Integration of bacterial RNA-Seq & human gut microbiota metaproteomic datasets highlights Faecalibacterium duncaniae A2-165's import systems

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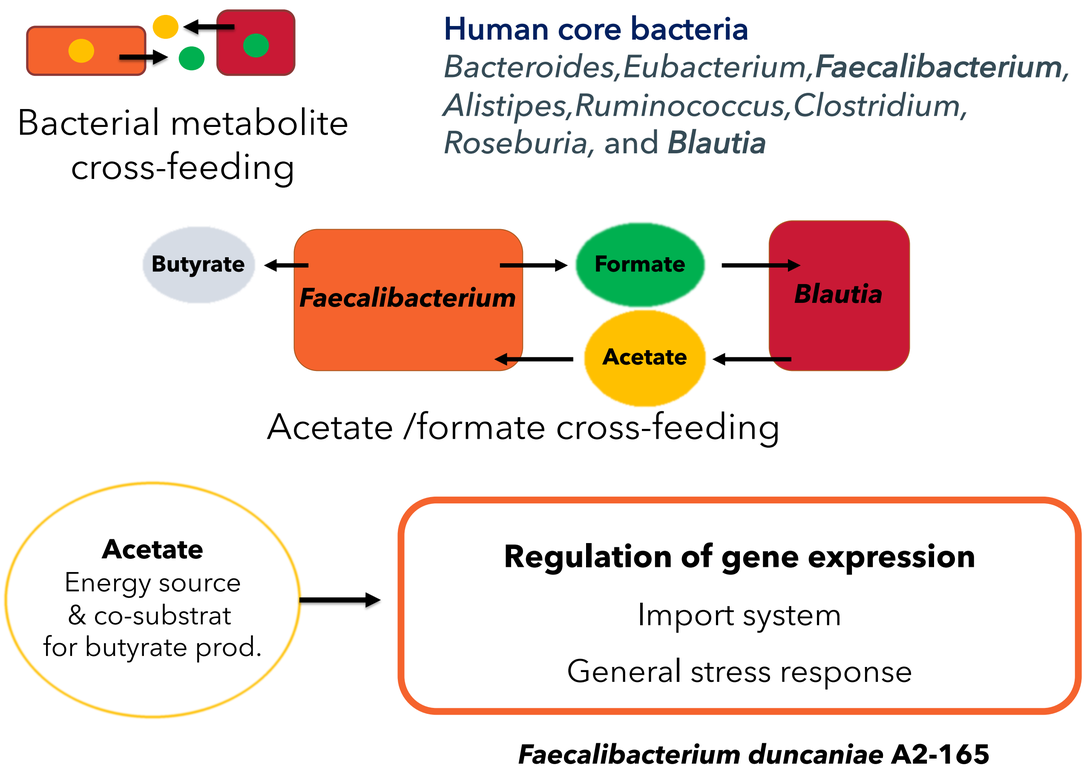
Introduction

Faecalibacterium commensal bacteria are among the main bacteria responsible for the consumption of acetate and the production of butyrate, which has anti-inflammatory properties beneficial to intestinal health (1-4). Production of selected strains as next-generation probiotic is in progress. Yet, little is known about how acetate availability affects this bacterium's gene expression strategies either in a pure culture, in coculture models with acetate-producing bacteria such as *Blautia* sp. (5) or in the complex ecosystem: human gut microbiome.

Objectives

- ✓ Transcriptional responses to low/high acetate level (RNA-seq, pure culture)
- ✓ Deciphering import systems expression *in vivo* at the protein level (human gut metaproteomic dataset from stool samples).



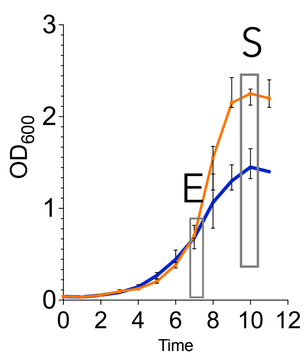
Method RNA-seq

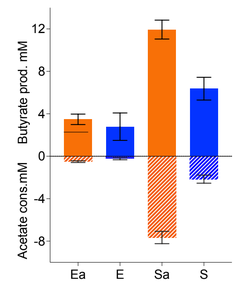
Comparison of gene expression patterns between 2 growth phases (late exponential vs. early stationary) and 2 acetate levels (low: 3 mM vs. high: 23 mM) in BHIS medium. Cultures in triplicat were performed in an anaerobic chamber.

Results

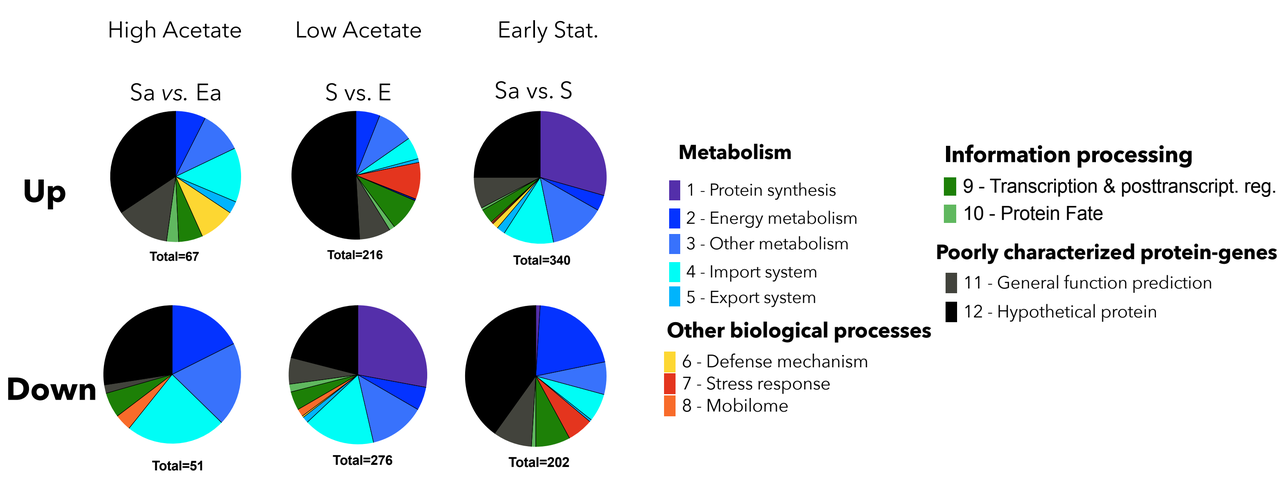
At low-acetate levels, a general stress response was activated, and protein synthesis expression was down-regulated. At high- acetate levels, there was greater expression of genes related to butyrate synthesis and to the importation of B vitamins and ferrous iron.

1. Overview of RNA-Seq data





Measures of SCFAs

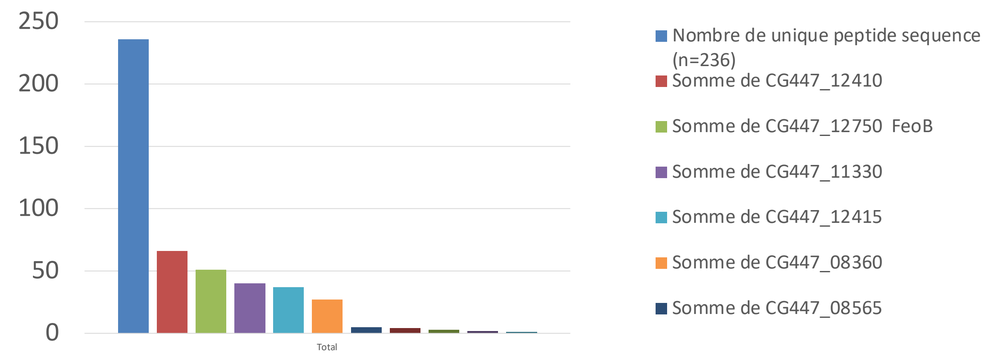


2. Overview of Metaproteomic data (6)

8 Healthy controls → 16 Faecal Samples (duplicat) → Microbiota proteins extraction → Envelope-enriched fraction → Identification & Analysis of peptides → Bacterial Envelope peptide hits **123 425**

3. Integrating RNAseq & metaproteomic data

Envelope peptides **123 425** → Import System Protein sequences **42** → Envelope Peptides **236** ⟷ (Matched hits) → Import System Protein sequences **10**



Conclusion- perspectives

Using RNA-Seq, we characterized 2 early stationary lifestyles of *F. duncaniae* A2-165 related to acetate consumption and butyrate production. Through multiomics and targeted approaches, we characterized the regulation of FeoB transporter expression involved in ferrous iron uptake. Currently, we analyze the metatranscriptome of the coculture *Blautia faecis* (7) & *F. duncaniae* A2-165. These data should decipher the acetate cross-feeding in a synthetic gut commensal community (BP acetate project-Qualiment).

References: 1. Sokol H, et al (2008) PNAS. 2. Miquel S, et al (2013) Curr Opin Microbio. 3. Martin R, et al (2018) Front Microbio. 4. Langella P. et al (2019) Front Microbiol 5. D'Hoe K et al (2018) 6. Henry C, et al. (2022) Cells. 7. Verstraeten et al (2022) Nutrients

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