

METAGENOMIC STUDY TOWARDS PHAGE THERAPY IMPROVEMENT: WHAT DO WE LEARN FROM BALANCE AND BACTERIA / PHAGE RATIO?

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Phage therapy can be most simply understood as an alternative to antibiotics, especially in the context of bacterial infections that have developed resistance to conventional treatments. However, a more refined approach—based on the study of natural bacterial-phage equilibria—can help avoid common errors that may lead to therapeutic failure. This strategy distinguishes phage therapy from traditional pharmacological methods. In fact, both approaches can be effectively combined, including in compassionate use cases.

After possible outbreak and equilibrium change a thorough understanding of the environmental conditions in which treatment is applied allows for more precise therapies, helping to achieve specific goals. The bacteria-to-phage (B/P) ratio is directly influenced by microbial variability and results from multifactorial interactions (Ref 1). These interactions determine whether a system is in a healthy or pathological state, influenced by internal or external factors.

Introducing new phages into an existing microbial system does trigger changes, potentially reducing the B/P ratio (typically by increasing the phage population, i.e., the denominator). In vivo, using phage cocktails allows for targeting multiple bacterial species simultaneously, helping to reduce also the development of resistance.

Therefore, information on microbiota composition and environmental conditions is essential to move toward a stable ecosystem, ideally mirroring a healthy state characterized by a positive B/P equilibrium. To achieve this, expanding our knowledge of unknown phage populations—the so-called "phage dark matter"—is critical (Ref 2). This would broaden the available phage repertoire and increase the chances of therapeutic success.

Both **in vitro** and **in vivo** studies are needed (Ref 3) to track microbiota changes during phage therapy, particularly by monitoring metagenomic variations and infer microbial interactions.

We present two examples that illustrate these concepts: 1) A metagenomic comparison of natural marine environments affected or not by long-term human activity. 2) An experimental study involving mice, comparing untreated microbiota to microbiota altered by DSS-induced colitis.

These examples confirm the high variability in bacterial and phage populations under different environmental conditions. For instance, cyanobacteria and their associated phages (cyanophages of Myoviridae) are nearly absent in the Arctic Ocean but are highly abundant in the Persian Gulf.

The mice gut microbiota analysis detected a diverse range of bacteria and phages. However, in DSS-treated mice, the B/P ratio decreased, with a significant increase in total phage counts. Specifically, *Bacteroides* became more abundant but with a lower phage ratio, while *Clostridioides* became less abundant, with their associated phages increasing. Increase of Caudovirales (bacteriophages) is associated with a decrease in enteric bacteria in inflammatory gut condition. Another important variable—prophage induction—was not measured in this study but should be considered in future research.

References:

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Centenarians have a diverse gut virome with the potential to modulate metabolism and promote healthy lifespan.

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