# The Role of $H_2$ in the Gut Microbiome: Findings and MMPC Applications

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# UM Microbiome Project

## Overall Goal: Link the gut microbiome to host phenotypes

### First Question: How does the host respond to short chain fatty acids?

1. Increased host respiration
2. Increased respiration in colonic epithelium
   (↑cytochrome oxidase, ↑succinate dehydrogenase, ↓$O_2$ in mucus)
3. Absence of SCFAs disrupts $O_2$ gradient in gut mucosa
   (Hif concentrations)

### Next Question: What regulates production of SCFA in the gut microbiome?

# Human gut microbiota

- Dense microbial community colonizing the human gut
- Broadly implicated in human health
  - Focus on role as a “metabolic organ”
- Who’s there?

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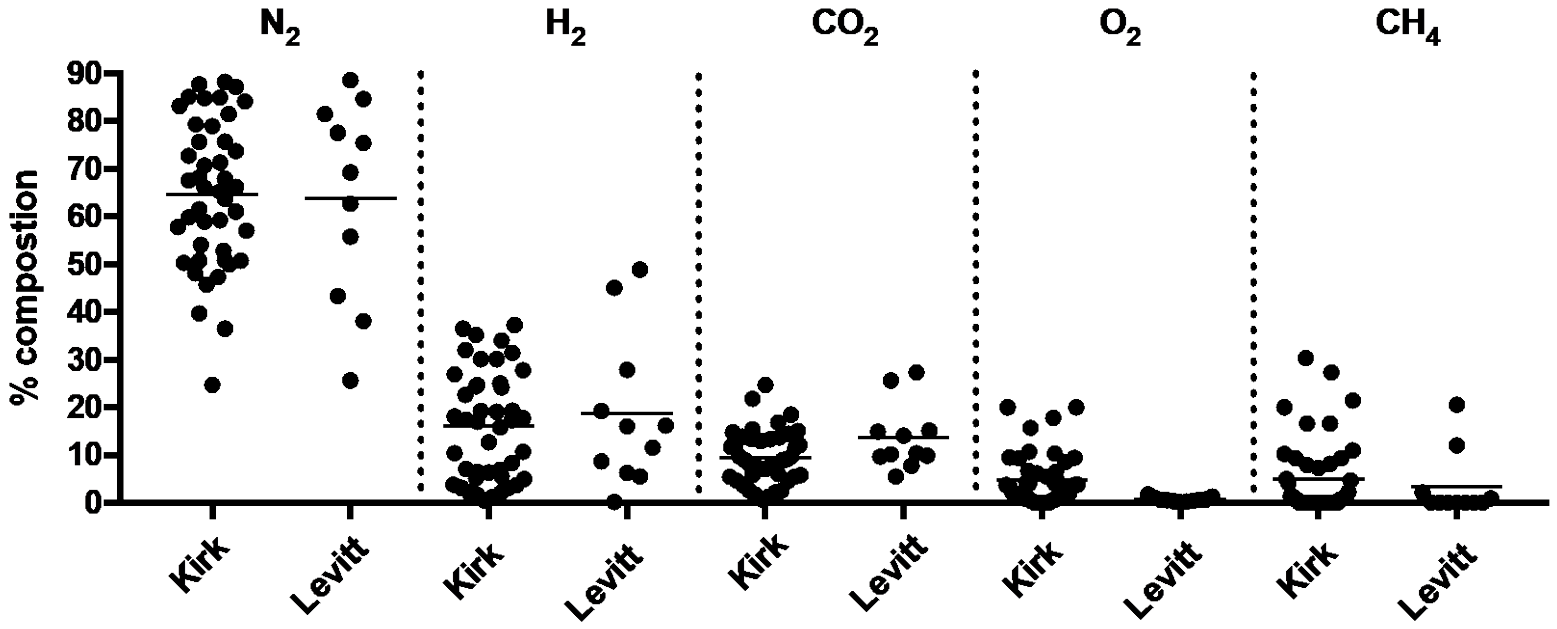
- Broadly implicated in human health
  - Focus on role as a “metabolic organ”
- Who’s there? → What’s going on?
  - Metabolic activity of individual microbes
  - Inter-microbial interactions
  - Gut environmental factors influencing microbial metabolism

# Human colonic gas




- Esben Kirk, The Quantity and Composition of Human Colonic Flatus (1949), *Gastroenterology* **12**: 782-94.
- Michael D. Levitt, Volume and Composition of Human Intestinal Gas Determined by Means of an Intestinal Washout Technic (1971):, *New England Journal of Medicine* **284**: 1394-8.

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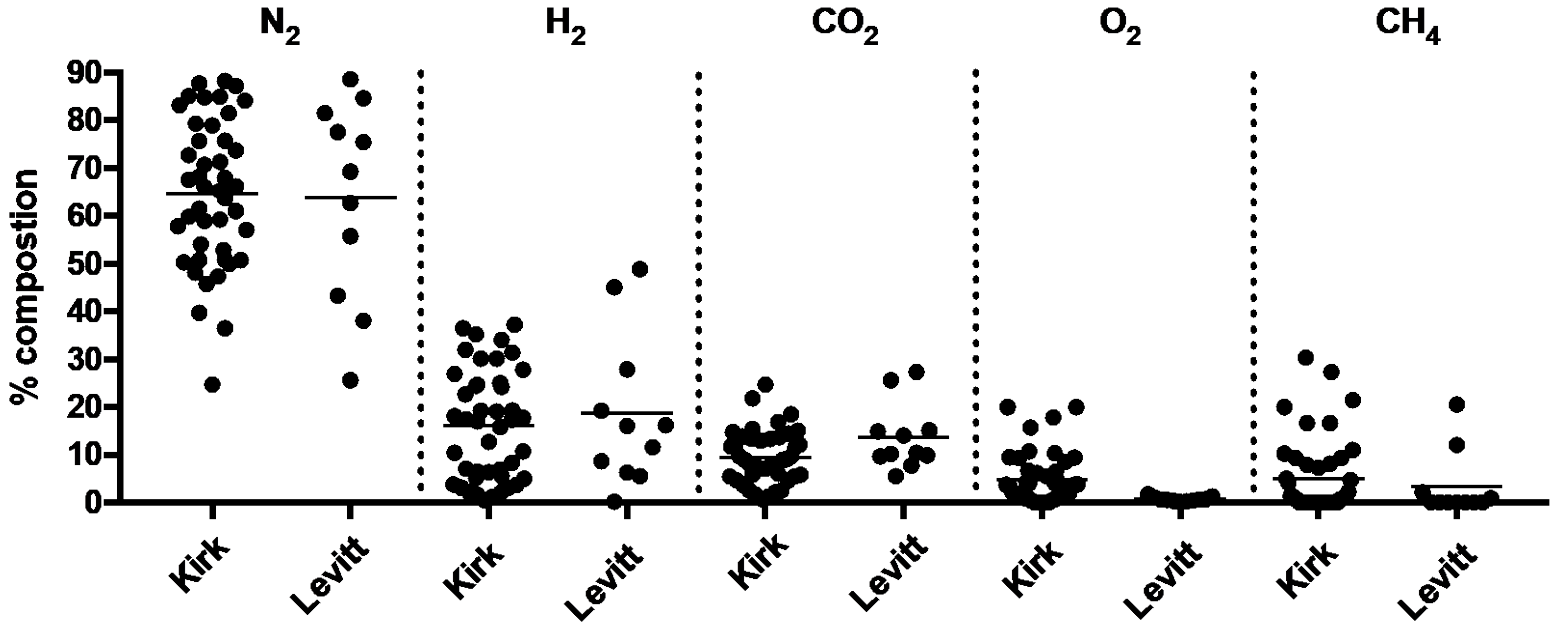
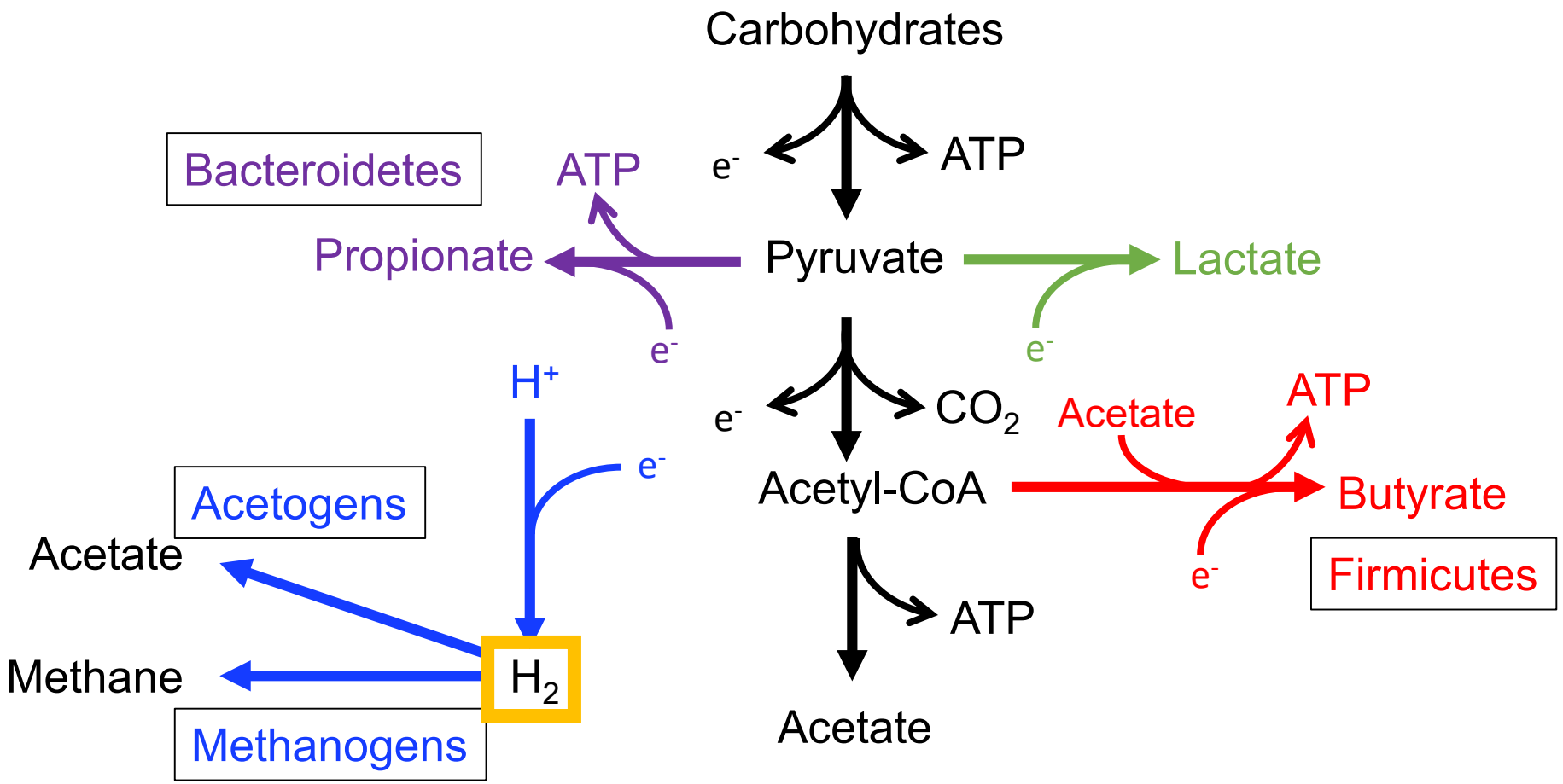


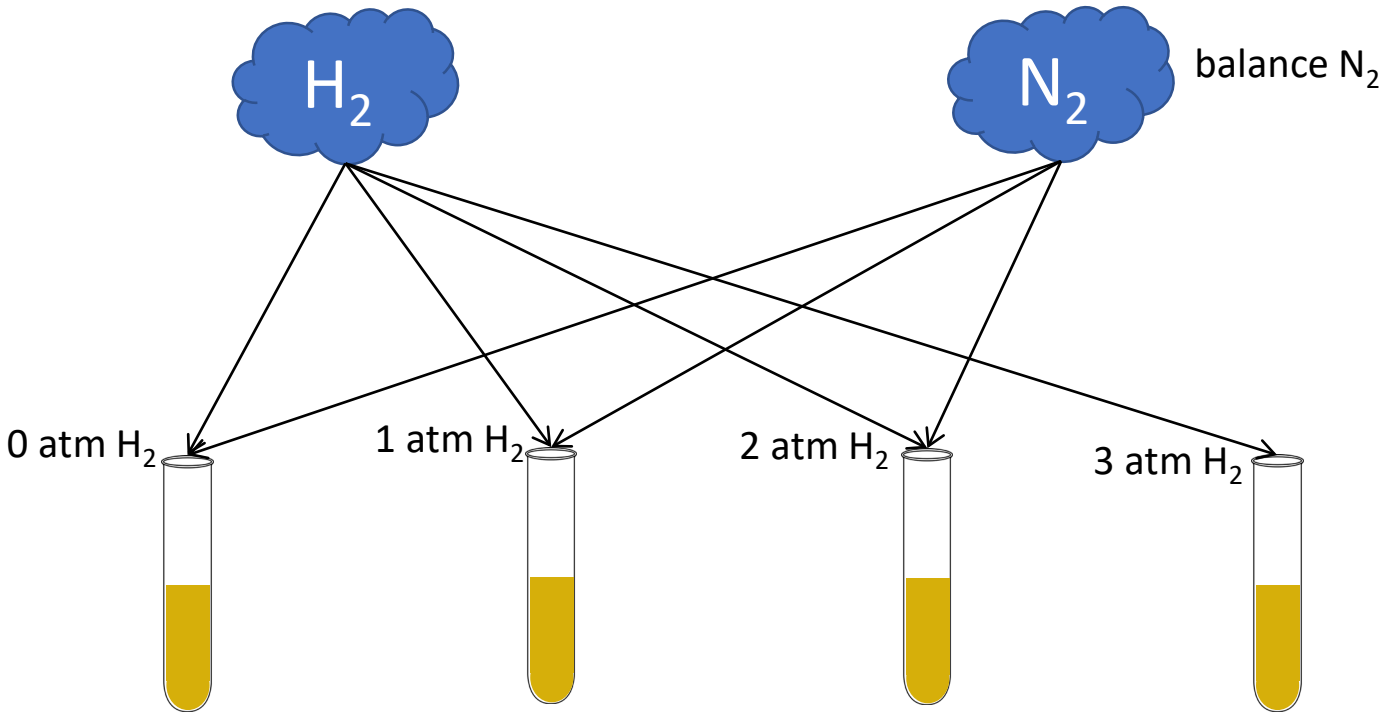

- Significant, variable, and understudied feature of the colon environment!
- Focus on $H_2$
  - Completely dependent on microbiota
  - Highly variable between individuals
  - Theoretical effects of $H_2$ on short-chain fatty acid (esp. **butyrate**) production

# A major challenge for microbes in anoxic environments is to relieve themselves of reducing power ($e^-$)



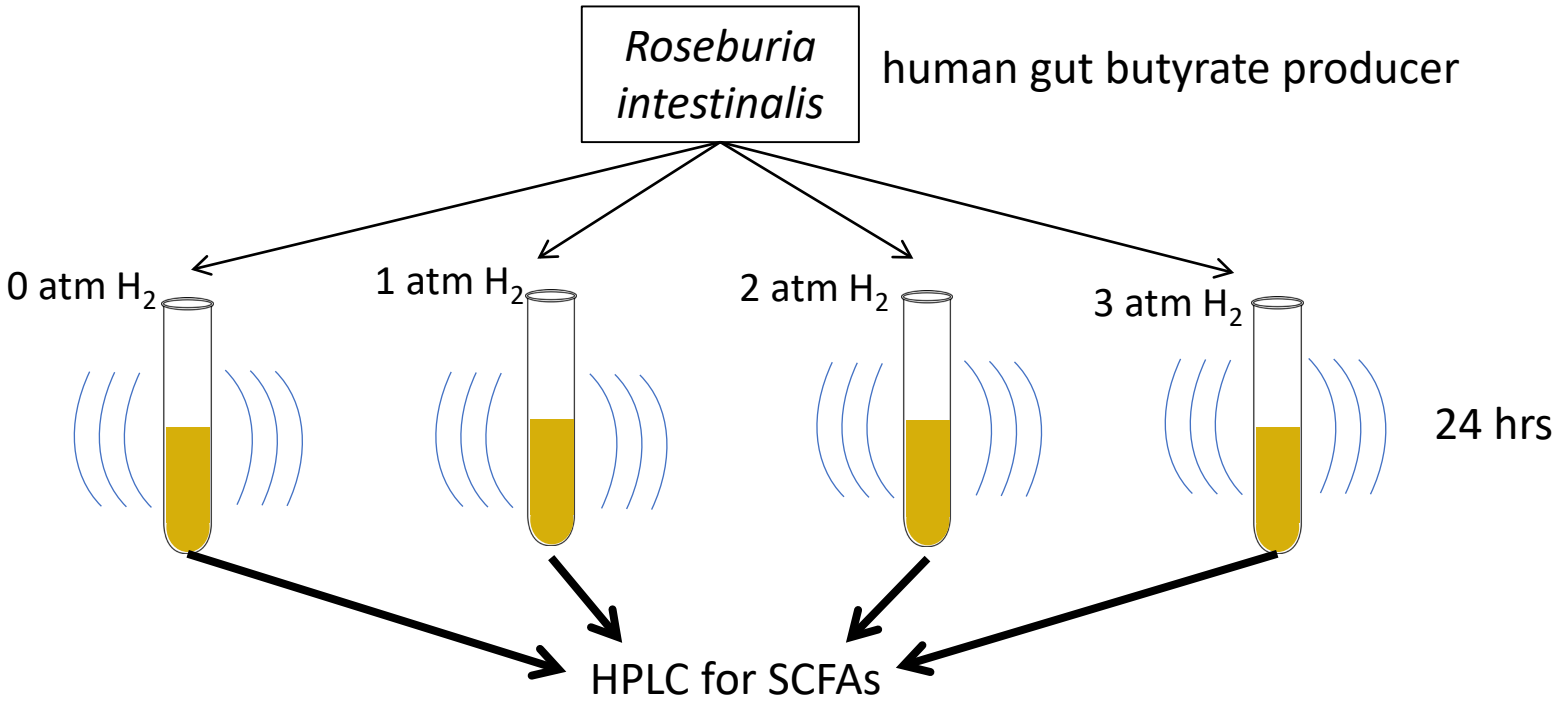

Hypothesis: More $H_2$ → more butyrate & propionate
Less $H_2$ → more acetate

# Experimental design: High [$H_2$]

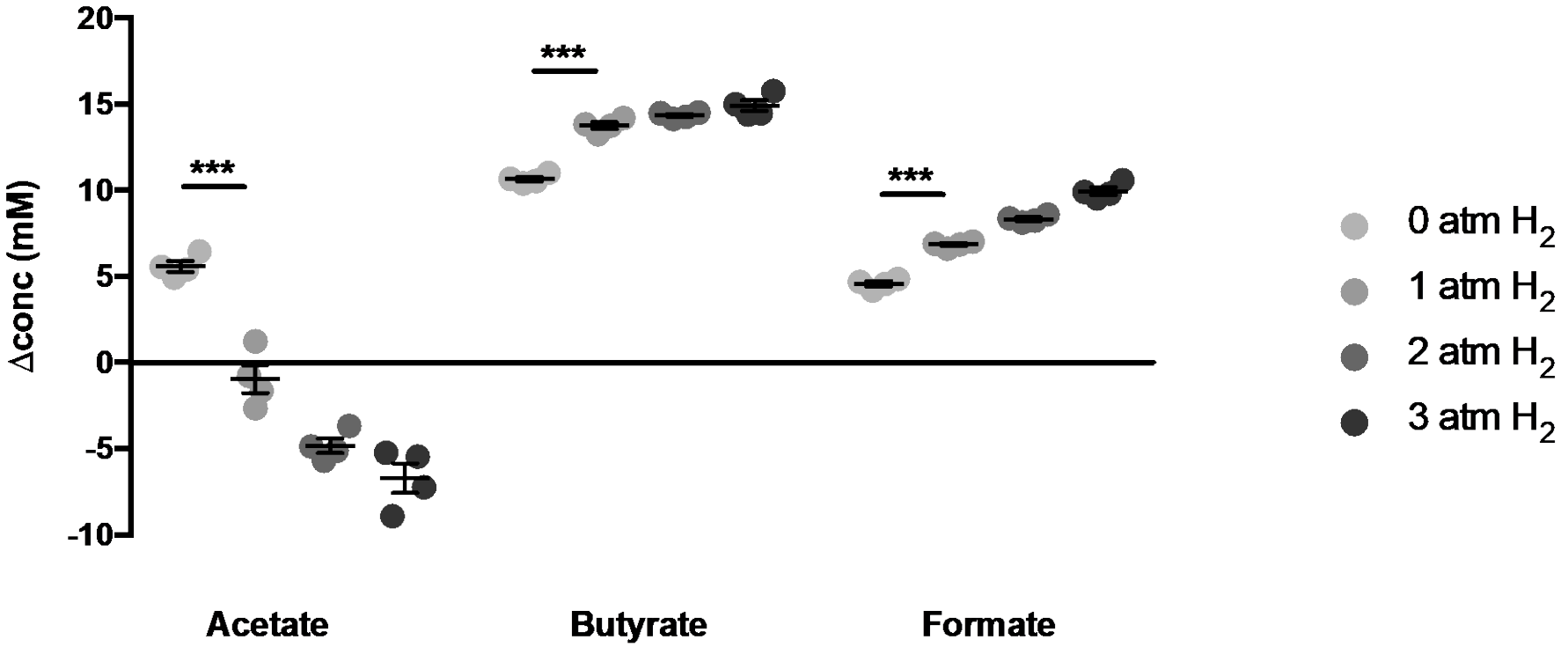


# Experimental design: High [$H_2$]

- Prediction: Human gut butyrate producer (*R. intestinalis*) will show increased butyrate and decreased acetate under higher [$H_2$]



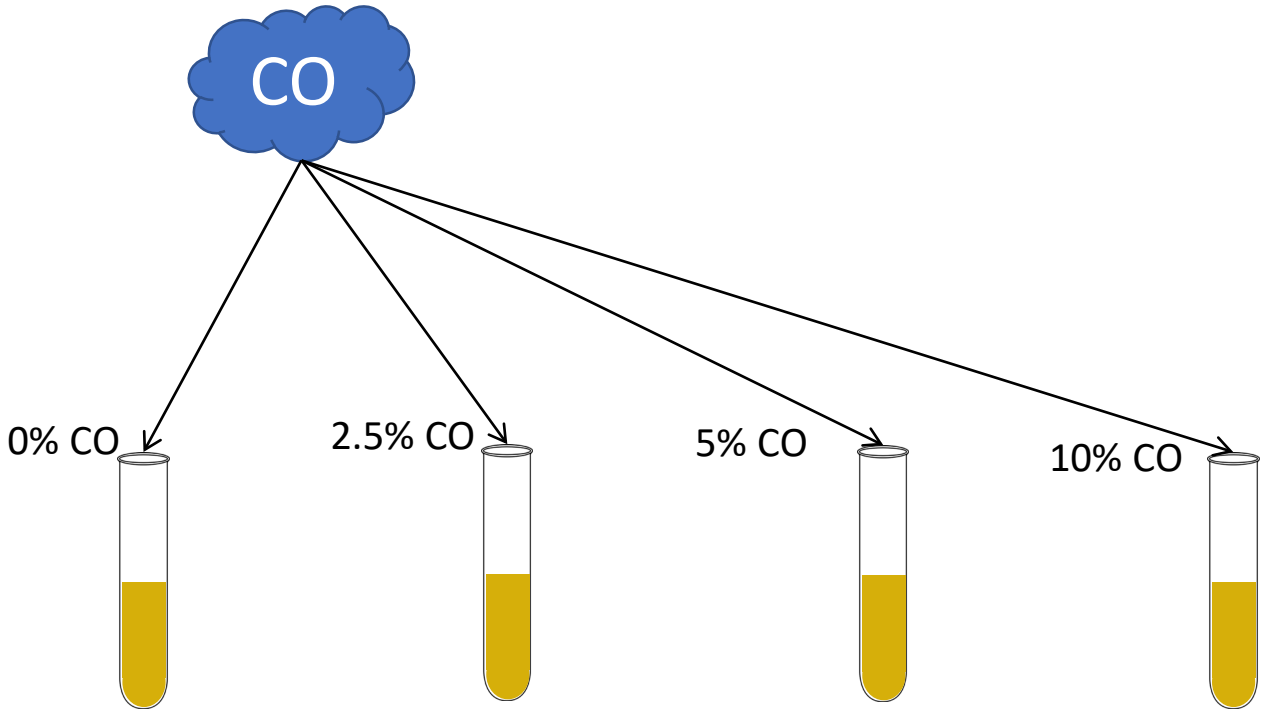
# Results: *R. intestinalis* +$H_2$




How to verify effect is from hydrogenase inhibition?

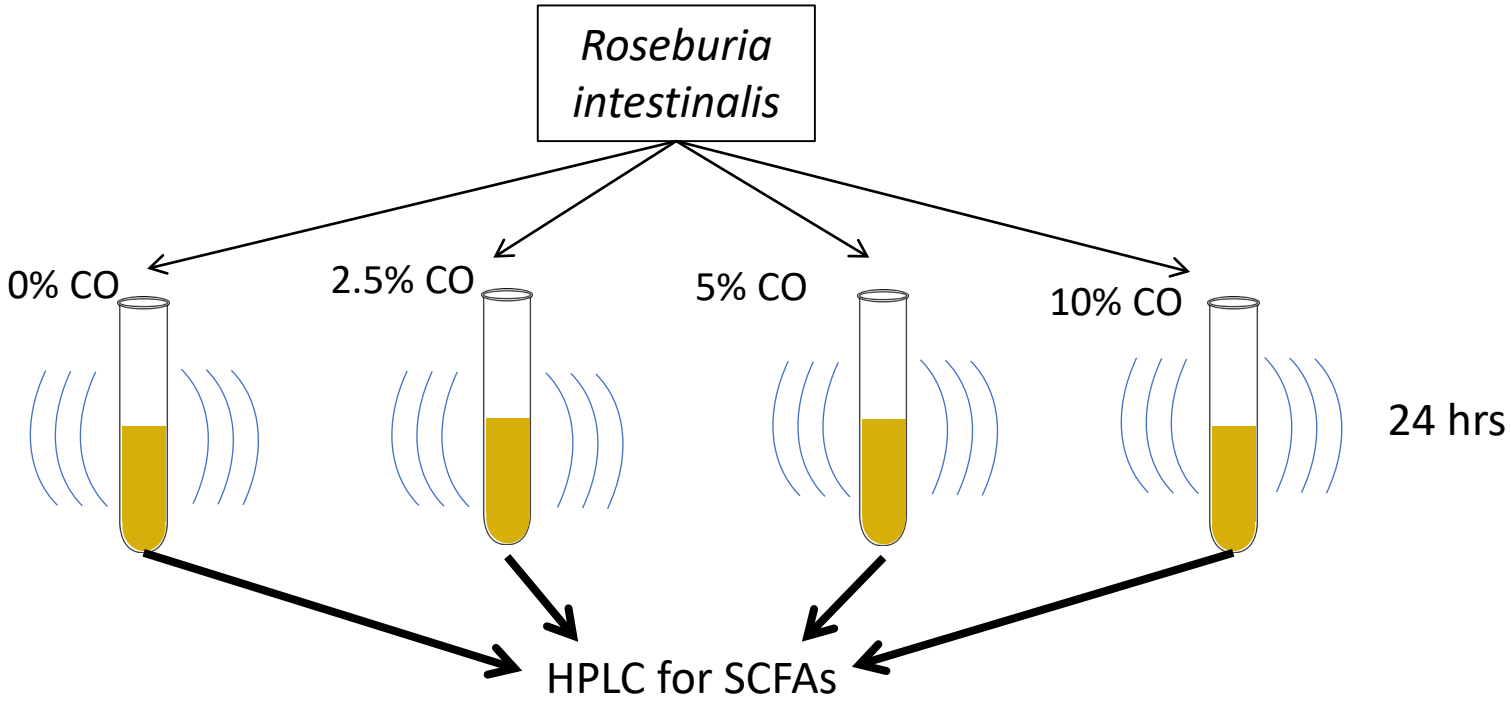
# Experimental design: Carbon monoxide (CO)

- Carbon monoxide is a known inhibitor of hydrogenase



# Experimental design: Carbon monoxide (CO)

- Carbon monoxide is a known inhibitor of hydrogenase
- Prediction: effects of added CO on SCFA production will mirror those of high [$H_2$], validating hydrogenase inhibition as mechanism



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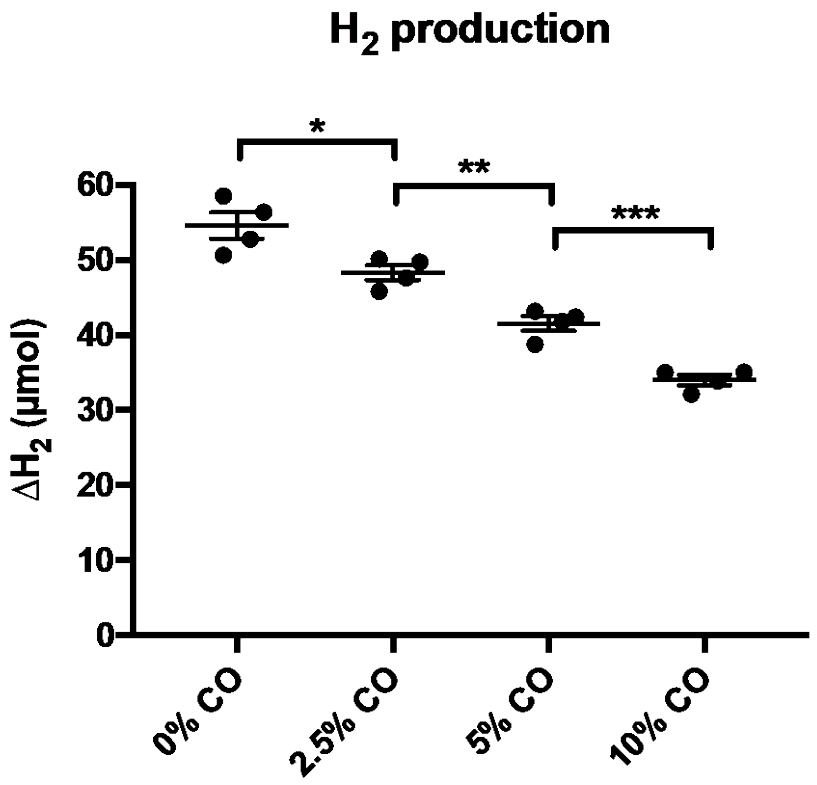


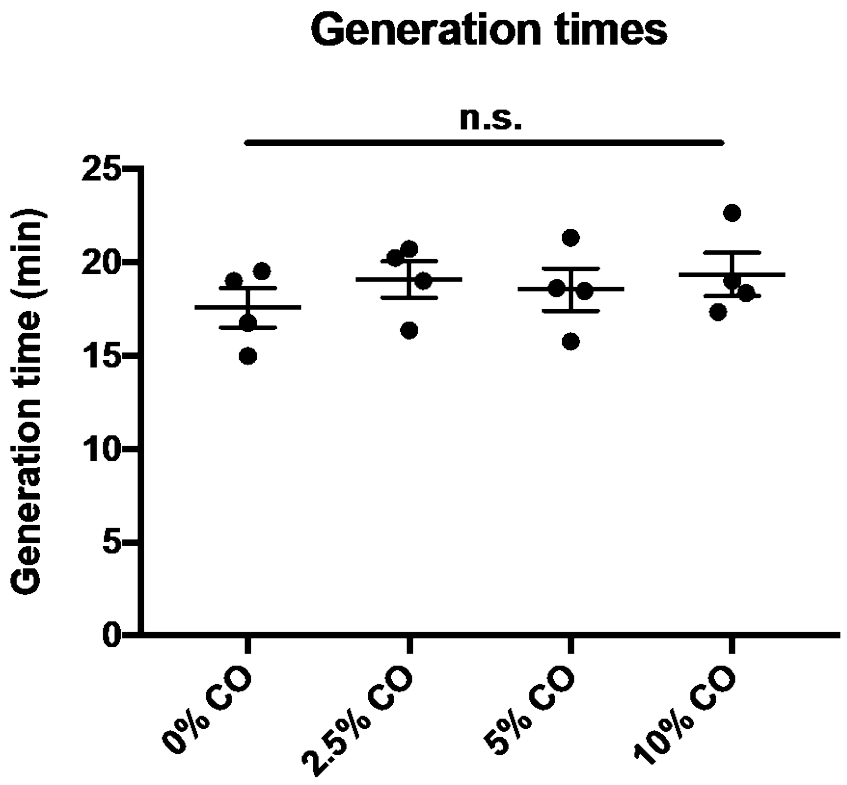

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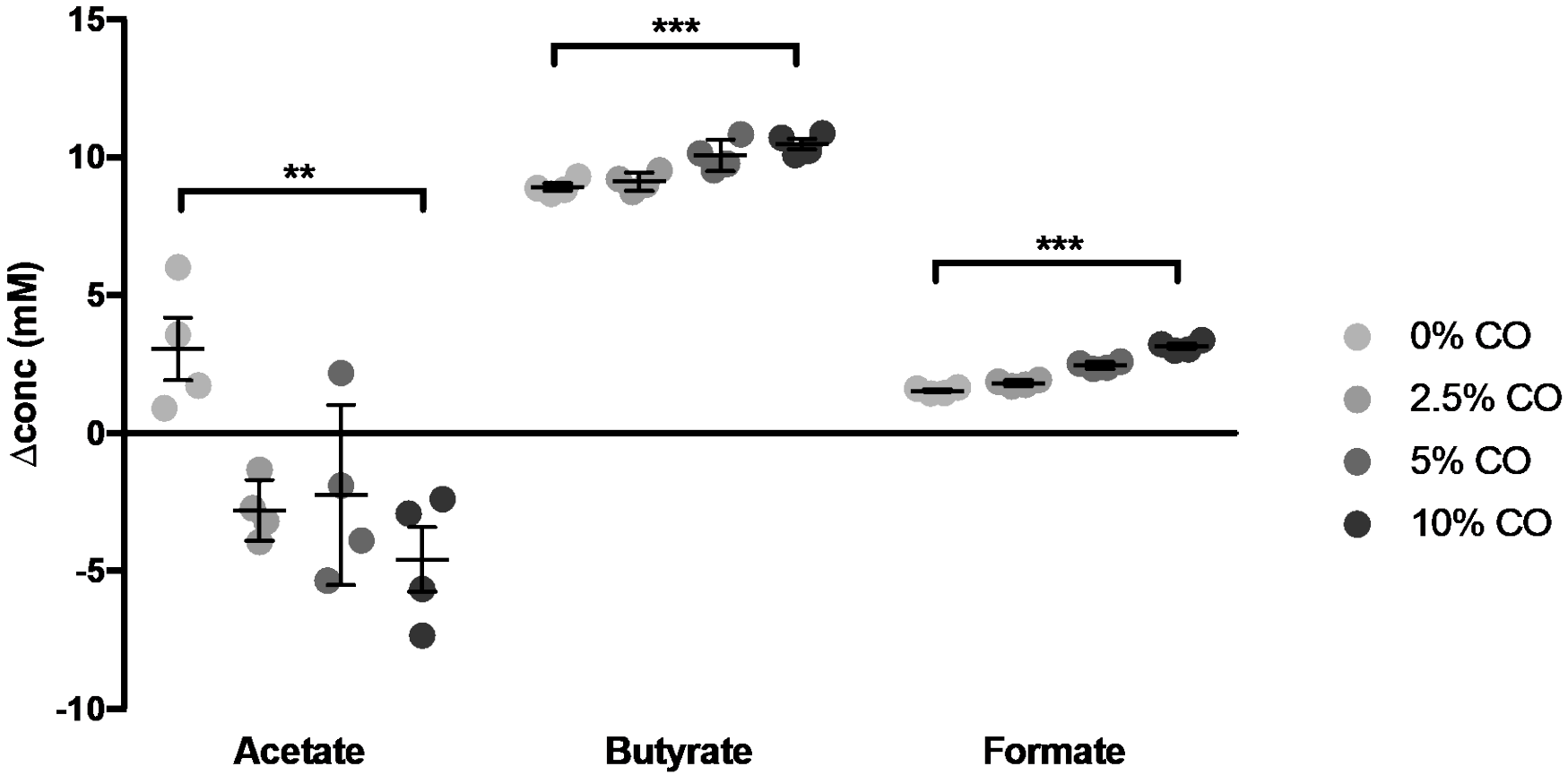
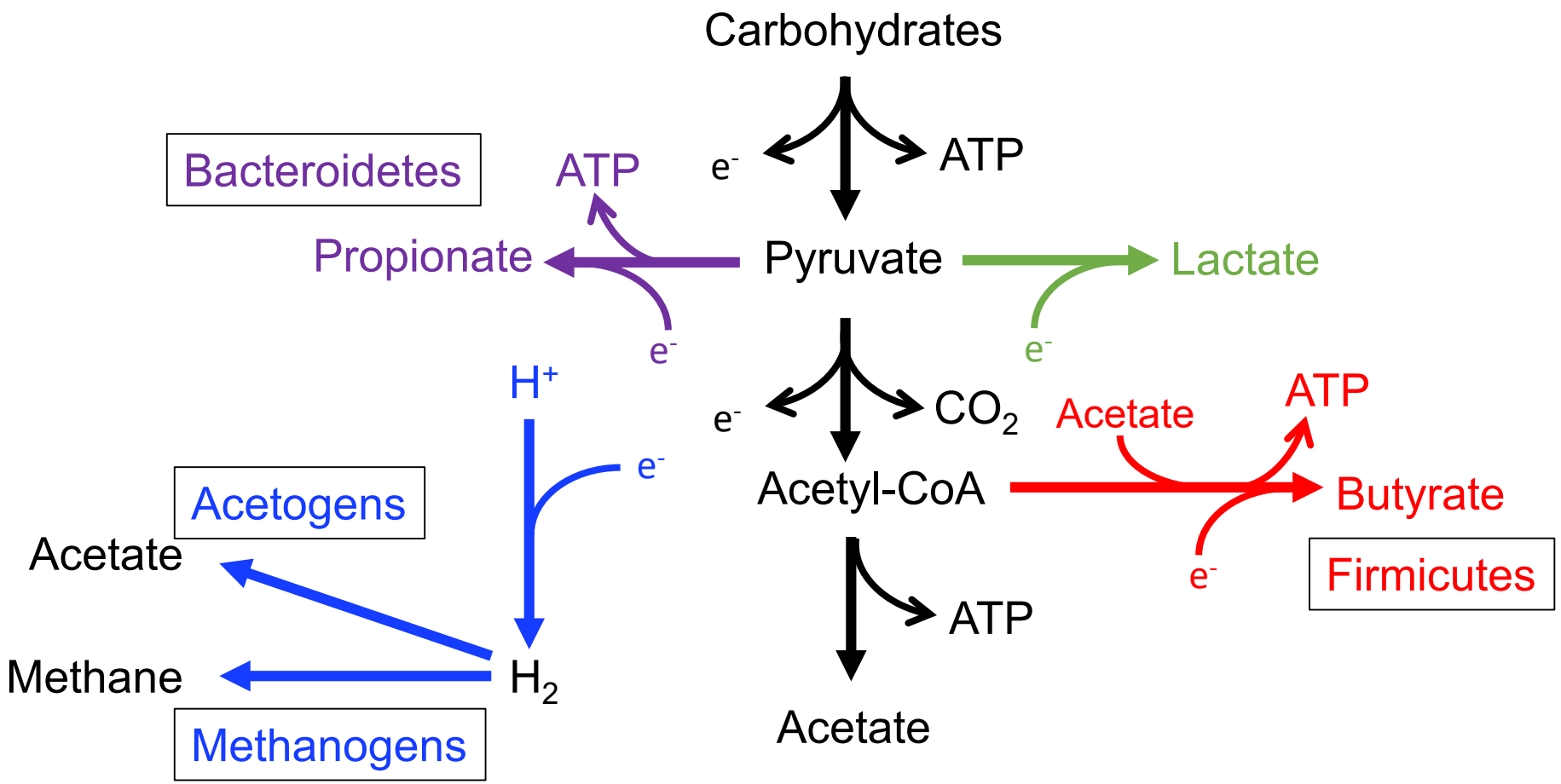


# A major challenge for microbes in anoxic environments is to relieve themselves of reducing power ($e^-$)



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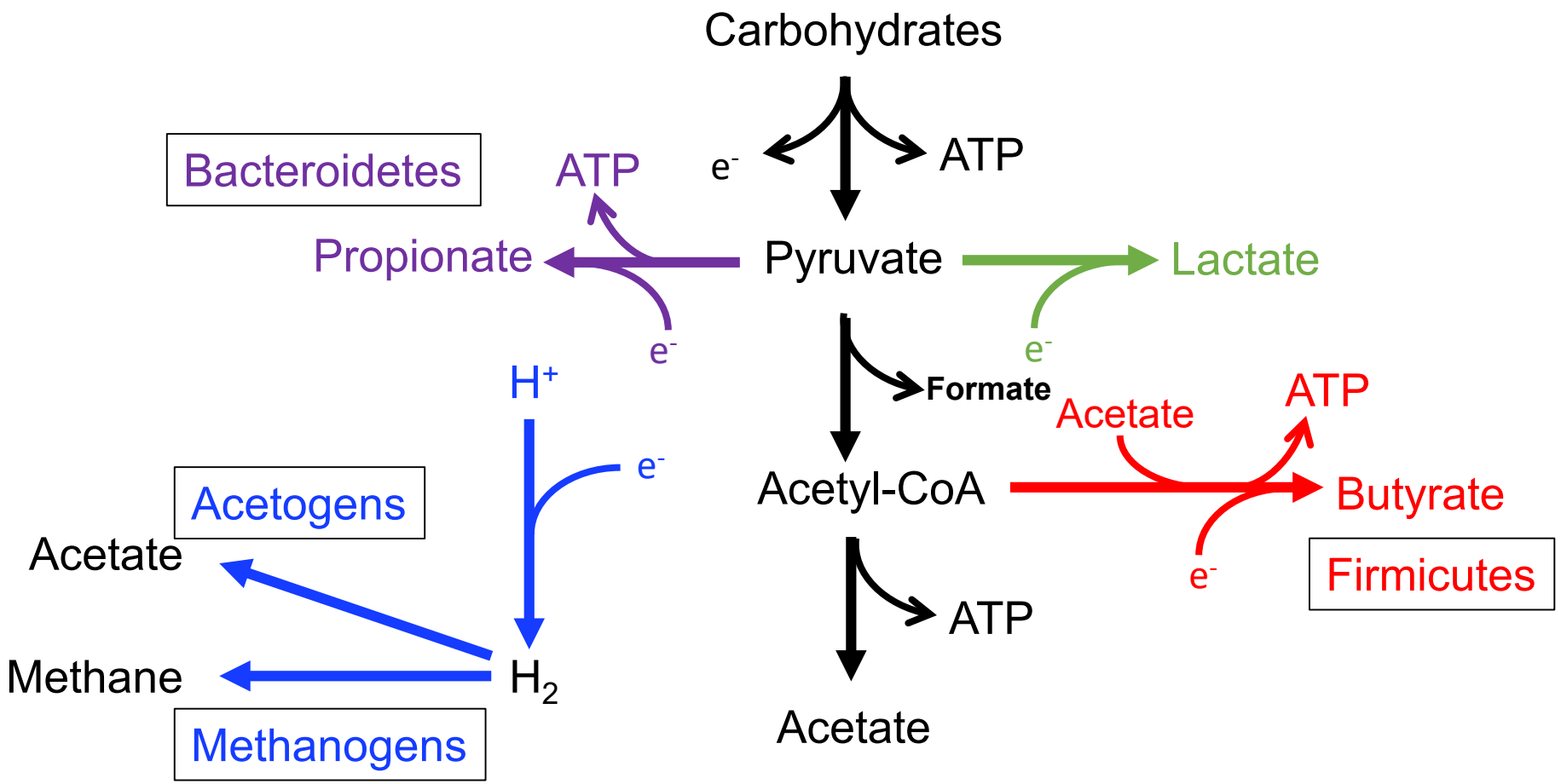

# *Faecalibacterium prausnitzii*

- Human gut butyrate producer lacking hydrogenase

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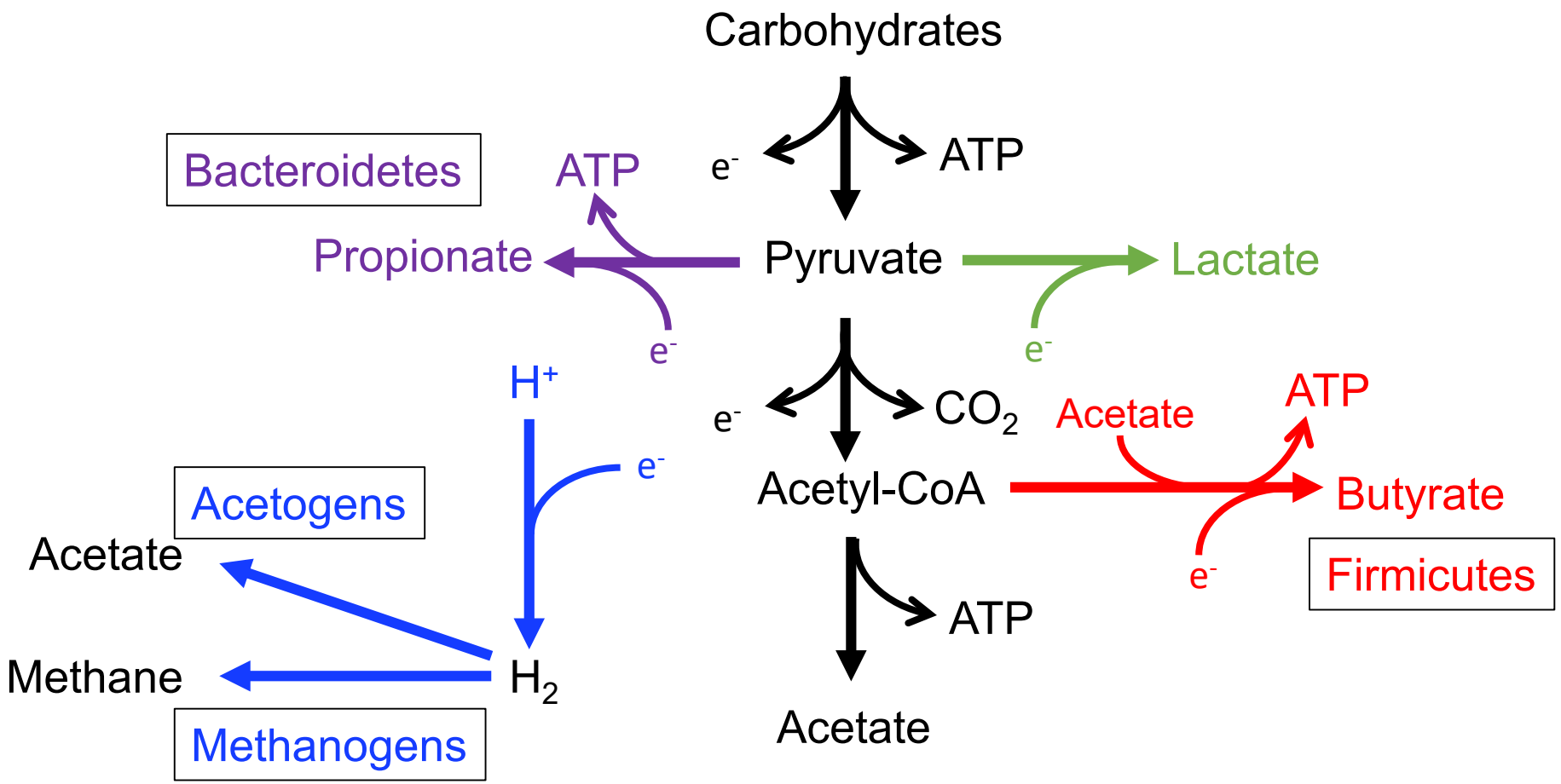

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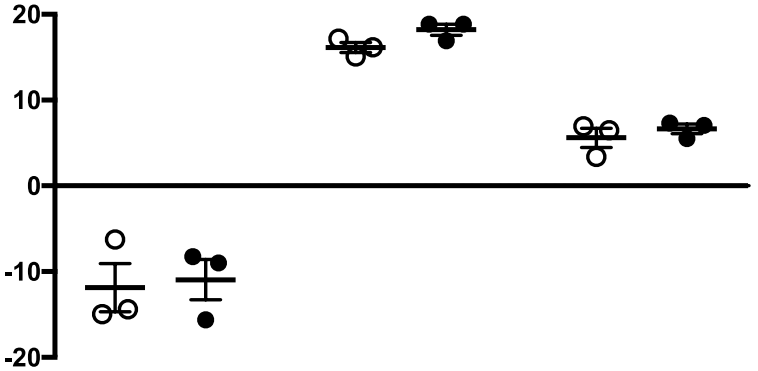
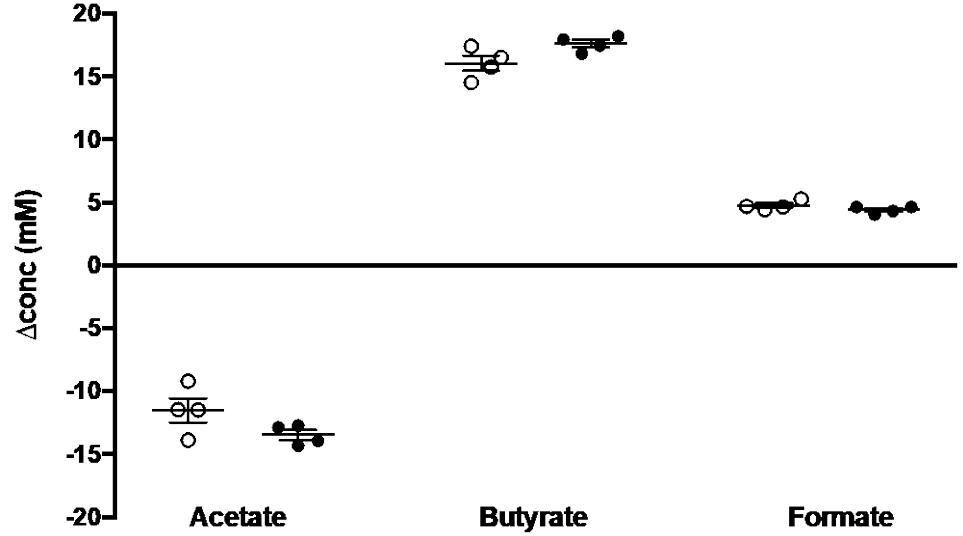



+$H_2$

○ 0 atm $H_2$

● 3.2 atm $H_2$




+CO (hydrogenase inhibitor)

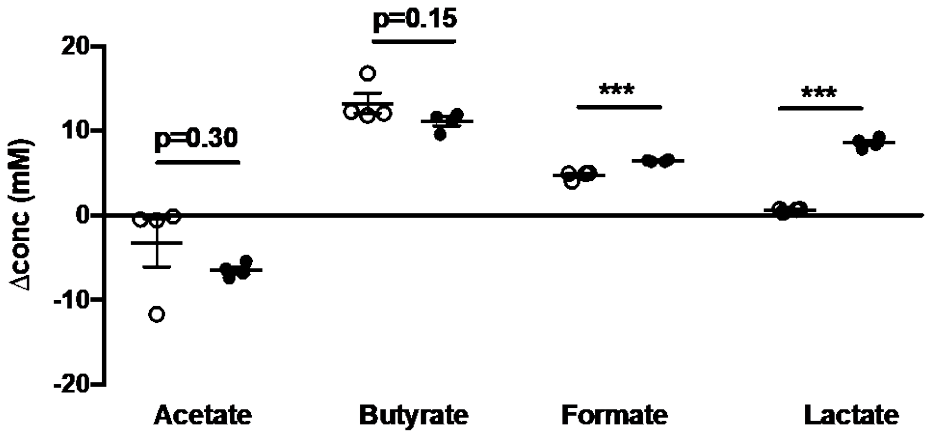
○ 0% CO

● 10% CO

Further validation of hydrogenase inhibition hypothesis

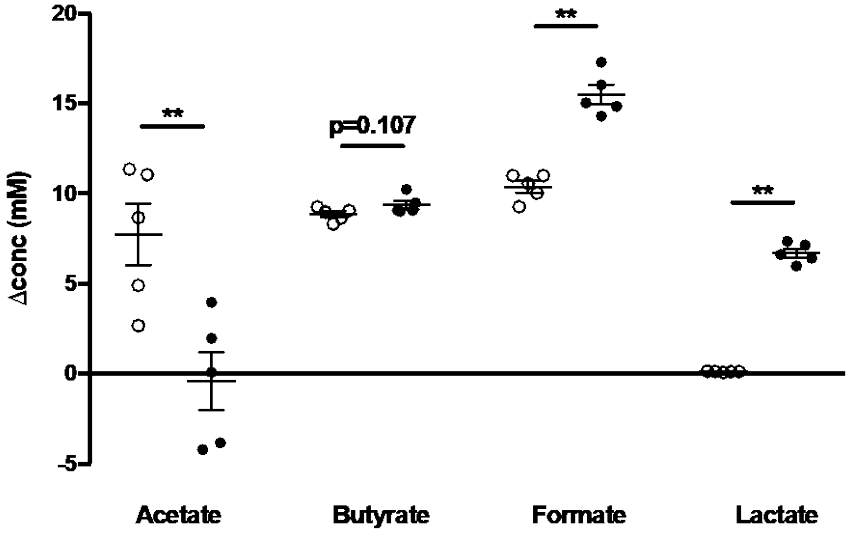
# *Eubacterium rectale*

- Human gut butyrate producer, close relative of *R. intestinalis*




+$H_2$

- 0 atm $H_2$
- 1 atm $H_2$

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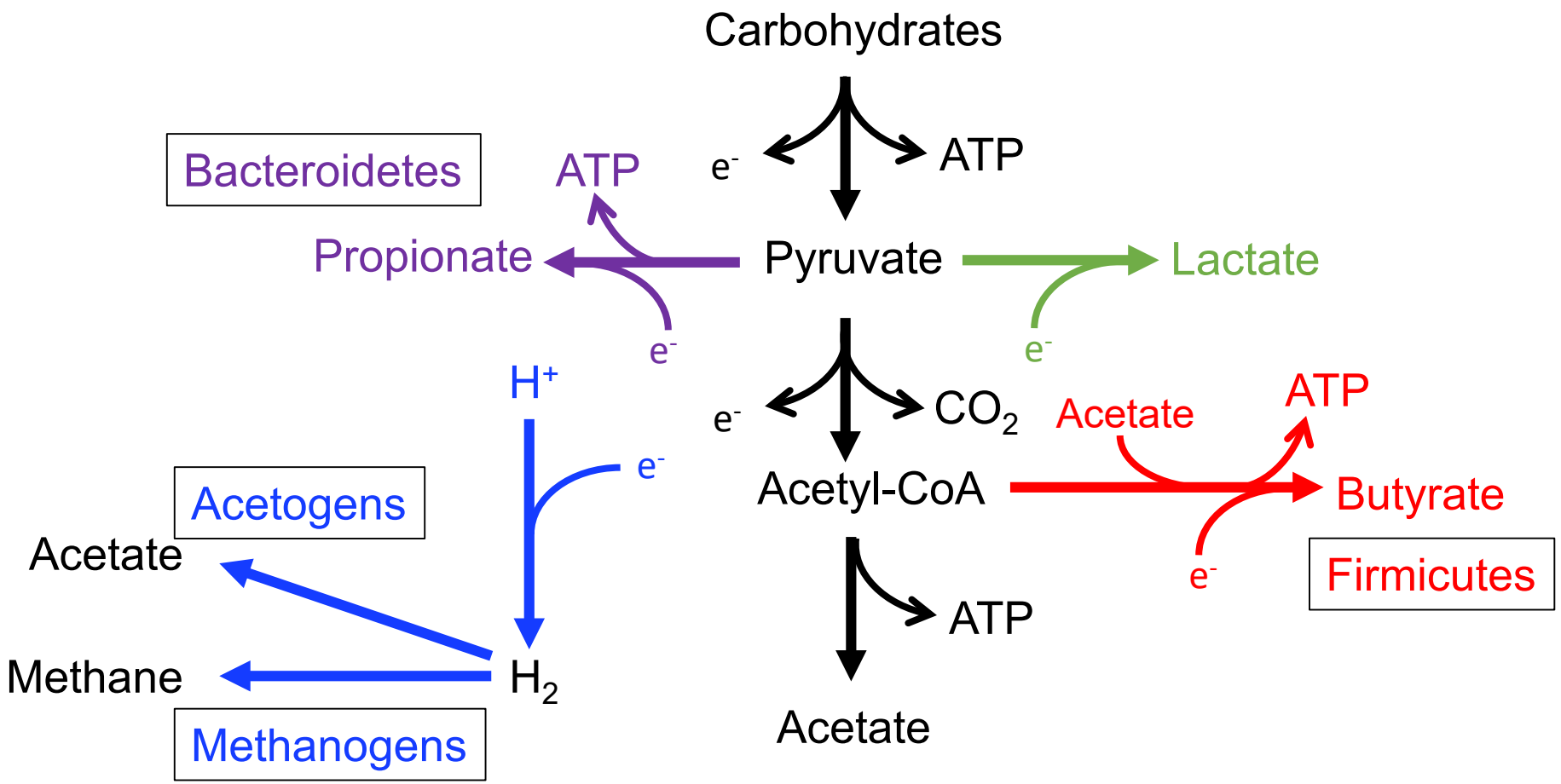


# Conclusions so far

- High [$H_2$] increases butyrate & lactate production and decreases acetate production by important human gut fermenters

- High [$H_2$] influences fermentation by inhibiting hydrogenase, forcing formation of reduced carbon end products

# Forward with MMPC Microbiome Project

1. Establish synthetic communities of microbes in gnotobiotic mice

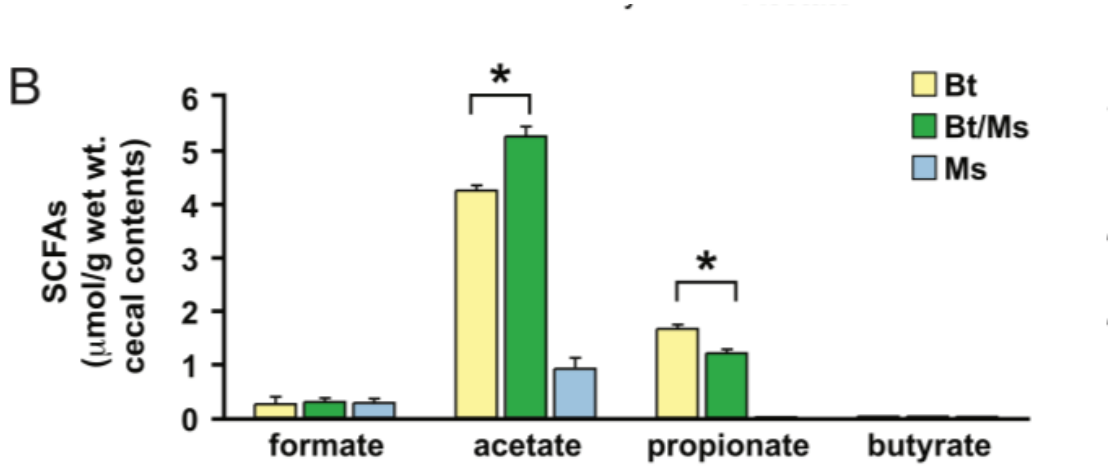
2. Find determinants of $H_2$ production in the gut
   - Promethion cages continuously measure $H_2$ production in gnotobiotic mice
   - High-$H_2$ and low-$H_2$ microbiota?
   - High-$H_2$ and low-$H_2$ diets?

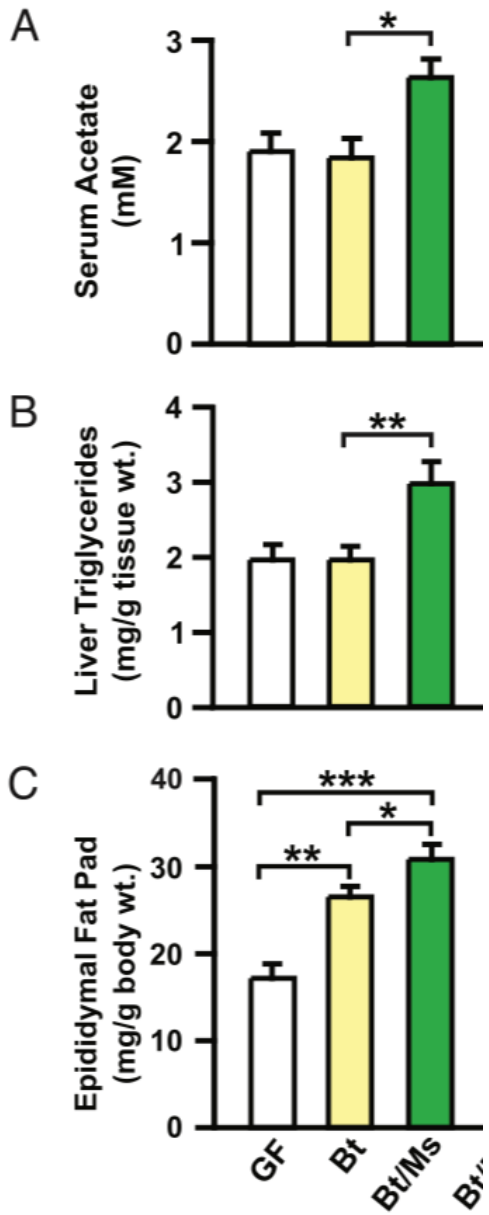
3. Validate *in vitro* effect of high $H_2$ on SCFA profile *in vivo*
   - Does fecal butyrate increase & fecal acetate decrease in high-$H_2$ vs low-$H_2$ microbiomes?

4. Measure effect of SCFA profile on mouse phenotypes

# A humanized gnotobiotic mouse model of host–archaeal–bacterial mutualism

Buck S. Samuel and Jeffrey I. Gordon* PNAS 2006



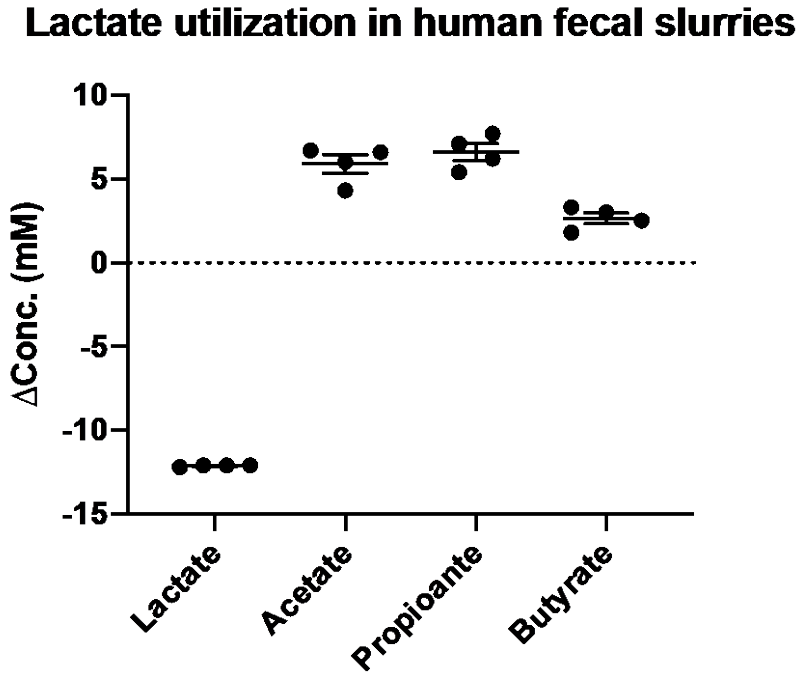




NMRI inbred strain, ‘adult mice’
n = 5 per group per experiment; three independent experiments;
Bt or Ms alone present for 14 days then Ms added to Bt

# Infusion study: Lactate cross-feeding

- Although it is expected to be produced by microbes, lactate is always low or absent in feces
- Lactate added to fecal cultures *in vitro* is rapidly converted to SCFAs

Belenguer et al., Impact of pH on Lactate Formation and Utilization by Human Fecal Microbial Communities (2007), Appl. Environ. Microbiol. **73**: 6526-33




A limiting substrate for SCFA production in the gut?

Investigate fate of lactate in mouse colon by infusing into terminal ileum?

# Acknowledgements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Questions?