Potential transcription factors of the *Clostridioides difficile* prophage phiCDKH02 influencing host virulence

C. difficile is an opportunistic pathogen that can be the main cause of antibiotic-associated diarrhea, due to prolonged antibiotic treatment, especially in a situation when healthy intestinal microbiota is disturbed. Infection by this pathogen can lead to more serious conditions like colitis, toxic megacolon, or even colon cancer. *C. difficile* has been considered a pathogen of great concern for a few reasons including the rapid increase in the number of *C. difficile* infections, the growing number of deaths among the elderly, the emergence of hypervirulent strains resistant to antibiotics, and the increasing number of infections outside healthcare settings.

Bacteriophages or phages are viruses that specifically attack bacteria. Scientists have discovered that many phages can influence the phenotype of their bacterial hosts. This interaction is evident in various bacterial species, where prophages – viruses integrated into the host genome - can significantly affect their virulence.

The genomes of *C. difficile* phages have a modular structure and there are modules responsible for processes such as DNA replication, transcription and recombination, among others. Studies have shown that these phages can encode a variety of potential transcription factors. However, the role of many of these proteins is still not fully understood, raising questions about whether these viral transcription factors interact with host proteins, particularly those that can enhance the virulence of the pathogen. Despite the growing number of sequenced *C. difficile* phage genomes, the direct impact of phage-encoded proteins on the phenotype of *C. difficile* remains largely unknown. Some studies suggest that prophages may influence toxin production in *C. difficile,* even if these prophages do not directly carry toxin genes.

The present project builds on previous studies that indicate that the phiCDKH02 *C. difficile* prophage affects host cell virulence. The planned research in the project is a continuation of studies on phage phiCDKH02 and aims to verify whether deletion of selected phage genes encoding potential transcription factors can affect the virulence of the bacterial host. The results obtained from this project are expected to serve as a prelude to a better understanding of the biology of phage phiCDKH02 and contribute to its use in the fight against this dangerous pathogen.