there is often substantial linkage disequilibrium between adjacent blocks (data not shown), allowing fewer markers to be used without loss of power. It will likely be productive to perform initial haplotype mapping in populations whose history contains one or more bottlenecks, because longer-range LD may make initial localization more efficient and favorable. Conversely, populations with shorter-range LD and greater haplotype diversity may offer advantages for fine mapping. In suggesting that block boundaries and common haplotypes are largely shared across populations, our data suggest that many common disease alleles can be studied—and likely will be broadly relevant—across human populations. In the future, comprehensive analysis of human haplotype structure promises insights into the origin of human populations, the forces that shape genetic diversity, and the population basis of disease.

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28. A low rate of genotyping error is critical to obtaining an accurate measure of haplotype diversity and the proportion in common haplotypes. Even a modest (1 to 2%) genotyping error will create a substantial number of false rare haplotypes; for example, with a 10-marker haplotype and a 2% error rate, 18% of chromosomes will contain at least one error and, thus, not match the few common haplotypes.
29. Within blocks, the common haplotypes showed little evidence for historical recombination. For example, we performed the four gamete tests using SNPs drawn only from haplotypes with frequency 5% or higher in each block. One or more violations of the four gamete tests were observed in only 5% of the blocks.
30. To maximize power, these comparisons were made only for SNP pairs spaced 5 to 10 kb apart. At shorter distances, nearly all SNP pairs are in a single block; at greater distances, most SNP pairs are in different blocks.
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Pseudomonas-Candida Interactions: An Ecological Role for Virulence Factors

Deborah A. Hogan and Roberto Kolter*

Bacterial-fungal interactions have great environmental, medical, and economic importance, yet few have been well characterized at the molecular level. Here, we describe a pathogenic interaction between *Pseudomonas aeruginosa* and *Candida albicans*, two opportunistic pathogens. *P. aeruginosa* forms a dense biofilm on *C. albicans* filaments and kills the fungus. In contrast, *P. aeruginosa* neither binds to nor kills yeast-form *C. albicans*. Several *P. aeruginosa* virulence factors that are important in disease are involved in the killing of *C. albicans* filaments. We propose that many virulence factors studied in the context of human infection may also have a role in bacterial-fungal interactions.

Interactions between prokaryotes and eukaryotes are ubiquitous. Although the pathogenic and symbiotic relationships bacteria have with plants and animals have garnered the most attention, the prokaryote-eukaryote encounters that occur among microbes are likely far more common. Many of the virulence factors that we study in the context of human disease may also have an ecological role within microbial communities.

Department of Microbiology and Molecular Genetics, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115, USA.

*To whom correspondence should be addressed. E-mail: rkolter@hms.harvard.edu

Bacteria and unicellular eukaryotes, such as yeasts and filamentous fungi, are found together in a myriad of environments and exhibit both synergistic and antagonistic interactions (*1*, *2*). Here, we describe a pathogenic relationship between a fungus, *Candida albicans*, and a bacterium, *Pseudomonas aeruginosa*, that involves genes important for bacterial virulence in mammals. *P. aeruginosa* is prevalent in soils and is often found on the skin and mucosa of healthy individuals (*3*). In compromised hosts, however, *P. aeruginosa* uses an arsenal of virulence factors to cause serious infections associated with burns, catheters, and implants. *C. albicans* is also a benign member of the skin and mucosal flora. When host defenses falter, however, *C.*

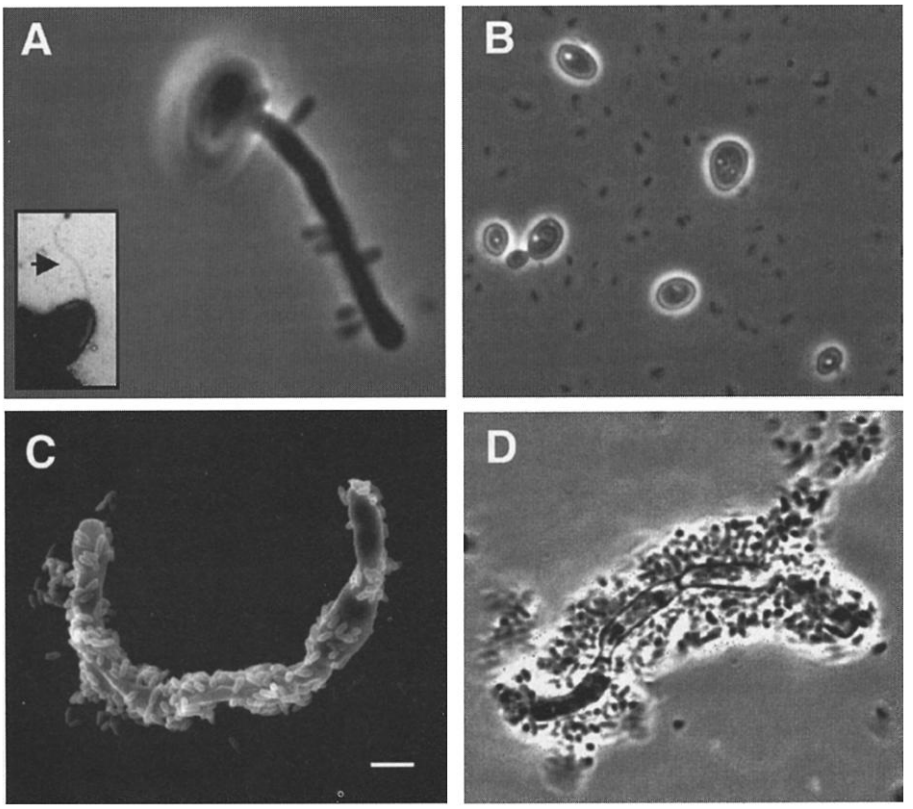

Fig. 1. Microscopic examination of the physical interactions between *P. aeruginosa* and *C. albicans*. (**A**) Phase-contrast image shows a single filamentous *C. albicans* cell after incubation with *P. aeruginosa* for 1 min. (Inset) Transmission electron micrograph shows polar attachment to the fungal filament. The flagellum is indicated by a black arrow. (**B**) Phase-contrast image shows *P. aeruginosa* incubated with yeast-form cells for 24 hours. (**C**) Scanning electron micrograph shows a fungal filament that was incubated in the presence of *P. aeruginosa* for 48 hours. Scale bar, 2 μm. (**D**) A phase-contrast image shows a biofilm surrounding a fungal filament after incubation for 72 hours. See *Science* Online for experimental details (*12*).

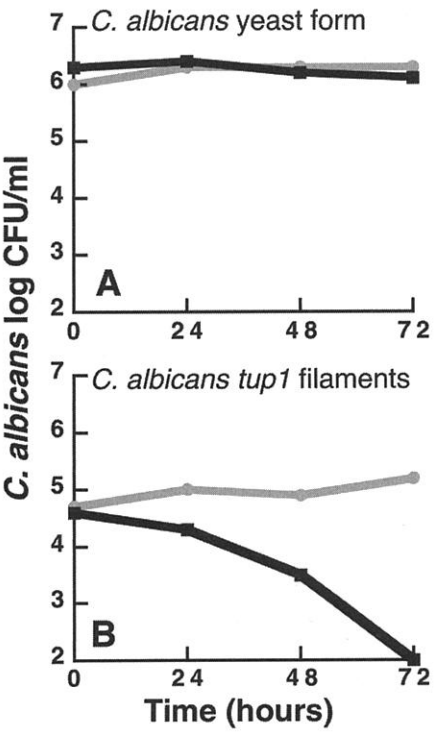

Fig. 2. Survival of *C. albicans* in the presence of *P. aeruginosa*. The viability of (**A**) yeast-form and (**B**) the constitutively filamentous *C. albicans tup1* mutant was measured in the presence (squares) or absence (circles) of *P. aeruginosa* cells. The total volume was 2 ml in a 13-mm by 100-mm tube, and cells were incubated with mild shaking at 30°C. *C. albicans* titers were determined by plating on YPD medium supplemented with tetracycline (60 μg/ml), gentamicin (30 μg/ml), and chloramphenicol (30 μg/ml) to suppress the growth of *P. aeruginosa*.

albicans initiates invasive growth that can lead to severe disease (*4*). In the host, *C. albicans* exists as both yeast-form and filamentous cells, and the ability to induce filamentation is important for its virulence (*5*). Several studies suggest that *P. aeruginosa* and *C. albicans* interact with each other in the human body (*6–11*). A molecular understanding of bacterial-fungal interactions, such as those between *P. aeruginosa* and *C. albicans*, should allow us to more effectively explore the interface between bacterial pathogenesis and microbial ecology.

Upon mixing cultures of *P. aeruginosa* and *C. albicans*, we observed that *P. aeruginosa* readily attached to *C. albicans* filaments (Fig. 1A), but almost never adhered to yeast-form *C. albicans* cells even after prolonged incubation (Fig. 1B) (*12*). Differences in the cell walls of yeast-form and filamentous *C. albicans* likely explain the selective attachment of *P. aeruginosa* to fungal filaments (*13*). Initial contact with the filament was usually made by one pole of the bacterial cell (Fig. 1A), and at least some cells were attached to the filament by the pole opposite the flagellum (Fig. 1A, inset) (*14*). Attachment to the fungus was affected by the physiological state of *P. aeruginosa*. For example, many more bacteria attached to filaments after a short period of coincubation when *P. aeruginosa* were taken from stationary-phase (WT) rather than from exponential-phase cultures (WT-EP) (Table 1) (*12*). Over the course of 24 to 48 hours, bacteria attached along the filamentous portion of *C. albicans* cells (Fig. 1C) and ultimately formed biofilms containing bacterial cells at high density surrounded by phase-bright material suggestive of an extracellular matrix (Fig. 1D). In the conditioned medium used in these experiments, biofilm formation occurred predominantly on the fungal filaments and not on the underlying glass coverslip (Fig. 1D). Given the scarcity of nutrients under these conditions, it is likely that forming a biofilm on fungal filaments enables *P. aeruginosa* to obtain nutrients from the fungus.

Plate count assays showed that *C. albicans* filaments were killed by *P. aeruginosa*, whereas yeast-form *C. albicans* retained full viability (Fig. 2). The viability of *C. albicans* filaments was measured using the constitutively filamentous *tup1* mutant (*15*) to circumvent the difficulties associated with measuring the survival of *C. albicans* filaments in the presence of a population of yeast cells. The number of surviving *C. albicans tup1* decreased after 24 hours in the presence of *P. aeruginosa* (Fig. 2B), but not after incubation in conditioned medium from stationary phase *P. aeruginosa* cultures without bacteria, showing that the presence of *P. aeruginosa* was necessary for fungal killing. Furthermore, the death of the fungal cell occurred after the onset of biofilm formation, suggesting a causal relationship between biofilm formation and fungal killing.

To investigate the role of *P. aeruginosa* virulence genes in *P. aeruginosa–C. albicans* interactions, we analyzed three classes of *P. aeruginosa* mutants (*12*) for their ability to form biofilms on *C. albicans* filaments and to kill the fungus (Fig. 3). In these experiments, we included *P. aeruginosa* mutants defective in (i) surface structures, (ii) secreted factors, or (iii) regulatory molecules. Although most of the mutants formed biofilms that were indistinguishable from those of the wild type, the biofilms produced by the *P. aeruginosa flgK* and *lasR* mutants were less robust than the wild type even after 72 hours (Fig. 3, A and B). *P. aeruginosa* mutants in the *las*-quorum sensing pathway are also known to produce thinner, less differentiated biofilms on glass (*16*), suggesting that the genetic control of biofilm formation on fungal filaments may share some elements with the

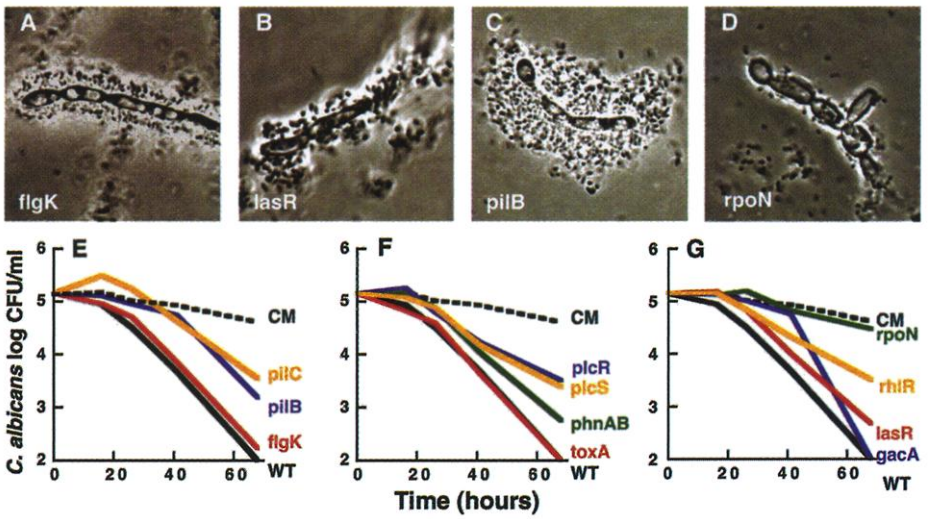

Fig. 3. (**A** to **D**) Representative phase-contrast images show *P. aeruginosa* mutant biofilms on *C. albicans tup1* after 72 hours of coincubation. (**E** to **G**) *C. albicans tup1* survival in the presence of isogenic *P. aeruginosa* mutants. *C. albicans tup1* viability (measured as CFU/ml) was followed in the presence of wild-type cells (black) and conditioned medium without cells (black dashed). Same results plotted in (E), (F), and (G). (E) *C. albicans* viability was monitored in the presence of mutants lacking type IV pili, *pilB* (blue), *pilC* (yellow), or lacking the polar flagellum, *flgK* (red). (F) *C. albicans* viability was monitored in the presence of mutants defective in the production of virulence factors including phospholipase, *plcS* (yellow) and *plcR* (blue); phenazines, *phnAB* (green); and exotoxin A, *toxA* (red). (G) *C. albicans* viability was measured in cultures with *P. aeruginosa* mutants defective in regulators that control the production of virulence factors including *rpoN* (green), *gacA* (blue), *rhlR* (yellow), and *lasR* (red). The values plotted represent the averages of four replicate cultures, and the experiment was performed multiple times with similar results. At 42 hours, the differences between the wild type and all mutants except *flgK* and *toxA* were statistically significant at $P < 0.05$ as determined by a *t* test analysis.

regulation of biofilm development on inert surfaces. *P. aeruginosa* PA14 mutants lacking pole-localized type IV pili (*pilB* and *pilC*) also do not form mature biofilms on abiotic substrates (*17*, *18*), yet these mutants still formed robust biofilms on filamentous *C. albicans* (Fig. 3C). Because some *P. aeruginosa* strains use type IV pili to attach to epithelial cells (*17*), we assayed their initial attachment to *C. albicans* filaments and found a decreased rate of adherence (Table 1). These data indicate that pili somehow participate in the initial attachment to *C. albicans* filaments, but they are not required for biofilm formation at later time points (compare Fig. 1D with Fig. 3C). The *rpoN* mutant forms extremely poor biofilms on *C. albicans* filaments (Fig. 3D), likely owing to multiple factors, including the lack of a flagellum and a decreased growth rate in minimal media (*19*). With the exception of the *rpoN* mutant, all *P. aeruginosa* mutants had the same planktonic growth rate as the wild type (*20*).

Pseudomonas aeruginosa mutants were assayed for their virulence toward *C. albicans*. First, we analyzed *P. aeruginosa* mutants lacking type IV pili and the polar flagellum. Although the type IV pili mutants made robust biofilms surrounding the filament (Fig. 3C), they did not kill *C. albicans tup1* until after 48 hours (Fig. 3E). The mechanisms by which type IV pili influence fungal killing remains unknown, but may involve pilus retraction to bring the bacterium in close contact with the fungal cell or the use of pili as sensors that signal attachment to the fungal surface (*17*, *21*). The flagellar mutant kills *C. albicans* filaments with kinetics similar to those of the wild type (Fig. 3E), even though it forms slightly smaller biofilms on fungal filaments (Fig. 3A) (*22*). The second class of *P. aeruginosa* mutants was defective in the production of broad-spectrum secreted factors that contribute to virulence toward diverse organisms including mammals, plants, and insects (*23*, *24*). Several of these mutants were also attenuated for their virulence toward *C. albicans* filaments. *P. aeruginosa* mutants unable to produce the hemolytic phospholipase C (because of disruption of either *plcS*, the structural gene, or *plcR*, which is required for phospholipase C secretion) were significantly attenuated in their ability to kill *C. albicans* filaments (Fig. 3F) (*25*, *26*). The same was true for the *phnAB* mutant, which is unable to synthesize phenazine antibiotics (Fig. 3F) (*27*). A *P. aeruginosa* mutant lacking exotoxin A, a type II–secreted toxin that targets translation in eukaryotic cells, was not significantly affected in its virulence toward *C. albicans* (Fig. 3F) (*23*). Last, inactivation of several virulence-factor regulators including GacA, LasR, RhlR, and RpoN, resulted in either delayed or attenuated virulence toward *C. albicans* filaments (Fig. 3G). The *P. aeruginosa rpoN* mutant, which was not capable of biofilm formation on fungal filaments, was also unable to kill *C. albicans*. *P. aeruginosa* mutants defective in *gacA*, *lasR*, and *rpoN* are also significantly attenuated in other virulence models (*19*, *23*, *28*). The less virulent phenotypes of pleiotropic regulatory mutants (*gacA*, *lasR*, *rhlR*, and *rpoN*) likely resulted from the decreased expression of multiple genes. In addition, some genes, such as those involved in phenazine production, are controlled by multiple regulators (*19*, *27*, *29*). However, the attenuated virulence of multiple mutants defective in single traits (*plcR*, *plcS*, *pilB*, *pilC*, or *phnAB*) more clearly shows that multiple mechanisms act in concert to kill *C. albicans* filaments. This explains the eventual decrease in *C. albicans* CFU/ml in almost all cultures after 60 hours (Fig. 3, E to G).

Table 1. Initial attachment of *P. aeruginosa* to *C. albicans* filaments. See *Science* Online for experimental details (*12*). *P. aeruginosa* wild type (WT), *pilB*, and *pilC* were taken from early stationary-phase cultures (OD_{600} of 1.2). WT-EP cells were taken from exponential phase cultures (OD_{600} of 0.4). The percentage of filaments colonized was determined by microscopic examination of 150 filaments per sample after a 20-min incubation. Each value represents the average of triplicate samples. In the last column, number represents the average number of bacteria attached to 300 filaments.

P. aeruginosa strain	Percent filaments colonized	Bacterial cells per filament
WT	80.9 ± 3.1	4.4
WT-EP	29.1 ± 0.4	1.2
pilB	39.1 ± 8.2	1.6
pilC	37.8 ± 8.2	1.5

Our data suggest a link between biofilm formation and the activity of some eukaryotic-specific virulence factors toward fungal cells. Both clinical and environmental isolates of *P. aeruginosa* produce a similar spectrum of virulence factors including type IV pili, phospholipase C, and phenazines (*30*, *31*). Thus, we speculate that antagonism between bacteria and microscopic fungi has contributed to the evolution and maintenance of many pathogenesis-related genes. Furthermore, we propose that the interactions between *P. aeruginosa* and *C. albicans* reflect the relationships of bacterial and fungal species that coexist in other environments. A deeper understanding of bacterial-fungal interactions may provide a new perspective on the role of known virulence determinants and may lead to the discovery of new factors involved in pathogenicity in multiple hosts.

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Disassembly of Transcriptional Regulatory Complexes by Molecular Chaperones

Brian C. Freeman[1] and Keith R. Yamamoto[2]*

Many biological processes are initiated by cooperative assembly of large multicomponent complexes; however, mechanisms for modulating or terminating the actions of these complexes are not well understood. For example, hormone-bound intracellular receptors (IRs) nucleate formation of transcriptional regulatory complexes whose actions cease promptly upon hormone withdrawal. Here, we show that the p23 molecular chaperone localizes in vivo to genomic response elements in a hormone-dependent manner, disrupting receptor-mediated transcriptional activation in vivo and in vitro; Hsp90 weakly displayed similar activities. Indeed, p23 and Hsp90 also disrupted the activities of some non–IR-containing transcriptional regulatory complexes. We suggest that molecular chaperones promote disassembly of transcriptional regulatory complexes, thus enabling regulatory machineries to detect and respond to signaling changes.

Many physiological signal transduction pathways culminate in selective modulation of specific gene transcription rates. For example, glucocorticoid or thyroid hormones in the bloodstream enter target cells and associate with the glucocorticoid receptor (GR) or thyroid hormone receptor (TR), respectively. These receptor-hormone complexes bind selectively to specific genomic sites termed hormone response elements (HREs) and trigger formation of large, heterotypic transcriptional regulatory complexes, which in turn activate or repress transcription from nearby promoters (*1*, *2*). The effects of hormonal signaling are quite rapid: Rates of transcription at inducible genes increase with a half-life ($t_{1/2}$) of ~5 to 10 min (*3*, *4*). Similarly, hormone withdrawal leads promptly to hormone release from the receptor and cessation of activation (*5*).

How does an intracellular receptor (IR), bound to hormone and in the cell nucleus as part of a large regulatory complex, detect and respond to a decline in extracellular hormone levels? Recent studies suggest that certain factors, when assembled into regulatory complexes, may become targets for proteasomal degradation (*6*). However, IRs do not appear to be degraded upon hormone withdrawal; indeed, unliganded IRs commonly display longer intracellular half-lives than their liganded counterparts (*7*, *8*). Rather, we have proposed that IR-containing regulatory complexes may be actively and reversibly disassembled by molecular chaperones (*9*).

We tested directly whether the p23 molecular chaperone may be involved in the disassembly of transcriptional regulatory complexes, by (i) assessing the actions of p23 on functional regulatory complexes in vitro, (ii) developing a general approach to determine in vivo the effects of chaperone components on regulatory complexes containing IRs or non-IR regulators such as nuclear factor kappa B (NF-κB) and activator protein 1 (AP1), and (iii) monitoring the recruitment of chaperone components to natural response elements in vivo.

The effect of purified p23 on TR-containing regulatory complexes and TR-mediated transcriptional activation in vitro is shown in Fig. 1 (*10*). In these experiments, receptor-DNA complexes were formed on two thyroid response element (TRE)–containing templates: the four-spaced direct repeat (DR_4) TRE favors TR/RXR heterodimer binding, whereas the palindromic TRE (TREpal) favors TR homodimer binding. In one experiment, we added p23 to the receptor-DNA complexes before the addition of HeLa nuclear extract (Fig. 1A). On the basis of our findings with purified components, we predicted that p23 should dissociate TR/RXR or TR from the template, unless factors in the extract reverse or preclude that process, and that transcriptional activation by the receptor should be reduced. Indeed, receptor-mediated activation, but not basal transcription (as shown by a control template, ADHp, lacking response elements), declined as a function of increasing p23.

In a second experiment, we addressed whether p23 could affect fully elaborated transcriptional regulatory complexes, which may correspond to its substrates in vivo. Specifically, we examined the effect of adding p23 to reactions in which receptor-DNA complexes were first preincubated for 30 min with nuclear extract (Fig. 1B). Under these conditions, fully functional regulatory complexes should assemble at the response elements, and as a result, competent preinitiation complexes should form at the promoter. We used Sarkosyl, which inactivates RNA polymerase II that is not in competent preinitiation complexes (*11*), to confirm that such complexes were indeed formed during the preincubation (Fig. 1B; compare Sa with Sb). We found that added p23 abrogated transcriptional activation by these preformed complexes, suggesting that p23 is a limiting factor for disassembly of functional regulatory complexes in vitro.

[1]Department of Cell and Structural Biology, University of Illinois, Urbana-Champaign, 601 South Goodwin Avenue, Urbana, IL 61801, USA. [2]Department of Cellular and Molecular Pharmacology, University of California, San Francisco, 513 Parnassus, San Francisco, CA 94143–0450, USA.

*To whom correspondence should be addressed. E-mail: yamamoto@cgl.ucsf.edu