国内シンポジウム 2

腸内常在菌の生体への影響を代謝産物の視点から考える

松本光晴
協同乳業株式会社研究所技術開発グループ

ヒト大腸内に生息する腸内常在菌は宿主の健康に強く関与している．長年，培養法により腸内常在菌の解析が行われてきたが，1990 年代後半より実施されている 16S rRNA 遺伝子を用いた系統分類学的解析により明らかにされた腸内常在菌の構造的複雑性（大部分を占める難培養性細菌の存在と個体差の大きさ）は，結果解釈をより困難な状況にしている．一方で，メタゲノム解析は，遺伝子情報の組成や機能を解析することが可能になり，腸内常在菌の役割解明に大きく貢献しているが，全ゲノム情報が得られない難培養性細菌の存在を含めた解釈は困難であり課題は多い．演者らは，これらの背景より，健康と腸内環境の関連性を研究する場合，腸内常在菌の代謝産物の方が，その菌種構成およびその遺伝子情報の組成や機能より直接的で重要と考え，代謝産物に着目し研究を進めてきた．

2010 年時点で，腸内環境をターゲットとしたメタボロミクス解析は，NMR，GC-MS および LC-MS を用いた研究は幾つか存在していたが，一部の代謝産物に焦点を当てた内容であり，全貌解明という視点からは不十分であった．また，これらの報告は菌体も破壊した抽出物を用いており，腸管内に存在し遊離している代謝産物とは言えないものであった．そこで演者らは，CE-TOFMS を用いて，同腹の雄性マウスから無菌（GF）マウスと通常菌叢定着（Ex-GF）マウスを作製し，水溶性の低分子代謝産物を広範囲に調べた（*1*）．結腸内容物より 179 成分が検出され，その内 46 成分は GF マウスが Ex-GF マウスより有意に高濃度，77 成分は GF マウスが Ex-GF マウスより有意に低濃度であり，それぞれ腸内常在菌が吸収する物質，産生する物質であることが示唆された．また，生理機能を有するアミン類は前駆体アミノ酸から腸内常在菌の脱炭酸作用で生じること等，腸内常在菌の作用も確認できた．

また，腸内常在菌の代謝産物は血中に移行し，全身を巡り各臓器に運ばれる．また，腸脳相関を介して，腸内常在菌が脳の代謝系にまで影響を与えている可能性がある．これらを調べるために，上記 GF マウスおよび Ex-GF マウスの大脳皮質および血液も CE-TOFMS メタボロミクス解析した（*2*）．その結果，大脳皮質から 196 の代謝産物が検出され 38 成分が GF マウスと Ex-GF マウスで有意差が認められた．この中には，統合失調症との関連性が示されている Ser，多発硬化症やアルツハイマーと関連がある *N*-Acetylaspartic acid が含まれていた．更に解糖系代謝産物（Glucose 6-phosphate，Fructose 6-phosphate，Fructose 1,6-diphosphate）にも差が認められ，大脳のエネルギー利用にも腸内常在菌が影響を与える可能性が示唆された．また，メタボロミクス用糞便試料調製における課題も紹介する．

本研究は生研センター・イノベーション創出基礎的研究推進事業の研究支援を受けて実施したものである．

【引用文献】

（*1*）Matsumoto M, Kibe R, Ooga T, Aiba Y, Kurihara S, Sawaki E, Koga Y, Benno Y. 2012. Impact of Intestinal Microbiota on Intestinal Luminal Metabolome. Sci. Rep. **2** : 233.
（*2*）Matsumoto M, Kibe R, Ooga T, Aiba Y, Sawaki E, Koga Y, Benno Y. 2013. Cerebral low-molecular metabolites influenced by intestinal microbiota: a pilot study. Front. Syst. Neurosci. **7** : 9.

Metabolomic approach for elucidating the relationship between intestinal microbiota and host health

Mitsuharu Matsumoto
Dairy Science and Technology Laboratory, Kyodo Milk Industry Co. Ltd.

Intestinal microbiota play a fundamentally important role in health and diseases. Many molecular biology approaches, including next-generation sequencing involving 16S rRNA gene sequencing, have been used for the analysis of intestinal microbiota. The findings have shown that gut microbiota are structurally complex in that they have great diversity and inter-individual differences, and 60–80% of the total bacterial species in the microbiota are difficult to culture. Recently, metagenomic techniques have been used to characterize both the composition and the potential physiological effects of the microbial community. However, since metagenomics databases do not contain information about the genome of uncultured bacteria, creating a comprehensive database is essential for understanding the intestinal environment. Therefore, we have been focusing on bacterial metabolites because low-molecular-weight metabolites produced by intestinal microbiota are absorbed constantly from the intestinal lumen and carried to systemic circulation; these metabolites play a direct role in health and disease.

Metabolomics is a rapidly evolving field involving comprehensive measurement of many metabolites in biological fluids. As of 2010, several metabolomics studies have focused on specific metabolites, such as bile acids, by using NMR and LC-MS; however, these studies did not focus on a wide spectrum of intestinal luminal metabolites. In addition, in these studies, metabolomes that included bacterial intercellular metabolites were analyzed because the sample was prepared by sonication of feces. For clarifying the relationship between health and gut bacterial metabolites, only free metabolites in the intestinal luminal content should be analyzed.

In this study, we divided male mice bred from sister-brother mating into two groups, germ-free (GF) mice and Ex-GF mice; these mice were inoculated twice with a suspension of feces obtained from SPF mice. We analyzed the colonic luminal, cerebral, and blood metabolomes of GF mice and Ex-GF mice by using capillary electrophoresis with time-of-flight mass spectrometry (CE-TOFMS). From the colonic luminal metabolome, 179 metabolites were isolated and 48 metabolites were found to be present at significantly ($p < 0.05$) higher concentrations and/or frequency in the germ-free (GF) mice than in the Ex-GF mice; 77 metabolites were present at significantly lower concentrations and/or frequency in the GF mice than in the Ex-GF mice, indicating that the intestinal microbiota highly influenced the colonic luminal metabolome and that a comprehensive understanding of the intestinal luminal metabolome is critical for clarifying host-intestinal bacterial interactions (*1*). For instance, the concentrations of all primary amines, which have physiological functions, in the Ex-GF mice were higher than those in the GF mice, although no significant differences were noted between the concentrations of most of the precursors for the GF mice and the Ex-GF mice. From the cerebral metabolome, 196 metabolites were identified in both GF and Ex-GF mice. The concentrations of 38 metabolites significantly differed between GF and Ex-GF mice (*2*). Approximately 10 of these metabolites, such as serine and *N*-acetylaspartic acid, are known to be involved in brain function, whilst the functions of the remainder are unclear. The concentrations of several cerebral glycolysis intermediates were higher in GF mice than in Ex-GF mice. I will also be discussing methods for obtaining the metabolome from human fecal samples during my presentation.

【References】

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