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Tarefas	2023								2024			
	mai	jun	jul	ago	set	out	nov	dez	jan	fev	mar	abr
Tarefa 1: Avaliação das vias metabólicas associadas ao metano												
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Figure 1. Work plan timeline

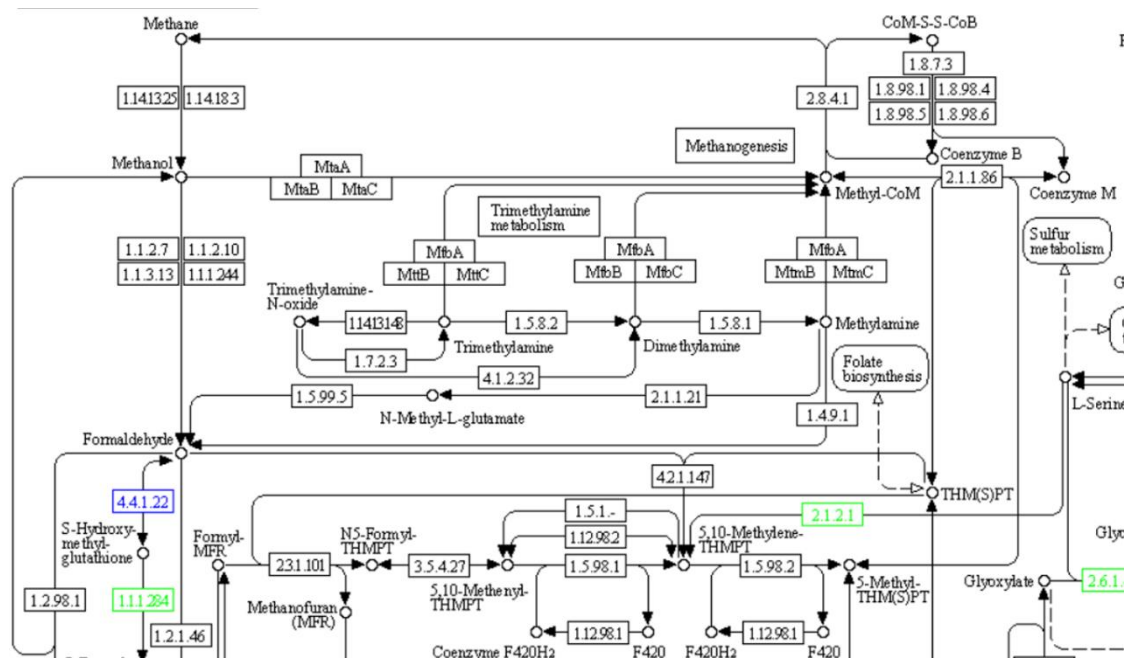


Figure 2. Segment of the methane metabolism of *C. vulgaris*, highlighting the absence of reactions and enzymes associated with it colored by *merlin*. Image extracted from KEGG.

The colors provided by *merlin* are interpreted as follows:

Green: The enzyme and reaction are present in the model;

Dark Blue: The reaction is present in the pathway, but the correspondent enzyme is missing; however, another enzyme present in another pathway might be associated with the reaction;

Cyan Blue: The enzyme is present in the model, but the reaction is connected to a dead-end;

Red: The enzyme is present in the network, but the reaction is a blocked and associated with a dead-end metabolite;

Colorless (Black): The enzyme is not present in the network (gap)

Table 1. Tools available for assisting the reconstruction of GSM models and generic biological databases

	Name	Web address	Reference
<i>Databases</i>	NCBI	https://www.ncbi.nlm.nih.gov/	[1]
	GOLD	https://gold.jgi.doe.gov/	[2]
	BRENDA	http://www.brenda-enzymes.org/	[3]
	KEGG	http://www.kegg.jp/	[4]
	KBase	https://www.kbase.us/	[5]
	BIGG models	http://bigg.ucsd.edu/	[6]
	BioCyc	https://biocyc.org/	[7]
	TCDB	https://www.tcdb.org/	[8]
	CCAP	https://www.ccap.ac.uk/	
<i>Tools</i>			
	BLAST		[9]
	LocTree3	https://roslab.org/services/loctree3/	[10]
	DeepLoc	https://services.healthtech.dtu.dk/services/DeepLoc-2.0/	[11]
	COBRA Toolbox		[12]
	MEWpy		[13]
Aiding software			
	Merlin	http://www.merlin-sybio.org/	[14]
	AureMe		[15]
	ModelSeed		[16]
	Raven Toolbox		[17]
	CarveMe		[18]
	Pathway Tools		[19]

Table 2. Biomass composition

Macromolecule	mass % (g MM/gDW)					Mean	Normalized
Protein	0,52	0,54	0,37	0,51	0,29	0,400	<i>0,457</i>
DNA	-	-	-	-	0,001	0,001	<i>0,001</i>
RNA	-	-	-	-	0,028	0,0028	<i>0,032</i>
Lipid	0,09	0,018	0,26	0,12	0,11	0,158	<i>0,180</i>
Carbohydrates	0,09	0,015	0,3	0,12	0,53	0,240	<i>0,274</i>
Pigments	-	-	-	-	0,04	0,040	<i>0,045</i>
Cofactors	-	-	-	-	0,01	0,010	<i>0,011</i>
Total						0,08795	<i>1</i>
Reference	[20]	[21]	[22]	[23]	[24]		

Table 3. RNA composition [25]

Metabolite	Chemical Formula	mmolmonomer/gDW	mmolmonomer/ge-RNA
UTP	$C_9H_{11}N_2O_{15}P_3$	0,058615616	1,841158
GTP	$C_{10}H_{12}N_5O_{14}P_3$	0,104205539	3,27317
CTP	$C_9H_{12}N_3O_{14}P_3$	0,104205539	3,27317
ATP	$C_{10}H_{12}N_5O_{13}P_3$	0,058615616	1,841158

Table 4. Carbohydrates composition [26].

Metabolite	MW - dTDP or UDP or H₂O (g/mol)	g M/ gDW (%)	g M/ gDW	mmol M / gDW	g M/ gDW (normalized)	mmol M / g MM
<i>UDP-L- arabinose</i>	186,18	0,72	0,0072	0,038672253	0,010464834	0,14122732
<i>UDP-D-Xylose</i>	436,29	0,57	0,0057	0,013064705	0,00828466	0,047711036
<i>UDP-alpha-D- galactose</i>	145,13	4,36	0,0436	0,300420313	0,063370385	1,097105876
<i>GDP-mannose</i>	144,1211	0,68	0,0068	0,047182543	0,009883454	0,172306076
<i>UDP-glucose</i>	147,1418	9,91	0,0991	0,673499984	0,144036815	2,45955669
<i>Starch</i>	48660,2	2,6	0,026	0,000534318	0,037789679	0,001951276
Total	49848,1929	18,84	0,1884	1,073374115	0,273829828	3,919858274

Table 5. Fatty-acid composition [27].

Metabolite	Molar fraction	MW (g/mol)	MW of Each Fatty Acid in the final Fatty Acid (g/mol _{Fatty Acid})
Myristic acid	0,01	227,37	2,2737
Pentadecanoic acid	0,004	241,4	0,9656
Palmitic acid	0,164	255,41	41,88724
Palmitoleic acid	0,015	253,41	3,80115
Margaric acid	0,012	269,45	3,2334
Oleic acid	0,042	281,47	11,82174
5-Octadecenoic acid	0,003	281,5	0,8445
7,10-Hexadecadienoic acid	0,204	251,4	51,2856
11-Octadecenoic acid	0,02	281,46	5,6292
Linoleic acid	0,351	279,45	98,08695
Stearic acid	0,028	283,48	7,93744
Nonadecylic acid	0,002	297,5	0,595
Linolenic acid	0,052	277,43	14,42636
Arachidic acid	0,057	311,53	17,75721
9-Eicosenoic acid	0,004	310,5	1,242
Eicosadienoic acid	0,013	308,5	4,0105
Homolinolenic acid	0,004	306,48	1,22592
Heneicosylic acid	0,002	325,56	0,65112
Arachidonic acid	0,006	303,47	1,82082
Behenic acid	0,009	339,58	3,05622
Total	1,002		

Supplementary text

Biomass composition

The overall composition of biomass is given on table 2. The determination of the composition of DNA and protein is automatically provided by merlin through the tool e-BiomassX [28]. Composition of RNA, carbohydrates and fatty acids is stated on tables 3, 4 and 5, respectively. Future work will involve gathering experimental data for lipids and cofactors on *Chlorella vulgaris*.

Medium composition

The common medium composition for *C. vulgaris* is: H₂O, oxygen, orthophosphate, CO₂, sulfate, H⁺, photon, nitrate, magnesium cation and Fe²⁺. The missing compounds were added as drains to the model which then automatically generated transport reactions.

Scripts

The research group involved in this project has developed scripts to facilitate the gap-filling process. In these scripts, the EC number of a missing reaction is inputted in the python file, which then retrieves BLAST results containing genes associated with corresponding e-values. (search_ec_in_genome.py)

Additionally, a jupyter notebook for simulating the flux on pathways was created to determine whether each pathway was functional and producing the required metabolites. (pathway_fluxes.ipynb)

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