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Enterococcus faecalis: *E. coli*'s Siderophore-Inducing Sidekick

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Abstract

Many infectious diseases involve polymicrobial infections, which are characterized by synergistic interactions between different microorganisms colonizing a host. In this issue of *Cell Host & Microbe*, Keogh et al. (2016) show that *Enterococcus faecalis* promotes *Escherichia coli* biofilm formation in low-iron conditions, thus facilitating polymicrobial growth.

Polymicrobial infections play important roles in gastrointestinal, urinary tract, wound, and lung infections, and their impacts can be more detrimental to the host than infections involving the individual microbes alone. The phenomenon is termed polymicrobial synergy and defined as "an interaction of two or more microbes in an infection site that results in enhanced disease compared to infections containing the individual microbe acting alone" (reviewed in Murray et al., 2014). While polymicrobial interactions can affect the host, the host can also impact the environment in which these interactions occur. However, the mechanisms shaping the complex interplay between the host and its multiple microbial populations are poorly understood.

During infection, an important and well-studied strategy of host defense is iron limitation (reviewed in Cassat and Skaar, 2013). As an excellent redox catalyst in many fundamental cellular processes, including respiration and DNA replication, iron is essential for both humans and bacteria. Not surprisingly, the control of iron is a key battle between host and pathogen during bacterial infection. To limit bacterial access to iron, the host stores iron intracellularly, and extracellular iron is bound by the host proteins transferrin or lactoferrin. Conversely, bacterial pathogens have evolved to use a number of different strategies to overcome iron starvation inside the host. For example, bacteria use alternative metals in metalloenzymes, utilize heme uptake systems, directly acquire iron from transferrin and lactoferrin, efficiently import any free iron ions, and produce iron-chelating siderophores. While the battle for iron in monobacterial infections has been well described, its role in polybacterial infections remains poorly understood.

Enterococcus faecalis and *Escherichia coli* are frequent causes of catheter-associated urinary tract infections, surgical site infections, and wound infections, either as monomicrobial or polymicrobial infections. In this issue of *Cell Host & Microbe*, Keogh et al. (2016) investigate potential synergistic interactions between *E. faecalis* and *E. coli* using in vitro co-

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culture models and a murine model of wound infection. *E. faecalis* enhanced *E. coli* growth in a mouse model of wound infection, suggesting a synergistic polymicrobial interaction. This finding is consistent with previous reports (Montravers et al., 1994; Lavigne et al., 2008). The synergy observed in vivo was recapitulated in vitro when the two bacteria were co-cultured under iron limiting conditions, giving the authors an opportunity to mechanistically dissect this phenomenon. Through the elegant combination of unbiased omics approaches, including RNA-seq, metabolomics, and classical transposon mutagenesis, the authors found that *E. faecalis* secretes a simple metabolite, L-ornithine, which acts as a cue for *E. coli* to enhance production of siderophores under iron-starvation conditions (Keogh et al., 2016).

Based on these findings, the following scenario could unfold (Figure 1): during wound infection, *E. faecalis* utilizes the arginine deiminase pathway to generate energy from arginine. The end product of this pathway, L-ornithine, is secreted into the environment. Under iron-limiting conditions, an increase in L-ornithine levels induces a transcriptional response in *E. coli*, including increased transcription of gene clusters involved in siderophore biosynthesis. Production of siderophores such as enterobactin, salmochelin, and yersiniabactin enhances growth of *E. coli* as part of a mixed biofilm with *E. faecalis*.

The present study by Keogh et al. (2016) breaks new ground in our understanding of polymicrobial interactions and opens intriguing avenues for future research. Simple metabolites are often involved in synergistic interspecies interactions—for example, as part of syntrophic relationships in which the end product of one microorganism is utilized as a major nutrient for another microbe. In contrast, inter-bacterial communication heavily relies on quorum sensing through highly specific secondary metabolites (Murray et al., 2014). In the work reported by Keogh et al., (2016), the metabolic end product L-ornithine produced by one microbe appears to serve as a signal about the environment for a neighboring organism rather than acting as a carbon, nitrogen, or energy source. At this point, the molecular mechanism by which L-ornithine induces a transcriptional response in *E. coli* warrants further investigation. Is L-ornithine linked to enterobactin production via feedback inhibition of the arginine biosynthesis pathway or other metabolic pathways? Is L-ornithine acting simply as a signal, or does it need to be metabolized first, producing a breakdown product that serves as the actual signal? Why is this regulatory effect restricted to biofilm growth, as opposed to planktonic growth?

In addition to limiting availability of free iron, the host also directly interferes with bacterial iron uptake systems during infection. Lipocalin-2 (also known as neutrophil gelatinase-associated lipocalin) is abundantly produced by neutrophils. In the context of a skin wound, lipocalin-2 expression is induced at the wound's edge and promotes cell migration and wound healing (Miao et al., 2014). Upon release, lipocalin-2 binds and sequesters enterobactin, thus rendering it inactive (Goetz et al., 2002) (Figure 1). Curiously, the uropathogenic *E. coli* strain studied in this article encodes operons for the biosynthesis of several siderophores: enterobactin, salmochelin, and yersiniabactin. Salmochelin is a glucosylated derivative of enterobactin and is thus not bound by lipocalin-2 (reviewed in Müller et al., 2009). The in vitro data support enterobactin as the sole siderophore in this scenario. However, it is plausible that salmochelin (and possibly yersiniabactin), whose

production was also induced by co-culture with *E. faecalis* (Keogh et al., 2016), may play an additional role in vivo. In particular, the relative contribution of each siderophore may depend on the context of the host environment. For example, in the inflammatory environment of a skin lesion, neutrophilic lipocalin-2 may limit enterobactin activity, and thus salmochelin may be more important (Figure 1). Conversely, in the absence of overt inflammatory responses, enterobactin may be the preferred siderophore. Consistent with these ideas, Keogh et al. (2016) show that an *E. coli entB* mutant, which is deficient for the production of both enterobactin and its derivative salmochelin, poorly colonized in a mouse wound-excisional model and did not benefit from co-infection with *E. faecalis*. Future work on the contribution of siderophores in the context of local inflammatory responses will be of great interest.

Furthermore, it will be informative to investigate the consequences of the metabolic interaction between *E. coli* and *E. faecalis* on the host. Is the synergy of the interaction limited to increased bacterial colonization, or is disease severity also enhanced? Is there a potential benefit for *E. faecalis*, possibly linked to the biofilm production by *E. coli*? In addition, it will be interesting to study other host-modulated factors, such as inflammatory byproducts, micronutrients, and hormones, in the context of polymicrobial infections.

This intriguing study highlights the importance of investigating the role of metabolic interactions in common polymicrobial infections and serves as an example of the many different facets of microbe-microbe interactions still to be discovered.

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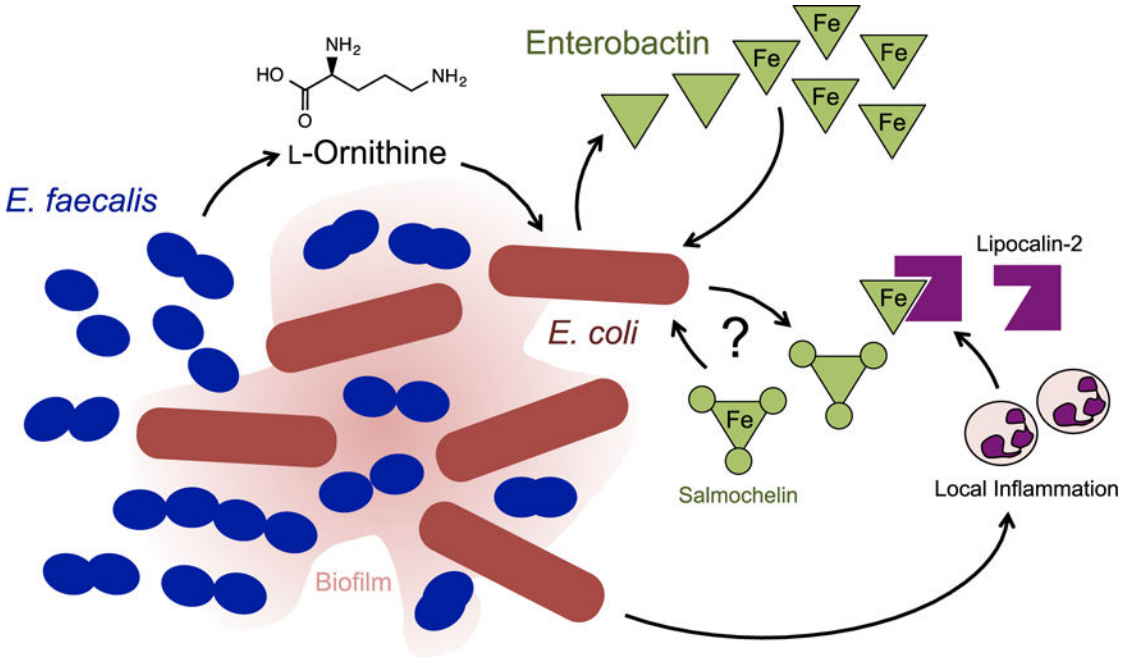

Figure 1. *E. faecalis* Enhances Growth of *E. coli* in a Polymicrobial Biofilm during Wound Infection

E. faecalis secretes L-ornithine as the end product of its arginine deiminase pathway. The presence of L-ornithine enhances the production of siderophores by *E. coli*. In the absence of inflammatory host responses, enterobactin-mediated iron uptake increases *E. coli* growth as part of the biofilm. During the inflammation that is induced in response to bacterial colonization, the host protein lipocalin-2 inactivates enterobactin. Lipocalin 2-resistant siderophores, such as salmochelin, may contribute to iron uptake in the context of polymicrobial wound infections.