Metabolic modeling PhenComm project biological results

5-25-23

Possible narrative for the introduction and throughout the results/discussion

* Our goal is to understand microbial community metabolic interaction **dynamics** and manipulate these dynamics to achieve a particular microbial community outcome
* One challenge in many areas of microbial community research is controlling metabolic dynamics to increase a particular organism’s prevalence/effects
* For example, pseudomonas fluorescens has emerged as a bioproduction chassis and plant growth promoting rhizobacteria. However, PF struggles to consume many complex sugars that are present in lignocellulosic or rhizosphere nutrient streams.
* Thus, bioproduction efficiency and crop growth/health can be improved by understanding how a secondary organism can be added to a microbial community to funnel intractable carbon sources towards beneficial and naturally occurring primary organisms such as PF. Furthermore, developing a fundamental understanding of these dynamics will enable engineering of the secondary organism to further increase benefits.
* Here we study mono- and cocultures of an easily engineered model organism, e coli, and the previously mentioned bioproduction and PGPR organism, pseudomonas fluorescens.
* One of the biggest challenges in studying coculture metabolisms is the lack of models that can translate easily obtained data into minutes-resolution molecular metabolic information. We thus focused on developing and validating a metabolic phenotype model [blah blah modeling justification, past, present… most of this is probably already written in the current introduction]
* In this work we demonstrate how this model reveals secondary metabolic interaction dynamics between EC and PF.
* These dynamics are rapidly parsed and studied due to the model’s predictions.
* Finally, we demonstrate how the models can predict means to manipulate the metabolic interactions between EC and PF to achieve increased PF growth.

Results

* Initial development of the model [this is also already written]
* Equipped with this new modeling tool, we investigated how an intractable glucose dimer, maltose, and a common lignin degradation product, 4-hydroxybenzoate, are consumed by EC and PF in monoculture and coculture.
* We observed significant cross feeding from EC to PF when EC consumes maltose. The model predicts EC produces acetate at timepoint X that PF then consumes.
* Experiments demonstrate that EC monoculture utilize acetate slowly, but acetate is primarily funneled to PF when cocultured with EC
* We studied how adding 4HB changes this cross feeding and observed xyz (rapid utilization of 4HB followed by consumption of acetate). These results show that the cross feeding results in additive PF growth and so may be a viable means to bolster PF growth in complex carbon streams
* The model suggested that if acetate production is KOed then lactate would be produced by EC instead. Lactate would represent a better carbon source for PF, resulting in higher yielding cross feeding
* We deployed a Pta KO cell line that is known to eliminate all acetate production and consumption in e coli. This KO is also known to produce excess acetate when consuming xyz carbon source (probably glucose)
* This KO impacted EC consumption of maltose to a minimal degree, but resulted in x % increased PF yield when in coculture
* We performed a time course metabolomics experiment with wt and PtaKO EC cocultured with PF and consuming either maltose or maltose and 4HB to validate the model’s molecular and temporal predictions.
* The metabolomics validated that lactate is indeed produced x(~7-10x I think) fold more in PtaKO cells than wt.
* The metabolomics also showed that there were only X metabolites detectable from a panel of ~200 common sugars/lipids/organic acids and blah. Moreover, only Y of these metabolites were detected at higher concentrations than base media and changed over the course of cell growth.
* Finally, we observed increased lactate accumulation and delayed consumption in the presence of 4HB, which can be explained by PF metabolic prioritization of 4HB combined with inhibition of EC lactate consumption via possible mechanisms.
* In sum, we rapidly gained a molecular level understanding of metabolic interactions between two organisms via modeling and used said modeling to engineer the interaction to produce a desired outcome (PF cross feeding).

These are the consistent results/observations that are exciting and concrete.

* E. coli growth on maltose is inhibited by increasing concentrations of acetate. Also, pseudomonas fluorescens consumes acetate much more quickly and effectively than e coli (excel file “PF-EC Acetate and Carbon 6-24-22” also “PF-EC-PFEC on Acetate 6-17-22” for relative acetate consumption rates and efficiency aka yields)
* Knocking out Pta eliminates acetate consumption and production (Excel file “Acetate KO Ecoli only 10-21-22”) and instead produces lactate (PMID 19852855).
* Pta KO increases growth on maltose, implying acetate production compared to lactate production is inhibitory (comparing F7 to F10 in Excel “Acetate KO Ecoli only 10-21-22”).
* Lactate production results in much greater pseudomonas fluorescens growth (comparing well G7 to G10, and column 7 to column 10 more broadly in Excel sheet “PF-ECKOs Ace maltose”)
* Based on metabolomics data, lactate is produced much more when Pta is knocked out (Excel sheet “TMS metabolomics with Graphs 3-28-23”).
* Moreover, when 4-hydroxybenzoate is present in the media there is a large increase in the total amount of lactate produced and a delay in its consumption (same excel sheet).
* There are no other metabolites that both increase over time and are produced in high enough concentrations to support observed pseudomonas growth

Conclusions

* Thus, 4-HB inhibits pseudomonas from consuming lactate and acetate (based on earlier metabolomics data from 08-2022), but E. coli does not consume these excess secondary metabolites either like they normally do. This dynamic could be caused by several mechanisms. 1. The pseudomonas may delay their consumption of acetate/lactate, but still transmit some signal to the e coli that prevents them from metabolizing the organic acids 2. The 4HB, which is an acid, could be inhibiting the E coli organic acid metabolism through physical interaction or other means (we know 4HB doesn’t inhibit e. coli consumption of maltose from earlier experiments – excel sheet needs to be found…). 3. The decreasing concentration of 4HB due to pseudomonas stimulates increased E coli acetate/lactate production that is not matched by increased psudomonas acetate/lactate consumption. Whatever the case, there is clearly a dynamic interplay between secondary metabolites and organism cocultures that has yet to be studied in depth.
* E. coli produces acetate/lactate when consuming maltose that results in pseudomonas cross-feeding
* PhenComm can use minimal data (OD and fluorescence) to generate qualitative and quantitative temporal and molecular hypotheses that, if pursued with simple experiments, lead to intriguing discoveries