Multidimensional Analysis of Prebiotic Substrate Utilization by Pathogenic and Probiotic Strains of *E. coli* Under Aerobic and Anaerobic Conditions

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Introduction

Escherichia coli (*E. coli*) is one of the most well-researched model organisms and a common constituent of human and animal gut microbiomes. Its versatility as a facultative anaerobe allows it to thrive in oxygen-rich environments and adapt to anaerobic environments, like portions of the human gut. Most *E. coli* strains are beneficial, but some Shigatoxigenic *E. coli* strains, such as *E. coli* O157:H7 (ECO), are zoonotic pathogens associated with gastrointestinal illnesses and are highly important to human health. While *E. coli* is perhaps the most extensively studied gut microbe, phenotypic profiling is needed to understand how prebiotic substrates modulate its influence on the gut microbiome and host. Novel solutions seeking to reduce the prevalence and impact of pathogenic *E. coli* go beyond antibiotics and leverage probiotic *E. coli* strains like *E. coli* Nissle 1917 (ECN), which can impede the growth of pathogenic bacteria, including ECO. Genomic analysis reveals that there are numerous variations between the ECO and ECN genomes; however, the functional differences in prebiotic substrate utilization are less understood. Here we sought to develop a mechanistic understanding of prebiotic substrate utilization by both strains, with the potential for developing prebiotic and symbiotic interventions to mitigate the impacts of pathogenic microbes. Using PreBioM™ plates for screening prebiotic substrate utilization, we challenged ECN and ECO strains against 90 different prebiotic substrates in both aerobic and anaerobic conditions, measuring growth and metabolic output.

Methods

Aerobic Experiments: Standard Biolog "PreBioM Protocol for Aerobic Organisms" was used to aerobically grow and phenotype ECN and ECO strains. Cells were cultured on BUG+B agar at 36° C for 24 hours. Cells were added to a tube containing 10 mL IF-0a, 0.4 mL 20% yeast extract, and 1.96 mL water to a density of 80% T. For metabolism experiments 0.12 mL Dye Mix A was substituted for an equal volume of water. The cell suspensions were inoculated at 100 µL per well onto each PreBioM plate (Figure 1). One plate per strain for each metabolism and growth assay was used. Plates were incubated in the Odin™ instrument at 36° C for 24 hours with reads every 20 minutes at 590 and 740 nm.

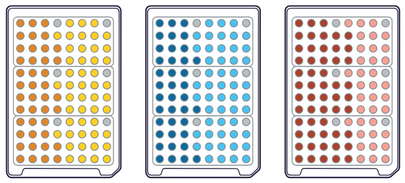

Figure 1. PreBioM1 (left) contains readily metabolized monosaccharides and disaccharides. PreBioM2 (center) includes oligo and polysaccharides which require a more intricate metabolism due to their structural complexity. PreBioM3 (right) features dietary fibers with varied branching to resist digestion and food extracts with rich mixtures of molecules.

Anaerobic Experiments: Standard Biolog "PreBioM Protocol for Facultative Organisms" was used to anaerobically grow and phenotype ECN and ECO strains. Cells were cultured on BUG+B agar at 36° C for 24 hours. In an anaerobic chamber, cells were added to a tube containing 15 mL IF-0a, 0.4 mL 20% yeast extract, and 2.96 mL water for growth to a final density of 80% T. For metabolism experiments 0.12 mL Dye Mix A, 0.022 mL 1M $C_6FeK_3N_6$, and 0.018 mL 0.5 mM menadione were substituted for an equal volume of water. The cell suspensions were inoculated at 150 µL per well onto deoxygenated PreBioM plates (Figure 1) and the plates were sealed. One plate per strain for each metabolism and growth assay was used. Plates were incubated in Odin at 36° C for 24 hours with reads every 20 minutes at 590 and 740 nm.

Data Analysis: Unpaired t-tests, adjusting for substrates using maximum optical density and corrected for multiple comparisons using the Hom-Sidak method, were performed alongside principal component analysis in GraphPad Prism v10.3.0. Log_2 fold change values and relative standard deviations were also calculated and compared. Significant difference in utilization was determined by a Log_2 fold change of |1.5| and an adjusted p-value of < 0.05.

Results

Plate	Substrate name
PB-M1	b-D-Allose
PB-M1	D-Mannitol
PB-M1	D-Ribose
PB-M1	D-Sorbitol
PB-M1	Lactulose
PB-M1	Sucrose
PB-M1	3-O Galactosylarabinose
PB-M2	D-Raffinose
PB-M2	Inulin
PB-M2	Lactitol monohydrate
PB-M2	Larch arabinogalactan
PB-M2	Fructooligosaccharide (FOS)
PB-M3	Gum arabic
PB-M3	Beta-Sitosterol
PB-M3	Vegetable fiber mix

Table 1. Prebiotic substrates exhibiting significant utilization differences between ECN and ECO in aerobic or anaerobic conditions

Fifteen of the substrates tested showed significant differential utilization for growth or metabolism between ECN and ECO in either atmospheric condition (Table 1). However, only allose and arabinogalactan, when measuring growth, and raffinose and sorbitol, when measuring metabolism, showed similar utilization in aerobic and anaerobic conditions. Genetic differences (Figure 2) and a gene ontology search revealed that genes involved in allose utilization are present in ECN, but are absent in ECO, offering a potential explanation of the phenotype. Segregation of substrate phenotypes by atmospheric condition supports the class separation displayed in the PCA, where 4 distinct groups were formed based on atmosphere and strain background (Figure 3).

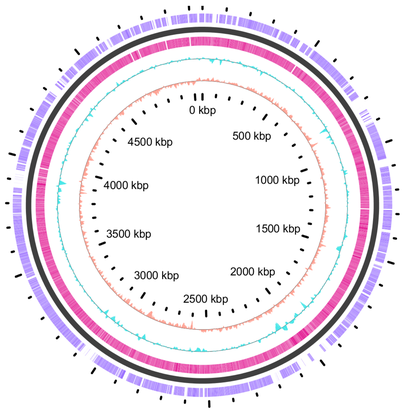

Figure 2. Comparative genome map showing genetic similarity between ECN (pink) and ECO (purple) generated in GView v1.7. GC content (cyan) and GC skew (orange) were also calculated. The ECO and ECN genomes show significant differences in both nucleotide variations and GC content

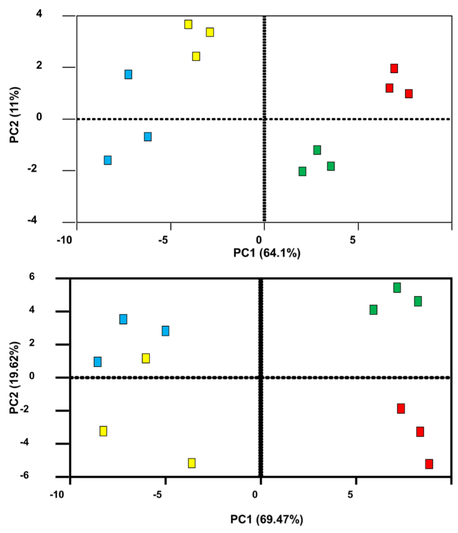

Figure 3. PCA of cell growth (top) and metabolism (bottom). ECO under aerobic conditions (blue), ECN under aerobic conditions (yellow), ECO under anaerobic conditions (green), and ECN under anaerobic conditions (red). Each dot represents a single interpolate replicate from each PreBioM plate. PCA reveals atmosphere-dependent and strain-dependent (PC2) class separation

Results, continued

Genomic data can offer some explanation for variation in substrate utilization, but the remaining differences are more difficult to explain. Allose supported growth and metabolic output in the probiotic ECN strain, while sucrose did the same for the pathogenic ECO strain, but only in anaerobic conditions (Figure 4), despite similar genetic potential for sucrose utilization in both. Interestingly, inulin fostered significantly more growth in ECN in contrast to ECO which generated more energy using inulin in anaerobic conditions, while the metabolic output was largely the same in aerobic conditions (Figure 4). Allose and inulin both showed reduced growth in ECO relative to ECN, indicating possible potential use for controlling ECO overgrowth in gut communities.

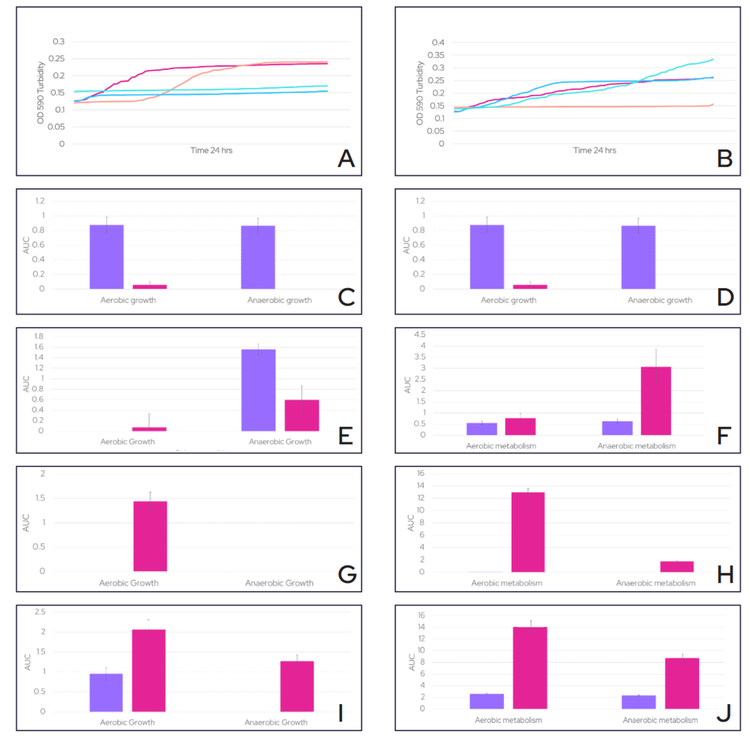

Figure 4. Allose (A) and raffinose (B) utilization for ECN aerobic (pink), ECN anaerobic (orange), ECO aerobic (blue), and ECO anaerobic (cyan) kinetic growth. (C-J) Area Under the Curve for ECN (purple) and ECO (pink) allose utilization: growth (C) and metabolism (D), inulin utilization: growth (E) and metabolism (F), sucrose utilization: growth (G) and metabolism (H), and raffinose utilization: growth (I) and metabolism (J).

Conclusions

- Significant substrate utilization differences were identified based on atmospheric condition and strain background
- Variation in substrate utilization underscores limitations of genetic information in predicting metabolic function
- Streamlined phenotypic profiling is key to accurately understanding and manipulating microbial interactions within the gut community
- Significant variation in microbial growth on different substrates demonstrates potential for prebiotic control measures for pathogenic gut microbiota