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Research Article

Metabolite Overproduction Potential of *Saccharomyces cerevisiae* S288C Explored Using its Genome-Scale Metabolic Model, iMM904

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Abstract

While many genome-scale metabolic models (GSMs) had been constructed with specific purpose, it can be used to explore the potential of the organism for metabolite overproduction. *Saccharomyces cerevisiae* is a widely used microorganism in biotechnology with many successful applications and a GSM, iMM904, has been constructed for *S. cerevisiae* S288C. In this study, explore the metabolite overproduction capabilities of *S. cerevisiae* S288C given single gene knockout using iMM904. Our simulation results suggest that 217 of the 1577 (13.76%) single reaction knockouts potentially leads to metabolite overproductions. This suggests the potential of overproducing native metabolites using only gene knockouts and forms the basis of future validation studies between *S. cerevisiae* S288C and its corresponding GSM.

Keywords: *Genome-Scale Metabolic Models; Yeast; Metabolic Engineering; Gene Knockouts; Overproduction; iMM904*

Introduction

Computational modelling and simulation are important to explore suitability of organisms and evaluate various engineering approaches to increase production of various metabolites of interests [1-4]. Genome-scale metabolic models (GSMs), which are based on steady-states of metabolites [5], have been used to inform many metabolic engineering requirements [6-8]; such as to optimize succinate and various alcohol productions in yeast [9]. In fact, several GSMs were constructed with the purpose of optimizing metabolite production in mind. For example, iVS1191 was constructed for optimizing polyunsaturated fatty acid productions [10]. iCN1361 was constructed for the purpose of polyhydroxybutyrate production [11]. iThaps987 was constructed with the intent of fucoxanthin production [12], and iZBM1060 was constructed to overproduce extracellular polysaccharide [13]. While GSMs may be constructed with specific purposes, it may be possible to use GSMs in reverse; that is, to ask what metabolites may be overproduced given a specific number of knockouts.

Saccharomyces cerevisiae is one of the most widely used microorganisms in biotechnology [14,15] with successful applications in a wide variety of purposes; including, food fermentation, production of various enzymes and chemicals [16]. iMM904 is a GSM for *S. cerevisiae* S288C, which has been recently used to explore squalene production [17]. In this study, we use iMM904 to explore the metabolite overproduction capabilities of *S. cerevisiae* S288C given single gene knockout. Our simulation results suggest that 217 of the 1577 (13.76%) single reaction knockouts potentially increase fluxes, leading to metabolite overproductions.

Materials and Methods

GSM for *S. cerevisiae* S288C, iMM904 [18], was obtained from BiGG database [19]. Single reaction knockouts [20] were performed using Cameo [21], which was available via cameo-mutant-fba command from AdvanceSyn Toolkit [22], and its predicted fluxome was obtained. A predicted fluxome is defined as the entire set of predicted fluxes obtained from a GSM. A flux can be then defined as the

production rate of a metabolite [23,24]. Using wildtype fluxome as baseline, the ability to increase the production of a metabolite can be estimated as the maximum positive difference of fluxes between fluxes from knock outs and its corresponding wildtype flux. For example, a metabolite (where the metabolite of interest is the reaction product) can be produced at 2x the rate if the wildtype flux is 10 millimoles per hour per gram dry weight (mmol/h/gDW) and the maximum flux achievable from each single reaction knock out is 20 mmol/h/gDW.

Results and Discussion

GSM iMM904 consists of 905 genes and 1226 metabolites in 1577 reactions [18]. However, the supplementary materials by Mo., *et al*. [18] only provided information for 1412 reactions, 1228 metabolites and 905 genes. Of the 1412 reactions, 326 (23.1%) reactions were encoded by 2 or more genes with 717 (50.8%) encoded by 1 gene (Figure 1). The mean and median number of genes per reaction are 1.256 and 1, respectively. This is similar to the average of 0.981 gene per reaction (706 genes in 720 reactions) in *Escherichia coli* MG1655 [25]. Six reactions encoded by 7 genes (BiGG reaction ID 2HMHMBQMTm, 2HP6MPMOm, 2HPMBQMTm, 2HPMMBQMOm, 3DH5HPB-MTm, and METt2r), 3 reactions encoded by 8 genes (BiGG reaction ID 13GS, G12MT1g, and G12MT2g), 2 reactions encoded by 15 genes (BiGG reaction ID FRUt2, and MANt2) and 1 reaction encoded by 17 genes (BiGG reaction ID GLCt1).

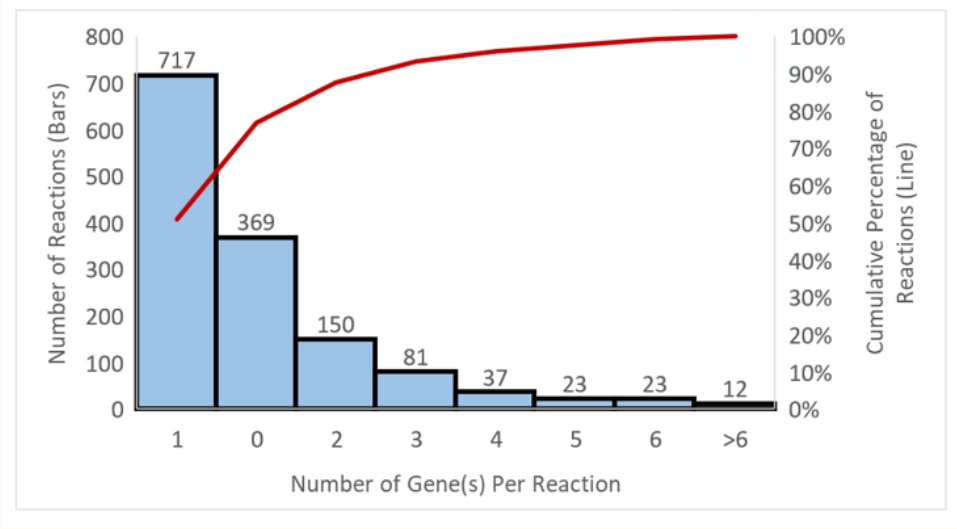

Figure 1: *Number of gene(s) per reaction.*

Reaction knockouts of each of the 1577 reactions were used identify potential increase in flux of a specific reaction as compared to wildtype. For example, the wildtype flux of BiGG reaction ID GALUi (UTP-glucose-1-phosphate uridylyltransferase for the reaction: D-glucose-1-phosphate + proton + UTP → diphosphate + UDP-glucose) is 0 mmol/h/gDW can be increased to the maximum of 0.483 mmol/h/gDW by one of the 1577 reaction knockouts. Our results suggest that 240 (15%) reaction knockouts potentially lead to increased fluxes in another reaction with a majority (75%) of the reaction knockouts resulting in no flux changes in any reactions as compared to wildtype (Figure 2). This is consistent with a study on *Synechocystis sp.* PCC 6803 using iJN678 showing that a majority of single reaction knockout have no significant fluxome differences as compared to wildtype [26].

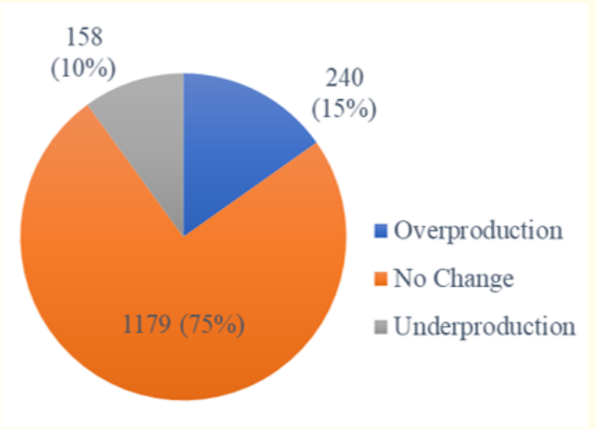

Figure 2: *Effects of single reaction knockouts on fluxes.*

Of the 240 increased fluxes suggesting potential overproductions of metabolites, 217 (90.4%) are considered potentially true overproductions, and 23 (9.6%) are considered false overproductions (Table 1). False overproductions are result of either negative flux for both wildtype and knockouts (56.5% of false overproduction and denoted as Class 1 false overproduction), which represents reduced usage rather than overproduction, or negative flux in wildtype and zero flux in knockouts (43.5% of false overproduction and denoted as Class 2 false overproduction), which represents reduced usage to no production.

Type	Count	Reaction List
Class 1 Overproduction (No production in Wildtype)	41	ACACT4p, ACACT5p, ACACT6p, ACACT7p, ACACT8p, ACOAO4p, ACOAO5p, ACOAO6p, ACOAO7p, ACOAO8p, ACRNtp, DDPAm, DUMPtn, E4Ptm, FA140COAabcp, FAO240p, FAO80p, FRDcm, G3PD1irm, G3PDm, GALUi, GK2, H2Otp, MALOAAtp, MCITL2m, MCITSm, MI13456PKn, MI1345PKn, MI145PKn, MI145Ptn, NMNt2p, NNMT, PI45BPP_SC, PMI12346PS, PPAm, RNDR1n, RNTR1, SERt2m, TRIGS_SC, TTCCOAtx, URIDK2rn
Class 2 Overproduction (Lower production in wildtype)	175	13GS, 2OBUTtm, 2OXOADPtim, AASAD2, AATA, ACACT1m, ACGKm, ACHBSm, ACLSm, ACONT, ACONT3m, ACONTm, ACOTAim, ADNK1, ADSK, AGPRim, AGTi, AHSERL2, AKGDam, AKGDbm, ANPRT, ANS, ARGSL, ARGSS, ASNS1, ASPCT, ASPK, ATPPRT, ATPS3m, ATPtm_H, BIOMASS_SC5_notrace, BPNT, C14STR, C24STRer, C3STDH1, C3STDH2, C3STKR1, C3STKR2, C4STMO1, C4STMO2, CATp, CBPS, CHLPCTD, CHLSTI, CHOLK, CHORM, CHORS, CRNtp, CSm, CSNATp,CTPS1, CYOOm, CYOR_u6m, CYSTGL, CYSTS, DAGCPT_SC, DB4PS, DHAD1m, DHAD2m, DHFR, DHORDfum, DHQS, DHQTi, DMATT, DOLPMMer, DOLPMTcer, DPMVD, DROPPRy, DRTPPD, ERGSTter, ERGTETROLter, EX_for_e, FBA3, FUMm, G5SADs, G5SD2, G6PDH2er, G6Pter, GCC2cm_copy1, GLCS2, GLU5K, GLUt7m, GMPS2, GRTT, GTPCII, HACNHm, HCITSm, HICITDm, HISTD, HISTP, HSDxi, HSERTA, HSK, HSTPT, ICDHym, ICDHyr, ICL, IG3PS, IGPDH, IGPS, ILEtmi, IMPD, IPDDI, IPMD, IPPSm, KARA1im, KARA2im, LNS14DMx, LNSTLS, MAN1PT, MCITDm, METAT, METS, MEVK2, MI1PP, MI1PS, MTHFR3, NDPK2, O2t, O2ter, O2tm, OAAt2m, OCBT, OMCDC, OMPDC, ORNt3m, ORNTACim, OXAGm, P5CR, PAPSR, PC, PDHm, PFK_3, PGI, PINOS_SC, PMDPHT, PMEVK, PPND2, PPNDH, PRAIi, PRAMPC, PRATPP, PRMICI, PSCVT, PYRt2m, RBFSa, RBFSb, RNDR2, RNDR4, RPE, SACCD1, SACCD2, SHK3Dr, SHKK, SLFAT, SO4ti, SQ23EPXter, SQLEr, SQLS, SQLter, SUCCtm, SUCD2_u6m, SULR, THRD_L, THRS, TKT1, TKT2, TMDS, TRDOXtp, TRDR, TRE6PP, TRE6PS, TRPS1, TYRTAi, UMPK
Class 3 Overproduction (Negative production in wildtype)	1	NADN
Class 1 False Overproduction (Negative production for both wildtype and knockouts)	13	ACt2r, ASPTA, CITtam, EX_nh4_e, EX_pi_e, FACOAL140, GLUDy, H2Ot, H2Otm, IMPC, OCDCEAt, PEtm_SC, RPI
Class 2 False Overproduction (Negative production for wildtype; no production from knockouts)	10	AMETtm, EX_nac_e, FA161tp, FACOAL161p, FAS240_L, G3PCt, GAT2_SC, HEXCCOAtx, NNAM, UGLT

Table 1: *List of knockouts resulting in overproduction.*

The 217 true overproductions can be separated into 3 classes. Class 1 overproduction is when there is no flux in the wildtype but positive flux in knockout(s), which accounts for 41 (18.9%) of true overproductions. An example of Class 1 overproduction is triglycerol synthesis (BiGG reaction ID TRIGS_SC for the reaction: decanoyl-CoA + dodecanoyl-CoA + hexadecenoyl-CoA + octadecenoyl-CoA + palmitoyl-CoA + stearoyl-CoA + tetradecanoyl-CoA + yeast-specific 1,2-diacylglycerol + octadecynoyl-CoA ↔ coenzyme A + yeast-specific triglyceride) with 0 mmol/h/gDW in wildtype and increased to the maximum of 0.0019 mmol/h/gDW in knockout(s). Similar work to overproduce fatty acids [27] and triglycerides [28] in *S. cerevisiae* had been attempted. Class 2 overproduction is when there is positive flux in the wildtype and higher positive flux in knockout(s), which accounts for 175 (80.6%) of true overproductions. An example of Class 2 overproduction is mitochondrial ubiquinone 6 succinate dehydrogenase (BiGG reaction ID SUCD2_u6m for the reaction: ubiquinone 6 + succinate ↔ fumarate + ubiquinol 6) with 0.268 mmol/h/gDW in wildtype and increased to the maximum of 0.344 mmol/h/gDW in knockout(s). Overproduction of fumarate in *S. cerevisiae* had also been attempted [29,30]. Class 3 overproduction is when there is negative flux in the wildtype but positive flux in knockout(s) and only one reaction in this class - NAD nucleosidase (BiGG reaction ID NADN for the reaction: water + nicotinamide adenine dinucleotide ↔ ADP-ribose + proton + nicotinamide) with -2.44E-15 mmol/h/gDW in wildtype and increased to the maximum of 1.74E-15 mmol/h/gDW in knockout(s).

Taken together, our results suggest a potential to engineer *S. cerevisiae* as a microbial factory for production of metabolites [31,32]. The main limitation of this study is that only single reaction knockouts, corresponding to an average of 1 to 2 gene knockouts, were considered while multiple gene manipulations had been considered in experimental studies, which may include gene knock-ins and over-expressions [33-37]. Furthermore, it has been shown that the predictive ability of iMM904 is much lower than that of *Escherichia coli*'s iAF1260 [38] despite a recent study illustrating a major limitation of iAF1260 [39]. Hence, this study can form the basis of future validation studies between *S. cerevisiae* S288C and its corresponding GSMs.

Conclusion

217 of the 1577 (13.76%) single reaction knockouts increase the metabolic flux across another reaction, suggesting the potential of *S. cerevisiae* S288C for overproduction of native metabolites.

Supplementary Materials

Supplementary material for this study can be downloaded at https://bit.ly/iMM904_opro.

Conflict of Interest

The authors declare no conflict of interest.

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