Gut Metabolome Helps to Understand the Relationship between Host and Microbe

Joint Graduate Student Seminar

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OUTLINE

- Introduction of microbial metabolomics

- Introduction of gut metabolomics

- Strategies to conduct a gut metabolomic profiling

- Summary

Background

High-throughput omics technologies

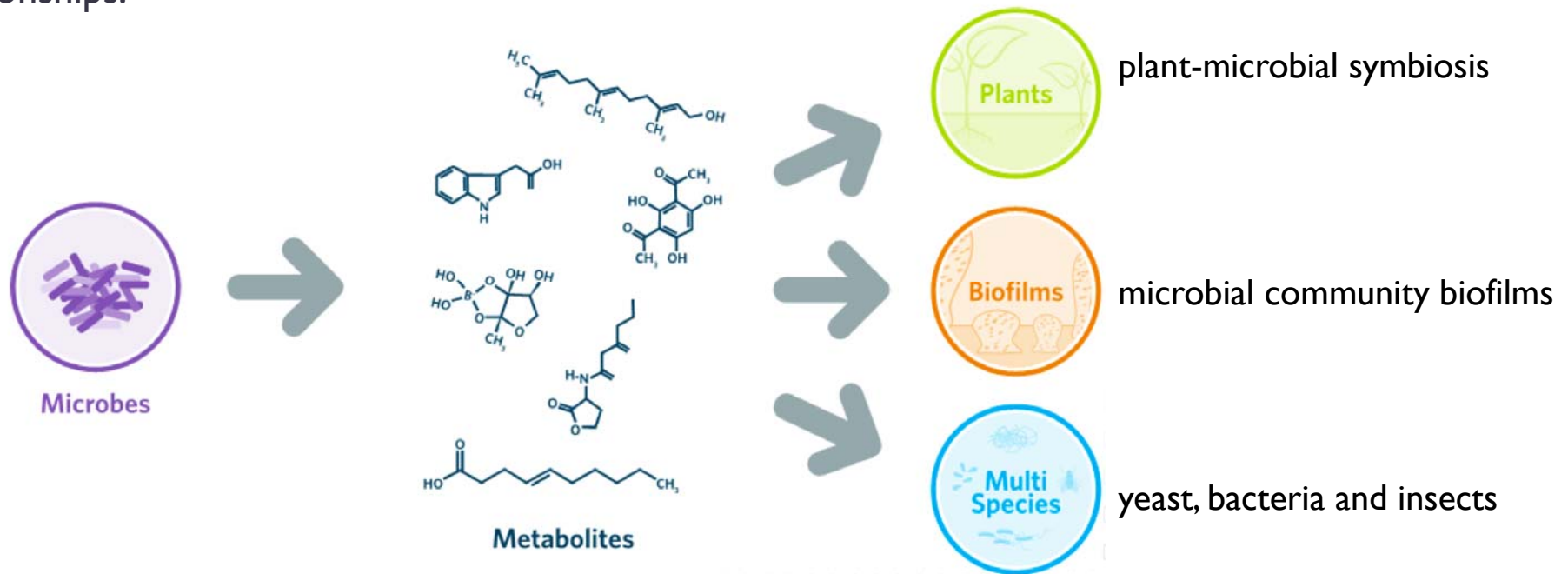- metagenomics
- meta-transcriptomics
- meta-proteomics

Metabolites are a surrogate of the physiological/phenotypic state making them an ideal way to track changes induced by disease or treatment. The assessment of the metabolic state is particularly important for understanding complex phenotypes where the drivers are numerous (i.e. genetics, environment and microbiota)
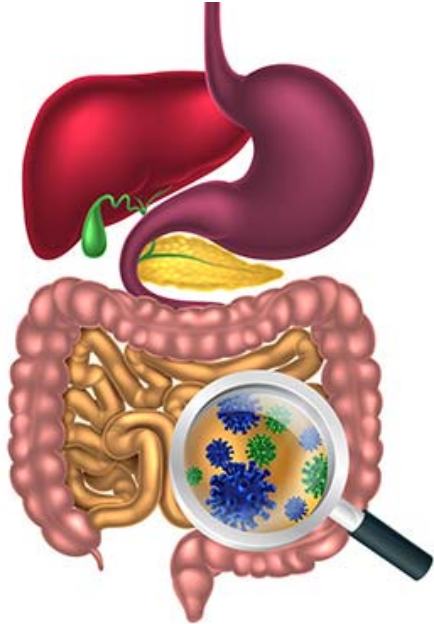
otype

The Microbe-host "Currency"

- The rich history of microbiology has established that, throughout nature, small molecules such as plant hormones or quorum-sensing metabolites serve as a "currency" and "language" that mediate cross-species relationships.

Microbes → Metabolites →

plant-microbial symbiosis

microbial community biofilms

yeast, bacteria and insects

Metabolites Also Link the human-microbiota Interaction

- At many junctions, small molecules/metabolites mediate function within this cross-species relationship. These metabolites can arise from host or microbial biosynthesis or small molecules such as drugs or xenobiotics. All have been reported to either affect the physiology of the host or the collective physiology of the "organ" of the microbiome - which in turn can affect the host.
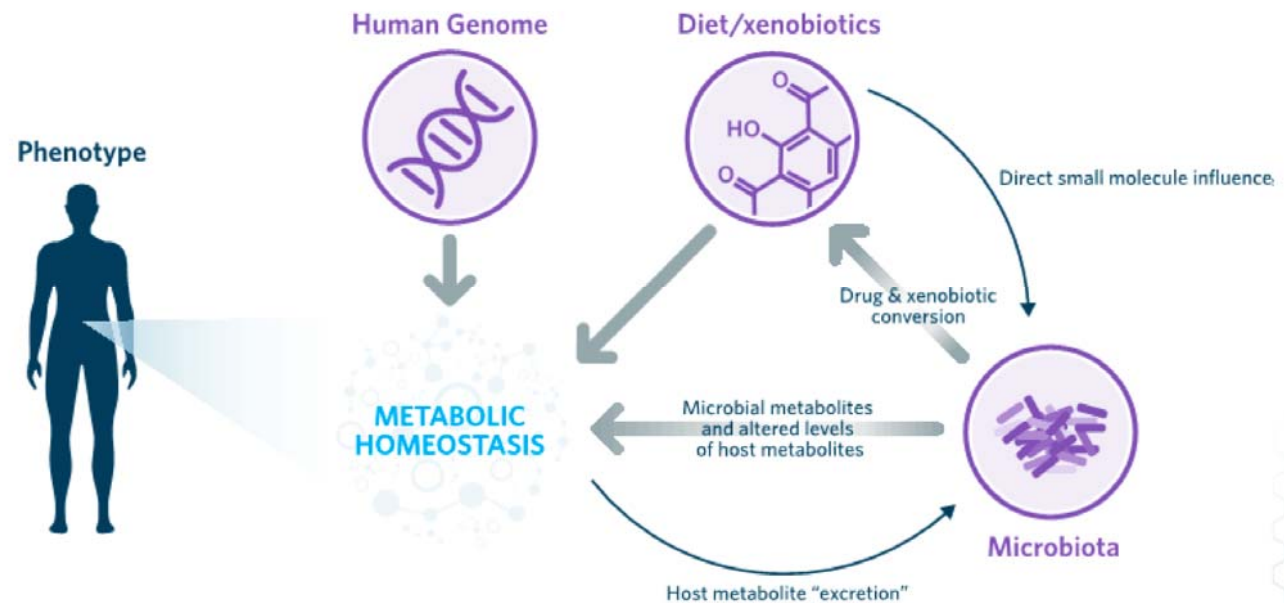
Gut Microbial Metabolite -- a Critical Regulator of Human Homeostasis

- Gut microbial metabolites serve as an additional layer of communication between the host and microbiota
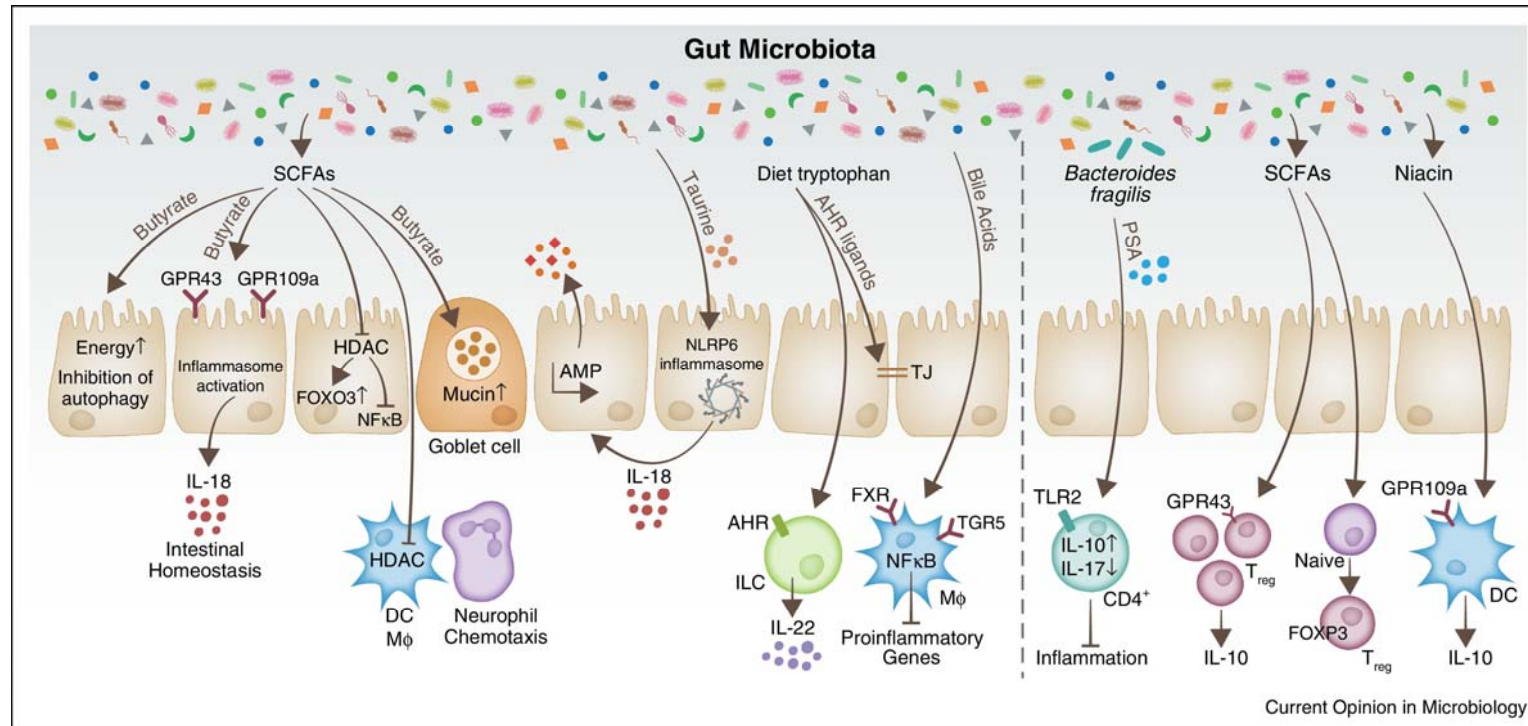
A Critical Regulator of Its Host Homeostasis

- When the balanced interaction between the host and the microbiota is disrupted, intestinal and extraintestinal diseases may develop.

Metabolite/Small Molecule	Physiology induced or associated with	Citation
Colibactin	Colitis-associated colorectal cancer (CRC)	(Arthur et al., Science, 2012)
Various dru...		...macol Res, 2013)
Metformin	...ut microbial community to result in an improved metabolic profile	(Shin et al., Gut, 2013)
Herbal medicines	Transformed by microbiota to deliver efficacy	(Kim et al., J Microbiol Biotechnol, 2008)
Deoxychol...		(Yoshimoto et al., Nature, 2013)
Cyanuric a...		..., Sci Transl Med, 2013)
Short chain...		(Liou et al., Sci Transl Med, 2013)
Guaiacol	Locust swarming	(Dillon, Vennard, and Charnley, Nature, 2000)
Trimethylamine N-oxide (TMAO)	Heart disease	(Wang et al., Nature, 2011)
Vitamin B metabolites	T-cell activation	(Kjer-Nielsen et al., Nature, 2012)
Medium chain fatty acids	Immune and inflammatory response	(Maslowski et al., Nature, 2009)
Portal circulation TLR agonists	Liver disease - NAFLD	(Henao-Mejia et al., Nature, 2012)
Polysaccharide A	Immunologic tolerance	(Round et al., Science, 2011)

short-chain fatty acids (SCFAs)

indoles

protective effect from the development of disease

trimethylamine N-oxide (TMAO)

4-ethylphenylsulfate (4-EPS)

directly drive the susceptibility to disease

A Few Examples of Small Molecules from the Human Gut Microbiota

- SCFAs

  result of non-digestible carbohydrate fermentation by anaerobic commensal bacteria

 - energetic substrates for epithelial cells

    utilize bacterially produced butyrate as a primary energy source

  - maintenance of the epithelial barrier

    protect stem cells from the anti-proliferative effect during homeostasis

    goblet cells up-regulate the expression of MUC genes in response to butyrate

    activate of the inflammasome, ↑ the downstream production of interleukin-18 (IL-18)
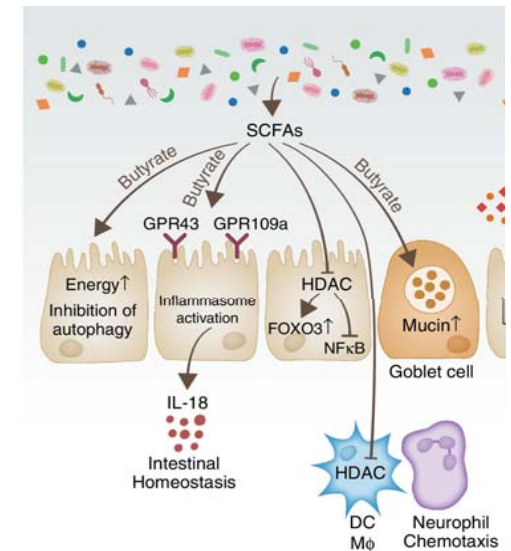
A Few Examples of Small Molecules from the Human Gut Microbiota

- ## Oligosaccharides

  - ### Polysaccharide A (PSA)

   From *Bacteroides fragilis*

    signal to the host's innate immune system through the Toll-like receptor 2 (TLR2)

    ⬇

    induction of regulatory T cells to produce the tolerogenic cytokine interleukin-10 (IL-10)

    restricts the activity of T helper 17 (TH 17) cells , promotes *B. fragilis* colonization, suppresses *Helicobacter hepaticus* induced colitis

Oligosaccharides

polysaccharide A
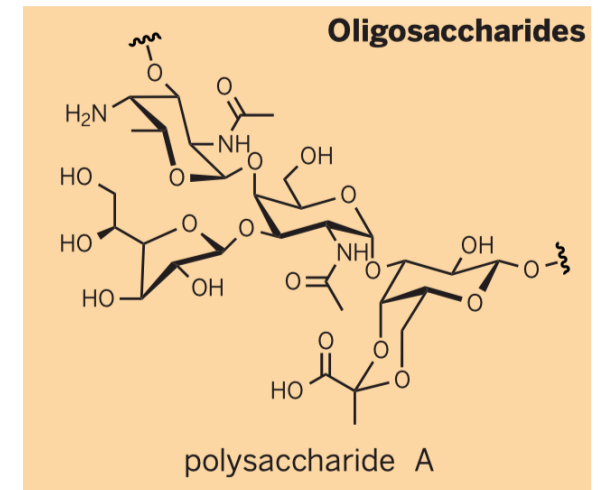
A Few Examples of Small Molecules from the Human Gut Microbiota

- Products of amino acid metabolism

  - **indole**

  derived from tryptophan by as-yet-unidentified enzyme(s)

  serves as a signaling agent in bacterial communities

  following absorption through the intestinal epithelium, indole in the liver becomes indoxyl sulfate, a well-known uremic toxin that is known from germ-free mice studies to be derived entirely from the gut microbiota

  Indoxyl sulfate occurs at a wide range of concentrations in humanurine (10 to200mg/day), likely reflecting differences among individuals in diet and in the level of indole-producing bacterial species in the gut community
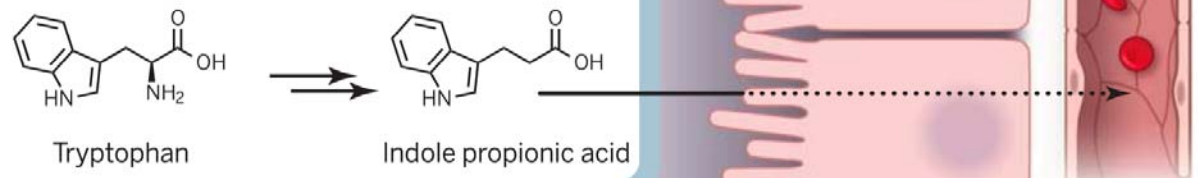
Materials for Gut Metabolic Profiling

luminal content collected in various sections of the gastro-intestinal track

- invasive

- representative of different sections of the gastro-intestinal track

faecal materials (faecal water/ fresh faecal)

- faeces are the ultimate end product of digestion processes taking place in the gut

- reliably reflect the microbial activity in distal colon

- easily accessible, non-invasive

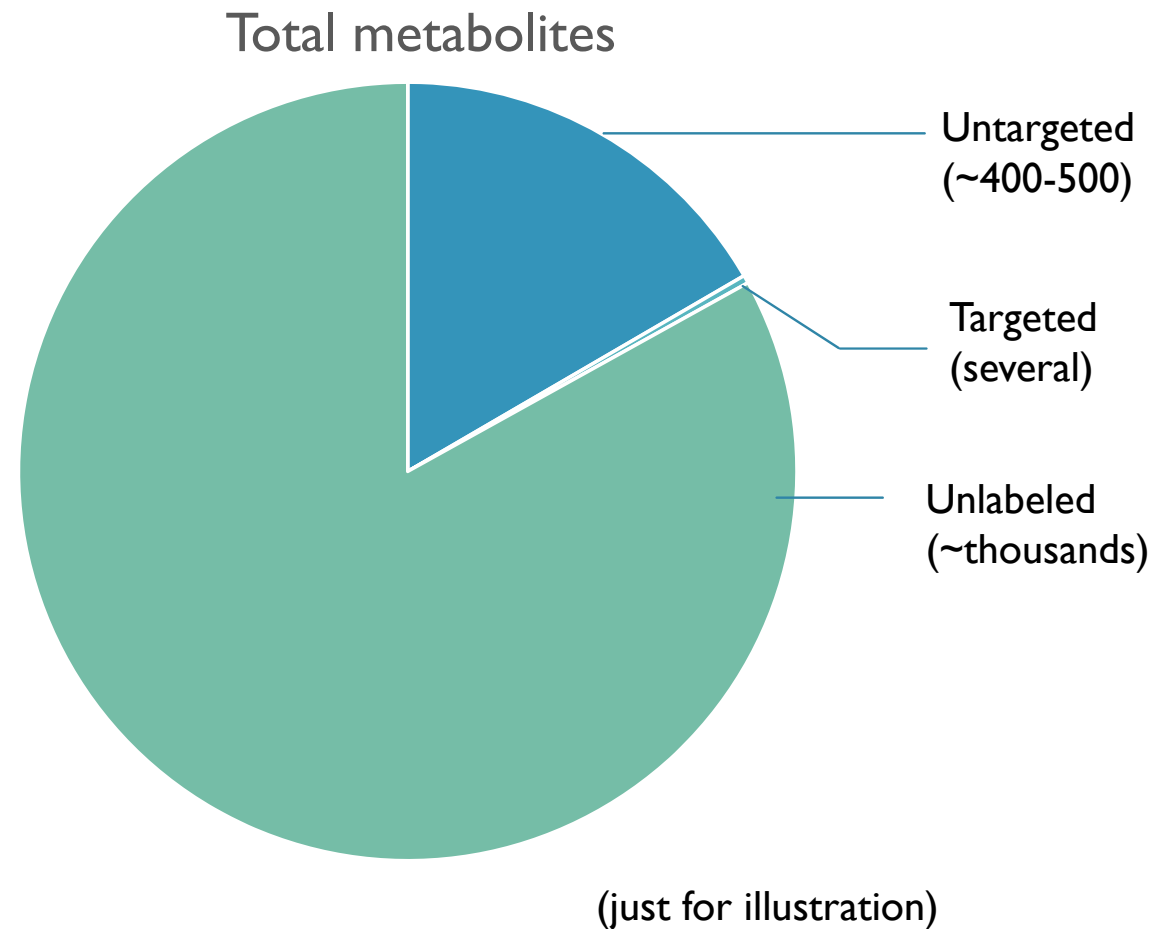Approaches of Metabolic Profiling

Untargeted metabolomics

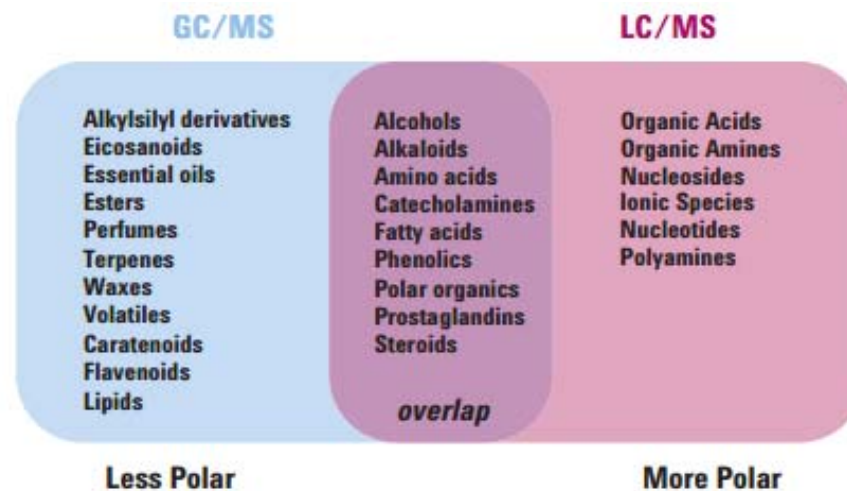comprehensive analysis of all the measurable analytes in a sample, including chemical unknowns

Targeted metabolomics

measurement of defined groups of chemically characterized and biochemically annotated metabolites (e.g. SCFAs, bile acid)

Total metabolites

Untargeted (~400-500)

Targeted (several)

Unlabeled (~thousands)

(just for illustration)

Approaches of Metabolic Profiling

- Metabolic profiling is mostly achieved using two analytical platforms:

  - nuclear magnetic resonance (NMR) spectroscopy

  - mass spectrometry (MS)

    gas chromatography (GC-MS)

    liquid chromatography (LC-MS)

GC/MS		LC/MS
Alkylsilyl derivatives	Alcohols	Organic Acids
Eicosanoids	Alkaloids	Organic Amines
Essential oils	Amino acids	Nucleosides
Esters	Catecholamines	Ionic Species
Perfumes	Fatty acids	Nucleotides
Terpenes	Phenolics	Polyamines
Waxes	Polar organics	
Volatiles	Prostaglandins	
Caratenoids	Steroids	
Flavenoids		
Lipids	*overlap*	
Less Polar		**More Polar**

Untargeted Metabolome

- NMR spectroscopy measurement

  all metabolites with nonexchangeable protons (micromolar range)

  - highly reproducible

  - cost effective

  - require a few simple preparation steps

❖ monitor the gut microbial metabolic activity in elderly   (Claesson et al., 2012)

   In this study, it was possible to cluster patients according to their community setting (length of hospital care) based on fecal water metabolic profiling

Untargeted Metabolome

- Mass spectrometry-based measurement

  more sensitive than NMR (in the nanomolar range)

  a large amount of unknown signals

❖ Cao et al. used UPLC-MS/TOF-MS to analyze the fecal metabolome in patients with liver cirrhosis and hepatocellular carcinoma (HCC)

    Chenodeoxycholic acid, 7-ketolithocholic acid, urobilinogen, urobilin, lysophosphatidylcholine (LPC) 16:0 and 18:0 were found to discriminate between healthy controls and patients with liver cirrhosis and HCC. Whereas LPC species were found in increased levels, the other discriminatory markers were decreased in the patient samples. The identities of these markers were confirmed by comparison of chromatographic retention and product ion spectra with authentic standards.

Targeted Metabolome

- MS method is optimized to the detection of a specific class of analytes

    - high analyte recoveries during sample preparation

    - reproducible

    - accurate quantification

Targeted Metabolome

❖ Han et al. presented a LC-MS/MS method for SCFA quantification in human feces

SCFAs are converted to 3-nitrophenylhydrozones (3NHPH) which are separated by reversed phase chromatography and detected in negative ion mode

covers 10 straight- and branched chain SCFAs

introduced an internal standard for every analyte by conversion of a standard mixture with a [13]C 6 -labeled derivatization reagent

This method showed a high reproducibility and analysis of human fecal samples revealed an increased fraction of branched-chain SCFA in T2D patient compared the other analyzed sample

Summary

- LC-MS based methods for targeted fecal metabolome analysis, for example bile acids and SCFAs determination

- NMR and GC–MS based metabolomics have been implied for untargeted fecal metabolome analysis

Take Home Messages

- The measurement of the fecal metabolome provides an easy estimate of the diet-gut microbiota-host metabolic interaction

- Both NMR and MS-based metabolic profiling are complementary techniques and none of them to date can holistically assess the fecal metabolome. Instead, it is recommended that a combination of methods is used to extend the metabolic coverage.
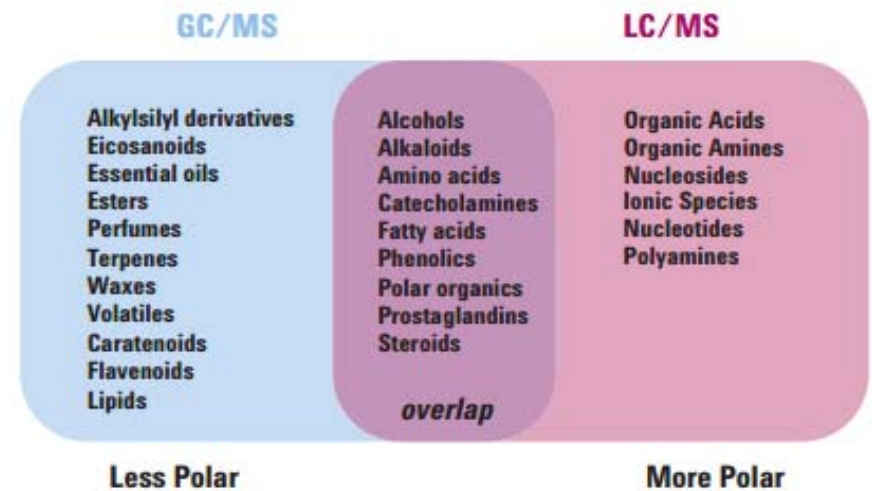
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GC vs. LC

	GC	LC
Mobile phase	Gas	Liquid
Sample state	Gas	Liquid
Stationary phase	Liquid or solid	Liquid

GC/MS

Alkylsilyl derivatives
Eicosanoids
Essential oils
Esters
Perfumes
Terpenes
Waxes
Volatiles
Caratenoids
Flavenoids
Lipids

Alcohols
Alkaloids
Amino acids
Catecholamines
Fatty acids
Phenolics
Polar organics
Prostaglandins
Steroids

overlap

LC/MS

Organic Acids
Organic Amines
Nucleosides
Ionic Species
Nucleotides
Polyamines

Less Polar   **More Polar**

- The gut microbiota is a highly metabolically active community of micro-organisms.