Supplementary material to: "The media composition as a crucial element in highthroughput metabolic network reconstruction"

Benedict Borer¹, Stefanía Magnúsdóttir²

1: Earth, Atmospheric and Planetary Sciences Department, Massachusetts Institute of Technology Cambridge, Massachusetts, USA

2: Department of Environmental Microbiology, Helmholtz Centre for Environmental Research-UFZ, Leipzig, Germany

Corresponding authors: Stefanía Magnúsdóttir, stefania.magnusdottir@ufz.de



MAG Stenotrophomonas maltophilia

		r ·	
0	0	0	0
0	0	0	0
5	5	0	1
4	4	0	0

Edwardsiella tarda

0	0	0	0
0	0	0	0
5	5	0	1
5	5	0	0

MAG Pantoea agglomerans

0	0	0	0
0	0	0	0
4	4	0	0
4	4	0	0

Abiotrophia defectiva				
0	13	0	0	
20	0	0	0	
20	20	0	2	
20	20	0	0	

Blautia producta

	,		
0	8	0	0
20	0	0	0
20	12	0	1
20	12	0	0

MAG Leclercia adecarboxylata

0	0	0	0
20	0	0	0
20	20	0	1
20	20	0	0

Yersinia frederiksenii				
0	0	0	0	

0	0	0	0	
3	3	0	0	
3	3	0	0	

Lactobacillus plantarum				
0	4	0	0	
20	0	0	0	
20	15	0	1	
20	15	0	0	

MAG Exiguobacterium

0	0	0	0
20	0	0	0
20	5	0	1
20	9	0	0

Pseudomonas putida

0	0	0	0
0	0	0	0
3	3	0	0
3	3	0	0

20 20 0 0

Clostridioides difficile

MAG Acinetobacter pittii

0	0	0	0
0	0	0	0
6	6	0	1
5	5	0	0

MAG Pseudomonas putida

0	0	0	0
0	0	0	0
-	-		
3	3	0	0
3	3	0	0

MAG Escherichia coli

0	0	0	0
0	0	0	0
3	3	0	0
3	3	0	0

Escherichia coli

Supplementary figure S1: Predicted number of auxotrophies for each species. Predicted auxotrophies are resolved be the media composition used for gap filling during network reconstruction (y-axis) and the media composition used for simulating growth (x-axis). For most species, reconstruction in complex media and simulation in defined media (lower left quadrant) resulted in the majority of predicted auxotrophies whereas reconstruction in minimal media and simulation in complex media (top right quadrant) resulted in no predicted auxotrophies.



Supplementary figure S2: Comparison of GapMind and GEM predicted auxotrophies. GEM predicted auxotrophies are taken from reconstructions on complex rich media (LB) simulated in glucose minimal media. NCBI genomes are used directly for the prediction of auxotrophies using GapMind for the same species using the web-interface. Overall, the GEM approach predicts a higher number of auxotrophies for the same species when compared with GapMind albeit at a similar trend.

	CTOTU49143	CTOTU49788	CTOTU50314	CTOTU46973	CTOTU47750
Glycolysis / Gluconeogenesis [0]				2	4
Citrate cycle (TCA cycle) [1]		3	3	3	
Pentose phosphate pathway [2] Pentose and glucuropate interconversions [3]	4			4	4
Fructose and mannose metabolism [4]	2				
Galactose metabolism [5]	2				4
Ascorbate and aldarate metabolism [6]		1			
Fatty acid biosynthesis [7]				4	
Fatty acid degradation [9]					
Synthesis and degradation of ketone bodies [10]					
Ubiquinone and other terpenoid-quinone biosynthesis [11]	16	16	16	8	24
Purine metabolism [12] Purimidine metabolism [13]	11		5	5	20
Alanine aspartate and glutamate metabolism [14]	4		7	8	8
Tetracycline biosynthesis [15]					
Glycine serine and threonine metabolism [16]		1	_	12	4
Cysteine and methionine metabolism [17]	1	1	5		1
Valine leucine and isoleucine biosynthesis [19]	4	4	4	4	4
Lysine biosynthesis [20]		4	4	10	16
Lysine degradation [21]					
Arginine and proline metabolism [22]			9	4	8
Histidine metabolism [23]			4	12	
Phenylalanine metabolism [24]					2
Benzoate degradation [26]					_
Tryptophan metabolism [27]					
Phenylalanine tyrosine and tryptophan biosynthesis [28]				4	12
Novobiocin biosynthesis [29] Benzovazipoid biosynthesis [30]					
beta-Alanine metabolism [31]					8
Taurine and hypotaurine metabolism [32]					-
Phosphonate and phosphinate metabolism [33]					
Cyanoamino acid metabolism [34]				0	0
D-Glutamine and D-glutamate metabolism [35]				0	0
Glutathione metabolism [37]	1			4	4
Starch and sucrose metabolism [38]	4				4
Amino sugar and nucleotide sugar metabolism [39]	2				20
Streptomycin biosynthesis [40]				12	
Butirosin and neomycin biosynthesis [41]				12	
Lipopolysaccharide biosynthesis [43]	16	16	36	20	36
Peptidoglycan biosynthesis [44]				32	20
Glycerolipid metabolism [45]					
Glycerophospholipid metabolism [46]					4
Pyruvate metabolism [48]					
Dioxin degradation [49]					
Xylene degradation [50]					
Aminobenzoate degradation [51]	1	F	1	1	2
Propanoate metabolism [52]	4	5	5	3	4
Butanoate metabolism [54]	4	3	2	2	3
One carbon pool by folate [55]			1	8	
Methane metabolism [56]				10	4
Carbon fixation in photosynthetic organisms [57]	3		1	4	
Thiamine metabolism [59]	1	1	1	13	5
Riboflavin metabolism [60]	2	4	11	8	6
Vitamin B6 metabolism [61]	4				
Nicotinate and nicotinamide metabolism [62]	1		10	4	8
Pantotnenate and COA biosynthesis [63] Biotin metabolism [64]	4	4	6	18	/
Folate biosynthesis [65]				8	4
Atrazine degradation [66]					
Porphyrin and chlorophyll metabolism [67]	12	8	7	25	29
Ierpenoid backbone biosynthesis [68]	16	12	16	16	24
Sulfur metabolism [70]	1		2		4
Isoquinoline alkaloid biosynthesis [71]					7
Tropane piperidine and pyridine alkaloid biosynthesis [72]					
Glucosinolate biosynthesis [73]			2		
Aminoacyl-tRNA biosynthesis [74]					1
Biosynthesis of siderophore group nonribosomal peptides [76]				4	4
Biosynthesis of vancomycin group antibiotics [77]				4	
1					
l					
C) 5	10	15 20	25	30 35

Supplementary figure S3: Pathway resolved gap filled reactions for low quality MAGs. Low quality MAGs that required negligible gap filling (CTOTU49143, CTOTU49788 and CTOTU50314) show no unique pattern when compared to medium quality MAGs where substantial gap filling was required for growth (CTOTU46971 and CTOTU47750). Gap filled reactions are shown as the sum for all reconstructions across all different media compositions used for gap filling.

Supplementary table S1: Average number of auxotrophies for all GEMs derived from MAGs depending on the reconstruction media and simulation media differentiated by the MAG quality. Each row corresponds to a reconstruction media, the corresponding simulated media are shown as columns. Data is shown as mean with standard deviation in brackets. HQ = high quality MAGs (\geq 90% complete), MQ = medium quality MAGs (<90% complete).

		Simulation media							
		Citrate Gluc		cose	NMS		LB		
		HQ	MQ	HQ	MQ	HQ	MQ	HQ	MQ
on	Citrate	0 (0)	0 (0)	0.18 (0.86)	0.80 (1.69)	0 (0)	0 (0)	0 (0)	0 (0)
tructi dia	Glucose	0.63 (3.50)	0.40 (1.26)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
const me	NMS	4.32 (3.83)	4.30 (2.79)	4.01 (2.76)	4.70 (3.20)	0 (0)	0 (0)	0.05 (0.22)	0.10 (0.32)
Rec	LB	4.36 (3.83)	4.30 (2.79)	4.08 (2.77)	4.90 (3.78)	0 (0)	0 (0)	0 (0)	0 (0)

Supplementary table S2: Fraction of GEMs derived from MAGs that are able to produce biomass depending on the reconstruction media and simulation media. Each row corresponds to a reconstruction media, the corresponding simulated media are shown as columns.

		Simulation media					
		Citrate	Glucose	NMS	LB		
Reconstruction media	Citrate	1	0.94	1	1		
	Glucose	0.96	1	1	1		
	NMS	0	0	1	0.94		
	LB	0	0	1	1		