Butyrate production pathways in phylogenetically diverse *Firmicutes* isolated from the chicken cecum

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Introduction and objectives

Although several molecular studies on the intestinal microbiota of chickens are published, the knowledge about the intestinal microbiota of poultry is incomplete and nonexistent when considering the bacteria producing butyric acid. Butyric acid, one of the quantitatively most important SCFAs, can in Clostridia be produced via 2 distinct pathways. One pathway uses butyrate kinase in the final step of butyrate production, while the other is using butyryl CoA-acetate CoA transferase. In this study the pathway was determined for strains isolated from the cecal content of chickens.

Materials and Methods

The route for butyrate synthesis was determined in 16 culturable and phylogenetically diverse butyrate producing strains. A part of the operon encoding phosphotransbutyrylase and butyrate kinase was amplified using degenerate primers PTBfor2 and BUKrev1 via a ramped annealing approach (Louis et al., 2004). Degenerate primers CoATDF1, CoATDR2, BCoATscrF and BCoATscrR were used to amplify part of the butyryl-CoA:acetyl CoA-transferase gene (Charrier et al., 2006; Louis and Flint, 2007). Degenerate primers PCTfor1 and PCTrev2 were designed against conserved regions of CoA-transferase genes related to a propionate CoA-transferase from *Clostridium propionicum* (AJ276553) (Charrier et al., 2006). PCR-products were purified and sequenced. Phylogenetic trees were constructed using the deduced protein sequences.

Results and Discussion

In only one of the 16 strains tested, the butyrate kinase gene could be amplified, while the butyryl-CoA:acetate CoA-transferase gene was detected in all cluster XIVa, XIVb and in the majority of the cluster IV strains. This gene could not be found in the cluster XVI strains, instead, a CoA-transferase gene more closely related to propionate CoA-transferases was found in all but one of the cluster XVI strains. Analysis of draft genome sequences from cluster XVI butyrate producers revealed that this gene is present directly downstream of the central pathway genes for butyrate formation, leading from acetyl-CoA to butyryl-CoA. Further, many butyrate producers have shown to exhibit a similar affinity for propionyl-CoA as for butyryl- CoA, while the propionate CoA-transferase from *Clostridium propionicum* also converts butyrate. Therefore it is conceivable that a CoA-transferase related to the gene from *C. propionicum* is responsible for butyrate formation in cluster XVI bacteria.