

Comparative evolutionary analysis of virulence-associated and housekeeping genes among species of genus *Clostridium*

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Recent studies that examined within-species divergence of housekeeping and virulence genes among isolates of such important human pathogens as *Clostridium difficile* and *C. perfringens* showed that a relatively modest amount of polymorphism exists among multiple isolates from the same species. It was suggested that purifying selection plays a rather big role in the evolution of both gene categories. However, it remains unclear whether this trend holds true over the long-term evolution of this genus. Thus, we examined patterns of nucleotide and amino acid sequence variability of several housekeeping and virulence genes from 7 species of *Clostridium* for which complete genomic sequences are available (either completed genomes or draft assemblies). Our results showed that significant heterogeneity of evolutionary rates exists among surveyed genes. Within housekeeping gene category, some genes (e.g., *tpi* and *gmk*) were found to be quite conserved at both the nucleotide and amino acid sequence levels. These genes also tend to exist as a single copy in each genome. Other housekeeping genes, such as *ddl* and *sodA*, were found to be relatively fast evolving, with a level of synonymous polymorphisms already reaching saturation. In some genomes multiple duplicated copies of these latter genes were detected. Unlike housekeeping genes, virulence genes were found to be much more variable at the inter-specific level, and tended to have multiple duplicated copies (both recent and ancient duplications) in almost every genome surveyed.