A comparison of two methods to extract bacterial DNA of the digestive tract

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Abstract

Bacterial species inhabiting the digestive tract vary greatly according to their location and a large proportion remains uncultured. In the bird, in the upper part of the digestive tract, from the crop to the end of the small intestine, the microflora is mostly aerobe or aerotolerant. In contrast, the main bacteria of the lower part of the digestive tract, i.e. the ceca, are anaerobes. In order to overcome cultural limits, digestive microflora can be studied by molecular methods that often require a prior DNA extraction. However, it has been demonstrated that the DNA extraction method can lead to a bias on our knowledge of microbiota [1]. Therefore the objectives of this work were to compare the suitability of two DNA extraction methods for qualitative and quantitative analysis of bacteria of the chicken digestive tract. DNA was isolated according to a currently widely used method, the QIAamp® DNA stool protocol (Qiagen) with minor modifications [2], or according to a new developed method, the G'NOME® kit protocol (BIO 101) with modifications one of which being bead-beating [3]. First one, the microbiota was qualitatively analyzed by a fingerprint technique (Temporal Temperature Gradient gel Electrophoresis, TTGE). Second one, quantitative analysis was performed by real-time PCR. Total bacteria and biologically important bacterial groups were quantified (*Clostridium leptum, C. coccoides*, *Bacteroides*, *Bifidobacterium*, *Lactobacillus*, *E. coli*). Studied digestive microflora was obtained from digestive contents and mucosa of small intestine and ceca collected on pool of 5 chickens. Qualitative analysis showed that the majority of bands were present in the profiles of both methods. However, some amplicons were less abundant or absent in the profiles generated by either the QIAamp or the G'NOME method. Quantitative analysis shows that the use of the G'NOME method resulted in an increase number of total bacteria detected compared to the use of the QIAamp one (x3 to x9). When regarding the different bacterial groups that were quantified, in most cases, the use of the G'NOME method resulted in an increase in bacterial quantification that differ according to bacterial groups and sample types (intestine / caeca; digestive content / mucosa). Moreover, several groups were under the threshold of detection for bacteria enumeration with good precision (10^6/g) with the QIAamp method while a high quantity was detected with the G'NOME one (10^6/g to 10^8/g). In conclusion, the G'NOME method resulted on a higher quantification of bacteria than the QIAamp one, but the differences between both vary depending on bacterial groups. That underlines a selective DNA extraction depending on bacteria species, which is consistent with the qualitative differences observed in this study.

References

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