP100 strR containing pDAL660Δ1-39 (cdiA+B+, triangles); and control E. coli K-12 EP100 strR (circles). The inhibitor-to-target ratio for these experiments was 10:1. At the times indicated, viable target cell counts were obtained. CFU, colony-forming units. (B) E. coli CDI+E. coli K-12 (DL4577) or CDI– E. coli K-12 (DL4527) were grown to logarithmic phase and added to the top chamber of a six-well plate containing either a 0.4-μm (solid bars) or an 8-μm (open bars) PET membrane. Target E. coli MG1655 tetR cells were added to the bottom well (in a 20:1 inhibitor-to-target ratio). Viable counts for the top and bottom chambers were measured after incubation (hours). Data are means ± SEM; n = 2.

Fig. 2. FACS analysis of contact-dependent growth inhibition. FACS and cell viability analyses of GFP- and DsRed-labeled E. coli were carried out as described (7). The relative fluorescence is indicated on the x and y axes with the filters shown. (A) CDI+E. coli inhibitor cells “I” constitutively expressing GFP (DL4905). (B) E. coli target cells “T” constitutively expressing DsRed (DL4920). (C) The inhibitory and target cells described above were mixed at a 1:4 inhibitor-to-target ratio and analyzed after 16 min of growth with shaking at 37°C. The aggregated cell population “A” contained at least one inhibitor cell and one or more target cells per particle. The target population contained free target cells that were not associated with inhibitor cells. (D) FACS sorting was used to isolate the free and inhibitor-bound target populations after the times of incubation indicated. Viability was scored as CFUs per particle sorted from each gated population. Free target cells are shown as open bars, and inhibitor-bound target cells are shown as solid bars. (E) As in (D), except that CDI+E. coli containing cdiA-FLAG1 (DL4955) was mixed with target cells (DL4920).
target ratio). Target cell viability was reduced more than a thousandfold after 1 hour of incubation (Fig. S4A), indicating substantial growth inhibitory activity. Conditioned medium from this 1-hour mixture, when added to fresh K-12 target cells, did not affect cell growth compared to conditioned medium from control cdiA–B– cell mixtures (Fig. S4B).

We tested the possibility that growth inhibition might occur through cell-to-cell contact by separating cdiA+/B+ inhibitory cells from target cells, using polyethylene terephthalate (PET) porous membranes in a six-well plate (Fig. 1B). Growth inhibition was not observed when contact between inhibitory cells and target cells was blocked by 0.4-μm pores; however, growth inhibition (~1000-fold) was observed when 8-μm pores were used, allowing inhibitor and target cell mixing. Addition of cdiA–B– negative control E. coli did not affect growth of target cells, regardless of pore size (Fig. 1B). We obtained similar results using EC93, from which the cdiAB genes were isolated for cloning into E. coli K-12 (5). A potential caveat is that a secreted inhibitory molecule might bind to the PET membrane and be sequestered or inactivated. However, addition of excess PET membranes did not affect the ability of cdiA+/B+. E. coli K-12 to inhibit cell growth (Fig. S5). Similar results were obtained with polycarbonate membranes (5). These results support the hypothesis that growth inhibition mediated by cdiAB requires cell-to-cell contact, designated as contact-dependent inhibition (CDI).

It is possible that the inhibitory factor could be an unstable secreted molecule that is only effectively delivered to target cells in close proximity. To address this possibility, we mixed fluorescently labeled cdiA+/B+ inhibitory cells [green fluorescent protein (GFP)–labeled] with E. coli K-12 target cells [Discosoma red (DsRed)–labeled], and after incubation with shaking, the cell mixtures were sorted by fluorescence-activated cell sorting (FACS). We observed three cell populations, corresponding to green inhibitory cells, red target cells, and cell aggregates containing at least one inhibitory and one target cell per aggregate particle (Fig. 2, A to C). The appearance of the aggregated particles was dependent upon cdiAB, indicating that CdiAB mediates intercellular binding (5). If cell-to-cell contact were required for growth inhibition, then the viability of target cells bound to inhibitory cells should decrease more rapidly than the viability of free target cells (Fig. 2D). The viability of aggregated targets compared with free targets decreased at time points of 8 min and longer, indicating that observable growth inhibition occurred after 4 to 8 min of contact. Although the viability of free targets was only marginally reduced at times up to 8 min, at later times viability was significantly reduced, albeit at a lesser rate than for aggregated targets (Fig. 2D). It is likely that the observed reduction in viability of “free” target cells was primarily the result of prior contact with cdiA-B+ inhibitory cells and release from cell aggregates.

The rapid decrease in viability of the aggregated targets might be due to a nonspecific effect of intercellular binding. FACS analysis was carried out using a cdiA mutant (Fig. 3A, cdiA–FLAG1) that no longer conferred CDI but retained intercellular adhesion. Under these conditions, the viability of aggregated target cells was not reduced compared to free target cells over the same time course (Fig. 2E), showing that the rapid decrease in viability of target cells bound to inhibitory cells required CDI activity. These results strongly indicate that CdiAB mediates growth inhibition through cell-to-cell contact.

We cloned a DNA region from E. coli EC93 that conferred a CDI+ phenotype to E. coli K-12 and generated 15 base-pair (bp) insertions within the region (7). Stop codon insertions within open reading frames (ORFs) designated cdiA and cdiB (GenBank accession no. DQ100454) abolished CDI activity (Fig. 3A), showing that the cdiA and cdiB ORFs were necessary for CDI. In addition, we identified a small ORF (Fig. 3A, cdiI) adjacent to cdiA that conferred full immunity to CDI (fig. S6), explaining why cells expressing cdiAB do not inhibit their own growth. The translated cdiA and cdiB ORFs showed significant amino acid sequence identity with two-partner secretion proteins (fig. S7) that are proteolytically processed during export to the cell surface (8). Using FLAG epitope tagging (Fig. 3A), we found that CdiA was expressed as a 303-kD protein on the cell surface (5), which was then processed to 284-kD and 195-kD proteins (Fig. 3B). A FLAG insertion in the cdiB ORF yielded a 56-kD protein, consistent with the predicted
size of CdiB. Proteolytic fragments of CdiA were detected in the growth medium (5) but were not growth-inhibitory (fig. S4), indicating that the secreted forms of CdiA are inactive.

High amino acid sequence identity was found between CdiA/CdiB and predicted proteins from uropathogenic E. coli (UPEC), including strain 536 (9). Complementation analysis indicated that UPEC 536 and four additional UPEC strains contain genes that are functional homologs of cdiB (fig. 3C) and cdiA (fig. S8). Bioinformatic analysis showed that Yersinia pestis (plague) and Burkholderia pseudomallei (meliodosis) also encode possible CdiAB homologs (fig. S7). Filamentous hemagglutinin from Bordetella pertussis (whooping cough) appeared more distantly related, sharing sequence identity to CdiA primarily in the N-terminal portion of the protein (fig. S7).

The cdiAB homologs in UPEC 536 are present within pathogenicity island II (10), but a cdili homolog is not present, nor is it found in the sequenced genome of UPEC CFT073, which also contains a cdiAB homolog (11). This observation suggests that cdiAB expression in UPEC strains would inhibit their growth. Pathogenicity island II in UPEC 536 also contains a pyelonephritis-associated pil (pap) operon closely linked to cdiAB. Because Pap pilis are expressed at the cell surface, we tested the possibility that pap expression might affect CDI because contact between CdiA and the target cell surface could be blocked. E. coli K-12 constitutively expressing P pilis (12) or S pilis (13) showed resistance to CDI, whereas cells expressing type 1 pilis were 1000- to 10,000-fold more sensitive to growth inhibition (Fig. 4). Thus, resistance to CDI conferred by P and S pilis involves specific interaction(s) and is not likely to be the result of nonspecific steric hindrance that blocks cell-to-cell contact.

Many UPEC strains contain fim (type 1 pilis), pap (P pilis), and sfa (S pilis) operons (10, 11). The expression of these pilis types is normally subject to reversible off/on switching, generating diversity within bacterial populations by a differentiation mechanism (14, 15). Such a mechanism might play a role in the temporal control of the differentiation observed for UPEC strains inside bladder cells, during which the bacteria progress through distinct developmental stages, including a quiescent growth state (16). E. coli K-12 cells inhibited by CDI appear to be nonviable because of their lack of growth on agar medium. However, CDI-inhibited cells appeared to be viable because they excluded propidium iodide (3), a standard criterion for distinguishing viable cells from nonviable cells (17). The identification of this sophisticated mechanism in E. coli, with possible homologs in a broad range of species, opens the door for exploration of the potential roles of CDI in controlling bacterial development and pathogenesis.

References and Notes
7. Materials and methods are available as supporting material on Science Online.
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Genome-Wide RNAi Screen for Host Factors Required for Intracellular Bacterial Infection
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Most studies of host-pathogen interactions have focused on pathogen-specific virulence determinants. Here, we report a genome-wide RNA interference screen to identify host factors required for intracellular bacterial pathogenesis.

Using Drosophila cells and the cytosolic pathogen Listeria monocytogenes, we identified 305 double-stranded RNAs targeting a wide range of cellular functions that altered L. monocytogenes infection. Comparison to a similar screen with Mycobacterium fortuitum, a vacuolar pathogen, identified host factors that may play a general role in intracellular pathogenesis and factors that specifically affect access to the cytosol by L. monocytogenes.

During bacterial infections, macrophages play a critical role in eliminating engulfed pathogens. However, intracellular bacterial pathogens have evolved varying strategies to avoid elimination by host macrophages (1). One strategy used by pathogens, such as Mycobacterium tuberculosis, is to modulate the phagosomal compartment to allow for vacular replication (2). Other bacterial pathogens, such as Listeria monocytogenes, escape the phagocytic vacuole to enter the host cell cytosol where replication occurs (3). Whereas numerous bacterial determinants that facilitate intracellular infection have been characterized from diverse bacterial species (4), less is known about the host factors that are exploited or subverted by intracellular bacterial pathogens. Here, we report the results of a genome-wide RNA interference (RNAi) screen conducted in Drosophila SL2 cells to identify host factors required for infection by L. monocytogenes, a cytosolic pathogen. In addition, we present the results of a comparison to a similar RNAi screen conducted with Mycobacterium fortuitum, a vacuolar pathogen (5).

Both Drosophila and cultured Drosophila cells are tractable models for analysis of L. monocytogenes pathogenesis (6, 7). We tested the ability of macrophage-like Drosophila SL2 cells to support intracellular infection by L. monocytogenes. DH-L1039, a green fluorescent protein (GFP)-expressing L. monocytogenes strain derived from wild-type 10403S, replicated within Drosophila SL2 cells (fig. S1). In contrast, GFP fluorescence of DH-L1137, a variant lacking the pore-forming cytolsin listeriolysin O (LLO), was punctate in appearance and growth was inefficient in SL2 cells (fig. S1, A and B). Next, we developed a microscopy-based, high-throughput RNAi screen to identify host factors required for intracellular infection by L. monocytogenes (Fig. 1).
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