Analysis of bacterial diversity and screening of novel lipolytic genes of dairy cattle rumen microflora [1]

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Received: Nov. 16, 2016; Accepted: Jan. 12, 2017

Abstract

The purposes of this study were to analyze the bacterial diversity of dairy cattle rumen microflora and to screen for lipolytic clones of a metagenomic library constructed from the dairy cattle rumen contents using function-driven approach. The metagenomic DNA was extracted from dairy cattle rumen contents using bead-beating method. The 16S rRNA genes of bacteria from rumen contents were amplified with bacterial specific sets of primers by PCR. The amplicons were ligated into TA cloning vectors to construct 16S rRNA gene libraries for DNA sequencing and bacterial diversity analyses. The clones were identified by comparing their sequences and GenBank database with BLASTn tool. The results showed that all the clones were recognized as uncultured and identified rumen bacteria. The rumen microbiome was dominated by *Bacteroidetes* (33/52), *Firmicutes* (18/52) and *Fibrobacteres* (1/52). By functional screening the metagenomic library of dairy cattle rumen microflora, a total of 3 unique clones conferring lipolytic activities were obtained using tributyrin plate assay. The DNA analysis revealed that there were 3 putative lipolytic ORFs of 3 clones. Based on the BLASTp analysis, the 3 putative lipolytic enzymes (LipoR1, LipoR2 and LipoR3) showed 39, 86 and 62% identity to the closest matches in the protein database, respectively. According to the classification of bacterial lipolytic enzymes proposed by Arpigny & Jaeger, LipoR1 might belong to Family I, LipoR3 was classified into the position between Family IV & VII and LipoR2 represented a new member of a new family (Family XV).

Keywords: Lipolytic genes, Metagenomic library, Rumen microflora, Dairy cattle.

(1) Contribution No. 2540 from Livestock Research Institute, Council of Agriculture, Executive Yuan.
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