学位論文内容の要旨

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学位論文題名

Identification and characterization of *Dorea ammoniilytica* as a novel deoxycholic acid-producing bacterial species in the human gut microbiota

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*Dorea ammoniilytica*の同定と特性の評価）

Primary bile acids (BAs) are synthesized in the liver and secreted into the duodenum to facilitate dietary fat absorption. In the large intestine, these primary BAs (cholic acid, CA; chenodeoxycholic acid, CDCA) are converted into secondary BAs (deoxycholic acid, DCA; lithocholic acid, LCA) by gut bacteria. DCA is the most abundant BA in the human gut and is produced from CA via a multistep 7α-dehydroxylation pathway that removes the C7 hydroxy group. Elevated DCA concentrations induce gut microbiota dysbiosis and are involved in metabolic disorders such as fatty liver disease, as well as colon and liver cancer. Despite the importance of DCA in our health, DCA-producing bacteria are not fully characterized: to date, only a few species, such as *Clostridium scindens*, *Clostridium hylemonae*, *Peptacetobacter hiranonis*, and *Claveliimonas bilis,* have been characterized as human-derived DCA producers. Recent metagenomic analyses of human gut microbiota revealed that bile acid-inducible (*bai*) genes, responsible for 7α-dehydroxylation, exist in different taxa not previously identified as DCA producers. This evidence suggests that additional, uncharacterized DCA-producing bacteria may exist in the human gut. Therefore, this study aimed to identify and characterize novel DCA-producing bacteria from human gut microbiota.

1. Screening of DCA-producing bacteria from human feces

Diluted human fecal suspensions (10^{-1} to 10^{-6}) were inoculated into GAM medium supplemented with 1 mM CA-Na and cultured to assess DCA production ability. Thin-layer chromatography and liquid chromatography-mass spectrometry analysis detected

DCA production in all fecal dilutions after 72 hours of incubation. Viable cell counts of the DCA-positive fecal cultures were determined, and cultures containing 10^7-10^4 cells were inoculated into 96-well plates containing GAM and 1 mM CA-Na to evaluate their DCA-producing ability. DCA production was detected in wells containing 10^7-10^5 cells, and the most diluted positive population (10^5) and negative populations were selected for 16S rRNA amplicon sequencing to characterize their microbial composition. Among the 79 detected amplicon-sequence variants, ASV_44 accounted for ~1.13% of the community in the DCA-positive population, and its sequence matched those of the 16S rRNA genes of *Dorea ammoniilytica* and *Ruminococcus* sp. 653 (JCM 18685).

2. Identification and characterization of novel DCA-producing bacteria

D. ammoniilytica possessed *bai* genes in its draft genome, supporting that it is a DCA-producing bacterium. Therefore, both *D. ammoniilytica* JCM 34808^T and *Ruminococcus* sp. JCM 18685 were selected for comparative characterization. Whole-genome sequencing and comparative genomic analyses showed that both isolates contain complete *bai* gene operons, supporting their 7α-dehydroxylation ability. Both strains exhibited strong DCA and LCA production (~100%), clarifying their high secondary BA-producing ability. Average nucleotide identity analysis indicated that *D. ammoniilytica*, *Ruminococcus* sp. JCM 18685, and other *Dorea* spp. (AM58-8 and AF36-15AT) shares values slightly above the commonly accepted intra-species cutoff (≥95%), suggesting they belong to the same species, *D. ammoniilytica*. Phylogenetic analysis showed that the *D. ammoniilytica* clade was distinct from other known DCA-producing bacteria, suggesting that this lineage comprises previously uncharacterized DCA-producers. Metabolic profiling revealed that both strains can utilize simple sugars, D-mannitol, and starch, which further differentiates them from other known DCA-producing bacteria.

In conclusion, this study characterized *D. ammoniilytica* as a potent DCA-producing bacterium that is phylogenetically different from known DCA producers. These findings expand the diversity of secondary bile acid-producing bacteria and reveal 7α-dehydroxylation capacity within the *D. ammoniilytica* lineage.