

Molecular enumeration of intestinal bacteria using quantitative PCR and the chaperonin-60 target

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ABSTRACT

Real-time quantitative PCR (qPCR) offers the sensitivity, specificity, and convenience of PCR and the ability to determine accurately the number of copies of a particular DNA sequence that are present in a complex mixture of sequences. Thus, with the appropriate choice of a target sequence, this method can be used to measure the number of copies of a given genome that are present in an environmental sample. We used qPCR and the target gene *chaperonin-60* (*cpn60*) to enumerate *Clostridium perfringens* genomes in DNA extracts from contents of the chicken gastrointestinal tract with the aim of optimizing this methodology to enumerate any bacterium of interest. To determine the most accurate protocols for determining target species abundance, we compared various DNA extraction methods in combination with four methods for producing standard curves. Test samples spiked with known numbers of *C. perfringens* cells were generated by adding cells to samples of pig ileum digesta prior to DNA extraction and qPCR analysis. Factors affecting accuracy included the co-purification of PCR inhibitors and/or fluorescence quenchers and the yield of target DNA in the extract. Anion exchange chromatography of the spiked test samples enabled accurate enumeration of *C. perfringens* using a standard curve comprised of purified *C. perfringens* genomic DNA or a plasmid containing a fragment of *C. perfringens cpn60*. We used qPCR to enumerate *C. perfringens* and other intestinal bacteria in ileum and cecum samples from chickens that had been challenged with *C. perfringens* and compared the results with viable counts on corresponding selective agars. We conclude that qPCR-based molecular enumeration of target species in the gastrointestinal tract is feasible, but care must be taken in order to mitigate the effects of confounding factors that can affect the apparent cell count.