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Research Article

Consistency between *Saccharomyces cerevisiae* S288C Genome Scale Models (iND750 and iMM904)

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Abstract

Saccharomyces cerevisiae is an important experimental organism for industrial and scientific research with *S. cerevisiae* S288C as the first eukaryote genome sequenced. Genome-scale metabolic models (GSMs) are computational tools to explore metabolic engineering requirements. Currently, there are 2 major GSMs of *S. cerevisiae* S288C, iND750 and iMM904, which raises the question of whether they are consistent to each other. Here, we compare iND750 and iMM904 by examining the fluxomic changes resulting from single reaction knockouts. 40.5% to 50.3% (n = 637) of the reactions are common in both GSMs. Of which, 64 (10.0% of common reactions, or between 4.1% and 5.2% of the total reactions in each GSM) reaction knockouts resulted in significant fluxomic changes. This is significantly lower (t = -15.882, df = 30, p-value = 3.82E-16) from expected using randomization test, suggesting that iND750 and iMM904 are likely to be consistent with each other from the perspective of common reactions.

Keywords: *Saccharomyces cerevisiae*; Genome-scale Metabolic Models (GSMs)

Introduction

Saccharomyces cerevisiae is a recognized "workhorse for industry and scientific research" [1] given its extensive use in fermentation [2], including biofuel production [3,4], and contribution to eukaryotic genetics [5,6]. *S. cerevisiae* S288C is a reference strain [7] and is the first eukaryote genome sequenced in 1996 [8,9]; as well as being the template for Synthetic Yeast Genome project, also known as the Yeast 2.0 or Sc2.0 project [10]. Hence, it is plausible to conceive that *S. cerevisiae* S288C has been examined for biofuel production [11-13].

Computational modelling and simulation are important to explore suitability of organisms and evaluate engineering approaches to increase production of biofuels [14-17]. Genome-scale metabolic models (GSMs), which is based on steady-states of metabolites [18], have been used inform many metabolic engineering requirements [19,20]. For example, Zhang., *et al*. [21] used GSM to examine bottlenecks in ethanol production by *Caldicellulosiruptor bescii*. Underpinning these computational approaches is the relationship between genotype and phenotype, commonly known as genotype-phenotype relationship [22-25], where genomic perturbations (such as knockouts) results in changes in the fluxome. Fluxome can be defined as the set of metabolite conversion rates in a metabolic network [26-28]. Therefore, genomic perturbations may result in metabolomic changes, leading to phenotypic changes.

Currently, there are 2 major GSMs of *S. cerevisiae* S288C, iND750 [29] and iMM904 [30]. GSM iND750 [29] was based off iFF708 [31], the first GSM of *S. cerevisiae*. iMM904 [30], on the other hand, is an extension of iND750 [29] by largely expanding lipid, transport, and carbohydrate subsystems. Hence, the question of whether

they are consistent to each other arises as a recent study had examined 58 GSMs constructed from various strains *Escherichia coli* and found significant differences between GSMs constructed for the same strain [32]. However, a comparison between iND750 [29] and iMM904 [30] has not been carried out. In this study, we compare iND750 [29] and iMM904 [30] by examining the fluxomic changes resulting from single reaction knockouts. Of the 637 reactions common in both GSMs, 64 (10.0% of common reactions, or between 4.1% and 5.2% of the total reactions in each GSM) reaction knockouts resulted in significant fluxomic changes, which is significantly lower ($t = -15.882$, $df = 30$, p-value = 3.82E-16) than that expected using randomization test, suggesting that iND750 [29] and iMM904 [30] are likely to be consistent with each other from the perspective of common reactions.

Materials and Methods

GSMs iND750 [29] and iMM904 [30] were obtained from BiGG database [33]. Growth rate on native media given as proxy as output from the objective function [34] and fluxes after flux balance analysis [35] using Cameo [36], which was available via cameo-fba command from AdvanceSyn Toolkit [37]. The entire set of predicted fluxes obtained from a GSM is known as a predicted fluxome. Single reaction knockouts [38] were performed using cameo-mutant-fba command as previously described [39]. Each flux from knockout was normalized by dividing with the corresponding wildtype flux. Common reactions between the 2 GSMs were identified and their normalized fluxes were compared between the 2 GSMs using paired t-test with p-value threshold was corrected using Bonferroni correction [40]. The number of reaction knockouts resulting in significant fluxomes were tested using randomization [41-43] from 30 randomized sets of fluxomes.

Results and Discussion

GSMs iND750 [29] and iMM904 [30] were obtained from BiGG database [33]. iND750 [29] consists of 1059 metabolites, 750 genes, and 1266 reactions whereas iMM904 [30] consists of 1226 metabolites, 905 genes, and 1577 reactions. Of which, 637 reactions (50.3% of reactions of iND750 [29], and 40.4% of reactions of iMM904 [30]) are common across both GSMs. Of the 637 common reactions, only 64 (10.05%) of the reactions show significant (p-value ≤ 5.4E-05) differences after Bonferroni correction [40] in normalized fluxomes when knocked out (Table 1).

Randomization procedure [41,42] was used to generate null hypothesis of average number of reaction knockouts resulting in significant fluxomes. Procedurally, the knockout normalized fluxomes from one of the GSMs were held constant while the knockout normalized fluxomes from one of the GSMs were randomized. This resulted in normalized fluxomes mismatched between knockouts; for example, UGLT (UDPglucose-hexose-1-phosphate uridylyltransferase) knockout normalized fluxome from iND750 [29] was mismatched to TALA (transaldolase) knockout normalized fluxome from the iMM904 [30] but analyzed using paired t-test as matched fluxomes. Each randomized and mismatched fluxome pairs were used tabulate average number of reaction knockouts resulting in significant fluxomes, which represents null hypothesis. This was repeated 30 times to generate 30 randomized fluxome sets. From these 30 randomized fluxome sets, the average number of reaction knockouts resulting in significant fluxomes is 187.167 with standard deviation of 7.7552. Hence, 64 reaction knockouts resulting in significant fluxomes is significantly different from randomized mean of 187.167 ($t = -15.882$, $df = 30$, p-value = 3.82E-16). This suggests that the two GSMs in question, iND750 [29] and iMM904 [30] are more similar than random in terms of fluxomes, which implies similar simulated metabolism [44,45].

This result can be expected as iMM904 [30] was constructed by extending iND750 [29] but our results also provide a list of 64 reactions (in Table 1) that may result in significantly different fluxomes when knocked out. Moreover, only about 40 to 50% of the reactions are common, demonstrating that iND750 [29] is not a subset of iMM904 [30]. This may suggest that although both GSMs are relatively similar, there are still substantial differences. Nevertheless, this pair of GSMs may be suitable to test algorithms given its similarity between common reactions. This concept had been used by Whelan and King [46] whom tested their algorithm using iFF708 [31] and iND750 [29], an earlier pair before the construction of iMM904 [30].

Conclusion

Of the 637 reactions common in both GSMs which accounts for 40.4% to 50.3% of the total reactions in the GSMs, the number reaction knockouts that resulted in significant fluxomic changes is significantly lower (p-value = 3.82E-16) than expected, suggesting that iND750 [29] and iMM904 [30] are likely to be consistent with

No.	BiGG Reaction ID	Descriptive Name from BiGG	RMSE	Paired t-test p-value
1	EX_nmn_e	NMN exchange	0.921	8.7E-195
2	AASAD1	L-aminoadipate semialdehyde dehydrogenase NADPH	0.762	2.8E-122
3	UGLT	UDPglucose-hexose-1-phosphate uridylyltransferase	0.710	1.5E-99
4	TALA	Transaldolase	0.696	4.6E-92
5	CITtcm	Citrate transport mitochondrial	0.686	2.0E-90
6	2DDA7Ptm	2-Dehydro-3-deoxy-D-arabino heptonate-7-phohsphate mitochondrial transport via diffusion	0.680	1.1E-88
7	CITtbm	Citrate transport mitochondrial	0.680	1.1E-88
8	DDPAm	2-deoxy-D-arabino heptulosonate-7-phosphate synthetase mitochondrial	0.680	1.1E-88
9	E4Ptm	D-erythrose-4-phosphate mtiochondrial transport via diffusion	0.680	1.1E-88
10	ACt2r	Acetate reversible transport via proton symport	0.659	4.2E-66
11	34HPPt2m	3,4-hydroxyphenyl pyruvate mitochondrial transport via proton symport	0.687	1.0E-62
12	TYRTAim	Tyrosine transaminase irreversible mitochondrial	0.687	1.0E-62
13	TYRt2m	Tyrosine mitochondrial transport via proton symport	0.687	1.0E-62
14	CYSTL	Cystathionine b-lyase	7E+14	1.1E-62
15	ASPt2m	Aspartate mitochondrial transport via proton symport	0.892	1.2E-61
16	ASPTAm	Aspartate transaminase	0.913	5.0E-60
17	DDPA	3-deoxy-D-arabino-heptulosonate 7-phosphate synthetase	0.552	7.1E-53
18	AHSERL2	O-acetylhomoserine thiol lyase	0.669	6.1E-51
19	ACOATAm	Acetyl-CoA ACP transacylase	1.538	3.5E-49
20	ACOAO7p	Acyl-CoA oxidase hexadecanoyl-CoA peroxisomal	1.538	3.7E-47
21	ECOAH7p	3-hydroxyacyl-CoA dehydratase 3-hydroxyhexadecanoyl-CoA peroxisomal	1.538	3.8E-47
22	ASPK	Aspartate kinase	0.720	1.5E-39
23	ASAD	Aspartate-semialdehyde dehydrogenase	0.720	1.5E-39
24	DHFR	Dihydrofolate reductase	0.607	6.6E-36
25	TYRTAi	Tyrosine transaminase irreversible	0.634	9.0E-30
26	FACOAL181	Fatty acid CoA ligase octadecenoate	0.422	1.0E-29
27	FACOAL140	Fatty acid CoA ligase tetradecanoate	0.833	2.4E-25
28	FACOAL180	Fatty acid CoA ligase octadecanoate	2E+16	4.2E-24
29	TRIGS_SC	Triglycerol synthesis	0.365	1.2E-22
30	3MOPtm	3-Methyl-2-oxopentanoate transport diffusion mitochondrial	0.365	1.2E-22
31	THRD_Lm	L-threonine deaminase mitochondrial	0.365	1.2E-22
32	THRt2m	Threonine mitochondrial transport via proton symport	0.365	1.2E-22
33	DESAT18	Stearoyl-CoA desaturase n-C18:0CoA n-C18:1CoA	0.365	1.2E-22
34	ACACT1r	Acetyl-CoA C-acetyltransferase	0.365	1.2E-22
35	AKGDbm	Oxoglutarate dehydrogenase dihydrolipoamide S succinyltransferase	0.381	6.2E-22
36	EX_for_e	Formate exchange	0.360	8.0E-22
37	3C3HMPtm	2-Isopropylmalate transport diffusion mitochondrial	0.356	1.5E-21

38	ACACT1m	Acetyl-CoA C-acetyltransferase mitochondrial	0.354	2.0E-21
39	AASAD2	L-aminoadipate semialdehyde dehydrogenase NADH	0.354	2.0E-21
40	THRD_L	L-threonine deaminase	0.357	2.4E-21
41	ETOHt	Ethanol reversible transport	0.359	3.2E-21
42	EX_etoh_e	Ethanol exchange	0.359	3.2E-21
43	DHORTS	Dihydroorotase	0.352	3.5E-21
44	DB4PS	3,4-Dihydroxy-2-butanone-4-phosphate synthase	0.352	3.5E-21
45	ACGKm	Acetylglutamate kinase mitochondrial	0.523	6.5E-21
46	ACOTAim	Acteylornithine transaminase irreversible mitochondrial	0.523	6.5E-21
47	AGPRim	N-acetyl g-glutamyl phosphate reductase irreversible mitochondrial	0.523	6.5E-21
48	THRS	Threonine synthase	0.353	6.5E-21
49	FA160COAabcp	Fatty acyl CoA peroxisomal transport via ABC system	2.589	7.0E-20
50	DHAPtm	Dihydroxyacetone phosphate transport mitochondrial	1.322	4.3E-17
51	ATPS	ATPase cytosolic	0.396	2.9E-16
52	ASPTA	Aspartate transaminase	0.975	2.3E-15
53	FACOAL161	Fatty acid CoA ligase hexadecenoate	4E+14	4.4E-13
54	ACS	Acetyl-CoA synthetase	0.446	4.7E-11
55	ASPt2n	Aspartate nuclear transport via proton symport	0.483	5.6E-11
56	CBPtn	Carbamoyl phosphate nuclear transport via diffusion	0.483	5.6E-11
57	ACONT	Aconitate hydratase	0.886	6.6E-11
58	THRA	Threonine aldolase	0.375	4.0E-10
59	SUCCtm	Succinate transport mitochondrial	2.774	3.6E-09
60	ALDD2y	Aldehyde dehydrogenase	1.361	1.0E-06
61	ATPtp_H	ADPATP transporter peroxisomal	3.982	3.0E-06
62	CYSS	Cysteine synthase	22.44	1.8E-05
63	CITtam	Citrate transport mitochondrial	2.617	5.3E-05
64	DESAT16	Palmitoyl-CoA desaturase n-C16:0CoA n-C16:1CoA	0.428	5.4E-05

Table 1: Reaction knockouts resulting significant fluxome. Paired t-test p-value threshold after Bonferroni correction (n = 637) corresponds to p-value ≤ 7.85E-5.

each other from the perspective of common reactions.

Supplementary Materials

Data files for this study can be downloaded at https://bit.ly/iND750-vs-iMM904.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgement

Bibliography

1. Binati RL., *et al*. "Non-Conventional Yeasts for Food and Additives Production in a Circular Economy Perspective". *FEMS Yeast Research* 21.7 (2021): foab052.

2. Steensels J and Verstrepen KJ. "Taming Wild Yeast: Potential of Conventional and Nonconventional Yeasts in Industrial Fermentations". *Annual Review of Microbiology* 68 (2014): 61-80.

3. Liu Z., *et al*. "Yeast Synthetic Biology Advances Biofuel Production". *Current Opinion in Microbiology* 65 (2022): 33-39.

4. Lacerda MP., *et al*. "The Model System *Saccharomyces cerevisiae* Versus Emerging Non-Model Yeasts for the Production of Biofuels". *Life (Basel, Switzerland)* 10.11 (2020): 299.

5. Landry CR., *et al*. "Ecological and Evolutionary Genomics of *Saccharomyces cerevisiae"*. *Molecular Ecology* 15.3 (2006): 575-591.

6. Adames NR., *et al*. "Yeast Genetic Interaction Screens in the Age of CRISPR/Cas". *Current Genetics* 65.2 (2019): 307-327.

7. Mortimer RK and Johnston JR. "Genealogy of Principal Strains of the Yeast Genetic Stock Center". *Genetics* 113.1 (1986): 35-43.

8. Engel SR., *et al*. "The Reference Genome Sequence of *Saccharomyces cerevisiae*: Then and Now". *G3 (Bethesda, Md)* 4.3 (2014): 389-398.

9. Goffeau A., *et al*. "Life with 6000 Genes". *Science* 274.5287 (1996): 546, 563-567.

10. Pretorius IS and Boeke JD. "Yeast 2.0 - Connecting the Dots in the Construction of the World's First Functional Synthetic Eukaryotic Genome". *FEMS Yeast Research* 18.4 (2018): foy032.

11. Maurer MJ., *et al*. "Quantitative Trait Loci (QTL)-Guided Metabolic Engineering of a Complex Trait. *ACS Synthetic Biology* 6.3 (2017): 566-581.

12. Jacobus AP., *et al*. "Comparative Genomics Supports That Brazilian Bioethanol *Saccharomyces cerevisiae* Comprise a Unified Group of Domesticated Strains Related to Cachaça Spirit Yeasts". *Frontiers in Microbiology* 12 (2021): 644089.

13. McIlwain SJ., *et al*. "Genome Sequence and Analysis of a Stress-Tolerant, Wild-Derived Strain of *Saccharomyces cerevisiae* Used in Biofuels Research". *G3 (Bethesda, Md)* 6.6 (2016): 1757-1766.

14. Jin X., *et al*. "Modeling, Design Guidelines, and Detection Limits of Self-Powered Enzymatic Biofuel Cell-Based Sensors". *Biosensors and Bioelectronics* 168 (2020): 112493.

15. Leow S., *et al*. "A Unified Modeling Framework to Advance Biofuel Production from Microalgae". *Environmental Science and Technology* 52.22 (2018): 13591-13599.

16. Ali H., *et al*. "CFD and Kinetic-Based Modeling to Optimize the Sparger Design of a Large-Scale Photobioreactor for Scaling up of Biofuel Production". *Biotechnology and Bioengineering* 116.9 (2019): 2200-2211.

17. Pham N., *et al*. "Genome-Scale Metabolic Modeling Underscores the Potential of Cutaneotrichosporon oleaginosus ATCC 20509 as a Cell Factory for Biofuel Production". *Biotechnology for Biofuels* 14.1 (2021): 2.

18. van Rosmalen RP., *et al*. "Model Reduction of Genome-Scale Metabolic Models as a Basis for Targeted Kinetic Models". *Metabolic Engineering* 64 (2021): 74-84.

19. Xu C., *et al*. "Genome-Scale Metabolic Model in Guiding Metabolic Engineering of Microbial Improvement". *Applied Microbiology and Biotechnology* 97.2 (2013): 519-539.

20. Kim B., *et al*. "Applications of Genome-Scale Metabolic Network Model in Metabolic Engineering". *Journal of Industrial Microbiology and Biotechnology* 42.3 (2015): 339-348.

21. Zhang K., *et al*. "Genome-Scale Metabolic Model of Caldicellulosiruptor bescii Reveals Optimal Metabolic Engineering Strategies for Bio-based Chemical Production". *mSystems* 6.3 (2021): e0135120.

22. Li BT., *et al*. "Analyzing Transcriptome-Phenotype Correlations". *Encyclopedia of Bioinformatics and Computational Biology* (Elsevier) (2019): 819-824.

23. Jakutis G and Stainier DYR. "Genotype-Phenotype Relationships in the Context of Transcriptional Adaptation and Genetic Robustness". *Annual Review of Genetics* 55.1 (2021): 71-91.

24. Kemble H., *et al*. "Recent Insights into the Genotype-Phenotype Relationship from Massively Parallel Genetic Assays". *Evolutionary Applications* 12.9 (2019): 1721-1742.

25. Kabimoldayev I., *et al*. "Basics of Genome-Scale Metabolic Modeling and Applications on C1-Utilization". *FEMS Microbiology Letters* 365.20 (2018): fny241.

26. Cortassa S., *et al*. "From Metabolomics to Fluxomics: A Computational Procedure to Translate Metabolite Profiles into Metabolic Fluxes". *Biophysical Journal* 108.1 (2015): 163-172.

27. Winter G and Krömer JO. "Fluxomics - Connecting 'Omics Analysis and Phenotypes. *Environmental Microbiology* 15.7 (2013): 1901-1916.

28. Babele PK and Young JD. "Applications of Stable Isotope-Based Metabolomics and Fluxomics Toward Synthetic Biology of Cyanobacteria". *Wiley Interdisciplinary Reviews Systems Biology and Medicine* 12.3 (2020): e1472.

29. Duarte NC., *et al*. "Reconstruction and Validation of *Saccharomyces cerevisiae* iND750, A Fully Compartmentalized Genome-Scale Metabolic Model". *Genome Research* 14.7 (2004): 1298-1309.

30. Mo ML., *et al*. "Connecting Extracellular Metabolomic Measurements to Intracellular Flux States in Yeast". *BMC Systems Biology* 3 (2009): 37.

31. Förster J., *et al*. "Genome-Scale Reconstruction of the *Saccharomyces cerevisiae* Metabolic Network". *Genome Research* 13.2 (2003): 244-253.

32. Tan FL., *et al*. "Significant Differences in Media Components and Predicted Growth Rates of 58 *Escherichia coli* Genome-scale Models". *Acta Scientific Microbiology* 5.2 (2022): 56-68.

33. King ZA., *et al*. "BiGG Models: A Platform for Integrating, Standardizing and Sharing Genome-Scale Models". *Nucleic Acids Research* 44.D1 (2016): D515-D522.

34. Feist AM and Palsson BO. "The Biomass Objective Function". *Current Opinion in Microbiology* 13.3 (2010): 344-349.

35. Orth JD., *et al*. "What is Flux Balance Analysis?" *Nature Biotechnology* 28.3 (2010): 245-248.

36. 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.

37. Ling MH. "AdvanceSyn Toolkit: An Open Source Suite for Model Development and Analysis in Biological Engineering". *MOJ Proteomics and Bioinformatics* 9.4 (2020): 83-86.

38. Castillo S., *et al*. "Yeast Genome-Scale Metabolic Models for Simulating Genotype-Phenotype Relations. *Progress in Molecular and Subcellular Biology* 58 (2019): 111-133.

39. Wee YY., *et al*. "Genome-Scale Metabolic Model-Based Reactome-Phenome Map of Synechocystis sp. PCC 6803, A Potential Biofuel Producer". *Medicon Microbiology* 1.4 (2022): 2-8.

40. VanderWeele TJ and Mathur MB. "Some Desirable Properties of the Bonferroni Correction: Is the Bonferroni Correction Really So Bad?" *American Journal of Epidemiology* 188.3 (2019): 617-618.

41. Hooton JWL. "Randomization Tests: Statistics for Experimenters". *Computer Methods and Programs in Biomedicine* 35.1 (1991): 43-51.

42. Rodgers JL. "The Bootstrap, the Jackknife, and the Randomization Test: A Sampling Taxonomy". *Multivariate Behavioral Research* 34.4 (1999): 441-456.

43. Chua MT., *et al*. "Gene Co-Expressions Cannot Predict Protein-Protein Interactions in *Escherichia coli*". *EC Microbiology* 18.3 (2022): 102-109.

44. Aon M and Cortassa S. "Systems Biology of the Fluxome". *Processes* 3.3 (2015): 607-618.

45. Emwas A-H., *et al*. "Fluxomics - New Metabolomics Approaches to Monitor Metabolic Pathways. *Frontiers in Pharmacology* 13 (2022): 805782.

46. Whelan KE and King RD. "Using a Logical Model to Predict the Growth of Yeast". *BMC Bioinformatics* 9 (2008): 97.