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Identifying Bacterial Menu Choices from the Host Buffet during Infections

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During his development of the germ theory of disease, Louis Pasteur compared cholera and anthrax infections and remarked "Quelle différence donc entre le vibrion septique et cette bactéridie, et n'est-il pas remarquable de voir se multiplier dans 1′organization animale des êtres aussi dissemblables par leur mode de nutrition!" (1). An abridged English translation of this phrase reads "So what's the difference between the septic vibrio and this (anthrax) bacillus, and is it not remarkable to see the organization of microscopic beings so dissimilar in their mode of nutrition multiply in the animal!" While Louis Pasteur made many seminal contributions to microbiology, one concept that has been largely overlooked is the role of pathogen nutrition and physiology during infection, specifically with regard to carbon nutrition (2). In this context, Jorth et al. (3) have explored the use of RNA sequencing (RNA-seq) and next-generation sequencing to obtain a high-resolution transcriptome of *Aggregatibacter actinomycetemcomitans* during growth *in vitro* and in a murine abscess infection. They identified a large number of metabolic genes whose expression is differentially upregulated during infection, including those for formate dehydrogenase (*fdhF1F2*) and fumarate reductase (*frdA* to *frdD*), which are associated with fermentative and anaerobic metabolism, respectively. Mutants lacking these genes retained *in vitro* growth characteristics but experienced a 10-fold reduction in bacterial numbers in a mouse abscess, thus confirming the RNA-seq results. Previous studies of *A. actinomycetemcomitans* abscess formation by other investigators concentrated on virulence properties (4), so metabolic genes were not identified. Overall, the experimental strategy described by Jorth et al. (3) provides a nice template for future investigations of bacterial physiology during infectious processes.

A. actinomycetemcomitans has a small 2.1-Mb genome with an estimated 2,134 coding sequences (5). Although *A. actinomycetemcomitans* has been traditionally associated with periodontal disease (5), it has also been associated with other infections, notably, endocarditis (6) and abscesses (7, 8). In their study, Jorth et al. (3) used the murine abscess model as the infectious dose could be better controlled and tissue sampling more easily performed than would be the case in a periodontitis infection. One other advantage of *A. actinomycetemcomitans* as a model for initial technique development is the small genome of this organism (5). This would minimize complications in data interpretation due to the potential redundancy of metabolic pathways seen in other bacterial pathogens with larger genomes, such as *Pseudomonas aeruginosa* (9) and *Staphylococcus aureus* (10).

The use of a chemically defined culture medium is extremely useful during investigations of bacterial physiology (11) to ensure *in vitro* experimental reproducibility and provide a point of reference. In this particular study, Jorth et al. (3) used a chemically defined Socransky medium (12), which was developed to model the oral environment. To our knowledge, no other defined medium for *A. actinomycetemcomitans* exists. While one might argue that Socransky medium does not mimic the conditions in abscess environments, this medium does provide a basis for reproducible physiological investigations of *A. actinomycetemcomitans*. While fermentative and anaerobic metabolism genes were identified in their most recent study, previous work by these same investigators showed that *A. actinomycetemcomitans* exhibits aerobic metabolism when grown in mixed culture *in vitro* under microaerophilic conditions mimicking periodontitis (6) and *in vivo* in the murine abscess model (13). As shown in Fig. 1, one would anticipate that physiological changes in *A. actinomycetemcomitans* may accompany its move from monoculture to mixed infections and manifestations in endocarditis. As this *in vivo* knowledge is gained, we will be able to investigate this organism under more clinically relevant laboratory conditions.

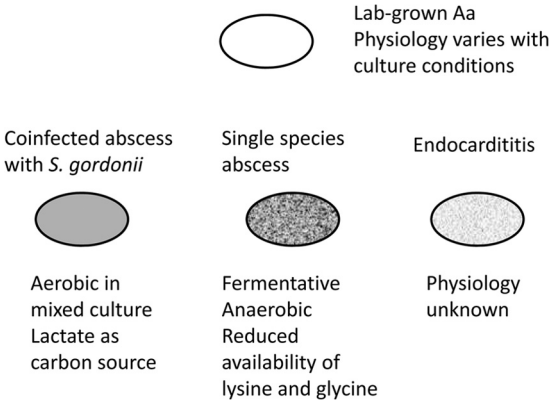

FIG 1 In contrast to the often predictable nature of laboratory culture growth, an organism such as *A. actinomycetemcomitans* likely adopts different physiological profiles as it moves from one tissue environment to another. In spite of the many potential nutrients present in host tissues, pathogen physiology during infection remains a largely unexplored field (2). The RNA-seq approach used by Jorth et al. (3) provides a mechanism whereby pathogen physiology can be explored during infections.

In 1878, Pasteur et al. recognized the importance of the host as a culture medium during infections (1). The study of Jorth et al. (3) provides a useful experimental strategy whereby pathogen nutrition and physiology can be monitored during infections by us-

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ing next-generation sequencing and genetic analysis. One would anticipate that tissue chemistry, the immune system, and interactions with other microorganisms would all influence physiology. In all likelihood, *in situ* bacterial physiology will be recognized as a major factor contributing to the onset and control of infectious disease, as are recognized virulence factors (14).

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