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Isolation of the novel secondary bile acid-producing bacteria from rat cecal contents and clarification of their interaction related to deoxycholic acid formation

（ラット盲腸内容物からの新規二次胆汁酸生成細菌の単離と
デオキシコール酸生成に関わる細菌間相互作用の解明）

Primary bile acids are synthesized in the liver and secreted into the duodenum to facilitate dietary fat absorption. Primary bile acids are converted to secondary bile acids (SBAs) by SBA-producing bacteria in the colon. Recently, bile acids have been reported to be involved in controlling host metabolism through nuclear receptors. Moreover, bile acids cause changes of the gut microbiota composition due to their bactericidal activities. Since the gut microbiota have been known to affect host health, identification of novel SBA producers and understanding of their characteristics in the bile acid metabolism are prerequisites for elucidating not only dynamics of gut microbiota and bile acid profiles but also their effects on host health. Therefore, two objectives were set in this study. Firstly, this study aimed to enrich the list of SBA-producing bacteria by isolation and characterization of novel SBA producers from rat cecal contents. Secondly, this study aimed to elucidate the reason why deoxycholic acid (DCA), a representative SBA, accumulates to a high level regardless of a low population of the DCA producers in the gut.

1. Isolation of six novel 7-oxo- or urso-type SBA-producing bacteria from rat cecal contents

Our knowledge of the SBA producers is limited in light of the diversity of gut microbes. This study reported the successful isolation of six novel SBA producers belonging to known species of gut microbes from rat cecal contents. *Anaerostipes caccae* D10, *Bacteroides nordii* C5, *Clostridioides difficile* D7, and *Clostridium*

cadaveris G11 and their type strains were capable of oxidizing primary bile acids, cholic acid (CA) and chenodeoxycholic acid, into their 7-oxo-derivatives with varying yields. Among them, *B. nordii* C5 and its type strain JCM 12987^T showed the highest molar yields of conversion, ~90%. *Clostridium disporicum* F4 and *Clostridium subterminale* C4 epimerized CA into ursocholic acid with yields of ~85%, while their type strains lacked epimerization activity. In addition, although not novel as an SBA producer, *Clostridium scindens* G10 that produced DCA from CA was isolated for the first time from rodents.

2. Identification of 7-oxo-DCA as an alternative substrate for formation of DCA

The co-culture study between DCA producer, *C. scindens* G10, and 7-oxo-DCA producer, *B. nordii* C5, was conducted to investigate their interaction in the SBA formation. The co-culture showed higher yield of DCA from CA than that in the single culture of *C. scindens* G10, suggesting that DCA formation was enhanced by the co-culture with *B. nordii* C5. Importantly, 7-oxo-DCA was detected at lower yields in the co-culture than those in the single culture of *B. nordii* C5, allowing us to hypothesize that 7-oxo-DCA might be a substrate for formation of DCA. A two-step culture was thus conducted to verify this hypothesis; *B. nordii* C5 was cultured for the production of 7-oxo-DCA from CA in the first-step culture, which was followed by the second-step culture to see if 7-oxo-DCA was used as the substrate for DCA formation by *C. scindens* G10 or *C. hiranonis* JCM 10541^T. The results showed that the 7-oxo-DCA was consumed as a substrate for DCA formation by the DCA producers. Thus, 7-oxo-DCA was firstly identified as an alternative substrate for DCA formation.

In conclusion, this study successfully identified six novel SBA producers from rat cecal contents and clarified the interaction between SBA producers, which will be important for further study on both the bacterial bile acid metabolism and the dynamics of the SBA formation in the gut.