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Research Article

A Simulation Study on the Effects of Media Composition on the Growth Rate of *Escherichia coli* MG1655 Using iAF1260 Model

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Abstract

Media compositions are important determinants of growth rate and genome-scale models (GSMs) had been used for optimizing media for metabolite production and growth. Recently, iAF1260, a GSM based on *Escherichia coli* MG1655, was used to study the effects varying glucose concentration in media on growth rate and metabolic fluxes. In this study, the effects of other media components in the presence of varying glucose concentrations on the predicted growth rate of *E. coli* MG1655 were examined. Our results show that 10 media components (ammonium, calcium, chloride, copper, glucose, manganese, magnesium, molybdate, phosphate, and potassium) demonstrate substantial impact on the predicted growth rate of *E. coli* MG1655. Of which, 4 components (glucose, ammonium, magnesium, and phosphate) have the most impact. However, our results also demonstrate the limitations of iAF1260 as media components that had been shown to affect *E. coli* growth rate were not reflected by the model.

Keywords: Growth Rate; Genome-Scale Models; Media Optimization

Introduction

Growth rate of cells in different media [1] is an important physiological parameter as it can affect various experiments [2]; such as, antibiotics [3,4] and antibacterial [5] susceptibilities. A recent study has also shown that the types of growth media can affect the metabolomics of *Escherichia coli* [6]. This is supported by studies showing that media compositions can affect the production of enzymes [7]. This led to studies on media optimization for various purposes: such as, production of uricase from *Bacillus subtilis* [8], production of succinic acid by *Enterobacter sp.* LU1 [9], and production of isocitric acid by *Yarrowia lipolytica* [10].

Genome-scale models (GSMs) had been used in various applications [11]; such as, metabolic engineering for specific metabolite production [12-14]. Since bacterial growth rate can be affected by media composition [1,15], it is plausible to estimate the effects of media compositions on growth rate [16] as that demonstrated by Chen., *et al* [17].

Previously, the GSM iAF1260 [18] had been used to show correlation of *E. coli* growth rate and metabolic fluxes on various glucose concentrations [19] as *E. coli* is an experimental organism used in various applications [20]. In this study, we examine the effects of other media components in the presence of varying glucose concentrations on predicted growth rate of *E. coli* MG1655. Our results suggest that 4 media components (glucose, ammonium, magnesium, and phosphate) have the most impact on the predicted growth rate of *E. coli*. However, our results also demonstrate limitations of GSM iAF1260 [18] as media components that had been shown to affect *E. coli* growth rate were not reflected by the model.

Materials and Methods

GSM Model: Simulated metabolism data was obtained by Parsimonious Flux Balance Analysis (pFBA) [23] using Cameo [21] via AdvanceSyn Toolkit (https://github.com/mauriceling/advancesyntoolkit) on iAF1260 model [18], a GSM based on *E. coli* MG1655 [18], from the BiGG database [22].

Sensitivity analysis of media components: Sensitivity analysis on 19 media components [(i) calcium (EX_ca2_e), (ii) chloride (EX_cl_e), (iii) carbon dioxide (EX_co2_e), (iv) cobalt (EX_cobalt2_e), (v) copper (EX_cu2_e), (vi) ferrous (EX_fe2_e), (vii) ferric (EX_fe3_e), (viii) water (EX_h2o_e), (vix) proton (EX_h_e), (x) potassium (EX_k_e), (xi) magnesium (EX_mg2_e), (xii) manganese (EX_mn2_e), (xiii) molybdate (EX_mobd_e), (xiv) sodium (EX_na1_e), (xv) ammonium (EX_nh4_e), (xvi) phosphate (EX_pi_e), (xvii) sulfate (EX_so4_e), (xvii) tungstate (EX_tungs_e), and (xiv) zinc (EX_zn2_e)] defined was performed to investigate the relative effects of each component on the growth rate of the cell as determined by the corresponding objective value from the model using pFBA [23] Cameo [21]. The sensitivity of each media component is $\sqrt{\sum_{i=0}^{N}(x_i - 599.768)^2}$, where is the objective value after changing one media influx rate at a time (24,25) and 599.768 is the objective value using the native media composition. The intake fluxes used for the different compounds were 0, 0.01, 0.1, 1, 10, 100, and 1000 mmol per gram dry weight per hour (mmol/gDW/hr). Hence, the sensitivity score is directly proportional to the impact of the media component to the growth rate of the cell.

Effects of multiple media components on growth rate: The effects of media components on growth rate was determined using stepwise regression using Akaike information criterion [26] from MASS [27] as $\text{Objective value} = \sum_{i=1}^{N}\beta_i Media_i + \beta_0$, where β_i is the coefficient of i-th media component, and β_o is the constant value. A total of 200 combinations of concentrations with regular intervals of 0.05 mmol/gDW/hr ranging from 0.025 to 9.975 mmol/gDW/hr were determined using Latin Hypercube Design [28] in pyDOE (https://pythonhosted.org/pyDOE/).

Results and Discussion

Our results show that glucose cannot be absent as it results in an error in pFBA [23], suggesting a mandatory requirement for carbon source. Of the 19 non-carbon media components analyzed for impact on *E. coli* growth rate using one-factor-a-time sensitivity

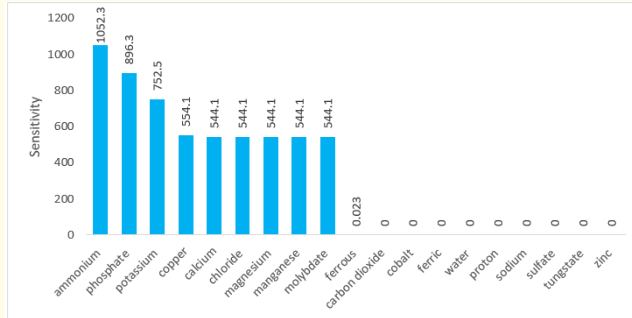

Figure 1: Effects of 19 non-carbon media components on growth rate.

analysis [24,25] suggests that only nine components exhibit substantial impact on growth rate (Figure 1). These nine components are (i) ammonium, (ii) calcium, (iii) chloride, (iv) copper, (v) manganese, (vi) magnesium, (vii) molybdate, (viii) phosphate, and (ix) potassium. These components largely corresponds to (Figure 2) the standard M9 minimal media [29], which contains (i) ammonium, (ii) calcium, (iii) chloride, (iv) magnesium, (v) phosphate, (vi) potassium, (vii) sulfate, (viii) sodium, and (vix) glucose.

Of the 200 combinations of media components determined using Latin Hypercube Design [28], 193 combinations gave non-error objective values, which were then used in regression analysis. Regression analysis on the 10 media components, including glucose, with impact on *E. coli* growth rate (Table 1; full model) suggests significant correlation (adjusted $r^2 = 0.6052$, $F = 30.43$, p-value $< 2.2e-16$) between these 10 media components and the predicted growth rate. However, only two coefficients (glucose and ammonium) are significant (p-value $< 2e-16$). Stepwise regression using Akaike information criterion [26] from MASS [27] suggests four media components; namely, glucose, ammonium, magnesium, and phosphate; with the largest impact to *E. coli* growth rate (Table 2; reduced model). The correlation between the reduced model is slightly higher than that of the full model (adjusted $r^2 = 0.6137$, $F = 77.27$, p-value $< 2.2e-16$). This is consistent with a review by Peterson., *et al*. [30] suggests that starvation of one of the three nutrients; namely, carbon (in the form of glucose), nitrogen (in the form of ammonium), or phosphorus (in the form of phosphate); triggers *E. coli* into dormancy. Thus, suggesting the importance of glucose, ammonium, and phosphate to *E. coli* growth. The dependence of *E.*

Regressor	Coefficient	Standard Error	t-statistic	p-value
(Intercept)	-107.174	43.3698	-2.471	0.0144
Glucose	27.749	2.7887	9.951	<2e-16
Manganese	0.8339	2.723	0.306	0.7598
Copper	1.0141	2.7096	0.374	0.7087
Potassium	0.9016	2.7018	0.334	0.7390
Magnesium	5.4352	2.6568	2.046	0.0422
Calcium	-2.985	2.6552	-1.124	0.2624
Chloride	1.0118	2.6852	0.377	0.7067
Molybdate	0.3652	2.724	0.134	0.8935
Ammonium	40.7948	2.6902	15.164	<2e-16
Phosphate	3.7352	2.6984	1.384	0.1680

Table 1: Impact of media components on growth rate (Full Model).

Regressor	Coefficient	Standard Error	t-statistic	p-value
(Intercept)	-100.522	27.754	-3.622	0.0004
Glucose	27.282	2.708	10.075	< 2e-16
Magnesium	5.641	2.608	2.163	0.0328
Ammonium	40.757	2.63	15.497	< 2e-16
Phosphate	3.858	2.609	1.479	0.1409

Table 2: Impact of media components on growth rate (Reduced Model).

Figure 2: Commonalities between growth sensitive components and m9 minimal media [29] components. The 4 media components having the most impact on the predicted growth rate of E. coli are highlighted.

coli growth on extracellular magnesium is illustrated in the 1960s by McCarthy [31] and Lusk., *et al*. [32] demonstrating the importance of magnesium in maintaining ribosome function.

Given that M9 minimal media can be considered as the minimum set of nutrients required for *E. coli* growth, it is conceivable to expect that all components of M9 minimal media should impact on *E. coli* growth rate. That is, M9 minimum media components should be a subset of growth sensitive components. Hence, the presence of M9 minimal media components (sodium and sulfate) not identified by iAF1260 [18] to be impacting on growth rate (Figure 2) may indicate limitations of the model.

Importantly, our results show an objective value rather than an error in pFBA [23] when sulfate intake is set to zero to indicate the void of sulfate in the media, which suggests that sulfate is a non-requirement. This contradicts Kertesz and Cook [33], whom suggest an absolute requirement for sulfate and found a set of proteins induced as a result of sulfate limitation in *E. coli*. Similarly, sodium chloride has been shown to affect the growth rate of *E. coli* [34-36]. Although it may be possible to argue that effects of sodium chloride on growth rate can be confounded by chloride, which is a growth sensitive component; a study by Dupree., *et al*. [37] suggest differential effects of sodium chloride and calcium chloride on the growth of *E. coli*. This suggests that the effects of calcium, sodium, and chloride on *E. coli* growth rate is likely to be independent; hence, the effects of sodium being confounded by the effects of chloride is not likely.

Conclusion

Therefore, this study highlights a fundamental and crucial divergence between experimental results and simulation results. This is supported by a study by Khodayari and Maranas [38] suggesting poor correlation between experimental yields and simulated yields using iAF1260 [18]. This may have implications on the applications of iAF1260 [18] for media optimizations. However, this study also provides a direction to improve on iAF1260 [18] by focus on improving the correlation and minimizing the error between experimental and predicted growth rates in different media.

Author's Contribution

KCC, RYH, CJS, IZA, and JHF contributed equally to this study.

Conflict of Interest

The authors declare no conflict of interest.

Bibliography

1. Miyashira CH., *et al*. "Comparison of radial growth rate of the mutualistic fungus of *Atta sexdens rubropilosa* forel in two culture media". *Brazilian Journal of Microbiology* 41.2 (2010): 506-511.
2. Bich GA., *et al*. "Isolation of the symbiotic fungus of *Acromyrmex pubescens* and phylogeny of *Leucoagaricus gongylophorus* from leaf-cutting ants". *Saudi Journal of Biological Science* 24.4 (2017): 851-856.
3. Haugan MS., *et al*. "Comparative Activity of Ceftriaxone, Ciprofloxacin, and Gentamicin as a Function of Bacterial Growth Rate Probed by *Escherichia coli* Chromosome Replication in the Mouse Peritonitis Model". *Antimicrobe Agents Chemotherapy* 63.2 (2019): e02133-02118.
4. Greulich P., *et al*. "Growth-dependent bacterial susceptibility to ribosome-targeting antibiotics". *Molecular Systems Biology* 11.3 (2015): 796.
5. De Leersnyder I., *et al*. "Influence of growth media components on the antibacterial effect of silver ions on *Bacillus subtilis* in a liquid growth medium". *Science Report* 8.1 (2018): 9325.
6. Ratiu I-A., *et al*. "The effect of growth medium on an *Escherichia coli* pathway mirrored into GC/MS profiles". *Journal of Breath Research* 11.3 (2017): 036012.
7. Tavafi H., *et al*. "Screening of alginate lyase-producing bacteria and optimization of media compositions for extracellular alginate lyase production". *Iran Biomed Journal* 21.1 (2017): 48-56.
8. Pustake SO., *et al*. "Statistical media optimization for the production of clinical uricase from *Bacillus subtilis* strain SP6". *Heliyon* 5.5 (2019): e01756.
9. Podleśny M., *et al*. "Media optimization for economic succinic acid production by *Enterobacter* sp. LU1". *AMB Express* 7.1 (2017): 126.
10. Kamzolova SV., *et al*. "Fermentation Cconditions and media optimization for isocitric acid Production from ethanol by *Yarrowia lipolytica*". *BioMed Research International* (2018): 2543210.
11. McCloskey D., *et al*. "Basic and applied uses of genome-scale metabolic network reconstructions of *Escherichia coli*". *Molecular Systems Biology* 9 (2013): 661.
12. Mishra P., *et al*. "Genome-scale model-driven strain design for dicarboxylic acid production in *Yarrowia lipolytica*". *BMC System Biology* 12 (2018): 12.
13. Iranmanesh E., *et al*. "Improving l-phenylacetylcarbinol production in *Saccharomyces cerevisiae* by in silico aided metabolic engineering". *Journal of Biotechnology* 308 (2020): 27-34.
14. Kim M., *et al*. "In silico identification of metabolic engineering strategies for improved lipid production in *Yarrowia lipolytica* by genome-scale metabolic modelling". *Biotechnology and Biofuels* 12 (2019): 187.
15. Ashino K., *et al*. "Predicting the decision making chemicals used for bacterial growth". *Science Report* 9.1 (2019): 7251.
16. O'Brien EJ., *et al*. "Using genome-scale models to predict biological capabilities". *Cell* 161.5 (2015): 971-987.
17. Chen Y., *et al*. "An unconventional uptake rate objective function approach enhances applicability of genome-scale models for mammalian cells". *npj Systems Biology and Applications* 5 (2019): 25.

18. Feist AM., *et al*. "A genome-scale metabolic reconstruction for *Escherichia coli* K-12 MG1655 that accounts for 1260 ORFs and thermodynamic information". *Molecular Systems Biology* 3 (2007): 121.
19. Chang ED and Ling MH. "Explaining Monod in terms of *Escherichia coli* metabolism". *Acta Scientific Microbiology* 2.9 (2019): 66-71.
20. Cronan JE. "E*scherichia coli* as an experimental organism". In: John Wiley and Sons Ltd, editor. eLS [Internet]. Chichester, UK: John Wiley and Sons, Ltd (2014).
21. Cardoso JGR., *et al*. "Cameo: A Python library for computer aided metabolic engineering and optimization of cell factories". *ACS Synthetic Biology* 7.4 (2018): 1163-1166.
22. King ZA., *et al*. "BiGG Models: A platform for integrating, standardizing and sharing genome-scale models". *Nucleic Acids Research* 44 (2016): D515-522.
23. Lewis NE., *et al*. "Omic data from evolved E. coli are consistent with computed optimal growth from genome-scale models". *Molecular Systems Biology* 6 (2010): 390.
24. Abou-Taleb KA and Galal GF. "A comparative study between one-factor-at-a-time and minimum runs resolution-IV methods for enhancing the production of polysaccharide by Stenotrophomonas daejeonensis and Pseudomonas geniculate". *Annuals of Agriculture Science* 63.2 (2018): 173-180.
25. Razavi S and Gupta HV. "What do we mean by sensitivity analysis? The need for comprehensive characterization of "global" sensitivity in Earth and Environmental systems models: A Critical Look at Sensitivity Analysis". *Water Resource Research* 51.5 (2015): 3070-3092.
26. Akaike H. "Information theory and an extension of the maximum likelihood principle". In: Selected Papers of Hirotugu Akaike. Springer (1998): 199-213.
27. Ripley B., *et al*. "Package 'mass'". *Cran R* (2013)
28. Viana FAC. "A tutorial on Latin Hypercube Design of experiments". *Quality and Reliability Engineering International* 32.5 (2016): 1975-85.
29. M9 minimal medium (standard). *Cold Spring Harbor Protocol* (2010): pdb.rec12295.
30. Peterson CN., *et al*. "*Escherichia coli* starvation diets: Essential nutrients weigh in distinctly". *Journal of Bacteriology* 187.22 (2005): 7549-7553.
31. McCarthy BJ. "The effects of magnesium starvation on the ribosome content of *Escherichia coli*". 55.6 (1962): 880-889.
32. Lusk JE., *et al*. "Magnesium and the growth of *Escherichia coli*". *Journal of Biological Chemistry* 243.10 (1968): 2618-2624.
33. Kertesz MA., *et al*. "Proteins induced by sulfate limitation in *Escherichia coli*, Pseudomonas putida, or *Staphylococcus aureus*". *Journal of Bacteriology* 175.4 (1993): 1187-1190.
34. Omotoyinbo O and Omotoyinbo B. "Effect of varying NaCl concentrations on the growth curve of *Escherichia coli* and *Staphylococcus aureus*". *Cell Biology* 4.5 (2016): 31-34.
35. Goh DJ., *et al*. "Gradual and step-wise halophilization enables *Escherichia coli* ATCC 8739 to adapt to 11% NaCl". *Electron Physician* 4.3 (2012): 527-535.
36. How JA., *et al*. "Adaptation of *Escherichia coli* ATCC 8739 to 11% NaCl". *Dataset Papers in Biology* (2013).
37. Dupree DE., *et al*. "Effects of sodium chloride or calcium chloride concentration on the growth and survival of *Escherichia coli* O157:H7 in model vegetable fermentations". *Journal of Food Protection* 82.4 (2019): 570-578.
38. Khodayari A and Maranas CD. "A genome-scale *Escherichia coli* kinetic metabolic model k-ecoli457 satisfying flux data for multiple mutant strains". *Nature Communication* 7 (2016): 13806.