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# The Fruit Fly as a Meeting Place for Microbes

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**Many infectious diseases of humans are caused by polymicrobial communities, but there are few in vivo models to study such communities. In a recent issue of *PLoS Pathogens*, Sibley and colleagues (Sibley et al., 2008a) report the development of a fruit fly infection model to investigate polymicrobial interactions and their effects on the host.**

The reductionist approach in microbiology has resulted in an extraordinary amount of knowledge about individual microorganisms, but in this age of "systems biology" thinking, new tools are needed to investigate complex, natural interactions. Indeed, microbes hardly ever grow as single species in nature. Rather, they live as members of microbial communities consisting of multiple species (Buckley, 2003).

Body surfaces and cavities of mammals contain mucosal surfaces harboring an extensive microflora. In these communities, the whole is much more than the simple sum of its parts since the interactions between the different constituents result in many new physiological functions that cannot be observed with individual components.

These polymicrobial populations can be important determinants of the organism health, as many infectious diseases are caused by mixed communities containing several organisms from different species or in some cases from different kingdoms (Brogden et al., 2005). However, despite the abundance of polymicrobial diseases, extraordinarily little is known regarding microbial interactions within polymicrobial communities.

The potentially important roles of bacterial interspecies interactions in virulence and response to therapy lead to a number of questions. How do microbial members interact? How does the host respond to the presence of these polymicrobial communities? New approaches are *de rigueur* to investigate these issues.

One of the most studied polymicrobial communities colonizes the airways of individuals with the disease cystic fibrosis (CF), the most common and severe monogenic recessive disorder in Caucasian populations. In virtually all patients with CF, a chronic infection with multiple microbial species is established during infancy. This colonization, with the resulting associated persistent inflammation, lead to progressive, and ultimately lethal, lung injury and destruction (Lyczak et al., 2002).

Of the multiple opportunistic bacteria that may colonize CF airways, the Gram-negative bacterium *Pseudomonas aeruginosa* is commonly considered the most significant pathogen. Therefore the primary focus of CF microbiological research has been on this microbe. However, a number of recent studies, using culture-independent molecular approaches, have revealed that complex communities composed of multiple microbial species are actually present in CF airways, most usually not detected by traditional culture techniques but some probably playing a significant pathogenic role (Harris et al., 2007; Sibley et al., 2008b). Moreover, little is known about the roles in CF pathogenesis of non-*Pseudomonas* bacteria, or about the interspecies interactions between the members of this polymicrobial association (Hoffman et al., 2006).

A major challenge for current studies on polymicrobial infections is the development of in vivo models that make it possible to easily explore microbe-microbe interactions as well as the host response. Mammalian model hosts are typically used to investigate the mechanisms of pathogenesis (from mono- or polymicrobial infections). However, use of these models is usually costly, time consuming, and ethically objectionable.

Alternatively, the use of simpler, more ethical, inexpensive, and practical surrogate hosts to study interactions with pathogens provides a way of overcoming these obstacles. Studies from several groups have clearly established the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* as model systems to study the virulence mechanisms of human pathogens. The fruit fly is simple to handle, genetically tractable, and has a well-studied innate immunity system. Moreover, its relevance as a suitable alternative to mammalian hosts has been confirmed in vertebrate organisms (Vodovar et al., 2004). *D. melanogaster* has been used to identify numerous *P. aeruginosa* virulence factors and to analyze the interactions between this bacterium and the innate host defenses.

Why not use the fruit fly to study polymicrobial infections? In an exciting paper recently published in *PLoS Pathogens*, Sibley and colleagues report the use of *D. melanogaster* as an alternative host to dissect the complex interactions between *P. aeruginosa* and bacterial isolates from the oropharyngeal microflora colonizing the airways of CF patients (Sibley et al., 2008a). The authors chose the ingestion route of microbial entry in their fly model and demonstrate that *P. aeruginosa* establishes a chronic infection in flies fed with the bacterium. The contribution of the oropharyngeal microflora to the lung disease of individuals with CF is ill defined and certainly underestimated (Sibley et al., 2008a).

Recently, the same team has reported that isolates from the *Streptococcus milleri* group play a significant role as pathogens in adults suffering from CF and that these bacteria can establish chronic pulmonary infections (Sibley et al., 2008b). A crucial unanswered question is how these bacteria interact with *P. aeruginosa* in CF airways. Forty oropharyngeal isolates were fed to *Drosophila,* alone or in combination with *P. aeruginosa,* and fly survival was assessed. Based on the observed infectious interactions, these isolates were

grouped into three classes: [I] the virulent strains, which are pathogenic to the flies by themselves and add to the killing by *P. aeruginosa*; [II] the avirulent strains, which have no effect on fly mortality, alone or in combination with *P. aeruginosa*; and most intriguingly [III] the synergistic strains, which are not pathogenic to the flies by themselves but increase the virulence of *P. aeruginosa* (Figure 1). The Surette laboratory had previously reported, using an agar bead model of infection in rats, that polymicrobial infections with *P. aeruginosa* and a *Streptococcus* sp. strain caused a synergistic enhancement of lung inflammation (Duan et al., 2003). Interestingly, with the same *Streptococcus* strain, a synergistic polymicrobial infection (class III) behavior was also observed in the fly feeding assay, validating the use of *Drosophila* as a surrogate host for polymicrobial infections.

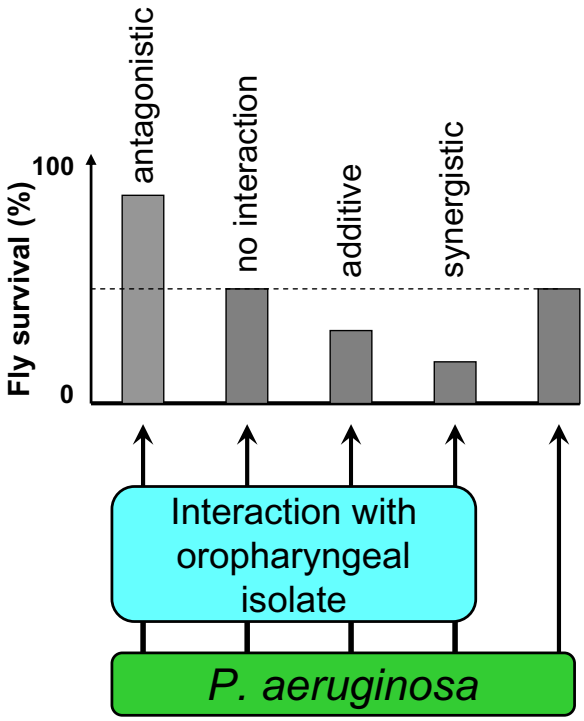


**Figure 1. A *Drosophila* Infection Model of Interactions between *P. aeruginosa* and Oropharyngeal Microflora from CF Patients**

Potential outcomes of these interactions, as reflected by the percentage of fly survival, are summarized above the bar graph.

An additive effect of the oropharyngeal microflora isolates on *P. aeruginosa* is the most plausible explanation for class I infections. On the other hand, explaining the synergistic interaction obtained with the class III isolates is less straightforward. Several mechanisms may occur; for instance, class III bacteria might alter *P. aeruginosa* virulence gene expression within the host. To investigate this question, the authors devised a clever procedure to follow the expression of 24 selected *P. aeruginosa* virulence factors in vivo by direct observation of infected flies. Taking advantage of the relative low opacity of these insects, they used reporter fusions between a virulence factor promoter and the *lux* operon (encoding luciferase activity) to directly measure, in real time, bacterial gene expression (by the light output) in individual flies. Several *P. aeruginosa* quorum-sensing-regulated genes were upregulated in the presence of *Streptococcus* isolates belonging to class III, including genes predicted to be regulated by interspecies bacterial communication via the extracellular signal autoinducer-2 (Duan et al., 2003). Thus, such interspecies signaling might modulate *P. aeruginosa* gene expression during polymicrobial infection of the fly.

Diverse interspecies interactions ranging from cooperation to antagonism exist between microorganisms. For instance, in another recently published paper, Peleg et al. have developed the use of *C. elegans* as an alternative host to investigate a polymicrobial interaction occurring this time between a prokaryote, the emerging pathogen *Acinetobacter baumannii*, and an eukaryote, the yeast *Candida albicans*. When the worm was infected with both pathogens, an antagonistic relationship between the two was found, which resulted in reduced *C. albicans* pathogenicity (Peleg et al., 2008). In the fly model, it is conceivable that some strains could decrease the virulence of *P. aeruginosa*. However, antagonistic interactions were not observed in the study of Sibley et al. although it is likely that the co-feeding assay developed in this study could reveal such interactions.

How does the host respond to polymicrobial infections? A powerful feature of *D. melanogaster* as an infection model is that the innate immune defense system of this animal is very well characterized. Sibley et al. could therefore investigate the polymicrobe-host interactions by monitoring the host innate immune response. The antimicrobial defense system of the fruit fly displays significant functional similarities with the vertebrate innate immune system. The *D. melanogaster* immune system discriminates between different classes of microbes and responds with the production of an array of antimicrobial peptides. Expression of these preeminent defense effectors is mediated via activation of the Toll and/or Imd signaling pathways (Vodovar et al., 2004). Sibley et al. note that *P. aeruginosa* induced the transcription of the three antimicrobial peptides tested (diptericin, cecropin, and drosomycin) and that expression of the immune response (antimicrobial peptides) to the mixed infection was complex, notably taking the form of additive or synergistic activation. Two fascinating observations illustrate the intricacy of the situation: the *P. aeruginosa* strain suppressed diptericin expression when co-fed with most oropharyngeal isolates, while on the other hand, a synergistic activation of drosomycin was observed with some isolates in association with *P. aeruginosa*.

This paper presents a compelling demonstration of the power of the fruit fly model for deciphering polymicrobial interactions in the context of a host but also highlights the complexity of these infections. Our understanding of the interactions occurring between microbial community residents is still rudimentary—and even more so inside a host! Thus, it is maybe not so surprising that infection control therapies and vaccination strategies targeting specific, apparently obvious, bacteria may not give the expected results. New approaches designed to simultaneously investigate multiple properties within microbial communities are necessary to provide information that could then be used for modulating the interactions between polymicrobial constituents, providing novel approaches for controlling infections.

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# Host Interception of Bacterial Communication Signals

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**Pathogenic *Staphylococcus aureus* employs a cell-to-cell communication system to control virulence factor expression in the host during infection. In this issue, Peterson et al. (2008) report that host lipoproteins can sequester the *S. aureus* communication signal and antagonize virulence factor expression and pathogenesis in a murine model of infection.**

Cell-to-cell communication allows bacterial populations to coordinate gene expression and synchronize cellular events. In bacterial pathogens, this regulatory switch relies on the secretion of a signaling molecule that is sensed by the cell population and triggers the expression of virulence determinants. Communication signals reach the critical concentration at a specific population density, or "quorum" of cells, and thus this regulatory mechanism is often termed "quorum sensing." To talk with neighbors, bacterial pathogens need to secrete these signaling molecules into the environment, providing an extracellular avenue for interrupting communication mechanisms. The lack of membrane barriers has allowed researchers to develop creative strategies for inhibiting quorum-sensing through direct inactivation of the signal or the identification of compounds that compete for the signal receptor. Therefore, quorum-sensing antagonism is viewed as a promising strategy for the discovery of antipathogenic therapeutics that can aid traditional approaches toward fighting bacterial infection (Hentzer and Givskov, 2003).

Like many innovative ideas for combating bacterial pathogens, nature has already developed intriguing mechanisms to antagonize quorum-sensing and thus disrupt communication among microbes. Gram-negative bacteria employ acylhomoserine lactone (AHL) signaling mechanisms, and marine alga produce furanone compounds that compete for the AHL signal receptors on marine bacteria as well as pathogens such as *Pseudomonas aeruginosa* (Hentzer and Givskov, 2003). Soil bacteria produce lactonase enzymes that degrade the AHL compounds by opening the lactone ring, and other bacteria produce acylases that remove the AHL fatty acid tail. Mammalian immune systems are not to be outdone in this regard, as airway epithelia produce paraoxanase enzymes that inactivate AHL signals through a lactonase mechanism (Ozer et al., 2005).

Gram-positive bacteria also utilize quorum-sensing for virulence factor regulation, but the sensing mechanism differs in that the signals are peptide based and the signal receptors are surface localized. An important subclass of peptide quorum-sensing signals possesses an embedded cyclic thiolactone or lactone ring structure and is produced by diverse members of the bacterial genera *Staphylococcus* and *Enterococcus* (Lyon and Novick, 2004), which include a number of prominent opportunistic pathogens. One of the best studied of these cyclic peptide-like structures is the autoinducing peptide (AIP) signal produced by *Staphylococcus aureus*. AIP activates a regulatory cascade that results in the repression of surface adhesins and upregulation of secreted toxins and invasive enzymes. This regulatory system is often termed accessory gene regulator (*agr*), and the *agr* response is especially strong in emerging methicillin-resistant *S. aureus* (MRSA) isolates. Considering that *S. aureus* is now the most