氏　　名	SOULIPHONE SIVIXAY
授与した学位	博　士
専攻分野の名称	農　学
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学位授与の要件	環境生命科学研究科　　農生命科学専攻 （学位規則第４条第１項該当）
学位論文の題目	Gut microbiota of rats and mice fed different sources of dietary protein and fat （種々のタンパク質及び脂質を摂取したラット及びマウスの腸内細菌叢）
論文審査委員	教授　齋藤　昇　　教授　森田　英利　　教授　西野　直樹　　准教授　鶴田　剛司

学位論文内容の要旨

The research exploring the health-promoting and disease-preventive effects of the gut microbiota is gaining momentum and any entities to which the gut microbiota sensitively respond are now considered valuable tools to exploit and develop new strategies to promote systemic health. Substrates for gut fermentation are fundamentally non-digestible dietary components and host secretions mainly comprising mucin. Thus, highly digestible protein and fat have been considered to have little influence on the shaping of the gut microbiota. However, a small amount of non-digestible protein and fat may reach the large intestine, being a potential factor affecting bacterial metabolism. In the present study, three experiments were conducted using rats and mice to examine whether different sources of protein (soy, meat, fish, milk, and egg) and fat (sacha inchi, soybean, olive, lard, canola, and sesame) affect the gut microbiota and their metabolism.

In the first experiment, a total of 60 rats were used in two separate feeding trials. Soy, meat (a mixture of beef and pork), and fish proteins were fed to rats with cellulose (CEL) and raffinose (RAF) in trial 1, and soy, milk (casein), and egg proteins were given to rats with CEL and RAF in trial 2. Egg protein feeding decreased the concentration of acetic acid and the richness and diversity of the cecum microbiota. When fed with CEL, the abundances of *Ruminococcaceae* and *Christensenellaceae*, *Akkermansiaceae* and *Tannerellaceae*, and *Erysipelotrichaceae* enhanced with soy protein, meat and fish proteins, and egg protein, respectively. The effects of dietary proteins diminished with RAF feeding and the abundance of *Bifidobacteriaceae*, *Erysipelotrichaceae*, and *Lachnospiraceae* increased and that of *Ruminococcaceae* and *Christensenellaceae* decreased regardless of the protein source. These results indicate that, although the effect of prebiotics is more robust and distinctive, dietary protein sources may influence the composition and metabolic activities of the gut microbiota.

In the second experiment, six different oils, i.e. sacha inchi, soybean, olive, lard, canola, and sesame, were fed to 30 mice. Casein and CEL were used as exclusive protein and fiber sources in this trial. No distinctive differences were seen between six oil treatments in the cecum short-chain fatty acids concentration, except that lard feeding lowered the propionic acid concentration compared with sacha inchi, soybean, and olive oil feedings. The abundances of *Enterobacteriaceae*, *Mogibecteriaceae*, and *Peptostreptococcaceae* appeared to increase when mice fed sacha inchi compared with sesame oil, and the abundance of *Ruminococcaceae* appeared to enhance when mice fed sacha inchi and olive oils compared with soybean oil and lard. Meanwhile, six oil treatments exerted substantial changes in lipid metabolism; sacha inchi oil feeding lowered the total cholesterol and phospholipid concentrations, while not affecting the triglyceride concentrations in the serum and liver. Dietary oils with different fatty acids composition greatly affected lipid metabolism, but the changes may have not be mediated via gut microbiota and their metabolism.

In the third experiment, mixed effects of dietary protein and oil were examined using 30 mice. Casein, soy, and egg proteins were chosen as protein sources from the first experiment and sacha inchi and canola oils were selected as fat sources from the second experiment. The concentration of cecum butyric acid was increased by egg protein than soy protein feeding and by sacha inchi oil than canola oil feeding. The abundances of Firmicutes and *Bifidobacterium* were lowered when egg protein was fed with sacha inchi oil, and that of Bacteroidetes was increased when egg protein rather than soy protein was fed. Moreover, although plasma total cholesterol, triglyceride, and phospholipid concentrations were not affected by the sources of dietary protein and fat, the concentration of liver total cholesterol was increased by soy protein compared with casein, and further increased by egg protein compared with soy protein feeding.

Several protein and oil effects on the gut microbiota and metabolism have been shown in this study, encouraging further works to gain further insights into the potential of macro- and micro-nutrients in facilitating the growth of gut microbiota and mediating healthy metabolic homeostasis.

論文審査結果の要旨

本研究は，食餌タンパク質及び脂質の違いが，腸内細菌叢及び栄養素代謝にどのような影響するかを調べたものである。栄養，免疫，疾病等に腸内細菌叢が関わっていることは広く知られているが，未消化で大腸に到達する量が少ないタンパク質や脂質といった食品成分は，腸内細菌叢の変動因子とはとらえられていない。本研究では，アジア人の標準的な食餌内容に相当するタンパク質 20%及び脂質 10%という条件で，腸内細菌叢及び栄養素代謝の変動を調査した。

実験 1 では，牛乳カゼイン（CA），精製ダイズタンパク質（SP），肉タンパク質（MP），魚タンパク質（FP）及び卵白アルブミン（EA）をラットに給与し，盲腸内短鎖脂肪酸濃度と腸内細菌叢の差異を調べた。SP は *Ruminococcaceae* を，MP と FP は *Christensenellaceae*，*Akkermansiaceae* 及び *Tannerellaceae* を，EA は *Erysipelotrichaceae* を増加させることが明らかとなり，EA 給与による変化は酢酸濃度の低下としても表れた。マウス，ラットを用いた食品機能解析では，CA を単一タンパク質源とする飼料が標準食とされており，MP，FP 及び EA といった多様なタンパク質源を組み合わせることが望ましいと判断された。

実験 2 では，タンパク質を CA だけとし，サチャインチ油（SI），ダイズ油（SB），オリーブ油（OL），ラード（LD），菜種油（CL）及びゴマ油（SE）という 6 種類の油脂をマウスに給与した。SI は *Enterobacteriaceae* と *Mogibacteriaceae* を，OL は細菌叢の *Ruminococcaceae* を，SE は *Streptococcaceae* を増加させたが，細菌叢全体の変化は小さく，短鎖脂肪酸濃度は油脂の種類による影響を受けなかった。

実験 3 では，タンパク質として CA，SP，EA を，油脂として SI と CL をマウスに給与した。SP は Bacteroidetes を，EA と SI の組み合わせは Firmicutes と *Bifidobacterium* を減少させることが明らかとなり，EA の作用は *iso*-酪酸濃度の低下及び *n*-酪酸濃度の上昇としても表れた。

これらの結果は，標準的な食餌条件でもタンパク質や脂質成分が腸内細菌叢に少なからぬ影響を与えることを示しており，CA 以外のタンパク質源で食品機能解析をする必要性も示唆している。得られた知見及び成果は関連分野で十分な新規性及びオリジナリティがあるものと評価されており，SIVIXAY Souliphone 氏は環境生命科学研究科の博士（農学）の学位を受ける資格があるものと判断した。